June 1988

SGR-92

Proceedings

Twelfth Annual Conference

of the

Tropical and Subtropical Fisheries Technological Society

of the

Americas

Florida Sea Grant College Program

Ordering Conference Proceedings

Copies of annual proceedings from the second through the eleventh are available from the Texas Sea Grant College Program. Please note that the tenth annual proceedings contains a cumulative index and that no copies of the first annual proceedings are still available for purchase. Prices for early Proceedings follow: second through eighth, $10; ninth, $12; tenth and eleventh, $15. Please make checks payable to Texas A&M University and mail with your order to **Texas Sea Grant College Program, Texas A&M University, College Station, TX 77843-4115.**

This twelfth proceedings may be purchased by a $15 check made payable to the University of Florida. Please request SGR-92 and mail your check and order to the **Florida Sea Grant Extension Program, G022 McCarty Hall, University of Florida, Gainesville, FL 32605.**

Twelfth Annual Conference

Tropical and Subtropical Fisheries Technological Society of the Americas

Proceedings

from the first joint conference with

Atlantic Fisheries Technological Society

November 9–11, 1987
Orlando, Florida

compiled by
W. Steven Otwell

Seafood Technology Specialist
Florida Sea Grant Extension Program
Food Science and Human Nutrition Department
Institute of Food and Agricultural Sciences
University of Florida, Gainesville

The Tropical and Subtropical Fisheries Technological Society of the Americas is a professional, educational association of fishery technologists interested in the application of science to the unique problems of production, processing, packaging, distribution, and utilization of tropical and subtropical fishery species.

These papers are compiled and reproduced with limited editions and without formal review by the Florida Sea Grant College Program. Content is the sole responsibility of the author(s). The Conference intent is to compile preliminary results from ongoing research and advisory services to facilitate communication between regional investigators, industries, and government entities. Reproduction in this Proceedings does not preclude supplementation and further publication of work in reviewed journals or similar publications.

Florida Sea Grant College Program
G022 McCarty Hall, University of Florida, Gainesville, FL 32611

June 1988

Price: $15.00

TABLE OF CONTENTS

Abstracts

The following abstracts represent conference presentations not submitted as full length papers. The Tropical/Subtropical Fisheries Technological Society encourages publication of all presentations, but publication is an author's option.

PURIFICATION AND CHARACTERIZATION OF PHENOLOXIDASES IN CRUSTACEANS: SHRIMP AND LOBSTER

Maurice R. Marshall, Benjamin K. Simpson[1], Obdulio Ferrer[2], and W. Steven Otwell
Food Science and Human Nutrition Department, University of Florida, Gainesville, FL 32611

[1]Department of Food Science & Agric. Chem., Macdonald College of McGill Univ., St. Anne de Bellevue, P.Q., Canada H9X 1C0.

[1]Facultad de Agronomia, Universidad del Zulia, Maracaibo, Venezuela.

INTRODUCTION

Phenoloxidases' (PO) from crustacean species (shrimp, lobster and crab) have been studied in some detail in this laboratory (Marshall et al. 1984; Ferrer et al. 1986; Simpson et al. 1987). It has been suggested by various researchers that PO's are responsible for: melanosis (Wagner and Finne, 1986), sclerotization (hardening) after molting (Cobb, 1977), wound repair in the integument, and may be indirectly involved in the calcification of the cuticle (Stevenson, 1985).

Melanosis or blackspot is a dark discoloration which is unattractive to consumers and reduces the market value of crustaceans. In a study by Koburger et al. (1985), melanosis developed in lobster abdomens stored on ice at 4°C. An intense blackening of the epidermis occurred with the dark pigment migrating into the muscle tissue. In some lobsters, the appearance of melanosis was obvious after 2 days of storage, while other lobsters took up to 5 days to blacken. Sulfiting agents have been used to prevent melanosis in shrimp. However, consumer and government concerns about the safety of sulfites added to foods have triggered a search for suitable alternatives. At this time, no suitable alternative other than bisulfite has been found (Taylor and Bush, 1983; Otwell and Marshall, 1986).

The solution to this problem may lie in the investigation of kinetic and other properties of the purified enzyme(s) to obtain a better understanding of the biochemical basis of phenoloxidase action and the melanosis phenomenon. The present paper describes kinetic and some other properties of PO from crustaceans (two species of shrimp and one species of lobster) which undergo melanosis to varying degrees.

MATERIALS AND METHODS

Dihydroxyphenylalanine (DOPA), CNBr-activated Sepharose 4B and bovine pancreas trypsin (type III) were purchased from Sigma Chemical Company. Para-amino benzoic acid (PABA) was purchased from Fisher Scientific Company and standard low molecular weight markers for polyacrylamide gel electrophoresis were obtained from Bio-Rad. The shrimp obtained from Key West, Florida, were kept on ice and transported (<1 day) to the laboratory. The carapace was removed and rapidly frozen in liquid nitrogen, comminuted to a fine powder in a Waring blender, and then stored at -30°C until needed. Lobsters (Panulirus argus) were obtained from the Florida Keys and maintained in tanks with flow-through sea water circulation at the Whitney Marine Laboratory, Marineland, FL. Water temperature was not controlled, and varied according to the season. Every month, during the 1985-86 season, 2-3 lobsters were selected at random for PO analysis; a total of 26 lobsters were analyzed for seasonal variation of PO levels. PO levels were also measured in lobsters (24 specimens) at different molt stages: molt (newly molted lobsters), postmolt, intermolt and premolt as described by Stevenson (1985). The different molt stages were determined by morphological changes in the epidermis, by the hardness of the exoskeleton, by following the development of setae in the pleopods and by the appearance of an ecdysial line along the branchiostegites, which is characteristic of a premolt lobster (Travis, 1954). Abdomens of the lobster were removed and immediately frozen and stored at -20°C. Only cuticle on the dorsal surface of the abdomen was used for the PO determinations.

Extraction of phenoloxidase:

The method used for extracting PO was a slight modification of one described previously (Simpson et al., 1987). Extraction involved stirring the shrimp powder in 0.05 M sodium phosphate buffer (pH 7.2, containing 0.5 M NaCl) with 0.2% "Brij 35" at a ratio of 1:3 (w/v) for 3 h at 4°C. The homogenate was centrifuged at 8,000xg (4°C) for 30 min and the clear supernatant was fractionated with solid ammonium sulfate. The fraction precipitating between 0% to 40% saturation was collected by centrifugation at 12,500xg (4°C) for 30 min. The precipitate was redissolved in extraction buffer and dialyzed against 3 changes of 6 L of 0.05 M sodium phosphate buffer (pH 7.2).

For lobsters the crude PO extract was prepared by homogenizing (Waring blender) lobster cuticle with 0.05 M potassium phosphate buffer (1:4 w/v; pH 7.2) for 2 min (four homogenizations of 30 sec each) at 4°C. The homogenate

was then centrifuged at 10,000g for 20 min at 4°C. The supernatant (crude extract) was filtered through Whatman # 1 filter paper and stored at -20°C until assayed (<24 h).

Affinity chromatography:

The dialyzed ammonium sulfate fraction was pumped (12 mL/h) onto a column packed with Sepharose 4B-p-aminobenzoate material. Unbound material was thoroughly washed off the column using extraction buffer and PO was eluted with 0.05 M sodium phosphate buffer (pH 6.5, containing 1.0 M NaCl). The affinity fraction was dialyzed against 6L of 0.05 M sodium phosphate buffer, pH 6.5. This technique was used to purify shrimp PO.

Protein Determination:

Protein content of the crude extract was determined using the biuret method (Clark and Switzer, 1977): 1 mL of the crude extract was placed in a test tube and 4 mL of biuret reagent added, vortexed for a few seconds, and allowed to stand for 30 min at room temperature. The absorbance was then measured at 540 nm using a spectrophotometer. Bovine serum albumin (BSA) was used as the standard protein.

Determination of Phenoloxidase Activity:

PO activity was quantified by spectrophotometric analysis of the chromophore generated at 475 nm under the following conditions: 0.1 mL of the crude enzymatic preparation, 2.8 mL of 5 mM DL-ß-3,4-dihydroxyphenylalanine (DL-DOPA, substrate) in 0.05 M phosphate buffer (pH 6.5), with and without trypsin at 25°C (Savagaon and Sreenivasan, 1978). PO activity was defined as moles of DL-DOPA transformed min^{-1} under the test conditions. The assay of shrimp PO activity, the influence of pH and the effect of temperature on the activity and / or stability of PO, were investigated using the spectrophotometric procedure described previously (Simpson et al., 1987), with DOPA as substrate.

Kinetic properties of phenoloxidases:

The kinetic parameters, V_{max} and K_m' were estimated at 25°C by the least squares method of Johansen and Lumry (1961) using DOPA (0.5 mM to 4.0 mM) as substrate. The "physiological efficiencies" (V_{max}/K_m') of the shrimp enzymes were determined using the procedure by Pollock (1965) and Mihalyi (1978).

Electrophoresis and molecular weight determination:

Electophoresis of the purified shrimp and lobster enzyme was performed under reducing (Laemmli, 1970) and non-reducing (Davis, 1964) conditions. Protein identification on the gel was by staining with Coomassie Brillant Blue (R-250) in 25% propan-2-ol, 10% acetic acid, 0.1% cupric acetate. The standard protein markers used to estimate the molecular weights of the enzymes were phosphorylase b, 92.5 kDA; bovine serum albumin, 66 kDA; ovalbumin, 45 kDA; carbonic anhydrase, 31 kDA; soybean trypsin inhibitor, 21 kDA; lysozyme, 14.4 kDA.

RESULTS AND DISCUSSION

Phenoloxidases' (PO) were isolated and purified from white (Penaeus setiferus), pink (P. duorarum), and Florida spiny lobster (Panulirus argus). PO from both shrimp sources migrated as a single protein band in polyacrylamide gels. Lobster PO showed a large protein band at the top of the gel which disappeared during refrigeration storage while activity increased (Table 1). Electrophoresis of the extract at each storage time showed the development of a smaller protein band (EAPO, R_f=0.48) of high activity (based on activity staining). Trypsin also caused the disappearance of the large PO band and the subsequent appearance of two smaller protein bands on electrophoresis, one of R_f=0.46 (TAPO1) having low activity and one of R_f=0.52 (TAPO2) having activity similar to EAPO.

Table 1. Lobster PO activity in extracts stored in the refrigerator at 4°C for 4 days.

Day	Relative PO Activity (%)
0	2
1	36
2	51
3	100
4	77

Kinetic studies performed on the purified enzymes are presented in Table 2. PO's from various crustaceans differ in size. The shrimp PO's are the smallest in size while the inactive PO (IPO1) from lobster is very large. After activation, the PO's formed from IPO1 either spontaneously or by trypsin are still twice as large as the shrimp PO's.

The pH optima for the oxidation of DOPA by all crustacean PO's was around neutral. Pink shrimp PO and EAPO were slightly higher with optima around pH 8.0. The stability of the PO's was very different depending on the source and form. White shrimp PO was stable at slightly acidic to neutral pH while pink shrimp PO was stable over a broader pH range (6.5 to 9.0). The pH stability curve for pink shrimp PO was similar to that of white shrimp PO (Simpson et al., 1987), however, pink shrimp PO appeared to be less susceptible to inactivation at alkaline pH (>8.0) than its white shrimp counterpart. IPO1 from lobster was the most stable at higher alkaline pH of 10 to 12. The activated forms TAPO2 and EAPO were also stable at alkaline pH but the TAPO2 had a very narrow stability region while EAPO had the largest region for stability. The observed differences in pH stability supports the inference made from the molecular weight differences that there probably exists some structural differences between pink and white shrimp PO's and the various forms of PO's from lobster.

The influence of temperature on the stability of PO from crustaceans indicates that the enzymes appeared to have similar stability regions (20 - 50°C). Pink shrimp PO was more heat-labile than white shrimp PO. For instance, about 35% of the original activity of pink shrimp PO was destroyed after 30 min at 50°C while the original activity of white shrimp PO remained practically unchanged after the same treatment. Of the 3 PO lobster forms, IPO1 was the most stable with TAPO2 and EAPO having similar temperature stabilities. Although IPO1 was the most stable of the lobster PO forms, it was still lower than the white shrimp PO. The difference in the susceptibility to heat inactivation also points to possible differences in the structures of the two protein molecules.

The V_{max} for the oxidation of DOPA was greater for the reaction catalyzed by pink shrimp PO than that catalyzed by its white shrimp counterpart (Table 2). The K_m' for the pink shrimp PO-DOPA reaction was also relatively lower than that of white shrimp PO-DOPA reaction (Table 2). The V_{max}/K_m' of pink shrimp PO was determined to be greater than that of white shrimp PO. The findings from the kinetic study, a higher substrate turnover, a greater substrate binding affinity as well as a superior physiological efficiency for the pink shrimp PO, all support the practical observation that pink shrimp undergo dark discoloration or melanosis much more rapidly and extensively after catching and during iced storage than white shrimp. The energies of activation (E_a) estimated for the reaction catalyzed by the two enzymes were 11.5 Kcal/mole (pink shrimp PO) and 13.9 Kcal/mole (white shrimp PO). The thermodynamic parameters for the reaction catalyzed by the two enzymes were similar (Table 2). Previous works by Low et al., (1973) and Simpson

Table 2. Kinetic and thermodynamic properties of crustacean PO.

Parameter	White Shrimp PO	Pink Shrimp PO	Lobster PO IPO1	Lobster PO TAPO2	Lobster PO EAPO
Molecular weight (kDA)	30	40	>300[a]	64	62
K_m' (mM)	2.83	1.63	0.81	0.36	0.92
pH optima	7.5	8.0	7.0	7.0	8.0
pH stability	6.0-7.5	6.5-9.0	10.0-12.0	7.0-9.0	6.0-10.0
Temp. stability	25-50	20-40	25-40	20-30	25-35
V_{max} X 10^3 (Units/mg PO)	3.10	5.60	NO[b]	NO	NO
V_{max}/K_m' X 10^3	1.10	3.51	NO	NO	NO
Entropy (e.u.)	-3.70	-8.87	NO	NO	NO
E_a (kcal)	13.90	11.54	12.2	7.8	10.3
Enthalpy (Kcal/mole)	14.40	12.25	NO	NO	NO
Free Energy (Kcal/mole)	15.90	14.94	NO	NO	NO
Trypsin Activation	-	-	+		
Copper	+	-	-	-	-

[a]Estimated by column chromatography on Sephacryl-300.
[b]Not Observed (NO).

and Haard (1984) have shown that 'free energy' values for reactions catalyzed by homologous enzymes are similar, and it may be assumed from these observations that the free energies of activation for reactions catalyzed by homologous enzymes are relatively fixed. However, the relative contributions by 'entropy' and 'enthalpy' to 'free energy' were different for the two phenoloxidases. Entropy appeared to be more important in lowering the free energy barrier in the case of pink shrimp PO, while for white shrimp PO, enthalpy was relatively more important.

Thermodynamic parameters were not performed on the 3 lobster PO forms (IPO1, TAPO2, and EAPO). However, K_m' and E_a values indicated that all three forms were different in their ability to catalyze the PO-DOPA reaction (Table 2). Of interest is the comparison between TAPO2 and EAPO in terms of K_m' and E_a. For the DOPA catalyzed reaction it appears that TAPO2 is more suitable for this reaction, however, TAPO2 being activated by trypsin is unlikely to be naturally present in the lobster shell. Because PO is physiologically important in molting and sclerotization, DOPA may not be the most appropriate substrate for this enzyme. N-acetyldopamine and 3,4-dihydrobenzoic acid have been reported as tanning agents and may be the actual substrates for EAPO (Pryor et al. 1946; Pryor, 1962); neither substrate was examined in this study.

There was a wide variation in the specific activity of PO in the shell of the lobsters tested (Table 3). Addition of trypsin to the PO crude extract increased the PO activity from 2-fold to over 120-fold in some cases. Values ranged from 0.01 to 0.80 and from 0.32 to 37.00 mmoles of DL-DOPA min^{-1} mg $protein^{-1}$ for free active and trypsin-activated PO's, respectively (Table 3). An increase in the levels of trypsin-activated PO (inert PO or prophenoloxidase) was observed during the period from May to September (Table 3), which were the warmest months of the year.

During the course of this study, it was observed that little molting occurred during the cold months while most molting took place in the summer. A relationship between molting and water temperature has been observed by Travis (1954), and it was found that molting is directly correlated with water temperature. Thus, a relationship between molt cycle and PO levels could exist. It is known that PO is involved in tanning the cuticle (in the case of a molting animal, the newly forming cuticle) by catalyzing the formation of quinones which form cross-links with cuticular proteins (Stevenson, 1985; Andersen, 1979). Therefore, the levels of free PO activity (endogenously activated PO or EAPO) should be higher during the warm months (May-September period) when lobsters molt more frequently. However, this was not the case as shown in Table 3. Unlike inert PO, there was no increase in free PO activity during the May-

Table 3. Free PO and Trypsin-Activated PO (TAPO) Specific Activity in Cuticle Extracts of Lobsters Collected During the 1985-86 Season

	TAPO ACT.	FREE PO ACT.	PROTEIN CONTENT	SP. ACT TAPO	SP. ACT* FREE PO
SAMPLE	EU/mL**	EU/mL	mg/mL	EU/mg	EU/mg
10-01-85	0.35	0.15	0.50	0.70	0.30
10-08-85	2.37	0.17	0.84	2.82	0.20
10-21-85	0.30	0.05	0.64	0.47	0.08
10-29-85	0.54	0.14	0.66	0.82	0.21
01-08-86	0.59	0.04	0.31	1.90	0.13
01-20-86	0.87	0.05	0.28	3.11	0.18
01-21-86	0.17	0.03	0.28	0.61	0.11
10-22-85	0.07	0.01	0.19	0.37	0.05
10-23-85	0.14	0.03	0.24	0.58	0.13
12-12-85	0.94	0.05	0.40	2.35	0.13
12-16-85	0.27	0.03	0.40	0.68	0.08
04-19-86A	3.32	0.03	0.40	8.30	0.08
04-19-86B	0.49	0.02	0.32	1.53	0.06
04-19-86C	3.70	0.05	0.48	7.71	0.10
05-15-86A	7.54	0.06	0.40	18.85	0.15
05-15-86B	8.88	0.19	0.24	37.00	0.79
05-15-86C	2.80	0.05	0.33	8.48	0.15
06-16-86A	0.06	0.002	0.19	0.32	0.01
06-16-86B	0.17	0.02	0.28	0.61	0.07
06-16-86C	0.11	0.02	0.30	0.37	0.07
06-16-86D	0.11	0.02	0.21	0.52	0.10
08-20-86A	10.77	0.09	0.46	23.41	0.20
08-20-86B	7.41	0.06	0.41	18.07	0.15
09-16-86A	6.72	0.06	0.38	17.68	0.16
09-16-86B	2.22	0.04	0.36	6.17	0.11

*Phenoloxidase activity

**EU (enzyme units) are expressed in mmoles DL-DOPA/min

September period. This was contradictory because the prophenoloxidase must become activated before it can efficiently act on the substrate.

A closer evaluation of Table 3 revealed that there was one lobster which had a high free PO activity during the sampling from May-September. This lobster was in the premolt stage, as determined by the presence of the new cuticle beneath the old one. The new cuticle looked like the old one (same color and spots), but was very thin, uncalcified and very fragile, having no hardness. The part of the cuticle formed at this stage may have been the epicuticle. It was hypothesized that IPO1 was being activated at this premolt stage in order to provide the cross-linking agents (quinones) for the cuticle sclerotization (tanning). To confirm these results, several other lobsters at the premolt stage were selected and analyzed for trypsin-activated (inert PO's or prophenoloxidases) and free PO activity as previously described. The premolt lobsters were identified by looking for the development of the setae on pleopods under the dissecting microscope, by the state of development of the epidermis, and by the appearance of an ecdysial line along the branchiostegites. Such a line becomes visible 3-4 days or 2-3 weeks before ecdysis in the summer months and cold months, respectively (Travis, 1954). The lengthening of the premolt stage in the winter months may be due to a decrease in the rate of physiological activities associated with molting.

Phenoloxidase activities for lobsters at different molting stages are presented in Table 4. An activation of phenoloxidase in premolt lobsters, especially in the late premolt stage was observed. Free and trypsin-activated PO activities were identical at the late premolt stage, suggesting the occurrence of a fully activated phenoloxidase. This was confirmed by analysis of the exuvia of newly molted lobsters where addition of trypsin to the PO extracts did not affect the PO activity levels, indicating the existence of a fully active PO. However, lobsters in early premolt and other stages did not present a fully activated prophenoloxidase (Table 4).

CONCLUSIONS

In conclusion, PO's from different shrimp species exhibit different kinetic and thermodynamic properties which could in part explain the differences in susceptibility of these species to melanosis. Activation of PO occurs in Florida spiny lobster and not in shrimp. The kinetic properties of the various PO forms also indicate unique differences between them. PO activity also appears to be related to molting.

Table 4. Free PO, Trypsin-Activated PO Levels and Protein Content in Cuticle Extracts from Lobsters at Various Molting Stages

	TAPO	FREE PO	PROTEIN CONTENT	TAPO	FREE* PO
SAMPLE	EU/mL**	EU/mL	mg/mL	EU/mg	EU/mg
Newly molted 1	0.11	0.02	0.21	0.52	0.10
Newly molted 2	0.06	0.04	0.19	0.32	0.21
Newly molted 3	0.07	0.01	0.20	0.35	0.05
Newly molted 4	0.05	0.04	0.23	0.22	0.17
Newly molted 5	0.08	0.02	0.21	0.38	0.10
Newly molted 6	0.23	0.01	0.55	0.42	0.02
Postmolt 1	0.47	0.02	0.33	1.42	0.06
Postmolt 2	0.38	0.05	0.35	1.09	0.14
Postmolt 3	0.21	0.02	0.38	0.55	0.05
Postmolt 4	0.07	0.03	0.27	0.26	0.11
Postmolt 5	0.10	0.01	0.25	0.40	0.04
Postmolt 6	0.08	0.03	0.20	0.40	0.15
Intermolt 1	1.98	0.01	0.43	4.60	0.02
Intermolt 2	5.14	0.04	0.39	13.18	0.10
Intermolt 3	3.27	0.10	0.26	12.57	0.38
Early premolt 1	3.49	0.12	0.13	26.85	0.92
Early premolt 2	7.60	0.09	0.24	31.67	0.38
Early premolt 3	3.30	0.04	0.11	30.00	0.36
Early premolt 4	7.67	0.09	0.26	29.50	0.35
Early premolt 5	5.52	0.10	0.33	16.72	0.30
Early premolt 6	5.31	0.11	0.27	19.67	0.41
Late premolt 1	1.82	2.05	0.30	6.07	6.83
Late premolt 2	0.96	1.24	0.14	6.86	8.86
Late premolt 3	0.93	0.88	0.18	5.17	4.89

*Specific activity, EU/mg protein
**EU (enzyme units) are expressed as mmoles DL-DOPA/min

REFERENCES

Andersen, S.O. 1979. Biochemistry of the insect cuticle. Ann. Rev. Entomol. 24:29.

Clark, J.M. and Switzer, R.L. 1977. In Experimental Biochemistry. W.H. Freeman and Co. San Francisco, CA.

Cobb, B.F., III. 1977. Biochemistry and physiology of shrimp: effect on use as food. In Proceedings of the conference on the handling, processing and marketing of tropical fish. pp. 405-411. Tropical Products Institute, London.

Davis, B.J. 1964. Disc electrophoresis-II. Method and application to human serum proteins. Ann. NY. Acad. Sci. 121:404.

Ferrer, O.J., Marshall, M.R. and Koburger, J.A. 1986. Biochemical basis for accelerated melanosis in the Florida spiny lobster (Panulirus argus). In Proceedings 11th. Annual Trop. Subtrop. Fish. Technol. Conf. of the Americas. 11:7.

Johansen, G. and Lumry, R. 1961. C. R. Trav. Lab. Carlsberg. 32:182-214.

Koburger, J.A., Miller, M., and Otwell, W.S. 1985. Thawed lobsters tails: some quality changes. In Proceedings of the 10th Annual Trop. Subtrop. Fish. Technol. Conf. Americas. 10:201.

Laemmli, U.K. 1970. Cleavage of structural proteins during assembly of the head of bacteriophage T4. Nature. 227:680-685.

Low, P.S., Bada, J.L. and Somero, G.N. 1973. Temperature adaptation of enzymes: roles of the free energy, the enthalpy, and the entropy of activation. Proc. Natl. Acad. Sci. 70:430-432.

Marshall, M.R., Otwell, W.S.,, and Walker, B. 1984. Preliminary study on the isolation and activation of polyphenoloxidase from deep sea crab (Geryon sp.). In Proceedings of the 9th Annual Trop. and Subtropic. Fish. Conf. Americas. 9:118.

Mihalyi, E. 1978. In "Application of proteolytic enzymes to protein structure studies". 2nd. ed. pp 57-59. CRC. Press, Boca Raton, FL.

Otwell, W.S. and Marshall, M.R. 1986. Screening alternatives to sulfiting agents to control shrimp melanosis. In Proceedings of the 11th. Annual Trop. Subtrop. Fish. Technol. Conf. Americas. 11:35.

Pollock, M.R. 1965. Purification and properties of penicillases from two strains of Bacillus lichenformis: a chemical, physicochemical and physiological comparison. Biochem. J. 94:666-675.

Pryor, M.G. 1962. Sclerotization. In "Comparative Biochemistry." (Volume IV): Constituents of life (Part B). M. Florkin and H. S. Mason, Editors. Academic Press, New York.

Pryor, M.G., Russell, P.B., and Todd, A.R. 1946. Protocatehuic acid, the substance responsible for hardening of the cockroach ootheca. Biochem. J. 40:627.

Savagaon, K.A., and Sreenivasan, A. 1978. Activation mechanism of pre-phenoloxidase in lobster and shrimp. Fishery Technol. 15:49.

Simpson, B.K. and Haard, N.F. 1984. Purification and characterization of trypsin from Greenland cod (Gadus ogac). 1. Kinetic and thermodynamic characteristics. Can. J. Biochem. Cell Biol. 62:894-900.

Simpson, B.K., Marshall, M.R. and Otwell, W.S. 1987. Phenoloxidase from shrimp (Penaeus setiferus). Purification and some properties. J. Agric. Fd. Chem. 35:918-921.

Stevenson, J.R. 1985. Dynamics of the integument: In The Biology of Crustacea (Volume 9): Integument, pigments, and hormonal processes. D.E. Bliss and L.H. Mantel, Editors. Academic Press, New York.

Taylor, S.L. and Bush, R.K. 1983. Prepared for : International Food Additives Council. Washington D.C.

Travis, D.F. 1954. The molting cycle of the spiny lobster Panulirus argus Latreille. I. Molting and growth in laboratory-maintained individuals. Biol. Bull. 107:433.

Wagner, T. and Finne, G. 1986. Evaluation of alternatives to sulfiting agents as melanosis inhibitors in raw shrimp. In Proceedings of the 11th. Annual Trop. Subtrop. Fish. Technol. Conf. of the Americas. 11:23.

A COMPREHENSIVE OVERVIEW OF THE USE AND TESTING OF SODIUM BISULFITE ON SHRIMP

Martha Hudak-Roos
3209 Frederic Street
Pascagoula, MS 39567

and

Dr. W. Steven Otwell
University of Florida
467 Food Science Building
Gainesville, FL 32611

Sulfites in various forms, especially sodium bisulfite, have been added to foods for centuries. One of the earliest recorded uses was the disinfection of wine vessels with sulfur dioxide in early Roman times. Since then, the use of sulfites have been extended to a variety of food products, including seafoods.

Sodium bisulfite is used in the shrimping industry to control melanosis on shrimp. Melanosis, commonly known as "blackspot," is a discoloration of the surface of the shrimp. This discoloration is caused by naturally occurring enzymes reacting in the presence of oxygen. The enzyme system is thought to involve a polyphenoloxidase. The reaction starts from the time the shrimp are removed from the water and continues through refrigeration or ice storage. Although harmless, blackspot is extremely objectionable to consumers and will result in a lower price for the product.

The use of sodium bisulfite to control discoloration was learned from the potato industry in the 1950's when shrimp vessel harvest began to expand. A recommendation to use a 1.25% sodium bisulfite dip for one minute was introduced in 1956 (Camber et al). Working aboard shrimp vessels in Key West, Florida, Camber used a variety of concentrations, 1.25 to 15%,and time intervals, 1 to 15 minutes, to develop this recommendation. This recommendation was the basis for Food and Drug Administration's (FDA) prior sanctioned use for sulfiting agents on shrimp (Federal Register 1959).

A 1.25% dip is equivalent to 125 grams of sodium bisulfite in 10 liters of water or 1 pound of powder per 10 gallons. A basket of headed and rinsed shrimp is placed into this dip for one minute. The shrimp should then be drained and rinsed and then stored in ice. By the prior sanction, all shrimp thus treated must be labeled such.

REGULATIONS

In addition to the FDA's prior sanction for the use of sodium bisulfite on shrimp, five other sulfiting agents have been listed by the

FDA as Generally Recognized As Safe (GRAS). This GRAS status indicates that these sulfiting agents may also be used to control blackspot in shrimp. In addition, sulfiting agents may also be used as GRAS in lobsters, scallops, dried cod, and clams. Sulfites may not be used in any food source recognized as a source of Vitamin B1, such as crabs (Pippin, 1986).

Thus, the FDA has established that six sulfiting agents may be used on seafood that is not a recognized source of Vitamin B1. In addition to these regulations, the FDA has established requirements for the use of sulfiting agents called Good Manufacturing Practice Guidelines (GMPG). So far, only one GMPG exists, and this GMPG is for sulfite use on shrimp (Federal Register, 1985). Very simply, the GMPG states that sulfite residuals as sulfur dioxide on the raw edible meat portion must be less than 100 parts per million (ppm). If the residual value is greater than 100 ppm, the product is actionable and subject to seizure. If the residual value is less than 100 ppm, the product must be labeled as containing sulfites, i.e., "Sodium bisulfite added as a preservative" or "Sulfite present as a preservative." Shrimp products containing less than 100 ppm sulfur dioxide on raw meat and not labeled as containing sulfites are considered misbranded.

This information reflects the current state of U.S. regulatory affairs. However, due to the widespread public health concern, the sulfite situation is under very close scrutiny in the U.S. and many other countries. The U.S. FDA is reviewing GRAS status of all food additives and may rescind the GRAS status for some additives. The use of sulfites have already been banned for raw vegetables and fruits and there exists the possibility that this ban may be extended to the seafood industry. In addition, the FDA has sampled other seafoods, possibly to establish GMPGs for other sulfited fishery products.

Foreign requirements, as developed by the Codex Alimentarius Commission of the Food and Agriculture Organization of the United Nations and the World Health Organization, differ slightly from the U.S. requirements. Nations that use the Codex Alimentarius guidelines will require less than 100 ppm sulfur dioxide on raw edible meat and less than 30 ppm on cooked meat (Codex, 1984). No current guidelines for residual sulfur dioxide on cooked meat exists in the U.S.

HEALTH RELATED ASPECTS

As previously mentioned, sulfites are found in a variety of food products. From potatoes, dried fruits and vegetables to wine, sulfites have a variety of uses. Sulfites are used as antimicrobial agents, to prevent discoloration, to inhibit enzymes, as antioxidant and reducing agents, and as bleaching agents. The differing sulfited products also exhibit varying degrees of residual sulfur dioxides, with shrimp at 100 ppm sulfur dioxide at the lower end of the range and dried fruits at 3000 ppm sulfur dioxide at the higher end.

Keeping in mind the wide range of products, uses, and concentrations of sulfiting agents, it is easy to understand how sulfiting agents have become suspect in causing allergic type reactions when present in consumed foods. This public health problem has grown into a major concern since the 1984 report of several deaths associated with consumption of fresh salads containing sulfites. It is this highly emotional public health concern that has caused the U.S. government to reevaluate the GRAS status of sulfiting agents. However it should be noted that none of the reported fatalities have been definitively associated with shrimp.

The FDA has kept record of sulfite consumer complaints by product types (Table 1). Table 1 indicates that "seafood" has been responsible for approximately 10% of adverse reactions to sulfite.

Table 1. Sulfite Adverse Reactions by Associated Product Type

Product Type	Count	Percentage
Salad Bar	277	27.5
Other	136	13.5
Non-salad bar fruits and vegetables, fresh (excluding potatoes)	135	13.4
Wine	108	10.7
Seafood	97	9.6
Potatoes, other than fresh	55	5.5
Fresh Potatoes	51	5.1
Fruit, dried	41	4.1
Baked goods	35	3.5
Drugs	28	2.8
Beer	14	1.4
Fruit and vegetable juice	14	1.4
Other alcoholic beverages	13	1.3

Of the 97 seafood sulfite complaints, 58 were reported as Type One or severe, for a 60% report figure. Others were reported as Type Two (moderate).

Research into the allergic reactions brought on by sulfited food consumption has been on-going at the University of Wisconsin's Food Research Institute by Dr. Steve Taylor (Taylor and Bush, 1986). His research has brought to light some important conclusions:

1) There is no evidence that the current usage level of sulfites represents a health hazard to the majority of the U.S. population,

2) Reproductive processes are not adversely effected by sulfites, nor do sulfites elicit carcinogenic response in chronic feeding studies,

3) Sulfite sensitive individuals represent a small percentage of the total population. Of the greater than 9 million asthmatics in the U.S. population, only about 20% of these are severe asthmatics and about 8% of the severe asthmatics are sulfite-sensitive asthmatics, and

4) Some sulfited foods do not elicit asthmatic attacks in confirmed sulfite-sensitive individuals. One of these foods is shrimp.

CURRENT SULFITE RESEARCH

Current research indicates that there is no superior alternative to the 1.25% one-minute sodium bisulfite dip for control of blackspot (Otwell and Marshall, 1986). If this dip is applied correctly, then the product should not only have little or no blackspot, but should also contain less than 100 ppm sulfur dioxide (Finne et al, 1986). The only exception to this is the addition of the sodium bisulfite to the brine aboard freezer boats. This type of dip may not yield product with less than 100 ppm residues. If the product was incorrectly treated and contains greater than 100 ppm sulfite residue, then reclamation of the product might be possible by thawing, peeling and washing in water for 30 minutes or by high intensity heat cooking (Marshall et al, 1986).

TESTING FOR SULFITES

Shrimp suspected of being treated with sulfites will often be tested by processors and regulatory agencies. Testing of shrimp can be done on a quantitative, on a semi-quantitative, or on a qualitative basis.

There are three different quantitative tests used most often for determining sulfite residue on shrimp. Only one of these, the Monier-Williams method, is approved for use in determining U.S. regulatory compliance. The Monier-Williams analysis is an Association of Official Analytical Chemists' (AOAC) approved final action procedure. This method is long, tedious, and expensive. It requires a complicated distillation apparatus with a sulfur dioxide gas collector. The product is placed into the reaction flask with water and acid and refluxed for 1 hour and 45 minutes. After refluxing, the solution in the sulfur dioxide gas collector is titrated from pink to yellow with a base solution and a value is calculated. The FDA has slightly modified and optimized the official method for ease in use. This modification was collaboratively studied by the NFPA in 1987. The results of the collaborative study recommend that the method be adopted official first action for the quanitation of sulfites. The report is currently under review prior to publication in the "Journal of the AOAC."

Another quantitative method is the ion chromatography method (Cooper et al, 1986; Kim and Kim, 1986). This method is much more rapid but requires an extremely expensive, sensitive piece of equipment. This method has not been approved as an official regulatory compliance method.

The third quantitative test is a rapid distillation method (DeVries et al, 1986). Again, this test requires a sensitive, moderately

expensive piece of equipment, but takes approximately only 10 minutes to run. This method also has not been approved as an official regulatory compliance method, but attempts are underway to examine the feasibility of a collaborative study for such approval.

There are a variety of semi-quantitative and qualitative tests available. The majority of these tests were not designed for shrimp and consequently require further examination before routine use by the shrimping industry. These methods include:

1) The p-rosaniline test, which is an AOAC (Section 20.101) recognized method for detection of sulfites on ground beef. This method requires a spectrophotometer. Attempts to modify this method for use with shrimp have not been successful.

2) The micro-diffusion test, which is very similar to the p-rosaniline test. This test uses a reaction vessel called a Conway diffusion dish. Experiences with this method indicate that it gives low values. In addition, this method requires use of a mercury compound which is not recommended for food processing facilities.

3) The enzyme test kit (i.e., Mannheim Boehringer), is relatively inexpensive. However, this kit requires the use of an ultraviolet spectrophotometer, which can be expensive to operate and maintain. Further research is currently being conducted in the U.S. to adapt this kit for use with shrimp.

4) Iodate - Iodide titret kits (i.e., Chemetrics, Inc.) which employ a standard chemical reaction within a disposable glass cell. Again modifying this kit to use with shrimp has presented problems and does not appear promising.

5) Drop count and titration kits (i.e., Lamotte) which are manufactured specifically for the waste water treatment industry. These kits require a water solution for their use, so application to the shrimping industry is limited.

6) Ion electrodes which specifically measure the sulfur dioxide ion. Initial studies on these electrodes were extremely discouraging (Wood et al, 1976). However, studies continue, and a possible application may be developed in the near future.

7) A permanganate distillation method that requires an acid distillation of the product (Hudak-Roos et al, 1986). The sulfur dioxide gas formed is carried into a permanganate solution. If the product contains less than 100 ppm, the permanganate retains its color. If the product contains greater than 100 ppm, then the permanganate becomes a clear (colorless) solution. More expensive and time consuming than the below mentioned malachite green test, this test permits greater sensitivity. An attempt was made to seek AOAC approval of this

method through a collaborative study. However an accurate evaluation of the study was compromised because of the disparity in Monier-Williams values among participants. This test was not put forth for AOAC approval and further collaboration was halted in order to more closely examine other methods.

Although these tests are available, most facilities qualitatively testing for sulfites use one of two methods: test papers or a malachite green test.

By far, the most common method of testing is to use test papers or strips. These test papers are distributed by a variety of manufacturers and are inexpensive to use. Each vial of test strips contains a comparison chart for determining approximate values. Some of these charts range from 0-400 ppm, others range from 0-1000 ppm. The strip is applied until moist to either the peeled meat or the peeled minced meat. This wet test paper is then compared to the chart on the vial and a value determined.

Experience with these test strips at industry workshops conducted in September, 1986 have shown that their accuracy is extremely limited (Tables 2-4). The workshop participants, the majority of which were industry Quality Control personnel, applied each of two different brands of test strips to two different shrimp forms: raw peeled and raw peeled minced. The tests were conducted during three workshops by a total of 21 groups of two or three each industry participants.

Table 2. Sulfite Test Strips, Brand A

Level (ppm)	% More	% Less	% Correct
0	94	0	6
40 - 50	54	17	29
80 - 110	49	25	26
180 - 210	44	54	2

At the critical range of 80-110 ppm a correct value was obtained less than 30% of the time, and rarely was a non-sulfited product detected as such. In terms of buying decisions, 48% of the time product in the 180-210 ppm range would be accepted, while 29% of the time product in the 0 and 40-50 ppm level would be rejected.

Table 3. Sulfite Test Strips, Brand B

Level	% More	% Less	% Correct
0	90	0	10
40 - 50	44	17	39
80 - 110	48	30	22
180 - 210	24	56	20

At the critical range of 80-110 ppm a correct value was obtained less than 25% of the time, and 90% of the time was a non-sulfited product detected as such. For buying decisions, acceptance of 180-210 ppm product would be 52%, while rejection of 0 ppm or 40-50 ppm product would be 22%.

Table 4. Sulfite Test Strips, Brands Combined

Level	% More	% Less	% Correct
0	92	0	8
40 - 50	49	17	34
80 - 110	48	28	24
180 - 210	34	55	11

The combination of the brands indicates more of the same where acceptance of 180-210 ppm product would be 50% ($\pm$9%) while rejection of 0 ppm or 40-50 ppm product would be 25% ($\pm$8%).

The problems with the sulfite test strips have encouraged investigations into more sensitive methods. One of these is the malachite green method. This method uses a modified AOAC method for sulfite determination on ground beef. By developing a series of standards with malachite green dye, product can be compared and a qualitative determination be made.

Again, experience at the same industry workshops demonstrated difficulties with accuracy (Table 5).

Table 5. Malachite Green Test

Level	% <100 ppm	% >100 ppm
0	85	15
40 - 50	75	25
80 - 110	45	55
180 - 210	40	60

Using this example, product in the 180-210 ppm range would be accepted 40% of the time. Product at 0 and 40-50 ppm would be rejected 20% of the time. While better than either brand of sulfite test strips, this method can also lead to a great number of faulty buying decisions.

SUMMARY

The use of food additives, while sometimes necessary, can create many problems and complications for an industry. Such is the case with sodium bisulfite on shrimp. Properly applied, sodium bisulfite can help produce a high profit, esthetically pleasing product. Misapplied, it can create regulatory compliance problems and possible serious public health concerns. Through industry efforts in education, research, and development of new methodology, the use of sulfites on shrimp maintains regulatory approval while providing reasonable consumer protection.

BIBLIOGRAPHY

1. Camber, C. I., Vance, M. H. and Alexander, J. E. 1957. The use of sodium bisulfite for the control of blackspot in shrimp. In: "State of Florida Board of Conservation," Technical series #20, E. Mitts, Director, The Marine Laboratory, Virginia Key, FL.

2. Federal Register, 1959 (Nov. 20), Sulfiting Agents: Final Rule - GRAS Status (24 FR 9368).

3. Pippin, Howard. July 1986. Letter to Marjorie Lewis, Lewis Financial Services, Inc. FDA Center for Food Safety and Applied Nutrition, Division of Regulatory Guidance.

4. Federal Register, 1985 (Jan, 23), Notice to Shippers, Distributors, Packers, and Importers of Shrimp Containing Sulfites. 50 (15), p. 2947-3306.

5. Food and Drugs Code of Federal Regulations 1985. Title 21. Part 182. Office of the Federal Register, National Archives and Records Service, General Services Administration, Washington, D.C.

6. Recommended International Code of Practices for Shrimp or Prawns. 1984. Codex Alemintarius Commission. Food and Agriculture Organization of the United Nations/World Health Organization Food Standards Programme. Vol. B, 2nd Edition.

7. Taylor, Steve and Robert Bush. June 1986. Sulfite as Food Ingredients. In: "A Scientific Status Summary by the IFT Expert Panel on Food Safety and Nutrition."

8. Otwell, W. Steven and Marty Marshall. April 1986. Screening Alternatives to Sulfiting Agents to Control Shrimp Melanosis. In: "Studies on the Use of Sulfites to Control Shrimp Melanosis (Blackspot)." Florida Sea Grant College Technical Paper 46.

9. Finne, Gunnar, Tom Wagner, Bernard DeWitt, and Roy Martin. 1986. Effect of Treatment, Ice Storage and Freezing on Residual Sulfite in Shrimp. Journal of Food Science; 51(1), pgs. 231-232.

10. Marshall, Marty, W. Steven Otwell and Roy Martin. April 1986. Influence of Washing and Cooking on Sulfite Residuals on Treated Shrimp. In: "Studies on the Use of Sulfites to Control Shrimp Melanosis (Blackspot)." Florida Sea Grant College Technical Paper 46.

11. A.O.A.C. 1985. "Official Methods of Analysis," 14th ed. Association of Official Analytical Chemists, Washington, D.C.

12. Cooper, P., M. Marshall, J. Gregory and W. Otwell. 1986. Ion Chromatography for Determining Residual Sulfite on Shrimp. Journal of Food Science. 51(4), p. 924-928.

13. Kim, H. J. and Y. K. Kim. 1986. Analysis of Free and Total Sulfites in Food by Ion Chromatography with Electrochemical Detection. J. Food Sci. 51:1360-1361.

14. DeVries, J., H. Ge, J. Ebert, J. Magnuson, and M. Ogawa. 1986. Analysis for Total Sulfites in Foods by Using Rapid Distillation Followed by Redox-Titration. Journal of the Association of Official Analytical Chemists. 69(5).

15. Wood, B. J., S. Badgen, and S. Garrett. 1976. Sodium bisulfite and Its Residual Use in Controlling Black Spot on Shrimp. First Annual Tropical and Subtropical Fisheries Technology Conference of the Americas. 1:383-394.

16. Hudak-Roos, M., B. J. Wood, and J. Carey. Screening of Sodium Bisulfite on Shrimp: A Modified Monier-Williams Approach. Eleventh Annual Tropical and Subtropical Fisheries Technical Conference of the Americas. 11:1-6.

17. Food Chemical News, 1985. 100 ppm Action Level for Sodium Bisulfite on Shrimp Sought by NFI, Jan. 7, p. 15.

18. Food Chemical News, 1985. FDA to Raise Action Level in Shrimp from 40 to 100 ppm, Jan. 21, p. 2.

19. Finne, Gunnar and R. Miget. 1985. The Use of Sulphiting Agents in Shrimp -U.S. Regulations for Domestically Produced and Imported Products. Infofish Marketing Digest, No. 4.

20. Nickelson, Ranzell and Bruce Cox. Stop Shrimp Blackspot. April 1977. Marine Advisory Bulletin, Texas A & M University, TAMU-SG-77-504.

THE QUANTITATIVE DETERMINATION OF EDTA IN FROZEN CRAWFISH

Meganne W. Finerty and Michael W. Moody
Louisiana State Univeristy, Department of Food Science
and Louisiana Cooperative Extension Service
Baton Rouge, LA 70803

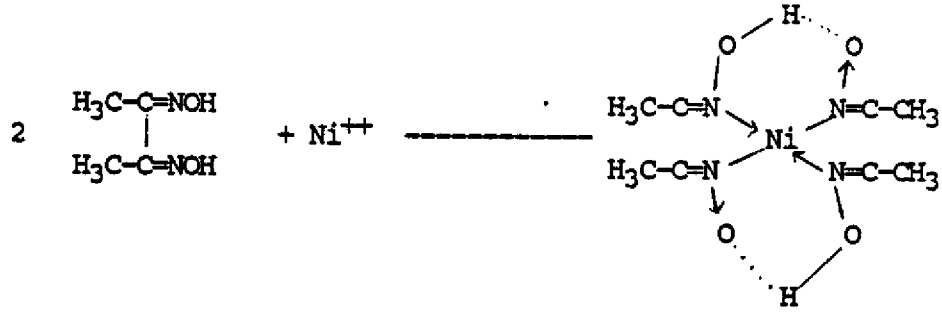
INTRODUCTION

Crawfish production and processing, limited to at most November through June, needs to supply meat to a year round market. In order to meet the demand for crawfish meat when fresh crawfish is unavailable, the hepatopancreas is washed from the tail meat and then the tail meat is frozen. Properly packaged and frozen tail meat usually retain good quality. However, unpredictable bluish gray discoloration may appear when the tail meat is thawed and reheated. This discoloration is unappetizing although harmless. Citric acid dips prior to packaging and freezing have been recommended as precautionary measures since citric acid dips are GRAS (generally regarded as safe). However, citric acid can causing toughening and bleaching of tail meat without always preventing the discoloration. A previous study (Marshall, 1986) showed that treatment of tail meat with 1% citric acid and 0.1% EDTA or 0.2% EDTA alone was more effective in preventing bluing.

Discoloration in canned cooked shrimp, canned cooked crabmeat and canned cooked clams has been effectively controlled with EDTA dips, which has been approved by the U.S. Food and Drug Administration. Modifications of the Darbey procedure has been used as the analytical method for each of these products. Since calcium disodium EDTA is a food additive regulated for use under 21 CFR 172.120, it is necessary to submit a food additives petition as described in 21 CFR 171.1. Therefore, the objectives of this study were to modify existing procedures for the accurate determination of residual concentration of calcium disodium EDTA in crawfish tail meat, which would support a food additives petition providing for the use of EDTA in frozen crawfish tail meat.

EDTA is a sequestering agent used to prevent discoloration, off-flavor formation and texture deterioration (struvite formation) caused or promoted by the presence of divalent cations. The basis of this method is to add a divalent metal ion, not present or present in low concentrations in the tail meat being analyzed, that can be determined colorimetrically. The metal chosen is nickel. Nickel has a good affinity for EDTA and has several properties that make it attractive for the determination. Several researchers have developed methods for determining nickel concentrations. The Darbey (1952) procedure based on these properties was modified for quantifying EDTA in canned cooked crabmeat and canned cooked shrimp. Since then numerous researchers have improved the procedure. The procedure developed herein is modifications of the Darbey procedure based on suggestions reported in Marczenko (1976).

Darbey made a nickel derivative using dimethylglyoxime (H_2Dm), which is a rather specific compound that can be separated from interfering substances. If the reaction is carried out twice, copper and cobalt can be eliminated. Oximes, such as dimethyl glyoxime, are more selective than the more sensitive dioximes: 1-(2-pyridylazo)-2-naphthol (PAN) and pyridine-2-aldehyde-2-quinolyl hydrazone (PAHQ). The reaction is:



H_2Dm $Ni(HDm)_2$

This reaction releases two protons, which combine with the anionic part of the nickel salt to produce an acid. The solution is then acidic and the complex is insoluble in this solution. The $Ni(HDm)_2$ complex is a pink flocculent precipitate in neutral or ammoniacal mediums, and is soluble in chloroform and other nonpolar organic solvents. After the complex is decomposed in the presence of 0.5M HCl, the chloroform layer discarded, and the nickel reacted with H_2Dm, an oxidant, and ammonia, $[Ni(Dm)_3]^{--}$, a red-brown, water soluble complex forms.

Additional steps are needed to eliminate interferences from other ionic species: hydroxylamine is added to eliminate the manganese and copper from forming $(HDm)_2$ complexes; the primary extraction with dimethylglyoxime followed by chloroform extraction eliminates interference from copper, cobalt, iron, chromium, aluminum and manganese; endogenous EDTA eliminates interference from iron (II), cobalt and copper ions; and finally potassium acid tartrate eliminates interference from phosphates.

PROCEDURE

Samples of 25 grams tail meat are blended at high speed with 75 mL saturated NaCl for 60 sec. Both water and tail meat controls are spiked with $CaNa_2EDTA$ (0 to 25 mg per sample) to be run simultaneously. The homogenate is centrifuged at 2500 rpm (1020 x g) for 20 min. The supernatant is decanted into another centrifuge bottle. The residual tissue is reextracted with 50 mL saturated NaCl, centrifuged, decanted, reextracted with 50 mL distilled water, centrifuged, and decanted. The supernatants are combined into another 250 mL centrifuge bottle.

The NiEDTA complex is formed by adding 7.5 mL NiSO4 (13.3 g/L $NiSO_4.6H_2O$) to the pooled supernatant solutions, and shaking or mixing well. The complex requires 10 min to form, but is stable. In order to remove excess nickel, add 2 to 5 mL concentrated ammonia to the controls and 5 to 8 mL to the other samples until the pH is atleast 10, and precipitate the nickel by adding 8 mL 1.5% DMGO and mixing well. Spin solution in GSA rotor at 5000 rpm (2040 x g) for 20 min. The supernatant is filtered through Whatman No. 40 paper into a 500 mL Erhlenmeyer flask. Discard precipitate.

Interfering ions are removed by reacting the filtrate with 5 mL 20% potassium sodium acid tartrate, and 4 mL 10% $NH_2OH.HCl$ (hydroxylamine hydrochloride). The ions are formed by the addition of at least 3 mL concentrated HCl (to free ions) until approximately pH 1. This solution is relatively stable.

The ions are derivatized with 5 mL 1.5% DMGO. In order to solubilize the derivative, at least 5 mL concentrated NH_3 are needed to reach at least pH 9. This solution is extracted 3x with $CHCl_3$ (50 mL) in 500mL separatory funnel. If an emulsion forms, filter through glass wool. Discard aqueous fraction. Wash chloroform extract with 25 mL NH3/water (1:50) and discard aqueous wash.

Shake chloroform extract with 20 mL 0.5M HCl and 15 mL 0.5M HCl collect aqueous layers in 50 mL or 200 mL volumetric flask. A larger volume of hydrochloric acid may be used if the dilution is to 200 mL, but all samples must be treated the same. The size of flask depends on the amount of EDTA expected. All samples and controls were diluted to 200 mL except where noted in this study.

In order to detect Ni bound by EDTA, 1 mL 1.5% DMGO, 2 mL 4% potassium persulfate (must be prepared fresh), and 5 mL concentrated ammonia are added in this sequence, mixing well between additions. Dilute to volume. Within 10-30 min the absorbance was read at 445 nm against water.

ANALYTICAL RESULTS

1. Determination of Linearity of Standard Solutions

The above procedure was used with aqueous solutions containing 100 mL water and 0, 0.25, 0.5, 0.75, 1.0, 1.25, 1.50 mg nickel (a correction factor must be used to determine the amount of nickel in the nickel sulfate solution); and 0, 5.0, 10.0, 20.0 mg $CaNa_2EDTA.2H_2O$. This was the calculated range of expected residues. Triplicate samples were analyzed. The average values were plotted in Figure 1. These lines demonstrate the linearity and the relative concentrations of Nickel and $CaNa_2EDTA$ useful for this assay. Tables 1 and 2 show the mean values and the standard deviations for the nickel and EDTA standards. Table 3 shows the reactivity ratio of EDTA to nickel. Approximately 2 moles of $CaNa_2EDTA$ react with 1 mole of nickel.

TABLE 1. Mean Absorbance of Nickel Standards

CONCENTRATION mg	ABSORBANCE Units	STANDARD DEVIATION
0	-0.0075	0.0034
0.25	0.1105	0.0453
0.50	0.2499	0.0099
0.75	0.3464	0.0680
1.0	0.3732	0.0668

TABLE 2. Mean Absorbance of $CaNa_2EDTA.H_2O$ In Aqueous Solution

CONCENTRATION mg	ABSORBANCE Units	STANDARD DEVIATION
0	0.4102	0.1006
5	0.5110	0.0363
10	0.7541	0.0623
20	0.7016	0.0398

TABLE 3. Reactivity Ratio of $CaNa_2EDTA$ to Nickel

ABS Units	NI mg	$CaNa_2EDTA.2H_2O$ mg	$CaNa_2EDTA.2H_2O$/NICKEL mg/mg	mole/mole
0.00	0	3.66		
0.15	0.25	8.05	17.56	2.53
0.22	0.50	10.10	12.88	1.85
0.34	0.75	13.46	13.07	1.88
0.45	1.00	16.82	13.16	1.89

2. Recovery Study

The same amount of $CaNa_2EDTA.2H_2O$ (0, 5, 10, 15, 20 mg) was added to both tail meat and aqueous solutions. The tail meat was homogenized as described in the procedure. The aqueous solution was reacted with nickel sulfate and continued through the remainder of the procedure. The raw data is presented in Table 4. The concentrations used to calculate the recovery were obtained from the regression line for the standard curve in Figure 1, which is represented as the second y-axis in Figure 2. The absorbance of the tail meat samples were used to interpolate the aqueous concentration. The % recovery is then calculated as the difference in the EDTA in the aqueous controls divided by the concentration of EDTA added to the tail meat and multiplied by 100.

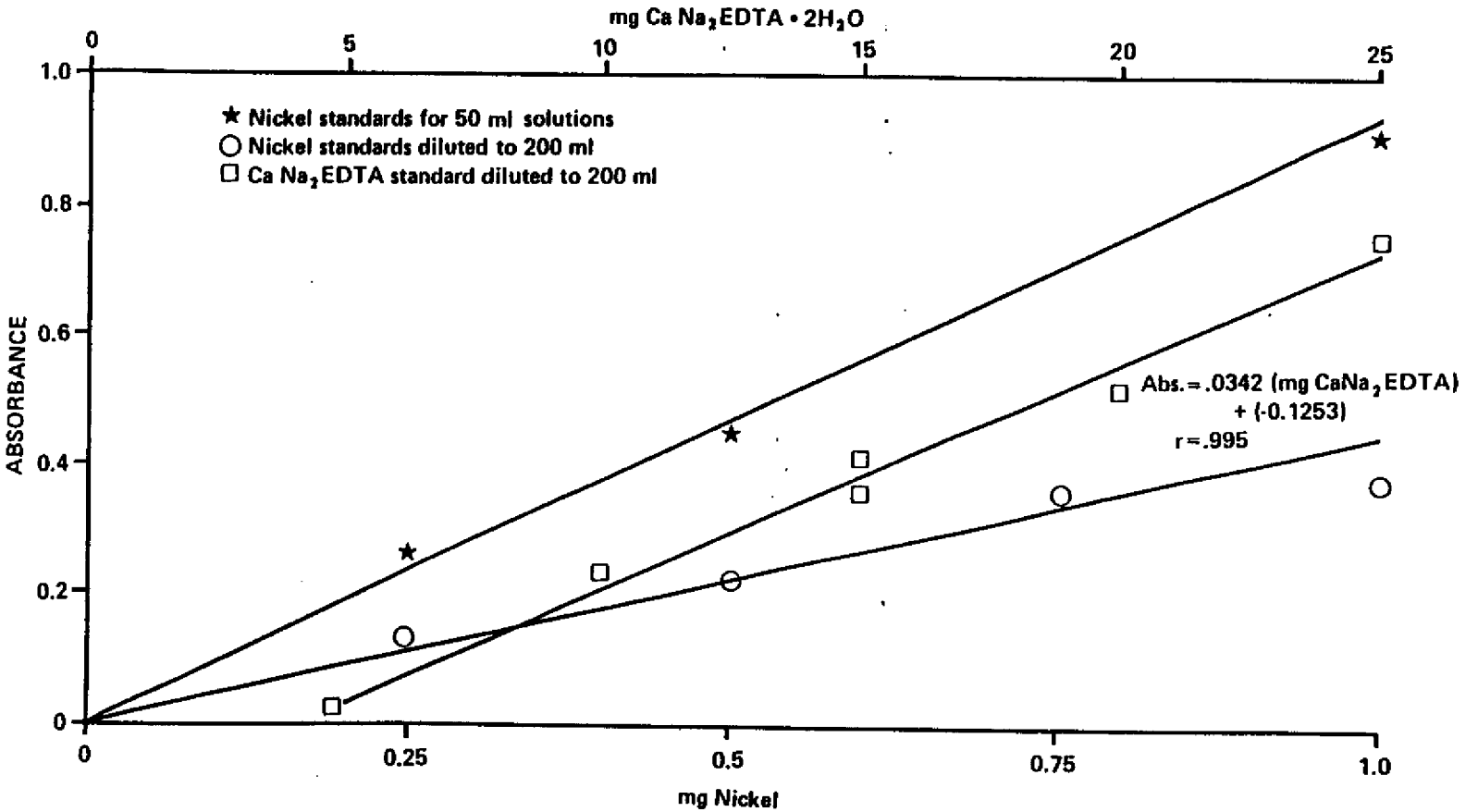


Figure 1. Calibration lines for nickel and $CaNa_2EDTA.2H_2O$. The absorbance of nickel and EDTA indicate the useful concentration region for both compounds.

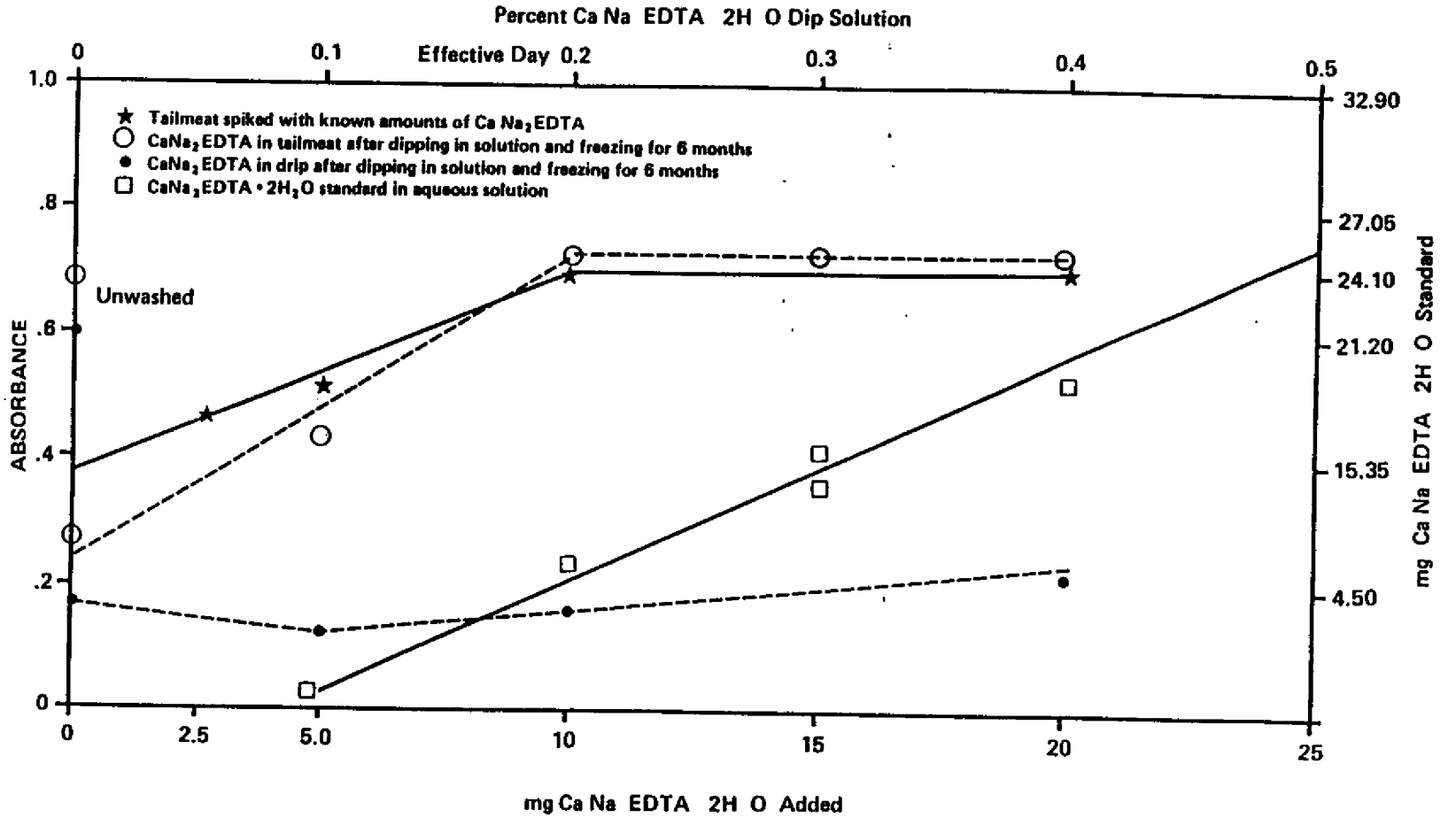


Figure 2. Dashed lines show $CaNa_2EDTA$ residues in tail meat and in drip of thawed tail meat after dipped in known concentration solutions and frozen for 6 months. Solid lines show the recovered concentration of $CaNa_2EDTA$ in spiked aqueous solution and tail meat.

TABLE 4. Absorbance of $CaNa_2EDTA.2H_2O$ in Tail Meat and Aqueous Solutions

CONCENTRATION $CaNa_2EDTA.2H_2O$ mg	MEAN ABSORBANCE Tail Meat Units (S.D.)[1]	MEAN ABSORBANCE Aqueous Solution Units (S.D.)[1]
0	0.0680 (0.0178)	0.4102 (0.1006)
5	0.1805 (0.0046)	0.5110 (0.0363)
10	0.4541 (0.0330)	0.7571 (0.0623)
20	0.5264 (0.0457)	0.7016 (0.0398)

[1]S.D. is the standard deviation of the mean of at least 3 values.

TABLE 5. Recovery of $CaNa_2EDTA$ from Crawfish Tail Meat

ABSORBANCE Units	TAIL MEAT $CaNa_2EDTA.2H_2O$ mg	AQUEOUS $CaNa_2EDTA.2H_2O$ mg	RECOVERY %
0.41	0.0	15.4	
0.54	5.0	19.5	82
0.73	10.0	24.1	87

3. Residue Study

Three 25 gram (+/- 0.2) samples of crawfish tail meat were dipped into 1000 mL solutions containing 0, 0.1, 0.2, or 0.4% $CaNa_2EDTA.2H_2O$. Three samples were not dipped as controls. These samples were analyzed using the above procedure. The absorbance readings were used to determine the concentration based on the regressed line in Figure 2. The anhydrous concentrations were determined by multiplying the dihydrate concentration by 0.91226 (374.3/410.3). These values are shown in Table 6.

TABLE 6. Anhydrous $CaNa_2EDTA$ Residues in Crawfish Tail Meat

$CaNa_2EDTA.2H_2O$ Dip (%)	Anhydrous $CaNa_2EDTA$ mg/25 g sample	Anhydrous $CaNa_2EDTA$ parts per million
0.0	-4.288	-171
0.1	2.098	84
0.2	8.758	350
>0.2	8.758	350
unwashed	7.161	286

DISCUSSION

The linear range for detection of nickel (II) is from 0 to 25 mg per liter and for calcium disodium ethylene diamine tetraacetic acid is from 25 to 200 mg per liter in aqueous solutions. The EDTA was extracted through the entire procedure. This partially accounts for the lower absorbance values for the EDTA compared to the nickel standards shown in Figure 1. From this data, 5 mg EDTA is the detection limit due to losses inherent in the procedure, absorbance properties, and reactivity during derivative formation. The correlations between concentration and absorbance for both curves is above 0.99 using the mean values. As also shown in Figure 1, the response of both the nickel and EDTA to the reagents coincide, showing a linearity between the nickel and EDTA permitting us to quantify nickel to determine the concentration of EDTA over this range.

In the initial study using 50 mL volumetric flasks, nickel above 1.0 mg in 50 mL could not be accurately quantified. Therefore, when the volume was later increased to 200 mL, the same samples were used.

The plateaus in EDTA at 0.2% dip or 10 mg $CaNa_2EDTA$ indicate that the tail meat can at most absorb 350 ppm $CaNa_2EDTA.2H_2O$. The 0.2% dip has 350 ppm, which if put on an anhydrous basis would be 320 ppm. The 10 mg $CaNa_2EDTA.2H_2O$ in 25 grams is 0.400 mg/g or 400 ppm (x 0.87 recovery) and (x 0.91 for anhydrous basis) would also be 320 ppm. This limit is the maximum amount that can be absorbed.

The crawfish are dipped for 1 minute and drained for 1 minute. The small amount of EDTA left in the drip that is packaged and the EDTA syneresed during the 6 months storage is approximately the same regardless of the amount of EDTA in the dip solution. This value is at the 5 mg concentration of EDTA, which is the limit of quantification. The real concentration of EDTA is most likely much lower than 5 mg, but this method is not sensitive in that concentration region. Quantification can be done by concentrating the sample into a smaller volume and quantifying the amount of EDTA using the procedure above.

The EDTA added may bind to the trace minerals already present in the crawfish tail meat. However, the contribution of this should be relatively low since the content of the trace metals is for the most part relatively low. There is considerable variation in the mineral content of the tail meat. This variation is probably responsible for the variation, although insignificant between concentration of dip solutions, observed in the EDTA present in the liquid part of the frozen crawfish. Table 7 presents the concentration and range of mineral content of the tail meat. These concentrations were obtained using an ICP on 100 samples over a two year period pooling samples from both pond-raised and wild crawfish.

TABLE 7. MINERAL CONTENT OF CRAWFISH TAIL MEAT

MINERAL	MEAN VALUE parts per million mg/kg (as is)	RANGE parts per million mg/kg (as is)
Cobalt	0.569	0.000-1.335
Copper	3.354	0.820-5.753
Iron	17.37	1.711-21.04
Nickel	0.967	0.000-2.141

REFERENCES

Darbey, A. 1952. Colorimetric Determination of the Sodium Salts of Ethylenediaminetetraacetic Acid. Anal. Chem. 24(2):373-378.

Marczenko, Z. 1976. Nickel, pp 369-379. In "Spectrophotometric Determination of Elements," Marczenko, Z. (ed.). Halsted Press, New York.

Marshall, G. 1986. Personal communication. Louisiana State University, Department of Food Science, Baton Rouge, LA 70803.

ACKNOWLEDGEMENTS

Partial support for this project was from the Louisiana Crawfish Research and Promotion Board, Baton Rouge, Louisiana.

INTRAMUSCULAR COLLAGEN OF FRESH AND FROZEN ATLANTIC COD (GADUS MORHUA)

C. G. Walton and T. A. Gill
Canadian Institute of Fisheries Technology
Technical University of Nova Scotia
P. O. Box 1000
Halifax, Nova Scotia
B3J 2X4

INTRODUCTION

Frozen storage is an important method of fish preservation, however, freezing and prolonged frozen storage result in deterioration of texture, flavour, and colour (Shenouda, 1980). Loss of water holding capacity, and a spongy, rubbery, or fibrous texture are sometimes observed in products held at high frozen storage temperatures. Product alterations have been attributed to the effects of interrelated physical, chemical and enzymatic changes (Shenouda, 1980).

There has been a great deal of research directed toward understanding the toughening phenomenon. Reduced extractability of myofibrillar proteins after frozen storage has been confirmed. However, the molecular basis of toughening remains unclear (King, 1966; Dyer and Morton, 1956; Dyer et al. 1956; Dyer and Dingle, 1961).

Interest in collagen as a protein contributing to the toughness of frozen-stored fish stems from the resistance of muscle cells to homogenization after frozen storage. This resistance is the basis of the "Cell Fragility Test" (Love, 1958) which related toughness and resistance to homogenization. Love et al. (1965) suggested that an increase in the strength of the sarcoplasmic reticulum or other cementing agent was responsible for the ability of the cells to withstand disruptive forces. It is suggested here that the collagen layer of the cell wall (endomysium) may be the cementing agent responsible for cell strength.

Early reports by Connell (1962), and Banks (1965) state that denaturation or changes in the connective tissue elements of the muscle likely cause certain textural defects. Tanaka (1965) noted the sarcolemma of Alaskan pollack became tougher with frozen storage although no work detailing the changes in connective tissue resulting from frozen storage has been reported (Connell, 1968). Sikorski et al., (1984) acknowledged that collagen may be involved in the toughening of the fish muscle with frozen storage.

Intramuscular collagen consists of the myocommata and fibrils of collagen extending from the myocommata. These fibrils, composed of the same collagen as found in the myocommata (Love, Lavety and Garcia, 1972), are part of the cell wall (endomysial collagen) and connect the myocomma to form a continuous muscle structure. Factors which must be considered when studying cod connective tissue are seasonal

variations, age, (Love et al., 1976) and sampling region within the fish (Love, 1970).

The objective of the present study was to determine the effects of frozen storage on collagen by chemical and microstructural methods.

METHODS

Fresh whole cod was purchased locally within two days of landing, and held on ice throughout rigor-mortis. Fish were measured, weighed and filleted. One fillet from each fish was frozen at -30°C, the other analysed immediately. Frozen fillets were held at -12°C for 5 weeks prior to analysis. Tissue pH and water content were determined as indicators of the nutritional status of the fish (Love, 1960; Love, 1969).

Myofibrils were prepared from fresh and frozen tissue (Olson et al., 1976) and incubated at 10°C with bacterial collagenase (Type VII: Sigma Chem. Co.) in a buffered $CaCl_2$ solution. Treated and untreated samples were fixed for scanning electron microscopy (SEM) in 2.5% glutaraldehyde in Sorensons phosphate buffer pH 7.0. Samples were subsequently frozen in liquid freon and freeze dried. Gold, in an argon atmosphere, was used to sputter coat the samples. Scanning electron microscopy was performed using JEOL Model JAX-35 Electron Probe X-ray Microanalyzer with an accelerating voltage of 25KV.

Tissue was dissected to separate the myocommata and myotomal tissue containing endomysial collagen (Love and Lavety, 1972), however, residual myotomal tissue remained attached to the myocommata. Dissected tissue was placed immediately into a 4°C solution of 0.6 M NaCl, 0.5 M Tris pH 7.4 containing protease inhibitors (20 mM ethylenediamine tetraacetic acid, 1.0 mM phenylmethylsulfonyl fluoride, 2.0 mM N-ethylmaleimide, 10 mM pepstatin, 1.0 mM sodium azide). Tissue suspensions were held in an ice-water bath while the dissections were completed. The extraction scheme is depicted in Figure 1.

Dissected tissue was dispersed in 20 volumes of salt solution using a Waring blender equipped with a baffle (Dyer, et al., 1950). The solution was blended for four 30 second periods, each followed by a 30 second rest period. Samples were stirred at 4°C overnight followed by centrifugation (1000 xg, 15 min) and insoluble material re-extracted with 10 more volumes (wet weight) of salt solution. These two salt extractions separated salt soluble myofibrillar, sacroplasmic and collagenous protein from salt insoluble protein (Figure 1B) in the pellet.

After washing the salt-insoluble pellet several times with cold distilled water, it was suspended in 10 volumes (wet weight) of cold 0.5 Macetic acid and disrupted for 15 sec using a Polytron Model PT10-/35 (Brinkman Instruments, Rexdale, Ont.). The solution was stirred

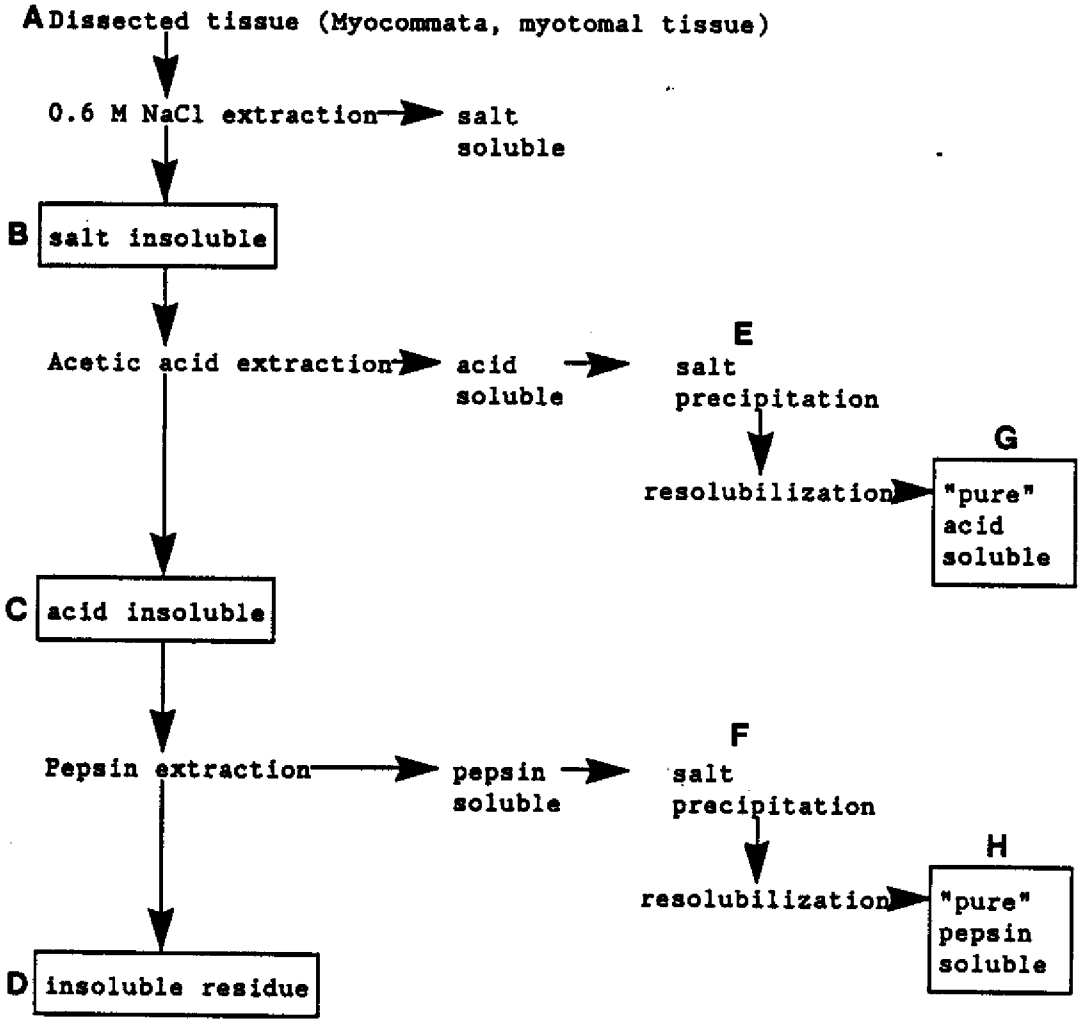


Figure 1. Extraction scheme - Sequential protein solubilization and collagen fractionation

at 4°C overnight and centrifuged at 1000 xg for 15 min to separate acid soluble and insoluble protein. Acid soluble collagen was precipitated by the slow addition of crystalline NaCl to 2 M and allowed to settle overnight (Figure 1E).

Acid insoluble tissue (Figure 1C) was suspended in 10 volumes (wet weight) of 0.5 M acetic acid containing 1 mg ml^{-1} pepsin (Sigma No. P-7125) to give about 1 mg enzyme to 100 mg protein. Extraction and precipitation were performed as for acid soluble protein (Figure 1F). Salt-precipitated protein was collected by centrifugation at 1000 xg for 15 min, washed 2-3 times in cold distilled water to remove salt, and resolubilized in 0.5 M acetic acid (Figures 1G and H).

Protein content was calculated at each stage of the extraction procedure (Lowry et al., (1951). Bovine serum albumin (Sigma Fraction V) was used as a standard and protein content expressed as mg BSA-protein per ml.

Hydroxyproline content was determined by the Woessner (1961) method in order to quantitate collagen content throughout the procedure. An hydroxyproline level of 8% was assumed for calculation of the collagen content of each fraction. This figure was determined for cod skin collagen in a previous study (Young and Lorimer, 1960).

Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) using 7.5% polyacrylamide gels containing 1.4% bisacrylamide crosslinker was used to monitor the isolation and purification process (Porzio and Pearson, 1977). Samples were prepared in the presence and absence of the disulfide reducing agent, 2-mercaptoethanol (ME). Collagen peptides were identified by their staining characteristics with Coomassie Blue R250. Apparent molecular weights were determined using standard heavy molecular weight marker proteins (Bio-Rad Labs., Richmond, CA).

Insoluble fractions were examined with the scanning electron microscope after each solvent extraction in order to see the effects of frozen storage and changes during the extraction process. Samples were prepared as described for myofibrils.

RESULTS AND DISCUSSION

Condition of Fish

Fish used were between 50 and 70 cm in length and were landed in March and August. Table 1 gives the average tissue pH and water content and establishes that the fish caught in March were poorly nourished.

Myofibrils

Homogenization resulted in fragmentation of individual myofibrils in fresh muscle but not in frozen. Muscle fibres prepared from frozen

tissue were resistant to shear as illustrated in Figure 2. The fibrous surface matrix of intact frozen muscle cells loses its porous appearance after frozen storage (Figure 3).

Table 1. pH and moisture levels of cod used in the study

	Fish Condition	
	average pH	percent moisture
March fish (3)*	7.15	85.6
August fish (4)*	6.90	83.5

*Figures in parantheses represent number of individuals while data represent overall averages of duplicate analyses of each individual fish

Digestion of the frozen cells with bacterial collagenase clearly disrupted the sheath surrounding the cell (Figure 4). This indicates that the collagen surrounding each muscle cell remained intact after frozen storage suggesting that perhaps the endomysial collagen is at least in part responsible for the resistance to fragmentation exhibited by frozen muscle fibres.

Solubility

Protein (Table 2) and collagen (Table 3) solubilization was reduced after the cod had been frozen stored at -12°C for 5 weeks. Seasonal variations are evident in collagen extractability as noted by Love et al. (1976).

Collagen of the fish caught in March, during the period of starvation, is 31% salt soluble and that of the fish caught in August, when fish are well nourished, is 54% salt soluble. Love et al. (1976) report collagen of cod is more highly crosslinked during periods of starvation, consequently reducing salt solubility as noted in the present study.

Scanning Electron Microscopy

Following removal of salt soluble protein in 0.6 M NaCl, the insoluble material (Figure 1B) was visualized using scanning electron microscopy (SEM). Collagen fibres isolated from fresh tissue were distinct and smooth. After frozen storage, material appeared to be deposited/associated with the fibres resulting in a loss of the distinctly fibrous appearance (Figure 5). One explanation for this could be the migration of water soluble sarcoplasmic proteins to the surface of the cell wall as a result of freezing/frozen storage. Ice crystal damage to the sarcoplasmic membrane may result in "leakage" of these proteins and subsequent deposition on the cell surface. Following

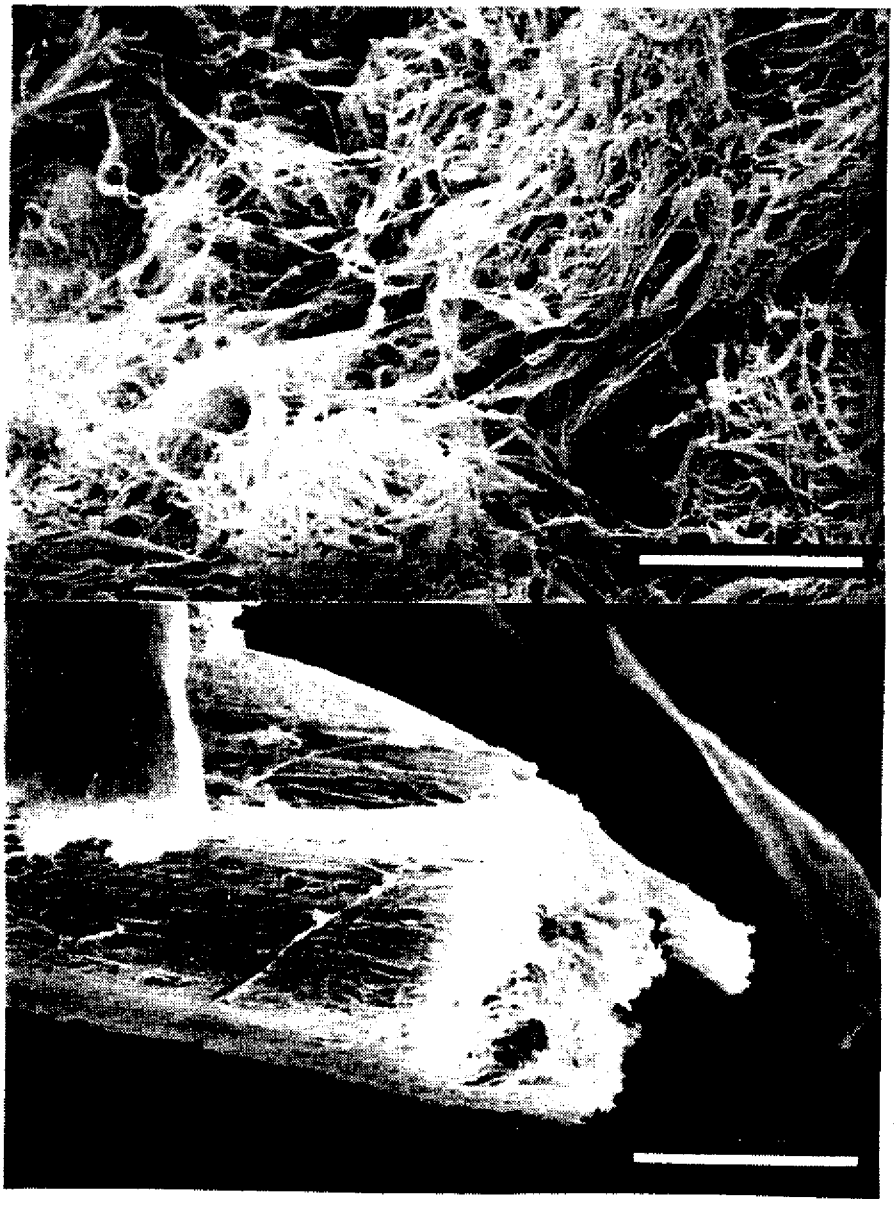


Figure 2. Scanning electron micrographs of myofibrils prepared from (A) fresh and (B) frozen cod muscle. Markers 100μm.

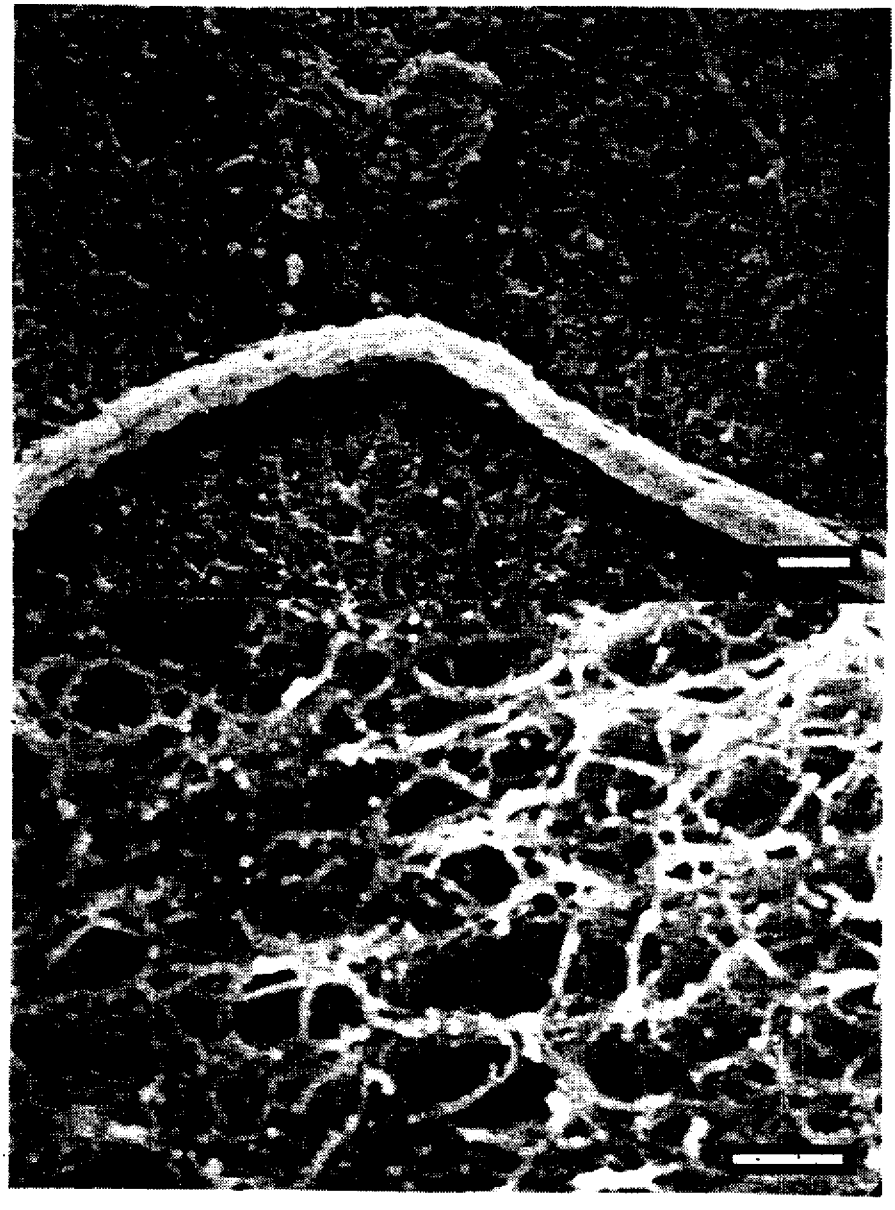


Figure 3. Scanning electron micrographs of cell surface ultrastructure from (A) fresh and (B) frozen cod muscle. Markers 1.0μm.

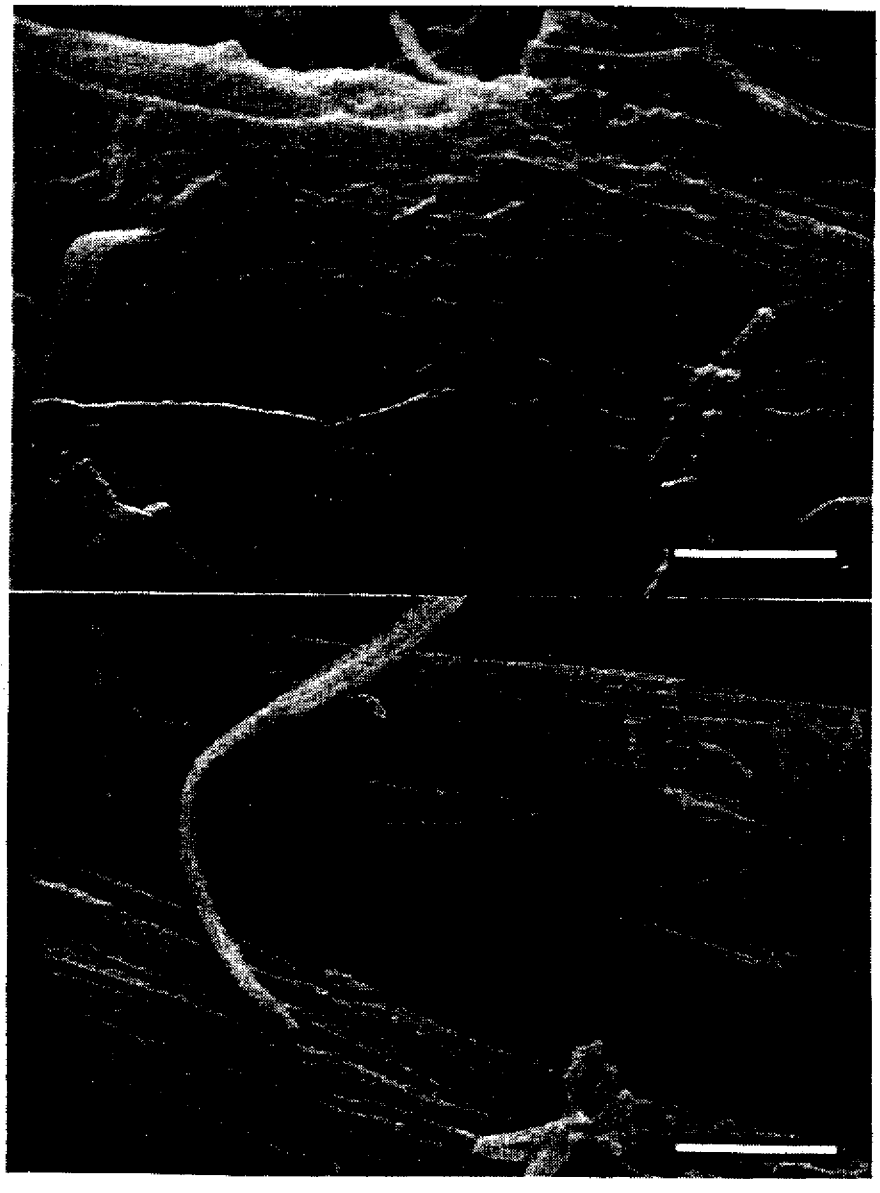


Figure 4. Scanning electron micrographs of frozen cod muscle cells (A) control, (B) incubated with collagenase. Markers 10μm.

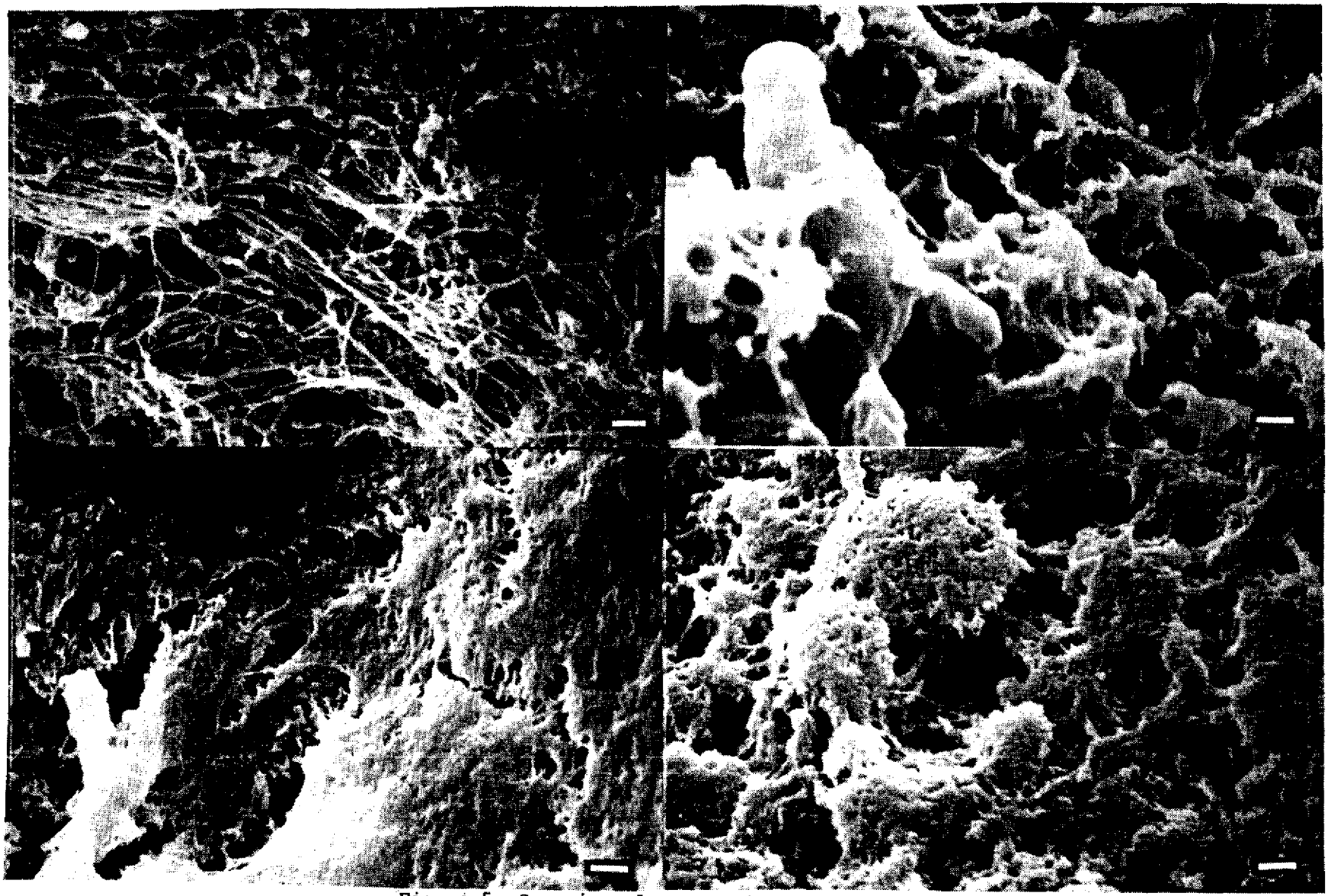


Figure 5. Scanning electron micrographs of salt insoluble material (A) fresh myocommata, (B) frozen myocommata, (C) fresh endomysium, (D) frozen endomysium. Markers 1.0μm.

acid solubilization (Fig 1C), thickened fibres were still apparent in the residue of the frozen sample (Fig 6).

Table 2. Protein solubility of fresh and frozen cod tissue using a sequential solubilization procedure.*

Extraction Medium	Endomysium			Myocommata		
	A	B	C	A	B	C
salt	97.9	97.5	87.8	83.7	81.8	80.7
acid	1.54	1.85	5.32	14.7	17.0	14.2
pepsin	0.47	0.52	6.12	1.54	1.14	4.70
insoluble	0.079	0.91	0.75	0.070	0.086	0.41

*Protein solubility in each extraction medium shown as a percentage of total protein in all steps.
A - fresh cod, landed February
B - fresh cod, landed August
C - frozen cod, landed August

Table 3. Solubility of fresh and frozen cod collagen using a sequential solubilization procedure.*

Extraction Medium	Endomysium			Myocommata		
	A	B	C	A	B	C
salt	88.3	92.46	65.77	31.03	54.27	35.80
acid	1.34	3.47	5.32	55.60	41.03	39.0
pepsin	10.11	3.74	28.8	13.09	4.51	24.8
insoluble	0.22	0.06	0.06	0.27	0.20	0.36

*Collagen solubility in each extraction medium shown as a percentage of total collagen recovered in all steps.
A - fresh cod, landed March
B - fresh cod, landed August
C - frozen cod, landed August

SDS-PAGE

Collagen bands stained with Coomassie blue R-250 appeared as red bands on the gels. All other proteins stained blue. Micko and Schlaepfer (1973) noted the metachromasy, characteristic of collagen and McCormick et al. (1979) reported that all bands staining red were sensitive to collagenase.

Electrophoresis of salt soluble protein recovered from isolated myocommata, with adhering cellular protein, resulted in banding patterns which were similar to those of muscle fibres from which the

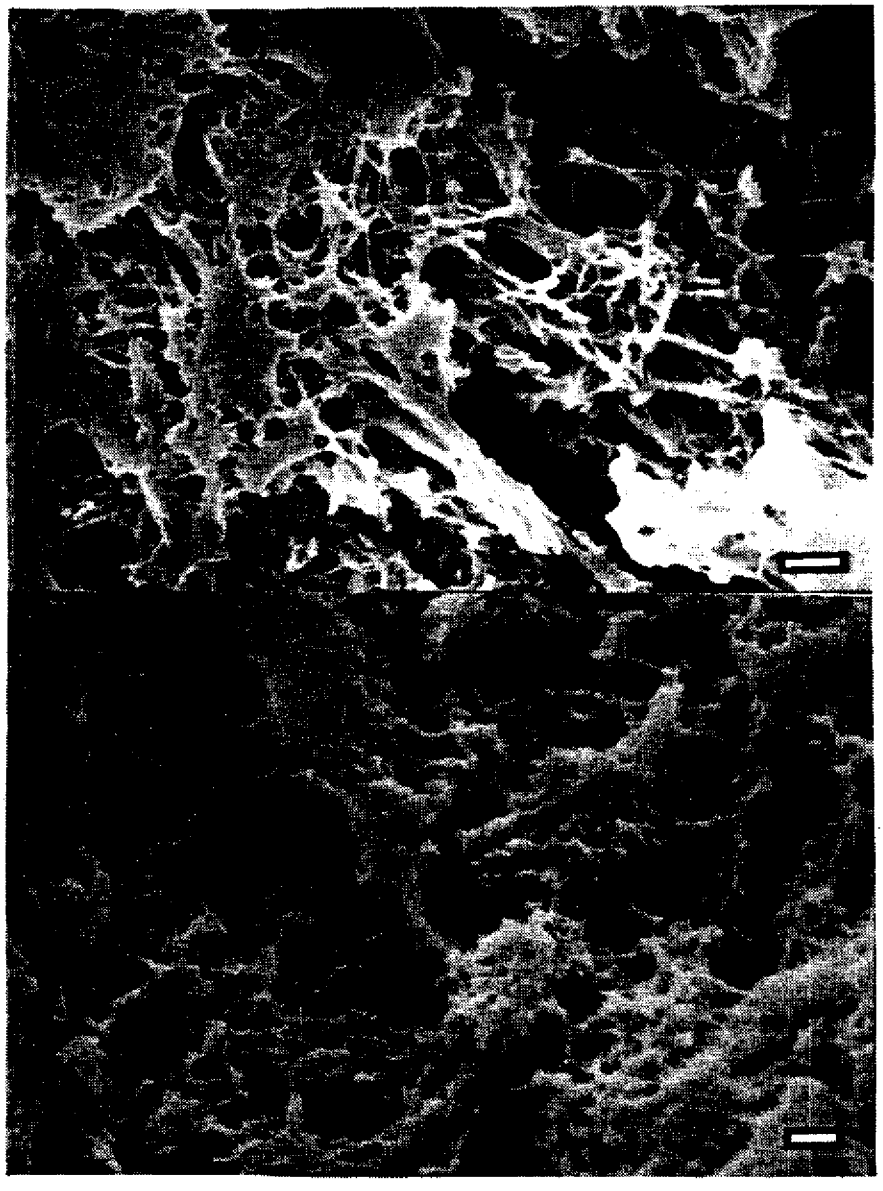


Figure 6. Scanning electron micrographs of acid insoluble myocommata from (A) fresh and (B) frozen cod tissue. Markers 1.0μm.

myocommata had been removed (Figure 7A). After frozen storage, a high molecular protein was present in both samples and appeared as a band barely entering the gel (Figure 7B).

SDS-PAGE of the salt insoluble frozen myotomal/endomysial and myocommatal tissues revealed an increase in high molecular weight (HMW) non-collagenous proteins and loss of intermediate (40-200 kdal) molecular weight species after frozen storage (Figure 8). Myocommatal tissue also contained unique intermediate molecular weight species after frozen storage. Two possibilities exist to account for these high and intermediate molecular weight salt insoluble proteins: (1) covalent interactions among non-collagenous proteins; or (2) interaction of collagen and myofibrillar or sarcoplasmic proteins resulting in high molecular weight complexes which are unable to induce metachromasy.

Type I collagen appears as one or two dimers and two monomers with calculated molecular weights of 200,000 and 96,000, respectively (Miller and Gay, 1982). Purified acid soluble endomysial collagen exhibited this banding pattern and no difference between the fresh and frozen samples were apparent. This implies no covalent changes occur in collagen which remains soluble in acid after frozen storage (Figure 9).

Acetic acid extracted a range of collagenous polypetides from the myocommata (Figure 10). Several peptides with molecular weights less than the typical monomers appeared in both fresh and frozen samples as well as HMW collagen dimers and trimers. After frozen storage, these HMW complexes are not depolymerized with lengthy exposure to acidic conditions thus appearing more stable than similar polymers in the fresh samples. This suggests that the effect of frozen storage on myocommatal acid soluble collagen is crosslink production or stabilization.

The effect of frozen storage on non-collagenous acid soluble protein of the endomysium and myocommata shows an increased proportion of HMW protein. An example of this is shown in Figure 11 displaying densitometer scans of acid soluble endomysial protein from fresh and frozen tissue. No HMW protein, including myosin, was present in the fresh sample in contrast with the frozen sample which is 27% HMW protein (greater than 150,000 daltons). These complexes are stable in the presense of SDS, urea, and mercaptoethanol (ME) suggesting covalent protein aggregation has occurred during frozen storage. The salt insoluble protein banding pattern is different than that of acid soluble protein. The presence of unique intermediate molecular weight (IMW) protein after acid extraction suggests that acid labile bonds are responsible for some of the HMW protein aggregates of the salt insoluble tissue (data not shown).

SDS-PAGE of the frozen, acid insoluble myocommata displayed high and low molecular weight (LMW) proteins which were not present in the fresh sample (Figure 12). This would imply association of high and

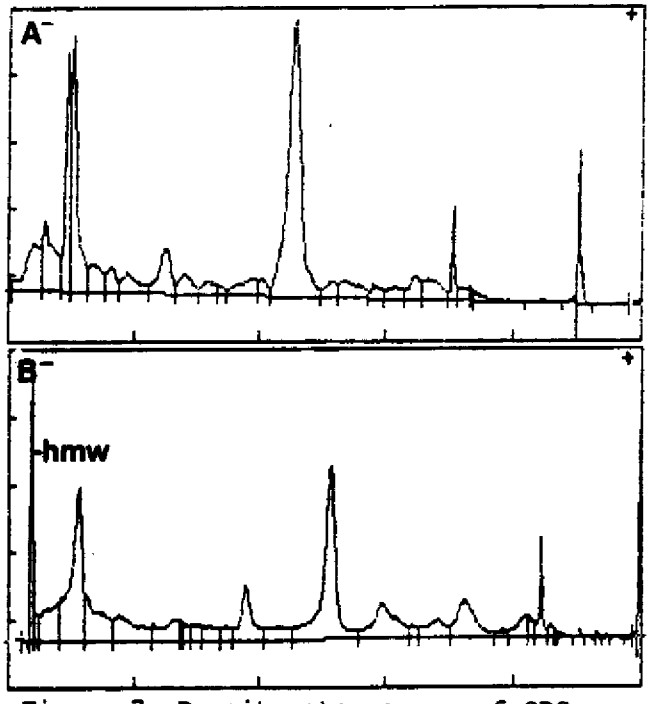


Figure 7. Densitometer scans of SDS electrophoretic gels of salt soluble myotomal protein from (A) fresh and (B) frozen cod myotomes.

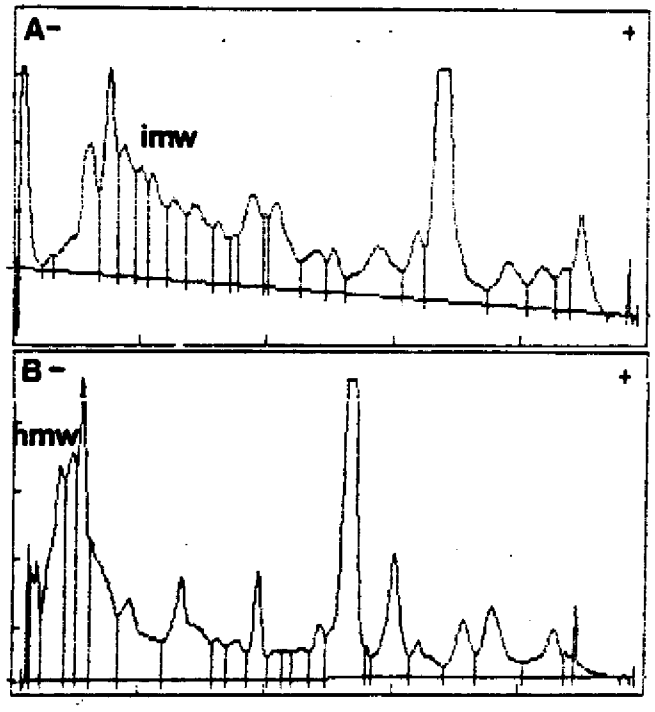


Figure 8. Densitometer scans of SDS electrophoretic gels of salt insoluble myotomal protein from (A) fresh and (B) frozen cod tissue, (Fig. 1b).

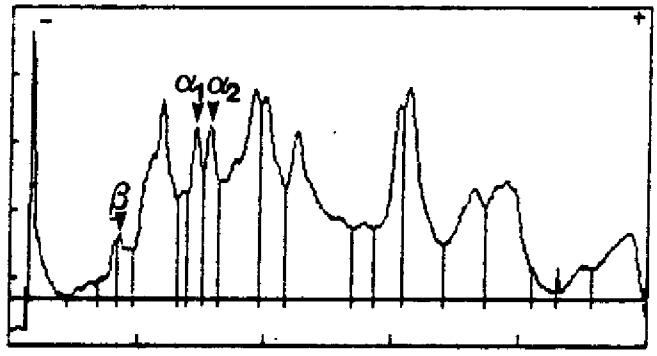


Figure 9. Densitometer scan of an SDS electrophoretic gel from acid soluble fresh endomysial protein. Red staining collagen bands are indicated by arrows.

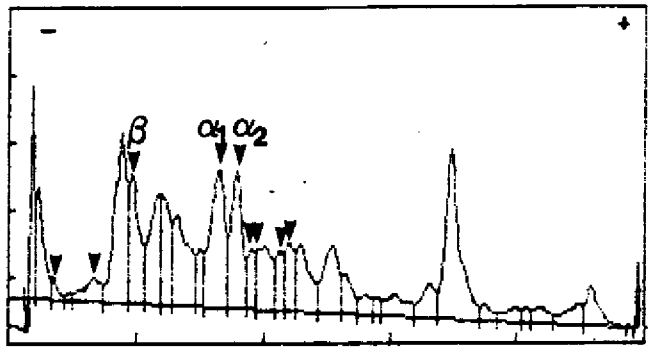


Figure 10. Densitometer scan of an SDS electrophoretic gel from acid soluble fresh myocommatal protein. Red staining bands are indicated by arrows.

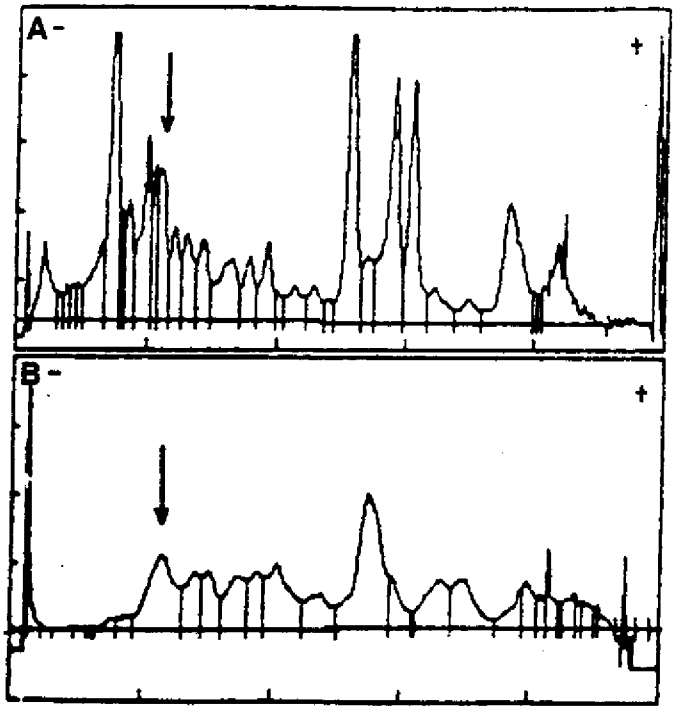


Figure 11. Densitometer scans of SDS electrophoretic gels. Acid soluble endomysial protein from (A) frozen and (B) fresh tissue (Fig. 1f). Arrow represents 150,000 daltons.

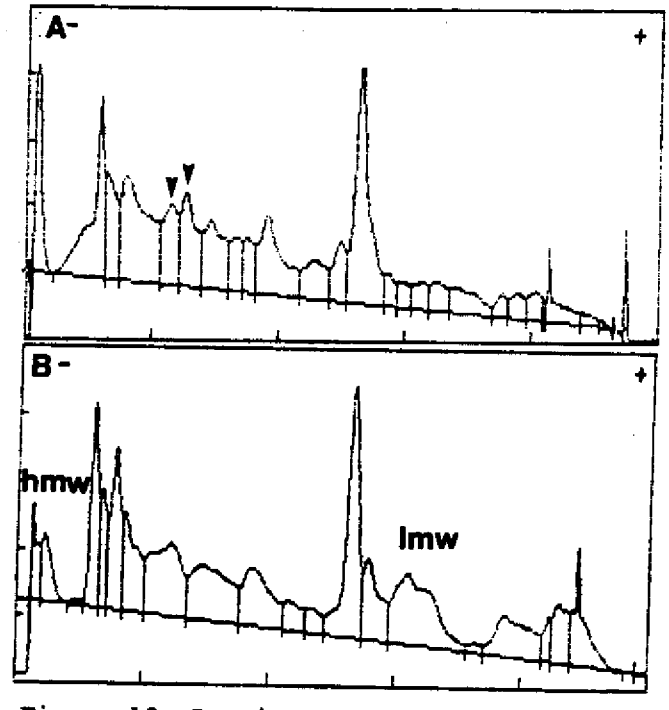


Figure 12. Densitometer scans of SDS electrophoretic gels. Acid insoluble myocommata (Fig. 1c), (A) fresh (B)frozen. Red staining collagen bands are indicated by arrows.

low molecular weight protein with the collagen matrix and that these interactions are resistant to breakdown in acid.

Pepsin treatment of acid insoluble tissue, from both the myocommata and endomysium, fresh and frozen, resulted in many collagen peptides ranging in molecular weight from 230,000 to 17,000 suggesting collagen cleavage by pepsin. Fresh and frozen, pepsin soluble endomysium revealed 3 major collagen components in addition to the two monomers observed in the acid soluble fraction. These additional components, designated x and y and the dimers βx, y (MW 190,000), suggest that an additional collagen type exists within the endomysium (Figure 13A). Several HMW bands appear in the fresh pepsin soluble sample in the presence of a disulfide reducing agent and appear as an HMW protein band barely entering the gel in the absence of ME (Figure 13B). In the frozen pepsin soluble sample, a dimer is associated by disulfide bonding as evidenced by reduction with ME (Figure 13C and D). This suggests breakdown during frozen storage or extraction of the HMW complexes resulting in lower MW crosslinked complexes which migrate as dimers (200 kdal.).

Pepsin released monomers and dimers as well as many lower molecular weight peptides from the myocommata. Unlike the endomysium, myocommatal pepsin soluble collagen is not influenced by the presence of a disulfide reducing agent.

The banding pattern of the frozen pepsin soluble myocommatal protein is qualitatively similar to that of the fresh. However, the proportion of LMW collagenous peptides in the frozen sample is far greater (Figure 14). These peptides are not the result of pepsin activity on native collagen, being less significant in the fresh sample. This implies cleavage of intact collagen peptides is altered by frozen storage.

Purification of the pepsin soluble extracts of both tissues by salt precipitation, resulted in a significant loss of hydroxyproline in the salt supernate. A greater percentage of collagen extracted from the frozen samples did not precipitate (Table 4). A greater quantity of non-precipitable collagen in the frozen sample suggests increased pepsin sensitivity after frozen storage, resulting in small collagen peptides.

Table 4. Pepsin soluble collagen remaining in the salt supernate as a percentage of pepsin soluble collagen.

	Fresh	Frozen
endomysium	69.1	98.9
myocommata	82.4	98.9

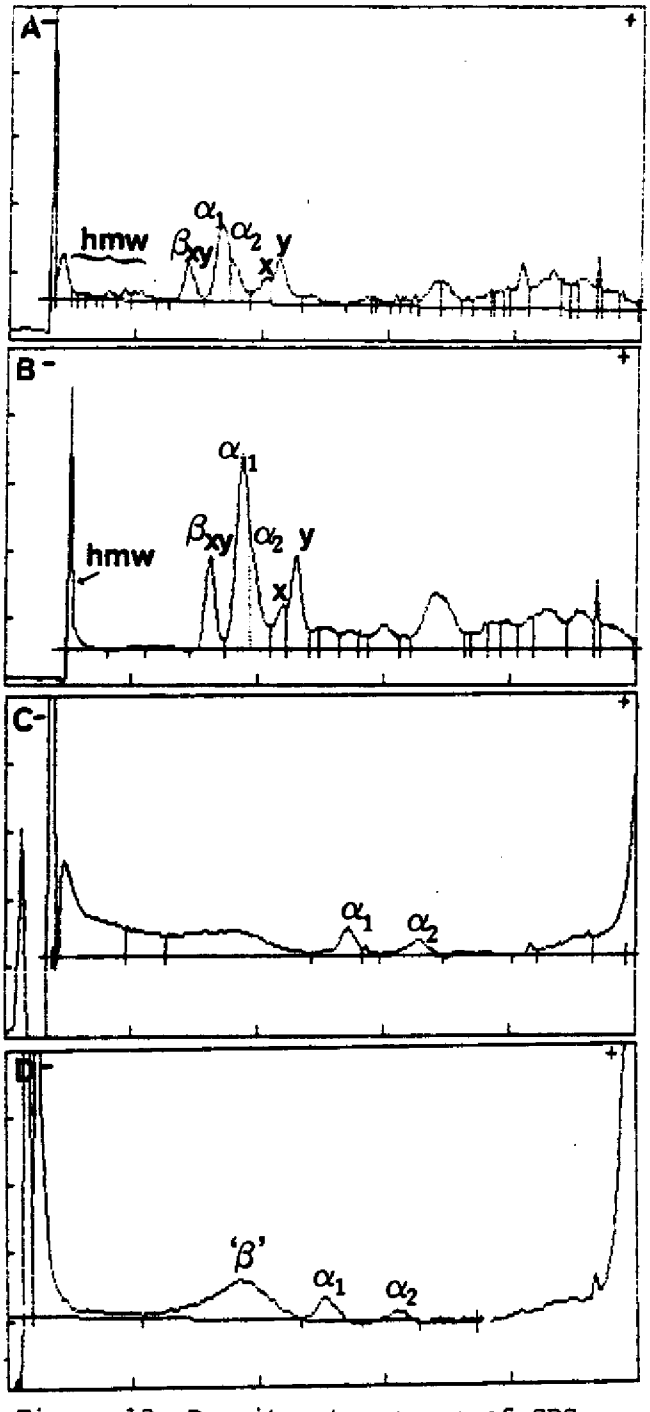


Figure 13. Densitometer scans of SDS electrophoretic gels of pepsin soluble endomysial protein. (A)fresh tissue, with ME; (B)fresh tissue, without ME; (C)frozen tissue, with ME; (D)frozen tissue without ME. All bands stained red.

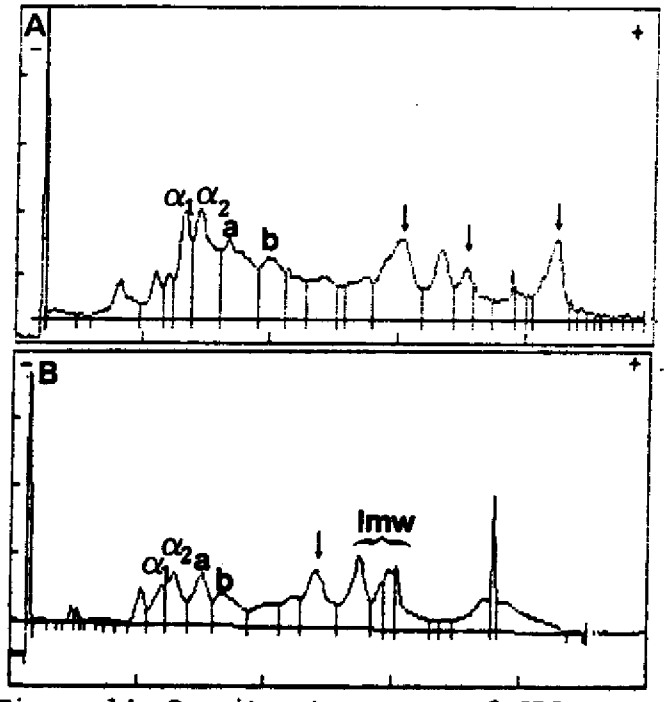


Figure 14. Densitometer scans of SDS electrophoretic gels of pepsin soluble myocommatal protein (A) fresh, (B) frozen. Non-collagen, blue bands indicated by arrow.

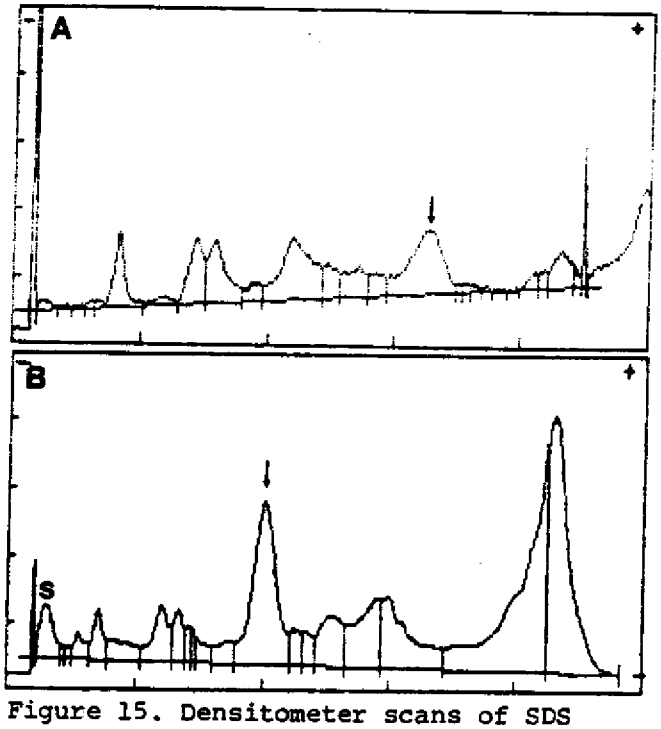


Figure 15. Densitometer scans of SDS electrophoretic gels of pepsin insoluble (Fig. 1d) endomysial material (A) fresh (B) frozen - peak "S" is composed of several bands. Non-collagen, blue staining bands indicated by arrows.

The electrophoretic pattern of the pepsin insoluble residue (Figure 1D) from the fresh endomysium, showed a similar banding pattern to pepsin soluble collagen (Figure 1B). Frozen endomysium displayed the same banding pattern as the fresh with the addition of several major HMW bands (270,000 to 300,000 daltons) representing 25% of the protein on the gel. These additional high molecular weight species appear as a result of crosslinking during frozen storage, or an increased stability of naturally occurring crosslinks.

Insoluble residue from fresh and frozen myocommata did not appear different and was composed of a range of collagenous proteins with low molecular weights to trimers.

CONCLUSIONS

Collagen in the endomysium and myocommata of Atlantic cod is primarily salt soluble. Acid and pepsin soluble collagen is also present in smaller amounts, indicating the presence of acid labile and acid stable crosslinks, respectively. Pepsin sensitivity within the collagen chains is evident. Polymerized peptides remaining after pepsin extraction appear on SDS gels as minor components implying extensively crosslinked collagen.

A series of LMW collagen peptides exist within the myocommata suggesting the presence of more than one type of collagen within this structure. The endomysium also revealed the presence of more than a single type of collagen. Endomysial collagen was different from the myocommata and exhibited disulfide bonding.

Insoluble collagen of the endomysium displays HMW complexes after frozen storage and HMW complexes of acid soluble myocommata become more stable. Salt soluble collagen decreased as a result of frozen storage. Crosslink production or stabilization during frozen storage is implied by these results. Pepsin sensitivity is increased as a result of frozen storage. Evidence for this includes the presence of a covalently bonded dimer in pepsin soluble endomysial collagen as well as an increase in the proportion of LMW collagenous proteins in pepsin digests of myocommata recovered from frozen muscle. Also, the portion of pepsin digested myocommata and endomysium which is not precipitated by salt increases if prepared from frozen fish, indicating increased levels of LMW collagen peptides.

Electron micrographs demonstrated thickened collagen fibres from frozen stored cod suggesting the possibility that associated material may prevent solubilization of cellular proteins by acting as a barrier to solvent entry. These changes are likely reflected in toughening of sarcolemma, reduced protein extractability and are probably the basis for Love's cell fragility test. Further research using several time and temperature storage periods is necessary to determine the quantitative relationship to sensory evaluation of toughness.

REFERENCES

1. Banks, A. 1965. Technological Problems in the Control of Deteriorative Changes in Frozen Fish Requiring more Knowledge from "Fundamental Research in the Technology of Fish Utilization". R. Kreuzer (ed). p. 89.

2. Connell, J. J. 1962. Recent Advances in Food Science. Vol. 1. (London: Butterworths) p. 136.

3. Connell, J. J. 1968. The Effect of Freezing and Frozen Storage of the Proteins of Fish Muscle from "Low Temperature Biology of Foodstuffs". Hawthorne, J. (ed). Pergamon Press p. 333.

4. Dyer, W. J. and J. R. Dingle. 1961. Fish Proteins with Special Reference to Freezing in "Fish as Food". p. 275.

5. Dyer, W. J., H. V. French and J. M. Snow. 1950. Proteins in Fish Muscle I. Extraction of Protein Fractions in Fresh Fish. J. Fish Res., Bd. Can. 7(10)585.

6. Dyer, W. J., and M. L. Morton. 1956. Storage of Frozen Plaice Fillets. J. of Fish. Res. Bd. Canada. 13(1)129.

7. Dyer, W. J., M. L. Morton, D. I. Fraser and E. G. Bligh. 1956. Storage of Frozen Rosefish Fillets. J. Fish. Res. Bd. Canada 13(4)569.

8. King, F. J. 1966. Ultracentrifugal Analysis of Changes in the Composition of Myofibrillar Protein Extracts Obtained from Fresh and Frozen Cod Muscle. J. Food Science 31:649.

9. Lavety, J. and R. M. Love. 1972. The strengthening of cod connective tissue during starvation. Comp. Biochem. Physiol. 41A:39.

10. Love, R. M., K. Yamaguchi, Y. Creac'h and J. Lavety. 1976. The Connective Tissues and Collagens of Cod during Starvation. Comp. Biochem. Physiol. 55B:487.

11. Love, R. M., J. Lavety and N. G. Garcia. 1971. The Connective Tissue of Fish. VI Mechanical Studies on Isolated Myocommata. J. Food Tech. 7:291.

12. Love, R. M. 1979. Toward a Valid Sampling Technique in "The Chemical Biology of Fishes". Academic Press. p. 1.

13. Love, R. M. 1969. In: Freezing and Irradation of Fish. R. Kreuzer (ed). Fishing News (Books), London. p. 40.

14. Love R. M. 1960. Water Content of Cod (Gadus callarias L.) Muscle. Nature. 185:692.

15. Love, R. M. 1958. Measurement of toughness in cold-stored fish. Nature. pp. 108-109.

16. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein Measurement with the Folin Phenol Reagent. J. Biol. Chem. 193:265.

17. McCormick, P. J., S. Chandrasekhar, and A. J. T. Millis. 1979. Direct Visualization of Collagens and Procollagens in Polyacryloamide Gels. Anal. Biochem. 97:359-366.

18. Micko, S. and W. W. Schlaepfer. 1978. Metachromasy of periferal nerve collagen on polyacrylamide gels stained with Coomassie Brilliant Blue R250. Anal. Biochem. 88:566.

19. Miller, E. J. and S. Gay. 1982. Collagen: An overview. In "Methods in Enzymology". Vol. 82, part A, p. 3.

20. Olson, P. G., F. C. Parrish, Jr., and M. H. Stromer. 1976. Myofibril fragmentation and shear resistance of three bovine muscles during postmortem storage. J. Food Sci. 41(5) 1036.

21. Porzio, M. A. and A. M. Pearson. 1977. Improved Resolution of Myofibrillar Proteins with Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis. Biochem. Biophys. Acta. 490:27.

22. Shenouda, S. Y. K. 1980. Theories of Protein Denaturation during frozen storage of fish flesh. Advances in Food Research. 26:275.

23. Sikorski, Z. E., D. N. Scott and D. H. Buisson. 1984. The Role of Collagen in the Quality and Processing of Fish. CRC. Critical Rev. in Food Sci. and Nutrition. 20(4)301.

24. Tanaka, T. 1965. Electron Microscopic Studies of Toughness in Frozen Fish. The Technology of Fish Utilization. R. Kreuzer (ed). p. 5.

25. Woessner, J. F. 1961. The Determination of Hydroxyproline in Tissue and Protein Samples Containing Small Proportions of this Amino Acid. Arch. Biochem. Biophys. 93:440.

26. Young, E. G., and J. W. Lorimer. 1960. The Acid-soluble collagen of Cod Skin. Arch. Biochem. Biophys. 88:373.

IN VITRO LIPID OXIDATION OF SARCOPLASMIC RETICULUM BY IN SITU CONCENTRATIONS OF Fe AND Cu

Eric A. Decker and Herbert O. Hultin

Massachusetts Agricultural Experiment Station
Department of Food Science and Nutrition
Marine Foods Laboratory
University of Massachusetts
Gloucester, Massachusetts 01930, U.S.A.

INTRODUCTION

Lipid oxidation is a major pathway of chemical deterioration in frozen fish muscle which leads to decreased quality. An enzyme found in winter flounder (Pseudopleuronectes americanus) is an active catalyst of lipid oxidation at temperatures as low as -20°C (Apgar and Hultin, 1982). This enzymic system, which is located in the sarcoplasmic reticulum (Borhan et al., 1984), catalyzes lipid oxidation in the presence of iron and NADH and is stimulated by the presence of ADP (McDonald and Hultin, 1987). The ability of copper to interact with this enzyme system is not well understood.

Halliwell and Gutteridge (1986) have suggested that a low molecular weight pool of soluble iron chelates are capable of stimulating lipid oxidation in vivo. Thirty percent of the iron of winter flounder is found in the water-soluble (press juice) fraction (Kramer, 1987). Addition of the soluble metals with a molecular weight less than 10,000 daltons as the sole source of metal to a model system containing 0.1 mM ADP and 0.5 mg sarcoplasmic reticular (SR) protein resulted in stimulation of lipid oxidation after 6 days (Kramer, 1987).

The purpose of this paper was to determine the soluble and low molecular weight (LMW) Fe and Cu concentrations of winter flounder and Atlantic mackerel (Scromber scrombrus) muscle and to determine if these concentrations of Fe and Cu could promote lipid oxidation. Varying concentrations of NADH and ADP were also tested to see their effects on SR-catalyzed lipid oxidation at in situ Fe and Cu concentrations.

MATERIALS AND METHODS

Winter flounder and Atlantic mackerel were obtained from fish processors in Gloucester, Massachusetts. The fish were immediately iced and used within 24 hr. Mackerel were frozen by placing whole in a -80°C freezer and thawed by placing under running tap water. Nicotinamide adenine dinucleotide, reduced (NADH) and adenosine-5'-diphosphate (ADP) were obtained from Boehringer Mannheim Biochemicals. Baker Instra-analyzed nitric acid and Gold-label magnesium nitrate used for metal determinations were purchased from J.T. Baker. All other

chemicals were reagent grade. All glassware used in metal determinations were acid washed.

Sarcoplasmic reticulum was isolated from flounder muscle using the method of Borhan et al. (1984) except that the minced tissue was homogenized three times at 30 s intervals, the resulting homogenate was adjusted to pH 7.3, the ratio of supernatant:45% sucrose:20% sucrose used for the discontinuous sucrose gradient was 50:9:3 and the gradient was centrifuged for 60 min. SR protein was determined using the Lowry procedure as modified by Markwell et al. (1978).

A "press juice" representing the soluble components of flounder muscle was prepared by centifuging coarsely cut up tissue at 15,000 rpm in a type 19 rotor (22,000 x g) for 15 hr in a Beckman L5-65B preparative ultracentrifuge at 5°C. The LMW fraction of the press juice was isolated by ultrafiltration using an Amicon Stirred Cell Model 52. The filtrate was collected until the press juice volume was reduced by one half. YM series membranes with molecular weight cutoffs (MWCO) of 10,000, 5,000 and 1,000 were used to isolate the LMW fraction. The LMW fraction (10,000 MWCO) of aged flounder muscle was isolated after storing the fillets on ice for 6 days.

Iron and copper concentrations of the press juice and the LMW fractions were performed on a Perkin-Elmer 3030B Atomic Absorption Spectrophotometer with a HGA-400 Graphite Furnace accessory. Iron concentrations were determined at 248.3 nm with a slit width of 0.2 nm. Pretreatment temperature was 1400°C with a ramp time of 5 s and a hold time of 20 s. Atomization temperature was 2400°C for 4 s with the gas flow shut off. All samples for iron determination contained 0.5 mg% magnesium nitrate. Copper contents were analyzed at 324.8 nm with a slit width of 0.7 nm, a pretreatment temperature of 1200°C with a ramp time of 5 s, a hold time of 20 s and an atomization temperature of 2300°C for 4 s with stop flow. Both iron and copper samples contained 0.2% nitric acid.

SR-catalyzed lipid oxidation in a model system was measured by determining the production of thiobarbaric acid-reactive substances as described by McDonald and Hultin (1987). The model system contained 0.5 mg SR protein in 5 mM histidine, 0.12 M KCl buffer, pH 6.8 with varying concentrations of metals, ADP and NADH. All reactions were run for 20 h at 6°C in triplicate unless otherwise indicated.

RESULTS AND DISCUSSION

Iron concentrations of the press juice and LMW fractions from flounder and mackerel are listed in Table 1. Flounder and mackerel white muscle had very similar levels of iron in the press juice and the LMW fraction. Approximately 10% percent of this water-soluble iron was found to have a molecular weight of 1,000 daltons or less. Storing

flounder muscle on ice for 6 d did not cause any changes in the iron content of the press juice or the LMW fraction. Freezing the mackerel muscle resulted in an increase in the iron concentration of the press juice. Mackerel red muscle had approximately 6 times as much iron in the press juice and about 4 times as much iron in the LMW fraction as flounder and mackerel white muscle. The LMW iron of the red muscle also represented about 10% of the total soluble iron. Freezing had very little effect on the total soluble iron of the mackerel red muscle.

Table 1. Iron concentrations (ppb) of the water-soluble fractions of mackerel and flounder muscle.

Muscle Fraction	Flounder Muscle	
	Fresh	Aged[1]
Press Juice	347	--
<10,000 daltons	33	33
<5,000 daltons	43	--
<1,000 daltons	48	--
	Mackerel White Muscle	
	Fresh	Frozen (-80°C)
Press Juice	400	718
<10,000 daltons	38	54
<5,000 daltons	49	37
<1,000 daltons	42	36
	Mackerel Red Muscle	
	Fresh	Frozen (-80°C)
Press Juice	2356	2244
<10,000 daltons	--	206

[1]Fillets stored on ice for 6 days.

Table 2 shows the copper concentration of press juice and the LMW fractions of flounder and mackerel muscle. The LMW copper in flounder muscle was found in 2 fractions. Approximently 60% of the copper was between 1,000 and 10,000 daltons and the remaining 40% was less than 1,000 daltons. These 2 fractions represented approximately 37% of the total soluble copper. Storing flounder fillets on ice for 6 d did not change the copper concentration in the press juice or the LMW fraction. The total soluble copper concentration of mackerel white muscle was very similar to flounder muscle. The LMW copper of mackerel white muscle had a molecular weight of less than 1,000 daltons and represented approximently 40% of the total soluble copper. Freezing the mackerel resulted in an increase in the copper concentration of the press juice. The soluble copper concentration of the mackerel red muscle was approximately 3 times as high as in flounder and mackerel white muscle. The concentration of LMW copper in red muscle was similar to the concentrations found in white muscle and flounder, but this only represented about 10% of the total soluble copper. Although copper tends to be found in lower concentrations than iron in fish

muscle (Gordon and Martin, 1982) its concentration in the LMW fractions is similar to iron suggesting that it could play an important role in SR-catalyzed lipid oxidation.

Table 2. Copper concentrations (ppb) of the water-soluble fractions of mackerel and flounder muscle.

Muscle Fraction	Flounder Muscle	
	Fresh	Aged[1]
Press Juice	98	—
<10,000 daltons	34	36
<5,000 daltons	37	—
<1,000 daltons	15	—
	Mackerel White Muscle	
	Fresh	Frozen (-80°C)
Press Juice	109	178
<10,000 daltons	44	54
<5,000 daltons	49	43
<1,000 daltons	39	44
	Mackerel Red Muscle	
	Fresh	Frozen (-80°C)
Press Juice	368	394
<10,000 daltons	—	44

[1]Fillets stored on ice for 6 days.

Fig. 1 shows the oxidation of the SR membrane by the concentrations of copper and iron found in the LMW fraction of flounder and mackerel muscle. Iron was a more powerful catalyst than copper at all concentrations. At the concentrations found in the LMW fraction of flounder and mackerel white muscle (approximately 40 ppb) there is not much difference between the activity of copper and iron, but at the concentration of iron found in red muscle (200 ppb) approximently 4 times as much oxidation occurred. This suggests that the concentrations of copper and iron found in the LMW fractions of fish muscle are capable of promoting lipid oxidation.

Fig. 2 shows the production of thiobarbituric-reactive substances (TBARS) over a 27 h period by 0.79 uM copper and iron. Iron stimulated SR-catalyzed lipid oxidation more than copper at all times examined. A lag phase occurred for 16-20 hrs for both iron and copper. This is much different than a model system containing 15 uM iron in which substantial oxidation occurred within 30 min (Hultin *et al.*, 1982).

Catalysis of lipid oxidation by iron (0.36 uM or 20 ppb) and copper (0.79 uM or 50 ppb) in the absence of ADP with varying NADH concentrations is shown in fig. 3. Maximum lipid oxidation occurred at 50 uM NADH for both copper and iron. The observed NADH optimum was lower than previously observed for the model system (McDonald and

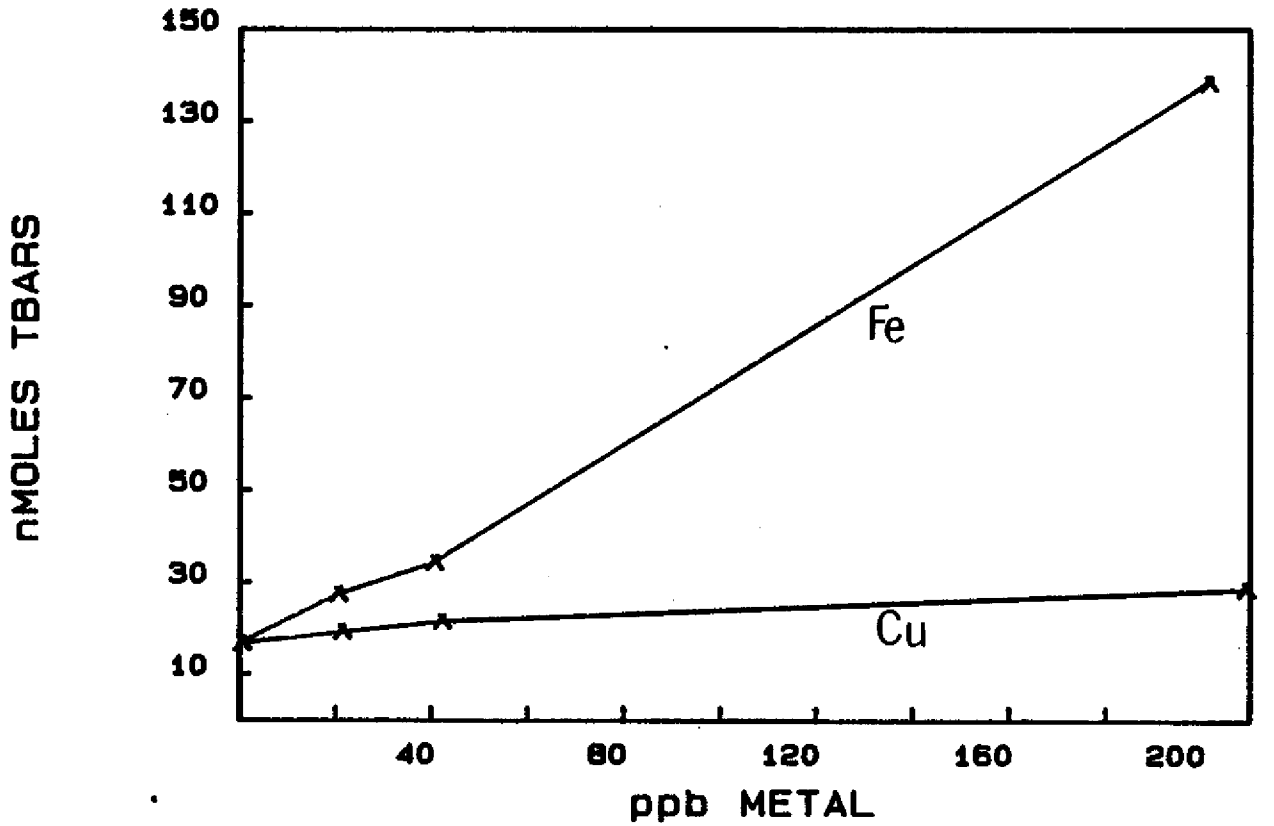


Figure 1. Production of TBA reactive substances by varying concentrations of iron and copper. The reaction was run at 6°C for 20 hr with 0.1 mg sarcoplasmic reticular protein per ml 0.12 MKCl, 5 mM histidine buffer, pH 6.8 and 0.1 mM NADH.

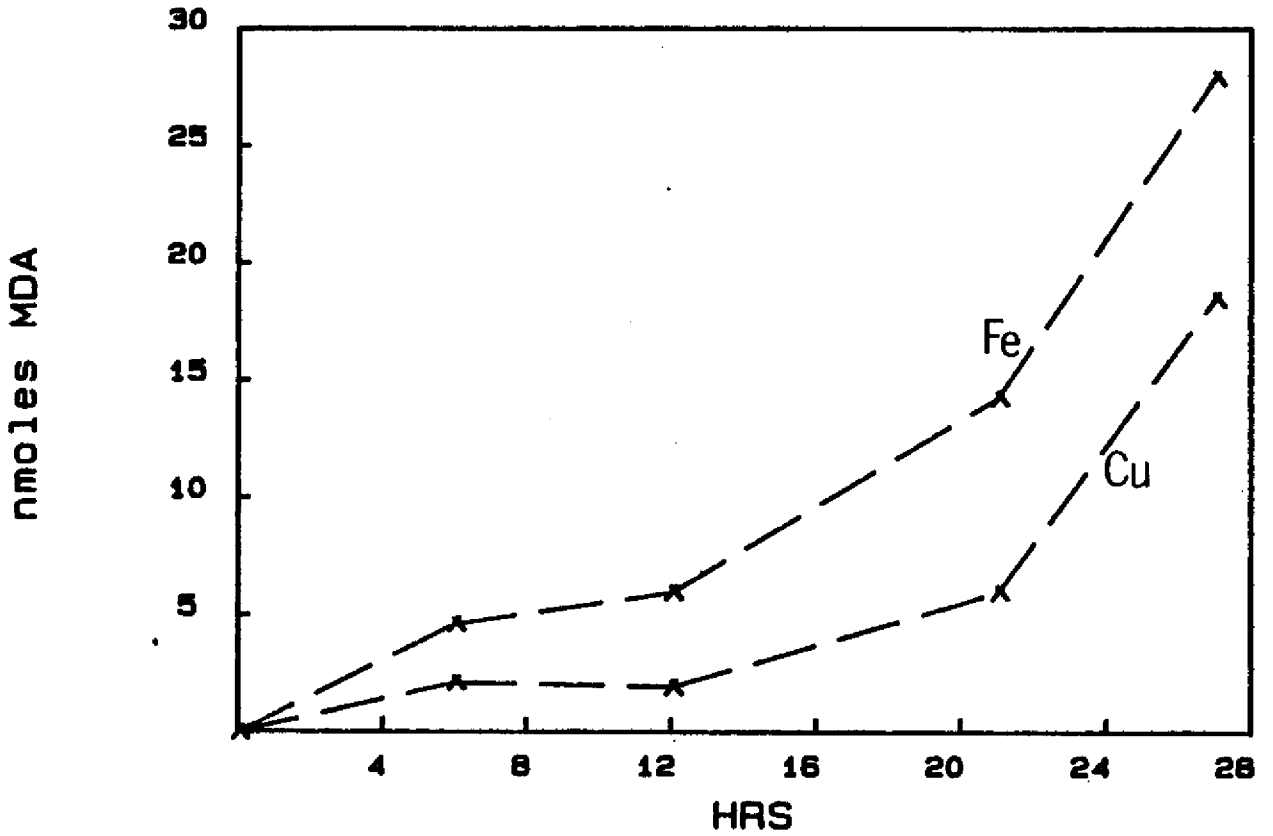


Figure 2. Production of TBA reactive substances as a function of time in the presence of 0.79 uM Fe and Cu and 0.1 mM NADH. The reaction was run at 6°C with 0.1 mg sarcoplasmic reticular protein per ml 0.12 MKCl, 5 mM histidine buffer, pH 6.8.

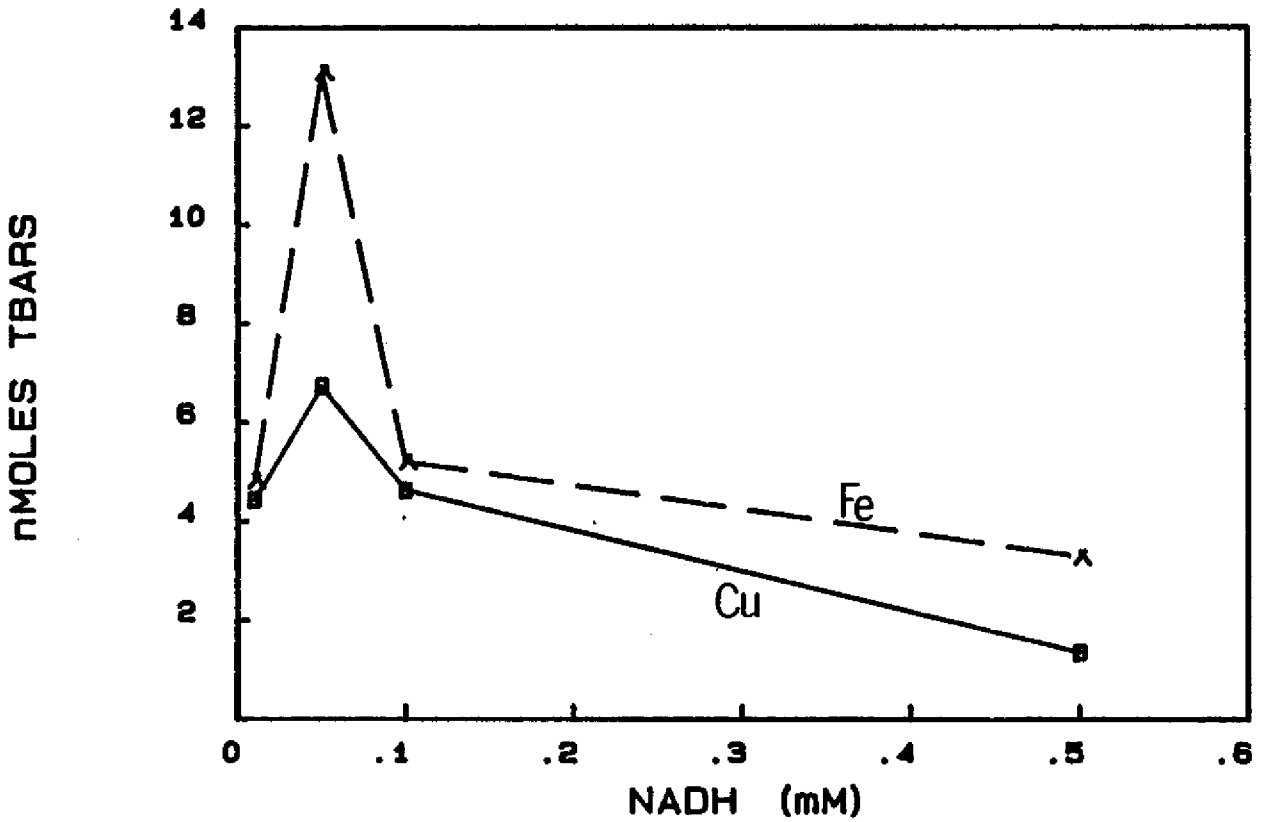


Figure 3. Production of TBA reactive substances by 0.36 uM Fe and 0.79 Cu, with varying NADH concentrations. The reaction was run at 6°C for 20 hr with 0.1 mg sarcoplasmic reticular protein per ml 0.12 MKCl, 5 mM histidine buffer, pH 6.8.

Hultin, 1987). The conditions for the two experiments differed in iron concentration (0.36 uM vs. 150 uM), assay time (20 h vs. 30 min) and by the presence of a NADH regeneration system in these experiments, but an optimal NADH concentration existed for lipid oxidation in both cases. At the optimal concentration of NADH the iron activity was much higher than the copper activity, but as the NADH concentration was increased or decreased the difference between the two activities decreased. This suggests that at NADH concentrations other than optimal, copper could have an important role in SR-catalyzed lipid oxidation.

The effect of ADP concentrations on copper- and iron-catalyzed enzymic lipid oxidation is shown in fig. 4. Maximal activity was observed at an ADP concentration approximately equal to the metal concentration (0.36 uM Fe or 0.79 uM Cu). This agrees with other observations in our laboratory where optimal enzymic lipid oxidation occurred when iron:ADP concentrations were 1:1 (Erickson, 1987). Iron was a more powerful catalyst than copper at the optimal ADP concentration and it remained more effective at all ADP concentrations examined. Iron-catalyzed SR oxidation fluctuated more with varying ADP concentrations than copper-catalyzed lipid oxidation suggesting that ADP is more important in iron-catalyzed enzymic lipid oxidation. ADP could be stimulating the Fe-catalyzed lipid oxidation by maintaining the ferric ion in a soluble, mobile state. This again suggests that copper could be an important catalyst of SR-stimulated lipid oxidation when <u>in situ</u> ADP concentrations are not optimal.

Table 3 shows the stimulation of SR-catalyzed lipid oxidation using the LMW fractions of flounder and mackerel muscle as the sole source of iron and copper. Very little oxidation occurred in the presence of the LMW fractions. Only the LMW fraction of mackerel red muscle catalyzed any substantial oxidation in 5 days. The lack of activity could be due to the presence of antioxidants in the LMW fractions or it could be due to the chelation of the metals to compounds which prevent metal-enzyme interactions or change the oxidation-reduction potential of the metals.

Table 3. Lipid oxidation of flounder SR in the presence of low molecular weight fraction of flounder and mackerel.

	nmol TBARS/mg SR protein	
Metal Source	1 day	5 days
Flounder LMWF (20 ppb Fe; 20 ppb Cu)	--	2
Mackerel LMWF (white muscle) (30 ppb Fe; 30 ppb Cu)	--	0
Mackerel LMWF (dark muscle) (120 ppb Fe; 30 ppb Cu)	—	11
20 ppb $FeCl_3$	5	—
50 ppb $CuSO_4$	1	—

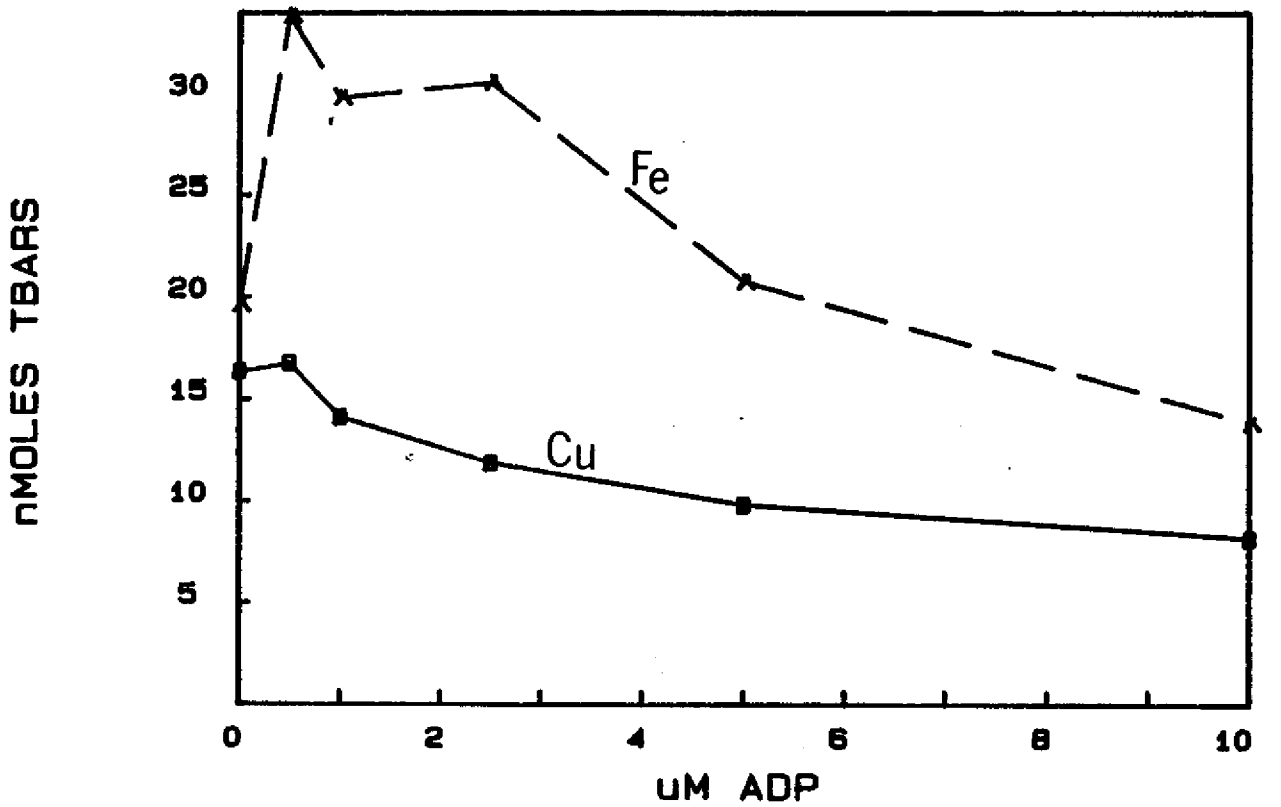


Figure 4. Production of TBA reactive substances by 0.36 uM Fe and 0.79 uM Cu with varying ADP concentrations. The reaction was run at 6°C for 20 hr with 0.1 mg sarcoplasmic reticular protein per ml 0.12 MKCl, 5 mM histidine buffer, pH 6.8.

The iron and copper concentrations found in the low molecular weight water-soluble fraction of winter flounder and mackerel white muscle muscle were very similar. At this concentration (40 ppb) iron produced slightly higher levels of TBARS than copper. Mackerel red muscle contained approximately 4 times as much LMW iron but had about the same concentration of LMW copper as flounder and mackerel white muscle. The concentration of iron found in the LMW fraction of red muscle was a much more effective catalyst of SR-stimulated lipid oxidation than the concentrations found in flounder and mackerel white muscle.

Lipid oxidation catalyzed by both copper and iron showed optimal concentrations of NADH and ADP, but iron was more strongly inhibited than copper when NADH and ADP were not at optimal levels. This suggests that even though iron is a more powerful catalyst than copper under optimal conditions, copper could be an important contributor to *in situ* lipid oxidation.

REFERENCES

Apgar, M.E. and Hultin, H.O. 1982. Lipid peroxidation in fish muscle microsomes in the frozen state. Cryobiology 19:154.

Borhan, M., Shewfelt, R.L. and Hultin, H.O. 1984. Sarcoplasmic reticulum from flounder muscle having improved lipid peroxidative activity. Anal. Biochem. 137:58.

Erickson, M.C. 1987. Private communication. University of Georgia Experiment Station, Griffin, GA.

Gordon, D.T. and Martin, R.E 1982. Vitamins and minerals in seafood of the Pacific northwest. Ch. 21. In "Chemistry and Biochemistry of Marine Food Products" R.E. Martin, G.J. Flick, C.E. Hebard and D.R. Ward (Eds.), p. 429 AVI Publishing Co., Westport, CT.

Halliwell, B. and Gutteridge, M.C. 1986. Iron and free radical reactions: two aspects of antioxidant protection. TIBS 11, 372.

Hultin, H.O., McDonald, R.E. and Kelleher, S.D. 1982. Lipid oxidation in fish muscle microsomes. Ch. 1. In "Chemistry and Biochemistry of Marine Food Products" R.E. Martin, G.J. Flick, C.E. Hebard and D.R. Ward (Eds.), p. 1-12 AVI Publishing Co., Westport, CT.

Kramer, K.M. 1987. Form of iron in flounder muscle and its relation to sarcoplasmic reticular lipid oxidation. M.S. Thesis, Univ. of Massachusetts, Amherst.

Markwell, M.A.K., Hass, S.M., Bieber, L.L. and Tolbert, N.E. 1978. Modification of the Lowery procedure to simplify protein determination in membrane and lipoprotein samples. Anal. Biochem. 87:206.

McDonald, R.E. and Hultin, H.O. 1987. Some characteristics of the enzymic lipid peroxidation system in the microsomal fraction of flounder skeletal muscle. J. Food Sci. 52, 15.

EFFECT OF CO_2-O_2 MODIFIED ATMOSPHERE WITH PRESSURIZATION ON THE KEEPING QUALITY OF COD FILLETS AT 2°C

F.M. Bartlett and E.G. Bligh
Canadian Institute of Fisheries Technology
Technical University of Nova Scotia
P. O. Box 1000
Halifax, Nova Scotia
B3J 2X4

INTRODUCTION

The highly perishable nature of fresh fish is well known. The primary cause of spoilage of unfrozen lean fish is the growth and metabolic activities of bacteria. The most common spoilage bacteria of fish, and other fresh muscle foods, are gram-negative, aerobic psychrotrophs, especially Pseudomonas spp. (Ogrydziak and Brown, 1982). Such bacteria are capable of causing deterioration of fish with the production of obnoxious odours and off flavours, during refrigerated storage.

Recently there has been increased interest by the fishing industry in modified atmosphere (M.A.) packaging using a carbon dioxide (CO_2)-enriched environment to extend the shelf life of refrigerated fresh fish. Carbon dioxide gas is a relatively safe and effective bacteriostatic agent which has been used for the preservation of fresh meats, poultry and produce since the 1930's (Baker et al., 1986; Wolfe, 1980). A number of studies have reported that a CO_2-enriched atmosphere can have a beneficial effect on the shelf life of various seafoods. Barnett et al. (1982) found that Pacific salmon stored in an atmosphere containing 90% CO_2 were still acceptable up to 21 days at 0°C. Rock cod fillets in 80% CO_2 had lower levels of ammonia and trimethylamine (TMA), as well as lower bacterial numbers, in comparison to samples stored in air (Mokhele et al., 1983; Brown et al., 1980). Cod (Cann et al., 1983), haddock (Stansby and Griffiths, 1935), swordfish (Lannelongue et al., 1982), turbot (Gauthier et al., 1986), shrimp (Layrisse and Matches, 1984) and crab (Parkin and Brown, 1983) have also been reported to have improved keeping quality (40-200%) under CO_2-enriched storage conditions.

A previous study by our lab (Woyewoda et al., 1984) was unable to show such a significant improvement in the shelf life extension of cod fillets using modified atmosphere storage. Inadequate exposure of the fillets to the gas mixture may have been a factor. In this study we attempted to maximize the surface area of the fillets exposed to the gas mixture by placing them on support pads. In addition, an initial pressurization treatment was evaluated in an attempt to accelerate the uptake of CO_2 by the fish flesh.

MATERIALS AND METHODS

Fresh Atlantic cod (Gadus morhua) fillets were purchased from a local fish plant the day after catching. They were packed in 10 lb polyethylene containers surrounded with ice and immediately transported to the laboratory (< 1 h). Upon arrival, each fillet was trimmed to a weight of approximately 200 g, washed under cold, running tap water and drained.

Modified Atmosphere

The fresh fillets (5 per bag) were placed on a porous nylon pad (24 x 15 x 0.5 cm) and put in a cryovac barrier bag (40 x 24 cm; 3 mil polyethylene/1 mil nylon with oxygen transmission rate - 88 cc/24 h/100 in^2/mil thickness at 25°C, 50% RH; carbon dioxide transmission rate = 549 cc/24 h/100 in^2/mil thickness at 25°C, 50% RH). Each package was evacuated and sealed with vacuum sealer, back flushed with a 60% CO_2:20% O_2:20% N_2 gas mixture, inflated and resealed. Half of the MA packaged fillets were subjected to a pressurization treatment, while the inflated bags were packed in ice, using a retort to facilitate maximum absorption of the gas mixture by the fillets. The fillets were pressurized at 45 p.s.i. above normal atmospheric pressure (total 4 atmospheres) for 1 h. Control fillets were similarly packaged in barrier bags except that they were filled with air and not pressurized.

All of the bags were stored at 2±1°C until assessed for quality at 0, 2, 5, 7, 9, 12 and 15 days.

Microbiological Analyses

A single bag from each treatment was selected at random each sampling day. Thirty gram samples were excised aseptically from each of three fillets per bag, diluted in sterile 0.1% peptone with 0.5% NaCl (w/v) and stomached for 1 min in a model BA 6021 Stomacher (A. J. Seaward; Edmunds, England). Serial dilutions were prepared and plated in duplicate on Plate Count Agar (Difco, Detriot, MI) with 0.5% NaCl to enumerate total aerobes, anaerobes and psychrotrophs. Lactic acid bacteria were enumerated on MRS agar (Difco) with 0.5% NaCl.

All plates were incubated at 21°C for 72 h except for the psychrotroph plates which were held at 7°C for 10 days. The anaerobe plates were incubated in Gaspak anaerobic jars (BBL; Cockeyville, MD).

Trimethylamine Analysis

Fifty gram samples were removed from each of three fillets per treatment and extracted wiht 7.5% trichloroacetic acid (TCA) and analyzed for trimethylamine according to the method of Dyer (1945).

Surface pH Determination

Surface pH measurement of the fillets were obtained using an

Orion surface pH electrode and Acument pH meter. pH values were obtained for at least two sites on each side of the fillets.

Drip Measurement

The volume of fluid released upon storage of the fillets was measured by pouring the liquid into a graduated cylinder as each bag was opened. Drip was expressed as a percentage of the initial weight of the fillets.

Raw Quality Grading

Raw fillets were judged for overall acceptability by four expert graders on each sampling day according to the following scheme:

Acceptability
1. excellent
2. highly acceptable
3. low acceptability
4. reject

Taste Panels

An informal taste panel evaluation of the fillets was carried out by four expert graders. On each sampling day, two fillets from each pack were removed, wrapped in plastic film, placed in 1 lb cardboard packages and stored at -30°C until evaluated (approximately one week after the 15th day). For each session the fillets were thawed, wrapped in aluminum foil and baked at 230°C for 15 min. Each panelist received four samples, along with a fresh reference sample (R). The reference samples were untreated fillets frozen at -30°C on day 0 of the study. The panelists were asked to compare the samples to the reference on the basis of flavour and overall acceptability.

RESULTS AND DISCUSSION

Those fillets stored in the presence of the CO_2-enriched modified atmosphere (MA/MAP) were found to have significantly lower total aerobic plate counts (TAPC) than the controls packaged in air (Fig. 1). The TAPC of the controls exceeded 10^6 colony forming units per gram (cfu/g) after 5 days at 2°C, while the MA/MAP stored fillets required 15 days to reach this level. The TAPC for the pressurized and non-pressurized MA stored fillets were not statistically different which suggests that the pressurization treatment did not enhance the bacteriostatic effect of the CO_2 gas.

The psychrotroph counts (results not shown) were similar to the TAPC for each group of samples indicating that those counts obtained at the mesophilic temperature were representative of the numbers of bacteria capable of growth during the storage of the fish at 2°C.

The major safety concern with the use of modified atmosphere packaging is the potential it has for creating conditions favourable for anaerobic bacteria. The growth and toxin production of Clostridium botulinum is of particular concern (Hintlian and Hotchkiss, 1986).

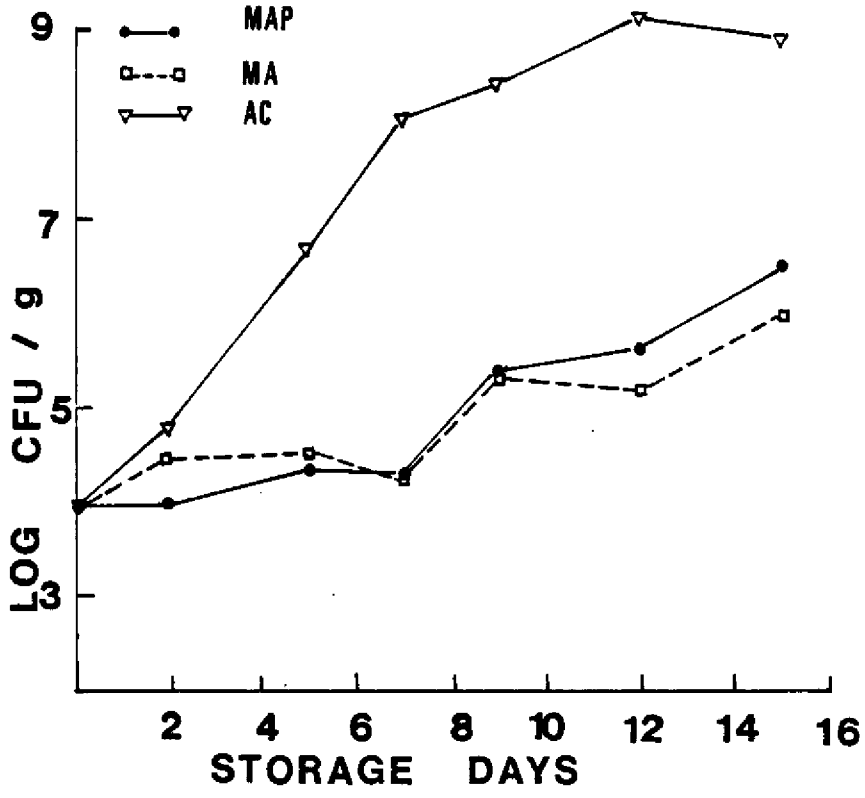


FIG. 1. Total aerobic bacterial counts on cod fillets at 2° C. ●, Modified atmosphere with pressurization (MAP); □, modified atmosphere (MA); ▽, air control (AC).

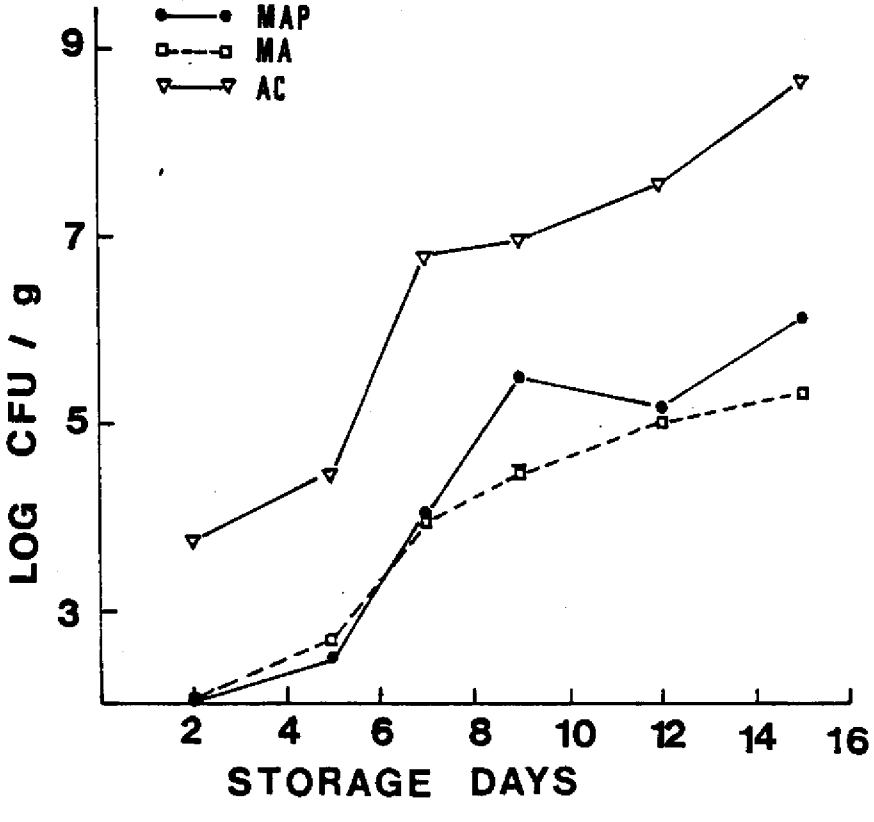


FIG. 2. Anaerobic bacterial counts on cod fillets at 2° C. ●, Modified atmosphere with pressurization (MAP); □, modified atmosphere (MA); ▽, air control (AC).

The results for the anaerobic bacterial counts showed that those fillets stored in the presence of 60% CO_2:20% O_2:20% N_2 (MA/MA) had lower counts than the control fillets on each sampling day (Fig. 2). The reduction of anaerobic counts was probably a result of the bacteriostatic effect of CO_2 and the relatively high concentration of O_2 (20%) which was used.

A number of studies of meat, poultry and fish stored under modified atmosphere conditions have noted a shift in the microflora from primarily gram-negative bacteria to a the predominance of a gram-positive flora, especially lactic acid bacteria (Lannelongue et al., 1982; Enfors et al., 1979; Sanders and Soo, 1978). The lactic acid bacterial (LAB) counts were generally found to be lower for the fillets packaged in the CO_2-enriched environment than the controls (Fig. 3). However, the LAB counts of the MA/MAP fillets increased steadily after 5 days and their numbers at 9 days (10^5 cfu/g) was at the same level as their total aerobic counts at 9 days. These results indicate that the microbial flora of the MA/MAP samples had indeed shifted towards a predominance of LAB during the latter part of the storage period. Conditions which are selective for the growth of LAB can be beneficial since members of this group are capable of inhibiting the growth of other types of bacteria which contribute to spoilage (Roth and Clark, 1975). It has been suggested that high numbers of LAB contribute to the beneficial effect of CO_2 on the shelf life of meat and seafood.

Chemical analysis of fish quality consisted of the measurement of trimethylamine (TMA) production. TMA is a bacterial breakdown product of trimethylamine oxide (TMAO). Based on an unacceptability level of 15 mg TMA-N/100 g (Woyewoda et al., 1984) the control fillets were considered spoiled at day 9 while the MA/MAP fillets took at least 15 days to reach this level (Fig. 4).

One of the theories concerning the mechanism by which CO_2 inhibits bacterial growth, is the formation of a weak organic acid (carbonic acid) on the surface of the flesh which lowers the substrate pH (Stathan, 1984). This was not found to be the case in this study. Measurement of the surface pH failed to show any dramatic decrease in pH which might account for the bacterial inhibition by CO_2 (Fig. 5). This is probably a result of the high buffering capacity of the fish flesh.

Storage of fish muscle in high concentrations of CO_2 has been reported to cause excessive drip (Fey and Regenstein, 1982; Villemure et al., 1986). Fish stored in the presence of 60% CO_2 did have higher drip losses, particularly on day 12 and 15, than the controls (Fig. 6). However, at no time during this study did the drip exceed 5 percent of the total weight, and would not be considered excessive.

The raw quality grading (overall acceptability) was consistent with the TMA values. The control fillets were judged to be spoiled after 9 days, while the MA/MAP fillets were acceptable up to and including day 15 (Fig. 7). The taste panel assessments (data not shown) of the samples were also in agreement with the raw grading scores and TMA values.

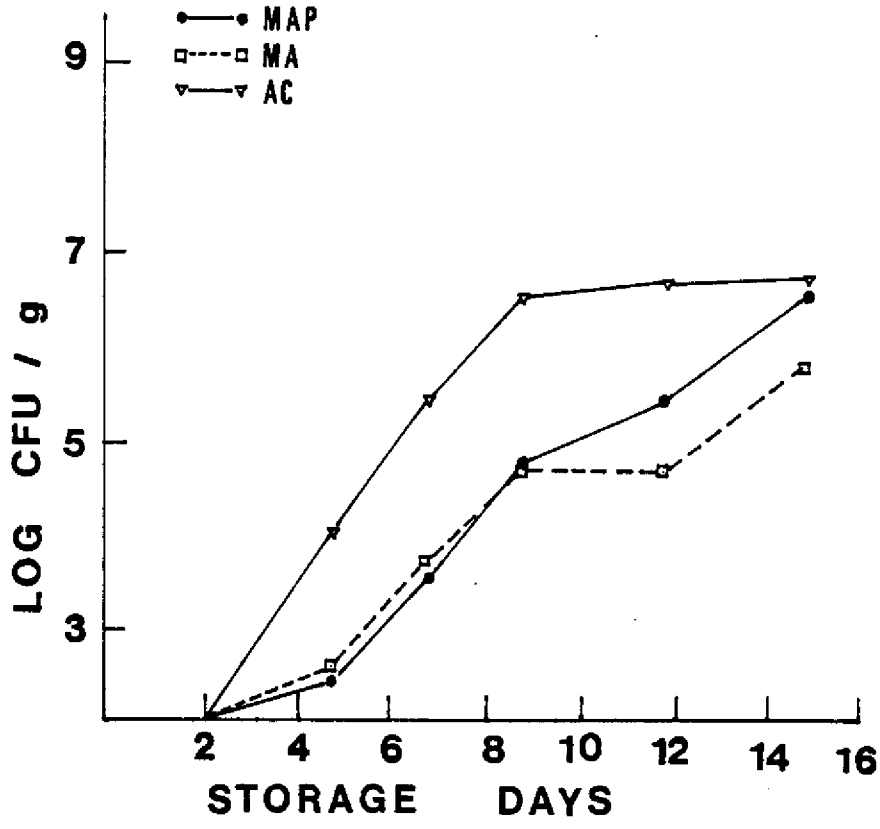


FIG. 3. Lactic acid bacterial counts on cod fillets at 2° C. ●, Modified atmosphere with pressurization (MAP); □, modified atmosphere (MA); ▽, air control (AC).

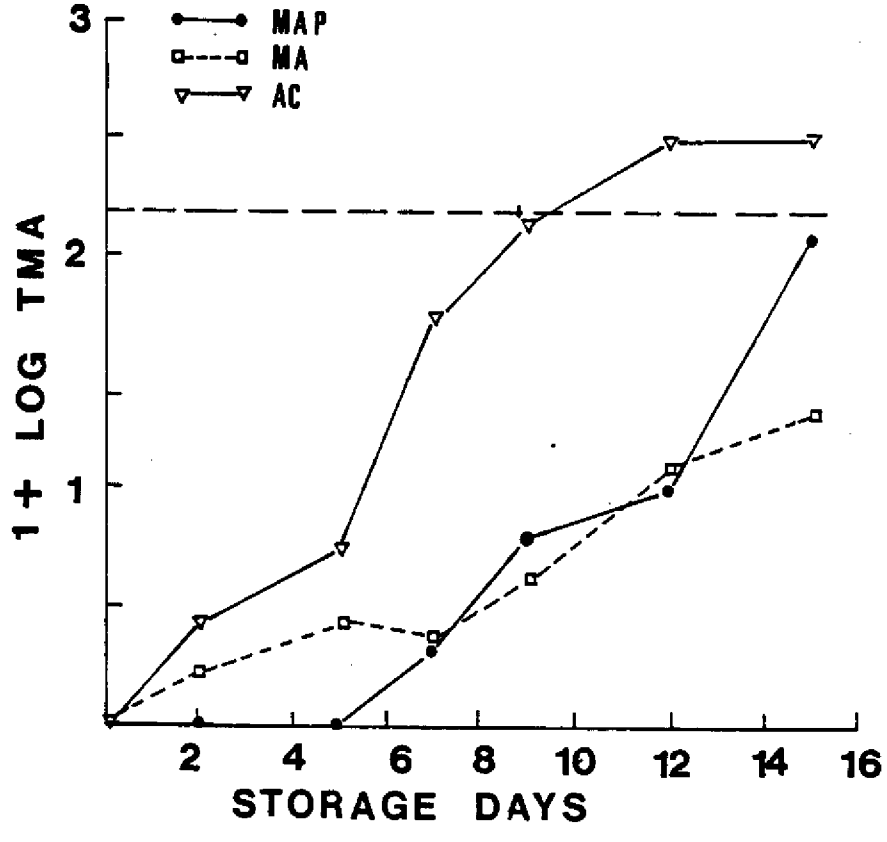


FIG. 4. Trimethylamine production in cod fillets at 2°C. ●, Modified atmosphere with pressurization (MAP); □, modified atmosphere (MA); ▽, air control (AC).

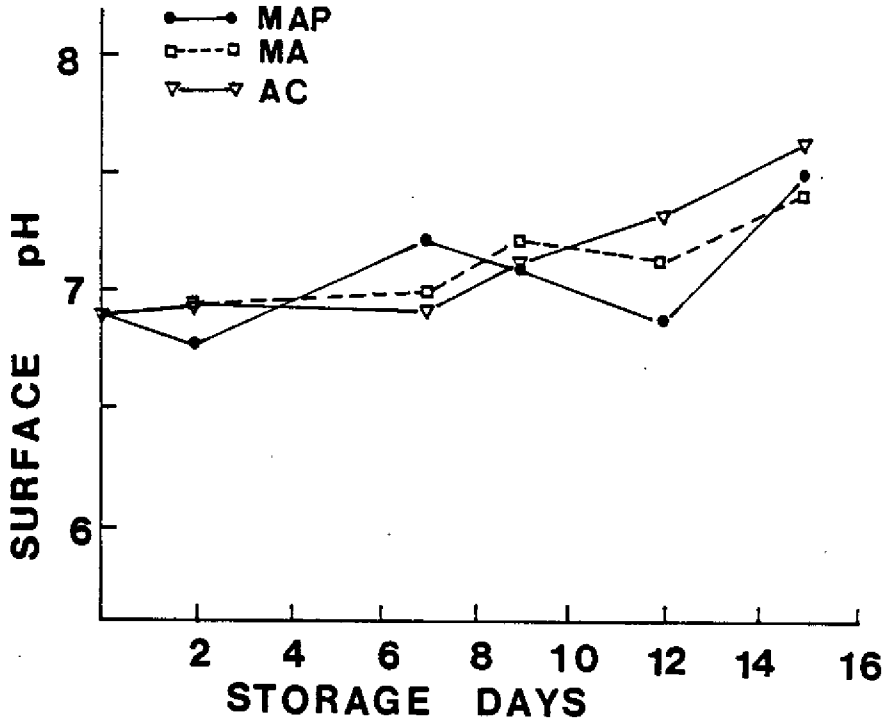


FIG. 5. Surface pH values of cod fillets at 2° C. ●, Modified atmosphere with pressurization (MAP); ◘, modified atmosphere (MA); ▽, air control (AC).

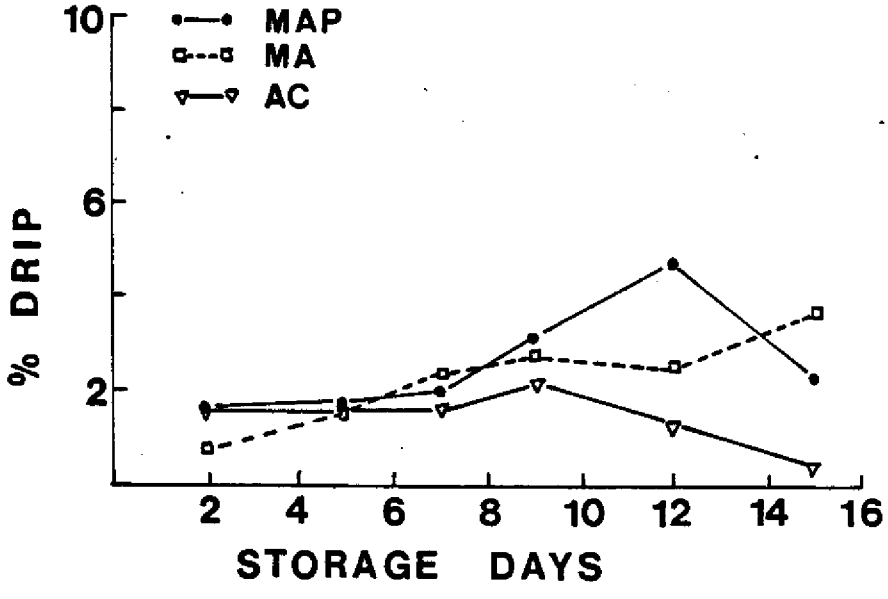


FIG. 6. Drip production as percentage of initial weight of cod fillets at 2° C. ●, Modified atmosphere with pressurization (MAP); ◘, modified atmosphere (MA); ▽, air control (AC).

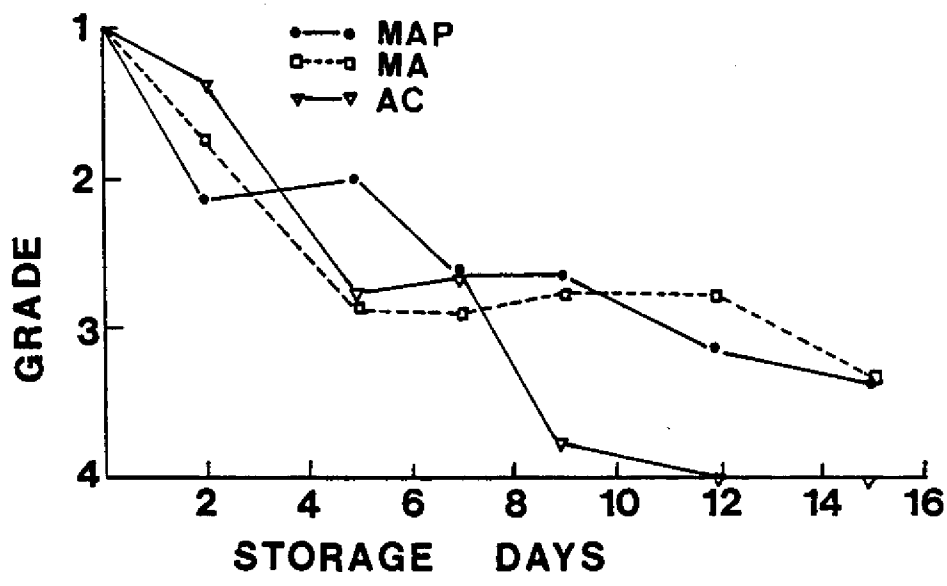


FIG. 7. Raw quality grading of cod fillets at 2° C (1 - excellent; 2 - highly acceptable; 3 - low acceptable; 4 - reject). ● Modified atmosphere with pressurization (MAP); □, modified atmosphere (MA); ▽, air control (AC).

CONCLUSIONS

1. The M.A. of 60% CO_2:20% O_2: 20% N_2 was effective at reducing total aerobic bacterial counts.
2. The M.A. did not appear to enhance the growth of anaerobic bacteria.
3. Lactic acid bacteria appear to predominate in M.A. packaged fillets.
4. Trimethylamine production was inhibited by the M.A.
5. M.A. did not cause a significant decrease in surface pH of fillets or result in excessive drip.
6. Sensory evaluation generally confirmed the beneficial effect of the M.A. as indicated by reduced bacterial counts and TMA production.
7. There was no significant difference between pressurized and non-pressurized M.A. packaged fillets.

REFERENCES

1. Baker, R.C., R.A. Qureshi and J.H. Hotchkiss 1986. Effect of elevated level of carbon dioxide containing atmosphere on the growth of spoilage and pathogenic bacteria at 2,7 and 13°C. Poultry Sci. 65:729-737.
2. Barnett, H.J., F.E. Stone, G.G. Roberts, P.J. Hunter, R.W. Nelson and J. Kwok 1982. A study in the use of high concentration of CO_2 in a modified atmosphere to preserve fresh salmon. Mar. Fish. Rev. 44(3):7-11.
3. Brown, W.D., M. Albright, D.A. Watts, B. Heyer, B. Spruce and R.J. Price 1980. Modified atmosphere storage of rockfish (Sebastes miniatus) and silver salmon (Oncorhynchus kisutch). J. Food Sci. 45:93-96.
4. Cann, D.C., G.L. Smith and N.C. Houston 1983. Further studies on marine fish stored under modified atmosphere packaging. Aberdeen: Ministry of Agriculture. Fisheries and Food.
5. Dyer, W.J. 1945. Amines in fish muscle I. colorimetric determination of trimethylamine as the picrate salt. J. Fish. Res. Bd. Can. 6:351.
6. Enfors, S.O., G. Molin and A. Ternstrom 1979. Effect of packaging under carbon dioxide, nitrogen or air on the microbial flora of pork stored at 4°C. J. Appl. Bacteriol. 47:197-208.
7. Fey, M.S. and J.M. Regenstein 1982. Extending shelf life of fresh wet red hake and salmon using CO_2-O_2 modified atmosphere and potassium sorbate at 1°C. J. Food Sci. 47:1048-1054.
8. Gauthier, S., R.E. Simard and J. Amiot 1986. Conservation en vrac du fletan du Groenland (Reihardtius hippoglossoides) sour atmosphere modifiee. Can. Inst. Food Sci. Technol. J. 19(5):249- 253.

9. Hintlian, C.B. and J.H. Hotchkiss 1986. The safety of modified atmosphere packaging. A review. Food Technol. 40:70-76.

10. Lanngelongue, M., G. Finne, M.O. Hanna, R. Nickelson and C. Vanderzant 1982. Microbiological and chemical changes during storage of swordfish (Xiphias gladius) steaks in retail packages containing CO_2-enriched atmospheres. J. Food Prot. 45:1197-1203.

11. Layrisse, M.E. and J.R. Matches 1984. Microbiological and chemical changes of spotted shrimp (Pandalus platyceros) stored under modified atmospheres. J. Food Prot. 47(6):453-457.

12. Mokhele, K., A. Johnson, E. Barrett and D.M. Ogrydziak 1983. Microbiological analysis of rock cod (Sebastes spp.) stored under elevated carbon dioxide atmospheres. Appl. Environ. Microbiol. 45(3):878-883.

13. Ogrydziak, D.M. and W.D. Brown 1982. Temperature effects in modified atmosphere storage of seafoods. Food Technol. 36:86-96.

14. Parkin, K.L. and W.D. Brown 1983. Modified atmosphere storage of dungeness crab (Cancer magister). J. Food Sci. 48:370-374.

15. Roth, L.A. and D.S. Clark 1975. Effect of lactobacilli and carbon dioxide on the growth of Microbacterium thermosphactum on fresh beef. Can. J. Microbiol. 21:629-632.

16. Sanders, E.H. and H.M. Soo 1978. Increasing shelf life by carbon dioxide treatment and low temperature storage of bulk pack fresh chickens packaged in nylon/surlyn film. J. Food Sci. 43:1519-1523.

17. Stansby, M.E. and F.P. Griffiths 1935. Carbon dioxide in handling fresh fish (haddock). Ind. Eng. Chem. 27:1452-1458.

18. Stratham, J.A. 1984. Modified atmosphere storage of fisheries products: The state of the art. Food Technol. Austral. 36(5):233-239.

19. Villemure, G., R.E. Wimard and G. Picard 1986. Bulk storage of cod fillets and gutted cod (Gadus morhua) under carbon dioxide atmosphere. J. Food Sci. 51(2):317-320.

20. Wolfe, S.K. 1980. Use of CO- and CO_2-enriched atmospheres for meats, fish and produce. Food Technol. 34(3):55-58.

21. Woyewoda, A.D., E.G. Bligh and S.J. Shaw 1984. Controlled and modified atmosphere storage of cod fillets. Can. Inst. Food Sci. Technol. J. 17:24-27.

PURIFICATION OF OMEGA - 3 FATTY ACIDS FROM FISH OILS USING HPLC: AN OVERVIEW

Judith Krzynowek, Daniel L. D'Entremont,
Laurie J. Panunzio and Richard S. Maney
National Marine Fisheries Service, NOAA, NEFC
Gloucester Laboratory, 30 Emerson Avenue
Gloucester, MA 01930

INTRODUCTION

The National Marine Fisheries Service(NMFS), and the National Institutes of Health(NIH) have entered into a joint research venture which is anticipated to span the next decade. The agreement assures researchers, who are studying the therapeutic value of fish oils, a source of fish oils and fish oil concentrates produced and quality controlled/quality assured by the NMFS Charleston SC Laboratory. The NMFS laboratories at Gloucester, MA and Seattle, WA were tasked to find a method for the production of highly purified fractions of the two major fatty acids of interest to the medical community, eicosapentaenoic acid(EPA) and docosahexaenoic acid(DHA). This paper offers some considerations for the separation of the ethyl esters of these and three other fatty acids at >90% purity using reverse phase high performance liquid chromatography.

RESULTS AND DISCUSSION

The stationary phase of choice for the separation of fatty acid esters is a hydrocarbon(C18) bonded to a solid support (Scholfield, 1975, Wille et al, 1987, Tokiwa, 1981, Bascetta et al, 1984). Columns prepacked with this packing are available through several suppliers. Columns 1 and 2 (Table 1) are examples of the columns obtained from two different suppliers. Columns with small pore size solid support, such as Column 1 with 8 micron pore size, have greater resolution and higher purity of eluted fractions than columns with larger pore sizes such as Column 2 with 20-30 micron pore size. Purities are given as percent of the total fatty acids as determined by capillary GLC analyses. Therefore, impurities in the fractions are other fatty acids.

Solvent choice depends on desired purity and end use of the eluted fractions. Ethanol and water are the solvent system chosen for this research, because they are food grade materials. This is a consideration for an end product which might be consumed by humans. The

solvents require no special handling or waste disposal measures and do not oxidize. If, however, none of these considerations is of import to a particular application, tetrahydrofuran(THF), methanol, and water will yield higher purity fractions of EPA and DHA (Table 1). THF is potentially explosive and requires special OSHA considerations for its safe handling and waste disposal, is toxic, as is methanol, and oxidizes readily, which is undesirable when in contact with fish oil which is prone to rapid oxidation.

Table 1. Purity of eluted EPA/DHA fractions from a feedstock of 57% EPA/31% DHA using different solvent systems on an analytical column.

Solvent	%EPA/%DHA	
	Column 1 8μ	Column 2 25μ
THF/MeOH/H20	99/95	99/89
EtOH/H20	98/92	97/87

The greater the concentration of EPA and DHA in the feedstock, the higher the purity of the eluted EPA and DHA (Table 2). We are working with a feedstock which has been highly concentrated in EPA and DHA by urea fractionation at the Charleston Laboratory and contains almost 50% EPA and 25% DHA. When using a feedstock furnished by the Seattle Laboratory, which has been cycled through their super-critical CO2 system and is 79% EPA or 79% DHA, purities of close to 100% can be recovered.

Table 2. Purity of eluted EPA/DHA using THF:methanol:water as the solvent system on an analytical column with differing purity of feedstock.

%EPA/%DHA	
Feedstock	Eluant
32/19	99/82
57/31	99/89
79% EPA	99.9% EPA
79% DHA	96% DHA

If purity is of paramount importance, peak slicing is recommended. The back sides of the eluted peaks yield the highest purity. This is definitely the case with DHA (Figure 1b).

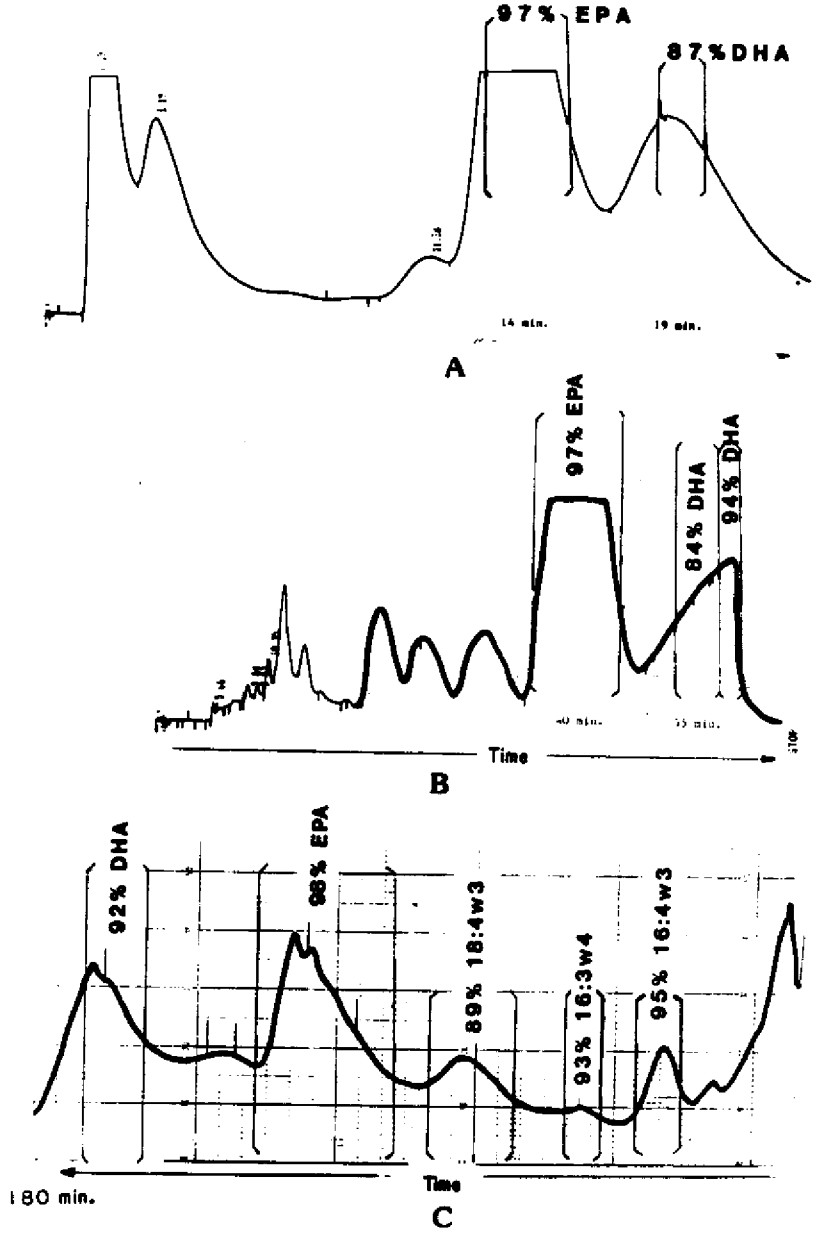


Figure 1. HPLC separation of fish oil concentrate (48%/24%, EPA/DHA) using ethanol:water on a.) analytical columnn, b.) preparative column, and c.) production column.

The first portion of the eluant can be discarded in favor of the higher purity eluant at the end of the separation. Yields are sacrificed to purity in this mode of collection. Yields of about 70% are the norm when collecting the entire peak.

The column size and loading will depend on production requirements. Five fatty acids have been successfully separated on analytical, preparative, and production scale columns (Figure 1). Production requirements for the analytical scale are 20mg of fish oil at a flow rate of 3ml/min with a run time of 25 min. On a preparative column, 10 grams can be loaded at a flow rate of 50ml/min with a run time of 65 min. On a large column, 60g were successfully separated at 250ml/min. with a run time of 180 min.

This report is intended as an overview of the possibilities for HPLC in the production of high purity fractions of EPA and DHA. Capital equipment costs for fully automated sample injection and fraction collection systems range from $28K to $120K. There are obviously a wide range of options, including the imaginative deployment of multiple small systems, to achieve desired production goals.

REFERENCES

Bascetta, E., Gunstone, F.D., and Scrimgeour, C.M., 1984. The purification of fatty acid methyl esters by high pressure liquid chromatography. Lipids, 19(10):801-803.

Scholfield, C.R., 1975. High performance liquid chromatography of fatty methyl esters: Preparative separations. Anal. Chem. 47(8):1417-1420.

Tokiwa, S., Kanazawa, A. and Teshima, S., 1981. Preparation of eicosapentaenoic and docosahexaenoic acids by reversed phase high performance liquid chromatography. Bull. Jap. Soc. Sci. Fisheries, 47(5):675.

Wille, H.J., Traitler, H., and Kelly, M., 1987.Production of polyenoic fish oil fatty acids by combined urea fractionation and industrial scale preparative "HPLC". Revue Francaise de CORPS GRAS, 34(2):69-73.

HEALTH MESSAGES ABOUT SEAFOOD

Joyce A. Nettleton
Nutrition Consultant, P.O. Box 39, Arlington Heights, MA 02175

INTRODUCTION

Enthusiasm about the health benefits associated with consuming omega-3 fatty acids from seafoods has generated media attention, marketing flamboyance and new product development, especially fish oil concentrates (1-7). Processors and retailers, usually hesitant to tout health benefits of foods are finding health a handy hook for promoting seafood (8). Nutrition has never had it so good (9). Nutrition information and health messages are currently flourishing in a sea of regulatory leniency and public confusion. Yet, in spite of or perhaps because of the hype, where there is still mass misunderstanding of basic nutrition issues, as fundamental as total fat intake for example, seafood is enjoying unprecedented publicity and vigorous demand. It is the latest "health food"(10).

By and large, consumers seldom select foods primarily for their health benefits. Quality and convenience consistently outrank nutrition as reasons for a purchase decision. Yet new awareness of the fact that eating seafood is good for you, wider availability of fresh seafood and improved seafood retailing are all contributing to an increased demand for seafood in supermarkets and restaurants. Presumably, bold health claims are also strengthening the demand for seafood products and supplements (11).

OBSERVATIONS

Health Claims: Health claims are an important aspect of communicating nutrition and health information. The permissive climate surrounding health claims for foods follows the much ballyhooed Kellogg's bran cereal campaign (12). With the endorsement of the National Cancer Institute, which has no jurisdiction over food labels, nutrition or health claims, Kellogg's advertised its bran cereal as an aid to deterring some forms of cancer. Although many dispute the quality of the data suggesting a protective effect of dietary fiber against certain cancers, this widely publicized advertising with its attendant controversy had at least three effects. First, it focussed attention on the possible benefits of wise food choices. In a sense, it made the gravel road between food and health a paved pathway. Second, it undermined the authority of the FDA to regulate health claims by sidestepping the usual approval procedures and involving another federal agency, The National Cancer Institute. Third, The Kellogg controversy hastened the publication of proposed regulations for health messages on food labels.

In August, the FDA proposed regulations which set forth criteria for evaluating health claims and information on food labels and point-of-purchase materials (13). This proposal recognizes the need for greater dissemination of health and nutrition information pertaining to food.

Advertising Messages: Advertising is a key factor in product marketing. Advertising and labeling communicate image and information about a product. Product attributes may be conveyed indirectly by association or directly with statements or comparative data. Statements about health benefits and nutritive value are frequently misleading or untrue. Take the example of this ad (14). Often what is unsaid is as important as what is stated. Some statements capitolize on consumer confusion or ignorance. For example, the claim that a product is made using only vegetable oil belies the fact that the oil may be highly saturated or constitutes more than fifty percent of the energy value of the food.

While the nutritional merits of unprocessed seafood obviate the need for advertising subterfuge, nevertheless, seafood advertising shares the troubles of other product categories when it comes to nutrition confusion. Sometimes the facts are wrong, as in the previous example. Sometimes the issue is misunderstood (15). And sometimes freedom of expression just tells half the story (16).

Marketing Magic: Marketing magic is another facet of health messages. An oft repeated bit of folklore regarding omega-3s is that they are present in cold water fish (8, 17). This wisdom presumably comes from romanticizing the Eskimo environment or marketing Arctic fish. The fact is, all fish and shellfish have some omega-3s. Some have more than others and some have higher proportions than others even if when the total fatty acid content is fairly low. A person wishing to choose fish for their abundance of omega-3s however, will find the greatest amounts in the oiliest fish, no matter whether the fish lives in marine or fresh water or both, as illustrated by tuna, rainbow trout and salmon.

The relationship between total fat content and omega-3 fatty acid content is illustrated in these scatter diagrams of finfish from the Northern Hemisphere and the Southern Hemisphere (18, 19).

Experts: Authority figures and experts, like everyone else, make mistakes (20). Quotes are taken out of context; source material may be out-of-date; and inappropriate extrapolations or conclusions may be drawn (21). And if you're not careful, the fish gets thrown in with the veal (20). Understandably, consumers are confused.

Wishful Thinking and the Application of Imaginary Data: There is currently a good deal of wishful thinking about omega-3s which is aided by the application of imaginary data. The publicity being given to fish oil supplements is an ideal case in point (6, 16). Its advertising is misleading, the citation of data selective and biased, and it substitutes

wish for information. In their rush to cash in on the good news generated by the Eskimo studies and other clinical data, fish oil promoters have decided we do not have to to wait for controlled studies. They are satisfied with results from clinical trials on small numbers of subjects. They have no qualms extrapolating from epidemiological data, some of it very weak, to giving advice to individuals for the management of blood cholesterol.

Fish oil supplements are marketed mainly on the assumption that taking them will lower a person's cholesterol level (16). In some individuals it may. In others however, fish oil supplements may have no effect on blood lipids and in still others they may actually be accompanied by an increase in cholesterol levels. You do not hear about these possibilities from the fish oil floggers. And we have very little information about the long term effects of fish oil in diets as rich in total fat as ours.

The sellers of fish oil supplements have also eschewed the need to define a safe and effective dose. Can everyone benefit? Are benefits available only with high doses as some studies suggest? Is long term consumption safe in the presence of other medications especially anti-coagulants and aspirin? Let's face it, caution doesn't sell.

As promising as omega-3 fatty acid research appears, worrisome safety issues have not been resolved. Processing techniques exist to remove virtually all nasty chemicals that may be present in fish oils, especially those from fish liver, but different brands vary in the amount of undesirable residues that may be present. They also differ in how they have been refined. Here is what FDA has found (22, 23).

These data make it clear that the assumption of adequate refining may not be justified. The problem with many nasty residues and contaminants is that just as fish do, we store them in our liver. Amounts that seem vanishingly small on an individual dose basis, may accumulate with long term supplement use. Presently, a purchaser of fish oil preparations has no way of knowing whether or not he is enriching his own contaminant reserves.

The FDA studies have also shown that label information is inadequate in other ways. In nearly all samples analyzed the amount of EPA and DHA was demonstrably below the level stated on the label, in some instances as much as one third below. It would appear that regulatory standards and careful monitoring are in order.

Guilt by Association: When facts are missing, perhaps a little fear will do the trick. Call it guilt by association. Fear generated by unsubstantiated claims about the safety of our seafood supply undermine consumer confidence as surely as reassuring nonsense about nutritional or health value. The difference is that the falsity works in different directions.

Last June, The Coast Alliance, a non-profit public interest group, issued a report with a press release that began:

> "The almost unmonitored and unchecked polluting of New England's coastal waters poses a serious threat to the nation's fish eaters and - ultimately- the sea itself and the life that inhabits it" (24).

This alarming statement achieved its purpose: the attraction of media attention with press articles and media interviews. The issue quickly and quietly died. But how many people turned away from seafood, all seafood? Just when people are re-thinking their red meat consumption they are being frightened about the safety of the fish supply.

Let me emphasize that I decry as loudly as anyone the use of any waterways as sewers and I recognize the potential threat to health of these extremely nasty substances but we have very little evidence to suggest that eating fish will make you ill. In fact, the evidence is just the opposite.

What is objectionable about this sensationalism is that there is no evidence that people in America are getting ill from the chemicals unfortunately being dumped into our waterways. We may be living on borrowed time, no doubt, but there is a quantum leap between chemicals in the waters and illness from eating cooked fish muscle.

Unquestionably people get ill; very ill, from eating seafoods harvested from contaminated waters. But the contaminants that we know make people sick come from raw sewage. Without doubt, the seafood preparation guaranteed to do the trick is eating such seafood raw. But that is a far cry from a "serious threat to the nation's fish eaters."

SUMMARY AND CONCLUSIONS

In summary, people are becoming aware of the benefits of eating sefood, thanks in part to health claims, advertising, journalism, and retailing activities. All these endeavors can benefit from awareness of facts, including what we know and don't know; refusal to endorse or commission mythology and wishful thinking; and the avoidance of sensationalism masquerading as truth.

What to Do: To protect against false claims and misleading or fraudulent advertising, we as health professionals must ensure that the seafood business has:

1. a clear understanding of the nutrition issues being addressed

2. an understanding of the complexities underlying both health and marketing issues

3. the facts

Where data do not exist, the seafood industry can support efforts to commission the appropriate research. They must resist the temptation to invent the facts they seek or wish.

Lest I leave you with the impression that all seafood and health messages are fraudulent or misleading, here are some examples that have avoided the pitfalls mentioned above.

1. Responsible journalism (25, 26);
2. Facts (27, 28);
3. Health by association (29);
4. Appropriate use of experts.

After all, the data support the health claim implied in the bumper sticker from the Massachusetts Division of Marine Fisheries: "Is health your wish? Eat more fish."

REFERENCES

1. Burros, M. 1985. High-fat fish: Healthful and tasty. The New York Times. May 9.

2. Bishop, J.E. 1985. Eating fish reduces heart-attack risk, researchers assert. The Wall Street Journal. May 9.

3. Winick, M. 1985. Why you should learn to love fish. Woman's Day. December 3.

4. ___ 1986. Glamour Magazine.

5. Rovner, S. 1986. Fish oil's new fame. Washington Post. March 26.

6. ___ 1986. Advertisement for Proto-chol. Boston Globe. October 26.

7. ___ 1986. Advertisement for MaxEPA. American Health. November.

8. ___ 1987. Wall advertisement in Long Island seafood shop. October.

9. ___ 1986. Advertisement for Fishery Products. More fish, less calories.

10. ___ 1986. Table tent from University of Maine, Sea Grant. Fish is brain food.

11. Hales, D. 1986. How fish can save your life. Parade Magazine. November, 9.

12. ___ 1984. Advertisement for Kellogg's Bran Flakes. October.

13. ___ 1987. Food labeling: Public health messages on food labels and labeling. Federal Register 52: 28843, August 4.

14. ___ 1987. Advertisement for catfish. Seafood Business. September/October.

15. ___ 1986. Advertisement for fish sausages. Seafood Business. November/December

16. ___ 1987. Advertisement for new Proto-chol. Seafood Business.

17. ___ 1986. Living off the fat of the sea. Consumer Reports. June.

18. Hepburn, F.H., J. Exler, J., and J.L. Weihrauch. 1986. Provisional tables on the content of omega-3 fatty acids and other fat components of selected foods. J. Am. Diet. Assoc. 86:788-793.

19. Nettleton, J. 1985. Seafood Nutrition: Facts, Issues and Marketing of Nutrition in Fish and Shellfish. Osprey Books, Huntington, NY.

20. Brody, J.E. 1987. Separating fats from fiction. The Good Health Magazine, New York Times Magazine, March 29.

21. Jenkins, N.H. 1986. More on fatty fish. The New York Times, June 4.

22. Plakas, S.M. and A.M. Guarino. 1986. Omega-3 fatty acids: Is the news all good? Proceedings of the Eleventh Annual Tropical and Subtropical Fisheries Technological Conference of the Americas 11: 275-283.

23. Guarino, A.M. 1987. Composition of commercially available fish oil capsules. J.Am. Oil Chem. Soc. 64: Abs.

24. Coast Alliance. 1987. Press release. Washington, D.C. June 24.

25. Brody, J.E. 1987. Personal health column. The New York Times, February 4.

26. ___ 1987. Fish oil supplements: Helpful or harmful? Good Housekeeping, p.70. January.

27. ___ 1986. Advertisement for Fishery Products. Flounder continental supreme: Waist watcher's dream.

28. Hackman, E. 1987. Shellfish: Heart healthy and low in salt. Richmond Times-Dispatch, March 1.

29. ___ 1987. Creamless salmon mousse and Japanese shellfish soup are low in fat and high in nutrients. The New York Times, November 1.

NOMENCLATURE AND LABELING OF FISHERY PRODUCTS A MARKETING DILEMMA

Donald R. Maher, NMFS
National Seafood Inspection Laboratory
Pascagoula, MS

James R. Brooker, NMFS
Office of Trade and Industry Services
Washington, DC

Mary J. Snyder, FDA
Center for Food Safety and Applied Nutrition
Washington, DC

This paper will describe the nomenclature problems that industry, government and consumers have in properly identifying fishery products in national and international trade. It also will discuss the steps taken by the National Marine Fisheries Service (NMFS), the Food and Drug Administration (FDA), and the National Fisheries Institute (NFI) to address the nomenclature problem.

PROBLEM IDENTIFICATION

The lack of standardized market name nomenclature in the interstate marketing system has created confusion at all levels of the market strata from processor to the ultimate consumer. The confusion is caused by several reasons. The main reason is the variation in common market names used for a given species from different areas or regions of the country. Another problem arises when industry markets a less commercially known species using an existing well-established market name of a more desirable fish which has a higher value in the market. Still another problem is created when, in some instances, state regulatory authorities have authorized the use of a well-established market name (applied and recognized to a species from another state or region) to be applied to a species from a totally different family or genera harvested and marketed in their state.

When such products enter interstate commerce, they are considered mislabeled by FDA and subject to seizure and legal action as misbranded products. Further, the lack of standardized market names creates confusion in the orderly worldwide marketing of United States produced and processed seafood.

FDA POLICY AND PROCEDURES FOR DETERMINING ACCEPTABLE COMMON OR USUAL NAMES FOR FISHERY PRODUCTS

Current provisions of the Federal Food, Drug and Cosmetic Act and the Fair Packaging and Labeling Act offer guidelines as to the names by which fishery products or other foods may be designated. The Act

require the label to bear a statement of the identity of the food by its common or usual name and prohibits labeling which is false or misleading in any particular. Where there are no established common or usual names in the United States, the label must bear an appropriately descriptive term informatively revealing the identity and nature of the food. FDA considers the question of what constitutes "the common or usual name" or "an appropriate descriptive term" for a food as a name or term in general usage and readily understood by the ordinary individual or consumer in the United States. Often the common name associated with its systematic biological classification or the common market name applied to the species in the country of origin, for an imported fish, is helpful in determining what does constitute an appropriately descriptive non-misleading name. Neither the common name applied by ichthyologists nor the common name used in another country may be used, however, if it conflicts with the common or usual name used by consumers for another species in the United States or if it is in any way misleading to United States consumers.

In considering whether or not a designation may be misleading, FDA looks to the courts for clarification and guidance as well as to the Act and its regulations. The comments of the Supreme Court of the United States, June 2, 1924, provide such clarification and guidance. The Court said,"... The Statute is plain and direct. Its comprehensive terms condemn every statement, design, and device which may mislead or deceive. Deception may result from the use of statements not technically false or which may be literally true. The aim of the statute is to prevent that resulting from indirection and ambiguity, as well as from statements which are false. It is not difficult to choose statements, designs, and devices which will not deceive. Those which are ambiguous and liable to mislead should be read favorably to the accomplishment of the act..."

FDA in considering the question of what constitutes an appropriate label designation for a seafood, must determine appropriateness based on the basic facts in each instance. Actually determining an acceptable name where none previously exists is not easy. It is much easier, however, than the problems encountered when FDA is called upon to settle a controversy concerning the name to be used throughout the United States when the same fish is known by a different name in different parts of the country.

Another problem arises when people wish to market a less well known or less commercially acceptable fish under the name of a more desirable fish. When called upon to settle controversies or when asked for guidance in developing acceptable names for seafood, FDA looks to several areas for resource material. The main resource for nomenclature of fish found in this country is currently the 4th edition (1980) of Common and Scientific Names of Fishes from the United States and Canada, published by the American Fisheries Society. Should the fish not be

indigenous to North America, or if it is some other type of seafood, recognized experts such as those at the Smithsonian Institution in Washington, D.C. or the National Marine Fisheries Service may be contacted.

Also, the FDA refers back to previous correspondence relating to the specific issue in which a precedence has been established for a particular species.

When working with controversial problems, it would be desirable to have an established standardized market name for every type of fishery product. However, since this luxury is not yet available, each issue must be decided on a case by case basis on its own merit. To illustrate this point, FDA often receives requests to label fish other than Lutjanus campechanus as "Red Snapper." The name "Red Snapper," however, has been preempted by many years of consistent consumer usage as meaning only the fish Lutjanus campechanus. Because of the high esteem in which this fish is held by consumers and the relatively limited catch, numerous attempts have been made to substitute other less expensive fish for this species. Substituted, less desirable species have included other members of the family Lutjanidae, groupers, and a number of West Coast rockfish of the genus Sebastes. The West Coast rockfish have, until recently, been distributed locally and thus have been beyond the reach of the Federal Food, Drug and Cosmetic Act. Some of the states on the Pacific coast have officially sanctioned "Pacific Red Snapper" or "Pacific Snappers" as an alternative name for such members of Sebastes genus. These fish are quite different from Lutjanus campechanus (Red Snapper) in appearance, flavor and texture, and are generally regarded as inferior by consumers familiar with Lutjanus campechanus. The current policy of FDA is that the labeling or sale of any fish other than Lutjanus campechanus as "Red Snapper" constitutes a misbranding in violation of the Federal Food, Drug and Cosmetic Act. Further, labeling of rockfish as "Pacific Red Snapper" is acceptable only in those West Coast states which have sanctioned that term.

Economic deception is not the only problem connected with misnamed seafood. Sometimes issues of public health are very much involved. As reported in the FDA Consumer (July-August 1982 issue) in 1982, the Health Department of Ulster County, NY, reported to FDA a double mystery when it asked the agency to assist in an apparent incident of food poisoning. The first question: Why did two men become ill shortly after eating a fish dinner at a restaurant? The answer: The two were suffering from scombroid poisoning, a severe gastric illness resulting from eating fish containing high quantities of the compound histamine. After the diagnosis, the Health Department placed the large lot of fish still remaining at the premises of the dealer who had supplied the restaurant under embargo. An analysis of the fish from the restaurant and the dealer showed the presence of histamine, with the sample from the restaurant containing the highest amount. The second question then

arose: Why did the fish shown on the menu as "Red Snapper," an ocean delicacy, have high levels of histamine, unusual for this kind of fish? The answer: It was not "Red Snapper," but Mahi-Mahi in a fish taken off the Pacific coast of South America. Mahi-Mahi is so predictably high in toxic histamine that FDA samples and examines each lot imported into the United States or automatically detains it upon entry.

The FDA found that the fish mislabeled "Red Snapper" had been shipped from Ecuador and processed in Panama. Had the fish been labeled Mahi-Mahi, it would never have made it ashore since FDA has an automatic detention for Mahi-Mahi from Ecuador.

While consumer usage has given a narrow meaning to some common fish names, other names have come to identify more than one species. An example is the term "Sardine." Commercially the name "Sardine" has come to signify almost any small, canned clupeoid fish. To avoid confusion, FDA recommends that all "Sardines" bear labels showing the place where produced and the nature of the ingredients used in preserving and flavoring the product. Thus, a small fish of the clupeoid family, caught upon or near the shores of Norway and packed in oil, or smoked and packed in oil, is properly labeled with the phrase "Norwegian Sardines in Oil," or "Norwegian Smoked Sardines in Oil" with the type of oil being designated. It also is suggested that the particular fish to which the term sardine is to be applied should be placed upon the label, for example, "Pilchard," "Herring," etc. Also, not all fish of the family Clupeidae are entitled to be called "Sardines." Many members of this family have well established identities of their own, i.e, Scotch herring, Polish herring, Alewives or River herring, and others. FDA would regard the labeling of these other fish as "Sardines" as misbranding and in violation of the Federal Food, Drug and Cosmetic Act.

In addition to its regulations, FDA also issues policy statements to provide and identify appropriate designations for seafood. These policy statements are issued as official "Compliance Policy Guides and Import Alerts." To date they have been issued to cover caviar, crabmeat, common or usual names of crustaceans, capelin versus smelt and red snapper.

A number of seafood items are also covered by standards of identity, or common or usual name regulations. Standards of Identity exist for various styles of pack of oysters, salmon, shrimp and tuna. Common or usual name regulations have been established for Pacific whiting, bonito, crabmeat, Greenland turbot and several other seafood items.

When interested parties wish to receive an opinion from FDA as to an acceptable name to use for a seafood item (or any other food item under FDA jurisdiction), they may ask for an informal opinion. Generally this only involves correspondence between the party and either one of the FDA field offices or the Division of Regulatory Guidance in the Center for Food Safety and Applied Nutrition (formerly the Bureau of Foods). A more formal response may be requested in the form of an advisory opinion

which will carry the designation of official agency policy. Anyone may file a citizen's petition to establish a new regulation such as a Standard of Identity. More likely in the case of seafood names, a petition may be filed to propose or amend an existing regulation for a common or usual name. Often there is a difference of opinion about the appropriate common or usual name of a food. For example, a new engineered seafood, such as imitation crab, may not have a common name, or a species of seafood which has not previously been offered as food in this country may not have an English name. In such cases, FDA welcomes suggestions for appropriate common or usual names. These suggestions would most likely be in the form of a citizen's petition. The names suggested should be descriptive of the food and not be misleading or deceptive. The names should represent the new food rather than be suggestive of the name of another already accepted food.

NATIONAL MARINE FISHERIES SERVICE POLICIES AND PROCEDURES FOR DETERMINING COMMON OR USUAL NAMES FOR FISHERY PRODUCTS.

Currently individuals or firms can request guidance from the NMFS in establishing an acceptable or preferred common name for a species described by its scientific name. After the request is made, the NMFS will check to see if the species is listed in the American Fisheries Society's "List of Common and Scientific Names for Fishes from the United States and Canada," or in FDA's precedence letter files. If the species is not listed in these sources, the NMFS will work with FDA's Division of Regulatory Guidance to determine an acceptable common or usual name and/or market name for the species.

To date, NMFS policy has been to encourage the industry to voluntarily comply with all federal and state laws and regulations as they relate to properly labeled fishery products. In an effort to aid the industry to comply with such laws in the most equitable manner, the agency continues to develop closer working relationships with FDA in all areas of food safety and labeling.

THE NATIONAL IDENTIFICATION AND LABELING PROGRAM PLAN FOR FISH AND FISHERY PRODUCTS

The increasing demand for both foreign and domestic fishery products will only continue to aggravate an already existing nomenclature problem. For example, at the consumer level, where acceptance of fishery products is of major importance, fishery product labeling can be confusing and sometimes misleading. Furthermore, the majority of consumers will not be likely to spend the time to inform themselves about the increasing number of species now available in the retail market place.

In view of these current and certainly inevitable future difficulties, the National Marine Fisheries Service (NMFS), the National Fisheries

Institute (NFI), and the Food and Drug Administration (FDA) have undertaken a comprehensive project that will aid in resolving future nomenclature problems. The primary objective of this project is to develop an improved nomenclature system and standardize market names for fishery products.

The final result of the project will be to implement an improved seafood products identification program which should yield two important benefits:

(1) A plan designed to build consumer familiarity and acceptance of seafood products which will promote seafood consumption, and

(2) a plan which will provide a comprehensive identity and labeling framework which will provide the tools needed for voluntary compliance by both industry and government.

The national program on seafood product identification has the following components:

I. NMFS, FDA and USDA Study Group -- Coordination and Regulatory Task:

Through an established Interagency Study Group at the agency's headquarters levels, review and propose resolutions to complex seafood labeling issues for consensus decision making by NMFS, FDA and United States Department of Agriculture (USDA) policy makers.

There is no specific budget for the study group and the issues to be addressed will be those provided from various sources as identified.

NMFS Contact Person: James R. Brooker
National Marine Fisheries Service
1825 Connecticut Avenue, N.W.
Washington, DC 20235

II. Official List of FDA/NMFS Approved Market Names for Fishes -- Development Project:

Develop, publish, distribute and maintain a list of acceptable market names of food fish species for use by government, industry and consumers.

CURRENT STATUS:

Work on the list is almost completed and is scheduled for release as a joint FDA/NMFS Interim Guideline in the Federal Register early

1988 as a notice of availability for use and comment by all interested parties. It is proposed at this time to be available for public comment and use for a period of six months.

Contact Persons: Donald R. Maher, NMFS Project Leader, National Seafood Inspection Laboratory Pascagoula, MS

Mary Snyder, FDA Project Leader, Division of Regulatory Guidance, Center for Food Safety and Applied Nutrition, Washington, DC

III. Edibility Data Bank -- Research Project:

Test the major U.S. food species and establish edibility profiles for each species. Publish the profiles for industry and consumer use and publish the protocol and test methods as the formal methodology for determining the edibility characteristics of species. The edibility profiling of species is carried out at the NMFS technology laboratories in Gloucester, MA, Charleston, SC, and Seattle, WA.

CURRENT STATUS:

Work has been completed on about 100 species, and others are being completed as species are identified and made available for testing.

Contact Person: John Wekell, Project Coordinator, Seattle Laboratory, Seattle, WA

IV. Universal Product Code for Seafood -- Development Project:

Develop and implement a standard nomenclature and numbering system for random weight fish and fishery products sold at retail for inclusion in the Universal Product Code System.

The project is being executed under a multi-year (FY86, 87 and 88) S-K Grant #NA 85AAHSK145, by NFI's National Fisheries Education and Research Foundation.

CURRENT STATUS:

Work on schedule with contract requirements.

Principal Investigator: Robert Collette, NFI, Washington, DC

Technical Monitor: James R. Brooker, NMFS, Washington, DC

As part of this program the project leaders for the "Official List of FDA/USDC Approved Market Names for Fishes" have provided the Foundation

a draft list for food fish containing some 1000 commercial food fish, of world wide distribution, which are marketed in the United States.

CONCLUSION

The National Marine Fisheries Service considers that the successful completion of all phases of the National Identification and Labeling Program Plan for Fish and Fishery products will (1) provide a system containing the framework and tools necessary for increasing consumer awareness and wider usage of fish and fishery products, (2) will provide both industry and government with a tool for orderly marketing through standardized market names and eliminate conflicts between government regulatory agencies and industry in interstate commerce, (3) will provide a mechanism for authorizing and approving market names for fish and fishery products traded in national and international commerce and (4),most importantly, the program will provide the ultimate consumer with properly identified products in the retail market.

While the competitive nature of the food industry will continue to produce nomenclature problems, the successful completion and publication of the "National Identification and Labeling Manual for Fish and Fishery Products" should provide both government and industry with the tools required to resolve these problems.

APPENDIX 1

PRINCIPLES DEVELOPED AND USED BY FDA AND NMFS IN SELECTING ACCEPTABLE COMMON MARKET NAMES

The principles developed by the NMFS and FDA in selecting common and market names for each species are listed in order of priority as follows:

1. The list shall be a compendia of existing scientific, common and market names for food fish, shellfish and fishery products only. Common and scientific names shall not be originated for this list.

2. The "American Fisheries Society's (AFS) List of Common and Scientific Names of Fishes and Aquatic Invertebrates from the United States and Canada" shall be the primary authority for scientific and common names of fishes and shellfish. In the absence of an AFS assigned scientific and common name other authorities will be used in the following priority of use:

    - o FAO Species Catalogues and/or Species Identification Sheets.
    - o Source country primary authority for scientific and common names of fishes.

3. The Food and Drug Administration and the NMFS jointly shall be the authority for the market names of fish and fishery products.

4. The National Oceanographic Data Center shall be the authority for the twelve (12) digit Taxonomic Code assigned to each species.

5. Market names shall reflect broad, current, and appropriate usage of vernacular nomenclature in the labeling of food fish, shellfish and fishery products that will add to stability and the universality of names applied to food fish and fishery products.

6. Market names are established for (a) individual species, and (b) species grouped together that are recognized in trade and commerce, and by consumers as having similarities, such as edibility characteristics, that make them indistinguishable from each other as products for human food purposes.

7. A market name representing multiple species will apply only at scientifically discrete levels of taxonomic classification, however, it may also comprise multiple species classified in two or more families and/or genera.

8. Individual species within a family or genus for which a market name has been established may be identified more precisely by the addition of a "modifier" to the market name when conditions or unique characteristics warrant more precise identification of the product. Reasons and conditions for more precise identification follow:

    - o Prior (grandfather) labeling regulations.
    - o Demonstratively significant characteristics (for food purposes) which distinguish one species in commerce from other closely (taxonomically) related species.

9. Species for which there are no acceptable and assigned common names in the English language will bear the market name in use for other taxonomically similar species until such time as a more precise market name is assigned. Reasons and conditions detailed in Item 7 shall prevail in assigning more precise market names.

10. Fish and fishery products intended for use as food may bear either the assigned market name or the complete common name of the species on the label.

THE GOLDEN CRAB (GERYON FENNERI) FISHERY OF SOUTHEAST FLORIDA

Robert B. Erdman and Norman J. Blake
Department of Marine Science
University of South Florida
St. Petersburg, FL 33701

INTRODUCTION

The golden crab, Geryon fenneri (Manning and Holthuis, 1984) is a large brachyuran inhabiting the continental slope off the southeast United States including the eastern Gulf of Mexico. Prior to its description in 1984, studies in the eastern Gulf of Mexico by Otwell, Bellairs and Sweat (1984) examined the potential development of a trap fishery for this species in depths exceeding 210 meters. Various trap designs were tested along with methods of on-board handling and processing (Sweat and Otwell, 1983); Bellairs and Otwell, 1983). Particularly attractive was the reported high meat yield of male crabs, ranging from 17 to 23 percent of total body weight.

Interest in the commercial exploitation of golden crab led to three vessels (two from Alaska and one from New England) initiating a fishery along the west coast of Florida during late 1984. Male crabs were butchered, cooked and blast frozen at sea. The final product of clusters, cocktail claws and split legs were delivered frozen to the market. However, because of marketing problems, compounded by distances in excess of 100 miles to the fishing grounds, gear loss and the absence of information on the distribution and biology of this species, commercial operations in the eastern Gulf of Mexico ceased by mid-1985.

In late 1985, continued interest in the commercial potential for G. fenneri led to the initiation of exploratory fishing and research in Bermuda (Luckhurst, 1986), South Carolina (Wenner and Ulrich, 1986), Georgia (D. Harrington, personal communication) and Florida. Additionally, in late 1985 a small commercial fishery developed along the southeast Florida coast with the catch delivered live to a local market in Ft. Lauderdale, Florida.

In February 1986, we began a study of the biology of Geryon fenneri as collected from this southeast Florida fishery. Reproductive biology, size and weight relationships, trap design and catch per unit effort were examined to ascertain additional information relative to the study of this potentially valuable species.

METHODS

Gear previously utilized by the eastern Gulf of Mexico golden crab fishery consisted of deep water long lines of between 30 and 60 traps attached to a ground line connected via a float line to large floats and radar reflectors on the surface (Otwell, Bellairs and Sweat, 1984).

This method requires the capability to retrieve upwards of one mile of line from depths in excess of 250 meters. Trap designs have included Dungeness crab pots, King crab traps, New England and Florida lobster traps and plastic Fathoms Plus traps (Otwell, Bellairs and Sweat, 1984; Wenner and Ulrich, 1986). Three or four trap lines were fished with soak times averaging 24 hours between set and retrieval.

Because of the close proximity to shore (less than 10 miles) of water depths in excess of 200 meters, the small fishery that has developed in southeast Florida employs a different strategy in harvesting golden crab. Four to six large traps are attached approximately 140 to 180 meters apart to a ground line which is fitted with concrete weights on each end. The large traps called Neilsen traps, measure approximately 6' x 3' x 3' and are made from steel round stock covered with 2" x 2" nylon stretch mesh (Figure 1). Traps are fitted with 5" diameter escape rings and a large side door providing access to the center bait well and easy removal of the catch. Four to six strings of traps may be fished, with each string reset immediately after it is pulled.

As the present fishing grounds are adjacent to commercial shipping lanes and affected by variable currents associated with the Gulf Stream, trap lines are deployed without a surface float system. Loran coordinates are recorded during deployment along with bottom profiles and relative position using shoreline landmarks. Soak time varies from three to six days depending on market demand. This allows the fishermen to pursue other commercial opportunities. Trap recovery involves grappling for the ground line, with the vessel moving from offshore to onshore and the grapnel dragged perpendicular to the ground line.

Samples were collected monthly during the period February 1986 through January 1987 from fishing depths ranging from 215 to 230 meters. Total numbers of crabs caught per trip were recorded in three categories: females, market size males in excess of 130 mm carapace width (CW), and small males less than 130 mm CW. Crabs were randomly selected from the catch, packed in crushed ice and returned live to the laboratory.

For each crab, carapace width (CW, the distance between the fifth lateral spine tips) and carapace length (CL, midline distance from the diastema between the rostral teeth to the posterior carapace edge) were recorded to the nearest millimeter. Weight was recorded to the nearest gram and missing appendages noted. The presence of eggs in pleopods were noted and molt stages were estimated according to a modification of stages presented in Byers and Wilke (1980) for G. quinquedens (probably G. maritae; see Manning and Holthuis, 1981). Weight-width relationships of animals in the intermolt stage and with no appendages missing were calculated using a log-log transformation expressed in the form $Y = aX^b$.

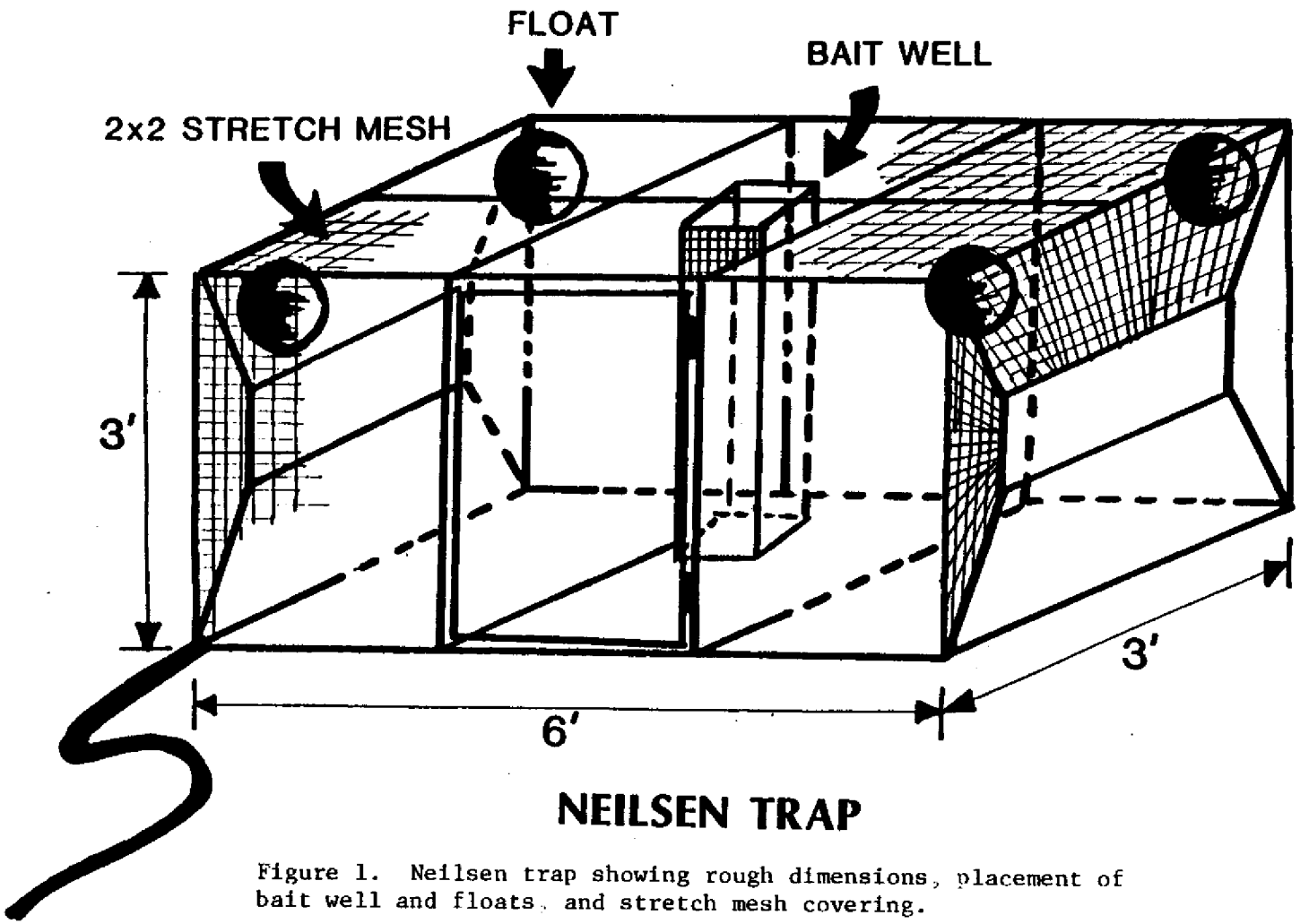


Figure 1. Neilsen trap showing rough dimensions, placement of bait well and floats, and stretch mesh covering.

RESULTS

Throughout the study period, the catch of male crabs greatly outnumbered females. Size frequency distributions of 508 males and 347 females examined (Figure 2) indicates a unimodal distribution for each sex, with no suggestion of distinct year classes. Males are considerably larger than females, with overlap between the largest females and smallest males. Carapace width of males ranged from 111 to 190 mm with a mean of 158 mm, whereas females ranged from 89 to 156 mm with a mean of 123 mm. Animals less than 89 mm CW were not collected, possibly due to bias from trap design and the use of escape rings. The narrow range of fishing depths also precluded the collection of information on segregation of sex and size with depth as has been reported for other Geryon species (Byers and Wilke, 1980; Intes and Le Loueff, 1976; Haefner, 1978).

Monthly size frequency distribution of female crabs is shown in Figure 3. The incidence of ovigerous females collected throughout the study period indicates an annual reproductive cycle with a single brood of eggs produced each year. Oviposition begins in late August and continues through early October. Eggs are carried for six months until larvae hatch during late March and early April of the following spring. Eggs are dark purple following deposition, gradually becoming dark brown prior to hatching. Size range of ovigerous females examined ranged from 96 to 147 mm CW.

Figure 4 shows the monthly size frequency distribution of male crabs. Monthly mean carapace widths ranged between 152 and 162 mm; however, the incidence of smaller males decreased beginning in July 1986. This decrease in small males coincided with the fitting of all traps with 5" diameter escape rings. Although data collected precluded statistical analysis, it was apparent from the overall catch of both sexes that fewer females and small males were present in traps fitted with escape rings.

Weight frequency distributions of 262 males and 136 non-ovigerous females is shown in Figure 5. Weight of male crabs, ranging from 230 to 1930 g with a mean weight of 1116 g and greatly exceeded that of females. Mean weight of females was 449 g, ranging from 207 to 880 g. Although weights of both sexes show a unimodal distribution, the greater incidence of females in a narrower range of weight classes is due to the great increase in mass associated with developing ovarian stages. The largest male examined was 190 mm CW and weighed 1930 g while the largest non-ovigerous female was 156 mm CW and weighed 800 g.

Weight on carapace width relationships were calculated separately from each sex. the relationship for 262 males illustrated in Figure 6, is described by $WT = 5.27 \times 10^{-5} (CW^{3.328})$. The relationship for 136 females is $WT = 5.599 \times 10^{-4} (CW^{2.812})$ as shown in Figure 7.

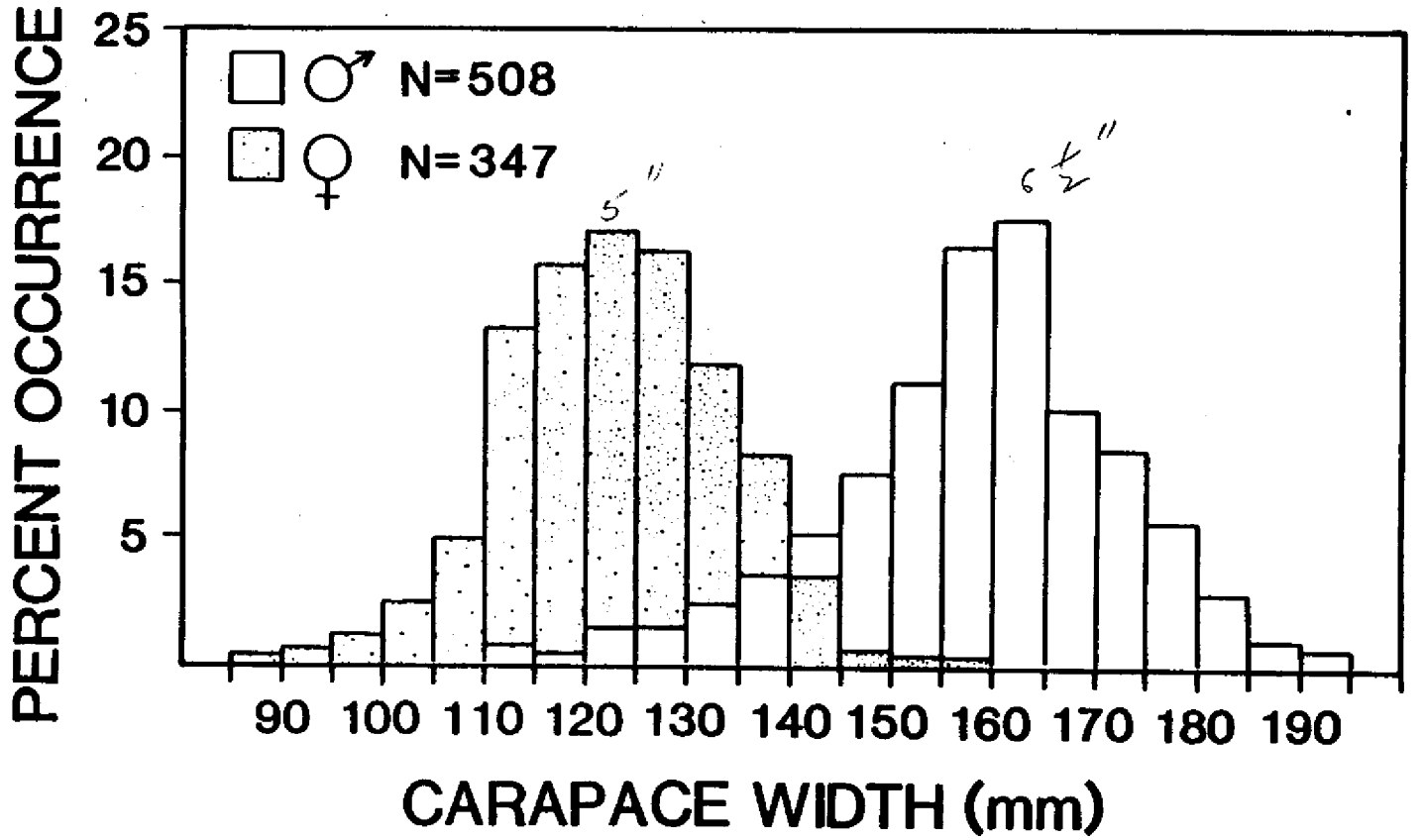


Figure 2. Cumulative size frequency distribution of male and female Geryon fenneri collected during the period February 1986 through January 1987 from Ft. Lauderdale, Florida.

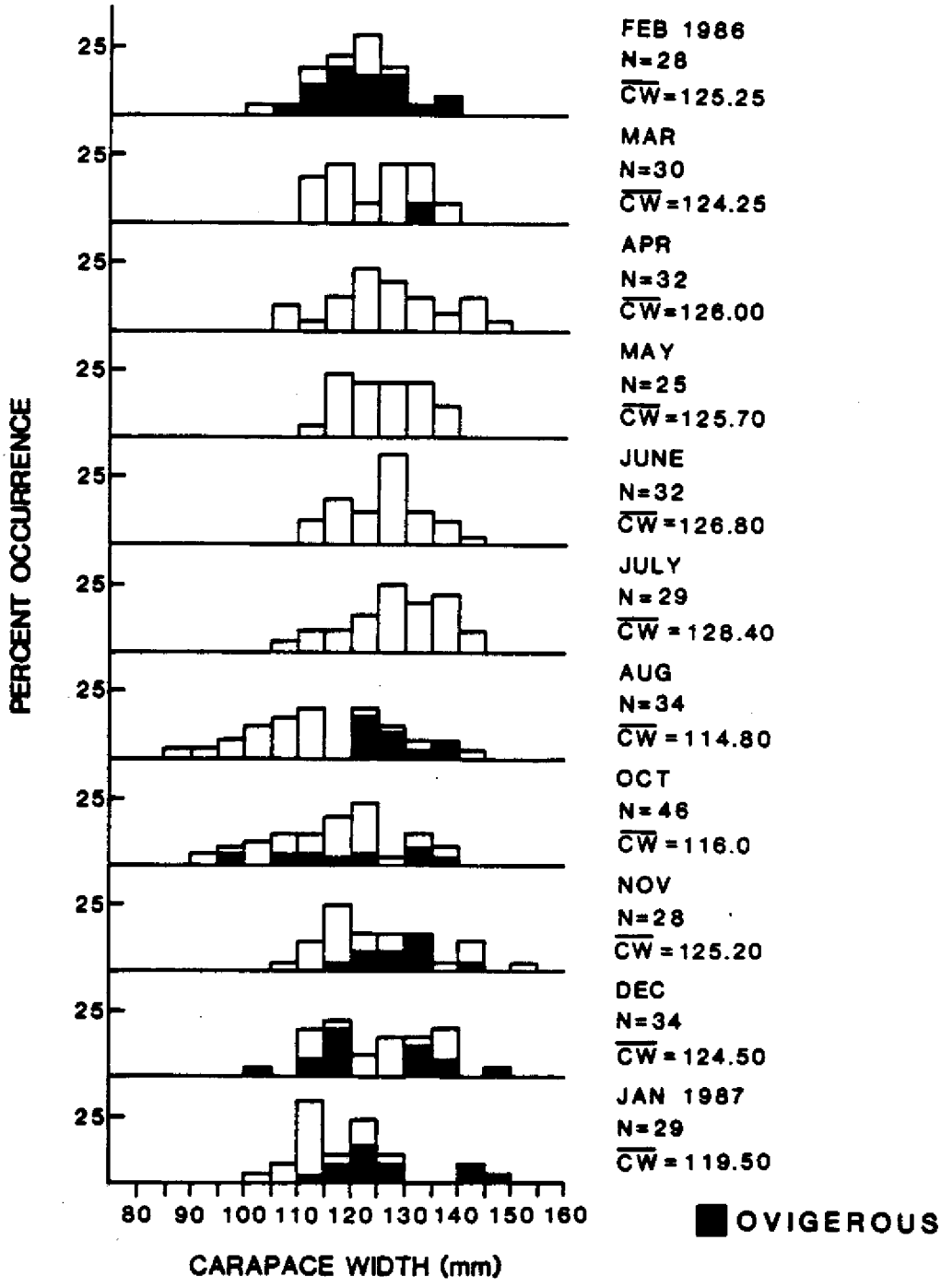


Figure 3. Monthly size frequency distributions of female Geryon fenneri including number of individuals and mean carapace width. Ovigerous females are shaded.

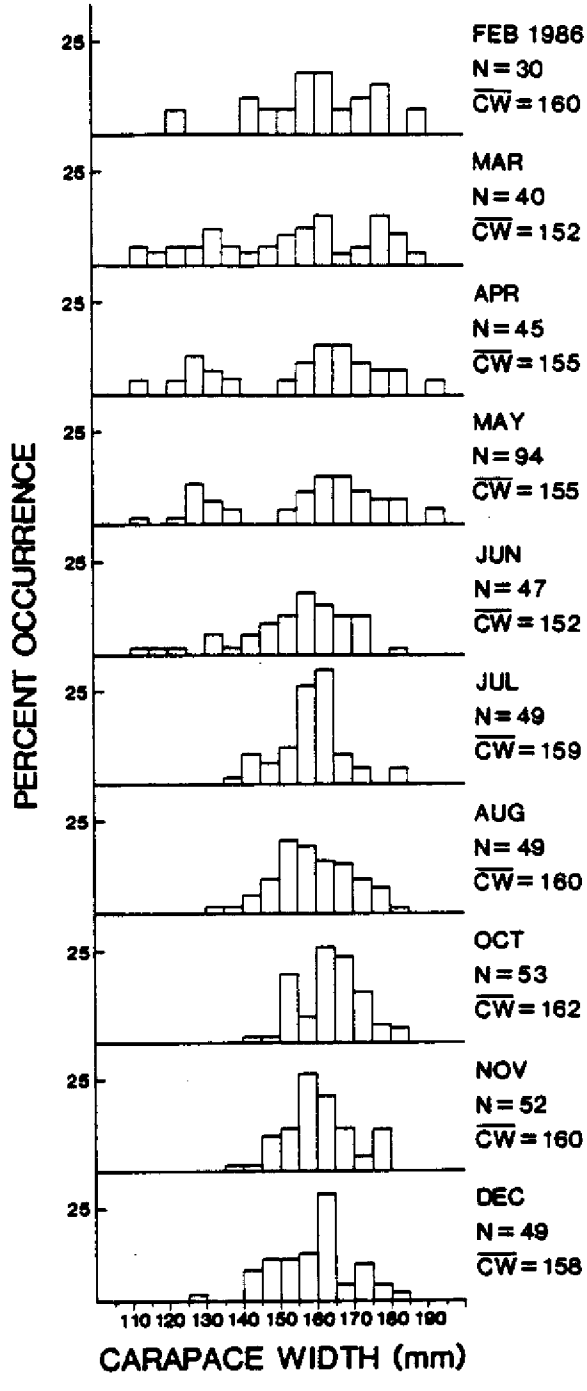


Figure 4. Monthly size frequency distributions of male Geryon fenneri including number of individuals and mean carapace width.

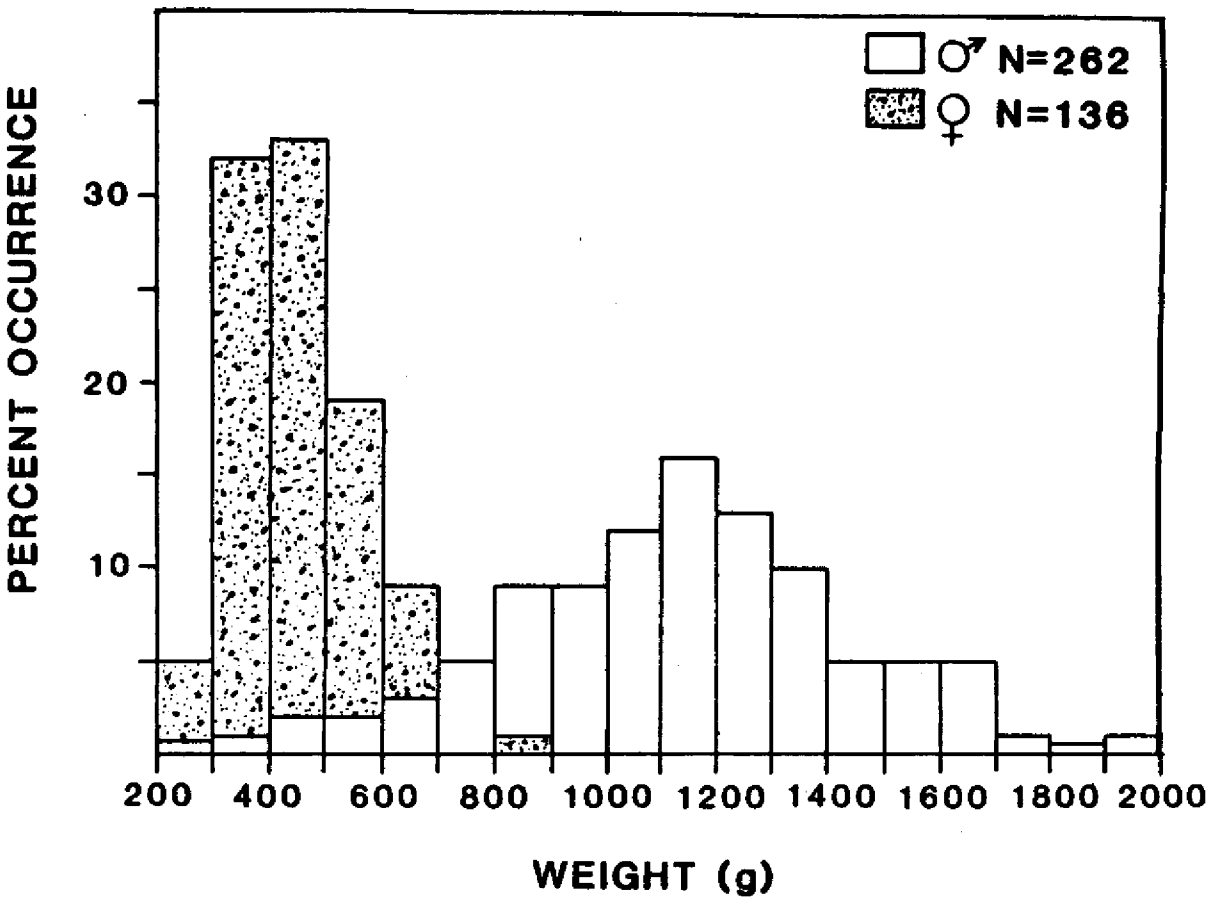


Figure 5. Cumulative weight frequency distribution of male and female Geryon fenneri collected during the period February 1986 through January 1987 from Ft. Lauderdale Florida.

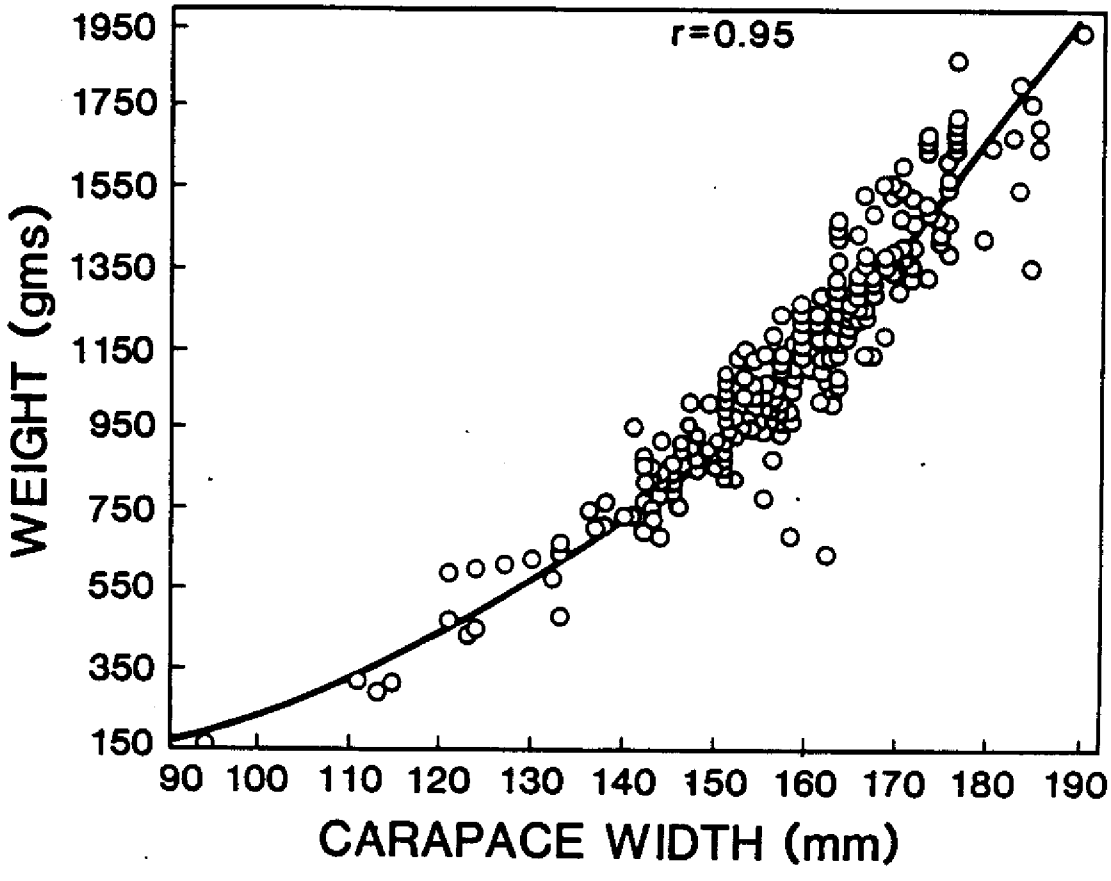


Figure 6. Weight on carapace width relationship for 262 male Geryon fenneri as described by WT = 5.27 x $10^{-5}(CW^{3.328})$.

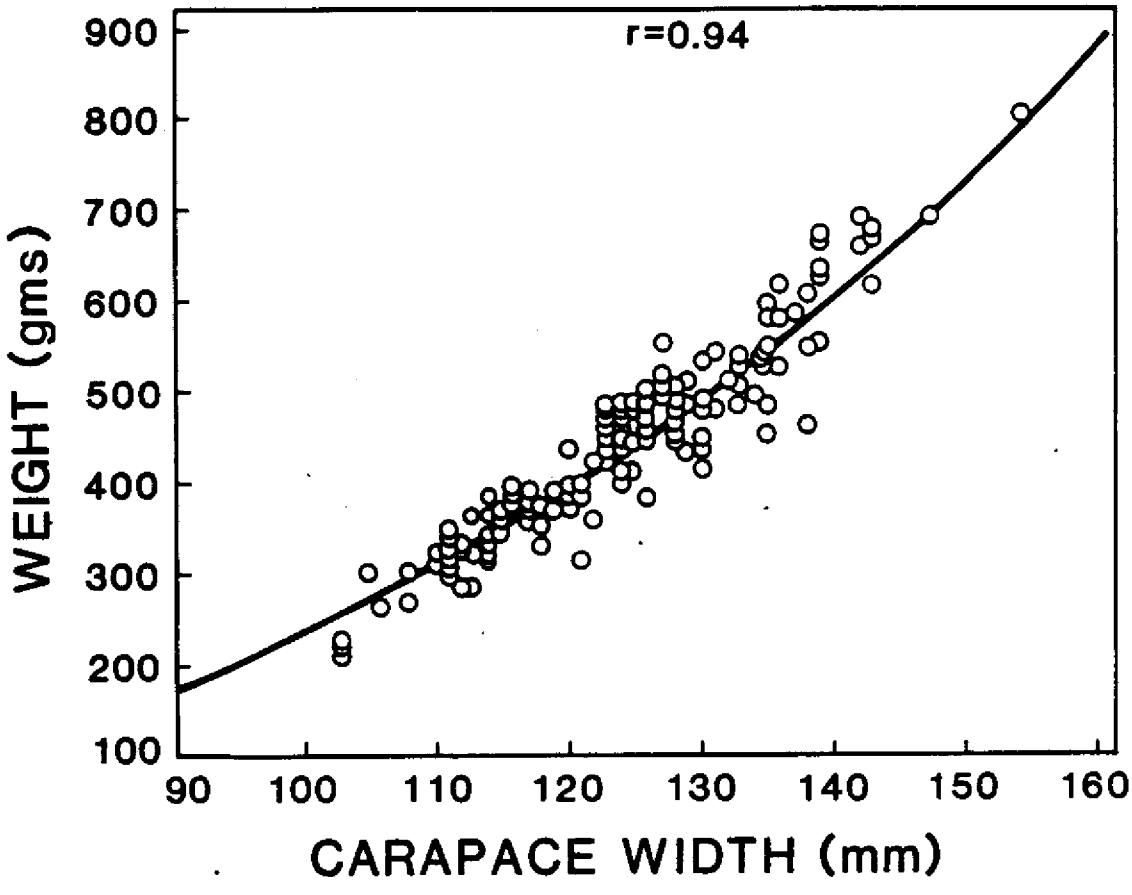


Figure 7. Weight on carapace width relationship for 136 female Geryon fenneri as described by $WT = 5.599 \times 10^{-4}(CW^{2.812})$.

DISCUSSION

Geryon fenneri is considerably larger than G. maritae and G. quinquedens, two additional species of commercial value. As male G. fenneri attain a maximum size in excess of 190 mm CW and weight up to 2000 g, interest in commercial utilization is warranted. At present, the small fishery for this species is unregulated, with no closed season, quota or minimum size limits on harvestable animals. In the southeastern Florida fishery, only males greater than 130 mm CW are utilized, and all females and small males returned to the water. Considering the annual reproductive pattern of females and the slow growth associated with organisms of large size from this deep water environment, the voluntary practice of selective harvest is undoubtedly of benefit in protecting the potential longevity of this fishery.

The strategy employed by the golden crab fishermen of southeast Florida has proven successful in providing a sufficient amount of product to local markets. The use un-buoyed trap lines with few large traps and long soak times has resulted in the development of a small scale local fishery, with fishermen able to pursue other commercial opportunities rather than fish crab exclusively. The close proximity of the fishing grounds to shore permits delivery of live product thus eliminating on-board processing and storage. This in turn reduces the cost per trip on the basis of fuel, labor and processing equipment. Live product is also more attractive to the consumer, who is able to purchase whole live crab or freshly butchered and cooked clusters rather than pre-cooked, frozen crab.

The full commercial potential of this species will remain unknown until such biological data as population density, reproduction patterns and geographical distribution, which are still under study, are collected and fully analyzed. Additionally, future research should continue to address gear development, trap design and the use of escape rings. Although the small southeast Florida fishery has been relatively successful, the longevity and potential expansion of the fishery for this valuable species remains unknown and over-capitalization by fishermen wishing to enter this fishery should be discouraged.

REFERENCES

1. Bellairs, J.J. and W.S. Otwell. 1983. Preliminary observations from on-board handling of deep-sea crabs (Geryon sp.) harvested from the Gulf of Mexico. Proceedings of the 8th Annual Tropical and Subtropical Fisheries Technological Conference of the Americas. 8:63-72.

2. Byers, C.J. De B. and C.G. Wilke. 1980. Quantitative stock survey and some biological and morphometric characteristics of the deep-sea red crab Geryon quinquedens off Southwest Africa. Fishery Bulletin of South Africa. 13:9-19.

3. Haefner, P.A., Jr. 1978. Seasonal aspects of the biology, distribution and relative abundance of the deep sea red crab Geryon quinquedens in the vicinity of the Norfolk Canyon, Western North Atlantic. Proceedings of the National Shellfish Association. 68:49-61.

4. Intes, A. and P. Le Loueff. 1976. Etude du crabe Rouge profund Geryon quinquedens en Cote D'Ivoire. Centre de Recherches Oceanographique, Abdijan. Document Scientifique. 7:101-112.

5. Luckhurst, B. 1986. Discovery of deep-water crabs (Geryon sp.) at Bermuda - A new potential fishery. Proceedings of the Gulf and Caribbean Fisheries Institute, 37th Meeting, November 1984. pp. 209-211.

6. Manning, R.B. and L.B. Holthuis. 1981. West African Brachyuran Crabs (Crustacea: Decapoda). Smithsonian Contributions to Zoology. 306:1-379.

7. Manning, R.B. and L.B. Holthuis. 1984. Geryon fenneri, a new deep-water crab from Florida (Crustacea: Decapoda: Geryonidae). Proceedings of the Biological Society of Washington. 97:666-673.

8. Otwell, W.S., J. Bellairs and D. Sweat. 1984. Initial development of a deep-sea crab fishery in the Gulf of Mexico. Florid Sea Grant Report No. 61. 29 pp.

9. Sweat, D. and W.S. Otwell. 1983. Experimental gear for harvesting deep-sea crab (Geryon sp.) from the Gulf of Mexico. Proceedings of the 8th Annual Tropical and Subtropical Fisheries Technological Conference of the Americas. 8:73-74.

10. Wenner, E.L. and G. Ulrich. 1986. Exploration for golden crab, Geryon fenneri, in the South Atlantic Bight: distribution, population structure and gear assessment. Final Report Year 1. South Carolina Wildlife and Marine Resources Department. 58 pp.

ACKNOWLEDGEMENTS

This article was partially developed under the auspices of the Florida Sea Grant College Program with support from NOAA, Office of Sea Grant, U.S. Department of Commerce, Grant No. R/LR-B-18. The authors wish to thank Dick, Rich and Dave Neilsen of Ft. Lauderdale, Florida for their invaluable assistance in collecting the material examined and their continued interest in developing a fishery for this species.

DEVELOPMENT OF A SWIMMING CRAB FISHERY IN ECUADOR

Kathleen M. Castro and Joseph T. DeAlteris

Department of Fisheries, Animal & Veterinary Science
International Center for Marine Resource Development
University of Rhode Island
Kingston, R.I. 02881

INTRODUCTION

During the last decade, Ecuador's fisheries have been dominated by the explosive growth of the shrimp farming industry. In 1983, Ecuador became the world's leading producer of farmed shrimp with an estimated 36,600 mt production (Sonu, 1985). However, this increase in shrimp production has been accompanied by several depressions in the trend caused by environmental changes and technological problems. Since shrimp is Ecuador's leading non-petroleum export commodity this decrease has caused considerable concern and promoted the development of hatchery operations, as well as a search for alternative fisheries.

In 1986, a project was initiated by the University of Rhode Island (URI) with their Ecuadorean counterpart, Escuela Superior Politecnica del Litoral (ESPOL) to investigate the potential for the development of a fishery for underutilized species of swimming crabs. Based on previous field observations and conversations with commercial and artisanal fishermen, it appeared that swimming crabs were a non-utilized by-catch of the shrimp trawlers and artisanal fishermen. No information on the identification, life history, abundance and distribution of these crabs was available in Ecuador, nor were these crustaceans considered in previous investigations for the development of alternative fisheries of underutilized species (FAO, 1978; Moran and Lopez, 1984; U.S.Dept. of Commerce, 1982.)

Callinectes sapidus, a member of the Portunidae family or better known as the blue crab, is the basis for the third largest fishery of the United States which in 1985, produced more than 83,200 mt of hard crab with a landed value of 60 million dollars (Vondruska, 1986). Demand exceeds the supply of domestic crab meat and USA processors have supplemented their supply with imported crab meat from Brazil, Venezuela and Mexico (W.F.Conley, personal communication).

Several basic criteria must be satisfied before a successful crab fishery can be established (Van Engel, 1974): the species must be present in consistent levels of high abundance; the species should be vulnerable to fishing gear; the species must be relatively large, easy to handle, transport, process and market; and the product must have consumer acceptance and potential market. In order to evaluate each of these criteria the project was divided into two phases with the following objectives:

PHASE I

(a) conduct a preliminary resource survey of coastal and estuarine waters,
(b) study the abundance & distribution of swimming crabs in Guayas Estuary,
(c) determine the catchability of the swimming crabs in the Guayas Estuary using a variety of fishing gear.

PHASE II

(a) develop processing technology
(b) investigate potential markets for the product

Phase I is nearing the end of its first year of investigation, preliminary results are summarized in the sequel. Phase II initiation is dependent on the results of Phase I.

MATERIALS AND METHODS

Ecuador extends 950 kms along the western coast of South America between the latitudes of 1° 00'N to 3° 20'S (McPadden, 1985). The configuration of the coastline is irregular, terminating in the Guayas Estuary and the Gulf of Guayaquil (Figure 1), the largest estuarine system on the Pacific coast of South America (Murray, 1975). The ocean is defined as sub-tropical with water temperatures exceeding 25° C and salt content below 33.5 ppt. The climate, however, is considered tropical and is divided into two distinct seasons. The wet season is characterized by heavy rainfall and extends from December through April. The dry season is cooler and includes the period from May through November.

The preliminary resource survey was conducted during the dry season month of July, 1986. Seven locations were chosen as sampling sites along the Ecuadorean coast covering the 3 major estuarine systems: Esmeraldas (Esmeraldas River), Bahia de Caraquez (Chone River) and the Guayas Estuary (Guayas River). A total of 41 trap samplings were made in these areas. Biological

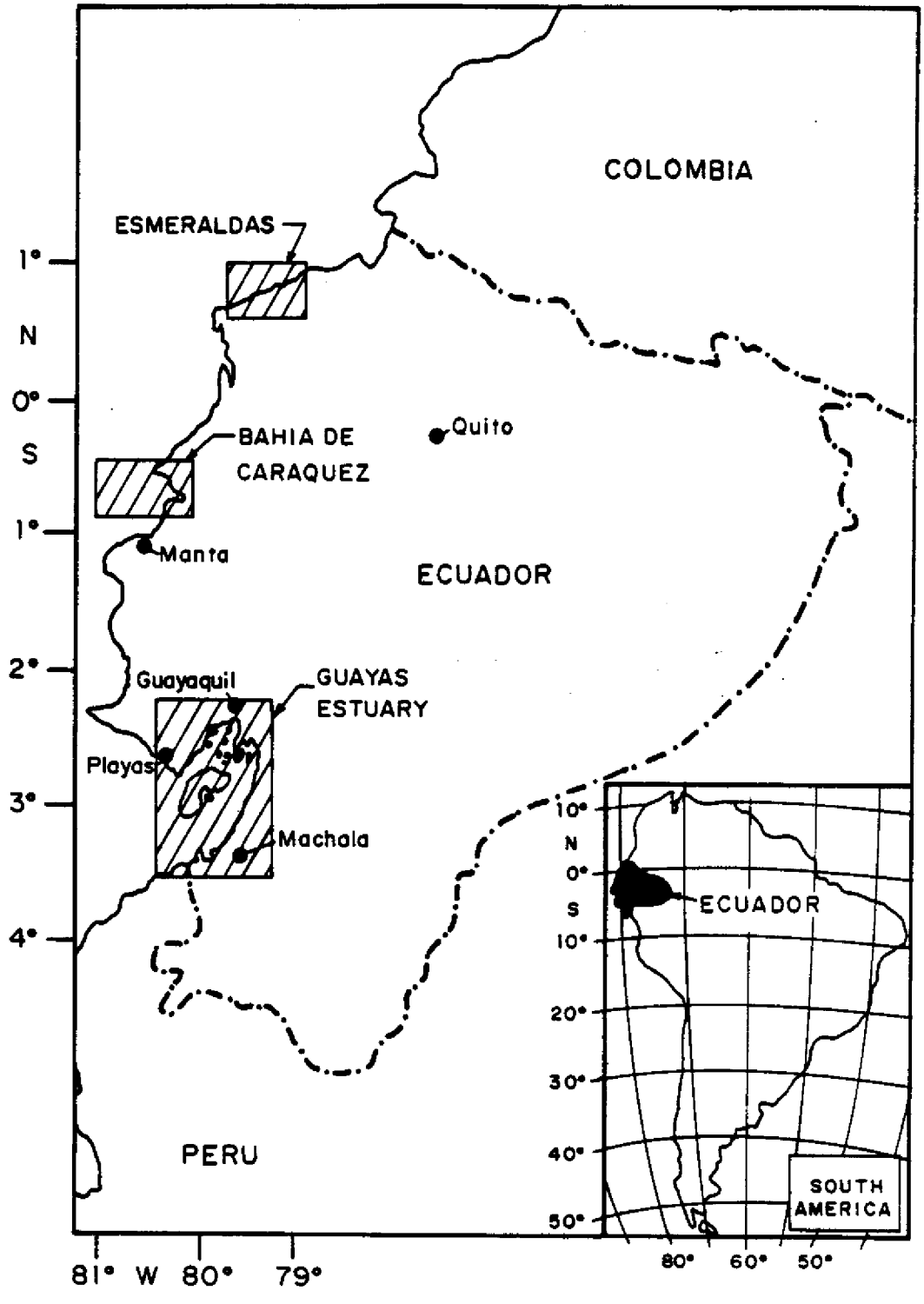


Figure 1. Map of Ecuador showing the three study areas.

and environmental data were recorded and crabs were identified according to available keys (Williams, 1974; Garth & Stevenson, 1966).

Experimental fishing for Callinectes spp. crabs was conducted in the Guayas Estuary during the wet season (January) and the dry season (June-August) of 1987. Sites were chosen in three areas based on expected salinity differences in the upper, middle and lower estuary (Figure 2). Four types of fishing gears were randomly fished at each site: Chesapeake Bay style crab traps (Van Engel, 1962), trotline, gillnet and lift nets. Five Chesapeake Bay style baited crab traps were constructed of locally available galvanized rectangular mesh wire with a mesh opening of 2.5 x 5 cm. Each trap measured 65 x 65 x 52.5 cm and had four entrance funnels. A smaller trap with a mesh size of 2.5 x 2.5 cm was also employed during the January sampling to capture juveniles, however, use of these was discontinued due to the possible exclusion of the larger animals. Two 30 m trotlines baited every 2 m were fished twice per hour. These were constructed of 1 cm diameter, hard lay nylon and anchored at both ends. Five baited lift nets were constructed with a square iron frame, covered with 1.2 cm webbing. These were lifted twice per hour. Two 30 m gillnets with 7.6 cm stretched mesh were constructed of soft lay polyamide twine. Traps, trotlines and lift nets were baited for each set with locally available species. These included Chloroscombrus orquesta, Selene perviance and Scomber japonicus. All gears were fished simultaneously for 3 hour periods. Unit fishing effort is expressed as gear-hour which represents one unit of gear with a one hour soak.

All crabs collected were identified, measured (carapace width) and weighed. Carapace width refers to the distance between the tips of the longest cephalothorax spines. General appearance of the crabs were noted including sex, maturity, and loss and regeneration of appendages. Environmental data including sediment type and bottom and surface temperature, salinity and dissolved oxygen levels were recorded.

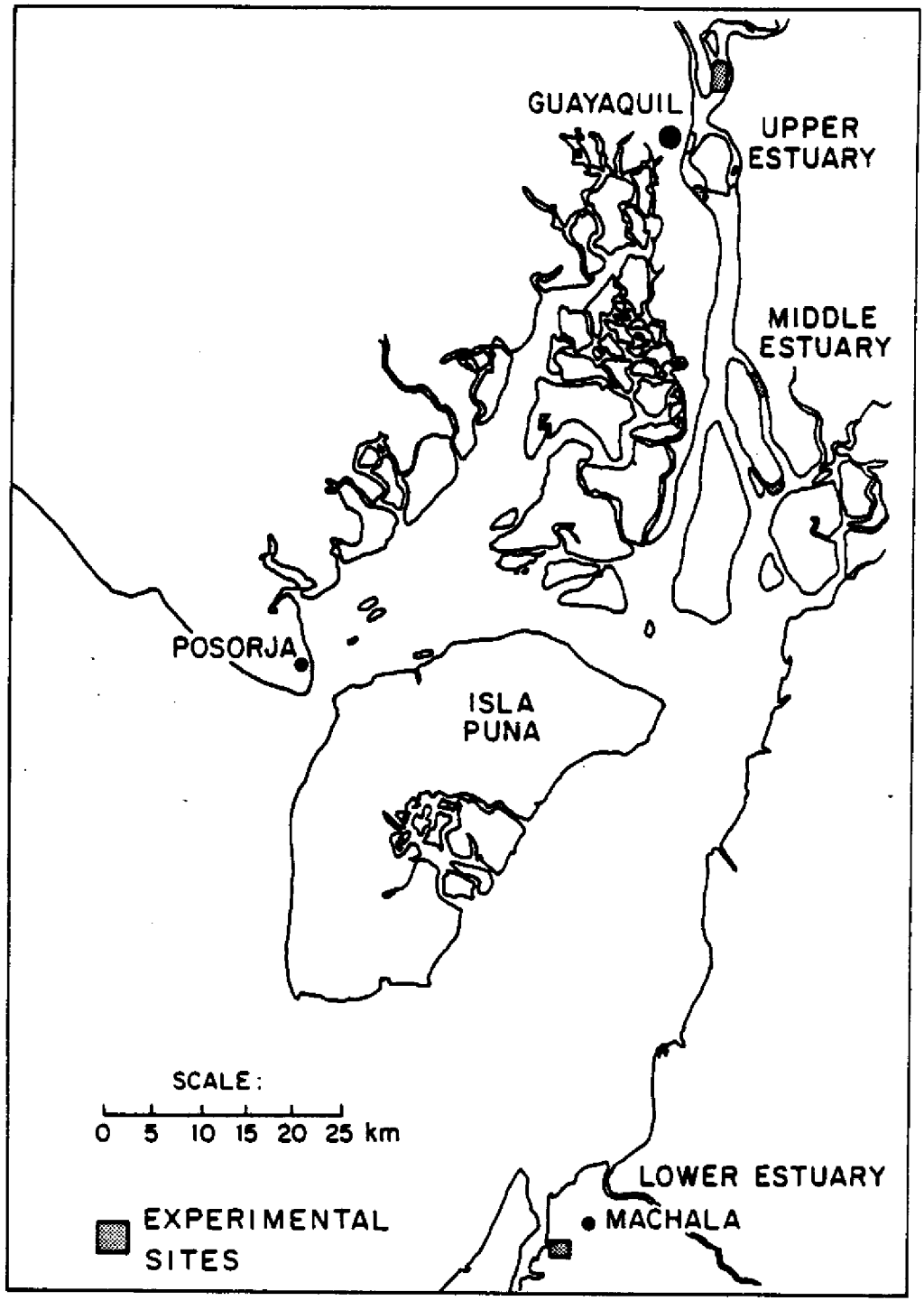


Figure 2. Map of Guayas Estuary showing the sampling sites.

RESULTS AND DISCUSSION

Resource Survey

The existence of five species of Portunid crabs inhabiting the coastal and estuarine waters of Ecuador as suggested by Williams (1974) was confirmed during these investigations. Callinectes toxotes and Callinectes arcuatus were captured in estuarine areas while samples of Euphylax robustus, Portunus asper and Cronius ruber were obtained from fishermen working in offshore areas. Initial indications suggest that these latter three species are not of sufficient size or quantity to support a fishery. However, the apparent abundance and size of the two Callinectes species suggests the presence of a large unexploited resource in Ecuador.

Callinectes toxotes was the largest of the swimming crabs found in the Guayas Estuary and have been described as the largest species in the genus (Williams, 1974). The largest specimen captured measured 22 cm in width, and weighed 660 gr. The average size of C.toxotes captured was 14.5 cm, with average weight of 211 gr. Callinectes arcuatus, a smaller species, had mean carapace widths of 10.3 cm and weights of 60 gr. Average carapace width and weight for both species for each area are summarized in Table 1.

Table 1. Average Carapace Width (CW) in cm and Weight (WT) in gr for Callinectes toxotes (TOX) and Callinectes arcuatus (ARC) Crabs Captured by Site and Season.

	WET SEASON				DRY SEASON			
SITE	TOX		ARC		TOX		ARC	
	CW	WT	CW	WT	CW	WT	CW	WT
UPPER GUAYAS ESTUARY	11.9	113			14.7	198		
MIDDLE GUAYAS ESTUARY	15.1	320	11.3	69.5	13.4	219		
LOWER GUAYAS ESTUARY					15.3	250	9.1	48.7

Abundance and Distribution

A detailed investigation of the abundance and distribution of the Callinectes spp. in the Guayas Estuary is currently being conducted by ESPOL. However, limited observations to date have indicated that the species distribution found in Ecuador is similar to that described by Norse and Estevez (1977) along the Pacific coast of Columbia, where C.toxotes dominated the less saline estuarine areas but was replaced by C. arcuatus in the higher salinity areas with some overlap in intermediate and high salinity areas (Figure 3). They suggest that salinity plays an important role in the distribution of the Callinectes species and describes both as being euryhaline.

A spatial and temporal shift of the species distribution occurred during the wet season, presumably following the shift of the salinity structure of the estuary due to increased fresh water input from the rivers. At the beginning of the wet season while salinity was still high (19 ppt) large populations of C. arcuatus were found in the middle estuary. At the end of the season, salinity dropped (15-17 ppt) and the population consisted entirely of C. toxotes. No shift in species composition was seen in the fresher upper estuary (0.1- 5 ppt), although there was an indication of size differences. No data is available for the wet season 1987 for the lower Guayas estuary.

If C. toxotes and C. arcuatus follow a similar pattern of distribution as Callinectes sapidus, a differential distribution by sex and maturity can be expected. Mating would occur in the fresher areas of the estuary, with females later migrating to estuary mouths to spawn resulting in the dispersion of the larvae into the offshore high salinity waters (Hail, 1984). Little data is available on the spawning habits of C. toxotes, although Williams (1974) mentions that the distribution of ovigerous females is presumed to be similar to that to C. sapidus.

Preliminary results indicate differential distribution of C. toxotes by sex and maturity in the three sites during the two seasons (Figure 4). In the wet season, immature males and females dominated the population in the upper estuary; while mature males and females were found in the middle estuary. During the dry season, mature males and females dominated in the upper estuary; while mature males were found in the middle estuary and the lower estuary. Preliminary data are insufficient to verify female

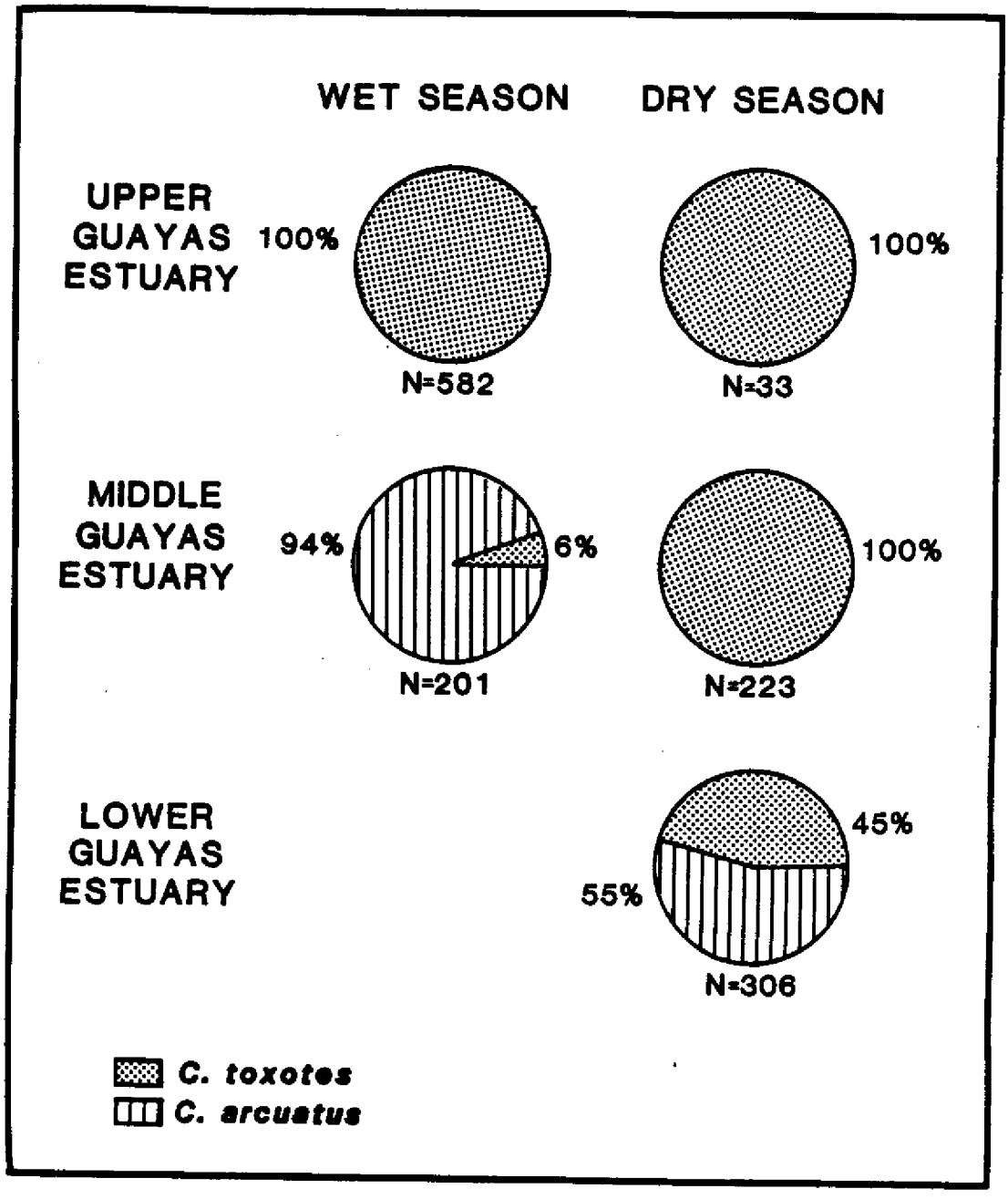


Figure 3. Species Composition in the Guayas Estuary.

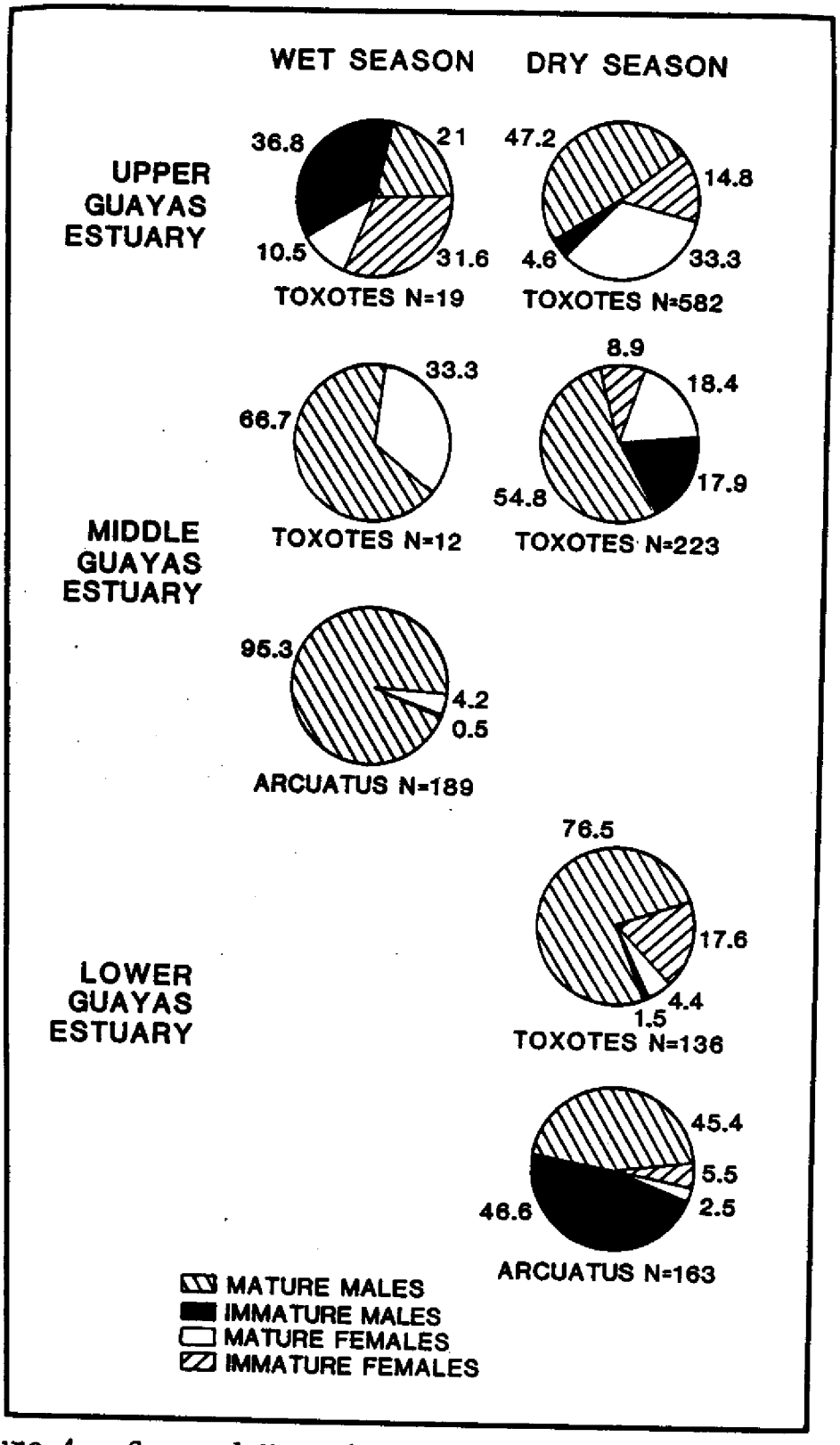


Figure 4. Sex and Maturity of Callinectes spp. crabs in the Guayas Estuary.

migration to higher salinity areas for spawning. There appeared to be a greater number of immature females in the upper estuary during the wet season, and a larger number of mature females during the dry season. Very few ovigerous females have been captured to date.

The majority of the C. arcuatus captured were males; principally mature males were found in the middle Guayas estuary, and mature and immature males were found in the lower estuary. Very few females were captured which suggests their absence from the estuary areas sampled. Dittel and Epifanio (personal communication) in their investigations in Costa Rica, have captured mostly female C. arcuatus but their sampling has been conducted offshore in the Gulf of Nicoya area.

The offshore waters were not sampled but literature supports the theory that C. arcuatus and C. toxotes may form large breeding populations in coastal waters throughout the year (Rosales, 1976) and that the estuarine phase of the life cycle is a growth phase as suggested for C. latimanus in Ghana (Kwie, 1974). Paul (1982) found that in Mexico, the female C. arcuatus continue their migration out of the estuary and continue spawning on the continental shelf.

Catchability

The blue crab in the United States may be subject to the most diverse kinds of fishing gear for any single species (Haefner, 1985). Gears can range from a simple baited hand line to the 50 kg crab dredge used in the winter months in the Chesapeake Bay. Only a few gears have proven to be economically practical on a commercial basis (Sholar, 1979): crab traps (80%) trotlines (10%) and dredges (10%) . In Ecuador, there are technological, social, cultural and environmental factors that must be considered in the selection of the appropriate harvesting gear. The final recommendations for the most appropriate harvesting gear will be a compromise between these factors as well as catch per unit effort or performance data for each gear type in the Guayas Estuary.

Catch per unit effort for each gear type by site and season are summarized in Table 2. Environmental conditions caused great variability in the CPUE for some gears. The trotlines and lift nets worked best when fished in quiet waters such as protected mangrove areas or during slack

tides. Traps performed well until current reached speeds causing them to "walk" or vibrate. The Guayas estuary has a tidal range of between 4 and 5 meters and tidal currents often reach speeds of 2-3 knots. Gillnets could only be placed in carefully selected sites due to debris in the water, boat traffic or currents.

Table 2. Catch per Unit Effort (CPUE) by Gear Type and Size.

	WET SEASON		DRY SEASON			
	LRG MESH TRAPS	SML MESH TRAPS	LRG MESH TRAPS	GILLNET	LIFTNET	TROTLINE
UPPER GUAYAS ESTUARY	.27(4)	.094(2)	.77(123)	1.03(8)	.49(25)	.85(10)
MIDDLE GUAYAS ESTUARY	.82(7)	.95(5)	.55(62)	.23(8)	1.5(20)	.92(8)
LOWER GUAYAS ESTUARY	-	-	.37(131)	.42(4)	.67(10)	.08(4)
AVERAGE CPUE	.62(11)	.70(7)	.56(316)	.59(20)	.89(55)	.73(22)

Note: Number of Replicates are indicated by parenthesis.

Traps and gillnets as passive gear offer the advantage of "self-fishing." Unless theft is a major problem in the area, these gears can remain unattended until the fisherman returns to remove the catch. US fishermen use a 24 hour soak for the traps, replacing the bait each time the trap is lifted. A 24 hour soak is not recommended for the gillnet since tidal currents and debris tend to cause the net to tangle. The trotline and lift nets must be continuously fished to harvest the crabs and the fisherman must remain the entire time with the gear. Therefore, a shorter total fishing time is recommended. CPUE may vary depending on soak time chosen for these gears.

If the assumption is made that the catch rate will be constant during the entire fishing time, ignoring effects of gear saturation, and species interaction, CPUE's can be extrapolated into expected daily catches for an artisanal

fishermen working from a non-motorized vessel such as a canoe, and for a small scale commercial fishermen with a motorized vessel less that 6 m in length (Table 3).

Table 3. Expected Catch

ARTISANAL LEVEL				SMALL SCALE COMMERCIAL LEVEL			
GEAR	UNITS	SOAK TIME (hrs)	CATCH	GEAR	UNITS	SOAK TIME (hrs)	CATCH
TRAP	10	24	134	TRAP	100	24	1350
LIFTNETS	10	8	58	LIFTNET	100	8	584
TROTLINE	200m	8	43	TROTLINE	1600m	8	376
GILLNET	200m	12	43	GILLNET	1600m	12	374

At present a very small local market exists for Callinectes spp. crabs. They are captured by handlines or lift nets by artisanal fishermen and vendors sell them on the streets of Guayaquil for 20 to 100 sucres each (10-50 cents US). Demand for swimming crab increases during the closed season of the preferred red mud crab, Ucides occidentalis, and prices up to 500 sucres each ($2.50 US) are common. Crab processors from the United States and Japan have visited Ecuador during this past year and a new Ecuadorean crab processing facility "Jaiba Azul", has recently received funding to begin operations.

It is difficult to ascertain the resource potential of Callinectes spp. from this preliminary study, although the results do offer some encouragement. Crabs were captured in all of the areas fished, although variability in the catch rates was due in large part to the exploratory nature of the investigation. From the preliminary data obtained thus far, the Callinectes spp. crab resource is sufficient to sustain an artisanal level fishery. However, it is recommended that more detailed investigations be conducted before any large scale commercial level fishery be established for this resource.

ACKNOWLEDGEMENTS

Support for this project was provided by U.S. AID through a cooperative agreement with the International Center for Marine Resource Development (ICMRD) at URI to provide fishery development support services to lesser

developed countries. The authors gratefully acknowledge the active participation and assistance of the Escuela Superior Politecnica del Litoral (ESPOL) located in Guayaquil, Ecuador. We also extend a special thanks to Ing. Bernardo Zapata and Tec. Daniel Castillo for their invaluable assistance in the collection of the samples in the field.

REFERENCES

FAO, 1978. Fishery Country Profile, FID/CP/ECU Rev. 2

Garth, J.S. and W. Stevenson, 1966. Brochure of the Pacific Coast of America. Brachyrhyncha: Portunidae. Allen Hancock Managr. Mar. Biol. 1, 1-154.

Haefner, P.A. 1985. The Biology and Exploitation of Crabs. In The Biology of Crustacea, Economic Aspects: Fisheries and Culture, Vol. 10. Editor: D. Bliss and A. Provenzano. Academic Press. Pp 111-165.

Hail, W.R. 1984. Delaware Blue Crab. University of Delaware Sea Grant College Program #11.

Kwei, E.A. 1978. Size Composition, Growth and Sexual Maturity of C. latimanus Rathbun in Two Ghanian Lagoons. Zool. J. Linn. Soc., 64, pp. 151-175.

McPadden, C. 1985. A Brief Review of the Ecuadorean Shrimp Industry. Publication of the Instituto Nacional de Pesca, Ecuador, funded by the Overseas Development Administration, UK, pp. 42-68.

Moran, F. and E. Lopez. 1984. Investigacion y Desarrollo de Nuevos Productos Pesqueros en el Ecuador. Rev. Lat. Tec. Alim. Pesq. Lime, Peru, 1, 1-32.

Murray, S., D. Conlon, A. Siripong and J. Santoro. 1974. Circulation and Salinity Distribution in the Rio Guayas Estuary, Ecuador. In Estuarine Research, Vol II, Editor: J. Cronin, Academic Press, NY, pp. 345-363.

Norse, E., and M. Estevez. 1977. Studies on the Portunid Crabs from the Eastern Pacific I. Zonation Along Environmental Stress Gradients from the Coast of Columbia. Marine Ecology, 40, 365-373.

Paul, R.F. 1982. Abundance, Breeding and Growth of C. arcuatus Ordway and C. toxotes Ordway in a Lagoon System on the Mexican Pacific Coast. Estuarine Coastal and Shelf Sci., 14, 13-26.

Ponce, J. and I.N. Dobrea. 1986. El Fenomeno El Nino y sus Implicaciones Agrometerologicas. ERFEN (Estudio Regional del Fenomeno el Nino), Boletin #16.

Rosales, F.J. 1976. Contribucion al Conocimiento de la Fauna de Acompanamiento del Camaron de Alta Mar Frente a la Costa de Sinaloa, Mexico. In Las Memorias de la Reunion sobre los Recursos de Pesca Costera de Mexico, Veracuruz, Mexico, pp. 25-80.

Sholar, T.M. 1979. Blue Crab Fisheries of the Atlantic Coast. In Proceedings of the Blue Crab Colloquium Gulf States Marine Fisheries Commission No. # 7. August 1982. Editor H Perry and W.A. Van Engel. pp. 111-127.

Sonu, S.C. 1985. Ecuadorean Shrimp, Culture and Exports, Marine Fisheries Review, 47, pp. 52-55.

United States Department of Commerce, 1982. Annual Fisheries Report for Ecuador, National Technical Information Service ITA-83-01-003.

Van Engel, W.A. 1962. The Blue Crab and the Fishery in Chesapeake Bay. Pt. 2. Types of Gear for Hard Crab Fishing. Commercial Fisheries Review , 24, pp. 1-10.

Van Engel. W.A. 1974. Underutilized Crustaceans of the Chesapeake Bay and Chesapeake Bight. Manuscript for Presentation at Fish Expo, 1974. Norfolk, Virginia. 6 pgs. Vir. Inst. Mar. Sci., Gloucester Pt., Virginia.

Vondruska, J. 1986. US 1985 Fish Landings: The Blue Crab Market 1984-1985. Marine Fisheries Review , 48, pp. 38-41.

Williams, A.B. 1974. The Swimming Crabs of the Genus Callinectes, Fish. Bull. US, 72, 685-798.

EFFECTS OF PROCESSING ON THE QUALITY AND TENDERNESS OF THE FLESH OF THE COMMON WHELK (BUCCINUM UNDATUM L.)

L.T. Adambounou
University of Quebec in Rimouski
Department of Biology and Health science
300 des Ursulines, Rimouski (Québec), Canada G5L 3A1

Lavallée, J. and Castaigne, F.
Department of Food Science and Technology
Laval University, Sainte-Foy (Québec), Canada, G1K 7P4

ABSTRACT

Three different cooking methods and six cooking times, mechanical tenderization and ten different salt solutions are investigated in an attempt to improve tenderness and shelf-life of whelk (Buccinum undatum L.) flesh. Tenderness, as evaluated by the Universal Testing Machine (INSTRON), was significantly influenced both by the duration and the method of cooking (Sheeffe Test $P < 0.05$). Samples tenderized mechanically by 200 g and 400 g loads were significantly more tender than untreated control and significantly less tender than hand tenderized samples ($P < 0.05$). Brines giving the lower microbial counts at 10°C storage were NaCl (2%) and $MgCl_2$ (6%), NaCl (2%), $MgCl_2$ (2%) and KCl (2%) and NaCl (8%). But there were no significant differences between the ten brines investigated ($P < 0.05$).

INTRODUCTION

The common whelk (Buccinum undatum L.) is a carnivorous marine snail widely distributed throughout the North Atlantic and Arctic Oceans. It provides a locally important fishery on the atlantic coasts of Canada, particularly in the St-Lawrence estuary, on the east and south coasts of Britain, and on the coasts of Normandy and Brittany. Santerelli and Gros (1986) estimated the French landings of whelk at 4 000 metric tons per year, with a commercial value of 17 millions french francs in 1983; Quebec landings are estimated at 300 metric tons per year with a commercial value of 100 000$ Can. (D.F.O. 1985).

In Quebec, the commercial forms of whelk are: untreated whelk (live in the shell); canned whelk (whelks are removed from the shell, canned and heat treated); and brined whelk (with about 8% NaCl) or preserved with vinegar. One of major characteristic of whelk is tough flesh texture and the consumer commonly complains of this. Like most gastropods, the locomotor activity of whelks is assumed by a pedal muscular organ. Such activity is energetically expensive (DaSilva and Hodgson, 1987) and implies a high degree of sophistication of the muscles. The organization of pedal muscles probably affects the tenderization modes during the processing.

Studies on the fine structure of the pedal musculature of gastropods are few and to date have been carried out either on terrestrial snails (Rogers, 1969) or on whelk (Hunt, 1976; DaSilva and Hodgson, 1987).

Salt, or sodium chloride (NaCl) is the most frequently used compound in food processing, and has been use for centuries for flavoring and as a bacteriostatic or bactericidal agent. The preservative effect of salt is primarily due to its ability to lower water activity by drawing water from tissue. In recent years sodium chloride consumption has become a major issue in the food industry, primarily because the sodium ion seems closely linked to human hypertension (Freis, 1976; Altschul and Grommet, 1980) and because the average consumption of sodium is 10-20 times that necessary for physiological balance (NAS, 1980). Sodium compounds occur naturally in many foods, including meats, fish, dairy products, grains, and vegetables. For the health of persons suffering from hypertension or other sodium related disorders FDA (1982), AMA (1972) and several other professionnal groups (Shank et al.,1983) have made formal recommendations concerning the use of sodium chloride. Some studies have been made concerning the optimization of functional and economic parameters when sodium chloride (NaCl) levels in processed foods are reduced by replacement with other chloride salts (Marsh, 1983, Maurer, 1983, Terrell, 1983).

It is known that some parameters such as: age (Snowden et al., 1978); muscle composition (Marsh et al., 1974, Marsh, 1977, Olson et Parrish, 1977), conservation processes (Bouton et al., 1973), cooking time and temperature (Bouton et al., 1981, Robertson et al, 1984, Findlay et Stanley, 1984) influence the toughness of animal muscle and therefore its texture.

The purpose of this paper is to report the effects of cooking methods, time/temperature parameters, enzymatic treatment and mechanical tenderization on the toughness of whelk flesh, as evaluated by the Universal Testing Machine. Some work was also done to evaluate the effect of partial replacement of sodium chloride by other chloride salts on the shelf-life of brined whelk.

MATERIALS AND METHODS

Sampling: Live welks bought from local commercial fishermen (Pecherie Bocard of Matane, or Poissonnerie Lucien Doucet of Bic) were kept in cold storage (4°C) until experimentation. Only live whelks with total shell length of 7-9 cm, as mesured with a vernier, were selected for experimentation as showed on the diagram of figure 1. Preliminary studies (Adambounou et al.) showed no significant difference between the tenderness of the pedal muscle of the samples of this group; while Santarelli and Gros (1986) reported the relationship between the age and shell length of the whelk.

EXPERIMENTAL DIAGRAM

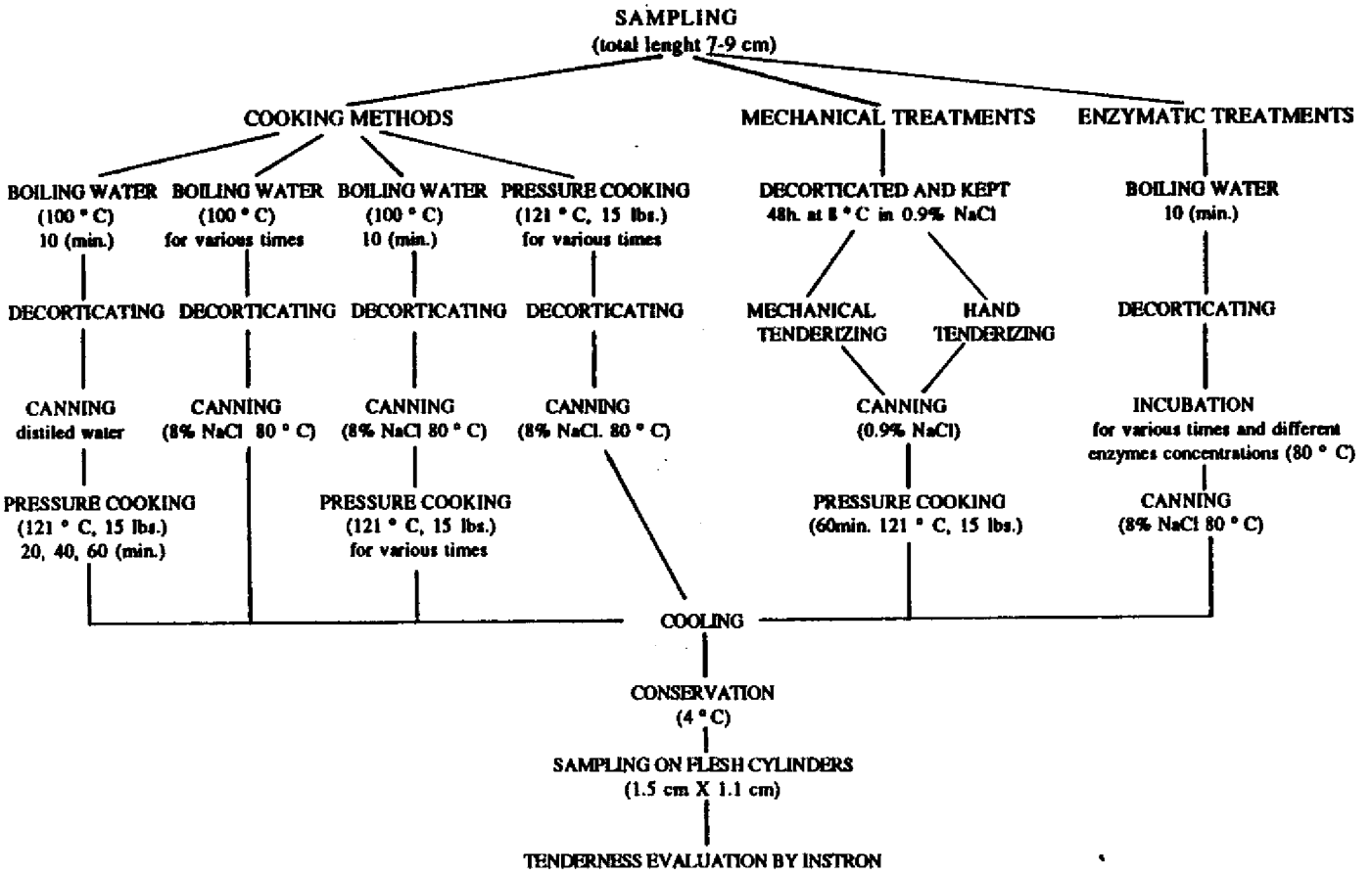


Figure 1. Experimental diagram.

Cooking methods: three cooking methods were investigated:

1) Boiling in water: About twenty whelks weighing approximately of 1 500 g were boiled in 1 000 g water (100°C) for fixed times (10, 20, 30, 40, 50 or 60 minutes). Cooked whelk were removed from the shells with a table fork, and pedal muscles sampled with a knife, washed and brined in a glass jar with warm sodium chloride solution (8% and 80°C), cooled at ambiant temperature (25°C) and stored at 4°C until evaluation of tenderness by Instron.

2) Ten minutes precooking followed by pressure cooking for various times. About 1 500 g of whelk were boiled in 1 000 g of water for ten minutes and the flesh removed from the shell with a table fork. Pedal muscles were sampled with a knife, washed and brined with warm 8% sodium chloride solution (80°C) in glass jars and pressure cooked (121°C, 15 lbs) for fixed times (10, 20, 30, 40, 50 or 60 minutes), cooled at ambient temperature (about 25°C) and stored at 4°C until evaluation of tenderness.

3) Pressure cooking (121°C, 15 lbs). 1 500 g of sample were pressure cooked for fixed times (10, 20, 30, 40, 50 or 60 minutes). Whelk were picked from the shell, brined in glass jars (8% NaCl, 80°C) cooled at 25°C and stored at 4°C for tenderness evaluation.

A control was prepared according to the cooking method (2) and the brine solution was replaced by distilled water.

Mechanical tenderization. Live whelk were obtained by breaking the shells with a hammer. Pedal muscles were selected, washed and kept in dilute sodium chloride solution (0,9%) at 0°C for 48 hours before mechanical tenderizing, with the apparatus shown in figure 2. The effect of two weights (400 g and 200 g), falling from a height of 80 cm was investigated; for comparison hand tenderizing was performed with a wooden mallet as used in the home for meat tenderizing. The samples were washed, slightly brined (NaCl solution 0,9% and 80°C) and pressure cooked for an hour (121°C, 15 lbs) in glass jars. They were cooled at 25°C and stored at 4°C until evaluation of tenderness.

Enzymatic tenderization. 1 500 g of whelk were boiled (100°C) in 1 000g of water for 10 minutes, removed from the shell with a table fork and the pedal muscle incubated at 80°C for fixed times (15, 30 or 45 minutes) in enzyme solutions of various concentrations (15, 30 or 45 g/l). The two enzymes investigated were crude papain powder type II and crude ficin (Sigma Chemical Co. Ltd). Pintauro, 1979, reported that the optimum temperature of these enzymes was from 65°C to 80°C. Samples were then brined (8% NaCl, 80°C), cooled (25°C) and stored (4°C) until tenderness evaluation.

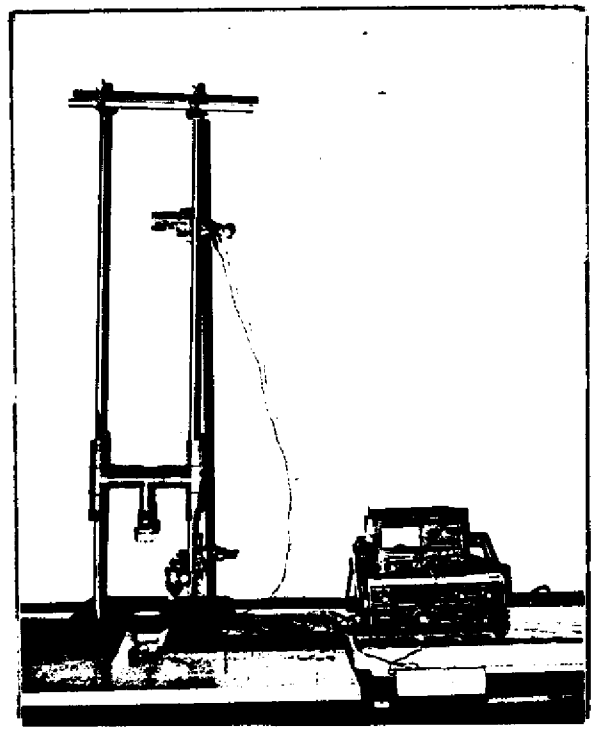


Figure 2. Experimental apparatus used for mechanical tenderizing.

Tenderness evaluation. Compression measurements were made using an Instron (Universal Testing Machine) Model TMS equipped with a 2 000 g load cell. For each treatment, thirty cylinders of 1,1 cm diameter were selected in the horizontal axis of pedal muscles with a circular knife and cut to 1.5 cm length. To eliminate sample mouvement during compression measurements, cylinders were placed in a plexiglass block drilled according the size of samples. The initial peak referred to as firmness was obtained by compressing cylinders to 90% of the original length. Preliminary investigations on calibration of Instron showed that 20 cm/min. is the optimal speed for both the crosshead and the chart (Lavallée et al., 1987). The method used was similar to the Instrumental Texture Profile Analysis with only the initial peak, referred to as firmness, being used (Breeene, 1975, Findlay and Stanley, 1984) (Figure 3).

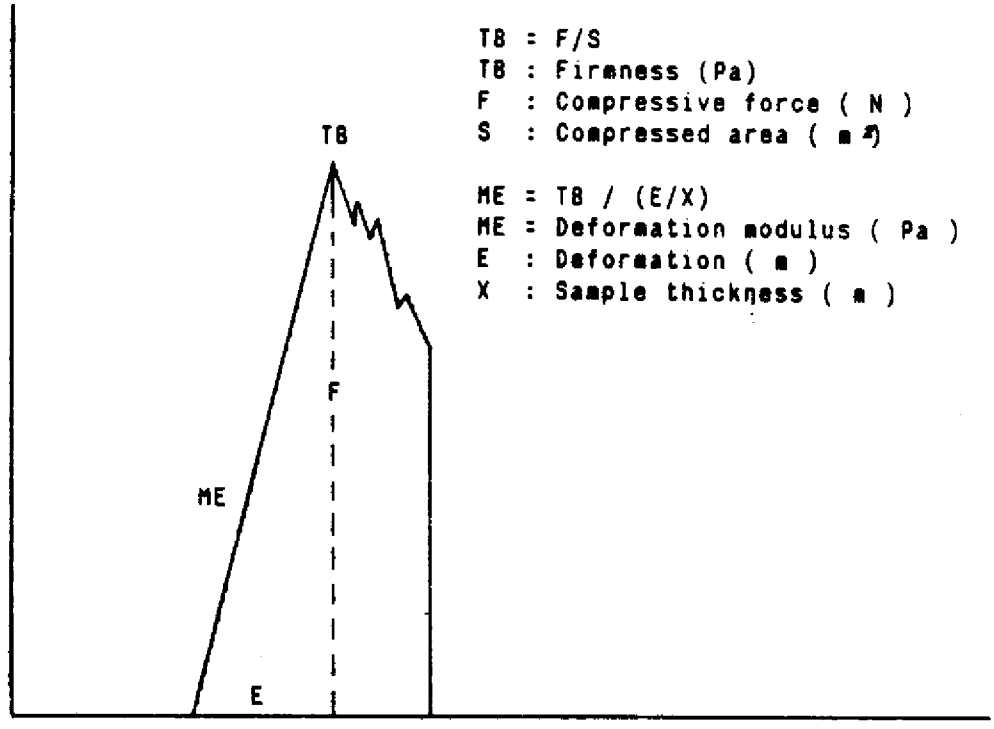


Figure 3. Typical deformation curve observed on Instron.

Microbial analysis. To evaluate the effects of different brines on the shelf-life of whelk, a total plate count using P.C.A. was made on the sample prepared as in cooking method (2), stored at 10°C for 60 days. The plating were done on triplicate.

Experimental plan and statistical analysis. Complete Random Factorial experimental design was followed, and all analyses shown in the experimental diagram (Figure 1) and microbial analyses were done in duplicate and the entire experiment repeated twice. Data were analysed by standard parametric analysis of variance technique. Degrees of significance were evaluated by the Scheeffe test (Kirk, 1968).

RESULTS AND DISCUSSION

Effect of enzymatic treatment. The influence of enzyme concentration on whelk toughness is shown in figures 4 and 5. The combination of 15 minutes incubation time and 15 g/l papain gave a acceptable tenderness of whelk (Figure 4). The tenderness of whelk flesh increase significantly ($P < 0,5$) with increasing papain concentration, whereas increasing the time of incubation decreases tenderness. In the

case of ficin, increasing either incubation time or enzyme concentration increases significantly the firmness of whelk (Figure 5). After 15 minutes incubation, both enzymes (papain or ficin) rendered whelk flesh more tender, as compared to untreated samples (Figures 4 and 5). The increase in firmness as function of duration of thermal treatment agrees with the results of Martens et al (1982). This change of texture depends of the composition of muscle (Khan, 1977). Enzyme treatment decreases pigmentation and this appearance might possibly influence the consumer.

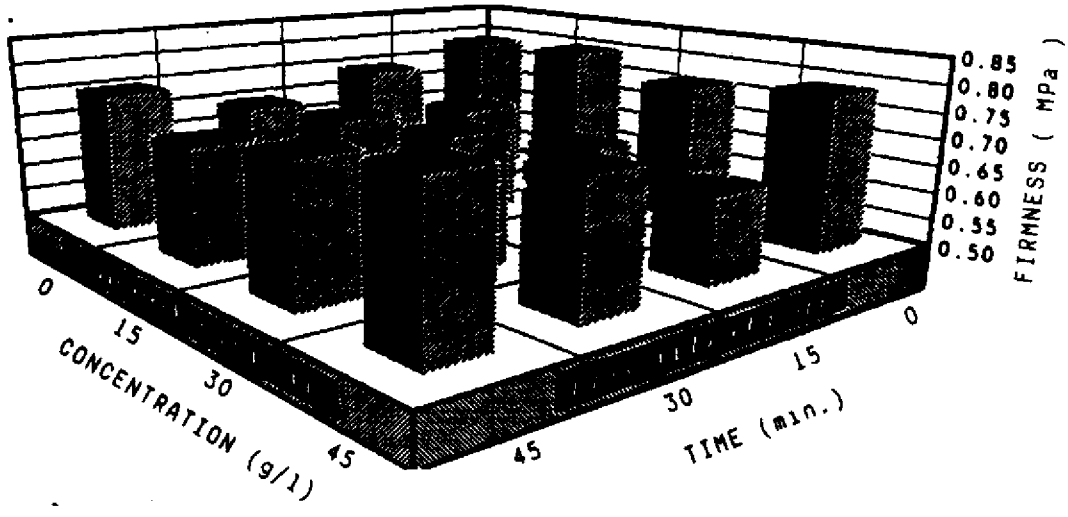


Figure 4. Influence of papain concentration and incubation time on whelk firmness.

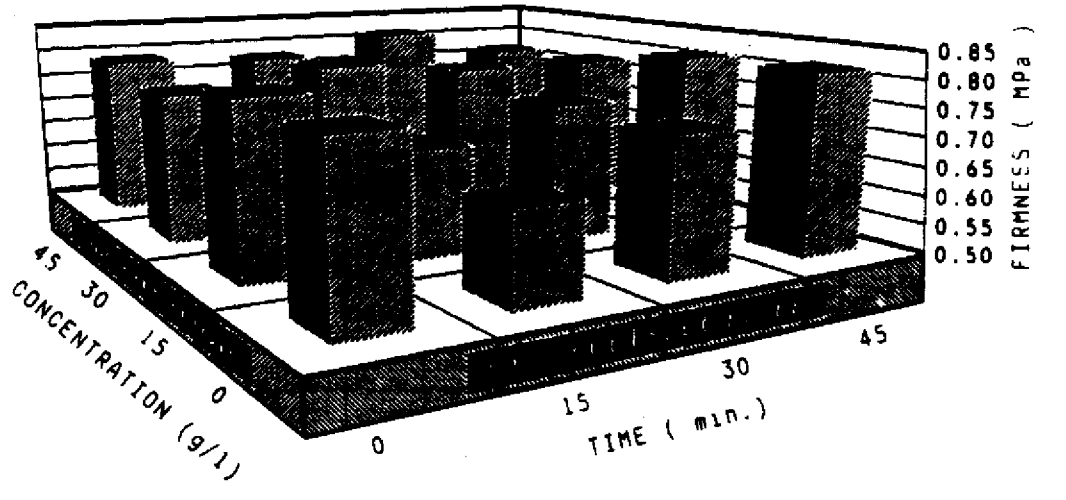


Figure 5. Influence of ficin concentration and incubation time on whelk firmness.

Effect of cooking time. For the three cooking methods investigated, the compression values of whelk decreased significantly after 20 minutes and after 40 minutes (Table 1). There is no significant difference between 10 and 20 minutes, nor between 30 and 40 minutes, nor between 50 and 60 minutes ($P < 0.05$). However in the case of cooking method (1), the difference was not significant between 40 and 60 minutes. These finding agree with those of Bouton and Harris (1972). These results indicate the longer the cooking time, the more tender whelk flesh becomes. This is not the case for enzyme treated flesh, where restructuration phenomena of hydrolysed tissue constituents probably take place with the duration of incubation.

Table 1. Influence of cooking times on whelk tenderness for different cooking methods.

COOKING METHODS	TIMES (min.)					
	10	20	30	40	50	60
Boiling water	$.775^{a}$ (1) $.146^{a}$ (2)	$.758^{a}$ $.142^{a}$	$.614^{b}$ $.120^{b}$	$.565^{b}$ $.107^{c}$	$.567^{b}$ $.108^{c}$	$.572^{b}$ $.106^{c}$
Whole + pressure cooking	$.765^{a}$ $.136^{a}$	$.757^{a}$ $.128^{a}$	$.741^{a}$ $.125^{a}$	$.689^{b}$ $.118^{b}$	$.538^{c}$ $.101^{c}$	$.496^{d}$ $.098^{c}$
Decorticate + pressure cooking	$.779^{a}$ $.149^{a}$	$.785^{a}$ $.151^{a}$	$.765^{a}$ $.152^{a}$	$.690^{b}$ $.134^{b}$	$.630^{c}$ $.128^{c}$	$.513^{d}$ $.108^{d}$

(1) Firmness in MPa
(2) Deformation modulus in MPa

a any means in the same horizontal line with same superscript are note significantly different (Scheffe Mutiple Range Test) at $P < 0.05$.

Effect of cooking method. The results obtained for the firmness and deformation modulus, indicate no significant difference ($P < 0.05$) between the three cooking methods for 10 minutes cooking time (Table 2). For 30 and 40 minutes, cooking in boiling water made whelk significantly ($P < 0.05$) more tender than cooking methods 2 and 3. For 50 minutes, pressure cooking (method 3) gave the best results for the firmness and deformation modulus. According to these results (Tables 1 and 2), decorticating whelks before pressure cooking increased firmness of samples. Between 30 and 40 minutes cooking whole whelk in boiling water gave the most tender flesh. In the pressure cooking method with decorticated whelks, the increase of flesh toughness could be related to the increasing rigidity of myofibrillar structure which is a function of cooking temperature (Bouton and Khan, 1975). In the case of cooking method (2),

the shell could reduce the effect of temperature on denaturation of myofibrillar proteins by reducing the rate of heat transfer.

Table 2. Influence of cooking methods on whelk tenderness for different times.

TENDERNESS PARAMETERS	COOKING METHOD (*)	TIME (min.)					
		10	20	30	40	50	60
TB (en MPa)	1	$.774^{a}$	$.757^{a}$	$.613^{b}$	$.564^{b}$	$.567^{b}$	$.571^{a}$
	2	$.765^{a}$	$.756^{a}$	$.737^{a}$	$.688^{a}$	$.539^{b}$	$.495^{b}$
	3	$.779^{a}$	$.784^{a}$	$.765^{a}$	$.691^{a}$	$.630^{a}$	$.512^{b}$
ME (en MPa)	1	$.146^{a}$	$.142^{a}$	$.119^{a}$	$.106^{a}$	$.108^{a}$	$.105^{a}$
	2	$.135^{a}$	$.128^{b}$	$.123^{a}$	$.118^{b}$	$.102^{a}$	$.097^{a}$
	3	$.148^{a}$	$.151^{c}$	$.151^{b}$	$.134^{c}$	$.127^{b}$	$.107^{a}$

(*) 1 = whole whelk cook in boiling water
2 = whole whelk cook in a pressure cooker
3 = precooked 10 min., decorticate and cook in a pressure cooker

a any means in same vertical line with same superscript are note significantly different (Scheffe .ultiple Range Test) at $P < 0.05$.

Effect of sodium chloride. Table 3 shows the effect of sodium chloride in cooking water on whelk firmness. These results agree with those of others who have demonstrated the use of salt to improve food texture or as a binding agent (Marsh, 1983). Sodium chloride interacts with proteins and others constituents to improve the texture of foods (Whiting and Richards, 1978).

Mechanical tenderization. The results obtained from mechanical treatments indicate no significant difference between the 200 g load and 400 g load (Figure 6), but both treatments rendered whelk flesh significantly more tender than untreated control and significantly less tender than hand tenderized ($P < 0.05$). There were no visual differences between the mechanically tenderized whelk and the untreated controls.

Table 3. Influence of NaCl in the cooking water on whelk tenderness.

TENDERNESS PARAMETERS	COOKING METHODS(*)	TIME (min.)		
		20	40	60
TB (en MPa)	1	.631[a]	.546[a]	.451[a]
	2	.787[b]	.690[b]	.505[b]
ME (en MPa	1	.115[a]	.094[a]	.097[a]
	2	.151[b]	.133[b]	.108[b]

(*) 1 pressure cooking in a glass jar with water only.
2 pressure cooking in a glass jar with NaCl 8%.

[a] any means in the same vertical line with same superscript are note significantly different (Sheffe Multiple Range Test) at P < 0,05.

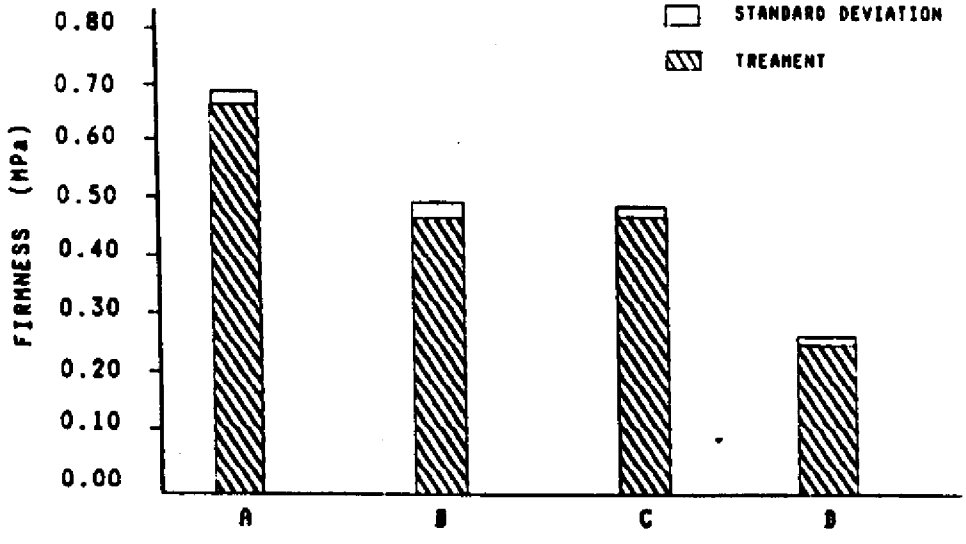


Figure 6. Effect of mechanical treatments on whelk firmness A - control, B - Stroked with 200 g weight, C - stroked with 400 g weight, D - hand hammering.

Effect of various brines on whelk shelf-life. As shown in figure 7, the maximum shelf-life is seven days. The microbial count increases exponentialy from 7 days to 30 days and becomes stable thereafter. The exponential proliferation of microorganisms could be related to osmotic phenomena: the extraction of water from tissue by the salt involves the diffusion of water soluble nutrients into the brine, and this provides the medium supporting the typical pattern of microbial growth. No significant difference was observed concerning the preservatif effect of the ten salts investigated. In other words, sodium chloride could be partialy replaced effectively by any of the other salts. This agree with the finding of various authors (Marsh, 1983, Seman et al, 1980, Seperich et al, 1983).

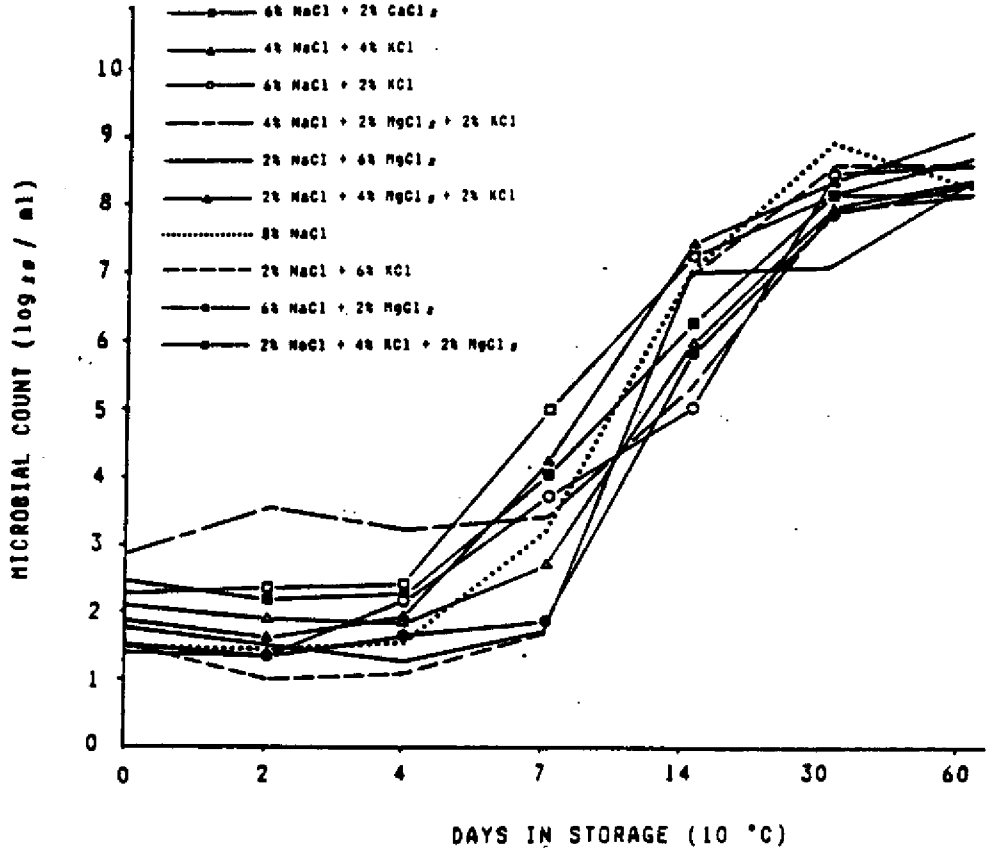


Figure 7. Effect of different salts on total microbial counts (PCA) for whelks stored during a 60 days period at 10°C.

— □ — 6% NaCl + 2% $CaCl_2$; — ▲ — 4% NaCl + 4% KCl;
— o — 6% NaCl + 2% KCl; — - — 4% NaCl + 2% $MgCl_2$ + 2% KCl;
——— 2% NaCl + 6% $MgCl_2$; — Δ — 2% NaCl + 4% $MgCl_2$ + 2% KCl;
..... 8% NaCl; ——— 2% NaCl + 6% KCl;
— • — 6% NaCl + 2% $MgCl_2$; — ■ — 2% NaCl + 4% KCl + 2% $MgCl_2$;

CONCLUSION

Optimal tenderizing of whelk can be obtained by cooking in boiling water for 30-40 minutes. Tenderness is better if whelk is mechanically tenderized. Enzymatic tenderizing reserves some surprises (effective concentration of enzyme, overall cost for industrial production and final appearance of the product). Some correllation with sensorial tests are needed before final conclusions can be drawn. The microbial test conducted in this work confirmed the possibility of partial replacement of sodium chloride by other chloride salts without affecting the duration of shelf-life of brined whelk.

REFERENCES

1. A.M.A. 1979. American Medical Association concepts of nutrition and health. Councilor or scientific affairs of A.M.A. J.Am. Med. Assoc. 242 (21): 2335.

2. Adambounou, T.L., J. Lavallée, and F. Castaigne. 1986. Optimisation de la tendreté de la chair de buccin (Buccinum undatum L.). Session Poster. 29e Congrès de I.C.S.T.A.

3. Altschul, A.M. and J.K. Grommet. 1980. Sodium intake and sodium sensitivity. Nutr. Rev. 38: 393.

4. Bouton, P.E. and P.V. Harris. 1972. The effect's of cooking temperature and time on same mechanical properties of meat. J.Food Sc. 37: 140-143.

5. Bouton, P.E., P.V. Harris, and R.I. Baxter. 1973. A comparison of the effects of some post-slaughter treatments on the tenderness of beef. J. Food Technol. 8: 39.

6. Bouton, P.E., P.V. Harris, and D. Ratcliff. 1981. The effect of cooking temperature and time on the shear properties of meat. J. Food Sci. 46: 1082.

7. Breene, W.H. 1975. Application of texture profile analysis to instrumental texture evaluation. J. Texture Studies, 6, 53-82.

8. DaSilva, F.M. and A.N. Hodgson. 1987. Fine structure of the Pedal Muscle of the whelk Bullia Rhodostrowa Reeve: correlation with function. Comp. Biochem. physiol. Vol.87A. No 1, pp. 143-149.

9. Department of Fisheries and Oceans. 1985. L'industrie des pêches maritimes du Québec. FS. 23-71/1985F. Ministère des Approvisionnements et Services du Canada.

10. F.D.A. 1982. Food Labeling. Declaration of Sodium content of Foods and Label Claims for Foods on the Basis of Sodium Content. Fed. Regist. 47: 26580-26589.

11. Findlay, C.J. and D.W. Stanley. 1984. Texture-structure relationship in scallops. J. of Texture studies. 75-85.

12. Freis, E.D. 1976. Salt, volume and the prevention of hypertension circulation 53 (4): 589.

13. Hunt, S. 1972. The fine structure of the smooth muscle in the hypobranchial gland of the gastropod Buccinum undatum L. Tissue and cell. 4(3) pp. 479-492.

14. Khan, A.W. 1977. Some factors causing variation in the texture of similar muscles from comparable animals. Meat Sci. 1, 169-176.

15. Kirk, R.E. 1968. Experimental design: procedures for the behavioral sciences. Brooks-cole, Belmont, Calif. 577p.

16. Lavallée, J., F. Castaigne, and L.Adambounou. 1987. Évaluation de la tendreté de la chair de buccin par Instron. Présentation orale. 55e Congrès de l'ACFAS. Annales de l'ACFAS. Vol.55. pp.247.

17. Marsh, B.B. 1977. Symposium: The basis of quality in muscle foods. J. Food Sc. 42(2): 295-299.

18. Marsh, B.B., N.G. Leet, and M.R. Dickson. 1974. The ultrastructure and tenderness of highly cold-shortened muscle. J. Food Technol. 9, 141.

19. Marsh. A.C. 1983. Processes and formulations that affect the sodium content of foods. Food Technol. 37(7): 45-49.

20. Martens, H., E. Stabursvik and Martens. 1982. Texture and colour changes in meat during cooking related to thermal denaturation of muscle proteins. J. Texture Studies 13: 291-309.

21. Maurer, A.J. 1983. Reduced sodium usage in poultry muscle foods. Food Technol 37(7): 60-65.

22. N.A.S. 1980. Toward healthful diets, National Research Council, Natl., Acad. of Sci., Washington, D.C.

23. Olson, D.G. and F.C.J.R. Parrish. 1977. Relationship of myofibril fragmentation, index to measures of beef steak tenderness. J. Food Sc. 42(2), 506-509.

24. Pintauro, N.D. 1979. Food Processing Enzymes: Recent developments. Food Techno. rev.no 52, p.231. Nayes Data Corporation.

25. Robertson, D., P.E. Ratcliff, P.E. Bouton, P.V. Harris, and W.R. Shorthorse. 1984. Effect of cooking temperature and animal age on the shear properties of beef and buffalo meat. J. Food Sci. 49(4) 1163-1166.

26. Rogers, D.C. 1969. Fine structure of smooth muscle and neuromuscular junctions in the foot of Helix aspersa. Z. Zellforsh 99, 315-335.

27. Santarelli, L. and P. Gros. 1986. Modélisation bioéconomique de la pêcherie de buccin (Buccinum undatum L.: Gastropoda) du port de Granville (Manche Ouest). Rev. Trav. Inst. Pêches marit., 48(1 et 2): 23-32.

28. Seman, D.L., D.G. Olson and R.W. Mandigo. 1980. Effect of reduction and partial replacement of sodium on bologna characteristics and acceptability. J. Food Sci. 45: 1116.

29. Seperich, G.J. and G.H. Ashoor. 1983. The effect of partial and total replacement of sodium chloride with calcium chloride in a bologna product. Progress report to the American Meat Inst. from Ariz. State Univ. Tempe.

30. Shank, S.F., L. Larsen, F.E. Scarbrough, J.E. Vanderveen, and A.L. Forbes. 1983. FDA Perspectives on sodium. Fd Technol. 37(7): 73-77.

31. Snowden, J. McK., P.E. Bouton, and P.V. Harris. 1978. Influence of animal age on the mecanical properties of restrained, heat-treated collagenous tissue. J. Food Sc. 43(2) 178-201.

32. Terrel, R.N. 1983. Reducing the sodium content of processed meats. Food Technol. 37(7): 66-71.

33. Whiting, R.C. and J.T. Richards. 1978. Influence of divalent cation on poultry meat emulsions and sausages. J. Food Sci. 43: 312.

AKNOWLEDGMENTS

The authors would like to express their appreciation to Mr Alexander Strachan of Department of Biology and Health Science for his help in the preparation of the manuscript. This work was supported in part by the National Science and Engineering Research Council and Institutionnal Research Fund of the University of Quebec in Rimouski.

EVALUATION OF A CONTAINERIZED SYSTEM FOR THE RELAYING OF POLLUTED CLAMS (MERCENARIA MERCENARIA)

William D. DuPaul and Michael J. Oesterling
Virginia Institute of Marine Science
School of Marine Science
College of William and Mary
Gloucester Point, VA 23062

INTRODUCTION

Bivalve molluscs are well-known for their ability to bio-concentrate polluting microorganisms and their equal capability to cleanse themselves when placed in unpolluted environments. This latter fact has led to the development of entire industries based upon taking shellfish from marginally polluted water and either moving them to approved harvesting areas for natural cleansing (termed relaying) or sending them to onshore depuration facilities where water quality can be rigorously controlled.

Of the two processes, depuration has received the greater emphasis from scientists, industry and regulatory agencies as evidenced by the volume of information available (for examples see Arcisz & Kelly 1955, Furfari 1966, Haven et al. 1978 and Neilson et al. 1978). The reasons for this include the shorter periods of time involved to depurate compared to relaying (48-72 hours versus 15 days or longer), ease of operation and product recovery, and a greater assurance for a cleansed product. Relaying, on the other hand, has received only limited attention, despite the fact that it is widely practiced in both the oyster and hard clam industries (Blogoslawski and Stewart 1983).

The relaying of hard clams (Mercenaria mercenaria) from marginally polluted waters to approved harvesting waters for natural cleansing represents a multi-million dollar fishery in Virginia. By law, relaying has been confined to direct on-bottom placement of clams in approved areas. This process is extremely inefficient and prone to product losses as high as 30% owing to mortality associated with handling, predation, stressful environmental conditions and inherently poor recovery procedures. This loss is significant considering that approximately 25 million clams were harvested and relayed in Virginia during 1986 (Virginia Marine Resources Commission). Containerized relaying offers a potentially superior alternative to natural relaying. However, prior to 1987, it had not been approved by the Virginia Department of Health due to lack of documentation on effective purification of hard clams within containers. All previous containerized relaying experiments have been conducted using oysters (Crassostrea virginica or C. gigas).

One of the earliest studies to employ containers in a relaying study was conducted using oysters (C. virginica) in Biloxi Bay (Cook and

Childers 1968). While containerization demonstration was not an objective of the study, their use of 30 x 18 x 3-inch racks covered with 1-inch wire mesh yielded valuable data on the potential for container relaying. When racks with 6-inch legs to support the rack off-bottom were compared to racks placed directly on a mud bottom, no significant differences were found and cleansing was complete by the eighth day. Similarly there was no difference between oysters held in racks and oysters placed directly on mud bottom. However, cleansing was not completed until the tenth day, indicating that cleansing may proceed faster in racks.

Quayle and Bernard (1976) used wire-mesh baskets to demonstrate coliform bacteria purification of Pacific oysters (Crassostrea gigas). Open top galvanized iron mesh baskets 62 x 62 x 30 cm (24 x 24 x 12 in), containing approximately 55 kilograms (121 pounds) of oysters were used. Within 48 hours of being placed on the bottom, the oysters reached an equilibrium with ambient bacteriological conditions. Ninety percent of the original bacterial load was eliminated within 24 hours and an additional 8% reduction experienced by 48 hours after being placed in approved harvesting waters. Oysters sampled from varying depths within the basket should no statistically significant differences between samples. The authors suggested that containerization offered an economical alternative to traditional on-bottom relaying.

Supan and Cake (1982) determined the effects of containerized relaying on oyster (C. virginica) survival and purification under varying environmental conditions, differing seasons and different loading configuration; and, to investigate various designs of rafts, racks, trays and other containers for commercial relaying. They categorized their container systems as suspension relaying, on bottom (longline) relaying, or rack-relaying. Successful cleansing varied, but according to the authors, commercial quantities of oysters in multilayers could eliminate indicator bacteria.

In an effort to document the effectiveness of bacterial cleansing of hard clams when held in containers, a series of experiments were conducted during 1985 and 1986 (Kator and Rhodes, unpublished data). This project had two main objectives: document the bacteriological cleansing of containerized hard clams in a commercial setting and demonstrate the commercial advantages of containerized relaying over traditional on-bottom relaying. In this paper the authors will summarize the bacteriological results and concentrate on the results that relate to advantages over traditional relaying.

MATERIALS AND METHODS

The full-scale commercial container relaying experiments were conducted in cooperation with an established industry relayer using the traditional on-bottom method. Containers were built of expanded steel, diamond shaped mesh (2.54 cm along longest axis) supported on a

framework of 2.54 cm L-shaped steel bars. The containers measured 1.2 x 1.2 x .3 m (4 x 4 x 1ft) and were fitted with 2, 15.2 x 15.2 cm (6 x 6 in) wooden skids on the bottom to support the container off the bottom. The container was constructed with a hinged edge midway across the top to permit one half of the top to be raised. The free edge of the top was fitted with a hasp closure to facilitate a seal for the container.

Results from the preliminary packing experiment indicated that hard clams representative of the size to be relayed (littlenecks and cherrystones) should not be packed more than 15.2 cm (6 in.) deep (Kator and Rhodes, unpublished data). Packing to this depth, each container held approximately 4500-5000 hard clams.

During the 1986 relaying season, five experiments were conducted utilizing naturally contaminated hard clams. Containers were deployed on approved bottom adjacent to ongoing bottom relaying operations. On the day of deployment, clams were randomly selected and returned to the laboratory for bacterial analysis. Water temperature and salinity were measured and a water sample taken for bacterial analysis. After a minimum of 14 days purification time, bagged clam samples were retrieved for analysis and the container returned to the bottom. If, upon analysis, the bagged clams met a product standard of 50 fecal coliform per 100 g of meats, then the remainder of the clams in the container were released for sale.

Once a container was released for sale, all clams were removed and counted to determine mortalities. Clams were separated into the following categories:

1. Marketable - no shell damage; still alive
2. Dead Clams - shells with both valves intact and attached;
3. Broken Clams, Old - broken shells with both valves attached, no meat inside; assumed damaged at time of harvest or packing in containers.
4. Broken Clams, New - broken shells with both valves attached, with meat inside; assumed damaged during reharvesting operation.

RESULTS AND DISCUSSION

Results of the bacterial analysis (Kator and Rhodes, unpublished data) for the five deployments of the relaying container indicated that effective depuration was accomplished. When containers were carefully deployed and clams exposed to favorable water quality, a product standard of 50 fecal coliforms/100 g meats was met.

Water temperatures fluctuated during the course of the experiments between a low of 17.5°C (63.5°F) on May 6, 1987 and a high of 29.5°C (85.1°F) on July 29, 1987. Salinity ranged from 18 and 22 ppt. Recovery data for 5 experiments using naturally polluted clams are

presented in Table 1. Results of each individual experiment will be discussed separately prior to summarization.

Experiment 1, May 6-27, 1986

The total number of clams held within the container was 4,835. This included 585 clams held in bags for microbiological analysis. Twenty-one days after initial placement, 4250 clams were released to market. For this experiment, distinctions between dead clams and old broken clams were not made. The significance of distinguishing dead from broken (old and new) is an indication of mortality that can be attributed directly to handling (initial harvest, placement in container or harvest from container) and other sources of mortality (smothering in container or unexplained). Excluding bagged clams for microbiological analysis, total mortality was 3.89%. Water temperature (17.5°C) and salinity (18.0 ppt) were recorded during this experiment.

Experiment 2, June 10-30, 1986

Container loading, deployment and retrieval went smoothly. A total of 4615 clams (585 held in sample bags) were loaded into the container. Upon release, 4030 clams were marketed. For this experiment, mortalities were separated into dead clams (43), old broken clams (114) and new broken clams (21). Total mortality (excluding bagged samples) was 4.23%. Of this total mortality, 3.21% could be attributed to handling effects.

Experiment 3, June 24-July 11, 1986

Results from this experiment are very similar to the previous one. A total of 4645 clams, including sample clams, were held in the container. At the end of 17 days 4060 clams were retrieved. Dead clams numbered 64; old broken clams, 90; and new broken clams, 31. Total mortality, excluding samples, was 4.36%. Mortality from handling was 2.85%.

Experiment 4, July 15-August 1, 1986

During the initial loading and deployment of the container, a mechanical problem caused the clams within the container to shift position on two occasions. Each time this happened the container had to be reopened, the bagged clams for microbiological analysis were repositioned and remaining clams redistributed. The increased handling was reflected in higher mortalities. Additionally, this sampling period experienced the highest water temperatures (29.0°C - 29.5°C), potentially stressing the clams and adding to the mortality. A total of 4723 clams were held in the container. Seventeen days later 4138 were released for sale. Total mortality was 6.84%. The number of dead clams increased to 123, about double the previous experiment; old broken clams increased to 151 (67% higher than the previous experiment); and there

Table 1. Recovery data for naturally polluted clams held in containers.

Sampling Period (Days Cage On-Bottom)	Total # Clams in Cage[1]	Total # Mortalities	# Of "Dead" Clams[2]	# Of "Old" Broken Shells[3]	# Of New Broken Shells[4]	% Handling Mortality[5]	% Total Mortality
MAY 6-27 (21)	4,250	172	-	132	40	3.89	-
JUNE 10-30 (20)	4,030	178	43	114	21	4.23	3.21
JUNE 24-JULY 11 (17)	4,060	185	64	90	31	4.36	2.85
JULY 15-AUG 1 (17)	4,138	304	123	151	30	6.84	4.07
AUG 5-28 (23)	3,881	339	114	207	18	8.03	5.33
TOTAL	20,359	1,178	334	694	140	5.47	3.87

1. Does not include clams used for microbiological analysis.
2. "Dead" clams, valves intact, no broken pieces.
3. "Old" broken shells, valves attached, no meat inside.
4. "New" broken shells, valves attached, with meat inside.
5. Broken clams are indicative of handling mortality - a distinction could be made between initial harvesting/cage loading and cage harvesting by looking at "old" and "new."

were 30 new broken clams (similar to the previous experiment). Handling mortality increased to 4.07% mainly on the basis of old broken clams. The higher number of old broken clams was attributed to the container shifts during initial deployment.

Experiment 5, August 5-August 28, 1986

This experiment was also beset with complications caused by unusually heavy rainfall. For a six-day period (August 11 - August 17), the relaying site and surrounding area experienced heavy rainfall associated with a passing tropical depression. As a possible consequence, sampled clams failed to reach acceptable fecal coliform levels and, therefore, were not released for sale. The entire container was left on the bottom for an additional 7 days, after which more clams were sampled for microbiological analysis. After this additional cleansing time, the acceptable fecal coliform level was achieved and the remaining clams released for sale. However, this entire procedure increased the amount of handling, disturbance and increased the length of time that the clams were held (23 days). Together, the increased handling and length of holding, combined to yield the highest total mortality and handling mortality. Initial clam container stocking was 4526. After 23 days and the removal of 2 samples, 3881 clams were marketed. Total mortality for the period was 8.03%. Dead clams numbered 114, old broken clams 207 and new broken clams 18. Handling mortality was 5.33%.

SUMMARY

The use of containers for relaying clams clearly is superior to direct on-bottom relaying in terms of recovery rate and ease of recovery. Over the course of the container experiments, average total mortality was 5.5%. During the 1986 relaying season, the adjacent on-bottom relayed clam loss was 22% (personal communication, Roy Davis Seafood). The term "loss" as opposed to mortality is used when referring to on-bottom clams because a distinction cannot be made between clams that actually died and clams that failed to be reharvested.

This difference in losses amounts to sizeable sums of money when large numbers of clams are involved. Over the course of a season a large scale relaying operation can relay 8 million clams. Using the simplest of economic evaluations employing only total gross sales loss without taking into account labor or other operating expenses the magnitude of this monetary loss may be illustrated. A comparison based upon 8 million relayed clams can be made between a totally containerized operation with a 5.5% loss and a totally on-bottom operation with a 22% loss. A 5.5% loss from 8 million clams represent a loss of 440,000 clams; at 22%, 1,760,000 clams are lost; a difference of 1,320,000 clams. If we assume a $0.15 per clam sales price, the on-bottom

operation had a total gross sales loss of $198,000 greater than the containerized operation.

Other benefits would accure from containerization, one of which would be labor reduction at reharvesting. Current on-bottom reharvesting requires extensive man-hour expenditures using either hand tongs (shaft tongs) or patent tongs. With a container, close to 5000 clams can be harvested in a matter of a few minutes. The actual savings in time and labor expense must be examined more closely in order to obtain a more accurate accounting.

An outgrowth of the ease of recovery would be a more efficient business plan in terms of sales planning or in meeting unforeseen sales opportunities. By knowing the number of clams needed to meet a day's sales, it would be a simple matter of harvesting the required number of containers. Additional sales requests could be quickly met merely by increasing the number of containers harvested.

An additional benefit from containerization comes from the overall appearance and condition of the clams. On-bottom clams, as a result of their physical contact with the sediment, can become discolored (shells darkened) and take on a gritty taste. On the other hand, containerized clams, since they never come in direct contact with the sediment, have cleaner, whiter shells and none of the grit problem associated with clams taken directly from the bottom. These points, in themselves, could be used as marketing tools, perhaps resulting in a premium price being received for containerized clams.

In conclusion, containerization of relayed clams can reduce clam losses and hence, increase revenues. In addition, containerization can offer to the market a superior product in appearance and taste, while satisfying health and regulatory requirements.

LITERATURE CITED

1. Arcisz, W. and C. B. Kelly. 1955. Self-purification of the soft clam, Mya arenaria. U.S. Public Health Report 70:605-614.

2. Becker, R. E. 1977. A basket relaying study off the coast of Alabama: reduction of coliform bacteria as a function of time and basket loading. In, Wilt, D. S. (ed). Proceedings 10th Annual National Shellfish Sanitation Workshop. U.S. Dept. Health Educ. & Welfare, FDA, 174-181.

3. Blogoslawski, W. J. and M. E. Stewart. 1983. Depuration and public health. J. World Maricul. Soc. 14:535-545.

4. Cook, D. W. and G. W. Childers. 1968. Depuration of Biloxi Bay oysters by relaying. Minutes of the Gulf States Marine Fisheries Commission, March 26, 1968, Panama City, FL. 18 pp.

5. Furfari, S. A. 1966. Depuration plant design. U.S. Dept. Health Educ. & Welfare, FDA, 119 pp.

6. Haven, D. S., F. O. Perkins, R. Morales-Alamo and M. W. Rhodes. 1978. Bacterial depuration by the American oyster (Crassostrea virginica) under controlled conditions. Volume I. Biological and technical studies. Virginia Institute of Marine Science, Special Scientific Report No. 88. 160 pp.

7. Kator, H. and M. Rhodes. 1986. Unpublished results. Virginia Institute of Marine Science, Gloucester Point, Va.

8. Neilson, B. J., D. S. Haven, F. O. Perkins, R. Morales-Alamo and M. Rhodes. 1978. Bacterial depuration by the American oyster (Crassostrea virginica) under controlled conditions. Volume II. Practical considerations and plant design. Virginia Institute of Marine Science, Special Scientific Report No. 88. 48 pp.

9. Quayle, D. B. and F. B. Bernard. 1976. Purification of basket-held Pacific oysters in the natural environment. Proc. N. Shellfisheries Assoc. 66:69-75.

10. Supan, J. E. and E. W. Cake, Jr. 1982. Containerized-relaying of polluted oysters (Crassostrea virginica [Gmelin]) in Mississippi sound using suspension, rack and onbottom-longline techniques. J. Shellfish Res. 2(2):141-151.

11. Virginia Marine Resources Commission. 1986. Landings Statistics.

ACKNOWLEDGEMENTS

This work is a result of research sponsored in part by the National Sea Grant College Program of the National Oceanic and Atmospheric Administration, U.S. Department of Commerce, under Grant No. NA81AA-D-00025 to the Virginia Graduate Marine Science Consortium and the Virginia Sea Grant College Program. The U.S. Government is authorized to produce and distribute reprints for governmental purposes notwithstanding any copyright notation that may appear hereon.

Contribution No. 1436 from the Virginia Institute of Marine Science, School of Marine Science, College of William and Mary, Gloucester Point, Virginia 23062.

EFFECT OF SHIPPING SHUCKED OYSTERS LONG DISTANCES ON DRAIN WEIGHT AND FREE LIQUOR CONTENT

Cameron R. Hackney and Lisa K. Sanders

Virginia Polytechnic Institute and State University
Seafood Agricultural Experiment Station
102 S. King St., P.O. Box 369
Hampton, Virginia 23669

INTRODUCTION

A major problem facing oyster processors is free liquor or drain weight. Free liquor in shucked oysters ranges from near zero to greater than thirty percent (3,4,6,7,9). The current national standard for free liquor content of shucked oysters is a maximum of 5% within 15 minutes after shucking (8). This standard is not enforced because it is well established that oysters will lose more than 5% (3,6,7). Also, this is an in-plant standard and it cannot be utilized in the market place. Concerns over adulation of oysters with added water has lead to recommendations of a 15% drain weight standard.

The Shellfish Institute of North America (SINA) conducted a study on the effect of seasonal variations, geographic area of harvest, NaCl levels, total solids, and storage time on free liquor content. One factor not considered in the SINA study was the effect that vibration, caused by trucking shucked oysters long distances to markets, has on free liquor content.

Shellfish processed in one state are often repacked in another. Shellfish for repacking are usually sold by weight rather than volume. Thus, processors often pack a 7 pound pack per gallon. Processors from the Gulf area claim that the oyster drain weight decreases because of shipping. The loss is reported to vary between 10-20%. Of course, repackers want to know of the loss in weight is real. The objective of this study was to determine if shipping shucked oysters by truck for long distances affects drain weight and free liquor content.

MATERIALS AND METHODS

The oysters were bought at a processing plant. Twenty-one jars were purchased for each trial. The oysters were shucked by hand and washed by blowing. In this process air is injected from the bottom of the tank to agitate the oysters to remove grit and shell particles adhering to the oysters. After 10-15 minutes the air was shut off and water was constantly added and allowed to overflow the tank. The shell particles and grit settled to the bottom of the tank and the foam generated by the agitation was carried away in the overflow. The oysters remained in the tank for approximately 15 minutes after the air was shut off. The oysters were removed and packaged without added water into either 12 ounce or 16 ounce glass jars. The oysters were taken to the laboratory in ice.

Seven of the jars were tested upon arrival at the laboratory for free liquor content, drain weight, pH, total solids, NaCl content and microbiological quality. Free liquor content, drain weight, pH and total solids was tested by AOAC procedures (2). The analyses were done in triplicate.

Sodium chloride was determined by the method of Hackney, et al. (5). For this procedure, ten grams of oyster homogenate were transferred to a flask and diluted with 50 to 70 mls of distilled water. This was titrated with 0.171 N silver nitrate solution (29.063 g/l) using 10 to 25 drops of 0.5% dichloroflurescein in ethanol as an indicator. Agitation was necessary to keep the oyster homogenate in solution. When salmon pink color developed the titration was stopped. When one gram of oyster homogenate is used each ml of silver nitrate solution is equal to 0.1 g of NaCl per 100 ml.

Microbiological analysis included testing for aerobic plate count, coliforms, fecal coliforms, and E. coli by Standard Methods (1). For the APC the appropriate dilution was pour plated with plate count agar and incubated for 5 days at 20°C. Coliforms were enumerated by the 5 tube most probable number (MPN) technique using lauryl sulfate broth and incubation at 35°C for up to 48 hours. An inoculum from all positive tubes was transferred to E.C. broth and further incubated at 44.5°C for an additional 24 hours to test for fecal coliforms. E. coli was determined by streaking each positive EC tube to eosine methylene blue agar and testing suspect colonies by IMViC (1).

The remaining 14 packs were divided into two equal lots. Seven packs were placed in ice and held at the laboratory. The other seven jars were placed in ice and shipped round trip by refrigerated truck. The truck traveled a distance of between 1000 and 2000 miles and was gone 4-7 days. When the trucked samples arrived back at the laboratory all 14 packs were analyzed for free liquor content, drain weight, pH, NaCl content, total solids and for microbiological analysis as described above. This study was repeated 7 times. In two of the trials, oysters from the Gulf Coast (Louisiana) which had been transported to Virginia for repacking, were obtained and tested as described.

Statistical analyses were performed by the method of Snedecor and Cochran (10).

RESULTS AND DISCUSSION

It is common practice to process oysters in one state and repack them in another state. These oysters are often sold by weight. Processors have long claimed that at certain times the oysters may lose considerable weight during shipping. According to processors, oysters from the Gulf Coast are particularly prone to losing weight during shipping. The weight loss has been blamed on vibrations encountered during shipping. The results of this work showed that the weight loss is a real phenomenon; however, the loss was due to the age of the oysters after packing and not vibrations encountered during shipping (Table 1; compare control at day 0 vs stored samples). Statistical analysis of the

7 trials with 7 replicates in each trial could not detect significant differences in free liquor content, pH, total solids and microbiological numbers in samples which had been held in the laboratory and samples shipped for up to one week for distances of over 1000 miles (Tables 1, 2, 3, 4, 5, 6). However, there were significant differences ($p > 0.01$) before and after storage with respect to % free liquor, aerobic plate counts and pH, especially with the Gulf Coast samples (Trials 6 and 7 of each of the tables).

This work clearly points out the importance of keeping the time interval between processing and repacking as short as possible. From an economic standpoint, older product will have a decreased drain weight which could affect profits, especially if a packer is repacking oysters processed on the Gulf Coast. The oysters processed in Virginia, did not lose as much weight as oysters processed in Louisiana and thus regional differences are important.

Table 1. Effect of storage and shipping on the % free liquid of shucked oysters.

Trial[a]	Control Day 0	Control not shipped[b]	Transported by Truck[c]
1	6.30	5.55	11.21
2	6.30	6.02	4.57
3	11.97	11.93	11.68
4	15.50	11.68	13.13
5	8.00	6.63	4.63
6	3.52	15.06	11.34
7	2.50	14.73	14.11

[a] each number is mean of 7 replicates per trial
[b] controls were held in ice at 0°C
[c] samples were shipped in ice on round trip run of 1000-2000 miles

Table 2. Effect of storage and shipping on the % total solids of shucked oysters

Trial[a]	Control Day 0	Control not shipped[b]	Transported by Truck[c]
1	12.07	12.15	12.36
2	12.09	12.20	12.19
3	7.95	7.88	7.68
4	9.00	8.34	8.40
5	12.09	11.67	11.84
6	13.51	14.49	14.17
7.	13.11	14.24	13.93

[a] each number is mean of 7 replicates per trial
[b] controls were held in ice at 0°C
[c] samples were shipped in ice on round trip run of 1000-2000 miles

Table 3. Effect of storage and shipping on the pH of shucked oysters

Trial[a]	Control Day 0	Control not shipped[b]	Transported by Truck[c]
1	6.82	6.50	6.36
2	6.80	6.40	6.38
3	6.67	6.52	6.57
4	6.86	6.60	6.63
5	6.38	6.14	6.12
6	6.16	5.54	6.03
7	6.32	5.77	5.89

[a] each number is mean of 7 replicates per trial
[b] controls were held in ice at 0°C
[c] samples were shipped in ice on round trip run of 1000-2000 miles

Table 4. Effect of storage and shipping on the aerobic plate count of shucked oysters

Trial[a]	Control Day 0	Control not shipped[b]	Transported by Truck[c]
1	4.94[d]	5.35	4.94
2	4.80	4.79	5.03
3	4.90	5.94	5.80
4	3.96	5.12	4.74
5	4.92	5.93	6.34
6	5.30	8.26	7.13
7	5.31	6.92	6.87

[a] each number is mean of 7 replicates per trial
[b] controls were held in ice at 0°C
[c] samples were shipped in ice on round trip run of 1000-2000 miles.
[d] numbers are given in logarithm of the colony forming units per gram

Table 5. Effect of storage and shipping on the coliform counts/gram of shucked oysters

Trial[a]	Control Day 0	Control not shipped[b]	Transported by Truck[c]
1	45	10	15
2	23	27	26
3	22	8	7
4	9	4	3
5	0	22	57
6	44	1500	525
7	23	43	23

[a] each number is mean of 7 replicates per trial
[b] controls were held in ice at 0°C
[c] samples were shipped in ice on round trip run of 1000-2000 miles

Table 6. Effect of storage and shipping on the Escherichia coli counts/100 g of shucked oysters

Trial[a]	Control Day 0	Control not shipped[b]	Transported by Truck[c]
1	110	60	0
2	10	6	1
3	0	70	70
4	0	0	0
5	0	0	0
6	100	0	30
7	0	0	0

[a] each number is mean of 7 replicates per trial
[b] controls were held in ice at 0°C
[c] samples were shipped in ice on round trip run of 1000-2000 miles

REFERENCES

1. American Public Health Association. 1984. Compendium of Methods for the Microbiological Examination of Foods. Am. Pub. Hlth, Assoc. Washington, Dc.

2. Association of Official Analytical Chemists. 1980. "Official Methods of Analysis of the Association of Official Analytical Chemists. Washington, DC.

3. Feiger, E.A., Novak, A.F., and Bailey, M.E. 1958. Composition of Gulf oysters, Manual of Seafood Merchandising.

4. Galtsoff, P.S. 1964. The American Oyster, Crassostrea virginica, gmelin. U.S. Government Printing Office, Washington, DC.

5. Hackney, C., Sanders, L., Rippen, T., and Reily, L. 1988. Improved Method for Determination of Salt in Oysters by Direct Titration of Chloride Ion. J. Food Sci. 53:

6. Hoff, J.C., Beck, W.J., Erickson, T.M., Vasconcelos, G.J., and Pressnell, W.M. 1967. Time-temperature effects in the bacteriological quality of stored shellfish. II. Bacteriological changes in shucked Pacific oysters and Olympia oysters. J. Food Sci. 32:125-128.

7. Novak, A.F., Fieger, E.A., and Liuzzo, J.A. 1962. Free liquid content of Gulf oysters and suggested change in standards. U.S. Fish and Wildlife, Bureau of Commercial Fisheries, Fishery Industrial Research. 2:1-3.

8. Office of the Federal Register. National Archives and Records Service, General Services Administration. 1985. Codes of Federal Regulations. Food and Drugs. U.S. Government Printing Office, Washington, DC.

9. Sidwell, V.D., Loomis, A.L., and Grodner, R.N. 1979. Geographic and monthly variations in composition of oysters, Crassostrea virginica. Marine Fisheries Review, March, 13-17.

10. Snedecor, G. and Cochran, W. 1967. "Statistical Methods" 6th ed. The Iowa State University Press, Ames, Iowa.

ACKNOWLEDGEMENTS

This work was supported by a grant from the Gulf and South Atlantic Fisheries Development Foundation Incorporated (Tampa).

LIQUID LOSS IN SHUCKED EAST AND GULF COAST OYSTERS DURING STORAGE

David W. Cook
Gulf Coast Research Laboratory, P. O. Box 7000
Ocean Springs, MS 39564

Tuu-jyi Chai
University of Maryland, P. O. Box 775
Cambridge, MD 21613

Cameron R. Hackney
Virginia Cooperative Extension Service, P. O. Box 369,
Hampton, VA 23669

W. Steve Otwell
University of Florida, 467 Food Science Building
Gainesville, FL 32611

Donn R. Ward
Texas A & M University, 442 Kleberg Center
College Station, TX 77843

Roy E. Martin
Shellfish Institute of North America, 2000 M Street, N.W.
Washington, D. C. 20036

INTRODUCTION

Oysters have traditionally been sold by volume with the most common retail packs being 10, 12 and 16 fluid ounces. To protect the consumer from the fraudulent practice of substituting water for oyster meats in the container, the Code of Federal Regulations (CFR) [1] describes the processing, draining and packing requirements. However, because processing plants are not under continual inspection and because there are no National Standards applied to the oyster after they leave the plant, it is impossible to insure that the CFR requirements are adhered to.

A different approach to protecting the consumer would be to establish a market level standard for the maximum amount of drained liquid allowable in packs of oysters. Any such standard should be national in scope because oysters harvested in one region may be shucked and packed in another region. To set such a standard, adequate knowledge of liquid loss by oysters packed under CFR requirements is necessary. That data should evaluate regional, seasonal and environmental effects on liquid loss during storage.

Several studies [2, 3, 5, 6] have been conducted on liquid loss from shucked oysters during storage. That data, however, has

generally been limited to studies on oysters harvested from a few areas, oysters harvested during one season of the year, oysters harvested from unknown or unreported sources, oysters by unreported procedures and data which included only a few time points.

In spite of limited scientific data, several states have promulgated market level standards for free liquid in oysters (Maryland, 5%; New York, 10%; Florida, 15%). The Interstate Shellfish Sanitation Conference and The National Conference on Weights and Measures have adopted a national market standard of 15% free liquid but the groups have no regulatory authority and must rely on individual states to promulgate and enforce regulations.

This study was undertaken to determine the appropriateness of the proposed 15% drained liquid standard for oysters at the retail market level.

METHODS

To insure that this research would be applicable to the industry, all harvesting, shipping, shucking and packing procedures were those normally used in the industry with the exception that CFR processing requirements were adhered to as near as possible. Regional differences in the liquid loss were evaluated by testing oysters packed in three East Coast and six Gulf Coast plants. In this study, only oysters harvested in the region of the plant were processed at that plant. Seasonal differences were tested by sampling oysters at the same plant at intervals of approximately six weeks from March through December, 1986.

On each sampling day the plant was visited, checked to see that the oysters being processed were harvested from the area where the plant was located and that the processing met the CFR requirements. None of the data reported here was gathered from oysters which had been processed with blowing.

Thirty five containers of oysters were then collected and packed in crushed ice until they were examined. Oyster packs were 12 oz. size in all plants except plant A which used 10 and 12 oz. size and plant D which used 16 oz. size. An initial drained liquid content and count of meats was measured on 5 containers within fifteen minutes after packing where possible. In some cases, these measurements could not be made in the plant, but were done within 3 hours, when the oysters were returned to the laboratory. The drained meats were homogenized and analyzed for pH, total solids and salt. Samples were collected and archived for glycogen analysis. All analytical procedures followed those outlined by AOAC [4]. At three day intervals for 18 days after packing, 5 containers were removed and examined for % drained liquid, count and pH.

Attempts were made to sample each plant at 6 week intervals, thus collecting 7 samples during the study period. Unfortunately, some samples could not be collected because of the unavailability of oysters from the desired source at that time.

RESULTS AND DISCUSSION

Figure 1 presents drained liquid (D.L.) data from East Coast plants. Plant A was in Virginia and plants B and C in Maryland. Data was combined over all samples and replicates for each storage day. The heavy bars represent one standard deviation and the whiskers extend to the maximum and minimum values recovered for that day. The mean for each day are connected so that the trends can be observed. The number in parenthesis above the whisker on day-0 represents the percent of samples above 5% and the numbers above the whisker on other days represents the percentage above 15%.

Mean day-0 %D.L. ranged from 3.3 at plant B to 6.6% at plant C. The large percentage of samples exceeding 5% on day-0 was attributed to the fact that the measurements were made 1 to 3 hours after packing and/or that the drain time for the oysters was not the full 5 minutes. During storage the oyster packs decreased in drained liquid before they began to increase. At day-18, mean %D.L. were 7.8, 2.6 and 4.8 at plants A through C, respectively. Similar trends in liquid loss had been reported for Chesapeake Bay oysters [2].

In East Coast region, data was collected on 700 containers of oysters representing 20 separate lots. Of these, only 6 containers (0.86%) exceeded 15% D.L. with the highest value being 20.9%. One of these containers was encountered on storage day-15 and 5 at day-18.

An entirely different picture was obtained with Gulf Coast plants (Figure 2). Plants F, G, and H were located in Louisiana. Plant I was located in Mississippi but processed Louisiana oysters. All plants averaged below 3% drained liquid 15 minutes after packing and only a small percentage exceeded 5%. There was a rapid increase in %D.L. during the first three days of storage followed by a continual but more gradual increase. At day-18, the mean D.L. ranged from 6.6 at plant E to 9.8% at plants F and G. In these 4 Gulf Coast plants, 26 lots of oysters consisting of 908 containers were tested. Ten containers (1.1%) exceeded 15% D.L. with the highest value reached being 17.1%. One of those containers was encountered at day-12 and the others at day-15 and 18.

Oysters harvested in Florida (plant D) and Alabama (plant E) showed the same trend in liquid loss as did the other Gulf plants, i.e. large initial liquid loss followed by a steady increase (Figure 3). At plant D, the mean day-0 D.L. value was 6.3% by day-6 the mean D.L. exceeded 15% and by day-18, the mean was 20.7%. The highest D.L. value recorded was 33.1%. Plant E data consisted of only four

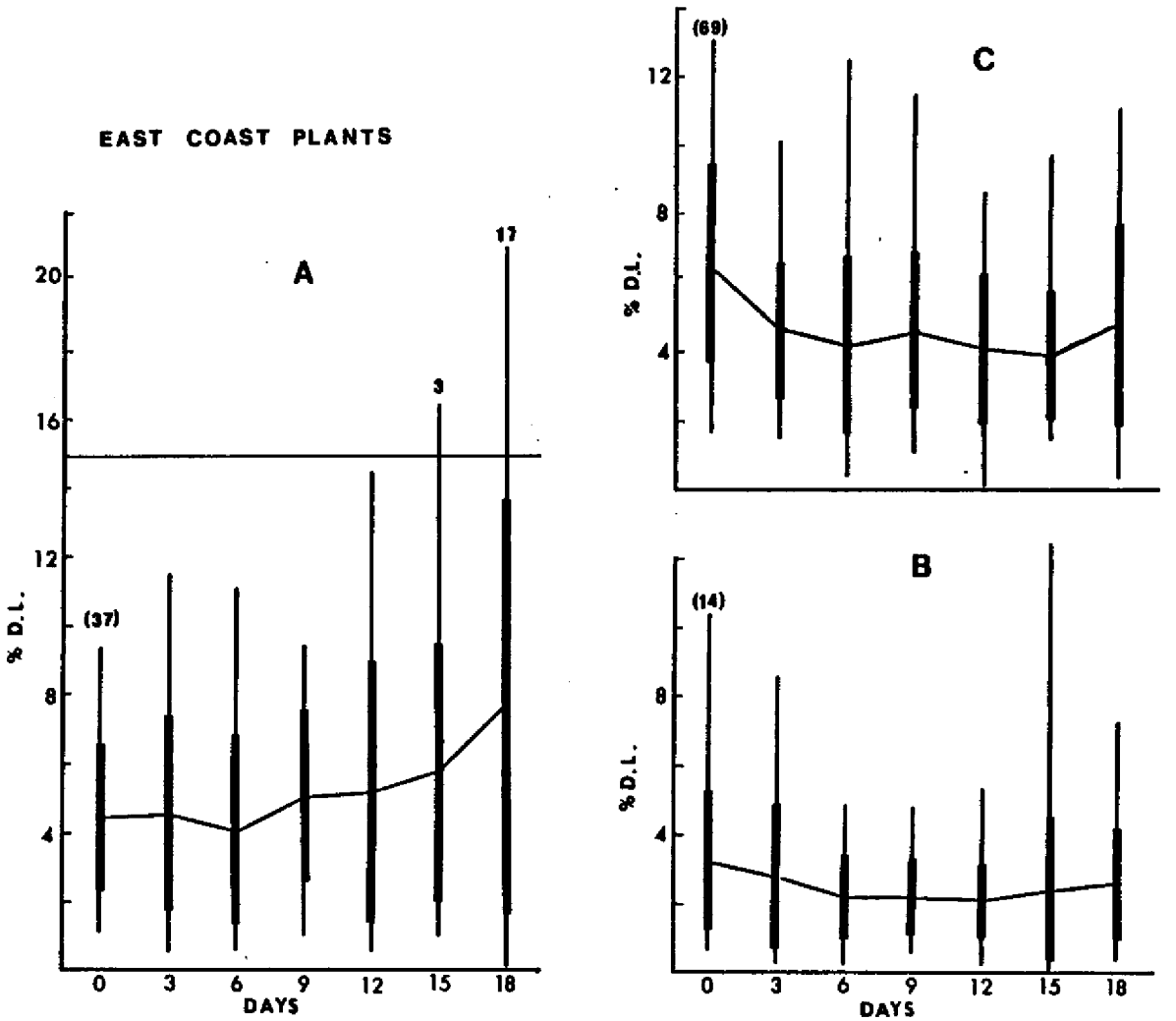


Figure 1. Percentage of drained liquid (% D.L.) in containers of oysters packed in East Coast plants. Data for each day of storage were combined over samples and replicates. See text for explanation of figure.

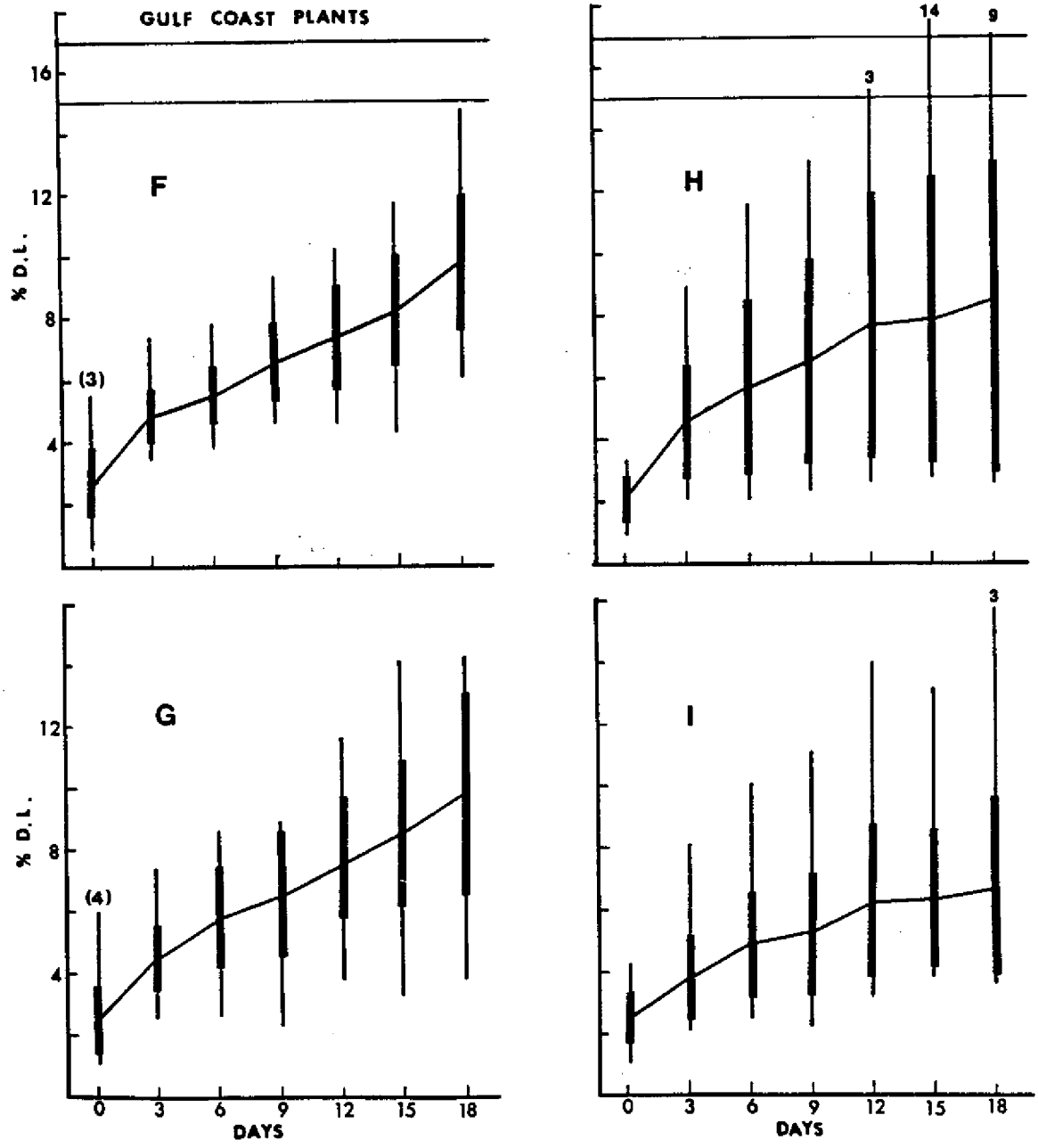


Figure 2. Percentage of drained liquid (% D.L.) in containers of oysters packed in Gulf Coast plants. Data for each day of storage were combined over samples and replicates. See text for explanation of figure.

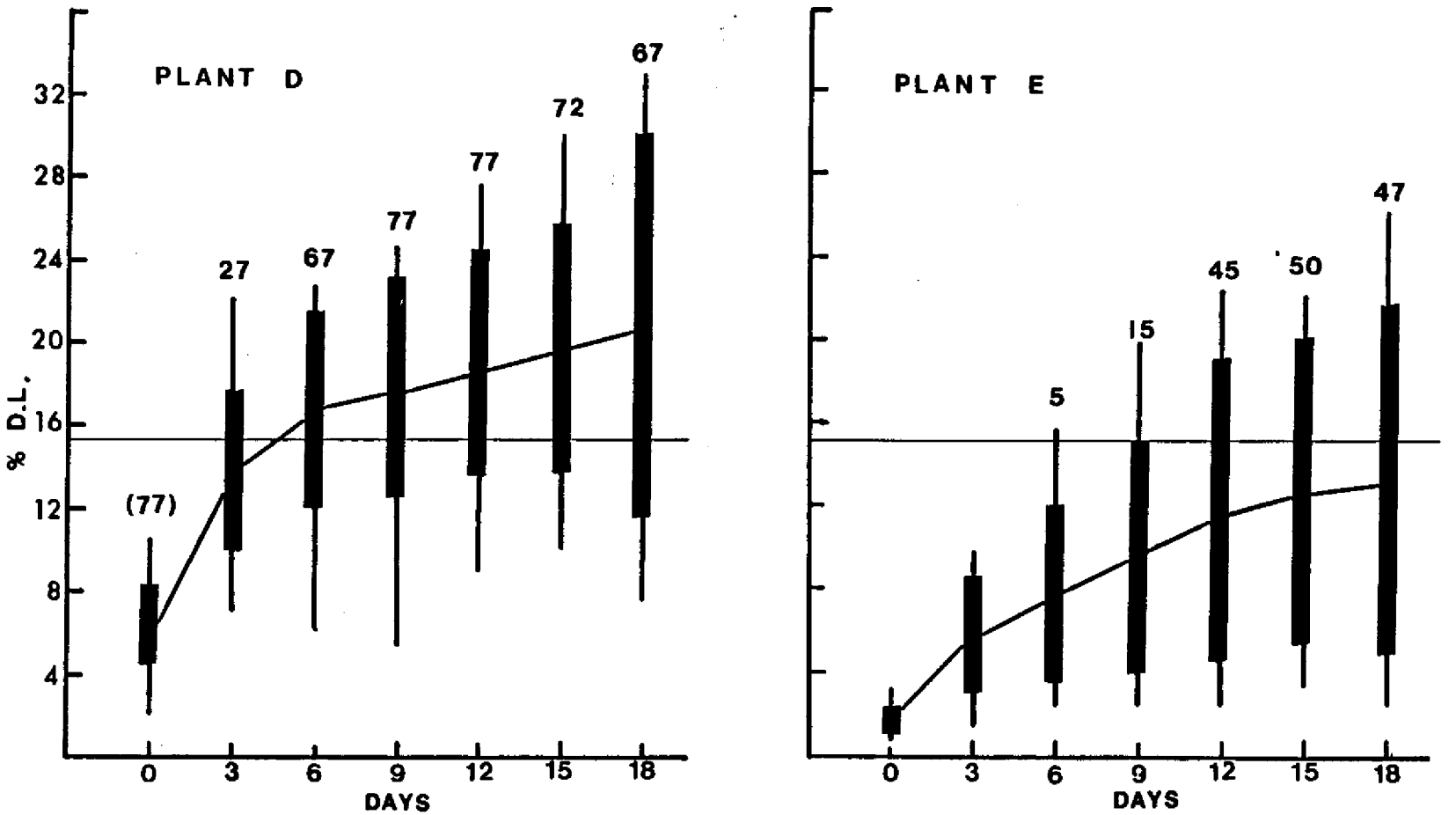


Figure 3. Percentage of drained liquid (% D.L.) in containers of oysters packed in Plant D (Florida) and Plant E (Alabama). Data for each day of storage were combined over samples and replicates. See text for explanation of figure.

samples, two of which had very high %D.L. and two of which had very low %D.L. Although the mean for that plant was less than 15%, almost 50% of the samples exceeded 15% D.L. during the latter portion of storage. Possible reasons for these plants differing from other Gulf plants will be discussed later.

These data suggest a regional difference in liquid loss from oysters during storage. These differences are emphasized in Figure 4 which presents the mean %D.L. for each plant averaged over samples and replicates for each day minus the initial (day-0) %D.L. Drained liquid values below 0 indicate that the oysters were absorbing some of the liquid in the container. East Coast plants (A, B, and C) showed slight declining levels of D.L. from day-0 to day-6 and then increasing levels from day-12 to day-18. In contrast, all Gulf plants (D through I) show the greatest increase in D.L. between day-0 and day-3 with approximately linear increase in D.L. after day-3.

The data from the East Coast plants did not suggest any seasonal trend in D.L. However, a plot (Figure 5) of mean day-15 increase in %D.L. for each sample suggests a seasonal trend among Gulf plants.

In the Gulf Coast region it was found that the percent salt was negatively correlated to the %D.L. When this information was included in the statistical analysis, it was clearly shown that the mean of %D.L. for samples 1-3 were significantly larger than the means for samples 5-7. Sample 4 was not significantly different from any other sample means. Thus, the spring and early summer oysters from the Gulf Coast might be expected to show higher D.L. than late summer and fall samples.

The noted correlation between salt concentrations and %D.L. may help to explain the large D.L. values at plants D and E. Samples 2 and 6 at plant E had high salt content (>0.50%) and low D.L., whereas samples 1 and 3 had low salt content (<0.21%) and high D.L. Salt data are not yet available from all samples from plant D, but samples 3 and 4 which had the lowest D.L. had very high salt values. Oysters from the other samples at plant D were archived and will be analyzed for salt content. That data will be useful in assessing plant D data.

It should also be noted that the oyster beds in Florida and Alabama were significantly disturbed by a hurricane about 6 months before the March samples were collected. That event weakened the oysters and may have contributed to the high D.L. in sample 1 from plants D and E.

To summarize, these data have demonstrated that a regional difference existed in the rate at which oysters lost liquid and within the Gulf Coast region, there were seasonal differences in liquid loss. Further, within the Gulf Coast region, there was an inverse correlation in salt content and liquid loss.

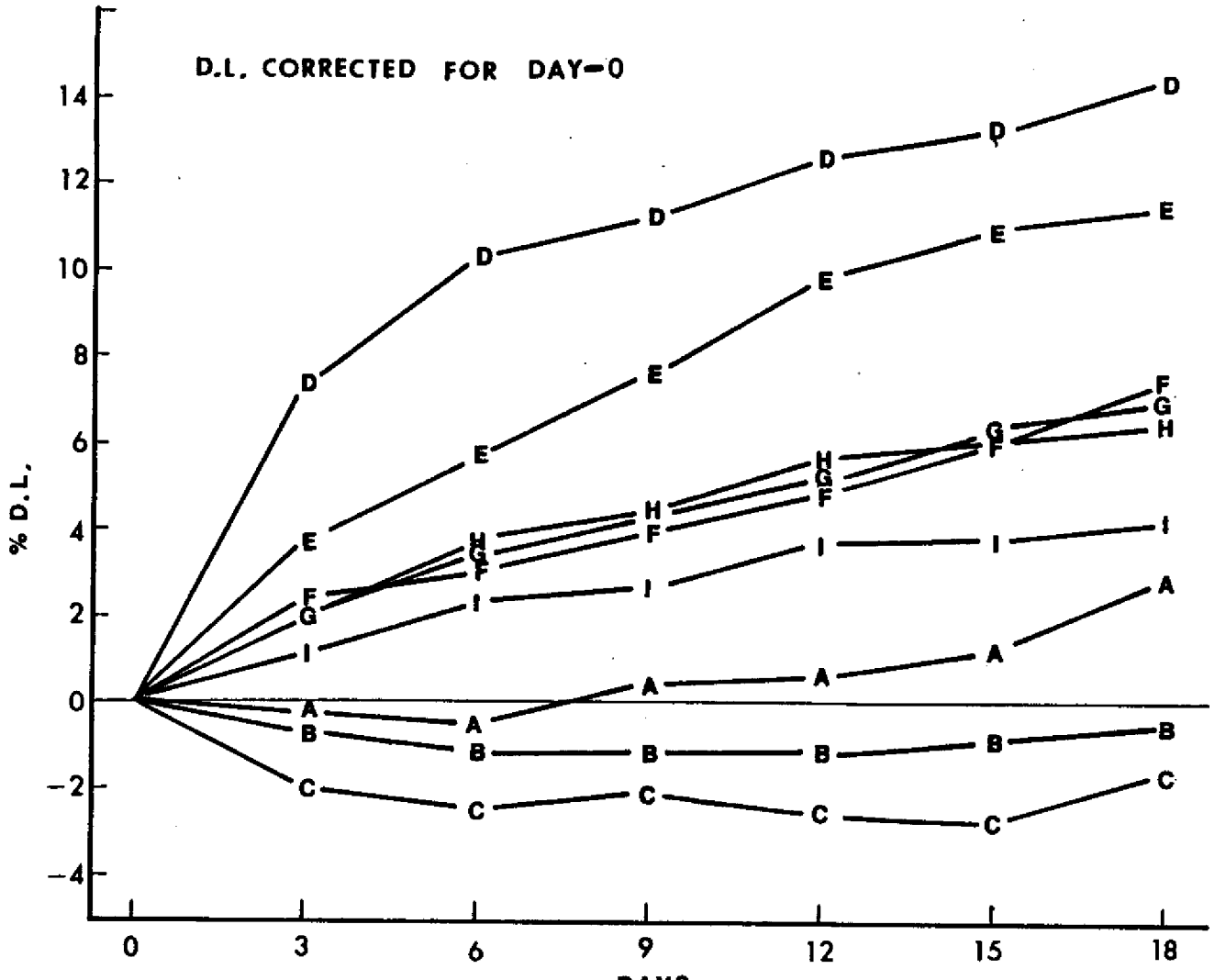


Figure 4. Increase in percentage of drained liquid (% D.L.) in containers of oysters during storage. Letters indicate processing plants. Data for each day of storage were averaged over samples and replicates.

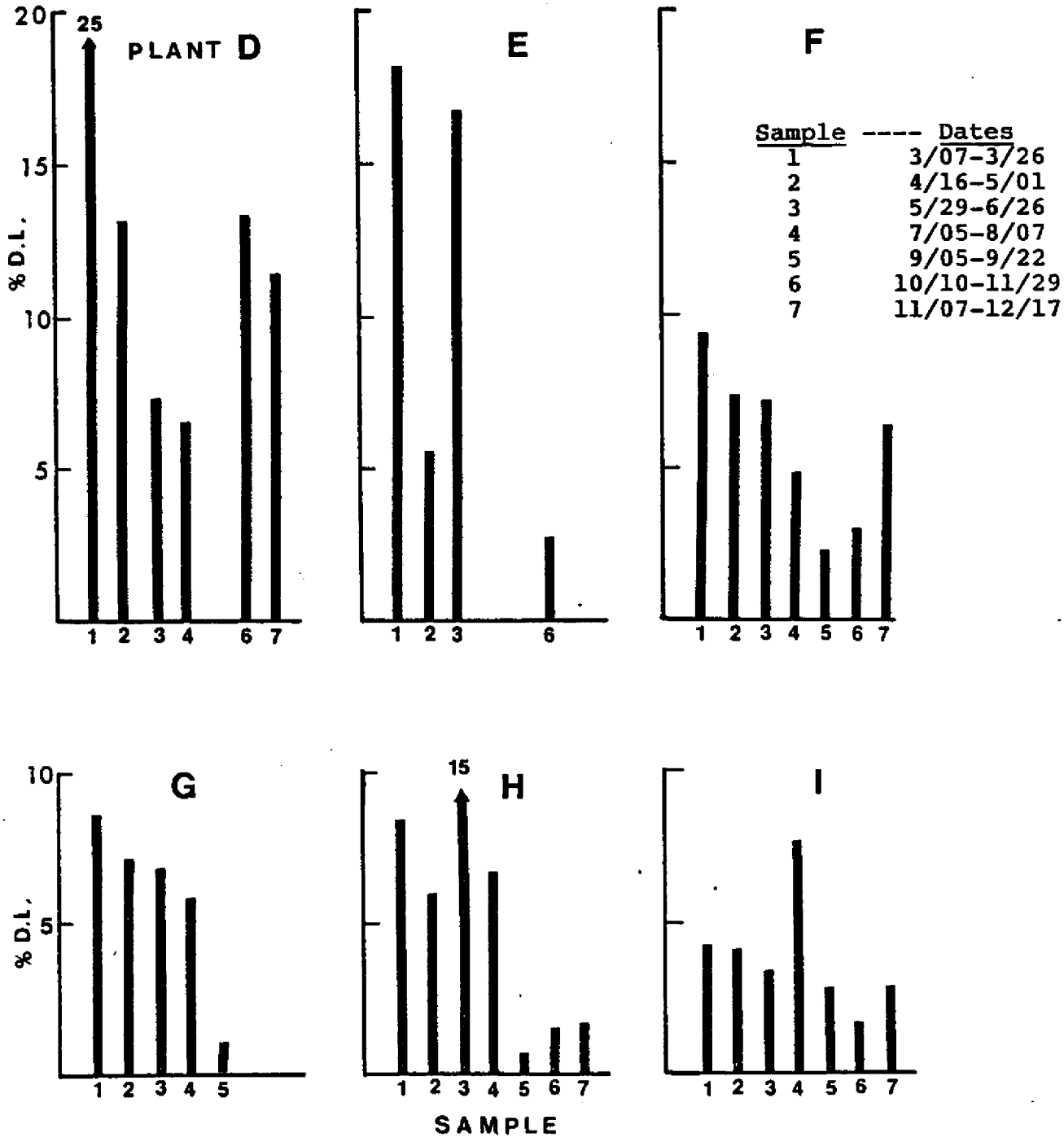


Figure 5. Mean percentage increase in drained liquid (% D.L.) in each sample of oysters during 15 days of storage. Data averaged over replicates.

Table 1. Observed percentage and estimated probability of oysters packed under CFR requirements exceeding 15% drained liquid.

	EAST COAST REGION		GULF COAST REGION		
DAY	OBSERVED	ESTIMATE	OBSERVED	ESTIMATE	(EXC. P-D)* OBSERVED
0	0.0%	0.0%	0.0%	0.0%	0.0%
3	0.0%	0.0%	4.4%	1.9%	0.0%
6	0.0%	0.0%	11.7%	8.1%	0.7%
9	0.0%	0.0%	14.4%	12.4%	2.0%
12	0.0%	0.0%	18.3%	19.6%	6.7%
15	1.0%	0.0%	20.1%	21.9%	10.0%
18	5.0%	1.2%	18.6%	26.6%	8.8%

* - Excluding data from plant D.

Considering these factors, let us now look at the appropriateness of a 15% drained liquid standard for retail market level oysters. Table 1 shows the observed percentages and statistically estimated probability of oysters packed under CFR requirements exceeding 15% drained liquid.

East Coast oysters rarely exceeded 15% D.L. and there should be no problem with East Coast plants which process East Coast oysters meeting the 15% standard. Data from the Gulf Coast region suggest that under certain conditions, oysters may not be able to meet the 15% standard. This data was greatly influenced by results from plant D. When plant D data was removed, 98% of the samples could meet the 15% standard after 9 days of storage and 90% could meet the standard after 15 days of storage.

Since plant D produced oysters that exceeded the 15% D.L. on a frequent basis, one may draw the conclusion that there is something different about the operation of that plant. However, other researchers [3] have observed that oysters harvested from Apalachicola, Florida reefs in the spring lost up to 28.1% liquid during 7 days of storage. These findings indicate oysters from that area may warrant further investigation.

ACKNOWLEDGEMENTS

This research represents a portion of a larger project entitled Molluscan Shellfish Market Standards which was developed by Roy E. Martin of the Shellfish Institute of North America (SINA) and which was funded in part by the National Marine Fisheries Service (NMFS) under the S/K program.

REFERENCES

1. Code of Federal Regulations, 21:161.130. U. S. Government Printing Office, Washington, D.C.

2. Kramer, A., Willis, B. H., Edge, R., Smith, H. R., Tubman, A. W., Lee, C. F., and Tompas, C. 1962. The "Government-Industry Oyster Research Program, Part III, Processing Studies." Journal of the Association of Official Agricultural Chemists, 45(4):1011-1037.

3. Miles, B. J. 1979. "Free Liquid Content of Oysters Harvested from Apalachicola Bay During the Month of March, 1978." Proceedings of the Fourth Annual Tropical and Subtropical Fisheries Technological Conference of the Americas, Texas A & M University, College Station, TX, p. 38-47.

4. Official Methods of Analysis, 13th Ed., Association of Official Agricultural Chemists, Washington, D. C.

5. Rychener, J. H., Rhodes, M., and Woodward, B. 1985. "Oyster Free Liquid Content and Related Physical and Chemical Characteristics." Proceedings of the Tenth Annual Tropical and Subtropical Fisheries Conference of the Americas, Texas A & M University, College Station, TX, p. 275-290.

6. Waters, M. E., Wood, B. J., and Haines, G. J. 1978. "Oyster Drip Loss Part I, Variability of Drip Loss in Commercially Shucked Oysters from Gulf States." Proceedings of the Third Annual Tropical and Subtropical Fisheries Technological Conference of the Americas, Texas A & M University, College Station, Tx, p. 116-225.

AN ECONOMIC ANALYSIS OF THE GULF OF MEXICO SHELLFISH PROCESSING INDUSTRY[1]

Walter R. Keithly Jr.
Center for Wetland Resources
Louisiana State University
Baton Rouge, LA 70803

Kenneth J. Roberts
Center for Wetland Resources &
Cooperative Extension Service
Louisiana State University

and

Charles M. Adams
Dept. of Food and Resource Economics
University of Florida

INTRODUCTION

Among National Marine Fisheries Service statistical reporting regions (New England, Mid-Atlantic, South Atlantic, Gulf of Mexico, Pacific, and Alaska), the Gulf of Mexico region (hereafter referred to as the Gulf region) has traditionally been the largest contributor to the total value of U.S. processed fishery products.[2] With annual employment in this region's processing sector exceeding 16 thousand full-time and part-time workers, approximately 25 percent of the U.S. processed fishery value is attributable to the Gulf region.

Shellfish products, averaging about three-quarters of the total Gulf region processed fishery value, represent the bulk of production (by value) among processing establishments.[3] The information and related analysis in this report pertain to the shellfish processing sector. Specifically, industry stability, size, sales concentration, and diversification among establishments in the region are examined.

A more complete understanding of the Gulf of Mexico's shellfish processing sector, while appealing from a theoretical viewpoint, is essential from a practical viewpoint. This is because industry and government efforts aimed at evaluating market legislation, management alternatives, and long-term trends all require an understanding of the processing sector. Mandatory seafood inspection and generic promotion of seafood provide prospects for change within the seafood sector. The objective of this paper is thus to furnish an analysis of the Gulf region shellfish processing sector to enable sound evaluations of such prospects.

The following information and discussion is directed toward total shellfish processed value and key species (shrimp, oysters, and blue crabs). It is derived from unpublished processed fishery products statistics provided by the National Marine Fisheries Service. In achieving the overall goal of this study, the authors focus on the pattern of concentration and stability among processing establishments. This is because concentration and stability can affect (a) the ability of

processors to acquire raw material, (b) entry of new processing firms, (c) sales growth, and (d) addition and/or deletion of species from company offerings.

The paper is organized as follows. Background information on the Gulf region seafood processing sector is given first, using available published data. Following this background information, a discussion pertaining to the stability, size, concentration, and diversification among Gulf region shellfish processing establishments is provided. The paper concludes with a discussion of implications of the analysis which companies, agencies, and trade groups may wish to consider when trying to estimate the impacts of regulatory and market changes.

BACKGROUND INFORMATION

The Gulf region seafood processing sector has been experiencing growth in both the number of establishments and the value of processed products (Table 1). The number of establishments, averaging 392 annually during 1971-74, increased to 412 during 1976-80, and rose again to 473 during 1981-85. More than 16 thousand seasonal workers were employed annually by, these 473 establishments (Table 1).

The total value of processed products increased from an annual average of about $421 million during 1971-75 to almost $1.3 billion during 1981-85, or about 200 percent. Much of this increased value can be attributed to inflation, i.e., the upward trend in prices throughout the U.S. economy. If one removes the upward trend in value caused only by inflationary effects[4] this gives the value in what is referred to as real or deflated terms. Expressed in this manner, the total value of the Gulf region processed fishery products increased slightly less than 40 percent between 1971-75 and 1981-85, from $308 million to $422 million.

Shellfish have consistently averaged at least three-quarters of the total value of processed products in the Gulf region (Table 1). The majority of shellfish processing, at least by value, originates from the processing of shrimp (Table 2). Shrimp, oysters, and blue crabs account for more than 95 percent of the Gulf region shellfish processed value (Table 2). Stone crabs, spiny lobsters, scallops, and crawfish comprise most of the remaining processed value.

The value of Gulf region processed shrimp products increased from an annual average of $279 million during 1971-75 to $822 million during 1981-85, or almost 200 percent (35% after removing inflation). There was, however, a slight decline in the deflated Gulf region processed shrimp value between 1976-80 and 1981-85 which is somewhat unexpected given the rapid increase in shrimp imports. Many of these imports are, however, already canned which requires little or no additional domestic processing. Also, the deflated price of the processed product may have declined slightly in recent years, largely the result of increased imports. Gulf region processed oyster value increased about 175 percent, but less than 30 percent (28.7%) after removing inflation. Only marginal growth, however, was observed between 1976-80 and 1981-85, indicating that most of the increase in real value occurred during the decade of the 1970's. The largest relative growth in processing among the three key Gulf region shellfish species is that related to blue crab activities. From an annual $12.4 million dollar industry during 1971-75, revenues

Table 1. Descriptive Statistics of the Gulf of Mexico Seafood Processing Sector, 1971-85.

Time Period	Number of Establishments	Seasonal Employees	Value of Processed Products			
			Finfish	Shellfish	Unclassified[a]	Total
			---------- Mill. doll. ----------			
1971 - 75 avg.	392	14,399	61.4 (44.1)[b]	315.4 (231.4)	44.0 (32.2)	420.8 (307.7)
1976 - 80 avg[c].	412	14,711	130.8 (63.6)	679.3 (336.5)	42.4 (21.5)	852.5 (421.6)
1981 - 85 avg.	473	16,250	250.8 (83.3)	957.7 (321.3)	53.0 (17.9)	1,261.5 (422.5)

Source: Processed Fishery Products, Annual Summary (various issues).

[a] Unclassified processed products include both finfish and shellfish products.

[b] Numbers in the parentheses refer to the deflated value (i.e. corrected for inflation) of processed products with 1967 equalling the base year (i.e., 1967 = 1.00).

[c] Data prior to 1977 may not be exactly comparable to earlier data. This is because processing establishments in the Mississippi River Region of Alabama, Louisiana, and Mississippi were not included in the Gulf of Mexico region processing statistics in the earlier years. The discrepancy is, however, probably quite small with the processed value in the Mississippi River Region of Alabama, Mississippi, and Louisiana equalling just $11.7 million in 1975.

Table 2. Gulf of Mexico Processed Shellfish Value for Individual Species and in Total, 1971-85 (5-yr. average).

Time Period	Value of Processed Products			
	Blue Crabs	Oysters	Shrimp	Total Shellfish[a]
	---------- Million dollars ----------			
1971 - 75 avg.	12.4 (8.9)[b]	18.8 (13.6)	279.0 (205.3)	315.4 (231.4)
1976 - 80 avg.	25.0 (12.4)	32.3 (16.3)	597.9 (296.2)	679.3 (336.5)
1981 - 85 avg.	41.4[c] (13.8)	52.0 (17.5)	822.1 (276.0)	957.7 (321.3)

Source: Processed Fishery Products, Annual Summary (various issues)

[a] Total shellfish values exceed the horizontal summation of individual components in the table because of inclusion of shellfish species other than shrimp, oysters, and blue crab.

[b] Numbers in parentheses refer to the deflated value (i.e., corrected for inflation) of processed products with 1967 representing the base year (i.e., 1967 = 1.00).

[c] The 1981-85 average blue crab value reported here is less than that provided in Processed Fishery Products, Annual Summary. This difference reflects errors in the data base from which statistics reported in Processed Fishery Products, Annual Summary are derived. This correction was not carried over to total shellfish processed values.

from blue crab processing activities increased more than 230 percent (55% after removing inflation) to $41.4 million annually during 1981-85.

The ability of the Gulf region shellfish processing sector to maintain the long-term growth trend established during the past several years will depend on at least two factors. Demand for seafood and value added processing must first remain strong. Second, processors must be able to secure adequate raw material supplies. Shrimp processors, for example, have probably turned increasingly to imports in their processing activities in order to supplement relatively stable domestic supplies. Prochaska and Andrew (1974), in fact, have shown that Florida shrimp processors have traditionally relied heavily on imports. Gulf region oyster and blue crab processing activities, on the other hand, appear to rely almost, if not entirely, on domestic production. Most of the raw materials in these activities are of Gulf origin and there has been little tendency to secure oysters and blue crabs from import sources. The ability to expand real processing revenues in these activities, assuming there is no increase in real price, will therefore depend upon more domestic landings and better utilization of landings.

STRUCTURAL CHARACTERISTICS

Structural characteristics of the Gulf of Mexico shellfish processing sector are presented in this section of the report. These characteristics are grouped according to (1) stability among establishments in the industry, (2) establishment size and changes over time, (3) concentration among establishments in the shellfish processing industry, and (4) diversification among establishments in the industry.

Industry Stability

Industry stability can be measured in a number of ways. One way is by analyzing patterns of entry and exit among establishments in the industry. These patterns are presented for the Gulf region shellfish processing sector in Table 3.

The total number of Gulf region shellfish processing establishments, as indicated in Table 3, increased by more than one-hundred between 1970 and 1985 (310 to 411). This increase occurred during a time period when the number of establishments processing the most valuable Gulf region shellfish species, shrimp, declined in number (126 to 114). Establishments processing blue crabs showed the greatest relative and absolute increases in number; 94 in 1985 compared to only 56 in 1970. The number of oyster processing establishments increased only marginally during 1970-85, from 174 to 185.

There is, as indicated in Table 3, considerable movement within the Gulf region shellfish processing industry. This movement is the result of entry and exit as establishments add and delete species from processing lines or, in the extreme, begin or cease all shellfish processing activities[5]. This movement is considerably more than what would be anticipated when observing only the 1970-85 change in number of processing establishments. Among establishments processing shrimp, for instance, the average five-year entry rate was roughly 57 percent, while the exit rate exceeded 60 percent. The 1976-1980 period appears to be the most volatile, possibly the result

Table 3. Entry and Exit of Gulf of Mexico Shellfish Processing Establishments in Total and for Individual Species, 1970-85.

	Blue Crab			Oyster			Shrimp			Total Shellfish[a]		
	Total	Entry[b]	Exit	Total	Entry	Exit	Total	Entry	Exit	Total	Entry	Exit
	No. of Estab.											
1970	56			174			126			310		
1971-75		59	33		100	102		60	62		164	135
1975	82			172			124			339		
1976-80		60	65		157	145		82	84		322	253
1980	77			184			122			408		
1981-85		78	61		159	158		65	73		285	282
1985	94			185			114			411		
Total		197	159		416	405		207	219		771	670
Average	77.2	65.7	53	178.5	138.7	135	121.5	69	73	367	257	223.3
Rate of Change[c]		85.1	68.7		77.6	75.5		56.8	60.1		70.0	60.8

Source: Compiled from unpublished data provided by National Marine Fisheries Service.

[a] The total number of shellfish processors and related entry and exit statistics do not equal the summation of individual species because (1) some processing establishments utilize more than one shellfish species in their business activities, and (2) the total shellfish column includes species other than shrimp, oysters, and blue crabs.

[b] Establishments that exited and subsequently reentered are included as an exit and an entrance in the analysis.

[c] The rate of change represents that occurring over a five-year period.

Note: Data in this table include processors of unclassified shellfish products (see Table 1).

of rising energy costs and a recession towards the end of the decade. Of the original 126 establishments processing shrimp in 1970, only 45, or slightly more than one-third, also processed shrimp in 1985 (not shown in Table 3).

The rate of movement among Gulf region establishments processing oysters has averaged about 75 percent over a five-year period, with the rate of entry (77.6 percent) slightly exceeding the five-year rate of exit (75.5 percent). Exit and entry among establishments processing oysters exceed that for shrimp, likely the result of two factors. First, oyster processing activities tend to be less capital-intensive than shrimp processing activities which, theoretically, would allow for more mobility in the oyster processing sector. Second, there tends to be considerable annual variation in the Gulf region oyster harvest which can impact processing activities. Unlike shrimp, this variation is not mitigated to any large extent by using imported oysters in processing activities. Fifty-nine of the 174 companies processing oysters in the Gulf region in 1970 were also processing oysters in 1985 (not shown in Table 3).

Ninety-four establishments processed blue crabs in the Gulf region in 1985; only 19 of these same establishments were also processing blue crabs in 1970. The average five-year rate of entrance (85.1%) has greatly exceeded the rate of exit (68.7%), explaining the large increase in establishments processing blue crabs.

The total Gulf region shellfish processing sector, as would be expected based on the discussion of the individual shellfish species, can be characterized by considerable movement in terms of entry and exit. Only 128 of the 310 establishments processing shellfish in 1970 continued doing so in 1985. Overall, the five-year entry rate has equalled about 70 percent compared to a 61 percent exit rate with a net result of about 100 more establishments between 1970 and 1985. While the total number of shellfish processing establishments increased by more than 100 between 1970 and 1985, establishments processing key species (shrimp, oysters, and blue crabs) increased by only 37, i.e., shrimp -12, oysters 11, and blue crabs 38. Hence, the majority of growth in the number of shellfish processors between 1970 and 1985 can be attributed to the processing of "non-key" species such as scallops, clams, etc.

Establishment Size

Establishment processing revenues associated with key shellfish species and total shellfish are presented in Table 4. Also presented are revenues for establishments entering and exiting processing activities. Total shellfish processing revenues per establishment have increased from an average of $947 thousand annually during 1971-75 to $2.2 million during 1981-85, or about 135 percent. After removing inflation, however, establishment revenues have increased by only about 10 percent. Deflated establishment revenues from the processing of shellfish during the 1981-85 period ($754.8 thousand) were, in fact, below those earned during the 1976-80 period ($883.5 thousand). New processing establishments, as would be expected, have sales below the industry average. Establishments which exit the seafood processing industry have annual sales below the industry average and, in fact, have sales below the average of entering

Table 4. Average Processing Revenues for Gulf Shellfish Establishments, 1971-85 (5-yr. avg.).

Time Period	Value of Processed Products Per Establishment											
	Blue Crabs			Oysters			Shrimp			Total Shellfish[a]		
	Exiting Estab.	Entering Estab.	All Estab.	Exiting Estab.	Entering Estab.	All Estab.	Exiting Estab.	Entering Estab.	All Estab.	Exiting Estab.	Entering Estab.	All Estab.
	Thous. dolls.											
1971 - 75 Avg.	43.3 (31.4)[b]	77.4 (56.2)	182.3 (132.3)	42.6 (30.9)	63.9 (46.4)	132.8 (96.4)	370.0 (268.7)	1,428.3 (1,037.2)	2,057.9 (1,494.5)	187.7 (136.3)	570.0 (413.9)	947.2 (687.9)
1976 - 80 Avg.	120.3 (59.5)	179.4 (88.7)	303.9 (150.2)	86.1 (42.6)	64.0 (31.7)	201.2 (99.4)	1,344.3 (664.5)	2,052.6 (1,014.6)	4,658.9 (2,303.0)	522.2 (258.1)	606.7 (299.9)	1,787.4 (883.5)
1981 - 85 Avg.	115.6 (38.8)	185.9 (62.4)	454.1 (152.4)	64.0 (21.5)	129.6 (43.5)	290.1 (97.4)	1,291.2 (433.4)	2,020.0 (678.1)	6,715.4 (2,254.2)	380.3 (127.7)	602.0 (202.1)	2,248.6 (754.8)

Source: Compiled from unpublished data provided by the National Marine Fisheries Service.

[a] Total shellfish values do not equal the horizontal summation of individual components in the table because some establishments process more than one shellfish species and the total includes species other than shrimp, oyster, and blue crabs.

[b] Numbers in parentheses refer to the deflated value (i.e. corrected for inflation) of processed products with 1967 equalling the base year (ie. 1967 = 1.00).

Note: Revenues in this table include those related to unclassified shellfish products (see table 1). Also, establishment revenues may tend to slightly underestimate company revenues to the extent that some companies may operate two or more establishments.

establishments. For example, while revenues among all shellfish processing establishments averaged about $2.2 million annually during 1981-85, revenues among entering establishments averaged only $602 thousand. Revenues among exiting establishments averaged just $380 thousand. This information indicates that stability in the Gulf region shellfish processing sector is directly related to establishment size with larger establishments exhibiting greater stability.

Average per establishment revenues from shrimp processing activities greatly exceed those earned from either oyster or blue crab processing. Establishment revenues from the processing of shrimp have more than tripled from the 1971-75 average ($2.06 million) to the 1981-85 average ($6.72 million). In real terms, establishment revenues from the processing of shrimp have increased from an annual average of $1.49 million during 1971-75 to $2.25 million during 1981-85, or about 50 percent. Deflated revenues for the 1981-85 period ($2.25 million) are, however, slightly less than those earned during 1976-80 ($2.30 million). This decrease could be a result of a slight decline in processed poundage, failure of shrimp product prices at wholesale to keep pace with general price increases, increased competition among companies, or combinations of the above.

Establishment revenues from blue crab processing have increased from an average of $132 thousand during 1971-75 to $454 thousand during 1981-85, or about 240 percent. In real terms, however, establishment revenues associated with blue crab processing have increased only about 15 percent, from $132.3 thousand to $152.4 thousand. Entering and exiting establishments have much smaller operations than those observed for the blue crab processing industry as a whole when revenues are used as a proxy for size of operation.

Average establishment revenues from the processing of oysters tend to be the smallest among major shellfish species processed in the Gulf region. While establishment revenues from the processing of oysters have more than doubled between 1971-75 and 1981-85, from $132.8 thousand to $290.1 thousand, establishment revenues from the processing of oysters have remained virtually constant when evaluated in real terms.

Industry Concentration

Industry concentration can affect a company's ability to acquire raw materials and market the finished product. Concentration is also thought to be related to company research expenditures, innovation, and promotion. One of the more frequently used indices of industry concentration is the measure of industry output in relation to the largest companies in the industry. This index is applied to the Gulf region shellfish industry and the results are provided in Table 5.

Although more than 400 establishments processed shellfish products in the Gulf region during 1985, five of these establishments, or about one percent, accounted for almost 30 percent (29.6%) of the total shellfish processed value. The largest 10 establishments represented slightly less than 50 percent of the 1985 total shellfish processed value, while the largest 50 establishment accounted for more than 80 percent of the total. Stated somewhat differently, 12.2 percent (50 establishments) of the

Table 5. Concentration in the Gulf of Mexico Shellfish Processing Sector in Total and for Individual Shellfish Species, 1970 and 1985.

Largest n Processing Estab.[a]	Percentage of Processed Value							
	Blue Crab		Oysters		Shrimp		Total Shellfish	
	1970	1985	1970	1985	1970	1985	1970	1985
n = 5	48.4	42.4	26.9	22.5	35.6	33.7	36.8	29.6
n = 10	65.9	57.6	41.9	35.8	57.6	53.4	51.6	46.4
n = 20	86.2	73.6	57.8	52.4	73.7	72.0	66.0	62.2
n = 50	99.8	93.3	81.9	78.3	94.6	93.7	85.5	82.2
Number of Estab.	56	94	174	185	126	114	310	411

Source: Compiled from unpublished data provided by the National Marine Fisheries Service.

[a]Establishments are ranked from the largest to the smallest on the basis of sales of individual shellfish species and in total.

Note: To the extent that some companies may operate more than one processing establishment in the Gulf region, the numbers in this table will tend to underestimate the degree of concentration in the industry. Multi-establishment companies in the Gulf region are, however, not thought to be common.

shellfish processing establishments accounted for more than 80 percent of the Gulf region shellfish production by value in 1985.

Among individual shellfish species, the five largest blue crab processors in 1985 contributed 42.4 percent of the value of blue crab production, while the five largest oyster processing establishments contributed 22.5 percent of the total value of oyster processing, and the five largest shrimp processors accounted for 33.7 percent of the total shrimp processing activities. Caution should, however, be exercised when using this measure of concentration to compare level of concentration among the different shellfish processing activities. This is because of the different number of establishments associated with each activity. Overall, however, the oyster processing sector appears to have the least degree of concentration in the Gulf region while the blue crab processing sector has the largest degree. This is not to infer, however, that larger companies in any sector can control the market in any way. They must compete on a national level with many other processors in other regions of the country and possibly even in the international market.

Because of the change in number of processing establishments between 1970 and 1985, it is difficult to assess the absolute change in concentration through time. A few general statements can, however, be made. It appears, for example, that the large increase in establishments processing blue crabs has notably lessened concentration in that industry. There also appears to be less concentration in both the oyster and shrimp processing sectors in 1985 than in 1970. Such a finding in the shrimp processing sector is somewhat different than what would be anticipated in light of the fact that the number of shrimp processing establishments have declined in number. Industry concentration in the total shellfish processing sector appears to have declined in association with an increase in number of establishments.

Industry Diversification

Sixteen different shellfish species were processed in the Gulf region in 1985, including four types of crabs, two types of shrimp, two types of scallops, oysters, spiny lobster, crawfish, clams, squid, conch, turtle, and unclassified shellfish. While the majority (274) of the 411 establishments processed only one species, many (84) processed two species, some (25) processed three species, and five establishments processed ten or more species. While the industry in total processed an average of 1.45 shellfish species per establishment, the largest ten establishments (by value) processed an average of 6.6 shellfish species per establishment during 1985. The largest 20 and 50 establishments, processed an average of 4.9 and 3.7 species per establishment, respectively. It can be concluded that, in general, there is a strong positive relationship between the size of the establishment and its diversification in terms of the number of shellfish species processed.

IMPLICATIONS

The previous analysis provides several implications which companies, agencies, and trade groups may wish to consider when trying to estimate impacts of change. Consider, for example, the rapid rate of turnover (i.e., entry and exit) in the Gulf region shellfish processing sector.

Shrimp processing is the more stable of the industry sectors. Ability to successfully adapt to regulatory and market changes is more likely for shrimp firms. Oyster dynamics indicate more entry and exit. Additional regulatory and market changes will impact the documented instability in the oyster sector. Developing and successfully implementing a mandatory seafood inspection program is likely to be much more difficult and costly in a situation where there is considerable instability in the industry.

The structure of the Gulf region shellfish processing sector will further add to the complexity and cost of a mandatory seafood inspection program. A large proportion of shellfish processing activities, as mentioned, is concentrated among a relatively small number of establishments. The remaining establishments are large in number but relatively small in size. A mandatory inspection program designed to monitor processing activities at each establishment will be quite costly due only to the sheer number of Gulf region shellfish processing establishments. This is especially true in, say, the oyster processing sector where there is the greatest number of establishments and a relatively low degree of concentration. If the monitoring program is sponsored by the Federal government, the taxpayers will be forced to cover the costs of an inspection program. If individual processing companies are forced to cover monitoring costs, it is likely that some of the smaller companies would be forced to cease processing activities due to an inability to cover inspection costs. Such a situation is especially likely to occur if an inspection program does not result in an increase in demand and, hence, the price of seafood. The oyster and blue crab processing sectors, as opposed to the shrimp processing sector, will have a particularly difficult time covering monitoring costs due to their relatively small scale of operations. This would likely lead to increased concentration in these particular sectors.

The previous analysis also indicates that the Gulf region shellfish processing sector is well suited for a generic promotion program. Since the industry is comprised of a large number of relatively small companies with a large degree of turnover, it is evident that most of these companies do not have the financial resources needed to develop a successful promotion program on their own. A generic promotion program utilizes the resources of all, or at least the majority, of the companies in the industry by instituting a check-off program whereby each company is assessed a small fee in relation to its output or sales[6]. Revenues from this check-off program are then used to increase demand for an industry's product through promotion. This increased demand can enhance the Gulf region processing sector through the expansion of processing activities (using either domestic or imported raw materials), an increase in the price for the processed product, or some combination of the above. With respect to particular sectors within the Gulf region shellfish processing industry, the oyster sector may be the most suited and in the most need of a generic promotion program. This is because their small scale of operation and large degree of turnover would make it very difficult for many of the individual companies to successfully develop their own promotion programs. Also, deflated revenues in this sector have remained virtually constant during the past 15 years. The relatively large size (as measured by sales) and stability of shrimp processors, on the other hand, suggest that many of these companies have the resources to develop and implement promotion programs of their own. However, the decline in

real revenues in this sector between 1976-80 and 1981-85 suggests that this sector may be able to benefit from a generic promotion program.

FOOTNOTES

[1]This project was supported by the Louisiana Sea Grant College Program, a part of the National Sea Grant College Program, maintained by NOAA, USDC.

[2]The Gulf region includes the coastal states from Texas though Alabama and the west coast of Florida.

[3]An establishment, or plant according to National Marine Fisheries Service terminology, is a unique processing entity. It is possible that two or more establishments may be under sole ownership of a company . To be considered a processing establishment, as opposed to a wholesaling establishment, some physical change of the product must be made. This change can range from, say, the heading of shrimp to producing soups or gumbos. Activities such as the repacking of shrimp from 100 pound boxes to five pound containers or the boxing of oysters are not considered processing.

[4]Deflated or real values throughout this report are obtained by dividing the current value by the Consumer Price Index using 1967 as the base year. Thus, deflated or real values reported throughout this paper represent the value of the product expressed in 1967 dollars.

[5]Turnover, or entry and exit, can result from three factors. First, existing establishments can add or delete species from their product lines. Second, establishments can change ownership which results in both an exit (the previous owner) and an entrance (the new owner). Finally, establishments that cease operations are recognized as an exit and new processing facilities are recognized as an entrance.

[6]As the "Fish and Promotion Act of 1986" is written, the assessment is levied on the first receiver of the product. This first receiver is often a processor.

REFERENCES

1. Prochaska, F. J. and C. O. Andrew. "Shrimp Processing in the Southeast: Supply Problems and Structural Change." Southern Journal of Agricultural Economics, 6 (I974):247-252.

2. United States Department of Commerce, Processed Fishery Products Annual Summary (various issues from 1970 through 1985).

IMPORTANT CONSIDERATIONS IN OPTIMIZING THE FORMULATION OF SURIMI-BASED PRODUCTS

Chong M. Lee, Jon-Shang Chen and Kang H. Chung
Department of Food Science and Nutrition
University of Rhode Island, Kingston, R.I. 02881

INTRODUCTION

The paper deals with formulation optimization of surimi-based products with focus on the selection of ingredients and optimization techniques. Surimi is a source of the most functional protein among proteins available in the present market. It is not only an excellent source of protein, but also can be used as a carrier of vital nutrients such as omega-3 fatty acids. According to our recent animal feeding study (Gerber and Lee, 1987), a red hake (Urophycis chuss) surimi protein showed the highest biological quality among known dietary proteins and was equally as good as egg protein in terms of protein efficiency ratio (PER), i.e. 3.0 compared to 2.5 for casein. It was also found that there were no significant changes in omega-3 fatty acid contents in the fish mince after washing, a main step of surimi processing; and that washing caused concentration of myofibrillar proteins in the washed mince, resulting in an improvement of essential amino acid profile (Leibovitz et al, 1987). As for omega-3 fatty acids, the level in the finished mince was not significantly different from that in the original mince, although there were some losses during washing. Such an insignificant change was due to the concentration effect of washing (Leibovitz et al, 1987). This suggests that surimi has potential to become an important commercial protein ingredient to be used as a source of functionality as well as nutrition. It means that surimi will play an important role in formulated products where surimi is used as a main ingredient of protein source. The purpose of this paper is to discuss how various types of commercial ingredients interact with surimi protein ; how they affect the textural properties ; what are factors which influence the textural properties of ingredient-added surimi products ; and from above information, how one can optimize formulation of surimi-based products.

In manufacturing a variety of surimi-based formulated products, various types of ingredients are incorporated into surimi. A study of the physicochemical properties of ingredients during thermal gelation and the ingredient-matrix interaction is important to understanding the gel-strengthening and texture-modifying effects of ingredients. The information from this type of study will be utilized in formulation optimization with the aid of appropriate optimization techniques. The important role of formulation technology in surimi-based formulated products is in the optimization of texture and physical properties (thermal and freeze-thaw stability), as well as in the development of new formulated products such as high protein formula. A high protein surmi product receives attention from manufacturers since bringing the level of protein to that in the natural product may ease the imitation labeling requirement by satisfying nutritional equivalency proposed by the Food and Drug Administration (FDA).

Classification and selection of ingredients

The commercial ingredients may be classified as below acccording to their functions to which their properties best fit.

Gel strengthening : starch, both native and modified (1 - 10%), protein (0 - 1%)
Texture-modifying : protein (> 1%), cellulose (0 - 4%) and fat/oil
Freeze-thaw stabilizing : modified starch (1 - 10%)

Starch : The selection of starch is determined by the formulation needs, primarily gel strength and freeze-thaw stability requirements. Both unmodified and modified starches possess gel strengthening ability, although there are considerable variations among starches. Those gel-strengthening starches include potato, corn(75% amylopectine) and wheat starch which yielded a good swelling and high viscosity upon gelatinization, and formed a relatively firm gel at room temperature (Kim and Lee, 1987). An excessive addition of starch (> 8% on a surimi weight basis) resulted in a sticky and somewhat cheese-like texture with short of elasticity and bite, although the texture still remained firm (Chen, 1987). Modified starch, mostly hydroxypropylated, has a good freeze-thaw stabilizing property. This type of modified starch did not undergo retrogradation and was not subject to freeze syneresis. This was supported by a microstructure analysis which showed that addition of modified starch kept the ice crystal growth to a minimum during frozen storage (Lee and Kim, 1987).

Protein : The selection of protein should be based on its texture-modifying properties rather than gel-strengthening properties. Addition of nonfish proteins of nonmyofibrillar nature always tends to reduce gel strength in terms of cohesiveness and rigidity in molded products and rubberiness in fiberized products. Such a reduction in gel strength and rubberiness leads to modification of texture, and often results in an imrovement of sensory quality. A certain type of nonfish protein, especially wheat gluten, prevents surimi gel from getting excessively rubbery during frozen storage, although it is not as effective as modified starch. As to the relationship of functional properties of nonfish protein to surimi gel properties, there was strong correlation($r = 0.97$) between the water binding ability of nonfish protein during heating (termed "thermal hydration") and the cohesiveness of nonfish protein-incorporated surimi gel (Chung and Lee, 1987). Unlike the conventional method which determines water binding in a cold state, the measurement of thermal hydration is done after the sample is heated to simulate the actual cooking process. Therefore, the water binding value obtained by this method correctly reflects a true gelation behavior of the protein during thermal process.

Cellulose : Cellulose is a kind of biopolymer that is chemically inert and thermally stable and is able to absorb water as much as seven times its weight (Miller, 1986). Owing to this unique physical properties, cellulose has been used in a variety of formulated products where water binding is less affected by heat treatment. Cellulose is avaliable in a powder form and, in the surimi-based products, unlike starch it reduced gel strength slightly due probably to the lack of swelling power. However, it has a desirable texture-modifying properties. Addition at 1-2% levels significantly improved the frozen-storage quality of products by keeping them from getting exccessivley rubbery and drying during extended frozen storage (Yoon et al, 1987). Since modified starch alone tends to yielded a starchy and less moist texture, a combined use of modified starch and cellulose is recommended. Cellulose also contributes as a fiber a nutritional benefit, especially where formulation calls for a complete nutrition.

Fat/Oil : Addition of fat or oil tends to weaken the gel texture, thus reducing rubbery and chewy texture. The extent of such a textural modification depends largely upon the hardness (solid fat contents) of fat and the dispersion of fat or oil. In contrast to fat dispersion in the comminuted meat protein gel, a more uniform dispersion is expected with low solid fat or oil (Lee and Abdollahi, 1981). The addition of fat was found to improve a freeze-thaw stability by preventing the development of a sponge-like texture due to freeze syneresis (Lee and Toledo, 1977).

Selection of Formula

In selecting a formula, one needs information on : what the product requirements are in terms of : form of product, texture requirement, freeze-thaw stability, and ingredient level (minimum and maximum level). These factors are interrelated to one another. Understanding of functional properties and behavior of ingredients is prerequisite to a proper formulation based on a multi-ingredient system.

Selection of optimization techniques

Several techniques can be used to achieve an optimum formulation. They are stepwise optimization, linear programming(Bender et al, 1982 ; Evans, 1982 ; Saguy, 1983), and response surface methodology (Giovanni, 1983 ; Gacula and Singh, 1984).

1. Stepwise optimization : Initially, the optimum level of each ingredient is determined in a single ingredient system. For additional ingredients, the optimum level of the second ingrdient is determined at a constant combined level using a fraction curve, and similarly for the next ingredient in a stepwise fashion. Because this method works on a limited number of variables, and the optimum level is determined on a one-to-one basis, this technique is used as a screening technique.

For flexibility and versatility in data analysis, a computer-aided optimization is being widely used in the manufacturing industry which is involved with formulation. The techniques include linear programming and response surface methodology. These techniques have been successfully used in formulation optimization, where cost, ingredient availability, target composition, and sensory quality are the main decision variables.

2. Linear programming : It is considered as a mathematical technique to solve a series of multivariate equations that is consisted of objective function, independent variables and a set of constraints. It can be effectively used when a specific objective function needs to be minimized or maximized, for instance where a minimum cost or an increase of one of ingredients to a maximum is required. Two examples are given below with step-by-step procedures.

a. High protein formula - targeted for a 17% protein level

Independent Variables :
X1 = surimi ; X2 = nonfish protein ; X3 = starch ; X4 = water

Objective Function : protein level (to be maximized)
Maximize --> 0.15(X1) + 0.87(X2) + 0.0(X3) + 0.0(X4)

Constraints :

weight : X1 + X2 + X3 + X4 = 96 (salt + flavor = 4)
moisture : 0.76(X1) + 0.08(X2) + 0.15(X3) + 1.00(X4) <= 78%
gel formability (cohesiveness index) : 1.0(X1) - 0.1(X2) + 0.25(X3) + (-1)(X4) >= 50 kg

Min - Max Limit :

X1 = 65 - 70% ; X2 = 6 - 8% ; X3 = 3 - 4% ; X4 = 15 - 20%

Execution : at 4 and 6 for loop and iteration number, respectively.

Solution : X1 = 66.7% ; X2 = 7.9% ; X3 = 3.5% ; X4 = 17.8% --> protein level 16.9%

* Note : Coefficients for gel formability (cohesiveness index) are given as relative functionality contributed by each ingredient and obtained from the slope (compressive force, kg/ % ingredient) of a curve, changes in gel cohesiveness as a function of % ingredient in surimi.

b. Least-Cost Formula - targeted for a cost less than $ 1.00/lb.

Objective Function : cost (to be minimized)

1.20(X1) + 0.30(X2) + 0.50(X3) + 0.0(X4) --> minimize
where X2 is frozen raw egg white

Constraints :

Moisture : 0.76(X1) + 0.88(X2) + 0.15(X3) + 1.00(X4) <= 78%
Gel formability : 1.0(X1) + 0.03(X2) + 0.25(X3) +(-1)(X4) >= 50 kg

Min - Max Limit :

X1 = 65 - 70% ; X2 = 15-20% ; X3 = 4-8% ; X4 = 10-15%

Solution : X1 = 65 ; X2 = 15 ; X3 = 4 ; X4 = 11 --> minimum cost = $ 0.845

Source : Lee (1987)

In addition to deciding objective function and identifying independent variables, establishing the appropriate constraints, especially the ingredient's ability to contribute to gel formation, are critical to obtaining a workable solution. Obtaining the working coefficients for each ingredient in the gel formability contraint is essential to the successful solution of a linear programming. It is also equally important to use the right method for evaluation of gel properties which performs with sensitivity in a consistent manner.

3. Response-surface methodology (RSM) : It is a statistical method designed for the optimization of variable-dependent systems where various independent variables (factors) affect the response variable (Evans, 1982 ; Saguy, 1983). Unlike the previously mentioned stepwise optimization, this method runs on a three dimensional basis. RSM enables one to determine the interrelationships among variables with respect to the response variable. A step-by-step procedure of RSM is to first identify the factors, usually two, and one response variable ; define the range of factor levels, usually three or more ; execute the experiment and obtain response data at varying levels of factors ; obtain a quadratic response surface by least-square regression : using coefficients from multivariate equations ; locate the optimum combination of factor levels which yields an optimum response.

REFERENCES

1. Bender, F.E., Kramer, A. and Kahn, G. 1982. Linear programming and its applications in food industry. Food Technol. 36(7) : 94.

2. Chen, J-S. 1987. Optimization in the formulationm of surimi-based extruded products. M.S. Thesis. University of Rhode Island, Kingston.

3. Chung, K.H. and Lee, C.M. 1987. Texture-modifying effect of various commercial proteins on surimi gel. 1st Joint Conference of Tropical and Subtropical Fisheries Technology and Atlantic Fisheries Technology, Orlando, FL. Nov. 9-11.

4. Evans, L.B. 1982. Optimization theory and its application in food processing. Food Technol. 36(7) : 88.

5. Gacula, M.C.Jr. and Singh, J. 1984. Response surface designs and analysis. In "Statistcal methods in food and consumer reseasrch", Academic Press, Inc. Orlando, Chap. 7, p. 214.

6. Gerber, L. and Lee, C.M. 1986. Unpublished data. Department of Food Science and Nutrition, University of Rhode Island, Kingston.

7. Giovanni, M. 1983. Response surface methodology and product optimization. Food Technol. 37(11) : 41.

8. Kim, J.M. and Lee, C.M. 1987. Effect of starch on textural properties of surimi gel. J. Food Sci. 52 : 722.

9. Lee, C.M. and Toledo,, R.T. 1979. Processing and ingredient influences on texture of comminuted fish mince. J. Food Sci. 44 : 1615.

10. Lee, C.M. and Abdollahi, A. 1981. Effect of physical properties of plastic fat on structure and material properties of fish protein gels. J. Food Sci. 40 : 1755.

11. Lee, C.M. and Kim, J.M. 1987. Structural analysis of gel reinforcing and freeze-thaw stabilizing properties of various starches in surimi gels. Intern. Meeting of Scanning Electron Microscopy, Hamilton, Ontario.

12. Leibovitz, H., Morizen, R.D. and Lee, C.M. 1987. Changes in the fatty acid and amino acid profiles of fish mince during surimi processing. 1st Joint Conference of Tropical and Subtropical Fisheries Technology and Atlantic Fisheries Technology, Orlando, FL. Nov. 9-11.

13. Miller, W.B. 1986. Cellulose in reduced-calorie baked goods. Bakers Digest. July.

14. Saguy, I. 1983. Optimization methods and applications. In "Computer-aided techniques in food technology" (Saguy, I., Ed.), Marcel Dekker, Inc., New York, Chap. 10, p. 263.

15. Yoon, KiSun., Lee, C.M. and Chung, K.H. 1987. Effect of powdered cellulose on the texture and water binding properties of surimi-based products. 1st Joint Conference of Tropical and Subtropical Fisheries Technology and Atlantic Fisheries Technology, Orlando, FL. Nov. 9-11.

MODIFICATION OF FUNCTIONAL PROPERTIES OF SURIMI BY CHANGES IN PROTEIN COMPOSITION

John S. French
Fishery Industrial Technology Center
School of Fisheries and Ocean Sciences
University of Alaska

INTRODUCTION

Surimi, as a minced, washed fish muscle preparation is primarily composed of myofibrillar proteins. The nature of these proteins, especially myosin, determine the properties of the surimi. This paper discusses some of the changes that occur in these proteins during surimi processing.

The physiological organization of muscle myofibrils involves a mole fraction of 0.17 myosin (50-55% (w/w)), 0.62 actin (15-20%), 0.10 tropomyosin (2-4%), and 0.10 troponin (2-4%). The myosin forming the thick filaments and the actin with tropomyosin and troponin forming the thin filaments (Goll, et al., 1977). Tropomyosin and troponin are wrapped around actin in a helical ribbon and regulate the calcium dependent hydrolysis of ATP and the interaction between the thick and thin filaments during contraction. Whereas actin is virtually spherical in shape, most of the myofibrillar proteins have a high degree of alpha-helical structure. In addition ionic bonds have a major role in the polymerization of tropomyosin, the association of troponin and tropomyosin with actin and the dimerization of myosin (Asghar, et al., 1985).

In addition to data on protein structure, there is general agreement that stimulation of actomyosin ATPase activity requires the presence of the major regulatory proteins (Goll, et al., 1977). There is a direct relationship to the remaining ATPase activity, the extractability of surimi proteins, and the gel forming ability of surimi (Okada and Tamoto, 1986). Thus it appears that gel forming ability is highly sensitive to enzyme denaturation and conformational changes in addition to overall protein composition.

METHODS

Alaskan pollock (Theragra chalcogramma) and pollock surimi were obtained from Alaska Pacific Seafoods, Kodiak, Alaska as part of an Alaska Fisheries Development Foundation sponsored study. Pollock were caught in Shelikof Strait and held in either slush ice or refrigerated seawater (RSW). Surimi samples were the highest grade products (SA grade).

Differential extraction of Alaskan pollock or surimi was carried out as described previously (French, 1987b). Samples were blended with either cold distilled water or phosphate buffered 0.5 M sodium chloride, pH 6.8,

incubated for 60 minutes at 4°C and centrifuged 10 minutes at 30,000 x g. Protein concentrations were determined by the Coomassie Blue binding method of Bradford (1976) using bovine serum albumin as the standard.

Polyacrylamide gel electrophoresis (SDS-PAGE) was performed in the presence of 0.2% SDS (Laemmili, 1970) using 12% acrylamide. Filtered soluble protein samples were denatured by heating 5 minutes at 95°C in the presence of SDS and 2-mercaptoethanol. Liquid chromatographic analysis was carried out on a BioRad HPLC using a UV detector at 280 nm and a model 3392A reporting integrator. Separation was by molecular exclusion chromatography on a 25 cm BioRad TSK 400 column. Isocratic conditions were maintained at 0.10 M sodium sulfate 0.05 M phosphate buffered to pH 6.8 at a flow rate of 0.7 ml/min and a column temperature of 25°C.

Proximate analysis and rheological tests on pollock surimi were performed by the standard methods (Cheng, et al., 1979; Hamann and Webb, 1979; Montejano, et al., 1985). The punch tests were done on a Food Checker (Sun Kagaku Co., Tokyo). Surimi sausages were cooked with either a 20 minutes presetting at 40 or 60°C followed by 20 minutes at 90°C, or 40 minutes at 90°C with no presetting, after addition of 2.5% salt to partially thawed surimi.

RESULTS

Changes in extractability of pollock muscle proteins have been studied at various stages in the conversion of pollock to surimi and cooked kamaboko including holding conditions of the fish, washing the mince, presetting and cooking the kamaboko.

Total protein extractability and the relative water and salt extractabilities of myofibrillar proteins changed with holding time and whether the pollock were held in ice or refrigerated seawater. HPLC analysis has been used to separate water (circles) and salt (squares) extractable myofibrillar proteins from pollock fillets after holding in slush ice (open symbols) or refrigerated seawater (closed symbols) for up to seven days are shown in Figure 1.

It has been shown (French, 1987a,b) that the stiffness and the water binding potential of surimi are related to the amount of water extractable tropomyosin remaining in surimi after washing. Stiffness was inversely correlated to tropomyosin content with a correlation coefficent of -0.68 and water binding potential directly related with a correlation coefficient of 0.87.

To study the effects of added salt and cooking on surimi, samples were blended with distilled water in the presence or absence of 0.5 M sodium chloride and centrifuged as above or the samples were cooked at 90°C for 40 minutes and centrifuged. The proteins extracted into the supernatant were analysed by HPLC. The extractability of myosin, actin and

tropomyosin into distilled water all decreased substantially after cooking (cross hatched bars) as shown in Figure 2. Similarly, the uncooked (open bars) actin was the most extractable with salt.

Figure 1. Extractability of pollock muscle proteins after holding in RSW or slush ice. Slush ice: water extract (○), salt extract (□). RSW: water extract (●), salt extract (■).

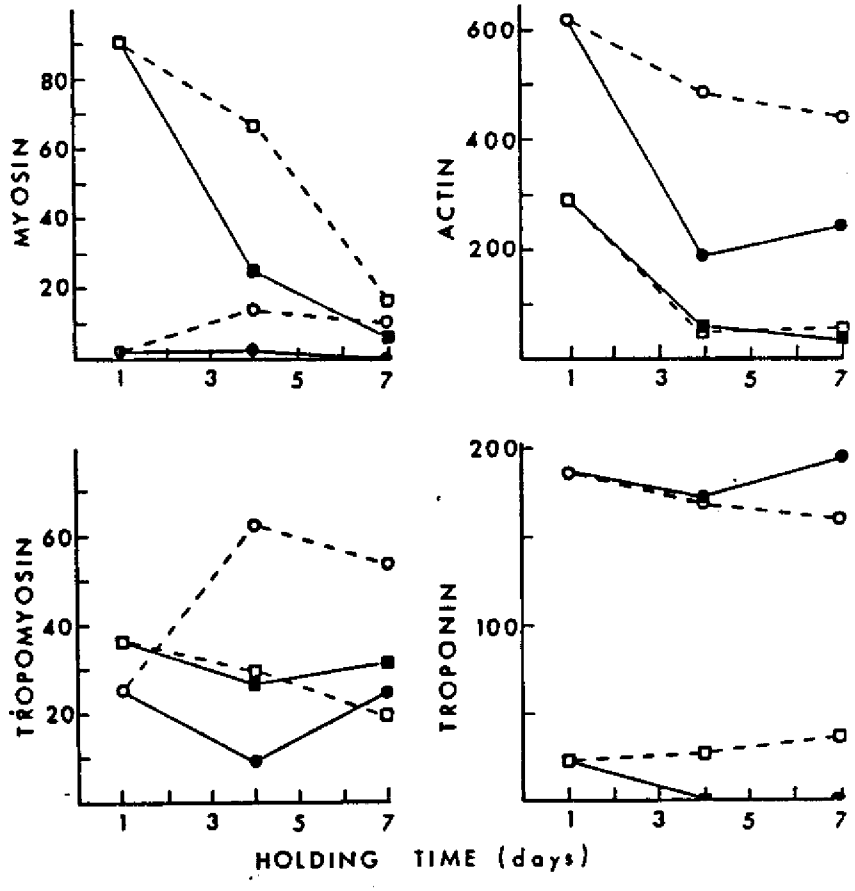
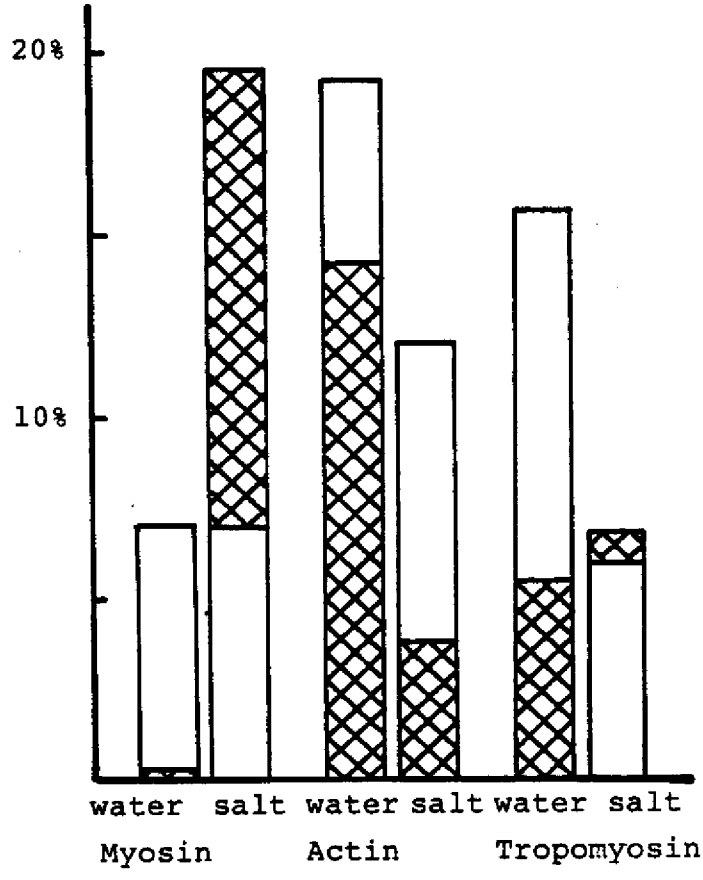
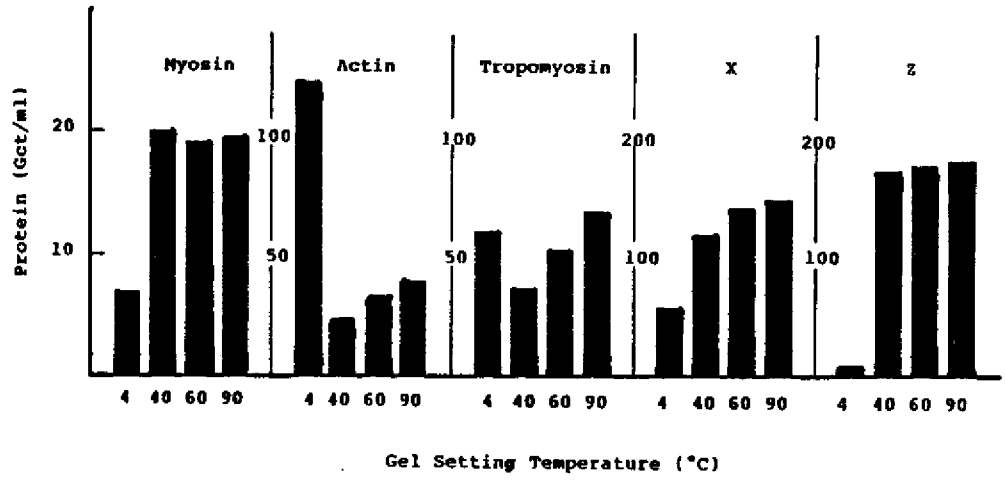


Figure 2: Effects of salt and cooking on extractability of pollock muscle proteins. Uncooked surimi: open bars. Cooked surimi: cross hatched bars.



To study the effects of presetting in addition to cooking on protein extractability, surimi samples were prepared with 2.5% salt and allowed to set at 4, 40, 60, or 90°C. The low temperature samples were not cooked whereas the remaining samples were cooked at 90°C. Substantial changes were seen in five of the salt extractable proteins, as shown in Figure 3.

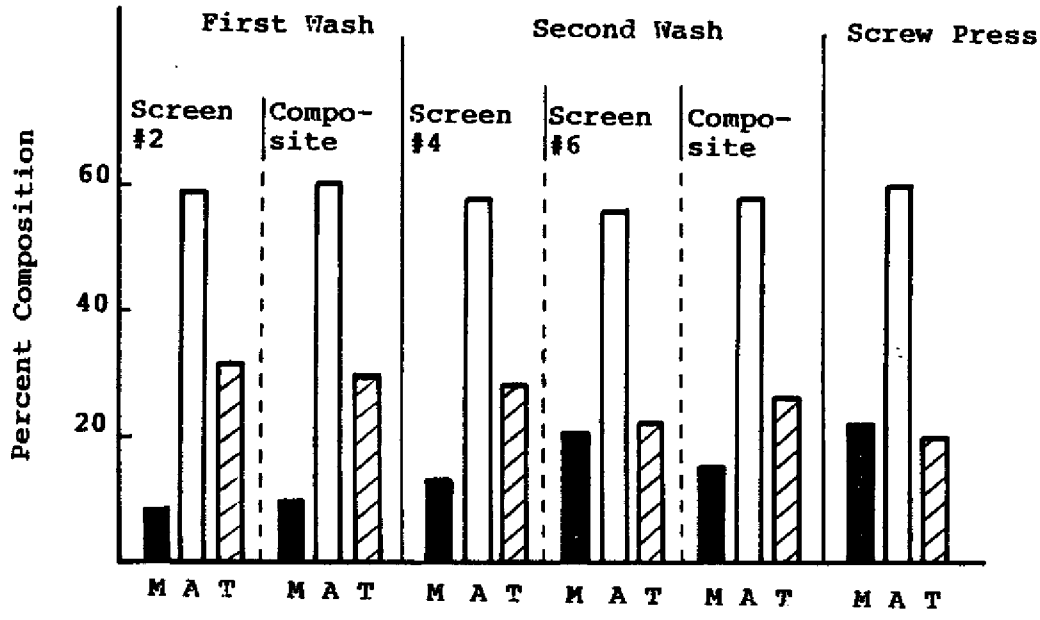
Figure 3: Role of 40 and 60°C setting on extraction on pollock muscle proteins by 0.5 M sodium chloride.



It is clear from these results that the cooked gels are not just a tangled mass of denatured proteins. With the exception of actin and possibly tropomyosin, the remaining major protein peaks showed substantial increases in extractability after cooking. Also note that while myosin and Z-peak proteins showed a constant increase irrespective of setting temperature, actin and tropomyosin showed the greatest changes in gels set at 40°C prior to cooking. This provides additional evidence that actin and tropomyosin contribute to the stronger "suwari" gels formed when surimi is preset before cooking.

The study of the protein composition of the waste water from various parts of the surimi processing line shows that different proteins are removed at different stages of the process. Results from Figure 4 show a peak containing myosin (M) increased from 9.9% in the first wash to 15.5 and 22.0% in the water from the second wash and screw press, respectively, while a fraction containing troponin (T) decreased form 29.7 to 26.3 and to 17.7%, respectively, the actin (A) fraction remained relatively constant near 60%.

Figure 4: Distribution of pollock muscle proteins in waste water during surimi manufacturing.



DISCUSSION

Actin and myosin, and their interaction as actomyosin, are critical for the formation of surimi protein gels (Asghar, et al., 1985). The nature and the degree of the dependency on actin is not fully resolved and the results presented here indicate roles for other myofibrillar proteins. Much of the conflicting data relating to the roles of these minor proteins may be due to the impurity of the preparations and possible denaturation of proteins during the isolation of individual proteins (Rowlerson, et al., 1985). Sano and co-workers (1986) report that natural actomyosin isolated from Alaskan pollock consisted of myosin heavy chain, light chains, actin, tropomyosin, troponin and other unidentified minor components. Croaker actomyosin has also been shown to contain protein components other than actin and myosin (Wu, et al., 1985). Even "pure" myosin preparations often show 5-10% impurities (Egelandsdal, et al., 1985) when analysed by SDS-PAGE. These relatively small amounts become significant when compared to tropomyosin or troponin being only 4-6% each in native muscle (Goll, 1977) or water extractable tropomyosin being 2-7% of the protein in pollock surimi (French, 1987b). The degree of tropomyosin degradation in fish muscle minces during thermal processing has been related to the gel texture (Cheng, et al., 1979). Paramyosin has

also been shown to have significant effects of the stretching strength of kamaboko when added at the 5% level (Sano, et al., 1986). The gel characteristics of actomysin with varying ionic strengths are reported to be different from that of myosin alone (Asghar, et al., 1985).

There are four major types of intermolecular attractions which dictate the fundamental structure of a protein system whether in a functioning fish muscle at 5°C or surimi gel at 90°C. These are covalent bonds, most frequently seen as disulfide linkages; ion pairs, such as those in polymerized tropomyosin; hydrogen bonds, such as those that stabilize alpha-helical structures; and hydrophobic bonds, usually seen in the interior of water soluble proteins (McGilvery, 1983). Disulfide bonds are probably of limited significance in fish protein gels since gel strength increases when disulfide bond reducing agents are present. There is good evidence that both ion pairs and hydrophobic bonds are important in both native muscle and cooked muscle gels (Asghar, et al., 1985). Hydrogen bond formation is an enthalpy driven process with a negative change in entropy (Cantor and Schimmel, 1980). Therefore, as the cooking temperature rises, hydrogen bonds will break and the alpha-helical and beta-sheet structures will become less important. On the other hand, ion pair formation is strongly entropy driven and greatly increases as the temperature rises. For example, the free energy driving the association of magnesium ions with ATP will become 1.7 kcal more favorable when heated from room temperature (25°C) to 90°C (Cantor and Shimmel, 1980). Hydrophobic bond formation is generally entropy driven but is often associated with a large change in heat capacity also (Tanford, 1980). Therefore, at gel setting temperatures, hydrophobic portions of the myofibrillar proteins will tend to associate with other hydrophobic portions but the extent this occurs will depend on the exact nature of the protein structures involved. Thus the thermodynamic forces causing of gelation of surimi proteins are entirely consistent with the good ionic bonding agents tropomyosin and troponin forming ion pairs and hydrophobic bonds between the remains of the thin filaments and myosin. To maintain these interactions surimi should contain the proper ratios of these constituents.

REFERENCES

Asghar, A., K. Samejima and T. Yasui. 1985. Functionality of muscle proteins in gelation mechanisms of structured meat products. CRC Crit. Rev. Food Sci. Nutr. 22, 27-105.

Bradford, M. 1976. A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248-262.

Cantor, C.R. and P.R. Schimmel. 1980. Biophysical Chemistry, part I: The conformation of biological macromolecules. W.H. Freeman and Co. San Francisco.

Cheng, C.S., D.D. Hamann and N.E. Webb. 1979. Effect of thermal processing on minced fish gel texture. J. Food Sci. 44, 1080-1086.

Engelandsdal, B., K. Fretheim and O. Harbitz. 1985. Fatty acid salts and analogs reduce thermal stability and improve gel formability of myosin. J. Food Sci. 50, 1399-1402.

French, J.S. 1987a. Relationship of protein composition to water holding properties of pollock surimi. J. Food Sci. (in preparation) (presented at Pacific Fisheries Technologists Meeting, Vancouver, B.C.).

French, J.S. 1987b. Postmortem changes in muscle protein structure as fish quality indices. in Kramer, D.E. and J.T. Liston (Eds.) Seafood Quality Determination. Elsevier Publishing, Amsterdam. (in press).

Goll, D.E., R.M. Robson and M.H. Stromer. 1977. Muscle proteins, in: Whitaker, J.R. and S.R. Tannenbaum (Eds.). Food Proteins. AVI Publishing, Westport CT, 121-174.

Hamann, D.D. and N.B. Webb. 1979. Sensory and instrumental evaluation of material properties of fish gels. J. Texture Studies 10, 117-130.

Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227, 680-684.

McGilvery, R.W. 1983. Biochemistry a Functional Approach, 3rd ed. W.B. Saunders Co., Philadelphia.

Montejano, J.G., D.D. Hamann and T.C. Lanier. 1985. Comparison of two instrumental methods with sensory texture of protein gels. J. Texture Studies 16, 403-424.

Okada, M. and K. Tamoto. 1986. Introduction to Surimi Manufacturing Technology. Overseas Fishery Cooperation Foundation. Tokoyo, Japan.

Rowlerson, A., P.A. Scapolo, F. Mascarello, E. Carpene and A. Veggetti. 1985. Comparative study of myosins present in the lateral muscle of some fish: species variations in myosin isoforms and their distribution in red, pink and white muscle. J. Muscle Res. Cell Motility 6, 601-640.

Sano, T., S.F. Noguchi, T. Tsuchiya and J.J. Matsumoto. 1986. Contribution of paramyosin to marine meat gel characteristics. J. Food Sci. 51, 946-950.

Tanford, C. 1980. The Hydrophobic Effect: Formation of micelles and biological membranes, 2nd ed. John Wiley, New York.

Wu, M.C., T.C. Lanier and D.D. Hamannn. 1985. Rigidity and viscosity changes of croaker actomyosin during thermal gelation. J. Food Sci. 50, 14-19 & 25.

THE PRODUCTION OF SURIMI FROM PACIFIC WHITING (Merluccius productus)

Ramon Pacheco-Aguilar and David L. Crawford
Oregon State University - Seafoods Laboratory
250 - 36th Street
Astoria, OR 97103

INTRODUCTION

Washing with water is the initial step in the conversion of minced fish flesh into what the Japanese refer to as surimi. Mechanical dehydration is carried out to produce a white, odorless and bland flavored product; to remove substances that promote protein denaturation during frozen storage; and to enhance the functional properties of surimi proteins. Surimi is an intermediate that is used as a raw material to prepare a wide variety of gel-type food products; it possesses excellent frozen shelf life providing a year round base for production of surimi-based shellfish products, fish sausages and fish ham (Buck and Fafard, 1985; Lee, 1984).

In the conventional process, minced fish is washed with two to eight times its volume in fresh water from one to several times depending upon the species and freshness of the fish (Lee, 1984). This procedure results in a high quality surimi, but requires large amounts of water and produces low yields and considerable water soluble waste materials. Surimi showing strong gel-forming characteristics is required to produce the rubbery and turgid texture that is highly accepted in Japanese products (Okada et al., 1983). Since textural preferences of U.S. consumers vary markedly from those of Japanese, the gel-strength required for surimi-based products designed for domestic acceptance could be considerably less. This can markedly alter the species, raw material conditions, and processing requirements for a surimi intermediate that would produce textural characteristics accepted by domestic consumers. The objectives of this study were to evaluate modifications in the conventional surimi processing and to convert species of underutilized fish into a surimi intermediate. Efforts were directed towards: a) utilizing Pacific whiting, b) reducing the amount of water required in the washing unit operation, c) optimizing the yield of washed product, and d) evaluating the gel-forming ability of surimi thus produced.

METHODOLOGY

In preliminary studies intended to optimize flesh recoveries, ten different washing regimes were evaluated in which water-flesh ratios, number of exchanges, dewatering techniques between exchanges (screening/pressing), and pH of the washing water were varied. Recovery of total solids and protein, and changes in washed and dewatered minced flesh composition (moisture, protein, lipids, ash, and TMAO) were used as criteria to evaluate washing regime efficiency. Washing at reduced pH was carried out by adjusting the first exchange of a 1:1 water-flesh mixture to an acidic pH in the range 4.9-5.8 with phosphoric acid, followed by a second exchange using potable water. The pH of the acidic dewatered mince was adjusted to neutrality with a solution of sodium carbonate. A cryoprotectant mixture of sorbitol, sucrose, and polyphosphates (4%, 4%, and 0.5%, respectively) was added into the surimi formulation. The final surimi moisture was adjusted to 77% with an ice-water mixture. Temperature during the mixing operation was constantly held under 10^{o}C. Prepared surimi (700 g units) was packed into polyethylene lined cardboard boxes, frozen and held at -30^{o}C. For the preparation of fish gels, partially thawed surimi was cut into small pieces and chopped; ice was added to keep the batter temperature at 5^{o}C and to bring the moisture content to 82.5%. Based upon the weight, 2.5% sodium chloride, 5% egg white and 5% potato starch were added to yield a batter with a moisture content of 74%. The batters were packed in glass tubes (30 X 150 mm), sealed and cooked at 90^{o}C for 60 minutes.

Texture profile analysis (TPA), expressible water (EW) and folding test (FT) determinations of heat-set gels were carried out to evaluate the gel-forming ability of surimi from the 3:1 water:flesh ratio, one exchange and the 1:1 water-flesh ratio, low pH water (pH 5.0-5.3) first exchange, potable water second exchange, washing regimes. TPA of gel samples of uniform geometry and weight were measured using an Instron Universal Testing Machine at 50% maximum compression. Results were defined in terms of hardness, elasticity, cohesiveness, gumminess and chewiness (Abbot, 1972). FT was conducted by folding a gel slice between thumb and index finger; if no cracking occurred along the fold, the slice was folded again perpendicular to the first fold. Rating scale for the test was based upon the degree of cracking occurring along the folds (Kudo et al., 1973). A Laboratory Carver Press was used to determine EW (Lee and Toledo, 1976). Uniform gel samples were pressed at 1000 psi for 20 seconds on Whatman #1 filter paper between plexiglass plates. The wetted area on the filters was

measured using a planimeter and the amount of EW expressed as cm of wetted area per unit weight of product.

RESULTS AND DISCUSSION

Single exchanges in potable water produced superior solids and protein recoveries to those observed for multiple exchanges utilizing the same total amount of water. A single exchange of a water:mince ratio of 3.0 produced the highest recoveries (73.32 and 74.07% of flesh solids and proteins, respectively). Washing at ratio 1.0 under acidic conditions followed by a potable water wash at ratio 1.0 produced recoveries superior or equal to all of the wash regimes of equal total water volume (73.02 and 76.2% of flesh solids and proteins, respectively). Washing under acidic conditions resulted in the most efficient removal of the original content of TMAO and lipid in muscle (91.4 and 31.1%, respectively). Superior removals were accomplished since acidic conditions favored the extraction of amines and produced greater pressures during screw-pressing due to the altered physical characteristics of the flesh. Pressing between multiple potable water exchanges showed better removals of TMAO, lipids and ash than single exchanges involving only one pressing operation or multiple exchanges involving screening in between. Water requirements were reduced (>80%) and yield was improved by up to 34% over the conventional process.

Addition of potato starch and dry egg white into the gel formulation overcame the lack of gel-forming ability of surimi samples without masking the effect of washing treatments. Surimi gels from potable water washed surimi were easily assigned the maximum grade SA according to the folding test; surimi gels produced from the low pH washing regime fell approximately equally within the A and B grade. An average grade of A-B was assigned to them. Surimi gels from both washing regimes were equal ($p>0.05$) in EW. TPA revealed no significant difference in gel hardness ($p>0.05$) between treatments. Gels derived from low pH treatment yielded poorer elasticity and cohesiveness ($p<0.01$) than gels from the potable water treatment. Gels with lower elasticity and cohesiveness produced lower folding test grades. No significant difference between treatments was found in gumminess or chewiness ($p>0.05$).

The study suggests that less water could be required in surimi processing, thus reducing the waste water management problems and increasing the low yield recoveries associated with surimi production. Washing under acidic conditions near the isoelectric point of flesh proteins reduced protein functionality somewhat over that observed for flesh washed in

potable water. This effect was determined for gel attributes considered most important for the fabrication of food products through restructuring procedures: elasticity and cohesiveness. The low moisture content of the washed and pressed flesh from the washing regimes evaluated would allow considerable flexibility in formulating surimi intermediates of optimum and uniform moisture contents. This study could establish a technical base for surimi process optimization and product formulation and to prove the feasibility of surimi production from Pacific whiting.

REFERENCES

Abbot, J.A. 1972. Sensory assessment of food texture. Food Technol. 26(1): 40.

Buck, E.M. and Fafard, R.D. 1985. Development of a frankfurter analog from red hake surimi. J. Food Sci. 50(2): 7.

Kudo, G., Okada, M. and Miyauchi, D. 1973. Gel-forming capacity of washed and unwashed flesh of some pacific coast species of fish. Marine Fisheries Review 35(12): 10.

Lanier, T.C., Lin, T.S., Liu, Y.M. and Hamann, D.D. 1982. Heat gelation properties of actomyosin and surimi prepared from Atlantic croaker. J. Food Sci. 47(6): 1921.

Lee, C.M. and Toledo, R.T. 1976. Factors affecting textural characteristics of cooked comminuted fish muscle. J. Food Sci. 41(2): 391.

Lee, C.M. 1984. Surimi process technology. Food Technol. 38(11): 69.

Okada, M., Miyauchi, D. and Kudo, G. 1973. "Kamaboko" - The giant among Japanese processed fishery products. Marine Fisheries Review 35(12): 1.

STUDIES ON FLOW AND GEL FORMING BEHAVIOR OF SQUID SURIMI IN RELATION TO FORMULATION

J. M. Kim
Department of Food Science
University Of Maine
Orono, ME 04469

INTRODUCTION

Little is known about the food uses of North Atlantic squid, although it is being described as a major underutilized marine species. It has been used as an important source of food in Mediterranean and oriental countries. In few other countries of the world is the squid as much utilized and appreciated in various forms of food as in Japan, whereas it has been limited for marketability in the United States because of a tough, rubbery texture. Most consumption of squid in the U.S. centers around certain ethnic groups. The lack of a domestic market means that virtually all North Atlantic squid are being consigned for export.

Recently, U.S. markets for squid are growing as consumers become aware of its versatility as a food and there have been numerous publications on squid in the United States dealing with TMAO degradation for quality factor, textural characterization, squid protein concentrate, the use in meat emulsion, and tentacle protein.

The characteristic tough texture of squid is caused by the peculiar structure of the tissue. The mantle is composed of several layers of muscle fibers and connective tissue. Each fibrous muscle layer (circumferential muscle fibers) is about 200-500 um thick and composed of very slender fibers, each about 5 um in diameter. Between every other layer, a narrower layer (radial muscle fibers) consisting of short fibers runs perpendicularly to the circumferential muscle fibers. Squid contains a relatively large amount of collagen, up to about 11% of the total protein in the case of the muscle of *Illex argentinus*. Therefore, it is believed that the collagen plays a significant role in the textural changes during cooking.

Squid is readily distributed throughout the world oceans, yet it is one of the least expensive protein foods. In ordinary fish, the ratio of edible parts to the whole is roughly 40-70% and the ratio in squid is as large as 80% (trunk 50%, arms 30%). Squid meat is equal to fish meat in protein content (16-20%) and amino acid composition, and can be considered an excellent source of protein.

The flesh of raw squid is tough and rubbery in texture, but chopping with salt in a silent cutter produced a soupy texture. Apparently, the squid protein is so soluble in the presence of salt that most of the squid goes into solution leaving very little material for structure or texture. Eventually soft and mushy fish sausage products were obtained.

The purpose of this study was (1) to investigate the relationship of composite characteristics with rheological properties of squid surimi sol and gel in relation to ingredients and formulation and (2) to examine the correlation of textural properties with gel gross microstructure before and after frozen storage of the gel.

MATERIALS AND METHODS

Preparation of surimi

Fresh skinned and cleaned North Atlantic squid (*Loligo pealei*) was purchased from a local supplier and processed into surimi within 24 hr. The mantle was passed through a mincer with a die having 5 mm diameter perforations. The minced meat was washed four times with ice-chilled water using 1 part squid meat to 4 parts water (W/W). The minced meat was allowed to settle for 10 min followed by removal of washing water. After the fourth washing and draining, the slurry was dewatered by centrifugation at 3000 xg. The meat pellet was chopped in a silent cutter with sugar, sorbitol, and sodium tripolyphosphate (4%, 4%, and 0.2%, W/W, respectively) and subsequently packed in cryobags to be stored at -20°C until used.

Preparation of surimi sol

All of the thawed surimi (overnight in a refrigerator) was chopped with 2% (W/W) salt except sodium alginate-containing paste, which was chopped with 1% salt in a silent cutter for 3 min and a calculated amount of ice-chilled water was added to adjust the final moisture level to 78% and chopping was continued for 5 min. At this point, ingredients were added and chopping was continued 4 min more. Twelve types of samples were prepared with different ingredients incorporated as shown in Table 1.

Table 1. Formulations of surimi sol/gel prepared.

Types	Ingredients
I	Squid surimi without any ingredient
II	Squid surimi, 2% cellulose
III	Squid surimi, 1.4% Na-alginate, 0.6% $CaSO_4$
IV	Squid surimi, 1% cellulose, 0.7% Na-alginate, 0.3% $CaSO_4$
V	Squid surimi, 6% freeze-thaw stable modified starch (MS)
VI	Squid surimi, 2% soy protein concentrate, 6% MS
VII	Squid surimi, 2% dried egg white powder, 6% MS
VIII	Squid surimi, 2% cellulose, 6% MS
IX	Squid surimi, 1.4% Na-alginate, 0.6% $CaSO_4$, 6% MS
X	Squid surimi, 1% cellulose, 0.7% Na-alginate, 0.3% $CaSO_4$, 6% MS
XI	Blended surimi:2 parts squid with 3 parts Atlantic pollock, 6% MS
XII	Blended surimi:1 part squid with 4 parts Atlantic pollock, 6% MS

Evaluation of flow behavior of surimi sol

For the measurement of viscosity, a small portion of the resulting paste was placed in a 150 ml plastic cup without any large air pockets. In less than 2 min after completion of chopping, a measurement was made by a Brookfield viscometer at 1, 2.5, 5, 10, 20, 50, and 100 rpm using a #7 spindle (3 mm dia and 30 mm depth), except the paste containing sodium alginate. Viscosity measurements on the sodium alginate-containing paste were performed after the paste was left for 24 hr in a refrigerator. Consistency, flow behavior, and fluidity indices were obtained from shear stress vs shear rate.

Measurement of textural properties

The chopped surimi paste was extruded into 30 mm diameter cellulose casings and cooked at 90°C for 40 min in a water bath followed by cooling in running tap water for 20 min. The prepared gels were left overnight at room temperature and cut into cylindrical shapes (30 mm diameter and 25 mm long).

Textural properties measured were compressive force, percent expressible moisture and penetration force as an index of cohesiveness, water holding ability and firmness. Detailed procedures are mentioned in another paper of these proceedings. Gel samples were subjected to three freeze-thaw cycles to evaluate the changes in texture during accelerated and adverse conditions of frozen storage. Samples frozen at -20°C for five days were thawed and equilibrated to room temperature before texture measurement was performed for compressive force, percent expressible moisture, and penetration force.

Light microscopic study

Small surimi gel blocks were frozen in liquid nitrogen and sectioned at 5-8 um with a cryomicrotome. Prepared sections were mounted on slides and stained for examination.

RESULTS AND DISCUSSION

Flow curves of the surimi sols containing various ingredients are shown in Fig. 1. As more ingredients were added, the surimi sols became less pseudoplastic, indicated by a moderation of the flow curve (Fig. 1) as well as by an increased and a decreased flow behavior index and consistency index, respectively (Table 2). This is attributed to the fact that 6% modified starch was incorporated with the addition of ice-chilled water to adjust the moisture level to 78%. When other ingredients were incorporated, again, more calculated water was added for the moisture adjustment. This indicates that flow behavior of the paste is moisture-dependent. Squid surimi chopped with salt became soupy in texture and actually could be poured from one container to another. Sodium alginate significantly increased viscosity of the surimi paste (Fig. 1). This was because calcium ions react with the carbonylic groups of the alginates and crosslink the molecules. Thus, increasing the molecular weight and viscosity. Flow curves of Atlantic pollock surimi paste prepared with and without starch are illustrated in Fig. 1 to compare with the soupy texture of squid surimi paste prepared with various ingredients.

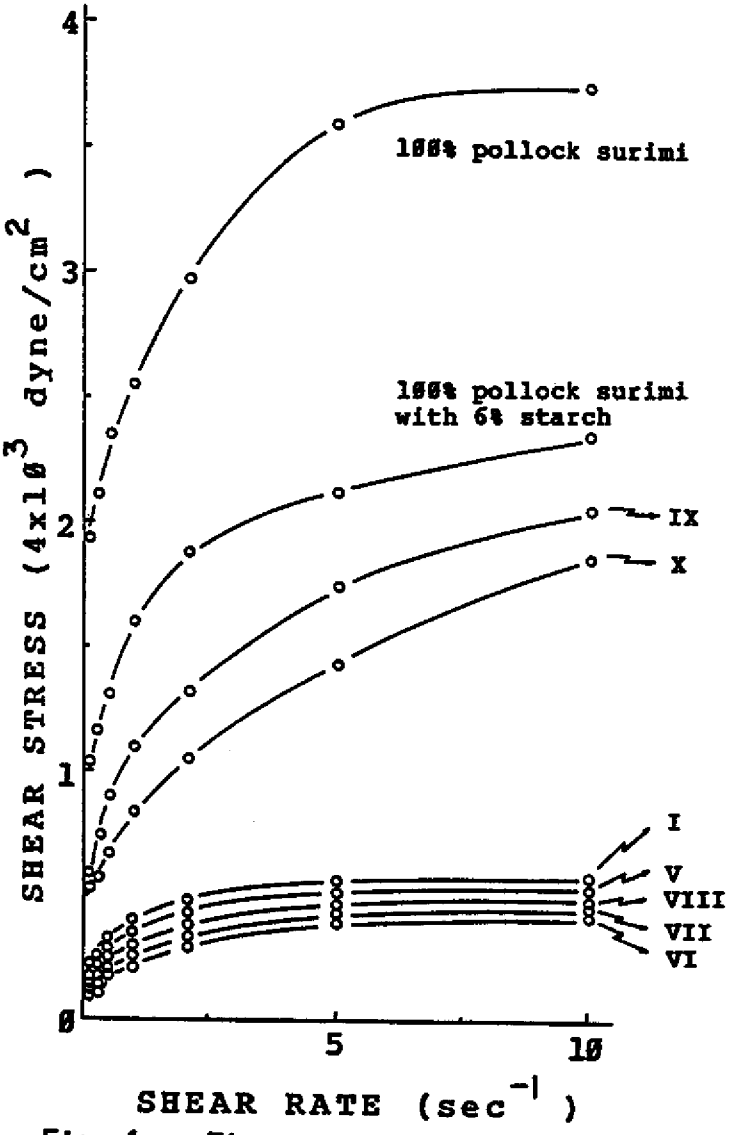


Fig. 1. Flow curve of squid surimi sols containing various ingredients.

Table 2. Flow behavior, consistency, and fluidity indices of surimi sol containing various ingredients.

Sol types	Sol characteristics[a]		
	n	K	F
I	0.325	3.20	0.313
V	0.329	3.18	0.314
VI	0.344	3.04	0.329
VII	0.336	3.06	0.327
VIII	0.333	3.08	0.325
IX	0.296	3.64	0.275
X	0.301	3.52	0.284
pollock	0.154	4.02	0.249
pollock with 6% modified starch	0.173	3.80	0.263

[a] n = flow behavior index; K = consistency index; F = fluidity index (K^{-1}).

Starch used in this study is a blend of potato and tapioca starches (about 1:6 ratio, W/W) and modified by hydroxyalkylation for freeze-thaw stability. In Figs. 8a-8c, bigger granules are potato starch and smaller ones, tapioca. Intact starch granules before cooking show characteristic maltese cross in polarized light (Fig. 8d). Muscle associated collagen can still be seen even though preparation of surimi and surimi paste involves a vigorous chopping in a silent cutter (Fig. 8c). Apparently, squid muscle fibers are not solubilized by chopping with salt (Figs. 8a-8c) and when viewed between crossed polars, the stained fibers show polarization color (Fig. 8d). Microcrystalline cellulose also shows bright birefringence in polarized light. After cooking at 90°C for 40 min, starch and fibers lost maltese cross and birefringence (Fig. 9g). Cellulose, however, still retained its crystallinity after cooking and show bright birefringence even during the freeze-thaw cycles (Figs. 9g, 10g, and 11g).

During heat-induced gel formation, squid surimi gel prepared without starch showed extreme shrinkage and expelled large amounts of water from the gel matrix, resulting in many broken areas on the surface of the gel. Incorporation of 6% modified starch improved the problem associated with thermal shrinkage of squid muscle protein holding the moisture in the gel during cooking and eliminated the broken areas on the surface of the gel. The effects of various ingredients with or without starch on compressive force, percent expressible moisture, and penetration force on squid surimi gels before and during successive freeze-thaw cycles are shown in Figs. 2-7. Although Type I-IV gels prepared without starch expelled large amounts of water during cooking, percent expressible moisture was still significantly high before and after freezing of the gel (Fig. 4) as evidenced by many air cells before freezing (Figs. 9a and 9b) and by many areas of ice crystals during successive freeze-thaw cycles (Figs. 10a, 10b, 11a and 11b). Incorporation of the freeze-thaw stable modified starch lowered compressive (Fig. 3) and penetration (Fig. 7) forces, while improving

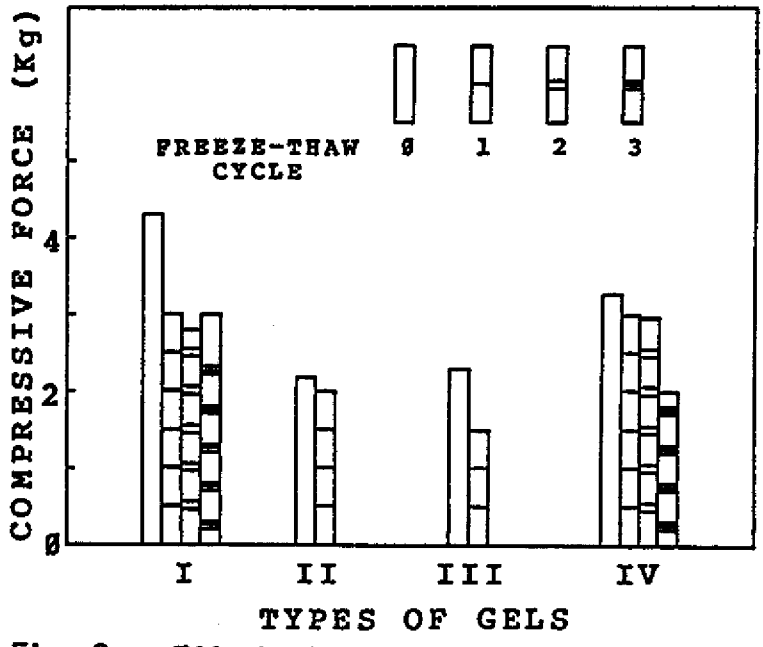


Fig. 2. Effect of various ingredients without starch on compressive force of surimi gel during freeze-thaw cycles.

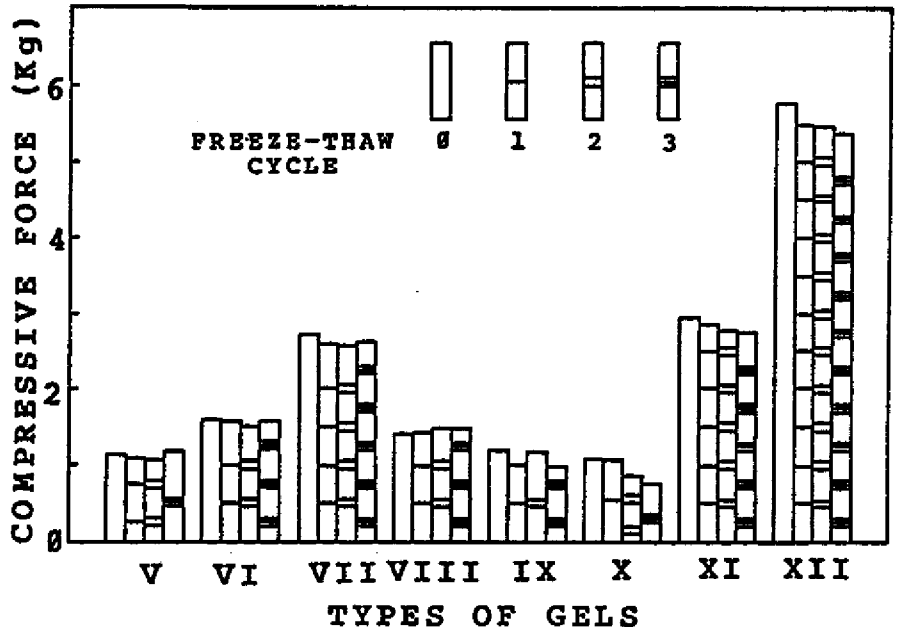


Fig. 3. Effect of various ingredients with 6% modified starch on compressive force of surimi gel during freeze-thaw cycles.

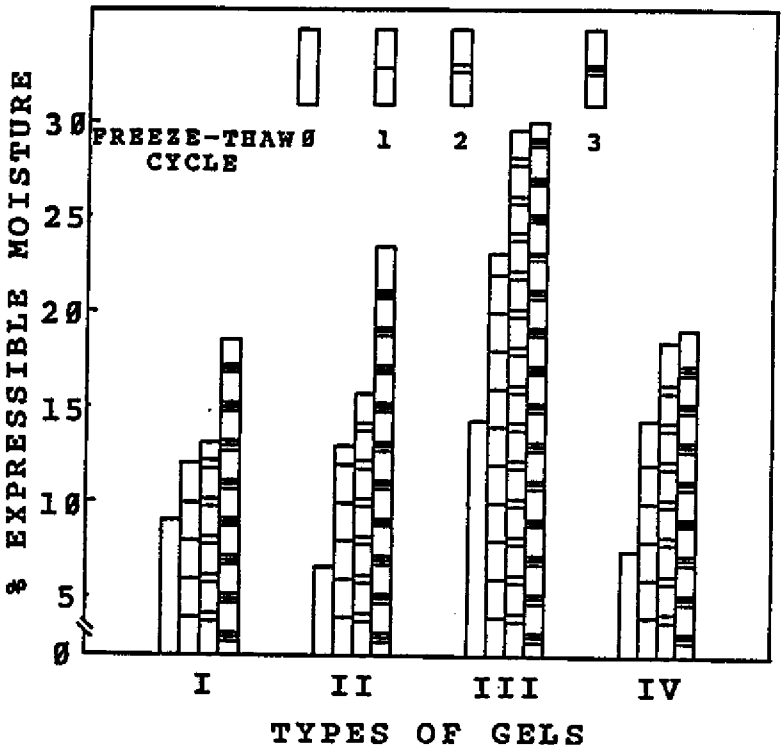


Fig. 4. Effect of various ingredients without starch on % expressible moisture of surimi gel during freeze-thaw cycles.

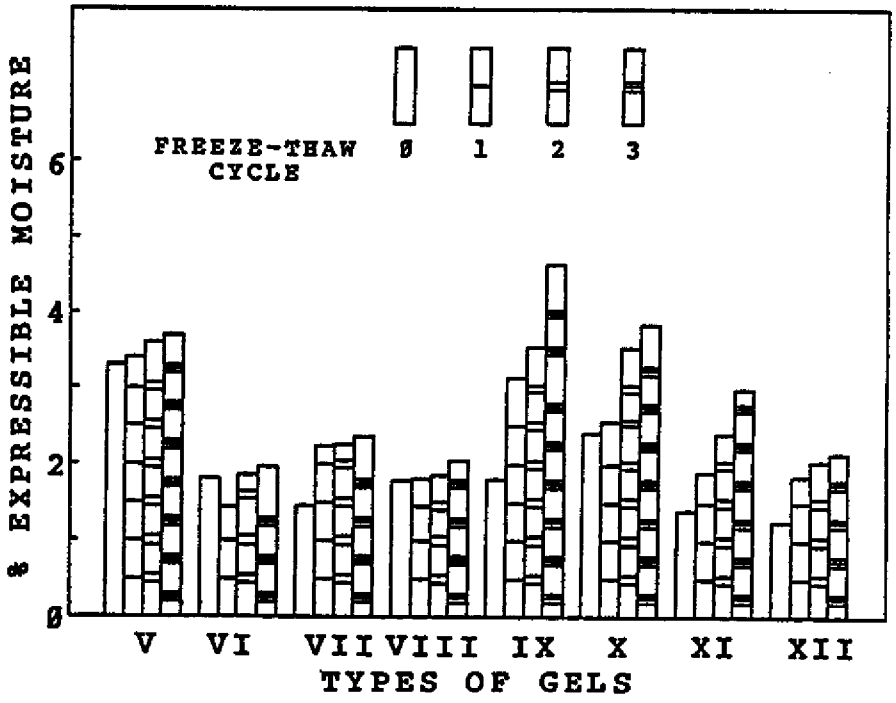


Fig. 5. Effect of various ingredients with 6% modified starch on % expressible moisture of surimi gel during freeze-thaw cycles.

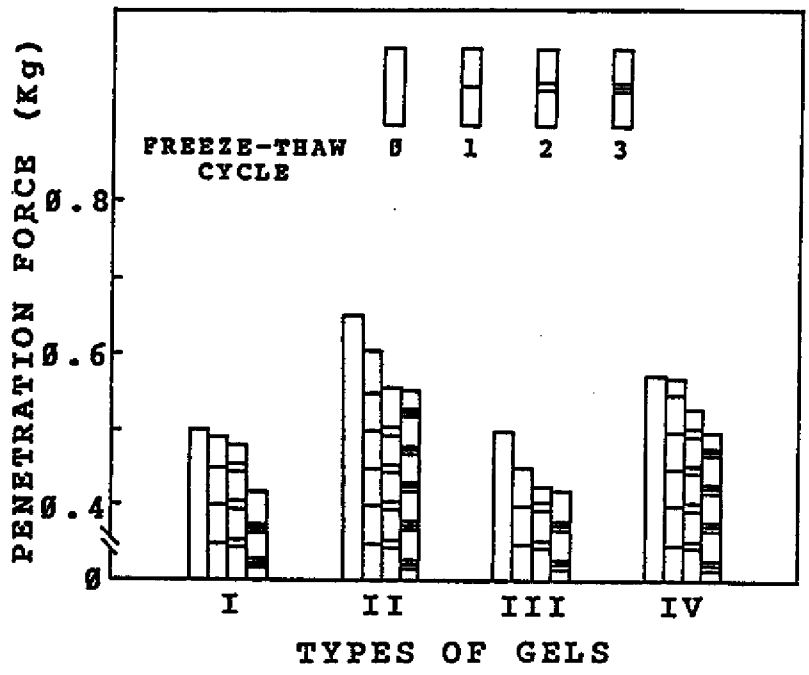


Fig. 6. Effect of various ingredients without starch on penetration force of surimi gel during freeze-thaw cycles.

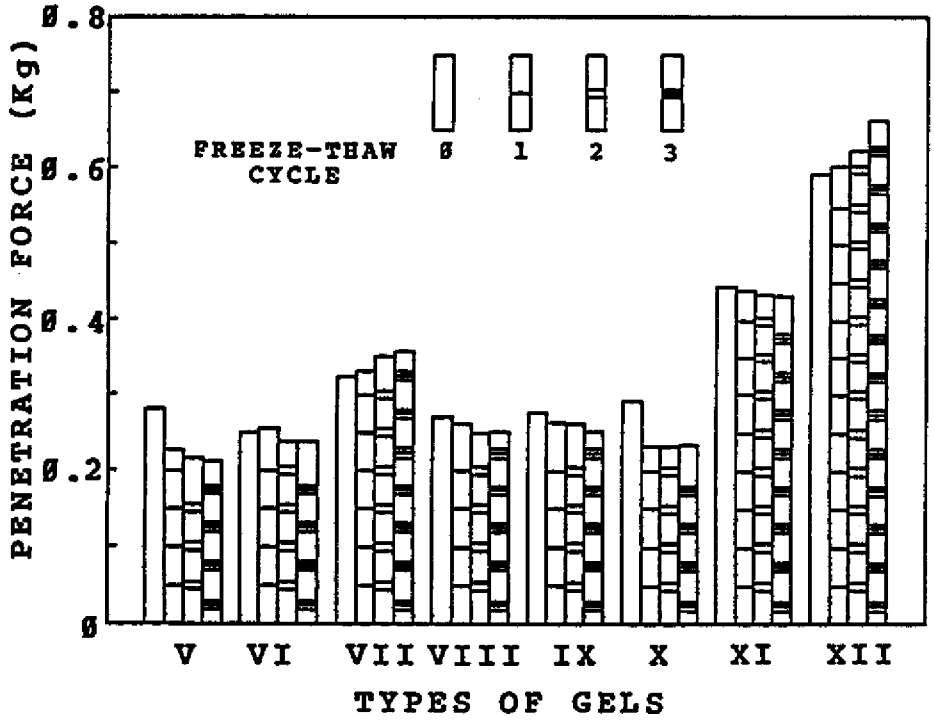


Fig. 7. Effect of various ingredients with 6% modified starch on penetration force of surimi gel during freeze-thaw cycles.

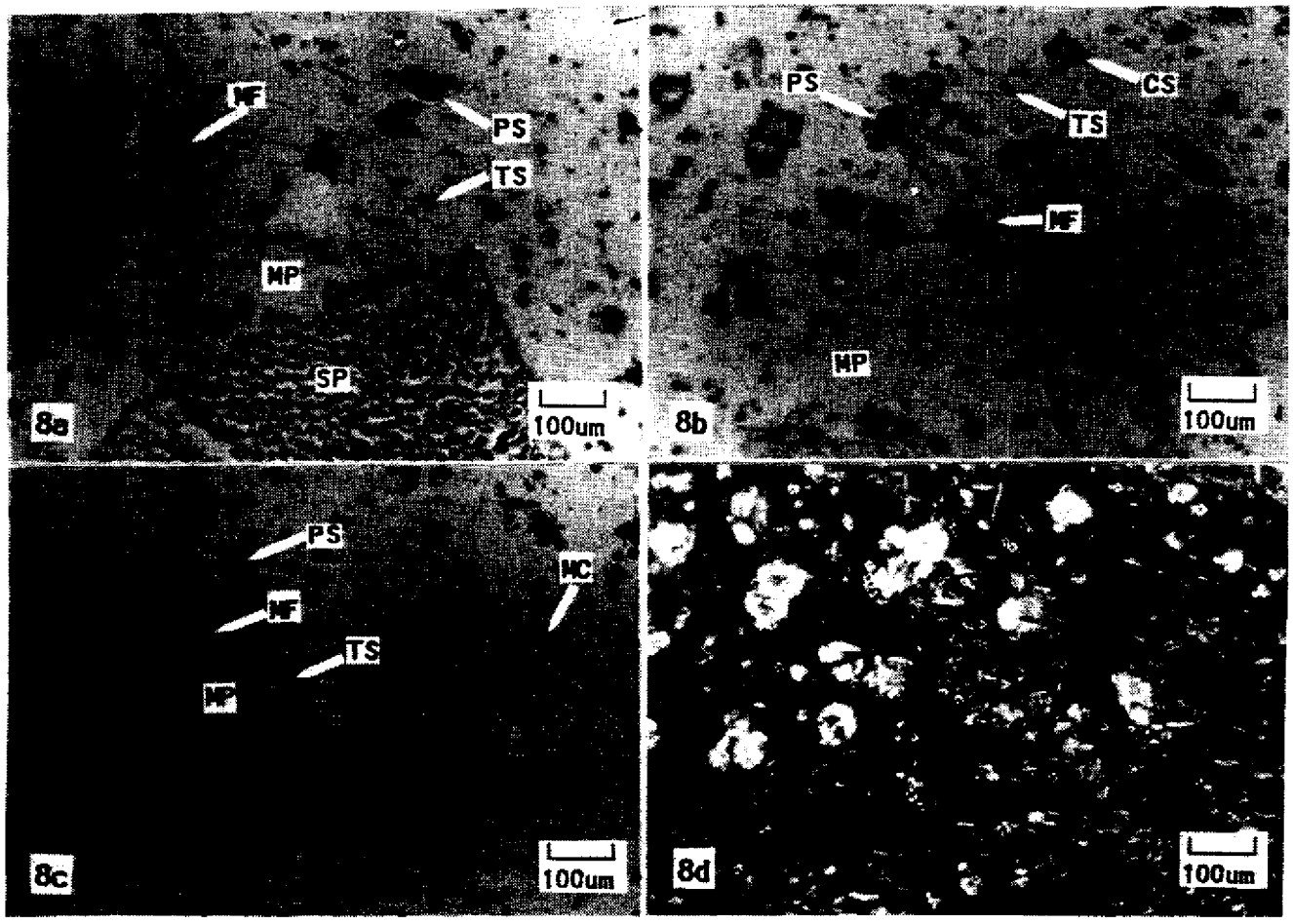


Fig. 8. Squid surimi pastes prepared with various ingredients. CG: collagen; CS: cellulose; EP: excessively gelatinized potato starch; MC: muscle associated collagen; MF: muscle fibers; MP: muscle protein; PS: potato starch; SP: soy protein concentrate; TS: tapioca starch. (a) Type VI; (b) Type VIII; (c) Type XI; (d) same as Fig. 8b, but photographed in polarized light.

percent expressible moisture (Fig. 5) by preventing air cell formation caused by the increased volume of swollen starch, which resulted in a decrease in the volume of the gel matrix. This reduced the space for air cell formation (Fig. 9c-9e). There were relatively insignificant changes in compressive (Fig. 3) and penetration (Fig. 7) forces during freeze-thaw cycles in the gels prepared with the modified starch compared to the ones prepared without the starch. By holding the water absorbed from the gel matrix during cooking, modified starch controlled the moisture migration and thus ice crystal formation during freezing (Figs. 10c-10e and 11c-11e). Control of ice crystal formation, i.e., percent expressible moisture during freeze-thaw cycles was synergistically supported by soy protein concentrate and cellulose (Fig. 5). More about this phenomenon will be mentioned later. Accordingly, the water holding ability was improved by the incorporation of freeze-thaw stable modified starch (Fig. 5). Decreased compressive and penetration forces before freezing caused by the incorporation of starch may be explained by two reasons: 1) prolonged heating of squid meat causes excessive shrinkage of the muscle fibers such that the fibers become hardened and densely packed as shown in Figs. 9a-9e; 2) starch granules in squid protein tend to be gelatinized and solubilized excessively during cooking due to the large amount of water resulting from dehydration of squid muscle tissue. Thus, molecular arrangement within the gelatinized starch embedded in the squid gel is loose enough to lower the gel-strength.

On the other hand, gel-strengthening ability of starch in fish protein gel has been well known. The composite gel-reinforcing effect of starch in fish protein gel is due to starch granules embedded in protein gel matrix which, when swollen, exerted pressure on and drew moisture from the matrix. This caused the gel matrix to become more compact and firm. There is a competition for water between starch granules and fish protein system during cooking. Water binding in the protein gel reduced the availability of water for the gelatinization of starch more so in the fish protein gel than in the squid protein gel. This was evidenced by the difference in water holding ability among Type V, Type XI, and Type XII gels. Addition of pollock surimi to squid surimi significantly reduced percent expressible moisture (Type XI). Increasing in pollock surimi further decreased in percent expressible moisture (Type XII, Fig. 5). In this way, swollen starch in fish protein gel acts as an elastic mass, whereas that in squid protein does not. This also reflected the difference in the shape of the gelatinized starch. As can be seen in Fig. 9c, there are many excessively gelatinized potato starch granules in Type V gel as evidenced by the solubilization and the leaching of part of the gelatinized starch. In contrast, there are few excessively gelatinized potato starch granules in Type XI gel (Fig. 9f).

When squid gels prepared without freeze-thaw stable modified starch were subjected to successive freeze-thaw cycles, they showed significantly increased freeze-thaw instability such that the gel became more brittle as indicated by great decreases in compressive (Fig. 2) and penetration (Fig. 6) forces, and increases in percent expressible moisture (Fig. 4). Type II and III gels even did not show break points at 90% deformation for the measurement of compressive force after second freeze-thaw cycle (Fig. 2).

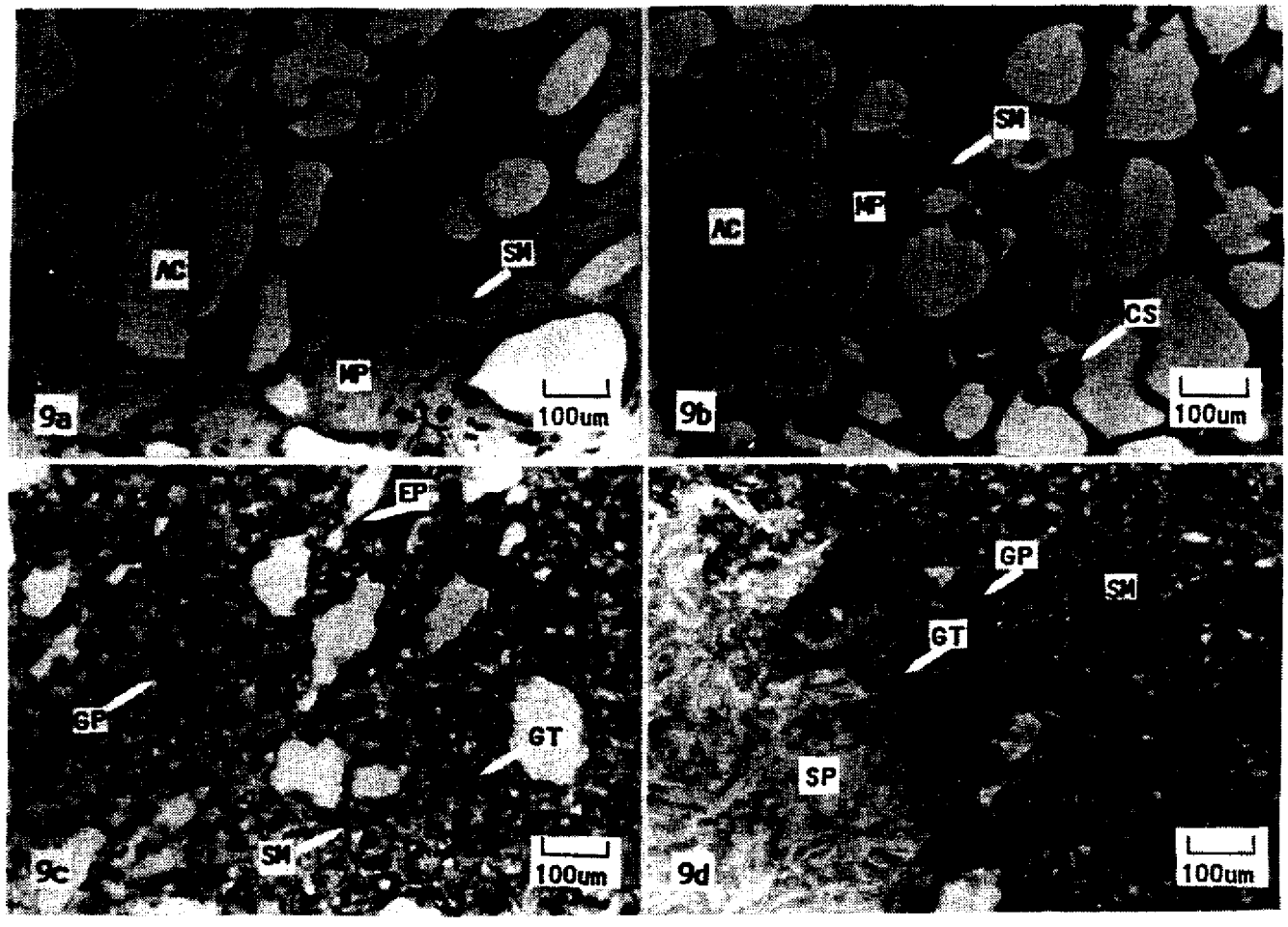
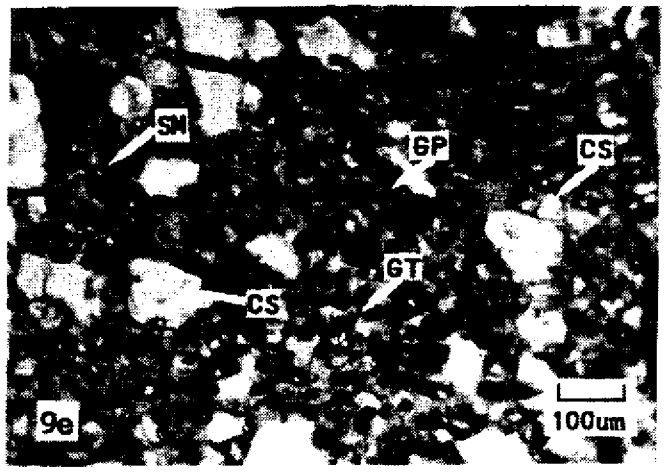
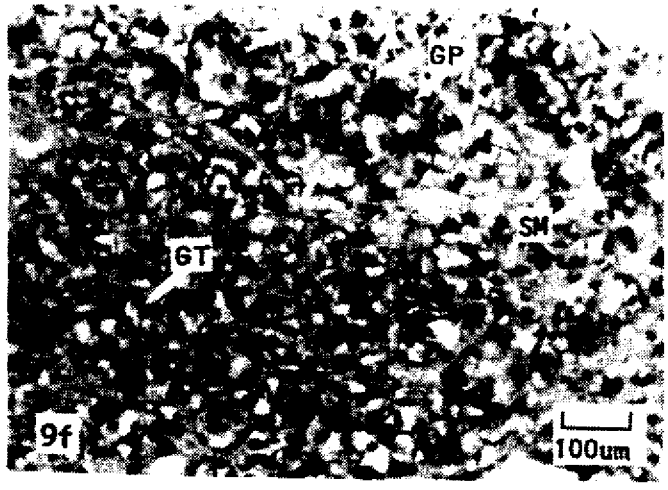
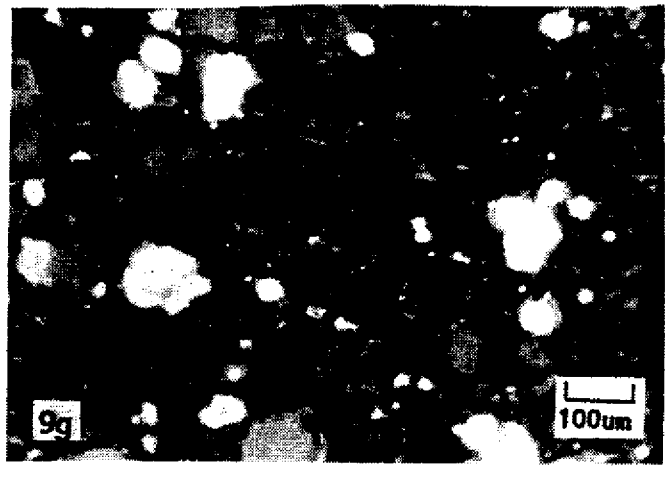


Fig. 9. Surimi gels prepared with various ingredients. GC: gelatinized collagen; GP: gelatinized potato starch; GT: gelatitapioca starch; SM: shrinked collagen or muscle fibers. (a) Type I; (b) Type II; (c) Type V; (d) Type VI; (e) Type VIII; (f) Type XI; (g) same as Fig. 9e but photographed in polarized light.


SM
GP
CS
GT
CS
100um
9e


GP
GT
SM
100um
9f


100um
9g

Soy protein concentrate (SP) did not demonstrate gel-strengthening ability as much as egg white (Figs. 3 and 7), but showed good water holding ability (Fig. 5) as is visualized in Figs. 10d and 11d by controlling ice crystal formation during freeze-thaw cycles. This may explained by the fact that SP imbibed water from the protein gel matrix, swelled during cooking (Fig. 9d), preserved its original structure (Figs. 10d and 11d) during freeze-thaw cycles, and held moisture within the structure to prevent ice crystal growth. However, it did not reinforce gel strength like an elastic mass of gelatinized starch due to loosely arranged internal structure as is shown in Fig. 9d. Gel prepared with egg white (EW) and starch (Type VII) demonstrated the highest cohesiveness (Fig. 3) and firmness (Fig. 7) with good water holding ability (Fig. 5) except those prepared with pollock surimi. Although sodium alginate-containing paste was highly pseudoplastic (Fig. 1), its heat-induced gels (Types III, IV, and X) did not show greater gel strength compared to others (Fig. 2-7). This may be explained by the fact that gel formed by ion exchange between sodium and calcium, in other words, insoluble calcium alginate formed from soluble sodium alginate apparently is not stable at the cooking conditions used in this study. Furthermore, gel matrix of the calcium alginate in the paste is disrupted during cooking by the excessive shrinkage of squid protein.

It has been claimed that microcrystalline cellulose (MC) helps prevent moisture migration and also inhibits the irreversible aggregation of protein and other solids during cooking and frozen storage of foods. MC alone in the gel (Type II) could not control the aggregation of squid protein leading to the formation of many areas of air cells (Fig. 9b) and high percent expressible moisture before freezing of the gel. MC was not very helpful in the prevention of ice crystal growth (Figs. 10b and 11b) resulting in high percent expressible moisture during freeze-thaw cycles (Fig. 4). MC, its role as a physical barrier to aggregation of protein and moisture migration, cannot play a significant role alone if the protein shrinks excessively. If used with 6% modified starch (Type VIII), MC synergistically demonstrated significantly low percent expressible moisture before and during freeze-thaw cycles (Fig. 5). After a third freeze-thaw cycle, MC still maintained its homogeneous state of the system during drastic physical changes in the gel (Fig. 11b and 11e) and demonstrated bright birefringence in polarized light (Fig. 11g). Being located in air cells or in ice crystal area, MC physically can prevent the free water migration upon thawing. Results of percent expressible moisture before and during the freeze-thaw cycles and microstructure of gels (Types VI and VIII) suggest that absorption of water from surrounding gel matrix, retainment of the absorbed water, and preservation of its original structure are essential for an ingredient to provide a freeze-thaw stability.

Squid surimi gels prepared with those ingredients discussed so far (Types I-X) had poor textural properties and would not be accepted commercially. In an attempt to improve texture, various levels of Atlantic pollock surimi were added to squid surimi with 6% modified starch. Formulations prepared with more than 60% pollock surimi (Types XI and XII) provided desirable texture for compressive force (Fig. 3), percent expressible moisture (Fig. 5) and penetration force (Fig. 7). Textural properties were supported by the gel microstructure before

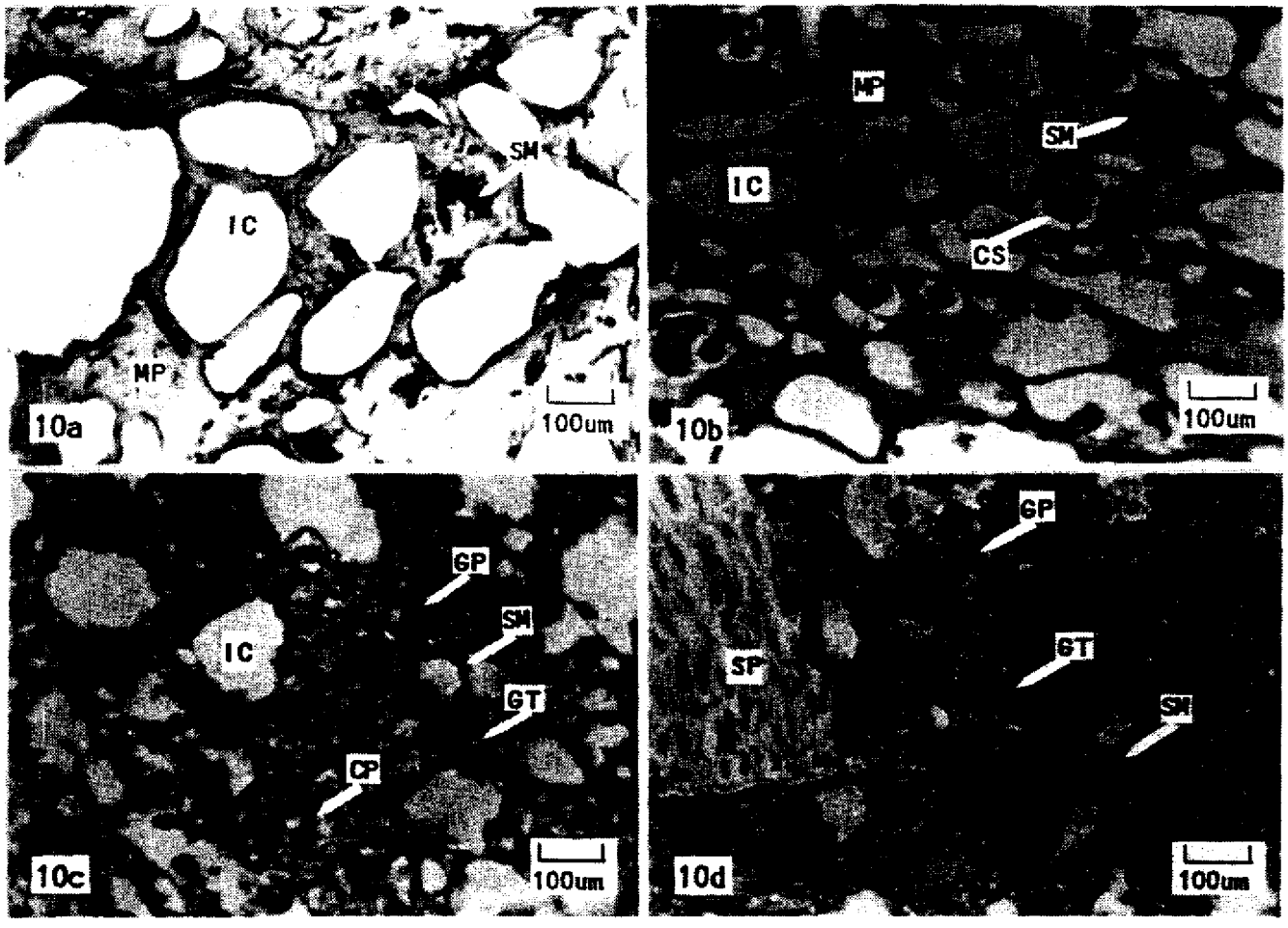
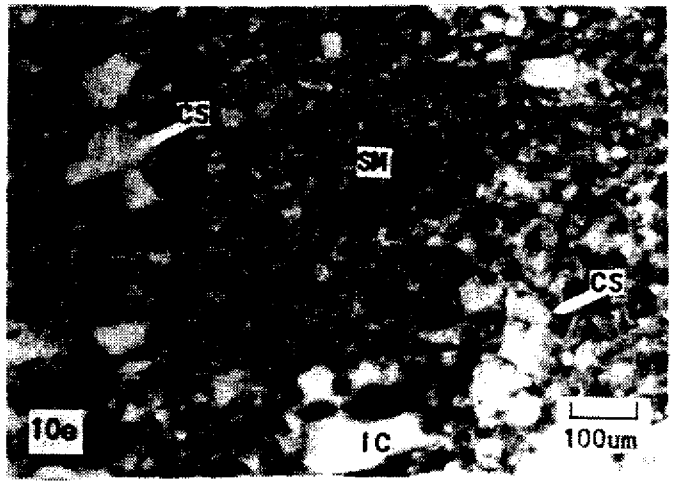
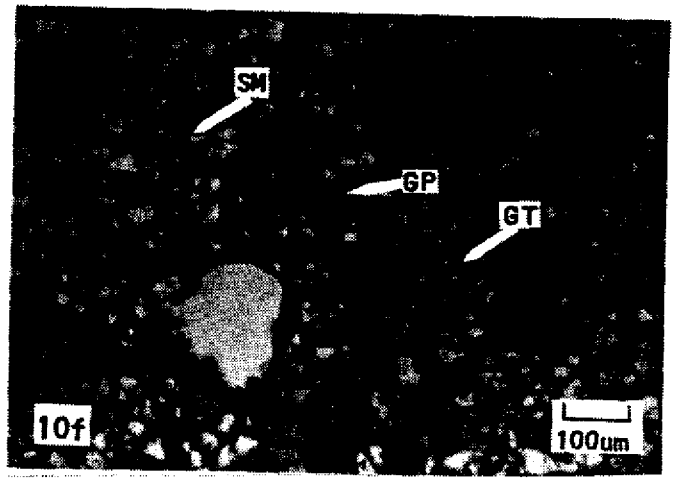


Fig. 10. Surimi gels after first freeze-thaw cycle. CP: collapsed potato starch; IC: ice crystal. (a) Type I;(b) Type II; (c) Type V; (d) Type VI; (e) Type VIII; (f) Type XI; (g) same as Fig. 10e, but photographed in polarized light.



10e



10f

10g

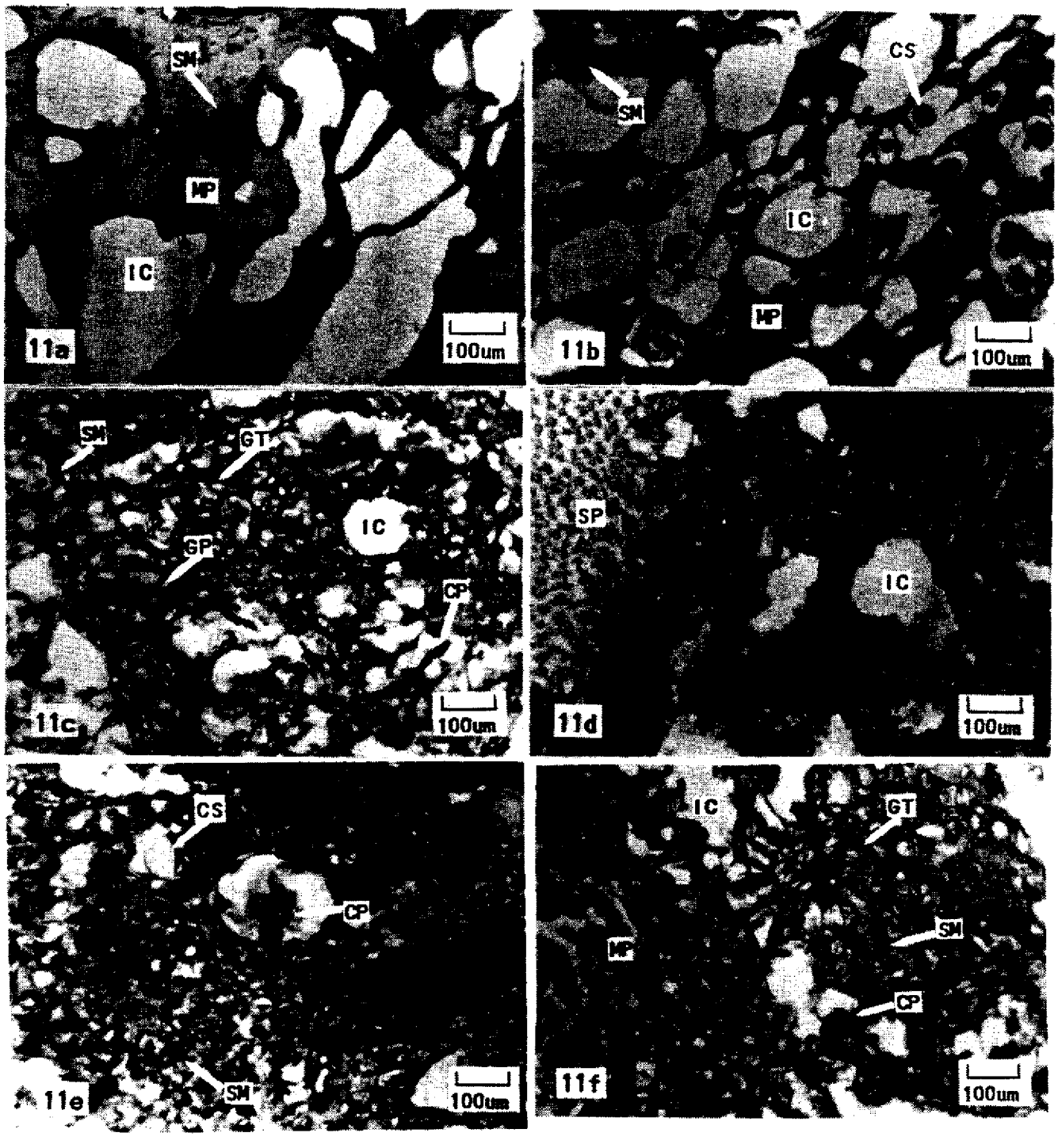


Fig. 11. Surimi gels after third freeze-thaw cycle. (a) Type I; (b) Type II; (c) Type V; (d) Type VI; (e) Type VIII; (f) Type XI.

(Fig. 9f) and during successive freeze-thaw cycles (Figs. 10f and 11f).

In summary, 1) there is no significant difference in sol characteristics among squid surimi pastes containing various ingredients except those containing sodium alginate; 2) sodium alginate containing sol showed significantly higher consistency and lower flow behavior indices than EW, MC, and SP containing sol; 3) incorporation of starch in the squid gel decreased compressive and penetration forces due to excessive gelatinization of starch granules; 4) alginate gel matrix did not demonstrate the gel-strengthening ability probably due to excessive shrinkage of squid protein; 5) in order to reinforce gel strength, an ingredient must absorb moisture from gel matrix, swell to exert pressure on the surrounding gel matrix, and maintain internal structure to some extent to be able to act as an elastic mass in the gel matrix; 6) in order to act as a physical barrier to freely moving water and to provide a freeze-thaw stability, ingredient must withdraw moisture from gel matrix during cooking and retain its original state of structure and the absorbed water within the structure during drastic physical changes due to successive freeze-thaw cycles; 7) squid surimi gel prepared with more than 60% pollock surimi and 6% modified starch had commercially acceptable texture; 8) structure study reflected the textural properties of gels.

REFERENCES

1. Anonymous. 1987. Technical Information. Protan, Inc. North Hampton, NH.

2. Anonymous. 1987. Product description. Bulletin G-34. FMC Corporation, Philadelphia, PA 19103.

3. Kahn, L.N., Berk, Z., and Pariser, E.R. 1974. Squid protein isolate: effect of processing conditions on recovery yields. J. Food Sci. 39:592.

4. Kim, J.M. and Lee, C.M. 1986. Effect of various combinations of protein and starch on freeze-thaw stability of surimi gels. Presented at 31st Atlantic Fisheries Technological Conference, Halifax, NS, Canada, August 17-20.

5. Kim, J.M. and Lee, C.M. 1986. Mechanisms of gel reinforcing effect of starch in fish protein gel. Paper No. 460, presented at 46th Annual Meeting of Inst. of Food Technologists, Dallas, TX, June 15-18.

6. Kim, J.M. and Lee, C.M. 1986 Comparison of various commercial starches for their effects on freeze-thaw stability of surimi gels. Paper No. 461, presented at 46th Annual Meeting of Inst. of Food Technologists, Dallas, TX, June 15-18.

7. Kim, J.M. and Lee, C.M. 1987. Effect of starch on textural properties of surimi gel. J. Food Sci. 52(3):722.

8. Kim, J.M., Lee, C.M., and Hufnagel, L.A. 1987. Textural properties and structure of starch-reinforced surimi gels as affected by heat-

setting. Food Microstruct. 6:81.

9. Kolodziejska, I., Sikorski, Z.E., and Sodowska, M. 1987. Texture of cooked mantle of squid Illex argentinus as influenced by specimen characteristics and treatments. J. Food Sci. 52(4):932.

10. Lang, E.R. and Rha, C. 1981. Determination of the yield stress of hydrocolloid dispersions. J. Text. Studies 12(1):47.

11. Lanier, T.C. 1986. Functional properties of surimi. Food Technol. 40(3):107.

12. Leach, H.W. 1965. Gelatinization of starch. In "Starch: Chemistry and Technology," vol. 1, R.L. Whistler, E.F. Paschall (eds.), Academic press, New York, p.289-307

13. Lee, C.M., Lee, T.C., and Chichester, C.O. 1974. The potential use of squid as a protein resource. In "Fishery products", R. Kreuzer (Ed.), Fishing News (Books) Ltd. Surrey, England, p.243.

14. Lee, C.M. and Kim, J.M. 1985. Texture and freeze-thaw stability of surimi gels in relation to ingredients and formulation. In "proceedings of the international symposium on engineered seafood including surimi." R.E. Martin and Collette (ed.), National Fisheries Institute, Washington, D.C., p.168.

15 Lee, C.M. and Kim, J.M. 1986. The relationship of composite characteristic to rheological properties of surimi sol and gel. In "Food engineering and applications, vol. 1," M. Lemaguer and P. Jelen (eds.), Elsevier Applied Science publishers, London and New York, p. 63.

16. Martin, R.E., Flick, G.J., Hebard, C.E., and Ward, D.R. 1982. Chemistry & biochemistry of marine food products, AVI Publishing company, Westport, CT.

17. Martin, R.E. 1986. Developing appropriate nomenclature for structured seafood products. Food Technol. 40(3):127.

18. Matsumoto, J.J. and Okuyama, E. 1958. On the solubility in water of the fibrous proteins of the squid muscle;phase-contrast and electron microscopic study. Bull. Tokai Reg. Fish. Res. Lab. 24:81.

19. Matsumoto, J.J. 1958. Some aspects on the water-soluble proteins of squid muscle. Bull. Tokai Reg. Fish. Res. Lab. 20:65.

20. Matsumoto, J.J. 1958. Studies on muscle proteins of the squid. Bull. Tokai Reg. Fish. Res. Lab. 23:51.

21. Matsumoto, J.J. 1959. Identity of M-actomyosin from aqueous extract of the squid muscle with the actomyosin-like protein from salt extract. Bull. Jap. Soc. Sci. Fish. 25(1):38.

22. Nitisewojo, P. and Hultin, H.O. 1986. Characteristics of TMAO

degrading systems in Atlantic short finned squid (Illex illecebrosus). J. Food Biochem. 10:93.

23. Okada, M. and Yamazaki, A. 1957. Enhancing effect of starch on jelly strength of fish meat jelly. 1. Enhancing effect and gelatinization of starch. Bull. Jap. Soc. Sci. Fish. 22:583.

24. Otwell, W.S. and Hamann, D.D. 1979. Textural characterization of squid (Loligo pealei Lesuer): scanning electron microscopy of cooked mantle. J. Food Sci. 44:1629.

25. Otwell, W.S. and Hamann, D.D. 1979. Textural characterization of squid (Loligo pealei L.): instrumental and panel evaluations. J. Food Sci. 44:1636.

26. Saffle, R.L. 1973. The use of squid in meat emulsions. J. Food Sci. 38:551.

27. Stanley, D.W. and Hultin, H.O. 1982. Quality factors in cooked North Atlantic squid. Can. Inst. Food Sci. Technol. J. 15(4):277.

28. Tanaka, T. 1958. Histological and histochemical study on processing the squid meat - I. Histological properties of the squid meat. Bull. Tokai Reg. Fish. Res. Lab. 20:88.

29. Tsuchiya, T., Shinohara, T., and Matsumoto, J.J. 1980. Physico-chemical properties of squid tropomyosin. Bull. Jap. Soc. Sci. Fish. 46(7):893.

30. Wu. M.C., Hamann, D.D., Lanier, T.C. 1985. Rheological and calorimetric investigations of starch-fish protein systems during thermal processing. J. Text. Studies. 16:53.

31. Wu, M.C., Lanier, T.C., and Hamann, D.D. 1985. Thermal transition of admixed starch/fish protein systems during heating. J. Food Sci. 50(1):20.

32. Yamanaka, H., Shiomi, K., and Kikuchi, T. 1987. Agmatine as a potential index for freshness of common squid (Todarodes pacificus). J. Food Sci. 52(4):936.

33. Yang, T.C.S. and Yang, A.P.P. 1986. Squid tentacle protein: extraction and its effects on the quality of Atlantic pollock surimi gels. Can. Inst. Food Sci. Technol. J. 19(4):158.

34. Yoshitomi, B., Kamiya, S., and Konno, K. 1984. Heavy meromyosin from squid mantle muscle. Bull. Jap. Soc. Sci. Fish. 50(9):1555.

ACKNOWLEDGEMENTS

This work was supported in part by the Maine Agricultural Experiment Station under project No. ME 08911 and ME 08553. Maine Agricultural Experiment Station Contribution No. 1233.

U.S. FISHERY BYPRODUCTS: A SELECTIVE UPDATE AND REVIEW

Susan H. Goldhor
Center for Applied Regional Studies
67 Highland Avenue, Cambridge, MA 02139
and
Joe M. Regenstein
Cornell University, Ithaca, NY 14853-5601

In this paper we would like to discuss three types of fish byproduct utilization, two of which originated in Northern Europe and the third in Canada, and all of which are being actively developed and commercialized in this country today. Our three topics are: composting of fish wastes with peat; fish protein hydrolysis; and utilization of fish meal in dairy cow rations. Each represents a very different approach towards the handling of fish processing plant wastes and industrial fish, and each of these approaches will make sense for some processors and fisheries.

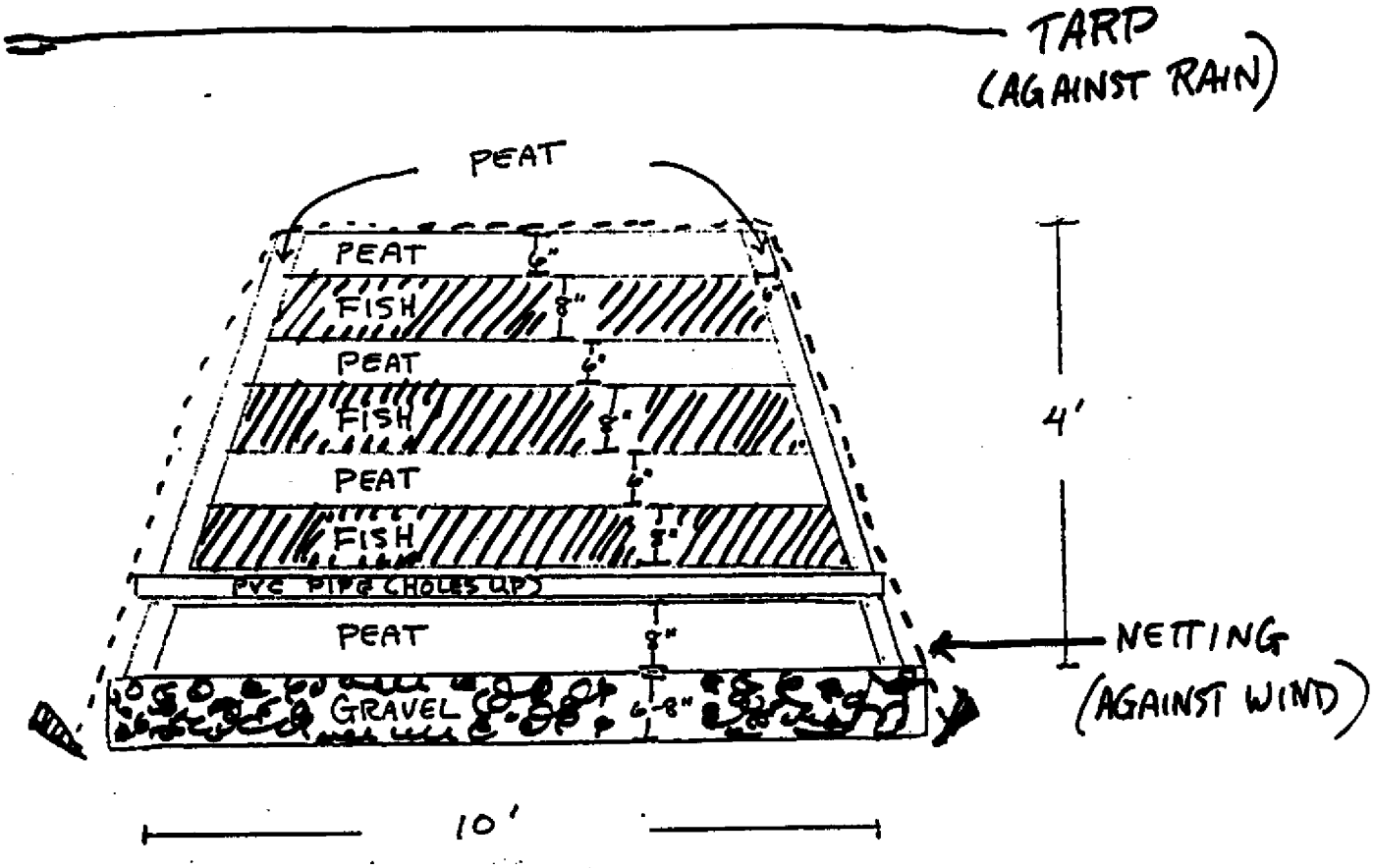
Composting fish wastes with peat: On the surface, composting fish wastes with peat is the simplest of the three technologies under discussion and, indeed, of all fish waste disposal methods, with the exception of dumping which is becoming both economically and environmentally unacceptable. Composting requires minimal capital investment, no engineering, and little training on the part of the operator. It is particularly attractive for three reasons. First, if properly handled it offers a completely clean method of waste disposal, generating no leachate and very little odor. Second, it produces an odorless, organic, nitrogen enriched soil amendment, which is saleable to home gardeners, highway departments, and others. Third, it is capable of handling not only standard finfish processing wastes but also extremely intransigent wastes which are unacceptable for rendering, such as those from dogfish or shellfish processing. On the other hand, composting, like many biological technologies, can be quite variable. Experienced operators get a "feel" for building and maintaining workable piles and can diagnose and cure most problems. Inexperienced operators are advised to start small and use an experienced consultant.

The initial work on composting fish, shellfish, and seaweed wastes with peat was done by Dr. Sukhdev Mathur of Agriculture Canada and his group (Mathur, 1986). Dr. Mathur's group has made two major contributions. First is the use of peat with which to compost fish. Under aerobic conditions, rotting fish generate significant quantities of ammonia, while sphagnum-dominated peats have high exchangeable acidity which permits the peat to adsorb ammonium ions up to 3% of dry weight. Air-dried peat also has an enormous water absorbing capacity — up to thirty times its dry weight — and this is a key point in preventing leachate. Their second major contribution is the design of a compost pile which is self-aerating and thus saves the labor and equipment costs associated with turning or mixing.

Mathur's work was subsequently repeated and extended by Joan Brooks (Brooks, 1986) of the University of Maine, who showed that even dogfish wastes could be effectively handled by this technology. At the end of the composting period almost all of the wastes have disappeared; the small amounts of bone, fin, or

shell left in the pile are removed by screening if the product is to be bagged and sold retail.

The standard dimensions of the pile are 10' wide by (minimally) 6' long by 3 to 4' high. The pile is trapezoidal in shape so that the top is significantly smaller than the base. The bottom of the pile rests on a base of gravel which aids air flow. However, the major sources of air flow are the PVC sewer and drainage pipes with holes, which go through the pile at about 2' intervals. Fig. 1 shows a cross-sectional view of such a pile.



FISH - PEAT COMPOST PILE IN CROSS SECTION

Figure 1. A Typical Peat/Fish Compost Pile in Cross-section

1. The ratio of fish to peat by volume is about 3:2. Since the peat is fluffed out before use, this means that a relatively small weight of peat can compost a relatively large weight of fish.

2. Note that there is no gurry left unsurrounded by peat once the pile has been constructed. This is important as any gurry left exposed will attract animals and repel humans. The netting is to protect the pile from wind which could expose the gurry.

3. Each fish layer must have compost starter inoculum added to it. This may be purchased commercially. After the first batch has composted, a portion of it may be used to start subsequent piles.

4. Experimental piles should be monitored for temperature; this is generally done by burying a thermistor in the pile. The temperature should rise to between 45°C and 65°C in a pile of this size. Composting is complete when the temperature falls to near ambient; generally eight to fourteen weeks in the Northeast. Variations exist with the material composted and with the weather; composting has been carried out in cold, wet weather but not in winter.

5. Peat used for composting must have a water content of 30 to 60%; if the water content is lower than this, the pile could combust.

This technology is said to be in commercial use in Quebec. At least two Maine processing companies (one handling dogfish and one packing sardines) are investigating and developing composting as an environmentally innocuous and potentially profitable means of waste disposal.

Maine has been a center for research on fish processing waste composting. One of the most active workers over the past year has been Phil Averill of the Maine Department of Marine Resources (DMR), who has concentrated on developing alternatives to peat for composting with fish. Currently, Maine envisions large scale regional composting centers where most processors would bring their wastes; only a relatively small number of isolated processors would do their own composting. One area of interest to the Maine DMR at this time is the materials handling aspects of such centers, where extremely large piles requiring turning and aerating would be built, and where stocks of carbohydrate sources would be maintained.

Among the questions currently being asked by composting researchers, including those mentioned above, the authors of this paper, and others, are:

*Can composting be done outdoors in Northern winters?

*What problems arise from composting indoors?

*Can sawdust, manure, pomaces, etc. replace some or all of the peat?

*Can the process be speeded up?

*Can a higher ratio of fish to other components be composted?

*What are the size limits of a static pile using pipes for aeration?

* * * * *

Fish protein hydrolysis: Like composting, fish protein hydrolysis (FPH) is an old technology which started out as a simple, low capital cost method for waste utilization. However, unlike composting, this technology has developed and expanded enormously, and has become complex and costly in some

applications. Balancing this increase in cost is an increase in value of the end products, which are finding new market niches in this country (Seafood Management Corporation, 1986).

All hydrolysing technologies use proteolytic enzymes to digest the fish protein and liquefy the mass of gurry. The major differences lie in the enzymes used, the extent to which digestion is controlled, the separation of one or more components of the hydrolysate, and the methods used to stabilize the final product.

The direct ancestor of FPH was fish silage, a product developed in Norway during World War II. For silage, the fish is ground and then preserved by acidification, usually with organic acids such as formic. The proteolytic enzymes in the fish are active at the levels of acidity used, and gradually digest and liquefy the fish. This technology is related to the ancient process by which fish sauces are produced; however, fish sauce is preserved during autolysis by salting rather than acidification.

Fish silage is still manufactured; in fact, the advantages which suited it to wartime Europe make it a useful technology for Third World countries and small fish processors. The equipment can be quite simple. All it takes is a grinder, a wooden tub, paddles and acid-proof storage containers. If the proper amount of acid has been added, fish silage may be stored for years with no odor or spoilage. Fish silage of this sort can be further processed into a high quality fertilizer or utilized as a livestock feed of rather mediocre quality (Raa, 1982; Windsor and Barlow, 1981; Winter, 1983).

In the next stage of complexity, heat can be added to one or more steps. Heat added to the digestion stage speeds up liquefaction. Heating also permits oil to be separated off. An additional advantage of the heat applied for oil separation is that it is sufficient to inactivate the enzymes. One reason why the primitive silages are poor feedstuffs is that they tend to be overdigested, since the viscera provide a mix of enzymes which continue to work on the protein present until a significant proportion of single amino acids and even non-protein nitrogen is generated. Stopping the digestion at a set point, before digestion has progressed too far, gives a product which is both of higher nutritional value, and more consistent from batch to batch.

Many wastes lack viscera entirely and, even when viscera are present, the quantity and quality of their enzymes varies. Thus, some of the most recent hydrolysis technologies involve the use of commercially available proteases. These provide limited, consistent, highly controllable digestions. Since digestion occurs so rapidly, spoilage is not a problem and digestion may be carried out at a neutral pH. When the process is completed, the product may be stabilized by drying or by acidification. Particularly if the product is to be dried or concentrated, equipment costs for this process can be quite high, and plant design becomes both complex and expensive (American Composite Technology, 1986; Windsor and Barlow, 1981). In addition to controlling digestion by the use of specific enzymes under set conditions of time, pH and temperature, recent advances include the use of anti-oxidant and anti-mold ingredients to improve palatability and shelf-life.

Hydrolysates are still minor products in the U.S. compared to, say, fish meal, but they are increasing rapidly. They are also extremely variable

compared to a commodity like fish meal. Because hydrolysates are so new and so varied, it is appropriate to list the unique qualities which specific hydrolysates can offer the marketplace.

*Palatability enhancement for a wide range of monogastric species, ranging from cats and dogs to salmon.

*Improved digestibility for very young animals.

*No antigenic reactions in the immature gut.

*High solubility.

*A choice of oil contents.

*A choice of wet, semi-moist, or dry product forms.

*A choice of peptide size ranges.

*Extremely high protein contents.

*Low ash contents.

*Sprayability.

To processors, hydrolysis offers the advantages of being environmentally benign, of having lower capital costs than fish meal, and of having potentially higher profits than fish meal. However, there are disadvantages as well. Some of the emerging markets for hydrolysates are small and hard to locate and/or define. Machinery and engineering are less well defined and harder to locate than that for older, better known technologies. Offal may need to be separated into streams in some operations. And, as with many other byproducts, the rule of "garbage in; garbage out" still holds true.

At this time there is probably more commercial work on hydrolysates in the U.S. than there is academic research. However, Dr. Robert E. Levin of the University of Massachusetts in Amherst, and the senior author, under contract to the New England Fisheries Development Foundation, looked at a cross-section of variables in hydrolysis technology. Basic hydrolysis procedures are not complex. However, the fact that there are so many potential variables in hydrolysis, and that each of them affects the suitability of the product for its end market -- plus the fact that these markets are young and still changing rapidly -- creates complexity in hydrolysis.

Much of the more general aspects of what was learned from the above project will be published by the Foundation in the form of a "how-to-do-it" handbook for fish processors and other non-specialists (Goldhor, 1988).

The major investment and interest in North American commercial fish hydrolysing has been in producing a feed ingredient for the booming salmon aquaculture industry. However, industrial approaches vary. There are single-minded companies like some aquaculture feed or fertilizer companies or a California company which opened a plant about a year ago to produce a spray dried, enzyme hydrolysed whitefish product aimed at the weaner pig market and

patterned after a classic French product made by Soprapeche. At the other end of the spectrum are companies like Bioproducts (Oregon), which produces a wide variety of aquaculture feeds and pet food palatability enhancers, and which hydrolyses a number of feed stocks, or Stinson Canning Company (Maine) which has mounted an impressive R & D operation resulting in a group of imaginative new products, ranging from a moist baby pig product acidified by a lactobacillus fermentation, to pet food palatability enhancers, to a product which is mixed with dry ingredients and extruded under heat and pressure to produce a dairy cow supplement.

* * * * *

Fish meal for dairy cows: The first mention of proposed U.S. research on feeding fish meal to cattle was at the Boston AFT conference two years ago. Since that time, support for this project has come from the New England Fisheries Development Foundation, the Center for Applied Regional Studies, Cornell University, the International Association of Fish Meal and Oil Manufacturers, and the Zapata Haynie Corporation.

Why feed fish meal to dairy cattle? The answer to this question lies in the nature of the rumen. The rumen of a cow or sheep is best viewed as an enormous vat full of culture medium for a zoo of microorganisms. The cow eats grass, the microbes digest its cellulose and protein, and the cow gets her protein from the bodies of the microbes. This arrangement worked well up until recently; however, for a modern, high producing dairy cow, the microbial protein produced on a grass diet is inadequate in both quantity and quality. Such a cow needs high quality rumen undegradable protein (sometimes inaccurately called "by-pass protein") which will pass through the rumen and arrive intact in the gut where it can go to the cow instead of to the microbes.

Fish meal is a particularly good source of such protein. Only 30 to 40% of fish meal degrades in the rumen, which is less than most other protein sources fed to cattle. Current thinking in the field holds that the portion of fish meal that is rumen degradable has an amino acid profile particularly useful for stimulating bacterial growth, and a slow rate of release helpful in maintaining rumen nitrogen levels, while the portion of fish meal which is digested in the gut has an amino acid profile which complements that provided to the cow by the rumen microorganisms. Fish meal also appears to increase the efficiency with which cows utilize their feed. Most impressive, all of these benefits are conferred by feeding low levels of fish meal averaging one pound per cow per day. There are close to ten million dairy cows in the U.S. so that even if only, say, a quarter of those cows got a pound of fish meal for a quarter of the year, that would account for close to 250 million pounds of fish meal per year at a time when sales to the poultry industry have been declining. Indeed, this has been the economic rationale behind our work on this project.

The British have been feeding fish meal to dairy cows for about a decade but British fish meal, British dairy cow diets, and British milk are all different from ours. Two years ago, when we started this project, most U.S. dairy nutritionists knew almost nothing about fish meal and were very suspicious of it. The major fears were first, that fish meal would be unpalatable to cattle so that they would go off feed, and second, that fish meal would taint the milk. We are pleased to report that work by Drs. David Barbano and Charles Sniffen at Cornell has just been completed which demonstrates that even

quantities of fish meal significantly higher than those recommended for diary feeding will not taint milk. A relevant point here is that the use of the anti-oxidant, ethoxyquin, in fish meal is a relatively recent improvement. Some of the earlier animal feeding experiments were done with unprotected meals which had turned rancid.

It is true that even good quality fish meal is unpalatable to cows when it is first introduced. Once cows become used to it, however, they seem to like it. The problem is that while they are getting used to it, milk production drops dramatically. After we had learned this -- the hard way -- we started recommending that cows be introduced to fish meal at least two weeks before they started the lactation cycle. This way, if they do cut down on feed, it does not affect milk production.

Although fish meal is a more consistent product than hydrolysates, it is far from uniform. Fish meal is made from different types of fish or fish processing wastes with widely differing oil and ash contents; the stick water or solubles pressed out of the initial cooked presscake may or may not be returned to the meal, and no two plants in the U.S. seem to use precisely the same method of drying the meal.

Two years ago, visiting British fish meal specialists suggested that the best meal to feed to dairy cows was a whitefish meal with no solubles added back. The reasoning behind their suggestion was, First, that the long chain polyunsaturated fatty acids of fish oil are toxic to rumen microbes, while whitefish meal is extremely low in oil. Second, solubles, because they would be expected to literally be soluble in the rumen, do not add to the rumen undegradable protein, so they should be eliminated. These reasons were logical -- so much so that we ought to have been suspicious. But, since the major U.S. fish meal is a high oil, high-to-moderate solubles menhaden meal, we entered the arena believing that a potential opportunity for a specialized ruminant meal made of whitefish processing wastes might exist here. However, when Cornell researchers, Drs. D.E. Hogue and M.L. Thonney, supplemented their earlier work in feeding menhaden meal to sheep and cattle with feeding trials using whitefish meal, no advantages and some potential disadvantages were seen (Hogue and Adam, 1982; Thonney and Hogue, 1986; Thonney et al., 1987). Meanwhile, we had become curious about the bypass value of fish meal. Was it really as uniform as implied by standard feed tables, which list a single value? Samples of fish meals were solicited from the U.S. industry which responded more generously than analytical capabilities could handle. Rumen degradability was measured by Dr. C.J. Sniffen's laboratory at Cornell using a variant of the method of Krishnamoorthy (Krishnamoorthy et al., 1983) and the values differed enormously. In general, although not invariably, meals with a higher bypass value had a higher oil content. (In fact, E.R. Orskov, at the Rowett Research Institute in Aberdeen, Scotland, had seen this earlier (Orskov, 1982), but his work had escaped our notice. It also turns out that the British do not feed mainly whitefish meal to cattle but, rather, intermediate oil herring meals.)

We then obtained, from the Zapata Haynie corporation, meals with and without added solubles. There was no consistent relationship between solubles content and rumen degradability except in meals which had been vacuum dried, the gentlest drying method. Our current theory is that the more common, harsher

drying methods impart a reasonable degree of rumen undegradability to the solubles.

There are still a number of challenges remaining and we are still involved with trying to solve them.

1. Fitting fish meal to the economics and management practices of U.S. dairy farms. Fish meal is by far the most expensive source of protein a dairy farmer can use. It is not economical for all cows, all the time, on all diets, and under all management practices. We need to find out when it is economical and get that information to farmers, nutritionists, extension agents, and feed manufacturers. In general we believe that highly rumen degradable diets, such as those high in alfalfa, hay, and their silages will benefit the most from fish meal.

2. Solving the fish oil problem. The polyunsaturated fatty acids in fish oil are toxic to the rumen microorganisms, in such a way that although milk production may not be affected, butterfat production may go down. The reasons are not understood, although a number of theories exist. We have just started to work on ways to diminish or eliminate the butterfat depression by changing the cow's diet while continuing to feed a reasonably high oil meal.

3. Working with the fish meal industry. In Great Britain and Scandinavia, fish meals are labelled and sold as specialty products -- not as a generic commodity. We believe that this is the right approach. Ruminant feed markets want to know accurate oil levels and rumen undegradability as well as total protein, ash, etc. They also want meals selected for lower oil content and moderately high undegradability. We believe that such selection and labelling will exist in this country within two to three years.

REFERENCES:

American Composite Technology. 1986. Fish Waste Handling Systems for New England: Engineering Specifications. New England Fisheries Development Foundation, 280 Northern Ave, Boston, MA 02210.

Brooks, J. L. 1986. Composting fish and shellfish scrap with peat. Report, Department of Civil Engineering, University of Maine, Orono, ME 04469.

Goldhor, S. H. 1988. Fish Protein Hydrolysis: A User's Guide. New England Fisheries Development Foundation, 280 Northern Ave, Boston, MA 02210.

Hogue, D. E. and Adam, A. A. 1982. Protein sources for young rapidly growing lambs. Proc. Cornell Nutr. Conf., pp. 103-106.

Krishnamoorthy, U., Sniffen, C.J., Stern, M.D., and Van Soest, P.J. 1983. Evaluation of a mathematical model of rumen digestion and an in vitro simulation of rumen proteolysis to estimate the rumen-undegraded nitrogen content of feedstuffs. British J. Nutr. 50:555-568.

Mathur. S. P., Daigle, J.-Y., Levesque, M., and Dinel, H. 1986. The

feasibility of preparing high quality composts from fish scrap and peat with seaweeds or crab scrap. Biol. Agric. and Hortic. 4:27-38.

Orskov, E. R. 1982. Protein Nutrition in Ruminants. Academic Press, New York, p. 58.

Raa, J. and Gildberg, A. 1982. Fish silage: a review. CRC - Critical Reviews Series in Food Sci. and Nutr. 16:383-419.

Seafood Management Corporation. 1986. New Markets for Maximizing New England Fisheries Byproduct Values. New England Fisheries Development Foundation, 280 Northern Ave, Boston, MA 02210.

Thonney, M. L. and Hogue, D. E. 1986. Fish meal or cottonseed meal as supplemental protein for growing Holstein steers. J. Dairy Sci. 69:1648-1651.

Thonney, M. L., Fox, D. G., Duhaime, D. J., and Hogue, D. E. 1987. Fish meal and apple pomace for growing cattle. Proc. Cornell Nutr. Conf., pp. 106-114.

Windsor, M and Barlow, S. 1981. Introduction to Fishery By-Products. Fishing News Books, Ltd., Farnham, Surrey, England.

Winter, K. A. and Feltham, L. A. W. 1983. Fish silage: the protein solution. Contribution 1983-6E, Agriculture Canada, Research Branch, P. O. Box 1210, Charlottetown, Prince Edward Island, C1A 7M8, Canada.

RECOVERY OF PROTEIN FROM MECHANICAL SHRIMP PEELER EFFLUENTS: MICROBIOLOGICAL QUALITY OF EFFLUENT AND RECOVERED PROTEIN

Brian E. Perkins
Alabama Sea Grant Extension Service
3940 Government Boulevard
Mobile, AL 36693

and

Kimberly P. Harper
Biology Department
University of South Alabama
Mobile, AL 36688

ABSTRACT

Protein was recovered from mechanical shrimp peeler effluent by HCl precipitation and centrifugation. Percent recovery of solids was 1% - 2% of the untreated effluent by weight and was predictable from untreated effluent turbidity. Total aerobic plate counts of bacteria recovered from the protein and unprocessed shrimp were 10^5 - 10^6 CFU/g, approximately 1.5 logs higher than peeled shrimp and untreated effluent. The acid precipitation and centrifugation process reduced effluent total organic nitrogen, biochemical oxygen demand, and turbidity by 45.0%, 53.8%, and 92.4%, respectively, compared with the untreated effluent.

INTRODUCTION

Disposal of seafood processing effluents is one of the most serious problems facing Bayou La Batre seafood processors. Currently, most seafood processing effluents are directed to the Bayou La Batre municipal sewage treatment plant, which also receives domestic sewage. The Bayou La Batre municipal sewage treatment plant was designed and constructed to provide tertiary treatment for 1 million gallons per day (1 MGD). However, best estimates indicate that seafood processors send over 2 million gallons of effluent to the sewage treatment plant each day during peak months (Polysurveying, 1986). Added to that amount is another 600,000 gallons of domestic sewage per day.

The sewage treatment plant does not have adequate capacity to treat between 2.5 MGD and 3 MGD, and has been forced to bypass any volumes of domestic sewage and seafood processing effluents which it receives that are in excess of 1 MGD. This situation led to regulatory actions by state and federal environmental agencies, which stipulated that Bayou La Batre municipal and seafood processing effluents achieve compliance with National Pollution Discharge Elimination System (NPDES) limitations by June 30, 1988.

Several solutions were proposed by the City of Bayou La Batre and effected processors. The final alternative resulted in the formation of BJV, Inc., composed of three shrimp processors. BJV has funded a $1 Million project (currently underway) to construct a new seafood processing effluent collection and outfall line to serve the needs of its principals and the other 15 seafood processing plants located along the route of the outfall line. While this method of disposal will improve on the present arrangement by segregating seafood processing effluents from domestic sewage, it will not provide any treatment of the seafood processing effluent beyond in-plant screening of solids. The effluent will be treated as a wastewater, not as a processing stream which contains recoverable byproducts. Prior research has shown that seafood processing effluents generated by plants which operate mechanical shrimp peelers contain substantial amounts of recoverable shrimp protein (CRESA, 1971), (Johnson and Lindley, 1982), (Johnson and Gallanger, 1984), (Mauldin and Szabo, 1974), (Meyers and Sonu, 1974), (Perkins, 1977), (Perkins and Meyers, 1977), (Soderquist, 1973), (Toma and James, 1975), (Toma and Meyers, 1975).

METHODS

Fifteen collections of mechanical shrimp peeler effluent were made from June 23, 1987 through August 17, 1987. The effluent was derived from a Laitram Model A mechanical shrimp peeler.

All liquid effluent samples were collected in 2 gal Nalgene carboys which had been previously sanitized with a solution of 1600 ppm sodium hypochlorite and rinsed with deionized water.

Samples of mechanically peeled raw shrimp tail meat were taken on eleven occasions. Since the peeled shrimp meat was a portion of the mechanical peeler discharge flow, it was included in the Nalgene carboys along with the liquid effluent. The carboys containing either liquid effluent or the liquid effluent/peeled shrimp combination were placed on ice in an insulated styrofoam cooler.

Raw head-on shrimp samples were taken on four occasions during the last seven collections. Samples were collected by hand (previously sanitized in 400 ppm HTH solution) from the mechanical shrimp peeler feed hopper. The shrimp were placed in sterile Nasco Whirl-Paks, sealed, and placed on ice in an insulated styrofoam cooler.

Potable influent (city water) samples were taken on three occasions during the last seven collections. Samples were collected by filling sterile Nasco Whirl-Paks from a thawing tank fill pipe. Filled bags were sealed and placed on ice in an insulated styrofoam cooler.

All samples were immediately transported by automobile to the laboratory (12 miles from the sample collection location). This trip usually took 20 minutes. Because the amount of time required for sample collection averaged 15-20 minutes, none of the samples were more than 45

minutes old upon arrival at the laboratory. All samples were immediately re-iced upon arrival at the laboratory, and held on ice until analyzed.

Note: Throughout all succeeding methods, all laboratory glassware, plastic ware, and stainless steel implements were sterilized prior to use.

Precipitation and Centrifugation

Carboys containing liquid effluent or the liquid effluent/peeled raw shrimp combination were shaken to assure uniformity of liquid effluent. The liquid effluent/peeled raw shrimp samples were first screened on a 5 - mesh stainless steel screen to remove peeled raw shrimp. (Peeled raw shrimp samples were placed in sterile Nasco Whirl-Paks and re-iced). A 2,000 ml aliquot of each liquid effluent sample was measured in a graduated cylinder and transferred to a 4,000 ml glass beaker. A magnetic stirring bar was inserted, and the sample beaker was placed on a magnetic stirrer.

Initial pH measurements were taken with an Orion Model 201 digital pH meter, and the values recorded. A 50 ml buret containing 1.0 N Reagent - Grade HCl (C.A.S. Reg. 7647 - 01 - 0) was positioned over the sample beaker. The magnetic stirrer was turned on and set to operate at a slow speed. Slow (drop-by-drop) addition of the hydrochloric acid was initiated. The pH was continually monitored until a pH in the range of 4.65 - 4.80 was reached, at which point addition of hydrochloric acid was discontinued. Values for final pH and amount of hydrochloric acid added were recorded.

200 ml aliquots of acidified liquid effluent samples were immediately transferred to sterile 250 ml centrifuge bottles which had been previously tared on a Mettler Model PE - 3600 digital scale, and the weights recorded. Filled centrifuge bottle weights were taken and recorded.

Bottles of acidified liquid effluent were centrifuged at 7,000 rpm for 1 min at 40 ° F in a Sorvall Model RC-5B centrifuge. Supernatant fractions were pooled, placed in sterile containers, and returned to iced storage. Weights of centrifuge bottles containing precipitate were taken and recorded. Precipitate fractions were pooled, placed in sterile containers, and returned to iced storage.

Turbidity

Measurements for turbidity of both liquid effluent and supernatant (clarified wastewater) were taken on fourteen of the sampling dates and the readings recorded. Turbidity measurements were made with a Hach Model 2100 A turbidimeter which had been previously calibrated with standard tubes.

Total Kjeldahl Nitrogen, Ammonium Nitrogen, and Biochemical Oxygen Demand

Subsamples of liquid effluent, supernatant (clarified wastewater), and precipitate were collected on each of the fifteen sampling dates, placed in sterile Nalgene plastic bottles, and stored in a -20 ° C freezer. Subsamples were maintained at -20 ° C until after three or four sampling runs had been completed.

The frozen subsamples were removed from frozen storage, iced in an insulated plastic cooler, and transported to a commercial chemistry laboratory in Pascagoula, MS (45 minutes away) on four occasions. The subsamples were allowed to thaw overnight in a 38 ° F refrigerator. All subsamples were subjected to Total Kjeldahl Nitrogen and Ammonium Nitrogen analyses according to standard methods (AOAC, 1980). Subsamples of liquid effluent and supernatant were subjected to Biochemical Oxygen Demand analyses on three occasions. Biochemical Oxygen Analyses were conducted according to the standard Winkler Azide Modification (EPA, 1983).

Total Aerobic Plate Count and Fecal Coliforms

Analyses for total aerobic plate count and fecal coliforms were conducted on the following samples for the indicated collections:

Collection Numbers	Sample Types
1-15	Liquid Effluent
1-15	Supernatant
1-15	Precipitate
1,2,4-15	Peeled Raw Shrimp
9,11,13,15	Raw Head-On Shrimp
9,11,13	Municipal Water

All sample handling and preparation, total aerobic plate count, and fecal coliform methods conformed to standards prescribed in the F.D.A. Bacteriological Analytical Manual (A.O.A.C., 1984).

RESULTS AND DISCUSSION

Precipitation and Centrifugation

Percent recovery of solids from the peeler effluent stream was calculated by the following formula:

$$\frac{\text{Weight of Recovered Solids}}{\text{Weight of 200ml Untreated Effluent}} \times 100 = \text{Percent Recovery}$$

Percent recovery data are presented in Table 1:

Table 1. Percent Recovery of Solids.

%Recovery, Range	%Recovery, Mean	Standard Deviation
1.16 - 1.94	1.55	0.26

The number of milliliters of 1.0 N HCl required to attain a pH in the range of 4.65 - 4.80 varied considerably. Hydrochloric acid data are presented in Table 2:

Table 2. Hydrochloric Acid Addition.

ml 1.0 N HCl added, Range	ml 1.0 N HCl added, Mean	Standard Deviation
2.7 - 7.0	4.9	1.26

Figure 1 plots the relationship of the percent recovery of solids from peeler effluent samples to the number of milliliters of 1.0 N HCl required to attain a pH in the range of 4.65 - 4.80. Regression analysis yielded a correlation coefficient of $R = 0.8544$. This indicates that, despite the extremely variable nature of the peeler effluent samples, the number of milliliters of 1.0 N HCl required to attain a pH in the range of 4.65 - 4.80 was a good predictor of the percent recovery which might be expected.

Turbidity

Turbidity measurements, expressed as Fluorometric Turbidity Units (FTU's), indicated the relative levels of liquid effluent and supernatant clarity. Turbidity data (excluding Collection 3, which was simply forgotten) are presented in Table 3:

Table 3. Turbidity.

	Range	Mean	Standard Deviation
Peeler Effluent, FTU	20 - 75	42.8	15.6
Supernatant, FTU	1.5 - 7	2.8	1.6
Percent Reduction	75 - 98	92.4	5.6

The extreme variability of the peeler effluent and supernatant turbidity data (note large standard deviations) illustrates very well the variable conditions under which shrimp are mechanically processed. The peeler effluent turbidity data simply indicate that at any given time, an unscheduled "grab sample" can yield turbidity readings which may vary by as much as 3.75 times. A composite (flow vs. time) sampler would help to alleviate this variability. Likewise, supernatant tubidities which result from subjecting variable peeler effluent samples to precipitation and centrifugation may vary by as much as 4.6 times.

However, percent reduction of turbidity

$$\frac{\text{(PEELER EFFLUENT TURBIDITY - SUPERNATANT TURBIDITY)}}{\text{PEELER EFFLUENT TURBIDITY}} \times 100$$

data reflect that, regardless of the peeler effluent turbidity value, the turbidity of the supernatant will be reduced to a mean value which is only 7.6% of the effluent. The consistency of this capability to reduce turbidity by a mean of 92.41% is supported by the rather low standard deviation of 5.61.

Figure 2 plots the relationship of peeler effluent turbidity to the percent recovery of solids from peeler effluent samples. Regression analysis yielded a correlation coefficient of $R = 0.80$. Although turbidity is a slightly less accurate predictor of percent recovery of solids from the peeler effluent than the number of milliliters of HCl added (Figure 1), it provides an estimate of the range of recoverable solids.

Figure 3 plots the relationship of peeler effluent turbidity to the number of milliliters of 1.0 N HCl required to attain a pH in the range of 4.65 - 4.80 (Collections 1,2,4-15). Regression analysis yielded a correlation coefficient of $R = 0.8775$. This indicates that turbidity is a very good predictor of the amount of HCl required to attain a pH in the range of 4.65 - 4.80. More importantly the use of turbidity measurements to predict the amount of HCl necessary for isoelectric precipitation would provide an effective method of on-line process control for a pilot or production - scale effluent treatment and byproduct recovery system.

Figure 1. Percent Recovery of Shrimp Solids due to Addition of HCl.

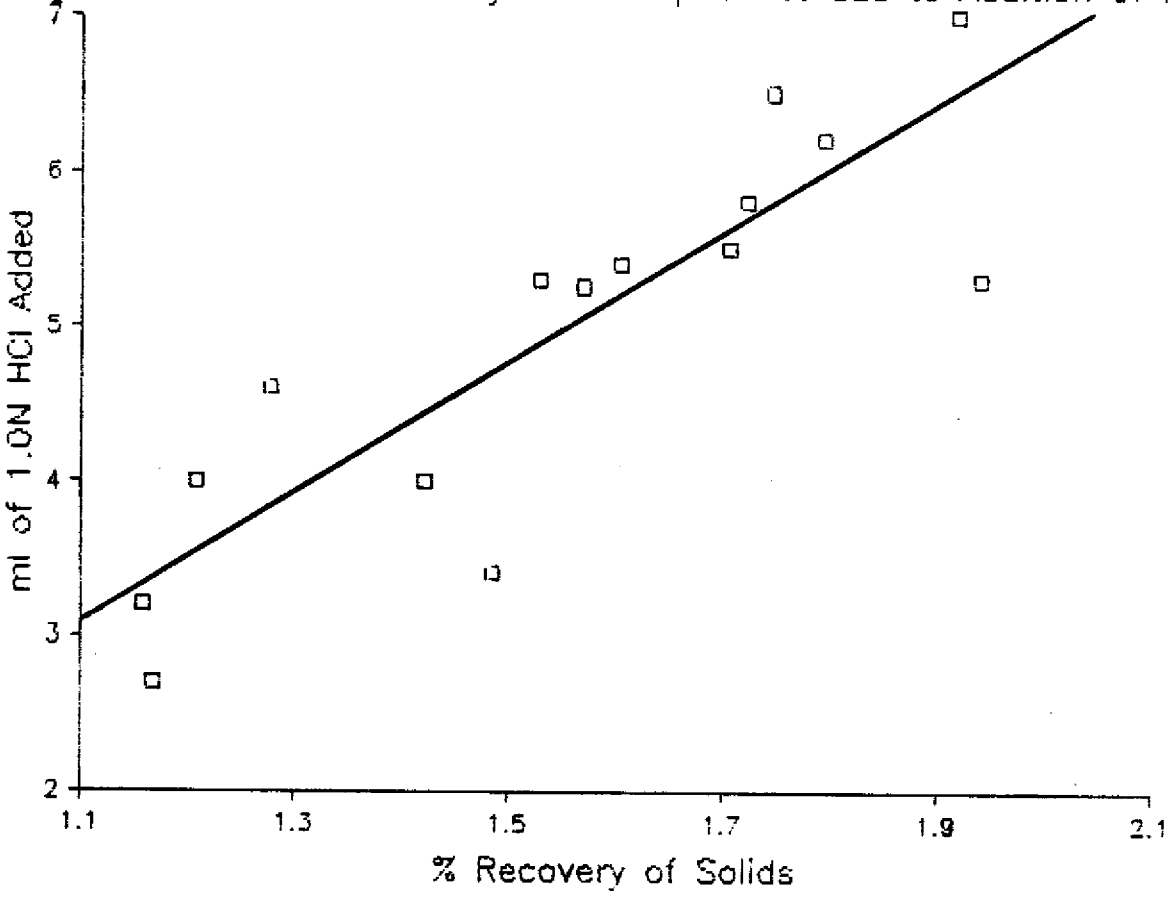


Figure 2. The Effect of Effluent Turbidity on Percent Recovery

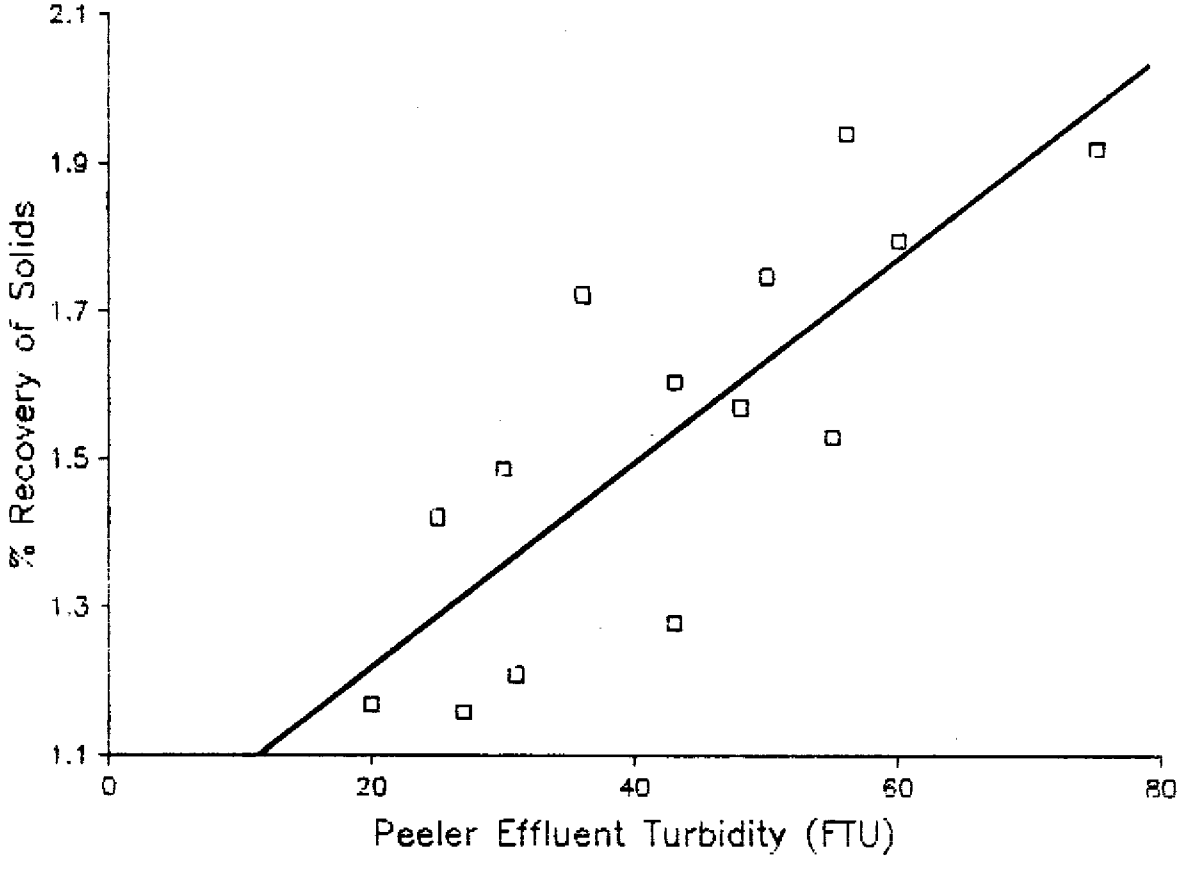
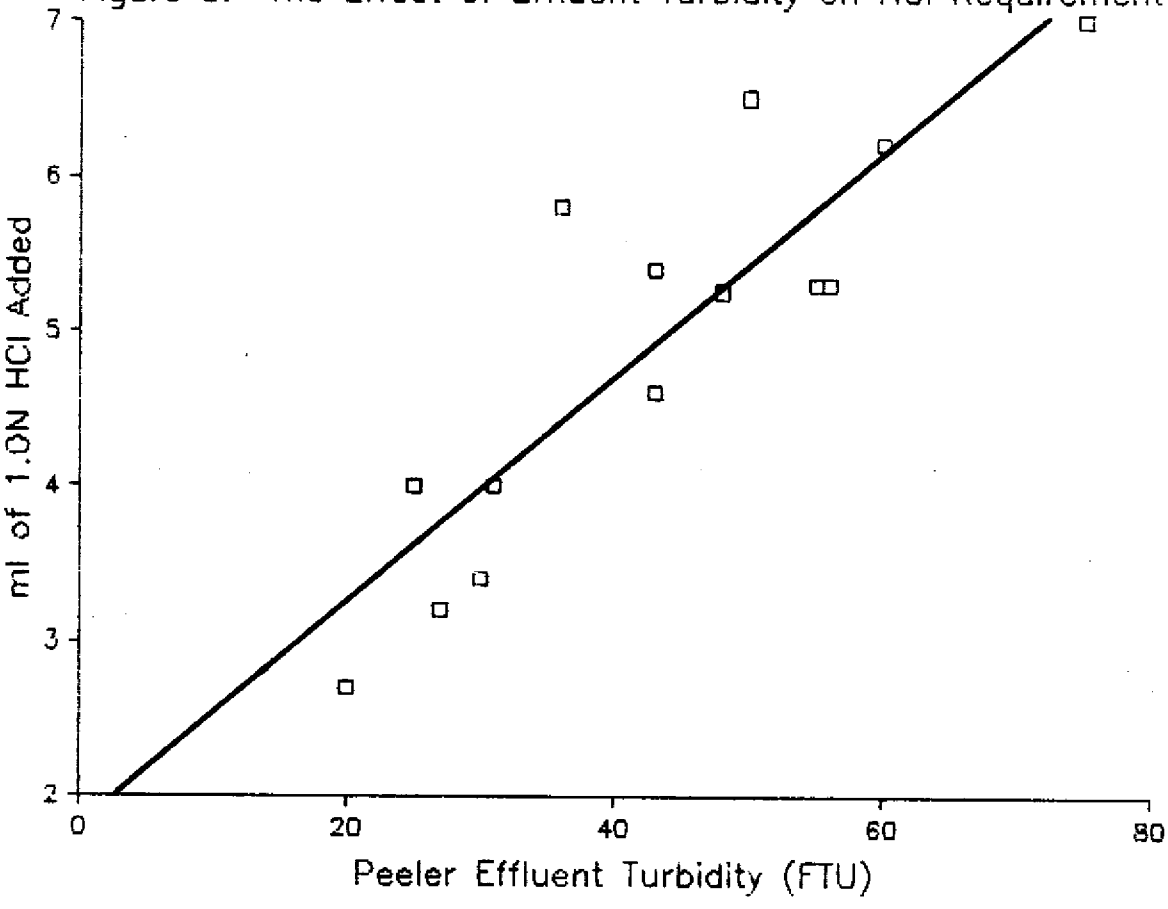


Figure 3. The Effect of Effluent Turbidity on HCl Requirement



Nitrogen

The results of Total Kjeldahl Nitrogen (TKN) and Ammonium Nitrogen analyses, plus calculated Total Organic Nitrogen (TON) values (Calculated as TON = TKN - Ammonium), are presented in Table 4:

Table 4. Nitrogen.

		Untreated Effluent, mg/l	Supernatant mg/l	Precipitate, ppm
TKN	Range	81 - 675	46 - 188	9094 - 25,200
	Mean	233.9	118.7	15,913
	Std. Dev.	139.2	40.2	4,228.6
Ammonium	Range	1 - 9	1 - 6	7 - 3710
	Mean	4.9	4.3	475.1
	Std. Dev.	2.1	1.6	1,132.5
TON	Range	80 - 666	45 - 181	9068 - 25,174
	Mean	229	114.3	15,394
	Std. Dev.	137.6	38.7	4127

Total Kjeldahl Nitrogen values were extremely variable, as evidenced by the wide ranges and large standard deviations listed in Table 4. The variability in untreated effluent TKN was probably due to uneven product flow (with constant water flow) through the mechanical shrimp peelers. Supernatant fraction and precipitate fraction TKN values were equally variable. This was a logical result since those two fractions were derived from the untreated effluent.

Ammonium Nitrogen, an indicator of age or degree of spoilage in shrimp, varied somewhat more than did TKN. This is to be expected since the age and quality of shrimp encountered during the processing season would likewise tend to vary.

Mean percentages of TKN recovered as total organic nitrogen from untreated effluent, supernatant, and precipitate samples were 97.8%, 96.4%, and 97.3%, respectively. Although standard deviations of calculated mean TON values for untreated effluent, supernatant, and precipitate samples were large, the percentage of TKN recovered as ammonium nitrogen exceeded 4.5% on only 2 occasions. This means that, in 43 of 45 instances, TON values were no more varible than their corresponding TKN values.

Precipitation and centrifugation proved an effective method for recovering TON from mechanical shrimp peeler effluents. Table 5 presents TON data for untreated effluent and supernatant samples, plus percent reduction of TON values:

Table 5. Percent Reduction of Peeler Discharge TON.

	Range	Mean	Standard Deviation
Untreated Effluent, mg/l	80 - 666	229	137.7
Supernatant, mg/l	45 - 181	114.3	38.7
Percent Reduction	29.3 - 72.8	45.0	13.7

These data suggest that roughly half of the available TON was left behind in the supernatant following precipitation and centrifugation. This is consistent with prior work in the area of shrimp protein precipitation (Perkins, 1977). The investigators are unsure why this mean percent reduction cannot be exceeded. Perhaps the isoelectric precipitation range (pH = 4.65 -4.80) was too selective. The need for further experiments to examine the effectiveness of a two - phase precipitation and centrifugation process appears to be in order.

Biochemical Oxygen Demand

Biochemical oxygen demand (BOD) determinations were conducted on three occasions to provide another indication of the effectiveness of removal of organic matter from shrimp peeler effluent. The results are presented in Table 6:

Table 6. Biochemical Oxygen Demand.

	Range	Mean	Standard Deviation
Untreated Effluent, mg/l	1800 - 2600	2066	461.8
Supernatant, mg/l	800 - 1000	933	115.5
Percent Reduction	44.4 - 61.5	53.8	8.7

These data suggest that roughly half of the demand for oxygen remained in the supernatant following percipitation and centrifugation. This is consistent with prior work in the area of shrimp protein precipitation (Perkins, 1977). The investigators are unsure why this reduction cannot be exceeded. Perhaps the isoelectric precipitation range (pH = 4.65 - 4.80) was too selective. The need for further experiments to examine the effectiveness of a two - phase precipitation and centrifugation process appears to be in order.

Total Aerobic Plate Count.

Total aerobic plate count (APC) determinations were conducted for all liquid samples, the various fractions derived after precipitation and

centrifugation, plus raw head-on and peeled raw shrimp samples. The results, expressed as Log10 APC, are presented in Table 7.

Table 7. Log10 Total Aerobic Plate Count.

Untreated Effluent	Range, Log10 APC/ml	3.11 - 5.00
	Mean, Log10 APC/ml	4.12
	Standard Deviation	0.56
Supernatant	Range, Log10 APC/ml	0.30 - 2.83
	Mean, Log10 APC/ml	1.51
	Standard Deviation	0.51
Precipitate	Range, Log10 APC/g	4.98 - 6.86
	Mean, Log10 APC/g	5.70
	Standard Deviation	0.51
Raw Head-on Shrimp	Range, Log10 APC/g	4.52 - 5.52
	Mean, Log10 APC/g	5.06
	Standard Deviation	0.50
Peeled Raw Shrimp	Range, Log10 APC/g	3.78 - 5.04
	Mean, Log10 APC/g	4.30
	Standard Deviation	0.42

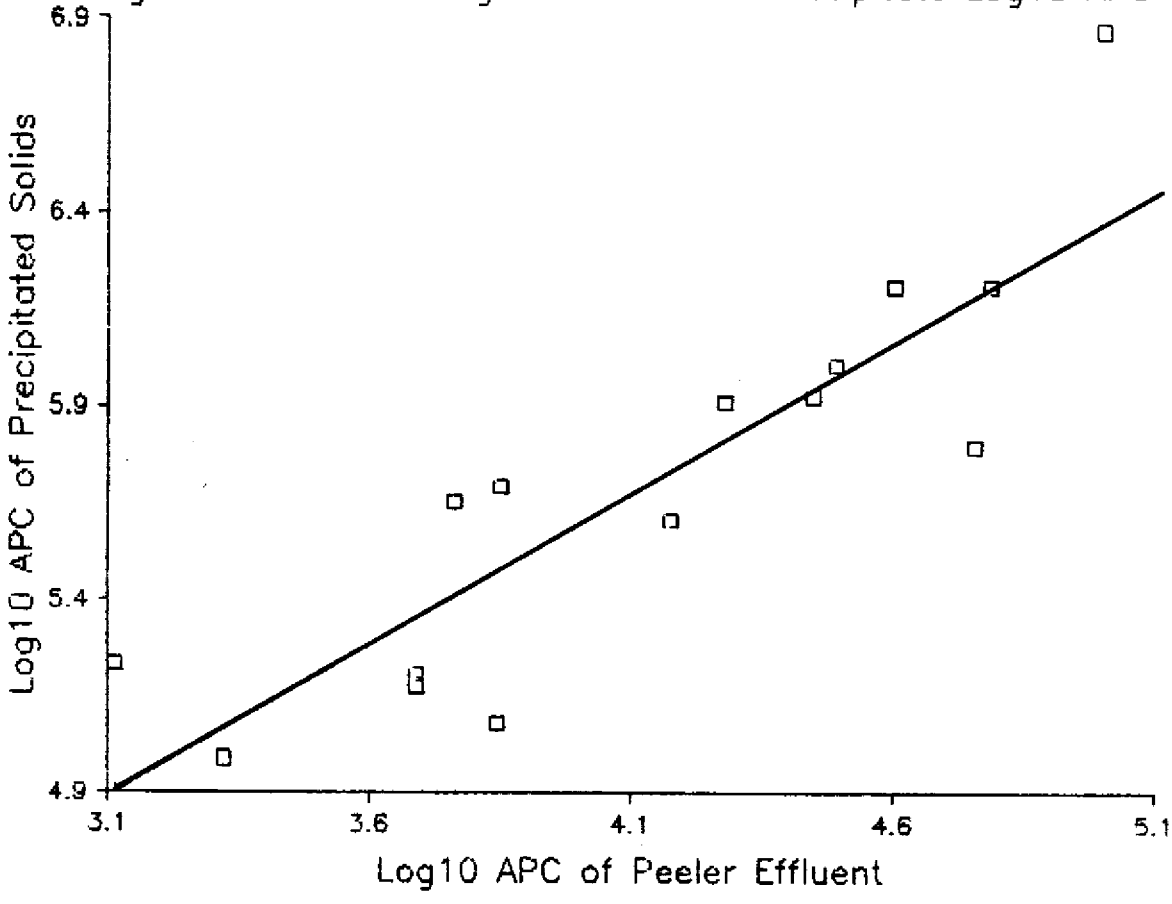
All municipal water APC determinations were negative (no growth).

Several trends can be noted based on the Log10 APC data. Mean log 10 APC/ml of the supernatant fraction was 2.61 logs less than the mean log 10 APC/ml of the untreated effluent from which it was derived. Correspondingly, mean log10 APC/g of the precipitate fraction was 1.58 logs greater than the mean log10 APC/ml of the untreated effluent from which it was derived. These data indicate that the precipitation and centrifugation process concentrates colony forming units in the precipitate fraction while reducing their numbers in the supernatant fraction.

The mean log10 APC/g of the precipitate fraction was only 0.64 log greater than the mean log10 APC/g of raw, head-on shrimp, and was within the same order of magitude (10^5). Likewise, the mean log10 APC/g of the peeled raw shrimp was only 0.18 log greater than the mean log10 APC/ml of the untreated effluent in which it was contained. Mean log10 APC/g of peeled raw shrimp and mean log10 APC/ml of untreated effluent were also within the same order of magnitude (10^4).

Figure 4 plots the relationship of untreated effluent log10 APC/ml to precipitate log10 APC/g. Regression analysis yielded a correlation coefficient of R = 0.8775. These data indicate that there is a definite, linear relationship between log10 APC of untreated effluent samples and the log10 APC of solids precipitated from them. This is especially

Figure 4. Effluent Log10 APC versus Precipitate Log10 APC



remarkable when consideration is given to the widely variable (2 logs) range of APC values noted in the untreated effluent.

Figure 5 plots the relationship of untreated effluent log10 APC/ml to supernatant log10 APC/g. Regression analysis yielded a correlation coefficient of R = 0.8185. These data indicate that there is also a linear relationship between log10 APC of untreated effluent samples and the log 10 APC of the supernatant remaining after precipitation and centrifugation. The data contained in Figures 4 and 5 provide good models for predicting rates of recovery of colony forming units in the precipitate and supernatant fractions following precipitation and centrifugation of peeler discharge samples.

Fecal Coliforms

Fecal coliform determinations were conducted for all liquid samples, the various fractions derived after precipitation and centrifugation, plus raw head-on and peeled raw shrimp samples. Fecal coliform ranges are presented in Table 8:

Table 8. Fecal Coliform Ranges,

Sample Type	Range, Fecal Coliforms/ml or /100g
Untreated Effluent	0 - <18
Supernatant	0 - <1.8
Precipitate	0 - 1700
Raw Head-On Shrimp	<18 - 3500
Peeled Raw Shrimp	<18 - 20

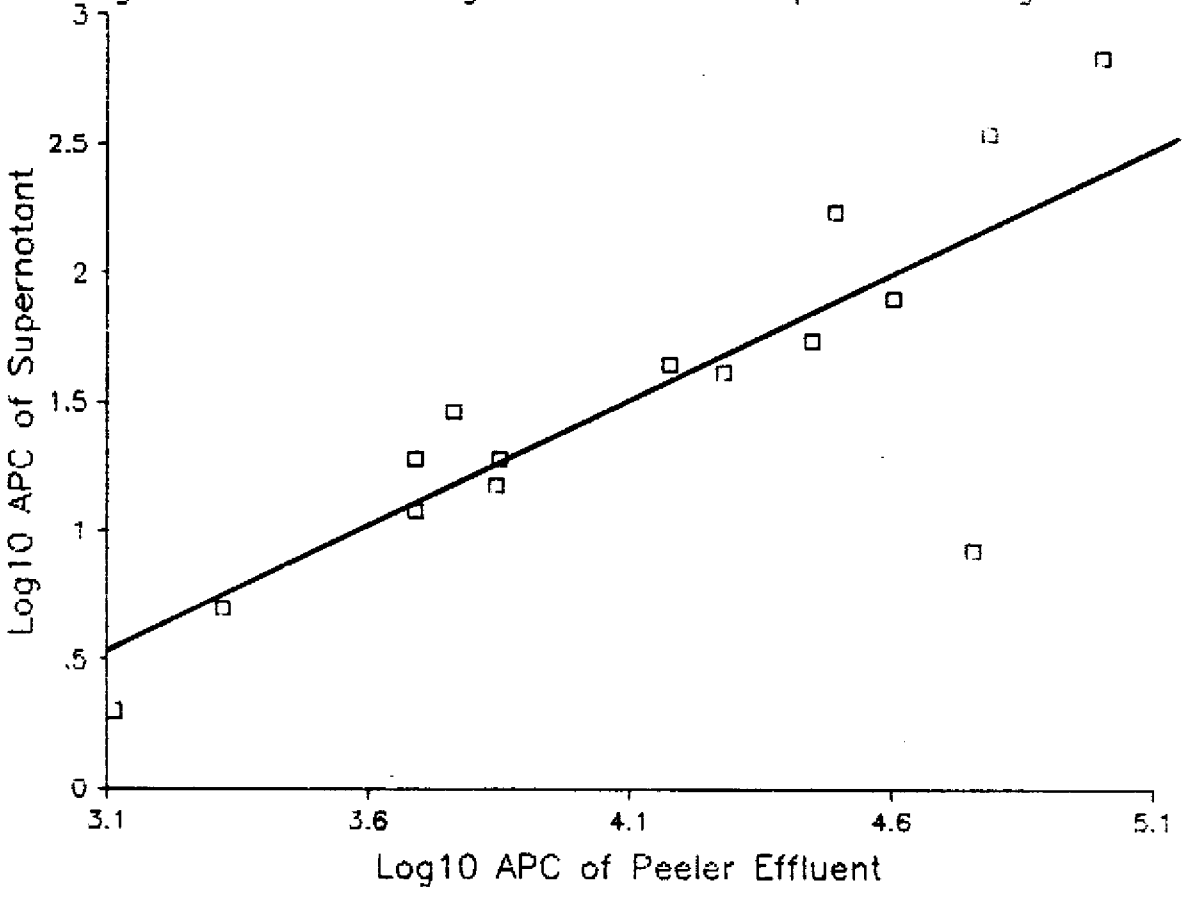
All municipal water fecal coliform determinations were negative (no growth).

Fecal coliform counts for untreated effluent samples ranged from 0 - <18/ml. Specifically, counts of 0 were noted on 5 occasions, and counts of <1.8/ml were noted on 7 occasions. Otherwise, counts of 4/ml, 7.8/ml, and <18/ml were each noted on one occasion.

Fecal coliform counts for supernatant fractions ranged from 0 - <1.8/ml. Specifically, counts of 0 were noted on 6 occasions, and counts of <1.8/ml were noted on 9 occasions. This represents a slight improvement over untreated effluent fecal coliform counts.

Fecal coliform counts for precipitate fractions ranged from 0 - 1700. Specifically, counts of 0 were noted on 3 occassions, counts of <18/100g were noted on 4 occasions, counts of 18/100g were noted on 3 occasions, and counts of 45/100g were noted on 3 occasions. Otherwise, counts of 20/100g and 1700/100g were each noted on one occasion. Discounting the

Figure 5. Effluent Log10 APC versus Supernatant Log10 APC



one high count of 1700/100g, these data suggest a slight reduction in quality from untreated effluent fecal coliform counts.

It should be noted that the precipitate fecal coliform count of 1700/100g occurred on the same collection date (07/27/87) when a fecal coliform count of 3500/100g was noted in a sample of raw, head-on shrimp. This leads the investigators to assume that raw product contaminated with fecal coliforms will logically produce precipitated shrimp protein which is also contaminated with fecal coliforms.

Fecal coliform counts for raw, head-on shrimp samples of <18/100g, 45/100g, 110/100g, and 3500/100g were each noted on one occassion. These data serve to provide a base of understanding about the wide diversity in quality of raw product entering the mechanical shrimp peeler.

Fecal coliform counts for samples of peeled raw shrimp ranged from <18/100g - 20/100g. Specifically, counts of <18/100g were noted on 9 occasions, and counts of 18/100g and 20/100g were each noted on one occasion. Obviously, passage of shrimp through a mechanical peeler (at the rate of one pound of shrimp per 8.6 gallons of water) serves to reduce the amount of fecal coliforms found in the final product.

RECOMMENDATIONS

Based on the results of this initial bench-scale project, the following recommendations are made:

- Reductions in supernatant TON, BOD, and turbidity are significant enough to warrant continued experiments to increase the effectiveness of the precipitation and centrifugation process.

- Precipitated shrimp byproduct quality is sufficient to warrant continued recovery efforts.

- Scale-up to a pilot plant installation to recover larger quantities of solid material from mechanical peeler effluent.

- Assess quantity and quality of solid materials recovered from pilot - scale recovery system.

- Assess effectiveness of reduction of organic matter from mechanical shrimp peeler effluent by pilot - scale recovery system.

- Assess market potential of recovered solid materials by allowing key food research and development laboratories to further test samples.

- Determine economic viability of the pilot - scale effluent treatment and byproduct recovery system.

ACKNOWLEDGEMENTS

The contributions of the following organizations and individuals are gratefully acknowledged:

- Alabama Department of Economic and Community Affairs for providing project funding.

- Auburn University/Alabama Cooperative Extension Service for providing matching funds.

- Dr. A.M. Guarino, Chief, U.S. Food and Drug Administration Fisheries Research Branch for providing laboratory facilities.

- Angelo DePaola, U.S. Food and Drug Administration Fishery Research Branch for assistance with all aspects of project design and performance.

- The cooperating shrimp processor for allowing sample collection.

- Micro-Methods Laboratories of Pascagoula, MS. for conduct of nitrogen and BOD analyses.

- Ms. Cecelia Formichella for conducting the processor survey.

- Dr. Merrill MacPhearson, U.S. Food and Drug Administration Fisheries Research Branch for assistance with statistical analysis.

LITERATURE CITED

AOAC. 1980. Official Methods of the Association of Official Analytical Chemists. 13th Edition. Arlington, VA.

AOAC. 1984. Bacteriological Analytical Manual of the Division of Microbiology. Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration. 6th Edition. Arlington, VA.

CRESA. 1971. Pollution Abatement and By-Product Recovery in Shellfish and Fisheries Processing. EPA Water Pollution Control Research Series. 12130 FJQ 06/71. U.S. Government Printing Office. Washington, D.C. 85pp.

EPA. 1983. Methods for Chemical Analysis of Water and Wastes. Cincinnati, OH.

Johnson, R.A. and K.L. Lindley. 1982. Use of hydrocyclones to treat seafood processing wastewaters. Journal WPCF. 54 (12): 1607 - 1612.

Johnson, R.A. and S.M. Gallanger. 1984. Use of coagulants to treat seafood processing wastewaters. Journal WPCF. 56 (8): 970 - 976.

Mauldin, A.F. and A.J. Szabo. 1974. Shrimp Canning Waste Treatment Study. EPA Environmental Protection Technology Series. EPA - 660/2-74-061. U.S. Government Printing Office. Washington, D.C. 130pp.

Meyers, S.P. and S.C. Sonu. 1974. Nucleotides and amino acids in shrimp blanching water. Feedstuffs 46:23.

Perkins, B.E. 1977. Recovery of proteinaceous by-products from shrimp cannery effluents. Masters Thesis. Louisiana State University. Department of Food Science. 94pp.

Perkins, B.E. and S.P. Meyers. 1977. Recovery and application of organic waste from the Louisiana shrimp canning industry. Proceedings: Eighth National Symposium on Food Processing Wates. EPA - 600/2-77- 184. pp.292 - 307. EPA. Cincinnati, OH.

Polysurveying. 1986. City of Bayou La Batre Industrial Outfall Collection System: Flow Requirements and Financial Schedules. 19pp. Polysurveying. Mobile, AL.

Soderquist, M.R. 1973. Canned and Preserved Fish and Seafoods Processing Industry Development Document for Effluent Limitations Guidelines and Standards of Performance. EPA Contract #68-01-1526. U.S. Government Printing Office. Washington, D.C.

Toma, R.B. and W.H. James. 1975. Nutritional evaluation of protein from shrimp cannery effluent (shrimp waste protein). J. Agric. Food Chem. 23:1168 - 1171.

Toma, R.B. and S.P. Meyers. 1975. Isolation and chemical evaluation of protein from shrimp cannery effluent. J. Agric. Food Chem. 23:632 - 635.

FREEZE-THAW CONCENTRATION OF SHELLFISH EXTRACT AND ITS FLAVOR PROFILE

Charles N. Jayarajah and Chong M. Lee
Department of Food Science and Nutrition
University of Rhode Island
Kingston, RI 02881.

INTRODUCTION

Shellfish flavor imparts distinctive taste to surimi-based products, soups and souces. However, the method currently followed in the extraction process often results in the loss of aromatic and flavor-giving compounds. The flavoring agents presently marketed are in either natural or artificial versions. Natural flavor extracts are often used in combination with artificial source to achieve maximum efffect. Due to consumer preference, natural flavor extracts are added as a principal constituent.

The first and one of the most important stages of extraction process is the selection of cost efficient and relatively abundant supply of raw materials. Shellfish waste weighing thousands of pounds are generated from seafood processing plants annually (Hood et al, 1976). Lippincott and Lee (1984) reported that meat of lobster bodies comprising 14% of the overall flesh is often discarded as waste. These bodies also resulted in waste disposal problem. The meat of these bodies are suitable for flavor extraction process. In an earlier work done by Hayashi et al (1981) on omission and addition tests of sensory analysis stated that the characteristic taste of shellfish is contributed by 44 water soluble extractive components. The major water soluble extractive components of shellfish are amino acids, nucleotides, organic bases, sugars, organic acids and minerals (Konosu et al, 1978, Hayashi et al. 1978 and Hayashi, 1979). The commercial natural flavorings at present are produced by a traditional evaporation technique which culminated in the development of an off-flavored and dark product. To overcome these draw backs as well as to meet the ever increasing demand for natural shellfish flavors, a non-thermal process was developed. Freeze-thaw concentration and freeze concentration processes were compared to determine the possibility of recovering shellfish notes in the preconcentrate. Although one is concerned with the retention

of all the flavor giving components in the preconcentrate, in this paper only the important amino acids are quantitatively identified to evaluate the feasibility of the approach.

MATERIALS AND METHODS

Lobster bodies (*Homorus americanus*) were collected from shellfish processing plants and were frozen stored until a reasonable quantitity was reached. A flow diagram of the method followed is shown in Fig. 1. These bodies were thawed in water to fascilitate the removal of carapace and sandy substances. The carapace removed body was washed in a jet stream of water to discard the unwanted entrails. The cleaned bodies were ground at moderate speed without the perforated cutting plate. This procedure enables water to get in contact with increased meat area. The minced bodies were combined with water weighing half the weight of mince and steam-cooked for 20 minutes. This step destroys spoilage causing enzymes and may also help in reducing microorganisms. The cooked mince with water was filtered through a cheese cloth and the filtrate was collected. This product was referred to as the first extract. The retentate containing minced meat was pressed in a hand press using a pressure of 2000 psig to recover the juice from the meat. The juice from this step was combined with the first extract to obtain final pool of extract. The pooled extract was centrifuged at 4500 rpm in a refrigerated centriguge for 10 minutes. The supernatant was collected and further subjected to freeze-thaw concentration and freeze concentration processes.

In freeze-thaw concentration study, the feed was frozen in different size blocks at varying temperatures. The frozen blocks were thawed on the next day at different time intervals. The partially thawed blocks were filtered under vacuum pressure. The resulting concentrate was referred to as freeze- thaw concentrate. In freeze concentration, the feed was crystallized by dry ice in isopropanol cooling medium. The slurry was cenrtrifuged and the concentrate was separately collected from ice crystals. This was referred to as freeze concentrate. The recovery rate for flavor giving constituents was calculated by using equation as follows:

$$\% \text{ solids recovered} = \frac{\% \text{ solute X volume of con.}}{\% \text{ solute X feed volume}} \text{ X } 100$$

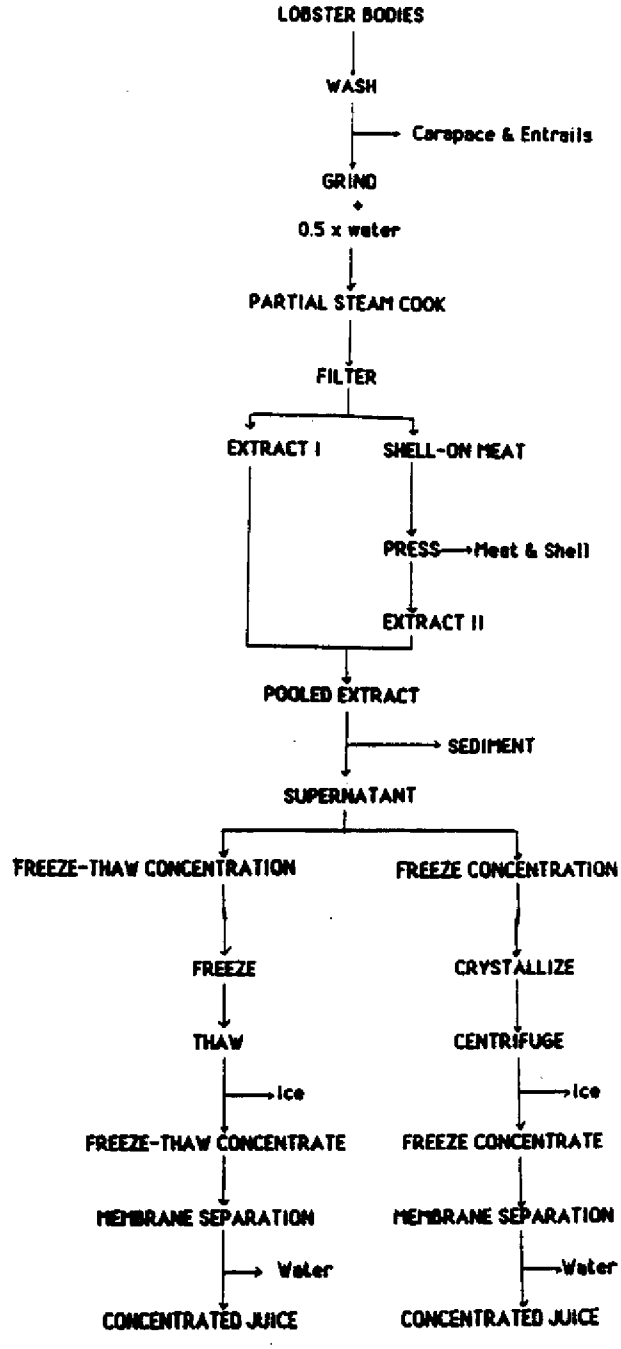


Fig. 1-Flow chart of flavor recovery process.

Chemical analysis

Proximate analysis was done according to AOAC (1975) on raw body flesh, feed stream, freeze concentrate and freeze-thaw concentrate. Total protein nitrogen content of the products was obtained by Kjeldahl method. Non-protein nitrogen content was also determined by Kjeldahl method after precipitating the protein with 10% trichloroacetic acid.

Amino acid analysis

Acid hydrolysed as well as the non-hydrolysed portions of flesh, feed and concentrates were analysed for amino acids by Perkin-Elmer computerized HPLC unit equipped with a reverse phase cation exchange column.

RESULTS AND DISCUSSION

The proximate analysis results obtained for raw lobster flesh, feed stream, freeze concentrate and freeze-thaw concentrate are shown in Table 1.

Table.1-Proximate composition of sample

SAMPLE	MOISTURE	PROTEIN	NPN	FAT	ASH
Flesh	82.73	13.56	5.33	1.43	2.16
Supernatant	97.28	1.65	1.06	0.09	0.90
Freeze Con.	96.55	2.08	1.46	0.04	1.30
Freeze-Th. Con.	84.43	13.31	7.36	0.05	2.11

The total protein nitrogen content of flesh was 13.56%. Out of this non-protein nitrogen portion constituted 5.33% of the total protein nitrogen. The fat content of feed and concentrates was very much lower than the flesh. This has an added advantage as it has been reported that the flavor components with fat in aqueous medium undergo hydrolysis

(Chang and Reineccius, 1985). A seven percent increase in non-protein nitrogen was observed in case of freeze-thaw concentrate compare to freeze concentrate. Here non-protein nitrogen fraction is of primary interest since it constituted peptides and free amino acids.

The effect of recovery rate on freeze-thaw concentration is shown in Table 2. The percent total solids in the concentrate increased up to 375 ml size frozen block. Beyond this size, an increment in the block size drastically reduced the total solid content. Percent volume reduction in concentrate and the ratio of concentrate volume to feed volume also showed a minimum value for the block size of 375 ml.

Table 2-The effect of recovery rate on freeze-thaw concentration.

Supnt. (ml)	Concent. (ml)	Vol. Con. Ratio	%Vol. Rdnt.	Total Solids (%)	Recovery Rate (%)
10	0.4	25	96	4.78	4.60
50	2.6	19	95	8.77	10.80
100	6.8	15	93	11.75	18.62
200	18.7	11	91	12.14	26.46
375	47.5	8	87	13.40	39.59
400	53.5	7	86	11.69	36.45

Fig. 2 shows the optimum thawing time for uniform sized frozen blocks. The blocks thawed for 30 minutes, recovered relatively, a good quantitiy of solids within a short time. Among the temperatures studied, blocks frozen at -12° C gave a maximum recovery rate.

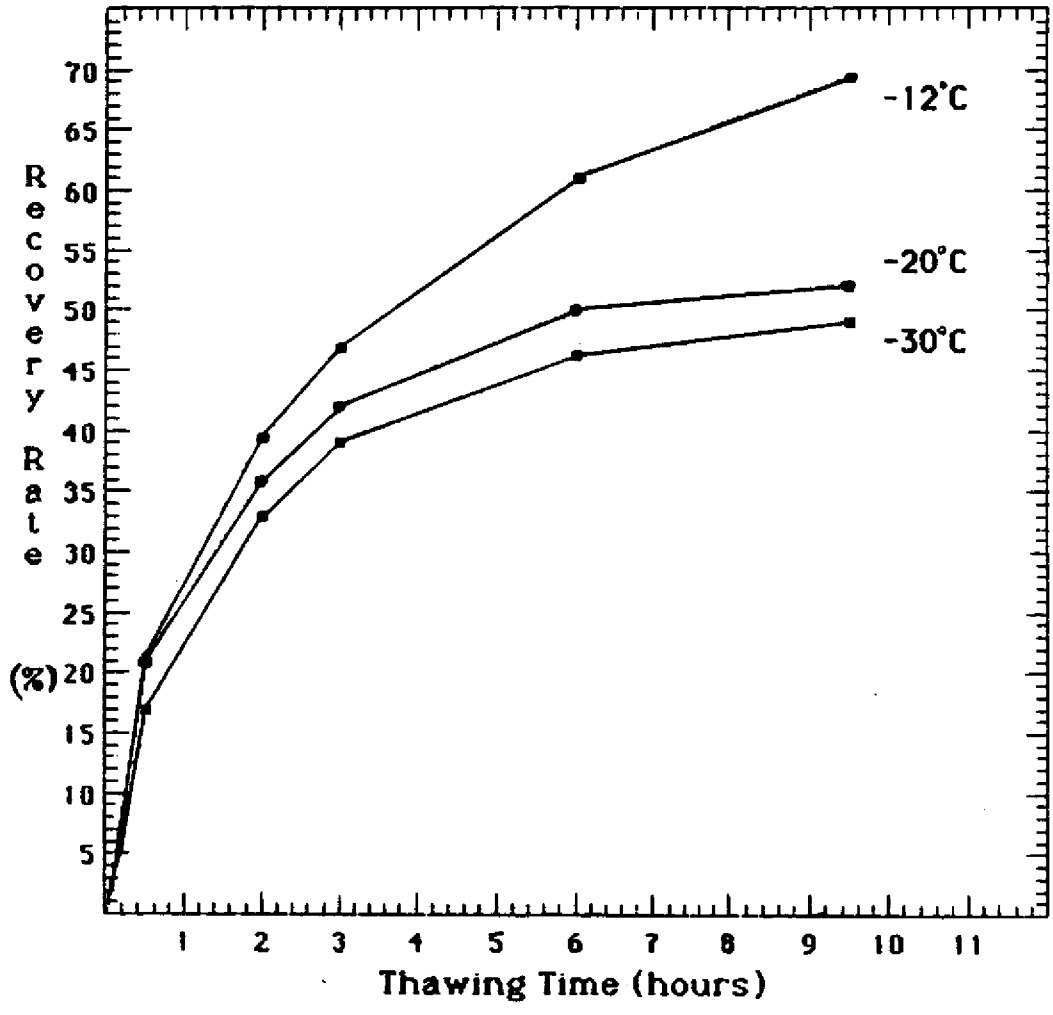


Fig. 2-The effect of thawing time on recovery rate.

The effect of block size on flavor recovery rate is illustrated in Fig. 3. The block size with the capacity of 375 ml showed maximum recovery rate. A decline in flavor recovery was noticed when the block size exceeded 375 ml. Freeze concentration was compared with freeze-thaw concentration with regard to performance.

For this purpose, the concentrate from the freeze concentration was recrystallized five times to reach a recovery rate closer to the value of freeze-thaw concentrate done under optimum conditions. Freeze-thaw concentration showed a two fold increase in total solid content with substantially reduced water content.

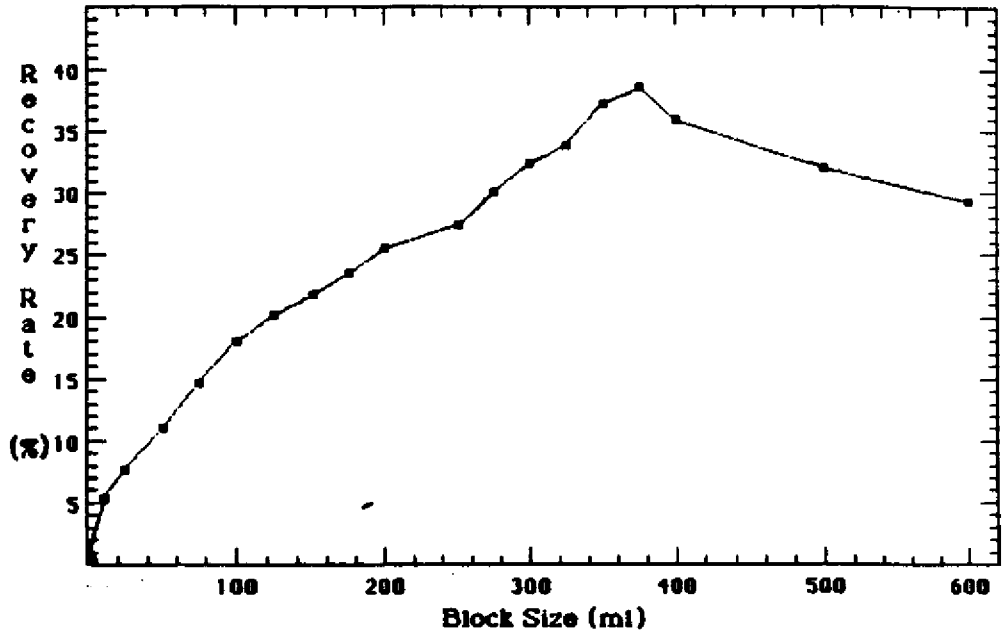
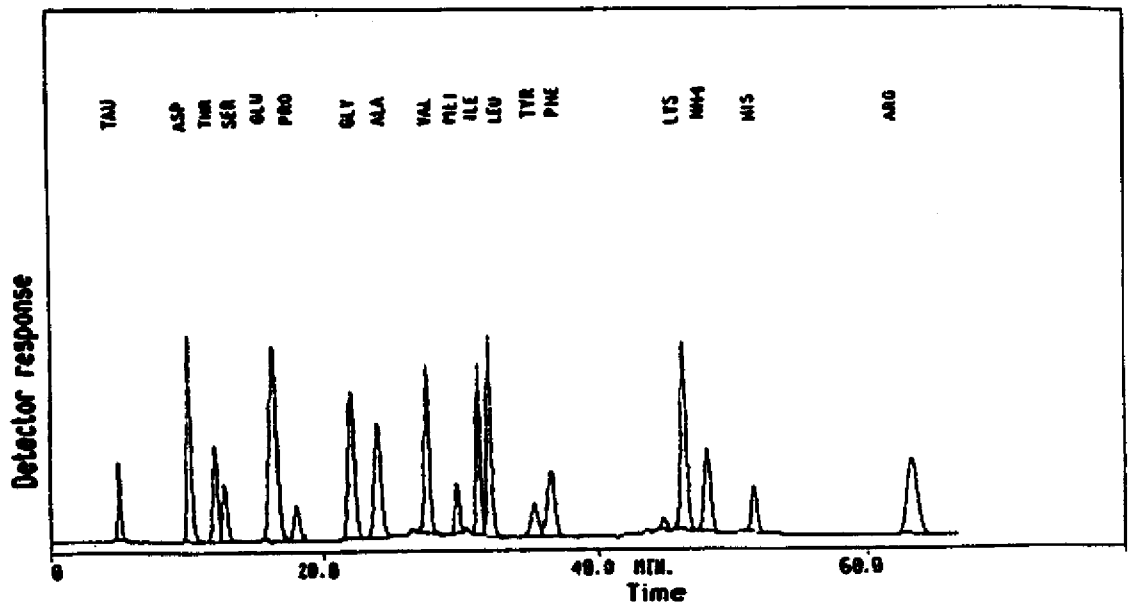


Fig. 3-The effect of block size on recovery rate.

The HPLC profile of acid hydrolysed lobster body flesh is presented in Fig. 4. The number of major amino acids quantified were 18. The most abundant amino acid in lobster body muscle was glutamic acid, followed by aspartic acid, lysine, arginine, leucine, alanine, proline, glysine, isoleucine, valine, phenyl alanine, threonine, tyrosine, serine, methionine and histidine. The least abundant was taurine. Among these only three amino acids namely, glycine, arginine and glutamic acid are reported to play a key role in producing shell fish-like taste (Take et al, 1964 and Hayashi et al, 1981).

The quantitative data of these three acid hydrolysed amino acids from the flesh, crude water extract, supernatant and sediment are shown in the form of bar diagram in Fig. 5. The water soluble extract was observed to recover 20% of glycine, 10% of arginine and 8% of



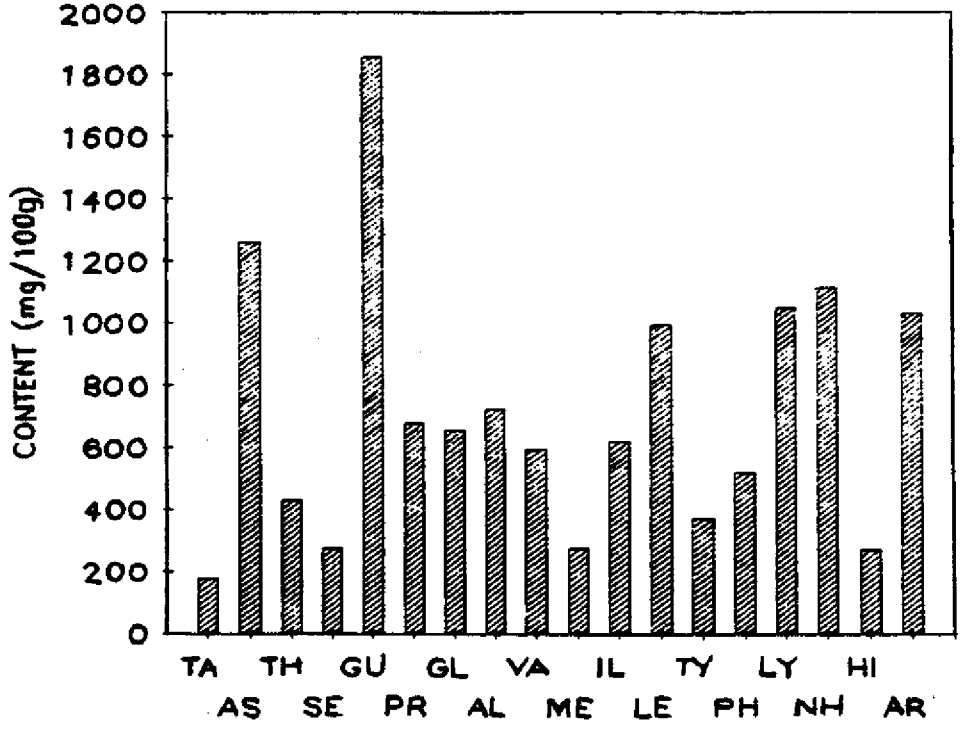


Fig. 4-Amino acids of acid hydrolysed lobster body muscle.

glutamic acid from the lobster body meat. A 10% decrease in the levels of all three amino acids was noticed in the feed when it was compared with extrat. The sediment collected from the centrifugation step. as such contains a slightly reduced quantities of amino acids to that of flesh.

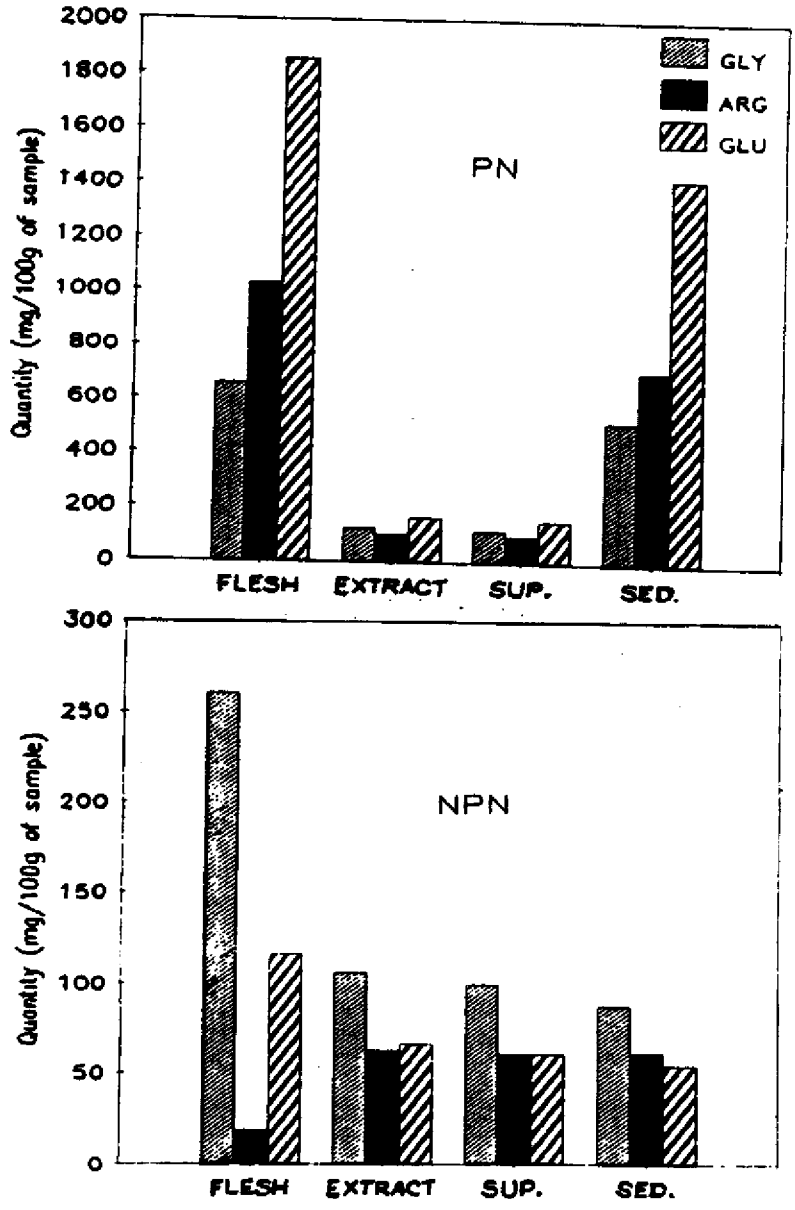


Fig. 5-The important flavor giving amino acids of samples analysed.

The bar diagram of fig. 5 also, illustrates the amount of hydrolysed amino acids obtained from non-protein nitrogen fractions of flesh, extract, feed and sediment. Glycine was the predominant amino acid of the three quantified. Camien et al (1951) analysed non-protein nitrogen fraction of lobster muscle and reported that glycine was the most abundant amino acid. Reduced levels of arginine and glutamic acid were noticed. This could mean that these amino acids were present in the form of protein nitrogen and glycine in free and short peptide chains.

The performance of freeze concentration and freeze-thaw concentration in terms of retaining amino acids are shown in in fig. 6.

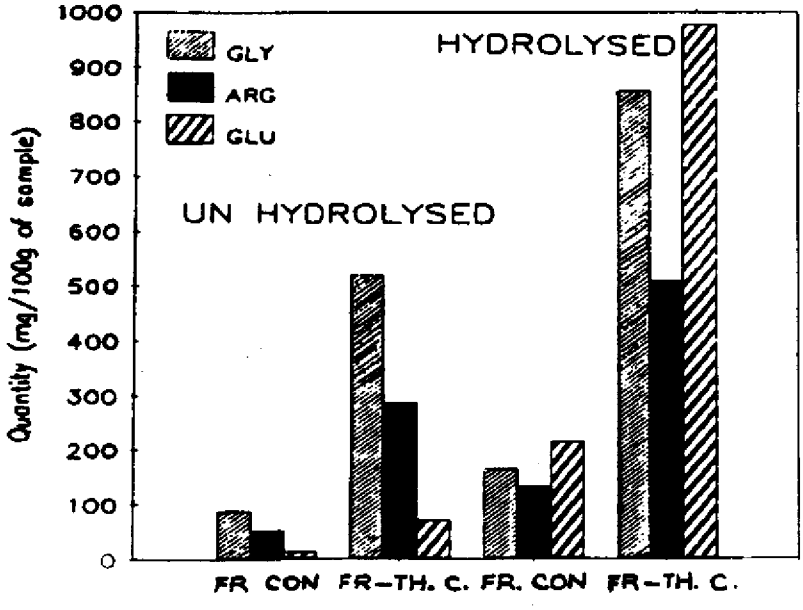


Fig. 6- Comparison of freeze-thaw concentration and freeze concentration processes.

Freeze-thaw concentration was observed to concentrate 10 times of that of freeze concentration method. The bar chart of hydrolysed fraction of concentrates shows a 15 fold increase in glutamic acid.

CONCLUSIONS

Among the three main processes currently used, namely (1)

freeze concentration, where water is separated in ice form (2) membrane separation, where water is removed in liquid state as water and (3) evaporation with water removal in the gaseous state as vapour, the former two are suitable for shellfish flavor extraction process. The freeze-thaw concentration process which was developed in this study recovered fairly highly levels of flavor giving constituents compare to freeze concentration. The freeze-thaw concentration method is diferent from the continuous freeze concentration technique with regard to ice crystallization and solid phase separation process. In the former technique the solid phase transfer is accomplished by slow melting of a large ice block, whereas the latter one is performed by continuous formation of small ice crystals from the water phase. A prelimiary study conducted with plate and frame reverse osmosis membrane separation unit which is of the type used by Ohya et al (1981), indicated that a relatively good quantity of water could be separated (Jayarajah and Lee, 1986). Considering the fact that the demand for surimi-based products will increase in the years to come, an increased requirement for a good natural flavoring agent is anticipitated.

REFERENCES

AOAC. 1975. Official Methods of analysis, 12th ed. Assoc. Off. Anal. Chem, Washington, D. C.

Camien, M. N., Sarlet, H., Duchateau, D and Florkin, M. 1951 Non-protein amino acids in muscle and blood of marine and fresh water crustacea. J. Biol. Chem. 193: 881-885.

Chang, Y. T., and Reineccius, G. A. 1985. Isolation of flavor components in model systems by counter current continuous dialysis. J. Agric. Food Chem. 33: 1168-1173.

Hayashi, T., Yamaguchi, K., and Konosu, S. 1978. Studies on flavor components in boiled crabs-II. Bull. Japan Soc. Sci. Fish. 44(12): 1357-1362.

Hayashi, T.,Asakawa, A. and Konosu, S. 1979. Studies on flavor components in boiled crabs-III. Bull. Japan Soc. Sci. Fish 45(10): 1325-1329.

Hayashi, T., Yamaguchi, K., and Konosu, s. 1981. Sensory analysis of taste active compounds in the extract of boiled snow crab meat. J. Food Sci., 46: 479-493.

Hood, L. F., Zall, R. R., and Conway, R. L. 1976. Conversion of minced clam wash water into clam juice: Waste handling or product development? Food Prod. Dev. 10: 86-88

Jayarajah, C. N. , and Lee, C. M. 1986. Concentration of

flavor components from lobster extract by a plate and frame reverse osmosis unit (unpublished data).
Konosu, S., Yamaguchi, K., and Hayashi, T. 1978. Studies on flavor componets in boiled crabs-I. Bull. Japan Soc. Sci. Fish. 44(5): 505-510.
Lippincott, R. K., and Lee, C. M. 1984. Mechanical extraction of meat from lobster and crab bodies. Aust. Fish 3: 51-52.
Ohya, H,. Negishi, Y., Kamato, K, Matsui, K and Inove, H. 1981. Prediction method for the life of reverse osmosis membranes. vol I Turbak, K. K. (ed.) ACS Symp. Ser. 153: 113-130.
Take, T., Honda, R., and Otsuka, H. 1964. Studies on the tasty substances of various foods (part 2). J. Japan Soc. Food Nutr. 17: 268-274.

Acknowledgement

Authors would like to thank the National Fisheries Institute (NFI) for providing a scholarship fund to support this study.

CHARACTERIZATION OF MINCED MEAT RECOVERED FROM BLUE CRAB BY-PRODUCTS

Keith W. Gates, Jackie G. EuDaly, Amanda H. Parker
and Carole A. Greene
The University of Georgia
Marine Extension Service
P. O. Box Z
Brunswick, GA 31523

INTRODUCTION

Recovering and marketing products of higher value from fishery wastes can reduce rising disposal costs while increasing profits and employment for the nation's seafood industry. The blue crab industry which yearly generates approximately 180 million pounds of crab by-products has been particularly vulnerable to waste disposal problems (Murray and DuPaul, 1981). Steam-processed blue crabs yield approximately 10% picked meat by weight. Remaining by-products are discarded or further processed as crab meal which sells for $100 to $150 per ton (Murray and DuPaul, 1981). Mechanical extraction of minced meat from crab picking by-products could recover an additional 15 to 20% edible meat. Nationally, annual recovery of minced crab meat could approach 30 million pounds (Thompson, 1985). Minced meat currently sells for $0.70 to $1.00 per pound and is used as an extender in deviled crab, seafood stuffings, soups, and chowders.

Grey-to-brown appearance and high microbial levels limit marketability. Minced meat produced in Georgia is packed in ring-sealed, five-pound plastic tubes and pasteurized in hot water, which further darkens the product. High tube-failure rates have forced processors to freeze meat for wholesale distribution. Processors market meat with poor knowledge of nutritional, sensory, and storage qualities. Improved quality would increase market demand, and new products could expand sales through production of white and claw meat analogs.

The following describes preliminary findings of a Sea Grant research project to improve quality and appearance of minced blue crab meat. Yields, chemical, sensory, microbiological, and nutritional qualities were determined for meats extracted from picking-room by-products. In-plant methods to reduce microbial loads were investigated. Low-temperature pasteurization and chemical additives were evaluated for potential reduction of heat-related darkening or "blueing." Chemical, sensory, microbiological, and nutritional changes in mixed minced meat and minced claw meat were monitored monthly during frozen storage at less than -4°F (-20°C).

METHODS

Minced meat was extracted from picking-room by-products of mechanically-backed (C. and K. Lord Backing Machine, Cambridge, MD), hand-picked, steam-retorted blue crabs using a Baader 694

deboning machine. Drum perforations were 1.3 mm. Two cooperating blue crab processors provided plant time and equipment for the project.

Picking-room by-products were separated into four components to evaluate extracted meat types and yields for the following:

1. Mixed minced meat - recovered from all picking-room by-products except claws

2. Minced white meat - recovered from "slabs" removed by the pickers first dorsal cut, containing only white body meat

3. Minced leg meat - recovered from separated legs and swimming legs

4. Minced claw meat - recovered from separated claws

Chemical and nutritional parameters determined in duplicate for minced meat samples included: percent moisture, percent Kjeldahl protein, percent ash, and percent fat (Williams, 1984). Microbiological quality was assessed through duplicate standard plate counts, MPN total coliforms, MPN E. coli, and MPN coagulase positive staphylococci analyses (Food and Drug Administration, 1978; Speck, 1984). An ACS Spectro Sensor was used to read minced meat Hunter L, a, b color values (Hunter and Harold, 1987).

A trained five-member sensory panel determined appearance, flavor, odor, and textural characteristics of extracted minced meat (Cardello, 1981; Civille and Szczesniak, 1973; Civille and Liska, 1975; Gates et al., 1984a; Jellinek, 1985). Sensory profiles were developed for unpasteurized and pasteurized minced meat samples:

1. Blueing: No obvious blueing is 0, 100% blueing is 6.

2. Wet-to-dry appearance: 0 is dry, 6 is free liquid draining from sample.

3. Ammonia odor: 0 is none detectable, while 6 is the odor of free ammonia that would strongly irritate the nose and eyes.

4. Cooked-crab odor: 0 is none detectable, 6 is an overwhelming crab aroma reminiscent of the odors evolved from steaming crabs.

5. Putrid: 0 is none detectable, 6 is the odor associated with rotten meat.

6. Fish or TMA odor: 0 is none detectable, while 6 indicates the "fish" odor associated with old fish that are getting "off" and are barely edible.

7. Cereal odor: 0 is none detectable, while 6 indicates a strong cereal-bread-yeasty aroma.

Taste and textural profiles were evaluated for pasteurized minced crab meat:

1. Moistness: The perceived degree of oil and/or water in the sample during chewing. 0 is a very dry sample, 6 indicates free liquid readily oozing from the sample.

2. Fibrousness: The perceived degree (number x size) of fibers evident during mastication. 0 is no fibers evident, 6 indicates many large fibers.

3. Adhesiveness: The force required to remove material that adheres to the mouth during the normal eating process.

   0 = no adhesion
   3 = cream cheese
   6 = peanut butter

4. Chewiness: The length of time required to masticate a sample at constant rate of force to reduce it to a consistency suitable for swallowing.

   0 = Rye bread
   2 = Jujubes
   4 = Black cow candy
   6 = Tootsie rolls

5. Particle size: Average size of particles detected during mastication.

   0 = smooth
   1 = chalky
   2 = gritty
   3 = grainy
   4 = coarse
   6 = chunky

6. Cooked-crab taste: Relative strength of crab taste. 0 = none detected, 6 = overwhelming crab taste.

7. Astringent: 0 = none detected, 6 = the feeling of pure alum.

8. Sourness: Relative strength of acidic components, 0 = none detected, 6 = pure lemon juice or vinegar.

9. Rancidity: The after taste associated with country ham. 0 = none detected, 6 = objectionable rancidity (old country ham).

10. Freezer-burn: The taste associated with a stale refrigerator or freezer that has been used to store food. 0 = none detected, 6 = overwhelming taste.

11. Old-seafood flavor: The aromatics associated with cooked seafood that is getting "off" but still acceptable. 0 = none detected, 6 = overhwelming taste of seafood that has developed strong off flavors and is barely edible.

Bacterial levels in mixed picking-room by-products were evaluated during four hours of iced- or room-temperature storage to determine the most effective holding condition and maximum acceptable storage period before extraction.

Pasteurization times, temperatures, and F-values were determined for meat packed in five-pound plastic tubes using a Digitec temperature recorder linked with an IBM-XT (Gates et al., 1984b). Initial pasteurization temperatures were reduced to 182°F (83.3°C), because processors noted excessive blueing of meat pasteurized at 186°F (85.5°C). Product color was evaluated by the sensory panel and by Hunter L, a, b color values (Boon, 1975; Strasser et al., 1971; Waters, 1971).

Effects of low-temperature pasteurization, 177°F (80.5°C), and blueing inhibitors on minced meat color were determined for mixed minced and minced white meat samples pasteurized in eight-ounce aluminum cans. The following buffer developed by the National Marine Fisheries Service was used in the additive portion of the study (Waters, 1971).

Na_2HPO_4	20.79 g
$H_3C_6H_5O_7$	16.64 g
NaCl	21.99 g

Sodium phosphate, citric acid, and sodium chloride were diluted to one liter with deionized water to complete the buffer. Five low-temperature pasteurization treatments of minced white meat and mixed minced meat were evaluated at 177°F (80.5°C):

1. 8 ounces (226.8g) of minced meat
2. 8 ounces (226.8g) of minced meat plus 64 ml buffer
3. 8 ounces (226.8g) of minced meat plus 64 ml buffer well mixed
4. 8 ounces (226.8g) of minced meat plus 91 ml buffer
5. 8 ounces (226.8g) of minced meat plus 91 ml buffer well mixed

Buffer was either poured into a can of meat without mixing or thoroughly stirred into the meat prior to sealing. Meat was pasteurized for three hours at 177°F (80.5°C) to $F_{185}^{16} = 37.65$. Cooling was 1.5 hours in an ice slurry at 37.4°F (3°C). Three cans of meat were composited for duplicate chemical, microbiological, color, and sensory analyses.

Mixed minced meat and minced claw meat used for the frozen-storage study were packaged in 5 mil, low-density, polyethylene tubes containing approximately one pound of meat. Tubes were sealed at each end with steel rings, pasteurized in a hot water bath at 182°F (83.3°C) for 180 minutes, and cooled in an ice slurry for 90 minutes. The mean F_{185}^{16} value was 44. Meat was blast-frozen at -11.2°F (-24°C). Samples were held in a walk-in freezer at less than -4°F (-20°C). Chemical, sensory, microbiological, and nutritional changes were monitored monthly for nine months to date. Three tubes of meat were composited for duplicate analyses.

Chemical, sensory, microbiological, color, and nutritional differences in minced meat samples were statistically compared using Personal Computer SAS (Joyner, 1985; Sasser, 1985). Differences among means were determined using the GLM procedure and Duncan's multiple-range test (Joyner, 1985). In the remainder of the paper, "significant" refers to statistically significant differences among means at the 0.05 level.

RESULTS AND DISCUSSION

Minced meat yields based on uncooked green crabs were:

minced white meat	3.18%
mixed minced meat	13.89%
mixed minced meat less "slabs"	10.71%
minced leg meat	2.62%
minced claw meat	6.39%

Total recoverable minced meat was approximately 22% of an uncooked crab's weight. Yields based on by-product type as the starting point were:

minced white meat	76.63%
mixed minced meat	59.45%
minced leg meat	40.44%
minced claw meat	38.07%

Mean proximate analyses of the four meat types are presented in Table 1. Minced leg meat had significantly higher moisture levels than minced white or claw meat. Moisture contents of minced leg and mixed minced meat were significantly greater than minced claw meat. Minced white meat moisture content was significantly less than that of minced leg meat. Minced leg meat had significantly lower ash content than other meat samples. Minced claw protein levels were significantly greater than mixed minced meat. Fat levels were low for all minced meats, but claw meat had significantly less fat than leg meat which had significantly lower fat levels than white or mixed minced meat. Mixed minced meat had significantly greater moisture-free ash content than other meats. Leg meat had significantly greater moisture-free protein levels than mixed or white minced meat.

Table 1. Mean Proximate Analyses with Duncan's Analyses of Differences Among Means for Minced Crab Meat*

ANALYSES		ANALYSES	
Meat	% Moisture	Meat	% Ash
Leg	81.54A	Mixed	2.39A
Mixed	81.02AB	White	2.04AB
White	78.29 BC	Claw	1.85AB
Claw	77.99 C	Leg	1.57 BC
Meat	**% Protein**	**Meat**	**% Fat**
Claw	18.54A	White	1.73A
White	16.79AB	Mixed	1.12A
Leg	16.75AB	Leg	0.29B
Mixed	14.91 B	Claw	0.13C
Meat	**% Moisture-Free Ash**	**Meat**	**% Moisture-Free Protein**
Mixed	11.48A	Leg	90.71A
White	9.40B	Claw	84.23AB
Leg	8.53B	Mixed	79.31 B
Claw	8.36B	White	77.42 B

*Means with the same letter are not significantly different at the 0.05 level

Table 2 presents mean Hunter color L, a, b results for the four meat types. Mean L values are significantly different for all minced meats. Order of decreasing whiteness is: white, mixed, claw, and leg. Mean Hunter a values show mixed minced to be significantly more red than other meats. Blue components (b values) of mixed and white minced meat are significantly greater than blue components of claw and leg meat.

Table 2. Mean Hunter Color L, a, b Values with Duncans Analyses of Differences Among Means for Minced Crab Meat*

MEAT	L	MEAT	a	MEAT	b
White	61.43A	Mixed	4.88A	Mixed	13.06A
Mixed	54.96B	Leg	3.11B	White	12.34A
Claw	46.25C	Claw	2.87B	Claw	5.25B
Leg	41.21D	White	2.49B	Leg	4.70B

* Means with the same letter are not significantly different at the 0.05 level

Mean sensory appearance and odor ratings for minced meat samples are reported in Table 3. Leg meat appeared to be significantly more wet than white meat. Leg meat had significantly

stronger ammonia odors than white meat. No significant differences were determined for cooked crab or putrid odors. Leg meat had significantly greater TMA odor ratings than other minced meat. White meat cereal odor was significantly less intense than other meats.

Table 3. Mean Hedonic Appearance and Odor Ratings with Duncan's Analyses of Differences Among Means for Minced Crab Meat*

HEDONIC RATING		HEDONIC RATING	
Meat	Wet-Dry Appearance	Meat	Ammonia Odor
Leg	4.07A	Leg	1.93A
Claw	3.57AB	Mixed	1.14AB
Mixed	3.00 B	Claw	1.07AB
White	2.50 C	White	0.43 B
Meat	**Cooked-Crab Odor**	**Meat**	**Putrid Odor**
Claw	3.29A	Leg	0.29A
Leg	2.64A	Claw	0.14A
White	2.57A	Mixed	0.14A
Mixed	2.57A	White	0.00A
Meat	**TMA Odor**	**Meat**	**Cereal Odor**
Leg	1.43A	Claw	3.57A
Claw	0.57B	Mixed	3.43A
Mixed	0.36B	Leg	3.14A
White	0.14B	White	1.93B

*Means with the same letter are not significantly different at the 0.05 level

Textural profiles of minced meat determined by the sensory panel are presented in Table 4. Claw and leg meat were significantly more moist than white meat. Claw meat was rated as significantly more fibrous than mixed minced meat. No significant differences were determined for adhesiveness or chewiness. White and claw minces had significantly larger particle sizes than mixed minced meat.

Mean hedonic flavor profiles for the four minced meats are shown in Table 5. Mixed mince meat had a significantly greater astringent feeling than other meat. Claw meat rated significantly higher old-seafood flavors than other minces. No significant differences were determined among minced meats for sour, rancid, or freezer-burn flavors.

Table 4. Mean Hedonic Textural Ratings for Pasteurized Minced Meat with Duncans Analyses of Differences Among Means*

HEDONIC RATING		HEDONIC RATING		HEDONIC RATING	
Meat	Moistness	Meat	Fibrousness	Meat	Adhesiveness
Claw	3.58A	Claw	3.00A	Leg	2.08A
Leg	3.33A	White	2.67AB	Claw	2.00A
Mixed	3.08AB	Leg	1.92AB	White	1.75A
White	2.25 B	Mixed	1.75 B	Mixed	1.58A

Meat	Chewiness	Meat	Particle Size
White	1.92A	White	2.58A
Claw	1.33A	Claw	2.50A
Leg	1.00A	Leg	1.58AB
Mixed	0.83A	Mixed	0.83 B

* Means with the same letter are not significantly different at the 0.05 level

Table 5. Mean Hedonic Flavor Ratings for Pasteurized Minced Meat with Duncan's Analyses of Differences Among Means*

HEDONIC RATING		HEDONIC RATING	
Meat	Cooked-Crab Taste	Meat	Astringent Taste
Leg	3.33A	Mixed	2.33A
Mixed	2.92A	Leg	1.08B
White	2.58A	Claw	1.08B
Claw	2.42A	White	0.92B

Meat	Sour Taste	Meat	Rancid Taste
Mixed	0.17A	Claw	0.50A
Leg	0.00A	Mixed	0.17A
Claw	0.00A	Leg	0.00A
White	0.00A	White	0.00A

Meat	Freezer-Burn Taste	Meat	Old-Seafood Taste
Claw	0.00A	Claw	0.67A
Leg	0.00A	Mixed	0.50AB
Mixed	0.00A	Leg	0.17AB
White	0.00A	White	0.00 B

*Means with the same letter are not significantly different at the 0.05 level

Minced meat exhibited excessive microbial levels, ranging from 10^5 to 10^7 organisms per gram (Figure 1).

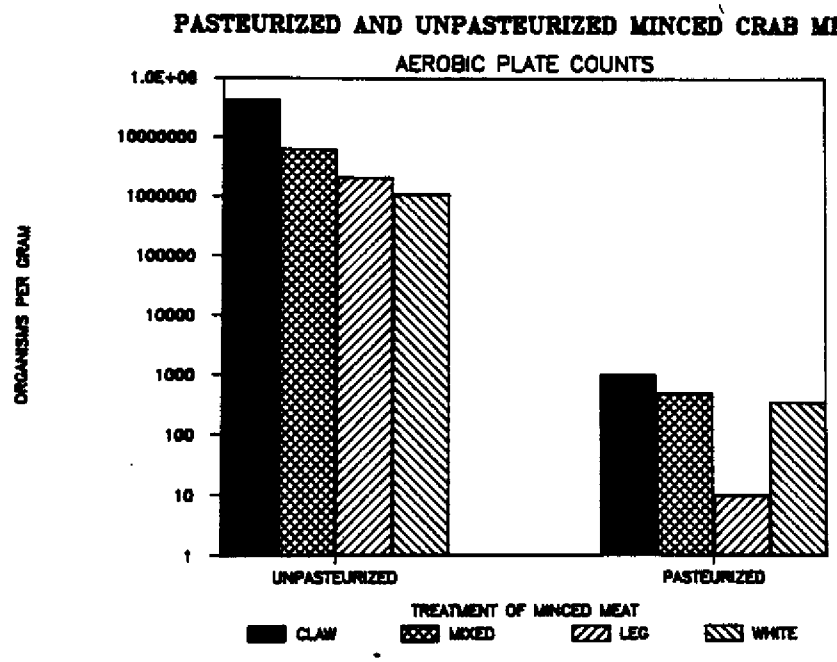


Figure 1. Mean minced meat aerobic plate counts before and after pasteurization

Pasteurization at 182°F (83.3°C) ($F_{185}^{16} = 44$) reduced plate counts to less than 3,000 organisms per gram (Figure 1). No total coliform, E. coli, or coagulase positive staphylococci were detected in pasteurized meats. Hourly clean-up and sanitation of the Baader machine improved product quality; however, by-product microbial levels increased rapidly when held in the picking room at room temperature. Extraction within 1.5 hours of picking showed little increase in the microbial population of mixed by-products. Delayed extraction beyond 1.5 hours required icing of picking-room by-products within plastic bags at a ratio of 2:1 ice-to-product to control microbial growth (Figure 2). By-product temperature dropped below 40°F (4.4°C) within 70 minutes of icing (Figure 3).

Pasteurization at 182°F (83.3°C) effectively reduced microbial levels for all minced meats (Figure 1); however, meats darkened following pasteurization. Hunter color L, a, b values before and after pasteurization are presented in Figures 4, 5, and 6, respectively. Hunter L or whiteness decreased for all pasteurized meats (Figure 4).

Hunter a or redness decreased for all pasteurized samples (Figure 5). Hunter b values decreased for all pasteurized samples, indicating increased levels of blueing (Figure 6).

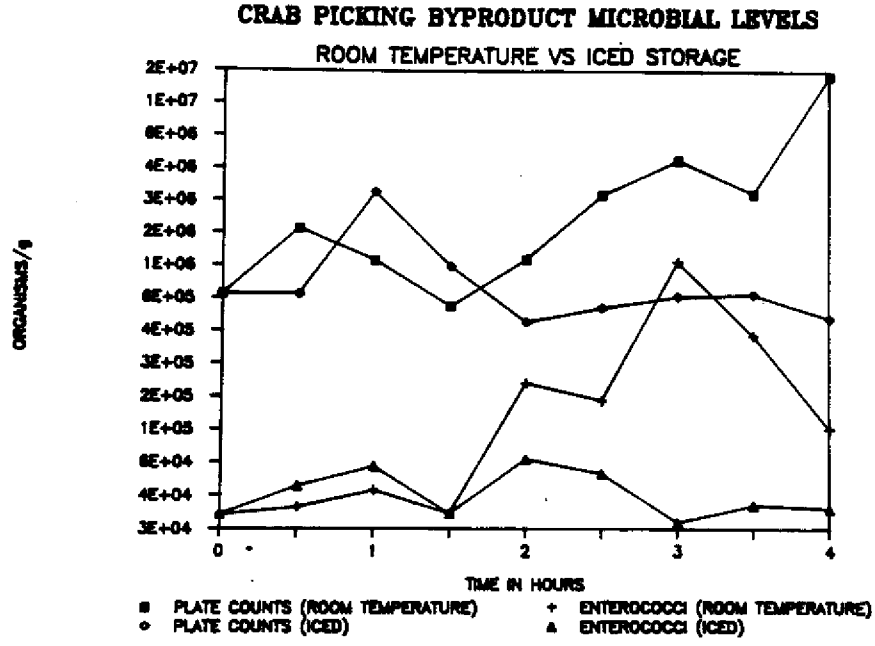


Figure 2. Mean microbial levels of mixed picking-room by-products held on ice and at room temperature

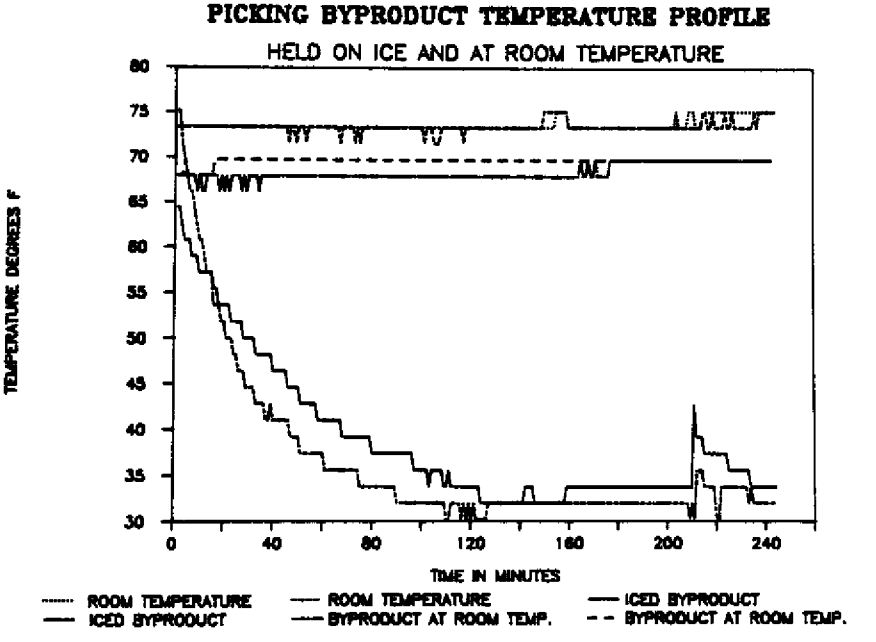


Figure 3. Temperatures of mixed picking-room by-products held on ice and at room temperature for four hours

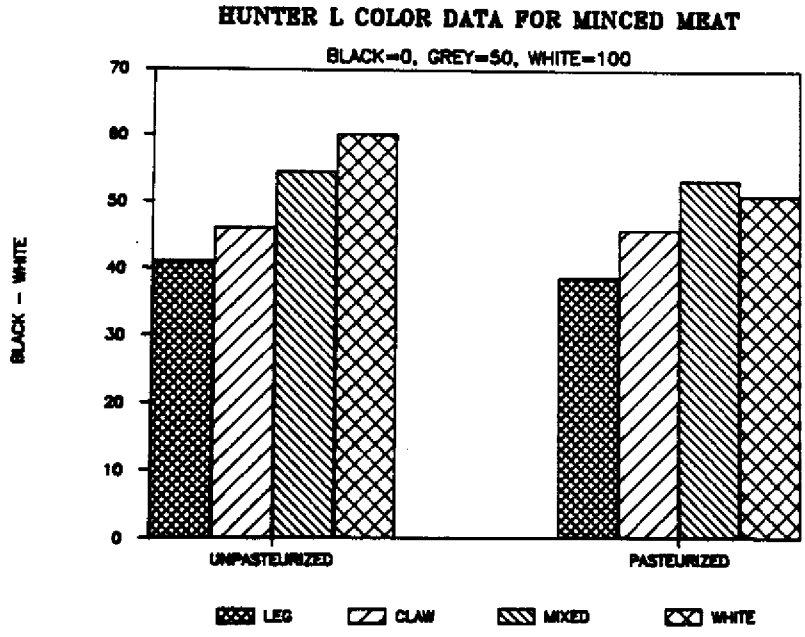


Figure 4. Mean Hunter L color values for leg, claw, mixed, and white minced meat before and after pasteurization at 182°F (83.3°C)

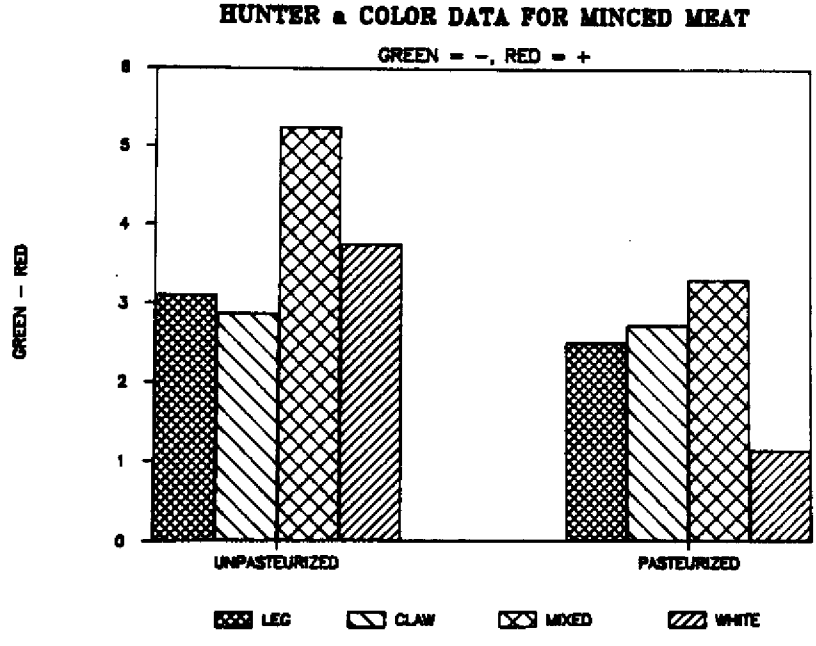


Figure 5. Mean Hunter a color values for leg, claw, mixed, and white minced meat before and after pasteurization at 182°F (83.3°C)

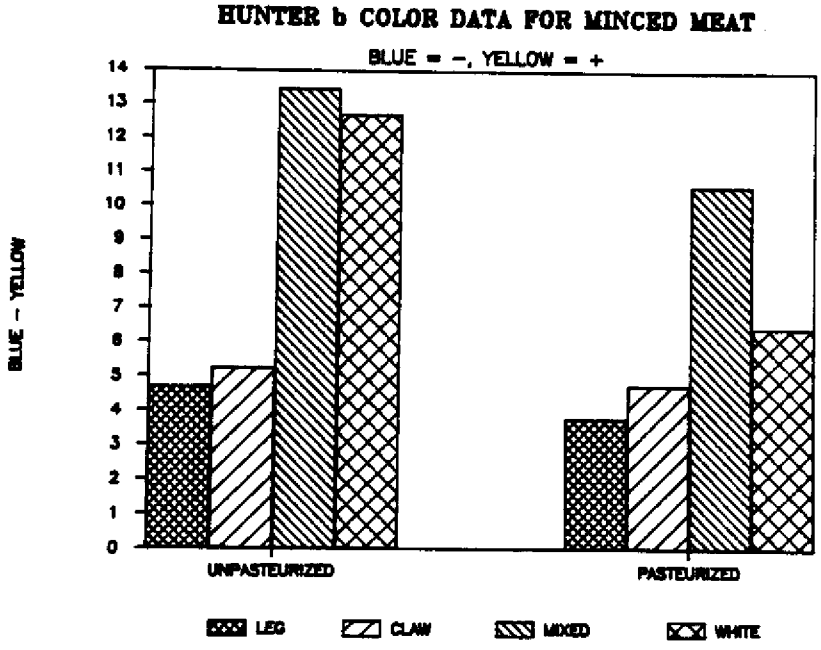


Figure 6. Mean Hunter b color values for leg, claw, mixed, and white minced meat before and after pasteurization at 182°F (83.3°C)

Hunter color L, a, b values for minced white meat and mixed minced meat pasteurized at 177°F (80.5°C) and 182°F (83.3°C) are presented in Figures 7 and 8, respectively. Minced white meat pasteurized at 177°F (80.5°C) was significantly more white and less blue than meat pasteurized at 182°F (83.3°C) as indicated by Hunter L and b values, respectively (Figure 7). White minced meat blended with 64 ml of buffer was significantly whiter than untreated white minced meat when both were pasteurized at 177°F (80.5°C). Unpasteurized white minced meat was more red or had greater a values than all pasteurized white meat. All treated white minced meat except for the unblended sample containing 64 ml of buffer exhibited less blueing or higher b values than untreated meat pasteurized at 177°F (80.5°C). There was no significant difference between unpasteurized white minced meat and meat treated with 91 ml of phosphate buffer.

Mixed minced meat pasteurized at 177°F (80.5°C) was significantly whiter as indicated by greater Hunter L values than meat cooked at 182°F (83.3°C) (Figure 8). Hunter b values showed significantly more blueing at 182°F (83.3°C) than at 177°F (80.5°C). Mixed minced meat treated with buffer and pasteurized at 177°F (80.5°C) was significantly whiter than untreated meat pasteurized at the same temperature. Mixed minced meat treated with phosphate buffer was significantly more red than unpasteurized mixed minced meat.

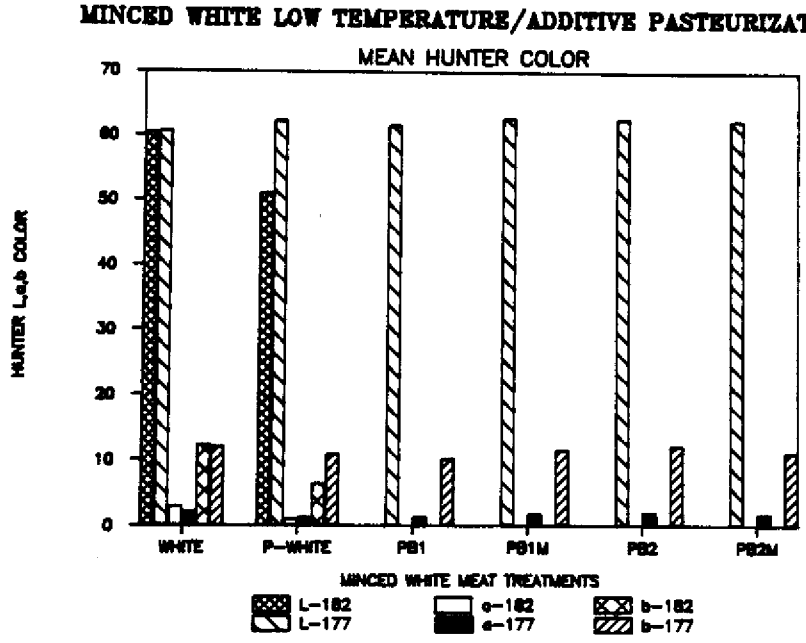


Figure 7. Mean hunter L, a, b color values of minced white meat pasteurized at 182°F (83.3°C) and 177°F (80.5°C), including added buffers at 177°F (80.5°C); white = unpasteurized, P-white = pasteurized; PB1 = 64 ml phosphate buffer, PB1M = 64 ml buffer mixed, PB2 = 91 ml phosphate buffer, PB2M = 91 ml phosphate buffer mixed

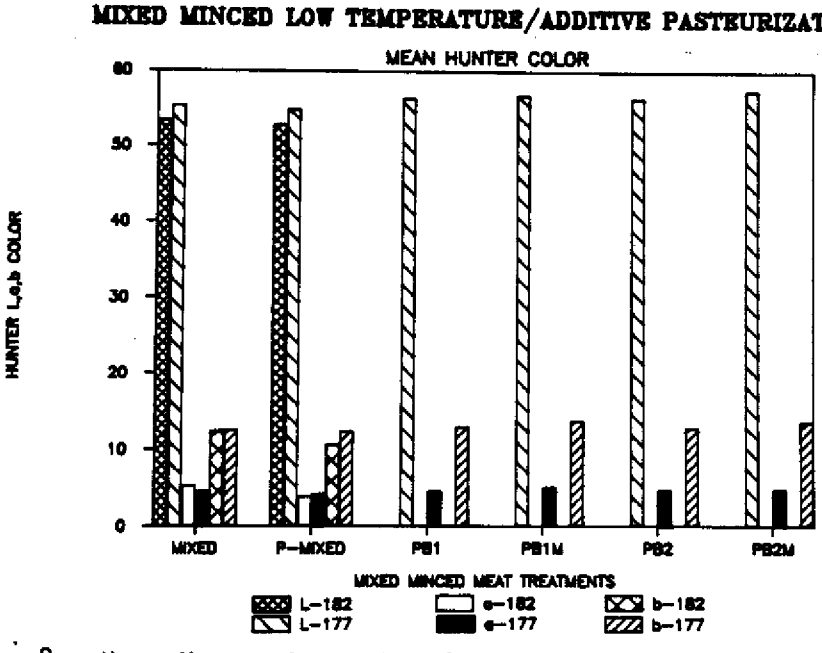


Figure 8. Mean Hunter L, a, b color values of mixed minced meat pasteurized at 182°F (83.3°C) and 177°F (80.5°C), including added buffers at 177°F (80.5°C); mixed = unpasteurized, P-mixed = pasteurized, PB1 = 64 ml phosphate buffer, PB1M = 64 ml buffer mixed, PB2 = 91 ml phosphate buffer, PB2M = 91 ml phosphate buffer mixed

Pasteurized minced meat was separated into three significantly different levels of increasing blueing by Hunter b values: (1) meat blended with phosphate buffer, (2) meat treated with phosphate buffer without blending, and (3) untreated meat. Pasteurization at 177°F (80.5°C) effectively reduced bacterial populations. Total aerobic plate counts for pasteurized mixed minced and minced claw meat ranged from none detected to 160 organisms per gram. No total coliform, E. coli, or coagulase positive staphylococci were detected.

Mixed minced and minced claw meat stored at less than -4°F (-20°C) exhibited no significant differences in the following parameters during nine months of frozen storage: (1) proximate composition, (2) blueing, (3) wet-to-dry appearance, (4) ammonia odor, (5) cooked-crab odor, (6) TMA odor, (7) cereal odor, (8) perceived moistness, (9) fibrousness, (10) adhesiveness, (11) chewiness, (12) particle size, (13) cooked-crab taste, (14) astringent taste, or (15) sourness.

The sensory panel rated minced claw meat significantly more putrid in the ninth month of frozen storage than meat evaluated in months zero through seven (Figure 9). Claw rancidity was significantly greater in month nine than all other samples (Figure 9). Figure 10 shows minced claw freezer-burn levels to be significantly greater in month nine than levels found in months one through three. Old-seafood flavors were significantly greater in month nine than all other sampled months. Mixed minced meat rancid levels were significantly greater at months six and nine of frozen storage. Old-seafood flavor levels peaked for mixed minced meat during month nine of the frozen-storage study.

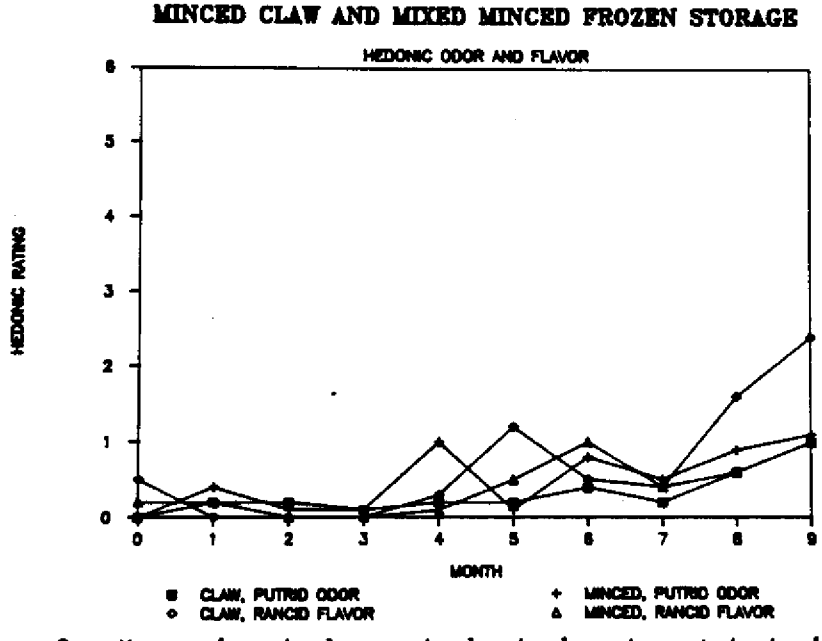


Figure 9. Mean minced claw and mixed minced meat hedonic odor and flavor ratings that were significantly different during nine months of frozen storage

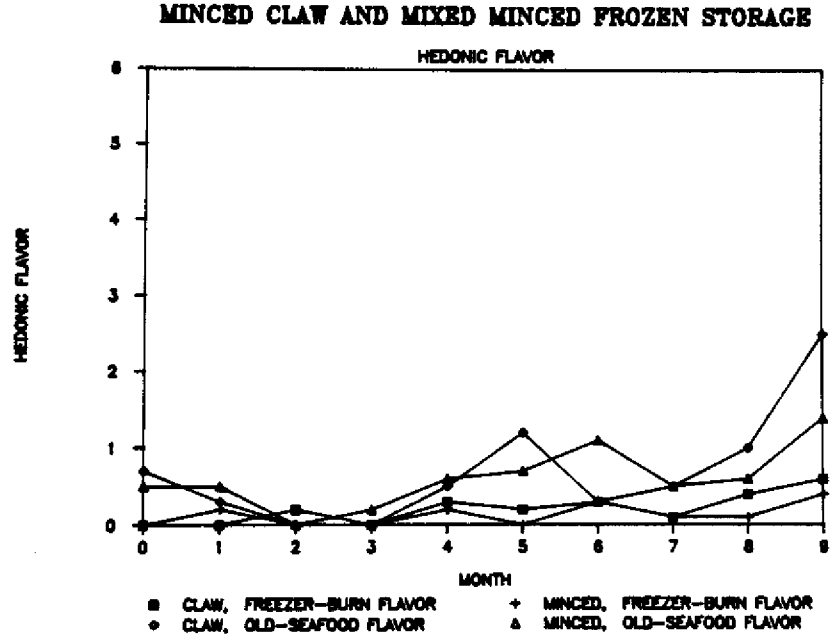


Figure 10. Mean minced claw and mixed minced meat hedonic flavor ratings that were significantly different during nine months of frozen storage

Both claw and mixed minced meat darkened with time as indicated by decreasing Hunter L values (Figure 11). Significant negative regressions were determined between time and Hunter L values. Claw and minced meat had r^2 values of 0.41 and 0.48, respectively.

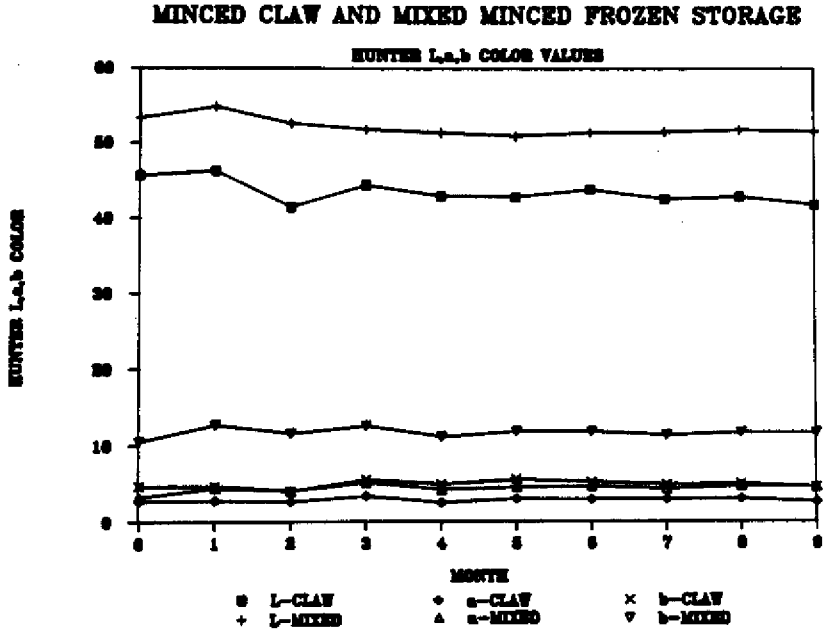


Figure 11. Mean Hunter L, a, b values determined for minced claw and mixed minced meat during nine months of frozen storage

CONCLUSIONS

Blue crab picking-room by-products were separated into four components prior to extraction with a Baader 694 machine. By-product types were: (1) "slabs," the portion of hand-picking by-product containing only white body meat; (2) mixed by-product that includes all picking-room by-products but claws; (3) separated legs; and (4) separated claws. Minced meat yields based on the weight of an uncooked green crab were: white meat, 3.18%; mixed minced meat, 13.89% (10.71% if slabs are separated); minced leg, 2.62%; and minced claw, 6.39%. Total recoverable minced meat is approximately 22% of an uncooked crab's weight. Yields based on by-product type as the starting point were: 76.63, 59.45, 40.44, and 38.07% for "slab," mixed, leg, and claw by-products, respectively.

Four meats with distinct chemical compositions, colors, flavors, and textures were extracted. Leg and mixed minced meat had the highest moisture contents. Ash contents were low, ranging from 1.57% for leg meat to 2.14% for mixed minced meat, indicating little shell contamination. Fat content was low, ranging from 0.12% to 1.73%. Mixed minced and white meat had significantly higher fat contents than minced leg or minced claw meat. Minced claw had the highest protein content, 18.54%. "Slabs" produced a dry, white, textured mince; mixed by-product produced a moist, golden-brown mince; legs produced a smooth, flavorful, dark-brown meat; and claws produced a highly-textured, less-flavored, chewy, brown mince.

Minced meat exhibited excessive microbial levels, ranging from 10^5 to 10^7 organisms per gram. Hourly clean-up and sanitation of the Baader machine improved product quality; however, by-product microbial levels increased rapidly when held in the picking room at room temperature. Extraction of by-products within 1.5 hours of picking showed little increase in the microbial populations of mixed by-products. Delayed extraction beyond 1.5 hours required icing of picking-room by-products within plastic bags at a ratio of 2:1 ice-to-product to control microbial growth. Iced by-product temperature dropped below 40°F (4.4°C) within 70 minutes.

Reduced pasteurization temperatures significantly improved the appearance of minced meat and effectively reduced microbial populations. Initial reduction of pasteurization temperatures from 186°F (85.5°C) to 182°F (83.3°C) ($F_{185}^{16} = 44$) improved the appearance of pasteurized meats. However, all minced meats still darkened following pasteurization at 182°F (83.3°C) as indicated by Hunter L values. Pasteurization of minced slab meat and mixed minced meat at 177°F (80.5°C) ($F_{185}^{16} = 38$) significantly improved appearance of pasteurized meats. Both minces were significantly more white and less blue than meat pasteurized at 182°F (83.3°C). The addition of citric-acid-phosphate buffer to minced slab and mixed minced meat pasteurized at 177°F (80.5°C) produced pasteurized product that was significantly whiter and

less blue than non-buffered meat cooked at 177°F (80.5°C). Buffered meats were not significantly darker or more blue than unpasteurized minced slab or mixed minced meat as indicated by Hunter L, a, b color values.

Local processors adopted a five-pound, ring-sealed, low-density, polyethylene plastic tube for pasteurized minced meats. Bag failure rates approaching 50% during shipping and handling required processors to blast-freeze pasteurized products. Monthly chemical, microbiological, and sensory monitoring of frozen minced claw and mixed minced meat showed initial sensory deterioration in both meats at nine months of a continuing frozen-storage study.

LITERATURE CITED

Boon, D. D. 1975. Discoloration in processed crab meat. A. Review. J. of Food Sci. 40:756-761.

Cardello, A. 1981. Psychophysical basis for the classification of fish by flavor, texture, and appearance. Gordon Research Conference on the Chemical Senses, Andover, NH.

Civille, G. V. and Szczesniak, A. S. 1973. Guidelines to training a textural profile panel. J. of Textural Studies. 4:204-223.

Civille, G. V. and Liska, I. H. 1975. Modifications and applications to foods for the General Foods sensory texture profile technique. J. of Textural Studies. 6:19-31.

Food and Drug Administration. 1978. Bacteriological Analytical Manual. pp. IV-1 t IV-10, V-1 to V-6, XI-1 to XI-7. Association of Official Analytical Chemists, Washington, DC.

Gates, K. W., EuDaly, J. G., Parker, A. H., and Pittman, L. A. 1984a. Nutritional and quality changes in frozen breaded shrimp within the wholesale-retail marketing chain. Georgia Marine Science Center Technical Report Series No. 84-2.

Gates, K. W., EuDaly, J. G., Parker, A. H., and Pittman, L. A. 1984b. Microcomputers improve quality and safety for blue crab heat processing. Georgia Marine Science Center Technical Report Series No. 84-3.

Hunter, R. S. and Harold R. W. 1987. The Measurement of Appearance. John Wiley and Sons, New York, NY.

Jellinek, G. 1985. Sensory Evaluation of Food, Theory and Practice. Ellis Horwood, Chinchester, England.

Joyner, S. P. 1985. SAS/STAT Guide for Personal Computers. SAS Institute, Inc., Cary, NC.

Murray, T. J. and DuPaul, W. D. 1981. Feasibility of crab meal processing in the Chesapeake Bay region. Special Report in Applied Marine Science and Ocean Engineering No. 248, July 1981. Virginia Institute of Marine Science, Gloucester, VA.

Sasser, M. 1985. SAS Procedures Guide for Personal Computers Version 6. SAS Institute, Cary, NC.

Speck, M. L. 1984. Compendium of Methods for the Microbiological Examination of Foods. American Public Health Association, Washington, DC.

Strasser, J. H., Lennon, J. S., and King, F. J. 1971. Blue Crab Meat II. Effect of chemical treatments on acceptability. Special Scientific Report - Fisheries, No. 630. U. S. Dept. of Commerce, Washington, DC.

Thompson, B. G. 1985. Fisheries of the United States. 1984. Current Fisheries Statistics No. 8360. U. S. Dept. of Commerce, Washington, DC.

Waters, M. E. 1971. Blueing of Processed Crab Meat. I. A study of processing procedures that may cause blue discoloration in pasteurized crab meat. II. Identification of some factors involved in the blue discoloration of canned crab meat (Callinectes sapidus). Special Scientific Report - Fisheries, No. 633. U. S. Dept. of Commerce, Washington, DC.

Williams, S. 1984. Official Methods of Analysis of the Association of Official Analytical Chemists, Fourteenth Edition. Association of Official Analytical Chemists, Arlington, VA.

ACKNOWLEDGEMENTS

The authors greatfully acknowledge Lewis Crab Factory, Inc. and Sea Garden Seafoods, Inc. for supporting the project, providing deboning machinery, pasteurization baths, and needed plant time. We wish to thank the O'Brien Corporation for use of their ACS Spectro Sensor.

The technical assistance of the following individuals was greatly appreciated: Andy Amason, Jack Amason, David Lewis, and Lura Relihan.

This work is the result of research sponsored by NOAA Office of Sea Grant, Department of Commerce, and the University of Georgia under Federal Grant #NA84AA-D-00072, Project #R/SS-10. The U. S. Government is authorized to produce and distribute reprints for governmental purposes notwithstanding any copyright notation that may appear hereon.

The Menhaden Surimi Demonstration Plant- A Progress Report

Malcolm B. Hale
National Marine Fisheries Service
Charleston, SC 29412

Anthony P. Bimbo
Zapata Haynie Corporation
Reedville, VA 22539

INTRODUCTION

Menhaden landings comprised approximately 40% of the total volume of U.S. commercial fisheries landings in 1986, but accounted for less than 3.5% of the ex-vessel value (NMFS, 1987). With the exception of a small fraction used for bait, the menhaden harvest is used only for the production of fish meal and oil. Continuing economic pressure by cheaper vegetable-based products has encouraged increased efforts to develop higher valued products for direct human food use of menhaden (Hale and Ernst, 1986). Both Lanier et al. (1983) and Regier et al. (1985) have reported on laboratory and small pilot scale investigations of surimi production from menhaden. With a rapid growth in demand and imports, surimi was targeted as a potential food use of menhaden. The remarkable growth in the U.S. supply of surimi products in recent years is shown in Figure 1.

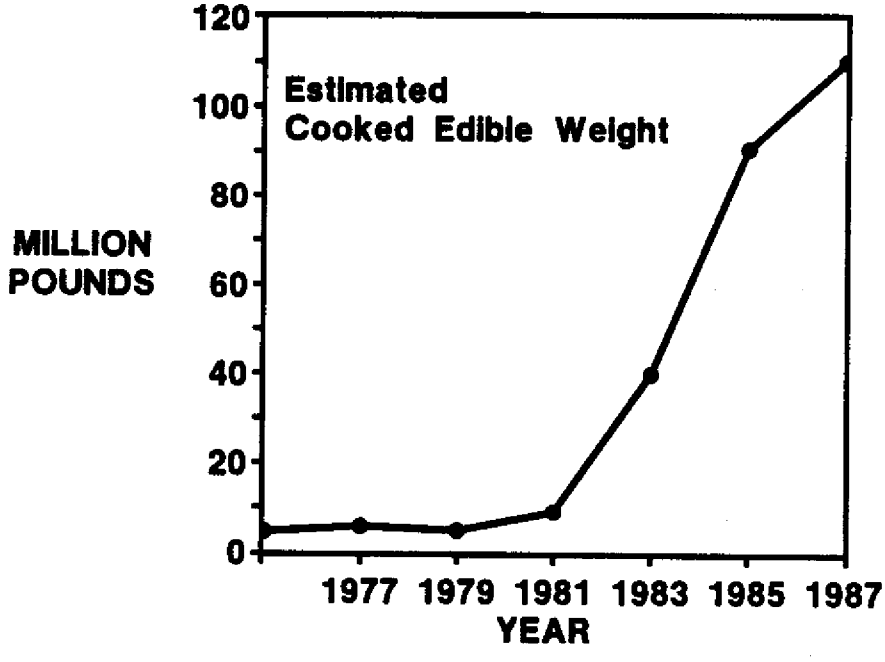


Figure 1. United States supply of surimi products, 1975-1987.

In response to industry needs and the opportunity for true economic growth, the U.S. Congress designated increased FY 1985 funding for menhaden research and development in the conversion of whole menhaden into surimi and minced forms. The National Marine Fisheries Service in cooperation with industry was directed to use the funds to achieve the following objectives: (1) determine the economic and technical feasibility of producing menhaden surimi, (2) produce developmental quantities of test menhaden surimi material for formulation and evaluation of end products by food scientists, and (3) determine onboard and inplant procedures necessary to maintain menhaden quality for human consumption.

R & D Contract

In February of 1986 a research and development contract for a 2-year, $2 million menhaden surimi project was awarded to the Zapata Haynie Corporation. About $1.3 million in U.S. Government funds was supplied for the contract, with technical monitoring performed by the National Marine Fisheries Service. Zapata Haynie Corporation provided in excess of $600,000. The functional and financial responsibilities of the two parties were specified as follows:
Zapata Haynie, Inc. must provide:

* An appropriate physical site with immediate access to fresh supplies of Atlantic menhaden.

* An appropriate food grade building with walls, floors, drains etc. meeting sanitation standards for food fish plants.

* Refrigerated holding facilities for menhaden between delivery and processing.

* A plate freezer of sufficient capacity to freeze surimi block production.

* Frozen storage facilities to hold surimi products at a temperature of -10 F (-23°C) or lower.

* Utilities installations to support plant operations.

* Amounts of fresh food grade menhaden to meet surimi production requirements; harvested, handled, and delivered according to specified procedures.

* An acceptable means for the disposal/utilization of both solid wastes and processing waste waters.

U.S. Government funds may be used for:

* The purchase of fish processing equipment.

* Installation and maintenance of processing equipment.

* Plant operating expenses.

* Collection of data required for technical and economic evaluation of the process and products.

* Storage and distribution of menhaden surimi samples.

* Preparation of interim and final reports.

* Conducting public access/technology transfer activities.

* Project overhead costs.

The contract calls for a plant capable of producing at least 1 ton of surimi per 8-hour day. Although the contract spells out the steps needed to produce surimi, it recognizes that the processing requirements to make a quality surimi from menhaden are not well defined and prescribes a flexible plant design to allow evaluation of a number of different processing options through experimental runs. The contract requires that at least 30 tons of menhaden surimi be produced under U.S. Department of Commerce inspection and that samples be made available for public distribution.

THE PROCESS

Harvesting

A top quality, food-grade processing facility was constructed by Zapata Haynie Corporation on a site adjacent to their fish meal and oil plant at Reedville, Virginia. Fresh Atlantic menhaden are supplied by either a small bait purse seiner, modified to quickly chill and hold the fish below 40°C using a recirculating ice water spray system, or by boats of the Zapata Haynie fishing fleet. On the decks of the Zapata boats, the fish are chilled and held in slush ice in 35 cubic foot insulated containers. On one boat, the EARL J. CONRAD, the containers are connected to a circulating refrigerated water system. All fish for the surimi plant are pumped directly from the water to the insulated storage containers, bypassing the main fish hold. The containers are taken off the vessel with a crane and are trucked from the dock to a refrigerated holding room adjacent to the processing area in the surimi plant. Plant chilled water can be circulated through the containers during the holding period. When required for processing, the individual containers are hoisted and dumped into a water-filled tank where the ice is removed by flotation. The fish are lifted from the tank by an inclined conveyor to a belt scale which weighs them as they are transported into the processing area.

Processing

In the processing area the fish are partially hand sorted as time permits on the feed belt leading to the two Baader 33[1] filleting machines. Solid waste from the filleters and rejected fish are conveyed from the building and trucked to the fish meal plant. The butterfly fillets (backbones removed) are washed with chilled water in a rotary washer before they are fed to the mechanical deboner. Initially the deboned mince was washed three times in the batch wash tank followed by rotary screening and refining. In July an inline washing system was installed. Menhaden mince is fed into the wash system by a Moyno pump while chilled water is metered in at a 5 to 1 ratio of water to mince. Static mixers inside a series of stainless steel retention loops provide good contact between the water and mince for extraction of soluble protein and color bodies. The slurry exits to a decanter centrifuge for recovery of the washed mince.

The washed mince is refined and then dewatered in a screw press. It is mixed with cryoprotectants (4% each sugar and sorbitol plus 0.2% polyphosphate) in a cutter-mixer to produce the surimi. The surimi is extruded into polyethylene bags and formed into blocks in metal trays. Some 1 and 2-kg blocks as well as the standard 10-kg blocks are produced from each batch. The formed blocks of surimi are frozen in a plate freezer and then stored in a holding freezer at -30 F (-34°C).

OPERATIONS

First Year

Equipment was ordered and installed and much of the basic work on harvesting methods was done during the spring and summer of 1986. Plant construction was completed in early October. After several shake down runs, a decision was made to produce and store frozen fillets for use during the winter. A full-scale production run was made in November which demonstrated the 1 ton per day capacity of the plant.

The winter work provided the opportunity to test the administrative structure of the project as well as the operation of the various pieces of equipment. Most batches run during the winter, with frozen fillets, were small and the press was not operated until near the beginning of the 1987 fishing season. The deboner, washing cycle, and refiner were extensively tested.

---

[1] The mention of trade names does not imply endorsement by National Marine Fisheries Service, NOAA.

Current Year

Initially, lab studies were made to determine the best quality surimi that could be produced from the white flesh of the menhaden. The results from those runs were averaged and used as the initial benchmark for menhaden surimi. The first extended production runs were made in June and were followed by more experimental runs alternated with production runs. All production runs are federally inspected and all product for distribution bears the U.S. Department of Commerce inspection seal.

Other types of equipment and procedures were evaluated including Beehive and Yield Master deboners, replacing the refiner with a strainer, smaller screen sizes in the deboner, and different wash cycles. In late July, we began testing the Alfa Laval inline wash system and decanter centrifuge. Most of the fat is removed with the liquid phase from the centrifuge. The solids are refined and dewatered before mixing with the cryoprotectants.

RESULTS AND DISCUSSION

Many of the equipment tests were inconclusive, but some changes were indicated and results from the inline washing system look promising. Surimi quality is at least as good as that from the batch system, yields are improved, water use is reduced, and there is a definite savings in labor. The inline wash system has remained in operation since late July.

The surimi process is essentially an isolation of the muscle protein of the fish from the other components of the flesh. Changes in protein, fat and ash through the processing steps are shown (moisture-free basis) in Figure 2. There is an increase in protein through the process but the level drops in the surimi block because of the addition of 8% cryoprotectants (24% dry basis). These are carbohydrates and thus are not reflected in the ash content.

Data collected so far indicates that residual fat in the surimi is related to the fat content of the starting material. In Figure 3 the % fat in the frozen surimi block is plotted versus % fat in the whole fish from which it was made. However, more data points from fatty fish are needed to confirm this positive correlation. Of more significance perhaps is the question of what fat level is acceptable in the final product. The menhaden surimis produced at Reedville have exhibited excellent gel strengths. Based on some earlier work at the Charleston Laboratory, the fatty acid profile in the residual lipids is similar to that of the fish fillet lipids and should have positive nutritional

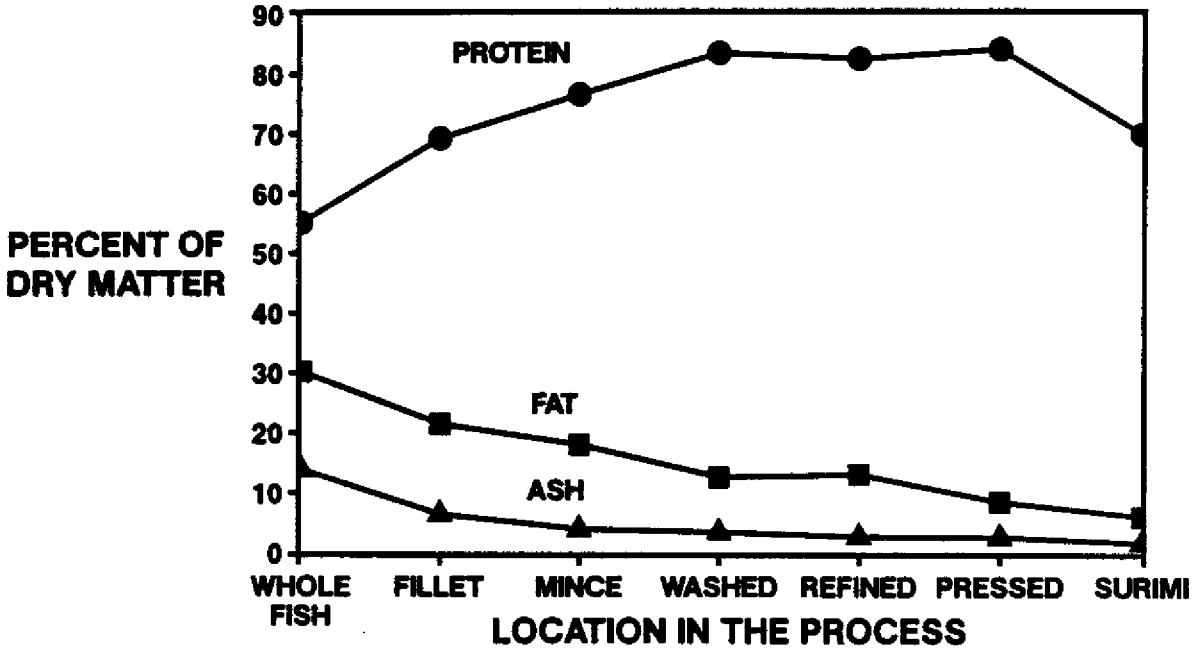


Figure 2. Changes in protein, fat, and ash content (dry basis) through the menhaden surimi process.

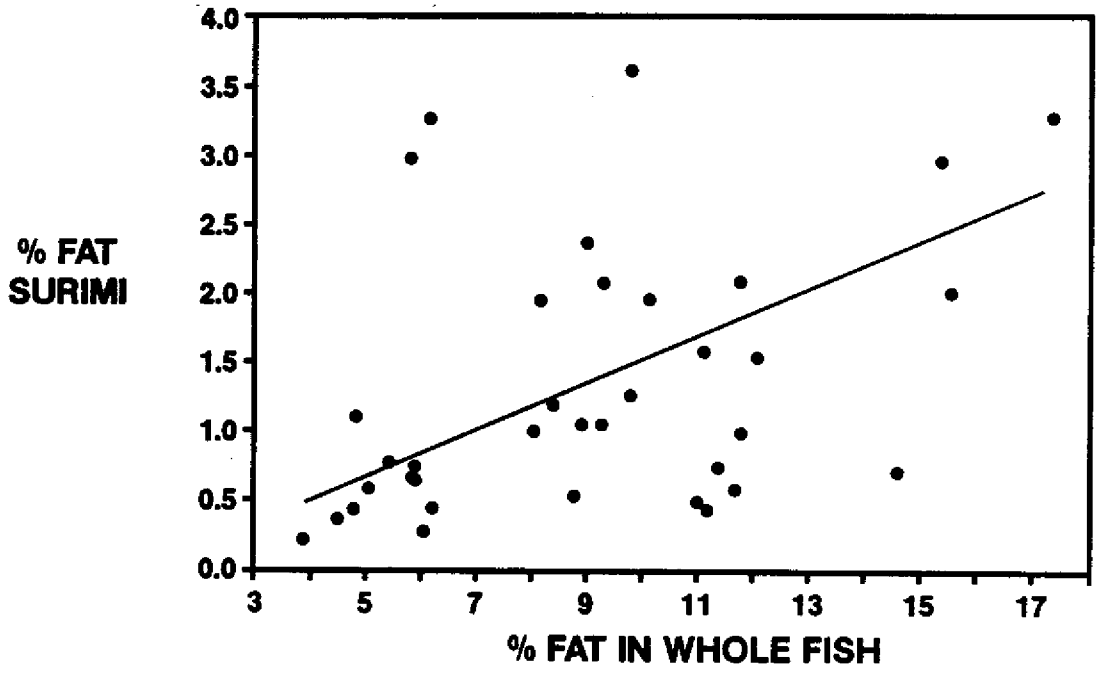


Figure 3. Fat content of surimi versus fat content of whole menhaden.

value. This is indicated by the data in Table 1. The calculations of grams of fatty acid per gram of tissue are based on the method of Weirauch et al. (1977). We did not measure whole fish fat levels, but the level in these Atlantic menhaden fillets was low. The fat levels in the gulf menhaden fillets and surimi were higher, but there were no significant flavor changes during 6 months of frozen storage at -20°C.

Table 1. Omega-3 fatty acids and lipid content of two menhaden surimis processed at NMFS, Charleston.

Species:	Atlantic Menhaden		Gulf Menhaden	
Form:	Fillets	Surimi	Fillets	Surimi
% Total Lipid:	0.93	0.54	4.61	1.85
Omega-3 Fatty Acids Area %:	40.7	40.3	30.4	26.5
gm/100 gm Tissue:	0.29	0.16	1.26	0.42

There have been wide fluctuations in yields for menhaden surimi at the Reedville plant, as shown in Figure 4. This has been due to large variations in fish size and also to experimental changes in equipment and procedures. Mechanical sorting before filleting should improve yield significantly. The goal is to improve yields without sacrificing quality.

The primary quality problem is color, assuming that the surimi is to be used in analog products. Changes in instrumental color values through the process are shown in Figure 5. Washing removes blood and pigment, increasing the lightness (L value) and decreasing the red color (a value). Significant changes in the L and a values also occur when the surimi is cooked into a kamaboko gel. The yellowness (b value) remains essentially unchanged through the processing steps, however.

A 400-lb sample of menhaden surimi from the Reedville plant was processed at Sea Fest Products in Motley, Minnesota into a crab analog product. The color was darker than desired but the flavor was acceptable and the texture of the product was very good.

Samples of menhaden surimi are currently available from Surimi Project, Zapata Haynie Corporation, P.O. Box 175, Reedville, Virginia 22539. The product may be obtained free for evaluation or product deve-

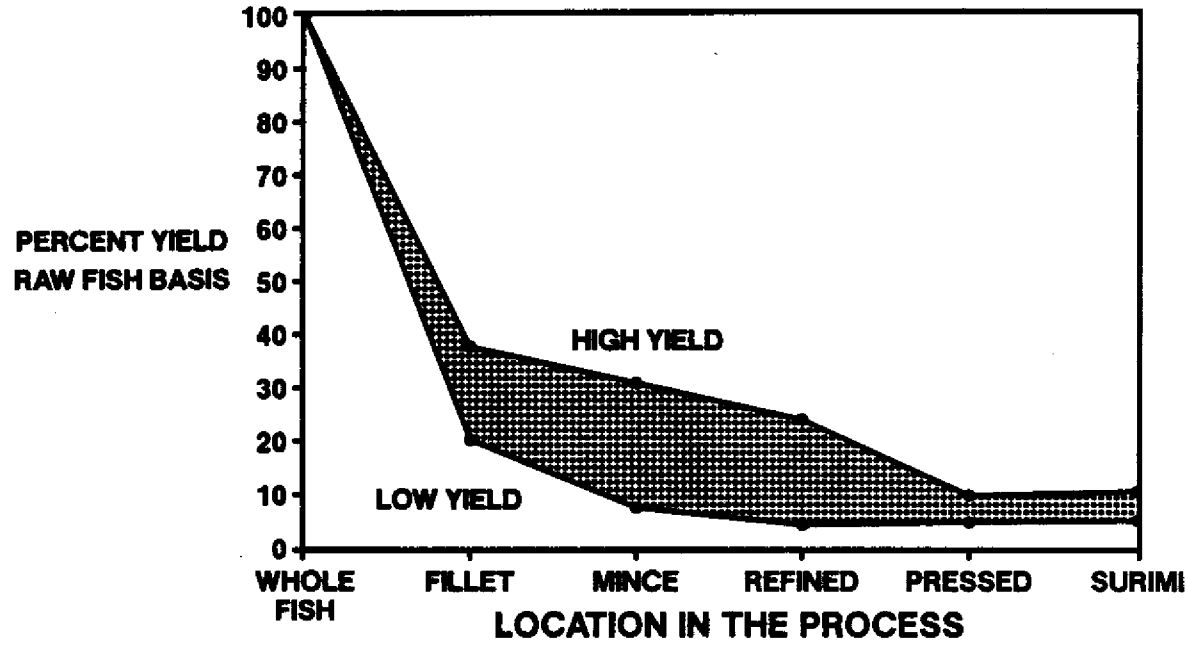


Figure 4. Range of yields versus menhaden surimi process step.

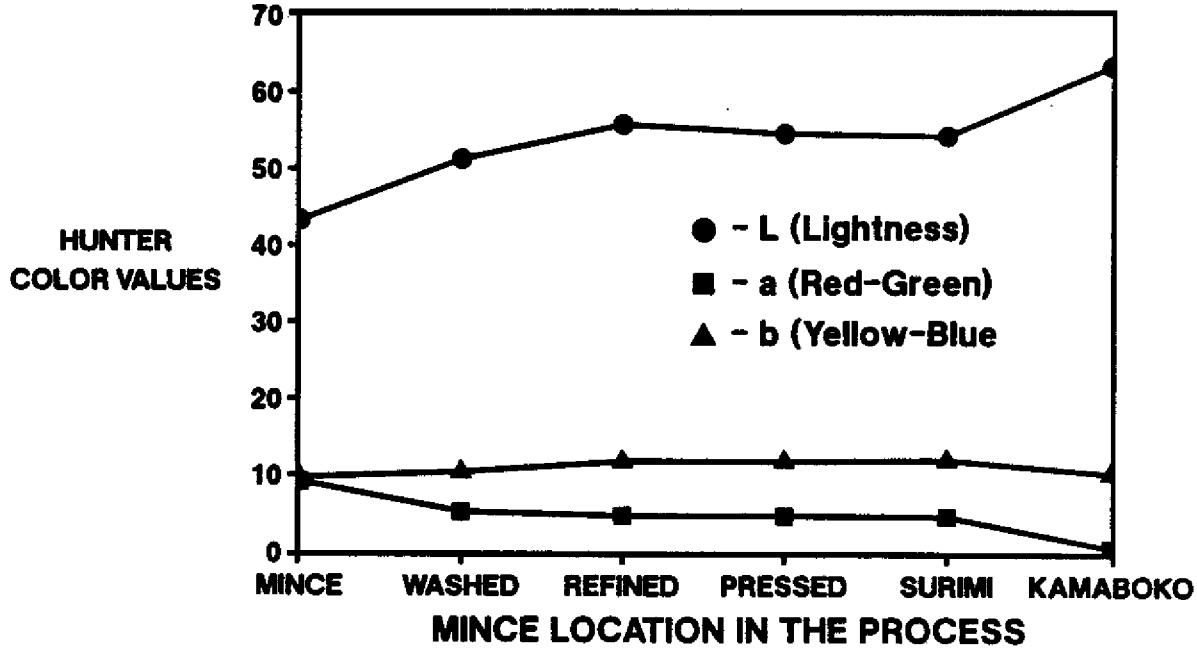


Figure 5. Changes in Hunter color values through the menhaden surimi process

lopment, but the recipient must pay shipping charges (by overnight air express) and is asked to provide feedback information on product applications and results.

Some additional contract money was provided to continue operation of the processing line through the fall, to test the use of higher fat fish. The current contract runs through January. It is anticipated that Congress will appropriate money for the additional work that is needed during the next fishing season. This would include:

* Determine methods to improve the color of the surimi and ways to further reduce fat content.

* Determine the flavor stability of the surimi and the need to remove additional fat.

* Establish reproducible specifications for menhaden surimi.

* Optimize yields at acceptable quality levels.

* Evaluate Atlantic mackerel as a surimi source.

* Evaluate gulf menhaden as a surimi source.

REFERENCES

1. Hale, M. and R. Ernst, Jr. 1986. Processing menhaden for conventional food products, minced intermediates and surimi. Proc. 11th Ann. Trop. Subtrop. Fish. Tech. Conf. Amer. pp. 293-304.

2. Lanier, T., T. Akahane, and R. Korhonen. 1983. Exploration of menhaden as a resource for surimi production and use in simulated shellfish products. Proc. 8th Ann. Trop. Subtrop. Fish. Tech. Conf. Amer. pp. 222-233.

3. National Marine Fisheries Service. 1987. Fisheries of the United States, 1986. Current Fishery Statistics No. 8380. U.S. Dept. Commerce, NOAA, NMFS.

4. Regier, L., R. Ernst and M. Hale. 1985. Processing requirements for the preparation of minced intermediates or surimi from menhaden. Proc. Intl. Symposium on Engineered Seafoods, Seattle, WA.

5. Weirauch,J., L. Posati, B. Anderson and J. Exler. 1977. Lipid conversion factors for calculating fatty acid contents of foods. J. Amer. Oil Chem. Soc. 54:36-40.

INTERACTION OF FROZEN STORAGE TEMPERATURE AND FOOD ADDITIVES WITH COD FILLET AND FRAME MINCE

Jorge Toro, Tim Hwang, and Joe M. Regenstein
Department of Poultry and Avian Sciences
and Institute of Food Science
Rice Hall, Cornell University, Ithaca, NY 14853-5601

INTRODUCTION

As the Northeast U.S. examines its alternatives for the use of its fishery by-products, the need to produce human grade products from these wastes becomes obvious. The simplest alternative in many cases may be the production of mince or mechanically deboned fish flesh from various filleting wastes. This would reduce the waste generated by 15 to 30% and would produce a more valuable product that could help offset the cost of removing the remaining waste. The available by-product raw material will vary from the very white mince of the V- or J-cut of a fillet to the very red frame mince obtained by deboning the fillet rack (assumed to be headed and gutted). The latter, however, contains much more iron and possibly more omega-3 fatty acids. The nutritional availability of iron and the actual content of omega-3 fatty acids in various minces are both areas that require further research.

One of the obvious uses of this redder fish mince would be in the manufacture of red meat-like products. In this case the presence of the iron would be of great nutritional benefit as iron is one of the nutrients that is often lacking in the American diet including many pre-menopausal middle and upper class females and red meats are one of our best sources of this nutritionally important mineral. Thus, it becomes important to establish the effect of switching from red meat to minced fish on the iron status of potential consumers.

The frame mince, although excellent for many new food product development efforts, is difficult to store frozen. Particularly the gadoid minces, i.e., those from cod, haddock, hake, whiting, pollock, and cusk, undergo a textural change that leads to a toughening of the fish meat. The texture is often characterized as "spongy" or "cottony." The textural change is believed to be due to a series of chemical changes initiated by enzymes specific to gadoids (and a few other species) that takes the naturally present trimethylamine oxide (TMAO) and converts it to dimethylamine (DMA) and formaldehyde (FA). The FA is a reactive compound that is believed to then go on to crosslink the proteins, causing the undesirable texture changes.

Previous work in our laboratory has indicated that the measurement of DMA, however, does not always follow the observed texture changes well, particularly in the presence of additives. Thus, we have developed various tests that specifically follow the textural changes; these include instrumental texture measurements, human panel texture evaluations, and protein functionality tests that can under some circumstances predict textural behavior. For example, with cod minces, the expressible moisture of the raw fish predicted the human panel

determined binding scores of cooked product quite well for a large number of different salts.

From work with surimi, researchers have identified compounds that seem to protect surimi from texture changes during frozen storage. These chemical additives are often generically referred to as cryoprotectants, i.e., materials that prevent changes during freezing. Surimi differs from mince in that most of the soluble compounds have been washed out and, thus, the level of compounds like TMAO is much lower. However, it is possible that the same cryoprotectants would have a beneficial effect if they were added into mince as is or to mince which has been washed once simply to reduce the level of soluble components. Some of these cryoprotectants might also be appropriate ingredients for further processed final products that may be made from the mince and, therefore, their presence in the mince might not provide a barrier to the further use of the mince.

The reactions leading to texture changes occur at different rates when fish is stored at different temperatures. The colder the temperature the slower the reaction. However, the reaction of TMAO to form DMA and FA does have a measurable rate down to -29°C or so. Thus, long term cold storage below that temperature is recommended, but is currently not commonly practiced in this country. Discussions of the possible benefit of making extra-cold storage available to the fishing industry are occurring in the Mid-Atlantic region. Some newer technologies, such as the use of a stored energy carbon dioxide system (i.e., making dry ice at night and using the cold during the day) might make lower temperature cold-storage economically feasable, by permitting more of the energy needs to be met in the evening when electricity is cheaper. Lowering of the day-time electricity demand would also help the economics of such a system.

We are also studying the possibility that minces stored at -40°C or so for a few months will not undergo as rapid a change in texture at normal cold storage temperatures (-15 to -20°C) as would mince placed directly into normal cold storage.

In the work presented here, we have attempted to take a look at various food-type additives added to cod fillet and frame mince that were then stored at different temperatures (-7, -20 and -40°C). Among the additives tested were:

1. Sorbitol. This compound is often used as a cryoprotectant with surimi. It is not as sweet as sugar, the other compound normally found in surimi.

2. Polydextrose. This compound has been proposed for use as a cryoprotectant by Dr. Lanier's group at North Carolina State. It is lower in calories and less sweet than sorbitol.

3. Starch. Starch is a traditional ingredient in further processed surimi. Preliminary data from our lab suggests that this compound does have a cryoprotectant action. (See appendix I for a copy of this report.) Also, some of the further processed minced fish products which we have developed require starch as an ingredient. Starch of the four compounds

tested in this study would also be expected to meet with the least amount of consumer resistance with respect to labeling etc.

4. Soy Protein Isolate. We have chosen to examine an extremely functional soy protein with no detectable flavor contribution to the mince.

METHODS AND MATERIALS

Samples preparation

Very fresh cod (estimated to be less than three days old on arrival in Ithaca) was obtained from Connolly Bros. in Boston. Both fillet trim and frames were shipped. The heads were removed from the frames.

The fish samples (fillets and frames) were minced using a Bibun deluxe model 15 mechanical deboner with 5 mm holes. The fillets were done before the frames.

Half of each type of mince was washed with tap water with a ratio water to fish of 2:1 (v/w). The mince was agitated for 10 minutes while being kept at 4°C. The water, after washing, was drained out and further moisture was removed by pressing the washed mince in cheesecloth. These samples are referred to as washed fillets and washed frames. The other pair of minces are referred as non-washed fillets and non-washed frames.

The following additives were used in this study. Starch 806 (Amaizo; Hammond, Indiana), isolated soy protein Ardex SP6 (Archer Daniels Midland Co.; Decatur, Illinois), polydextrose FCC (Pfizer Inc.; New York, N.Y.), and sorbitol N.F., F.C.C. (Pfizer Inc.; New York, N.Y.). Each additive was added to samples of the various minces so that the final additive concentration was 2% w/w.

These additives were selected because:

1. These additives have been extensively studied and are known to have a beneficial effect on the water retention properties of food systems. Water retention deteriorates during the usual frozen storage of fish.

2. The change in texture associated with the frozen-storage of fish has been associated with physical changes of some of the fish muscle proteins. Some of the additives utilized (sorbitol, polydextrose) are considered to possess cryoprotectant properties and are utilized with that objective at the industrial level (mainly in the surimi industry).

One oz. Nalgene jars were filled to the top with minced fish samples; trying as the cups were packed to avoid air pockets. Each sample was then frozen at -20°C in a large walk-in freezer and transferred to freezers at -7, -20 and -40°C and stored for 90 days. Samples were taken at days 2, 10, 60 and 90. The samples were then thawed in a cold room at 10°C for 24 hr, and analyzed for cook loss, expressible moisture, human texture panel evaluation of springiness and cohesiveness of raw product, and texture profile (on the Instron Universal Testing Machine) to determine hardness, cohesiveness, and springiness.

Cook loss of the samples was measured by cooking the mince, while still in the cup, for 15 minutes in boiling water. The samples were drained, cooled to room temperature, and weighed. The weight difference, expressed as a percentage of the starting weight, is the cook loss.

Expressible moisture was measured by taking 0.8 to 1.0 g of raw fish and placing it on preweighed Whatman 1 filter paper. The sample was in contact with Whatman No. 5 paper and three additional layers of Whatman No. 3 were used. The sample and the filter paper were put into a Sorvall polycarbonate centrifuge tube and centrifuged in the RC2B refrigerated centrifuge at 4°C (SS-34 head) for 10 minutes at 3,000 rpm. The weight gain of the filter paper divided by the starting weight of the fish is the percentage expressible moisture.

The human raw texture panel evaluated a 1/4 pie shaped piece of the mince removed from the cups. Springiness and cohesiveness were measured on a 11 point scale with 0 representing the absence of any springiness or cohesiveness and 10 representing an extremely springy or cohesive sample.

The General Foods Texture Profile on the Instron involves placing the minced fish sample removed from the plastic cup between two parallel plates. The upper plate is lowered onto the sample which is then compressed to 65% of its original height. The instrument reverses and returns to its original position. It then goes through a second compression cycle. The chart records the amount of force on the upper plate as a function of the time during the compression/relaxation cycle. From these curves, the brittleness (fracturability during the first compression), hardness (maximum compression force on the first compression), the instrumental cohesiveness (change in area under the compression part of the curve for the second compression divided by that from the first compression), and the instrumental springiness (distance on the second curve from the beginning of compression until the maximum force is reached) were measured. The cohesiveness is a measure of the percentage of work needed to compress the sample during the second compression in comparison to the first compression. The springiness is a measure of how much height the sample recovered between the first and second compression (elasticity). With all of the samples studied, no brittleness peak was observed. In addition, if the sample adheres to the upper plate an adhesion value can also be calculated. However, this value depends heavily on the nature of the surface of the upper and lower compression plates. A small amount of adhesion was observed with the fish samples, but the detailed results will not be reported.

RESULTS AND DISCUSSION

A storage temperature of -15 to -20°C is most commonly used in the fish industry. Thus storage at -20°C represents the best commercial storage conditions. At -40°C, the biochemical reactions that affect the organoleptic acceptability of the fish are theoretically stopped. Other changes should be minimized. However, we have observed on various occassion that some changes, such as in expressible moisture, can still occur even at this temperature. Thus although this temperature can usually be considered as an invariant control; caution must be exercised in interpreting results which are compared to samples stored at this temperature. At -7°C we are looking at an abuse temperature. This temperature would be expected to highlight any changes observed with the

different samples within a reasonable amount of time. Generally this would be a condition that is worse than might be expected in even mediocre frozen storage conditions. (However temperature variation in frozen storage may be even more important than the actual temperature. These studies do not address this important point.)

The first property examined with each sample was the cook loss. These are shown in Table 1 and 2.

The washed minces had a much greater cook loss, with the fillets possibly having a bit more cook loss than the frames. This suggests that the original moisture removal may not have been as complete as compared with the original material. Within the time-frame of the experiment and because of the high variability of the measurement, no differences as a function of time are obvious except possibly for the non-washed fillets at -7°C where a peak at day 10 is observed. Limitations in the availability of sample prevents one from establishing whether any differences actually exist for the different temperatures, but the absence of change at the higher storage temperature would suggest that changes would not be observed at the lower temperatures. In all cases the soy protein isolate and starch have the lowest cook loss. In the case of the washed fillets the results are particularly obvious and also to some degree in the washed frames. The starch would be expected to gelatinize on cooking, which should certainly aid in holding moisture.

The next series of experiments involve the expressible moisture of the raw mince. These are shown in Table 3 and 4.

The difference between the expressible moisture for the washed and non-washed samples seem to reflect moisture differences of the same order of magnitude as the cook loss data, i.e., the actual difference is about the same although the actual amount of expressible moisture is about double that of the cook loss. The soy protein isolate again seems to suggest some benefit in minimizing expressible moisture changes. Unlike the cooked samples, the starch shows no particular benefit. This is not surprising since the starch effect would only be expected in cooked samples after gelatinization.

The results of the raw human texture panel work is shown in Tables 5 and 6. The change in texture is clearly indicated after 60 days and 90 days. With washed frame mince the best improvement in quality is seen with the polydextrose. Soy protein seems to aggravate the texture change slightly. In general the non-washed minces did surprisingly better than the washed frame minces at -7°C. This was certainly an unexpected result. The process of washing may have disrupted the fish more and the probably presence of more water in the washed samples might have also influenced the effectiveness of the various additives.

The same pattern of washed doing more poorly than unwashed is seen with the fillets. With fillets, at 60 days but not at 90 days the polydextrose seemed to have the greatest beneficial effect. We also again see that the soy protein was detrimental to the quality of the raw mince.

The General Foods Texture Profiles on the Instron on shown in Tables 7 and 8. Notice that the hardness of the washed frames and fillets were

TABLE 1. COOK LOSS FOR FRAMES

TIME
(Days)
WASHED FRAME MINCE

	2			10			60			90		
T	-7	-20	-40	-7	-20	-40	-7	-20	-40	-7	-20	-40
N	20.2	19.4	23.4	22.2	-	-	19.3	23.0	-	22.5	24.2	-
S	14.3	15.0	-	15.4	-	-	13.8	-	-	15.5	16.8	-
So	22.4	24.7	-	17.1	-	-	16.0	-	-	18.1	19.8	-
P	14.5	17.8	-	25.6	-	-	22.7	-	-	25.1	27.4	-

NON-WASHED FRAME

	2			10			60			90		
T	-7	-20	-40	-7	-20	-40	-7	-20	-40	-7	-20	-40
N	7.4	6.0	7.6	11.1	-	-	4.3	-	-	6.5	-	-
S	4.9	5.8	5.1	7.7	-	-	3.0	-	-	6.7	-	-
So	5.1	5.0	3.6	8.7	-	-	3.7	-	-	3.1	-	-
P	5.8	10.1	6.7	10.9	-	-	5.8	-	-	8.0	-	-

Each value represents the mean of 3 sub-samples of the same batch of fish. T, temperature in degree Celsius; N, no additives; S, starch; So, isolated soy protein; and P, polydextrose. Each additive at 2% w/w.

TABLE 2. COOK LOSS FOR FILLETS

TIME
(Days)
WASHED FILLETS

	2			10			60			90		
T	-7	-10	-40	-7	-20	-40	-7	-20	-40	-7	-20	-40
N	26.3	25.8	24.4	24.9	25.8	-	24.0	23.9	-	24.7	-	-
S	18.2	17.6	19.9	19.2	20.1	-	16.4	-	-	16.0	-	-
So	19.4	17.4	15.8	18.3	17.7	-	17.6	-	-	17.0	-	-
P	27.8	28.7	22.8	28.6	29.4	-	26.3	-	-	25.8	-	-
Sr	25.9	26.5	24.9	27.1	26.1	-	22.6	-	-	23.3	-	-

TIME
(Days)
NON-WASHED FILLETS

	2			10			60			90		
T	-7	-10	-40	-7	-20	-40	-7	-20	-40	-7	-20	-40
N	2.6	4.2	1.6	14.1	14.1	-	7.7	11.7	-	6.2	-	-
S	2.5	3.2	5.5	8.8	9.7	-	3.3	6.3	-	-	-	-
So	1.6	0.3	0.8	11.8	11.9	-	4.4	2.2	-	-	-	-
P	3.6	4.1	3.3	14.9	15.2	-	11.2	11.9	-	-	-	-
Sr	1.7	1.2	0.8	13.4	15.2	-	7.9	11.4	-	-	-	-

Each value represents the mean of 3 subsamples of the same batch of fish. T, temperature in degree Celsius; N, no additives; S, starch; So, isolated soy protein; P, polydextrose; and Sr, sorbitol. Each additive at 2% w/w.

TABLE 3. EXPRESSIBLE MOISTURE FOR FRAMES

TIME
(Days)
WASHED FRAME

	2			10			60			90		
T	-7	-20	-40	-7	-20	-40	-7	-20	-40	-7	-20	-40
N	43.9	46.2	43.0	44.2	-	-	51.2	47.0	-	46.2	45.7	-
S	46.0	49.8	-	47.2	-	-	45.8	46.1	-	46.7	47.5	-
So	43.6	-	42.6	43.6	-	-	44.7	-	-	46.4	44.7	-
P	44.8	43.8	-	47.8	-	-	47.4	-	-	46.4	48.2	-

TIME
(Days)
NON-WASHED FRAME

	2			10			60			90		
T	-7	-20	-40	-7	-20	-40	-7	-20	-40	-7	-20	-40
N	37.7	38.3	35.9	39.8	-	-	43.0	-	-	40.6	-	-
S	40.4	40.5	39.8	42.7	-	-	44.5	-	-	43.1	-	-
So	35.0	40.0	34.9	35.3	-	-	38.8	-	-	41.2	-	-
P	37.6	40.0	36.2	39.4	-	-	39.6	-	-	40.7	-	-

Each value represents the mean of 3 subsamples of the same batch of fish. T, temperature in degree Celsius; N, no additives; S, starch; So, isolated soy protein; and P, polydextrose. Each additive at 2% w/w.

TABLE 4. EXPRESSIBLE MOISTURE FOR FILLETS§

TIME
(Days)
WASHED FILLETS

	2			10			60			90		
T	-7	-20	-40	-7	-20	-40	-7	-20	-40	-7	-20	-40
N	41.6	42.2	42.3	43.1	48.4	-	50.9	42.9	-	45.3	-	-
S	48.9	44.9	47.4	48.0	45.7	-	44.8	-	-	50.6	-	-
So	41.2	37.4	42.5	42.3	39.9	-	43.1	-	-	44.2	-	-
P	40.1	42.4	42.6	41.3	46.2	-	46.3	-	-	47.7	-	-
Sr	40.4	42.4	37.3	45.2	39.8	-	44.9	-	-	42.7	-	-

TIME
(Days)
NON-WASHED FILLETS

	2			10			60			90		
T	-7	-20	-40	-7	-20	-40	-7	-20	-40	-7	-20	-40
N	34.2	34.3	38.7	32.5	36.1	-	37.2	37.1	-	-	-	-
S	38.0	40.4	39.0	40.2	41.6	-	43.9	40.6	-	-	-	-
So	29.4	28.2	31.8	36.4	33.2	-	37.3	39.1	-	-	-	-
P	37.0	36.9	34.3	39.4	37.3	-	40.8	33.4	-	-	-	-
Sr	34.5	31.0	33.5	37.3	29.8	-	42.4	37.1	-	-	-	-

Each value represents the mean of 3 subsamples of the same batch of fish. T, temperature in degree Celsius; N, no additives; S, starch; So, isolated soy protein; P, polydextrose; and Sr, sorbitol. Each additive at 2% w/w.

TABLE 5. HUMAN RAW TEXTURE PANEL EVALUATION OF FRAME MINCES

TIME (Days)
WASHED FRAMES

T		2		10		60		90	
		-7	-20	-7	-20	-7	-20	-7	-20
N	Si	1.0	2.3	1.8	-	6.0	3.2	7.2	3.1
	Co	1.0	2.0	1.0	-	5.2	2.7	5.7	2.0
S	Si	1.8	2.0	1.5	-	5.2	3.0	6.9	3.0
	Co	1.3	1.0	1.0	-	5.2	2.2	5.2	1.7
So	Si	2.5	1.5	2.5	-	5.7	-	7.7	3.0
	Co	2.0	1.0	1.8	-	5.2	-	6.2	1.7
P	Si	1.5	1.8	1.3	-	5.0	-	6.7	2.0
	Co	1.0	1.0	1.0	-	4.2	-	4.7	1.2

TIME (Days)
FRAME NON-WASHED

T		2			10	60	90
		-7	-20	-40	-7	-7	-7
N	Si	1.0	1.3	1.5	3.3	2.7	2.2
	Co	1.0	1.0	1.0	2.8	2.2	1.5
S	Si	2.5	1.6	1.3	2.8	3.0	2.2
	Co	1.3	1.3	1.0	2.5	2.2	2.2
So	Si	1.3	1.3	2.3	3.8	3.7	-
	Co	1.0	1.0	1.5	3.0	3.2	-
P	Si	1.0	-	1.0	2.8	2.0	-
	Co	1.0	-	1.0	2.0	1.7	-

Each value represents the mean of 4 subsamples of the same batch of fish. T, temperature in degree Celsius; Si, springiness; Co, cohesiveness; N, no additives; S, starch; So, isolated soy protein; and P, polydextrose. Each additive at 2% w/w.

TABLE 6. HUMAN RAW TEXTURE PANEL EVALUATION OF FILLET MINCES

TIME (Days) FILLETS WASHED

		2			10		60		90	
T		-7	-20	-40	-7	-20	-7	-20	-7	-40
N	Si	2.8	2.5	3.5	2.5	1.5	4.8	2.5	3.2	-
	Co	1.8	1.5	2.0	1.5	1.3	4.0	1.8	3.0	3.2
S	Si	3.3	4.3	3.8	2.8	2.0	4.3	-	5.2	-
	Co	2.0	2.4	2.3	2.0	1.3	3.5	-	3.7	-
So	Si	3.5	3.0	3.3	2.5	1.3	5.3	-	5.7	2.5
	Co	2.5	1.8	1.8	2.0	1.3	4.8	-	4.0	2.0
P	Si	2.5	2.3	2.5	4.0	1.3	3.5	-	4.7	-
	Co	2.0	1.0	1.5	1.3	1.0	2.8	-	3.7	-
Sr	Si	2.0	1.0	1.3	1.8	1.0	3.5	-	-	-
	Co	1.5	1.0	1.3	1.0	1.0	2.8	-	-	-

TIME (Days) NON-WASHED FILLETS

		2			10		60	
T		-7	-20	-40	-7	-20	-7	-20
N	Si	1.0	1.0	1.3	1.3	1.5	1.8	1.8
	Co	1.0	1.0	1.3	1.3	1.0	1.5	3.3
S	Si	1.3	1.3	1.5	1.3	1.3	2.3	3.0
	Co	1.0	1.5	1.5	1.0	1.8	1.3	2.3
So	Si	1.5	1.8	1.8	1.5	1.0	2.3	3.3
	Co	1.0	1.8	1.8	1.0	1.3	2.0	3.8
P	Si	1.0	1.0	1.3	1.0	1.0	1.0	2.3
	Co	1.0	1.0	1.5	1.3	1.0	1.0	2.3
Sr	Si	1.0	1.0	1.0	1.3	1.0	1.8	2.3
	Co	1.3	1.3	1.0	1.3	1.5	1.5	1.8

Each value represents the mean of 4 subsamples of the same batch of fish. T, temperature in degree Celsius; Si, springiness; Co, cohesiveness; N, no additives; S, starch; So, isolated soy protein; P, polydextrose; and Sr, sorbitol. Each additive at 2% w/w.

TABLE 7. TEXTURE PROFILE ANALYSIS OF FRAME MINCES

TIME (Days)
WASHED FRAMES

		2			10	60		90	
T		-7	-20	-40	-7	-7	-20	-7	-20
N	Si	4.83	2.20	1.3	1.69	2.23	1.61	1.90	1.60
	Hl	13.80	8.08	22.8	27.94	144.80	60.80	64.78	39.45
	Co	.35	.76	.9	1.04	.81	.46	.85	.52
S	Si	4.30	4.07	-	1.67	2.14	-	1.82	1.38
	Hl	23.80	11.81	-	27.64	94.50	-	55.31	28.48
	Co	.25	.44	-	.94	.81	-	.87	.75
So	Si	3.90	1.35	1.3	1.76	2.26	-	1.95	1.62
	Hl	11.55	22.25	-	42.16	171.73	-	69.63	33.30
	Co	.16	.76	.6	1.01	.77	-	.87	.60
P	Si	2.45	1.31	-	1.59	2.07	-	1.87	1.51
	Hl	12.55	15.67	-	23.36	114.80	-	55.40	36.05
	Co	.66	.67	-	.97	.71	-	.88	.67

TIME (Days)
NON-WASHED FRAMES

		2			10	60	90
T		-7	-20	-40	-7	-7	-7
N	Si	-	1.23	.75	1.18	1.25	1.25
	Hl	-	14.49	5.91	9.39	30.76	18.63
	Co	-	.91	.50	.61	.42	.79
S	Si	.90	.93	.93	1.70	1.30	1.29
	Hl	10.02	10.75	10.68	16.93	59.93	29.11
	Co	.50	.50	.43	.77	.47	.46
So	Si	-	.98	1.09	1.19	1.23	1.38
	Hl	-	18.74	15.62	23.65	63.85	38.34
	Co	-	.43	.53	.65	.43	.59
P	Si	.84	.70	.73	1.06	1.19	1.27
	Hl	7.94	6.20	6.68	11.18	42.36	21.30
	Co	.49	.46	.46	.81	.43	.59

Each value represents the mean of 3 subsamples of the same batch of fish. T, temperature in degree Celcius; Si, springiness (mm); Co, cohesiveness (work); Hl, maximum compresion force on the first compression cycle (Newtons). N, no additive; S, starch 806; So, isolated soy protein; and P, polydextrose; Each additive at 2 % w/w. The samples were compresed 65 % of their original height in each of the compression cycles.

TABLE 8. TEXTURE PROFILE ANALYSIS OF FILLET MINCES

TIME
(Days)
WASHED FILLETS

T		2			10		60	90
		-7	-20	-40	-7	-20	-7	-7
N	Si	1.31	1.46	1.4	1.33	1.24	1.95	1.84
	Hl	-	26.10	23.2	25.47	21.62	87.21	44.32
	Co	.61	.74	.7	.81	.79	.59	1.02
S	Si	1.42	1.42	1.4	1.31	1.5	2.40	1.76
	Hl	25.60	17.65	19.9	26.37	26.01	113.21	49.89
	Co	.69	.67	.7	.79	.95	1.00	.78
So	Si	1.53	1.50	1.5	.94	-	1.84	1.83
	Hl	29.12	25.00	27.1	28.10	-	109.27	64.31
	Co	.47	.65	.8	.69	-	0.58	.86
P	Si	1.19	1.25	1.2	1.26	-	-	1.67
	Hl	18.30	17.45	16.6	26.19	-	-	45.43
	Co	.59	.66	.6	.66	-	-	.70
Sr	Si	1.16	1.11	1.1	1.27	.98	1.44	1.34
	Hl	14.30	14.56	16.9	16.73	21.95	70.08	38.28
	Co	.55	.53	.5	.77	.60	.43	.67

TIME
(Days)
NON-WASHED FILLETS

		2			10		60		90
T		-7	-20	-40	-7	-20	-7	-20	-7
	Si	.89	.90	.89	1.12	1.01	1.24	.98	1.41
N	Hl	9.69	11.84	12.63	16.78	11.17	49.03	42.14	32.71
	Co	.41	.37	.37	.58	.44	.33	.29	.44
	Si	.91	.89	-	1.17	.95	1.20	1.02	-
S	Hl	12.56	14.00	-	15.23	15.54	62.44	49.35	-
	Co	.46	.37	-	.53	.47	.39	.35	-
	Si	1.0	-	-	1.26	1.04	1.34	1.22	-
So	Hl	16.64	-	-	22.42	18.69	72.24	49.65	-
	Co	.43	-	-	.51	.38	.32	.35	-
	Si	.82	.73	.79	1.03	.86	1.09	.77	-
P	Hl	12.51	15.40	8.79	14.71	10.16	46.93	39.75	-
	Co	.44	.30	.44	.47	.51	.33	.23	-
	Si	.79	.69	.77	.98	.87	1.19	.74	-
Sr	Hl	12.53	10.53	9.81	15.84	11.98	46.83	33.42	-
	Co	.40	.35	.37	.45	.38	.34	.28	-

Each value represents the mean of 3 subsamples of the same batch of fish. T, temperature in degree Celcius; Si, springiness (mm); Co, cohesiveness (work); Hl, maximum compresion force on the first compression cycle (Newtons). N, no additive; S, starch 806; So, isolated soy protein; P, polydextrose; and Sr, sorbitol. Each additive at 2 % w/w. The samples were compresed 65 % of their original height in each of the compression cycles.

higher than those for the unwashed. Generally sorbitol seems to be among the lower samples, while the soy protein is generally among the additives giving the greatest hardness. In general the springiness was helped slightly by the additives with polydextrose followed by sorbitol having the greatest beneficial effect. Cohesiveness of the non-washed fillets seems to be the lowest, while cohesiveness seems to be highest for the washed frames.

CONCLUSIONS

The results to date are consistent with the observation that a high temperature is detrimental to the storage of the mince. The length of storage time used in these experiments does not permit one to distinguish between -20 and -40°C. The detrimental effect of washing on the flesh was unexpected while the beneficial effects of polydextrose is at least what one might have expected. With respect to whether the cryoprotectants used for surimi would work with minces, it was hoped that they would but little experimental evidence existed, and the results to date suggest that they do not function the same way. The results of Xu and Regenstein (1986) and this work both suggest that starch, if beneficial works only on the cooked material. The results in this work suggest that further studies of the role of additives with cooked and raw texture are in order. Other work, currently coming out in an informal reports from South Africa and with gums from Holland suggest that the benefits of additives are less than the benefits of improved temperature of storage.

Overall these results indicate that the problem of stabilizing minces is sufficiently different from those encountered with surimi that results from one system to the other cannot be extrapolated, but rather that mince requires its own experimental work.

Acknowledgements

We wish to thank Seafood Management, Cos Cob, CT, the New England Fisheries Development Foundation, Boston, MA, and the New York Sea Grant Institute for support of this work.

REFERENCE

Xu, X. and J.M. Regenstein. 1986. Preventing Textural Changes in Frozen Gadoid MInces with Corn Starch. Appendix 1 of Regenstein, J.M. and C.E. Regenstein. 1986. Using Frozen Minced Fish. International Symposium on Engineered Seafoods Including Surimi. Seatttle, WA., p. 74-83.

THE BENEFITS OF CARRAGEENAN AS A SEAFOOD PROCESSING AID

CHARLES W. BULLENS, JAMES J. MODLISZEWSKI, AND JAMES J. NAVARRO
Marine Colloids Division of FMC Corporation
2000 Market Street, Philadelphia, PA 19103

INTRODUCTION

Carrageenan is a natural chondrus seaweed extract classified as GRAS (21 CFR - 182.7255) in accordance with FDA regulations. Applications using specially formulated carrageenan have been developed to enhance the quality of processed seafood. Seafood analogues, from surimi, benefit from using carrageenan as a binder which also serves to customize texture and provide freeze/thaw stability. Thawed raw shrimp can be returned to their natural moisture level by injecting a brine solution containing carrageenan which then entraps moisture normally lost during cooking thereby enhancing sensory benefits.

RESULTS AND DISCUSSION

Seafood analogues

Seafood analogues are an ever increasing new application which uses surimi (washed, minced fish) as the main food matrix component. Diverse products, such as fabricated shrimp, lobster and crab legs have found great acceptance by the consumer today.

Transportation of finished products is in the cooked, frozen state. Problems occur upon thawing due to ice crystal formation which dehydrates and denatures the protein matrix structure resulting in excessive moisture loss and poor taste.

Additional problems occur due to utilization of inferior quality surimi which contains enzymatically denatured protein with poor binding/strength characteristics. Using carrageenan based systems help improve textural qualities providing increased firmness, better shape retention after cooking and improved storage stability of the seafood analogue.

Carrageenan's unique property of complexing with proteins helps produce better textured, more organoleptically acceptable products. In addition, carrageenan's ability to entrap moisture in a gel matrix helps to control the detrimental effect of ice crystal formation during frozen storage.

An experimental seafood analogue formula using Marine Colloids* carrageenan-based stabilizer is displayed in Table 1.

Table 1. Experimental Seafood Analogue Formula

INGREDIENTS	CONCENTRATION (%)
Surimi	50.0-60.00
Carrageenan-based Stabilizer	0.2-1.0
Potato starch	4.0-8.0
NaCl	2.0
Flavors, Preservatives	q.s.
Water (Ice)	to 100%

Optional ingredients: Proteins, humectants, colorants

The experimental procedure for preparing the seafood analogue is described below:

1. Add pre-weighed frozen (-20°C) surimi to a vacuum cutter/mixer and process until a paste is formed.
2. Add ice water and continue to mix until uniform*.
3. Add dry ingredients except the NaCl and mix.
4. When uniform, add NaCl last and mix until product reaches a consistent paste texture (dough-like).
5. Deaerate mixture and carefully fill into casings (~25 mm.).
6. Seal ends, taking care to remove any trapped air.
7. Place filled casings into a water bath of ~ 85°C for 40 minutes.
8. After cooking, cool with a tap water spray and then refrigerate overnight (~ 4°C).
9. Product can then be evaluated for textural and organoleptic properties.

* Note: Process temperature should be controlled to below 10°C (preferrably 5°C).

Processed Shrimp

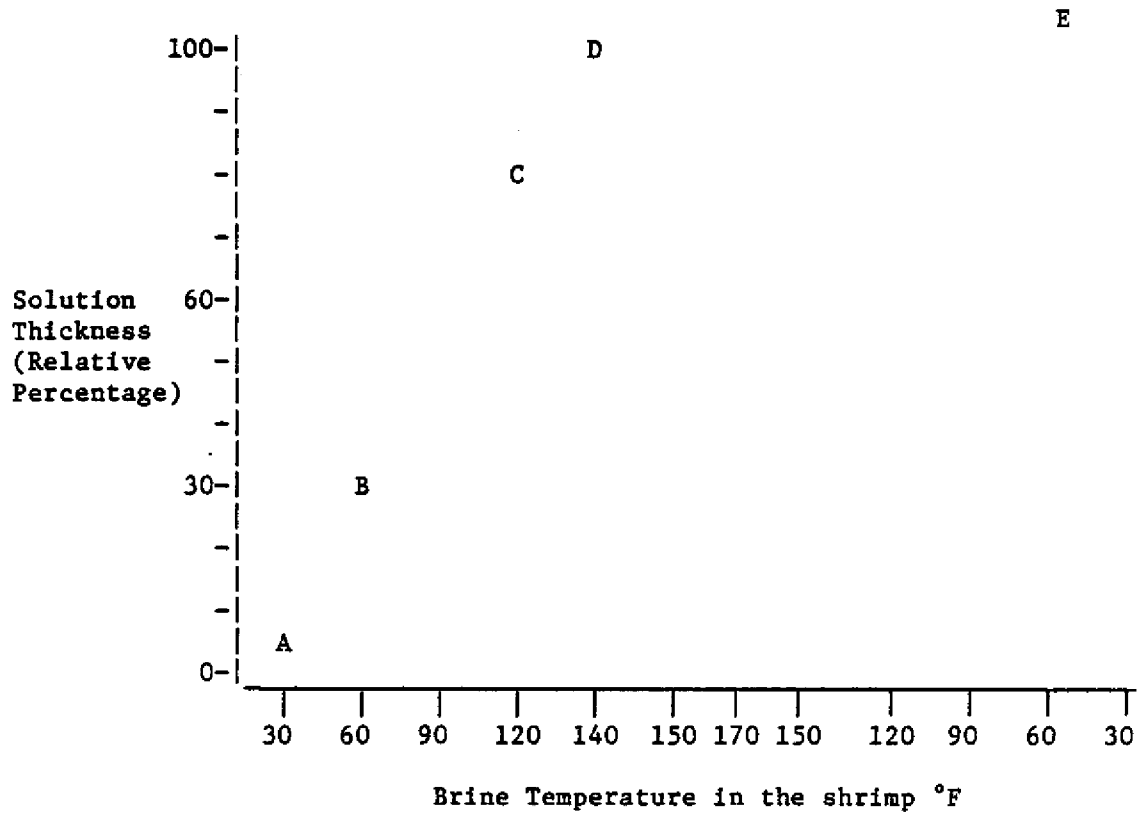
Raw shrimp go through a freeze/thaw cycle before processing which results in drip loss and changes in texture. Unaided processing of the shrimp results in additional moisture loss during cooking and cooling as well as additional texture changes reducing the organoleptic acceptability of the product.

Injecting a brine solution containing carrageenan into the shrimp after the raw product is thawed aids the shrimp in maintaining its natural moisture content. As the shrimp cooks and cools, carrageenan forms a unique matrix which prevents additional moisture loss. The yield depends upon the amount of brine injected and the size of the shrimp. Yield increases of 20 percent have been achieved in experimental trials using carrageenan solutions.

Carrageenan also serves to improve the texture and organoleptic appeal of the shrimp due to its moisture retentive properties.

Figure 1 illustrates how Gelcarin® SA 911 carrageenan functions during the injection, cooking, and cooling process.

Figure 1. How Gelcarin® SA 911 Works



A Gelcarin® SA 911 particles disperse in brine and are injected in shrimp
B Gelcarin SA 911 particles absorb moisture
C Gelcarin SA 911 particles begin to unravel
D Gelcarin SA 911 starts forming network entrapping moisture
E Gelcarin SA 911 gell network controls purge and maintains yield

The formula for preparing a brine solution containing carrageenan for injection is displayed in Table 2.

Table 2. Brine Formula For Injecting Shrimp to Replace Ten Percent Moisture Loss

INGREDIENTS	CONCENTRATION (%)
Gelcarin® SA 911 carrageenan	3.2
Sodium chloride	1.5
Water	69.8
Ice	25.5
Total	100.0

Note: Ten pounds of the above formulation would be injected into 100 pounds of shrimp resulting in a finished weight of 110 pounds.

The procedure for preparing a brine solution containing carrageenan is described below.

1. Dissolve the sodium chloride in the water and add ice. Solution temperature should be maintained below 5°C.
2. Agitate the brine and disperse Gelcarin SA 911.
3. Maintain brine containing carrageenan at a temperature below 5°C with agitation during injection.

Additional trials are recommended to customize and optimize the experimental formulas and procedures.

MECHANICAL PROPERTIES OF FISH GELS MADE FROM MIXED SPECIES WITHOUT ADDED SODIUM CHLORIDE

K. Vareltzis, E.M. Buck, and H.O. Hultin
Dept. of Food Science and Nutrition, University of Massachusetts
Amherst, MA 01003

INTRODUCTION

There has been much interest, in recent years, in the traditional Japanese process of making fish gels and as a result, a significant industry has developed, and is continuing to develop, relative to the production of surimi and various seafood analogs.

One of the unique properties of fish protein is its ability to form highly elastic gels. It has generally been thought that 2.5 to 3% sodium chloride must be added to the minced fish prior to gel formation in order to attain the desired elasticity. We have recently found and reported however, that it is possible to produce excellent protein gels from certain fish proteins without added sodium chloride. The nutritional advantages of such a process are obvious.

In this study we investigated the affect on gel quality of adding a species previously demonstrated to produce good gels in the absence of sodium chloride, namely red hake (Urophycis chuss), to a species which did not possess this property to the same extent, winter flounder (Pseudopleuronectes americanus).

MATERIALS AND METHODS

Red hake and winter flounder were purchased from day boats operating out of Gloucester, Massachusetts during the period October through December, 1986. The whole fish were transported on ice to the University of Massachusetts at Amherst where they were hand skinned and filleted on the same day, then held on ice overnight.

The boneless fillets were ground through the 1/4" plate of a table grinder. Mixing of species was done immediately after grinding by vigorously mixing by hand for 1 min. In those cases where a slurry was used, the appropriate amount of red hake was blended with 100 ml of distilled water for 1 min. The slurry obtained was then added to the flounder mince which was already being mixed in the bowl of a Hobart mixer set on high. Mixing was continued for 1 min. An equivalent amount of distilled water was mixed into red hake and flounder controls prior to further processing. Washing was accomplished by placing one

volume of minced fish in an aluminum container with three volumes of iced distilled water. The washing process was repeated two more times. The third wash contained 0.15% NaCl to facilitate de-watering.

De-watering was accomplished by placing the cheese cloth wrapped mince on the bed of a hydraulic press and applying pressure gradually over the next 2 min. until the gauge read 15,000 psi. It was then held at that pressure for an additional 15 min.

The de-watered mince was removed from the press and made into gels following a 2 hr chill period at 2°C. Cryoprotectants were not added since no storage was involved.

Gel Production

Gels were made in 220g batches using a Cuisinart Food Processor for chopping. The well-chilled mince was placed in the bowl of the food processor which had been previously chilled to -25°C. It was chopped at high speed for 2 min. This procedure ensured that the temperature of the fish paste was kept below 15°C at all times. No sodium chloride was added to any of the gels in this process.

The resulting pastes were immediately transferred to a hand sausage stuffer and stuffed into nylon cylinders (2.1 cm x 13 cm). The ends were stoppered with cork and the cylinders were immersed first into a 40°C water bath for 30 min. and then into a 90°C water bath for 20 min. The cylinders were removed from the second water bath and cooled by placing in a mixture of ice and water for 20 min.

The mechanical properties of the gels were determined by three different procedures.

Fold-Test. This procedure involved folding a 3 mm thick by 2.1 cm diameter slice of gel first in half and then into quarters, if possible (Kudo et al. 1973). A score of 5 (AA grade) was awarded to a gel slice that could be folded into quarters without cracking at the fold lines. No cracks on folding in half was equal to a 4 (A grade). Some cracking when folded in half was equal to a 3 (B grade). Breaking in half when folded in half was equal to a 2 (C grade) and breaking of the slice into fragments from finger pressure was equal to a 1 (D grade). Values reported are the average of four slices per gel.

Warner-Bratzler Shear. A Warner-Bratzler Shear (Model 2000) equipped with a dial calibrated in 0.02 lb. increments to 10 lbs. was used to shear gel cylinders perpendicular to their long axes. Values reported are the average of five measurements per sample.

Instron. Duplicate specimens, 1.5 cm in length by 2.1 cm in diameter were cut from each gel perpendicular to the long axis of the cylinder and tested on an Instron Universal Testing Machine (Model TM).

The deformability modulus, which is a measure of the resistance of the gel to deformation, or hardness, was calculated by methods described by Johnson et al. (1980). The deformation recovery was also determined from a compression-decompression cycle using a deformation rate of 1 cm/min. in both directions. The gels were subjected to 60% deformation since beyond this level specimens fractured. The recovery is expressed as a ratio between recovered and total deformation multiplied by one hundred.

RESULTS AND DISCUSSION

The first experiment was conducted to determine the effect on gel properties of mixing species, and the effect, if any, of holding mixed minces before washing. The effect of mixing species after separate washings was also investigated.

The pH and percent moisture contents of the initial minces used in experiment 1 are shown in Table 1. All values are in expected ranges and are typical of those recorded for the other experiments. In the interest of saving space, these values will not be reported again. It should be noted that the moisture content of the mince from mixed species shows an additive effect.

TABLE 1. pH AND PERCENT MOISTURE CONTENTS OF INITIAL MINCES. EXPERIMENT 1.

Treatments	pH	% Moisture
Red Hake	7.05	84.6
Flounder	6.83	78.1
$Fl+RH_{25}$(0 min.)[1].	6.79	80.2

1. Mixture of 75% flounder and 25% red hake mince held 0 time after mixing and before washing.

In subsequent experiments, only the moisture, fold test, and percent deformation values will be reported since they are the most significant, however, in Table 2 all mechanical properties measured are shown.

Flounder gels were approximately twice as hard or rigid as red hake gels as measured by shear force or Instron deformation values. Rigidity of mixed gels appears to be greater than that which can be

accounted for from additive effects alone. The greatest effect was achieved by mixing minces and holding for 30 min. prior to washing.

Measures of elasticity (fold test and Instron percent recovery) also do not show simple additive effects, however, in this case results are skewed towards the characteristics of the red hake.

Best results for elasticity were obtained by washing minces immediately after mixing (no holding) or mixing species after separate washings.

TABLE 2. PERCENT MOISTURE AND MECHANICAL PROPERTIES OF GELS-EXPERIMENT 1. (25% RED HAKE).

Treatments	% Moisture	Lbs. Shear Force	% Deformation	Fold Test Score	% Recovery
Red Hake	80.8	1.24	11.8	4.0	66.3
Flounder	74.0	3.28	29.0	2.0	50.0
$Fl+RH_{25}$(0 min.)	76.3	2.77	20.5	4.0	70.3
$Fl+RH_{25}$(30 min.)	75.3	3.05	24.8	3.5	58.9
$Fl+RH_{25}PW$[1](0 min.)	75.5	2.44	22.5	4.0	68.5

1. PW (post-wash) indicates species were mixed after each was separately washed and dewatered, rather than before.

Our original hypothesis was that perhaps an enzyme catalyzed reaction was involved in the ability of the fish proteins to form good gels in the absence of NaCl. The data shown from Experiment 1 are consistent with this hypothesis.

In our next experiment we examined the effect of adding smaller amounts of red hake, and the effect of holding mixed minces for various times before washing, since if an enzyme was involved, one would expect to see a change with time of holding. The results are shown in Table 3.

Holding time apparently affected moisture content as evidenced by the decrease with time. As might be expected, fold test scores also decreased with time. Although fold test scores did not reach those of the red hake control, both 0 and 30 min. holding produced scores better than that of flounder alone.

Five percent added red hake decreased the hardness of the mixed species gel at 0 time, however, hardness is apparently restored with holding.

As the concentration of red hake added to the mixture is reduced, mixing becomes a very critical process. This is particularly true since much of the enzymic activity in red hake muscle has been demonstrated to be associated with particulate fractions. Thus, in subsequent experiments the slurry procedure, described earlier, was used to facilitate distribution.

TABLE 3. MECHANICAL PROPERTIES OF GELS CONTAINING 5% RED HAKE - EXPERIMENT 2.

Treatments	% Moisture	Fold Test Score	% Deformation
Red Hake	78.5	4.0	11.8
Flounder	76.1	2.5	29.0
Fl+RH_5(0 min.)	76.2	3.0	20.5
Fl+RH_5(30 min.)	73.5	2.8	24.8
Fl+RH_5(60 min.)	69.9	2.5	22.5

In our next experiment we investigated the effects of using even smaller amounts of red hake, specifically, 5, 2, or 1%, without any holding period after mixing and before washing. The results are shown in Table 4.

The % moisture content of gels was significantly increased at all levels of added red hake as was fold test score. The elasticity as measured by the fold test score was superior at the 5% level of addition. It should be noted that at the 5% level (0 time) slurry mixing produced a superior gel (4.5) as compared to hand mixing (3.0, Table 3).

TABLE 4. MECHANICAL PROPERTIES OF GELS CONTAINING 1, 2, OR 5% ADDED RED HAKE - EXPERIMENT 3.

Treatments	% Moisture	Fold Test Score	% Deformation
Red Hake	81.6	5.0	5.3
Flounder	75.5	3.3	8.3
$Fl+RH_1$(0 min.)	80.3	3.8	8.5
$Fl+RH_2$(0 min.)	79.3	3.8	8.7
$Fl+RH_5$(0 min.)	79.8	4.5	7.7

Note: Red hake added as a slurry in this and subsequent experiments.

Hardness of flounder gels was apparently not significantly affected by the addition of red hake although there was a small decrease at the 5% level.

Since there appeared to be a distinct positive effect on elasticity at the 1% level, in the next experiment we investigated the effect of various holding times at the 1% level. The results are shown in Table 5.

Gels were clearly affected by the addition of only 1% red hake. Moistures increased for all holding times with the greatest increase occurring at 0 time. Fold test scores followed a similar pattern.

There was a tendency for the properties of the mixed gels to revert to values closer to those of flounder gels with increased time of holding. There was a general decrease in elasticity, and an increase in hardness.

TABLE 5. MECHANICAL PROPERTIES OF GELS WITH 1% RED HAKE HELD 0-60 MIN. BEFORE WASHING - EXPERIMENT 4.

Treatments	% Moisture	Fold Test Score	% Deformation
Red Hake	82.8	4.8	3.9
Flounder	78.9	3.0	12.2
$Fl+RH_1$(0 min.)	81.9	4.5	7.3
$Fl+RH_1$(30 min.)	80.8	4.0	10.0
$Fl+RH_1$(60 min.)	81.2	3.5	11.3

These results suggest that if what we are seeing is an enzymic effect, then it occurs relatively quickly since major changes are seen at 0 time.

Finally, in our last experiment we investigated the effect of holding a mince made-up of red hake only, for 0-60 min. before processing.

As may be seen in Table 6, moisture contents and fold test scores did not change significantly with holding, although there was some increase in hardness. This suggests that the results obtained in the foregoing experiments were due to an interaction of the red hake with that of the flounder.

TABLE 6. MECHANICAL PROPERTIES OF RED HAKE GELS MADE FROM MINCES HELD 0-60 MIN. BEFORE WASHING EXPERIMENT 5.

Treatments	% Moisture	Fold Test Score	% Deformation
Red Hake (0 min.)	82.8	5.0	3.2
Red Hake (30 min.)	82.9	4.8	3.9
Red Hake (60 min.)	82.3	4.8	3.8

It has been previously reported that good fish gels could be formed in the absence of salt if the muscle tissue was first washed (Hennigar et al. 1987). This phenomenon was found to be species dependent, with red hake yielding the best gels, scoring 5 on the fold test scale. Also, the ability to form these gels was not related to the amount of contractile protein present.

The data reported here demonstrate that a small amount of red hake tissue can significantly modify the properties of gels made from flounder without added salt. It is suggested that something other than the red hake protein itself is causing this effect.

The ability of red hake to form good gels in the absence of salt may be related to its ability to decompose TMAO to DMA and FA, since red hake has a very active system for TMAO breakdown. It has been reported that there is more than enough enzyme activity in red hake muscle to accomplish desired results (Parkin and Hultin, 1982) and this was evidenced by the small amount of muscle used in this study. In

practical terms then, efficiency of mixing may be at least as important as the amount of muscle used.

In summary, small amounts of red hake tissue can significantly modify the properties of gels produced without salt from flounder muscle. Generally, there is a greater effect on elastic properties of the gel than on hardness. Differences in hardness observed suggest that the properties of fish protein gels may be selectively modified, perhaps to give a wider range of physical properties than is now available. And finally, it would be expected that the phenomenon reported here would also occur in the presence of salt.

REFERENCES

1. Hennigar, C. J., Buck, E. M., Hultin, H. O., Peleg, M., and Vareltzis, K. 1987. The Effect of Washing and Sodium Chloride on the Mechanical Properties of Fish and Beef Muscle Gels. (Submitted to J. Food Sci.)
2. Johnson, E. A., Segars, R. A., Kapsalis, J. G., Normand, M. D., and Peleg, M. 1980. Evaluation of the compressive deformability modulus of fresh and cooked fish flesh. J. Food Sci. 45: 1318.
3. Kudo, G., Okada, M., and Miyauchi, D. 1973. Gel-forming capacity of washed and unwashed flesh of some Pacific coast species of fish. Mar. Fish. Rev. 35: 10.
4. Parkin, K. L., and Hultin, H. O. 1982. Fish muscle microsomes catalyze the conversion of trimethylamine oxide to dimethylamine and formaldehyde. FEBS Lett. 139: 61-64.

NATIONAL MARINE FISHERIES SERVICE SALTONSTALL-KENNEDY (S-K) FUNDING: TECHNICAL PEER REVIEW OF NATIONAL PRIORITY PROPOSALS

Paul Comar
National Marine Fisheries Service, NOAA,
National Seafood Inspection Laboratory,
P. O. Drawer 1207, Pascagoula, MS 39568-1207

INTRODUCTION

The Saltonstall-Kennedy (S-K) Act makes available to the Secretary of Commerce up to 30 percent of the gross receipts collected under the customs laws from duties on fishery products. The Secretary must use a portion of these funds each year to make available grants to assist persons in carrying out research and development projects which address aspects of United States fisheries, including, but not limited to, harvesting, processing, and associated infrastructures. This includes both the commercial and recreational sectors of U.S. fisheries. For FY 87, $7.4 million was appropriated for the S-K program. Approximately $559,000 was committed to fund the second and third years of previously approved multi-year projects, and about $750,000 was placed in the Fisheries Promotional Fund established by the Fish and Seafood Promotion Act of 1986. Therefore, about $6.1 million was used to fund new fisheries research and development projects.

The National Marine Fisheries Service (NMFS) identifies fisheries funding priorities in consultation with a wide cross section of the U.S. fishing industry. Priorities are identified on a regional basis according to specific fisheries. These funding priorities are listed by region annually in the Federal Register with a deadline for submission of proposals to address the needs. Priorities which cross regional fisheries bounds or include all fisheries or regions are listed as national priorities. One portion of the entire proposal review, evaluation and approval process is the technical peer review, which is coordinated through the various Regional Offices. This paper describes the process of the technical peer review for those proposals within the national priorities designation.

METHOD

Over the past three years an average of 43 proposals have been submitted annually addressing national priorities as listed in the Federal Register. These proposals are received, checked for completeness and grouped under major headings by the S-K office in Washington, DC. The proposals are then sent to the NMFS, National Seafood Inspection Laboratory (NSIL) in Pascagoula, MS which coordinates the technical peer review.

After receipt and review of the proposals at NSIL, a list of competent reviewers for each proposal is assembled. Through its conduct

of the technical peer review over the past several years, the NSIL has compiled a list of qualified and committed reviewers who can assess the technical merits of individual proposals. Because funding priorities are modified somewhat in the annual Federal Register solicitation, new reviewers are added and some experienced S-K reviewers may not be asked to participate as necessary to address current year priorities. Usually three to five reviewers are selected and asked to review each proposal. The proposals are reviewed for technical merit by NMFS staff members from the Washington Office of Trade and Industry Services; National Seafood Inspection Laboratory, Pascagoula, MS; Resource Utilization Laboratories in Gloucester, MA; Charleston, SC; and Seattle, WA. Additional reviewers experienced in the field of each proposal are scientists and professionals associated with other Federal agencies, universities and other qualified groups. During the 1987 S-K process, there were 76 reviewers who participated in the national priorities technical peer review. Care is taken to ensure no conflict of interest by those taking part in the review.

As detailed in the Federal Register solicitation, the proposals are evaluated under five general criteria:

1) Problem description and conceptual approach to resolve it;
2) Soundness of project design/technical approach;
3) Project management; experience/qualifications of personnel;
4) Project monitoring and evaluation; and
5) Project cost.

A standardized response/scoresheet is completed by each reviewer on each proposal. Reviewers provide responses to specific questions and then score the proposal up to a set maximum point value in each of the five criteria. The response/scoresheets are returned to the NSIL - nearly 200 in the 1987 S-K process.

The NSIL prepares a synopsis of each reviewer's comments along with the reviewer's technical score total for each proposal. A comprehensive briefing book is prepared, assembled and printed by the NSIL which includes among its various sections these synopses and a further single statement on the technical merit each of proposal synthesized from the reviewer comments.

Each regional review office conducts its own technical review of proposals submitted for funding under the Federal Register listing of regional priorities. What has just been described is the process used to conduct the peer review of proposals which address national priorities. The following comments in this section are added to complete the picture on the final decision making process on actual funding of proposals.

After the technical evaluation, each reviewing office solicits comments from the fishing industry, consumer representatives, and others, as appropriate, to rank the projects filed with the office.

This review is carried out by correspondence or by formal meetings of industry representatives. The reviewers rank each project in terms of importance or need for funding and provide recommendations on the level of funding NMFS should award to each project and the merits and benefits of funding each project. The recommendations are submitted to the Assistant Administrator for Fisheries, who determines the number of projects to be funded based on the recommendations provided, consistency of projects with the identified fisheries objectives, and the amount of funds available for the program. The Department of Commerce reviews all recommended projects and funding before final authority is given to proceed on the project.

DISCUSSION

Through the Saltonstall-Kennedy Act, the NMFS is able to fund proposals to develop, support, and benefit the U.S. fishing industry. Several million dollars is available to projects which address the established needs and priorities of the industry. The entire S-K review process helps ensure funding of the very best proposals of high technical merit and importance to the industry. The S-K program as a whole can be only as good as the technical review of proposals by qualified professionals in the various priority areas. The technical review for national priority proposals as outlined in this paper is conducted to ensure that the cream rises to the top.

It would be most difficult at a panel meeting to review and digest the many response/scoresheets generated - again nearly 200 from the 1987 national priority proposals. The briefing book of reviewer comments, scores and recommendations prepared by NSIL is used to describe the technical merits of proposals to an industry review panel. Through the cooperation of NSIL with the NMFS Washington Office of Trade and Industry Services, a solid review package is assembled and presented to the industry panel so that it can make informed funding recommendations to the agency.

REFERENCES

1. Federal Register. Part VII. Department of Commerce, Vol. 52, No. 106. pp. 20962-20972. June 3, 1987.

Quality Changes Occurring in Hybrid Striped Bass Skinless Fillets and in Headed and Gutted Hybrids During Iced Storage

Michael Jahncke, Malcolm B. Hale and Janet A. Gooch
National Marine Fisheries Service
P.O. Box 12607
Charleston, SC 29412

INTRODUCTION

The pond culture of hybrid striped bass has great potential in South Carolina. Personnel at the South Carolina Marine Resources Division have been able to produce, under intensive management, approximately 7400 pounds of hybrids per acre in 18 months (personal communication, T.S. Smith, 1987). Little information, however, is available on the sensory characteristics of hybrid striped bass. This information is necessary if hybrids are to be successfully marketed in the United States.

The objectives of this research were to determine the 1) sensory characteristics, and 2) to monitor the changes in sensory characteristics, TBA values and total aerobic plate counts of iced hybrid striped bass skinless fillets and iced headed and gutted (H&G) hybrids.

MATERIALS AND METHODS

Collection of Samples

The fish used in this study were cultured in ponds at the Waddell Mariculture Center near Bluffton, S.C. The fish averaged 36.0 cm in fork length (FL) and 765 g in weight. The fish, which were immediately iced after harvesting, were processed commercially and transported to the National Marine Fisheries Service Laboratory in Charleston, S.C.

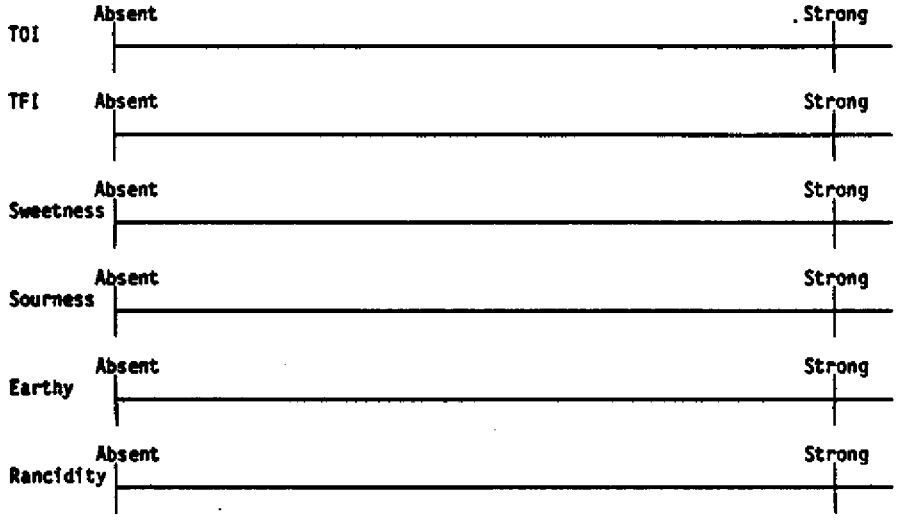
Storage of Samples

Sixty hybrid bass were divided into two treatments and prepared as skinless fillets or headed and gutted fish (H&G). The skinless fillets were rinsed with clean water, placed on styrofoam trays and over wrapped with saran. These trays were then covered with ice, placed in drainage trays, and stored in a refrigerator. The headed and gutted fish were rinsed, placed in an insulated chest, from which melt water was allowed to drain, covered with ice and stored in a refrigerator. Samples were randomly selected and evaluated beginning at one day of iced storage.

Sensory Evaluations

The panelists were trained, using several species of fresh marine fish as standards, according to the flavor and texture profile method of Kapsalis and Maller (1980). An unstructured 15 cm line scale, however, was used instead of a 0-7 category scale, since an unstructured scale generates data which can be analyzed by standard statistical methods which assume a normal distribution.

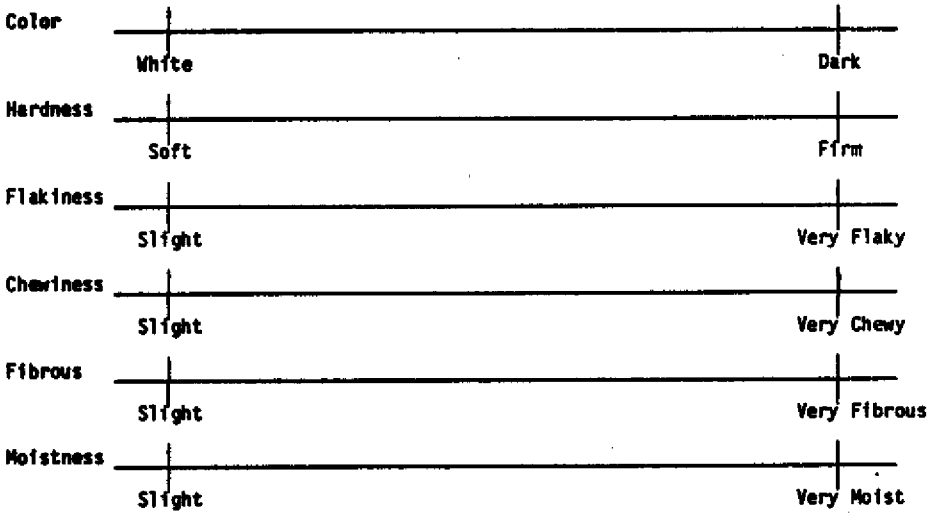
Place a vertical mark across the horizontal line according to your judgement as to the intensity of the attribute. Mark only the attributes you detect.



Comments:

Table 1. Flavor Profile Score Sheet

Place a vertical mark across the horizontal line according to your judgement as to the intensity of the attribute. Your comments are encouraged.



Comments:

Table 2. Texture Profile Score Sheet

Term	Definition
Total Odor Intensity (TOI)	The initial or early total impact of odor.
Total Flavor Intensity (TFI)	The initial or early total impact of flavors.
Sweetness	The basic taste sensation of which the taste of sucrose is typical.
Sourness	The taste sensation produced by acids. The taste of vinegar or lemon are typical examples.
Earthy	The flavor associated with slightly undercooked boiled potato, soil, or muddy fish.
Rancidity	The flavor associated with strong, oxidized rank, acrid flavor.
Salty-briny	A combination of the taste sensations of sodium chloride and the other salt compounds found in ocean water.
Shellfish	The flavor associated with any cooked shellfish, such as lobster, clam, crab, or scallop.

Table 3. Definitions of Sensory Terms for The Flavor of Fish

Term	Texture Definition
Color	The perceived degree of darkness of the flesh, ignoring any concentrated dark lateral lines.
Hardness	The perceived force required to compress the sample using the molar teeth.
Flakiness	The perceived degree of separation of the sample into individual flakes when manipulated with the tongue against the palate.
Chewiness	The total perceived effort required to prepare the sample to a state ready for swallowing.
Fibrousness	The perceived degree (number x size) of fibers evident during mastication.
Moistness	The perceived degree of oil and/or water in the sample during chewing.

Table 4. Definitions of Sensory Terms for the Texture of Fish

Individual fillets (n=3 fillets/treatment) were randomly placed into boil-in bags with drainage pockets, and cooked to an internal temperature of 70°C. A six member trained sensory panel determined the flavor and texture profiles of the fillets. The score sheets are shown in Table 1 and 2, and the description of the sensory terms is presented in Table 3 and 4.

Proximate Composition

Protein, moisture and ash of the raw and cooked fillets were determined in duplicate by standard AOAC (1985) procedures. Lipid was determined in duplicate by a chloroform-methanol extraction method (Smith et al., 1964).

Thiobarbituric Acid Evaluation (TBA)

TBA values were determined in duplicate, on homogenized cooked fillet samples, by the method of Woyewoda et al., (1986).

Microbiological Evaluation

Microbial counts were determined on the same day that sensory and TBA analyses were performed. The microbiological analysis consisted of total aerobic plate counts (TAPC). Fifty gram samples (in triplicate), were aseptically removed from three raw skinless fillets and three raw H&G fish. These samples were serially diluted and plated on standard plate count agar. The plates were incubated at 20°C for 48 hours (AOAC, 1985).

Statistical Evaluation

Statistical Analysis System (SAS Institute Inc., Cary, NC) was used to do a two way analysis of variance on the results. Fisher's LSD Test was used to further evaluate the samples whenever the F test was found to be significant.

RESULTS AND DISCUSSION

Table 5 shows the proximate composition of raw and cooked hybrid striped bass fillets.

Table 5. Proximate Compositions (weight percent of tissue sample) of Raw and Cooked Hybrid Fillets

Protein		Fat		Moisture		Ash	
Raw	Cooked	Raw	Cooked	Raw	Cooked	Raw	Cooked
19.38	21.40	2.56	2.76	75.93	72.45	1.07	1.11

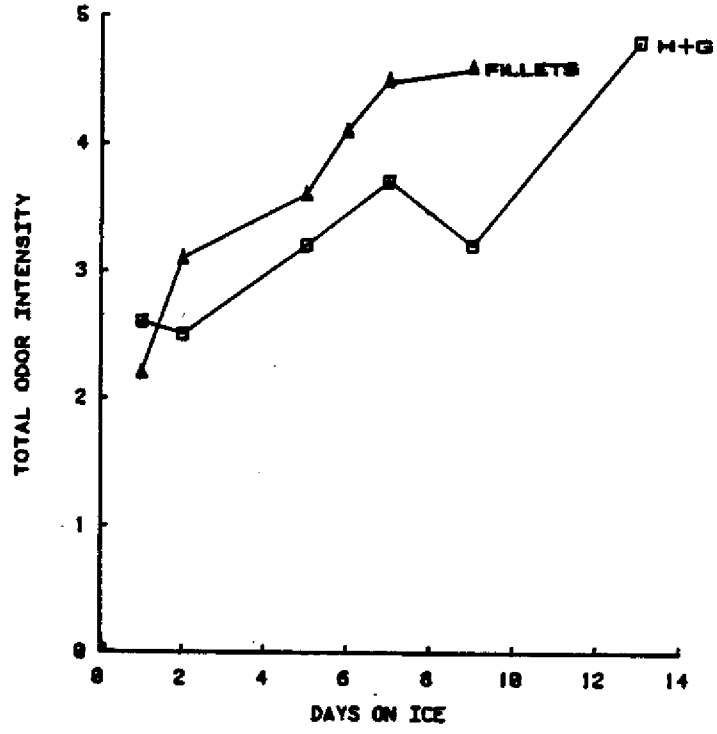


Figure 1. Total odor intensity score for cooked fillets prepared from iced skinless fillets or iced headed and gutted fish.

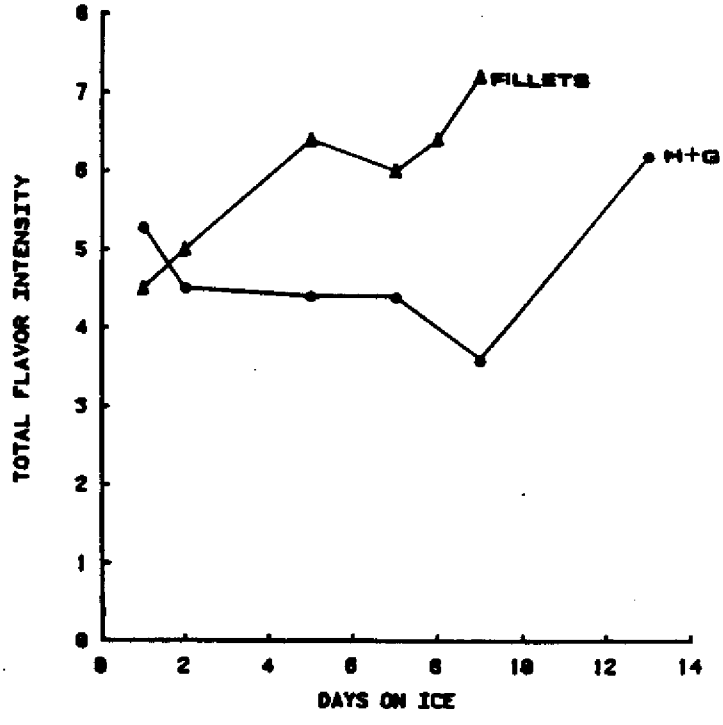


Figure 2. Total flavor intensity scores for cooked fillets prepared from iced skinless fillets and iced headed and gutted fish.

The sensory results indicated that initially the fillets had a mild total odor intensity (TOI) (Figure 1) and total flavor intensity (TFI) (Figure 2) with moderate sweetness (Tables 6 and 7). The fillets had no shellfish flavor. The dark flesh did, however, have a slight earthy flavor (Tables 6 and 7). The texture scores indicated that the fillets were moderately flaky, slightly fibrous, moist, soft and easy to chew (Tables 6 and 7).

Iced storage had no significant effect on any texture scores (Tables 6 and 7) ($P > 0.05$). Iced storage did, however, have an effect on flavor profiles (Figures 1, 2, and 3, Tables 6 and 7), TBA values (Figure 4) and total aerobic plate counts (Figure 5).

Figure 1 indicates that, at 6-7 days of iced storage, TOI scores increased significantly in cooked fillets prepared from iced skinless fillets and in cooked fillets prepared from H&G fish ($P < 0.05$). At 9 days of iced storage, TOI scores for cooked fillets prepared from iced skinless fillets were significantly higher than TOI scores of cooked fillets prepared from H&G fish ($P < 0.05$). Figure 2 shows that TFI scores increased significantly at 5 and 13 days of iced storage for cooked fillets prepared from iced skinless fillets and for cooked fillets prepared from iced H&G fish respectively ($P < 0.05$). In addition, at 5 days of iced storage, cooked fillets prepared from iced skinless fillets had significantly higher TFI scores than cooked fillets prepared from H&G fish ($P < 0.05$).

These higher odor and flavor intensity scores were primarily due to a combination of bacterial growth (Figure 5) and rancidity development (Figures 3 and 4). The TAPC for raw skinless fillets and raw H&G fish increased throughout iced storage (Figure 5). The bacterial counts on the skinless fillets were always higher than the H&G fish, as additional processing and larger cut surface area may have provided a better medium with more surface for growth.

Figure 3 indicates that at 7 days of iced storage, the sensory panel noted a significant increase in rancidity of cooked fillets prepared from iced skinless fillets ($P < 0.05$). In contrast, there was a gradual but not significant increase in rancidity scores of cooked fillets prepared from H&G fish ($P > 0.05$). Similarly, at 6 days of iced storage, TBA values were significantly higher in cooked fillets prepared from skinless fillets ($P < 0.05$), but not for cooked fillets prepared from H&G fish ($P > 0.05$) (Figure 4).

In addition, after five days of iced storage, sweetness scores had decreased significantly for cooked fillets prepared from iced skinless fillets (Table 6) ($P < 0.05$). Sweetness scores in cooked fillets prepared from H&G fish also decreased, but not significantly (Table 7) ($P > 0.05$). Earthiness scores, on the other hand, increased gradually but not significantly during iced storage ($P > 0.05$). Earthiness scores

Table 6. Flavor and Texture Profile of Cooked Fillets Prepared from Iced Skinless Fillets (Mean ± S.D.; 15 cm Descriptive Scale)

	Days on Ice				
	1	2	5	7	9
Flavor Profile					
Sweetness	3.7±1.6[a]	3.3±1.7[ab]	1.6±1.2[b]	2.0±1.9[b]	1.6±1.8[b]
Sourness	0.4±0.4	0.7±0.4	1.7±1.4[a]	1.3±1.2[a]	2.4±1.3[a]
Earthy	1.5±1.5	1.8±1.2	2.1±1.5	1.8±2.0	2.3±1.8
Salty-briny	1.8±0.7	1.6±1.1	1.5±0.7	1.8±1.2	1.5±0.8
Shellfish	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Texture Profile					
Color	6.5±1.1	5.8±0.9	5.3±1.0	5.8±1.0	5.6±1.0
Hardness	4.6±2.1	4.9±1.2	3.1±1.3	3.8±1.8	3.8±1.5
Flakiness	5.6±1.8	4.7±2.8	3.7±1.4	4.5±1.3	4.2±1.4
Chewiness	3.4±1.6	5.8±2.0	3.5±1.3	3.8±1.7	3.0±1.3
Fibrousness	3.0±1.3	4.1±0.9	3.3±1.3	3.1±0.7	2.8±1.0
Moistness	9.0±1.2	8.0±2.3	8.0±2.4	8.1±1.9	8.0±1.4

Means within the same row with the same superscripts are not statistically different ($P > 0.05$).

Table 7. Flavor and Texture Profile of Cooked Fillets Prepared from Iced H&G Fish(Mean ± S.D.; 15 cm Descriptive Scale)

	Days on Ice					
	1	2	5	7	9	13
Flavor Profile						
Sweetness	2.5±1.2[a]	2.1±1.6[ab]	2.3±1.3[ab]	1.6±1.1[ab]	2.0±0.9[ab]	1.4±1.2[b]
Sourness	0.5±1.1	0.7±0.6	0.8±1.0	1.4±0.7[a]	-	2.2±1.5[a]
Earthy	1.4±1.5	1.3±1.3	1.6±1.9	1.6±1.3	1.8±1.4	2.5±2.0
Salty-briny	1.5±1.3	1.5±1.1	1.5±1.0	1.8±1.1	1.2±0.8	1.4±1.1
Shellfish	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Texture Profile						
Color	5.9±1.0	5.1±0.9	4.8±0.7	5.4±1.1	4.9±1.5	5.6±1.1
Hardness	4.6±2.0	4.2±0.7	3.4±1.0	3.9±0.9	4.7±1.5	3.9±1.8
Flakiness	5.9±1.1	5.0±1.0	4.7±2.3	4.8±3.1	5.1±1.2	5.5±2.2
Chewiness	3.0±1.1	4.5±1.6	3.6±1.6	3.8±1.4	4.1±0.4	3.9±1.1
Fibrousness	3.4±1.7	4.0±0.9	2.9±1.0	2.9±2.1	2.9±0.9	3.3±0.7
Moistness	8.3±2.2	7.6±1.8	7.6±2.0	7.3±2.1	8.1±1.7	7.8±2.3

Means within the same row with the same superscripts are not statistically different ($P > 0.05$).

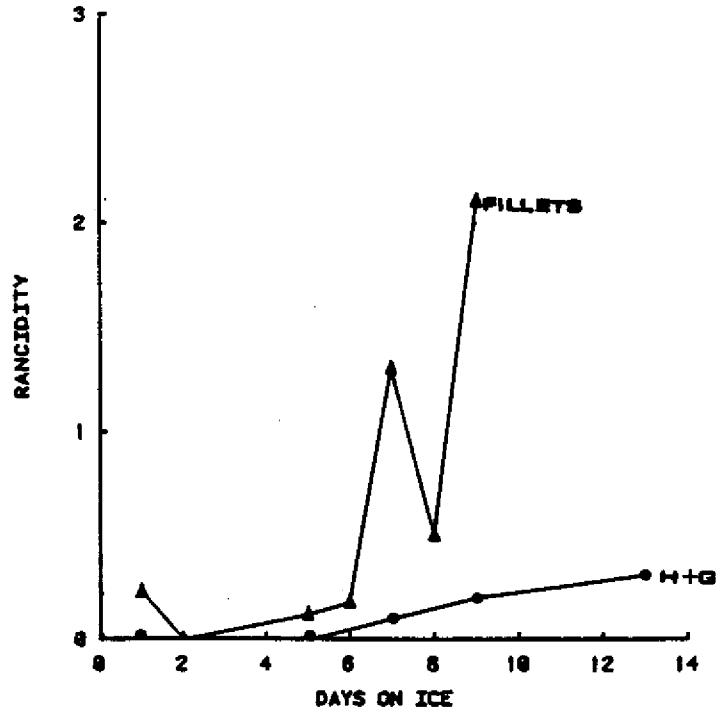


Figure 3. Rancidity sensory scores for cooked fillets prepared from iced skinless fillets and iced headed and gutted fish

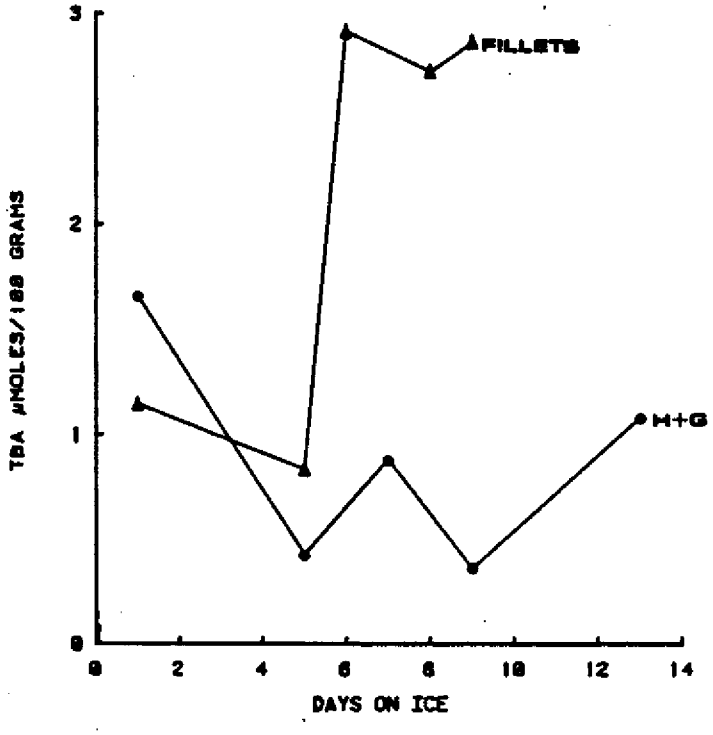


Figure 4. TBA values for cooked fillets prepared from iced skinless fillets and iced headed and gutted fish.

of cooked fillets prepared from skinless fillets were also higher, but not significantly higher, than earthiness scores for cooked fillets prepared from H&G fish ($P > 0.05$). This earthy flavor was primarily associated with the dark red muscle fibers of the lateral line. The red muscle fibers have been shown to contain more lipid than the white muscle fibers (George 1962, Thurston and MacMaster, 1960).

Tables 6 and 7 show that at 5 and 7 days of iced storage, sourness scores increased significantly in cooked fillets prepared from skinless fillets and in cooked fillets prepared from H&G fish ($P < 0.05$). This increase in sourness scores may have been a result of bacterial activity (Figure 5). At 13 and 16 days of iced storage, the study was terminated, since TAPC were 10^7/g and spoilage odors were present in both the iced raw skinless fillets and iced raw H&G fish respectively (Figure 5).

CONCLUSIONS

1. TOI scores increased significantly, at 6-7 days of iced storage, in cooked fillets prepared from skinless fillets and in cooked fillets prepared from H&G fish ($P < 0.05$).

2. At 9 days of iced storage, TOI scores of cooked fillets prepared from skinless fillets were significantly higher than TOI scores of cooked fillets prepared from H&G fish ($P < 0.05$).

3. TFI scores increased significantly at 5 days of iced storage for cooked fillets prepared from iced skinless fillets and at 13 days of iced storage for cooked fillets prepared from H&G fish ($P < 0.05$).

4. At 5 days of iced storage, cooked fillets prepared from iced skinless fillets had significantly higher TFI scores than cooked fillets prepared from H&G fish ($P < 0.05$).

5. TBA values and rancidity sensory scores increased significantly at 6 and 7 days of iced storage, respectively in cooked fillets prepared from iced skinless fillets ($P < 0.05$).

6. There were no significant changes in TBA values or rancidity sensory scores in cooked fillets prepared from H&G fish ($P > 0.05$).

7. Sweetness scores of all samples decreased during iced storage.

8. Earthiness scores of all samples increased gradually, but not significantly, during iced storage ($P > 0.05$).

9. There were no significant changes in texture scores of any samples during iced storage ($P > 0.05$).

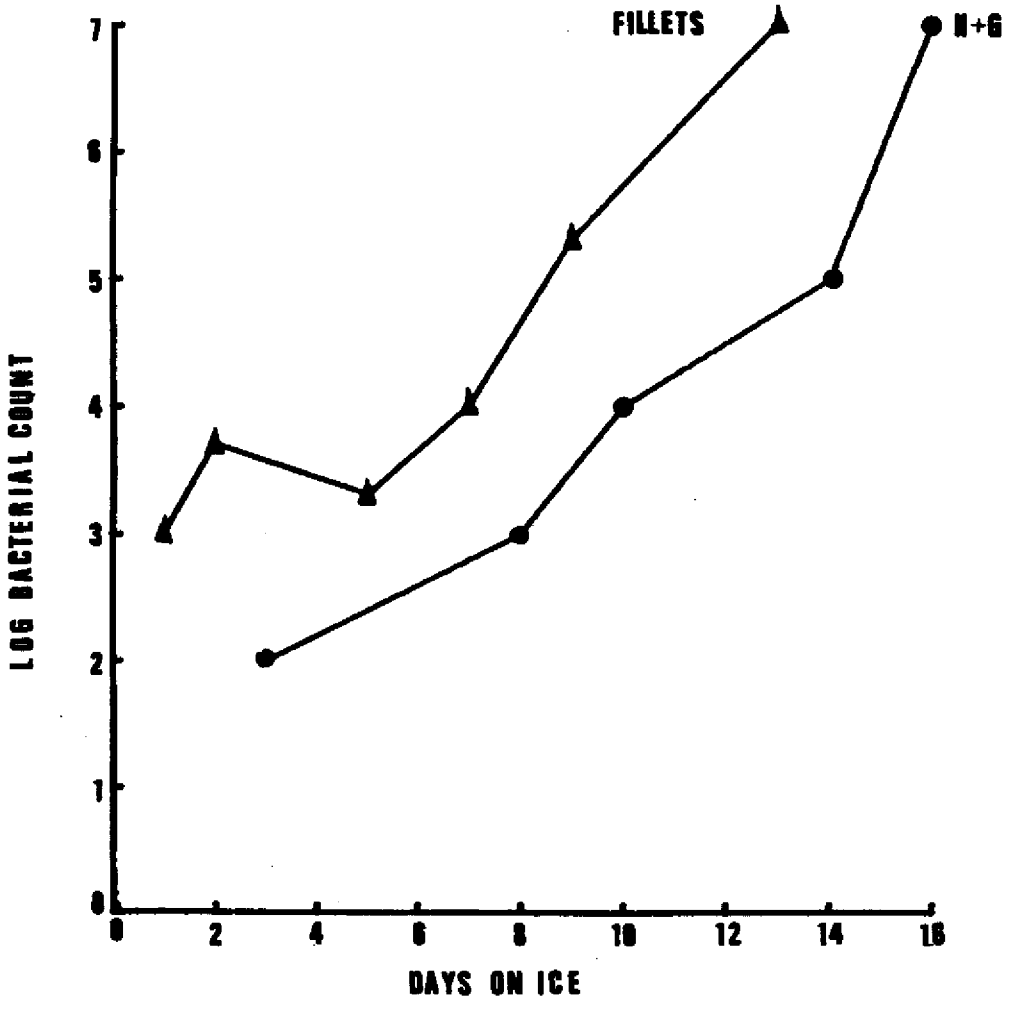


Figure 5. Total aerobic plate counts for iced raw skinless fillets and iced raw headed and gutted fish.

10. At 13 and 16 days of iced storage, respectively TAPC were 10^7/g and spoilage odors were evident in raw skinless fillets and in raw H&G fish.

REFERENCES

1. AOAC. 1985. "Official Methods of Analysis" 14th ed. Association of Official Analytical Chemists. Washington D.C.

2. George, J.C. 1962. A histophysiological study of the red and white muscles of the mackerel. Amer. Mid. Nat. 68:487.

3. Kapsalis, J.G. and O. Maller. 1980. Consumer and instrumental edibility measures for grouping of fish species. Final Report, U.S. Army Natick R&D Laboratories. Dept. of Commerce, National Technical Information Service Report PB82-150921.

4. SAS/STAT. 1985. "Guide for Personal Computers." Version 6. Edition. SAS Institute Inc., Box 8000, Cary, N.C.

5. Smith, P. Jr., M.E. Ambrose, and G.M. Knobl, Jr. 1964. Improved rapid method for determing total lipids in fish meal. Commer. Fish Rev. 26(7):1.

6. Smith. T.S. 1987. Personal communication.

7. Thurston, C.E., and MacMaster, P.P. 1960. Variations in chemical composition of different parts of halibut flesh. Food. Res. 25:229.

8. Woyewoda, A.D., S.J. Shaw, P.J. Ke and B.G. Burns. 1986. Recommended laboratory methods for assessment of fish quality. Canadian Technical Report of Fisheries and Aquatic Sciences. No. 1448.

THERMAL PROCESSING STUDIES OF PACIFIC HAKE

Boey Chee Cheong
Fisheries Division, Primary Production Department
Republic of Singapore

and

Marvin A. Tung
Canadian Institute of Fisheries Technology
Technical University of Nova Scotia
Halifax, Nova Scotia
B3J 2X4

INTRODUCTION

Pacific hake (Merluccius productus) is an underutilized fish resource in British Columbia. In 1982, the domestic catch was only 15% of the annual total allowable catch while the national and co-operative fisheries of Poland, the USSR and Japan exceeded their annual total allowable catch in Canada's 200 mile exclusive economic zone. Co-operative fishing, where domestic boats transfer their catch at sea to foreign vessels for processing and export, does employ Canadian fishermen but does not realize the potential profits associated with post harvest production and marketing of value added products.

The Pacific hake fishery in British Columbia occurs in two areas, the primary area is in offshore waters west of Vancouver Island along the edge of the continental shelf and a smaller secondary stock occurs in the Strait of Georgia which runs between Vancouver Island and mainland British Columbia. The fish in Georgia Strait have a slower growth rate (Stocker, 1981) with an average weight of 580 g as compared to 1080 g for offshore stock (Buechler and Proverbs, 1983).

Pacific hake in Canadian waters are known to be infested with two species of Myxosporean protozoan parasites: Kudoa thyrsitis and Kudoa paniformis. Kudoa thyrsitis has been found in hake harvested in offshore waters west of Vancouver Island and in Georgia Strait; however, Kudoa paniformis has been found only in the offshore stock (Kabata and Whitaker, 1981). Tsuyuki et al. (1982) associated the presence of Kudoa paniformis with mushiness in the cooked texture of Pacific hake. They found that the muscle tissue of fish infested with only Kudoa thyrsitis contained an enzyme with a strong proteolytic activity in the acid range. The acid protease was heat labile and did not lead to an unacceptable mushy texture upon cooking. However,

muscle tissue of west coast hake infested with Kudoa paniformis alone or together with Kudoa thyrsitis demonstrated strong proteolytic activity in both acid and neutral ranges. Muscle with proteolytic activity in the neutral pH range would become mushy during slow cooking and the enzyme concerned was relatively heat stable with an optimum activity at 50-60°C. Willis (1949) hypothesized that the proteolytic activity was highly localized within pseudocysts and while living, the host removed the enzyme by blood circulation. But, after death of the host, the active proteolytic enzymes diffused from the cyst to the adjacent muscle fibers and damaged the texture of the flesh.

If Pacific hake is to be utilized for human consumption it is critical that the cooked fish has acceptable textural qualities. The objective of this work was to study the textural properties of Pacific hake after quick heating by thermal processing in cylindrical and thin profile cans.

METHODS

Fish used in these experiments were obtained in three lots from two sources. West coast offshore samples, caught by a Polish midwater trawler, had been frozen on board, sealed in polyethylene bags and stored in paperboard cartons. Once obtained, the fish were stored at -29°C for later filleting. Fish obtained from Georgia Strait were stored on board in refrigerated seawater tanks at -1.1°C for up to 3 days prior to landing on shore. The first lot was then stored at -29°C awaiting further processing while the second lot was kept on ice in a 0°C cold room for up to four days prior to the completion of filleting. Both frozen sample lots were thawed in running tap water mixed with ice before filleting on both sides of the body. The paired fillets were cleaned, placed into barrier bags (Cryovac Division, W.R. Grace & Co. of Canada Ltd., Mississauga, ON), vacuum packed and stored at -29°C.

Kabata and Whitaker (1981) reported that the two species of myxosporean parasites in Pacific hake could be identified by differences in the shape and size of their spores. In this study, the occurrence and severity of parasitic infestation were evaluated by wet mount microscopic examination of fish muscle tissue using a light microscope (E. Leitz Wetzler) under phase contrast illumination at a magnification of 400X. The parasite spore intensity was evaluated by observing the average number of spores in three randomly selected fields. Severity of parasitization was arbitrarily categorized on the basis of the average number of spores per field as: light, less than 6; medium, 6 to 19; heavy, 20 to 29; and very heavy, greater than 29.

Three containers were used in the canning operations, three-piece tinplate half (307x200.5) and quarter-pound (301x106) salmon cans and two-piece aluminum quarter dingley (105x76x21.3 mm) cans. The fill weights were 175, 85 and 85 g, respectively. The half-pound salmon cans were vacuum seamed and processed in a pilot scale vertical steam retort (Patterson Boiler Works Ltd., Vancouver, BC), whereas the quarter-pound salmon and dingley cans were sealed at atmospheric pressure and processed in an FMC 500W water immersion pilot scale sterilizer (FMC Corporation, Madera, CA).

Thirty heat penetration histories were recorded for each can type to determine the processing times required to commercially sterilize the fish. Temperature measurements in the tinplate cans were made using Ecklund non-projecting thermocouples (O.F. Ecklund, Cape Coral, FL) with Teflon insulated copper/constantan leads (Type TT-T-24, Omega Engineering Inc., Stamford, CT). Thermocouples of the same type were introduced into the aluminum cans using Ecklund pouch packing glands and the environmental temperature in the retort was also monitored using type T thermocouples. All the thermocouples were calibrated in a circulating oil bath against an ASTM thermometer at each processing temperature.

Temperature measurements were recorded at one minute intervals on a Kaye Ramp II Scanner/Processor data logger (Kaye Instruments Inc., Bedford, MA) and stored on digital tape (Columbia Data Products Inc., Columbia, MD). The centerpoint temperature history data were analyzed using a microcomputer to evaluate the heating and cooling rate indices (f_h and f_c), and the heating and cooling lag factors (j_h and j_c) for each test can. The lag factors were adjusted to correct for the effects of the non-projecting thermocouple receptacles and Stumbo's formula method (Stumbo, 1973) was then applied to calculate estimates of the process times to achieve a target process lethality, F_o, of 6 min at an initial product temperature of 0°C and retort temperatures of 115, 120 and 130°C for each test pack. Estimates of the process times were then calculated as 3 standard deviations above the mean estimated process time determined for the individual test packs. This approach acknowledged the variabilities in the retort heating conditions and the can-to-can differences in the product, providing a statistically based level of probability that the target lethality would be delivered to the product during processing (Tung and Garland, 1978).

Fish samples from each lot were canned in three different container types at processing temperatures of 115, 120 and 130°C. Only fish with light or medium levels of parasite infection were used to ensure homogeneity in raw material quality. Weight loss during processing was assessed by comparing the weight of the cooked fish, after draining over a sieve (#10 mesh size, 2.0 mm) for 2 min, to the original product weight.

Mechanical textural analyses were completed in duplicate using an Instron 1122 Universal Testing Machine (Instron Corporation, Canton, MA) equipped with a standard Kramer Shear-compression test cell. The number of blades in the Kramer cell was reduced from 10 to 4 and the cell capacity was also reduced for the experiments. The crosshead speed was set at 50 mm/min, the chart speed at 100 mm/min and the sensitivity at 100 kg full scale. A 25 g sample of white muscle from the cooked fish was separated from the skin, bones, dark muscle and blood vessels, flaked and packed in the cell for each test. Thorough flaking of the fish improved the homogeneity of the test sample and increased the randomness of the fiber orientations with respect to the shearing blades. The compression cycle was terminated when the blades passed through the bottom of the cell. The force curve was examined to determine the hardness and firmness of each sample. Hardness was measured as the peak force observed and firmness as the slope of the initial straight line portion of the curve.

RESULTS AND DISCUSSION

Microscopic examination of the tissue samples revealed that fish harvested in west coast offshore waters were infected by two species of Myxosporea, Kudoa thyrsitis and Kudoa paniformis, whereas those from Georgia Strait were infected only by Kudoa thyrsitis. Among the offshore hake (n=281), 42.3% had mixed infection, 35.6% were infected only by K. paniformis, 14.9% were infected only by K. thyrsitis and 7.1% were non-infected, whereas 76.9% of the fish sampled from Georgia Strait were parasitized only by K. thyrsitis and the rest were free from infection. The severity of parasitization is indicated in Table 1 in terms of parasite spore intensity. In most of the mixed infection cases, both parasites had low spore intensities and very often, the number of spores observed in a selected field under the microscope was only one or two. Tissue samples had to be examined repeatedly in order to confirm mixed infection. Heavily infected fish with a high spore intensity could usually be identified without difficulty due to the presence of infected muscle fibers which varied in color from yellowish white to dark brown or black. The variation in the color of the infected muscle fiber was thought to be due to defensive mechanism of the fish in which melanin granules were deposited around the infected fibers (Kabata and Whitaker, 1981). Occasionally the presence of black pseudocysts did not coincide with a high spore intensity in the same tissue sample as most of the spores were deformed, probably due to melanin deposition, and confined within the pseudocysts. The west coast offshore fish fillets with high parasite spore intensity tended to show softness in texture and the muscle could be easily teased apart when the tissue sample was prepared for microscopic examination. For the purpose of this study, only fish with light and medium levels of infection, in terms of parasite spore intensity, were used.

Table 1. Occurrence and severity of parasitization of Pacific hake harvested in offshore waters west of Vancouver Island and in Georgia Strait

A. Fish samples from offshore waters west of Vancouver Island (n=281)

PARASITE SPORE INTENSITY[a]	TYPES OF PARASITIZATION			
	Single		Mixed[b]	Total
	K. thyrsitis	*K. paniformis*		
None	-	-	-	20
Light (L)	42	60	67	169
Medium (M)	-	15	30	45
Heavy (H)	-	8	7	15
Very Heavy (VH)	-	17	15	32
	42	100	119	281

B. Fish samples from Georgia Strait (n=363)

PARASITE SPORE INTENSITY[a]	TYPES OF PARASITIZATION			
	Single		Mixed[b]	Total
	K. thyrsitis	*K. paniformis*		
None	-	-	-	83
Light (L)	265	-	-	265
Medium (M)	14	-	-	14
Heavy (H)	1	-	-	1
Very Heavy (VH)	-	-	-	-
	280	-	-	363

[a] L: $\leq$5, M:6-19, H:20-29, VH: $\geq$30

[b] Parasite spore intensity based on counting of *K. paniformis* spores.

The heat penetration parameters were calculated from the center-point temperature histories (Table 2), and Stumbo's formula method was used to generate the process times used in experiments (Table 3). As expected the process time decreased as can thickness decreased and retort temperature increased.

The mean observed weight losses of the fish were 27.3, 21.2 and 19.7% for half and quarter-pound salmon and quarter dingley cans, respectively. Retort temperature was significant ($p<0.05$) in increasing weight loss only when processing in half-pound salmon cans at 115°C as compared to 120 and 130°C, and this increase was small (about 1%) in absolute terms.

Figure 1 shows typical force-deformation curves observed during the study and the results of the textural analyses of the different fish, packages and retort temperatures are summarized in Tables 4 and 5. The textural properties of Strait frozen (SF), Strait chilled (SC) and West Coast offshore (WC) samples packed in half-pound salmon cans were not significantly ($p>0.05$) different for different retort temperatures. Samples processed at 120 and 130°C in quarter-pound salmon and dingley cans were significantly ($p<0.05$) harder and firmer than those processed at 115°C. Similarly, WC samples processed at 130°C were harder and firmer than those processed at 115 or 120°C. The texture of SC samples was unaffected by retort temperature.

The fish type had a significant ($p<0.01$) influence on texture with the WC samples exhibiting the greatest firmness and the SC samples the least. Fish caught at different times in Georgia Strait which were infected only with the myxosporean parasite, K. thyrsitis but subjected to different postharvest handling conditions exhibited significantly ($p<0.01$) different textural characteristics. The processed SC fish texture was considered soft but not mushy when tested organoleptically. The softness may be a consequence of the time delay until initial freezing, the number of freezing cycles or the condition of the fish at harvest. For example, Dunajski (1979) reported that the texture of fish muscle could change during spawning season.

West coast offshore fish samples were parasitized with either light or medium infections of K. paniformis alone or together with K. thyrsitis. The presence of K. paniformis apparently did not cause mushiness in the cooked texture of canned west coast offshore hake. The muscle protease(s) associated with Pacific hake muscle tissue infected with K. paniformis is relatively heat stable with an activity optimum at 55-60°C (Tsuyuki et al., 1982). The heating rates were so rapid in all three container types that the duration of time in that temperature range was less than one minute. Patashnik et al. (1982)

Table 2. Heat penetration parameters for Pacific hake in three types of containers

CONTAINER TYPE	MEAN VALUES (STANDARD DEVIATIONS)			
	f_h (min)	f_c (min)	j_h	j_c
Half pound salmon	20.34 (2.08)	33.96 (2.51)	2.65 (0.77)	1.23 (0.08)
Quarter pound salmon	16.67 (1.68)	17.71 (1.42)	1.72 (0.43)	1.38 (0.11)
Quarter dingley	9.03 (1.18)	7.10 (1.71)	0.79 (0.24)	1.07 (0.20)

Table 3. Process schedules of Pacific hake processed in different containers and at different retort temperatures

CONTAINER TYPE	OPERATOR'S PROCESS TIME (min)		
	Retort temperature (°C)		
	115	120	130
Half pound salmon	76.1	56.5	41.4
Quarter pound salmon	57.9	39.4	25.8
Quarter dingley	40.4	23.5	13.0

*F_o=6 min, Initial temperature=0°C

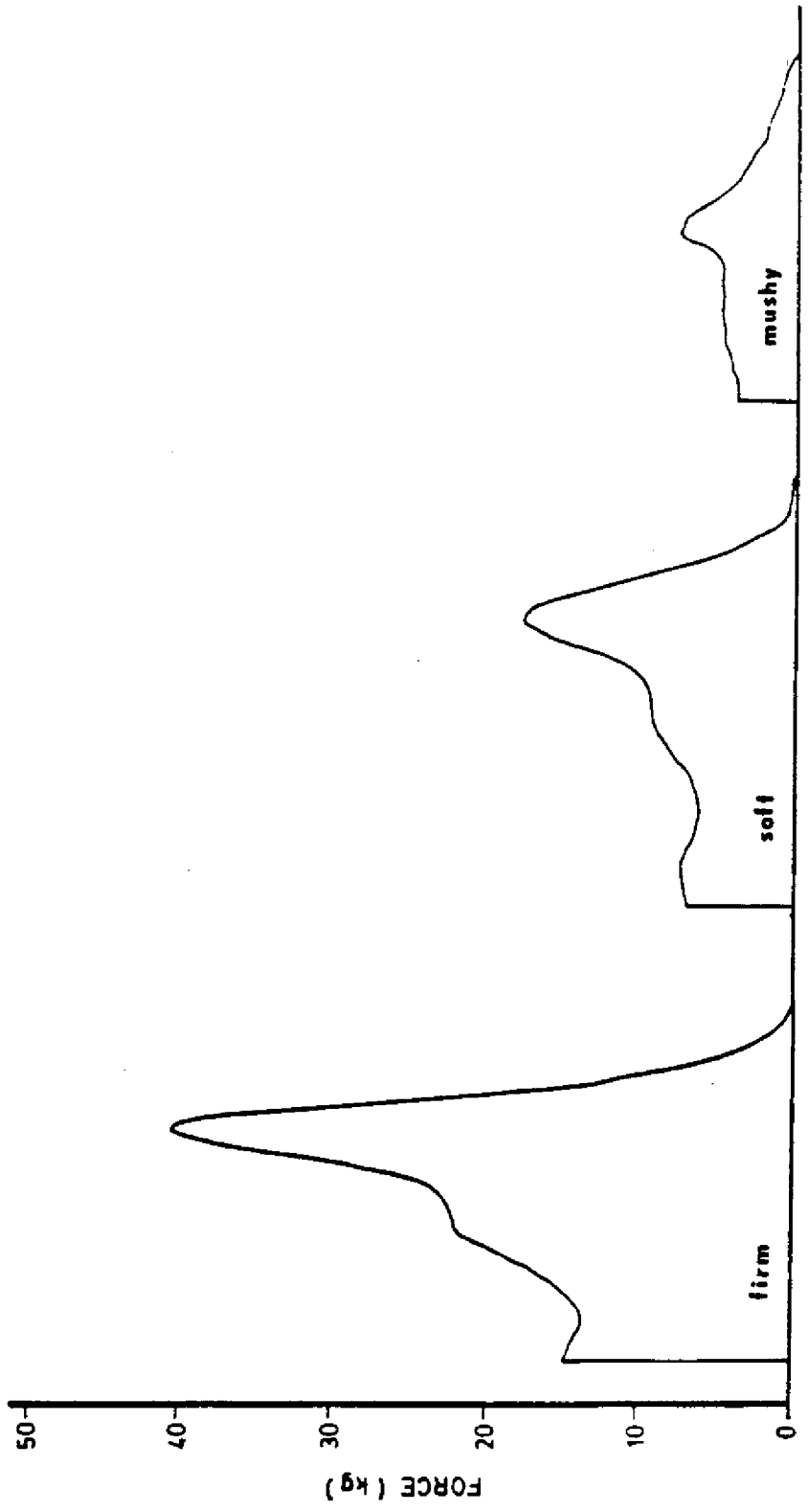


Figure 1. Typical force-distance curves of cooked Pacific hake muscle.

Table 4. Textural properties of thermally processed Pacific hake

FISH SOURCE	RETORT TEMPERATURE (°C)	HARDNESS (kg)			FIRMNESS (kg/mm)		
		1/2 lb Salmon	1/4 lb Salmon	1/4 Dingley	1/2 lb Salmon	1/4 lb Salmon	1/4 Dingley
Straight, Frozen	115	25.83 (2.84)	24.61 (1.89)	24.36 (2.10)	2.27 (0.31)	2.22 (0.22)	2.03 (0.23)
	120	26.42 (3.96)	27.98 (3.19)	28.15 (4.84)	2.29 (0.39)	2.35 (0.25)	2.40 (0.56)
	130	25.73 (5.26)	30.32 (3.51)	31.48 (7.81)	2.28 (0.51)	2.57 (0.30)	2.68 (0.75)
Straight, Chilled	115	19.05 (2.65)	18.35 (2.96)	18.10 (1.46)	1.67 (0.33)	1.46 (0.24)	1.35 (0.13)
	120	18.95 (2.48)	18.41 (2.79)	18.31 (1.97)	1.60 (0.21)	1.60 (0.33)	1.36 (0.19)
	130	19.03 (4.10)	19.93 (3.68)	18.61 (1.72)	1.70 (0.31)	1.67 (0.39)	1.42 (0.19)
Offshore, Frozen	115	30.04 (6.02)	29.09 (3.20)	26.13 (5.74)	3.11 (0.84)	2.97 (0.44)	2.38 (0.65)
	120	31.32 (4.97)	30.70 (4.11)	29.09 (6.06)	3.40 (0.68)	3.07 (0.46)	2.64 (0.64)
	130	31.65 (5.53)	33.59 (3.80)	30.70 (5.76)	3.39 (0.74)	3.48 (0.53)	2.91 (0.72)

Note: Data expressed as mean (standard deviation), n=15

Table 5. Summary of F values from an analysis of variance of the textural properties of thermally processed Pacific hake

TEST	SOURCE	F-VALUE		
		1/2 lb Salmon	1/4 lb Salmon	1/4 Dingley
Hardness	Fish Type (F)	84.96**	164.65**	67.28**
	Retort Temp (T)	0.27 n.s.	16.11**	8.40**
	FT	0.22 n.s.	1.77 n.s.	1.90 n.s.
Firmness	Fish Type (F)	113.82**	214.18**	78.67**
	Retort Temp (T)	0.51 n.s.	10.78**	7.61**
	FT	0.53 n.s.	0.98 n.s.	1.40 n.s.
Weight Loss	Fish Type (F)	3.51*	0.10 n.s.	0.21 n.s.
	Retort Temp (T)	7.60**	1.28 n.s.	0.24 n.s.
	FT	0.73 n.s.	2.34 n.s.	2.21 n.s.

** significant at $p<0.01$
* significant at $p<0.05$
n.s. not significant ($p>0.05$)

found that the enzyme(s) was completely inactivated by heating at 70°C for 10 min. This suggests that rapidly heating Pacific hake to an internal temperature higher than 70°C inactivated the enzyme during cooking.

In conclusion, Pacific hake harvested from Canadian waters and thermally processed exhibits acceptable texture when heavily parasitized fish are eliminated from the raw material supply and good postharvest handling practices are observed. Further studies are necessary to establish optimum process schedules for each container type based on other quality attributes such as color, flavor and appearance of the final product.

ACKNOWLEDGEMENTS

Financial support for this work was provided by the Canadian International Development Agency under the Asean-Canada Fisheries Post Harvest Technology Project and by the British Columbia Ministry of Agriculture and Fisheries. The use of facilities at the Department of Fisheries and Oceans Laboratory and the supply of fish arranged by Crest Packers Ltd. of Vancouver were invaluable to the project.

REFERENCES

Buechler, G.N. and Proverbs, T.B. 1983. A short history of the British Columbia Pacific hake fishery with an extensive review of activities in 1981 and 1982. Field Service Branch, Department of Fisheries and Oceans, Vancouver, BC.

Dunajski, E. 1979. Texture of fish muscle. J. Texture Studies 10:301.

Kabata, Z. and Whitaker, D.J. 1981. Two species of Kudoa (Myxosporea: Multivalvulida) parasitic in the flesh of Merluccius productus (Ayres, 1855) (Pisces: Teleostei) in the Canadian Pacific. Can. J. Zool. 59:2085.

Patashnik, M., Groninger, H.S., Barnett, H., Kudo, G. and Koury, B. 1982. Pacific whiting, Merluccius productus: 1 Abnormal muscle texture caused by myxosporidian-induced proteolysis. Marine Fisheries Review 44(5):1.

Stumbo, C.R. 1973. Thermobacteriology in Food Processing, 2nd Ed. Academic Press Inc., New York, NY.

Stocker, M. 1981. Groundfish stock assessments off the West Coast of Canada in 1981 and recommended total allowable catches for 1982. Canadian Manuscript Report of Fisheries and Aquatic Science No. 1626.

Tsuyuki, H., Williscroft, S.N., Kabata, Z. and Whitaker, D.J. 1982. The relationship between acid and neutral protease activites and the incidence of soft cooked texture in the muscle tissue of Pacific hake, Mercluccius productus, infected and held for varying times under different pre-freeze chilled storage conditions. Fisheries and Aquatic Sciences, Canadian Technical Report, No. 1130.

Tung, M.A. and Garland, T.D. 1978. Computer calculation of thermal processes. J. Food Sci. 43:365.

Willis, A.G. 1949. On the vegetative forms and life history of chloromyxum thyrsites Gilchrist and its doubtful systematic position. Australian J. Sci. Res., Ser. B., 2:379.

ON BOARD FISH HANDLING SYSTEMS FOR OFFSHORE WETFISH TRAWLERS
"Work Smarter, Not Harder"

by

W.K. Rodman, P. Eng.
Engineering Services
Fishery Development Division
Department of Fisheries and Oceans
P.O. Box 550
Halifax, Nova Scotia, Canada
B3J 2S7

ABSTRACT

Fish handling equipment and procedures employed on board three representative offshore trawlers were observed and analysed using elementary time-and-motion techniques and a video tape record of observations. These observations and analyses were used to develop an improvement strategy and to subsequently design a new fish handling system. This prototype system was installed on board the trawler, the "Cape Hunter" (46 m LOA) and its performance was evaluated. Results of this conversion have been very positive with a considerable increase in productivity and improvements in crew comfort. Four other trawlers have been converted to similar systems as a direct result of this work. Quality improvement evaluations are currently under investigation.

INTRODUCTION

Recent technological advancements on fishing vessels have focussed primarily on the ship's structure and sophisticated electronic equipment. Hull design, deck layout and detection equipment have contributed to much-improved catch efficiency. The processing and stowage systems, however, have received much less attention.

The increasing international demand for premium quality fish products provides ample incentive to improve fish handling systems that can impact directly on fish quality.

A previous study entitled, "Dockside Grading Project - Canso Seafoods" (Canadian Industry Report, Fisheries and Aquatic Sciences No. 148) has shown that there was a direct correlation between rapid handling and increased Grade A landings. Figure 1 shows the relationship between the percentage of fish assessed to be Grade A at dockside and lag time; that is, time between the codend being landed and iced storage. This curve suggests that a reduction in lag time of one hour for example, could result in as much as a 20% increase in the percentage of Grade A fish landed. Low crew productivity and systems that failed to consistently handle fish in an efficient and gentle manner were seen as an opportunity to enhance landed quality and to improve the utilization of our finite fishery resources.

METHOD

A contract was awarded to A. Orlic Associates Ltd. of Halifax, Nova Scotia, to investigate present on board fish handling practices, systems in use and to recommend possible improvements.

Between June and December 1984 three trips were made on board typical offshore trawlers employing different fish handling systems, procedures and equipment. Qualified observers prepared detailed logs, including time-and-motion data and made video tape records of their observations. This data was analysed and the results were presented to an industry Review Committee which had been formed to oversee and guide the study. These consultations resulted in a design strategy for a new fish handling system. A. Orlic Associates Ltd. then prepared a detailed design of a prototype system for installation and evaluation aboard a typical offshore trawler. This system was installed on board the "Cape Hunter", and the prototype fish handling system's performance, in terms of productivity and landed quality, was analysed.

CURRENT COMMERCIAL PRACTICE

The following summarizes the results of the analyses performed on the fishing trawlers "Cape Charles" (45 m LOA), "Cape Fame" (50 m LOA) and "Hillsboro" (41 m LOA).

(A) Manual Gutting

Two procedures were observed in use under commercial conditions and analysed in terms of productivity.

The 1-Step method, as the name implies, involved the entire gutting process being performed by one man. A variety of individual techniques were observed with varying gutting rates in the range of 6.3 to 11.2 fish/man-minute. It was noted that the "best" gutters were all of a similar height and when casually observed, they did not seem to be working any harder. Upon close examination of their technique, however, it was evident that there were few, if any, wasted movements. These individuals had learned to work smart which resulted in reduced effort and improved performance.

Utilizing the 1-Step method, the average worker performance observed was 8.8 fish/man-minute.

The 2-Step method consisted of two distinct operations. The first worker selected, oriented, and cut the throat and opened the belly of the fish. The fish was then deposited into a holding bin where a second worker removed the gut cavity contents. Two of the vessels investigated employed this process and achieved a combined gutting rate in the range of 7.1 to 7.9 fish/man-minute.

Utilizing the 2-Step method, the average worker performance observed was 7.5 fish/man-minute.

(B) Machine Gutting

Gutting machines were employed on board two of the vessels to process cod and pollock. Other species, such as haddock and halibut, were manually gutted as were cod and pollock judged to be outside the set operating range of the gutting machines.

The machines utilized on board both vessels were the Kronborg-Jutland MK III automatic gutters.

The gutting rate observed for the machines was in the range of 17.8 to 26.0 fish/machine-minute, for an average rate of 21.9 fish/machine-minute. This was approximately 73% of the machine's theoretical capacity.

There was some criticism of the gutting machine's performance as the cuts were not always straight and clean, and there was some requirement to inspect and remove bits and pieces of viscera not completely removed by the machine-gutting process. This problem was attributed to inadequate maintenance and/or poor adjustment.

ERGONOMICS

Generally speaking, ergonomics was not a prime consideration when the surveyed vessels were originally designed and equipped. Manual and and machine-gutting stations were located within the available space with interconnecting conveyors and chutes installed to establish a flow of fish through the processing area. Crew members were required to virtually "insert" themselves into their work stations. As a result, productivity was low and fish were often "forked" and thrown from one station to another.

The gutting machine operators were crowded and required to select from a bin of unsorted fish. Often the layout required the operator to bend and twist each time a fish was processed. The result was a fatiguing work pattern which tended to reduce productivity.

STRATEGY TO IMPROVE THE ON BOARD FISH HANDLING SYSTEM

A Review Committee, composed of industry representatives and various fishery professionals, was formed to review the analyses, video tapes and raw data gathered by the observers, and to formulate a strategy to design a new fish handling system that would enhance crew productivity and fish quality. The strategy agreed upon was as follows:

1. Optimize the use of automatic gutting equipment.

   It was agreed that the new system should attempt to fully utilize automatic gutting machines since they offered the most potential to rapidly gut large numbers of fish.

   In theory, the automatic gutting machine offers an increase in gutting rate (30 fish/minute) over the manual 1-Step method of more than 340%.

   It had been observed that when fish were machine-gutted, it was often necessary to inspect the gut cavity and remove any bits and pieces not completely removed by the machine. The Committee was of the opinion that if the operators processed only appropriate species within the correct size range and if the machines were well-adjusted and maintained, then the performance of the equipment would be acceptable. This opinion was corroborated to a large extent by the Torry Research

Station assertion that their fish handling system, which centered around the Kronborg-Jutland Mark III/s machines, had been very successful and that the gutting machines in particular, had performed very well.

2. Utilize the 1-Step manual gutting method.

   Since not all fish captured are suitable for machine gutting, either because of physical structure or size, it was considered essential that a significant manual gutting capacity be designed into the system.

   The 1-Step method of gutting offered a productivity improvement in the order of 17% over the 2-Step method and did not require any additional crew handling or facilities.

   Using the 1-Step method, by-catch could be segregated by the manual gutters and immediately processed.

   The 1-Step method also allowed for a good deal of flexibility as workers could be added or redeployed at will to optimize throughput as required.

3. Design the individual work stations using sound ergonomic design principles.

   It was the recommendation of the Review Committee that every effort be made to improve the quality of the working environment and to minimize the need for the workers to lift, throw and bend in order to accomplish their tasks.

   It was also felt that worker attitude could be improved by making the process area as bright and attractive as possible.

4. Eliminate multiple or repeated handling of fish.

   The Review Committee agreed that multiple handling of fish was damaging as well as wasteful.

   The immediate 1-Step method of gutting by-catch, for example, was an opportunity to reduce double handling and to begin the bleeding process as soon as possible.

5. Utilize conveyors, chutes, mechanical washers, flumes, etc., to ensure a gentle, steady and uniform flow of fish.

   Essentially, this strategy focussed on means of conveying fish from one station to another in a reliable, steady and gentle manner. Previous layouts often required fish to be thrown and/or forked from station to station, which resulted in the puncturing and crushing of flesh. Inappropriate slopes in the receiving areas and conveyors that did not self-feed required human intervention and clearly did not contribute to an efficient system.

COST/BENEFIT

It was estimated that the new fish handling system could increase at-sea productivity by as much as 19-56%. This estimate was obtained by comparing the likely performance of an integrated and ergonomically

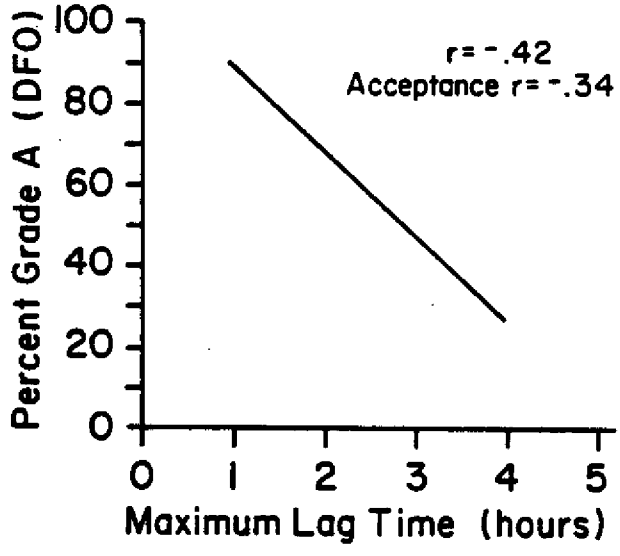


Figure 1. Relationship Between Percentage of Grade A Fish at Dockside and Lag Time.

Source: Dockside Grading Project - Canso Seafoods. Canadian Industry Report of Fisheries and Aquatic Sciences No. 148.

designed system with those rates actually measured at-sea on board the three analysed trawlers.

The benefits of improved productivity are considered to have two distinct aspects. The first is an enhanced work environment and work that is less physically demanding. Its value is almost intangible and so no attempt was made to quantify it in terms of dollars and cents. The benefits in terms of enhanced quality are very real but are difficult to isolate and quantify. It was estimated, however, that by reducing the handling time (Figure 1), a significant improvement in landed quality was likely and if the incidence of rough handling could be reduced, then improved yields and higher product grades were likely. Modest improvements in these two areas alone could justify an expenditure in the order of $150,000 with a payback period of about one year.

On this basis, it was decided to proceed with a prototype system and to evaluate its performance.

VESSEL SELECTION

The "Cape Hunter" was selected as the test vessel for the following reasons.

1. The "Cape Hunter" is one of nine vessels in the Fox class.
2. The captain and crew were supportive of the goals of quality enhancement and improved crew productivity. The Review Committee was therefore confident that the new system would be given a fair trial.
3. The vessel was already fitted with two automatic gutting machines and so the crew were experienced in the machines' operation and maintenance.
4. The Fox class represented a real challenge as there was very little room to install a completely integrated system. It was therefore evident, that if we were able to achieve a measure of success on this vessel, then deriving similar benefits on other classes would be considerably less difficult.

"CAPE HUNTER" - ORIGINAL LAYOUT

The original layout is shown in Figure 2.

In October 1985, a trip was made on board the "Cape Hunter" in order to document the existing fish handling system's performance.

Several "bottlenecks" and opportunities for improvement were noted as outlined below.

1. The gutting machine operator was crowded between two manual gutting stations.
2. The gutting machine operator was compelled to handle unsorted fish.

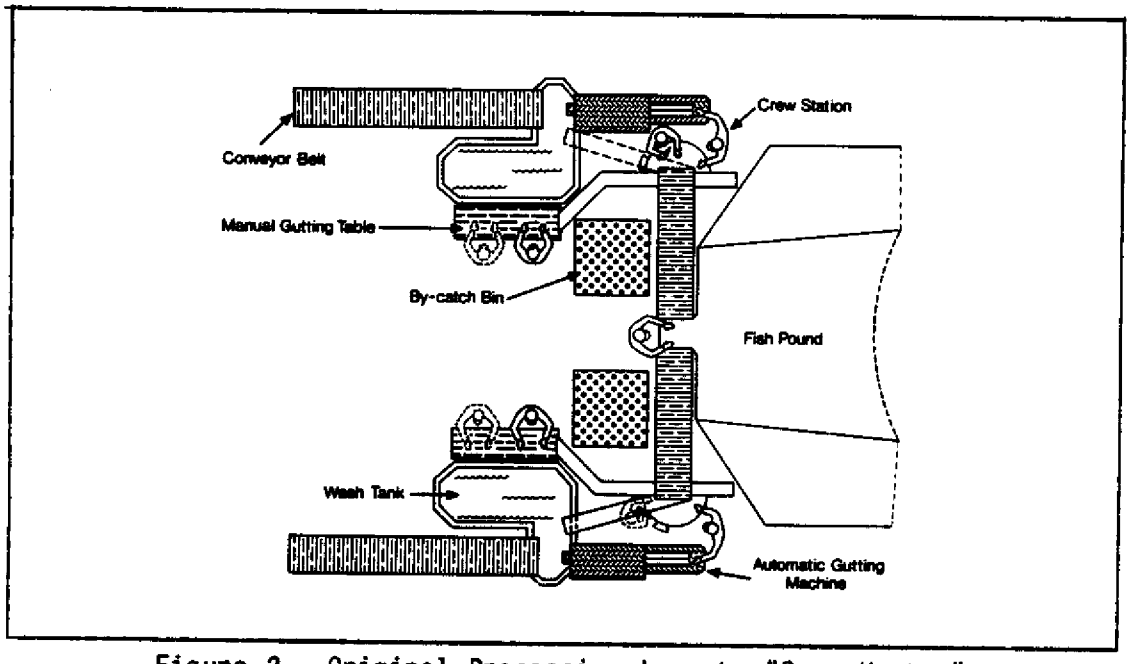


Figure 2. Original Processing Layout; "Cape Hunter".

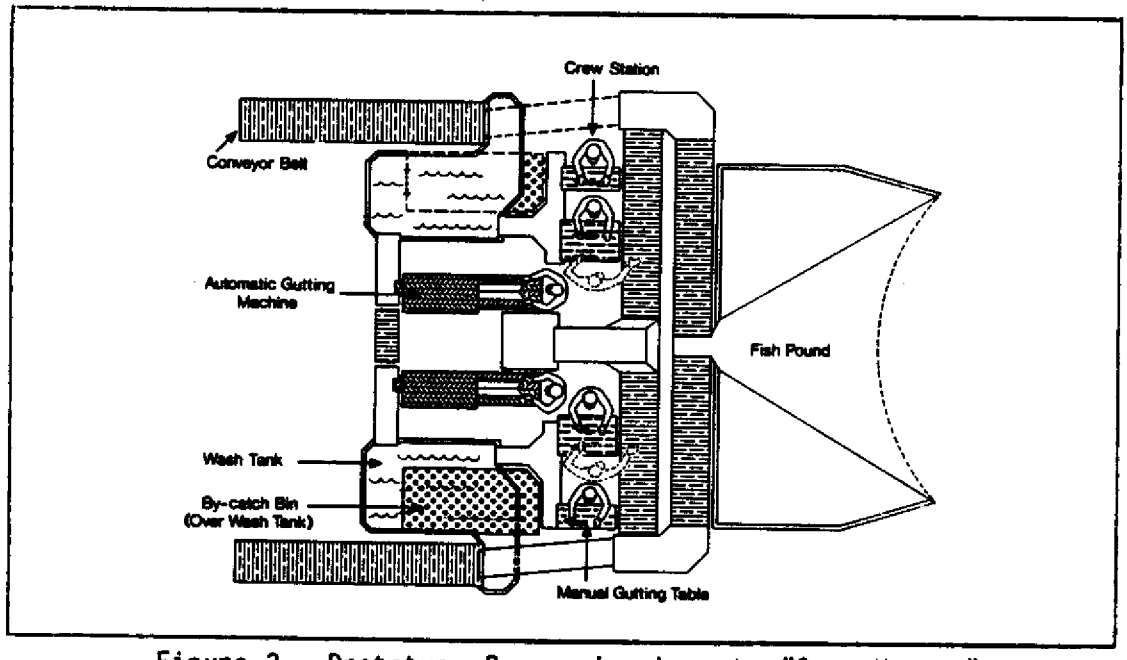


Figure 3. Prototype Processing Layout; "Cape Hunter".

3. The gutting machine operator was forced to stoop and twist in order to select fish.

4. Machine-gutted fish were often poorly washed as the fish were deposited into the washer at the washer discharge conveyor.

5. The receiving bin was poorly configured and inadequately sloped to empty itself and feed the incline conveyor to the processing area.

6. By-catch was repeatedly thrown and allowed to warm up while the primary species was processed.

7. Trash fish had to be moved by hand to the fish grinder for disposal.

8. The lighting was inadequate and harsh shadows were common in the work areas.

The following tables summarize the productivity performance of the "Cape Hunter's" processing deck operations in the "before" condition.

Gutting Rates	
Machine Gutting Rate	- 25.0 Fish/Man-Minute
Manual Gutting Rate (2-Step Method)	- 5.2 Fish/Man-Minute

Distribution of Processing Effort	
Manual Gutting (2-Step Method)	64.5%
Storage (Penned)	28.3%
Inspection	5.4%
Miscellaneous	1.1%
Machine Gutting	0.7%

It should be noted that the gutting machines were not utilized to any significant extent on this trip.

"CAPE HUNTER" - PROTOTYPE LAYOUT

The prototype layout is shown in Figure 3.

The modifications began, however, in the receiving bin. The bin was reconfigured and sloped at the expense of some loss in holding capacity to ensure that the incline feed conveyor would be self-feeding.

The Review Committee decided to adopt the 1-Step gutting method which had been observed to be more productive. The manual gutting stations were positioned upstream of the automatic gutting machines so that the manual gutters could remove all desirable by-catch and fish beyond the set operating range of the gutting machines.

The manual gutting boards were hinged on one end to facilitate crew accessibility and mounted on slides to accommodate either right or left-handed workers. Four workers could be comfortably accommodated using the four gutting boards which were adequately sized and arranged to accommodate up to eight workers on an intermittent basis.

The heights of the delivery/selection conveyor, gutting table and flume to the wash tank were convenient so that fish did not have to be picked up or otherwise lifted on a continuous basis. By-catch, when selected by a worker, was gutted and placed in a by-catch holding bin located beside the worker and above the wash tank. By-catch was held there until the main species had been processed, at which time the already gutted and bled by-catch could be dumped automatically into the wash tank for washing and delivery to the fish hold.

It has subsequently been recommended that a system of spray nozzles be installed over the by-catch bin in order to assist washing and cooling.

Fish which remained on the delivery/selection belt were conveyed to a common storage hopper located between the gutting machine operators. The operators were simply required to orient and slide the fish along the hopper edge into the gutting machine in a smooth, non-lifting motion.

Undesirable by-catch which accumulated in the hopper could then be dumped into a grinder by simply opening a sliding gate.

All gutted fish were flumed or dropped into a swirl tank washer which washed the gutted fish in large quantities of clean sea water before the rolling action of the boat deposited washed fish onto the incline conveyor leading to the fish hold. As the fish were lifted out of the wash tank, they were gently rinsed with clean sea water, prior to being delivered to the fish hold.

RESULTS AND DISCUSSION

After completion of the prototype fish handling system in July 1986, two trips were observed to evaluate the new system in terms of crew productivity. The following summarizes the performance of the Cape Hunter's processing deck operation in the "after" condition.

Gutting Rates	
Machine Gutting Rate	22.00 Fish/Machine-Minute
Manual Gutting Rate (1-Step Method)	6.75 Fish/Man-Minute

Distribution of Processing Effort	
Machine Gutting	42%
Inspection	28%
Storage (Penned)	27%
Manual Gutting	2%
Miscellaneous	1%

The following details the results of the observations.

1. The reconfigured receiving bin was able to accommodate the contents of the codends and reliably delivered fish to the incline conveyor supplying the processing area.

2. The 1-Step gutting method resulted in approximately a 30% improvement in productivity.

   This improvement in gutting rate was below the assumed figure of 8.8 fish/man-minute; however, the crew were still adjusting to the new system and had not fully learned to use the 1-Step method. Further improvements are anticipated as the crew becomes more familiar with the method.

3. The machine gutting rate decreased by approximately 12%, however, the utilization of the gutting machines increased by more than 41%.

4. The gutting machine did not perform well as it was necessary to inspect and clean machine-gutted fish. The inspection of machine-gutted product increased from 5% to approximately 28% of effort.

5. The overall system throughput increased by approximately 50%.

   This increase throughput was due primarily to the virtual replacement of hand gutting by the higher output gutting machines.

CONCLUSIONS

The new fish handling system has been in constant use since the conversion of the "Cape Hunter" was completed in August 1986. Crew comments have been very positive and National Sea Products Ltd. have converted four other vessels to similar systems.

Refinements including improved automatic gutting machines, washing equipment, inspection stations and the introduction of fish chilling equipment are presently under consideration.

Subjective assessment of improvements in the quality mix of fish landed by the "Cape Hunter" are very positive and estimated to be in the range of 5-10%. Dockside grading and plant utilization data are currently being analysed in an attempt to quantify the value of "Cape Hunter" landings versus conventionally equipped vessels.

REFERENCES

1. M.R. Desantis, R. Hoge, P.C. Fahie and D. Lemon. 1980. Dockside Grading Project - Canso Seafoods. Canadian Industry Report of Fisheries and Aquatic Sciences No. 148.

2. A. Orlic and Associates. 1985. On Board Fish Handling System for Offshore Wetfish Trawlers. Fisheries Development Branch Project Report No. 93.

3. A. Orlic and Associates. 1987. Time and Motion Analysis for On Board Fish Handling System - Cape Hunter. (Draft Report).

POLYPHOSPHATE DETERMINATION IN SHRIMP USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Melissa Sturno, Maurice R. Marshall,
and W. Steven Otwell
Food Science and Human Nutrition Department,
University of Florida, Gainesville, FL 32611

INTRODUCTION

Polyphosphates (POP) application in the seafood industry is expanding and includes both the fresh and processed market. Treatment reduces thaw-drip loss, results in improved texture and quality, and improves particle binding. POP are also used to soften underskins, thereby, facilitating peeling and increasing yields in the shellfish industry (Steinhauer, 1983).

Tripolyphosphate (TPP) is the predominant phosphate species used in the seafood industry, although hexametaphosphate may also be incorporated (Schwartz, 1987). Hydrolysis of POP, either enzymatically and/or nonenzymatically, readily occurs during fresh and frozen storage (Sutton, 1973; Reddy and Finne, 1986; Reddy and Finne, 1985; Tenhet et al., 1981b) forming orthophosphate (OP) which cannot be differentiated from the naturally occurring phosphate.

Existing procedures for POP determination in foods are not designed for quick and sensitive detection. Thin layer chromatography (TLC) is generally used for polyphosphate detection in both meat and seafood (Gibson and Murray, 1973; Tenhet et al.; 1981a; Reddy and Finne, 1986). Although high performance liquid chromatography (HPLC) has been used for POP detection in other chemical sectors, it has not been modified for application in the seafood industry. Fitchett and Woodruff (1983) developed a procedure for POP determination (of sequestering agents in detergents) involving an anion-exchange column and UV detection. An HPLC procedure which could simplify phosphate detection in seafood would have potential for widespread use in research and routine analysis by expanding their potential application, while discouraging abusive application. The main objectives of this study were to develop an HPLC, ion chromatography (IC) technique for the detection of orthophosphate, pyrophosphate and tripolyphosphate levels in shrimp.

MATERIALS AND METHODS

Double-distilled deionized water was used in all solution and extraction formulations. Reagent-grade chemicals were used unless otherwise stated. All equipment used was acid washed to remove any residual phosphate.

Shrimp samples

Fresh, headed, untreated, pink shrimp were received from Tampa, FL on various occasions. Samples were placed in quart-size plastic containers and stored at -20°C until treated. Prior to treatment, the desired sample size was retrieved from storage, placed in Whirl-Pak bags and thawed under running water. The shrimp were peeled, deveined and then treated. Shrimp were dipped in various concentrations (0%, 1.0%, 1.5%, or 2.0% w/v, TPP) for 6 min at room temperature. Commercial dip applications were 3% TPP for 6 min.

Standards

Commercial food-grade phosphates were donated by Food Machinery Corporation (FMC). Disodium hydrogen phosphate (OP) and tetrasodium pyrophosphate (PP) assayed at 100% purity and were employed directly in standards. Sodium tripolyphosphate (TPP) was purified using a water/ethanol recrystallization method (Greenfield and Cliff, 1975), which gave a reported assay of >99.8% and was then incorporated into standards.

Chromatographic Conditions

HPLC was performed using a Dionex Chromatographic System, 2010I following the procedure of Fitchett and Woodruff (1983) with a few modifications. The system was equipped with a Dionex Reagent Delivery Module with micromembrane reactor, a 50 ul injection loop, a Dionex UV/VIS detector and a Dionex strip chart recorder. A Dionex HPIC AS-7 anion-exchange column was the separator column, and HPIC AG-7 and HPIC NG-1 were guard columns. Eluent (flow rate: 0.05 ml/min) was 0.075 M HNO_3. Post column derivatization (PCD) with a ferric nitrate solution (1 g $Fe(NO_3)_3.9H_2O$/ L solution, with 2% (v/v) $HClO_4$, flow rate; 0.06 ml/min, Fisher Scientific). Detection was at 310 nm with the detector set at 0.02 absorbance units (AU), full scale.

Preparation of Shrimp Extract for HPLC Analysis

Sample extracts were prepared by homogenizing 2 g of shrimp with 150 mL of water for 30 seconds using a Polytron, speed setting 7, followed by a 15 min centrifugation at 10,000 x g at 4°C. The supernatant was then passed through a C-18 Sep-Pak (Waters) filter (acetonitrile activated). The sample was diluted 9:1 (sample:acid) with 0.75 M HNO_3 and injected twice through a 0.45 m filter (Gelman).

Phosphate Quantitation

Peak heights were measured and compared to standard curves using linear estimation to determine phosphate concentrations. The following equation was used to determine the phosphorus content of the shrimp:

$$\text{mg P/100 g shrimp} = (\text{mg species/L})(\text{mL } H_2O/\text{g shrimp})(\text{mole fraction P})(100).$$

Statistical Analysis

Statistical analysis was performed using SAS (1982) General Linear Model (GLM) procedure. Student's t test was used for comparison of means, unless otherwise noted.

RESULTS AND DISCUSSION

Chromatography Methodology

HPLC with conductivity detection was able to resolve standard aqueous solutions of OP, PP, and TPP. However, when standards were placed in shrimp extracts, no resolution occurred between OP and the solvent front. Since hydrolysis of polyphosphates ultimately produce OP, resolution of OP is essential. Various attempts to modify chromatographic conditions were unable to resolve OP from the solvent front.

An HPLC postcolumn derivatization procedure was developed for phosphate detection in shrimp. Nitric acid at 0.075 M HNO_3 was chosen as the eluent for determining polyphosphate residuals on shrimp (Figure 1). TPP was eluted in 11 min compared to 15 min when 0.065 M HNO_3 was used as eluent. The limitation of using the 0.075 M eluent was that PP was not baseline resolved from OP (Figure 1). A major advantage of using postcolumn reaction was that no interaction occurred between the shrimp extracts and the derivatizing reagent. Secondly, postcolumn derivatization

showed a good linear relationship with concentration for each species of polyphosphate (OP, PP, and TPP) (Figure 2), and limits of detection were as low as 10 ppm.

Recovery of Phosphate Species Added to Extract

Recovery of total P added was directly influenced by the added species of phosphate (Table 1). At combined high treatments of all three species and at all high TPP levels, total recovery of P added was approximately 94 - 96%. When TPP was low in the combination treatments, recoveries were reduced to 90% (Table 1).

Table 1. Total phosphorus recovered from combinations of polyphosphate species added to shrimp during extraction.

				% Recoveries[a]
Trt	OP	PP	TPP	Total P
1	L[b]	L	L	90
2	L	H	L	90
3	H	H	L	91
4	H	L	L	90
5	H	H	H	96
6	L	H	H	97
7	L	L	H	94
8	H	L	H	96

[a]Means of 4 observations
[b]Low (L) and High (H) levels: L-OP, H-PP, and L-TPP = 20 ppm; L-PP = 15 ppm; H-OP = 75 ppm; H-TPP = 150 ppm.

Comparison of HPLC Method to Spectrophotometric Method

The HPLC procedure was compared to the standard AOAC spectrophotometric method for quantifying total P. A significant difference ($p<0.05$) between both methods was observed; the spectrophotometric method assaying consistently higher P than HPLC (Table 2). The average P determined by the HPLC method was approximately 86% of what was determined by the spectrophotometric method. However,

the spectrophotometric method does involve an acid digestion step which would liberate organic phosphates. This step is not used for the HPLC procedure.

Table 2. Comparison of HPLC with AOAC spectrophotometric method for determining total P in shrimp.

	P content (mg/100 gm shrimp)[a]			
	HPLC		Spectrometric	
Control	101.8	3.6	117.9	6.8
Treated[b]	175.7	6.0	202.5	15.2

[a]Means s.d., n = 6.

Effect of Dip Concentration on Phosphate Uptake

The Duncan Multiple Range test was performed on the data to assess any significant differences between treatments. TPP treatment levels of 1.0%, 1.5% and 2.0% all resulted in significant (alpha = 0.05) differences in absolute P levels as compared to the untreated sample and to each other. The variation in levels resulted from the uptake of PP and TPP species (Figure 3) from the treatment solution. No TPP was detected in untreated shrimp (Figure 3). Baseline levels (intrinsic OP) in all treatments were similar. Upon storage, one could expect to observe an increase in the OP component of treated samples resulting from TPP hydrolysis.

Shrimp muscle uptake of TPP was observed to be concentration dependent, which agrees with the findings of Spinelli et al. (1968). TPP at the low level treatment (0.5% TPP) was detectable, the level of total P did not differ significantly from an untreated sample. This finding has been previously reported by Tenhet et al. (1981b). Assuming the TPP component has not yet hydrolyzed, HPLC would be able to identify a low treatment, where a less sensitive procedure may not.

Hydrolysis of TPP in Dipped Shrimp During Frozen Storage

Significant hydrolysis of TPP was observed over each 2-week analysis period ($P<0.05$) (Figure 4) which resulted in a concurrent increase in OP levels. PP seemed to increase slightly and then remain constant. However, variations in the detection of PP could have resulted due to poor resolution from OP, but combined standard curves suggest that the variation in PP levels detected are not significant. This suggests that the rate constant responsible for TPP hydrolysis may be of similar magnitude to the rate constant responsible for PP hydrolysis, which agrees with Sutton's (1973) report on the rate constants in cod muscle, but contradicts Reddy and Finne (1986).

The rate of TPP hydrolysis correlated well with the rate of OP and PP production as determined by comparing the slopes of the lines (see Figure 2). This reinforces previous reports that TPP hydrolysis results in PP and OP species levels, in combination or ultimately as OP, which are equal in total P content of the initial TPP species. After 8 weeks of storage, approximately 35% of the initial TPP present still remained.

Hydrolysis of TPP During Refrigerator Storage

The rate of TPP hydrolysis under refrigerated conditions was greatly increased, as compared to frozen storage. Following 3 days of refrigerated storage, no TPP remained, although an amount of PP was still present (Figure 5). This agrees with Sutton's (1973) observations that TPP was not detectable in cod muscle 30-40 hours after treatment. Complete hydrolysis to OP was observed on the fourth day of analysis after treatment. The rate of TPP disappearance was not significantly different than the rate of formation of OP and PP.

CONCLUSIONS

A faster procedure to identify potential phosphate treatment on shrimp has been developed using HPLC. The benefits of this procedure include isocratic elution, and short analysis time and quantitation. Furthermore, this procedure has the potential for application in other seafood products, such as scallops and fish fillets.

Recovery studies of added species seem to suggest a degree of interaction between them. The proposed procedure was successful in identifying phosphate treatment. It was also effective in monitoring the breakdown of TPP in shrimp muscle under refrigerated and frozen conditions. Under

refrigerated conditions, breakdown of TPP to OP was complete after 4 days of storage while frozen storage samples still had approximately 35% of the initial TPP after 8 weeks.

REFERENCES

Fitchett, A.W. and Woodruff, A. 1983. Determination of polyvalent anions by ion chromatography. Liq. Chromatog. 1(1).

Gibson, D.M. and Murray,C.K. 1973. Polyphosphates and fish: some chemical studies. J.Food Technol. 8:197-204.

Greenfield, S. and Clift, M. 1975. Preparation of pure samples. In "Analytical Chemistry of the Condensed Phosphates." p. 186. Pergamon Press, New York, NY.

Reddy, B.R. and Finne, G. 1985. A preliminary procedure for the detection of condensed phosphates in shrimp using thin-layer chromatography. Proceedings of the Tenth Annual Tropical and Subtropical Fisheries Conference of the America's 10th Year, New Orleans, FL. 149-156.

Reddy, B.R. and Finne, G. 1986. Hydrolytic and enzymatic breakdown of food grade condensed phosphates in white shrimp (Penaeus setiferus) held at different temperatures. Proceedings of the Eleventh Annual Tropical and Subtropical Fisheries Conference of the America's 11th Year, Tampa, FL. 201-212.

SAS. 1982. "SAS User's Guide: Statistics". SAS Institute, Inc., Cary, NC.

Schwartz, B. 1987. Personal contact. FMC Corporation. Philadelphia, PA.

Spinelli, J., Pelrog, G. and Miyauchi, D., 1968. Irradiation of Pacific Coast fish and shellfish. 6- Pretreatment with sodium tripolyphosphate. Fish. Ind. Res. 4,37.

Steinhauer, J.E. 1983. Food phosphates for use in the meat, poultry and seafood industry. Dairy and Food Sanitation. 3(7):244-247.

Sutton, A.H. 1973. The hydrolysis of sodium triphosphate in cod and beef muscle. J. Food Technol. 8:197-204.

Tenhet, V., Finne, G., Nickelson II, R., and Toloday, D. 1981a. Penetration of sodium tripolyphosphate into fresh and prefrozen peeled and deveined shrimp. J. Food Sci. 46:344-349.

Tenhet, V., Finne, G, Nickelson II, R., and Toloday, D. 1981b. Phosphorus levels in peeled and deveined shrimp treated with sodium tripolyphosphate. J. Food Sci. 46:350-352.

Enzymes of Commercial and Scientific Importance from Cold Water Fish Intestines

Robert D. Riley, Kelly M. Sledz, Jon B. Bjarnason, and Jay W. Fox*
University of Virginia School of Medicine
Microbiology Department
Charlottesville, VA 22908

*To whom correspondence should be sent

INTRODUCTION

Trypsin is a proteolytic enzyme found in the digestive tract of many organisms. This enzyme is a member of the serine proteinase class of proteolytic enzymes (Fersht, 1985). The active site of the serine proteinases has been shown to contain three critical residues for catalysis: serine 195, histidine 57, and aspartate 102. These residues comprise the catalytic triad of this proteinase class (Fersht 1985). Trypsin demonstrates a rather limited specificity for peptide bond hydrolysis. The primary sites of trypsin cleavage are carboxy to arginyl and lysyl residues

Enzymes generally have temperature and pH optima at which they are most active. The optimal temperature for enzymatic reactions in homothermic organisms is usually regulated via the maintenance of a constant body temperature. At this temperature, normal metabolic processes are possible. However, in the case of poikilothermic organisms (organisms which do not regulate their own body temperature) the body temperature is dependent upon the ambient environmental temperature. In poikilothermic organisms adaptation has occurred which allows for normal metabolic processes to take place at the temperature of the environment at which the organism inhabits. It has been demonstrated that poikilothermic organisms which inhabit cold environments often have enzymes which have greater catalytic efficiencies as compared to homologous enzymes isolated from homothermic organisms (Hochachko and Somero, 1987).

The Atlantic cod (*Gadus morhua*) is found off the coast of New England, primarily inhabiting waters where the temperature is approximately 4°C. Consequently, the enzymes of the cod must be capable of functioning reasonably well at lower temperatures. However, when considered in light of Arrhenius's law, one would expect a decrease in catalytic activity of the enzyme at lower temperatures. In this study we report on the temperature dependence of catalysis by cod trypsin as well as its temperature stability. This data is also compared to that found for the homologous enzyme, bovine trypsin. Finally, our data suggest that the higher activity of the cod trypsin at lower temperatures, as compared to the bovine enzyme, is related to a decrease in structural stability.

RESULTS AND DISCUSSION

Following a modification of the method of Simpson and Haard (1984b), we have isolated four trypsins from homogenized pyloric ceca of the Atlantic cod. The major modifications involved two additional chromatography steps following affinity chromatography on Benzamidine-Sepharose 6B. The first was anion exchange chromatography on a Mono-Q column (Pharmacia) and the second by hydrophobic interaction chromatography on a Spherogel-TSK phenyl-SPW column. Purity of the enzymes was determined by native and SDS PAGE (Pharmacia PhastGel electrophoresis). Only the major trypsin isolated by this procedure will be discussed.

The enzyme we isolated was classified as a trypsin on the basis of several experimental procedures. The enzyme bound to the p-aminobenzamidine Sephrose affinity column and demonstrated esterase activity on tosyl arginine methyl ester (TAME) (Worthington Enzymes, 1977) and amidase activity on benzoyl-DL-arginine p-nitroanilide (BAPA) (Erlanger *et al.*, 1961). These hydrolytic reactions were inhibited by PMSF and aprotinin. The molecular weight of the major trypsin was estimated by SDS PAGE to be approximately 24,000. By isoelectric focusing (Pharmacia PhastGel electrophoresis) the isoelectric point was estimated to be 6.5.

The amino acid composition of the protein was determined following hydrolysis of the protein in 6N HCl and derivatization of the resultant amino acids with phenylisothiocyanate (Table I). (Heinrikson and Meredith, 1983). The N-terminal amino acid sequence was determined on an Applied Biosystems 470A gas phase sequencer with an on-line 120 PTH amino acid analyzer (Table II) (Hunkapiller et al., 1983). A sequence homology search was performed using the FASTP program of Lipman and Pearson (1985). The results of the homology search are shown in Table III.

Figure 1 shows the relationship of the catalytic activity of bovine trypsin and cod trypsin to temperature. The temperature optimum for the bovine trypsin is approximately 5-10^{o}C higher than that of the cod trypsin. The cod trypsin appears to lose activity at a lower temperature than the bovine. However, at low temperatures, the cod trypsin is significantly more active than the bovine.

Table I. Comparison of Amino Acid Compositions of Cod Trypsins and Bovine Trypsin

Amino Acid	ACT[a]	GCT[b]	BT[c]
Ala	10	16	14
Arg	9	5	2
Asp	26	23	22
Cys	*ND	8	12
Glu	21	19	14
Gly	28	28	25
His	10	7	3
Ile	10	8	15
Leu	16	14	14
Lys	7	6	14
Met	3	3	2
Phe	3	4	3
Pro	8	10	9
Ser	27	32	33
Thr	11	10	10
Trp	ND	2	4
Tyr	15	7	10
Val	20	16	17
Residues per molecule	224	218	223

*ND is Not Determined. [a]Atlantic Cod Trypsin from present study. [b]Greenland Cod Trypsin from Simpson and Haard (1984). [c]Bovine Trypsin from Kiel (1971).

Table II. Comparison of Amino Terminal Sequences of Various Trypsins (ec3.421.4)

Bovine[a]	IVGGYTCGANTVPYQVSLNSGYHFCGGSLINSQWVVS
Atlantic Cod[b]	IVGGYECTRHSQAHQVSLNSGYHF
Pig[c]	IVGGYTCAANSIPYQVSLNSGSHFCGGSLINSQWVVS
Rat[d]	IVGGYTCPEHSVPYQVSLNSGYHFCGGSLIAPGWVVS
Spiny Dogfish[e]	IVGGYECPKHAAPWTVSLNVGYHFCGGSLIAPGWVVS
Crawfish[f]	IVGGTDAVLGEFPYQLSFQETFLGFSFHFCGASIYNE

[a]Titani *et al.* (1975). [b]Present study. [c]Smith and Liener (1967). [d]MacDonald *et al.* (1982). [e]Hermodson *et al.* (1971) and Titani *et al.* (1975). [f]Titani *et al.* (1983)

Table III. Comparison of Trypsin Sequences by FASTP[a]

Pig, trypsinogen:	82.5% identity in 228 amino acid overlap
Rat, trypsinogen II precursor:	74.7% identity in 229 amino acid overlap
Rat, trypsinogen I precursor:	73.4% identity in 229 amino acid overlap
Spiny dogfish, trypsinogen:	66.5% identity in 227 amino acid overlap
Crayfish, trypsin I:	41.5% identity in 236 amino acid overlap
Cod, *Gadus morhua*, NH_2-terminal:	70.3% identity in 37 amino acid overlap

[a]Sequences are compared to bovine trypsinogen (Lipman and Pearson, 1985)

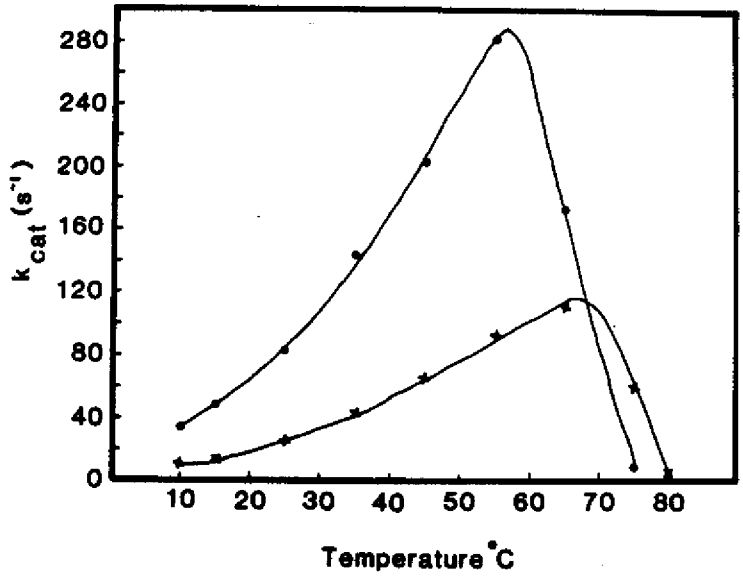


Figure 1. Turnover Number of Cod Trypsin (•) Compared to (x) Bovine Trypsin. The kcat was determined using TAME as the substrate while incubating the trypsins for 5 minutes at various temperatures, then reading spectrophotometrically their change in absorbance at 247 nm.

The temperature stability of the cod and bovine trypsins was examined by a set of experiments. The first experiment was designed to observe the rate of loss of esterolytic activity with time at a constant temperature (45°C). As can be observed in Figure 2, the loss of activity of the cod trypsin was faster than bovine trypsin. In the second experiment the trypsins were incubated at various temperatures for 30 minutes followed by esterolytic activity assay. Over the temperatures assayed the cod trypsin had lost approximately 50% of its activity at the 48°C incubation temperature whereas the bovine trypsin appeared to be stable over the temperature range tested (Figure 3). In the third experiment the melting points of the trypsin were assayed for by the change in the intrinsic fluorescence of the proteins. The enzymes were placed in thermostated quartz cells in the fluorometer and the temperature raised at a constant rate. As can be seen in Figure 4, the T_m for the cod trypsin is approximately 52°C and the T_m for the bovine trypsin is approximately 58°C.

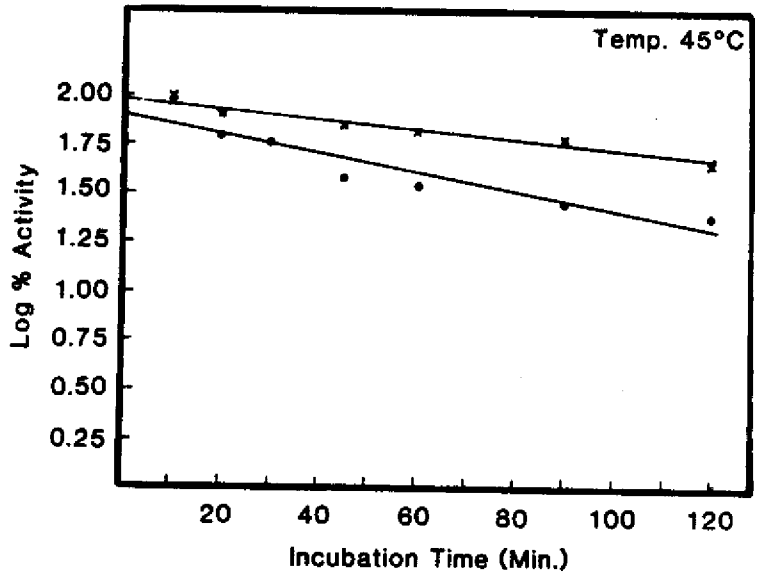


Figure 2. Comparison of the Stability Over Time of Cod (•) and Bovine Trypsin (x). The enzymes were incubated at 45°C for various times followed by assay of their rates of hydrolysis on TAME at 25°C. Change in absorbances were measured at 247 nm.

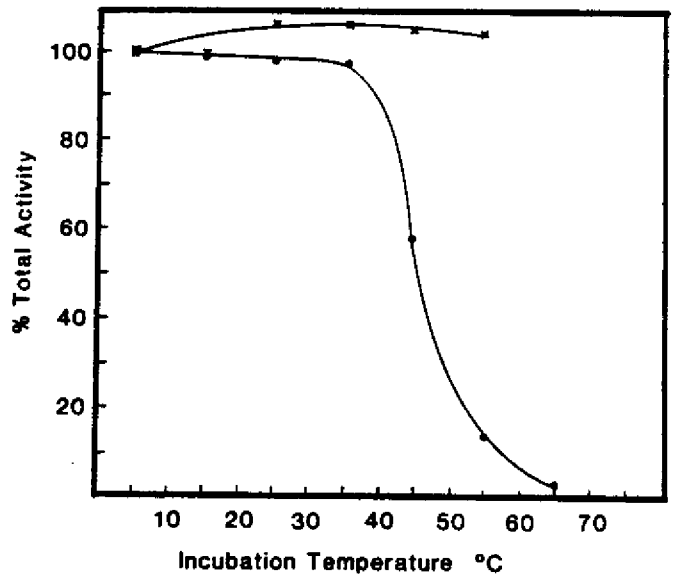


Figure 3. Comparison of the Thermal Stabilities of Cod (•) and Bovine Trypsin (X). The enzymes were incubated at various temperatures for 30 minutes and then assayed for their rate of hydrolysis of TAME at 25°C. Change in absorbances were measured at 247nm.

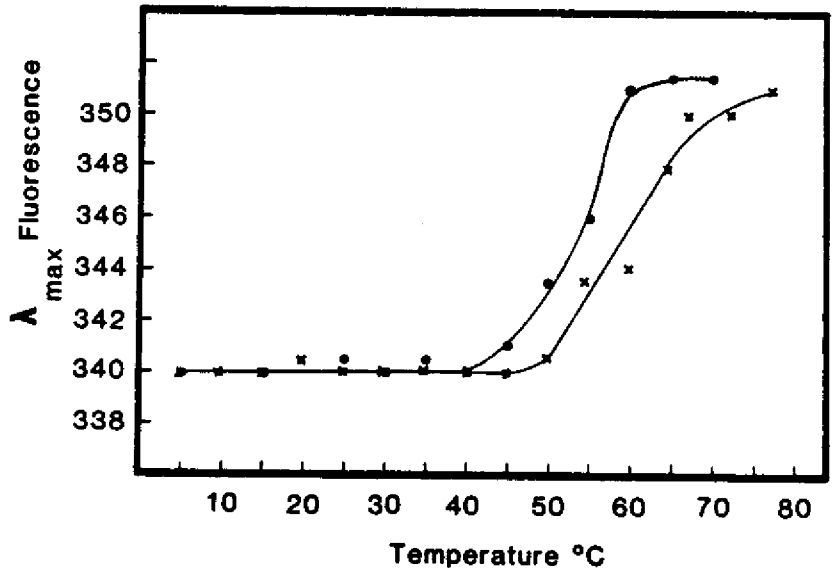


Figure 4. Thermal Denaturation of Cod (•) and Bovine Trypsins (x). The enzymes were subjected to increasing temperatures at a rate of 1°C/min. The temperature was measured in the cuvette. The excitation wavelength was 280nm and the change in the maximum emission wavelength was determined. The T_m of denaturaton for the cod trypsin is approximately 52°C while for bovine trypsin it is approximately 58°C.

All of the data we have presented in this paper demonstrate that the cod trypsin is thermally less stable than the homologous bovine enzyme. The interesting question is whether the decrease in thermal stability of the cod trypsin is related to its higher enzymatic activity at lower temperatures compared to the bovine enzyme. One possible explanation may rest in the fact that at lower temperatures most proteins become less flexible. Nevertheless, a certain amount of flexibility is often important in enzymatic reactions where the enzyme must bind substrate(s), carry out the catalysis, and then release the product(s). One can envision that all of these steps necessitate some structural flexibility on the part of the enzyme for optimal contacts to be made. At the lower temperatures, the optimal contacts may be restricted due to decreased structural flexibility. Therefore, enzymes which maintain a high degree of activity at lower temperatures may in fact be able to do so as a result of their overall increased flexibility. This increased relative flexibility is reflected in their lower thermal stability as is the case observed for the cod trypsin. Exactly what changes in the structure of the enzyme give rise to these catalytic properties are currently under investigation in our laboratory.

Finally, we wish to point out the commercial relevance of this research. Just as finding enzymes which maintain their activities at higher temperatures is an important endeavor in microbiology, biochemistry, and food science so is the search for enzymes which carry out certain reactions at lower temperatures; temperatures at which less thermal energy is necessary to put into the reaction system resulting in a more economical process. Also certain industrial enzymatic processes, due to lability of the reaction, would perhaps be best performed at reduced temperatures yet with a reasonable catalytic efficiency. Enzymes such as those isolated from cold adapted organisms may be useful in this role. Furthermore, the investigation of these enzymes will hopefully lead to the structural understanding of this cold activity and allow for the application of these structural rules to genetic engineering of other enzymes with altered temperature dependent catalytic efficiencies.

ACKNOWLEDGEMENTS

This work was supported by a grant to JWF from the Virginia Graduate Marine Science Consortium.

REFERENCES

Erlanger, B. F., N. Kokowsky, and W. Cohen. 1961. Archs. Biochem. Biophys. 95:271-278.

Fersht, A. 1985. Enzyme Structure and Mechanism. W. H. Freeman and Company, New York. pp. 17-22, 27-30.

Heinrikson, R. L., and S. C. Meredith. 1984. Analytical Biochemistry 136:65-74.

Hermodson, M. A., R. W. Tye, G. R. Reeck, H. Neurath, and K. A. Walsh. 1971. FEBS Lett. 14:222-224.

Hochachko, P. W., and G. N. Somero. 1984. Biochemical Adaptation. Princeton Univ. Press, Princeton, N.J. pp. 355-449.

Hunkapiller, M. W., R. M. Hewich, W. J. Dreyer, and L. E. Hood. 1983. Methods in Enzymology 91:399-413.

Kiel, B. 1971. The Enzymes. Vol. III, ed. P. D. Boyer, Academic Press, New York. pp. 249-275.

Lipman, D. J., and W. R. Pearson. 1985. Science 227:1435-1440.

MacDonald, R. J., S. J. Starym, and G. H. Swift. 1982. J. Biol. Chem. 257:9724-9732.

Simpson, B. K., and N. F. Haard. 1984a. Can. J. Biochem. Cell Biol. 62:894-900.

Simpson, B. K., and N. F. Haard. 1984b. Comp. Biochem. Physiol. 75B:613-622.

Smith, R. A., and I. W. Liener. 1967. J. Biol. Chem. B242:4033-4039.

Titani, K., L. H. Ericsson, R. G. Woodbury, and K. A. Walsh. 1975. Biochemistry 14:1358-1366.

Titani, K., T. Sasagawa, R. G. Woodbury, L. Ericson, H. Sorsam, M. Kraemer, H. Neurath, and R. Willing. 1983. Biochemistry 22:1459-1465.

Worthington Enzymes. 1977. ed. Decker, L. A. Worthington Biochemical Corporation, New Jersey. pp. 221-224.

BIOCHEMICAL FISH QUALITY EVALUATION WITH RECOMMENDED METHODS

P.J. Ke and B.G. Burns*
Department of Biochemistry (Food Science Program)
Memorial University of Newfoundland
St. John's, Newfoundland Canada A1B 3X9
(*Halifax Laboratory, Fisheries & Oceans Canada)

ABSTRACT

Biochemical methods for fish quality, evaluation have been reviewed and selected, and in most cases, tested for applicability to Atlantic species. The recommended procedures published in Canadian Technical Report of Fisheries and Aquatic Sciences (No. 1448, 1986) have been summarized and discussed. The results from both physical grading and biochemical tests have been compared for various quality operations in the field and laboratory. The biochemical fish quality assessment is specific, objective and reliable with the over-all error for no more than 16% by using the recommended methods. The guidelines of selecting biochemical quality parameters have been also discussed and recommended. The grading standards of various fish products can be determined correlated by applying the described procedures.

INTRODUCTION

Fish and seafood products are relatively unstable in fresh, frozen, and other forms of preparations. Quality changes occur readily for a variety of reasons. Accurate measurement of biochemical composition and freshness quality are important from both economic and processing efficiency aspects. In the laboratory, fish may be considered a biological-chemical entity. The meat is composed of about 70-80% water, 16-20% protein, 1-15% fat, and traces of inorganic salts and minerals. (Kreuzer, 1971). The first component, water, is the life-giving substance surrounding and suspending other molecules and acting as the medium in which biochemical reactions take place. The fat content of fish is relatively low and although fat oxidizes during storage to produce off-flavours. The lipids in fish may be considered as the body fluid for biochemical quality analysis, and becomes more important during frozen storage (Martin et al., 1982). The remaining component, therefore, which is responsible for the decrease of quality is protein.

Protein is an ordered array of very large molecules and predominantly of structural function. However, one protein class, the enzymes, is very active in quality indicating reactions. The delicate state of enzyme equilibrium and control maintained by body regulators fails when an organism dies and from that point enzymatic reactions proceed unhindered. Not only are these by-products foul smelling and putrid to the taste, but in fish some may produce undesirable texture denaturation and discolorations.

While living, all organisms have protective mechanism barring the entrance of bacteria. When life processes stop, bacteria quickly invade the body and secrete powerful enzymes to begin the digestion of tissues.

Therefore, at death, fish enter a state of uncontrolled enzymatic protein degradation from both natural and bacterial sources which results in the accumulation of peptides, amines, ammonia and other nitrogen containing compounds. While this chaotic state is undesirable for preservation, a study of the levels of quality indicating compounds makes it possible to develop various biochemical methods for the assessment of objective fish quality (Ke, et al., 1981 & 1984).

RECOMMENDED LABORATORY METHOD

To perform biochemical assessments accurately and with confidence, and to be able to compare results between testing facilities, it is necessary that standard procedures be used. This recommendations of twenty-one laboratory methods is an attempt to compile such a standard. The procedures have been drawn from current literature and experience and have been tested by Halifax Laboratory, Fisheries and Oceans Canada for their validity or appropriateness. Results of such testing were considered in the outlining of methodology.

The procedures have been divided into sections of proximate composition, quality indices of refrigerated or fresh storage, quality indices of frozen storage (lipid and non-lipid related) and physical attributes. Each method is followed by applicable references where additional information may be obtained regarding any aspect of the procedure, its application or interpretation. A brief of above selected methods have been listed in Table 1. The complete text of these laboratory methods for assessment of fish quality has been published by Fisheries & Oceans Canada (Woyewoda & Ke, 1986).

Table 1. Selected biochemical method for assessment of fish quality*

Method	Description
Trimethylamine (TMA)	Extract./photometry (Dyer, 1959; Regenstein, 1982)
Total Volatile base (TVB-1)	Direct with MgO (Botta, 1982 & 1984)
" " " (TVB-2)	$MgSO_4$ Distil. (Montgomery, 1960)
Hyposanthine (HX-1)	Enzymatic/photometry (Spinlli, 1964; Burt, 1977)
" (HX-2)	HPLC (Burns, 1985)
Thiobarbituric Acid Reactive substrates (TBARS)	Distil./photometry (Ke, 1984)
Peroxide Value (POV)	Extraction/titration (AOCS, 1971)
Carbonyl Value (CV)	photometry (Waternabe, 1974)
Free Fatty Acid (FFA)	MGP-titration (Ke, 1978)

Extractable Protein Nitrogen (EPN)	Extraction/photometry (Dingle, 1967)
Dimethyl Amine (DMA)	photometry (Costell, 1974; Dingle, 1977)
Formaldehyde (FA)	photometry (Costell, 1973; Connell, 1975)
G/P Value	HPLC (Burns, 1985)

*Summarized from "Recommended Laboratory Methods for Assessment of Fish Quality by Woyewoda et al., 1986. Can. Tech. Report Fish. Aquatic Sci. No. 1448.

For the proximate composition which is also important for various objective quality assessment of fish, eight procedures such as the contents of bone, salt, ash and fats, and the measurements of proteins, moisture and pH, have been also specified and recommended with those quality indices in the same publication (Woyewodn & Ke, 1986).

RESULTS AND DISCUSSION

In the national quality enhancement program from 1981, various grading operation at the selling points by the recommended physical and sensory evaluation for fresh fish have been conducted Some of these sample have been collected and analyzed in our laboratories by various biochemical methods. The selected results have been summarized in Table 2.

Table 2. Comparison of the Selected data of grading data of the data of the fresh groundfish

Grade	Relative deviation (%)	
	Physical (Sensory) method	Biochemical method
A (Excellent)	16	9
A/B	28	12
B (Acceptable)	14	10
B/R	13	11
R (Rejected)	15	13

The three grades, A (excellent), B (acceptable) and R (rejected) were used for this evaluation for iced fish at the dockside and the selling points. The reliability of biochemical examination for Grade A fish and on the determination between grade A or B, were better than the physical grading results. When the quality changes further, it seems that both physical grading and laboratory analysis can given about same results with relative deviations of about 15%.

For various fish products, a comparison of overall errors between physical and biochemical quality assessments have been listed in Table 3. Some of these data were calculated from the limited experiments. Thus these results can just give you some information and comprehensive work should be made to develop the better guideline and standards for various fish products.

Table 3. Comparison of the overall error between physical and biochemical quality grading.

Products	Overall Error (%)	
	Physical Grading	Biochemical evaluation
Frozen Cod Fillet	15	8
Frozen Herring Block	21	13
Fresh scallop	13	11
Frozen crab meat	16	9
Frozen squid	20	6
Fresh sea cucumber	>30	12

Scientific Quality Assessment

Various quality parameters which are sensitive and reproducible, and their changes as a function of quality deterioration and keeping time, can be selected and applied to fish quality evaluation. Comprehensive reviews on quality indicators have been initiated in our laboratory. The most useful quality parameters are total volative bases, total volatile acids, extractable proteins, hypoxathine, trimethylamine, dimethylamine, thiobarbituric acid-reactive substances, free fatty acid, peroxide, carbonyl, pH, dielectric behaviour, polyene index and texture index, etc.

In order to develop standards for fish quality grading, the following rules must be considered when selecting quality parameters for the laboratory evaluation:

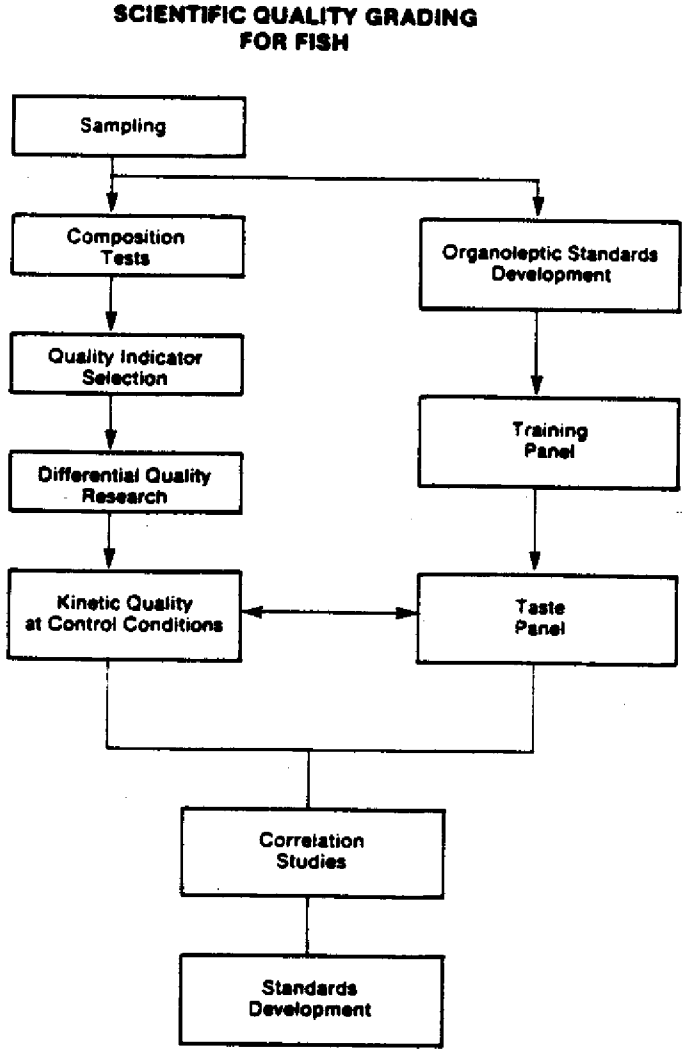
(1) Two or more of indicators from the recommended quality indicating reactions should be selected.
(2) Two of the three types of quality changes; bacterial, enzymatic and

chemical reactions must be covered from above indicators selection.

(3) The major reacting components are proteins, fats, waters, and indicating compounds. At least two of these components must be related by above selected two or more quality reactions.

(4) The parameter of a group of indicating compounds such as TVB, FFA would give better reliable results than from single compound.

Figure 1



A brief procedure for selecting quality indicators and developing the grading standards has been shown in Figure 1. The selected examples for biochemical evaluation of fish quality have been listed in Table 4 for your information and comparison.

Table 4. Selected quality indicators for some fish quality assessment.

Fish	Selected Quality Parameters
Fresh Cod	P & G value/FFA/TVB
Frozen Cod fillet	TBARS/EPN/TVB/TMA
Fresh mackeral	FFA/TVB/TBARS
Frozen mackeral	TBARS/FFA/HX
Fresh crab meat	FFA/TMA/EPN
Frozen squid	TVB/FFA/TBARS
Fresh sea cucumber	TVB/TMA

In addition to the grading operation, it can be concluded that physical grading can be employed satisfactorily for fresh groundfish and shellfish with less than 15% error on an accept/reject grade and 28% on quality differential evaluation. The Biochemical quality examinatons have demonstrated much better sensitivity and reproducibility (Table 2 and 3), with the overall error no more than 16%. Therefore, biochemical fish quality evaluation could be satisfactorily employed for developing quality guideline of fish for various field grading operations. Those methods would also provide a useful objective fish quality evaluation to meet the requirements from the fish inspection and industry.

REFERENCES

Kreuzer, R. 1971. Fish inspection and quality control. Fishing News Books Ltd., London, U.K.

Martin, R.E., G.J. Flick, D.R. Ward 1982. Chemistry and Biochemistry of marine products. Avi Publishing, Westport, Connecticut.

Connell, J.J. 1980. Advances in fish Sciences and Technology Fishing News Books Ltd., London, U.K.

Ke, P.J., B.G. Burns, and A.D. Woyewoda 1984. Recommended procedures and Guideline for quality evalutation of Atlantic Short-Fined Squid. Internal. J. Food Sci. Technol. 17:276-281.

Ke, P.J., B. Smith-Lall and A.B. Dewar 1981. Quality improvement investigation for Atlantic Ocean crab. Can. Tech. Report Fish. Aquatic Sci. No. 1002.

Woyewoda, A.D., S.J. Shaw, P.J. Ke and B.G. Burns 1986. Recommended Laboratory methods for assessment of Fish Quality. Can. Tech. Report Fish. Aquatic Sci. No. 1448. (143 pages).

DETERMINATION OF MINIMAL TEMPERATURES FOR HISTAMINE PRODUCTION BY FIVE BACTERIA

C-M. Chen, M.R. Marshall, J.A. Koburger, W.S. Otwell and C.I. Wei. Department of Food Science and Human Nutrition, University of Florida, Gainesville, FL 32611

Introduction

Scombroid poisoning, one of the most common foodborne diseases in the U.S. (Taylor, 1986), is caused by the ingestion of spoiled scombroid fish which contain unusually high levels of histamine. The so-called scombroid fish, such as tuna, mackerel, and skipjack are frequently involved because they possess large amounts of free histidine in their muscle (Lukton and Olcott, 1958). By enzyme action, histidine is converted to histamine by microbial histidine decarboxylase. The Food and Drug Administration has established a defect action level for histamine, 50 mg per 100 g canned tuna (Federal Register, 1982).

The bacteria that possess large amounts of histidine decarboxylase mainly belong to the family Enterobacteriaceae. Other bacteria, such as Vibrio, Clostridium and Lactobacillus have also been reported to possess this enzyme (Arnold and Brown, 1978; Arnold et al, 1980; Taylor, 1986). However, Proteus morganii, Klebsiella pneumoniae and Hafnia alvei are the only histamine-producing bacteria that have actually been isolated from incriminated fish involved in scombroid outbreaks.

During fish processing, bacterial histamine formation is controlled primarily by low temperatures. In the retail market, tuna fish are usually covered with ice or simply placed on ice to prevent spoilage. However, storage under these conditions for long periods of time can result in microbial growth and conversion of histidine to histamine. A better understanding of the lower temperature limit for bacterial growth and histamine production is therefore important to control fish quality and safety. This study was undertaken in tuna fish infusion broth (TFIB) to determine the low temperature limits of the five most implicated histamine-producing bacterial strains.

Materials and Methods

Bacteria

Five strains of histamine-producing bacteria, *Klebsiella pneumoniae* 111-5 and T_2, *Proteus morganii* 110SC-2 and JM, and *Hafnia alvei* T_8, provided by Dr. S. L. Taylor (Department of Food Microbiology and Toxicology, University of Wisconsin at Madison) were used. The cultures were maintained on trypticase soy-histidine (2%) agar slants and stored at refrigeration temperatures.

Media and growth conditions

Trypticase soy broth-histidine medium (TSBH) was used as the preinoculum broth. It contains per liter: 30 g trypticase soy broth, 1 g histidine monohydrochloride, and 5 mg pyridoxal hydrochloride. The final pH of TSBH was adjusted to 6.8 before sterilizing for 15 min at 121 °C.

Tuna fish infusion broth (TFIB) was prepared from fresh tuna muscle by homogenization with 2 volumes of distilled water (wt./vol.), steaming at 100 °C for 1 hr, filtration, supplementation with 1% glucose, and sterilization at 121 °C for 15 min (Taylor et al., 1979). The final pH was 5.7.

Before assaying, the bacteria were activated in TSBH and incubating at 28 °C for 24 hr. Aliquots of 0.2 ml were transferred into another fresh preinoculum broth and incubated for an additional 18 hr.

A 0.2 ml aliquot of the activated bacterial culture was inoculated into 70 ml of TFIB in duplicate Erlenmeyer flasks and incubated at different temperatures (3°, 5°, 7°, 10°, 15° and 25 °C) in a constant temperature refrigerated water bath shaker with a shaking speed of 100 rpm. At various time intervals (0, 24, 48, 120, and 240 hr), aliquots were removed from each sample for aerobic plate count (APC) and histamine analysis. A blank TFIB was used as a control.

APC was conducted using a spread plate method (Speck, 1984) of serial dilutions of the bacterial cultures in 0.9% saline. Plates were spread on aerobic plate count agar and incubated at 28 °C for 24 hr. Duplicate samples and plate counts were used throughout.

Histamine analysis

Quantification of histamine was accomplished by a modified ion-moderated partition high performance liquid chromatography method developed by Gill and Thompson (1984). A hp 1090 liquid chromatograph equipped with hp 85B personal computer, hp 9121 multichannel integrator and hp 7470A plotter were used. The chromatographic column was a custom-packed IMP type HPX-72-O (OH-form) with a column dimension of 300 X 7.8 mm. A guard column packed with the same material was also used. The chromatographic conditions were: flow rate, 0.8 or 1.0 ml per min; detector wavelengh, 210 nm; peak width, 0.5 min; chartspeed, 0.2 min; and injection volume, 20 µl. The mobile phase, 0.01 N NaOH (pH 11.5), was prepared fresh daily using HPLC grade water and further filtered through a 0.45 µm millipore filter.

Histamine standard solutions were prepared by dissolving 165.6 mg histamine dihydrochloride in 100 ml of 6% perchloric acid (PCA)-30% KOH solution (pH 7) to obtain an equivalent 100 mg% histamine stock solution. Histamine working standards of 0.1, 0.2, 0.4, 0.8, and 1.0 mg% were prepared from the stock standard. For HPLC analysis, an injection volume of 20 µl was used.

Five-ml aliquots of TFIB from the duplicate culture flasks at each incubation period were mixed with 10 ml of 6% PCA solution and homogenized with a polytron homogenizer at setting 5 for 1 min. The homogenate was centrifuged and the supernantant filtered with a Whatman # 1 filter paper. The filtrate was then brought to 10 or 25 ml with a 6% PCA solution followed by pH adjustment to 7.0 with a 30% KOH solution. Potassium perchlorate precipitate was removed by filtration and the final extracts were filtered again with 0.45 µm filter paper before subjecting to HPLC analysis. The concentrations of histamine in TFIB were calculated according to the formula:

$$C_2 = \frac{C_1 \times V_1/V_2 \times 10^6 \times 1/0.93}{MW}$$

where C_1= concentration of histamine derived from chromatographed standard curve (mg/ml); C_2= final concentration of histamine (nmoles/ml); V_1= the volume of the first filtrate (10 or 25 ml); V_2= total TFIB volume removed at each time interval; 0.93= the extraction recovery rate; MW= molecular weight of histamine (111.55). Each histamine sample was analyzed three times by HPLC.

Results and Discussion

Growth and histamine production of the five test strains at different temperatures are shown in Figures 1-5. K. pneumoniae 111-5 failed to grow and produce histamine in TFIB at 3 °C even after 10 days (Fig. 1). Although bacterial growth occurred at 5°, 7° and 10 °C, no histamine was produced. When the temperature was raised to 15° or 25 °C, this bacterium showed a normal growth pattern with the logarithmic phase occurring in the first 48 hr and the stationary phase thereafter. Large quantities of histamine, about 340 nmoles of histamine/ml, were produced within 24 hr at 25 °C. Thus, the minimal histamine production temperature for this bacterium was 15 °C.

Similar patterns of bacterial growth and histamine production occurred for K. pneumoniae T_2 (Fig. 2) However, this organism produced histamine at 7° and 10 °C after 5 days. Again, at elevated temperatures, histamine production was greatly increased. As incubation proceeded, the rate of production decreased.

Although K. pneumoniae 111-5 and T_2 showed similar growth patterns at low temperatures, their ability to produce histamine in TFIB is strain specific. For strain T_2, the lowest temperature for histamine production was 7 °C, while it is 15 °C for strain 111-5. Behling and Taylor (1982) found that K. pneumoniae T_2 was able to produce histamine at 7 °C after a 72 hr incubation period and suggested the lower temperature limit for histamine production to be 7 °C.

More histamine was produced by strain 111-5 than strain T_2 at 25 °C by day 5. Therefore, the histamine-producing system of K. pneumoniae T_2 appeared to be more tolerant to low temperatures, whereas K. pneumoniae 111-5 favored room temperature conditions. K. pneumoniae T_2 seems to possess a broader temperature range than strain 111-5 for histamine production.

Growth of P. morganii 110SC-2 was inhibited at 3° and 5 °C (Fig. 3). Although bacterial growth was apparent, no histamine was produced at 10 °C. The minimal temperature for strain 110SC-2 to produce histamine was 15 °C. Small amounts of histamine (38.8 nmoles/ml) were produced after 48 hr. Behling and Taylor (1982) also showed that 15 °C was

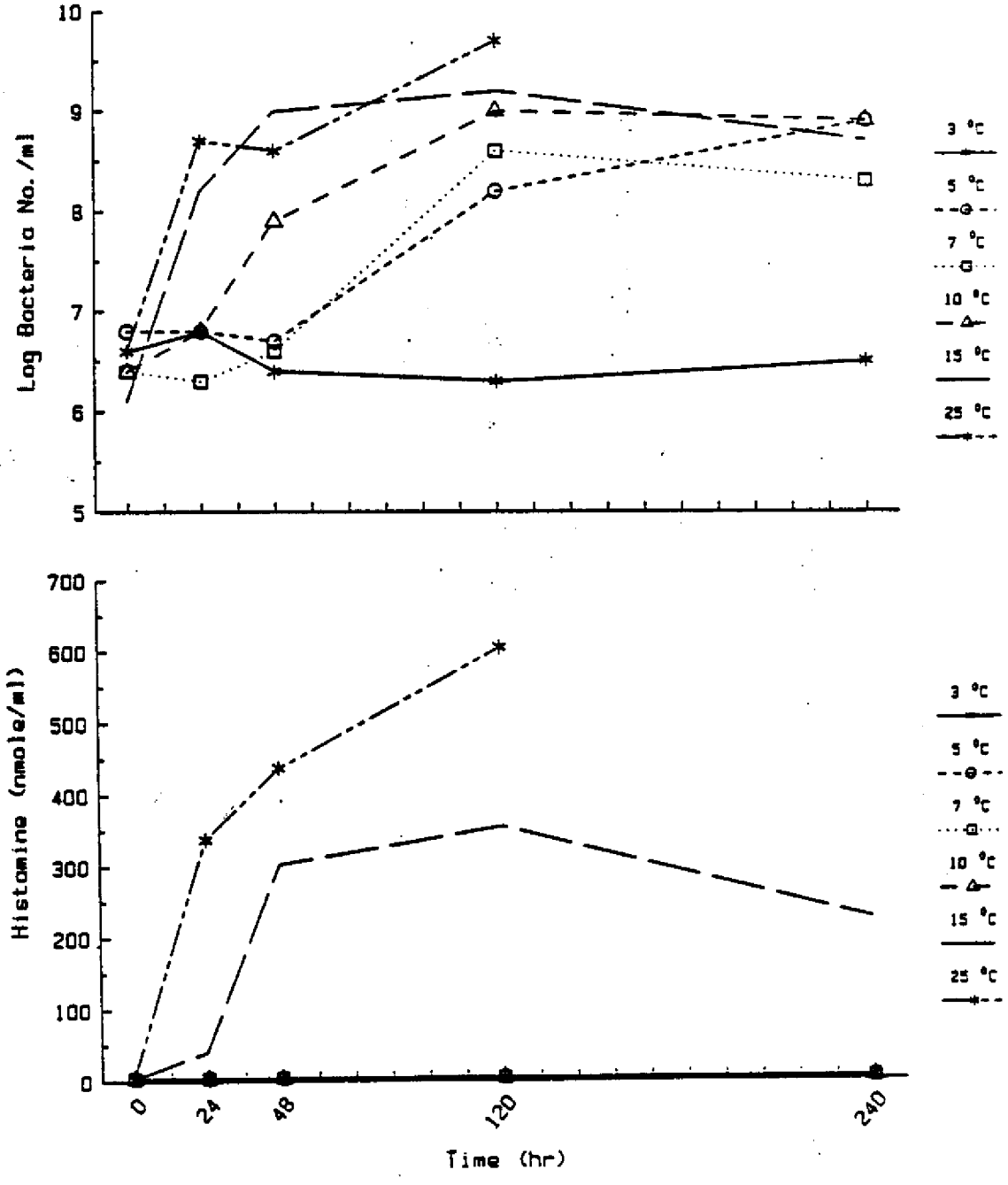


Figure 1. Time and temperature related growth and histamine production of Klebsiella pneumoniae 111-5 in tuna fish infusion broth.

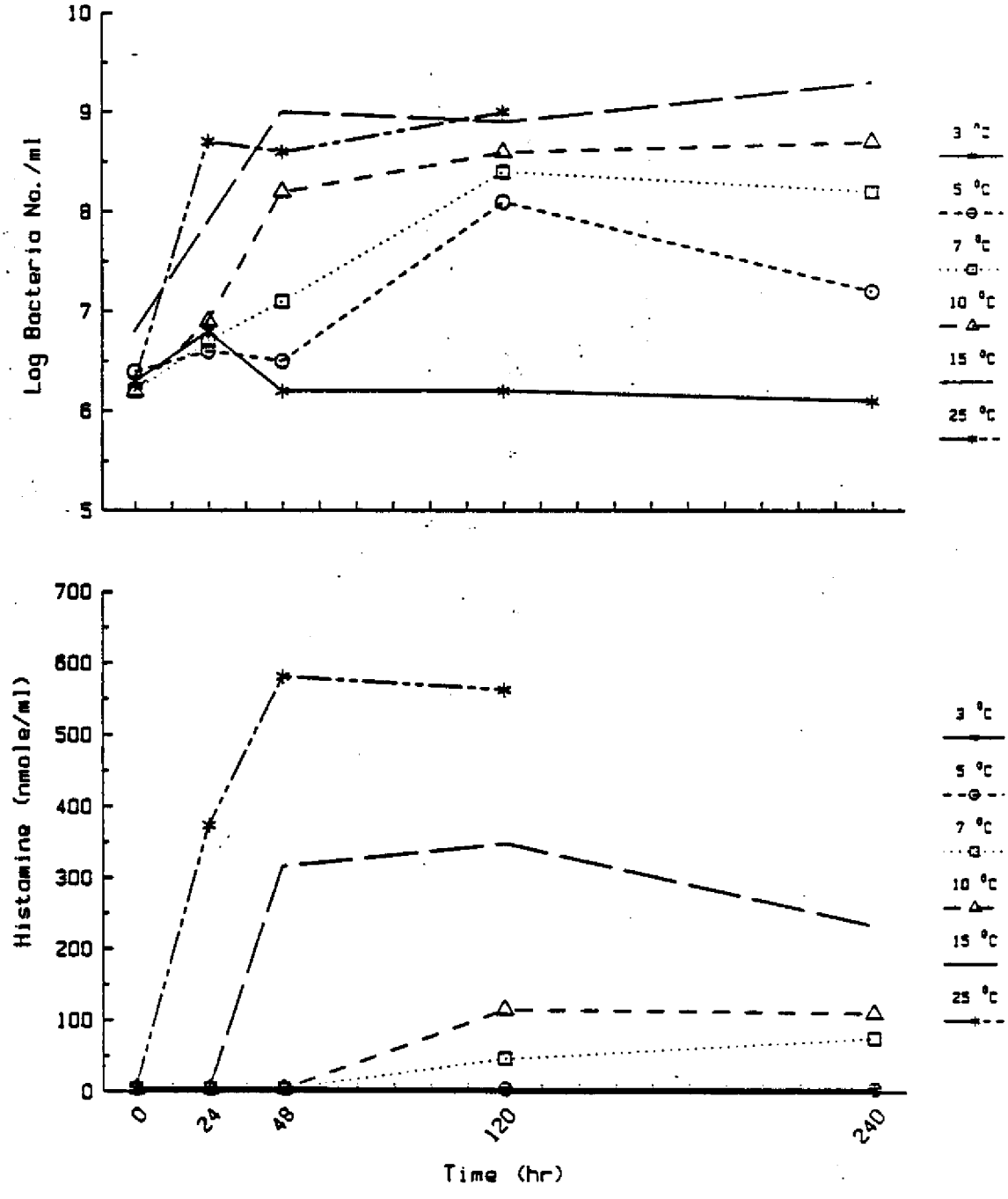


Figure 2. Time and temperature related growth and histamine production of Klebsiella pneumoniae T_2 in tuna fish infusion broth.

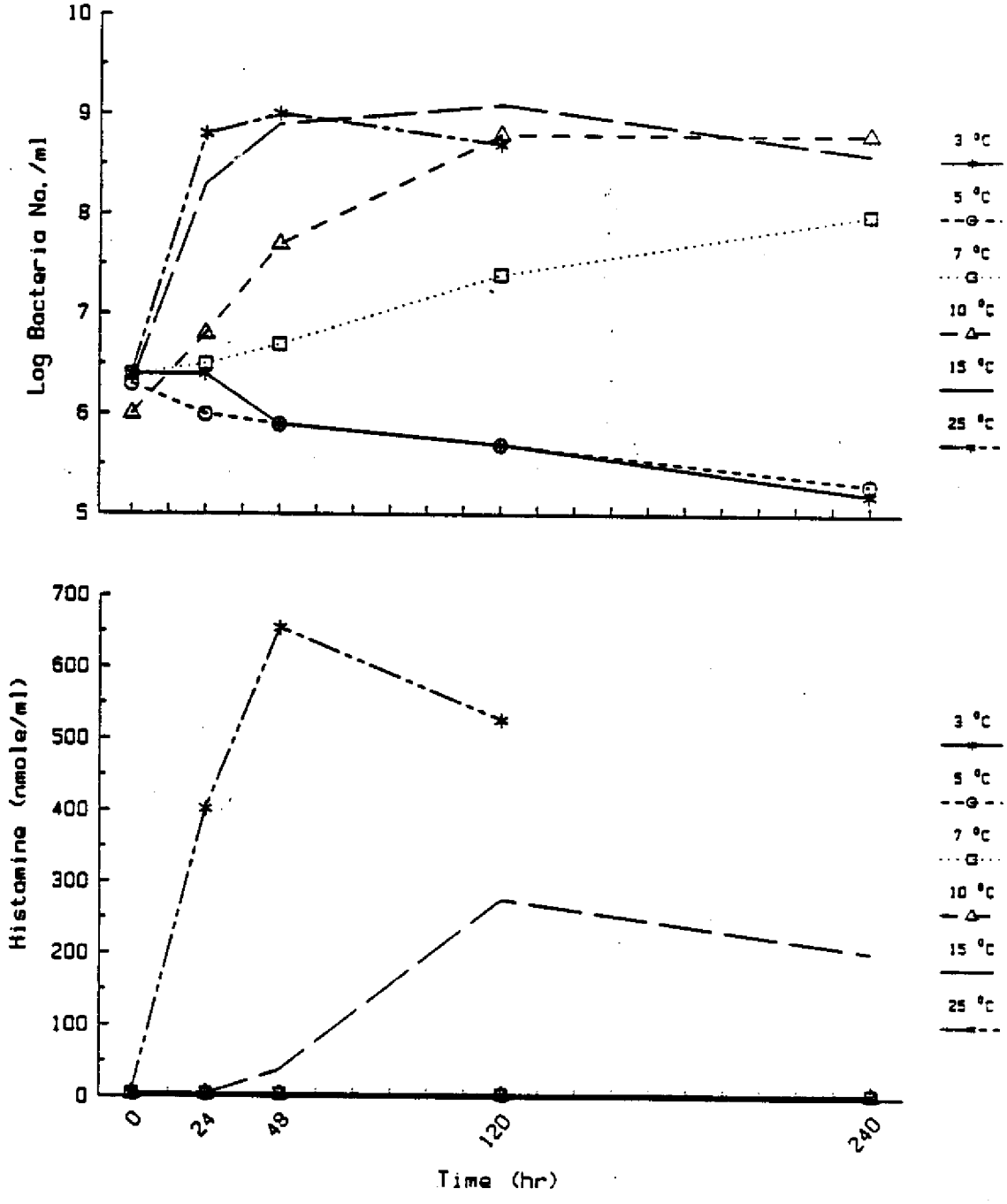


Figure 3. Time and temperature related growth and histamine production of Proteus morganii 110SC-2 in tuna fish infusion broth.

the lower temperature limit for this organism to produce substantial histamine levels (> 2.5 umoles/ml). In the present study, P. morganii 110SC-2 showed the highest histamine-producing capability among the five cultures tested at 25 °C. A level of 655 nmoles/ml was detected at 48 hr; it declined to 527 nmoles/ml after 5 days.

No bacterial growth and histamine production was observed for P. morganii JM incubated at 3 °C throughout the 10 days (Fig. 4). At 5 °C, only limited growth occurred after 10 days, however, this bacterium started to grow after 48 hr at 7 °C. At 7 °C, histamine production was 85 nmoles/ml after 5 days. Behling and Taylor (1982) reported that P. morganii JM and 110SC-2 had a lower temperature limit for histamine production of 15 °C. However, Arnold et al. (1980) reported P. morganii JM was able to produce histamine at 7 °C after 4 days. Thus, our results support the latter report, although a larger bacterial inoculum (10^7/ml) was used in their study. At 15° and 25 °C, histamine production increased until day 5 and 2, respectively, and then levels decreased.

The psychrotrophic bacterium, H. alvei T_8, was the only organism able to multiply at 3 °C (Fig. 5). Histamine production did not occur until the growth temperature was higher than 15 °C, although bacterial growth was readily apparent at temperatures below 5 °C. At 15 °C, only a negligible amount of histamine (8.1 nmoles/ml) was detected after 10 days. Higher histamine levels were produced at 20 °C while at 25 °C, about 300-400 nmoles/ml of histamine were produced in 5 days.

Histamine production by H. alvei T_8 was less and slower when compared to the other four bacterial strains. Behling and Taylor (1982) reported the lower temperature limit for production of histamine by H. alvei was 30 °C. Arnold et al. (1980) found that H. alvei was able to produce histamine at 19 °C under aerobic, microaerophilic or anaerobic conditions after 3 days.

Histamine-producing organisms are believed to produce histamine during the stationary or death phase of growth (Behling and Taylor, 1982). Our results with K. pneumoniae T_2 and 111-5 as well as P. morganii JM and 110SC-2 at temperatures above 15 °C do not support this hypothesis. Enhanced histamine production occurred during the rapid

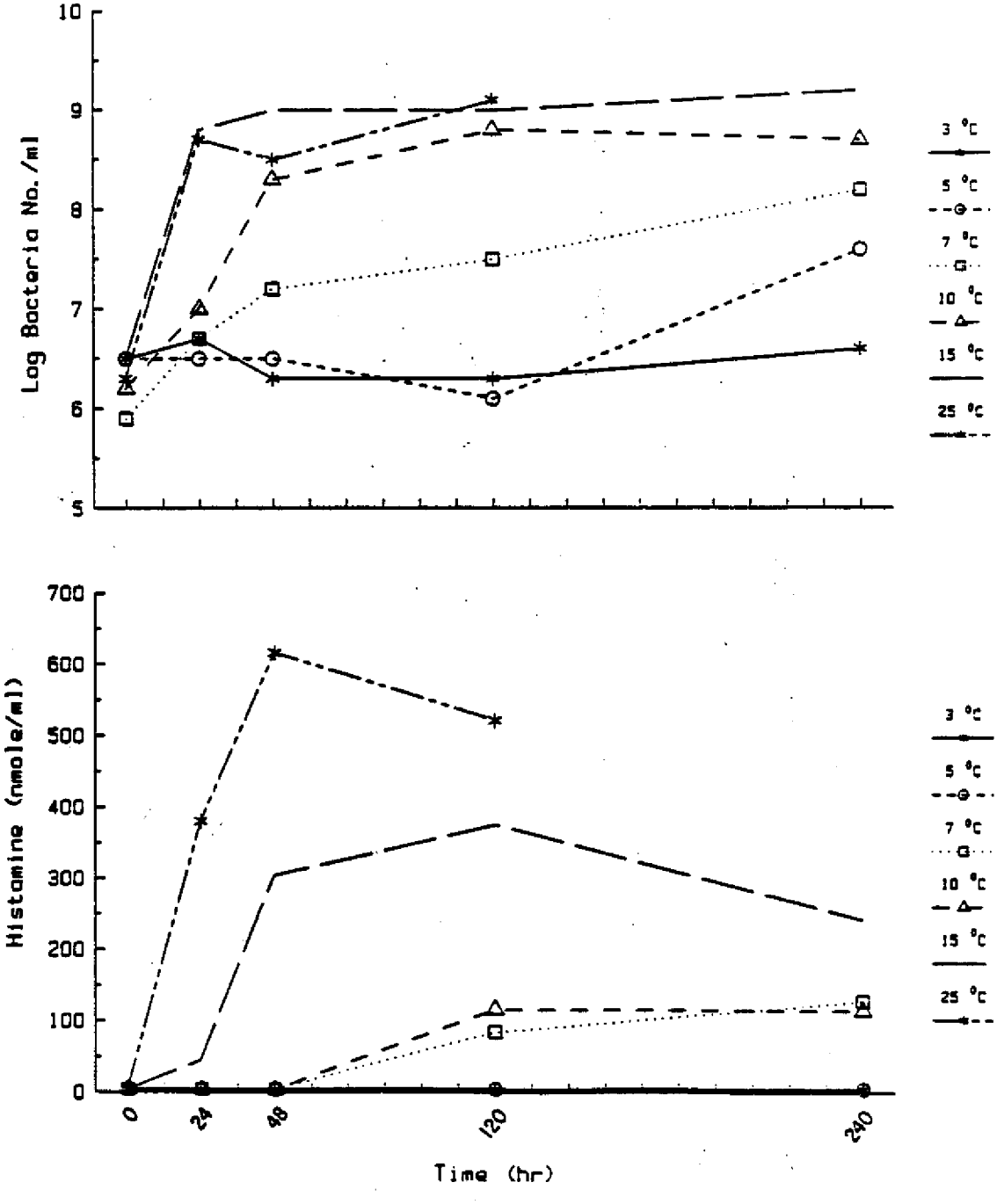


Figure 4. Time and temperature related growth and histamine production of Proteus morganii JM in tuna fish infusion broth.

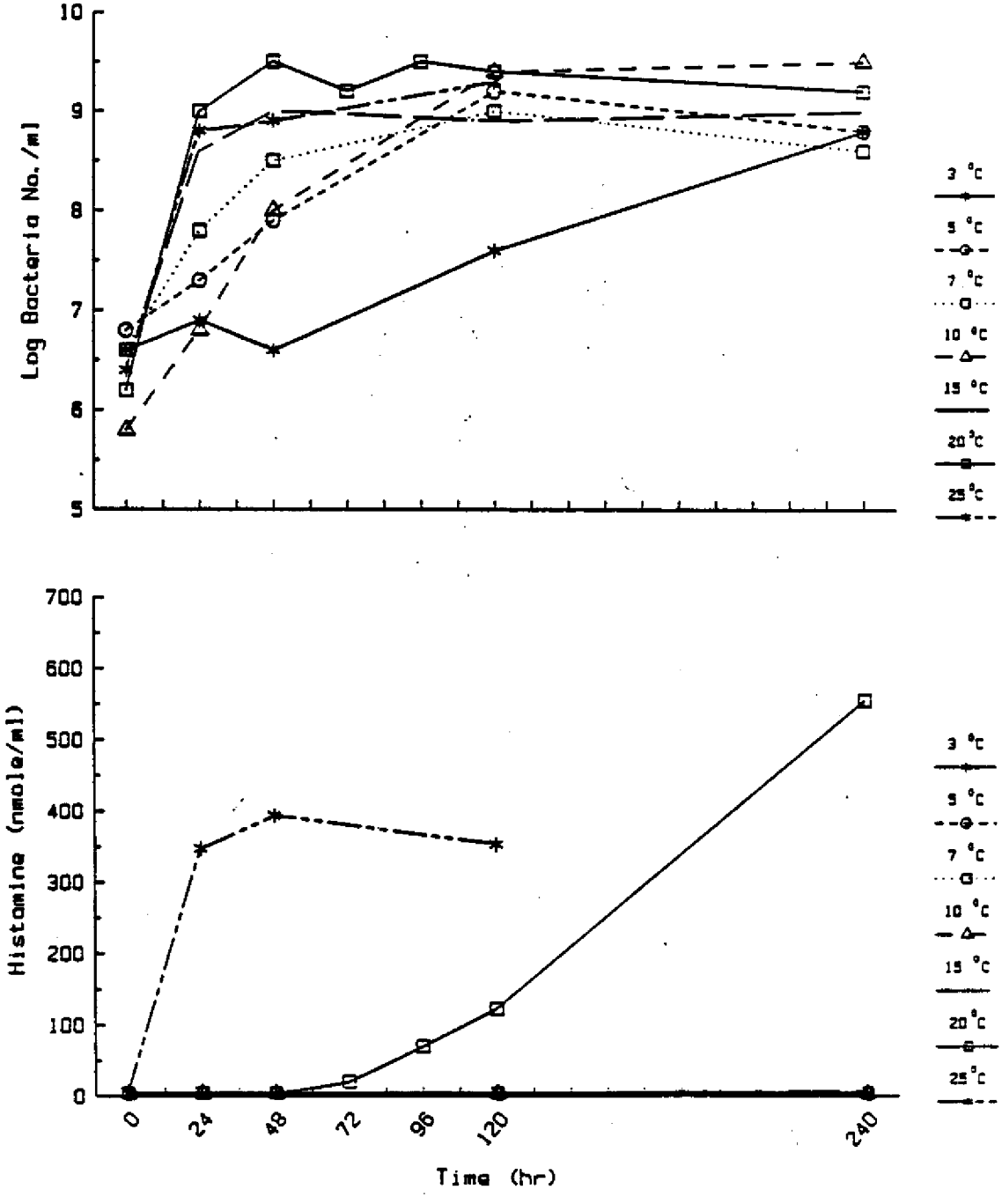


Figure 5. Time and temperature related growth and histamine production of Hafnia alvei T_8 in tuna fish infusion broth.

growth stage with maximal histamine levels were found at the start of the stationary or death phase. Ienistea (1971) suggested that some bacterial strains possess histaminase and thus limit histamine accumulation in TFIB. Omura et al. (1978) indicated that histamine at 0.05 M in culture medium depressed histidine decarboxylase activity. This phenomenon may have occurred in this study. Further research is needed.

At low temperatures, histamine production did not correspond to bacterial growth. Although bacterial growth occurred at temperatures below 10 °C, histamine was not produced by *K. pneumoniae* 111-5, *P. morganii* 110SC-2 and *H. alvei* T_8. *H. alvei* T_8 grew well at temperatures as low as 5° and 7 °C, however, histamine was not detected until the temperature was above 15 °C. Thus, low temperatures may inhibit the activity of histidine decarboxylase and therefore affect histamine production. Further study on the relationship between histidine decarboxylase activity and histamine formation by histamine-producing bacteria at low temperatures is warranted.

The low temperature limit for bacterial growth of the five test strains is 3 °C for *H. alvei* T_8, 5 °C for *K. pneumoniae* strains 111-5 and T_2 and 7 °C for *P. morganii* 110SC-2 and JM. Moreover, the low temperature limit for "potential" histamine production appears to be 7 °C for *K. pneumoniae* T_2 and *P. morganii* JM, 15 °C for *K. pneumoniae* 111-5 and *P. morganii* 110SC-2 and 20 °C for *H. alvei* T_8. To better maintain tuna quailty, the use of a temperature lower than 3 °C is recommended.

Acknowledgement

This project is supported by the US Department of Commerce grant number NA86WC-H-06106.

References

Arnold, S.H. and Brown, W.D. 1978. Histamine (?) toxicity from fish products. Adv. Food Res. 24: 114.

Arnold, S.H., Price, R.J., and Brown, W.D. 1980. Histamine formation by bacteria isolated from skipjack tuna, Katsuwonus pelamis. Bull. Jpn. Soc. Sci. Fish. 46: 991.

Behling, A.R. and Taylor. S.L. 1982. Bacterial histamine production as a function of temperature and time of incubation. J. Food Sci. 47: 1311.

Food and Drug Administration. 1982. Defect action levels for histamine in tuna; availability of guide. Fed. Regist. 470: 40487.

Gill, T.A. and Thompson, J.W. 1984. Rapid, automated analysis of amines in seafood by ion-moderated partition HPLC. J. Food Sci. 49: 603.

Ienistea, C. 1971. Bacterial production and destruction of histamine in foods and food poisoning caused by histamine. Nahrung 15: 109.

Lukton, A. and Olcott, H.S. 1958. Content of free imidazole compounds in the muscle tissue of aquatic animals. Food Res. 23: 611.

Omura, Y., Price, R.J., and Olcott, H.S. 1978. Histamine -forming bacteria isolated from spoiled skipjack tuna and jack mackerel. J. Food Sci. 43: 1779.

Speck, M.L. 1984. Compendium of methods for the microbiological examination of foods. APHA. Washington, D.C.

Taylor, S.L. 1986. Histamine food poisoning: Toxicology and and Clinical Aspects. CRC Crit. Rev. Toxicol. 17: 91.

Taylor, S.L., Guthertz, L.S., Leatherwood, M., and Ellen, R. 1979. Histamine production by Klebsiella pneumoniae and an incident of scombroid fish poisoning. Appl. Environ. Microbiol. 44: 447.

XENOBIOTIC RESIDUES IN CRAWFISH (PROCAMBARUS CLARKII) EDIBLE TISSUE

JD Madden[1], MW Finerty[2], RM Grodner[1]
[1]Department of Food Science
Louisiana Agricultural Experiment Station
Louisiana Agricultural Center
Baton Rouge, LA 70803

[2] Department of Food Science
College of Agriculture
Louisiana State University
Baton Rouge, Louisiana 70803

INTRODUCTION

Biomagnification, the transmission of metal residues from crayfish to consumer species higher up the food chain resulting in either a dangerous concentration or accumulation effect (Stinson and Eaton, 1983; Gillespie, et al., 1977), is a possibility since other detritus feeders are known to concentrate trace metals in their body tissues. Evans (1980) reported that Orconectes rusticus could concentrate cupric ion in the abdomen, claws and thorax accumulating 86 ug in the whole animal from exposure to 3 mg/kg in the water for 48 hours. Gillespie, et al. (1977) determined that Orconectes propinquus propinquus (Girard) in Erie, New York after exposure to 1 mg/kg cadmium, as Cd-109Cl, in the water for 190.5 hours accumulated 534.4 mg/kg in the whole crayfish with only 1/15 fatalities. This is of concern since concentrations above 10 ug/L in drinking water and 13 mg/kg in food (wet weight) are considered hazardous to human health (Anon., 1962).

The annual harvest for Louisiana crayfish in 1985 was 85 million pounds (de la Brettone, 1986, personal communication). The predominant species are Procambarus clarkii and P. acutus acutus. Since investigations of this nature have not been published for these species, the purpose of this study was to determine which metal residues were present in what quantities, whether the source of the metals was the sediment or water in the environs, and whether the concentration of the residues were dependent on the time of season (maturity) or the location of the sampling.

MATERIALS AND METHODS

Sampling

Samples of crayfish, both Procambarus clarkii and P. acutus acutus, were taken from the 'upper' and 'lower' Atchafalya River Basin and from

3 ponds the first year and 2 ponds the second year in different Parishes (counties). Forty pound samples were taken at each site at the beginning, peak and end of the season. Sediment and water were sampled in the environs of each sampling site at each time period for each of the 2 seasons. Crayfish were deheaded and immediately the hepatopancreatic tissue was separated from the abdominal muscle. The tissues were vacuum-sealed, frozen and stored at -20°C until analyzed.

Preparation of Samples

One gram samples of abdominal muscle or hepatopancreatic tissue were cold digested with 5 mL concentrated nitric acid overnight. Digestion was continued at 130°C for 1 to 3 hours until complete. Simultaneously, National Bureau of Standards (NBS) oyster and bovine tissue standards, National Research Council of Canada lobster hepatopancreas standard and reagent blanks were likewise digested. Digests were filtered through Whatman No. 42 paper and diluted to 50 mL with glass distilled water.

Sediment samples were dried to constant weight at 110°C, reweighed and ashed in a muffle oven at 400°C. The procedure of Jackson (1974) and Sridhar and Jackson (1974) as written in Page (1982) was used. Sediment standards, samples and blanks were digested with 1 mL aqua regia and 10 mL hydrofluoric acid for 500 mg samples by standing overnight followed by shaking for 12 hours. The digested samples were quickly solubilized with a sufficient quantity of saturated boric acid solution for the total sample weight including the boric acid to be 100 grams. The digested samples were capped tightly, left at ambient temperature overnight, and filtered through Whatman No. 42 paper.

The water samples were filtered through Whatman No. 42 paper. All samples for the ICP were prepared in triplicate.

Inductively Coupled Plasma Emission Spectrometry

The filtered digests were analyzed on an Applied Research Laboratory (Sunland, Calif.) Model 34100 Inductively Coupled Plasma Emission Spectrometer. Aliquots of 2 mL were analyzed in duplicate. The ICP was controlled by software from Applied Research Laboratory used on a DEC PDP1103 computer. The weights of all digested samples, accurate to 0.1 mg, were entered into the computer. Therefore, the printout reported the results for the sediment, hepatopancreas, and abdominal muscle as mg/kg and the water as mg/L. Reagent blanks, and standards were analyzed simultaneously with the samples. The samples were arranged in the autosampler such that the water samples were analyzed

first, followed by the abdominal muscle and hepatopancreas, and finally the sediment.

Statistical Analysis

The data were analyzed using the Statistical Analysis System (SAS, Cary, NC) generalized linear model program, which performs analysis of variance on the means and determines the probability of difference between the least square means for each metal in each tissue with the other locations or times of season. The alpha level of 0.01 was used to determine significance.

RESULTS

The program for the ARL Model 34100 ICP separates and quantifies 27 elements simultaneously. Some major elements found in crayfish, including calcium, magnesium, phosphorus, potassium, sodium, and sulfur, were not incorporated into this study. According to Duffs (1980), the metals hazardous to human health and found as residues in food are arsenic, beryllium, cadmium, copper, lead, mercury, manganese, nickel, tin, vanadium, and zinc. Others that are frequently monitored include chromium, iron and selenium. National and international legislatures monitor cadmium and lead more closely than the other metals since they are the most commonly publicized toxic metals.

In Tables 1 and 2 the values determined by us for the various metals in the standards for tissues and sediment are shown with those reported by the source. The data from this study are presented in Table 3. The results from the two years were pooled except when the statistics indicated that the samples were significantly different ($p=0.05$). In these cases, the data for the two years are presented on two separate lines. The concentrations of metals found in the water were extremely low. The water samples were not concentrated prior to analysis, and therefore, the metals were reported by range rather than by absolute values.

TABLE 1. COMPARISON OF MEANS FOR METAL STANDARDS

METAL	BOVINE		LOBSTER		OYSTER	
	Ours[ab]	NBS[c](S.D.)[d]	Ours[ab]	NRC[e](S.D.)[d]	Ours[ab]	NBS[c](S.D.)[d]
AS	1.82	0.055(0.005)	26.3	24.6(2.2)	28.2	13.4(1.9)
	<1.00[f]		38.9		13.3	
CD	<0.10	0.27(0.04)	24.7	26.3(2.1)	4.3	3.5(0.4)
	<0.10		26.4		7.5	
CR	<0.020	0.088(0.012)	1.17	2.4(0.6)	2.01	0.69(0.27)
	<0.020		3.15		<0.20	
CO	N/A		<0.25	0.42(0.05)	N/A	
			2.12			
CU	198	193(10)	370	439(22)	67.2	63.0(3.5)
	191		384		59.2	
FE	278	268(8)	159	186(11)	158	195(34)
	251		157		157	
PB	<2.50	0.34(0.08)	N/A		11.36	0.48(0.04)
	<2.50				<2.50	
MN	10.4	10.3(1.0)	N/A		17.1	17.5(1.2)
	9.8				16.3	
HG	<1.00	0.16(0.002)	N/A		<1.00	0.057(.015)
	<1.00				<1.00	
NI	N/A		0.7	2.3(0.3)	4.38	1.03(0.19)
			4.3		0.55	
V	N/A		<0.1	1.4(0.3)	4.0	2.3(0.1)
			2.0		2.0	
ZN	138	130(13)	154	177(10)	849	852(14)
	94		150		769	

N/A MEANS NOT ANALYZED FOR BY NATIONAL BUREAU OF STANDARDS OR NATIONAL RESEARCH COUNCIL OF CANADA.

[a]Concentration is in ng/mg.

[b]Mean concentration of triplicates determined in duplicate in our laboratory.

[c]Mean concentration determined by the National Bureau of Standards.

[d]Standard deviation from the mean.

[e]Mean concentration determined by the National Research Council of Canada.

[f]Less than values are based on detection limits and dilution ratio of samples.

TABLE 2. COMPARISON OF MEANS FOR METAL STANDARDS

METAL	RIVER SEDIMENT		ESTUARINE SEDIMENT	
	CURS[ab]	NBS[c](S.D.)[d]	CURS[ab]	NBS[c](S.D.)[d]
AL	2.05%	2.26(0.04)%	5.30%	6.25(0.2)%
AS	88.84ppm		33.92ppm	11.6(1.3)ppm
CD	7.742ppm	10.2(1.5)ppm	1.47ppm	0.36(0.07)ppm
CR	2.61%	2.96(0.28)%	69.34ppm	76(3)ppm
CO	2.594ppm	10.1(0.6)ppm	15.23ppm	10.5(1.3)ppm
CU	79.63ppm	109(19)ppm	11.07ppm	18(3)ppm
FE	9.32%	11.3(1.2)%	3.32%	3.35(0.1)%
PB	599.7ppm	714(28)ppm	105.9ppm	28.2(1.8)ppm
MN	657.2ppm	785(97)ppm	323.9ppm	375(20)ppm
HG	21.96ppm	1.1(0.5)ppm	15.23ppm	10.5(1.3)ppm
NI	19.01ppm	45.8(2.9)ppm	27.38ppm	32(3)ppm
V	21.62ppm	23.5(6.9)ppm	93.68ppm	94(1)ppm
ZN	0.138%	0.172(0.017)%	108.5ppm	138(6)ppm

[a]Concentration is in ppm (ng/mg) or %.
[b]Mean concentration of triplicate samples determined in duplicate in our laboratory.
[c]Mean concentration determined by National Bureau of Standards.
[d]Standard deviation from the mean.

Aluminum, barium, antimony and silicon are not certifiably quantitated by the National Bureau of Standards or the National Research Council of Canada. Aluminum and barium were present at levels less than 300 ug/L, antimony at levels less than 100 ug/L, and silicon at levels less than 10 ug/L water for both years in all samples. However, higher concentrations of these metals are commonly found in sediment, especially in waters near agricultural runoff or industrial effluents. The levels of these metals are generally very high in the sediment and in the mg/kg region in the tissues, with the levels higher in the hepatopancreatic tissue than in the abdominal muscle as can be seen in Table 3.

Arsenic used to be the active ingredient in defoliating agents. The use of arsenates diminished greatly until recently. The recent increase in use of arsenates may explain the discrepancy in residue concentrations in the sediment for the two years in the Basin locations and the increase in arsenic from less than 10 ug/L the first year to 100 ug/L in the lower Basin and Vermilion Parish water samples during the second year and less elevated levels in the other locations. Although the concentrations found in the sediment may be higher than

TABLE 3. CONCENTRATION OF METAL RESIDUES IN ABDOMINAL MUSCLE, HEPATOPANCREAS AND SEDIMENT OF CRAYFISH IN 6 LOCATIONS DURING 2 SEASONS.

METAL	LOCATION[a]	ABDOMINAL MUSCLE MEAN[bc] (RANGE)	HEPATOPANCREAS MEAN[bc] (RANGE)	SEDIMENT MEAN[bc] (RANGE)
AL	AP	7.63 (<1.50-21.32)[d]	7.35 (2.75-11.69)	41589 (36721-47648)
	CRS	7.60 (<1.50-24.78)	17.14 (<1.50-35.66)	41556 (24095-54145)
	LB	6.96 (<1.50-16.85)	8.48 (<1.50-29.43)	28726 (18480-42549)
	STM	8.81 (<1.50-28.26)	20.12 (7.04-31.97)	109057 (82889-127675)
	UB	13.68 (<1.50-27.31)	5.54 (<1.50-13.87)	25116 (16819-32578)
	VER	6.92 (<1.50-16.31)	7.98 (5.05-10.66)	34075 (24313-51349)
AS	AP	<1.00 (<1.00-<1.00)	2.23 (<1.00-3.19)	33.01 (14.88-49.76)
	CRS	2.99 (<1.00-4.49)	2.62 (<1.00-4.42)	31.80 (14.30-45.13)
	LB	3.23 (<1.00-4.35)	3.09 (<1.00-5.47)	13.92 (7.99-17.74) & 137.80 (98.07-186.90)
	STM	<1.00 (<1.00-<1.00)	1.56 (<1.00-2.584)	49.96 (29.86-65.21)
	UB	4.08 (<1.00-5.07)	3.65 (<1.00-5.577)	15.37 (6.764-23.10) & 88.91 (63.57-136.9)
	VER	3.59 (2.36-4.63)	5.28 (3.80-6.70)	13.39 (7.73-20.92)
BA	AP	<0.035 (<0.035-<0.035)	4.840 (2.036-9.713)	556 (497-624)
	CRS	0.532 (<0.035-3.150)	6.869 (1.200-9.623)	519 (337-2351)
	LB	1.288 (<0.035-5.770)	6.177 (2.371-8.924)	297 (215-468) & 2510 (2220-2733)
	STM	0.043 (<0.035-0.385)	2.193 (1.127-3.447)	945 (844-1054.)
	UB	2.383 (<0.035-8.902)	6.558 (3.805-9.402)	282 (205-352.8) & 1826 (830-3303.)
	VER	0.782 (<0.035-1.065)	8.223 (5.655-12.140)	333 (262-397.9)
CD	AP	0.262 (<0.100-1.941)	0.192 (0.105-0.358)	<0.400(<0.400-0.645)
	CRS	0.137 (<0.100-0.792)	0.158 (<0.100-0.551)	1.572 (<0.400-3.931)
	LB	0.844 (<0.100-4.325)	0.272 (<0.100-0.746)	2.892 (<0.400-6.243)
	STM	1.618 (<0.100-8.002)	<0.100 (<0.100-0.176)	1.147 (<0.400-2.156)
	UB	1.086 (<0.100-10.320)	0.296 (<0.100-0.796)	2.985 (1.947-6.811)
	VER	0.320 (0.137-0.496)	0.397 (0.203-0.491)	2.122 (1.562-2.668)

METAL	LOCATION[a]	ABDOMINAL MUSCLE MEAN[bc] (RANGE)	HEPATOPANCREAS MEAN[bc] (RANGE)	SEDIMENT MEAN[bc] (RANGE)
CO	AP	<0.250 (<0.250-<0.250)	0.857 (0.316-1.661)	10.79 (7.52-12.91)
	CRS	0.722 (<0.250-1.168)	0.401 (<0.250-0.713)	12.86 (9.99-1626)
	LB	0.717 (<0.250-0.910)	0.987 (<0.250-2.294)	13.46 (11.13-17.51) & 59.04 (41.03-76.27)
	STM	<0.250 (<0.250-0.250)	0.444 (0.254-0.776)	18.75 (16.55-22.39)
	UB	1.020 (<0.250-1.335)	1.274 (<0.250-2.022)	12.66 (9.96-13.83) & 51.13 (25.50-89.55)
	VER	0.916 (0.536-1.228)	1.219 (0.692-1.762)	11.85 (11.02-12.77)
CU	AP	1.867 (0.820-3.229)	7.373 (3.945-10.46)	3.178 (<0.400-16.86)
	CRS	3.241 (0.849-4.083)	14.73 (1.490-25.64)	8.471 (<0.400-19.31)
	LB	3.395 (1.459-4.146)	23.63 (5.137-51.19)	11.35 (6.814-16.19) & 101.3 (69.96-152.7)
	STM	2.179 (1.038-5.487)	27.06 (6.570-63.41)	25.04 (18.44-36.25)
	UB	4.296 (1.508-5.753)	11.91 (6.736-20.14)	9.047 (6.847-10.59) & 72.04 (20.47-169.6)
	VER	5.144 (3.785-5.672)	13.56 (10.46-18.14)	10.65 (7.168-17.05)
HG	AP	<1.00 (<1.00-<1.00)	<1.00 (<1.00-<1.00)	28.86 (25.12-34.86)
	CRS	<1.00 (<1.00-<1.00)	<1.00 (<1.00-<1.00)	10.13 (4.87-15.13) & 30.05 (25.64-34.19)
	LB	<1.00 (<1.00-<1.00)	<1.00 (<1.00-<1.00)	5.11 (4.49-5.70) &
		<1.00 (<1.00-<1.00)	<1.00 (<1.00-<1.00)	75.42 (55.04-96.10)
	STM	<1.00 (<1.00-<1.00)	<1.00 (<1.00-<1.00)	31.55 (27.36-36.51)
	UB	<1.00 (<1.00-<1.00)	<1.00 (<1.00-<1.00)	5.37 (<4.00-8.89) &
		<1.00 (<1.00-<1.00)		57.45 (29.09-103.5)
	VER	<1.00 (<1.00-<1.00)	<1.00 (<1.00-<1.00)	3.71 (2.37-5.40)
NI	AP	<0.400 (<0.400-<0.400)	<0.400 (<0.400-<0.400)	11.66 (8.354-19.02)
	CRS	1.086 (<0.400-1.880)	1.221 (<0.400-2.184)	17.04 (6.642-27.59)
	LB	1.257 (<0.400-2.043)	1.393 (<0.400-2.335)	18.70 (12.39-27.44) & 128.1 (84.08-173.9)
	STM	<0.400 (<0.400-0.909)	0.274 (<0.400-0.558)	36.13 (29.44-45.53)
	UB	1.667 (<0.400-2.100)	1.933 (<0.400-3.785)	14.64 (8.713-19.91) & 106.4 (58.81-192.5)
	VER	1.611 (1.108-2.141)	1.892 (1.311-2.721)	18.51 (11.79-25.37)
PB	AP	<2.50 (<2.50-<2.50)	<2.50 (<2.50-<2.50)	49.84 (29.14-74.21)
	CRS	<2.50 (<2.50-5.114)	<2.50 (<2.50-<2.50)	108.8 (90.65-128.5) &

METAL	LOCATION[a]	ABDOMINAL MUSCLE MEAN[bc] (RANGE)	HEPATOPANCREAS MEAN[bc] (RANGE)	SEDIMENT MEAN[bc] (RANGE)
	STM	<2.50 (<2.50-<2.50)	<2.50 (<2.50-<2.50)	88.51 (72.54-110.5)
	UB	5.06 (3.29-7.50)	<2.50 (<2.50-4.47)	84.05 (67.39-103.0) &
		<2.50 (<2.50-2.82)	<2.50 (<2.50-<2.50)	231.2 (125.0-394.1)
	VER	4.77 (2.56-6.22)	5.69 (3.76-7.27)	91.60 (70.98-114.0)
SB	AP	<2.00 (<2.00-<2.00)	4.52 (3.99-5.81)	48.12 (16.75-79.13)
	CRS	<2.00 (<2.00-3.43)	4.59 (<2.00-5.80)	46.53 (18.69-73.66)
	LB	2.66 (<2.00-5.20)	4.84 (<2.00-6.93)	18.13 (11.38-22.99) &
				123.5 (43.35-186.2)
	STM	<2.00 (<2.00-<2.00)	4.08 (3.34-5.59)	57.85 (26.18-81.37)
	UB	3.35 (<2.00-4.47)	4.43 (<2.00-5.64)	17.12 (8.495-29.47) &
				98.52 (46.40-242.8)
	VER	3.17 (2.19-5.13)	4.86 (3.58-7.27)	11.66 (3.426-18.67)
SI	AP	17.77 (11.84-30.52)	17.30 (13.04-23.38)	*
	CRS	15.33 (8.60-23.45)	18.81 (2.08-31.15)	*
	LB	13.76 (2.22-18.12)	16.10 (5.10-23.17)	*
	STM	19.04 (8.27-32.33)	25.38 (13.02-33.26)	*
	UB	14.35 (2.39-26.02)	24.02 (5.17-50.81)	*
	VER	3.93 (1.66-8.07)	27.70 (9.81-70.44)	*
V	AP	0.183 (<0.100-0.366)	0.245 (<0.100-0.344)	67.09 (57.47-73.73)
	CRS	0.533 (<0.100-0.691)	0.412 (<0.100-0.554)	78.68 (44.17-102.0)
	LB	0.718 (<0.100-0.946)	<0.100 (<0.100-0.285)	61.19 (42.54-89.29) &
				526.9 (404.8-727.2)
	STM	0.136 (<0.100-0.351)	0.596 (0.333-1.115)	192.5 (149.3-227.5)
	UB	0.959 (<0.100-1.078)	0.495 (<0.100-0.862)	52.59 (44.82-65.68) &
				411.3 (176.5-781.0)
	VER	0.868 (0.731-1.007)	0.904 (<0.100-1.101)	66.65 (39.47-105.8)

[a]Location abbreviations represent the following sampling sites: Acadia Parish (AP), Crowley Rice Station (CRS), Lower Atchafalaya River Basin (LB), St. Martins Parish (STM), Upper Atchafalaya River Basin (UB), and Vermilion Parish (VER).

[b]Each value is the mean of triplicate samples determined by duplicate analysis using the ICP. The mean values are reported in mg/kg with the range of values obtained in parentheses.

[c]The significant digits reported depended on the precision of the detection limit and the dilution factor.

[d]The < sign indicates that the value is less than the detection limit of the ICP. The

actual values, there appears to be no accumulating effect in the crayfish tissue.

The concentration of cobalt in the sediment is approximately that found in the standards. The two Basin sites with 50 mg/kg have high concentrations in the sediment. The levels of cobalt in the water were below 10 ug/L the first year and less than 190 ug/L the second year. The increased concentration of cobalt in the water was responsible for the increased concentration of cobalt in the tissues for the second year.

The determinations for cadmium in standards agree well with those reported by the source. The amount of cadmium in the water was below 10 ug/L. Therefore, the water did not contribute significantly to the concentration of residues found in the tissues.

Wright (1976) analyzed Cragon vulgaris (shrimp) and Leander serratus (prawn) for heavy metals. The C. vulgaris had 7 mg/kg copper in the exoskeleton, 25.9 mg/kg Cu and 8.80 mg/kg Ni in muscle, and 3.5 mg/kg Cd in the whole body. L. serratus had 2.80 mg/kg Cd in the whole body. Lyon et al. (1983) analyzed for heavy metals in crayfish (Austropotamobius pallipes) using radiolaballled zinc, cadmium and copper injected into the posterior cephalothorax. They reported that these metals accumulated in the hepatopancreas bound to different molecular weight proteins.

Gillespie, et al. (1977) determined the cadmium uptake by Orconectes propinquus propinquus (Girard) in Erie, New York. This species was chosen since it is an important link in aquatic food webs. Cadmium-109 chloride was dissolved in water at levels up to 1 mg/kg Cd. The animals were monitored for 190.5 hours, at which point the mean concentration in the crayfish exposed to 1 mg/kg was 534.4 mg/kg with 1/15 deaths. This is of concern since concentrations above 10 ug/L in drinking water and 13 mg/kg in food (wet weight) are considered hazardous to human health (Anon., 1962). Upper permissible limits for these and other elements in crayfish have not been established by federal or Louisiana regulatory agencies.

Ray (1984) reported that the base residue level for cadmium in the North Atlantic surface water is 40-60 ng/L and somewhat higher in estuarine and coastal waters.

The lower sediment concentration of copper in the pond samples resemble the concentration in the estuarine standard, while the higher values of the Basin samples more closely resemble the concentration in the river standard. The values for copper in the crayfish tissues are lower than those anticipated if the values in the standards are typical of physiological levels.

Since the concentration of copper in the water was less than 10 ug/L for both years in all samples, there was no significant accumulation or concentration of copper from the water by the crayfish.

Evans (1980) wanted to determine whether crayfish (Orconectes rusticus) could be used as a good biological monitor of heavy metal pollution. Cupric ion was dispersed in the water. Cupric ion concentration, determined using an electrode, for the abdomen, claws and thorax was combined to determine the amount of accumulation. The accumulation in body parts was 86 ug/animal from exposure to 3 mg/kg for 48 hours.

Bernard and Roy (1979) used X-ray fluorescence to monitor heavy metals in thoracic fatty material (hepatopancreas) and tail muscle of crayfish from marsh ponds, rice fields and the Atchafalaya River Basin at Catahoula, Louisiana. Of the copper, nickel, chromium, arsenic, lead, zinc and mercury monitored, only copper, zinc and iron were found in occasionally high levels in crayfish.

The quantification of mercury residues is difficult to interpret. Our analysis of the mercury in the river sample was approximately ten-fold the values reported by NBS, however, our values agree quite well with the estuarine standard. There was no quantification of mercury by the NBS in the tissue samples. However, the concentrations of mercury in our samples are in the same range as that for the bovine and oyster standards. All of the water samples for both years had less than 10 ug/L mercury.

Rincon, et al. (1987) reported that P. clarkii in Spain had approximately 100 ug/kg mercury in the whole animal. Sheffey (1978) reported 0.070 to 0.560 mg/kg mercury in the whole animal using Orconectes virilis in a contaminated portion of the Wisconsin River, where paper, pulp, chlorine and caustic soda plants are located.

The amount of nickel residues in the crayfish tissues was greater than those reported by Rincon, et al. (1987) and Sheffey (1978). The amount reported in the sediments falls within the range reported in the literature (Table 4) (Kirkham, 1979). The concentration of nickel in the water was less than 30 ug/L for both years.

The amount of lead in the Basin sediment samples was less than that found in the river sediment standard. Since the estuarine standard was quantitated to 106 mg/kg compared to the NBS quantification at 28, the actual values in the sediment are probably much lower than those reported. The inaccuracy in the analysis of lead impedes making conclusions about the quantity of lead in the tissues. However, since the quantity of lead in crayfish tissues are lower than the values obtained for the analysis of lead in the standards and the standards were quantified at 0-11 mg/kg for a reported 0.5 mg/kg lead in oysters,

TABLE 4. TRACE ELEMENT CONTENT OF SOILS

Element	Total (ppm)
Al[1]	71,000(10,000-300,000)
As[2]	6(0.1-40)
Ba[2]	500(100-3500)
Cd[2]	0.5(0.01-0.7)
Co[2]	8(1-40)
Cu[2]	20(2-100)
Hg[2]	0.03(0.01-0.8)
Ni[2]	40(5-5000)
Pb[2]	10(2-200)
Si[1]	320,000(230,000-350,000)
V[2]	100(20-500)

[1]number preceding the parenthesis is the mean value found in Lindsay (1979)
[2]number preceding the parenthesis is the mean value found in Kirkham (1979)

the relative amount of lead is very low. The level of lead in the water was less than 10 ug/L in all samples for the two years.

Bernard and Roy (1977) reported that P. clarkii in Louisiana had the following ranges: 5-69 mg/kg Cu; <0.1-3 mg/kg Ni; <0.1 mg/kg Hg, As, Cd; and <0.1-0.9 mg/kg Pb in the hepatopancreatic tissue. The concentrations of mercury, arsenic, lead and cadmium were higher in this study than this earlier study. The concentrations of copper and nickel residues reported by Wright (1976) were higher for prawns and shrimp than those obtained in this study.

The values for antimony and silicon in the standards were not quantified by either the NBS or NRC of Canada. Therefore, little can be said about the values obtained. The amount of antimony in all hepatopancreas tissue analyzed appears to average at 4 mg/kg. There is considerably more variation in the abdominal muscle and sediment. There does not appear to be any geographical explanation for the variation of values in the sediment. Other possible explanations are that the amount of antimony in the water may be a better variable, or the type of soil may affect the release of antimony.

Silicon is a major component of soil. The upper Basin (UB), lower Basin (LB) and Vermilion Parish (VER) sites had lower levels of silicon, since the sediment is primarily organic matter compared to the ponds. These lower silicon concentrations seem to correspond to the samples with more residues from other metals. The ponds, Crowley Rice Station (CRS), St. Martin's Parish (STM), and Acadia Parish (AP), had more silicon and less of the other metals in general since there is less organic matter to sequester the metals. Even though the levels of metals varied from one year to the next in the two Basin locations, the Basin sites had more metal residues than the pond sites.

Vanadium analyses in the sediment standards agreed well with the NBS. The unusually high values in the sediment of the two Basin locations and STM for the first year may be due to more industrial effluent bound to the sediment such that it could be easily hydrolyzed. The peak season upper Basin (UB) samples and the late season VER pond water samples had approximately 40 ug/L vanadium. The abdominal muscle from UB, LB, and VER samples and the hepato-pancreatic tissue from STM, VER, and UB had higher levels of vanadium than the others.

DISCUSSION

An attempt was made to discern changes in the residue levels due to the time of season, and therefore maturity of the animal. However, the animals mature at different rates in different sites depending on the environment and the density of the animal. In addition, the animals

were not separated on the basis of sex since Dickson, et al. (1979) found no significant difference in accumulation of metals in Cambarus tenebrosus and Orconectes australis australis. Only copper, iron, and silicon concentrations were significantly differ-ent depending on the time of the season ($p=0.01$). These changes may be due to physiological changes, molting or growth of the animal.

In addition, the wild and pond-raised crawfish were compared for residue levels. The concentration of metal residues in the pond-raised and the Atchafalaya River Basin crayfish did not signifi-cantly differ. The variability in metal content between crayfish in different locations was high, possibly a complication of the varia-tion in maturity of the animals, the former use of the land in the area, the rainfall as well as the current management practices. The highest residue levels generally were found in the hepatopancreas of UB and VER crayfish. Furthermore, the tissue samples with the high-est levels of residues did not necessarily come from the locations with the highest levels in the sediment or water.

As can be seen in Table 3, the majority of the differences that were observed between the samples was between the hepatopancreas and the abdominal muscle. As was anticipated, higher levels of most metals were found in the hepatopancreas, but the levels found were not toxic to crayfish. However, the concentration of antimony was higher in abdominal muscle (2-3 mg/kg) than in hepatopancreas (less than 2 mg/kg except in VER), as was aluminum 6-14 mg/kg in abdominal muscle and less than 7.5 mg/kg in the hepatopancreas. The levels of arsenic, cobalt, nickel and vanadium were similar in both tissues. Barium was present at 6-8 mg/kg, iron at levels up to 640 mg/kg, silicon at 5-28 mg/kg and copper at 11-15 mg/kg in the hepatopan-creas and these levels were found to be at significantly higher levels ($p=0.01$) in the hepatopancreas than in the abdominal muscle. These results agree with those reported by Bernard and Roy (1977), who found that the heavy metals bioaccumulate in hepatopancreas and that the levels of copper, iron and zinc are highly variable and may be found in high concentrations even in controlled environments.

Most of the previous studies were concerned about the toxicity of the various metals to the crayfish and whether the metals could be concentrated by the crayfish and other species in the food web, which ultimately could be toxic to humans or cause health problems. For instance, Hendrick and Everett (1965) reported that methyl mercury dicyandiamide has a 5 day median tolerance level of 83 ug/kg, as compared to that of aldrin at 38 ug/kg and methyl parathion at less than 10 g/kg, which indicates that crawfish are more susceptible to effects from aldrin than either methyl mercury dicyandiamide or methyl

parathion. In addition, the potency of these chemicals did not significantly increase during the molting cycle of crawfish.

Bagetto and Alikham (1987) analyzed Cambarus bortoni and Orconectes viriles in both industrially polluted lakes and unpolluted lakes for copper, cadmium and nickel. The copper levels in the hepatopancreatic tissue and abdominal muscle were approximately ten-fold higher in these animals caught in unpolluted water than in this study. The cadmium concentrations were similar between the studies when both are put on a dry weight basis. The concentration of nickel in this study was slightly higher than their samples from unpolluted waters, but much lower than their samples from polluted waters when put on a dry weight basis for comparison.

CONCLUSIONS

Crayfish in 6 locations were sampled at 3 different times during 2 seasons. There was no significant difference in the concentration of metal residues in the abdominal muscle and hepatopancreatic tissues between locations or between times of season or between wild and pond-raised crayfish. There was no correlation when samples were separated by location between the crayfish with the higher concentrations of metal residues in the tissues and the higher con-centrations of metal residues in the sediment and water. Further-more, there did not appear to be any accumulating or concentrating effect in the crayfish. The higher concentration of some metals in VER and UB tissue samples might be attributed to the higher concen-tration of clay and smectite in the sediment since both smectite and clay are good sequestering agents. The concentrations of metal residues were similar to those reported by other researchers for other species of crustaceans.

REFERENCES

Anon. (1962) Drinking water standards. US Dept. Health, Education and Welfare. Pub. 956:61.

Bernard, DL and Roy, KW (1977) Metals in Louisiana crayfish (P. clarkii) determined by x-ray fluorescence analysis. In: Lindqvist, OV (ed) Freshwater crayfish 3: Papers from the third international symposium on freshwater crayfish at the University of Kuopia, Finland. University of Kuopia, Finland, pp357-361.

Day, P.R. 1965. Particle fractionation and particle-size analysis. In C.A. Black (ed.). Methods of Soil Analysis. Am Soc. Agron. Monograph No. 9, pp545-567.

Duffs, JH (1980) Environmental toxicity. John Wiley and Sons, Inc., New York, New York, p68.

Evans, ML (1980) Copper accumulation in the crayfish (Orconectes rusticus). Bull. Environ. Contam. Toxicol. 24:916-920.

Gillespie, R, Reisine, T, Massaro, EJ (1977) Cadmium uptake by the crayfish Orconectes propinquus propinquus (Girard). Envion. Res. 13:364-368.

Hendrick, RD, Everett, TR (1965) Toxicity to the Louisiana red crawfish of some pesticides used in rice cultures. J. Econ. Entom. 58:958-961.

Hopkins, SP, Martin, MH, Moss, SJ (1985) Heavy metals in isopods from the supra-littoral zone on the southern shore of the severn estuary, UK. Environ. Pollut. 9B:239-254.

Lyon, R, Taylor, M, Simkiss,K (1983) Metal-binding proteins in the hepatopancreas of the crayfish (Austropotamobius pallipes). Comp. Biochem. Physiol. 74C:51-54.

Page, AL (1982) Methods of Soil Analysis Part 2: Chemical and Microbiological Properties, 2nd ed. American Society Agronomy and Soil Science Society of America, pp5-7.

Ravera, O (1984) Cadmium in freshwater ecosystems. Experientia 40:2-14.

Ray, S (1984) Bioaccumulation of cadmium in marine organisms. Experientia 40:14-23.

Stinson, MD, Eaton, DL (1983) Concentrations of lead, cadmium, mercury and copper in the crayfish (Pacifasticus leniusculus) obtained from a lake receiving urban runoff. Arch. Environ. Contam. Toxicol. 12:693-700.

Wright, DA (1976) Heavy metals in animals from the northeast coast (England). Marine Pollut. Bull. 7:36-38.

ACKNOWLEDGEMENTS

Partial support for this project was from the Louisiana Crawfish Research and Promotion Board, Baton Rouge, Louisiana.

PROPERTIES AND POTENTIAL USES OF OYSTER SHUCKING WASTE WATER

Chyuan-Yuan Shiau and Tuu-jyi Chai
Seafood Science and Technology Division
Horn Point Environmental Laboratories
University of Maryland
Cambridge, MD 21613

INTRODUCTION

Oysters are commonly marketed fresh, shucked or in the shell. Raw oysters are first shucked, then washed, drained and packaged. In this process, a large quantity of waste water is generated. It is estimated that the annual production of 50 million pounds of oyster meat in the USA will generate 55 million gallons of waste water or 2 million pounds of soluble solids (Kramer et al. 1962a). The soluble solids contain proteins, flavor components and other nutrients. This large amount of soluble solids lost in waste water creates a serious environmental pollution problem and tedious task for the seafood industry. The recovery of these soluble solids for human consumption can benefit both the industry and public health.

Recovery of other seafood processing wastes has been attempted. Potential products from effluents of surf clam and quahog processing industries have been studied by Barnett and Lin (1981) using foam fractionation. Protein and flavor components from clam processing wash water have been isolated and characterized (Hang et al. 1980; Joh and Hood, 1979). Removal of suspended solids from tanner crab, salmon and shrimp waste waters has been studied using hydrocyclone system (Johnson and Lindly, 1981). Recovery of functional protein from clam effluent processing with direct acid precipitation, ultrafiltration, microgas dispersion flotation and ion exchange chromatography has been evaluated (Jhaveri, 1984).

Little information has been reported about the potential use of oyster processing waste for human consumption. The recovered products of this oyster waste under sanitary conditions and good quality control can be processed for oyster broth, oyster-flavored concentrates or powder and oyster sauce. It can also be processed for oyster-flavored ingredients for use in formulated foods such as chips, snacks, and advanced fabricated seafoods. Further fermentation of recovered concentrate can be a more effective approach in processing the oyster waste into high quality flavor ingredients and food condiments. This report describes the composition, methodology and quality assessment of recovered concentrate from the oyster processing waste water. The potential use for human consumption of the final products processed from the oyster waste has been evaluated.

MATERIALS AND METHODS

Oyster liquor samples. Raw oysters were harvested from Choptank River and Nanticoke River in Maryland. The oysters were shucked in the laboratory to determine the yield of oyster meat and waste liquor. Oyster processing waste water was obtained from a large local oyster processing plant. Commercial oyster sauces of various brands were purchased from supermarkets in New York City and Cambridge, MD.

Concentration methods. Flash evaporation was applied to concentrate the oyster liquors to a paste at a temperature below 40°C. Millipore pellican ultrafiltration with membranes of 10,000 nominal molecular weight limits were used to concentrate the waste water to 10 to 20 fold concentration.

Fermentation. Ten percent salt was mixed into the recovered waste liquor concentrates and incubated at 15°C, 25°C and 35°C for fermentation with and without the addition of Aspergillus oryzae (purchased as malted rice form, commercially named "koji").

Quality assessment. Waste water quality including pH, total solids, total suspended solids, chemical oxygen demand (COD) and conductivity were determined according to the standard procedures (US EPA 1979). Microbiological analysis was performed as the procedures described in FDA method (1984). Heavy metals were measured by atomic absorption spectrophotometry.

Chemical analysis. Moisture, lipid, ash and salt were determined according to the AOAC method (1984). Total nitrogen and non-protein nitrogen separated from TCA precipitate fraction were determined by Kjeldahl method. Fatty acid composition was determined by gas chromatography of total lipid prepared from chloroform-methanol extraction. SDS-gel electrophoresis was performed for the determination of protein profile according to the method described by Chai and Foulds (1974). Amino acids were analyzed by an amino acid analyzer. Nucleotides and related components were determined by anion exchange chromatography (Hayashi et al., 1978). Volatile base nitrogen (VB-N) was measured with the method used by Gould and Peters (1971). Amino nitrogen was analyzed according to the method described by Cobb et al. (1973). Glycogen was determined using phenol-sulphuric acid extraction described by Dubois et al. (1956).

RESULTS AND DISCUSSION

The yield and composition of shell liquor and bled liquor

Freshly harvested oysters were shucked in the laboratory to determine the yield of shell liquor which originally existed inside the shell and the yield of bled liquor after shucking. As shown in Fig. 1

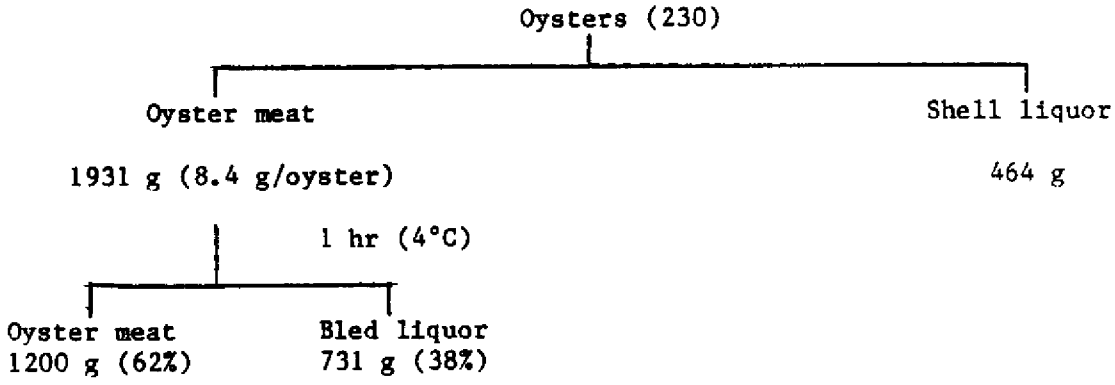


Figure 1. Yield of oyster meat and liquor

shell liquor and bled liquor occupied about 20% and 30% respectively of the initial oyster meat volume (drained meat plus liquor generated during shucking). The total waste liquor including both shell liquor and bled liquor which was released from oyster meat at 1 hr storage after shucking accounted for 50% of the initial oyster meat volume. Shell liquor and bled liquor contain 2.6% and 3.7% total solids and 1.1% and 1.8% of protein respectively (Table 1). During processing, proteins in oyster liquor would be lost by shucking, washing and blowing. In the U.S.A., oyster meat production is about 50 million pounds a year, and about 50 million pounds of liquor and 1.5 million pounds of protein would be lost in the waste water.

Table 1. Composition of oyster meat, shell liquor and bled liquor

Composition (%)	Oyster meat	Shell liquor	Bled liquor
Moisture	80.99	97.38	96.31
Total solid	19.01	2.62	3.69
Protein	13.68	1.05	1.84
Ash	1.60	1.22	1.22
Salt	0.54	0.72	0.73

Both liquors contained about 0.7% and 1.2% salt respectively. The salt content in liquor was higher than in oyster meat. Kramer et al. (1962b) indicated that the salt content of shell liquor was considered to be a reasonably close approximation to the actual salinity of the water where the oysters were harvested. However, our results showed that shell liquors had a lower level of salt than that of the oyster growing water (1.1%).

Based on the result of SDS gel electrophoresis, both shell liquor and bled liquor had an identical protein profile which was similar to that of the oyster meat (Fig. 2). During storage, the protein pattern of drained liquor collected at different storage time of 15 min and 2 hr was not changed. There were about 36 bands which appeared on the SDS-polyacrylamide gel. By comparison to the marker proteins, the molecular weights of major proteins were in four groups of 22,000, 32,000, 43,000 and 58,000 Daltons with the preliminary protein ranging from 20 to 25,000 Daltons.

The quality and composition of oyster processing waste water

In commercial oyster shucking plants, the shell liquor and bled liquor were mixed in the oyster pot. This mixed liquor formed original waste water and was collected when the oysters were first drained for weight determination. The original waste water was composed of shell liquor, bled liquor and rinse water. After draining, the freshly shucked oysters were normally washed in a blowing tank with 5 to 6 volumes of water resulting in a large quantity of wash water with certain amounts of soluble solids. It has been reported that the oyster lost about 14% solids to the wash water (Kramer et al. 1962b). Carawan and Thomas (1981) also indicated that much waste was generated in the blowing process. Both original waste water and wash water were used for recovering byproduct in this study as they would otherwise be sources of pollution.

The original waste water contained 0.65 - 2.24% of total solids and 0.24 - 0.96% of protein (Table 2). Due to the dilution with water, the total solids and protein in original waste water is lower than those of shell liquor and bled liquor (Table 1), but higher than those of wash water. The original waste water also had higher COD, conductivity, VB-N, aerobic and anaerobic count than those of wash water. Based on protein content, the recovery of the byproducts from original waste water seemed to be much more potential than wash water.

The parameters and compostion of both liquors varied depending on oyster quality, storage time, season, post harvest treatment and shucking processing method. As shown in Table 2, the waste water in May contained higher total solids, protein-N, nonprotein-N, ash and salt than those in February.

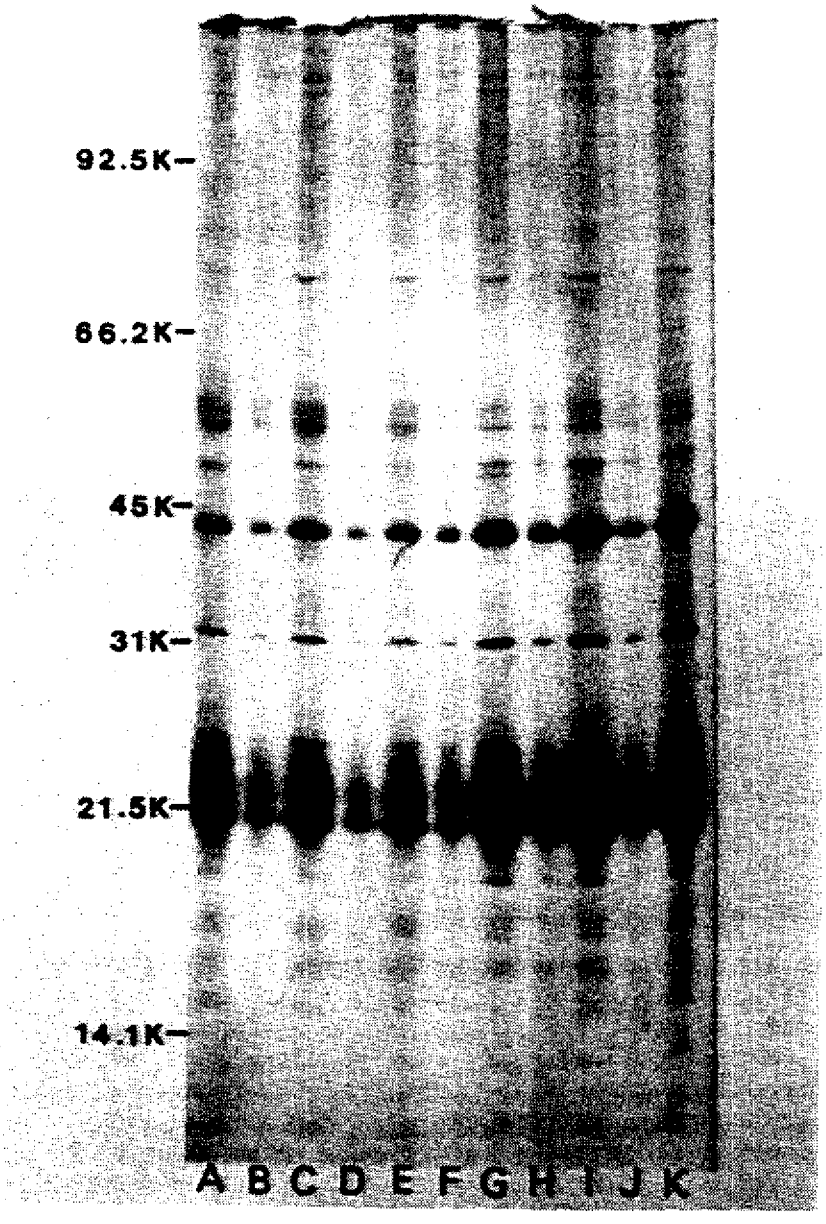


Figure 2. Stained SDS-polyacrylamide gel after electrophoresis:A-B, shell liquor; C-F, bled liquor collected at 15 and 120 min intervals of storage; and G-K, oyster meat of different samples.

Table 2. Quality assessment and composition of oyster plant waste water

Parameter	Original water			Wash water		
	Feb.	May	Dec.	Feb.	May	Dec.
pH	7.36	6.79	7.41	7.51	7.16	7.38
Total solids (%)	1.12	2.24	0.65	0.24	0.33	0.59
Total suspended solids (mg/l)	1,521	3,332	714	339	371	704
COD (mg/l)	6,500	8,250	1,890	930	1,080	1,440
Conductivity (μmhos/cm)	7,998	12,212	3,422	1,830	2,247	3,254
VB-N (mg %)	2.34	3.12	0.87	0.31	0.58	0.77
Aerobic count (PFU/ml)	2.2×10^5	2.6×10^6	1.2×10^5	2.5×10^3	4.7×10^5	4.5×10^4
Anaerobic count (PFU/ml)	1.2×10^5	8.4×10^5	1.1×10^4	6.9×10^2	1.7×10^5	7.5×10^3
Fungi (PFU/ml)	9.0×10^1	0	0	2.5×10^2	0	0
Crude protein (%)	0.45	0.96	0.24	-	0.19	0.24
Ash (%)	0.49	0.86	0.21	0.11	0.12	0.21
Salt (%)	0.43	0.76	0.15	0.08	0.10	0.14

Characterization of oyster waste paste

Oyster liquor was concentrated to paste by flash evaporation and the composition was analyzed. The approximate composition was 55.2% moisture, 4.2% total N, 3.7% protein-N, 0.5% non-protein-N, 0.35% amino-N, 0.3% lipid, 15.9% salt, 1.5% glycogen and 6.1% ash (Table 3). The final recovered waste paste contained lower organic matter than that of oyster meat except for ash and salt. Particularly, the paste had much lower level of lipid and glycogen than that of oyster meat. It has been reported that only 2.4% glycogen lost into bled liquor from oyster (Kramer et al., 1962b). Lipid lost in liquor may have the same situation. The high content of salt in concentrated paste indicated that most of the salt-content solid release into the oyster liuqor. The result agrees with that observed by Kramer et al. (1962b).

The amino acid analysis showed that the concentrated oyster paste had a full spectrum of amino acids (data not shown). The predominate amino acids, in descending order, were aspartic acid, histidine, glutamic acid, leucine, arginine, alanine and proline. However, the predominate amino acids in oyster meat were glutamic acid, arginine, alanine, aspartic acid, methionine, serine, lysine, glycine and luecine (Burnette et al. 1979).

Table 3. Approximate composition of oyster waste paste and oyster meat

Composition (%)	Concentrated paste		Oyster meat	
	Wet Wt. basis	Dry Wt. basis	Wet Wt. basis	Dry Wt. basis
Moisture	55.15		82.86	
Total-N	4.18	9.32	2.18	12.72
Protein-N	3.67	8.18	1.94	11.32
Nonprotein-N	0.51	1.14	0.24	1.40
Amino-N	0.35	0.78	0.16	0.93
Lipid	0.28	0.62	1.57	9.16
Ash	15.90	35.45	1.19	6.94
Salt	6.14	13.69	0.48	2.80
Glycogen	1.54	3.43	2.74	15.98

CMP, AMP, Hyp and Ino were the major nucleotides and related components which were similar to freshly shucked oyster except that AMP was slightly reduced and Hyp was increased (Table 4). AMP and CMP also had been detected as predominate nucleotides in boiled crab meat by Hayashi et al. (1978). AMP, GMP and CMP were considered as taste-active components to boiled snow crab meat (Hayashi, et al. 1981).

Table 4. Nucleotides and related compounds of oyster waste pastes and oyster meat (μmole/g)

Nucleotide and related compound	Liquor paste	Fresh oyster meat	Commercial oyster meat
CMP	5.64	2.84	4.36
AMP	1.03	1.66	0.62
GMP	-	0.19	-
UMP	-	0.19	0.08
IMP	-	0.30	-
ADP	-	0.07	-
Ino	0.79	0.33	1.31
Hyp	1.69	0.25	1.17

CMP - Cytidine 5'-monophosphate
AMP - Adenosine 5'-monophosphate
GMP - Guanosine 5'-monophosphate
UMP - Uridine 5'-monophosphate
IMP - Inosine 5'-monophosphate
ADP - Adenosine 5'-diphosphate
Ino - Inosine
Hyp - Hypoxanthine

It was interesting that the fatty acid profile of concentrated paste was different from that of oyster meat (data not shown). The former contained higher non-ω_3 fatty acids while the latter contained higher ω_3 fatty acids. It is possible that the lipid components were bound to the tissue membrane of the oyster meat so that they would be difficult to release during shucking process and refrigerated storage. Another possiblity is that the high level of eicosapentaenoic acid (20:5 ω_3) and docosahexaenoic acid (22:6 ω_3) could be the substrates for enzymatic conversion to aldehyde and alcohol volatile aroma compounds in oyster (Josephson et al. 1984).

Characterization of ultrafiltrated concentrate

By ultrafiltration (with membrane filter of 10,000 nominal molecular weight cutoff (NMWC) oyster wash water was concentrated to ten or twenty fold concentration. The total solids were four or seven fold of the original wash water when wash water was concentrated to 10 or 20 fold concentration respectively (Table 5). Twenty fold concentrate had 18 fold protein increase and 5 fold nonprotein increase as compared with those of original waste water. Most protein had been recovered by ultrafiltration, but most nonprotein compounds lost in the filtrate.

Table 5. Comparison of chemical changes of oyster wash water before and after ultrafiltration with 10,000 nominal molecular weight cutoff membrane filter

Parameter	Unconcentrated wash water	10 fold Concentrations	20 fold Concentrations	Filtrate
pH	7.62	7.62	7.66	7.92
Total solids (%)	0.58	2.27	4.03	0.38
Total suspended solids(mg/ℓ)	403	1,925	4,683	10
COD (mg/ℓ)	1,320	6,580	19,200	700
Conductivity (μmhos/cm)	3,427	3,726	3,521	3,378
VB-N(mg%)	0.85	1.48	1.90	0.66
Total-N (%)	0.036	0.240	0.464	0.012
Protein-N(%)	0.022	0.202	0.395	0.002
Nonprotein-N(%)	0.014	0.038	0.069	0.010
Ash (%)	0.21	0.25	0.29	0.19
Salt (%)	0.15	0.15	0.13	0.15

Ultrafiltration treatment reduced COD and total suspended solids from 1,320 mg/ℓ and 403 mg/ℓ to 700 and 10 mg/ℓ respectively. Zn and Cu accumulated in concentrates, indicating that these two heavy metals might bind to the protein in the waste liquor (Table 6). Nevertheless, as compared to the heavy metals in oyster meat, waste liquors contained lower heavy metals (Burnette et al. 1979).

Table 6. Metals in original liquor and wash water

Metal (ppm)	Original liquor		Washed water	
	Unconcentrated	Concentrated	Unconcentrated	Concentrated
Cu	0.97	6.80	0.34	3.25
Cd	<0.01	0.10	0.02	0.04
Pb	0.05	0.17	0.03	0.07
Cr	0.05	0.27	0.03	0.07
Zn	34.9	248	7.74	64.2
Hg	<0.01	<0.01	<0.01	<0.01

Utilization. Oyster waste concentrates contain an adequate amount of proteins and nonprotein nitrogen compounds. These components and other flavor constituents not yet identified are flavor potentiators which make oyster waste concentrate a potential flavoring agent. Oyster sauce prepared from concentrated oyster broth with other ingredients has been widely used world-wide for centuries. Three brands of commercial oyster sauce were chemically analyzed and found to contain variable amounts of major components (Table 7). Oyster waste concentrate can be prepared by addition of other ingredients to make oyster sauce. Other products such as oyster broth and oyster flavor ingredients can also be prepared. The wash water was concentrated to 3 times its original solid content by steam-jacketed kettle. The concentrate has been prepared into oyster broth which has been accepted by sensory evaluation (data now shown). Fish sauce, a naturally fermented fish juice prepared from whole small fish containing over 20% salt, is a strong flavoring food condiment widely used in Southeast Asia.

To enhance flavor intensity, fermentation was applied on oyster liquor concentrate. A temperature of 15°C and addition of 10% NaCl were found to be the optimum conditions for fermentation. During fermentation, pH was decreased by 1 to 2 units at 3 months and was then stable until 6 months (Table 8). Non-protein N and volatile base N were increased as fermentation progressed. The increase in these types of N components would enhance the flavor and odor of the products.

Table 7. Characteristics of commercial oyster sauce products

Test	Products		
	A	B	C
pH	4.97	4.56	5.20
Moisture (%)	51.50	75.47	40.32
Total-N (%)	1.11	2.87	2.02
Nonprotein-N (%)	0.29	0.77	0.50
Protein-N (%)	0.82	2.10	1.52
VB-N (mg %)	21.21	28.42	14.70
Lipid (%)	0.74	4.29	2.15
Ash (%)	10.97	5.60	11.94
Salt (%)	10.36	4.82	7.91
Price (¢/oz)	8.13	22.60	12.90

Table 8. Chemical evaluation of oyster waste concentrate during fermentation

Test	Storage time (month)					
	Sample I			Sample II		
	0	3	6	0	3	6
pH	6.52	5.61	5.31	7.83	5.38	5.39
Moisture (%)	87.89	87.14	-	89.68	89.49	-
Ash (%)	9.07	9.27	-	9.24	9.17	-
Total-N (%)	0.47	0.44	0.50	0.11	0.10	0.10
Protein-N (%)	0.44	0.40	-	0.09	0.07	-
Non-protein-N (%)	0.03	0.04	0.30	0.01	0.03	0.04
VB-N (mg %)	4.13	7.70	13.60	2.10	3.70	4.68

The chemical evaluation of oyster waste concentrate during fermentation with the addition of Aspergillus oryzae (Koji form) is shown in Table 8. The addition of "koji" to the concentrate significantly accelerated hydrolysis when compared to that observed without addition of "koji" (Table 9). In a week of incubation, there was a significant increase in the rate of digestion as judged by the nonprotein-N and amino-N. The product possessed oyster flavor in the early stage of fermentation. After the first week of fermentation, the digestion gradually slowed and a more soy sauce-like sweet taste developed in the

product. A similar result was reported by Huynh (1982) in the fermentation of herring with "koji."

Table 9. Chemical evaluation of oyster waste concentrate during fermentation with the addition of Aspergillus oraze (5% "koji" form)

Test	Fermentation time (weeks)								
	At 15°C					At 25°C			
	0	1	3	5	7	1	3	5	7
pH	6.82	5.97	5.51	5.42	5.24	5.04	4.17	4.37	4.46
Total-N(%)	0.95	1.21	-	1.33	1.29	1.32	-	1.54	1.35
Protein-N(%)	0.64	0.78	-	0.16	0.19	0.14	-	0.33	0.18
Nonprotein-N(%)	0.31	1.03	-	1.17	1.10	1.18	-	1.21	1.17
Amino-N(%)	0.19	0.69	-	0.73	0.84	0.73	-	0.94	0.98
VB-N(mg%)	2.57	5.13	-	6.32	6.81	5.43	-	6.57	8.03

SUMMARY

Oyster waste liquors contain valuable soluble solids including proteins, non-protein N substances, nuleotides and related components. Millipore pellican ultrafiltration was demonstrated to be an effective method to recover the soluble solids. Heavy metal analyses indicated that oyster waste concentrate was safe and suitable for human consumption. Oyster sauce, oyster broth and flavor ingredients would be potential products of this recovered waste. Fermentation of recovered waste concentrate could further enhance the flavor of oyster sauce for the use as a food condiment.

REFERENCES

AOAC. 1984. "Official Methods of Analysis," 14th ed. Assoc. of Official Analytical Chemists. Washington, DC.

Barnett, S.M. and S.F. Lin. 1981. Recovery of by-products from seafood processing wastes. Pages 176-184. In W.S. Otwell, ed. Seafood Waste Management in the 1980's: Conference Proceedings. Florida Sea Grant College. Univ. of Florida.

Burnette, J.A., G.J. Flick, D.R. Ward and R.W. Young. 1979. Comparison of composition and selected enzyme activities in Crassostrea virginica and Crassostrea gigas, Eastern and Korean oysters. J. Food Protection. 42:251-255.

Carawan, R.E. and F.B. Thomas. 1981. Assisting North Carolina seafood processors in meeting water pollution requirements. Pages 10-25. In W.S. Otwell ed. Seafood Waste Management in the 1980's. Conference Proceeding. Florida Sea Grant College. Univ. of Florida.

Chai, T. and J. Foulds. 1974. Demonstration of a missing outer membrane protein in tolG mutants of Escherichia coli. J. Mol. Biol. 85:465-474.

Cobb, III, B.F., I. Alaniz and C.A. Thompson. 1973. Biochemical and microbial studies on shrimp. Volatile nitrogen and amino nitrogen analysis. J. Food Sci. 38:431-436.

Dubois, M., K.A. Gilas, J.K. Hamilton, P.A. Rebers and S. Fred. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28:350-356.

Food and Drug Administration. 1984. Bacteriological Analytical Manual. 6th ed. Association of Official Analytical Chemists. Washington, DC.

Gould, E. and J.A. Peters. 1971. On testing the freshness of frozen fish. Fishing News Ltd., London.

Hang, Y.D., E.E. Woodams and G.F. Parsons. 1980. Isolation and chemical evaluation of protein from clam wash water. J. Food Sci. 45:1040-1041.

Hayashi, T., K. Yamaguchi and S. Konosu. 1978. Studies on flavor components in boiled crabs II. Nucleotides and organic bases in extracts. Bull. Jpn. Soc. Sci. Fish. 44:1357-1362.

Hayashi, T., K. Yamaguchi and S. Konosu. 1981. Sensory analysis of taste-active components in the extract of boiled snow crab meat. J. Food Sci. 46:479-483.

Huynh, M.D. 1982. Fish sauce production through fermentation: A method to utilize fishery waste. Technical Report No. 8. Fisheries Technology Division, B.C. Research. Vancouver, Canada.

Jhaveri, S.N. 1984. Evaluation of processes for recovery of functional proteins from clam processing effluent. Ph.D. Dissertation. University of Rhode Island.

Joh, Y. and L.F. Hood. 1979. Preparation and properties of dehydrated clam flavor from clam processing wash water. J. Food Sci. 44: 1612-1614.

Johnson, R.A. and K.L. Lindley. 1981. Removal of suspended solids from seafood processing waste waters. Pages 116-122. In: W.S. Otwell, ed. Seafood Waste Management in the 1980's: Conference Proceedings. Florida Sea Grant College. Univ. of Florida.

Josephson, D.B., R.C. Lindsay and D.A. Stuiber. 1984. Biogenesis of lipid-derived volatile aroma compounds in the emerald skiner (Notropis atherinoides). J. Ag. Food Chem. 31:326-330.

Kramer, A., B.H. Willier, R. Edge, H.R. Smith, A.W. Tubman, C.F. Lee and C. Toompas. 1962a. The government industry cooperative oyster research program. Part I. History and methodology. J. Assoc. Offc. Anal. Chem. 45:262-275.

Kramer, A., B.H. Willier, R. Edge, H.R. Smith, A.W. Tubman, C.F. Lee and Toompas, C. 1962b. The government industry cooperative oyster research program. Part II. Native oyster studies. J. Assoc. Offc. Anal. Chem. 45:565-577.

U.S. Environmental Protection. 1979. Method for Chemical Analysis of Water and Wastes. U.S. Environmental Protection Agency. Cincinnati, Ohio.

ACKNOWLEDGEMENT

This work was supported in part by a Sea Grant Project of Grant No. NA 86-AA-D-SG006 funded by the National Sea Grant Program, NOAA.

PRELIMINARY ASSESSMENTS OF MULLET CARCASS BYPRODUCTS GENERATED BY THE HOLLOWAY PROCESS

Brian E. Perkins
Alabama Sea Grant Extension Service
3940 Government Boulevard
Mobile, AL 36693

and

Michael H. Eley, Clifford C. Holloway, and Melvin V. Kilgore, Jr.
Johnson Research Center, Biotechnology Laboratory
The University of Alabama in Huntsville
Huntsville, AL 35899

ABSTRACT

Female roe mullet carcasses were digested in the Holloway Processor, a pressure chamber, by using saturated steam at 60 psig (300 ° F) with continuous mixing for 30 minutes. The resulting liquid (soluble) and solid (flesh, bone, and viscera) byproducts contained (on a wet weight basis) 6.25% and 15.7% protein, respectively. These two byproducts possess good potential for further refinement into feed and fertilizer ingredients.

INTRODUCTION

Processing of female roe mullet produces average roe and gizzard yields of 17% and 1% by weight, respectively. Over 1.35 million pounds of female roe mullet carcasses were generated by Alabama processors in 1986.

A small amount (6%) of the female roe carcasses found their way into human food channels, although their overall poor quality usually precluded this as a viable outlet. Traditional outlets for female roe mullet carcasses have included landfilling or freezing for future use as crab bait.

Landfilling of carcasses has become quite unpopular. Many municipalities and operators of private sanitary landfills have closed their gates to any type of seafood processing waste. Others have imposed substantial surcharges, which have made landfilling uneconomic. In the case of roe mullet processors in southern Mobile County, the nearest sanitary landfill is 50 miles away. The daily 100-mile round trip has likewise proven an economic hardship. Thus, no female roe mullet carcasses were landfilled by Alabama processors during 1986.

Over 1.2 million pounds of carcasses were placed in frozen storage by Alabama's mullet roe processors in 1986. Crab bait is worth only 6 cents - 7 cents per pound, and frozen mullet must sometimes be held for up to 6 months before requests from crab fishermen are received. With frozen storage costs averaging 12 cents/lb for six months, economic losses of 5 cents - 6 cents/lb are not unusual.

A cooperative project was undertaken by the Johnson Research Center and the Alabama Sea Grant Extension Service with funds provided by the Alabama Department of Economic and Community Affairs, Science, Technology, and Energy Division, under Contract No. ADECA-1EX-WWS-8607. The project objectives were to:

- Evaluate the effectiveness of the Holloway Process for treatment of female roe mullet carcasses.
- Quantify chemical composition of recovered materials.
- Determine economic potential of recovered materials for feed and fertilizer applications.

METHODS

The Holloway Processor

Diagrammatic representations of the Holloway Processor are presented in Appendix Figures 1 and 2. The patented process was developed by Urban Waste Resources (UWR) of Birmingham, Alabama for the treatment of municipal solid waste (MSW) and sewage sludge. The process includes a pressure steam pretreatment of MSW, with water or sewage sludge as the wetting agent, to yield a sterile cellulosic fraction and recyclable metals and glass. The process can potentially reduce the amount of processible materials currently being landfilled about 80% by weight and 90% by volume (Urban Waste Resources, No Date).

Energy to run the Holloway Process consists of electricity used to operate the loading conveyor and processor agitator (440 V, 3 phase) and to operate the boiler water pump and air induction fan (220V, single phase). Process energy also consists of saturated steam. The boiler is a 30 bhp package boiler fueled by No. 2 fuel oil. The boiler is operated at 100 psig, but the process steam is regulated down to 60 psig at the process unit. The quantity of steam used is measured by metering the boiler feed water. The saturated steam at 60 psig (300 ^{o} F) is injected directly into the process chamber.

Sample Collection and Processing

Five sample collections were made during the period from November 4, 1986 through December 4, 1986. The mullet had been netted along the Gulf

Coast and brought to a processing plant in the Mobile area. The roe sacks and gizzards were removed at the processing plant, and the remaining carcasses were transported from the fish processing plant to the University of Alabama in Huntsville (UAH) in 55-gallon drums in ice. After arriving in Huntsville, the ice and water were drained from the fish, and the fish were weighed before processing. The average weight of each fish carcass was 1.38 lb. A total of 2,556 pounds of fish were processed.

For processing at UAH, the Holloway Process unit was preheated, and the whole carcasses were loaded into the process unit. No water or other components were added since the moisture content of the fish was sufficient for processing. The process unit was sealed, and saturated steam was directly injected into the process unit containing the fish carcasses, with gentle agitation to insure uniform heat distribution. The steam was continously injected, and agitation maintained during the heat-up period to 55 psig, and during the cook cycle, which lasted 30-60 minutes after reaching 55-60 psig.

At the end of the cook cycle, the process unit was depressurized, and the unit contents were removed by draining into 55 gallon drums. In a cooking time of 30 minutes or more, the fish carcasses were cooked completely to a fine stew with fish scales and vertebrae being the only identifiable pieces. All of the material passed through a 1/2" screen.

The collected material was weighed and filtered through a 120-mesh screen to separate liquid and solid fractions. Small quantities of the liquid fraction were further separated into an oil layer and an aqueous layer by centrifugation. Generally, oil was collected by decanting from the top of the liquid fraction. Samples of liquid and solid fractions were collected in sterilized mason freezer jars. All samples were stored at -20 ° F until analyzed.

Electricity and fuel oil consumptions were recorded for each processing run. Steam usage was also monitored and recorded.

Total Kjedahl Nitrogen, Ammonium Nitrogen, and Moisture

Five TKN analyses were performed on samples of fish liquid and fish solids. All TKN analyses were performed according to standard methods (AOAC, 1980) and (EPA, 1983).

Three Ammonium Nitrogen analyses were performed on samples of fish liquid and fish solids. Ammonium Nitrogen analyses were performed according to a standard method (AOAC, 1980).

Three moisture determinations were performed on samples of fish solids. Moisture analyses were performed according to a standard method (EPA, 1981).

Total Organic Carbon

Samples of fish solids and fish liquid were analyzed for Total Organic Carbon (TOC) on two occasions. All TOC analyses were conducted

on an Astro Model 2001 analyzer (UV/persulfate decomposition with IR detection) according to Method #EPA-415.2 (EPA, 1983).

Chemical Oxygen Demand

Chemical Oxygen Demand (COD) determinations were conducted on two occasions on samples of fish solids and fish liquid. All COD determinations were performed according to Method #EPA-410.1 (EPA, 1983).

Elemental Analysis

Elemental (or mineral) analyses were conducted on two occasions on samples of fish solids and fish liquid. Elemental composition was determined by the Inductively Coupled Plasma (ICP) technique according to Method #EPA-200.7 (EPA, 1983).

Fatty Acids

Small amounts of oil recovered from fish liquid samples were analyzed for fatty acid composition. Extracts were prepared from the fish oil, and methyl esters of the extracts were analyzed on a Hewlett-Packard Model #5890-A GC - Flame Ionization detector using a 30 m X 25 mm capillary column according to instructions in the GC - FI operation manual (Hewlett - Packard, No Date).

RESULTS AND DISCUSSION

Energy Usage

Appendix Figure 3 provideds a schematic representation of average energy and material flows. For processing, the amount of steam required to pre-heat and cook the fish averaged 500-600 pounds per batch operation. Thus, steam usage averaged about 1 pound per pound of fish. During processing, about 50% of the steam was condensed into the final product, and the remaining 50% of the steam was vented to the atmosphere during depressurization.

The amount of electricity used averaged only 1-2 KWH per batch operation. The amount of #2 fuel oil consumed by the boiler, which operated at 80% efficiency, averaged 5-6 gallons per batch. Based on current prices of electricity and fuel oil, total energy costs averaged $5 per batch, or about 1 cent per pound of fish.

This cost, although small, can be reduced by increasing the quantity of fish per batch. Steam consumption was reduced to only 0.62 pounds of steam per pound of fish when 861 pounds of fish were processed. Reduced

steam usage also resulted in less aqueous liquid and higher solids yields in the products.

Energy consumption, both as electricity and fuel oil, averaged 1160 Btu per pound of fish. By increasing the quantity of fish per batch to 861 pounds, energy consumption was reduced to 750 Btu per pound of fish.

Material Recoveries

A combined total of 2,556 pounds of mullet carcasses were processed in five batch runs. The smallest quantity processed was 263 pounds, the largest quantity processed was 861 pounds, and the average was 511 pounds per run. A watery fish stew was initially obtained from the test runs. The material showed an increase in weight of 50% - 60% over the original fish weight due to the steam condensate.

Liquid and solid fractions were obtained after filtering on a 120-mesh screen. The yield of filtered solids obtained varied from one run to another, but was in the range of 200-300 pounds per run. The yield of the liquid fraction also varied from one run to another, but was approximately equal to the original weight of the fish processed.

The liquid fraction could also be separated into an oil layer and an aqueous layer upon centrifugation. The oil represented 0.5% - 1.0% of the liquid fraction. The aqueous layer also contained some additional suspended solids that precipitated during centrifugation.

Nitrogen

The results of Total Kjedahl Nitrogen (TKN) and Ammonium Nitrogen analyses of fish solid and fish liquid samples are presented in Table 1. Calculated Total Organic Nitrogen (TON) and protein contents are also presented in Table 1. Total Organic Nitrogen was calculated as (TON = TKN - Ammonium). Protein was calculated as (Protein = TON x 6.25).

Table 1. Nitrogen Contents.

Fraction		TKN	Ammonium	TON	Protein
Fish Solids	Range,ppm	6,300-32,400	177-198	6,114-32,217	38,212-201,356
	Mean,ppm	25,305	186	25,119	156,996
	Std Dev	10,854	7.6	10,855	67,847
Fish Liquid	Range,mg/l	10,000-10,750	224-291	9,743-10,494	60,894-65,588
	Mean,mg/l	10,250	257	9,993	62,455
	Std Dev	300	24	296	1850

Results from four out of five fish solid TKN analyses performed were in very good agreement, ranging from 26,400 ppm through 32,400 ppm. One

low data point (TKN = 6,300 ppm) lowered the mean TKN to 25,305 ppm. The low value may have resulted from high percent moisture in the fish solid fraction analyzed, or simply from analytical error. Fish solid fraction ammonium data, however, agreed very well. These data yield a calculated mean fish solid protein content of 15.69% on a wet weight basis.

Results from the five TKN analyses performed on fish liquid fractions were very close. Fish liquid ammonium data likewise agreed very well. These data yielded a calculated mean fish liquid protein content of 6.25% on a wet weight basis.

Moisture

The results of moisture determinations for fish solid fractions are presented in Table 2:

Table 2. Moisture.

	Range, %	Mean, %	Standard Deviation
Fish Solids	59.0 - 61.9	60.17	1.53

Table 1 lists fish solid mean protein concentration as 15.69% on a wet weight basis. That figure translates to a mean of 39.4% on a dry weight basis. Actual dry-weight protein contents would have been greater, but one poor TKN data point caused the mean percent protein of all other fish solid samples to be lowered from 18.6% on a wet weight basis and from 46.8% on a dry weight basis.

Total Organic Carbon

The results of Total Organic Carbon (TOC) determinations for fish solid and fish liquid samples are presented in Table 3:

Table 3. Total Organic Carbon.

	Range, mg/g	Mean, mg/g	Standard Deviation
Fish Solids	118 - 149	134	21.9
Fish Liquid	27 - 35	31	5.7

The TOC data for fish solids and fish liquid samples exhibited much less variability than did the corresponding TKN values. TOC may be extrapolated to represent the total amount of organic material present by the formula: Total organic material = (TOC x 2)

Therefore, fish solid and fish liquid samples contained calculated mean organic contents of 26.8% and 6.2%, respectively. Noting the close

agreement between percent protein and percent organic matter contained in fish liquid samples, it appears that almost all of the organic matter in the fish liquid was composed of soluble proteinaceous materials.

Chemical Oxygen Demand

The results of Chemical Oxygen Demand (COD) analyses for fish solid and fish liquid samples are presented in Table 4:

Table 4. Chemical Oxygen Demand.

	Range, mg/g	Mean, mg/g	Standard Deviation
Fish Solids	439 - 497	468	41.0
Fish Liquid	74.6 - 79.3	77	3.3

COD values for fish solid and fish liquid samples were quite reproducible (note small standard deviations). Furthermore, the ratio of mean fish liquid COD to total COD (77 mg/g)/(545 mg/g) = .141 was very nearly equal to the ratio of mean fish liquid TOC to total TOC (31 mg/g)/(165 mg/g) = .187. The relative proximity of these two ratios illustrates that the COD analyses and TOC analyses are mutually supportive, and indicates a high degree of reliability in both data sets.

Elemental Analysis

Elemental analysis indicated that the various products derived from subjecting mullet carcasses to the Holloway Process are good sources of nutritive elements which contain low concentrations of toxic minerals. The results of elemental analysis of fish solid and fish liquid samples are presented in Appendix Table 1.

Many of the ranges were variable, probably due to the small number of analyses performed. However, the values in Appendix Table 1 provide approximations of elemental concentrations which can be found in fish solids and fish liquid.

The fish liquid was quite similar in appearance to commercially available fish emulsion fertilizer. The ratio of Nitrogen to Phosphorus and Potassium in the commercial fish emulsion is 5:1:1. The ratio of TON to Phosphorus and Potassium in the mullet fish liquid derived from the Holloway Process was 20:1:4. This indicates that the fish liquid is a nutrient-rich product with good potential for further refinement into a competitive commercial liquid horticultural fertilizer.

Fish emulsion fertilizer is used to a lesser extent as an ingredient in rations fed to aquacultured larval crustaceans. The nitrogen (or protein) content is of primary interest in this application. Dietary

protein supplements reduce the degree of cannibalism in larval crustacean rearing tanks, thereby increasing overall production. However, the concentration of Potassium in the mullet fish liquid may preclude its use as an ingredient in larval crustacean diets. Further research would be necessary to determine its suitability for that application.

The fish solids were quite similar in appearance to canned cat foods. The mean Sodium content was 805 mg/1,000 grams, or slightly more than the Sodium content found in raw mullet flesh (700 mg/1,000 grams). The mean Iron content was 486 mg/1,000 grams, which was substantially more than is normally found in raw mullet flesh (17 mg/1,000 grams). The authors believe that the carbon steel wall of the Holloway Processor contributed to the elevated level of Iron noted in the mullet fish solids. The mullet fish solids also contain significant amounts of dietary Calcium (1,303 mg/1,000 grams). Thus, the types and amounts of minerals found in the mullet fish solid fraction indicate that it has good potential for inclusion in canned pet foods.

The ratio of Total Organic Nitrogen to Phosphorus and Potassium in the mullet fish solids was 18:1:2. Additional drying would yield a fertilizer - grade meal which would be excellent for lawn - greening applications. The rather low Phosphorus and Potassium contents would also allow the mullet fish solids to be used in catfish feed formulations as a supplement or replacement for menhaden meal.

Fatty Acids

The results of fatty acid analyses performed on sub - units of fish oil recovered from fish liquid samples are presented in Appendix Table 2. All values totalled 99.88% of the weight of fatty acids present.

The predominant fatty acid found in mullet fish oil samples was 17:1, which represented 64.9% of the total fatty acid content. Although this is basically consistent with prior research on mullet fatty acid composition (Deng et al, 1976), the previous study dealt with raw mullet flesh, and noted that 17:1 fatty acids were present as only 10.7% of the total fatty acid content. Another study (NMFS, 1987) failed to find 17:1 fatty acids present in concentrations greater than 1% in raw mullet flesh. They postulated that such a wide variation may have been due to differences in diets consumed in various geographic locations.

The second most prevalent fatty acid noted in the present study was 15:1, which accounted for 15.6% of the total fatty acid content. Neither of the prior studies (Deng et al, 1976) (NMFS, 1987) reported the presence of any 15:1 fatty acids. Several research efforts (Gruger, 1967) (Deng et al, 1976) (NMFS, 1987) indicate that mullet are, uniquely enough, composed of a large percentage of odd carbon - chained fatty acids which vary according to maturity, size, sex, and the availability and nature of the food supply. Thus, extremely variable mullet fatty acid contents do not appear unusual.

The third fatty acid found in oil extracted from the mullet fish liquid was 16:1, which accounted for 6.8% of the total fatty acid content. One prior study (NMFS, 1987) identified 16:1 fatty acids as the

second most prevalent in mullet, with 16:1 contents ranging between 4.1% and 18.0% of the total fatty acid content. The percentage of 16:1 fatty acids noted in the present study falls within the range noted in the prior study, although its degree of importance is reduced somewhat.

CONCLUSIONS

Based on the results of the research presented in this report, the authors offer the following conclusions:

- The energy costs for digesting mullet carcasses in the Holloway Processor are minimal (1 cent/lb).

- Screened solids resulting from digestion of mullet carcasses in the Holloway Processor occupy only half of the volume of the original mullet carcasses.

- The liquid fraction resulting from digestion of mullet carcasses in the Holloway Processor is equivalent in weight to the weight of the original mullet carcasses.

- The oil fraction derived from the mullet fish liquid was recovered at the rate of 0.5% - 1.0%.

- The protein and mineral contents of the mullet fish liquid fraction indicate that it has particularly good potential for further refinement into a commercial liquid horticultural fertilizer.

- The protein and mineral contents of the mullet fish solid fraction indicate that it has particularly good potential for inclusion as an ingredient in canned pet foodds. Additional drying to a meal would allow its inclusion in various aquaculture rations.

- The oil fraction contained substantial amounts of 17:1, 15:1, and 16:1 fatty acids. Although these predominant fatty acids are not the same as the predominant mullet fatty acids reported by other researchers, the natural variability of mullet fatty acid composition makes it difficult to hypothesize any commercially viable use for mullet oil.

ACKNOWLEDGEMENTS

The contributions of the following organizations and individuals are gratefully acknowledged:

- Alabama Department of Economic and Community Affairs, Science, Technology, and Energy Division, for providing project funding.
- Auburn University/Alabama Cooperative Extension Service, for providing matching funds.
- Robert Chen, A & A Seafood, for providing samples.
- Brenda D. Lukefahr, UAH, for assistance with sample processing and analysis.
- W. H. Tippins, Jr., UAH. for assistance with sample collection and processing.
- Tim Hester, UAH for assistance with sample processing and analysis.

LITERATURE CITED

AOAC. 1980. Official Methods of the Association of Official Analytical Chemists. 13th Edition. Arlington, VA.

Deng, J.C., F.T. Orthoefer, R.A. Dennison, and M. Watson. 1976. Lipids and fatty acids in mullet (Mugil cephalus): Seasonal and locational variations. J. Food Sci. 41:1479-1483.

EPA. 1981. Procedures for Handling and Chemical Analysis of Sediment and Water Samples. Cincinnati, OH.

EPA. 1983. Methods for Chemical Analysis of Water and Wastes. EPA - 600/4-79-020. Cincinnati, OH.

Gruger, E.H. 1967. Fatty acid composition. In: Stansby, M., ed. Fish Oils: Their Chemistry, Technology, Stability, Nutritional Properties, and Uses. pp. 3 - 30. AVI Publishing Company. Westport, CT.

Hewlett - Packard. No Date. Operations Manual for Hewlett - Packard Model #5890-A Gas Chromatograph - Flame Ionization Detector. Hewlett - Packard Corporation.

NMFS. 1987. Proximate and Fatty Acid Composition of 40 Southeastern U. S. Finfish Species. NOAA Technical Report NMFS 54. NTIS. Springfield, VA.

Urban Waste Resources. No Date. The Holloway Processor: An Economic Alternative to Landfill and Burning MSW. Urban Waste Resources, Inc. 1141 Regent Dr., Birmingham, AL 35226.

ACKNOWLEDGEMENT STATEMENT

"This (material) was prepared with the support of the U.S. Department of Energy (DOE) Grant No. DE FG-44-80R410074. However, any opinions, findings, conclusions, or recommendations expressed herin are those of the authors (s) and do not necessarily reflect the views of DOE."

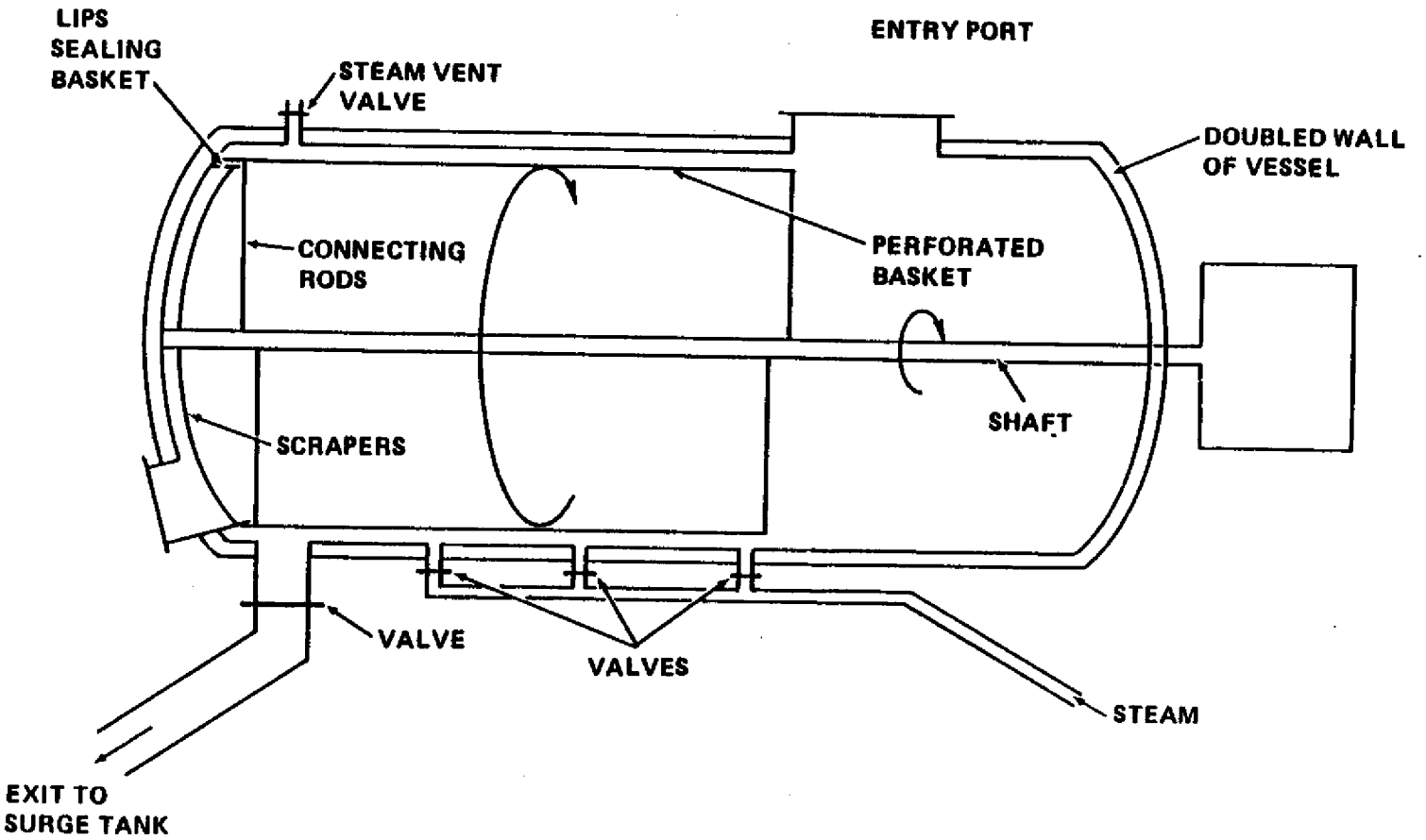
Appendix Table 1. Elemental Anaylsis.

		Fish Solids mg/kg	Fish Liquid mg/l
ZN	Range	26.0 - 45.3	.04 - 3.76
	Mean	35.6	1.9
	Std Dev	13.6	2.6
CD	Range	.04 - .08	.01 - .35
	Mean	.06	.18
	Std Dev	.03	.24
CU	Range	4.58 - 26.4	.08 - 6.87
	Mean	15.5	3.48
	Std Dev	15.4	4.8
NI	Range	.59 - .63	.02 - .14
	Mean	.61	.08
	Std Dev	.03	.08
FE	Range	257 - 716	16.4 - 17.1
	Mean	486	16.8
	Std Dev	324	0.5
CR	Range	.98 - 1.76	.01 - .02
	Mean	1.37	.015
	Std Dev	.55	.007
MG	Range	350 - 539	60.2 - 72.8
	Mean	444	66.5
	Std Dev	134	8.9
CA	Range	1032 - 1574	76.5 - 165
	Mean	1303	120.8
	Std Dev	383	62.6
P	Range	1229 - 1563	382 - 617
	Mean	1396	499
	Std Dev	236	166
K	Range	2467 - 2476	2153 - 2169
	Mean	2472	2161
	Std Dev	6.4	11.3
NA	Range	650 - 960	572 - 823
	Mean	805	698
	Std Dev	219	177

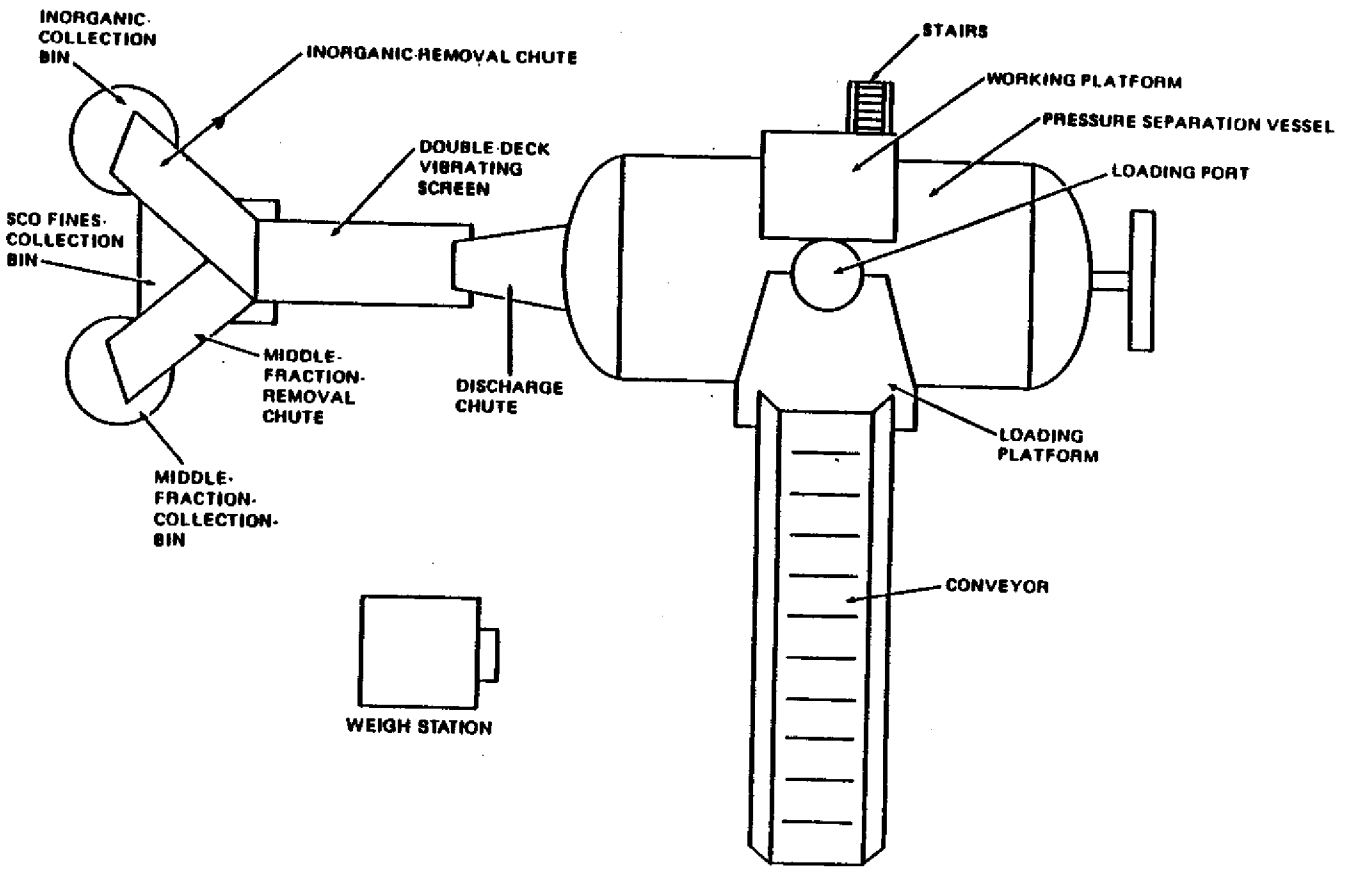
Appendix Table 2. Mullet Oil Fatty Acids.

	Fatty Acid	Weight%
	9:0	.02
R	10:0	.01
R	11:0 Antesiso	.01
R	11:0	.02
	12:1	.01
R	12:0	.06
	13:1	.02
R	13:0	.08
	12:1 3 OH	.55
R	14:0 Iso	.01
	13:0 Iso 2 OH	.02
	14:1	.08
R	14:0	1.69
	15:1 Iso F	15.48
	15:0 Iso	.09
R	15:0 Anteiso	.03
	15:1 A	.05
	15:1 B	.04
R	15:0	1.13
	14:0 2 OH	.53
	16:1 Iso E	6.18
R	16:0 Iso	.05
	16:0 Anteiso	.94
R	16:1 trans	.56
	16:1 cis	.06
	16:0	5.26
	17:1 Iso	64.48
	17:1 cis 10	.46
	17:0 cyclo	.01
	16:0 Iso 3 OH	.75
	18:1 Iso F	.35
	18:1 trans 11	.82
R	19:0 cyclo	.01
	18:0 2 OH	.02
	Total	99.88

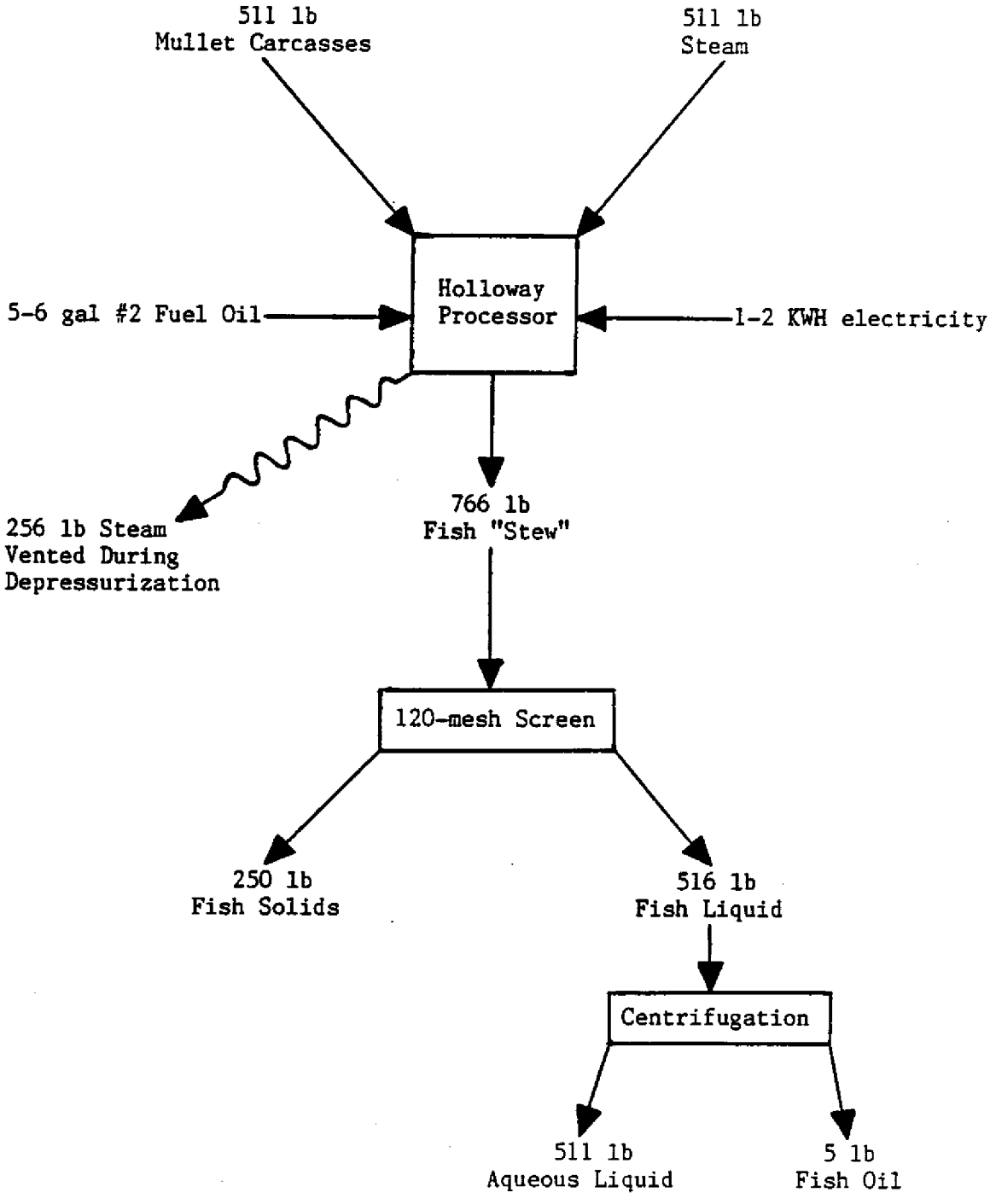
APPENDIX FIGURE 1. CROSS SECTION OF PROCESSOR



APPENDIX FIGURE 2. **Configuration of the Holloway processor.**



Appendix Figure 3. Average Energy and Material Flows.



QUALITY OF PREVIOUSLY FROZEN OYSTERS REPACKED FOR THE FRESH MARKET.

Cameron R. Hackney, Thomas E. Rippen and Lisa K. Sanders

Virginia Polytechnic Institute and State University
Seafood Agricultural Experiment Station
102 S. King St., P.O. Box 369
Hampton, Virginia 23669

INTRODUCTION

There are advantages to freezing oysters and repacking them for the fresh market when demand is high. The demand for oysters is seasonal, with the highest demand occurring during Thanksgiving and Christmas holidays. The price is of course very high at this time. If oysters are bought at lower prices during October and early November and frozen, they can be thawed and repacked to meet the holiday demand.

Prior to 1986, repacking of previously frozen oysters was not permitted by Part II of the National Shellfish Program's Manual of Operations (FDA 1986). The public health explanation given is "If shellfish are thawed during repacking, high bacterial counts and accelerated product deterioration may result". However, in reality freezing usually decreases the number of bacteria in a product. Certain changes do occur during freezing of oysters. Frozen oysters may darken during storage and the drained weight is decreased (Desrosier and Tressler, 1977). Packaging is an important consideration in the freezing of oysters. The packaging material must lend itself to freezing and must be impervious to moisture and oxygen transfer. Of course freezing should not be used as a means to salvage oysters near the end of their expected shelf-life. In order to have a good product, the oysters should be frozen as soon after shucking as possible.

The objective of this research was to compare the quality of previously frozen oysters to fresh during storage and to determine if it is preferable to freeze oysters before or after blowing.

MATERIALS AND METHODS

Oysters were purchased either fresh or frozen from a local processor. The oysters were shucked at anyone of several locations and brought to a central location for freezing. Fresh oysters were obtained from the same lots as the frozen oysters. They were brought to the laboratory in one gallon containers, drained, repacked in 12 ounce jars and used in shelf-life studies. For freezing, one half gallon of the shucked oysters were drained, and transferred to 3 ml-thick poly bags. The bags were impermeable to oxygen. Air was squeezed from the bags and the bags were sealed. The bags were laid flat and the maximum thickness was less than 3 inches. The bags were frozen at -40°C and were frozen in

less than two hours. The bags of oysters were allowed to stay overnight at -40°C and were then shipped frozen to the laboratory, where they were held at -20°C until thawing and repacking. Lot 1 was stored frozen for 2, 6, and 8 months before testing. Lots 2-5 were held for 1-3 months before testing.

Shelf-life studies

Fresh oysters were tested at approximately 2 day intervals for 12-13 days for their sensory and microbiological quality. In addition the samples were analyzed for pH. Raw and cooked samples were subjected to sensory analysis by a 9 member trained panel. The raw samples were placed into odorless, clear glass containers and the panelists were asked to evaluate them for odor, appearance and texture. The cooked samples were individually wrapped in aluminum foil and steamed for 15 minutes. The samples were evaluated for odor, appearance, taste and texture. The panelists used a 9 point scale ranging from excellent(9) to inedible (1).

The aerobic plate counts of the oysters were determined by the procedures of APHA (1984). The oysters were drained and blended without liquor and the appropriate dilution was pour plated with plate count agar and incubated for 4 days at 20°C. The pH of the samples was determined on the undiluted homogenate.

Frozen oysters were thawed in ice water and the drain weight and free liquor content were determined by the procedure of AOAC (2), using sterile skimmers. Samples included oysters that were blown prior to freezing and non-blown oysters. The oysters that were blown before freezing were drained two minutes and reweighed. The drained oysters were packed into 12 ounce jars for shelf-life studies described above.

Non-blown oysters were blown prior to repacking. One gallon of oysters were added to 35 l of water in a plastic carboy. The water was chlorinated to 50 ppm. Air was pumped into the carboy from the bottom. The oysters were agitated in the blow tank for 10 minutes; then air was shut off. Fresh water was added and allowed to overflow the tank for an additional 15 minutes. The wash water was clear before oysters were removed. The oysters were collected, drained and weighed to determine weight gain. The oysters were then packed into 12 ounce jars for shelf-life studies as described above.

A portion of each sample of non-blown, blown before freezing, blown after freezing, and fresh samples was tested for total solids by procedures of the AOAC (1980) and for NaCl content by the method of Hackney et al. (1988).

Statistical analysis was done by the method outlined by Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

The quality of previously frozen oysters was compared to fresh

oysters over the expected shelf-life of the products. The oysters were either blown before or after freezing. The non-blown oysters were significantly ($p>0.05$) higher in total solids and NaCl than the blown oysters (Data not shown). However, when the non-blown oysters were blown in the laboratory after freezing, there was no significant difference in either NaCl or total solids content. Also, there was no significant difference between the frozen and fresh oysters ($p <0.05\%$) with respect to NaCl and total solids.

The previously frozen oysters were evaluated in eight different storage trials. Trials one through four were from the same batch of oysters stored for different periods of time over 10 months. Trials four through eight were from different batches of oysters processed on different days. Since there were no differences between trials for the previously frozen, repacked oysters during storage, the trials were combined. There was no significant difference ($p <0.05\%$) in the APC of the previously frozen oysters over storage (Figure 1). The mean APC was significantly ($p>0.05$) higher for the fresh oysters, indicating that freezing was lethal to some of the bacteria. The initial count of the oysters that were frozen non-blown and blown in the laboratory after thawing, was lower. The wash water was chlorinated and this may have affected the counts.

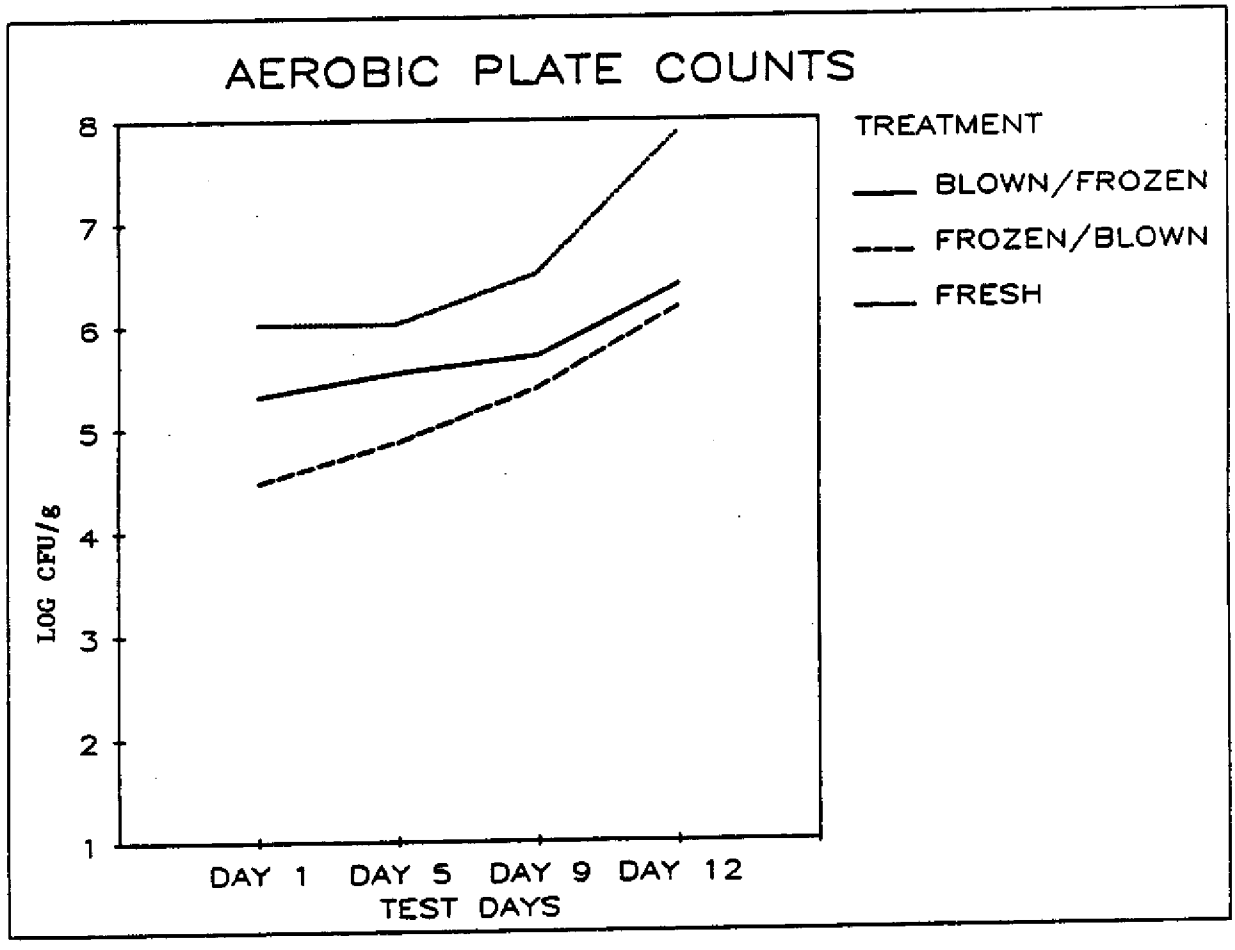
The initial pH of the frozen oysters was significantly lower than that of the fresh oysters (Figure 2). It is well known that solutes are concentrated during freezing, which can lower pH. The pH values of the previously frozen oysters remained about the same throughout storage; and the pH value of the oysters blown after freezing actually increased (although not significantly) during storage. This is the opposite pattern usually observed with oysters and may have been due to acid producing bacteria being sensitive to freezing and/or chlorine.

The overall sensory scale for raw oysters is a combination of the odor, appearance and texture scores. The overall sensory score was very similar, for the fresh and previously frozen oysters (Figure 3). The previously frozen oysters were a little darker, but not significantly so. The oysters which were frozen non-blown and later blown in the laboratory were lighter then the oysters blown before freezing. When the oysters were tested with a colorimeter, using the LAB scale, the laboratory blown oysters had higher L values and lower B values. Unfortunately this test was only performed during the last two trials and there wasn't enough data for statistical analysis.

The overall cooked sensory score, which was a combination of the odor, taste, texture and appearance scores, for the steamed oysters was also quite similar to the fresh and previously frozen oysters and differences were not significant ($p<0.01$) (Figure 4). This indicates that the previously frozen oysters would be acceptable to the consumer.

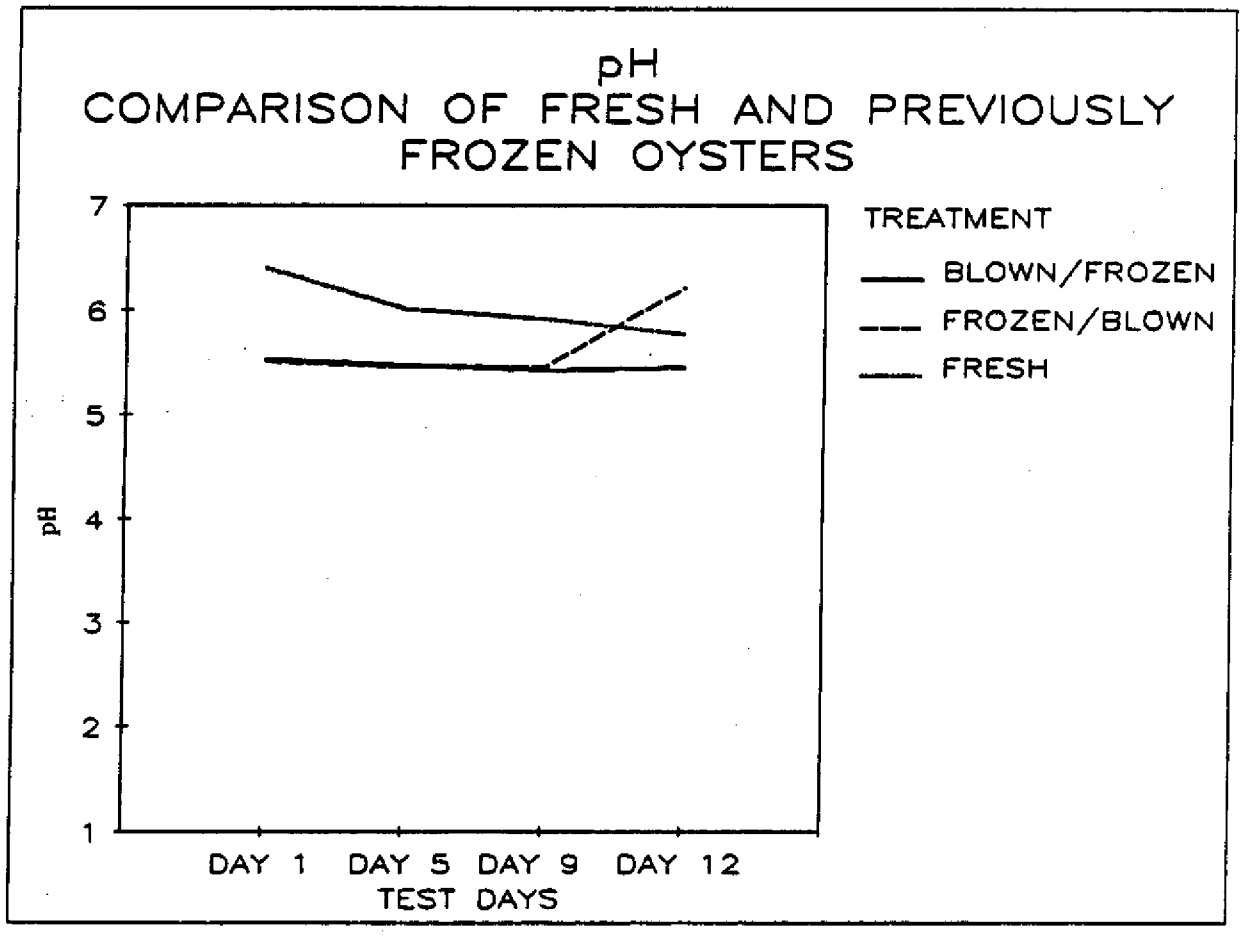
This work indicates that previously frozen oysters would be an acceptable product from a microbiological and sensory standpoint. The quick freezing method used in this study minimized the darkening often encountered with previously frozen oysters and a gentle blowing in 50-100 ppm chlorine could serve to lighten the color. The shelf-life of the

Figure 1. Overall mean of the aerobic plate counts for previously frozen and fresh oysters during storage[a].



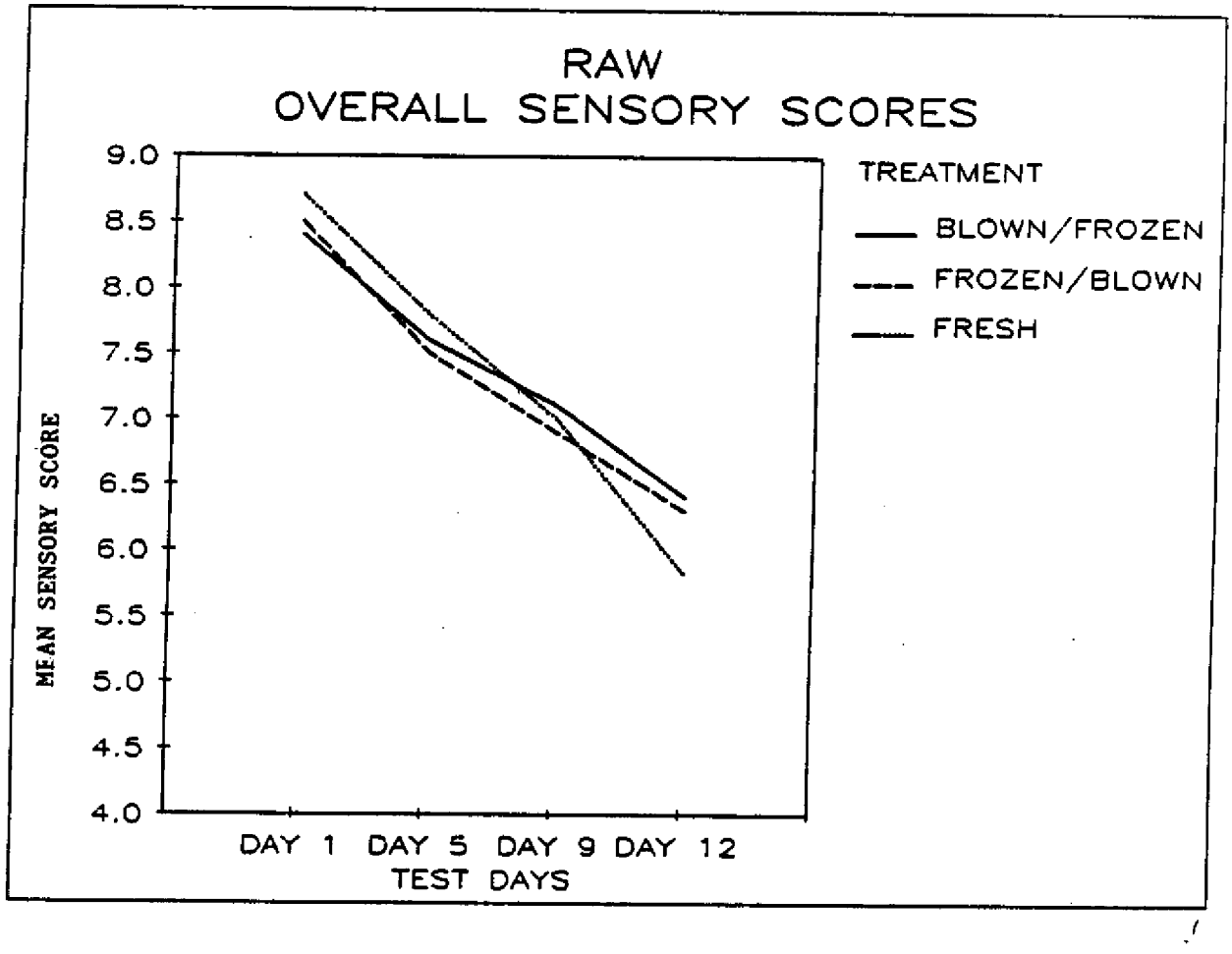
[a]Each data point is the mean of 8 replicates

Figure 2. Overall mean of the pH of previously frozen and fresh oysters during storage[a].



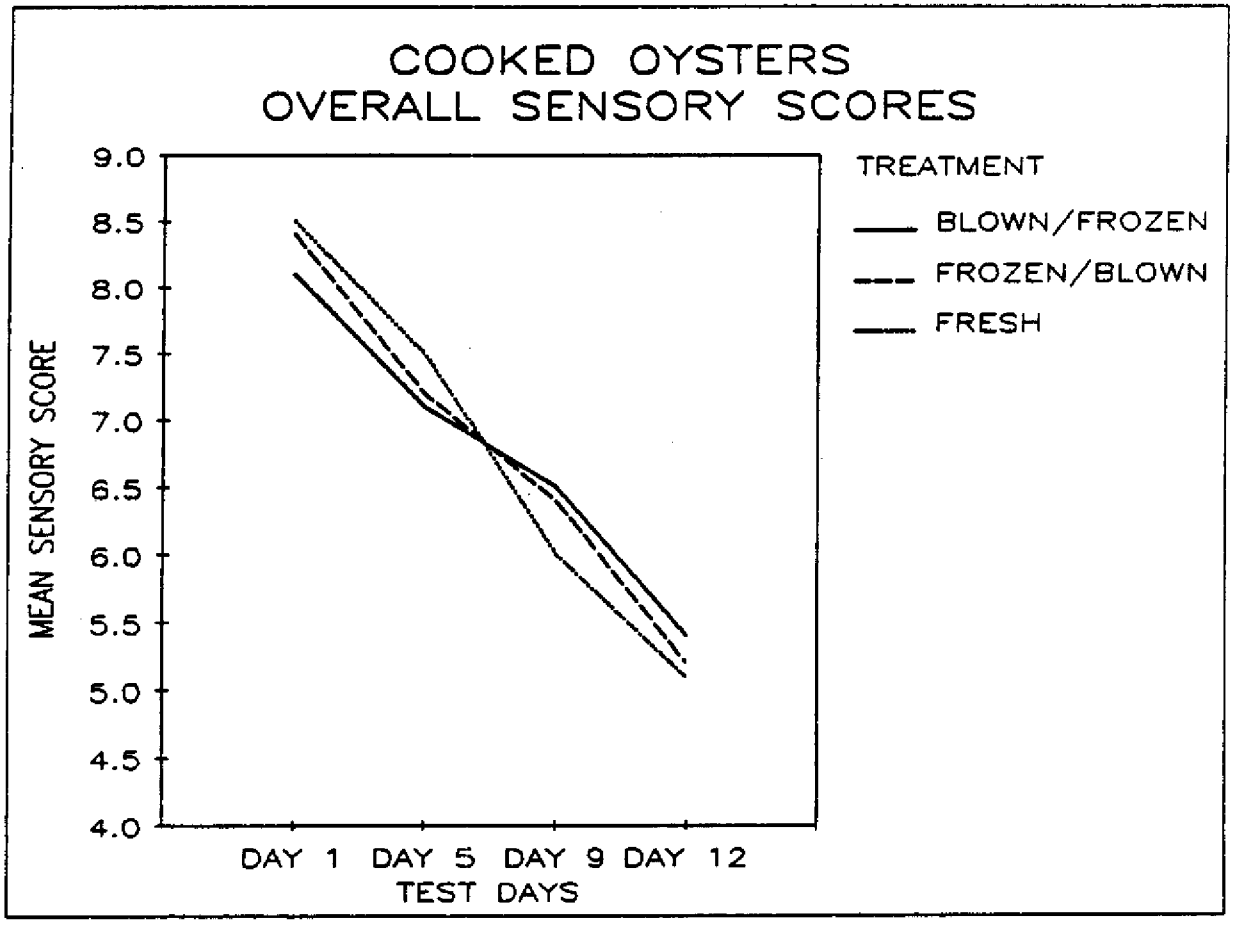
[a]Each data point is the mean of 8 replicates.

Figure 3. Overall mean raw sensory score of previously frozen and fresh oysters during storage[a].



[a]Each data point is the mean of 8 replicates.

Figure 4. Overall mean sensory score of previously frozen and fresh (cooked) oysters[a].



[a]Each data point is the mean of 8 replicates.

previously frozen oysters was similar to that of fresh oysters. It is, of course, important that only fresh product be frozen for later repacking.

The issue of labeling was not addressed by this investigator. The FDA Compliance Guide 7120.06 does define a fresh product and interpretation of this guideline may require that oysters that were previously frozen and later repacked for the fresh market be so labeled.

REFERENCES

1. American Public Health Association. 1984. Compendium of Methods for the Microbiological Examination of Foods. Am. Pub. Hlth, Assoc. Washington, Dc.

2. Association of Official Analytical Chemists. 1980. "Official Methods of Analysis of the Association of Official Analytical Chemists. Washington, DC.

3. Desrosier, N.W., and Tressler, D.K. 1977. "Fundamentals of Food Freezing". AVI Publishing Co., Westport, CT.

4. Food and Drug Administration, Shellfish Sanitation Branch. 1985. National Shellfish Sanitation Programs Manual of Operations, part II, Sanitation of the harvesting and processing of shellfish. U.S. Dept. of Health and Human Services. Government Printing Office, Washington, Dc.

5. Hackney, C., Sanders, L., Rippen, T., and Reily, L. 1988. Improved Method for Determination of Salt in Oysters by Direct Titration of Chloride Ion. J. Food Sci. 53:

6. Snedecor, G. and Cochran, W. 1967. "Statistical Methods" 6th ed. The Iowa State University Press, Ames, Iowa.

ACKNOWLEDGEMENTS

This work was supported by a grant from the Gulf and South Atlantic Fisheries Development Foundation Incorporated (Tampa).

GAMMA IRRADIATION OF *VIBRIO CHOLERAE* IN CRAYFISH (*PROCAMBARUS CLARKII* GERARD)

Robert M. Grodner and Arthur Hinton, Jr.
Department of Food Science
Louisiana State University Experiment Station
Louisiana State University Agricultural Center
Baton Rouge, Louisiana 70803

INTRODUCTION

Although freshwater crayfish may be found in every state in the United States, Louisiana grows, processes, and consumes 90% of all crayfish produced for human consumption (6). Today the demand for crayfish in Louisiana and the surrounding Southern states is increasing (3). Most commercially processed crayfish is marketed as hand peeled tailmeat (2). The tailmeat may be frozen, but it is usually stored at low temperatures above freezing. When the crayfish meat is properly handled, it has a shelf life of approximately one week (6).

The refrigerated shelf life of most seafoods can be increased by using pasteurizing levels of irradiation . Doses of 100 to 1000 krad destroy 90 to 99% of spoilage organisms present (1). A longer shelf life would mean that fresh crayfish could be stored for longer periods of time and marketed at greater distances from its source.

Crayfish are harvested from rivers, bayous, swamps, lakes or shallow ponds built especially for crayfish production (6). If the water from which the crayfish is taken is contaminated, there is a chance that the crayfish meat may harbor pathogens. Crabs taken from contaminated waters were responsible for 11 *Vibrio cholerae* infections in Louisiana in 1978 (5). Microorganisms of fecal origin have been isolated from crayfish waters, whole crayfish, and crayfish tailmeat (3).

The purpose of this study was to determine the effects of pasteurization levels of irradiation and cold storage on the survival of *V. cholerae* in nonsterile and sterile crayfish homogenates.

MATERIALS AND METHODS

Crayfish tailmeat homogenate was prepared by blending 3 parts fresh crayfish meat with one part sterile saline to form a smooth paste. The homogenate was then inoculated to produce a final concentration of 10^7 *Vibrio cholerae* 01/g (biotype *eltor*, serovar Inaba, Louisiana Strain #5875). The sterile crayfish meat homogenate was prepared in the same manner, except the crayfish meat was sterilized at 121°C for 15 minutes before blending. The inoculated samples were placed in 250 ml Nalgene bottles, packed in ice and transferred to the L.S.U. Nuclear Science Center.

Irradiation of the samples was performed by placing the Nalgene bottles containing the samples in a watertight chamber filled with ice and sealed. The chamber was then lowered into the 18 ft deep and 6 ft diameter lead lined pit which was filled with water. providing the effective radiation shield. The Colbalt-60 source dose rate was 1650 rad per minute. The samples were exposed to 25, 50 and 100 Krad based on the number of rads emitted per minute as a function of time to attain the desired dosage. The samples were then removed, packed in ice and transferred to the Food Science Department for analysis and examined for the presence of Vibrio cholerae at 0, 7, 14 and 21 days after cold storage at -8^{o}, 0^{o} or 4^{o}C. Control samples were treated in the same manner, except that the irradiation treatments were omitted. The number of V. cholerae surviving was calculated by determining the average number of V. cholerae recovered from triplicate samples subjected to the same irradiation treatment and cold storage.

Recovery methodology for V. cholerae on crayfish meat homogenates included recovery from a 6^{o} 8 hr alkaline peptone water (APW) enrichment and isolation thiosulfate-citrate-bile salts-sucrose (TCBS) and gelatin agar. After incubation at 35^{o}C, suspect V. cholerae colonies growing on the TCBS were transferred to and confirmed biochemically with Kleiger's iron agar (KIA) and lysine iron agar (LIA), and serologically with Polyvalent and Inaba Vibrio cholerae 01 antiserum.

After irradiation the samples were stored at 4^{o}, 0^{o}, or -8^{o}C for 21 days. The number of V. cholerae surviving the irradiation treatment and the cold storage were enumerated at 0, 7, 14 and 21 days respectively (4).

Irradiation of V. cholerae in sterile and nonsterile samples were performed in triplicate.

RESULTS AND DISCUSSION

Effect of irradiation. In the nonsterile homogenate each 25 krad dose of irradiation produced a 1 to 3 log reduction in the number of V. cholerae/g recovered (Figure 1). The nonsterile unirradiated homogenates' initial V. cholerae population of 4.3×10^{6}/g was reduced to 1.66×10^{5}/g by 25 krad. Only 8.01×10^{2} V. cholerae/g were recovered from the homogenates treated with 50 krads. No V. cholerae were recovered from the nonsterile homogenates treated with 100 krad.

In the sterile homogenates, V. cholerae seemed more susceptible to the irradiation than in the nonsterile homogenates. There were 3.67×10^{6} V. cholerae/g recovered from the homogenates which were not irradiated, but only 4.4×10^{0}/g were recovered from the homogenates treated with 25 krad. No V. cholerae were recovered from the homogenates treated with 50 or 100 krad.

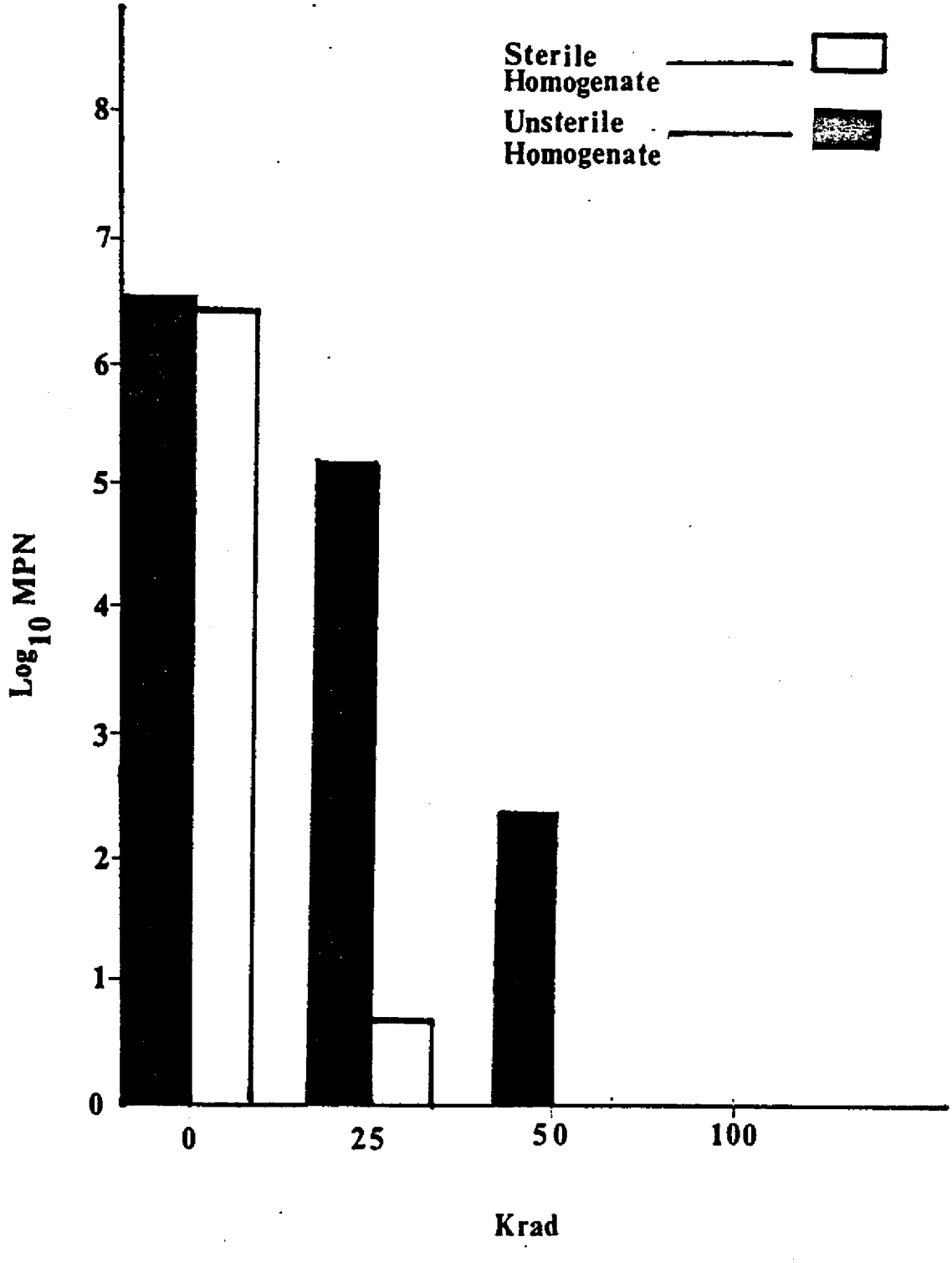


Figure 1. Effect of low dose gamma radiation on Vibrio cholerae #5875 in crayfish homogenates. MPN values are averages of 3 replications.

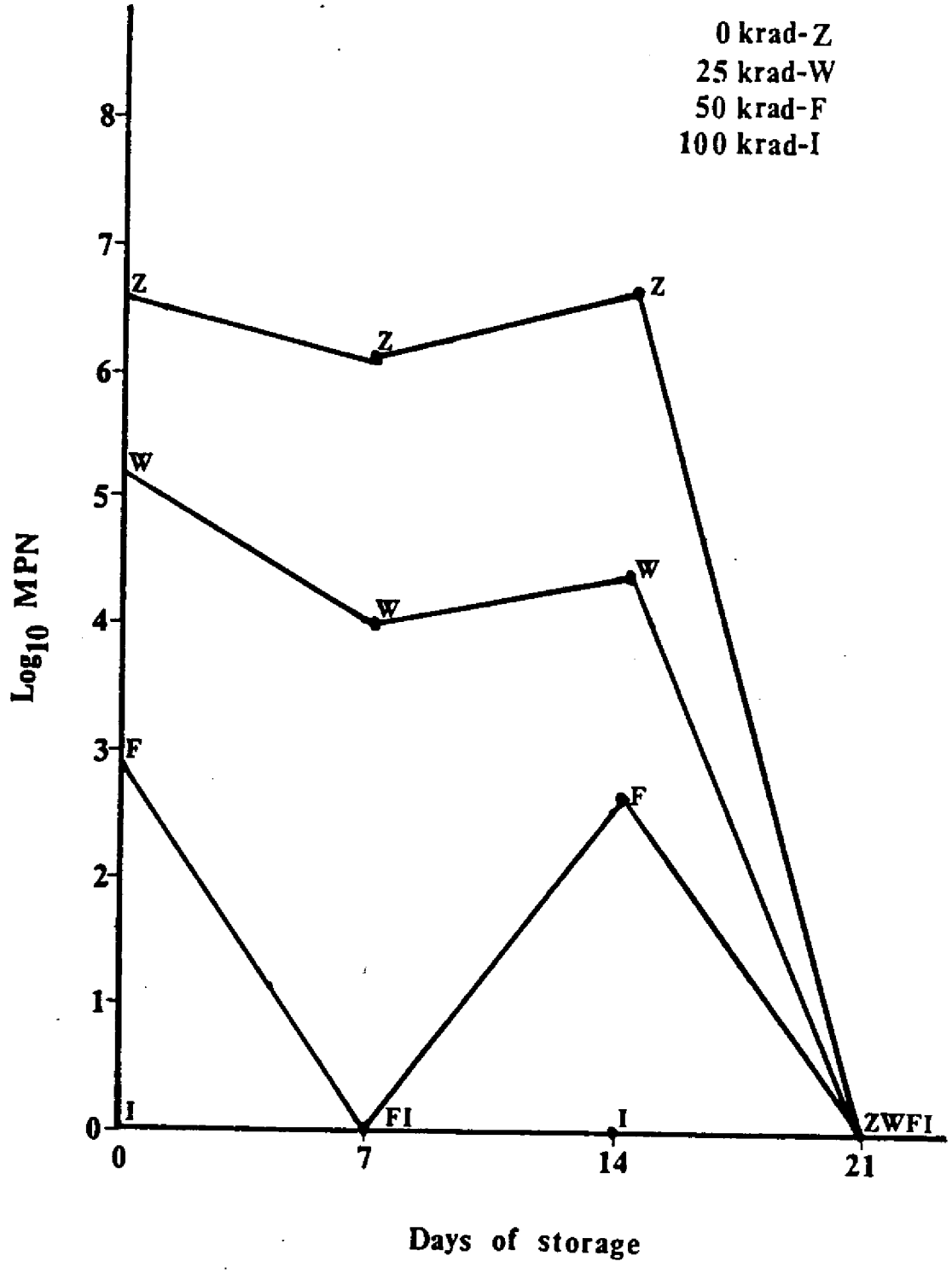


Figure 2. Survival of Vibrio cholerae #5875 in unirradiated and irradiated unsterile crayfish homogenates stored at 4C. MPN values are averages of 3 replications.

Effect of time. Vibrio cholerae were not able to survive for 21 days in the nonsterile homogenates stored at 4°C (Figure 2). The organism was able to survive for 14 days in the nonsterile homogenates treated with 0, 25, or 50 krad. After 14 days there was a large decrease in the V. cholerae population.

On day 0, 4.3×10^6 V. cholerae/g were recovered from the unirradiated nonsterile homogenates, and on day 14, 5.6×10^6 were recovered. No V. cholerae were recovered from the unirradiated nonsterile samples on day 21. The number of V. cholerae recovered from the nonsterile homogenates treated with 25 krad decreased from 1.66×10^5/g on day 0 to 3.87×10^4/g on day 14 before decreasing to 0 on day 21. The nonsterile homogenates treated with 50 krad showed results similar to those obtained with the homogenates treated with 0 krad or 25 krad. Though there was a decline in the V. cholerae population on day 7, approximately 8.0×10^2 V. cholerae/g were isolated from the homogenates on day 0 and on day 21. No V. cholerae were recovered from the homogenates on day 21.

After 21 days there was only a slight decrease in the V. cholerae population of the sterile crayfish homogenates treated with 0 krad or 25 krad and stored at 4°C (Figure 3). On day 0, 3.63×10^6 V. cholerae/g were recovered from the unirradiated sterile homogenates. There were 6.0×10^6 V. cholerae/g recovered on day 7. The population of the cholera organisms declined to 3.13×10^6/g on day 14 and declined further to 9.11×10^5/g on day 21.

The survival pattern of the organism in the sterile homogenate treated with 25 krad was similar to the survival of the organism in the sterile unirradiated homogenate. The V. cholerae population numbered 4.4×10^0/g on day 0 and 1.33×10^0 V. cholerae/g were recovered on day 21.

Vibrio cholerae were able to survive for 21 days in the nonsterile homogenates stored at 0°C (figure 4). In the homogenates treated with 0 krad or 25 krad the V. cholerae population decreased approximately 1 log cycle over the 21 day storage period. There were 4.3×10^6 V. cholerae/g recovered from the nonsterile unirradiated homogenates on day 0, and 6.25×10^5/g were recovered on day 21. The number of V. cholerae recovered from the nonsterile homogenates treated with 25 krad decreased from 1.66×10^5/g on day 0 to 4.86×10^4/g on day 21. In the homogenates treated with 50 krad and V. cholerae population decreased from 8.01×10^2/g on day 0 to 5.0×10^0/g on day 21.

The V. cholerae also appeared to survive well in the sterile crayfish homogenate stored at 0°C (Figure 5). The initial population of 3.63×10^6/g in the unirradiated homogenate decreased slightly to 1.16×10^6/g after 21 days of storage. The V. cholerae population of the homogenate treated with 25 krad decreased from 4.40×10^0/g on day 0 to 1.33×10^0/g on day 7. On day 14, 3.0×10^0 V. cholerae/g were recovered, and 2.67×10^0 V. cholerae/g were recovered on day 21.

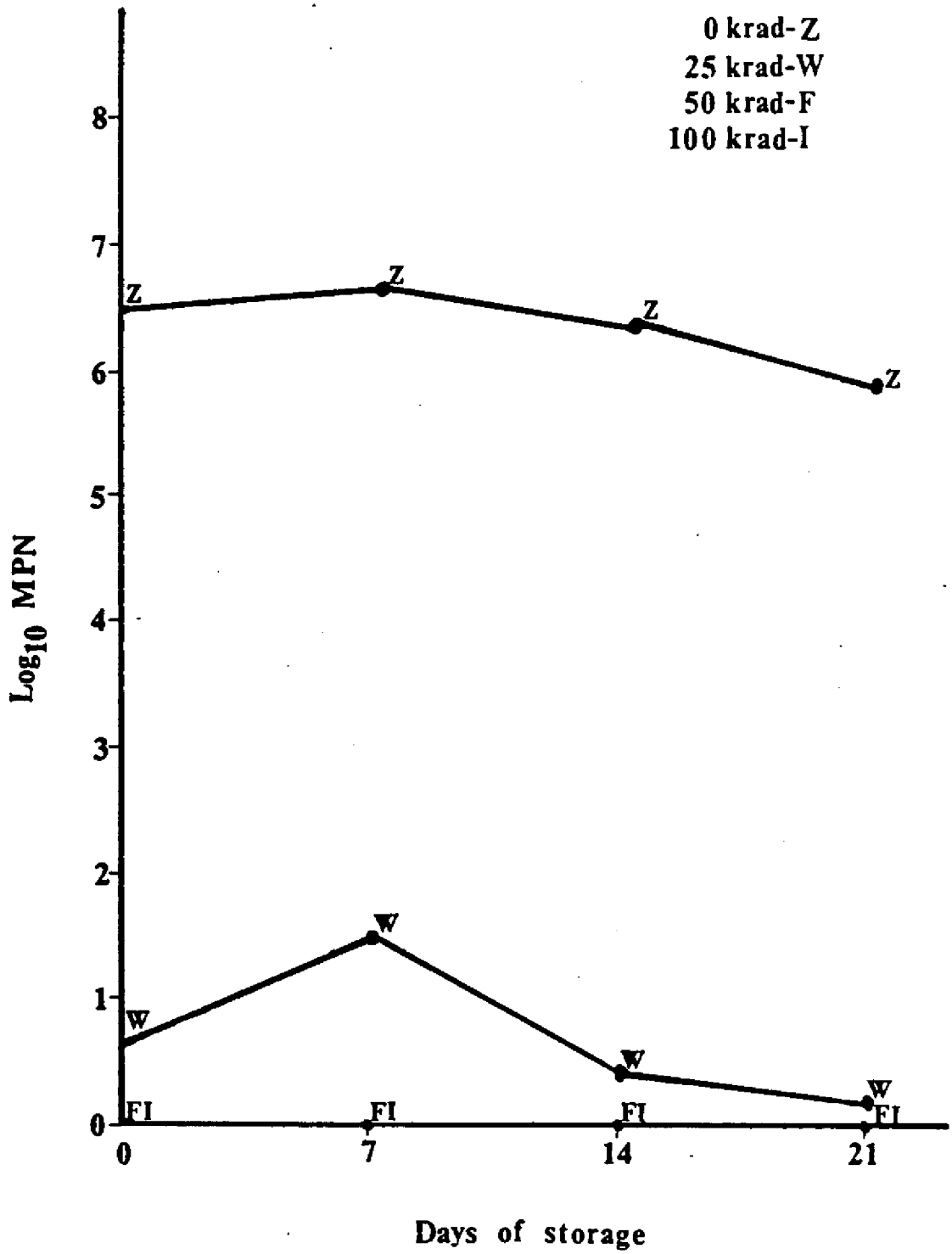


Figure 3. Survival of Vibrio cholerae #5875 in unirradiated and irradiated sterile crayfish homogenates stored at 4C. MPN values are averages of 3 replications.

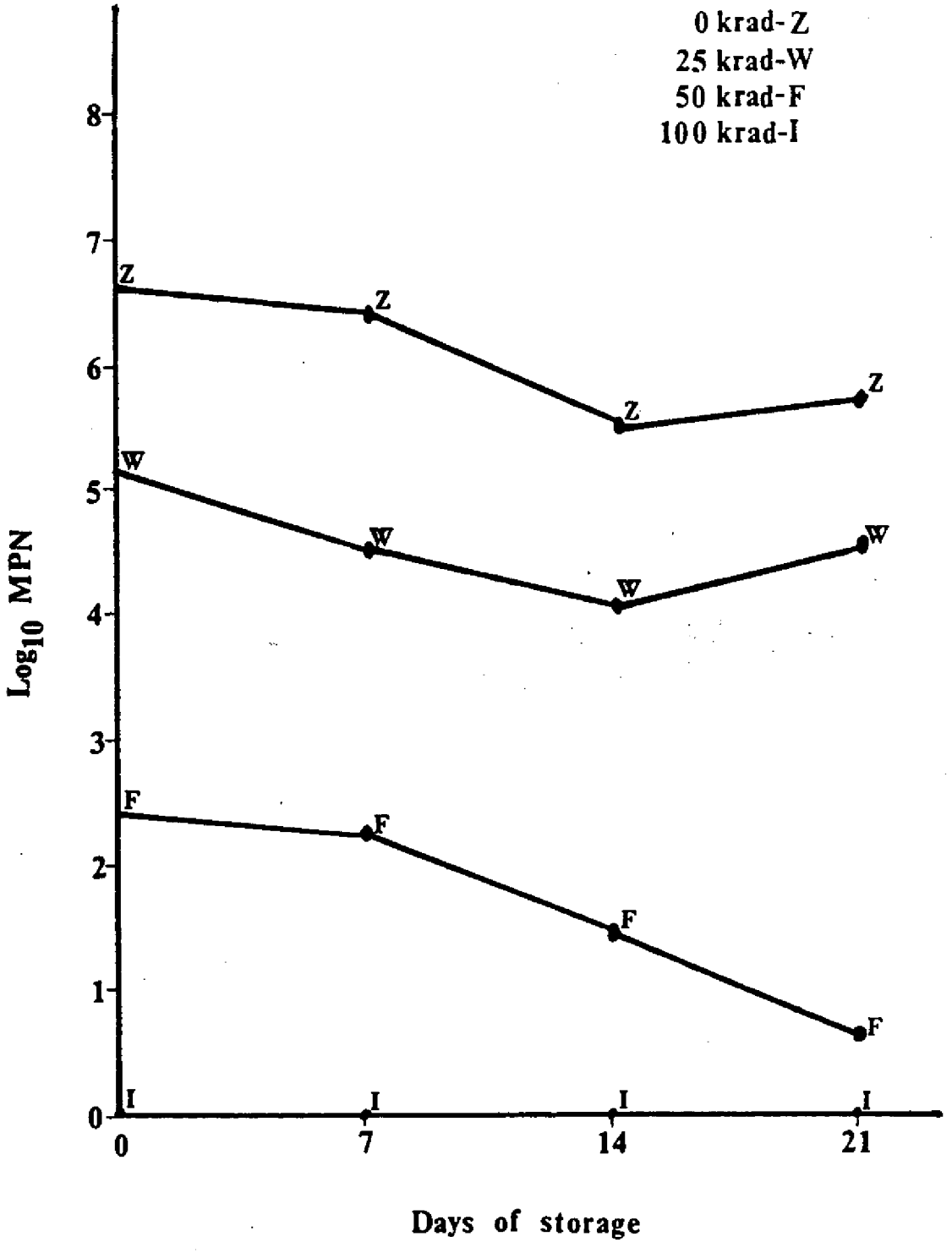


Figure 4. Survival of Vibrio cholerae #5875 in unirradiated and irradiated unsterile crayfish homogenates stored at 0C. MPN values are averages of 3 replications.

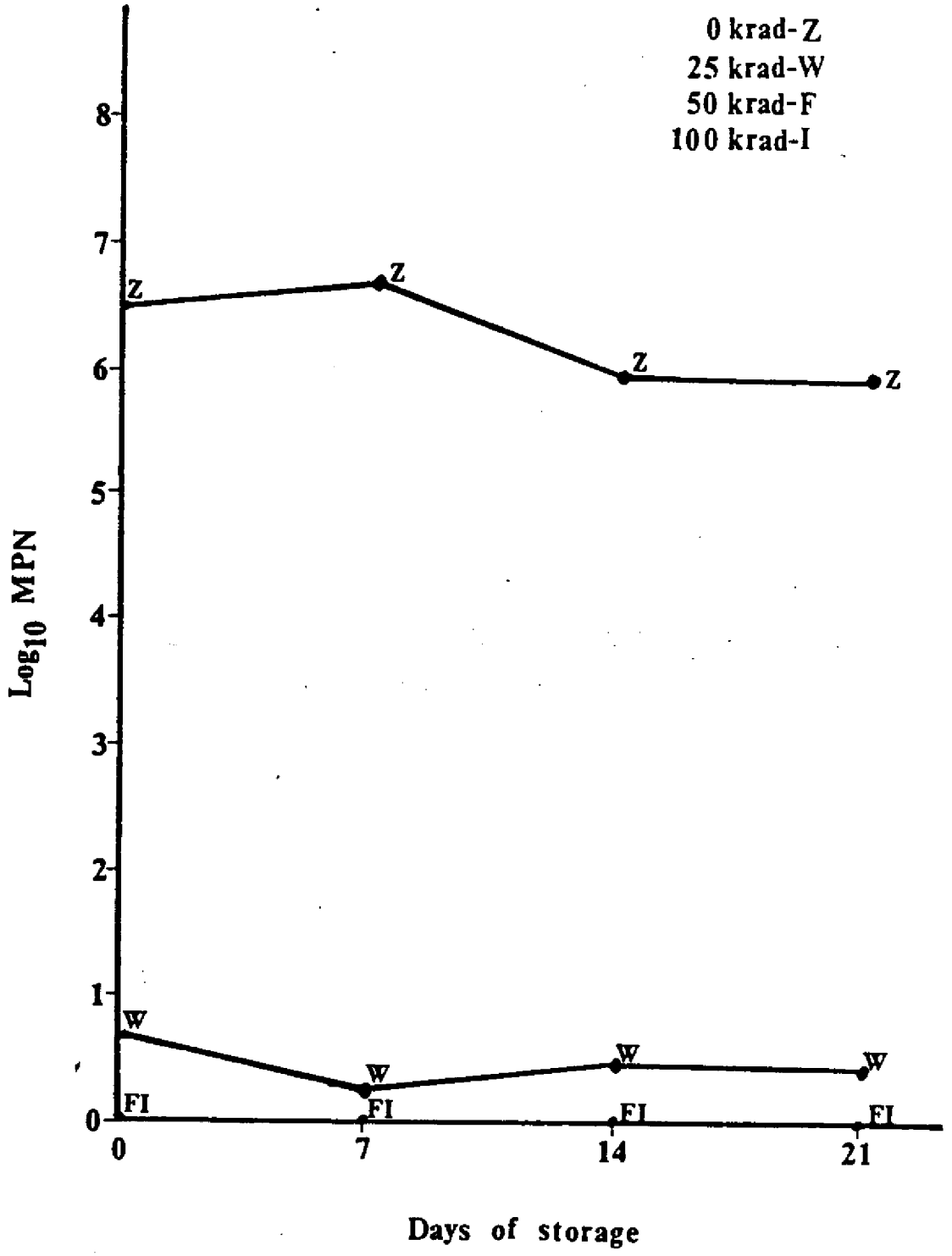


Figure 5. Survival of Vibrio cholerae #5875 in unirradiated and irradiated sterile crayfish homogenates stored at OC. MPN values are averages of 3 replications.

There were relatively small changes in the V. cholerae population of the nonsterile homogenates stored at -8°C for 21 days (Figure 6). On day 21, 1.14 x 10^6 V. cholerae/g were recovered from the unirradiated nonsterile homogenates which contained 4.3 x 10^6 V. cholerae/g on day 0. The number of V. cholerae recovered from the homogenates treated with 25 krad decreased from 1.66 x 10^5/g on day 0 to 9.2 x 10^3/g on day 21. On day 0, there were 8.01 x 10^2 V. cholerae/g recovered from the homogenates treated with 50 krad, and 8.15 x 10^2 V. cholerae/g were recovered on day 21.

There was a decline in the number of V. cholerae recovered from the sterile homogenates stored at -8°C for 21 days (Figure 7). The V. cholerae population of the unirradiated homogenates decreased from 3.63 x 10^6/g on day 0 to only 8.5 x 10^2/g on day 21. On day 0, although 4.4 x 10^0 V. cholerae/g were recovered from the sterile homogenates treated with 25 krad, no V. cholerae were recovered from these homogenates after 14 or 21 days of storage.

Effect of temperature. The survival of V. cholerae in unirradiated and irradiated sterile homogenates stored at 4° or 0°C was very similar. The organism was able to survive for 21 days in the sterile homogenates stored at both temperatures. In some cases there were slight increases and decreases in the V. cholerae population, but the number of organisms recovered during storage never varied more than 1 log cycle from the number of organisms recovered on day 0.

The organism did not survive as well as in the sterile homogenate stored at -8°C. The number of V. cholerae recovered from the frozen homogenates declined by over 3 log cycles during storage at -8°C. The homogenates treated with 25 krad contained 4.4 x 10^0 V. cholerae/g on day 0, but no V. cholerae were isolated from the homogenates on day 14 or 21.

Up to day 14, there was only a slight difference in the number of V. cholerae recovered from the nonsterile homogenates stored at 4°, 0°, or -8°C. Usually the number of organisms recovered from the nonsterile homogenates stored at the different temperatures differed by less than 1 log cycle.

On day 21, no V. cholerae were recovered from the nonsterile homogenates stored at 4°C, but the organism was recovered from the homogenates stored at 0° or -8°C. The possible reason for this is discussed below.

Survival in nonsterile and sterile homogenates. In fresh crayfish meat Micrococcus, Staphylococcus, and Alcaligine are the predominant genera of microorganisms present (2). After eight days of storage at 0°C, Pseudomonas becomes the dominant genera, but after eight days at 5°C Achromobacter becomes the dominant genera. At the end of the spoilage of the fresh crayfish meat, Pseudomonas and Achromobacter groups account for almost 100% of the microbial population.

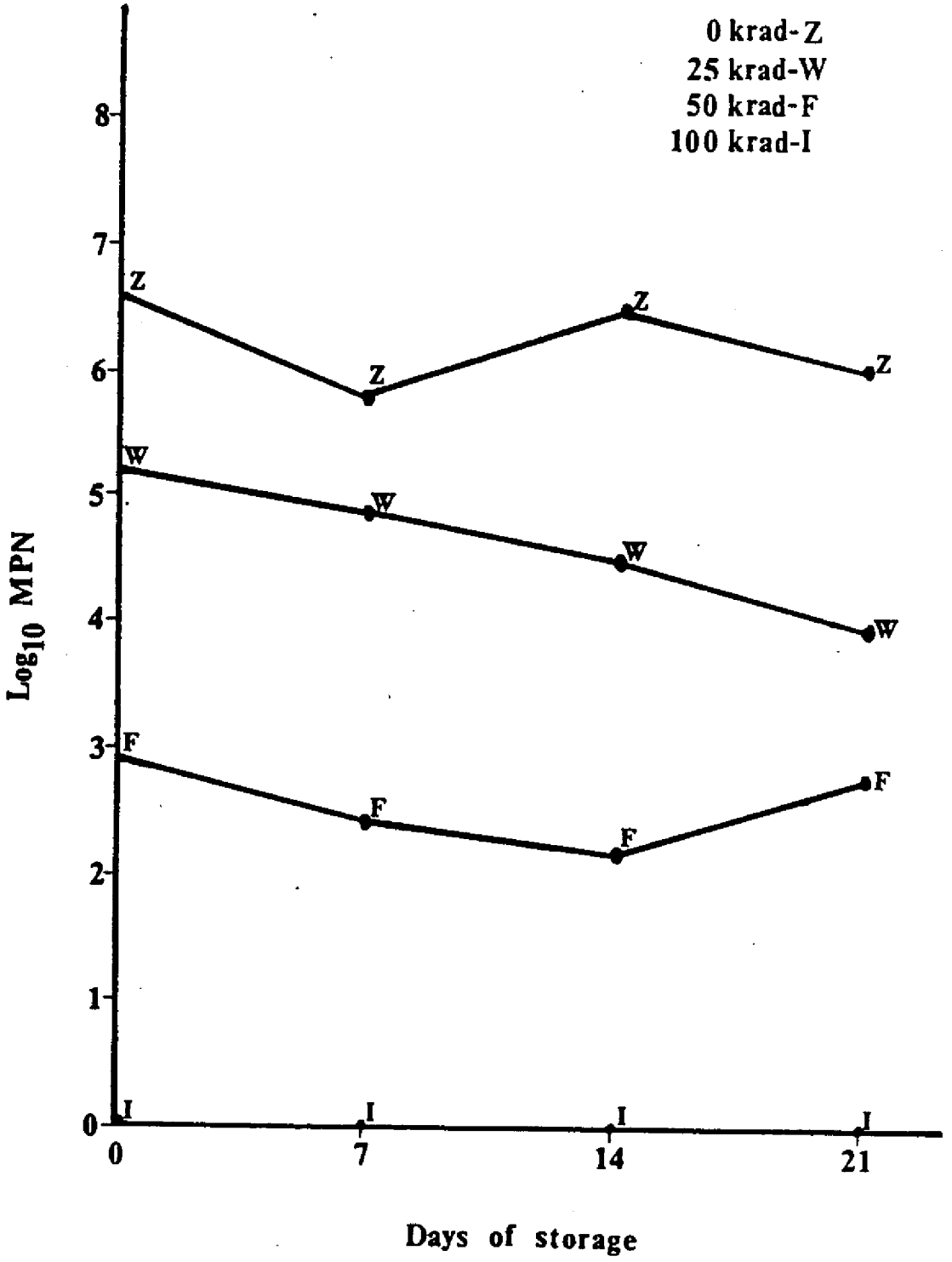


Figure 6. Survival of Vibrio cholerae #5875 in unirradiated and irradiated unsterile crayfish homogenates stored at -8C. MPN values are averages of 3 replications.

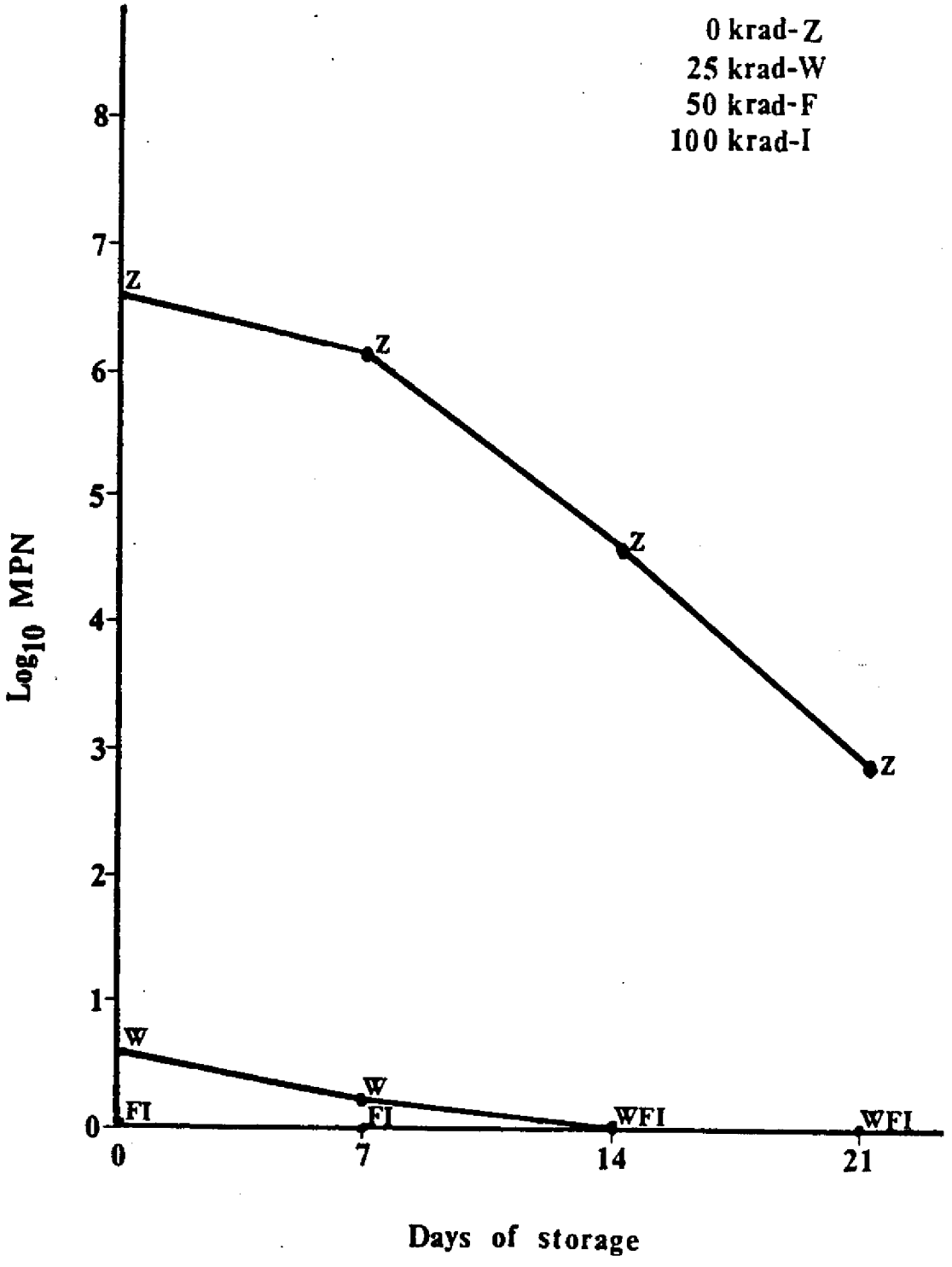


Figure 7. Survival of Vibrio cholerae #5875 in unirradiated and irradiated sterile crayfish homogenates stored at -8C. MPN values are averages of 3 replications.

In the sterile homogenates the natural microbial flora and the naturally occurring enzymes of the crayfish meat had been destroyed. The V. cholerae placed in the nonsterile crayfish meat had to compete with the crayfish's natural microflora to survive. Fresh crayfish meat has a total aerobic plate count (TPC) OF 1.6 X 10^5 organisms/g. After 24 days of storage at 0°C, the TPC increases to 3.2 x 10^9/g, and after 24 days of storage at 5°C the TPC increases to 2.8 X 10^9 organisms/g (2).

In the unirradiated sterile and nonsterile homogenates stored at 4°C and the survival pattern of V. cholerae was similar up to day 14. On day 21, 9.11 x 10^5 V. cholerae/g were recovered from the unirradiated sterile homogenates, but no V. cholerae were recovered from the unirradiated nonsterile homogenates. Similar results were seen in the sterile and nonsterile homogenates treated with 25 krad. At 4°C, the psychrotrophic microorganisms in the crayfish's natural microflora were able to grow. This competitive inhibition was probably responsible for the decline in the number of V. cholerae recovered from the homogenates.

Vibrio cholerae were able to survive for 21 days in nonsterile homogenates stored at 0° or -8°C. The lower temperatures retarded the growth of the psychotrophs which outgrew the V. cholerae at 4°C.

There was a large difference in the survival of V. cholerae in the sterile and nonsterile homogenates stored at -8°C. The V. cholerae population of the nonsterile homogenates remained relatively stable during the 21 days of storage. The proteins of the sterile homogenates had been denatured by the sterilization treatment. Native proteins are able to provide more protection to microorganisms during freezing because they have a greater effect on the rate of crystallization in the food (7). Native proteins bind more water molecules and reduce the rate of crystallization. Less dehydration of the bacterial cells is produced and the microorganisms can survive longer in the food.

These results indicate that V. cholerae can survive for some time in crayfish tissues stored at low temperatures. Pasteurization doses or irradiation may be successfully used to destroy this pathogen in crayfish.

REFERENCES

1. Banwart, G. 1979. Basic Food Microbiology. AVI Publishing Co., Westport, Conn.

2. Cox, N. A. and R. T. Lovell. 1973. Identification and characterization of the microflora and spoilage bacteria in freshwater crayfish Procambarus clarkii (Girard) J. Food Sci. 38:679-681.

3. Grodner, R. M. and A. F. Novak. 1975. Microbiological guidelines for freshwater crayfish (Procambarus clarkii Girard). P. 161-171. In J. W. Avault, Jr. (ed.), Freshwater Crayfish. Louisiana State University, Division of Continuing Education, Baton Rouge, LA.

4. Hinton, A., Jr. 1983. Irradiation of Vibrio cholerae in shrimp (Panaeus setiferus). Ph.D. Thesis, Louisiana State University, Baton Rouge, LA.

5. Louisiana Monthly Morbidity Report. Sept. 1978. Dept. of Health and Human Resources. New Orleans, LA.

6. Moody, M. W. 1980. The Crawfish. Louisiana Cooperative Extensions Service. Sea Grant Publication, LSU-TL-80-002.

7. Raj, H. and J. Liston. 1961. Survival of bacteria of health of significance in frozen seafoods. Food Technol. 15:429-433.

A STATISTICAL REVIEW OF THE INTERSTATE SHELLFISH SANITATION CONFERENCE'S SAMPLING PROTOCOL FOR SATISFACTORY SHIPPING CONDITIONS

Alvin P. Rainosek
NOAA, National Marine Fisheries Service,
National Seafood Inspection Laboratory,
P. O. Drawer 1207, Pascagoula, MS 39568-1207

INTRODUCTION

At the 1986 Annual Meeting of the Interstate Shellfish Sanitation Conference (ISSC), a sampling protocol for the microbiological analysis (Fecal Coliform, E. Coli and Aerobic Plate count) and acceptance of shellfish shipments was adopted. This protocol appears in Appendix A of the National Shellfish Sanitation Program (NSSP) Revised Manual - Part II (effective January, 1988). Prior to mandatory implementation, however, the ISSC at its 1987 Annual Meeting decided to refer the protocol to a subcommittee for additional study of its operational feasibility and statistical reliability. This subcommittee will report it findings at the 1988 ISSC Annual Meeting.

When evaluating any sampling protocol, several major issues should be addressed. One, is the sampling protocol feasible, practical, cost effective, etc? This is primarily a managerial/administrative decision and is not considered in this paper. Also not considered is the definition of a lot, what constitutes a laboratory sample (e.g., how many oysters make up a sample), and how will the samples be selected from a lot? What is presented, however, is a statistical review of the discriminatory power (performance/operating characteristics) of this multi-phase, multi-stage sampling protocol. That is, how well will this protocol identify shipments of varying microbial quality? Such information should be available and understood as part of the decision making process regarding the acceptability of the protocol for the intended purpose. The initial draft of this paper has been reviewed by FDA staff in the Division of Microbiology, the Shellfish Sanitation Branch, and the Division of Mathematics.

This statistical review is provided for the first and second shipment conditions as outlined in Appendix A of the NSSP Revised Manual Part II. Probabilities were calculated by using either the binomial or the trinomial probability distributions under the following two assumptions: (1) the number of samples analyzed is less than 10% of all possible samples within an entire lot, and (2) the test results for E. coli and Aerobic Plate Count are independent. Derivations of all probabilities presented are available upon request.

FIRST SHIPMENT SAMPLING - FECAL COLIFORM (FC) AND E. Coli (EC)

Step 1 - Analyze 2 random samples of shellstock or shucked product for FC. Decision rule - If one or both samples exceed 230 MPN FC, go to Step 2 (EC); otherwise, the lot is considered satisfactory.

Step 2 - For those samples in Step 1 exceeding 230 MPN FC, analyze for EC. Decision rule - If one or both samples exceed 230 MPN EC, notify the dealer, etc., and go to Second Shipment Sampling; otherwise, the lot is considered satisfactory.

For Steps 1 and 2 combined (i.e., FC then EC), what is the chance that the lot will be found satisfactory assuming various percentages of all possible samples in the lot that exceed 230 MPN EC?

Let p = the actual percent of all possible samples in the lot that exceed 230 MPN EC

P(EC) = the probability (in percent) that the lot will be found satisfactory for EC

p	P(EC)	p	P(EC)	p	P(EC)
0	100.0	35	42.2	70	9.0
5	90.2	40	36.0	75	6.2
10	81.0	45	30.2	80	4.0
15	72.2	50	25.0	85	2.2
20	64.0	55	20.2	90	1.0
25	56.2	60	16.0	95	0.2
‡ 30	49.0	65	12.2	100	0.0

‡Example Interpretation: For First Shipment Sampling (FC then EC) and when 30% of all possible samples in the lot have EC levels exceeding 230 MPN, there is a 49% chance that the lot will be found satisfactory.

The above table can also be used to determine the likelihood that FC screening will require testing for EC. For example, suppose that 30% of all possible samples in the shipment (lot) exceed 230 MPN FC, then there is a 49% chance that none of the two samples will require testing for EC (or a 51% chance that at least one of the two samples will require testing for EC).

For First Shipment Sampling, it may be of interest to have P(EC) values for different sample sizes (i.e., 1, 3, etc). For selected sample sizes, this information is presented in Appendix 1.

FIRST SHIPMENT SAMPLING - AEROBIC PLATE COUNT (APC)

Analyze the two samples selected in Step 1 above for APC. Decision rule - If one or both samples exceed 500,000/g APC, notify shipper state for investigative and corrective action.

What is the chance that the lot will be found satisfactory for APC assuming various percentages of samples in the entire lot that actually exceed 500,000/g APC?

Let q = the actual percent of all possible samples in the lot having APC levels exceeding 500,000/g

P(APC) = the probability (in percent) that the lot will be found satisfactory for APC

The P(EC) probabilities given on page 2 apply to APC as well. That is, when q = p, then P(APC) = P(EC). For example, when q = 30%, the probability that the lot will be found satisfactory for APC is 49.0%.

FIRST SHIPMENT SAMPLING - CHANCE OF LOT ACCEPTANCE CONSIDERING BOTH EC AND APC

What is the probability when considering both tests (FC/EC and APC), that the lot will be found satisfactory assuming various values of p and q? This will occur when neither EC nor APC (nor both) exceed their tolerances. The probabilities (rounded to whole percentages) that the lot will be found satisfactory when considering the results of both the EC and APC tests for various values of p and q are given below (again, p = the actual percent of all possible samples in the lot exceeding 230 MPN EC and q = the actual percent of all possible samples in the lot exceeding 500,000/g APC):

	q										
p	0	10	20	30	40	50	60	70	80	90	100
0	100	81	64	49	36	25	16	9	4	1	0
10	81	66	52	40	29	20	13	7	3	1	0
20	64	X 52	41	31	23	16	10	6	3	1	0
30	49	40	31	24	18	12	8	4	2	1	0
40	36	29	23	18	13	9	6	3	1	0	0
50	25	20	16	12	9	6	4	2	1	0	0
60	16	13	10	8	6	4	3	1	1	0	0
70	9	7	6	4	3	2	1	1	0	0	0
80	4	3	3	2	1	1	1	0	0	0	0
90	1	1	1	1	0	0	0	0	0	0	0
100	0	0	0	0	0	0	0	0	0	0	0

XExample Interpretation: When p = 20% (i.e., 20% of all possible samples in the lot have EC levels exceeding 230 MPN) and when q = 10% (i.e., 10% of all possible samples in the lot have APC levels exceeding 500,000/g), there is a 52% chance that the lot will be found satisfactory for both EC and APC. Or, there is a 48% chance that either the EC test or the APC test (or both) will exceed their tolerances.

SECOND SHIPMENT SAMPLING - FC AND EC

Step 1 - Analyze 5 random samples of shellstock or shucked product for FC. Decision rule - If any sample exceeds 330 MPN FC or 2 or more samples exceed 230 MPN FC but are less than or equal to 330 MPN FC, go to Step 2 (EC); otherwise, the lot is considered satisfactory. Note: This sampling plan represents a 3-Class plan where n = 5, c = 1, m = 230, and M = 330.

Step 2 - For those samples in Step 1 exceeding 230 MPN FC, analyze for EC. Decision rule - If any sample exceeds 330 MPN EC or 2 or more samples exceed 230 MPN EC but are less than or equal to 330 MPN EC, initiate action as indicated in Appendix A of the NSSP Revised Manual Part II; otherwise, the lot is found satisfactory. Note: Same 3-Class sampling plan as in Step 1 above.

For Steps 1 and 2 combined (FC then EC), what is the chance that the lot will be found satisfactory for EC?

Let p_1 = the actual percent of all possible samples in the lot having EC MPN levels greater than 230 but less than or equal to 330

p_2 = the actual percent of all possible samples in the lot having EC levels exceeding 330 MPN

P(EC) = the probability (in percent) that the lot will be found satisfactory for EC

The P(EC) probabilities assuming various values of p_1 and p_2 are given in the following table.

	p_2								
p_1	0	5	10	15	20	25	30	35	40
0	100	77	59	44	33	24	17	12	8
5	98	75	57	43	32	23	16	11	7
10	92	70	53	40	29	20	14	10	6
15	84	63	47	35	25	17	12	8	5
20	74	55	41	29	21	14	9	6	4
25	63	47	X 34	24	16	11	7	4	2
30	53	38	27	19	12	8	5	3	1
35	43	30	21	14	9	5	3	2	-
40	34	23	16	10	6	3	2	1	-
45	26	17	11	7	4	2	1	-	-
50	19	12	7	4	2	1	-	-	-

XExample Interpretation: For Second Shipment Sampling (FC then EC) and when p_1 = 25% (i.e., 25% of all possible samples in the lot have EC MPN levels greater than 230 but less than or equal to 330), and when p_2 = 10% (i.e., 10% of all possible samples in the lot have EC MPN levels exceeding 330), there is then a 34% chance that the lot will be found satisfactory for EC.

SECOND SHIPMENT SAMPLING - APC ANALYSIS

Analyze the 5 random samples for APC. Decision rule - If any sample exceeds 1,500,000/g or if 3 or more samples exceed 500,000/g but are less than or equal to 1,500,000/g, notify the shipper state for investigative and corrective action. Note: This sampling plan represents a 3-Class plan where n = 5, c = 2, m = 500,00/g, and M = 1,500,000/g

What is the chance that the lot will be found satisfactory for APC?

Let q_1 = the actual percent of all possible samples in the lot having APC levels greater than 500,000/g but less than or equal to 1,500,000/g

q_2 = the actual percent of all possible samples in the lot having APC levels exceeding 1,500,000/g

P(APC) = the probability (in percent) that the lot will be found satisfactory for APC

The probabilities (in percent) that the lot will be found satisfactory for APC for various values of q_1 and q_2 are given in the following table:

	q_2								
q_1	0	5	10	15	20	25	30	35	40
0	100	77	59	44	33	24	17	12	8
5	99	77	59	44	33	24	17	12	8
10	99	77	58	44	32	23	16	11	7
15	97	75	57	43	31	22	16	11	7
20	94	72	54	40	29	21	14	10	6
25	90	68	X 51	37	27	19	13	8	5
30	84	63	47	34	24	16	11	7	4
35	76	57	41	29	20	13	8	5	3
40	68	50	35	25	16	10	6	3	2
45	59	42	29	20	12	7	4	2	1
50	50	35	23	15	9	5	2	1	-

XExample Interpretation: For Second Shipment Sampling (APC) and when q_1 = 25% (i.e., 25% of all possible samples in the lot have APC levels exceeding 500,000/g but are less than or equal to 1,500,000/g), and when q_2 = 10% (i.e., 10% of all possible samples in the lot have APC

levels exceeding 1,500,000/g), there is a 51% chance that the lot will be found satisfactory for APC.

SECOND SHIPMENT SAMPLING - CHANCE OF LOT ACCEPTANCE CONSIDERING BOTH EC AND APC

What is the probability when considering both tests (FC/EC and APC), that the lot will be found satisfactory assuming various values of p_1, p_2, q_1 and q_2 ? This will occur when neither EC nor APC (nor both) exceed their tolerances. These probabilities can be easily determined by multiplying the second shipment P(EC) and P(APC) probabilities given above. Two examples follow:

Example 1: Let p_1 = 15%, p_2 = 20%, q_1 = 10%, and q_2 = 15%. Then the chance that the lot will be found satisfactory when considering both the EC and APC test results is given by (0.25)(0.44) = 11%.

Interpretation: When the lot has p_1 = 15% of all possible samples exceeding 230 MPN EC but less than or equal to 330 MPN EC, p_2= 20% of all possible samples exceeding 330 MPN EC, q_1 = 10% of all possible samples exceeding 500,000/g APC but less than or equal to 1,500,000/g APC, and q_2= 15% of all possible samples exceeding 1,500,000/g APC, there is an 11% chance that the lot will be found satisfactory for both EC and APC.

For reference purposes, when considering the two tests separately: (1) for p_1 = 15% and p_2 = 20%, there is a 25% chance that the lot will be found satisfactory for EC, and (2) for q_1 = 10% and q_2 = 15%, there is a 44% chance that the lot will be found satisfactory for APC. When considering the two tests together, the separate probabilities are multiplied; thus, (.25)(.44) = 11%.

Example 2: Let p_1 = 10%, p_2 = 5%, q_1 = 15%, and q_2 = 10%. Then the chance that the lot will be found satisfactory when considering both the EC and APC test results is given by (0.70)(0.57) = 40%.

When considering the two tests separately: (1) For p_1 = 10% and p_2 = 5%, there is a 70% chance that the lot will be found satisfactory, and (2) for q_1 = 15% and q_2 = 10%, there is a 57% chance that the lot will be found satisfactory.

FIRST AND SECOND SHIPMENT SAMPLING COMBINED - EC

Suppose First Shipment Sampling (two samples analyzed) is to be applied to a certain shipment. What is the probability, denoted by P (both), that both this shipment as well as the next shipment from the same shipper (Second Shipment Sampling - five samples analyzed) will be found unsatisfactory for EC. Note: Both unsatisfactory for EC thereby initiating SSRA action as noted in Appendix A of the NSSP Revised Manual Part II.

For various values of p (the actual percent of all possible samples in the lot having EC exceeding 230 MPN under First Shipment Sampling), and for Second Shipment Sampling, p_1 (the actual percent of all possible samples in the lot exceeding 230 MPN EC but less than or equal to 330 MPN EC) and p_2 (the actual percent of all possible samples in the lot exceeding 330 MPN EC); P(both) can be determined by using the First Shipment P(EC) and Second Shipment P(EC) probabilities given above. Two examples follow:

Example 1: Let p = 50%, p_1= 30%, and p_2 = 20%, then P(both) = (1-0.25)(1-0.12) = 66%. Note: This calculation assumes that the two shipments from the same shipper are independent.

Interpretation: If the first shipment has p = 50% of all possible samples exceeding 230 MPN EC, and the second shipment has p_1 = 30% of all possible samples exceeding 230 MPN EC but less than or equal to 330 MPN EC and p_2 = 20% of all possible samples exceeding 330 MPN EC, then there is a 66% chance that both shipments will be found unsatisfactory for EC.

Example 2: Let p = 20%, p_1 = 15%, and p_2 = 10%, then P(both) = (1-0.64)(1-0.47) = 19%.

Interpretation: If the first shipment has p = 20% of all possible samples exceeding 230 MPN EC, and the second shipment has p_1 = 15% of all possible sample exceeding 230 MPN EC but less than or equal to 330 MPN EC and p_2 = 10% of all possible samples exceeding 330 MPN EC, then there is a 19% chance that both shipments will be found unsatisfactory for EC.

SECOND SHIPMENT SAMPLING - UNSATISFACTORY CONDITIONS OF SHIPMENT

SSRA notifies shipper and state of origin by telephone. For product on hand and appears satisfactory, apply the same sampling plans used for satisfactory condition of shipment. Hence, all Second Shipment probabilities given above for satisfactory conditions of shipment apply to this case as well.

CONCLUSION

Among the several issues that should be considered when adopting a sampling plan for application in practice is its statistical reliability. The performance/operating characteristics of the ISSC's sampling protocol for satisfactory shipping conditions presented in this paper should enable responsible authority to judge the statistical acceptability of this protocol for the intended purpose.

APPENDIX 1

For first shipment sampling, the probabilities that a shipment will be found satisfactory for *E. coli* (EC) using samples sizes of 1, 2, 3, 4 and 5 are given below.

Let p = the actual percent of all possible samples in the lot having EC levels exceeding 230 MPN

n = the number of random samples (sample size) of shellstock or shucked product selected from the lot and analyzed

P(EC) = the probability (in percent) that the lot will be found satisfactory for EC (i.e., none of the n samples will exceed 230 MPN EC)

p	n=1 P(EC)	n=2 P(EC)	n=3 P(EC)	n=4 P(EC)	n=5 P(EC)
0	100.0	100.0	100.0	100.0	100.0
5	95.0	90.2	85.7	81.5	77.4
10	90.0	81.0	72.9	65.6	59.0
15	85.0	72.2	61.4	52.2	44.4
20	80.0	64.0	51.2	41.0	32.8
25	75.0	56.2	42.2	31.6	23.7
30	70.0	49.0	34.3	24.0	16.8
35	65.0	42.2	27.5	17.9	11.6
40	60.0	36.0	21.6	13.0	7.8
45	55.0	30.2	16.6	9.2	5.0
50	50.0	25.0	12.5	6.2	3.1
55	45.0	20.2	10.0	4.1	1.8
60	40.0	16.0	6.4	2.6	1.0
65	35.0	12.2	4.3	1.5	0.5
70	30.0	9.0	2.7	0.8	0.2
75	25.0	6.2	1.6	0.4	0.1
80	20.0	4.0	0.8	0.2	0.1
85	15.0	2.2	0.3	0.1	0.1
90	10.0	1.0	0.1	0.1	0.1
95	5.0	0.2	0.1	0.1	0.1
100	0.0	0.0	0.0	0.0	0.0

CONTROLLED FRESH WELL-WATER CLOSED SOFT CRAB SHEDDING SYSTEM

Wayne Wescott
UNC Sea Grant College Program, Marine Advisory Service
P.O. Box 699, Manteo, N.C. 27954

The shedding of blue crabs has become a multimillion dollar industry along the U.S. East Coast. The North Carolina industry alone grew more than 700 percent between 1983 and 1986 with landings rising from 87,570 pounds to 596,468 pounds.

Crabbers shed blue crabs in three types of systems: floating, flow-through and closed recirculating.

The oldest method of shedding soft crabs is the floating shedder --- a floating box that allows water to flow in and out. It is placed near the shoreline, and crabs are shed in the box.

Although inexpensive, floating shedders are difficult to work, dependent on good quality water, and subject to wave action and temperature fluctuations. Poor water quality, strong waves and temperature changes can cause mortalities.

In an onshore flow-through system, shedding boxes are built on land and water is pumped from a nearby creek or bay. This is a more expensive system, and it also can be affected by poor water quality and temperature changes.

Because the cost of waterfront property is expensive, its availability is limited and water quality is variable, crabbers are turning to closed recirculating systems.

Present recirculating systems use shedding trays, a reservoir, mechanical filters, biological filters, protein skimmers, pumps and plumbing to provide suitable water for shedding crabs.

These systems do not control water temperature, require constant monitoring and need 20 to 30 days start-up time for bacteria to grow before peelers can be shed. Bacteria are needed to convert harmful ammonia (from crab wastes) into harmless nitrate.

UNC Sea Grant, working with commercial shedders, developed a temperature-controlled, well-water shedding system. This system can be used anywhere sufficent well water is available. It uses cool groundwater and heating elements to regulate water temperatures between 70 F and 75 F.

This system requires little monitoring for ammonia, nitrate or nitrite. It needs no biological filters, protein skimmer or preliminary start-up because well water is used to replace water that is continuously drained from the system. The continuous dilution prevents the buildup of harmful substances.

TEMPERATURE CONTROLLED FRESHWATER SYSTEM

Figure I illustrates a typical closed freshwater shedder used for an onshore flow-through system. The trays are lighted for night work. They are plumbed with two control valves and drains per tray, an aerator at each valve to add oxygen, a rubber hose on each aerator to reduce spray on the crabs, and a screen on each drain to keep premolt and soft crabs out.

Water is pumped from a reservoir that is supplied by a well. PVC pipe, measuring 1 1/2 inches, supplies the water from the reservoir to the shedding trays, and a 3-inch PVC pipe returns it to the reservoir. A valve is added to the return line to drain the system if necessary.

The fresh well-water reservoir reduces the problems of water quality, oxygen content and water temperature. A variance in any of these factors can cause crab mortalities.

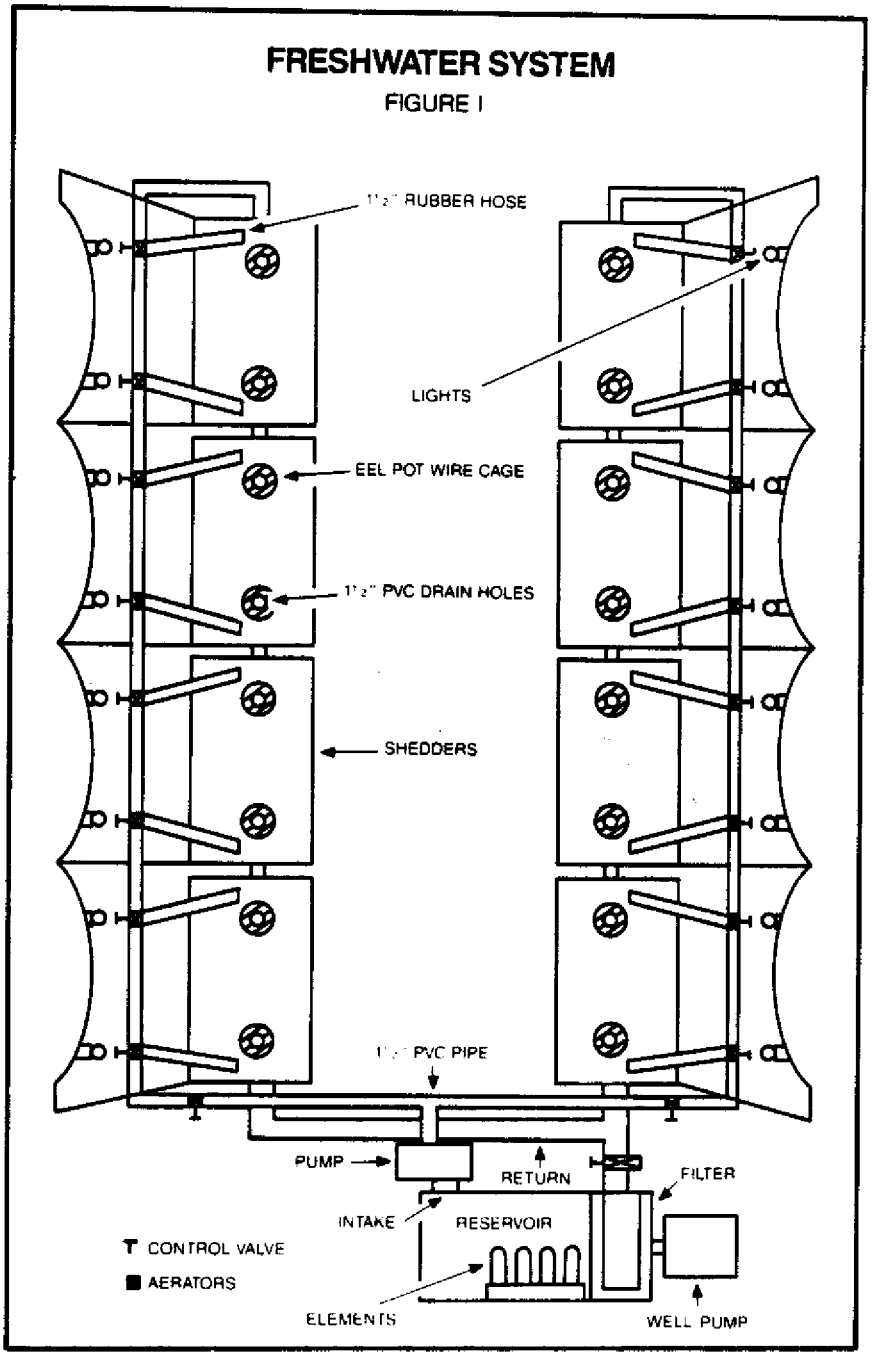
Other systems have maintained suitable oxygen levels, and some have attempted to manage water quality. But none have successfully managed all three factors.

To maintain water quality, the fresh well-water system allows water to seep into the ground through holes drilled in the bottom of the reservoir. This seepage eliminates the need for nitrifying bacteria.

Inexpensive test kits can be purchased to monitor water quality. If problems arise, the water can be diluted or

FRESHWATER SYSTEM

FIGURE I



completely replaced to eliminate problems. It is not as important to reuse the water as it is to have good water for the crabs.

Oxygen is added to the water as it returns to a filter box, as it passes through the filter box and by two aerators in the supply line of each shedding box.

The water temperature of groundwater ranges from 55 F to 65 F. This is too cold for crabs to shed. To warm the water to the ideal shedding temperature of 72 F, four 3000-watt heating elements, like those in a hot water heater, are used. To cool the water temperatures in June, July and August, well water is added to the reservoir.

MODIFICATIONS

The freshwater recirculating system has several design modifications. It requires the use of a groundwater well pump to supply the reservoir and a circulating pump to supply the shedding boxes.

Other items needed are a 220-volt power line for the 3000-watt elements, a thermostat to control the elements, a thermostat to control the well pump, a mercury switch or water level-control switch to maintain the water level in the reservoir, a valve to drain the system, an overflow drain in the reservoir and a filter box.

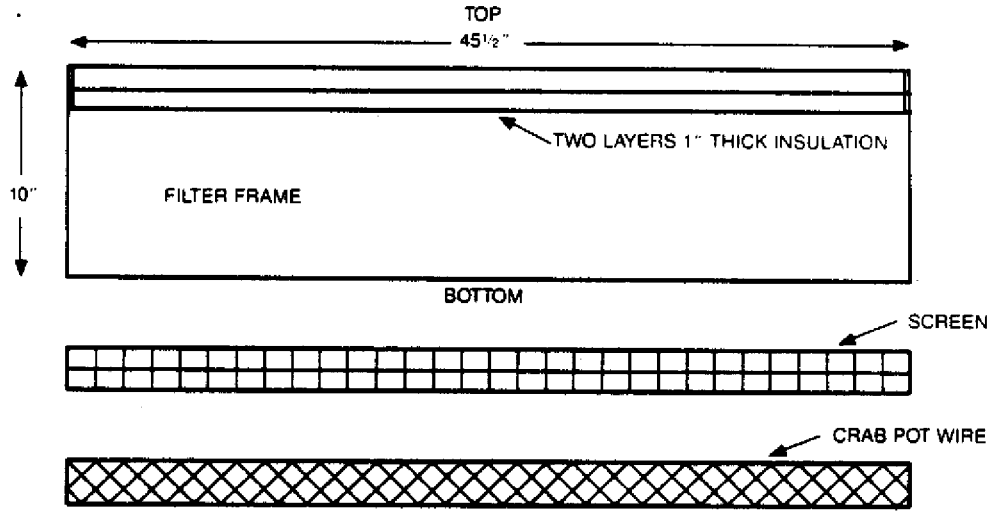
These modifications help maintain the proper water quality, oxygen content and temperature to successfully shed crabs any time peelers are available.

THE FILTER

To build the filter frame (Fig. 2), use 1-by-10 inch lumber. The frame is 45 1/2 inches long, 18 inches wide and 10 inches deep. The freshwater filter is nailed against the short side of the 4-by-8-by-4 foot plywood reservoir below the return line from the shedding boxes and about 4 inches from the top.

FRESHWATER FILTER

FIGURE II



The bottom is covered with window screen that may be nailed or stapled to the frame. To provide strength and support, a layer of crab pot wire is nailed or stapled under the screen.

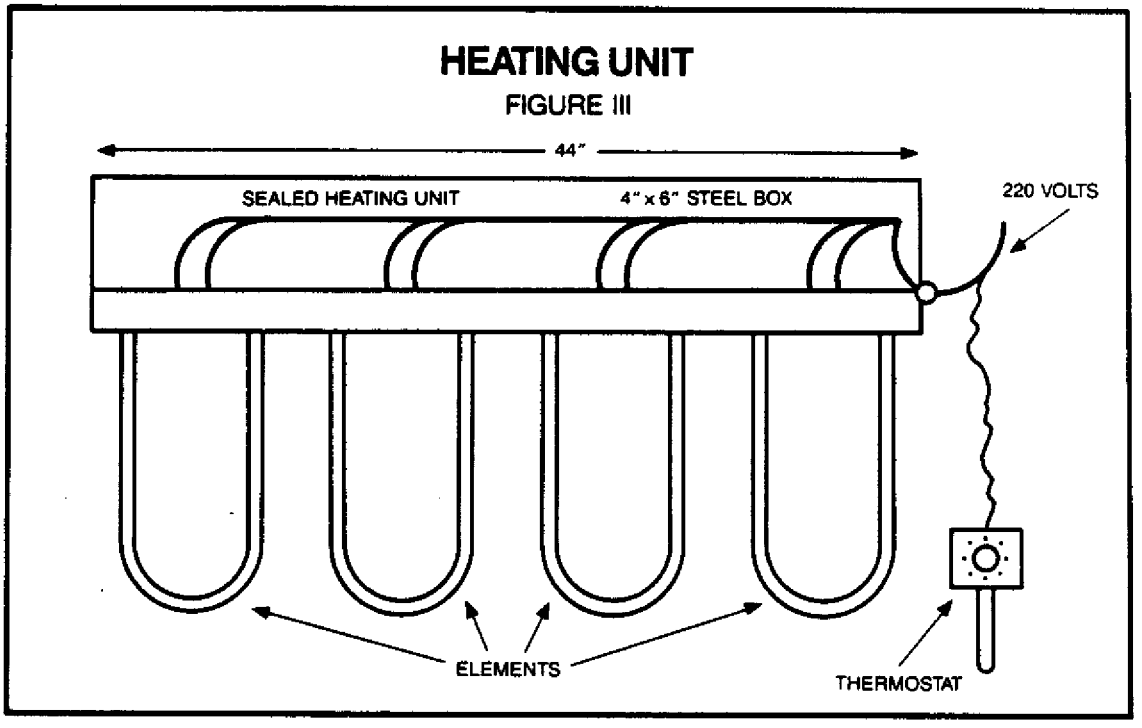
Then two layers of 1-inch thick foam rubber air conditioning insulation are cut to fit snugly within the top of the frame. A snug fit should force the water to filter through the insulation instead of passing through a crack along the edge.

Two layers of insulation are placed in the top of the frame. A third layer is also cut and used as a spare. Twice a week the top layer of insulation is removed, replaced by the spare and cleaned in a washing machine with regular detergent. The clean layer of insulation becomes the new spare.

Every two weeks the bottom layer of insulation is also removed and cleaned the same way.

HEATING ELEMENT

A homemade heating device shown in Figure 3, using four 3000-watt elements like those in a hot water heater, is inserted underwater in the reservoir.



These elements are attached to 4-by-44-by-6 inch box steel box. The elements are sealed with waterproof caulking or by welding. Or crabbers can buy an equivalent heating element at their local hardware store.

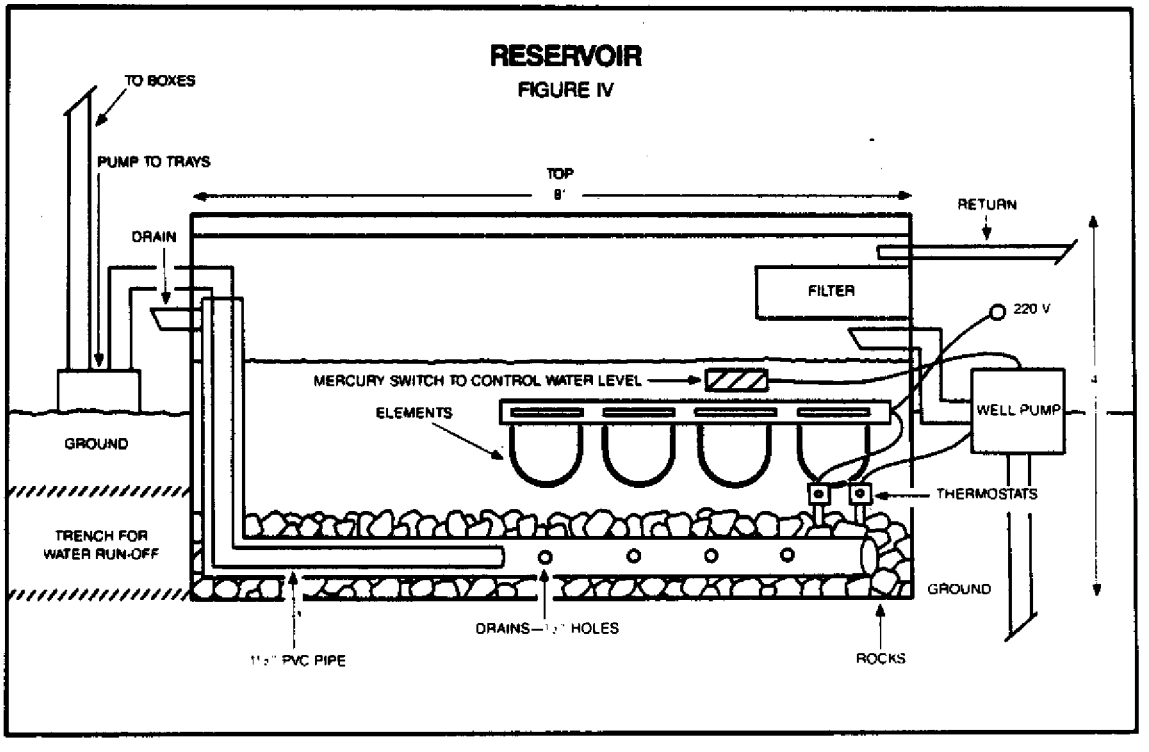
The element is mounted lengthwise in the reservoir about 2 feet from the bottom. The element should be covered by water at all times.

The heating device is activated by a thermostat switch on a probe near the reservoir bottom. When the water temperature reaches 72 F the elements are turned off by the thermostat.

The well pump has a manual switch for start-up and a thermostat to activate the pump if the water gets too hot. To prevent the heating elements from burning up, a water level switch is used to activate the pump if water level in the reservoir drops because of leakage.

THE RESERVOIR

The reservoir in Figure IV is built of 1/2-inch plywood 4 feet wide, 4 feet deep and 8 feet long. It is buried in the ground 2 feet to allow gravity drainage from the shedding tables. The ground also reinforces the box so that in most cases it does not need to be braced with 2-by-4 inch boards.



Six to eight 1 1/2-inch holes are drilled in the bottom of the reservoir to allow leakage into the ground.

The 1 1/2-inch PVC pipe intake is inserted in a 5-inch perforated sewer pipe covered with one ton of rock. The reservoir should have an overflow drain in case excess fresh water is added when cooling, and it should be covered to keep out trash.

It should be noted that 110 volts are required to operate the pumps and lights. A separate line of 220 volts is necessary to supply the heating elements.

The reservoir shown in Figure IV can store up to 600 gallons of water. That should be enough water for eight to 15 shedding tables. If you're considering a larger system, you will need to make adjustments in the size of the reservoir.

HOW THE SYSTEM WORKS

The well pump fills the reservoir and remains on until the desired water level in the reservoir and shedding trays is achieved. Initially this should take about two hours or less if your well has a pumping capacity of 10 gallons of water per minute or more.

The circulating pump forces water to the shedding tables. The heating device is activated only after it is completely submerged in water.

Water is pumped from the reservoir to the boxes and returns through the filter box to the reservoir. Water constantly seeps from the reservoir through the holes in the reservoir. The water level switch activates the well pump to replace lost water. Groundwater is pumped in to ensure good water quality.

If the water temperature falls below 70 F, the heating unit automatically warms cool water to 72 F. If the water temperature rises above 75 F, the pump automatically adds more cool groundwater to bring the temperature down. This cooling operation makes an overflow pipe necessary for release of excess water.

If you have a successful floating shedder system, flow-through system or closed recirculating system, don't change it. The freshwater shedding system is for those who want to shed crabs in cold and hot weather or for those who have poor surface or no natural surface supply nearby.

Freshwater systems require at least two pumps, two thermostats, a water control, mercury switch and heating units. These devices will increase the electric bill.

Also keep in mind that peeler crabs used in this system must be captured in waters with salinities of 15 ppt or less. Peeler crabs collected from water with higher salinities may suffer total mortality unless artificial salt is added to bring salinity within 15 ppt of the level where the crabs were caught.

Some people question the stress of fresh water upon blue crabs, but experience has proven it is not a factor. In fact, the largest soft crab shedding facility between North Carolina and Mexico successfully sheds soft crabs in zero salinity.

AN ECONOMIC ANALYSIS OF THE 1986 ALABAMA MULLET ROE INDUSTRY

William Hosking, Brian E. Perkins, and Richard K. Wallace
Alabama Sea Grant Extension Service
3940 Government Boulevard
Mobile, AL 36693

and

Hugh A. Swingle
Alabama Department of Conservation and Natural Resources
Marine Resources Division
P.O. Box 189
Dauphin Island, AL 36528

ABSTRACT

Alabama processors converted 2.05 million lbs of roe mullet into five products (roe, gizzards, whole male fish, carcasses for human consumption, and carcasses for bait) valued at $1.75 million, and employed 140 land-based personnel during the October - November 1986 season. Analyses of domestic and overseas product and value flows are presented.

INTRODUCTION

The Autumn of 1986 saw many newcomers enter the Alabama mullet roe industry. The previous year's strong prices (up to 75 cents per/lb of female roe mullet) provided incentive for additional Gulf Coast fishermen to direct their efforts toward roe mullet. Consequently, roe mullet gillnetted from Louisiana through Florida were processed in Alabama. Several new mullet roe processing facilities also opened their doors during 1986.

The Alabama Sea Grant Extension Service gathered the following poundage and value data as part of a larger project conducted in cooperation with the Alabama Department of Conservation and Natural Resources, Marine Resources Division. Funds for the overall project were provided by the National Marine Fisheries Service through the Marine Fisheries Initiative.

METHODS

The data presented in this report were collected from five Alabama mullet roe processors. In some instances, processors provided access to their purchase and sales receipts. In other cases, processors simply provided total product volume and value statistics for the various commodities purchased and sold.

RESULTS

Roe mullet purchased by Alabama processors were caught by fishermen in four Gulf Coast states. Total poundage and contributions from individual states are listed in Table 1:

Table 1. 1986 Sources of Roe Mullet Processed in Alabama.

State	Pounds	% of Total
Louisiana	905,263	44.1
Alabama	644,210	31.4
Florida	463,158	22.5
Mississippi	40,000	2.0
TOTALS	2,052,631	100.0

The number of fishermen responsible for the above production has proven impossible to ascertain.

Prices paid to fishermen for "95% pure" female roe mullet ranged from 75 cents/lb to 90 cents/lb. Therefore, roe mullet fishermen were paid between $1.54 million and $1.85 million during the seven- week season. Based on an average seasonal price of 82.5 cents/lb, the mean total price paid to fishermen was $1.69 million.

Although Alabama processors paid for "95 % pure" female roe mullet, the collected statistics indicate that the previously reported total of 2,052,631 lb was actually composed of 1,650,000 lb of female mullet and 402,631 lbs of male mullet. This translates into 19.6% males and 80.4% females.

Upon arriving at the processing facilities, the mullet were separated by sex. The whole (round) male "white roe" mullet were purchased by Egyptian buyers at an average price of 21 cents/lb. The 402,631 lb of male mullet generated a total gross profit of $84,552 for Alabama processors during 1986.

The female roe mullet were then split and stripped of roe sacks and gizzards. Roe sack and gizzard yields averaged 17% and 1%, respectively. Based on 1.65 million lb of female roe mullet, total roe and gizzard production was 280,000 lb and 16,500 lb, respectively. All gizzards and

female roe sacks were purchased by Taiwanese buyers. Gizzards were sold for an average of $1.60/lb, and yielded a 1986 total gross profit of $26,500. Female roe prices ranged from $2.40/lb for less-than-2-ounce or broken roe and up to $7.00/lb for 7-8 ounce roe. The mean sale price for all sizes of mullet roe was $5.50/lb, and generated a total gross profit of $1.54 million for Alabama processors during 1986.

The remaining 1,353,500 lb of female roe mullet carcasses were disposed of by either of two methods. Some of the carcasses (6%, or 83,500 lb) were of extremely high quality, and were sold to 5 or 6 smaller mullet smoking operations for 25 cents/lb, and yielded the mullet roe processors a total gross profit of $20,875. (The value added by the smaller mullet smoking firms could not be ascertained.) However, the majority of the carcasses (94% or 1.27 million lb) were sold as crab bait for a mean of 6.25 cents/lb, and generated a total gross profit of $79,455.

The 1986 Alabama mullet roe production and gross income statistics are summarized in Table 2:

Table 2. 1986 Alabama Mullet Roe Industry Products, Production, and Gross Income.

Product Type	Total Production, lb	Mean Value, $/lb	Gross Income, $
Mullet Roe	280,000	5.50	1,540,000
Male Mullet	402,631	.21	84,552
Gizzards	16,500	1.60	26,500
Bait Carcasses	1,270,000	.0625	79,455
Food Carcasses	83,500	.25	20,875
TOTALS	2,052,631	---	1,751,382

The gross income values are not exactly multiplicative due to rounding of mean $/lb values.

The five Alabama mullet roe processors employed an approximate total of 140 land-based personnel. Those personnel were manual laborers who were paid at or slightly more than minimum wage ($3.35/hour). The manual laborers were responsible for loading and unloading refrigerated transport trucks, splitting mullet, removing roe and gizzards, and packaging roe and other secondary products for transportation or storage. The 140 manual laborers were paid a total of $131,320 in wages by the five processors. That figure translates into $938 per employee, or an average processing labor cost of 6.4 cents/lb of roe mullet.

As was previously mentioned, Alabama processors paid a mean total of $1.69 million for roe mullet during 1986. That price was FOB plant, and no additional freight costs were borne by the processors on the

purchasing end. Likewise, no additional freight costs were paid by the processors on the selling end. Primary products (female roe sacks and gizzards) and a portion of the secondary products (whole, round male mullet) were purchased at the plants by Taiwanese and Egyptian buyers. Consequently, any additional freight costs were borne by the foreign buyers.

Several processors sold their byproducts (bait carcasses) to Florida buyers as the carcasses were produced. The bait carcasses were purchased on site, and no additional freight costs were borne by the processors. The same was true for processors who sold high-quality carcasses to local mullet smoking operations. The food carcasses were purchased at the processing plants, and no additional freight charges were incurred by the processors.

Several other processors stored their byproducts in a frozen storage facility in downtown Mobile. Approximately 520,000 lb of mullet carcasses had to be transported from the processing plants to the warehouse in downtown Mobile. Based on an average shipping cost of $150 per 10,000 lb, a total transportation cost of $7,800 was paid by Alabama processors.

The 520,000 lb. of mullet carcasses were held in frozen storage for various periods of time ranging from 5 - 7 months. Based on an average charge of 2 cents/lb/month, Alabama processors incurred total frozen storage expenses of $59,280. That figure translates to an average of 11.4 cents/lb of stored mullet carcasses.

All primary products, secondary products, and byproducts were packaged for transportation and storage. The usual packages were poly-lined 20-kilogram cardboard boxes, although sub-units of female roe sacks were additionally packed in individual plastic bags and 1-kilogram paper boxes. Packaging costs averaged .4 cent/lb. Therefore, it cost a total of $8,210 to package the 2.05 million pounds of mullet products.

The 1986 Alabama mullet roe production expense statistics are summarized in Table 3:

Table 3. 1986 Alabama Mullet Roe Industry Production Expenses.

Expense Type	Pounds	Mean $/lb Expenses	Expense, $
Cost of Mullet	2,052,631	.825	1,693,420
Labor Expense	2,052,631	.064	131,320
Frozen Storage	520,000	.114	59,280
Packaging	2,052,631	.004	8,210
Transportation	520,000	.015	7,800
TOTAL	---	---	1,900,030

Appendix Figure 1 provides a schematic representation of 1986 Alabama roe mullet industry product and value flows. The total expense and total gross income figures calculated in Tables 3 and 2, respectively, indicate that, based on a mean purchase price of 82.5 cents/lb of roe mullet, the 1986 Alabama roe mullet industry suffered a $148,648 mean net loss. Given that prices as low as 75 cents/lb and as high as 90 cents/lb were paid for roe mullet, the overall 1986 Alabama mullet roe industry economic situation actually could have ranged anywhere between a $5,299 net profit and a $302,596 net loss.

The $148,648 mean net loss was probably the result of several processors who profited, several who broke even, and several more processors who operated at a loss. This presumption is based on several situations which presented themselves during the October - November 1986 roe mullet season:

- Several firms discontinued processing once the price for "95 % pure" female roe mullet escalated beyond 80 cents/lb.

- Several firms, with specific contractual agreements to fulfill, had to continue processing regardless of exvessel price.

- The long-term frozen storage of mullet carcasses imposed an additional expense of $67,080, which contributed roughly 45% toward the overall industry mean net loss.

- Some processors felt that their "counts" were off. They expected a greater percentage of 7 - 8 oz roe (the product of greatest dollar value) than they actually recovered. Thus, their total gross profit and average price per lb of roe were both less than they anticipated.

CONCLUSIONS

Based on the data collected during the course of this project and presented in this report, the authors offer the following conclusions:

- Processors generally paid too much for roe mullet.

- Too many male mullet were purchased at female roe mullet prices.

- Exported mullet roe was of smaller size.

- Too much expense was incurred for long-term storage of bait carcasses.

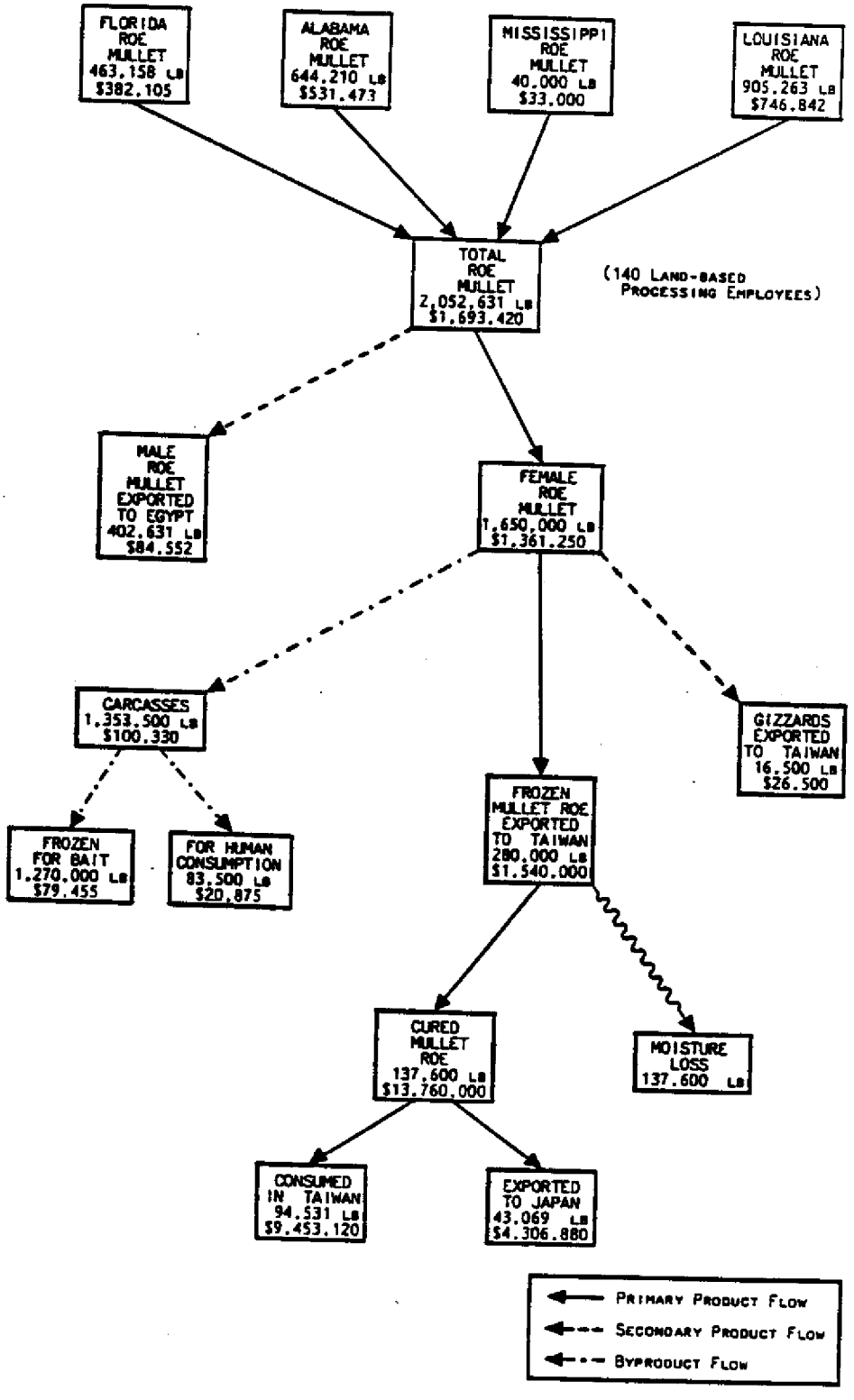
- The mullet roe industry, as a whole, operated at a loss.

ACKNOWLEDGEMENTS

The authors wish to express their appreciation to the following individuals and organizations for their support and cooperation.

- The National Marine Fisheries Service, Marine Fisheries Initiative, for providing project funding.
- The Auburn University/Alabama Cooperative Extension Service, for providing matching funds.
- The members of the Alabama roe mullet industry, for providing data and statistics.
- Mr. E. Moret Smith, for assistance with data collection.

APPENDIX FIGURE 1. 1986 ALABAMA ROE MULLET INDUSTRY PRODUCT AND VALUE SCHEMATIC



HARVEST AND PRESERVATION OF A SHRIMP BY-CATCH: CANNONBALL JELLYFISH (*Stomolophus meleagris*)

Yaowen Huang, Paul Christian and Dan Colson
The University of Georgia
Marine Extension Service
P. O. Box Z
Brunswick, GA 31523

INTRODUCTION

Jellyfish have been highly valued as a salted, dried seafood commodity in the Orient for centuries. In 1985, the world harvest of jellyfish was 182,662 metric tons. These were primarily caught from the Indian, Northwest Pacific, and Western Central Pacific Oceans by countries including Thailand, Indonesia, Malaysia, the Phillippines, and China (FAO, 1987). The species caught in these areas belong to the genus *Rhopilema*. Among them, *R. esculenta* is the most common species for processing (Mayer, 1910; Davidson, 1977). However, *R. asamushi*, *Stomolophus nomurai*, and *Dactylometra pacifica* are also used as food items. In recent years, jellyfish have been gaining importance in the international markets, especially in Asia (Huang, 1985). This has led countries not producing jellyfish to begin studying the production of other species. *Catostylus* spp. was, therefore, used in Australia (Wootten et al., 1982) and *Aurelia* spp. in India (Govindon, 1984).

In U. S. waters, there is a non-stinging species of jellyfish *Stomolophus meleagris*, commonly called cannonball jellyfish. They range from the mouth of the Chesapeake Bay to Texas. When abundant, they become a nuisance to shrimp fishermen. This species has been successfully developed into an edible form on a pilot plant scale (Huang, 1986, 1988). The prototype product was compared favorably to the existing imported products (Huang, 1987). In order to completely utilize this resource, the harvesting method, on-board handling, and preservation of cannonball jellyfish need to be understood. Objectives of this study are: (1) to determine the harvesting methods using smaller and larger fishing vessels, on-board handling, and preservation, and (2) to compare the catches of cannonball jellyfish by using different Turtle Excluder Devices (TEDs).

MATERIALS AND METHODS

Research was conducted aboard University of Georgia vessels in two phases. The first phase was conducted in the St. Simons Sound, Georgia (Study area A) (Figure 1) from May through June 1986 using the R/V UNDERDOG, a 12-m long lobster boat. The objectives were to study small-scale harvesting, on-board handling, and preservation.

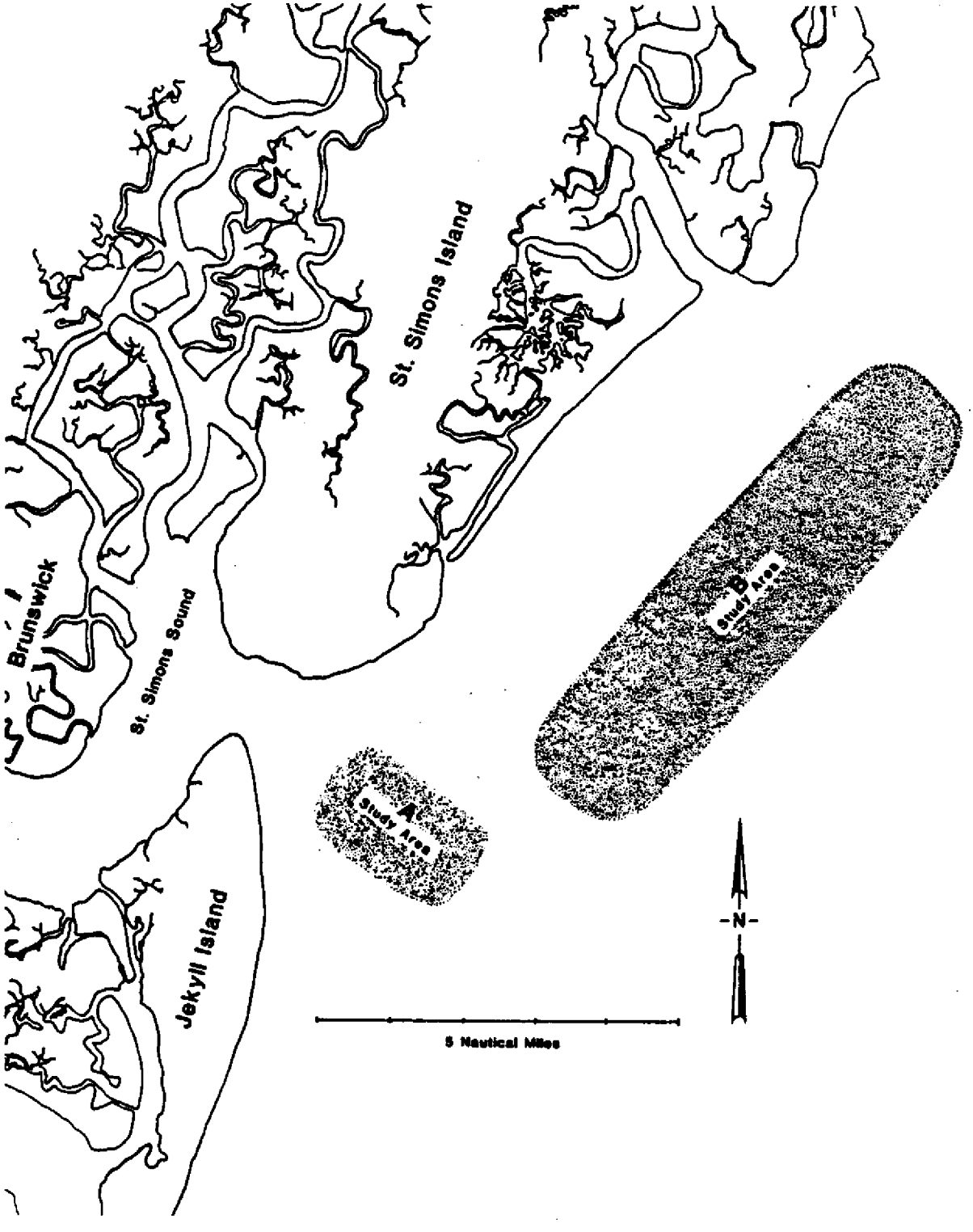


Figure 1. Map of Study Areas A and B in the waters around St. Simons Island, Georgia

A 6-m flat net was used for phase one studies. The net was constructed from 4.8-cm stretched mesh number 12 nylon webbing for the wings and body, with number 24 twine for the codend. Standard wooden trawl doors were used, and measured 0.9 x 0.38 m. Bridle length was 9.1 m. The net was equipped with three 15.2-cm Spongex floats on the headrope with standard loop chain spaced every 0.4 m on the footrope. A 4.8-mm tickler chain which was 45.7 cm shorter than the footrope was also used. The trawling time was 15 minutes.

Phase two was conducted 3 to 5 N miles east of the south portion of St. Simons Island, Georgia (Study area B) (Figure 1) from October through December 1986 using the R/V GEORGIA BULLDOG, a 22-m long commercial shrimp boat. One of the objectives was to determine the commercial scale catch and retention of cannonball jellyfish in nets equipped with Turtle Excluder Devices (TEDs).

Two identical 18.2-m flat nets were used for these studies. Each net was constructed from 4.8-cm stretched mesh number 15 twine for the wings and body, with number 36 twine for the codend. Standard wooden trawl doors were used, and measured 1.0 x 2.4 m. Bridle length was 75.8 m. Each net was equipped with ten 20.3-cm Spongex floats on the headrope and mud rollers spaced every 1.1 m on the footrope. In addition, 64-mm loop chain was used on the footrope at the center (1.9 m) and wing/corner piece sections (3.8 m).

The sampling technique was designed to fit the random block design (Mendenhall, 1968), and the trawling was in a manner similar to that used by commercial fishermen. The net on the side of the vessel to receive the TED was randomly chosen. All TED-equipped nets were pulled simultaneously against a control net with no TED. Four TEDs, including the Louisiana TED, Georgia TED, Texas TED, and NMFS TED, were used in this study (Christian and Harrington, 1987). Tow time remained constant at 1 hour.

Ice and seawater were used for testing the preservation of cannonball jellyfish on the boat. An innovative three-phase salting and pressing technique was tested (Huang, 1986) to produce and evaluate the finished jellyfish product.

RESULTS AND DISCUSSION

Cannonball jellyfish is the most abundant jellyfish in the waters off St. Simons Island, Georgia. It appears offshore in early April and moves in nearer the beaches in May and June. Some cannonball jellyfish, however, were washed onto the beaches of Jekyll Island and St. Simons Island during April 1986, but none were caught in our trawling test during that time. By July, they had disappeared from shrimp grounds (Figure 1), but they reappeared from late-October through mid-December (Table 1). During one of these periods, jellyfish filled the nets of our boat

Table 1. Total catches, catch per unit effort (kg of biomass per net per hour), percentage of jellyfish and shrimp and average weight of cannonball jellyfish by shrimp trawling

Date	No. of trawl tows	Total catch (kg)	CPUE	Avg. % of jellyfish	Avg. % of shrimp	Avg. wt. of jellyfish (kg)
10/29/86	7	3357	239.8 (79–653)	60.25 (36.67–84.91)	2.73 (0.17–6.41)	0.31 (0.25–0.40)
11/05/86	8	4082	255.2 (84–490)	77.25 (67.92–90.38)	2.42 (0.34–5.66)	0.33 (0.16–0.46)
12/04/86	12	3119	130.0 (30–508)	49.50 (6.24–93.20)	5.22 (0–17.65)	0.46 (0.23–0.57)
12/11/86	5	368	36.9 (29–44)	11.18 (8.25–17.75)	17.59 (11.60–26.30)	0.38 (0.23–0.64)
12/12/86	4	715	89.3 (59–143)	12.94 (6.16–23.50)	8.23 (3.92–15.30)	0.31 (0.17–0.44)
12/16/86	5	449	89.8 (21–24)	10.87 (1.15–23.20)	4.65 (0.29–14.66)	0.56 (0.42–0.64)

so quickly that trawling times were dramatically reduced. After mid-December, only the manubriums (the stem-like mouth-tube) of jellyfish were caught; jellyfish had nearly disappeared from the shrimp grounds. Results from our 1986 studies were similar to that of Kraeuter and Setzler (1975). They reported that cannonball jellyfish were collected in the waters around Sapelo Island (about 20 miles north of St. Simons Sound) from March through October. Both studies suggest that cannonball jellyfish are present along the Georgia coast during the spring and the fall seasons.

Cannonball jellyfish can be easily harvested using an otter trawl with any boat rigged for shrimping. Jellyfish primarily drift along the water's surface, and may be more easily caught using a top water or surface trawl. The otter trawl should work fine for surface trawling, but there is a need to modify the net as well as to design a floating trawl door.

In compliance with the new Turtle Excluder Device regulations, four TEDs were used to study the effect on the quantity and size of catch. Results show that use of the Louisiana TED reduced the percentage of jellyfish by 84%, followed by the Georgia TED at 78%, and the NMFS TED at only 3% (Table 2). However, during the entire study period, no cannonball jellyfish were present when the Texas TED was tested. The reason the NMFS TED did not affect the quantity of catch was because the bar spacing of the NMFS TED (114.3 mm) was larger than that of the Louisiana (50.8 mm) and Georgia TEDs (60.3 mm) (Christian and Harrington, 1987). The size of cannonball jellyfish caught in a TED-equipped net was also reduced (Table 2). Size was reduced by 50% using the Louisiana TED, while no difference was found with the NMFS TED. Since the TED will be mandatorily used by shrimpers off the South Atlantic coast from May 1 until August 31, 1988, it suggests that a TED with larger bar spacing and a net with larger mesh should be designed to catch cannonball jellyfish.

Ice and seawater were tested for holding cannonball jellyfish after harvesting during spring and fall. There are no significant differences in the eating quality of processed products. Therefore, seawater is suggested for use in preserving the catch during May and June. However, there is no need for preservation from late-October through mid-December for a day-long trip.

For further preservation of cannonball jellyfish, a salted and dried product was produced. Results show that a three-phase processing method is suitable for cannonball jellyfish. It takes eight days to process (Table 3), and the final product can be preserved at 5°C up to one year without any loss of eating quality. There are no significant differences in the texture of processed products using fresh and frozen cannonball jellyfish.

Table 2. Catch per units effort (kg of biomass per net per hour), percentage and weight of cannonball jellyfish by trawling with different TEDs

	Louisiana TED		Georgia TED		NMFS TED	
CPUE	Control	109.8 (31-210)	Control	190.7 (31-490)	Control	301.6 (118-653)
	TED	45.4 (19-82)	TED	35.6 (15-63)	TED	251.9 (109-551)
% of jellyfish	Control	44.08 (0-71.43)	Control	51.54 (8.25-90.38)	Control	78.55 (63.16-93.2)
	TED	7.16 (0-32.20)	TED	11.37 (0-29.58)	TED	76.29 (44.44-94.38)
Avg. weight of jellyfish (kg)	Control	0.30 (0.25-0.37)	Control	0.29 (0.14-0.46)	Control	0.40 (0.16-0.57)
	TED	0.15 (0.09-0.19)	TED	0.25 (0.20-0.34)	TED	0.41 (0.24-0.58)

Table 3. Recommended three-phase processing method of salted dried cannonball jellyfish

Salting	Water	Salt	Alum	Duration
First phase	1000 ml	75 gm	25 gm	two days
Second phase[1]	1000 ml	150 gm	10 gm	four days
Third phase[2]	---	dried salt	---	two days

[1] adding pressure on the top of brined jellyfish after salting for two days

[2] piling one above the other

CONCLUSION

1. Harvesting of cannonball jellyfish can be easily done by commercial shrimp vessels. However, when TEDs are required for shrimp trawling, nets with larger mesh and TEDs with larger bar spacing need to be designed. This will allow continued capture of larger sized cannonball jellyfish, while allowing for the ejection of marine turtles. This needs to be studied further.

2. Chilled seawater is suggested for use in preserving cannonball jellyfish caught during spring season. However, there is no need for preservation in the fall season.

3. Salted, dried cannonball jellyfish can be preserved at 5°C up to one year without any loss of quality.

LITERATURE CITED

Christian, P. and Harrington, D. 1987. Loggerhead turtle, finfish and shrimp retention studies on four Turtle Excluder Devices (TEDs). Proc. Nongame and Endangered Wildlife Symposium, September 1987. Georgia DNR, Social Circle, GA. (In Press)

Davidson, A. 1977. Seafood of South-East Asia. Publisher: Federal Publications (S) Pte Ltd., Singapore. p. 192.

FAO. 1987. Yearbook of fishery statistics. FAO. UN. Rome.

Grovindon, T. K. 1984. A novel marine animal with much export potential. Seafood Export J. 16(7):9-11.

Huang, Y. 1985. The utilization of American jellyfish and its markets. Paper presented at the 30th Annual Fisheries Technological Conference and International Symposium, August 25-29. Boston, MA.

Huang, Y. 1986. The processing of cannonball jellyfish (Stomolophus meleagris) and its utilization. Proc. 11th Annual Tropical and Subtropical Fisheries Conference of the Americas. p. 141.

Huang, Y. 1987. Acceptability and quality of cannonball jellyfish (Stomolophus meleagris) as a commercial product. Paper presented at the 7th World Congress of Food Science and Technology. September 28 - October 1. Singapore.

Huang, Y. 1988. Cannonball jellyfish (Stomolophus meleagris) as a food source. J. Food Sci. (In Press)

Kraeuter, J. N. and Setzler, E. M. 1975. The seasonal cycle of scyphozoa and cubozoa in Georgia estuaries. Bulletin of Marine Science. 25(1):66.

Mayer, A. G. 1910. Medusae of the world. Volume 3. "The Scynhomedusae." Carnegie Institute of Washington, Washington, DC.

Mendenhall, W. 1968. Introduction to linear models and the design and analysis of experiments. Wadsworth Publishing Co., Inc., Belmont, CA.

Wootton, M., Buckle, M. A., and Martin, D. 1982. Studies on the preservation of Australian jellyfish (Catostylus spp.) Food Technol. Australia. 34:398.

ACKNOWLEDGEMENTS

The authors greatly appreciate Lindsey Parker, David Daniels, Marty Higgins, and Lura Relihan for their technical assistance. This study was supported in part by The University of Georgia, the Gulf and South Atlantic Fisheries Development Foundation, and the National Oceanographic and Atmospheric Administration, U. S. Department of Commerce, through the National Sea Grant Program (NA84 AA-D-00072). The U. S. Government is authorized to produce and distribute reprints for Governmental purposes notwithstanding any copyright notation that might appear hereon.

Development and Evaluation of Skinless, Boneless Pink Salmon Products

Charles Crapo
University of Alaska, Kodiak, Alaska
Elisa Elliot
USDA, FSIS, Washington, D.C.
Jerry Babbitt and Kermit Reppond
National Marine Fisheries Service, Kodiak, Alaska
Paul Peyton
Office of Commercial Fisheries Development, Juneau, Alaska

INTRODUCTION

Pink salmon (Oncorhynchus gorbuscha) is the most abundant salmon species harvested in Alaska. Since 1980, pinks have accounted for 56 percent of the total salmon landings, averaging 71 million fish per year. During this period, ex-vessel prices have fallen from $0.44 per pound in 1981 to $0.23 per pound in 1986. Among the reasons for this decline have been limited product forms and markets.

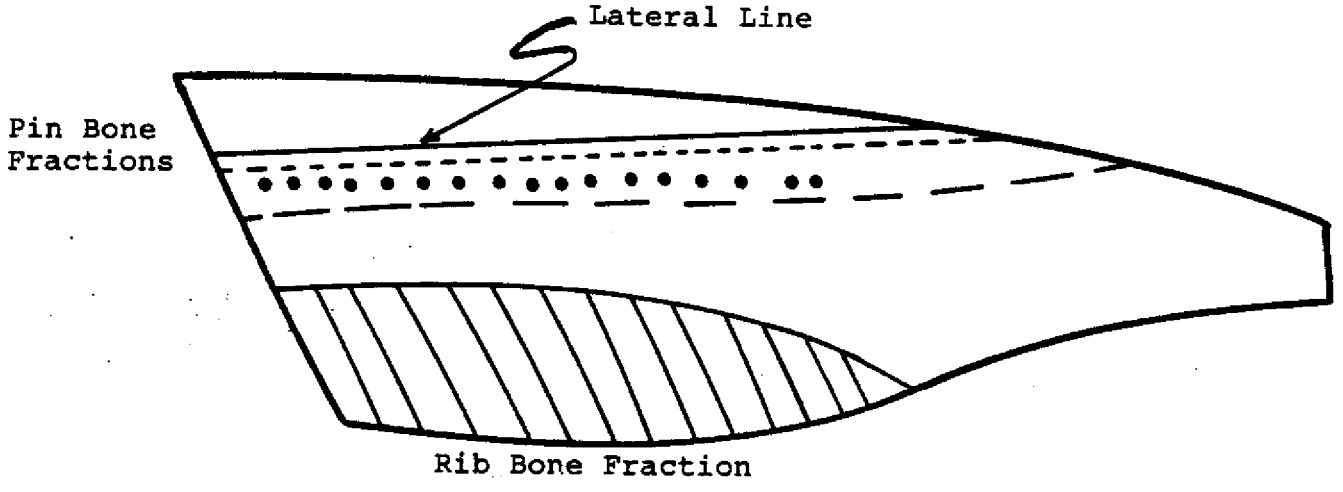
The traditional markets for pink salmon have been as canned products, but in recent years consumption has dropped while supplies have been at historic highs. The drop in consumption has been related to the change in consumer trends to convenience type foods. As a response, the canned salmon industry has developed a skinless, boneless canned product which has found new markets.

In an effort to broaden product forms for pink salmon, the State of Alaska Office of Commercial Fisheries Development (OCFD), University of Alaska Fishery Industrial Technology Center (FITC), Alaska Fisheries Development Foundation (AFDF) and the National Marine Fisheries Service (NMFS) have conducted a series of studies to evaluate alternate fresh/frozen pink salmon products made from skinless, boneless fillets. Studies included investigations into product form and fresh/frozen versus reprocessed shelf life.

METHODS

Fresh-Frozen Product/Shelf Life Evaluations. Fresh pink salmon were dressed, hand or machine filleted and skinned, then trimmed to produce boneless fillets. Trimmings, which included pin and rib bone fractions (Figure 1), were minced using a Baader 694 deboner. Using 18.5 pound metal forms, pink salmon blocks of 100 percent fillet, 100 percent mince, 75 percent fillet/25 percent mince, and 50 percent fillet/50 percent mince were produced, frozen and stored at -18°C (0°F) for 1, 3, 6, and 12

Figure 1. Cuts Used To Prepare Pink Salmon Fillets (Skinless, Boneless) and Trimmings (Pin and Rib Bones)



months. Fillet (100 percent) blocks were used as controls and stored at -36°C (-34°F).

At the end of each storage period, product forms were evaluated for sensory, oxidative and physical changes. Sensory evaluation scored color, flavor, chewiness, moistness and desirability on a seven point descriptive scale and texture on a five point scale (Table 1). Color and moisture descriptors were anchored to the control samples. Taste panel data was analyzed using a factorial design and least squares difference to determine the effect of storage and product form on shelf life. Fat oxidation was determined using Lemon's modified TBA test (1975) and thaw drip (AOAC, 1984) was used as a measure for some of the physical and sensory changes occurring during frozen storage.

Reprocessed Product/shelf Life Evaluation. Frozen, dressed pink salmon stored at -18°C (0°F) for 3, 6, and 12 months were thawed overnight at 10°C (50°F), hand filleted, skinned and trimmed to produce boneless fillets. The trimmings were minced using a Baader 694 deboner. Fillet, mince and combination fillet/mince blocks were produced, refrozen and stored at -18°C (0°F) for 0, 3, 6, and 9 months. At the end of each storage period, the reprocessed blocks were compared with the fresh/frozen samples and evaluated for sensory, oxidative, and physical changes.

RESULTS AND DISCUSSION

Recoveries. Fillet and mince yields from round fish varied with filleting methods (Table 2). The use of mechanical filleting equipment resulted in slightly higher recoveries than hand filleting. Its addition to the combination fillet/mince blocks maximized recovery and provided 40 percent more product.

Table 2. Recovery of Fillets (Skinless, Boneless) and Trimmings From Manual and Mechanized Operations

	% RECOVERY (WHOLE WEIGHT BASIS)	
	Manual (Deep-skinned)	Mechanical (Deep-skinned)
Fillets (skinless, boneless	33.26%	33.56%
Trimmings	12.24	14.76

One Month Evaluation. After one month storage, the fresh/frozen products compared favorably with the control samples. High sensory scores, low TBA values and thaw drip indicated the product forms had not deteriorated noticeably during storage. Taste panelists expressed significant

Table 1. Score Sheet Used for Sensory Evaluation

COLOR	FLAVOR	CHEWINESS
7-Much Lighter	7-Control, Excellent Fresh Salmon Flavor	7-Very Tender
6-Lighter	6-Good, but not Intense	6-Moderately Tender
5-Slightly Lighter	5-Only Mildly Pleasant	5-Slightly Tender
4-Same as Control	4-Trace of Bad Flavor, Rancid, Oxidized	4-Tender
3-Slightly Darker	3-Mild Bad Flavor	3-Slightly Tough
2-Darker	2-Strong Bad Flavor	2-Moderately Tough
1-Much Darker	1-Intense Bad Flavor	1-Very Tough

TEXTURE	MOISTNESS	DESIRABILITY
5-Fibrous	7-Much More Moist	7-Like Extemely
4-Grainy	6-More Moist	6-Like Moderately
3-Flaky, Firm	5-Slightly More Moist	5-Like Slightly
2-Soft	4-Moist as Control	4-Neutral
1-Mushy	3-Slightly Drier	3-Slight Dislike
	2-Drier	2-Moderate Dislike
	1-Much Drier	1-Dislike Extremely

preferences for the 100 percent fillet and 75 percent fillet/25 percent mince blocks (Table 3). TBA values revealed low amounts of fat oxidation (Table 4) and thaw drips were constant (Table 5).

Table 3. Mean[1] Desirability Scores for One Month Fresh/Frozen Pink Salmon Blocks

TREATMENT	FORM (% FILLET)			
	100%	75%	50%	0%
Fresh/Frozen Block	6.50	6.12	5.83	5.08
Control	6.60			
Analysis of Variance				
F Values		Ranking of Level Means		
Form (F)	5.28[2]	100% > 75% > 50% > 0%		

[1] n=10 [2] Sig p>.005

Level means with the same underline did not vary significantly (p=.05) from one another.

Table 4. TBA Values (umoles/100g) For One Month Fresh/Frozen Pink Salmon Blocks

TREATMENT	FORM (% FILLET)			
	100%	75%	50%	0%
Fresh/Frozen Block	0.83	0.82	0.80	0.84
Control	0.80			

Table 5. Thaw Drip from One Month Fresh Frozen Pink Salmon Blocks

FORM	% THAW DRIP
100 % fillet	2.42 %
75 % fillet	4.84
50 % fillet	2.98
0 % fillet	2.85

Three Month Evaluation. At three months storage, reprocessed blocks had lower sensory scores, TBA values and higher thaw drip than the fresh/frozen product. Taste panelists indicated a significant (p=.05) preference for the fresh/frozen product (Table 6). This product had better flavor and moistness than the reprocessed blocks. The 100 percent mince block scored significantly (p=.05) lower than other product forms. Taste panel scores revealed that the mealy texture of the mince block lowered overall desirability.

Table 6. Mean[1] Desirability Scores For Three Month Fresh Frozen and Reprocessed Pink Salmon Blocks

TREATMENT	FORM (% FILLET)			
	100%	75%	50%	0%
Fresh/Frozen Block	5.76	5.43	5.52	4.80
Reprocessed Block	5.35	4.75	4.65	4.15
Control	6.60			

Factorial Analysis of Variance

F Values		Ranking of Level Means
Treatment (T)	13.33[2]	Fresh Frozen > Reprocessed
Form (F)	5.28[3]	100% > 75% > 50% > 0%
T X F	0.29[4]	

[1] n=10 [2] Sig p>.001 [3] Sig p>.005 [4] NS p<.05

Level means with the same underline did not vary significantly (p=.05) from one another.

TBA values of the fresh/frozen blocks were much higher than reprocessed product (Table 7). It appeared that processed products were more susceptible to the development of rancidity during frozen storage. This was due in part to the protection of the product in frozen storage. The fresh/frozen products were stored in waxed liners and plastic lined master cartons while the dressed salmon had been glazed and double plastic wrapped.

Table 7. TBA Values (umoles/100g) for Three Month Fresh Frozen and Reprocessed Pink Salmon Blocks

TREATMENT	FORM (% FILLET)			
	100%	75%	50%	0%
Fresh/Frozen Block	1.61	1.31	1.50	1.48
Reprocessed Block	0.71	0.78*	0.85*	0.98
Control	0.80			

*Estimated Values

Thaw drips were highest for the reprocessed products as a result of double freezing (Table 8). Most were within reasonable limits although the reprocessed 100 percent mince block had a thaw drip of 5.39 percent, considered unacceptable by many Alaska seafood processors.

Table 8. Thaw Drip Values for Three Month Fresh Frozen and Reprocessed Pink Salmon Blocks

TREATMENT	FORM (% FILLET)			
	100%	75%	50%	0%
Fresh/Frozen Block	2.96%	4.46%	2.65%	3.06%
Reprocessed Block	4.38%	3.60%	3.64%	5.39%
Control	2.15%			

Six Month Evaluation. After six months of frozen storage, differences between fresh/frozen and reprocessed products were not significant (p=.05). Fresh/frozen products were generally more desirable than the three and six month reprocessed products (Table 9). The exception was the 100 percent mince block which had the lowest desirability scores of any sample. The high fillet products were significantly (p=.05) more desirable than the high mince products. Average sensory scores indicated a preference for the 75 percent fillet/25 percent mince and 100 percent fillet forms. These samples had better flavor, texture and moistness. The first rancid and oxidized flavors were noted in some of the samples.

Table 9. Mean[1] Desirability Scores for Six Month Fresh Frozen and Reprocessed Pink Salmon Blocks

TREATMENT	FORM (% FILLET)			
	100%	75%	50%	0%
Fresh/Frozen Block 3 Month	5.64	5.18	4.54	3.55
Reprocessed Block 6 Month	4.64	4.36	4.27	4.00
Reprocessed Block	4.00	5.18	4.00	4.18
Control	6.55			

Factorial Analysis of Variance

F Values		Ranking of Level Means
Treatment (T)	1.39[3]	Fresh Frozen > 6 Month > 3 Month
Form (F)	4.15[2]	75% > 100% > 50% > 0%
T X F	1.92[3]	

[1] n=11 [2] Sig p>.005 [3] NS p<.05

Level means with the same underline did not vary significantly (p=.05) from one another.

TBA values were highest for the three month reprocessed product that had been stored an additional three months and lowest for the six month reprocessed product (Table 10). This indicated that thawing, refreezing and subsequent storage accelerated the development of rancidity in the products. TBA values for the fresh/frozen products remained fairly constant.

Table 10. TBA Values (umoles/100g) for Six Month Fresh Frozen and Reprocessed Pink Salmon Blocks

TREATMENT	FORM (% FILLET)			
	100%	75%	50%	0%
Fresh/Frozen Block 3 Month	1.33	1.32	1.12	1.38
Reprocessed Block 6 Month	1.68	1.54	1.44	1.75
Reprocessed Block	1.26	1.02	1.21	0.87
Control	0.80			

Thaw drips were highest for the reprocessed products (Table 11) revealing the effect of thawing and refreezing. All reprocessed samples had excessive thaw drips of 5.6 percent or greater indicating changes in the texture and moistness that were confirmed by the taste panel evaluations. The fresh/frozen products had acceptable thaw drips less than 4.5 percent.

Table 11. Thaw Drip Values for Six Month Fresh Frozen and Reprocessed Pink Salmon Blocks

TREATMENT	FORM (% FILLET)			
	100%	75%	50%	0%
Fresh/Frozen Block 3 Month	2.70%	3.55%	3.56%	4.45%
Reprocessed Block 6 Month	8.04%	5.58%	5.77%	7.34%
Reprocessed Block	5.60%	5.77%	6.31%	6.29%
Control	2.06%			

Twelve Month Evaluation. At the end of twelve months frozen storage, sensory scores were significantly lower revealing the general deterioration of all products. No significant (p=.05) differences existed between the fresh/frozen and reprocessed forms (Table 12). The fresh/frozen and twelve month reprocessed products had the slightly higher desirability than other samples. The three month reprocessed samples, the longest stored in frozen storage, had the lowest scores. Panelists again preferred (p=.05) the 100 percent fillet block form over all others. A significant (p=.05) dislike of the 100 percent mince blocks were also

noted. No differences existed between the 75 percent fillet/25 percent mince and the 50 percent fillet/50 percent mince products.

Table 12. Mean[1] Desirability Scores for Twelve Month Fresh Frozen and Reprocessed Pink Salmon Blocks

TREATMENT	FORM (% FILLET)			
	100%	75%	50%	0%
Fresh/Frozen Block 3 Month	4.73	3.82	3.54	3.27
Reprocessed Block 6 Month	3.82	3.72	3.18	2.27
Reprocessed Block 12 Month	4.18	3.36	4.00	2.91
Reprocessed Block	4.64	3.82	3.55	3.09
Control	6.50			

Factorial Analysis of Variance

F Values		Ranking of Level Means
Treatment (T)	9.20[3]	Fresh Frozen > 12 Mo. > 6 Mo. > 3 Mo.
Form (F)	46.83[2]	100% > 75% > 50% > 0%
T X F	8.19[3]	

[1] n=11 [2] Sig p>.001 [3] NS p<.05

Level means with the same underline did not vary significantly (p=.05) from one another.

Thaw drips were high for all products ranging from 3.43 percent for the fresh/frozen fillet to 11.80 percent for the twelve month reprocessed 50 percent fillet block (Table 13). Most samples had excessive drip although they were the lowest for the fresh/frozen product. All reprocessed products had drips exceeding 6.91 percent which contributed to the poor texture scores. Most of these products would have been unacceptable for commercial packs.

Table 13. Thaw Drip Values for Twelve Month Fresh Frozen and Reprocessed Pink Salmon Blocks

TREATMENT	FORM (% FILLET)			
	100%	75%	50%	0%
Fresh/Frozen Block 3 Month	3.43%	5.85%	5.26%	4.61%
Reprocessed Block 6 Month	9.40%	9.49%	6.91%	8.28%
Reprocessed Block 12 Month	11.74%	8.29%	9.46%	10.90%
Reprocessed Block	7.78%	10.21%	11.80%	11.07%
Control	3.43%			5.33%

CONCLUSIONS

This project produced alternate pink salmon products that had acceptable shelf life and provided alternatives to processors needing to diversify. The major results from the study include:

1. Combination fillet/mince products provided the optimum mix of recovery and acceptability. Returning mince to the product increased yields by 12 to 14 percent on a whole weight basis.

2. Fresh/frozen product was slightly more desirable than the reprocessed forms although pink salmon stored for three months produced very acceptable blocks. After six months storage, all products were indistinguishable.

3. The most desirable product form was the 100 percent fillet block. The least desirable form was the 100 percent mince block. No more than 25 percent mince could be added back to the product and maintain acceptable sensory properties.

4. Thaw drip increased substantially in the reprocessed products and adversely affected texture and desirability. Thaw drip increased in frozen storage and became unacceptable in the reprocessed products at six months and in the fresh/frozen products at twelve months.

5. Frozen storage time for both fresh/frozen and reprocessed products should be limited to six months. At twelve months frozen storage, all products had deteriorated significantly. This was especially noticeable in the high mince products.

ACKNOWLEDGMENTS

This study was funded by the Alaska Fisheries Development Foundation (AFDF) under its Pink Salmon Development (Alternate Pink Salmon Product Forms) A component of the AFDF Full Utilization of American Surimi and Other Underutilized Species (NOAA Account # 86-ABH-00044) and supported by the State of Alaska, Office of Commercial Fisheries Development.

REFERENCES

AOAC. 1984. Offical Methods of Analysis, Fourteenth Edition. Association of Official Analytical Chemists, Washington, D.C.

Lemon, D.W. 1975. An Improved TBA Test for Rancidity. New Series Circular No. 51, Environment Canada, Fisheries and Marine Service, Halifax, N.S., Canada.

DEVELOPMENTS IN ENGINEERED SEAFOODS

James R. Brooker and Robert D. Nordstrom

National Marine Fisheries Service, NOAA
1825 Connecticut Avenue, N.W., Washington, D.C. 20235

INTRODUCTION

Engineered seafoods marketed in the United States in 1986 amounted to 100 million pounds with a retail value of $350 million. It is indeed safe to say that these products have become a well recognized line of food products in the US marketplace and enjoyed by many consumers. The arrival of these modern food products and their current prominence in the US marketplace was not by coincidence; rather it has been the result of a well planned, orchestrated, and executed series of actions undertaken by the Japanese fish harvesting, processing and marketing industries. Noting a very rapid consumer acceptance of these products, representatives of the U.S. Fishing Industries, Trade Associations, Fishery Development Foundations and US Government joined forces to evaluate the Japanese technologies and attempt to apply them to the living marine resources under US jurisdiction, to derive maximum economic and social benefits for U.S. fishermen and processors.

The National Marine Fisheries Service's (NMFS) involvement in stimulating the development of minced fish and surimi technology within the United States has centered around the Saltonstall-Kennedy (S-K) grant research program for fishery development coupled with some highly focused in-house research. For the past 6 years NMFS has been partners with the US industry providing funds through cooperative S-K agreements to conduct research and development projects in the areas of minced fish and surimi.

Three major technological breakthroughs in seafood processing and preservation have taken place during the past two and a half decades:

(1) Mechanical separation and recovery of edible fish meat from skin and bones.

(2) Stabilization of processed minced fish meat as surimi for good frozen storage shelflife.

(3) Fabrication that takes advantage of the functional (gel forming) ability of fish flesh.

These developments have stimulated a renewed interest in the fishing countries of the world for increasing the production of food from underutilized species. Research in all three areas by several countries over twenty five years has suggested that careful application of technical advancements in these three areas may make it technologically and economically feasible to achieve full and near-optimum utilization of most fishery resources for human food purposes. Further, the state-of-the-art fabrication technology suggests that the variety of end products is nearly limitless, since they can be engineered to meet product edibility preferences unique to many different consumer groups of the world.

Surimi as we have all come to know is a Japanese term meaning "a semi-processed wet fish protein", that is, mechanically deboned minced fish meat which has been washed to remove fat and undesirable matters (such as blood, pigments, and odorous substances) and subsequently mixed withh cryoprotectants (such as sugar, and/or sorbitol) to achieve good frozen shelflife. At this stage surimi is an intermediate product, like flour made from a cereal grain, that can be used in the production of a limitless variety of end products.

Two extremely important discoveries about surimi were made at a very early date in its development in the U.S. that continue to influence this expanding US industry.

(1) Surimi is the common thread that links many fisheries to gether -- pollock, menhaden, whitings, hakes and all other species used in its production. In the process of making surimi, the normal organoleptic and edibility characteristics of the particular species are lost resulting in a near-neutral fish protein product.

(2) Surimi is a basic link between the seafood industry and the food business in general. Its availability

as an animal protein and its unique functional properties have caught the attention of the entire food manufacturing complex in the US and abroad.

RESOURCE AVAILABILITY FOR SURIMI

The literature on surimi research and development abounds with reports and data about species with respect to gel-forming ability (GFA) which is the primary characteristic for determining suitability in making surimi. In Japan more than 50 species have been analyzed and their GFA established and ranked. Similar studies are being executed throughout the world and well over 100 species beyond those identified by Japan have been or are currently being tested to establish their GFA and thus their potential for making surimi.

In the United States, several target species have been the subject of similar investigation as shown in Table I. At this time no attempt has been made to generate, evaluate, and organize GFA data for many US species due in part, to the early stage of many of the investigations.

Table 1.--US Species under Investigation

Geographic Area	Species
New England and Northwest Atlantic	Red Hake Silver Hake Spiny Dogfish Ocean Pout Sand Larce Sculpin Sea Robin
South Atlantic and Gulf of Mexico	Crocker Atlantic Menhaden
West Coast	Rockfish Species Pacific Whiting
North Pacific and Alaska	Alaska Pollock Atka Mackerel

Three species (Alaska pollock, Atlantic menhaden, and Pacific whiting) which represent major US resources, are the target of extensive fishery development activities specifically for utilization in the production of surimi.

A brief description and comments about the status of development of these resources will highlight the stage of progress that has been achieved or not, and the existing challenges associated with the conversion of these resources into surimi.

Industrial development of the Alaska pollock resource in just a few years is hailed by all factions of the domestic fishing industry as "spectacular". The nature of the changes in this fishery are well illustrated in Table II. Much of the credit for this growth goes to a project funded by an S-K grant to the Alaska Fisheries Development Foundation (AFDF).

Table 2.--The Developing Alaska Pollock Market, 4-Year Growth Chart

	1982	1986
Pollock Catch, US Vessels	131,000 Metric Tons	1,077,000 Metric Tons
Pollock Processed by US Firms	2,325, Metric Tons	169,000 Metric Tons
Catch Value Ex-Vessel	$14.5 Million	$118.5 Million
U.S. Factor Trawler Processors	2	20
Processed Value Ex-plant	$830.000	$59.5 Million
Pollock Product Sales	???	$100 Million
US Sales of Surimi/Kamaboko	19 Million Lbs.	120 Million Lbs.
US Analogue Manufacturers	1	14

Source: Alaska Fisheries Development Foundation

The abundance of Alaska pollock is generally described in terms of abundance in both the Bering Sea and the Gulf of Alaska stocks which are believed to be almost exclusively separate from one another. There are substantial quantities of Alaska pollock from the US exclusive economic zone (EEZ) taken by Japan, and purchased by the Japanese from US boats, that can be directed into surimi by the US industry. In 1985 the US quota of Alaska pollock to Japan was 670,101 metric tons; and Japan purchased 430,076 metric tons from US catcher vessels. While the total harvest was not processed into surimi, the figures provide a useful benchmark to understanding the existing potential for continued US expansion in the Alaska pollock fishery at the same time as the overall developmental activities are shifting to improving the efficiencies in using this resource.

The second major US fishery resource subject to research and development via the surimi scenario is menhaden. This resource occurs along the Southeast Atlantic Coast and the Gulf of Mexico. Menhaden is the largest harvested US resource by volume of landings. Combined landings of Gulf and Atlantic menhaden purse-seine fisheries for reduction amount to about 1,202,000 metric tons annually (5 year average for 1981-86). In contrast to the non-oily, white-fleshed Alaska pollock, menhaden is an oily, darker fleshed species which historically has been used in the manufacture of fish meal for use in animal feed, and semi-refined fish oil that has been exported to Europe for use in margerine.

A third major resource available to U.S. fishermen in the EEZ off the West Coast of the US is Pacific whiting. Recent estimates put the harvestable biomass at between 300,000 - 400,000 metric tons, a significant quantity even when compared to Alaska pollock. The total tonnage of this resource fished annually by American and foreign fishermen in the territorial waters during the past five years amounted to 80-100,000 metric tons. most of which has been utilized by the Soviets. Historically there has been limited utilization of Pacific whiting in domestic products because a relatively large proportion of the fish are infected with a myxosporidian parasite. This infectious organism produces protease - an emzyme which induces undesirable textural changes in whiting products.

Investigations by Japanese researchers in the early 1970's included examination of the GFA of four species of

Merluccius including Pacific whiting. These early results showed that the GFA for these species was moderate to good, that is, the GFA's scored between 6 and 8 on a 10 point scale (very superior: 9-10): very low: 0-1). used by the Japanese at that time. There was one exception to this GFA rating for the four species, and that was parasitized Pacific whiting taken off the US Coast which scored very low.

Other less significant fishery resources exist in the US EEZ and inshore water which have the potential for utilization as raw materials for surimi. Without identifying and quantifying them, these fishery resources appear to have the potential to meet a continuing demand for surimi for the next 2-3 decades.

TECHNOLOGICAL DEVELOPMENTS AND PROCESSING RESEARCH

A stage of maturation in the processing of Alaska pollock has been achieved by the industry as a result of applying the results of technological investigations and processing research efforts that have been completed over the past 5 years. Early handling and storage studies, and investigation of functional properties impacted by handling and storage, provided essential information to the fishing sector on how to supply good quality fish to shore based surimi plants.

Armed with a working understanding of the Japanese surimi-making technology and the results of handling and storage studies, a pilot plant project was initiated in October 1984 with a requirement to produce a million pounds of surimi. The surimi production contract to Alaska Pacific Seafood in Kodiak, Alaska focused on quality, consistency and production economics, with the final product being surimi that met Japanese quality standards.

Technological improvements of the processing steps, performance of and use of different equipment was the next focus of research and development. This effort addressed improving processes, improving yields, lowering production costs, and assessment of waste stream materials including the potential for conversion of reclaimable material to marketable products. The contribution of data and

information from this work is expected to significantly improve the overall US surimi manufacturing process and US competitiveness.

Technology transfer to industry of the state-of-the-art surimi processing procedures from the Japanese industry, and US research and development experience was accomplished through a cooperative project among Federal, State, industry, and Japanese surimi processing experts. A manual, Introduction to Surimi Manufacturing Technology, was prepared and a training school was held in Alaska to transfer the technical know how, in a uniform manner, to production and quality control personnel of a number of US surimi processing firms. This has had a favorable impact of accelerating the industry application of the best available surimi processing technology and expanding domestic surimi production from Alaska pollock.

Shifting to surimi from menhaden, the US is still at a very early stage of research and development since there is no organized body of knowledge available from other scientists describing a good industrial process for producing surimi from fatty fishes. To generate this needed information, the NMFS awarded a research and development contract in February 1986, to the Zapata Haynie Corporation (ZHC) for a two year period to construct and equip a demonstration plant and investigate the technical and economic feasibility of producing surimi from Atlantic menhaden. A strategy similar to that used in Alaska calls for that project to supply 40 tons of menhaden surimi to a wide-range of researchers and food manufacturing firms for investigation of its functional and performance characteristics and to carry out new product development research. Because of problems with scheduling equipment deliveries, testing and operating-requirements, results were not conclusive in the first year. Menhaden from the Gulf of Mexico which is the same genus but somewhat different in size, composition and season of harvest, also likely will be investigated for use in the production of surimi following completion of the research on the Atlantic species. A technically-oriented conference on the manufacture of surimi from fatty fishes, tentatively scheduled to be held late in 1987, will provide an open forum for reporting progress on the surimi from menhaden project, and for exchanging results with international scientists investigating various aspects of producing surimi from other fatty fishes.

It is important to recognize that the processing equipment, its configuration, and the manufacturing process for the surimi from menhaden project was developed from the results of the Alaska pollock project and now is 4 years old. Newer equipment has been developed and alternative processing procedures more appropriate to menhaden, are evident from some experimental work already conducted at the demonstration plant. It is unclear at this time whether or not the technical and economic feasibility of producing surimi from atlantic menhaden can be adequately demonstrated within the established parameters and schedule of the project currently in progress.

Recent research data show that parasitized Pacific whiting, could, when properly processed, provide a new source of surimi. Bench level research results suggest that by proper processing and treatment, the earlier described protease induced changes can be easily and economically neutralized. Pacific whiting surimi tested following such treatment exhibited functional properties that are comparable to surimi from Alaska pollock. However, additional work is needed on Pacific whiting to validate these research findings and demonstrate what are the optimum processing parameters for producing consistently good quality surimi from this resource.

Other laboratory scale research results on producing acceptable quality surimi from Pacific whiting suggest that neither the processing procedures for surimi from Alaska pollock, nor the experimental level processing regime for making surimi from menhaden are directly applicable to Pacific whiting. A demonstration plant type research project may be required to fully develop and provide the necessary data and information on handling, treatment and processing techniques that will facilitate the conversion of this resource and other groundfish species off the West Coast into surimi.

Assuming that the research and development period for a fishery to industrial maturation is roughly 6 years, based upon the model for Alaska pollock, and assuming that a Pacific whiting development project is implemented in 1990, the U.S. production of surimi from pollock, menhaden and Pacific whiting could easily reach an estimated 150,000 tons by the year 2000.

PRODUCT SAFETY

There have been only scant reports of gastroenteritis resulting from the consumption of surimi based foods marketed primarily in the form of analogue products. This is a good record for the industry in view of the rate of increase and volume of consumption of these imitation products in the United States.

Because of the rapid growth of this industry and its future stability, it is extremely important that the industry continue to produce consistent high quality products free of microbiological or other public health problems.

In-house research has been completed by NMFS at its Northwest and Alaska Fisheries Center that defines the processing, product, packaging and storage requirements necessary for the inhibition and/or destruction of Clostridium botulinum (bacterium responsible for botulism) in surimi analogues. Results of experiments showed that imitation crab legs from seven different processors would not support growth and toxin production by nonproteolytic C. botulinum during a 67-day storage period at 50°F. When the products were stored at an abuse temperature of 77°F, toxin production was delayed between 3 and 6 days. The results of these severe tests indicate that the ingredients of the products (2.4% to 2.6% water-phase salt among other things), provide significant protection from nonproteolytic C. botulinum and that small increases of salt and other ingredients could result in complete inhibition of growth and toxin production.

Based on the results of the research on C. botulinum a set of interim recommendations by Dr. Mel Eklund has been developed, published and provided to the industry as a first effort to assure that good manufacturing, packaging, and distribution practices are implemented by the industry. These recommendations are provided in an Appendix.

Information from the Food and Drug Administration indicates that over 900 surimi based analogue samples of foreign and domestic origin, obtained at retail stores in the US, were tested during the past 3 years. Of the 921 samples tested, two samples of breaded scallops and 2 samples of imitation

breaded shrimp contained C. botulinum type A spores. Five samples of imitation lobster contained type D spores. None of the products to date, contained type E spores (the type normally associated with fishery products). Sampling at the retail level and testing will continue for some time.

A collaborative effort between the surimi industry, NMFS, USDA, FDA and the Alaska Fisheries Development Foundation has been initiated to develop a Hazard Analysis of Critical Control Points (HACCP) program for surimi during the next year. The program was conceived to (a) establish industry consistency in product quality and safety, and (b) to facilitate industry efforts to achieve utilization of surimi as a raw ingredient in other processed foods, including meat and poultry products. The question of a need for a HACCP for the secondary processing and fabrication of surimi into end products already has been raised. However, a decision and action plan for its development have not been made at this time.

The diversity in application of surimi as an ingredient in processed food products, as well as in pharmaceuticals and cosmetics, promises dynamic growth to the US surimi industry. The progressive posture exhibited by the surimi processors in assuming a participating role in the development of a HACCP shows a commitment to long term planning and exercise of leadership by this emerging industry.

DEVELOPMENT OF SURIMI BASED PRODUCTS

Surimi based analogues currently are the major end products marketed in the United States. Products in the form of imitation legs, flakes and chunks, shrimp, scallops and lobster tails continue to be the major products in the marketplace.

In 1986 the U.S. market consumed 45,000 tons of products and industry experts predict consumption will exceed 50,000 tons in 1987. If consumption continues at the same rate as the past 3 years it will hit 70,000 tons in 1990, which is consistent with the trends indicating increased overall consumption of seafoods in the US. A recent prediction by the U.S. Department of Agriculture is that US seafood

consumption could double to 30 pounds per capita by the year 2020.

Use of surimi as a functional protein material is the centerpiece concept behind much of the latest research and development underway in the United States. Food technologists and others have been experimenting with surimi in new product development activities in a variety of ways as follows:

- in traditional salmon products such as patties and jerky
- in combination with blue crab meat to formulate uniform units of lumped crab meat.
- in sausage and processed meat products
- in ice cream
- in pet food
- in cosmetics such as face cream.

Other completed research has demonstrated that analogues can withstand the more vigorous heat sterilization process of canning and maintain acceptable product texture, color, flavor and odor when preserved in retortable pouches. This research, however, was limited to imitation crab chunks in sauce, that was packed in retortable pouches, processed, and stored. No difference in the organoleptic and color characteristics were apparent due to storage for periods up to one year, the length of the storage studies.

SURIMI AS AN INTERMEDIATE

Introduction of fish-protein products in the form of intermediates, for use as ingredients in other foods, is a new area for exploration by the industry. A window of opportunity for the seafood industry is in producing and marketing high-quality intermediate fish protein products, such as minced fish meat and surimi (from various species and in many styles of intermediates), that can be offered at attractive prices to food processors. Some limited research results show that fish protein products can be added to traditional meat and poultry products to improve binding properties, improve nutritional composition, change flavor and color, and reduce costs. They may also be used as extenders and protein supplements. One on-going activity focusing on this type of use is a S-K project undertaken by the AFDF in 1986 to obtain USDA approval for using surimi in a surimi/meat product. Recently the AFDF achieved approval of a sketch label for pork/surimi nugget patties. Final approval hinges on assurance to USDA that surimi entering meat and poultry plants is microbiologically safe on a consistent basis.

On an international level, the Codex Committee on Processed Meat and Poultry Products has been examining the question of a need to develop guidelines for the use of other protein products such as milk powder, casein, caseinate and fish in processed meat and poultry products. The Codex Committee on Fish and Fishery Products identified minced fish meat and surimi as two fish protein products that merit consideration for inclusion in processed meat and poultry products. While no action has been taken on the need for guidelines addressing the use of other protein products in processed meat products, the assertive action of the Codex Fish Committee should encourage world wide consideration of formulating new end products composed of meat and fish ingredients.

The direction of new product development research clearly indicates that surimi used as an ingredient in other foods and non-foods for many different purposes will continue to create real opportunities for developing seafood industries. While the market forecast is for consumption of analogues to level off slightly about 1990, new applications for surimi should create growth opportunities for the forseeable future.

QUALITY AND FUNCTIONAL ASSESSMENT

Quality grades and grading procedures have been used by the Japanese surimi industry for several years. That system specifies (a) standard methodologies for the analysis of various compositional and functional properties of surimi and (b) application of the measurements to assignment of grades for surimi which are specific to the species of fish and location of the surimi process (factory ship or shore plant). At an early date, the emerging US surimi industry considered the Japanese approach, and then decided in favor of developing a different system of quality specifications rather than quality grades.

A research effort headed by Dr. Tyre Lanier of North Carolina State University was designed to critically evaluate both the current Japanese testing methodology and other available methodologies and to determine the optimum testing procedures to elaborate quality specifications for surimi in the U.S. The results of the three year research effort have been organized into a proposed standardized system of quality specifications for surimi that allows surimi users to purchase raw material according to its characteristics (compositional) and functional properties.

A decision has been made to prepare a manual of standard test methods for raw surimi that will contain the following level of detail:

- identity of properties to be tested
- identity of a "standard" method for testing each property
- a procedure for applying each method to achieve uniformity of measurement
- units of measurement to be used
- description of how results shall be expressed

The draft specification system presently is under review and consideration by a surimi technical committee for adoption as standardized test methods. It tends to parallel the system now used in the US meat industry by meat processors who purchase product (raw materials) by specifications based on product functionality.

The concluding efforts under this project will be to publish the standardized methods for quality and functionality assessment of surimi in a Manual of Methods for distribution and use by the industry. Steps have already been taken to familiarize industry quality control and inspection personnel with the methods and use of them.

END PRODUCT QUALITY STANDARDS AND PURCHASE SPECIFICATIONS

Secondary processing of surimi into seafood analogues has increased dramatically in the US from 1 plant in 1982 to 14 processing establishments in 1986. Additional plants and floating processers are scheduled to commence processing fish into surimi in 1987. As this industrial expansion was occuring in 1985, NMFS was requested to start the process of developing voluntary U.S. Quality Grade standards for the major end products, i.e., imitation crab legs, chunks and flakes, scallops, lobster tails, and shrimp.

An announcement of this request was published in the November 25, 1985 issue of the Federal Register and comments were invited from interested parties. In view of varied responses received and noting that the US surimi industry is is a very dynamic state at this time, it is unlikely that NMFS will proceed to develop voluntary quality grading standards for analogues in the near future.

Interest in using analogue products has been developing in the military services, motivated in part, by an evaluation of a crab food product by the Armed Forces Product Evaluation Committee (AFPEC). Additional product evaluation was carried out by the U.S. Army Natick Research Development, and Engineering Center (NRDEC) which resulted in a decision to develop a Commercial Item Description (CID) that can be used by the military services to purchase imitation crab products.

A draft CID has been prepared and circulated to interest groups and industry for views and comments. Once the CID is developed and approved, the product will be added to the Federal Supply Catalog as an approved item for military food service programs. Upon completion of these actions military purchasing of imitation crab products can commence, perhaps by the end of 1987. Assuming good acceptance by military personnel similar to that of US consumers generally,

opportunities for using analogues in a variety of prepared food items such as in half steam table tray packs, may be pursued on a timely basis.

INTERNATIONAL DEVELOPMENTS

The Codex Committee on Fish and Fishery Products responsible for elaborating international standards and codes of practice for fish and fishery products, at its 17th Session, took note of the global developments in the production of surimi and the implications for the Committee of the potential uses in fish and other food products covered by existing and draft international standards. In response to a request from the Committee, the US delegation agreed to prepare a background paper on the world-wide status of surimi and products manufactured therefrom for consideration by the Committee at its 18th session in 1988.

Other international developments in surimi and engineered seafoods have moved rapidly in the past year. Japan has substantial research underway investigating the technology for making surimi from oily fish, and may be somewhat ahead of the US at this time.

From investigations on making surimi from sardines and mackerels, Japanese scientists have announced the use of a new vacuum flow process which allegedly produces a better quality surimi resulting from a more thorough separation of oil and flesh. They visualize using this type of surimi in a number of the traditional fried kamaboko products for their domestic market. In addition, Japanese researchers and vessels have been active in several regions of the world conducting investigations as follows:

(a) in Peru, Chile, and Argentina researching jack mackerel and hakes

(b) in Europe working on blue whiting

(c) in New Zealand working on hoki and southern blue whiting

Recent curtailments of fishing in other countries'waters have prompted this flurry of Japanese activity.

The Koreans have stepped up activity in both surimi production and export of engineered seafoods. Korean exports of Alaska pollock surimi to Japan and the US have grown substantially in the last few years. This year, the Koreans outfitted several new factory vessels to process surimi off Alaska.

Much of the activity in Europe has concerned Japanese joint ventures, although the Faroe Island industry had undertaken surimi production on their own with blue whiting. Japanese analogue plants have been constructed in Great Britain and efforts to utilize blue whiting, cod frames, and Norway pout are in the test stages.

Norway has been conducting pilot plant operations on a number of species and has a mobile plant which moves from port to port as seasons and target species change. The rest of Europe has had little activity in surimi processing, but there are plans for analogue plants in several EEC countries.

In South America, Peru has made and distributed to school children, fish milk and fish cookies. These products combine processes reminiscent of fish protein concentrate as well as minced fish and surimi. Chile and Argentina have large quantities of mackerel and hake which they are planning to utilize in conjunction with Japanese interests.

Canada has one plant operating on the East Coast with a second one planned for operation under U.S./Canadian joint venture arrangement.

INTERNATIONAL TRADE

After Japan, the United States is the largest consumer of surimi based products. An estimated 110 million pounds will be sold in the U.S. in 1987. Until 1984, Japanese exports of surimi were small, while exports of analogue doubled each year. Now that the U.S. has close to 20 plants making either surimi or analogue products, the situation is changing. In 1986, Japan exported about 26,000 metric tons of analogue products to the US, a drop of about 6,000 tons from 1985. At the same time, US imports of surimi blocks, used for making the analogues, rose from less than 1000

metric tons in 1974-81 to 4,800 tons in 1985 and almost 8,000 tons in 1986. The increased secondary processing capacity built in the US in the last two years is the cause of this turnaround. In addition, there are three shore-based surimi plants in Alaska and three new processing vessels either operating or ready to produce surimi this year. The US recently started exporting surimi to Japan and this trade is expected to increase steadily.

Japan is exporting surimi and analogue products to about 24 countries. Over 80% of the volume comes to the United States with very small amounts entering Australia, Canada, U.K. and Europe. Some small amounts being shipped to Spain, Hong Kong and Singapore, are transhipped to the United States.

CONCLUSION

The efforts in the US and other countries for using underutilized fish species for human food purposes are largely concentrated on adapting raw material for processing into foods by controlling and engineering their appearance, flavor and texture keyed to consumer taste preferences. Much of this effort includes studying the characteristics of under-utilized species for GFA, thereby giving food processors the ability to accurately predict the performance and therefore the utility of the raw material in various food systems. Beyond these general conclusions there are others that can be drawn:

1. A wide-range of fatty and non-fatty species are being researched for potential use as food through the surimi and food processing technologies.

2. Many fishing countries of the world perceive that the surimi technology offers new opportunities for better uses of under utilized resources.

3. A single handling and processing technology is unworkable for all species. Technology is not directly transferrable between species groups. Technical developments and improvements in the surimi process are contining at a rapid rate and processing protocols are being refined depending upon the species being investigated.

4. Excellent equipment is readily available for producing surimi and end-products for some species; and improvement in both equipment, and process configuration continue to be made.

5. Process controls, and product safety and quality concerns are being addressed and implemented at a rate paralleling technological advancements.

6. The type and variety of surimi based end products will continue to expand well beyond the shellfish analogue that now dominate the market place.

7. Surimi as raw material for use in formulating all types of stand-alone foods and reformulating traditional foods is the next major wave of developments in the food industry. Some complex labeling issues may emerge from these developments.

8. International trade in both surimi and surimi based end products should continue to increase over the next two decades.

REFERENCES

1. Anonymous. 1987. Draft Report of the Technical Subcommittee, Surimi Committee of NFI: Standard Testing Methods for Raw Surimi, April 1987

2. Eklund, M. 1986. Careful Crabstick Makers Safe from Botulism. In the Lodestar Vol. IV, No 4, Alaska Fisheries Development Foundation, Anchorage, AK.

3. Elkins, E.R., Dudek, J. and Chin, H.B. 1986. Commercial Heat Sterilization of Seafood Packed in Retortable Pouches and its effect on Quality and Nutrient Retention. Report to National Marine Fisheries Service, Washington, DC.

4. Erkins, R.A. The Erkins Seafood Letter Vol. 22, Issue 3, March 1987.

5. Gordon, W., 1985. Letter to FDA Associate Commissioner for Regulatory Affairs dated April 10, 1985, regarding Compliance Policy Guidance for labeling surimi based products.

6. Groninger, H., Kudo, G., Porter, R., and Miller, R. 1985. Preparation and Evaluation of Surimi from Pacific Whiting. In Proceedings of the International Symposium

on Engineered Seafood Including Surimi, p 199-210. Edited by R.E. Martin and R. L. Collette, National Fisheries Institute, Washington, DC.

7. Lanier, T., Hamman, D.C., and Wu., M.C. 1985. Development of Methods for Quality and Functionality Assessment of Surimi and Minced Fish to be used in Gel-type Food Products. Report to Alaska Fisheries Development Foundation, Inc., Anchorage, AK.

8. Lee, C.M. Surimi Processing: Surimi Manufacturing and Fabrication of Surimi-Based products. Presented at the 45th IFT Annual meeting, Atlanta, GA., June 9-12, 1985.

9. Martin, R.E. Developing Appropriate Nomenclature for Structured Seafood Products, 1986. In Food Technology 40(3) p 127-124, March 1986.

10. McKnight, Jeanne, 1986 S-K Review -- An Overview of the Saltonstall-Kennedy Fisheries Development Program.

11. Mitchell, C. Surimi - The American Experience, 1986. In INFOFISH Marketing Digest. Kuala Lumpur, Malaysia.

12. Miyaucki, D., Kudo, G., and Patashnik, M. 1973, Surimi - A Semi-processed Wet Fish Protein. Mar. Fish. Rev. 35(12):7.

13. National Marine Fisheries Service 1986. Preliminary Review of the 1986 Atlantic and Gulf Menhaden Fisheries. Report to the National Fish Meal and Oil Association, Washington, DC

14. Okada, M. and Noguchi, E. 1974. Trends in the Utilization of Alaska Pollock in Japan. In Fishery Products. (Ed.) Kreuzer, Rudolf, Fishing News (Books) Ltd. Surrey, U.K.

15. Okada, M. and Tamoto, K. 1986. Introduction to Surimi Manufacturing Technology. Overseas Fishery Cooperation Foundation. Tokyo, Japan.

16. Yutaka, S. 1985. Biochemical and Functional Properties of Material Fish. In Processings of the International Symposium on Engineered Seafood Including Surimi p 148-167. Edited by R.E. Martin and R.L. Collette, National Fisheries Institute, Washington, DC.

17. Zapata Haynie Corporation 1986. Surimi from Menhaden, Technical Aspects of the Project. Report to US Government under Contract No. 59-EANF-6-00048.

TEXTURE AND FREEZE-THAW STABILITY OF SURIMI GELS PREPARED WITH A COMBINATION OF ATLANTIC POLLOCK AND WHITE HAKE

J. M. KIM
Department of Food Science
University of Maine
Orono, ME 04469

INTRODUCTION

It has been reported that species variation of muscle protein may be due to the differences in fishing ground, method, and season. Studies on the behavior of the purified fish actomyosin suggests there is no species difference in the intrinsic viscosity value, electrophoretic mobility, salting-in and salting-out range, etc., while the temperature at which denaturation takes place, and the velocity of denaturation differs from species to species. It is generally believed that actomyosin is related to the gel-strength of fish muscle products. From observations in the literature, it appears that hydrogen and hydrophobic bonding participate in species difference in "setting" temperature, i.e., formation and deformation of the setting and gel network formation, but the role of disulfide bonding is still uncertain.

Atlantic pollock and white hake are regionally abundant fish species off the coast of Maine and they demonstrate good gel-forming ability. Pollock muscle is brown to grayish and regarded as not really suitable for a crab or lobster meat-analog products. Little published information is available about the effects of blending surimi of different species and how variations of blending of surimi affect the gel characteristics of the finished product. Therefore, the objectives of this study were (1) to evaluate and compare rheological properties of surimi gels prepared with varying combinations of regionally abundant fish species, Atlantic pollock and white hake, and (2) to determine the relationships of their rheological properties to gross microstructure of the gels before and after successive freeze-thaw cycles.

MATERIALS AND METHODS

Preparation of surimi

Surimi was prepared separately from fresh Atlantic pollock (Pollachius virens L.) and white hake (Urophycis tenuis) within 24 hr after being caught off the coast of Maine. Fresh fillets were run through a mincer (Model 84141, Hobart MFG. Company, Troy, OH) with a die having perforations of 5 mm in diameter. The minced meat was washed four times with water, using 1 part fish meat to 4 parts water (W/W). After the fourth washing and draining, the slurry was dewatered by centrifugation (Model K, International Equipment Company, Needham Hts., MA) at 3000 xg. The meat pellet was chopped in a silent cutter (Model 84141, Hobart MFG. Company, Troy, OH) with sugar, sorbitol, and sodium tripolyphosphate (4%, 4%, and 0.2%, W/W, respectively) and subsequently packed in cryobags to be stored at -20°C until used.

Preparation of thermally induced surimi gel

The thawed pollock and hake surimi (overnight in a refrigerator, 78% moisture) were separately weighed and recombined in varying proportions. The blended surimi was chopped with 2% salt in a silent cutter for 9 min followed by 3 min additional chopping with or without 6% starch and with the addition of ice-chilled water to adjust the moisture level to 78%. The chopped surimi paste was extruded into 30 mm diameter cellulose casings and cooked at 90°C for 40 min in a water bath. These were immediately cooled in running tap water for 20 min. Ten types of samples were prepared using varying levels of pollock and hake surimi and incorporating either native potato or freeze-thaw stable modified starch, as shown in Table 1.

Table 1. Types of surimi pastes/gels prepared

Types	Formulations (%)		
	Atlantic pollock	White hake	Starch
I	100	---	---
II	80	20	---
III	60	40	---
IV	40	60	---
V	20	80	---
VI	---	100	---
VII	100	---	6 (modified)
VIII	---	100	6 (modified)
IX	50	50	6 (potato)
X	50	50	6 (modified)

Measurement of textural properties

The prepared gels were left overnight at room temperature to equilibrate and were cut into cylindrical shapes (30 mm diameter and 25 mm long). The textural properties of the gels measured were compressive force, percent expressible moisture, and penetration force using an Instron testing machine (Model 1000, Instron Engineering Corp., Canton, MA) as an index of cohesiveness, water holding ability, and firmness, respectively. Compression was done uniaxially at a crosshead and a chart speed of 50 and 100 mm/min, respectively.

Compressive force was evaluated at 90% deformation with failure using a compression head of 10 cm in diameter. At the same time, the amount of moisture expressed upon compression was measured by collecting the fluid on filter paper and recorded in terms of % expressible moisture on a sample moisture weight basis. Penetration force was measured at 90% deformation with failure using a plunger of 9.5 mm diameter.

Samples were subjected to three freeze-thaw cycles to evaluate the freeze-thaw stability. Gel samples were frozen at -20°C for five days and thawed to equilibrate to room temperature before texture measurement for compressive force, percent expressible moisture, and penetration

force.

A Hunter LabScan II spectrocolorimeter (Model PC100-B2, Hunter Associates Laboratory, Inc., Reston, VA) was used to measure the Hunter L, a, and b values of surimi pastes prepared with various combinations of pollock and hake surimi using a half inch aperature.

Structure analysis

For examination of surimi gels with a light microscope, small gel blocks were quickly frozen in liquid nitrogen and sectioned at 5 um with a Reichert cryostat microtome (Model 975C, AO Scientific Instruments, Buffalo, NY). Prepared sections were mounted on slides and stained for observation.

RESULTS AND DISCUSSION

Species differences in gel-strengthening ability have been well known. In this experiment, Atlantic pollock and white hake were used because they are abundant in the Gulf of Maine region. White hake, particularly, is an underutilized species. Even though preparation of surimi and surimi paste involves a vigorous chopping process, muscle associated collagen is seen (Fig. 1b). Collagen in the surimi paste show its birefringence enhanced in polarized light (Figs. 1d and 1f). Intact potato and modified starches demonstrated characteristic maltese cross patterns in polarized light (Figs. 1d and 1f).

The textural properties of surimi gels prepared with various combinations of Atlantic pollock and white hake, and with or without different types of starches, are shown in Figs. 2-4 for compressive force, percent expressible moisture, and penetration force, respectively.

Changes in color due to varying combinations of pollock and hake surimi are presented in Fig. 5. There were significant differences in whiteness and yellow and green colors between pollock and hake surimi.

Both pollock and hake surimi produced good surimi gels, but gels prepared with hake surimi, with or without starch, exhibited significantly higher compressive force, water holding ability (lower percent expressible moisture) and penetration force than those prepared with pollock surimi, with or without starch, respectively. It is well known that dark flesh, particularly red flesh meat, such as tuna, has weak gel strengthening ability. Usually, it has been said that the whiter the flesh, the stronger the gel-strengthening ability it has. Therefore, gradual increases in compressive force, water holding ability, and penetration force were observed with proportional increases in hake surimi (Figs. 2-4). Substantial increases in L (white) and b (yellow), and a (green) values were observed as the level of hake surimi increased (Fig. 5). In addition to improving the gel strength, the problem associated with the brown-gray color of pollock surimi can be alleviated by mixing with white hake surimi.

Modified starch used in this study is a blend of potato and taploca starches (about 1:6 ratio, W/W) and modified by hydroxyalkylation for freeze-thaw stability.

The structure of a protein gel matrix of Type IV (Fig. 6b) was more uniform than that of Type I (Fig. 6a). Many air cells were observed

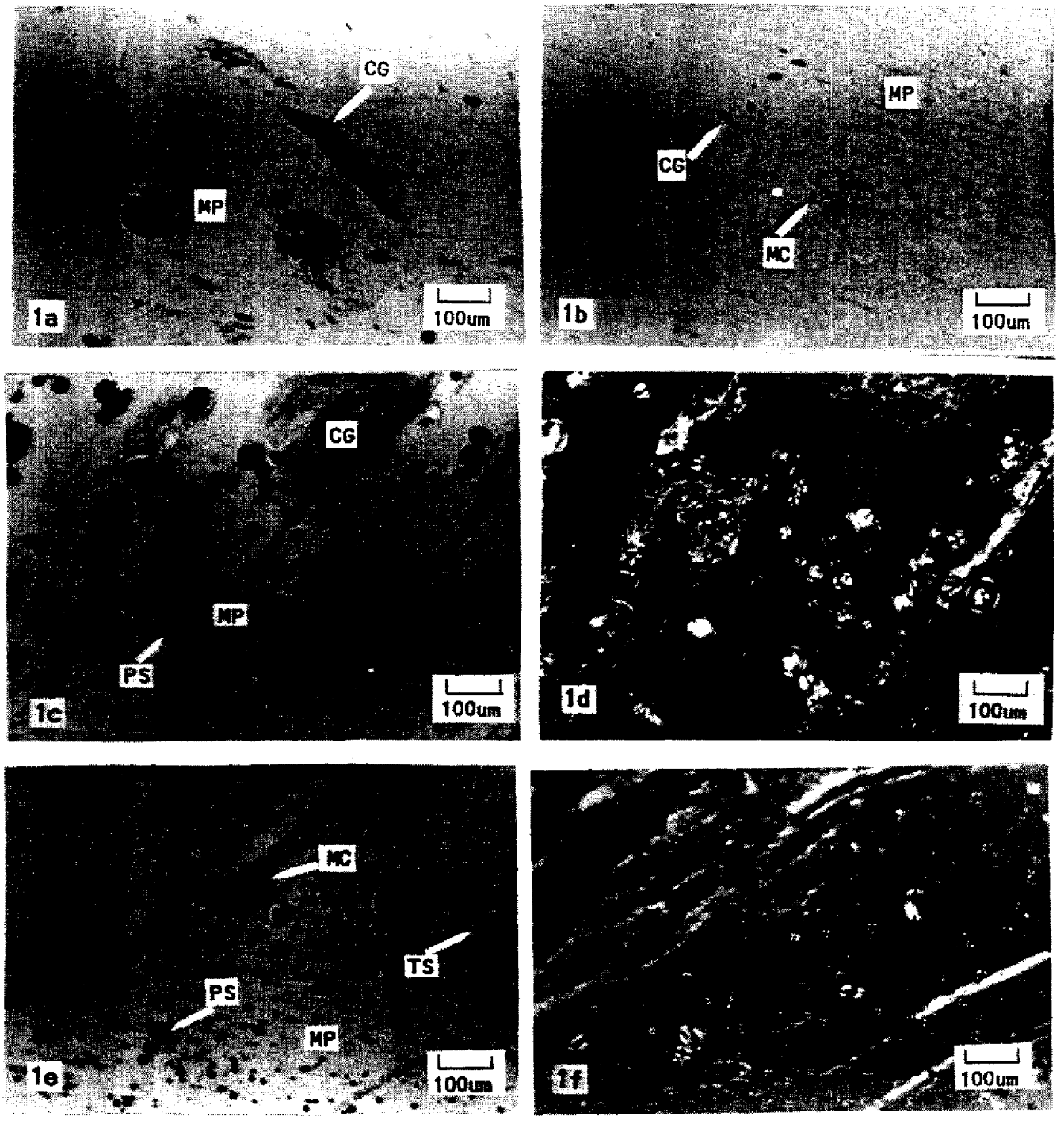


Fig. 1. surimi pastes before cooking. CG: collagen; MC: muscle associated collagen; MP: muscle protein; PS: potato starch; TS: tapioca starch. (a) Type I; (b) Type VI; (C) Type IX; (d) same as Fig. 1c, but photographed in polarized light; (e) Type X; (f) same as Fig. 1e, but photographed in polarized light.

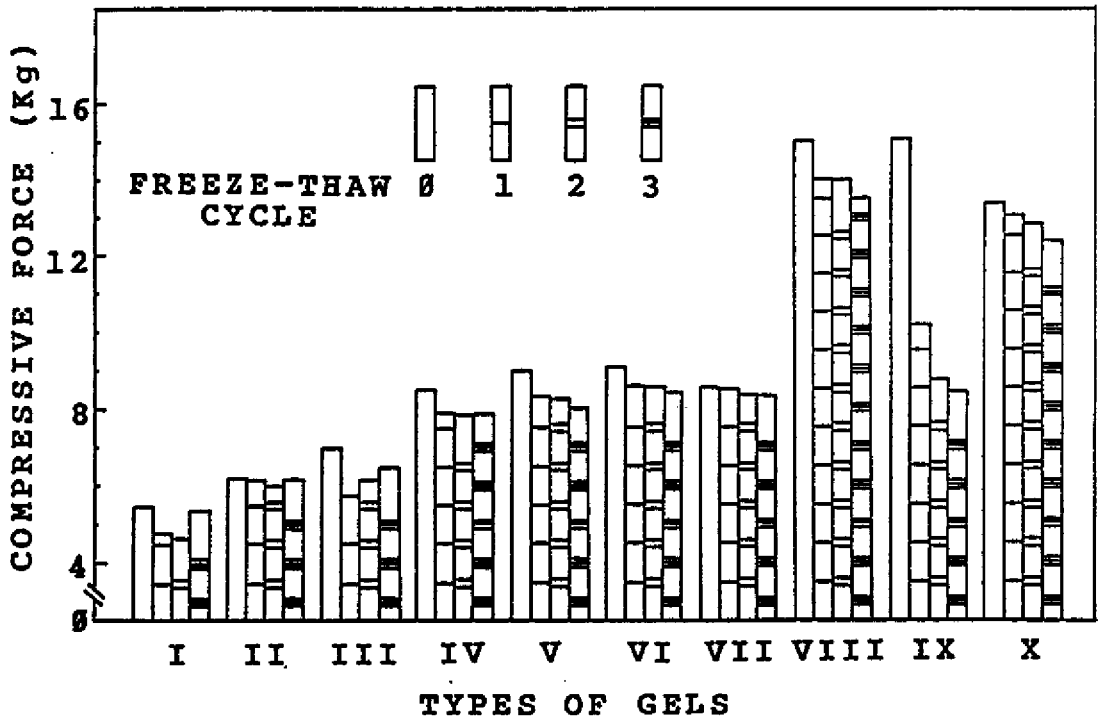


Fig. 2. Effect of varying combinations of pollock and hake surimi, with or without different types of starch, on compressive force of gel.

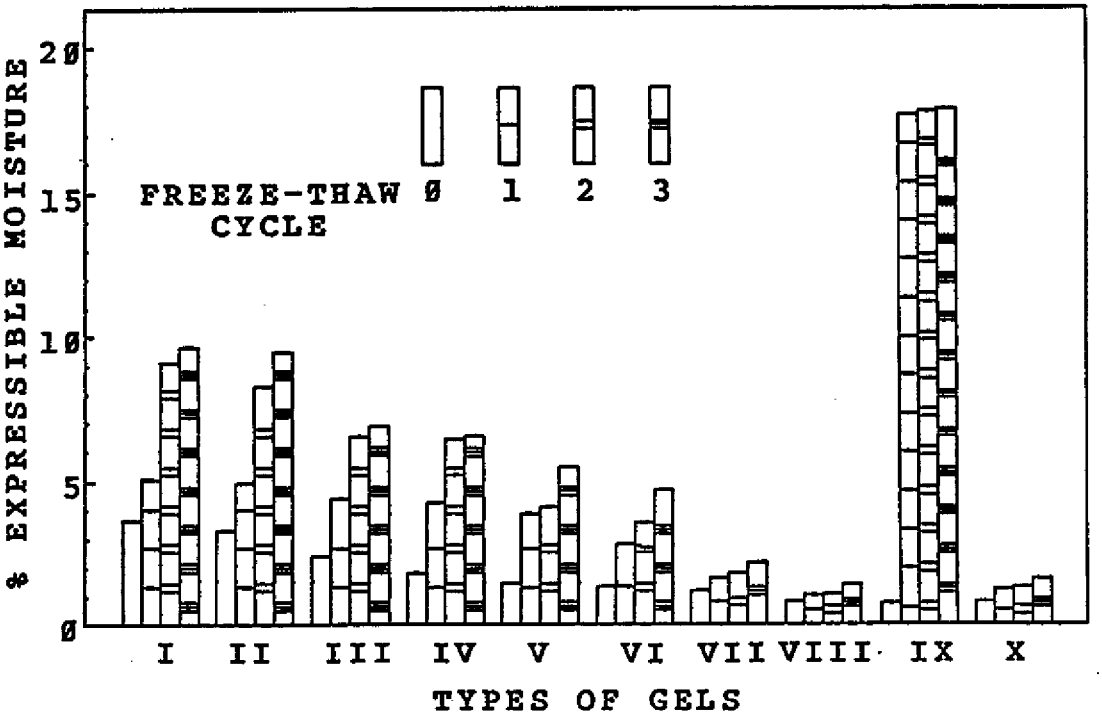


Fig. 3. Effect of varying combinations of pollock and hake surimi, with or without different types of starch on % expressible moisture of gel.

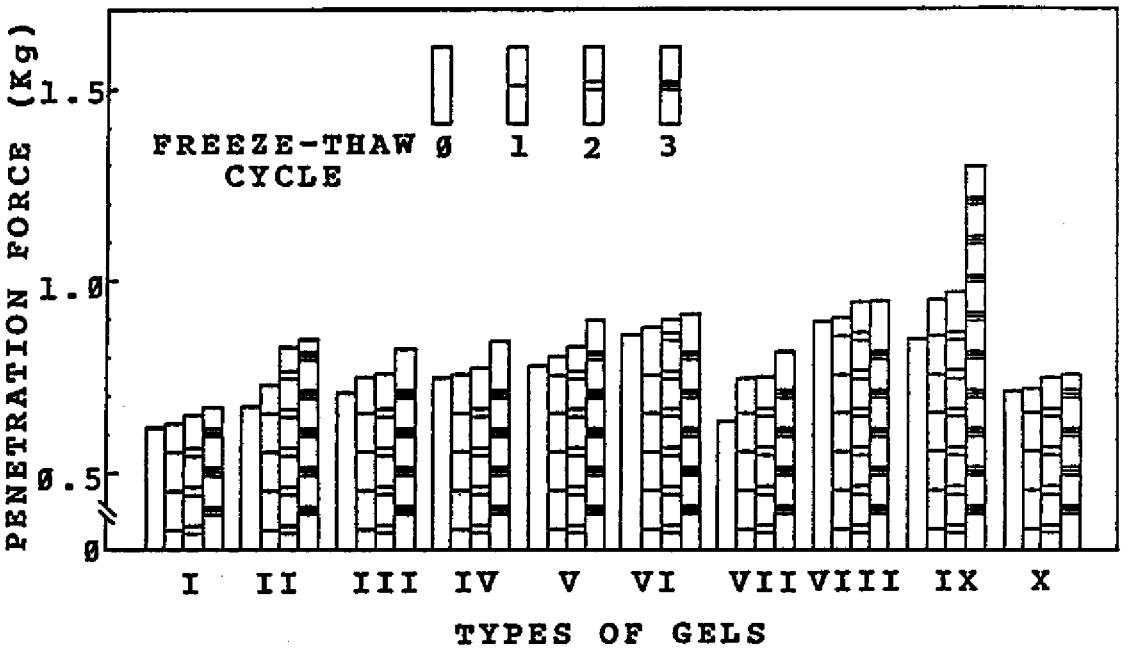


Fig. 4. Effect of varying combinations of pollock and hake surimi, with or without different types of starch, on penetration force of gel.

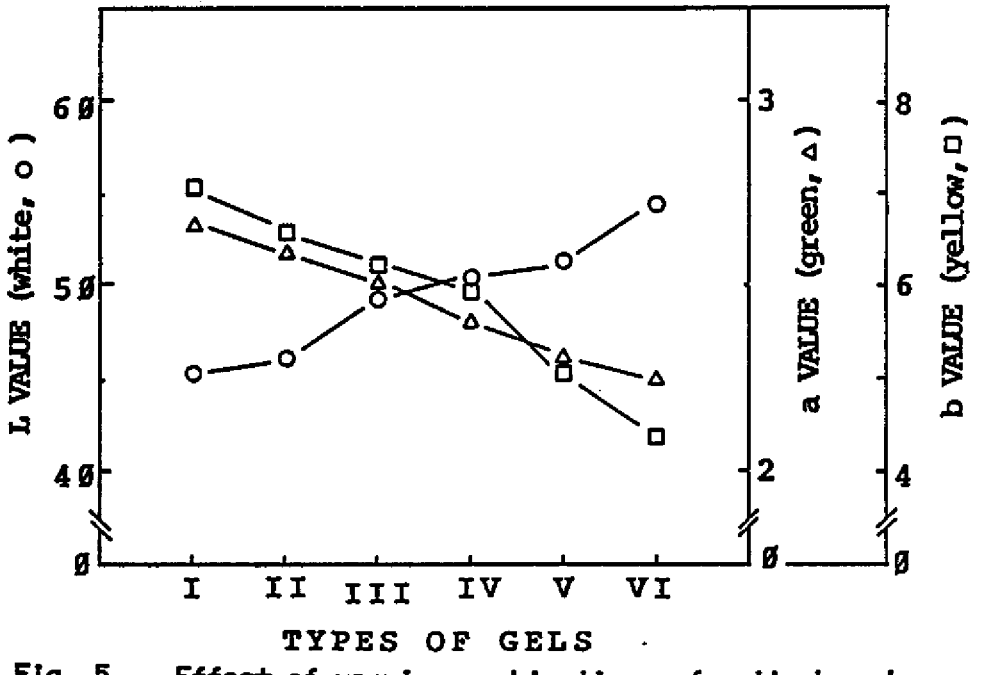


Fig. 5. Effect of varying combinations of pollock and hake surimi on color of the paste.

throughout the latter protein gel (Type I). These observed structural differences in the protein gels were reflected by the differences in gel strength (Figs. 2 and 4) and water holding ability (Fig. 3) before the gels were frozen. On heat treatment the polarization became dull and potato starch lost its maltese cross (Fig. 6d).

When gels were subjected to repeated freeze-thaw cycles, there were slight decreases in compressive force in the case of the gels prepared without (Types I-VI) and with (Types VII, VIII, and X) modified starch (Fig. 2). A gel prepared with native potato starch (Type IX), however, showed a significant decrease in compressive force. Percent water expression and penetration force were significantly increased in the case of the gels prepared with native potato starch and without starch (Fig. 3), while gels prepared with modified starch did not show significant changes. During freezing, mechanically entrapped water in the gel matrix migrated to form ice crystals (Figs. 7a, 7b, 8a, and 8b). In this way, the protein gel matrix is densely packed and hardened due to the pressure exerted and dehydration of gel matrix resulting from the development of ice crystals. This resulted in increased freely moving moisture and firmness of the gel upon thawing to cause increased percent expressible moisture and penetration force. These changes were more pronounced when the gel was prepared with native potato starch because of the increased availability of water for ice crystal formation from collapsed potato starch. This process was visualized in Figs. 7c and 8c. On the other hand, there were no significant changes in compressive force, percent expressible moisture, and penetration force in the case of the gel prepared with freeze-thaw stable modified starch. Commercial freeze-thaw stable starches are modified by acetylation or hydroxyalkylation. These types of modification stabilize the starch gel by mutual repulsion of amylose molecules and thus hold the moisture between the molecules. This retards or eliminates the association of amylose molecules, i.e., crystallization or retrogradation phenomenon during cold storage. Starch granules embedded in the protein gel matrix which, when swollen, exerted pressure on and drew moisture from the matrix. This caused the gel matrix to become more compact and firm. The composite gel-reinforcing effect of starch in heat-induced surimi gel was demonstrated in Figs. 2-4. When the gel is frozen, water for ice crystal formation is available from the protein gel matrix and gelatinized starch. Availability of water for ice crystal formation is greater in the gel prepared with native starch because this type of starch cannot withstand drastic physical changes, such as freeze-thaw cycle and is collapsed resulting in increased availability of water for ice crystal formation. Furthermore, calculated water was added to adjust moisture content to 78% during gel preparation. This fact explains why more pronounced changes in textural properties occurred in the gel prepared with potato starch than those prepared without it (Figs. 2-4). This also reflected the difference in gel structure during freeze-thaw cycles (Figs. 7a-7c and 8a-8c). In contrast, gels prepared with modified starch (Types VII, VIII, and X) did not show significant changes in the textural properties because moisture imbibed by starch granules from the gel matrix during cooking lowered the moisture level in the surimi gel. This moisture is not available for ice crystal formation because it is held within the gelatinized starch as mentioned above during accelerated freeze-thaw cycles of the gel (Figs. 2-4). Gel microstructure analysis supports this phenomenon (Figs. 7d and 8d).

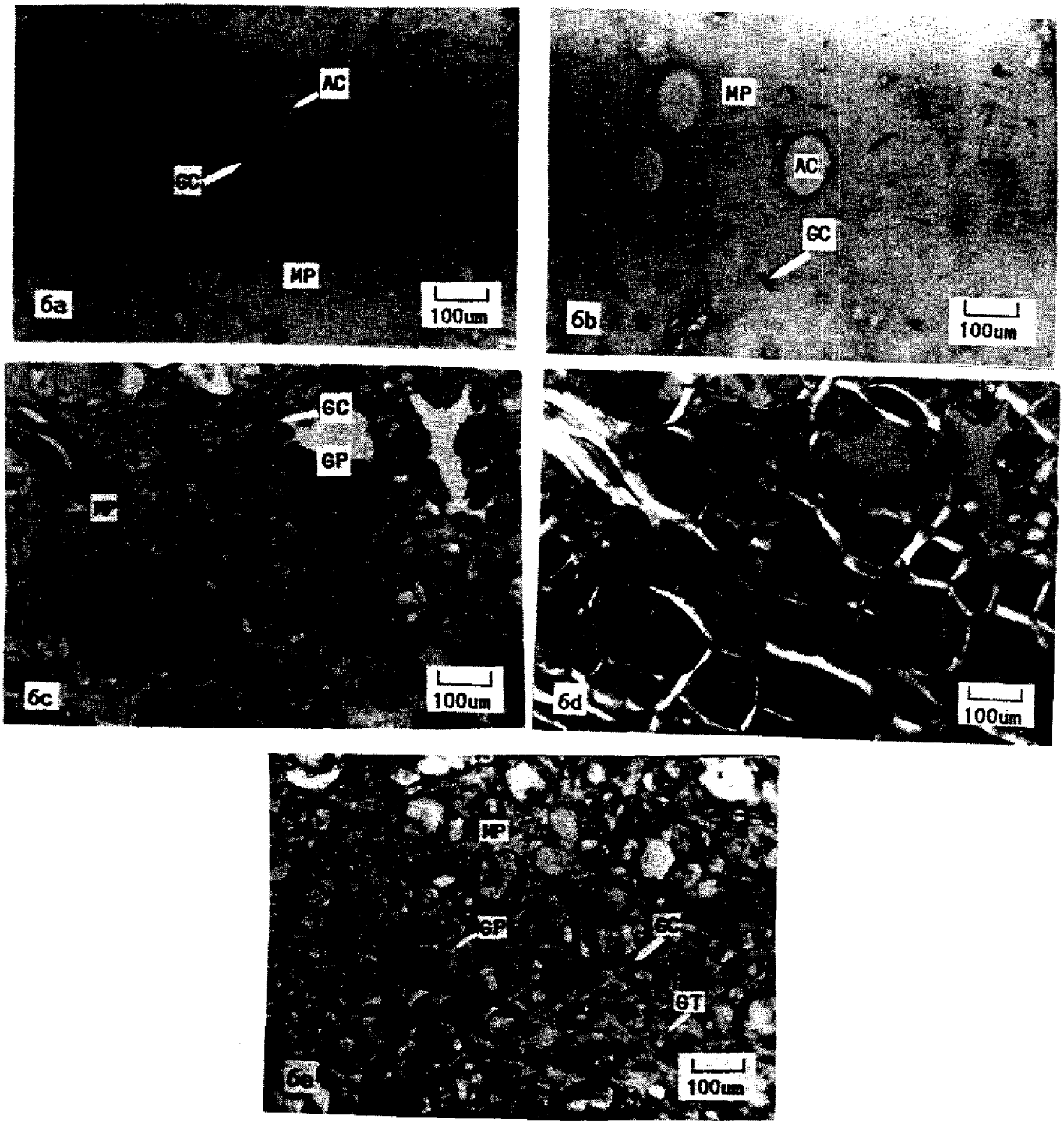


Fig. 6. Surimi gels after cooking at 90 C for 40 min. AC: air cell; GC: gelatinized collagen; GP:gelatinized potato starch; GT: gelatinized tapioca starch. (a) Type I; (b) Type VI; (c) Type IX; (d) same as Fig. 8c, but photographed in polarized light; (e) Type X.

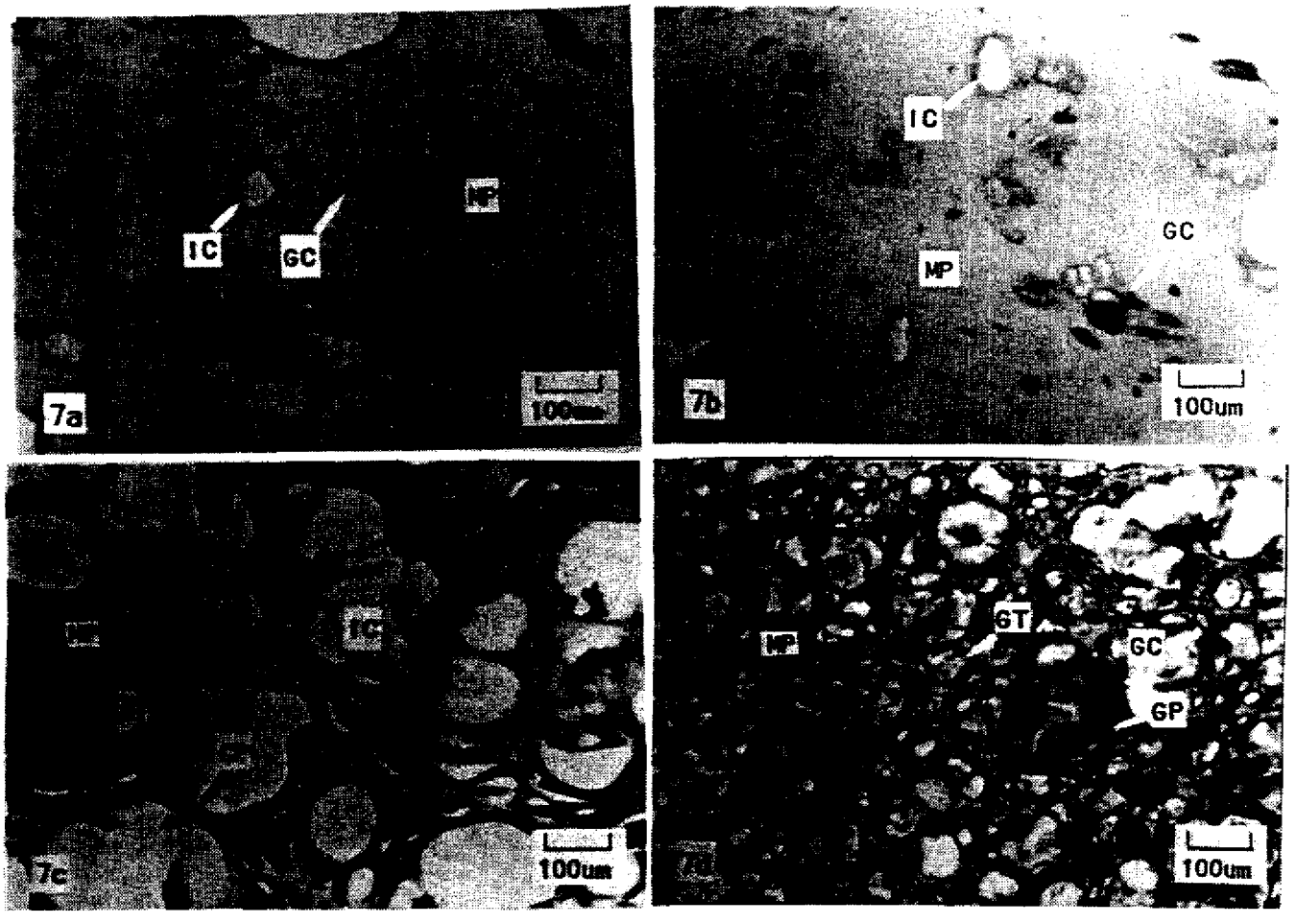


Fig. 7. Surimi gels after first freeze-thaw cycle. CP: collapsed potato starch; IC: ice crystal. (a) Type I; (b) Type VI; (c) Type IX; (d) Type X.

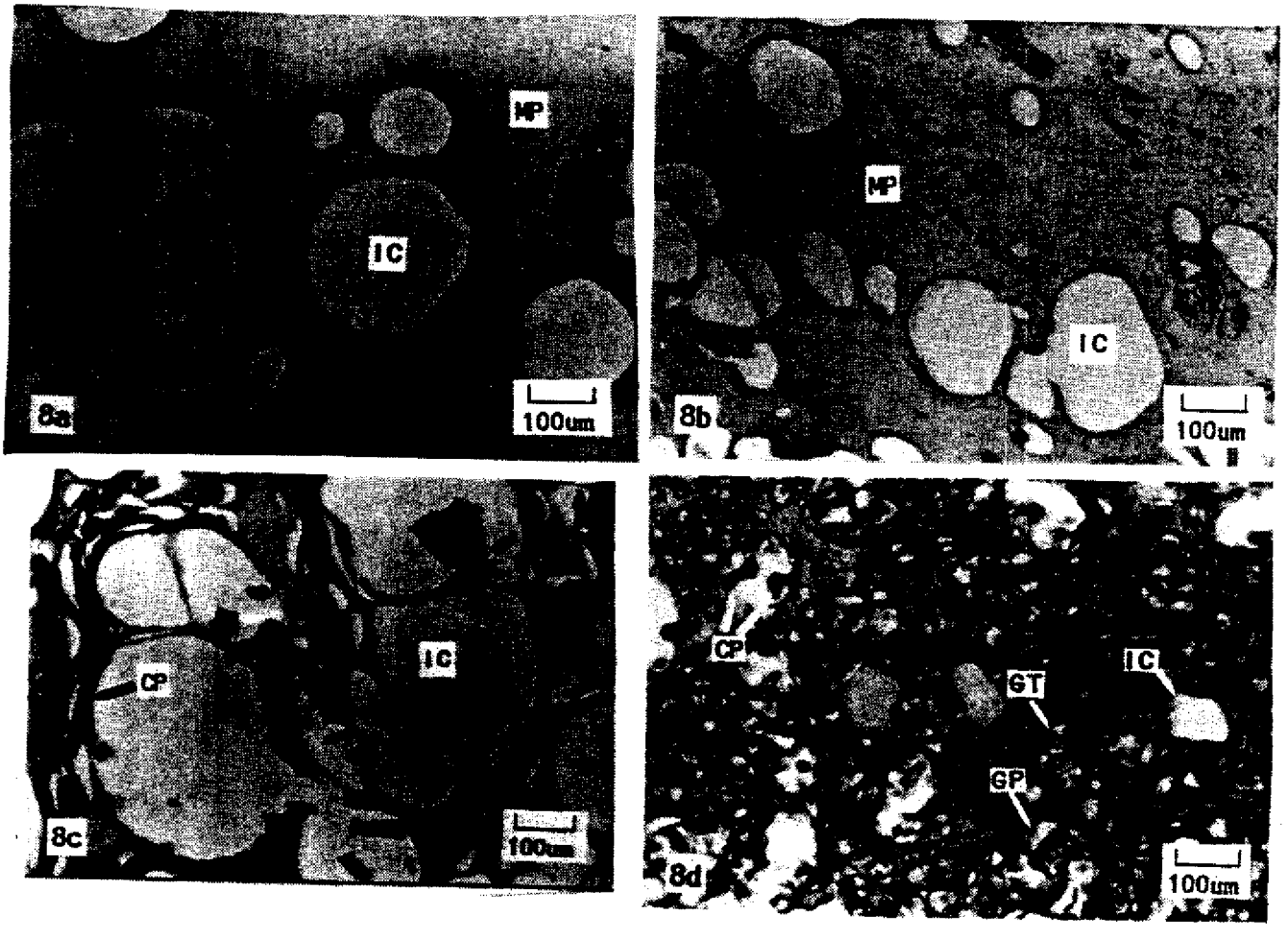


Fig. 8. Surimi gels after third freeze-thaw cycle. (a) Type I; (b) Type VI; (c) Type IX; (d) Type X.

In conclusion, 1) good surimi gels can be prepared from Atlantic pollock and white hake. The concept of the mixed species in this study was suggested by the brown to grayish color of the pollock muscle, 2) the problem associated with the color can be alleviated by mixing the pollock with white hake surimi, which also improves the texture, 3) presence or absence of starch and the type of the starch clearly influences the gel texture, and 4) changes in the textural properties were supported by the changes in the gross gel microstructure during the accelerated freeze-thaw cycles.

REFERENCES

1. Kim, J.M., Lee, C.M. and Hufnagel, L.A. 1987. Textural properties and structure of starch-reinforced surimi gels as affected by heat-setting. Food Microstruct. 6(1):81.

2. Kim, J.M. and Lee, C.M. 1987. Effect of starch on textural properties of surimi gel. J. Food Sci. 52(3):722.

3. Lee, C.M. 1984. Surimi process technology. Food Technol. 38(11):69.

4. Lee, C.M. and Kim, J.M. 1985. Texture and freeze-thaw stability of surimi gels in relation to ingredients and formulation. In "Proceedings of the International symposium on engineered seafood including surimi." R.E. Martin and R.L. Collete (eds.), National Fisheries Institute, Washington, D.C. p. 168.

5. Lee, C.M. and Kim, J.M. 1986. The relationship of composite characteristics to rheological properties of surimi sol and gel. In "Food engineering and applications, vol. 1," M. Lemaguer and P. Jelen (eds.), Elsevier Applied Science publishers, London and New York, p. 63.

6. Radley, J.A. 1976. Starch production technology. Applied Science publishers LTD, London, England.

7. Suzuki, T., Kanna, K., and Yamamoto, T. 1969. Variation of the muscle protein in horse mackerel. Bull. Jap. Soc. Sci. Fish. 35(5):451.

8. Ueda, T., Shimizu, Y., and Simidu, W. 1968. Species difference in fish muscle - I. The gel-forming ability of heated ground muscles. Bull. Jap. Soc. Sci. Fish. 34(4):357.

9. Whistler, R.L., BeMiller, J.N., and Paschall, E.F. 1984. Starch:Chemistry and technology, 2nd ed., Academic Press, Inc., Orlando, FL.

ACKNOWLEDGEMENTS

This work was supported in part by Maine Agricultural Experiment Station under project No. ME 08911 and ME 08553. Maine Agricultural Experiment Station Contribution No. 1232.

EFFECT OF POWDERED CELLULOSE ON THE TEXTURE AND WATER BINDING PROPERTIES OF SURIMI-BASED PRODUCTS

KiSun Yoon, C.M. Lee and K.H. Chung
Department of Food Science and Nutrition
University of Rhode Island, Kingston, RI 02881

INTRODUCTION

The overall textural characteristics of surimi-based products are affected by moisture, the functional properties of surimi, ingredients and the temperature-time relationship during heat setting(Lee, 1986). Lately, various types of ingredients were tested in surimi-based products for their ability to strengthen gel or to modify gel texture. Most of work has been focused in starch(Okada, 1963, Lee, 1984, Wu et al., 1985, Kim and Lee, 1987) and protein(Lee, 1986). Starch was found to have a gel strength effect, while protein does have texture modifying effect at appropriate levels. Recently, interest has been directed to powdered cellulose because it can contribute to binding, strength, structure in food systems (Labell, 1986).

Powdered cellulose is purified from specially treated wood pulp which has the lignins and impurities removed (Labell, 1986). Chemically, it is relatively inert which means it will not interfere with any reactions taking place in a food system (Miller, 1986). Among ingredients, the application of cellulose in surimi-based products has not been investigated. The objective of this research was to study the texture-modifying and water-binding properties of cellulose in surimi-based products.

MATERIALS AND METHOD

Sample preparation.

Frozen surimi prepared from Alaska Pollock(Theragra Chalcogramma) was manufactured by the Alaskan Pacific Seafoods(Kodiak, AK) and obtained from the North Pacific Seafood(Seattle, WA). Surimi was kept at -20 C until used. Cellulose powder and modified starch(Nustar®) were obtained from James River Co.(Berlin, NH) and Staley Manufacturing Co.(Decatur, IL), respectively.

Surimi-based products, both molded and fiberized forms, were prepared from a 800g surimi block. Surimi was partially thawed to -2 C and chopped for 2 min with 2%

salt(on a surimi basis) in a silent cutter, followed by an additional chopping for 8 min with other ingredients, namely, cellulose, starch or ice-chilled water to adjust the necessary moisture level. Modified starch was also added in combination with cellulose to determine the freeze-thaw stabilizing ability of cellulose when used together with modified starch.

One portion of the resulting surimi paste was stuffed into cellulose casings(25 mm dia.) to make a molded gel (shrimp analog) and steam-cooked for 20 min in an autoclave at 90 C and atmospheric pressure. Cooked gels were immediately cooled with cold water and left overnight at room temperature before gel forming properties were measured.

The other portion was used to make a fiberized product (crab analog). It was prepared by extruding the paste into a thin sheet through a reactangular nozzle having a narrow opening(1.5mm). The extruded sheets were then partially heat-set for 2 min in the oven at 110 C and scored into 1.5mm-wide strips by a cutter similar to a noodle cutter. The resulting sheet of strips was folded manually into a rope, wrapped with a satin wrap, and steam-cooked for 15 min in an autoclave at 90 C and immediately stored in a refrigerator at 4 C before being evaluated for textural properties.

Formulation and Processing.

The following experiments were conducted: 1) the different sizes ranging from 11 um to 55 um were tested for their binding properties in surimi gel; 2) using the best performing size of cellulose from the result of first experiment, the effect of cellulose levels on the texture of surimi-based products was studied; and 3) the effect of varying cellulose levels at a 6% starch level on gel texture. Along with water binding and gel texture, freeze-thaw stability of both molded and fiberized gels was also studied by measuring textural change, such as increases in rubberiness and firmness, as well as by analyzing sensory properties. For this purpose, all samples were subjected to 1 freeze-thaw cycle.

Texture analysis

The textural properties of gels measured were compressive force at 90% deformation, expressible moisture and penetration force using the Instron Testing Machine

(Model 1122). Testing was carried out following the procedures described by Lee (1984) except for shear test. The shear test was conducted only on the fiberized gel. In this test, the cylindrical fiber bundle was sheared longitudinally by a flat blade(1 mm thick) while the sample rested on a stationary plate. The maximum force required to cut the sample was measured as shear force and was used as an index of firmness and chewiness.

The texture evaluation was also conducted by a sensory panel of 7 people from the department who previously participated in the evaluation of the similar products. They were asked to score 4 textural characteristics (firmness, chewiness, rubberiness and moistness) for their intensity and desirability, as well as overall desirability using a structured 9-point scale(1:least, 9:greatest). Samples were presented in the flake form of fiberized crab leg products.

Statistical anlysis

The data were analyzed using the Statistical Analysis System Package(SAS, 1982). Correlation coefficients were calculated to determine relationships between sensory and instrumental parameters using the CORR program. Duncan's Multiple Range Test was used to determine the significance of the differences between treatments.

RESULTS AND DISCUSSION

Table 1 shows effect of various sizes of cellulose on the texture of surimi gel. Cellulose of the size from 11um to 20um performed better than ones larger than 20um in terms of gel cohesiveness and water binding. As the size of fiber increased beyond 20um, both gel cohesiveness and water binding ability decreased with increasing sense of chewing a thread.

Under the microscope, it was clearly seen that finer grade cellulose(11um) was dispersed finely throughout the matrix. Within the range of grade of 11um-20um, coarser grades tend to be better binders than the finer ones. Water absorption is also affected by the fibrous particles of powdered cellulose. The coarse grades have a more open structure, which allows them to absorb up to seven times their weight in water. Finer grades have a more dense structure, and they generally only absorb three to four times their weight in water(Miller, 1986). It can be suggested that gel forming of protein in surimi gel can be

affected by the amount of moisture held in cellulose.

Table 1. Effect of various sizes of cellulose on the texture of surimi gel.

SIZE OF CELLULOSE(μm)	expressible moisture(%)		GEL STRENGTH(kg) compressive force		penetration	
control	1.47	c	16.6	a	0.303	a
11	2.10	b	9.91	c	0.196	c
17	1.48	c	12.3	b	0.253	b
20	1.37	c	13.2	b	0.257	b
35	2.37	b	8.0	d	0.195	c
45	3.80	a	5.46	e	0.195	c
55	2.76	b	6.5	ed	0.172	d

* tested at 2% and 78% moisture
** means within each column with different letter are significantly different($P<0.05$).

Table 2. Effect of cellulose level on the texture of surimi based molded products

LEVEL*	expressible moisture(%)		GEL STRENGTH(kg) compressive force		penetration		%increase rigidity
control(0)	0.49 (4.70)	ab (c)	93.3 (45.8)	a (b)	0.446 (0.538)	a (a)	20.6
1	0.39 (3.0)	b (d)	80.3 (45.7)	b (a)	0.402 (0.430)	a (abc)	6.9
2	0.51 (2.36)	ab (e)	70 (46.5)	c (a)	0.377 (0.393)	b (c)	4.2
3	0.52 (5.24)	ab (b)	60.1 (36.3)	d (b)	0.365 (0.523)	bc (ab)	43.8
4	0.57 (5.50)	a (a)	44.8 (37.3)	e (b)	0.319 (0.418)	c (bc)	31.0

* 20 μm powdered cellulose at 78 % moisture.
** ( ) indicates the value after 1 freeze-thaw cycle

Effect of cellulose level on the texture of surimi based molded products is shown in Table 2. Overall, compressive and penetration forces of molded products gradually decreased with an increase in the cellulose level.

After 1 freeze-thaw cycle, the gel showed a good freeze-thaw stability at 1% and 2% level with a least amount of expressible moisture and a smallest increase in rigidity. Cellulose at levels greater than 2% may have prevented its ability to reduce ice crystal growth upon freezing. This is in turn caused increases in expressible moisture and penetration force(rigidity).

The results of effect of cellulose level on the textural characteristics of crab leg products are shown in Table 3. There were no significant changes up to 2%, but beyond this level, there were significant decreases in firmness, chewiness and rubberiness with an increase in cellulose level($P<0.05$).

Table 3 Effect of cellulose level on the textural characteristics of crab leg products

* LEVEL	SENSORY SCORE shear force(kg)	firm ness	chewi ness	rubberi ness	moist ness	overall
control	0.73 (1.48)	6 (7.0)	6.4 (7.25)	6.2 (7.5)	6 (5.75)	5.9 (6.5)
1	0.73 (1.26)	5.2 (7.38)	6.2 (7.5)	6.6 (7.13)	6 (5.75)	6.2 (7.5)
2	0.68 (1.14)	5.2 (6.13)	5.6 (7.13)	5.5 (7.0)	6.6 (6.5)	5.6 (8.0)
3	0.59 (1.02)	3.9 (5.38)	4.8 (6.13)	4.9 (6.0)	6.2 (6.5)	5.4 (6.63)
4	0.53 (0.98)	3.8 (5.25)	3.8 (5.75)	4.2 (5.75)	5.8 (5.75)	4.0 (6.63)

* 20 μm powdered cellulose at 78% moisture
** ( ) indicates the value after 1 freeze-thaw cycle.

No significant differences in texture score were noted between control and products with 1% level. However, the better overall texture value was obtained at a 1% level.

After 1 freeze-thaw cycle, there were slight decreases in firmness, chewiness and rubberiness with an increase in the cellulose level. There were no significant differences between control and at 1% level in the texture score of chewiness, firmness and rubberiness. However, the better overall texture score was marked at the 1% level cellulose due to a more meaty, less rubbery texture and a better mouthfeel. Crab leg products without cellulose showed the lowest desirability score due to a rubbery and dry texture. The highest overall texture score was received at the 2% level. The improvement in the texture of the cellulose-containing gel is probably due to the freeze-thaw stabilizing ability of cellulose. The microscopic examination revealed that cellulose was able to reduce ice crystal growth at a moderate moisture level during frozen storage.

Table 4 shows effect of addition of cellulose with 6% starch on the textural characteristics of molded products. Overall, cohesiveness and rigidity of molded products gradually decreased with an increase in the cellulose level with 6% starch. Gels without starch had a more rigid and less cohesive texture with high expressible moisture than the one with starch.

Table 4 Effect of addition of cellulose with 6% starch on the textural characteristics of molded products.

SAMPLE	GEL STRENGTH(kg)					
	expressible moisture(%)		compressive force		penetration	
control	0.68	a	43.6	bc	0.473	a
S-C:6-0	0.57	a	60.2	a	0.370	b
S-C:6-1	0.66	a	49.3	b	0.325	c
S-C:6-2	0.64	a	41.0	cd	0.328	c
S-C:6-3	0.57	a	40.3	cd	0.320	dc
S-C:6-4	0.64	a	35.5	d	0.280	d

* S-C:modified starch:powdered cellulose
** means within each column with different letter are significantly different ($P<0.05$).

Addition of cellulose above 1% significantly reduced the cohesiveness and rigidity of surimi gels despite their ability to bind moisture. This could be its disruptive effect on the composite-reinforcing effect of starch.

Table 5 shows the effect of addition of cellulose with 6% starch on the textural characteristics of crab leg products. The highest textural strength and desirability was obtained at 1% cellulose level. Beyond 1% level, the textural values gradually decreased with an increase in the cellulose level.

Table 5 Effect of addition of cellulose with 6% starch on the textural characteristics of crab leg products.*

SAMPLE	shear force(kg)	SENSORY SCORE firm ness	chewi ness	rubberi ness	moist ness	overall texture
control	0.57 (1.55) [172]	6.5 (7.13)	7 (7.13)	6.7 (7.13) [+6.4]	5.5 (6.0) [+9.9]	6.6 (4.75) [-28]
S-C:6-0	0.45 (0.75) [66.7]	5.3 (6.0)	5.8 (6.0)	5.2 (5.0) [-3.8]	5.7 (6.15) [+7.9]	5.92 (6.5) [+9.8]
S-C:6-1	0.45 (0.93) [107]	5.83 (5.88)	6.8 (6.5)	5.3 (5.5) [-3.7]	5.7 (6.38) [+11.9]	6.25 (6.75) [+8.0]
S-C:6-2	0.40 (0.91) [127]	5.0 (5.85)	5.5 (6.3)	5.1 (4.63) [-9.2]	5.2 (6.75) [+29.8]	6.13 (6.25) [+1.95]
S-C:6-3	0.38 (0.92) [142]	4.8 (5.0)	5.0 (5.75)	4.7 (4.5) [-4.2]	5.75 (6.75) [+17.4]	6.0 (7.13) [+18.8]
S-C:6-4	0.34 (0.63) [85.3]	4.5 (4.63)	4.6 (5.0)	4.0 (4.25) [+6.25]	5.6 (7.0) [+5.6]	5.6 (7.38) [+31.8]

( ) indicates the value of 1 freeze-thaw cycle
[ ] indicates % increase after 1 freeze-thaw cycle.

All starch-cellulose containing formulas (5.6-6.3) were slightly inferior to control(6.6) in the overall texture score. A moderate correlation(r=0.76) between shear force and rubberiness was observed at the 99% level of confidence. This suggests that shear test can be used to measure changes in rubberiness.

After 1 freeze-thaw cycle, the overall textural scores of cellulose-containing crab leg formulas at all levels were higher than those without cellulose. This was due to a decrease in rubberiness and an increase in moistness. As expected, the control without starch and cellulose became too rubbery and dry and received the lowest desirability score. As shown previously in Table 3, all textural values gradually decreased with an increase in the cellulose level. However, when crab leg products were formulated with starch, the ones having cellulose received better desirability score than those without cellulose. It means that cellulose prevents the toughening of fiberized crab leg products during frozen storage. This result demonstrates the advantage of adding cellulose to products to be distributed frozen.

CONCLUSION

Cellulose at 1-2% level helps keep products, both molded and fiberized forms, from becoming rubbery and dry during freeze-thaw cycles by effectively preventing freeze syneresis and improving water binding of a gel. Modified starch alone results in a less moist and starchy texture. However, with addition of cellulose, a product having a moist and less starchy texture can be produced after frozen storage.

REFERENCES

1. James river Corp., "Solka-Floc® powdered cellulose for the food industry"(brochure).

2. Kim, J.M. and Lee, C.M. 1987. Effect of starch of textural properties of surimi gel. J. Food Sci. 52(3):722.

3. Kim, J.M., Lee, C.M., and Hufnagel, L.A. 1987. Textural properties and structure of starch-reinforced surimi gels as affected by heat-setting. Food Microstruct. 6:81.

4. Labell, F. 1986. Powdered cellulose adds texture, high

fiber to reduced calorie foods. Food Processing 47:7.

5. Lee, C.M. 1984. Surimi process technology. Food Technol. 38(11):69.

6. Lee, C.M. 1986. Surimi manufacturing and fabrication of surimi-based products. Food Technol. 40(3):115.

7. Miller, W.B. 1986. Seeing the 'light' Cellulose in reduced-calorie baked goods. Bakers Digest. 7.

8. Morse, E. 1978. Cellulose:The versatile dietary fiber. Cereal Foods World. 23(11):645.

9. Okada, M. 1963. Studies of elastic property of Kamaboko. Bull. Tokai Reg. Fish. Res. 36:21.

10. Osman, E.M. 1975. Interation of starch with other components of food systems. Food Technol. 29(4):30.

11. SAS. 1982. "SAS User's Guide:Statistics." SAS Institute Inc., Cary, NC.

12. Vetter, J.L. 1984. Fiber as a food ingredient. Food Technol. 38(1):64.

13. Wu, M.C., Hamann, D.D., and Lanier, T.C. 1985. Rheological and calorimetric investigations of starch-fish protein systems during thermal processing. J. Text. Stud. 16:53.

ACKNOWLEDGEMENT

Authors would like to thank the James River Corporation for their financial support throughout the study. Thanks are also extended to A. E. Staley Manufacturing Co. and ICI America for providing starch and sorbitol samples. The use of trade names in this publication does not imply endorsement of the products named.

TEXTURE-MODIFYING EFFECT OF NONFISH PROTEIN IN SURIMI GEL

K.H. Chung and C.M. Lee
Department of Food Science and Nutrition
University of Rhode Island, Kingston, RI 02881

INTRODUCTION

As a result of increasing consumption of surimi-based shellfish analog products, a marked advancement in the surimi technology has been made primarily in the manufacturing of surimi and shellfish analog products.

Texture of the product produced with surimi alone tends to be rubbery and less desirable to the Americans' taste. Not only to ease the rubberiness but also to allow flexibility in formulation, textural modification of surimi-based product has been attempted. Modification of texture can be achieved not only by a mechanical texturization process, but also by the incorporation of gel-forming ingredients such as starch and protein. Addition of ingredients to the surimi-based product can contribute to the improvement of textural properties as well as economic and nutritional benifits (Lee, 1986 ; Lee and Kim, 1986).

When nonfish proteins are incorporated into surimi, each protein contributes different functional and textural properties to the surimi gel. Therefore, understanding of the physicochemical properties of nonfish proteins and their interaction with fish protein is important to optimizing the textural property of the surimi-based products. There appear to be potential demands for high protein surimi-based products which stemmed from the idea of bringing the protein level of the surimi-based product (11-12 %) to that of the natural product (16-17 %). This may waive manufacturers an "imitation" labeling requirement according to a recent FDA ruling on "nutrition equivalency". The formulation of a high protein product needs to be developed without reducing the desirable textural quality which is normally found in the ordinary surimi-based product.

The objectives of this study were : 1) to study texture-modifying properties of nonfish proteins in surimi gels, and 2) to determine the relationship between functional properties of nonfish proteins and nonfish protein-incorporated surimi gel.

MATERIALS AND METHODS

Preparation of nonfish protein gel

Nonfish proteins evaluated were soy protein isolate (SPI), whey protein concentrate (WPC), lactoalbumin (LA), egg white (frozen raw, EW), milk protein isolate (MPI) and wheat gluten (WG). SPI was obtained from Grain Processing Corporation (Muscatine, Iowa); EW from Hygrade Egg Products (Elizabeth, NJ); WPC, LA and MPI from New Zealand Milk Products (Petaluma, LA); and WG from Ogilvie Mills (Minnetonka, MN). Protein gels were prepared by blending the proteins for 10 min in a silent cutter with addition of 1.5 % salt and a proper amount of water to adjust the moisture level to 78 %. One portion of paste was stuffed into a 25 mm diameter cellulose casing and the other portion placed into centrifuge tubes. Casings were cooked for 20 min in a steam-cooker.

Evaluation of gel texture and water binding ability of nonfish protein

Gel properties of samples were evaluated by a method reported by Lee (1984) using an Instron Testing Machine(Model 1122). Protein content of the samples were determined by the micro-kjeldahl method (1975). Water binding ability (WBA) of the nonfish protein was determined by a centrifugation method and expressed in % amount of water retained in the sample. Centrifugation was done for 10 min at 3000 g using a Sorvall centrifuge (Model RC 2B) after cooking the paste for 20 min in a steam-cooker while it was in the tube. The water binding ability of nonfish protein during heating was referred to "thermal hydration". The water binding ability was also determined by measuring the amount of moisture expressed from the plug of cooked protein gel (25 mm x 25 mm) upon compression and expressed in % moisture retained in the sample.

Preparation of surimi gel

The Alaska pollock (Theragra chalcogramma) surimi obtained from the Alaska Fisheries Developement Foundation (Anchorage, AK) was used throughout the study. Half-thawed surimi was chopped for 10 min in a silent cutter with addition of 2% salt, 3% nonfish protein and a proper amount of water to adjust the moisture level to 78 %. All ingredients were added on a surimi weight basis. The resulting paste was divided into three portions. One part of

the paste was extruded into a sheet (1.5 mm thick, 25 mm wide and 70 mm long) and partially heat-set for 15 min at 50 C to run a tensile test. The second one was also extruded into a sheet, partially heat-set for 2 min at 110 C, and fiberized to run sensory evaluation and shear test. The third one was stuffed into a 25 mm diameter cellulose casing. Fiberized and casing-molded samples were cooked for 15 and 20 min, respectively, in a steam-cooker. The fiberized sample was cooled and kept in a refrigerater , while the casing-molded sample was cooled down in the running cold water for 10 min and kept overnight at room temperature prior to the evaluation of the textural properties.

Evaluation of textural and water binding properties

Textural properties of gels were evaluated following the method reported by Lee(1984) except for shear test using an Instron Testing Machine. Testing parameters included compressive force at failure(cohesiveness), penetration force(rigidity), and % expressible moisture(water binding ability) for the casing-molded samples; tensile and shear force for the fiberized samples. In the shear test, a fiberized sample was sheared longitudinally by a descending flat blade (1 mm thick) while the sample rested on a stationary plate. Water binding of casing-molded surimi gel was determined by expressible moisture measured by the compression test.

Sensory evaluation of fiberized products

Sensory evaluation of the textural properties of the fiberized surimi gel product was conducted by a group of 5 panelists. The textural characteristics evaluated were firmness, rubberiness, chewiness, moistness and overall texture acceptability. The panel was composed of graduate students and faculty of the department who had a prior experience in evaluating the quality of surimi gel products. The panelists were asked to score the intensity and desirability of each textural characteristic on a 9-point scale.

Analysis of data

Analysis of variance was used to determine the statistical significance of the sample variations in physicochemical properties of nonfish protein. The degree of correlation was determined between functional properties of nonfish protein and nonfish protein-incorporated surimi gel.

RESULTS AND DISCUSSION

The results of the textural evaluation of the nonfish protein-incorporated surimi gel was shown in Table 1. The control surimi gel exhibited a consistently and significantly higher compressive force (cohesivenes) than those gels with nonfish proteins ($p < 0.05$). There was a significant variation in cohesiveness among the nonfish protein-incorporated surimi gels ($p < 0.05$). A similar trend was found in the rigidity with exception of EW-containing surimi gel. On the other hand, the tensile force of control surimi gel was lower than that of the nonfish protein-incorporated surimi gel except WPC-containing gel. The higher gel strength shown by the control was attributed to the greater amount of gel-forming myofibrillar proteins in the control surimi gel than those in the nonfish protein-incorporated surimi gel. The decreased gel strength of the nonfish protein-incorporated surimi gel was due to a combination of reduced amount of myofibrillar proteins and the interference of nonfish protein, primarily albumin-type, with formation of cohesive gel matrix (Okada, 1964). On the

Table 1. Textural properties of nonfish protein-incorporated casing-molded surimi gel products

PROTEIN SOURCE	COMPRESSIVE FORCE(Kg)	PENETRATION FORCE(g)	% EXPR. MOIST.	TENSILE FORCE(g)
CONT.	51.0 a	390.0 a	0.39 bc	41.3 bc
SPI-1	41.0 b	373.0 a	0.34 cd	43.0 bc
SPI-2	35.3 b	323.0 bc	0.26 d	44.3 abc
WPC	19.3 d	296.7 cd	0.33 cd	37.0 c
LA	21.0 d	273.3 d	0.48 ab	44.7 abc
MPI	16.7 e	213.3 d	0.50 a	42.0 bc
EW	38.7 b	400.0 a	0.53 a	51.3 a
WG	28.7 c	360.0 ab	0.44 ab	48.3 ab

* Significantly different between values with different letters ($p < 0.05$)

RESULTS AND DISCUSSION

The results of the textural evaluation of the nonfish protein-incorporated surimi gel was shown in Table 1. The control surimi gel exhibited a consistently and significantly higher compressive force (cohesivenes) than those gels with nonfish proteins ($p < 0.05$). There was a significant variation in cohesiveness among the nonfish protein-incorporated surimi gels ($p < 0.05$). A similar trend was found in the rigidity with exception of EW-containing surimi gel. On the other hand, the tensile force of control surimi gel was lower than that of the nonfish protein-incorporated surimi gel except WPC-containing gel. The higher gel strength shown by the control was attributed to the greater amount of gel-forming myofibrillar proteins in the control surimi gel than those in the nonfish protein-incorporated surimi gel. The decreased gel strength of the nonfish protein-incorporated surimi gel was due to a combination of reduced amount of myofibrillar proteins and the interference of nonfish protein, primarily albumin-type, with formation of cohesive gel matrix (Okada, 1964). On the

Table 1. Textural properties of nonfish protein-incorporated casing-molded surimi gel products

PROTEIN SOURCE	COMPRESSIVE FORCE(Kg)	PENETRATION FORCE(g)	% EXPR. MOIST.	TENSILE FORCE(g)
CONT.	51.0 a	390.0 a	0.39 bc	41.3 bc
SPI-1	41.0 b	373.0 a	0.34 cd	43.0 bc
SPI-2	35.3 b	323.0 bc	0.26 d	44.3 abc
WPC	19.3 d	296.7 cd	0.33 cd	37.0 c
LA	21.0 d	273.3 d	0.48 ab	44.7 abc
MPI	16.7 e	213.3 d	0.50 a	42.0 bc
EW	38.7 b	400.0 a	0.53 a	51.3 a
WG	28.7 c	360.0 ab	0.44 ab	48.3 ab

* Significantly different between values with different letters ($p < 0.05$)

contrary, nonfish proteins tended to increase the tensile force of the fiberized products. It was not clear, however, whether such an increase in tensile force was an indication of stiffness or elasticity. The variation of gel properties among the nonfish protein-incorporated surimi gel was attributed to the differences in physical and functional properties of nonfish proteins (Table 3). Addition of nonfish protein having higher gel strength resulted in

Table 2. Sensory textural properties and shear force of the nonfish protein-incorporated fiberized surimi gel products

PROTEIN SOURCE	SHEAR FORCE(g)	TEXTURAL CHARACTERISTICS			
		FIRMNESS	CHEWINESS	RUBBERINESS	OVERALL TEXTURE
CONT.	933 a	7.3 a (6.0) a	7.2 a (6.0) a	7.0 a (6.0) a	(6.0) b
SPI-1	527 d	5.0 c (6.7) a	5.6 b (6.3) a	6.0 ab (6.7) a	(6.7) a
SPI-2	750 b	5.6 bc (6.7) a	5.7 b (7.0) a	5.3 b (7.00 a	(6.9) a
WPC	417 e	6.3 b (7.0) a	6.0 b (6.7) a	5.7 ab (7.0) a	(6.8) a
LA	550 d	5.3 bc (6.3) a	5.3 b (7.0) a	5.3 b (6.3) a	(6.5) a
MPI	650 c	5.5 bc (7.0) a	5.5 b (7.0) a	5.0 b (6.5) a	(6.8) a
EW	743 b	5.6 bc (7.0) a	5.7 b (7.0) a	5.3 b (6.7) a	(6.9) a
WG	600 cd	6.0 bc (7.0) a	5.7 b (7.0) a	5.3 b (7.0) a	(7.0) a

* Without parenthesis : intensity
With parenthesis : desirability
** Significantly different between values with different letters ($P < 0.05$)

formation of a weaker gel. It was reported that such a result was due to a disruptive effect of nonfish protein on the network formation of surimi gel (Lanier , 1986). Nonfish proteins react to various treatments in different manners depending on their physicochemical properties such as molecular size and weight, ionic effect, protein concentration, amino acid composition, and chemical bondings involved in gelation (Buttkus, 1974; Beveridge et al, 1980).

Table 2 shows the results of the textural evaluation of fiberized products. The shear force of the control surimi gel was significantly greater ($p < 0.05$) than all nonfish protein-incorporated surimi gels. The intensity of sensory score of the control followed the same trend as the shear force. The desirability in terms of three textural characteristics and overall texture, on the other hand, showed an opposite trend. The lower score shown by the

Table 3. Gel strength and water binding ability of nonfish protein gels

PROTEIN SOURCE	COMPRESSIVE FORCE(kg)	PENETRATION FORCE(g)	WATER 1 BINDING(%)	THERMAL 2 HYDRATION(%)
SPI-1	0.60 d	25.0 d	98.3 d	100
SPI-2	0.61 d	29.0 d	97.4 d	100
WPC	3.08 a	275.0 a	76.1 a	79
LA	1.2 c	107.7 c	78.2 b	80
EW	1.98 b	116.0 b	91.27 c	100
WG	No failure	108.0 c	Trace	60.3

* Significantly different between values with different letters ($p < 0.05$).

1. $\left(1 - \frac{\text{the amount of water upon compression}}{\text{moisture content of sample}}\right) \times 100$

2. $\left(1 - \frac{\text{the amount of water upon centrifugation}}{\text{moisture content of sample}}\right) \times 100$

control was due to a higher intensity, but less desirable sensory score. However, addition of nonfish protein reduced the intensity score and thus increased the desirability score.

In an effort to determine what caused such variations in the textural properties of nonfish protein-incorporated surimi gel, the relationship of the functional properties of nonfish protein to the properties of nonfish protein-incororated surimi gel was studied. The functional properties studied were water binding ability and gel strength. Woodward et al (1982) and Hermansson (1975) claimed that water binding ability of protein is one of the most important factors which control the textural and structural properties of heat-induced protein gel. There was a great deal of variation in water binding ability among proteins, where WPC showed the least water binding ability, but with the highest gel strength for both cohesiveness and rigidity (Table 3). Such a low moisture absorption by WPC may have facilitated more protein-protein interaction, resulting in the development of a firmer gel.

Table 4. Correlations among the water binding ability,gel strength of nonfish protein and nonfish protein-incorporated surimi.

1. r = -0.65 between thermal hydration (centrifugation) and compressive force of nonfish protein gel (Table 3).
2. r = 0.94 between water binding based on expressible moisture of nonfish protein by compression (Table 3) and compressive force of nonfish protein-incorporated surimi gel.
3. r = 0,97 between thermal hydration (Table 3) and compressive force of nonfish protein-incorporated surimi gel, WG excluded (Table 1).
4 r = - 0.68 for rigidity between nonfish protein-incorporated surimi gel and nonfish protein gel.
5. r = - 0.63 for cohesiveness between nonfish protein-incorporated surimi gel and nonfish protein gel.

Cohesiveness of nonfish protein-incorporated surimi gels highly correlated with thermal hydration ($r = 0.97$, WG excluded), as well as with water binding based on the expressible moisture ($r = 0.94$) of nonfish protein gels. This result suggests that both thermal hydration and expressible moisture of nonfish protein are good indices of protein functionality to be related to the textural properties of nonfish protein-incorporated surimi gel. Thermal hydration of nonfish proteins inversely correlated with cohesiveness ($r = -0.65$), rigidity ($r = -0.46$), and % expressible moisture ($r = -0.97$) of nonfish protein gel. In summary , nonfish protein with less thermal hydration and greater expressible moisture produced a stronger nonfish protein gel due to protein concentration, but it made surimi gel weaker when nonfish proteins were incorporated. Unlike cohesiveness, rigidity poorly correlated with thermal hydration ($r = 0.26$) as shown in Table 4. Cohesiveness is the degree of protein binding in the matrix and its value is better considered for the gel forming ability than rigidity value (Lee and Chung, 1987). This may explain the discrepancy between two correlation coefficients.

The result of protein analysis suggested that water binding ability of nonfish protein was dependent upon its protein content. WPC having 74 % protein content showed the least WBA, while SPI having 89 % showed the highest WBA. The protein concentration of SPI is higher than that of WPC. It means that SPI has more solid concentration and thus more surface area which allows more water molecules for absorption.

Conclusions

Addition of nonfish protein to the surimi-based products improved the textural properties by reducing the intensity of undesirable rubberiness, chewiness and firmness, thus increasing the desirability of overall texture. The reduction in the intensity of such undesirable textural characteristics resulted in a soft mouthfeel.

Thermal hydration of nonfish protein inversely correlated with the cohesiveness of nonfish protein gel, but it moderately correlated with the cohesiveness of nonfish protein-incorporated surimi gel. Gel strength of both cohesiveness and rigidity of nonfish protein gel showed an inverse correlation with those of nonfish protein-incorporated surimi gel. It was concluded that the main

cause of variations in the texture-modifying effects of different nonfish proteins was the differences in thermal hydration and gelation properties of nonfish proteins. Therefore, thermal hydration and gelation properties can be effectively used in determining texture-modifying ability of nonfish proteins

References

1. AOAC. 1975. Official Method of Analysis, 12th ed. Assoc. Off. Anal. Chem., Washington, D. C.

2. Beveridge, S., Ko, S.A.S and Chung, J.K.H. 1980. Firmness of heat induced albumem coagulum. Poultry Sci. 59: 1229

3. Buttkus, H. 1974. On the nature of the chemical and physical bond which contribute to some structural properties of protein foods: A hypothsis. J. Food Sci. 39: 484

4. Hermasson, A. M. 1975. Functional properties of added proteins correlated with properties of meat system. " Effect of concentration and temperature on water-binding properties of Model Meat Systems." J. Food Sci. 40: 603

5. Lanier, T. C. 1986. Functonal properties of surimi. Food Technol. 40(3) : 107

6. Lee, C. M. 1984. Surimi process technology. Food Technol. 38(11): 69

7. Lee, C.. M. 1986. Surimi manufacturing and fabrication of surimi-based products. Food Technol. 40(3): 115

8. Lee, C. M. and Kim, J. M. 1986. Texture and freeze-thaw stability of surimi gel in relation to ingredients and formulation. Proc. of Inter. Symp. on Engineered Seafood Including Surimi (R. Martin and R. Collete, Ed) National Fishies Institute, Washingston, DC, P 168

9. Lee, C. M. and Chung, K. H. 1987. Textural analysis of surimi gel properties by compression and punch tests. paper No. 572 , presented at 47th Annual Meeting of Institute of Food Technologists, Las Vegas, Nevada, June 16-19.

10. Okada, M. 1964. Effect of washing on the jelly forming ability of fish meat. Bull. Jap. Soc. Sci. Fish. 30: 255

11. Woodward, S. A. and Cotterill, O. J. 1982. Texture and microstructure of heat-formed egg white gel. J. Food Sci. 51: 333

CHANGES IN THE FATTY ACID AND AMINO ACID PROFILES OF FISH MINCE DURING SURIMI PROCESSING

Henry Leibovitz, Richard D. Morizen and Chong M. Lee
Department of Food Science and Nutrition
University of Rhode Island, Kingston, RI 02881

INTRODUCTION

Marine fish oils containing omega-3 fatty acids are increasingly important to health-minded consumers who believe that increasing their intake of omega-3 fatty acids will reduce their chances of heart disease by lowering blood cholesterol levels (Lands, 1986). Essential amino acid (EAA) profiles are of particular interest to nutrition oriented individuals and food producers because they are indicative of protein quality. Examination of the effects of processing on the EAA profiles of surimi is necessary in order to substantiate its nutritional value and the necessity of recovering amino acids currently discarded in the wash water and strainer reject.

The objectives of the study were: to measure the effect of washing on levels of total lipid, eicosapentaenoic acid (EPA, 20:5W3) and docosahexaenoic acid (DHA, 22:6W3) in red hake (Urophycis chuss) and mackerel (Scomber scrombrus) surimi, and to determine changes in the concentration of each amino acid in the mince and wash water at the different surimi production steps using red hake.

MATERIALS AND METHODS

The general procedure for preparation of fish mince was based on the method by Lee (1986). Red hake and mackerel fillets were ground through a plate having 4.5 mm holes and washed two times for the amino acid study and three times for the fatty acid study at a 3 : 1 water mince ratio at 10 C for 5 min. Following the second washing red hake mince was strained using a strainer (Bibun sum 420). A portion of the resulting slurry from each washing was pressed with a Carver press and used for subsequent analyses. Changes in the fatty acid profile of the mince were analyzed after each washing. The fatty acid analysis was conducted in the following manner. Red hake and mackerel flesh samples were collected at each washing cycle. Total lipids were extracted from the flesh using a solvent system of chloroform-methanol-water (1:2:0.8 V/V) (Bligh and Dyer, 1959). Lipid extracts were saponified and free fatty acids were converted into fatty acid methyl esters (Kates, 1972). Fatty acid methyl esters were identified by gas chromatography on a cyanosilicon column (SP-2330, Supelco Inc.) at 200 C in a Varian 1700 gas chromatograph.

Samples collected from each processing site were freeze-dried (Virtis Unitrap II) and analyzed for moisture, protein and amino acids. Samples included fresh fish fillets, wash waters, reject and strained mince. The moisture and protein contents of each sample collected were determined by using the oven drying and the micro-Kjeldahl method, respectively. Amino acids were analyzed in the following manner. Freeze-dried samples of quantities equivalent to 8 µg of protein were hydrolyzed in 6N HCl at 110 C for 22 hours followed by evaporation of the acid. The hydrolysates were diluted to 5 ml with 0.1N HCl and filtered through a 0.45 µ filter. Six µl of the resulting filtrate was injected into a high pressure liquid chromatograph (Perkin-Elmer Corp.) set up with a sulfonated polystyrene cation exchange column (Interaction Corp.). The separated amino acids were converted into thiolesters by a post-column derivatization reaction with fluoraldehyde (Pierce Co.). The secondary amino acids such as proline were converted into primary forms by a post-column reaction with sodium hypochlorite before deriving their thiolesters. Amino acid derivatives were identified using a fluorescence detector (model LC-10, Perkin-Elmer Corp.), and the concentration of amino acid was determined.

RESULTS AND DISCUSSION

No significant differences in total lipid content were seen following the washings. The total lipid, EPA and DHA remained similar on a dry weight basis in the red hake samples. In the mackerel samples, EPA was significantly ($p < 0.05$) more concentrated in the mince following the third washing (Table 2). Trends toward increasing total lipids and DHA after washings in mackerel samples and decreased levels of total lipids, EPA and DHA in the red hake samples were not significant ($p < 0.05$) (Table 1 and 2).

Table 1: Effects of Washing on Total Lipid, Eicosapentaenoic Acid and Docosahexaenoic Acid in Red Hake Surimi

Washing Number	Total Lipid		EPA		DHA	
	(mg / 100 mg fish dry wt.)					
0	3.39	0.58a	0.51	0.01a	1.06	0.14a
1	3.03	1.05a	0.51	0.18a	0.71	0.25a
2	2.72	0.11a	0.36	0.02a	0.82	0.02a
3	2.15	0.23a	0.31	0.02a	0.76	0.16a

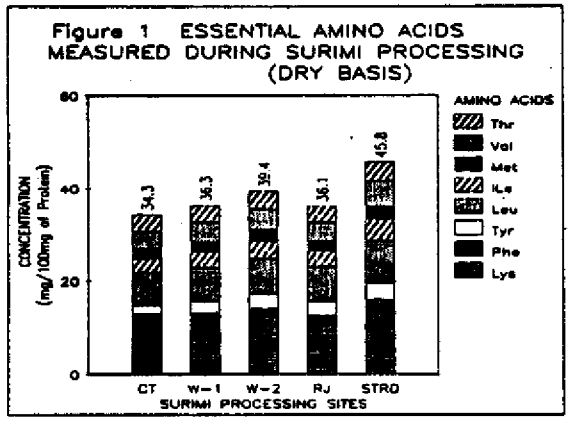
Means followed by different letters are significantly different ($p < 0.05$).

Table 2: Effects of Washing on Total Lipid, Eicosapentaenoic Acid and Docosahexaenoic Acid in Mackerel Surimi

Washing Number	Total Lipid		EPA		DHA	
	(mg / 100 mg fish dry wt.)					
0	8.94	0.09a	0.48	0.00a	1.19	0.02a
1	9.46	0.32a	0.51	0.02ab	1.29	0.02a
2	9.34	0.91a	0.52	0.05ab	1.22	0.07a
3	9.74	0.67a	0.60	0.02b	1.31	0.05a

Means followed by different letters are significantly different ($p < 0.05$).

There were significant differences in the amino acid profile between washed mince and wash water. Increased essential amino acid (EAA) levels in the mince increases after washing, notably, those of Val, Tyr, Ile, Leu and Lys (Fig. 1).

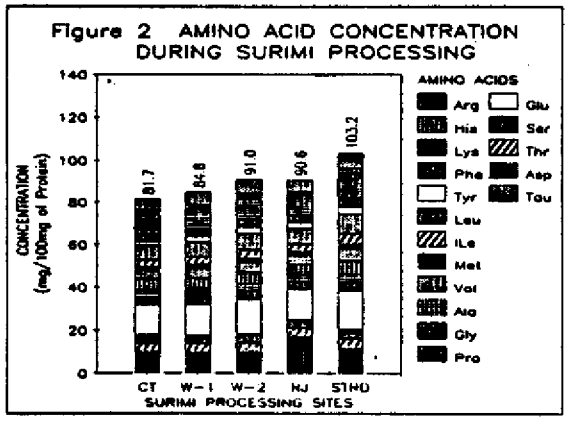


The results of the essential amino acids (EAA) analysis at different processing steps illustrates the concentration effect of the surimi washing process (Table 3) as indicated by an increase in the EAA concentration in mince with increased washing cycles. A similar trend was seen in the changes in EAA concentration in the wash water. However, this change was not due to the concentration effect, but due to the increase in tissue protein with a decrease in nonprotein nitrogen in the second washing. It was particularly noteworthy that there was significant difference in EAA concentration between the control fillets and the strained sample. Overall, the concentration of EAA in the strained mince was higher than that in the fillet.

Table 3: Essential Amino Acids Measured During Production Steps

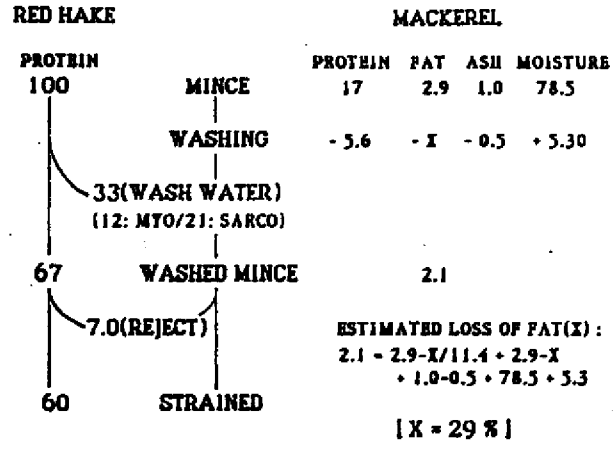
Amino Acids	Control (CT)	First Wash (W-1)	Wash Water One (LQ1)	Second Wash (W-2)	Wash Water Two (LQ2)	Reject (RJ)	Strained (STRD)
			(mg / 100 mg protein)				
THR	3.8	3.7	1.6	4.0	2.1	3.6	4.3
VAL	3.4	4.0	2.0	4.2	2.7	3.8	5.3
MET	2.4	2.4	0.8	2.7	0.9	2.4	2.9
ILE	3.1	3.5	1.6	3.8	2.3	3.5	4.7
LEU	6.8	7.0	3.0	7.5	4.4	7.2	9.1
TYR	2.0	2.7	---	3.3	1.0	3.1	3.6
PHE	3.8	3.5	3.2	3.6	4.7	3.3	4.5
LYS	9.0	9.5	4.6	10.3	6.1	9.2	11.4

The nutritional quality of the reject in terms of amino acid profile was equally as good as those of the control and first washed samples, and the amino acid levels in the pressed strained mince was far higher than those in the fresh fillet (Figure 2).

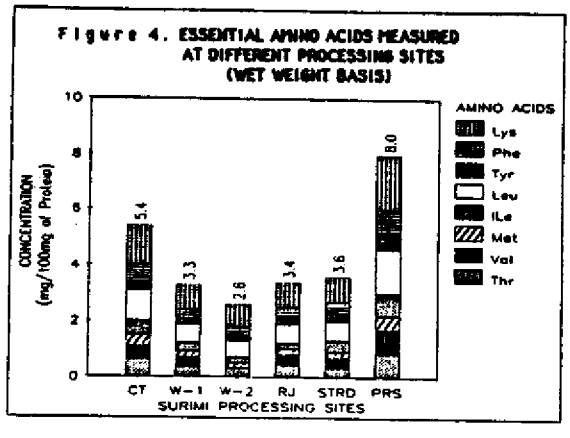


Using current knowledge, the expected percent fat and protein losses in red hake and mackerel samples are displayed in Figure 3 . As shown, the estimated loss of fat amounts to about 29% and the protein remaining is about 60 % of the original following washing and discards in the reject.

Figure 3. PROTEIN AND FAT LOSSES DURING WASHING



If any nutritional comments are to be made on the amino acid content and its value as a food source, we should consider the amino acid content on a wet basis instead of a dry basis. The moisture contents of the strained and the pressed mince were 92 % and 82 %, respectively, on a wet basis. The effect of the moisture reduction on the concentration of essential amino acids are shown clearly in Figure 4, where the concentration of amino acids in the pressed mince was much higher than that in the strained mince and the variations in the concentration are clearly reflected by differences in the moisture content.



CONCLUSIONS

The washing did not reduce the levels of either omega-3 fatty acids or total lipid in the fish flesh. This can be reasoned by the concentration effect of washing with losses of soluble components. A significant amount of lipids were estimated to be lost during washing, however, and further studies are needed to recover and measure the amount of total lipid actually lost in the wash water. Consumers should not consider surimi devoid of marine fish oil or omega-3 fatty acids.

Surimi processing does not appear to lower the protein quality of the final product. The washing increased the overall amino acid concentration in the fish mince. The amino acid profile of the wash water appears to be significantly inferior to that of the washed mince. The reject after straining is considered equally as nutritious as the final mince.

REFERENCES

Bligh E. G. and Dyer, W. J. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. and Physiol. 37:9, p. 911.

Kates, M. 1972. Identification of individual lipids and lipid moieties. In: "Techniques of Lipidology". Elsevier Science Publishers, Amsterdam, The Netherlands. p. 451.

Lands, W. E. M. 1986. Common factors in heart disease, heart attacks, and strokes. In: "Fish and Human Health". Academic Press, Inc. New York.

Lee, C. M. 1986. A pilot plant study of surimi making properties of red hake (Urophycis chuss). In " Proc. Intern. Symp. on Engineered Seafood Including Surmimi" (R. E. Martin and R. L. Collete, Ed.), National Fisheries Institute, Washington, D. C., p. 225.

SENSORY ANALYSIS OF EDIBLE FISH OILS

Janet A. Gooch and Sylvia B. Galloway
National Marine Fisheries Service, NOAA, SEFC
Charleston Laboratory, P.O. Box 12607, Charleston, SC 29412

INTRODUCTION

Considerable interest has arisen regarding the nutritional and therapeutic value of fish oils. Fish oils, with their relatively high concentration of long-chained omega-3 fatty acids, are linked with the prevention of coronary heart disease (Stansby, 1984; Sanders, 1985; Leaf, 1986). In conjunction with this, several studies have shown that the consumption of fish or fish oils alters blood lipid patterns in humans. Phillipson et al. (1985) found that, in patients with hypertriglyceridemia, a fish oil enriched diet (up to 30% of total calories) led to decreases in both plasma cholesterol and plasma triglyceride, as compared with control diets not containing fish oils. In addition to lowering lipid levels, certain fish oil fatty acids may also act to reduce platelet aggregation, decrease blood viscosity, and prevent ischemic damage as well as modify inflammation and immune responses (Stansby, 1984; Harris, 1985; Kinsella, 1986; Goetzl et al., 1986).

A conference was held on the "Health Effects of Polyunsaturated Fatty Acids in Seafoods" in 1985 in Washington, D.C. Session chairpersons voiced the crucial need for uniform test materials to be used for research on the biological mechanisms by which fish oils influence health and modulate disease processes (Simopoulos, 1986). This research will be conducted by qualified biomedical researchers who are supported by a variety of funding agencies including the National Institutes of Health (NIH), the National Science Foundation (NSF), and private universities. During 1986, the National Oceanic & Atmospheric Administration, National Marine Fisheries Service, Southeast Fisheries Center, Charleston S.C. Laboratory began its Biomedical Test Materials Program to provide refined menhaden oil and oil fractions of defined quality and composition for the NIH-approved studies.

Menhaden oil (Brevoortia spp.) was selected as the source for all test materials since eicosapentaenoic acid (EPA or 20:5n3) is one of its principal fatty acids (Joseph, 1985) along with moderate concentrations of docosahexaenoic acid (DHA or 22:6n3). Eicosapentaenoic acid and DHA are two of the most common omega-3 fatty acids in fish oils, and EPA is believed to be especially effective in reducing the risk of heart attack (Hirai et al., 1980; Kobayashi et al., 1981; Dyerberg, 1982). Menhaden oil is the primary commercial fish oil in the United States, and has been thoroughly analyzed (Ackman et al., 1976, 1981; Ackman, 1980; Dubrow et al., 1976; Joseph, 1985; Seaborn, 1986).

Since the biomedical test materials must be of defined quality and composition, quality control of the production processes and quality assurance of the products are of utmost importance. A number of stan-

dard methods for measuring the quality and composition of these products are currently in use, including sensory analysis. Sensory analysis is an integral part of our QA/QC program and is performed on fresh samples as well as oils undergoing storage to determine their flavor stability.

This paper outlines the methodology and presents results from sensory evaluation of the following fish oil products:

1) steam-stripped menhaden oil and placebo corn oil in soft gel capsules;
2) vacuum-stripped menhaden oil bulk packaged in bottles;
3) placebo corn oil, vacuum-stripped menhaden oil, and fish oil polyunsaturated ethyl esters, each microencapsulated in a matrix of 100% corn starch.

MATERIALS AND METHODS

Packaging and Antioxidants

The fish oil products have been bulk packaged in bottles, soft gel-encapsulated or microencapsulated and stored in amber bottles which were flushed with nitrogen prior to sealing. Placebo oils (corn, safflower, or olive) as well as ethyl esters of the fish oil were also packaged in the same manner. Antioxidants (Kodak 5-67, Tenox GT-1, and Tenox 20A[1]) were added to the various oils so that the final concentrations of antioxidants in all oils would be as follows: (1) alpha-tocopherol, 1.3 mg/g; (2) gamma-tocopherol, 1.2 mg/g; and (3) tertiary butyl hydroquinone (TBHQ), 0.2 mg/g. In this way, the antioxidant levels would be "balanced".

Panel Selection and Training

A preliminary examination of several freshly processed and deliberately abused (exposed to light and air in a glass petri plate) menhaden oil samples showed that the fish oils exhibited many of the same flavor and odor characteristics that are reported in the literature for other edible oils (Chang, 1972; Jackson, 1981, 1985; Waltking, 1982; AOCS, 1983; Lawson, 1985; Warner, 1985). To produce some of the flavor and odor attributes (i.e., painty, rancid, and oxidized), edible oils (i.e., sunflower, linseed, soybean, and cottonseed) were heated at 60°C over a period of days (up to 16) until they attained the desired off-flavor and odor (Warner, 1985). A series of triangle tests was used to screen panelists for the four basic tastes. Separate sessions were held over a period of several weeks to familiarize panel members with odors and flavors of different oils and then associate these characteristics with the description of each term on the evaluation form. Chemical reference standards and known oils were used in training the panelists to rate intensity and to discriminate among oil samples. Several publications (ASTM, 1968, 1981; IFT, 1981; Powers, 1982, 1984) provide background information and basic guidelines for sensory panel training.

---

[1] The use of trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

Odor and Flavor Evaluation Form

The procedure employed is a modification of the Quantitative Descriptive Analysis (QDA) method for sensory analysis (Stone et al., 1974; Mecredy et al., 1974; Zook and Wessman, 1977). The scale is an unstructured line 15 cm in length with one vertical line at the left end and a second vertical line 1 cm in from the right end of the line. The scale runs from absent to very strong in intensity. Each panelist records his/her evaluation by making a vertical line across the 15 cm horizontal line at the point that best reflects his perception of the intensity of each attribute. Only the attributes actually detected are marked on the score sheet.

Fig. 1 and Table 1 show an example of the evaluation form and definitions for each attribute (Stansby and Jellinek, 1965; Chang, 1972; Mounts and Warner, 1980; Jackson, 1981, 1985; Waltking, 1982; AOCS, 1983; Lawson, 1985; Warner, 1985).

Many researchers (Chang, 1972; Jackson, 1981, 1985; Waltking, 1982; AOCS, 1983; Warner, 1985) have utilized a 10-point flavor quality scale for sensory analysis of vegetable oils, with 10 as excellent and 1 as repulsive. However, we decided to use the QDA method because it has worked well for sensory evaluation of other fishery products; and the open line scale generates data that can be analyzed by standard statistical methods which assume a normal distribution.

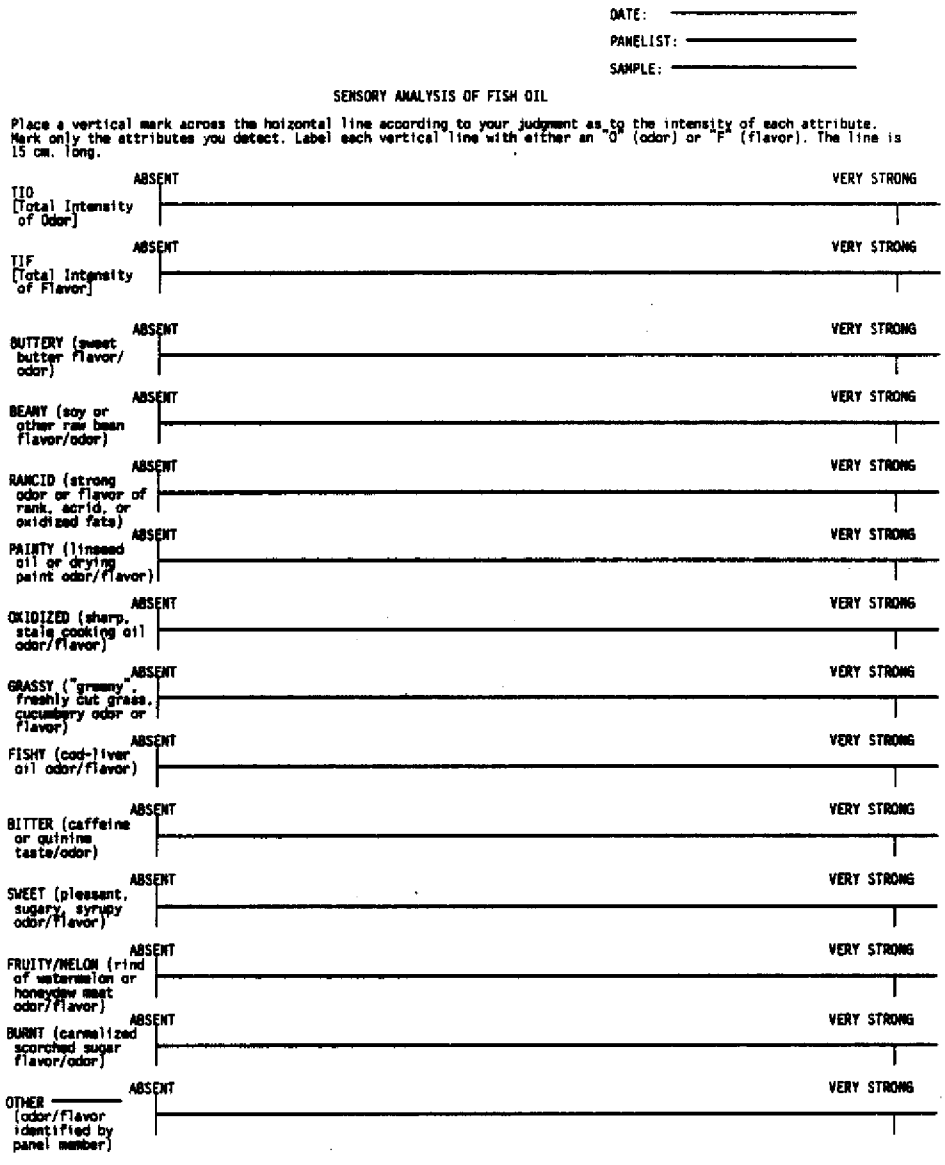


Figure 1. Example of evaluation form using the unstructured line scale.

Table 1. Definition of odors and flavors of fish oil.

TIO - Total intensity of odor.

TIF - Total intensity of flavor.

BUTTERY - Aroma and flavor of sweet, freshly-churned butter. A strong buttery flavor would refer to a pronounced, sweet butter flavor, not old or rancid.

BEANY - Characteristic of soybean oil products or fresh, raw soybeans or other raw beans.

RANCID - Strong, very oxidized, rank acrid flavor and odor, very old fats.

PAINTY - Resembling drying paint or linseed oil.

OXIDIZED - Sharp; characteristic of oils exposed to air, particularly cottonseed oil. A strong oxidized flavor is termed "rancid".

GRASSY - The "green" flavor or odor of freshly cut grass; slightly characteristic of "green" in cucumbers.

FISHY - The characteristic odor and flavor of fish oil, such as cod-liver oil.

BITTER - Taste and odor of caffeine or quinine; may be acrid.

SWEET - Pleasant, sucrose odor and flavor; slightly characteristic of syrup.

FRUITY/MELON - Characteristic of watermelon rind or honeydew melon; may be slightly citric; characteristic odor and flavor of fish oil esters.

BURNT - Scorched; like carmelized sugar; may be found in conjunction with "sweet".

OTHER - Any odor/flavor not defined above which is experienced by the panelist.

RESULTS AND DISCUSSION

Specially processed menhaden oil was purchased from Zapata Haynie Corp., Reedville, Virginia[1]. This oil was either steam-stripped or vacuum stripped, antioxidants were added, and the oil was shipped to commercial companies for encapsulation. The steam-stripped oil was soft-gel encapsulated, and the vacuum-stripped oil was microencapsulated; and then returned to NMFS for distribution to the various researchers.

Specific details of the sensory evaluation procedures used are outlined in Table 2. Table 3 shows the results of sensory analysis on soft-gel capsules of fish and corn oil. These data indicate only a slight increase in intensity of odor and flavor of the fish oil as a result of encapsulation. The sensory panel characterized the fish oil capsules as being bland in odor and flavor with very little "fishiness". Stansby and Jellinek (1965) characterized highly refined menhaden oils as having a slightly "green" or "grassy" odor and flavor. We found this to also be true of our menhaden oil samples.

Tables 4 and 5 show results of sensory evaluation of a vacuum-stripped menhaden oil packaged in bulk and three microencapsulated products (corn oil, vacuum-stripped menhaden oil, and fish oil ethyl esters). In Table 5 the microencapsulated fish oil and ethyl esters exhibited only a minute "painty" off-odor. The microencapsulating matrix was corn starch, as evidenced by panel members adding other attributes such as "chalky" and "starchy".

The results and samples presented in this paper are representative of the type of products that will be produced as part of the Biomedical Test Materials Program at the Charleston Laboratory. Sensory analysis is an important component of the QA/QC program. Panel members are capable of detecting off-odors and flavors long before chemical analyses will indicate oxidation of a product. It is hoped that we will be able to correlate sensory scores with analysis of volatiles by direct gas chromatography as well as other chemical analyses such as free fatty acids, peroxide values, iodine values, and anisidine values. Statistical analysis of the data will also be employed. In summary, sensory scores of the oils and oil derivatives show that they are of high quality with only slight "fishiness" and very few undesirable odors and flavors.

Table 2. NMFS Biomedical Test Materials Program Sensory Evaluation Procedures

SENSORY ANALYSIS OF OILS[a]

SAMPLE PREPARATION:

(1) A 40 ml glass beaker containing 5-6 ml of oil is covered with a watch glass and placed in a preheated 50°C water bath.

(2) The oils are heated 10 minutes prior to serving.

(3) A sample is coded with a 3-digit random number.

(4) A maximum of 3 samples per session are evaluated due to taste and olfactory fatigue of the panel members.

ODOR AND FLAVOR TESTING PROCEDURES:

(1) Panel members rate the oils for odor in the order in which samples are presented from left to right. Reference standards that characterize the attribute as well as the intensity were utilized during panel training and are available for review.

(2) A covered beaker is swirled and lifted to the nose. Panelist removes cover and sniffs the volatiles. The beaker is swirled again if necessary.

(3) The type and intensity of odor as well as total intensity of odor is recorded on the score sheet.

(4) For flavor attributes, the lightest aromatic sample is tasted first, followed by samples of increasing odor intensity.

(5) Disposable pipets are used to transfer an aliquot (50-100 µl) of oil into the mouth. The oil is held on the back of the tongue as air is drawn into the mouth and exhaled through the nose.

(6) If possible, the sample is not swallowed. Carbon-filtered tap water heated to 38°C is used to cleanse the palate after each sample.

(7) The type and intensity of flavor as well as total intensity of flavor are recorded on the same score sheet used for odor.

(8) Each product is sampled and evaluated, for odor and flavor, 3 times. The intensity data is measured numerically (in cm) and analyzed.

Table 2 continued

SENSORY ANALYSIS OF MICROENCAPSULATED OILS[b]

SAMPLE PREPARATION:

(1) A 40 ml glass beaker containing 5 g of the material and 25 ml boiling water is thoroughly mixed, covered with a watch glass, and placed in a preheated 50°C water bath to attain equilibrium.

(2) After 15-20 minutes, the temperature of the emulsion will have fallen to about 50°C and the odor is evaluated.

ODOR TESTING PROCEDURES:

Odor only is evaluated on microencapsulated oils since the microencapsulating matrix not only contributes flavors of its own, but also masks some oil flavors. See Steps 1-3 of Odor and Flavor Testing Procedures for methodology.

[a] Mounts and Warner, 1980; Jackson, 1981, 1985; Lawson, 1985; Chang, 1972; Warner, 1985; AOCS, 1983.
[b] Heath and Reineccius, 1986

Table 3. Mean scores (scale of 0-15) and standard deviations are listed for baseline data at the beginning of a storage study on fish oil and corn oil (placebo) capsules. Data was obtained from sensory evaluation of steam-stripped menhaden oil before and after encapsulation by a commercial company for NMFS.

	FISH OIL[a] (BEFORE ENCAPSULATION)	SOFT GEL CAPS FISH OIL CAPSULES[b]	SOFT GEL CAPS CORN OIL CAPSULES[b]
ODOR ATTRIBUTES:			
TIO	1.6 ± 0.7	2.4 ± 1.1	2.1 ± 1.2
BEANY	0.3 ± 0.3	0.2 ± 0.2	0.4 ± 0.5
GRASSY	0.2 ± 0.3	0.2 ± 0.5	0.0 ± 0.0
FISHY	0.5 ± 0.7	0.7 ± 0.7	0.1 ± 0.1
SWEET	0.1 ± 0.1	0.1 ± 0.2	0.4 ± 0.6
FLAVOR ATTRIBUTES:			
TIF	1.7 ± 0.6	2.6 ± 1.2	2.0 ± 1.1
BEANY	0.4 ± 0.3	0.1 ± 0.2	0.7 ± 0.6
GRASSY	0.5 ± 0.4	0.3 ± 0.5	0.0 ± 0.0
FISHY	0.3 ± 0.4	1.0 ± 0.8	0.0 ± 0.0
SWEET	0.0 ± 0.0	0.1 ± 0.1	0.4 ± 0.6
TYPICAL CORN	-	-	0.6 ± 0.9

[a] Mean from 4 separate panels; N = 7 panelists.
[b] Mean from 3 separate panels; N = 7 panelists.

Table 4. Mean scores (scale of 0-15) and standard deviations for a vacuum-stripped menhaden oil bulk packaged in bottles.[a]

Odor Attributes:		Flavor Attributes:	
TIO	2.8 ± 1.4	TIF	3.5 ± 1.3
Painty	0.2 ± 0.4	Painty	0.6 ± 1.1
Oxidized	0.2 ± 0.4	Oxidized	0.5 ± 0.6
Grassy	0.2 ± 0.4	Grassy	0.2 ± 0.3
Fishy	1.1 ± 1.4	Fishy	1.9 ± 1.4

[a] Mean from 3 separate panels; N = 7 panelists.

Table 5. Mean scores (scale of 0-15) and standard deviations are listed for baseline data at the beginning of a storage study. Data was obtained from sensory evaluation (odor only) of microencapsulated placebo corn oil, vacuum-stripped menhaden oil, and fish oil ethyl esters commercially microencapsulated for NMFS.

		MICROENCAPSULATED	
	CORN OIL[a]	FISH OIL[a]	FISH OIL ESTERS[a]
ODOR ATTRIBUTES:			
TIO	1.8 ± 1.4	3.0 ± 1.9	3.0 ± 1.4
BEANY	0.3 ± 0.5	0.0 ± 0.0	0.0 ± 0.0
PAINTY	0.0 ± 0.0	1.1 ± 0.8	1.4 ± 0.6
FISHY	0.0 ± 0.0	0.6 ± 0.8	0.2 ± 0.3
FRUITY/MELON	0.0 ± 0.0	0.0 ± 0.0	1.2 ± 0.7
CHALKY	0.6 ± 0.9	-	-
STARCHY	0.5 ± 0.6	0.4 ± 0.8	-
TYPICAL ESTER	-	-	1.3 ± 2.0

[a] Mean from 2 separate panels; N = 7 panelists.

REFERENCES

1. Ackman, R.G. 1980. Fish lipids. Part 1. In J.J. Connell (editor), Advances in Fish Science and Technology, p. 86-103. Fishing News Books, Ltd., West Byfleet.

2. Ackman, R.G., C.A. Eaton, and J.H. Hingley. 1976. Menhaden body lipids: Details of fatty acids in lipids from an untapped food resource. J. Sci. Food Agric. 27:1132-1136.

3. Ackman, R.G., W.M.N. Ratnayake, and C.A. Eaton. 1981. Considerations of fatty acids in menhaden from the northern limits of the species. Proc. N.S. Inst. Sci. 31:207-215.

4. AOCS. 1983. Flavor panel evaluation of vegetable oils. Am. Oil Chem. Soc. recommended practice No. CG 2-83; 4 p. Part of: Sampling and Analysis of Commercial Fats and Oils. In Official and Tentative Methods of the Am. Oil Chem. Soc., Am. Oil Chem. Soc., Champaign, IL.

5. ASTM Committee E-18. 1968. "Manual on Sensory Testing Methods", STP 434. Am. Soc. for Testing and Materials, Philadelphia, PA.

6. ASTM Committee E-18. 1981. "Guidelines for the selection and training of sensory panel members", STP 758. Am. Soc. for Testing and Materials, Philadelphia, PA.

7. Chang, S.S. 1972. Organoleptic evaluation of edible fats and oils. Unpublished data. Dept. Food Sci. Rutgers Univ., New Brunswick, NJ.

8. Dubrow, D., M. Hale, and A. Bimbo. 1976. Seasonal variations in chemical composition and protein quality of menhaden. Mar. Fish. Rev. 38(9):12-16.

9. Dyerberg, J. 1982. Observations on populations in Greenland and Denmark. In S.M. Barlow and M.E. Stansby (editors), Nutritional Evaluation of Long-chain Fatty Acids in Fish Oils, p. 245-261. Acad. Press, Inc. Ltd., Lond.

10. Goetzl, E.J., M.Y.S. Wong, D.G. Payan, T. Chernov-Rogan, W.C. Pickett, and V.A. Blake. 1986. Effects of eicosapentaenoic acid on immune responses and inflammation in humans. In A.P. Simopoulos, R.R. Kifer, and R.E. Martin (editors), Health Effects of Polyunsaturated Fatty acids in Seafoods, p. 239-246. Academic Press, Inc., Orlando, FL.

11. Harris, W.S. 1985. Health effects of omega-3 fatty acids. Contemp. Nutr. (General Mills) 10(8), 2 p.

12. Heath, H.B., and G. Reineccius. 1986. Flavorists and flavor creation. In Flavor Chemistry and Technology, p. 346-353. Van Nostrand Reinhold Co., New York.

13. Hirai, A., T. Hamazaki, T. Terano, T. Nishikawa, Y. Tamura, A. Kumagai, and J. Sajiki. 1980. Eicosapentaenoic acid and platelet function in Japanese. Lancet ii:1132-1133.

14. IFT. 1981. Sensory evaluation guide for testing food and beverage products. Sensory Evaluation Div., Inst. of Food Technologists. Food Technol. 35(11):50-59.

15. Jackson, H.W. 1981. Techniques for flavor and odor evaluation of soy oil. J. Am. Oil Chem. Soc. 58(3):227-231.

16. Jackson, H.W. 1985. Oil flavor quality assessment. In T.H. Applewhite (editor), Bailey's Industrial Oil and Fat Products, Vol. 3, p. 243-272. John Wiley & Sons, New York.

17. Joseph, J.D. 1985. Fatty acid composition of commercial menhaden, Brevoortia spp., oils, 1982 and 1983. Mar. Fish. Rev. 47(3):30-37.

18. Kinsella, J.E. 1986. Food components with potential therapeutic benefits: The n-3 polyunsaturated fatty acids of fish oils. Food Technol. 40(2):89-97, 146.

19. Kobayashi, S., A. Hirai, T. Terano, T. Hamazaki, Y. Tamura, and A. Kumagai. 1981. Reduction in blood viscosity by eicosapentaenoic acid. Lancet ii:197.

20. Lawson, H.W. 1985. Research and development. In Standards for Fats and Oils, p. 207-219. AVI Publishing Co., Westport, CT.

21. Leaf, A. 1986. Introduction: Approaches to prevention. In A.P. Simopoulas, R.R. Kifer, and R.E. Martin (editors), Health Effects of Polyunsaturated Fatty Acids in Seafoods, p. 81-86. Academic Press, Inc., Orlando, FL.

22. Mecredy, J.M., J.C. Sonnemann, and S.J. Lehmann. 1974. Sensory profiling of beer by a modified QDA method. Food Technol. 28(11)36-41.

23. Mounts, T.L. and K. Warner. 1980. Evaluation of finished oil quality. In Handbook of Soy Processing and Utilization. p. 245-266. Am. Soybean Assoc. and Am. Oil Chem. Soc., Champaign, IL.

24. Phillipson, B.E., D.W. Rothrock, W.E. Connor, W.S. Harris, and D.R. Illingworth. 1985. Reduction of plasma lipids, lipoproteins, and apoproteins by dietary fish oils in patients with hypertriglyceridemia. N. Engl. J. Med. 312:1210-1216.

25. Powers, J.J. 1982. "Materials and Training Exercises for Workshop on Sensory Analysis", South African Assoc. for Food Sci. and Technol., Johannesburg, 52 pp.

26. Powers, J.J. 1984. Current practices and application of descriptive methods. In J.R. Piggott (editor), Sensory Analysis of Foods, p. 179-242. Elsevier Applied Science Publishers, Lond.

27. Sanders, T.A.B. 1985. The importance of eicosapentaenoic and docosahexaenoic acids. In F.B. Padley and J. Podmore in collaboration with J.P. Burn, R. Burt, and B.W. Nicols (editors), The Role of Fats in Human Nutrition, p. 101-116. Ellis Horwood, Chichester, England.

28. Seaborn, G.T., J.D. Joseph, and P.E. Bauersfeld. 1986. Fish oil research aids fishing industry and consumers. Proc. Eleventh Annual Trop. and Subtrop. Fish. Technol. Conf. of the Americas held January 13-16, Tampa, FL. p. 263-274.

29. Simopoulos, A.P. 1986. Historical perspective, conference conclusions and recommendations, and actions by federal agencies. In A.P. Simopoulos, R.R. Kifer, and R.E. Martin (editors), Health Effects of Polyunsaturated Fatty Acids in Seafoods. p. 3-32. Academic Press, Inc., Orlando, FL.

30. Stansby, M.E. 1984. Fish or fish oil in the diet and heart attacks. Mar. Fish. Rev. 46(2):60-63.

31. Stansby, M.E. and G. Jellinek. 1965. Flavor and odor characteristics of fishery products with particular reference to early oxidative changes in menhaden oil. In R. Kreuzer (editor), The Technology of Fish Utilization, p. 171-176. Fishing News Books Ltd., London.

32. Stone, H., J. Sidel, S. Oliver, A. Woolsey, and R.C. Singleton. 1974. Sensory evaluation by quantitative descriptive analysis. Food Technol. 28(11):24-34.

33. Waltking, A.E. 1982. Progress report of the AOCS flavor nomenclature and standards committee. J. Am. Oil Chem. Soc. 59(2):16A-20A.

34. Warner, K. 1985. Sensory evaluation of flavor quality of oils. In D.B. Min and T.H. Smouse (editors), Flavor Chemistry of Fats and Oils, p. 207-221. Am. Oil Chem. Soc., Champaign, IL.

35. Zook, K., and C. Wessman. 1977. The selection and use of judges for descriptive panels. Food Technol. 31(11):56-61.

ELECTROPHORETIC IDENTIFICATION OF SHRIMP USING VARIOUS PROTEIN EXTRACTION SYSTEMS

Haejung An, Maurice R. Marshall, W. Steven Otwell
and Cheng-i Wei
Food Science and Human Nutrition Department
University of Florida, Gainesville, FL 32611

INTRODUCTION

Willful or unintentional adulteration by substituting lower quality and/or lower priced seafood products for higher priced products has led to an increasing demand for identifying species of fish or other seafood in the marketplace. However, only a limited number of methods, such as starch gel-zone electrophoresis, acrylamide disc electrophoresis, thin layer polyacrylamide gel isoelectric focusing, and cellulose acetate strip, has been accepted as official methods to differentiate species of seafood or seafood products (AOAC, 1984). In most test methods, the highly water-soluble sarcoplasmic proteins have been used for species identification (Lundstrom, 1981 and 1983; Hamilton, 1982). Protein solubility is affected by several factors, such as pH, ionic strength, temperature or the use of organic solvents (Thakker and Grady, 1984). Thus, by modifying the extraction conditions, species-related proteins can be more effectively extracted and used for species identification.

In addition, the solubility of sarcoplasmic proteins were affected significantly after heat treatment. The number of water-extractable proteins, as demonstrated by comparing the electrophoretic gels of heated to non-heated samples, was reduced after heat treatment (Dowdie and Biede, 1983; Lee et al., 1974). The solubility of heat-denatured proteins can be improved by using various solvent systems such as urea and SDS (Tanford, 1968; Thakker and Grady, 1984).

This study was carried out to compare the relative effectiveness of various solvent systems in extracting proteins for use in gel electrophoresis. Five solvent systems were used to extract proteins from three species of raw and cooked shrimp. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was applied to separate sarcoplasmic proteins and to differentiate and identify the species from raw and cooked shrimp. The best

protein extraction system was used with SDS-PAGE to check applicability in identifying shrimp species and their relative contents in fabricated samples by a blind study.

MATERIALS AND METHODS

Three shrimp samples, pink (Penaeus duorarum) (Key West, FL), white (Penaeus setiferus) (Jacksonville, FL), and rock shrimp (Sicyonia breviostris) (Port Canaveral, FL) stored at -33°C were thawed under tap water, peeled, and deveined. For the cooking treatment, shrimp muscle was placed in boiling water for 5 min.

Five solvents were compared for their effectiveness in extracting sarcoplasmic proteins from whole raw and cooked shrimp. They were water, water homogenate adjusted to pH 8.0 with 0.1 N NaOH, 0.1 M NaCl, 1% SDS (w/v), and 8 M urea. All five solvents contained 0.1 mM phenylmethylsulfonyl fluoride (PMSF), 10 mM EDTA and 0.01% (w/v) sodium azide. Shrimp samples were chopped, mixed with each solvent at a ratio of 1:3 (w/v), and homogenized at room temperature for 1 min at setting 5.5 using a Polytron (Brinkmann Instrument, Westbury, NY). After the samples were centrifuged at 48,000x g for 20 min at 20°C, the supernatants were collected, and the protein contents determined by the Lowry method (Lowry et al.,1951).

The percent yield of protein was determined for the supernatants of water, 1% SDS or 8 M urea extract. The percent yield was calculated as the ratio of total protein content in the supernatant to that in the homogenate.

SDS-PAGE was performed according to the modified procedure of Laemmli (1970) and O'Farrell (1975) using a Protean II (vertical slab) unit (Bio-Rad, Richmond, CA). Slab gels (1.5 mm thick) of 10.4 and 3.1% (w/v) total acrylamide were prepared to serve as a running and a stacking gel, respectively. Proteins at 35 µg were analyzed on SDS-PAGE. A constant current of 15 mA/slab was applied initially and increased to 30 mA/slab when the marker front reached the running gel. Following electrophoresis, the proteins were stained with Coomassie Brilliant Blue R-250 (Bio-Rad). Molecular weights of the protein bands were determined according to the method of Weber and Osborn (1969) and Weber et al. (1972) using a low molecular weight (MW) protein kit (Pharmacia, Piscataway, NJ).

A blind study was performed on fabricated mixtures of pink and rock shrimp. Raw pink and rock shrimp were individually chopped and then mixed in various ratios (w/w)

unknown to the investigators. For heat (boiled) treatment, the chopped shrimp mixture was boiled for 5 min in a 2 oz Whirl-Pak bag (Fisher Scientific). Shrimp proteins were extracted with water and SDS for the raw and heat-treated samples, respectively. After SDS-PAGE, electrophoretic protein patterns were compared to reference samples of pink and rock shrimp at various ratios. The percent content of rock shrimp in the unknown sample was determined by visual observation of the intensity of the species-specific protein bands.

RESULTS AND DISCUSSION

Comparison of the five protein extraction solvents

The five protein extraction systems used in this study can be categorized into two general groups: non-denaturing and denaturing agents. Characteristic SDS-PAGE patterns were obtained for all the extracts of the raw and boiled shrimp. Only the patterns of white shrimp were shown in Figures 1 and 2.

For all three raw samples, a low ionic strength salt solution (0.1 M NaCl) or the adjustment of the homogenates to pH 8.0 did not increase protein extractability (data not shown) or change the electrophoretic patterns when compared to the water extract (Fig. 1). When using two common protein-denaturing solubilizers, SDS and urea, more protein bands were observed (Fig. 1).

Low percent protein recovery was noted for water extract (Table 1). However, water was the most suitable solvent to extract proteins from raw shrimp for electrophoretic identification. Specific patterns were obtained with water extract for each species, and distinct bands could be recognized and used for species differentiation. The highest percent recovery was noted for 1% SDS (Table 1) which has been often used to solubilize membrane proteins.

Heat treatment of shrimp prior to water extraction resulted in a drastic change in SDS-PAGE patterns; the number of bands reduced greatly (Fig.2). High temperature causes both denaturation and dissociation of subunits (Dowdie and Biede, 1983; Lee et al., 1974). Thus, water was not effective in extracting proteins from cooked shrimp. A protein yield of only 8-11% was obtained (Table 1). This problem was alleviated by using SDS or urea which enhanced

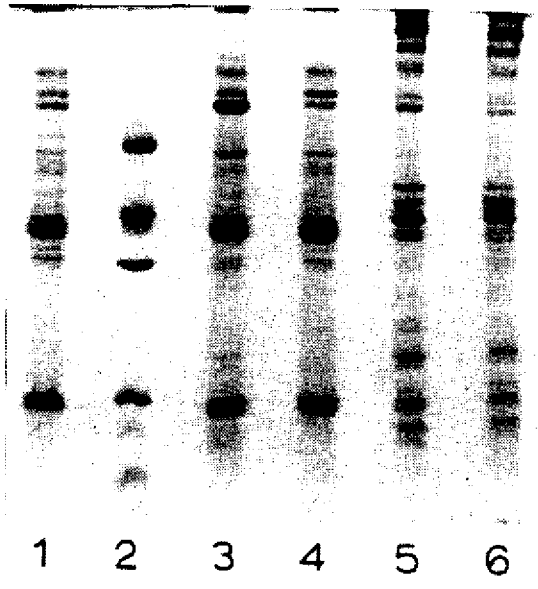


Figure 1. SDS-PAGE patterns of raw white shrimp extracted with water (1), water homogenates with pH adjustment to pH 8.0 (3), 0.1 M NaCl (4), 1% SDS (5), and 8 M urea (6). Protein standards are also included (2).

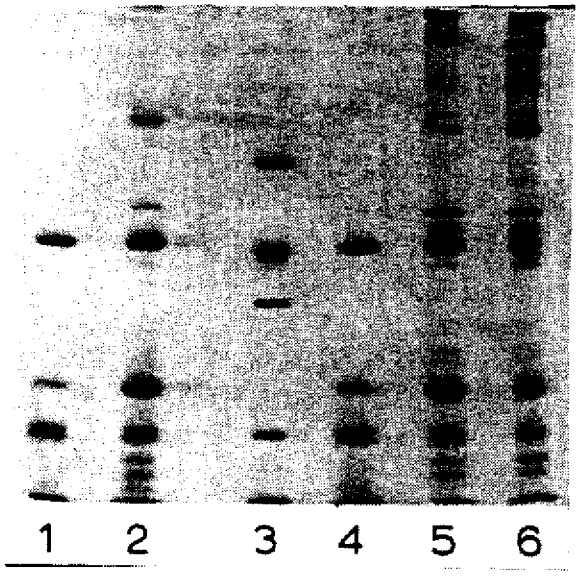


Figure 2. SDS-PAGE patterns of cooked white shrimp extracted with water (1), water homogenates with pH adjustment to pH 8.0 (2), 0.1 M NaCl (4), 1% SDS (5), and 8 M urea (6). Protein standards are also included (3).

protein extractability (Table 1). Additional proteins were extracted with these solvents, and provided greater information for species identification (Fig.2). Since the patterns of these two extracts were similar (Fig. 2), SDS was chosen to extract proteins from cooked shrimp for genus identification.

Species-specificity of the SDS-PAGE pattern

Characteristic gel patterns were obtained for all shrimp samples. For ease of comparison, the gel was divided into three equal segments, A (top), B (middle) and C (bottom).

The water extracts of the raw samples showed the most diversity between species (Fig. 3a). Four major bands with MW of 88.3, 81.5, 78.6 and 74.1 kD were found in section A of pink shrimp. For white shrimp, there were five bands in this section. Their MW were 88.6, 81.7, 79.9, 77.7 and 75.7 kD. The rock shrimp had six bands in section A with MW of 88.9, 82.1, 80.6, 79.5, 78.1 and 76.0 kD. A species-specific minor band was also found between section A and B. Its MW was 66.4, 65.7, and 63.8 kD for pink, white and rock shrimp, respectively.

Unlike those found in section A, the protein bands in section B showed no significant species difference. Two major thick bands were detected in section C with MW 19.1 and 18.3 kD for pink and white shrimps or 18.5 and 17.7 kD for rock shrimp.

Different banding patterns were observed for the SDS extracts of raw shrimp (Fig. 3b). Differences were found in section A and B for pink and white shrimp. The 78.6 kD band was only found in section A of pink shrimp. Two bands (80.5 and 76.7 kD) in section A and one band (41.1 kD) in section B were only present in white shrimp. For rock shrimp, the banding pattern at section C was obviously different from that of the other two shrimps. Three unique bands of 26.5, 23.7 and 21.5 kD were found in rock shrimp. The 58.4 kD band in section B was also unique for rock shrimp. The urea extracts of the three shrimps showed similar patterns as the SDS extracts.

In contrast to the raw samples, the species-specificity of the water extracts of boiled shrimp could not be easily detected. Only a few bands were detected and they were basically similar for all three species (Fig. 4a). Improved gel patterns were found for the SDS extracts (Fig. 4b). Pink shrimp was found to have similar patterns as white shrimp, possibly because they belong to the same genus. Two

characteristic thick bands (79.1 and 75.9 kD) found in section A of the rock shrimp extract were not as apparent in white or pink shrimp. The bands found in section C of rock shrimp could also be used for identification. Some variations were found in the rock shrimp as compared to the other two shrimp. The bands with MW of 27.7-31.3 kD were not present in rock shrimp, while three new bands of 21.6, 18.7 and 17.8kD were detected. A new band with a MW of less than 27.7 kD was also found in rock shrimp. Again, as in the case of raw samples, the urea extracts of boiled shrimp revealed the same patterns as the SDS extracts. Distinct differences in banding patterns could only be differentiated between white or pink and rock shrimp.

Comparison of SDS-PAGE patterns of mixed shrimp protein extracts

Characteristic banding patterns of each species were used in identifying the existence of that specific species in shrimp mixtures. For example, the 79.9kD band of water extracted raw white shrimp (Fig. 3a) could serve as an important indicator for the presence of white shrimp in a white-rock shrimp mixture. The minor band specific for each species (66.4, 65.7 or 63.8 kD for pink, white or rock shrimp, respectively) provided another useful indicator for species identification. The minor band of the pink and rock shrimp is resolved enough to distinguish these species (Fig. 3a). For example, the 58.3 kD band of the rock shrimp (Fig. 3a) provides additional information on the presence of rock shrimp in a mixture. This sharp band is intense and can be easily recognized despite the presence of other bands with similar MW's (56.5 and 57.0 kD for the pink and white shrimp, respectively).

The SDS extracts of boiled shrimp demonstrated a genus-specific pattern (Fig. 4a). The presence of two characteristic thick bands (79.1 and 75.9 kD) in section A, the 26.7 kD band in section B, and the 21.6 and 17.8 kD bands in section C would indicate the presence of rock shrimp in a mixture of sample. The 26.7 kD band is characterized by its intensity in mixture samples. The 21.6 and 17.8 kD bands are unique because they are only found in the SDS extract of rock shrimp.

Blind Study

Pink and rock shrimps of different genus were used in the blind study. By using SDS-PAGE, various extraction systems, and the information obtained in the previous studies, all unknown samples were correctly identified. The adulterated samples were detected at a maximum of 10%

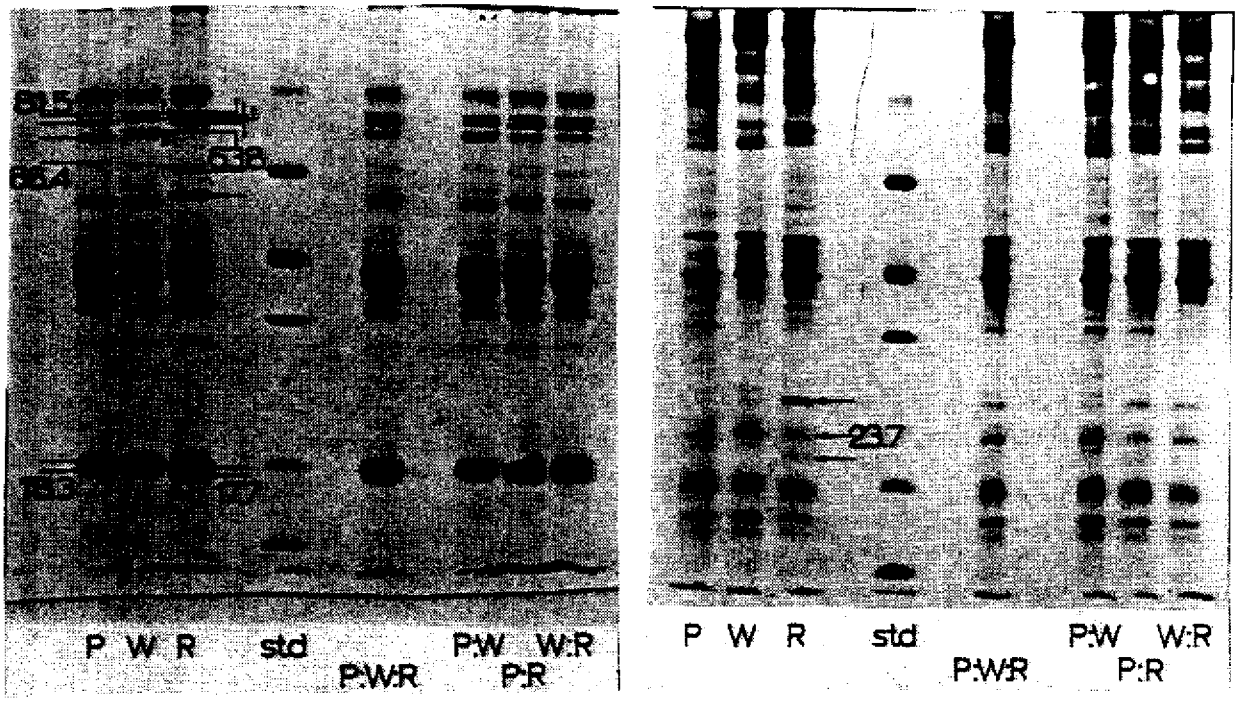


Figure 3. SDS-PAGE patterns of water extracts (a, left) and SDS extracts (b, right) of raw pink, white and rock shrimps and their mixtures (1:1 protein ratio). The numerical values are molecular weights of the specific proteins. P, pink; W, white; R, rock shrimp.

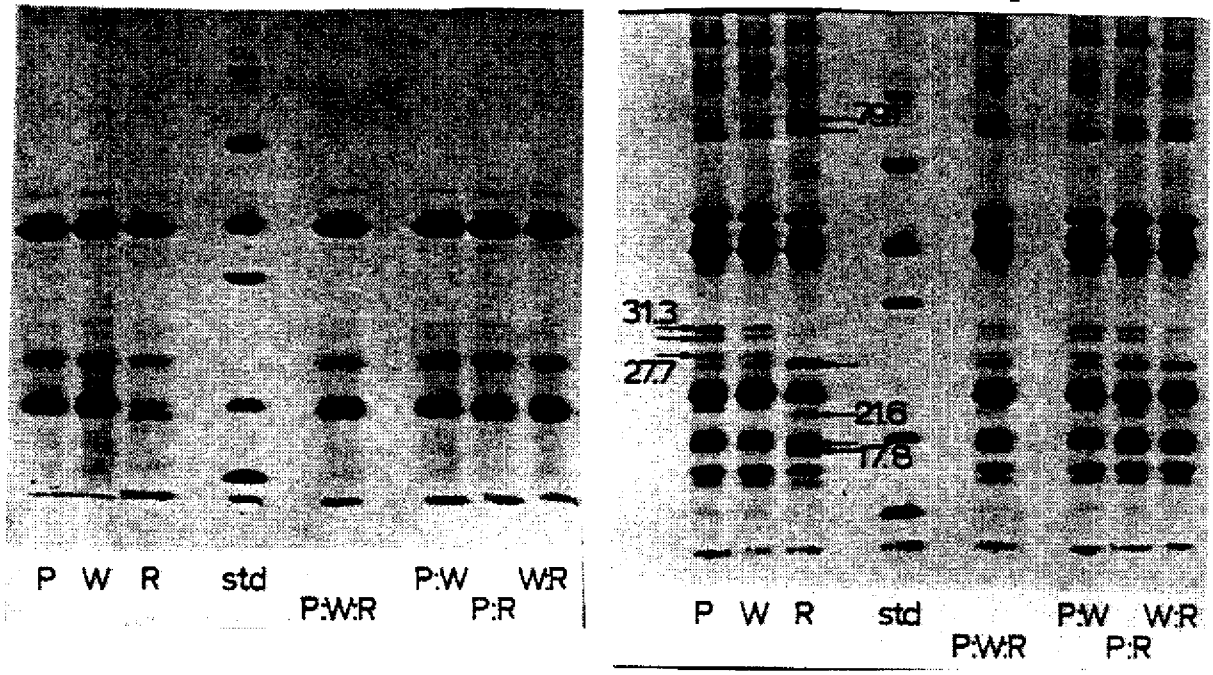


Figure 4. SDS-PAGE patterns of water extracts (a, left) and SDS extracts (b, right) of cooked pink, white and rock shrimps and their mixtures (1:1 protein ratio). The numerical values are molecular weights of the specific proteins. P, pink; W, white; R, rock shrimp.

difference from what was actually present for both raw and boiled shrimp samples (Table 2) as judged by direct inspection using the 20% increment ratio for the mixed extracts as a reference.

CONCLUSION

SDS-PAGE was an effective method to differentiate and identify species of raw or boiled shrimp. Each species of raw shrimp demonstrated a characteristic electrophoretic pattern when extracted with water. Heat-treatment of shrimp in boiling water for five min caused a loss of water-extractable proteins. No species-specific gel pattern was obtained for these samples. SDS greatly increased protein extractability and the number of proteins on the gel, thus making the identification of the genus of heat-treated shrimp possible. The technique to use SDS-PAGE with appropriate protein extraction systems to identify shrimp species was found to be very effective in a blind study. A maximum error of 10% was encountered to detect the actual amount of the individual shrimp in a rock-pink shrimp mixture.

ACKNOWLEDGEMENT

This work is the result of research sponsored by NOAA, Office of Sea Grant, Department of Commerce, under Grant No. IR-85-6.

REFERENCES

AOAC. 1984. "Official Method of Analysis" 14th ed. Association of Official Analytical Chemists, Washington, DC.

Dowdie, O.G. and Biede S.L. 1983. Influence of processing temperature on the distribution of tissue and water-soluble proteins in blue crabs (Callinectes Sapidus). J. Food Sci. 48: 804.

Hamilton, W.D. 1982. Fish species identification by thin layer agarose isoelectric focusing and densitometric scanning. J. Assoc. Off. Anal. Chem. 65: 119.

Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680.

Lee, Y.B., Rickansrud, D.A., Hagberg, E.C., and Brisky, E.J. 1974. Application of SDS-acrylamide gel electrophoresis for determination of the maximum temperature to which bovine muscle have been cooked. J. Food Sci. 39: 428.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 256.

Lundstrom, R.C. 1981. Rapid fish species identification by agarose isoelectric focusing of sarcoplasmic proteins. J. Assoc. Off. Anal. Chem. 64: 38.

Lundstrom, R.C. 1983. Fish species identification by agarose gel isoelectric focusing: Collaborative study. J. Assoc. Off. Anal. Chem. 66: 123.

O'Farrell, P.H. 1975. High resolution two-demensional electrophoresis of proteins. J. Biol. Chem. 250: 4007.

Tanford, C. 1968. Protein denaturation. Adv. Prot. Chem. 23: 121.

Thakker, K.D. and Grady, L.T. 1984. Solubility. Chap. 2. In "Food Analysis," D.W. Gruenwedel and J.R. Whitaker (Eds.), Marcel Dekker, Inc., New York, NY.

Weber, K. and Osborn, M. 1969. The reliability of molecular determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. J. Biol. Chem. 244: 4406.

Weber, K., Pringle, J.R., and Osborn, M. 1972. Measurement of molecular weights by electrophoresis on SDS-acrylamide gel. Meth. Enzymol. 26: 3.

FIGURE LEGENDS

Figure 1. SDS-PAGE patterns of raw white shrimp extracted with water (1), water homogenates with pH adjustment to 8.0 (3), 0.1 M NaCl (4), 1% SDS (5), and 8 M urea (6). Protein standards are also included (2).

Figure 2. SDS-PAGE patterns of cooked white shrimp extracted with water (1), water homogenates with pH adjustment to 8.0 (2), 0.1 M NaCl (4), 1% SDS (5), and 8 M urea (6). Protein standards are also included (3).

Figure 3. SDS-PAGE patterns of water (a) and SDS (b) extracts of raw pink, white and rock shrimps and their mixtures (1:1 protein ratio). The numerical values are molecular weights of the specific proteins. P: pink, W: white and R: rock shrimp.

Figure 4. SDS-PAGE patterns of water (a) and SDS (b) extracts of cooked pink, white and rock shrimps and their mixtures (1:1 protein ratio). The numerical values are molecular weights of the specific proteins. P: pink, W: white and R: rock shrimp.

SPECIES IDENTIFICATION BY ISOELECTRIC FOCUSING

Kellie Haynes
NOAA/NMFS/National Seafood Inspection Laboratory
P. O. Drawer 1207, Pascagoula, MS 39568-1207

Seafood is rapidly becoming more important in the American diet. In 1986 the per capita consumption reached a record 14.7 pounds and is expected to increase three percent annually (Table 1). With an increase in seafood also comes an increase in related problems. Species substitution, both intentional and unintentional, is one of the many problems. In addition to causing public health risks, species substitution is considered economic fraud. When lower economically valued species of fish are substituted for those of higher value, such as a grouper or rockfish being substituted for red snapper, consumer confidence is lowered. Many species carry inherent health risks; when these fishes are substituted a danger of illness and even death is introduced. Because of the many risks involved a great deal of people and public interest groups are becoming involved.

There are presently two bills in Congress dealing with seafood inspection. The National Marine Fisheries Service (NMFS) is presently working on a Model Seafood Surveillance Program mandated by Congress that would provide a plan for improved inspection and certification of seafood products in the U.S. Both the bills and the Model Program are intended to address all consumer hazards in the consumption of fishery products including the economic fraud issues. More specifically related to the problem of species substitution, is a joint project being done by NMFS and Food and Drug Administration (FDA) to develop standardized market name nomenclature in the interstate marketing of seafood products.

The seafood industry is not the only group to be interested in species substitution. According to Food Chemical News, USDA is instituting a program to monitor for undeclared species in cooked meat and poultry products. This indicates the problem of species substitution is not limited to the seafood industry.

BACKGROUND

There are presently four (4) methods of species identification approved by the "Official Methods of Analysis of the Association ofOfficial Analytical Chemists (AOAC) (1984 ed)", they are 1) Starch Gel-Zone electrophoresis, 2) Acrylamide Disc Electrophoresis, 3) Cellulose Acetate Strip method, and 4) Polyacrylamide Gel Isoelectric Focusing. Of the four, isoelectric focusing by polyacrylamide gel is by far the most desirable. The other three have demonstrated a problem of reproducability. With the isoelectric focusing method, it is not necessary to analyze a known sample and an unknown sample

simultaneously. A "bank" of known samples can be prepared and photographed for later comparison. This saves a great deal of time and effort.

The Isoelectric Focusing method utilizes an LKB 2117 multiphor for electrophoresis (or equivalent apparatus). The sample is prepared by grinding 1 gram of fish with 5 milliliters of water then it is centrifuged for 20 minutes to extract the proteins. The protein solution

Table 1. U.S. Per Capita Consumption of Commercial Fish and Shellfish, 1960-86.

Year	Civilian resident population July 1	Per capita consumption			
		Fresh and frozen	Canned	Cured	Total
	Million persons	Pounds, edible meat			
1960	178.1	5.7	4.0	0.6	10.3
1970	201.9	6.9	4.5	.4	11.8
1971	204.9	6.7	4.3	.5	11.5
1972	207.5	7.1	4.9	.5	12.5
1973	109.6	7.4	5.0	.4	12.8
1974	211.6	6.9	4.7	.5	12.1
1975	213.8	7.5	4.3	.4	12.2
1976	215.9	8.2	4.2	.5	12.9
1977 (1)	218.1	7.7	4.6	.4	12.7
1978 (1)	220.5	8.1	5.0	.3	13.4
1979 (1)	223.0	7.8	4.8	.4	13.0
1980 (1)	225.6	8.0	4.5	.3	12.8
1981 (1)	227.7	7.8	4.8	.3	12.9
1982 (1)	229.9	7.7	4.3	.3	12.3
1983 (1)	232.0	8.0	4.8	.3	13.1
1984 (1)	234.8	8.5	4.9	.3	13.7
1985 (1)	237.0	X9.0	5.1	.3	14.4
1986 (1)	239.4	9.0	5.4	.3	X14.7

XRecord.

Note:--These consumption figures refer only to consumption of fish and shellfish entering commercial channels, and they do not include data on consumption of recreationally caught fish and shellfish which since 1970 is estimated to be between 3 to 4 pounds (edible meat) per person annually. The figures are calculated on the basis of raw edible meat, i.e. excluding bones, viscera, shells, etc. From 1970 through 1980, data were revised to reflect the results of the 1980 census.

is placed on the gel and a constant power supply of 30 watts is applied for 90 minutes. When the proteins reach their isoelectric point a biochemical "fingerprint" is produced. The gel then undergoes a series of fixing, staining then destaining steps. At the end of this process the gel can be photographed using a specialized type of Kodak paper which can be easily stored.

There is one drawback to using the acrylamide gel isoelectric focusing method. The acrylamide gels contain traces of the acrylamide monomer which is harmful if absorbed through the skin. Because of this the AOAC method includes a warning. In 1983, Ronald C. Lundstrum of NMFS Northeast Fisheries Center in Gloucester, MA conducted a collaborative study using Agarose gels. These gels demonstrated an average of 84% accuracy and have the added advantage of a 30 minute protein separation step. The results of the study were not good enough to recommend the method for official first action in the AOAC, however this appears to be a promising method for future study.

RESULTS

As part of its routine laboratory mission, in 1986 the National Seafood Inspection Laboratory (NSIL) analyzed a total of 18 samples by isoelectric focusing. Of these, 15 were labeled incorrectly. In other words, some other species had been substituted for the species indicated on the label. In 1987 a total of 16 samples were analyzed with 14 of these being substituted. Table two summarizes these results in percentage form.

Table 2. Total Samples Analyzed for Species Identification by Isoelectric Focusing.

Year	Percent Substituted	Percent not Substituted
1986	83%	17%
1987	87%	13%

Here at NSIL we have developed a "bank" of authentic samples that are often substituted. We have amassed approximately 50 species from all regions of the country. With regard to samples analyzed here, red drum and red snapper have been substituted for most often.

SUMMARY

Our analyses show a large percentage of fish are being substituted. This indicates that more testing and inspection is anticipated. Also with the hazards involved with the polyacrylamide gel a new and safer method of analysis needs to be developed.

AVOIDANCE COSTS DUE TO PUBLIC AWARENESS OF CIGUATERA FISH POISONINGS IN FLORIDA AND HAWAII

Myles Raizin and Malcolm Meaburn
National Marine Fisheries Service, NOAA, SEFC
Charleston Laboratory, P.O. Box 12607, Charleston, SC 29412

INTRODUCTION

Ciguatera fish poisoning is a common food-borne illness which affects the health of consumers and the economy of fisheries around the world. Ciguatera is caused by eating fish that contain a virulent, naturally occurring toxin or toxins usually referred to as ciguatoxin. The toxin(s) accumulate under generally unpredictable environmental conditions in the flesh of commercially important reef-associated fishes from tropical and sub-tropical waters. The overall ciguatera problem has been summarized by Ragelis (1984). Studies on the impact of ciguatera on public health and local economies in endemic areas of the Caribbean (De Motta et al. 1986) and Pacific (Lewis, 1986) serve to illustrate the severity of the problem. Ciguatera was reported to be the most common seafood-borne illness in the United States between 1975 and 1979 (MMWR, 1980), with Hawaii and Dade County, Florida, accounting for the majority of documented cases during this period. It has been estimated that the annual incidence of ciguatera in the Miami, Florida, area alone may be as high as five cases per ten thousand population (Lawrence et al, 1980).

There is currently no reliable and easily applied test for detecting ciguatoxic fish. Protective measures are based primarily on resource management procedures, e.g., prohibition of fishing in waters believed to harbor the progenitors of ciguatoxin. The continuing outbreaks of ciguatera in the United States demonstrate the ineffectiveness of this approach. The provision of a test to prevent toxic fish from reaching the marketplace would do much to reduce the incidence of ciguatera and to minimize negative consumer perceptions about the safety of seafood.

The objective of this study is to determine if consumer avoidance due to the threat of ciguatera poisoning had resulted in a significant economic cost to producers at the ex-vessel grouper and snapper markets in Hawaii and on the east coast of Florida. Avoidance costs in the context of this paper are the costs to producers that accrue due to consumer avoidance of ciguatoxic finfish. We chose the snapper/grouper fisheries of eastern Florida and Hawaii for analysis. However, the methodology to determine these costs may be used to estimate the effects of consumer avoidance at the ex-vessel market for any species of finfish or shellfish due to adverse health effects.

Shulstad and Stoevener (1978) and Schwartz and Strand (1981) presented methods to determine the impacts that public news items may have had on demand for pheasant hunting and shellfish, respectively, by measuring changes in the intensity and scope of information relative to actual levels of avoidance in the marketplace. In our analyses, we were concerned only with the timing of the release of information, not its intensity and scope. The basic hypothesis tested in this study was: when the public is aware of a risk to its health from eating grouper or snapper which may contain ciguatoxin, it will avoid consuming these fish at prices which prevailed when the public was unaware of the risk.

We begin with a discussion of risk profiles, biased risk perception, and avoidance costs. While risk is not directly used in estimating avoidance costs it is the essential concept that determines the magnitude of these costs. This section is followed by the model estimation, including methodology and interpretation of the results, which in turn is followed by our summary and conclusions.

RISK PROFILES

Risk is a mathematically derived concept that uses probabilities. In the example of tossing a coin, the total probability is 1.0 and the probability of either heads or tails is 0.5. With these probabilities, we can quantify the coin toss and relate it to economic choice by determining expected gain or loss from the toss. If we assigned a prize of $100 for correctly guessing the coin toss, we can derive an expected gain of $50 (100 x 0.5) for each toss of the coin.

Let us introduce the human element to our game. Suppose the lottery master asked for the highest amount of money that each person in the audience would be willing to wager on the outcome of one toss to win the grand prize of $100. Let us further assume that secret bids are taken and that only the highest bidder will be able to play the game. It would be folly to bid over $50 for an infinite number of tosses, but in the case of a single toss our game will reveal to us three different risk profiles. Those who bid under $50 are considered to be risk averters. The risk averse person will never bid more than his expected gain. In fact, the risk averter might be willing to pay up to $50 not to play the game. This would allow him to transfer the risk, the basis for insurance. Alternatively, those who bid over $50 are considered risk takers. These individuals will assume risk rather than pass it on. Finally, we have the risk neutral individuals who bid exactly $50. These individuals neither assume nor transfer risk.

The risk profile as discussed above is influenced by individual choice and may be derived from one's utility function. An individual may be both a risk taker when smoking cigarettes and risk averter when buckling up his seat belt or vice-versa.[1]

BIASED RISK PERCEPTION

The perception of health risk and true health risk may vary depending upon toxicity of the transmitting agent, epidemiological data and projections, and media exposure (information). It was once thought that large populations would tend on the average to be risk neutral in regard to their perceptions of health risk, i.e., the mean risk profile of large population would tend to view the expected value of the gain from avoiding disease as a maximizing level of utility of their health.

Recent psychological studies on biased perceptions of risk, such as that of Lichtenstein et al. (1978), indicate that individuals tend to overestimate risks of relatively infrequent events, e.g., death from botulism, and underestimate risks of more frequent events, e.g., death from heart disease. Also, individuals have a tendency to overestimate certain risks characterized by wide media exposure, memorability, or uniqueness of various events.

Tversky and Kahneman (1981) conducted a study on preference reversal based on paired lotteries with unique expected values. They found that participants in the study when given a choice would prefer not to gamble on probable losses and accept sure gains even though the gains were quite less than the expected value of the lotteries. This study revealed that even though perfect information is available, the wording of the information may effect choice.

The theory of cognitive dissonance as advanced by Akerlof and Dickens (1982) may be the most important of the several theories concerning risk perception and economic consequences. It is based on the concepts that individuals not only have preferences with respect to the states of the world, but also have preferences regarding the specific beliefs they hold, even if these beliefs contradict available information. Individuals, after making a decision systematically, held stronger beliefs than those who were on the verge of making a choice even when there has been no new information. Akerlof and Dickens (1982) cite examples of benzene workers who denied they were working with dangerous chemicals and nuclear plant workers who were given badges but failed to wear them to measure radiation exposure. Cognitive dissonance, in part, explains why individuals' revealed preference in the market may not reflect true health risk and avoidance costs may include a risk aversion premium depending upon individual beliefs, regardless of the information available to them.

A problem in calculating a risk aversion premium for the threat of ciguatera poisoning is that the health risk associated with consuming snapper and grouper is not known. For example, it is assumed that for Canadians, only one case of ciguatera poisoning in twenty-five is reported (Hanschild and Bryan, 1980) and by assumption, only one case in ten during 1974 to 1976 was reported to the Dade county (Miami) Department of Public Health (Lawrence et al., 1980). We cannot expect

the consuming public to make informed decisions regarding their personal safety if true health risk is not known. Therefore, if we define a risk aversion premium as the difference between the cost of a health risk and avoidance cost, and health risk is underestimated, we may discover that the consuming public in aggregate has reacted in a risk neutral or even risk-taking manner vis-a-vis true health risk. Underestimation of health risk could thus have serious public health consequences. Alternatively, if health risk is overestimated, consumers may react in a more risk-averse manner which may have a severe economic impact on the fishing industry. Unfortunately, it is not possible to separate out that portion of the risk aversion premium that is caused by biased risk perception.

Economic theory tells us that the consumer will maximize his utility subject to his income and the prices of goods he desires. We assume that the consumer is acting in a rational manner vis-a-vis these parameters. When an element of health risk is introduced, the consumer's utility for a good may decrease if not diminish to zero. If his risk perception is biased, this may cause the consumer to act irrationally from the viewpoint of society even though he as an individual may believe he is maximizing his utility. This is especially true in light of the theory of cognitive dissonance where the consumer may derive satisfaction from not consuming.

AVOIDANCE COSTS

Swartz and Strand (1981) showed that avoidance costs vary with intensity of information. While this may be true, we recognize also the contributions of biased risk perception and cognitive dissonance to the magnitude of these costs. If we assume the general public to be risk averse in relation to their health, avoidance costs will certainly be larger than if they were risk neutral and such costs may be more influenced by information.

A classic example of avoidance costs associated with health risk is the recent pharmaceutical capsule scare. The actual risk of poisoning was quite small yet an entire industry was forced to alter its product form and packaging requirements. In fact, the entire food and pharmaceutical industries spent large sums of money to reduce avoidance costs due to biased risk perception. A further example of biased risk perception arose in Puerto Rico in 1981 when amberjack and barracuda were banned from sale due to several documented cases of ciguatera poisoning attributable to these species (San Juan Star Magazine, 1981). A poll of restaurateurs revealed that snapper and grouper sales decreased approximately 50% even though these species were not implicated in poisonings and consumers were well informed of this fact.

MODELS TO DETERMINE AVOIDANCE COSTS

1. The Snapper and Grouper Ex-Vessel Markets of Eastern Florida and Hawaii

Several species of snapper and grouper are landed with regularity on the east coast of Florida from Miami in the south to Jacksonville in the north. These fish are generally channeled through coastal dealers to wholesalers or retailers very quickly in either a fresh whole iced or fresh fillet basis (Prochaska and Keithly, 1984). In the absence of auction markets, prices generally are negotiated between buyer and seller ex-ante through forward contracting. A substantial portion of the snapper catch is marketed to the Midwest and Northeast while grouper is, for the most part, consumed on the East Coast and is likely to appear on the menus of popular restaurants in the coastal region.

In Hawaii, all snapper and grouper are consumed very close to their source. While bilateral exchange with negotiated pricing similar to Florida takes place in the Hawaiian Islands, the bottom fish market which includes snapper and grouper operates around an auction which is the basis for price determination (Pooley, 1978). Snapper has become economically important especially for the restaurant trade and for consumption during holiday periods. Alternatively, grouper is viewed to be of lesser quality and economically inferior to snapper. Therefore, the majority of grouper is consumed at home.

2. Methodology

Avoidance costs in these ex-vessel markets were estimated by observing demand shifts in the periods when the public was informed of articles of ciguatera poisoning incidents. Articles from the Miami Herald and the Miami News for the years 1977 to 1985 (Table 1), and the Honolulu-Advertiser and Honolulu Star Bulletin for the years 1977 to 1984 (Table 2), were used as proxies for public awareness of ciguatera poisonings.

Inverse demand curves were estimated using monthly data. These demand curves included a binary dummy variable with a value of 1 for the months when information on ciguatera poisoning was published and with a value of 0 for all other months. The dummy variable was lagged until its estimated coefficient was no longer significant. This technique is suggested by Johnston (1984) in cases where the number of lagged periods is indeterminate.

The model specifications for the two markets differed in that a partial equilibrium adjustment model (Wang, 1984) was used for the east coast of Florida while the traditional equilibrium model (Crutchfield, 1983) was used for Hawaii. The partial equilibrium model is used when prices do not adjust easily to changes in exogenous variables and are very much influenced by prices in previous periods. While this model

lacks simultaneity, the traditional model assumes that prices and quantities are instantly equilibrated. However, both models reflect the very different pricing structures of the two geographic areas selected for this study. Since price dependent models were used, by definition it was assumed that quantities were predetermined or fixed during each period. The estimation of inverse demand curves at the ex-vessel level is not unusual (Wang, 1984, and Crutchfield, 1983). It was also shown in a recent article by Squires (1986), that product supply curves for fishery producers tend to be extremely inelastic in the short run.

The equations were estimated using AR1, a maximum likelihood iterative technique (Beach and MacKinnon, 1978). This technique was used because ordinary least squares regression yielded first order serial correlation of the error term which produced biased estimators. Furthermore, the ordinary least squares technique will yield inconsistent estimators for an equation with a lagged dependent variable and autocorrelated disturbances (Johnston, 1984).

The models addressed the following hypotheses:

(1) Consumers significantly avoid eating snapper or grouper at prevailig prices when these species are implicated as poisoning agents.

(2) Consumers significantly avoid eating snapper or grouper at prevailing prices when these species are not implicated as the poisoning agent, but mentioned as possible transmitters of the toxin.

(3) Consumers significantly avoid eating snapper or grouper at prevailing prices when these species are not implicated as the poisoning agent and are not mentioned as possible transmitters of the toxin.

A sub-hypothesis for each of the above was also addressed as follows:

> There is a significant and measurable lag effect associated with this avoidance.

Table 1 gives the dates of newspaper articles for Florida and the species implicated or mentioned. Seven of the sixteen newspaper articles identified grouper as the species implicated in poisonings, while only three identified snapper. Other implicated species included barracuda and amberjack.

Table 2 provides the dates for Hawaii and the species implicated. Note that grouper was not implicated nor specifically mentioned in these newspaper articles and snapper was implicated only one time.

Table 1. Articles reporting ciguatera involvement on the east coast of Florida.[1]

Month/Year	Species Implicated	Snapper or Grouper Mentioned
11/77	Grouper	Snapper
12/77	Non-specific	Snapper, Grouper
2/78	Amberjack	Snapper, Grouper
5/78	Grouper	Snapper
6/79	Snapper	NA
11/79	Non-specific	Snapper, Grouper
1/80	Snapper	Grouper
5/80	Non-specific	Snapper, Grouper
7/80	Grouper	Snapper
3/82	Barracuda	Snapper, Grouper
5/82	Grouper	Snapper
10/84	Grouper	Snapper
4/85	Snapper, Grouper	NA
6/85	Grouper	Snapper
9/85	Barracuda	Snapper, Grouper

[1] Miami Herald and Miami News, 1977-1985.

Table 2. Articles reporting ciguatera involvement in Hawaii.[1]

Month/Year	Species Implicated	Snapper or Grouper Mentioned
9/78	Non-specific (small reef fish)	NA
10/78	Marquesian sardine	NA
11/78	Marquesian sardine	NA
3/79	Jack, Goatfish, Mullet Snapper	NA
4/79	Non-specific (reef fish)	NA
11/79	Non-specific	NA
6/80	Non-specific	NA
9/80	Bonito, non-specific	NA
12/80	Goatfish	NA
9/81	Wrasse	NA
10/83	Non-specific	NA
9/84	Surgeon fish	NA
12/84	Non-specific (reef fish)	NA

[1] Honolulu Star Bulletin and Honolulu Advertiser, 1977-1984.

According to a national survey of fish consumption conducted on behalf of the National Marine Fisheries Service in 1980-81, 60 percent of grouper was eaten away from home compared to 26 percent of snapper. This trend appears to reflect consumption patterns for fish landed in Florida. However, the trend is reversed in Hawaii where snapper is the fish of choice in restaurants (Pooley, 1978). Due to these different consumption patterns our measures of income are necessarily different. These characteristics are reflected in our models.

3. East Coast Florida Model

The following are functional form equations with expected signs used to estimate avoidance costs in the east coast of Florida. Table 3 indicates the variable specifications used in both models.

1) $GP_t = f(-GLBS_t, -SNPLBS_t, +Y_t, +GP_{(t-1)}, +S_t, -DG_t, \ldots -DG_{(t-n)}$

2) $GP_t = f(-GLBS_t, -SNPLBS_t, +Y_t, +GP_{(t-1)}, +S_t, -DDG_t, \ldots -DDG_{(t-n)}$

3) $SP_t = f(-SLBS_t, -GRPLBS_t, +RY_t, +SP_{(t-1)}, +S_t, -DS_t, \ldots -DS_{(t-n)}$

4) $SP_t = f(-SLBS_t, -GRPLBS_t, +RY_t, +SP_{(t-1)}, +S_t, -DDS_t, \ldots -DDS_{(t-n)}$

3.a. Estimated Equations[2/]

1) $GP = \underset{(-1.03)}{-5.94} - \underset{(-1.84)^*}{0.22}\, GLBS - \underset{(-4.84)^*}{0.13}\, SNPLBS + \underset{(4.55)^*}{1.06}\, Y$

$+\underset{(5.40)^*}{0.45}\, GP_{t-1} + \underset{(2.55)^*}{2.00}\, S - \underset{(2.76)^*}{3.06}\, DG - \underset{(-0.85)}{0.76}\, DG_{t-1}$

$R^2 = 0.84 \qquad F_{(7,99)} = 74.36^* \qquad h = 0.21$

RHO after 4 iterations = 0.12

2) $GP = \underset{(-1.31)}{-7.65} - \underset{(-2.16)^*}{0.26}\, GLBS - \underset{(-3.95)^*}{0.11}\, SNPLBS + \underset{(+4.34)^*}{1.02}\, Y$

$+\underset{(6.22)^*}{0.51}\, GP_{t-1} + \underset{(2.20)^*}{1.70}\, S - \underset{(-1.93)^*}{2.09}\, DDG - \underset{(-1.82)^*}{1.99}\, DGG_{t-1}$

$-\underset{(0.19)}{0.22}\, DGG_{t-2}$

$R^2 = 0.85 \qquad F_{(8,98)} = 65.40^* \qquad h = 0.22$

RHO after 4 iterations = -0.13

Table 3. Variable specifications[1/]

Variable	Specification
GP_t	Price of grouper in cents per pound in period t.
SP_t	Price of snapper in cents per pound in period t.
$GLBS_t$	Landings of grouper in thousands of pounds in period t.
$SLBS_t$	Landings of snapper in thousands of pounds in period t.
$SNPLBS_t$	Total landings of snapper in thousands of pounds in Florida in period t.
$GRPLBS_t$	Total landings of grouper in thousands of pounds in Florida in period t.
Y_t	Personal income spent in restaurants in thousands of dollars in period t.
RY_t	Personal income spent for fish in retail outlets in thousands of dollars.
DG_t	Dummy for avoidance costs in period t For ECF[2/], DG=1 when grouper mentioned, else=0 For Hawaii, DG=1 for ciguatera article, else=0
DGG_t	Dummy for avoidance costs in period t For ECF, DBG=1 when grouper indicated, else=0
DS_t	Dummy for avoidance costs in period t For ECF, DS=1 when snapper mentioned, else=0 For Hawaii, DG=1 for ciguatera article, else=0
DSS_t	Dummy for avoidance costs in period t For ECF, DSS=1 when snapper mentioned, elso=0
S_t	Seasonal dummy in period t For ECF, S=1 for February to June, else=0 For Hawaii, S=1 for December, else=0

1/ All income and price variables were deflated using the Consumer Price Index with 1967=0

2/ East Coast of Florida

3) $SP = \underset{(3.14)^*}{14.91} - \underset{(-3.64)^*}{0.91}\ SLBS - \underset{(-2.34)^*}{0.05}\ GRPLBS + \underset{(3.18)^*}{2.89}\ RY$

$$+ \underset{(9.53)}{0.70}\ SP_{t-1} + \underset{(1.17)}{1.04}\ S - \underset{(-.004)}{.005}\ DS$$

$R^2 = 0.88$ $\quad F_{(6,100)} = 112.58^*$ $\quad h = -0.16$

RHO after 5 iterations = 0.27

4) $SP = \underset{(3.12)^*}{14.80} - \underset{(-3.63)^*}{0.90}\ SLBS - \underset{(-2.37)^*}{0.05}\ GRPLBS + \underset{(3.21)^*}{2.85}\ RY$

$$+ \underset{(9.62)^*}{0.70}\ SP_{t-1} + \underset{(1.19)}{1.05}\ S - \underset{(-0.47)}{1.14}\ DSS$$

$\overline{R^2} = 0.88$ $\quad F_{(6,100)} = 112.87$ $\quad h = -0.16$

RHO after 4 iterations = -0.27

3.b. Interpretation of the Estimated Equations

All equations showed a high level of explanatory power of the exogenous variables on prices ($\overline{R^2}$), a statistically significant relationship (F), and an absence of serial correlation (h). All signs are consistent with economic theory. All variables are significant with the exceptions of the seasonality variables in Equations 3 and 4 and the final avoidance variables. All variables with the exception of the dummy variables represent the change in price caused by a one unit change in an external variable, e.g., in Equation 1, a one thousand pound increase in quantity demanded of grouper will cause the price of grouper to decrease 0.22 cents. However, the dummy variables represent the absolute change in price caused by seasonality or consumer avoidance. Table 4 shows the dollar amounts associated with consumer avoidance of grouper on the east coast of Florida. These were calculated by multiplying the estimated coefficient of the avoidance dummy by quantity demanded in the periods when newspaper articles appeared. The average decrease in price when grouper was only mentioned was approximately 10 cents[3/] for the months that the newspaper articles appeared while average prices decreased 7 cents for months when the species was implicated in a poisoning, followed by approximately 7 cents decrease in the first lagged month. Snapper, however, showed no significant decrease in price during periods of public awareness.

Table 4. Avoidance costs for grouper producers on the east coast of Florida (in 1986 dollars)

Month/Year	Grouper Mentioned	Grouper Implicated	Total Revenue
11/77		2432	39007
12/77	5087	3560	58596
2/78	4615		62566
5/78		7447	132817
6/78		6556	113811
11/79	5702		68737
1/80	7983		105344
5/80	8793		103642
7/80		5045	82738
8/80		2869	52026
3/82	7706		102150
5/82		6873	112620
6/82		3997	59523
10/84		1669	38148
11/84		1553	34407
4/85		5884	149628
5/85		4529	97722
6/85		4235	92252
7/85		4528	101228
9/85	3840		62520

Table 5. Avoidance costs for grouper producers in Hawaii (in 1986 dollars)

Month/Year	Other Species Mentioned or Implicated	Total Revenue
9/78	857	13429
10/78	2020	11627
11/78	3264	18657
12/78	2188	19696
3/79	1806	28299
4/79	2646	15129
5/79	745	6709
11/79	338	5294
12/79	698	6286
6/80	536	8403
7/80	733	6604
9/80	318	4986
10/80	198	1788
12/80	769	12043
1/81	441	3969
9/81	295	4621
10/81	885	7972
10/83	121	1897
11/83	1209	10884
9/84	641	10056
10/84	1266	11395
12/84	808	12674

4. Hawaiian Model

Functional form equations with expected signs used to estimate avoidance costs in Hawaii are:

1) $\ln GP = \ln(-GLBS_t, +RY_t, +S_t, -DG_t, \ldots\ldots\ldots -DG_{(t-n)})$

2) $\ln SP = \ln(-SLBS_t, +Y_t, +S_t, -DS_t, \ldots\ldots\ldots\ldots -DS_{(t-n)})$

4.a. Estimated equations

1) $GP = 1.69 - 0.13\ GLBS + 0.42\ RY + 0.04S - 0.06\ DG$

$(1.15)\quad (-5.80)^*\quad (2.38)^*\quad (0.81)\quad (-1.38)^*$

$-0.10\ DB_{t-1} + .05\ DG_{t-2}$

$(2.19)^*\qquad (1.11)$

$R^2 = 0.67 \qquad F_{(6,89)} = 30.74^* \qquad d = 1.92$

RHO after 2 iterations = 0.58

2) $SP = 3.76 - 0.28\ SLBS + 1.08\ Y + 0.10\ S - 0.01\ DS$

$(-0.64)\quad (-7.03)^*\quad (1,89)^*\quad (2.02)^*\quad (-0.37)$

$R^2 = 0.63 \qquad F_{(4,91)} = 39.18^* \qquad d = 1.96$

RHO after 2 iterations = 0.43

4.b. Interpretation of the Estimated Equation

Both equations showed an acceptable level of explanatory power of the exogenous variables on price ($\bar{R}^2$), a significant relationship (F), and an absence of serial correlation (d). With the exception of the dummy variables, the estimated coefficients for the double log form equation are interpreted as percentage changes in price caused by a one percent increase in an explanatory variable. The estimated coefficients of the dummy variables show us percentage changes in price caused by seasonal variation or public awareness of ciguatera poisonings, e.g., an estimated coefficient of -0.10 translates to a 10 percent decrease in price on average. Avoidance costs for grouper associated with ciguatera news releases are found in Table 5. These were calculated by estimating what prices would have been without decreases due to consumer avoidance. For example, to calculate a 10 percent decrease, prices must have been approximately 11.1% higher. The following equation was used to calculate a 10% decrease in price: $GP/0.9 - GP = x$, where x = price differential. These price differentials were then multiplied by quantity

demanded in each period. Unlike the Florida case, grouper was not mentioned or implicated during the eight year period, yet significant avoidance costs for producers of grouper occurred in the month the article appeared and the following month. This result is not surprising considering the aforementioned costs incurred by the pharmaceutical industry of the United States and the fishing industry of Puerto Rico for products not directly implicated in poisoning events. Decreases in snapper price were insignificant for months when the public was aware of poisoning events.

SUMMARY AND CONCLUSIONS

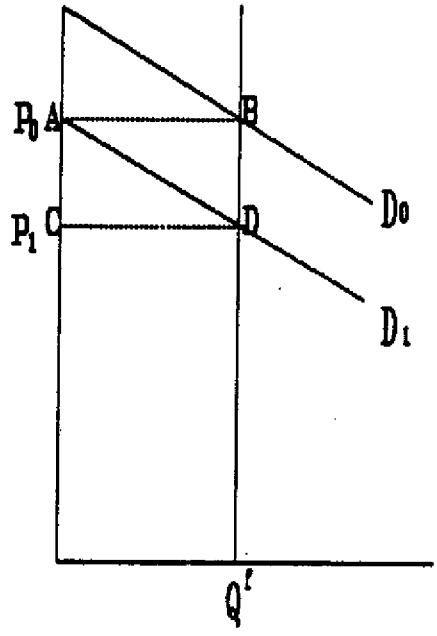
We conclude from our study that significant revenue losses associated with ciguatera do exist for grouper producers in Hawaii and on the east coast of Florida. During periods of public awareness consumers avoided purchasing grouper at prevailing prices, thus causing a decrease in the value of the product. While the results are conclusive, the reader should be aware that regression analysis yields only approximations. The true coefficient of our parameter may vary within the range of our confidence levels depending upon the standard error in relation to the estimated coefficient with the estimated coefficient being an average or mean value.

In regard to avoidance costs, we show in Figure 1 that producer willing-ness to pay to prevent avoidance costs can be estimated by measuring the decrease in producer rents (rectangle A-B-C-D) resulting from a downward shift of the demand curve. However, only the triangle A-B-D represents net welfare loss to society (social costs) while the triangle A-C-D represents the transfer of producer rents to consumer surplus. These avoidance costs could be drastically reduced if ciguatoxic fish were kept from the marketplace. This could be accomplished through an improved inspection system employing a reliable test for toxic fish.

If we were to use our results in a cost-benefit analysis, other factors beside welfare loss due to price changes may be included as social costs. For instance, days lost due to illness and medical costs may be viewed as social losses. In a cost benefit approach, the elimination of social costs are viewed as a benefit to society and the funding of projects are contingent upon the magnitude of these costs.

To truly assess the risk profile of the general population regarding the threat of ciguatera poisoning, we first need reliable epidemiological information regarding the incidence of ciguatera due to consumption of contaminated species of finfish. We believe that this information is vital to both the physical well-being of the consumer and the economic well-being of the affected segments of the fishing industry. We advise caution when making estimates of the incidence rate of ciguatera poisoning since overestimates may cause consumers to lose utility for the suspected species involved and may cause producers to experience diminished profitability.

Figure 1. The loss of producer surplus from a decrease in demand.



ACKNOWLEDGMENTS

We wish to thank Jim Waters, Jeff Cunningham, Stanley Wang, Sam Pooley, Joyce Nettleton, John Ward, John Vondruska, and Dale Squires for their help in constructing the models and their timely reviews of the draft manuscript.

FOOTNOTES

1/ For a more thorough discussion see Von Neumann and Morgenstern (1947).

2/ An asterisk indicates significance at the 0.10 level (one-tail t-test). The h-statistic is used to test for serial correlation in the presence of an exogenous lagged dependent variable (Johnston, 1984 and Wang, 1984).

3/ All price changes are expressed in 1986 cents.

LITERATURE CITED

Akerlof, George A. and William T. Dickens. 1982. The economic consequences of cognitive dissonance. American Economic Review 72:307-319.

Bagnis, R. 1968. Clinical aspects of ciguatera (fish poisoning) in Fresh Polynesia. Hawaiian Medical Journal 28:25-28.

Beach, Charles M. and James G. MacKinnon. "A Maximum Likelihood Procedure for Regression with Autocorrelated Errors." Econometrica. 46(1978). pp. 51-58.

Crutchfield, Steven R. 1983. Estimation of foreign willingness to pay United States fishery resources: Japanese Demand for Alaska Pollock. Land Economics 59:16-23.

Halstead, B.W. 1978. Poisonous and Venomous Marine Animals of the World. Darwin Press, Princeton, N.J. 1043 pp.

Hauschild, A.H. and F.L. Bryan. J. Food Protect. 43,435 (1980).

Henderson, J.M. and R.E. Quandt. 1980. Microeconomic Theory, a Mathematical Approach, McGraw-Hill, Inc., New York. pp 52-56.

Johnston, J. 1984. Econometric Methods. 3rd Ed. McGraw-Hill Book Company. New York. 568 p.

Lawrence, Dale N., Myriane B. Enriquez, Robert M. Lumish, and Antonio Maceo. 1980. Ciguatera Fish Poisoning in Miami. J. Amer. Med. Assoc., 244(3):254-258.

Lewis, Nancy D. 1986. Epidemiology and Impact of Ciguatera in the Pacific: A Review. Mar. Fish. Rev., 48(4):6-13.

Lichtenstein, Sarah, Paul Slovic, Baruch Fischhoff, Mark Layman, and Barbara Combs. 1978. Judged Frequency of Lethal Events. Journal of Experimental Psychology: Human Learning and Memory. 4:551-578.

Morbidity and Mortality Weekly Report, Centers for Disease Control. 1980. 29:610-611.

de Motta, G. Escalona, J.F. Felieu, and A. Izquierdo. 1986. Identification and Epidemiological Analysis of Ciguatera Cases in Puerto Rico. Mar. Fish. Rev., 48(4)14-18.

Pooley, Samuel G. 1978. Demand consideration in fisheries management - Hawaii's market for bottom fish. In: Tropical Snappers and Groupers (Jeffrey Polovina and Stephen Ralston, ed.) Westview Press, Boulder and London. pp. 609-633.

Prochaska, Fred J. and Walter R. Keithly. 1984. Region, market outlet and product form distribution of Gulf of Mexico landed grouper. In: Proceedings of the Ninth Annual Tropical and Subtropical Fisheries Technological Conference of the Americas.

Ragelis, E. 1984. Ciguatera seafood poisoning overview. In: Seafood Toxins (E. Ragelis, ed.). American Chemical Society, Washington, D.C. pp. 25-36.

Schulstad, Robert N. and Herber H. Stoevener. 1978. The effects of mercury contamination in pheasants on the value of pheasant hunting in Oregon." Land Economics. 34:39-49.

Schwartz, David G. and Ivan E. Strand. 1981. Avoidance costs associated with imperfect information: The case of Kepone. Land Economics 57:139-150.

Squires, Dale. 1981. Public regulation and the structure of production in multiproduct industries: an application to the New England otter trawl industry. Rand Journal of Economics 18(2):232-247.

Todd, E.C.D. Ciguatera poisoning in Canada. 1985. In: Toxic Dinoflagellates (D. Anderson, A. White and D. Baden, eds.). Elsevier Publishing Co., New York. pp. 505-519.

Tversky, Amos and Daniel Kahneman. 1981. The Framing of Decisions and the Psychology of Choice. Science 22:453-458.

Von Newmann, J. and O. Morgenstern. 1947. Theory of Games and Economic Behavior. 2nd Ed. Princeton University Press. 980 p.

Wang, D.H. 1984. Partial adjustment price models: a study of the impact of fish imports on ex-vessel prices on New England groundfish. In: Proceedings of the International Institute of Fisheries Economics and Trade. Christchurch, New Zealand.

ENHANCED ISOLATION OF PLESIOMONAS SHIGELLOIDES USING AN ENRICHMENT TECHNIQUE AND FACTORS INFLUENCING ITS RECOVERY FROM RAW OYSTERS

Susan M. Freund, John A. Koburger, W. Steven Otwell
and Cheng-i Wei
Food Science and Human Nutrition Department
University of Florida
Gainesville, FL 32611

INTRODUCTION

Plesiomonas shigelloides is a Gram negative, oxidase postive bacterial rod isolated from many aquatic sites worldwide (1,2,7). It is a suspected opportunistic pathogen causing the classic symptoms of gastroenteritis and more rarely meningitis or septicemia in infants (3,4,14). P. shigellloides has been found in Florida to be associated with outbreaks of gastrointestinal illness. Twenty-nine people became ill in a 6 day period after consuming raw oysters from Apalachicola Bay. Illness occurred approximately 24-30 hours after ingestion and symptoms lasted a median of 5 days (8).

P. shigelloides is generally considered to be a waterbourne pathogen, found most commonly in fresh surface waters (17) and it has also been isolated from a variety of animals including many types of fish and crustaceans as well as wild and domestic mammals and birds (1,9). Seasonal variation has been reported with Plesiomonas being found more frequently during the summer months (1,2,6).

Inositol brilliant green bile salts agar (IBB) has been recommended for Aeromonas and Plesiomonas (16). Miller and Koburger (9) recommended the use of both IBB and plesiomonas agar for environmental samples because P. shigelloides recovery from their samples was highest using IBB, but in laboratory controlled studies they found plesiomonas agar to be better at isolating injured organisms.

Various enrichment broths have been used to enhance the recovery of Plesiomonas. Gram negative (GN) broth in combination with some enteric agars has been tried (2,13), but these researchers indicated that direct plating was still more effective. Von Gravenitz and Bucher (20) suggested enrichment in alkaline peptone water (APW) was superior to direct plating at low coliform to Plesiomonas ratios. Millership and Chattophady (12) also reported that APW incubation before plating on IBB improved isolation for Aeromonas when its ratio to coliforms was $1;10^3$ or less, but in the case of Plesiomonas, no isolates could be recovered using APW. Direct plating in this instance was more effective. Tetrathionate broth has been shown to be helpful for the recovery of Plesiomonas. Comparison of tetrathionate broth (TT) with and without the addition of iodine showed that TT without iodine increased Plesiomonas recovery on Salmonella-Shigella agar, but highest isolation of this organism was achieved after direct

plating onto MacConkey's agar (19). A series of incubation times, ranging from 6-24 hours, and temperatures ranging from 24-45°C have been tried using these broths and plating media. The general trend seems to be toward the use of higher incubation temperatures in that they reduce the problem of competing bacteria growing in the various media (7,19).

In this study, five enrichment media were compared to the direct plating method described by Miller and Koburger (9) for the recovery of Plesiomonas from environmental water samples. The development of a more sensitive isolation procedure could enhance the detection of Plesiomonas in samples, such as oysters, where it may have previously been unnoticed. This would lead to a better understanding of this organism's ecology as well as its survivability and resusitation in both environmental and clinical situations.

MATERIALS AND METHODS

Water Samples

Water samples were collected from five freshwater sites in Gainesville, Florida. The sites included Lake Wauberg, Lake Alice, Newnan's Lake, Hogtown Creek, and a small pond. The samples were collected in sterile whirl-pak bags and transported immediately to the laboratory. Analysis was performed on the day of collection. This procedure was repeated on 5 different days between January and June of 1986.

Enrichment Broths

The five enrichment broths included: gram negative broth (GN), alkaline peptone water (APW, 18), tetrathionate broth without the addition of iodine (TT), a plesiomonas enrichment broth (PLE) and a modified plesiomonas enrichment broth (mod PLE). The PLE is comprised of ammonium nitrate 0.1%, inositol 0.2%, yeast extract 0.05%, potassium phosphate 0.5%, magnesium sulfate 0.02% and bile salts 0.1%. The modified PLE is the same as PLE, but contains less inositol (0.1%). The pH is adjusted to 7.6 for both PLE and mod PLE.

Plating Media

The two plating media used were inositol brilliant green bile salts agar (IBB, 17) and plesiomonas agar (PL, 9).

Enrichment Procedure

Ten ml of each water sample was inoculated in duplicate into 90 ml portions of each of the five test broths. After mixing, one of the duplicate broth samples was incubated at 35°C and the other at 40°C for 18 hours. The enriched samples were each streaked onto both

PL agar and IBB agar plates and incubated at 35°C for 24-48 hours. Presumptive counts of typical Plesiomonas colonies were done and the total number of colonies counted. The rate of isolation of Plesiomonas was expressed as the ratio of total number of Plesiomonas colonies to the total number of bacterial colonies on the plates.

Direct Plating Procedure

The water samples were serially diluted using Butterfield's phosphate buffer (18) before being surface inoculated directly onto IBB and PL agars. The plates were incubated at 35°C for 24-48 hours and presumptive Plesiomonas colonies were counted.

Confirmation

Presumptive Plesiomonas isolates obtained from broth enrichment and direct plating were confirmed. Plesiomonas is alkaline over acid on Triple Sugar Iron agar slants. It is able to ferment inositol without the production of gas and cannot hydrolyze gelatin in Inositol Gelatin Deeps (9,17). It is oxidase positive.

Oyster Samples

The enrichment method determined to be superior from the environmental water study was applied to both commercially packaged raw oyster meats as well as fresh unshucked raw oysters. The shell oysters were purchased from various retail stores and were shucked in the laboratory in as sterile a manner as possible. The commercially shucked oysters are packaged in plastic containers raw and without further processing.

Enrichment Broths

The oyster meats were diluted with Butterfield's phosphate buffer in a 1:1 ratio and blended for 3 minutes. Homogenates were then further diluted and 0.1ml was surface inoculated onto PL and IBB agars. Also 10 ml aliquots were inoculated into 90 ml portions of the various test broths and incubated for 18 hours at 40°C.

Pure Culture Studies

A series of tests using a pure culture inoculum of Plesiomonas were also performed to determine the sensitivity of the method. Oyster samples were spiked with various levels of Plesiomonas culture inocula. The Plesiomonas cultures were grown overnight in Brain Heart Infustion broth (BHI) before they were added to the oyster samples. The bacterium was added to oyster samples before and after blending. The spiked oyster samples were serially diluted and inoculated in tetrathionate broth as described above.

Competing Bacteria

Bacteria found commonly on the solid plating media were isolated and identified using an Oxi-Ferm or an Enterotube determination. Equal volumes of pure Plesiomonas culture and the competing organisms, Klebsiella and Pseudomonas, were mixed in and plated directly as well as inoculated into tetrathionate broth to determine the effects of competition.

Statistical Analysis

A 5 X 3 factorial design analysis of variance using the General Linear Models Procedure in the Statistical Analysis System (15) was applied to compare the relative effectiveness of the broths in enhancing the recovery of P. shigelloides. Significant differences between broths were determined by the Least Significant Difference (LSD) t-test.

RESULTS AND DISCUSSION

Recoveries of Plesiomonas on IBB and PL agars from the water samples for each of the five test broths are shown in Table 1. The values range from 0.04% to 69% of the total colonies being Plesiomonas. Comparison of the five enrichment broths revealed tetrathionate to be significantly ($p<0.05$) superior to all others tested, either at the two incubation temperatures or on the two isolation media. The only other broth that gave a significantly higher recovery rate was the alkaline peptone water (APW) at 40°C on plesiomonas agar, but this value was still significantly ($p<0.05$) lower than that of tetrathionate under the same conditions. Other researchers have noted some success using tetrathionate enrichment. As previously mentioned, VanDamme and Vandepitte (19) reported that tetrathionate broth without iodine was superior to tetrathionate with iodine as an enrichment step before plating on Salmonella-Shigella (SS) agar, although they indicated that direct plating on MacConkey's agar achieved a higher isolation rate. Alkaline peptone water is also cited in a number of references for Plesiomonas enrichment (2,12), but again these workers recommended direct plating over enrichment.

It is interesting to note that generally IBB appeared to be a better medium for the recovery of Plesiomonas when the numbers of Plesiomonas in the samples were low. This was true with GN, APW, PLE, and mod PLE. When P. shigelloides was present in greater numbers as was the case following tetrathionate enrichment, plesiomonas agar proved more effective at isolating this organism following both 35°C and 40°C enrichment. This observation may be attributed to the greater amount of bile salts in the IBB agar as well as the ability to recognize Plesiomonas colonies on PL agar. A higher concentration of bile salts helps to reduce the level of competing bacteria and this may allow a greater chance of recovering the Plesiomonas colonies that may be present (9).

Table 1. Evaluation of Enrichment Broth, Agar Plating Media, and Incubation Temperature on the Recovery of *Plesiomonas shigelloides* (% of Total Colonies) from 25 Water Samples

	35°		40°C	
Broth	PL	IBB	PL	IBB
GN	1.20	1.60	0.03	0.20
APW	0.81	2.04	14.52*[1]	6.88
PLE	0.04	2.32	1.40	2.64
PLE mod	0.20	0.00	0.17	1.76
TT	21.12*	15.68*	68.92*	53.96*

* Difference significant between broths at 0.05 level as determined by the LSD t-test

*[1] Difference significantly higher than GN, PLE and mod PLE, but significantly lower than TT at 0.05 level.

The elevated incubation temperature of 40°C was superior overall in enhancing the recovery of Plesiomonas. The inhibition of some of the competing microorganisms such as Pseudomonas and some of the Enterobacteriaceae which are often found in food and water may have contributed to this higher recovery rate (5,7,9,10). Miller and Koburger reported that of 40 Plesiomonas isolates tested, all were able to grow at 40°C, but that only 25% grew at 45°C (11). For environmental samples, therefore, it is suggested to incubate enrichment cultures no higher than 40°C to avoid the risk of inhibiting some Plesiomonas.

The advantage of using the tetrathionate enrichment technique to enhance the detection of Plesiomonas from environmental water samples is well demonstrated in Table 2. The value of enrichment is not as great for a eutrophic sample such as Sample 1 because by using direct plating only, about 35% of the colonies are easily detected as Plesiomonas. However, in a sample such as Sample 5, where only 3% of the colonies are Plesiomonas as shown by the direct plating method, the benefits of this enrichment step become obvious; the recovery rate for Plesiomonas was increased to 86% post-enrichment. The chances of finding a Plesiomonas colony when only 3% of the total colonies are Plesiomonas is low, but it can hardly be missed when it is present as 86% of the colonies. It should also be noted that in 12% of the samples tested, no Plesiomonas colonies could be detected by direct plating and yet it was easily isolated following enrichment. It is under such conditions, when the organism is present in low numbers that the enrichment technique can be a significant aid in the study of the ecology of this microorganism.

A similar experiment using both freshly shucked and packaged oyster meats was conducted based on the results of the environmental water samples. In preliminary studies with packaged oyster meats, direct plating detected no Plesiomonas positive samples, however following enrichment in tetrathionate broth some of the samples were positive. These results suggested that the enrichment method does work for oyster samples, but recovery seemed to be affected by a number of factors. Initially, the high positive recoveries of Plesiomonas detected with the water samples were not being achieved using oyster samples. Pure Plesiomonas cultures were spiked into the oyster samples and were plated directly and enriched. Table 3 shows the results of one such experiment. In the low dilutions of oyster, Plesiomonas was not detected, but as the sample was diluted more, Plesiomonas colonies were easily detectable. This indicates that Plesiomonas was present in the low dilutions, but was unable to be detected because of the presence of a high number of competing bacteria that were visually evident on the plates. As these competing bacteria were diluted out the Plesiomonas colonies became more apparent.

In order to better understand the effect of competing bacteria on the growth of Plesiomonas, a number of bacteria growing on these plates were isolated and identified. Table 4 lists the organisms recovered. As would be expected there is Pseudomonas, Aeromonas, Vibrio and Enterobacter. Isolate 1, Klebsiella was often recovered

Table 2. A Comparison of Direct Plating and Tetrathionate Enrichment in the Recovery of *Plesiomonas shigelloides* from 25 Water Samples

	Sample (% of total colonies)				
	1	2	3	4	5
Direct plating	36.0	3.6	26.2	25.4	3.2
Enrichment*	76.6	64.8	81.8	68.0	86.6

*The values of the enrichment group are significantly higher ($p<0.05$) than the direct plating group as determined by the LSD t-test.

Table 3. Detection of *Plesiomonas* using Direct Plating when Different Amounts of *Plesiomonas* are Added to an Oyster Homogenate

Number of *Plesiomonas* Added/ml	Dilution of Oyster Homogenate		
	1/5	1/50	1/500
10^6	25*	87	87
10^5	0	56	39
10^4	0	17	0

*Values reported are % of total colonies found to be *Plesiomonas*

Values are an average of three repetitions performed in duplicate

Table 4. Identification of Some Competing Bacteria Isolated from a Raw Oyster Homogenate following Direct Plating

	Color on Plating Media		
Isolate No.	PL Agar	IBB Agar	Enterotube Result
1	yellow	pink	Klebsiella
2	pink	light pink	Pseudomonas spp.
5	yellow	light pink	Aeromonas
6	yellow	light pink	Enterobacter
7	yellow	pink	Aeromonas
9	yellow	colorless	Edwardsiella
11	red	colorless	P. putrifaciens
12	yellow	beige	Vibrio spp., Flavobacterium
13	red	colorless	P. putrifaciens
*15	pink	pink	P. shigelloides

* Pure culture used as control

and this may have been due to the large size of its mucoid colonies. Pure cultures of these isolates were mixed with pure Plesiomonas cultures to study the effect of competition. Table 5 and Table 6 report the data when Klebsiella was mixed with Plesiomonas in varying concentrations. In this study enrichment greatly enhanced the recovery of Plesiomonas, causing it to be easily detected in samples where it was unable to be found before the enrichment step. The same experiment was performed using a Pseudomonas isolate (Tables 7 and 8). However, the enrichment step did not enhance the recovery of Plesiomonas. In fact, the organism was less detectable after enrichment. These results indicate that the type of organism present initially in the oysters can greatly affect the performance of the enrichment step. This is unfortunate, as there is no way of predetermining the type of bacteria that is going to be present in a given sample.

When comparing recovery before and after enrichment in packaged oysters (Table 9) to that of freshly shucked meats (Table 10), an obvious difference can be seen. The enrichment technique is a significant aid in the recovery of Plesiomonas from packaged meats whereas direct plating is more effective for freshly shucked samples. We attributed this to the different types and numbers of competing bacteria present in the samples. It is difficult to conclusively state a method which will enhance isolation of Plesiomonas from all oyster samples, but the present data indicates that enrichment can be used for environmental water samples and some packaged oyster meats.

Table 5. Detection of *Plesiomonas* in the *Plesiomonas*-*Klebsiella* Mixtures following Direct Plating

Number of *Plesiomonas* Added/ml	Number of Klebsiella Added/ml							
	10^8	10^7	10^6	10^5	10^4	10^3	10^2	10^1
10^8	-	-	-	+	+	+	+	+
10^7	-	-	-	+	+	+	+	+
10^6	-	-	-	+	+	+	+	+
10^5	-	-	-	+	+	+	+	+
10^4	-	-	-	-	+	+	+	+
10^3	-	-	-	-	+	+	+	+
10^2	-	-	-	-	-	-	+	+
10^1	-	-	-	-	-	-	+	+

+, *Plesiomonas* detected; -, No *Plesiomonas* detected

Table 6. Detection of *Plesiomonas* in the *Plesiomonas*-*Klebsiella* mixtures following Tetrathionate Enrichment

Number of *Plesiomonas* Added/ml	Number of Klebsiella Added/ml							
	10^8	10^7	10^6	10^5	10^4	10^3	10^2	10^1
10^8	+	+	+	+	+	+	+	+
10^7	+	+	+	+	+	+	+	+
10^6	-	+	+	+	+	+	+	+
10^5	-	+	+	+	+	+	+	+
10^4	+	+	+	+	+	+	+	+
10^3	+	+	+	+	+	+	+	+
10^2	+	+	+	+	+	+	+	+
10^1	+	+	+	+	+	+	+	+

+, *Plesiomonas* detected; -, No *Plesiomonas* detected

Table 7. Detection of *Plesiomonas* in the *Plesiomonas*-*Pseudomonas* Mixtures following Direct Plating

Number of *Plesiomonas* Added/ml	Number of Pseudomonas Added/ml							
	10^8	10^7	10^6	10^5	10^4	10^3	10^2	10^1
10^8	+	+	+	+	+	+	+	+
10^7	+	+	+	+	+	+	+	+
10^6	+	+	+	+	+	+	+	+
10^5	-	-	-	+	+	+	+	+
10^4	-	-	-	+	+	+	+	+
10^3	-	-	-	-	-	+	+	+
10^2	-	-	-	-	+	+	+	+
10^1	-	-	-	+	+	+	+	+

+, *Plesiomonas* detected; -, No *Plesiomonas* detected

Table 8. The Detection of *Plesiomonas* in the *Plesiomonas*-*Pseudomonas* Mixtures using Tetrathionate Enrichment

Number of *Plesiomonas* Added/ml	Number of Pseudomonas Added/ml							
	10^8	10^7	10^6	10^5	10^4	10^3	10^2	10^1
10^8	-	-	-	-	-	-	-	-
10^7	-	-	-	-	-	-	+	-
10^6	-	-	-	-	-	+	+	+
10^5	-	-	-	-	-	-	-	+
10^4	-	-	-	-	-	-	+	+
10^3	-	-	-	-	-	+	+	+
10^2	-	-	-	+	-	+	+	+
10^1	-	-	-	-	+	+	+	+

+, *Plesiomonas* detected; -, No *Plesiomonas* detected

Table 9. Effectiveness of Direct Plating versus Enrichment in Detecting *Plesiomonas* from Packaged Oyster Meats

	Presence of Plesiomonas Colonies	
Sample	Direct Plating	Enrichment
1	-	+
2	-	+
3	-	-
4	-	-
5	-	+
6	+	-
7	-	-
8	-	+
9	-	+
10	-	+
11	+	+
12	-	-
13	-	-
14	-	+
15	-	+
16	-	+

+, *Plesiomonas* was detected; -, No Plesiomonas was detected

Table 10. Effectiveness of Direct Plating versus Enrichment in Detecting *Plesiomonas* from Freshly Shelled Oyster Meat

	Presence of Plesiomonas colonies	
Sample	Direct Plating	Enrichment
1	-	-
2	+	-
3	-	-
4	-	-
5	+	-
6	+	-
7	-	-
8	+	+

+, *Plesiomonas* was detected; -, No *Plesiomonas* was detected

REFERENCES

1. Arai, T. and N. Ikejima. 1980. A survey of Plesiomonas shigelloides from aquatic environments, domestic animals , pets and humans. J. Hyg., Camb. 84:203-211.

2. Cooper, R. G. and G. W. Brown. 1968. Plesiomonas shigelloides in South Australia. J. Clin. Pathol. 21:715-718.

3. Davis, W. A., II, J. H. Chretien, V. F. Garagusi and M. A. Goldstein. 1978. Snake-to-human transmission of Aeromonas (Pl) shigelloides resulting in gastro-enteritis. Southern Med. J. 71:474-476.

4. Dudley, A. G., W. Mays and L. Sale. 1982. Plesiomonas (Aeromonas) shigelloides meningitis in a neonate - a case report. J. Med. Assoc. Ga. 71:775-776.

5. Dupray, E. and M. Cormier. 1983. Optimal enrichment time for isolation of Vibrio parahaemolyticus from seafood. Appl. Environ. Microbiol. 45:1234-1235.

6. Holmberg, S. D. and J. J. Farmer, III. 1984. Aeromonas hydrophila and Plesiomonas shigelloides as causes of intestinal infections. Rev. Infect. Dis. 6:633-639.

7. Koburger, J. A. and M. L. Miller. 1984. Plesiomonas shigelloides: A new problem for the oyster industry? Proc. 9th Ann. Trop. Subtrop. Fish. Conf. Am. 337-342.

8. Lieb, S. 1983. Outbreak of shellfish associated gastroenteritis, Bay County. Dept. of Health and Rehabilitative Services, Tallahassee, FL.

9. Miller, M. L. and Koburger, J. A. 1986. Evaluation inositol brilliant green bile salts and plesiomonas agars for recovery of Plesiomonas shigelloides from aquatic samples in a seasonal survey of the Suwannee river estuary. J. Food Prot. 49:274-278.

10. Miller, M. L. and Koburger, J. A. 1986. Plesiomonas shigelloides: an opportunistic food and waterbourne pathogen. J. Food Prot. 48:449-457.

11. Miller, M. L. and Koburger, J. A. 1986. Tolerance of Plesiomonas shigelloides to pH, sodium chloride and temperature. J. Food Prot. 49:877-879.

12. Millership, S. E. and B. Chattopadhyay. 1984. Methods for the isolation of Aeromonas hudrophila and Plesiomonas shigelloides from faeces. J. Hyg., Camb. 92:145-152.

13. Pauckova, V. and A. Fukalova. 1968. Occurrence of Aeromonas hydrophila and Plesiomonas shigelloides in feces. Zentralbl. Bakteriol. (Orig.) 206:212-216.

14. Rutala. W. A., F. A. Sarubbi, Jr., C. S. Finch, J. N. MacCormack and G. E. Steinkraus. 1982. Oyster-associated outbreak of diarrhoeal disease possibly caused by Plesiomonas shigelloides. Lancet, March 27:739.

15. SAS. Users guide: basic 1982 edition. SAS Institute Inc. Cary, North Carolina.

16. Schubert, R. H. W. 1981. On the ecology of Plesiomonas shigelloides. Zentralbl. Bakteriol. Hyg., I. Abt. Orig. B. 172:528-533.

17. Schubert, R. H. W. Genus IV. Plesiomonas pp. 548-550. In Krieg, N. R. and J. G. Holt (eds.). Bergey's Manual Volume I. Williams and Wilkins. Baltimore, Maryland.

18. Speck, M. L. (ed.). 1984. Compendium of methods for the microbiological examination of foods 2nd ed. American Public Health Association, Washington, D.C.

19. VanDamme, L. R. and J. Vandepitte. 1980. Frequent isolations of Edwarsiella tarda and Plesiomonas shigelloides from healthy Zairese freshwater fish: a possible source of sporadic diarrhea in the tropics. Appl. Environ. Microbiol. 39:475-479.

20. Von Graevenitz, A. and C. Bucherf. 1983. Evaluation of differential and selective media for isolation of Aeromonas and Plesiomonas spp. from human faeces. J. Clin. Microbiol. 17:16-21.

THE EFFECT OF LACTIC ACID STARTER CULTURES ON FISH SPOILAGE BACTERIA

S. M. Rose, F. M. Bartlett and E. G. Bligh
Department of Food Science and Technology
Technical University of Nova Scotia
Halifax, Nova Scotia Canada B3J 2X4

INTRODUCTION

Development of an antispoilage or an inhibitory agent which will delay deterioration of food would be of great importance from both an economic and public health standpoint. Lactic acid bacteria are known for their ability to repress bacterial growth, which is a major cause of food spoilage.

The use of lactic acid bacteria in food is not a new concept. In the dairy industry, cultures are commonly used to improve the shelf life of dairy products. Metabolic products of these bacteria, (e.g., lactic acid, propionic acid, diactyl and antibiotic-like substances) have a profound inhibitory effect on a variety of gram negative spoilage bacteria. Developing a dominant microflora of Lactobacillus spp would therefore be preferred due to its potentially inhibitory effect on spoilage bacteria.

Numerous studies have shown the beneficial effects of using lactic acid bacteria cultures for decreasing the spoilage organisms in both beef and poultry. Reddy and Chen, (1975) studied the effect of lactic cultures, (Streptococcus lactis plus Leuconostoc citrovorum) on changes in beef. Their data indicated a significant inhibitory effect on the growth of the gram-negative bacteria (Pseudomonas, Achromobacter, and Proteus) when treated with a 10% lactic culture. Another study with beef examined the effect of the application of lactobacilli and carbon dioxide on the growth of Microbacterium thermosphactum on fresh beef (Roth and Clark, 1975). Results were positive for the repression of the growth of the Microbacterium using lactobacilli on the vacuum-packaged meat. The authors concluded that the inhibition was caused by a substance produced by the lactobacilli.

Raccach and Baker (1978), studied the effect of two lactic acid bacteria (Pediococus acidilactici and Lactobacillus plantarum) on the inhibition of Pseudomonas species which are the primary spoilage bacteria of uncooked poultry meat. It was noted that Alteromonas putrefaciens was the most sensitive to the lactic acid bacteria, being repressed by four log cycles when the mixed culture was used.

There are a number of mechanisms by which lactic acid bacteria are able to repress the growth of other bacteria. The following antagonistic properties were summarized by Andersson (1986):

1. Acid production and pH reduction
2. Hydrogen peroxide production

3. Carbon dioxide production
4. Nutrient depletion
5. Decrease in reduction-oxidation potential
6. Production of antibiotic like compounds

The purpose of this study was to evaluate the potential use of lactic acid bacteria (L. plantarum) as an inhibitory agent of gram negative spoilage bacteria of fresh codfish. Also, the extracellular and intracellular fluids of Lactobacillus plantararum and Pediococcus acidilactici were examined in order to assess their ability to inhibit Alteromonas putrefaciens, a major spoilage organism of fish.

MATERIALS AND METHODS

Microorganisms

Freeze-dried cultures of Lactobacillus plantarum 8014 and Pediococcus acidilactici 8042 were obtained from the American Type Culture Collection. The bacteria were rehydrated in MRS broth (Difco) and incubated at 30°C for 24-48 hrs. MRS agar slants and plates were used to maintain the cutures which were then stored at 6°C.

Preparation of extracellular and intracellular extracts

L. plantarum and P. acidilactici were each grown in 1 L erlynmeyer flasks containing 400 mL of MRS broth media for 48 h at 25°C (without shaking). The growth medium was centrifuged at 5,000 rpm for 15 min at 5°C. The supernatant was decanted and filtered using a Millipore filter (0.45 µm pore size). This was the source of the extracellular extract.

The pelleted cells were washed by resuspending in 100 mL of sterile saline solution (0.85% NaCl) and centrifuging (5,000 rpm/15 min). The supernatant was discarded, and the cells were again washed with the saline solution (total of 3 washes). After the final wash the cells were suspended in a minimum amount of saline (≈ 5 mL) and put on ice. The cells were ruptured using a Biosonic III sonicator (Bronwill Scientific, Rochester, N.Y.) at the maximum recommended setting for one minute. This was repeated a total of 6 times to ensure complete breakage of the cells. The ruptured cells were centrifuged at 10,000 rpm for 30 min at 2°C. The supernatant was filtered and refrigerated. This was the intracellular extract.

The pH of the extracellular and intracellular extracts were adjusted to a value of ≈ 6.3 with 1N NaOH.

Disk assay procedure

Petri plates containing plate count agar (Difco) were inoculated with 24 h cultures of Alteromonas putrefaciens ATCC 8071 using sterile swabs. The plates were allowed to dry for 1 h at room temperature.

Sterile disks (0.7 cm in diameter) were placed on the agar surface and impregnated with the extracellular and intracellular extracts (10-100 µL) of the two lactic acid bacteria cultures. The plates were incubated at 22°C for 24-48 h. Antimicrobial activity was determined from the diameter of the zone of no growth around each disk.

Effect of LAB on cod fillets

Lactobacillus plantarum cultures were added to fresh cod fillets to attain a level of 10^8 cells/g. On the same day, codfish fillets were purchased and placed on ice. The bacteria was applied by means of a sterile non-aerosol spray. Fillets were wrapped and stored at 2°C for testing every second day. Control fillets left untreated were stored in the same way.

Fillets were sampled in duplicate for microbiological analysis, trimethylamine (TMA) analysis and surface pH. Gram-negative bacterial counts were performed on freshly prepared Crystal Violet TTC agar plus 0.5% NaCl. Serial dilutions were made with 0.1% (w/v) sterile peptone. Incubation for plates was at 22°C for 48 hrs. Fifty grams of tissue was extracted with 7.5% trichloroacetic acid (TCA) and analyzed for TMA (Dyer, 1945). Surface pH was obtained by taking four representative readings of each fillet using a surface pH electrode.

RESULTS

Disk Assay Procedure

The results of the inhibitory effect of the extracellular extracts of L. plantarum and P. acidilactici towards A. putrefaciens are summarized in Table 1.

Table 1. Inhibitory effect of extracellular extracts of L. plantarum and P. acidilactici toward A. putrefaciens

Volume extract (µL)	Zones of Inhibition[a] (cm)			
	L. plantarum extract		P. acidilactici extract	
	pH 3.81	pH 6.28	pH 4.32	pH 6.27
10	1.1	0.8	0.9	---
20	1.1	1.0	1.3	---
30	1.4	1.1	1.5	0.9
40	1.7	1.3	1.9	1.0
50	1.9	1.7	1.9	1.2
60	1.9	2.0	2.0	1.2
70	>2[b]	2.1	>2[b]	---
80	---	2.2	---	---
90	---	2.3	---	---
100	---	2.3	---	---

[a]Diameter of area of no growth; 0.7 cm sterile disks were used.
[b]Accurate measurements could not be determined beyond this point.

Growth of A. putrefaciens was inhibited by the extracellular extracts of both L. plantarum and P. acidilactici at their unadjusted pH values of 3.81 and 4.32 respectively. Inhibition was also evident by the extracts adjusted to pH 6.27 and 6.28. It is apparent that although the inhibition is greater in each case at the lower pH, the effect is still present at pH 6.27-6.28, particularly for the L. plantarum extract. Uninoculated MRS broth did not adversely affect the growth of A. putrefaciens.

No inhibition of A. putrefaciens was noted by the intracellular extracts of either lactic acid bacterial cuture at pH 6.2 (data not shown).

Effect of LAB on cod fillets

Gram-negative bacterial counts for the treated fillets were significantly lower than those for the control fillets on days 2 and 4 (Fig. 1). The surface pH of the treated fillets tended to be lower than the controls particularly towards the latter part of the storage period (Fig. 2).

The trimethylamine values of the treated and untreated fillets are presented in Fig. 3. It is apparent that the increase in TMA production is more rapid for the control fillets than the fillets treated with L. plantarum.

DISCUSSION

Both lactic acid bacterial cultures (L. plantarum and P. acidilactici) produced a substance(s) which effectively inhibited the growth of A. putrefaciens (Table 1). The antimicrobial compound(s) apeared to be exclusively extracellular, since no antimicrobial activity was associated with the intracellular fluids of either bacterium. Although the chemical nature of the compounds was not determined, it is unlikely that the inhibitory effect could be attributed solely to the production of lactic acid since the antimicrobial effect was evident at pH 6.3. Abdel-Bar et al. (1987) reported similar findings for L. bulgaricus. This bacterium produced an extracellular antibiotic-like compound which inhibited the growth of P. fragi. The production of an extracellular inhibitory agent by a L. plantarum isolate from fermented carrots has also been reported (Andersson, 1986).

Direct application of L. plantarum cells to cod fillets was found to have an inhibitory effect on the gram-negative bacterial population, particulary during the first six days of storage (Fig. 1). The introduction of L. plantarum appeared to be especially effective against TMA producing bacteria as indicated by the significantly lower TMA values for the treated samples as compared to the controls (Fig. 3). Raccach and Baker (1978) were able to show that the addition of L. plantarum and P. cerevisiae effectively extended the lag phase of

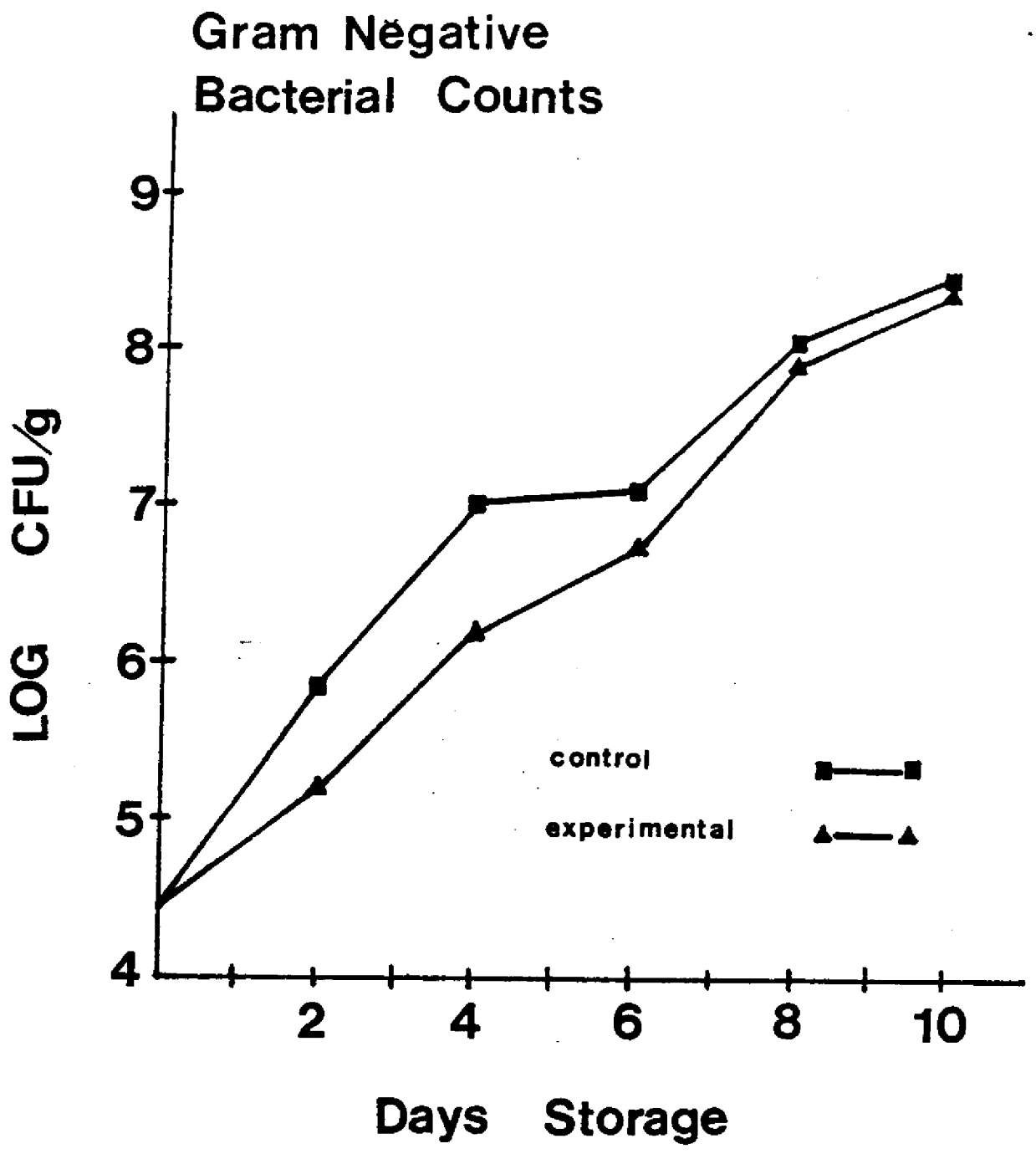


FIG.1 TOTAL GRAM NEGATIVE BACTERIAL COUNTS FOR COD FILLETS STORED AT 2C. CONTROL SAMPLES - UNTREATED. EXPERIMENTAL SAMPLES - TREATED WITH L. PLANTARUM AT A CONCENTRATION OF 10^8 CELLS/GRAM.

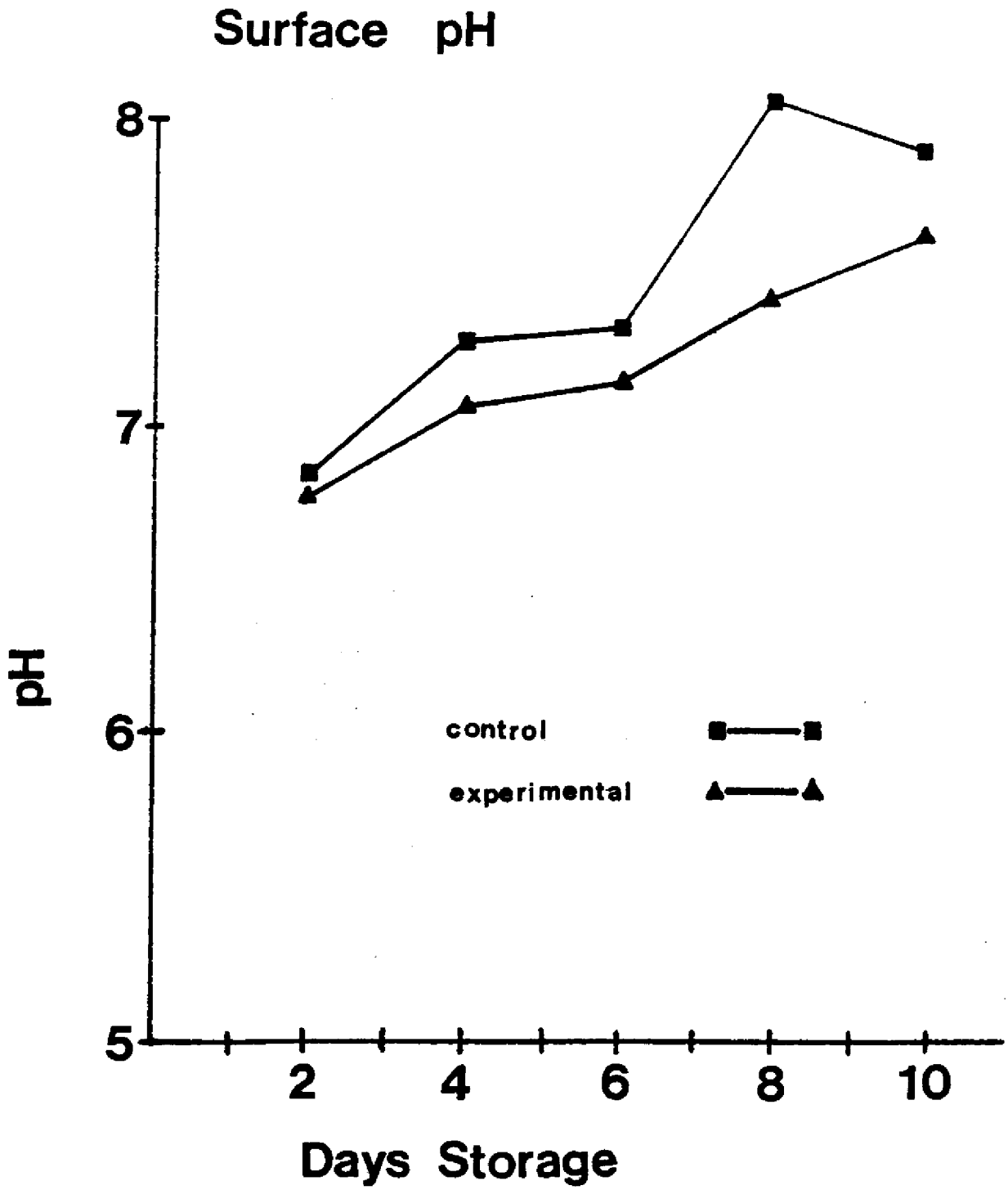


FIG.2 SURFACE pH OF COD FILLETS STORED AT 2C. CONTROL FILLETS - UNTREATED. EXPERIMENTAL FILLETS - TREATED WITH L. PLANTARUM AT A CONCENTRATION OF 10^8 CELLS/GRAM.

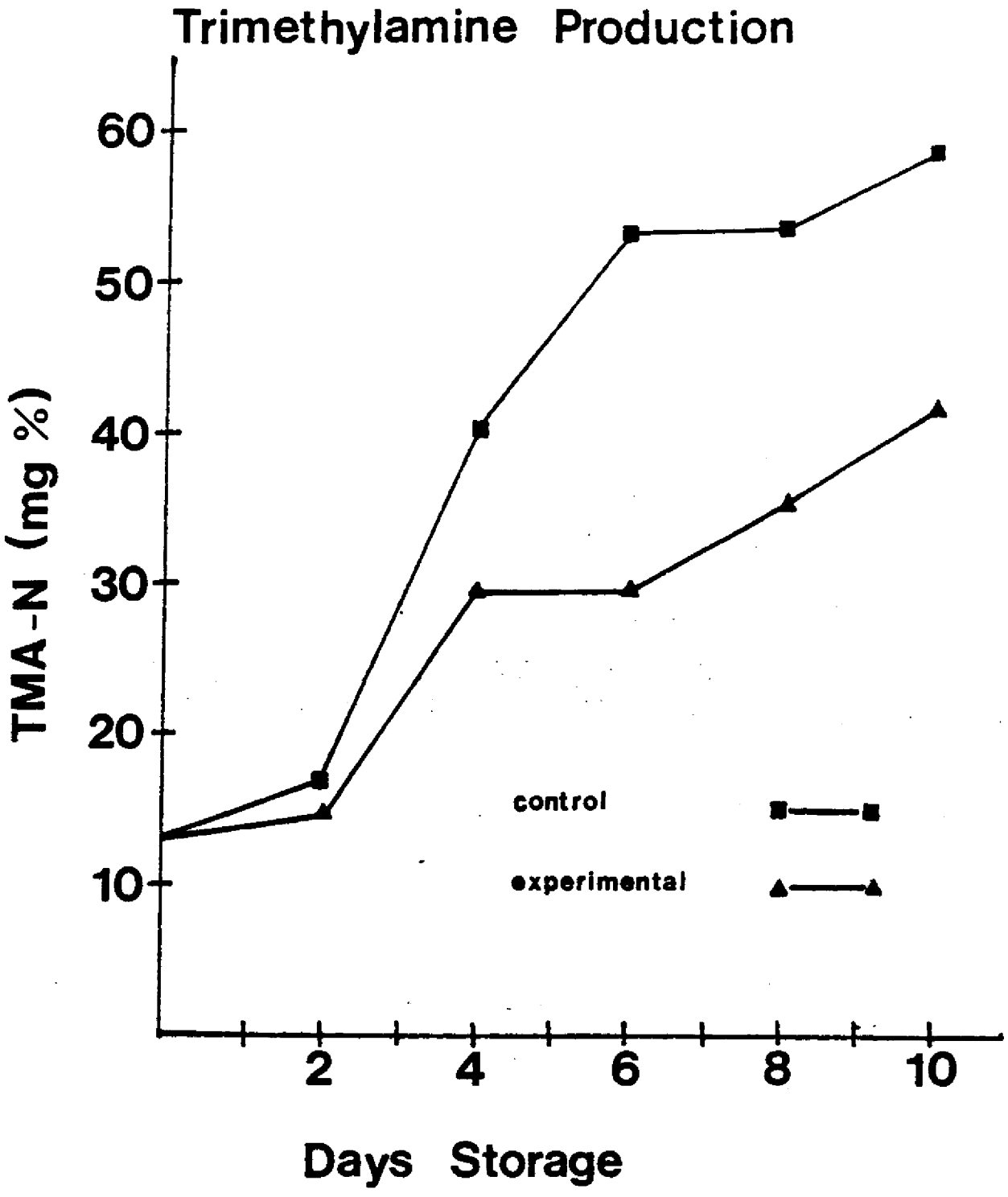


FIG.3 TMA VALUES FOR COD FILLETS STORED AT 2C. CONTROL SAMPLES - UNTREATED. EXPERIMENTAL SAMPLES - TREATED WITH L. PLANTARUM AT A CONCENTRATION OF 10^8 CELLS/GRAM.

pure cultures of Pseudomonas species in deboned poultry meat. As was the case in this study, they noted that the slight change in pH of the meat was not sufficient to explain this repression.

In conclusion, our study has shown that the use of LAB has potential as an inhibitor of fish spoilage bacteria. Further work is required to determine whether a significant shelf-life extension for fresh fish can be realized as a result of their use. In addition, evaluation of the extracellular extract as an alternative to the application of whole cells to fish needs to be investigated.

REFERENCES

Abdel-Bar, N., Harris, N. D. and Rill, R. L., 1987. Purification and Properties of an Antimicrobial Substance Produced by Lactobacillus bulgaricus. J. Food Sci. 52:411-415.

Andersson, R. 1986. Inhibition of Staphylococcus aureus and Spheroplasts of Gram Negative Bacteria by an Antagonistic Compound Produced by a Strain of Lactobacillus plantarum. Internat. J. Food Microbiol. 3:149-160.

Dyer, W. J. 1945. Amines in Fish Muscle; 1. Colorimetric Determination of Trimethylamine as the Picrate Salt. J. Fish. Res. Bd. Can. 6:351.

Raccach, M. and Baker, R. C. 1978. Lactic Acid Bacteria as an Antispoilage and Safety Factor in Cooked Mechanically Deboned Poultry Meat. J. Food Protect. 41:703-705.

Reddy, S. G. and Chen, M. L. 1975. Influence of Lactic Cultures on the Biochemical, Bacterial and Organoleptic Changes in Beef. J. Food Sci. 40:314-318.

Roth, L. A. and Clark, D. S. 1975. Effect of lactobacilli and Carbon Dioxide on the Growth of Microbacterium thermosphactum on Fresh Beef. Can. J. Microbiol. 21:629-632.

NUCLEOSIDE PHOSPHORYLASE AND ITS ROLE IN FISH SPOILAGE

T. A. Gill, Marc Surette and Stephen MacLean
Canadian Institute of Fisheries Technology
Technical University of Nova Scotia
P. O. Box 1000
Halifax, Nova Scotia
B3J 2X4

INTRODUCTION

Autolytic degradation in refrigerated fish muscle occurs as a result of enzymatic changes within the muscle. Enzyme decompartmentalization probably contributes to the rapid autolysis observed in fish muscle after death. The autolytic accumulation of ATP catabolites in postmortem fish muscle has been shown to be a reliable indicator of edible fish quality (Uchiyama et al., 1970; Hiltz et al., 1971). In a live fish, adenosine diphosphate (ADP) is produced in muscle contraction and can either be rephosphorylated by respiratory action or phosphocreatine, therefore the adenosine monophosphate (AMP) level remains low (Watts and Watts, 1974). After death, (Fig. 1) inosine monophosphate (IMP) accumulates via dephosphorylation and deamination of ATP. Jones and Murray (1964) suggested that the subsequent production of inosine (Ino) was rate-limiting in the ultimate formation of hypoxanthine (Hx), xanthine and uric acid.

Inosine nucleosidase (E.C. 3.2.2.2) and nucleoside phosphorylase (E.C. 2.4.2.1) were isolated from the muscle of Pacific lingcod by Tarr (1955, 1958) and were responsible, at least in part, for the postmortem production of Hx from Ino. Recently, LeBlanc (1987) found little or no inosine nucleosidase nor nucleoside phosphorylase activity in crude extracts of fresh cod. Ammonium phosphate fractionation of crude muscle extracts as described by Tarr (1955) resulted in no significant purification of inosine nucleosidase. However, measurable levels of this enzyme were detected in commercial cod fillets which had been stored on ice for several days.

The present study was initiated to examine the pattern of nucleotide catabolite accumulation in Atlantic cod (Gadus morhua) as well as the determination of the relative importance of autolytic and microbial enzymes in the production of Hx.

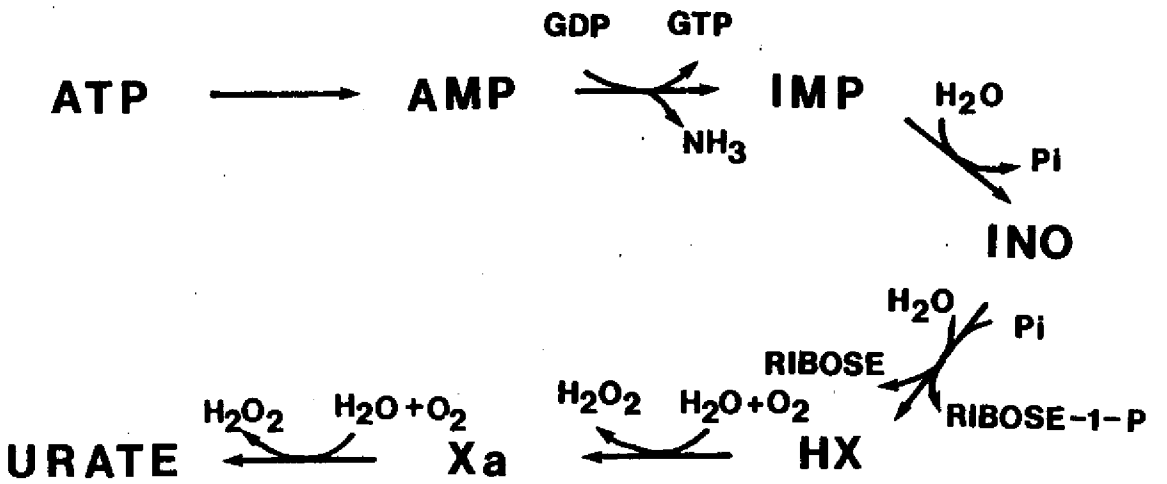


FIGURE 1. Sequence of postmortem nucleotide catabolism in fish muscle tissue. (ATP=adenosine triphosphate; AMP=adenosine monophosphate; IMP=adenosine monophosphate; Ino=inosine; Hx=hypoxanthine; Xa=xanthine).

MATERIALS AND METHODS

Studies of sterile cod muscle were performed by aeseptic removal of tissue from freshly killed fish, and subsequent storage of samples in sterile Petri dishes at 3°C. Sterile samples were checked for bacterial contamination at regular intervals, each time tissue was to be removed for perchloric acid extraction and subsequent chemical analysis. In order to compare autolytic with microbiological production of Hx, paired fillets were removed from the same fish described above but without precautions to preserve sterility.

Analysis of nucleotide catabolites was routinely carried out by homogenizing 50 g samples of tissue in 2 volumes of 6% perchloric acid for two, 1-minute intervals in a Waring blendor. Extracts were filtered, neutralized to pH 7.0 with 30% KOH, and chromatographed on a Waters HPLC system equipped with a μ Bondapak C-18 radial compression column using a 0.05 M, pH 7.0 potassium phosphate buffer pumped at 2.0 ml min^{-1}. Detection was carried out by absorbance at 254 nm. Quantitation was accomplished with external standards obtained from Sigma (St. Louis, MO).

Bacterial colonies recovered from spoiling cod were grown in trypticase soy broth for 3 days and cells harvested by centrifugation cell-free extracts were prepared by ultrasonic disruption for 7, 2-min intervals (Biosonik III, Bronwill Scientific, Rochester, N.Y.). Seven colonies were subsequently screened for their ability to produce Hx in the presence of Ino.

Activity assays for nucleoside phosphorylase were performed according to Boehringer Mannheim (1975), Activity assays for inosine nucleosidase were performed on bacterial cultures, cell-free extracts and muscle tissue. The reaction mixtures contained 3.0 ml 0.05 M veronal or TES buffer, 0.10 ml of 7.3 mM inosine solution, and 0.10 ml of a xanthine oxidase solution (Boehringer Mannheim) containing 20 units ml^{-1}. Reactions were initiated by the addition of 0.01 ml crude inosine nucleosidase. Activity was measured as the increase in absorbance at 293 nm and 22°C. Protein concentrations were determined by the Lowry et al. (1951) method.

Identification of spoilage bacteria producing significant quantities of Hx was performed using the api 20E enterobacteriaceae identification kit (api Anyltab products, Plainview, N.Y.).

For determination of pH optimum, reaction mixtures containing 1.8 ml of 0.05 M Pipes-HCl (Sigma, St. Louis, MO), pH.6.65-7.4; TES-NaOH at pH 7.0-7.5; or Tris-HCl at pH 7.4-8.4 were added to 0.10 ml of 7.3 mM inosine, 0.01 ml xanthine oxidase (0.4 U/ml, Boehringer Mannheim)

and 0.10 ml of 0.10 M sodium phosphate pH 7.3. Reactions were initiated by adding 0.10 ml of the enzyme preparation and activity measured as the increase in absorbance during the initial minute of reaction at 293 nm and 23°C.

For the determination of kinetic properties, the assay mixture contained 0.05 ml phosphate - free xanthine oxidase (0.04 U/ml), 0.05 to 0.20 ml of 2 mM inosine, 0.025 to 0.20 ml of 0.10 M sodium phosphate, 0.025 ml nucleoside phosphorylase and was made up to 2.0 ml with 0.05 M TES, pH 7.3. The activity was expressed in concentration units based on the molar absorbancy change for inosine to uric acid of 12,500 at 293 nm (Kalckar, 1947). The assay with guanosine as the substrate was determined spectrophotometrically by the decrease in absorbance at 257 nm due to the depletion of guanosine to guanine with a molor absorbancy decrease of 5000 (Fiers and De Bersaques, 1962). The reaction mixture contained 0.025 to 0.10 ml of 2 mM guanosine, 0.05 to 0.20 ml of 0.10 M sodium phosphate 0.10 nucleoside phosphorylase and was made up to 2.00 ml with 0.05 M TES, pH 7.3. Adenosine was also assayed as a substrate by monitoring the change in absorbance at 250 nm. The reaction mixture contained 0.25 ml of 2 mM adenosine, 0.2 ml sodium phosphate, 0.2 ml nucleoside phosphorylase and was made up to 2.0 ml with 0.05 M TES at pH 7.3. All assays of activity were performed at 25°C.

Purification of the enzyme catalyzing the production of Hx from Ino was carried out by a 3-step procedure starting with a cell-free extract of Proteus vulgaris recovered from spoiling cod. The first step involved gel filtration on a Sephacryl S-300 (Pharmacia) column (2.5 x 80 cm) with 0.05 M Tris-HCl, pH 8.0. Active fractions were collected, pooled and dialyzed against 0.05 M sodium citrate, pH 6.0. Activity was further purified on a DEAE-Sephacel (Pharmacia) column (1.8 x 8 xm) equilibrated with 0.05 M sodium citrate, pH 6.0. Samples were eluted by an 80 ml continuous gradient from 0.05 M to 0.12 M citrate at 0.4 ml min^{-1}. Active fractions were pooled, dialyzed against 0.01 M Na_2 Pipes-HCl, pH 7, and applied to an Agadenosine (Pharmacia) (1.1 x 7.5 cm) affinity column. Elution was accomplished with 20 ml 0.01 M Pipes, pH 7.0, followed by a continuous gradient of 0 to 1.5 M KCl in Pipes at a flow rate of 6 ml h^{-1}.

Enzyme purity was determined by isoelectric focussing in polyacrylamide gels (Pharmacia) using a Pharmalyte 3-10 pH range. Activity staining was carried out by immersing gels in 0.02% 3-[4,5 - dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide, 8 mM inosine and 0.008 U xanthine oxidase per ml, all in 0.05 M TES, pH 7.3. Gels were incubated at 35°C until a dark blue-black band appeared (within 30 min). Gels were fixed, stained with Coomassie Blue G-250 and compared with marker proteins for the determination of pI.

The molecular weight of nucleoside phosphorylase was determined by polyacrylamide gel electrophoresis on a 4-30% gradient slab in 0.09 M Tris, 0.08 M boric acid and 2.5 x 10^{-3} M Na_2 EDTA.

RESULTS AND DISCUSSION

Figures 2 and 3 illustrate the progression of formation of nucleotide catabolites in paired sterile and non-sterile cod fillets. It is clear that at 3°C, similar patterns emerge except that the conversion of Ino to Hx is delayed by about 2 days in the sterile samples. This suggests that the enzymes responsible for the production of Hx are both autolytic and microbiological in nature. As would be expected the rate of dephosphorylation of IMP to Ino was similar in both sterile and non-sterile samples, since few spoilage bacteria are normally present during the first four days of refrigerated storage.

Figures 4 and 5 illustrate the purine metabolite spoilage patterns for whole, gutted, bled and iced cod which were obtained fresh from a local aquarium and the headless, gutted cod available commercially. Notable were the differences in rates of nucleotide catabolism between intact aquarium fish and the aquarium fish which were filleted under sterile or non-sterile conditions. It is evident that Hx is produced much more rapidly in fillets (either sterile or non-sterile) than in headless, gutted cod. One explanation for the accelerated rate of decomposition in the fillets may be the mechanical damage received by the tissue during the filleting operation. This phenominon could be explained by the effects of enzyme decompartmentalization or the breakdown of barriers to microbial invasion within the muscle.

Initial attempts to recover either inosine nucleosidase or nucleoside phosphorylase from very fresh fish were fraught with difficulty. Only by using partially decomposed muscle tissue were we able to recover measurable amounts of activity. Seven bacterial cultures obtained from spoiling fillets were screened for their ability to produce Hx from Ino.

Only 2 cultures (Proteus vulgaris and Pseudomonas fluorescens) contained any measurable activity, with the specific activity of P. vulgaris cultures being approximately twice that of P. fluorescens. Little activity was observed prior to ultrasonic disruption of the cells, suggesting that the enzyme was primarily intracellular in nature. Upon extensive dialysis against phosphate-free buffers, little activity was observed, suggesting that nucleoside phosphorylase was far more important than inosine nucleosidase in the bacterial production of Hx.

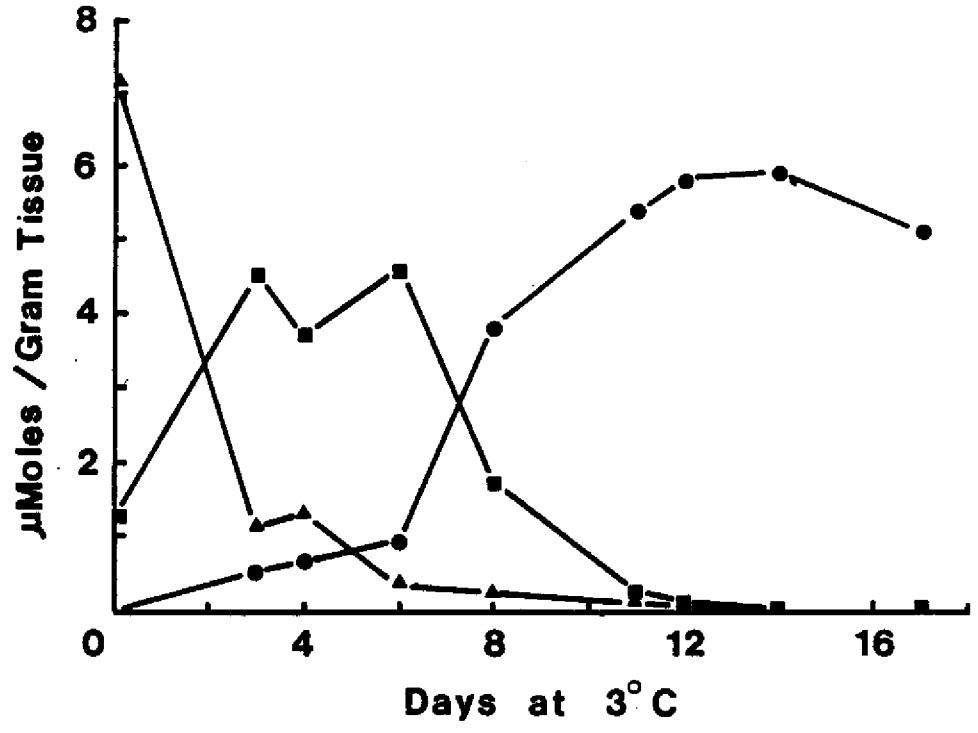


FIGURE 2. Metabolite concentrates (IMP▲, Ino ■ and Hx●) in sterile cod fillets stored at 3°C.

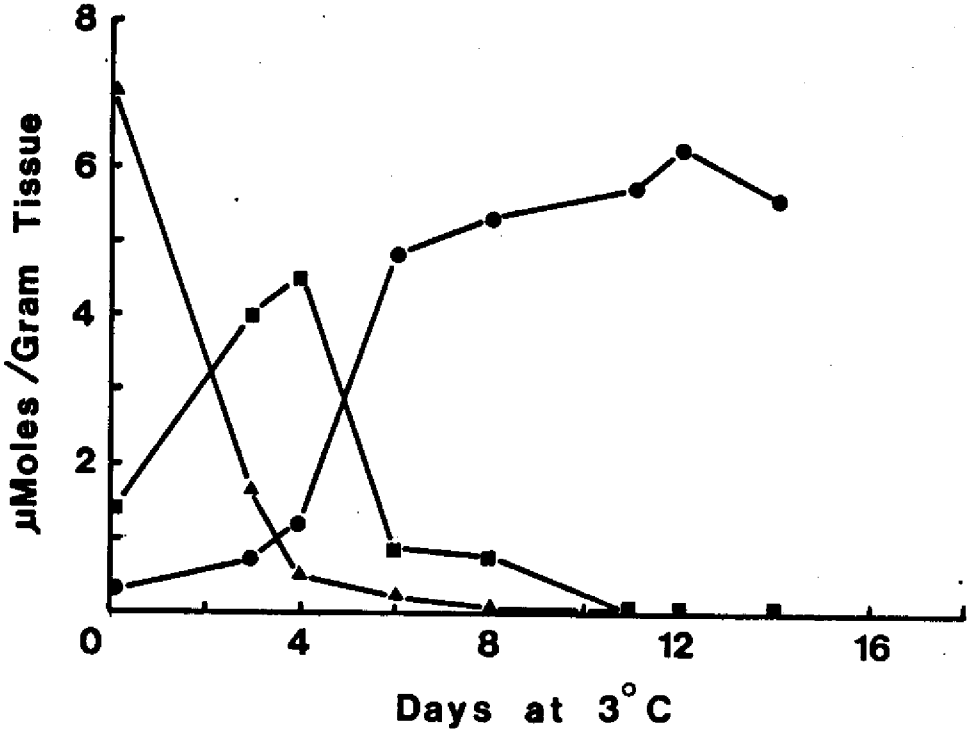


FIGURE 3. Metabolite concentrates (IMP▲, Ino■ and Hx●) in non-sterile cod fillets stored at 3°C.

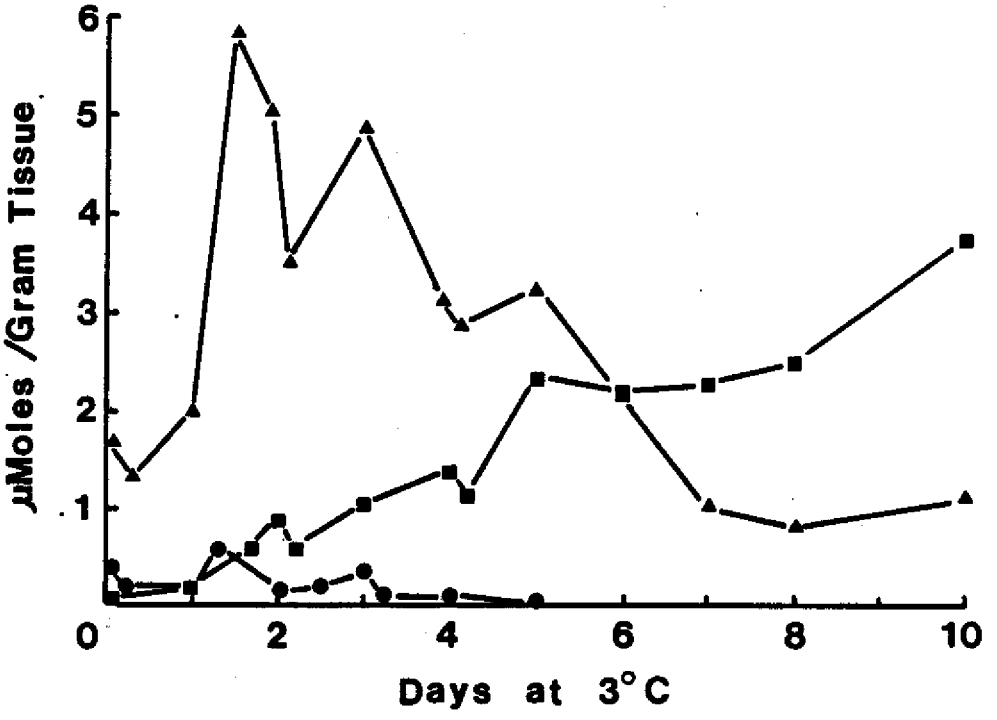


FIGURE 4. Metabolite concentrations (IMP▲, Ino■ and Hx●) in aquarium fish which were gutted, bled and stored at 3°C.

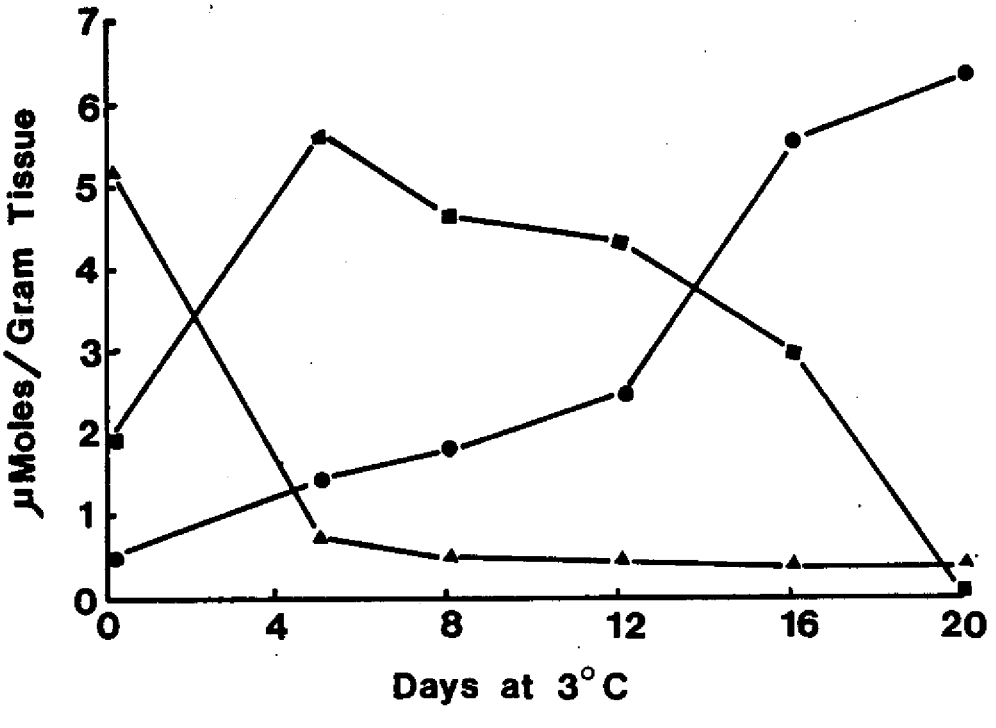


FIGURE 5. Metabolite concentrations (IMP▲, Ino■ and Hx●) in commercial gutted whole cod during storage at 3°C.

Figure 6 illustrates the pH profile for activity of nucleoside phosphorylase (NP) in three different buffer systems. A TES buffer at pH 7.3 was used routinely for the assay of NP activity.

A three-step purification of the cell-free extracts was performed by gel filtration, ion exchange chromatography and affinity chromatography using adenosine as the ligand. Gel filtration on Sephacryl S-300 (Fig. 7) resulted in the recovery of a single peak of NP activity, which when applied to a DEAE-Sephacel column (Fig. 8), resulted in a 5-fold increase in specific activity from the Sephacryl column and an overall purification of 23-fold from the crude cell-free extract. Affinity chromatography (Fig. 9) resulted in a further 23-fold purification from the ion exchange step (500-fold from the crude extract) yielding an overall recovery of 69% for all three steps. Table 1 summarizes the results of the purification procedure.

TABLE 1

Summary of the Purification of NP.

STEP	ACTIVITY (μmoles/min)	PROTEIN (mg)	SP. ACTIVITY (μmoles mg-1 min-1)	RECOVERY (%)
Crude extract	6.85	165.6	0.04	100.0
Sephacryl	6.90	37.7	0.18	100.0
Sephacel	5.88	6.4	0.92	85.7
Agadenosine	4.75	0.22	21.6	69.2

The nucleoside phosphorylase was recovered in isoelectrically-pure form with a pI of 6.8 and an apparent molecular weight of 120,000 ± 2000 daltons as determined by pore gradient electrophoresis.

The kinetics of NP were examined using Ino, and guanosine substrates. The enzyme exhibited typical Michaelis-Menten kinetics. Double reciprocal plots (not shown) with inosine or guanosine as the variable substrate and phosphate as the changing fixed substrate were also constructed. Kinetic parameters obtained by plotting the slopes and intercepts of the reciprocal plots against 1/[phosphate] are summarized in Table 2. Although inosine was used as the assay substrate during the purification of NP, the ability of NP to catalyze the phosphorylysis of guanosine indicates broad specificity. The Km values for both substrates were similar but the apparent specific activity toward inosine was double that obtained for guanosine. Adenosine was not used as a substrate for NP but is ability to bind the enzyme as a ligand in affinity chromatography has proven valuable in the purification procedure.

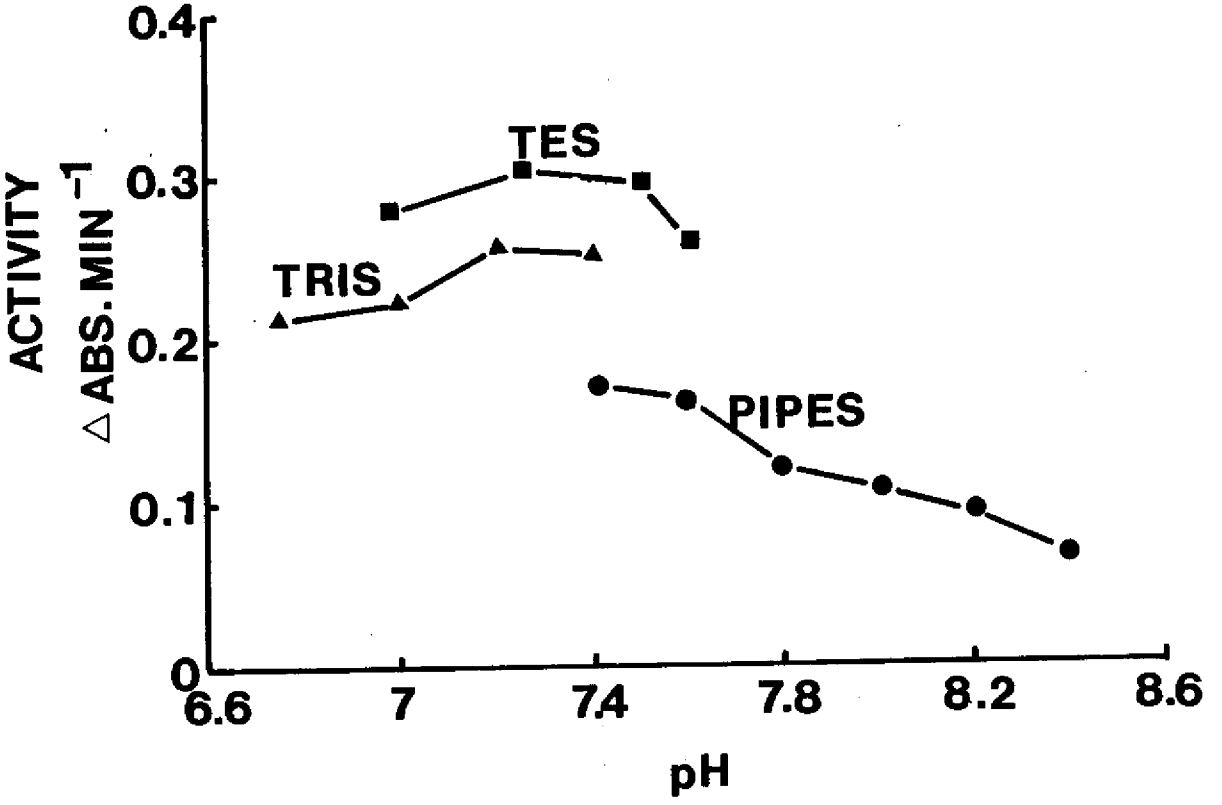
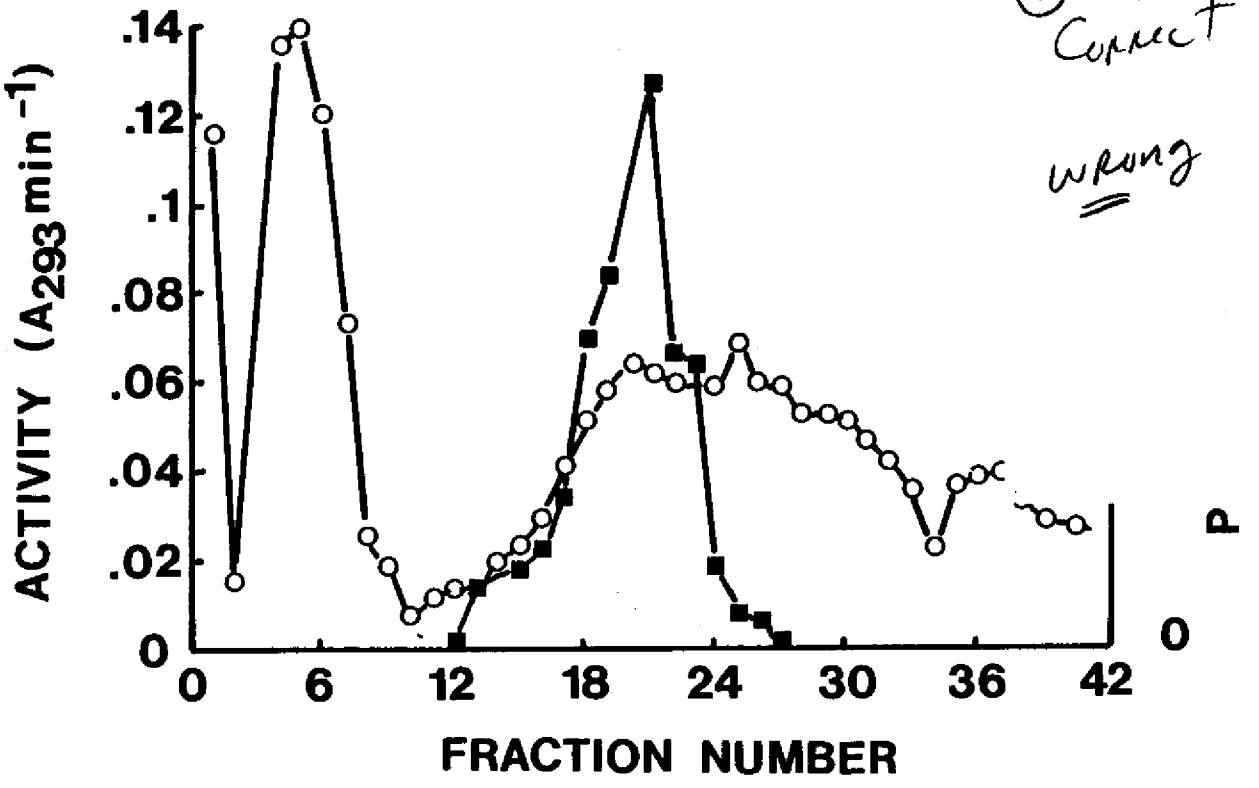


FIGURE 6. Activity assays for NP performed at various pH levels using 0.05 M. Pipes, TES or Tris buffers.

Fig 7 Lill et al, 1981



⑦–⑨ Correct figure wrong legend

FIGURE 7. Gel filtration of NP on a Sephacryl S-300 column with dimensions of 2.5 x 80 cm. Elution was performed with 0.05M Tris-HCl, pH 8.0 at a flow rate of 0.25 ml min^{-1}. Activity (■), Protein (O).

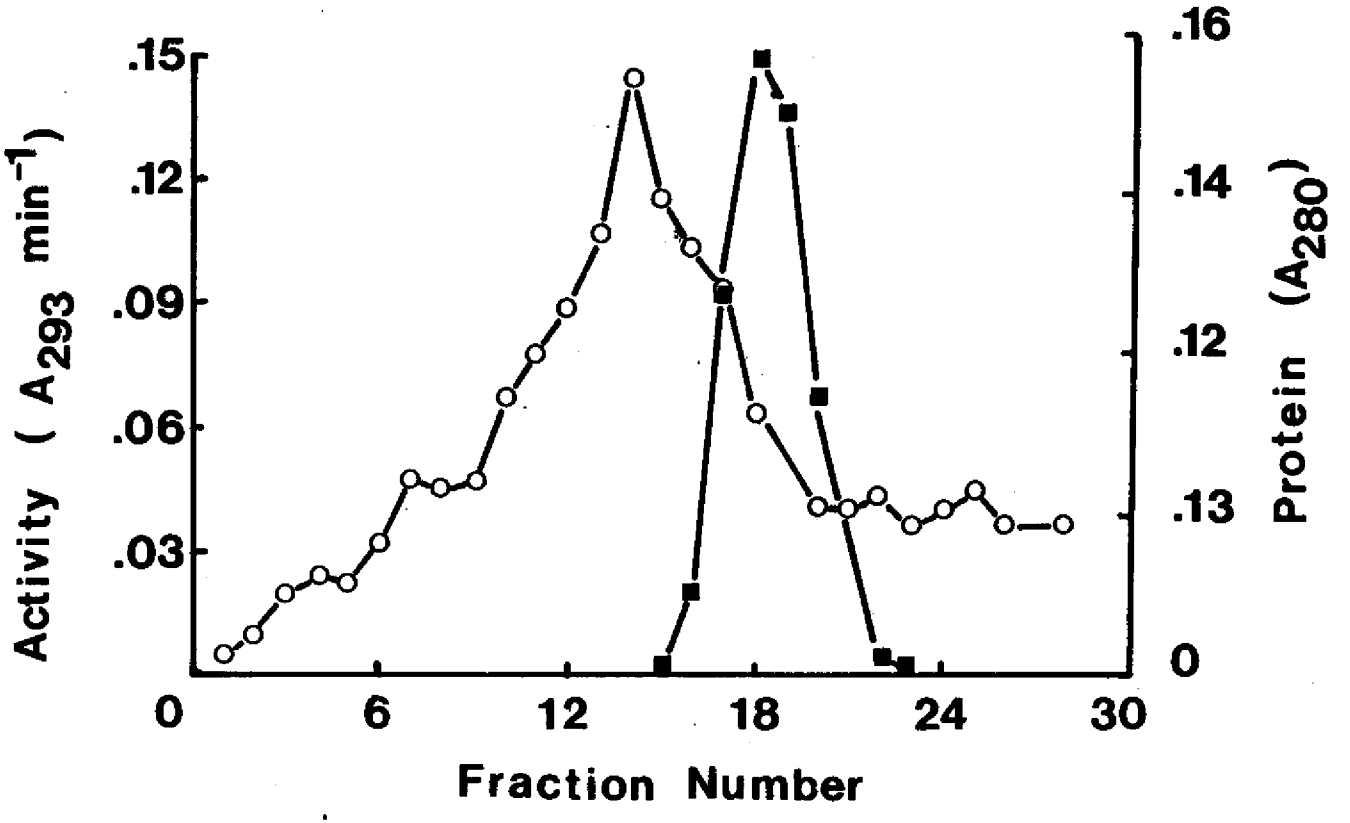


FIGURE 8. DEAE-Sephacel ion exchange chromatography of NP on a 1.8 x 80 cm column. Elution was obtained with an 80 ml gradient of from 0.05 to 0.12 M sodium citrate pH 6.0. Fraction volume was 4.0 ml. Activity (■), Protein (O).

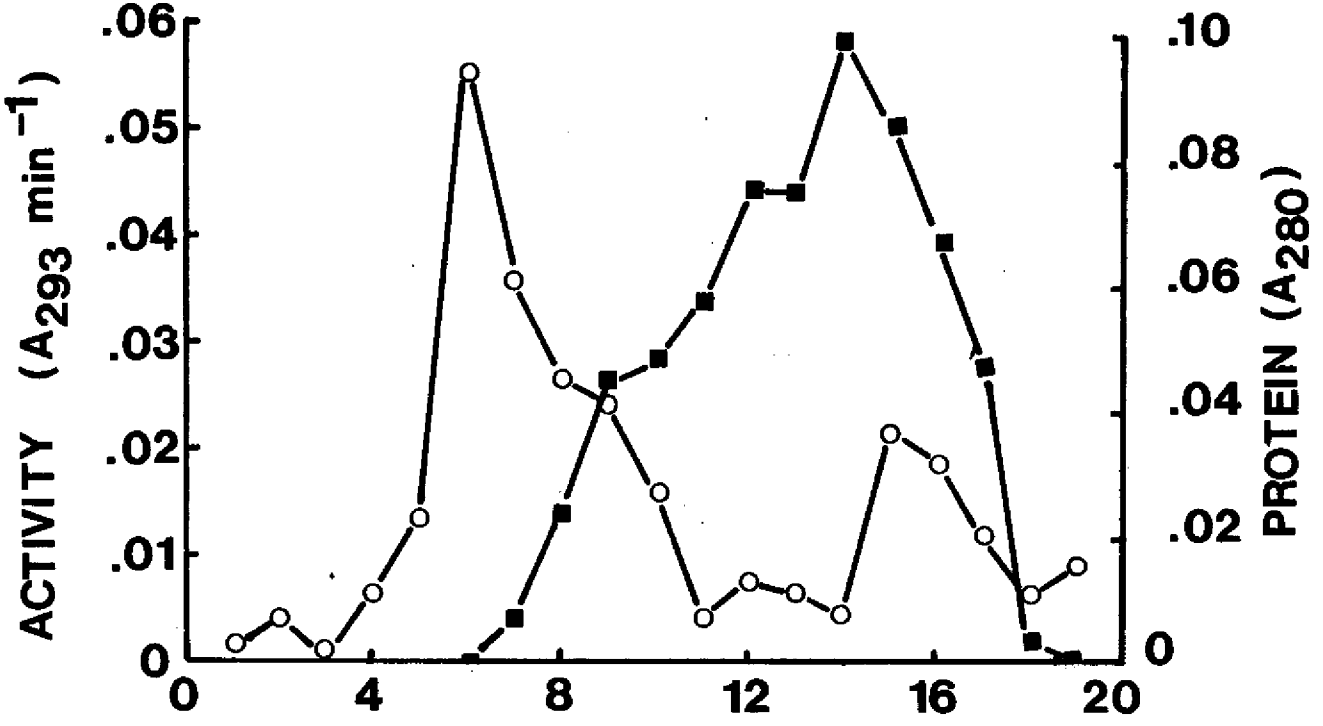


FIGURE 9. Affinity chromatography of NP on a 1.1 x 8.0cm Agadenosine column. Elution was accomplished with a KCl gradient of from 0 to 1.5 M in 0.01 M Pipes - HCl, ph 7.0. Fraction volume was 2.0 ml. Activity (■), Protein (O).

Fig 10 Gill et al, 1981

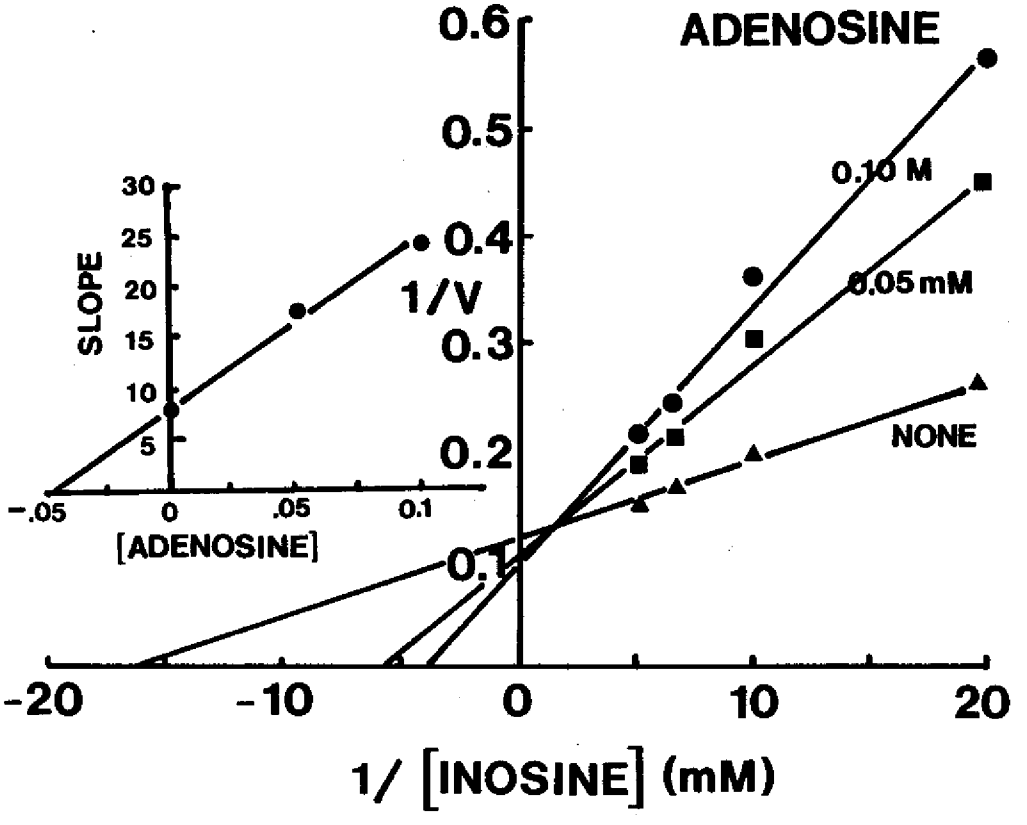


FIGURE 10. Double reciprocal plots of initial velocity with respect to inosine concentration at different adenosine concentrations. The replot of the slopes with respect to adenosine is given in the inset. All assays were performed in duplicate at 25°C.

TABLE 2

Kinetic Parameters Estimated from the Replots of the Slopes and Intercepts of the Lineweaver-Burk Plots in Figs. 7 and 8.

Parameter	M	μmoles mg^{-1} min^{-1}
Inosine, Km	3.8×10^{-5}	-
Inosine, Kia	1.4×10^{-4}	-
Guanosine, Km	2.9×10^{-5}	-
Guanosine, Kia	2.5×10^{-5}	-
Phosphate, Km		
with Inosine	1.30×10^{-5}	-
with Guanosine	1.45×10^{-5}	-
Vmax (Inosine)	-	10.26
Vmax (Guanosine)	-	5.80

Kinetic analysis with adenosine as the inhibitor of inosine phosphorolysis was undertaken to determine if the site of attachment was the same as the active site for inosine binding. Figure 10 illustrates the typical pattern of competitive inhibition, indicating that adenosine is attached to the active site of NP. The Ki of adenosine was determined to be 4.9×10^{-5} M from the replot (inset, Fig. 10).

SUMMARY AND CONCLUSIONS

The breakdown of Ino in Atlantic cod tissue is at least in part due to bacterial nucleoside phosphorylase. Very little nucleoside phosphorylase or inosine nucleosidase was recovered from sterile cod muscle and the production of Hx was delayed by at least 2 days in sterile fillets as compared to non-sterile fillets from the same fish. The fact that inosine breakdown occurred at a more rapid rate in sterile and non-sterile fillets than in whole, gutted cod, suggested that mechanical disruption of the tissue accelerated the formation of Hx.

Nucleoside phosphorylase recovered from spoilage bacteria was purified and characterized. NP had an apparent molecular weight of 120,000, an isoelectric point of 6.8 and catalysed the phosphorylysis of both inosine and guanosine. NP was competitively inhibited by adenosine.

ACKNOWLEDGEMENTS

This research was supported in part by a Strategic Grant provided by the Natural Sciences and Engineering Research Council of Canada. The grant was awarded to author Gill.

REFERENCES

1. Uchiyama, H., Ehira, S., Kobayashi, H. and W. Shimizu. 1970. The current studies on the freshness of fish with special reference to nucleic acids and their related compounds. Bull. Jap. Soc. Sci. Fish. 36:977-982.

2. Hiltz, D.F., Dyer, W.J., Nowlan, S. and J.R. Dingle. 1971. "Variations of biochemical quality idices by biological and technological factors in fish inspection and quality control" p.191. In Fish Inspection and Quality Control. R. Kreuzer, ed., Fishing News Books Ltd., London.

3. Watts, R.L. and D.C. Watts. 1974. "Nitrogen Metabolism in Fish" p.396. In Chemical Zoology, Vol. VIII. M. Florkin adn B. Sheer, eds., Academic Press, New York.

4. Jones, N.R. and J. Murray. 1964. Rapid measures of nucleotide dephosphorylation in iced fish muscle. Their value as indices of freshness and of inosine 5'monophosphate concentration. J. Sci. Food Agr. 15:684-689.

5. Tarr, H.L.A. 1955. Fish muscle riboside hydrolases. Biochem. J. 59:386-391.

6. Tarr, H.L.A. 1958. Lingcod muscle purine nucleoside phosphorylase. Can. J. Biochem. Physiol. 36:517-530.

7. LeBlanc, P.J. 1987. Approaches to the study of nucleotide catabolism for fish freshness evaluation. M.Sc. Thesis. Dept. of Food Science and Technology, Technical University of Nova Scotia.

8. Boehringer Mannheim. 1975. "Biochemica Information" p.111. Boehringer Mannheim GmbH, Penzberg, W. Germany.

9. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and R.J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265-275.

10. Kalckar, H.M. 1947. Differential spectrophotometry of purine compounds by means of specific enzymes. J. Biol. Chem. 167: 429-443.

11. Fiers, W. and J. De Barsaques. 1962. The reaction mechanism of a nucleoside phosphorylase. Enzymologia 24:197.210.

A Rapid Color Test Strip for Estimating Fish Freshness

Kwan Wong and Tom Gill

Canadian Institute of Fisheries Technology

Technical University of Nova Scotia

P.O. Box 1000

Halifax, Nova Scotia

B3J 2X4

INTRODUCTION

Traditionally, freshness of fish have been assessed by sensory methods which are subjective in nature. The criteria commonly used to grade fish freshness are texture, color, odor and taste. Ultimately sensory evaluation is the most satisfactory for the consumers. However, the procedure is both laborious and expensive since many trained graders and tasters are required to test large numbers of fish. Furthermore, the qualitative and quantitative characteristics of the criteria mentioned above are extremely difficult to assess and convey to others. For these reasons many objective tests, mostly chemical in nature, have been proposed.

A number of chemicals have been reported as useful indicators of fish freshness. Some of these include: nucleotides, hypoxanthine, total volatile bases, and trimethylamine. Trimethylamine is perhaps one of the most widely used. It has been used as an objective indicator for bacterial spoilage in seafood for over 40 years. Several studies have shown a high positive correlation between bacterial numbers and TMA values for various fish species, (Dyer et al., 1946; Laycock and Regier, 1971). Several studies have also reported excellent correlations between subjective determination of eating quality and TMA levels (Castell and Greenough, 1958; Hoogland , 1958).

The Dyer method (1945) as modified by Tozawa et al. (1971) has been used for many years for the determination of TMA in fish. Some other methods of analysis include gas chromatography (Ruiter, 1973; Miller et al., 1972; Kuwata et al., 1980); ion

specific electrode (Chang et al., 1976); high pressure liquid chromatography (Gill and Thompson, 1984); and enzyme assay (Large and McDougall, 1975; Wong and Gill, 1987). These methods share one or more of the following disadvantages: lack of specificity, cannot be performed outside the laboratory, expensive and time consuming.

Improvement over the above methods for estimating TMA have recently been developed in our laboratory. The method is also based on the TMA dehydrogenase reaction of Wong and Gill (1987), but using dry reagent chemistry on a test strip rather than a test tube reaction. The entire procedure requires 5 min at room temperature. The technique is sufficiently simple so that personnel with little or no laboratory experience can perform the test.

MATERIALS AND METHODS

Strictly fresh cod (Gadus morhua) and hake (Urophycus tenuis) were purchased locally, filleted and stored at 2°C. Samples of the fillets were used periodically for perchloric acid (PCA) or trichloracetic acid (TCA) extractions as described in Woyewoda et al. (1986). Fish juice was prepared with a modified 20 ml plastic syringe. The modification consisted of drilling 3 rows of holes (1.5 mm in diameter) 3-4 mm apart at the lower 1.5 cm of the barrel. A row of holes was also drilled at the end of the syringe. The opening of the syringe was plugged by the lock end of the needle. Squeezing 5-6 g of fish muscle in the syringe yielded 0.3-0.5 ml of juice. The extracts and juice were diluted to a workable level before use.

TMA ESTIMATION

Typically, a 10 ml disposable beaker contained 1 ml of a 0.2 M MOPS (3- (N-morpholino) propanesulfonic acid) buffer (pH 7.5) and 0.1 ml of the diluted fish juice, neutralized TCA or PCA extracts. A test strip was immersed in the solution, swirled once and then allowed to react at room temperature (19-21°C). After 5 min the color test strip was rinsed in water and the TMA concentration estimated by matching the intensity of the red color with the color of a set of TMA standards. Five inexperienced judges were asked to estimate the TMA levels using TMA standards of 0, 0.5, 1, 3, 5.5 and 7 µg TMA-N. Storing the reacted strips in 10% H_3PO_4 at 2°C in the dark preserved the color for a few days.

Trimethylamine in fish extracts was also estimated by the picric acid method of Dyer (1945) as modified by Tozawa (1971).

RESULTS AND DISCUSSION

The test strip was specific for TMA. Compounds such as trimethylamine oxide (TMA-O), dimethylamine (DMA), monomethylamine and ammonia did not react and did not interfere with the reaction. The minimum detectable level for TMA was 0.25 µg TMA-N. The best working range was between 0.5 and 3 µg with saturating level at 7 µg TMA-N.

Neutralized TCA extract, neutralized PCA extract or fish pressed juice worked equally well. Using 7 day old cod (2°C) the pressed fish juice (17 times diluted) showed a slightly darker pink color than either the neutralized TCA or PCA extracts of the same dilution. When diluted 35 times or more, however, no detectable color differences were observed for the 3 different methods of sample preparation. Similar results were found for hake extracts.

Figures 1 and 2 illustrate the estimation of TMA levels in aged cod and hake fillets with the test strip and picric acid assays. Excellent agreement was observed for the 2 methods. The TMA-N values for the picric acid method were averages of 2 determinations while the values for the test strip method were averages of 4 determinations from 5 inexperienced judges. Regression of these data gave a linear relationship between the 2 methods with a slope of 0.65, intercept of 0.86 and r^2 of 0.99 for cod samples and a slope of 1.37, intercept of 1.01 and r^2 of 0.96 for hake samples.

The test strip assay was more rapid than any published methods for determining TMA in fish extracts. By immersing test strips in samples at 15 second intervals, at least 12 fish samples can be assayed within 10 minutes. The method was simple and inexpensive. The only equipment required was a knife, a modified syringe, disposable beakers and pipettes. Neither electrical power nor potent protein precipitants such as TCA or PCA were required to determine TMA in fish samples. Because of its simplicity, rapidity and reproducibility, the test trips will be useful for routine testing of microbiological quality of fish.

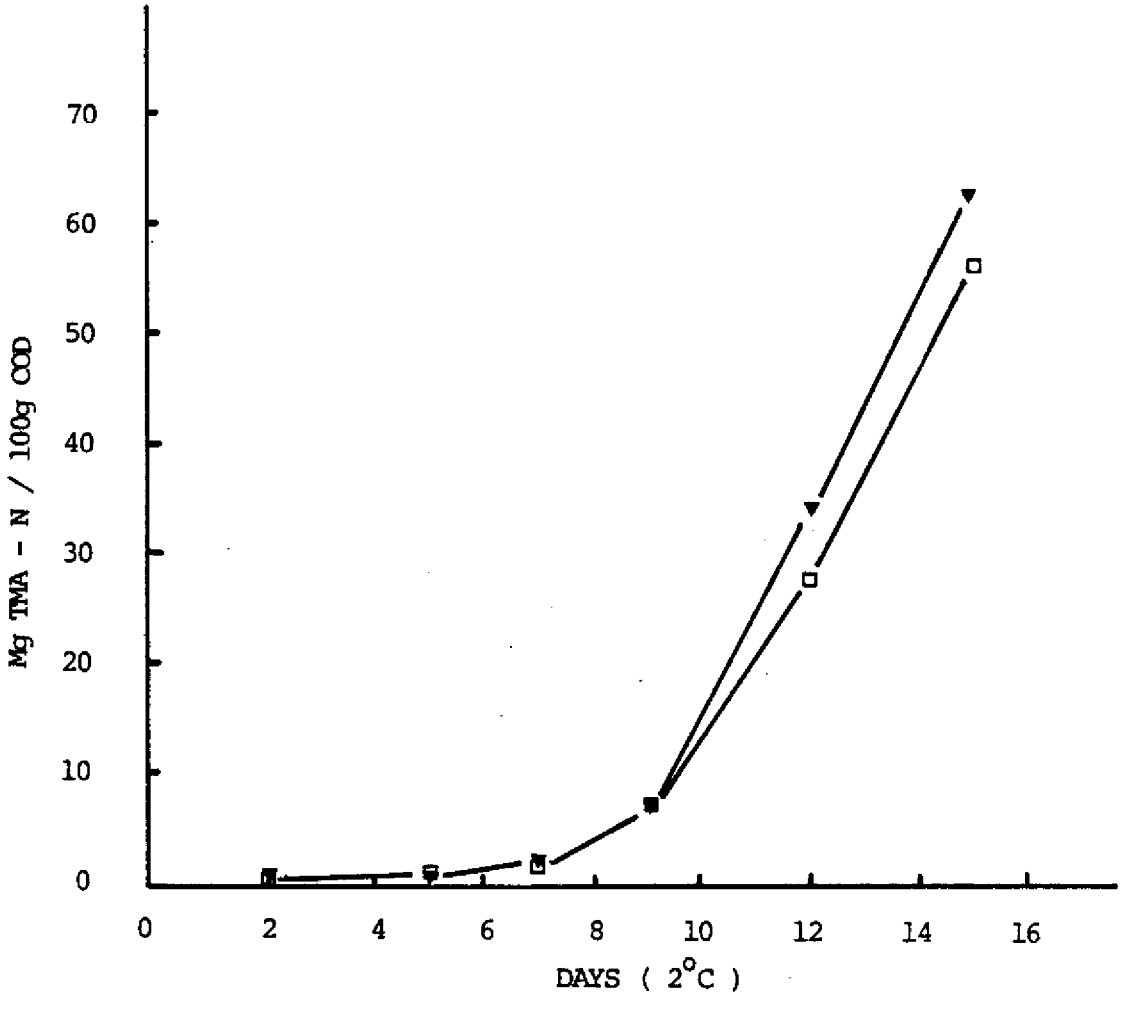


Figure 1. TMA determination by the picric acid (▼) and the test strip (□) methods.

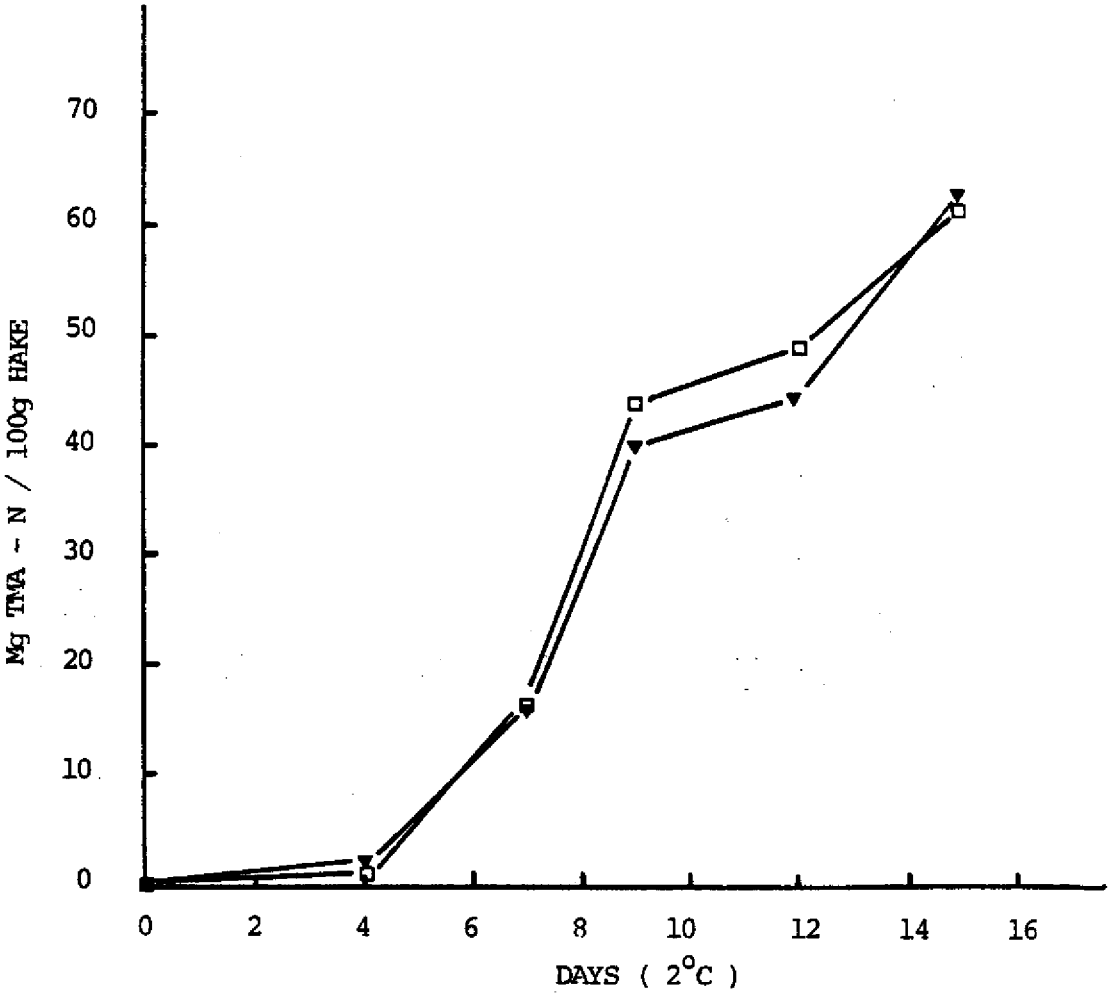


Figure 2. TMA determination by the picric acid (▼) and the test strip (□) methods.

REFERENCES

1. Castell, C.H., M.F. Greenough, R.S. Rogers and A.S. MacFarlane. 1958. Grading fish for quality. 1. Trimethylamine values of fillets cut from graded fish. J. Fish. R. Bd. Can. 15:701-716.

2. Chang, G.W., W.L. Chang and IK.B. Lew. 1976. Trimethylamine - specific electrode for fish quality control. J. Food. Sci. 41:723-724.

3. Dyer, W.J. 1945. Amines in fish muscle. I. Colorimetric determination of trimethylamine as the picrate salt. J. Fish. Res. Bd. Can. 6:351-358.

4. Dyer, W.J., F.E. Dyer and M. Snow. 1946. Amines in fish muscle. III. Spoilage of iced eviscerated cod. J. Fish. Res. Bd. Can. 6:403-413.

5. Gill, T.A. and J.W. Thompson. 1984. Rapid, automated analysis of amines in seafood by ion-moderate partition HPLC. J. Food Sci. 49:603-606.

6. Hoogland, P.L. 1958. Grading fish for quality. 2. Statistical analysis of the results of experiments regarding grades and trimethylamine values. J. Fish. Res. Bd. Can. 15:717-728.

7. Kuwata, K., Y. Yamazaki and M. Uebori. 1980. Determination of traces of low aliphatic amines by gas chromatography. Anal. Chem. 52:1980-1982.

8. Large, P.J. and H. McDougall. 1975. An enzymatic method for the microestimation of trimethylamine. Anal. Biochem. 64:304-310.

9. Laycock, R.A. and L.W. Regier. 1971. Trimethylamine - producing bacteria on haddock (Melanogrammus aegle-finus) fillets during refrigerated storage. J. Fish. Res. Bd. Can. 28:305-309.

10. Miller, A., R.A. Scanlan, J.S. Lee and L.M. Libbey. 1972. Quantitative and selective gas chromatographic analysis of dimethyl- and trimethylamine in fish. J. Agr. Food Chem. 20:709-711.

11. Ruiter, A. 1973. Determination of volatile amines and amine oxides in food products. Proc. Int. Symp. Nitrite Meat Prod. Zeist., Pudoc, Wageningen, pp 37-43.

12. Tozawa, H., K. Enokihara and K. Amano. 1971. Proposed modification of Dyer's method for trimethylamine determination in cod fish. In "Fish Inspection and Quality Control". Fishing News (Books) Ltd., London, pp. 187-190.

13. Wong, K. and T.A. Gill. 1987. Enzymatic determination of trimethylamine and its relationship to fish quality. J. Food Sci. 52:1-3.

14. Woyewoda, A.D., S.J. Shaw, P.J. Ke and B.G. Burns. 1986. Recommended laboratory methods for assessment of fish quality. Can. Tech. Rep. Fish. Aquatic Sci: No. 1448, pp 35-40. Dept. of Fisheries and Oceans P.O. Box 550, Halifax, N.S. B3J 2S7.

PERFORMANCE CHARACTERISTICS OF FDA'S SAMPLING PLANS FOR DECOMPOSITION OF FISH AND SEAFOOD

Alvin P. Rainosek
National Marine Fisheries Service, NOAA
National Seafood Inspection Laboratory
P. O. Drawer 1207, Pascagoula, MS 39568-1207

INTRODUCTION

Sampling plans are extensively used in practice as a means of gathering data to assist in determining whether or not the product produced (usually on a lot-by-lot basis) satisfies one or more specifications. Since the conclusions drawn from the sampling and analysis process are applied to the total production (lot) under evaluation, there always exist the possibility of making an incorrect decision on the actual lot status, i.e., rejection of a conforming lot or acceptance of a nonconforming lot.

Of interest then, or at least it should be, is to what extent does the sampling plan being used protect the producer/consumer against making incorrect decisions when faced with lots of varying quality. In this context, how a sampling plan performs, i.e., its so-called performance characteristics, is usually described by that plan's operating characteristic or OC curve. This curve indicates the chance of lot acceptance for lots of varying quality and can be presented in tabular and graphical form.

For the typical and simplest type of sampling plan, a 2 class plan (e.g., each sample unit selected is judged to be defective or nondefective relative to a certain specification), a few selected values on the OC curve are used to basically describe the performance of that plan. Conventionally, these selected values are the Acceptance Quality Level (AQL) and its associated Producer's or Seller's Risk of 5%, the Indifference Value (IV) and its corresponding Indifference Risk of 50%, and the Rejectable Quality Level (RQL) with its associated Consumer's or Buyer's Risk of 10%.

For example, suppose we are using a 2 class sampling plan that requires 12 sample units with an acceptance number of 1. This means that if 0 or 1 of the 12 sample units are defective, then the lot is accepted; but if two or more are defective, then the lot is rejected. This sampling plan (using the Binomial probability distribution) has the following performance characteristics:

AQL = 3.0% IV = 13.6% RQL = 28.8%

Interpretation: For a certain specification -

AQL: If the entire lot actually has 3.0% defective units, there is a 95% chance that this sampling plan will indicate lot acceptance (or a 5% chance of lot rejection).

IV: If the entire lot actually has 13.6% defective units, there is a 50% chance of lot acceptance (or a 50% chance of lot rejection).

RQL: If the entire lot actually has 28.8% defective units, there is a 10% chance of lot acceptance (or a 90% chance of lot rejection).

This paper presents the performance (operating) characteristics of sampling plans for decomposition used by the Food and Drug Administration (FDA) in four seafood categories: (1) Fish (Fresh or Frozen), Compliance Policy Guide (CPG) 7108.05, (2) Canned Salmon, CPG 7108.10, (3) Shrimp - Fresh or Frozen Raw Headless, Peeled or Breaded, CPG 7108.11, and (4) Canned Albacore, Skipjack and Yellow Fin Tuna, CPG 7108.24. These sampling plans, however, are not of the simple 2 class type as described above. For fish, shrimp and canned salmon, FDA uses several different 3 class plans corresponding to three classes of decomposition. For canned albacore, skipjack and yellow fin tuna, the FDA utilizes a 4 class sampling plan corresponding to four levels of histamine content.

For 3 and 4 class sampling plans, a simple description of their performance characteristics using one set of values for the AQL, IV and RQL is not appropriate. These values would have to be reported for one classification at various fixed levels of the other classifications and would quickly become complicated and of questionable practical use. Generally,the most useful presentation of the performance characteristics of 3 and 4 class sampling plans is to employ 2 and 3 - way tables. For example, the rows of a 2 - way table could represent various levels of Class 2 decomposition and the columns could denote different levels of Class 3 decomposition. The tabular value found in the intersection of each row with each column would then indicate for that sampling plan the chance of lot acceptance for those row and column values of Class 2 and Class 3 decomposition.

In this paper, 2 - way tables are presented which describe the performance characteristics of FDA's sampling plans for decomposition of fish and seafood. I want to thank and express my appreciation to the Division of Mathematics, FDA, for their cooperation and assistance in providing these tables.

EVALUATION OF FDA SAMPLING PLANS FOR DECOMPOSITION IN FISH AND SEAFOODS

The sampling plans of Compliance Policy Guides 7108.05, 7108.10, 7108.11, and 7108.24 were evaluated employing computer simulation and/or mathematical calculation involving either the uniform, binomial, multinomial, or double hypergeometric probability distributions or combinations of these in double and multi-stage sampling schemes.

A. Guide 7108.05 Fish (Fresh or Frozen) - Adulteration Involving Decomposition

1. Criteria for Decomposition - (1) 5% or more of the fish or fillets in the sample (but not less than 5) show Class 3 decomposition over at least 25% of their areas; or (2) 20% or more of the fish or fillets in the sample (but not less than 5) show Class 2 decomposition over at least 25% of their areas; or (3) the percentage of fish or fillets showing Class 2 decomposition as above, plus 4 times the percentage of those showing Class 3 decomposition as above, equals at least 20 percent and there are at least 5 decomposed fish or fillets in the sample.

2. Though the product is defined by three classes of decomposition, the rejection criteria involving these three classes was of sufficient difficulty to require computer simulation to evaluate the O. C. Curves. A program was written employing a uniform distribution (UD) with values ranging between 0 and 1. A lot, say, 80% Class 1, 10% Class 2, and 10% Class 3 decomposition was considered in one-to-one correspondence to UD; and, for example, if a randomly chosen value from UD times 100 was between 0 and 80 the value was considered in Class 1; if a randomly chosen value from UD times 100 was between 80 and 90, the value was considered in Class 2; and if a randomly chosen value from UD times 100 was between 90 and 100, the value was considered in Class 3. The random sampling from UD and the value transformations were replicated until 50 values (the number of fish up to 3 lbs in a sample) were chosen. The criteria for decomposition was then used to test the sample to assess the pass/fail status. Approximately 1000 random samples of 50 subsamples each were subjected to the criteria for decomposition for each specified percentage of Class 1, Class 2, and Class 3 decompositions.

3. The following table provides simulated O. C. values (%) for the probability of accepting a lot of bulk fish, each weighing up to 3 lbs., based on a random sample of 50 fish. In this table, Percent Class 2 represents the percent of fish in the lot with Class 2 decomposition over at least 25% of their areas, and Percent Class 3 denotes the percent of fish in the lot with Class 3 decomposition over at least 25 % of their areas.

		Percent Class 3						
		0	1	3	5	10	15	20
Percent Class 2	0	100.0	100.0	97.6	90.2	40.6	11.6	4.0
	1	100.0	99.8	94.6	83.0	32.0	10.8	2.8
	3	100.0	98.2	84.0	62.0	20.6	3.8	0.6
	5	100.0	94.4	75.4	47.8	14.4	1.4	0.2
	10	97.0	80.8	46.8	21.6	2.6	0.4	0.0
	15	80.6	61.8	23.2	11.2	1.2	0.0	0.0
	20	45.2	27.4	8.6	3.8	0.0	0.0	0.0

B. Guide 7108.10 Canned Salmon - Adulteration Involving Decomposition

Regulatory Action Guidance:

The following represents criteria for direct reference seizure to ACRA, and for direct citation by District Offices.

Examination of the entire sample by two qualified organoleptic analysts in the Seattle District in accordance with the sampling plans (Tables I and II) show either:

1. A number of defective cans that equals or exceeds the numbers required for action (Tables I and II), or

2. Two or more Class II cans in either the first or total sample. Note: One Class III can in the first sample of any lot size is a basis for resampling. The term "defective cans" as used in the criteria above and in Tables I and II includes both Class II cans and Class III cans.

3. The probabilities of acceptance were determined by using a multinomial in three classes converted to a double hypergeometric as the underlying probability distribution for a double sampling plan. Specified percentages of Class I, Class II, and Class III were defined and used as the parameters to calculate the probabilities for a double sampling plan. Table I, Plans 1-7 presents the sampling plans and probabilities of acceptance for cans of salmon weighing ¼ pounds through 1 pound. Table II, Plans 1-7 presents the sampling plans and probabilities of acceptance for cans of salmon weighing over 1 pound through 4 pounds.

FDA SALMON DECOMPOSITION SAMPLING PLANS
COMPLIANCE POLICY GUIDE 7108.10, CHAPTER 8 - FISH AND SEAFOOD
Table I

1/4 Pounds Through 1 Pound Cans

PLAN 1

Lot Size (Cases)	First Sample (Cans)	Defective Cans in First Sample For		Second Sample (cans)	Total Sample (cans)	Defective Cans in Total Sample For
		Legal Action	Resample			Legal Action
<100*	18	3	1-2	32	50	5

Note: a) Legal action numbers include both Class II and Class III cans.
b) Two or more Class III cans supports legal action.

Table (1) Probabilities of Acceptance (Percent).

		Percent Class II Cans in Lot							
		0	1	3	5	10	15	20	25
Percent Class III Cans in Lot	0	100	100	98.44	90.76	50.23	16.93	4.13	0.88
	1	94.88	93.66	89.18	78.52	38.52	12.04	2.87	0.62
	3	70.17	67.19	60.25	49.74	21.01	6.01	1.42	0.33
	5	47.08	43.47	36.53	28.59	10.97	3.03	0.74	0.18
	10	16.00	13.65	9.89	6.91	2.31	0.65	0.17	0.04
	15	5.43	4.44	2.94	1.92	0.59	0.17	0.04	0.01
	20	1.79	1.43	0.91	0.57	0.16	0.04	0.01	0.00
	25	0.56	0.44	0.27	0.16	0.04	0.01	0.00	0.00

* Calculations Based on 4752 cans (99 cases of 48 cans each).

FDA SALMON DECOMPOSITION SAMPLING PLANS
COMPLIANCE POLICY GUIDE 7108.10, CHAPTER 8 - FISH AND SEAFOOD
Table I

1/4 Pounds Through 1 Pound Cans

PLAN 2

Lot Size (Cases)	First Sample (Cans)	Defective Cans in First Sample For: Legal Action	Defective Cans in First Sample For: Resample	Second Sample (cans)	Total Sample (cans)	Defective Cans in Total Sample For: Legal Action
100-199*	20	3	1-2	48	68	9

Note: a) Legal action numbers include both Class II and Class III cans.
b) Two or more Class III cans supports legal action.

Table (2) Probabilities of Acceptance (Percent).

		Percent Class II Cans in Lot							
		0	1	3	5	10	15	20	25
Percent Class III Cans Lot	0	100	100	98.32	92.78	62.34	24.69	5.60	0.84
	1	92.21	90.50	86.10	78.94	48.05	16.81	3.48	0.52
	3	62.25	57.64	49.74	42.28	22.08	6.73	1.30	0.21
	5	39.06	34.16	26.48	20.57	9.19	2.59	0.51	0.10
	10	12.30	9.96	6.53	4.26	1.36	0.36	0.08	0.02
	15	3.87	3.06	1.89	1.16	0.32	0.08	0.02	0.00
	20	1.14	0.89	0.53	0.32	0.08	0.02	0.00	0.00
	25	0.32	0.24	0.14	0.08	0.02	0.00	0.00	0.00

*Calculations based on 9552 cans (199 cases of 48 cans each).

FDA SALMON DECOMPOSITION SAMPLING PLANS
COMPLIANCE POLICY GUIDE 7108.10, CHAPTER 8 - FISH AND SEAFOOD
Table I

1/4 Pounds Through 1 Pound Cans

PLAN 3

Lot Size (Cases)	First Sample (Cans)	Defective Cans in First Sample For: Legal Action	Defective Cans in First Sample For: Resample	Second Sample (cans)	Total Sample (cans)	Defective Cans in Total Sample For: Legal Action
200-499*	24	4	2-3	64	88	11

Note: a) Legal action numbers include both Class II and Class III cans.
b) Two or more Class III cans supports legal action.
c) One Class III can in first sample requires resample.

Table (3) Probabilities of Acceptance (Percent).

		Percent Class II Cans in Lot							
		0	1	3	5	10	15	20	25
Percent Class III Cans in Lot	0	100	100	99.61	97.07	69.53	25.12	5.24	1.02
	1	95.18	91.00	86.61	81.61	52.55	17.12	3.52	0.72
	3	63.35	56.62	48.90	42.17	22.60	6.97	1.59	0.36
	5	35.25	31.79	26.51	21.39	9.88	3.08	0.78	0.18
	10	8.18	7.83	6.50	4.92	1.92	0.59	0.15	0.03
	15	2.03	1.96	1.60	1.18	0.42	0.12	0.03	0.00
	20	0.47	0.45	0.36	0.26	0.09	0.02	0.00	0.00
	25	0.10	0.10	0.08	0.05	0.02	0.00	0.00	0.00

* Calculations based on 23952 cans (499 cases of 48 cans each).

FDA SALMON DECOMPOSITION SAMPLING PLANS
COMPLIANCE POLICY GUIDE 7108.10, CHAPTER 8 - FISH AND SEAFOOD
Table I

1/4 Pounds Through 1 Pound Cans

PLAN 4

Lot Size (Cases)	First Sample (Cans)	Defective Cans in First Sample For: Legal Action	Defective Cans in First Sample For: Resample	Second Sample (cans)	Total Sample (cans)	Defective Cans in Total Sample For: Legal Action
500-799*	30	4	2-3	80	110	17

Note: a) Legal action numbers include both Class II and Class III cans.
b) Two or more Class III cans supports legal action.
c) One Class III can in first sample requires resample.

Table (4) Probabilities of Acceptance (Percent).

		Percent Class II Cans in Lot							
		0	1	3	5	10	15	20	25
Percent Class III Cans in Lot	0	100	100	98.93	94.08	64.63	28.45	6.16	0.62
	1	84.08	83.43	79.44	72.08	44.47	16.58	2.96	0.28
	3	43.38	42.26	36.36	29.01	13.47	4.08	0.66	0.08
	5	22.03	21.23	17.15	12.49	4.46	1.14	0.20	0.03
	10	4.24	4.05	3.12	2.11	0.59	0.13	0.02	0.00
	15	0.76	0.73	0.54	0.36	0.09	0.02	0.00	0.00
	20	0.12	0.12	0.09	0.05	0.01	0.00	0.00	0.00
	25	0.02	0.02	0.01	0.01	0.00	0.00	0.00	0.00

* Calculations based on 38352 cans (799 of 48 cans each).

FDA SALMON DECOMPOSITION SAMPLING PLANS
COMPLIANCE POLICY GUIDE 7108.10, CHAPTER 8 - FISH AND SEAFOOD
Table I

1/4 Pounds Through 1 Pound Cans

PLAN 5

Lot Size (Cases)	First Sample (Cans)	Defective Cans in First Sample For: Legal Action	Defective Cans in First Sample For: Resample	Second Sample (cans)	Total Sample (cans)	Defective Cans in Total Sample For: Legal Action
800-999*	36	5	2-4	96	132	23

Note: a) Legal action numbers include both Class II and Class III cans.
b) Two or more Class III cans supports legal action.
c) One Class III can in first sample requires resample.

Table (5) Probabilities of Acceptance (Percent).

		Percent Class II Cans in Lot							
		0	1	3	5	10	15	20	25
Percent Class III Cans in Lot	0	100	100	99.71	96.94	71.23	34.70	9.32	0.99
	1	79.36	78.38	73.67	67.04	43.64	18.68	3.86	0.32
	3	35.41	33.99	27.25	20.20	8.57	2.83	0.46	0.10
	5	16.00	15.15	11.22	7.31	2.01	0.45	0.06	0.00
	10	2.25	2.11	1.49	0.90	0.18	0.03	0.00	0.00
	15	0.29	0.27	0.18	0.11	0.02	0.00	0.00	0.00
	20	0.03	0.03	0.02	0.01	0.00	0.00	0.00	0.00
	25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

* Calculations based on 47952 cans (999 cases of 48 cans each).

FDA SALMON DECOMPOSITION SAMPLING PLANS
COMPLIANCE POLICY GUIDE 7108.10, CHAPTER 8 - FISH AND SEAFOOD
Table I

1/4 Pounds Through 1 Pound Cans

PLAN 6

Lot Size (Cases)	First Sample (Cans)	Defective Cans in First Sample For: Legal Action	Defective Cans in First Sample For: Resample	Second Sample (cans)	Total Sample (cans)	Defective Cans in Total Sample For: Legal Action
1000-1499*	42	6	2-5	112	154	26

Note: a) Legal action numbers include both Class II and Class III cans.
b) Two or more Class III cans supports legal action.
c) One Class III can in first sample requires resample.

Table (6) Probabilities of Acceptance (Percent).

		Percent Class II Cans in Lot							
		0	1	3	5	10	15	20	25
Percent Class III Cans in Lot	0	100	100	99.96	98.41	76.18	37.38	8.99	0.70
	1	74.64	73.22	67.06	60.45	40.81	17.10	2.89	0.16
	3	29.02	27.35	20.10	13.56	5.13	1.60	0.20	0.01
	5	11.68	10.85	7.31	4.23	0.86	0.15	0.02	0.00
	10	1.20	1.10	0.71	0.38	0.05	0.00	0.00	0.00
	15	0.11	0.10	0.06	0.03	0.00	0.00	0.00	0.00
	20	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00
	25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

* Calculations based on 71952 cans (1499 cases of 48 cans each).

FDA SALMON DECOMPOSITION SAMPLING PLANS
COMPLIANCE POLICY GUIDE 7108.10, CHAPTER 8 - FISH AND SEAFOOD
Table I

1/4 Pounds Through 1 Pound Cans

PLAN 7

Lot Size (Cases)	First Sample (Cans)	Defective Cans in First Sample For: Legal Action	Defective Cans in First Sample For: Resample	Second Sample (cans)	Total Sample (cans)	Defective Cans in Total Sample For: Legal Action
>1500*	48	6	2-5	128	176	30

Note: a) Legal action numbers include both Class II and Class III cans.
b) Two or more Class III cans supports legal action.
c) One Class III can in first sample requires resample.

Table (7) Probabilities of Acceptance (Percent).

		Percent Class II Cans in Lot							
		0	1	3	5	10	15	20	25
Percent Class III Cans in Lot	0	100	100	99.71	96.84	65.32	25.09	4.63	0.25
	1	70.00	68.08	60.05	52.03	30.00	10.06	1.30	0.05
	3	23.88	22.03	14.69	8.81	2.51	0.62	0.06	0.00
	5	8.56	7.79	4.76	2.42	0.34	0.04	0.00	0.00
	10	0.64	0.57	0.33	0.16	0.02	0.00	0.00	0.00
	15	0.04	0.04	0.02	0.01	0.00	0.00	0.00	0.00
	20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

* Probabilities Stable for Lot Sizes Greater Than 1500 Cases of 48 Cans Each.

FDA SALMON DECOMPOSITION SAMPLING PLANS
COMPLIANCE POLICY GUIDE 7108.10, CHAPTER 8 - FISH AND SEAFOOD
TABLE II

(over 1 pound through 4 pound cans)

PLAN 1

Lot Size (Cases)	First Sample (Cans)	Defective Cans in First Sample For: Legal Action	Defective Cans in First Sample For: Resample	Second Sample (cans)	Total Sample (cans)	Defective Cans in Total Sample For: Legal Action
<100*	6	2	1	12	18	2

Note: a) Legal action numbers include both Class II and Class III cans.
b) Two or more Class III cans supports legal action.

Table II (1) Probabilities of Acceptance (Percent).

		Percent Class II Cans in Lot							
		0	1	5	10	15	25	35	45
Percent Class III cans in Lot	0	100	99.64	86.23	63.13	43.30	18.84	7.63	2.75
	1	99.64	97.10	81.51	58.69	39.96	17.26	6.92	2.46
	5	86.23	81.51	63.05	43.27	28.80	12.11	4.67	1.55
	10	63.13	58.69	43.27	28.80	18.83	7.63	2.75	0.82
	15	43.30	39.96	28.80	18.83	12.11	4.67	1.55	0.40
	25	18.84	17.26	12.11	7.63	4.67	1.55	0.40	0.07
	35	7.63	6.93	4.67	2.75	1.55	0.40	0.07	0.01
	45	2.75	2.46	1.55	0.82	0.40	0.07	0.01	0.00

* Calculations based on 1188 cans (99 cases of 12 cans each).

FDA SALMON DECOMPOSITION SAMPLING PLANS
COMPLIANCE POLICY GUIDE 7108.10, CHAPTER 8 - FISH AND SEAFOOD
TABLE II

(over 1 pound through 4 pound cans)

PLAN 2

Lot Size (Cases)	First Sample (Cans)	Defective Cans in First Sample For: Legal Action	Defective Cans in First Sample For: Resample	Second Sample (cans)	Total Sample (cans)	Defective Cans in Total Sample For: Legal Action
100-199*	7	2	1	16	23	2

Note: a) Legal action numbers include both Class II and Class III cans.
b) Two or more Class III cans supports legal action.

Table II (2) Probabilities of Acceptance (Percent).

		Percent Class II Cans in Lot							
		0	1	5	10	15	25	35	45
Percent Class III Cans in Lot	0	100	99.06	81.21	54.68	34.93	13.62	4.90	1.51
	1	99.06	95.79	75.57	50.09	31.86	12.35	4.39	1.33
	5	81.21	75.57	54.65	34.93	21.95	8.29	2.79	0.77
	10	54.68	50.09	34.93	21.95	13.62	4.90	1.51	0.37
	15	34.93	31.86	21.95	13.62	8.29	2.79	0.77	0.16
	25	13.62	12.35	8.29	4.90	2.79	0.77	0.16	0.02
	35	4.90	4.39	2.79	1.51	0.77	0.16	0.02	0.00
	45	1.51	1.33	0.77	0.37	0.16	0.02	0.00	0.00

* Calculations based on 2388 cans (199 cases of 12 cans each).

FDA SALMON DECOMPOSITION SAMPLING PLANS
COMPLIANCE POLICY GUIDE 7108.10, CHAPTER 8 - FISH AND SEAFOOD
TABLE II

(over 1 pound through 4 pound cans)

PLAN 3

Lot Size (Cases)	First Sample (Cans)	Defective Cans in First Sample For: Legal Action	Defective Cans in First Sample For: Resample	Second Sample (cans)	Total Sample (cans)	Defective Cans in Total Sample For: Legal Action
200-499*	8	2	1	22	30	2

Note: a) Legal action numbers include both Class II and Class III cans.
b) Two or more Class III cans supports legal action.

Table II (3) Probabilities of Acceptance (Percent).

		Percent Class II Cans in Lot							
		0	1	5	10	15	25	35	45
	0	100	98.35	75.38	46.79	28.30	10.04	3.18	0.83
	1	98.35	93.98	68.91	42.33	25.58	9.01	2.81	0.72
Percent	5	75.38	68.91	46.78	28.30	17.00	5.76	1.67	0.39
Class III	10	46.79	42.33	28.30	17.00	10.04	3.18	0.83	0.17
Cans in	15	28.30	25.58	17.00	10.04	5.76	1.67	0.39	0.07
Lot	25	10.04	9.01	5.76	3.18	1.67	0.39	0.07	0.01
	35	3.18	2.81	1.67	0.83	0.39	0.07	0.01	0.00
	45	0.83	0.72	0.42	0.17	0.07	0.01	0.00	0.00

* Calculation based on 5988 cans (499 cases of 12 cans each).

FDA SALMON DECOMPOSITION SAMPLING PLANS
COMPLIANCE POLICY GUIDE 7108.10, CHAPTER 8 - FISH AND SEAFOOD
TABLE II

(over 1 pound through 4 pound cans)

PLAN 4

Lot Size (Cases)	First Sample (Cans)	Defective Cans in First Sample For: Legal Action	Defective Cans in First Sample For: Resample	Second Sample (cans)	Total Sample (cans)	Defective Cans in Total Sample For: Legal Action
500-799*	10	2	1	27	37	3

Note: a) Legal action numbers include both Class II and Class III cans.
b) Two or more Class III cans supports legal action.

Table II (4) Probabilities of Acceptance (Percent).

		Percent Class II Cans in Lot							
		0	1	5	10	15	25	35	45
Percent Class III cans in Lot	0	100	99.42	79.03	43.87	22.15	5.70	1.34	0.25
	1	97.51	95.37	71.15	38.29	19.31	4.97	1.15	0.21
	5	67.80	63.71	42.16	21.92	11.20	2.83	0.60	0.10
	10	37.11	33.77	21.24	11.12	5.69	1.34	0.25	0.03
	15	20.10	17.96	10.98	5.68	2.83	0.60	0.10	0.00
	25	5.63	4.93	2.82	1.34	0.60	0.10	0.01	0.00
	35	1.34	1.15	0.60	0.25	0.10	0.01	0.00	0.00
	45	0.25	0.21	0.10	0.03	0.01	0.00	0.00	0.00

* Calculations based on 9588 cans (799 cases of 12 cans each).

FDA SALMON DECOMPOSITION SAMPLING PLANS
COMPLIANCE POLICY GUIDE 7108.10, CHAPTER 8 - FISH AND SEAFOOD
TABLE II

(over 1 pound through 4 pound cans)

PLAN 5

Lot Size (Cases)	First Sample (Cans)	Defective Cans in First Sample For: Legal Action	Defective Cans in First Sample For: Resample	Second Sample (cans)	Total Sample (cans)	Defective Cans in Total Sample For: Legal Action
800-999*	11	3	1-2	30	41	4

Note: a) Legal action numbers include both Class II and Class III cans.
b) Two or more Class III cans supports legal action.

Table II (5) Probabilities of Acceptance (Percent).

		Percent Class II Cans in Lot							
		0	1	5	10	15	25	35	45
Percent Class III Cans in Lot	0	100	100	88.54	51.09	23.01	4.43	0.88	0.14
	1	97.0	96.33	80.51	43.76	19.38	3.78	0.74	0.11
	5	63.98	60.93	43.60	21.66	9.75	2.00	0.36	0.05
	10	33.00	29.97	18.98	9.33	4.36	0.88	0.14	0.02
	15	16.97	15.01	8.89	4.30	1.99	0.36	0.05	0.00
	25	4.22	3.64	1.98	0.87	0.36	0.05	0.00	0.00
	35	0.87	0.74	0.36	0.14	0.05	0.00	0.00	0.00
	45	0.14	0.11	0.05	0.02	0.00	0.00	0.00	0.00

* Calculations based on 11988 cans (999 cases of 12 cans each).

FDA SALMON DECOMPOSITION SAMPLING PLANS
COMPLIANCE POLICY GUIDE 7108.10, CHAPTER 8 - FISH AND SEAFOOD
TABLE II

(over 1 pound through 4 pound cans)

PLAN 6

Lot Size (Cases)	First Sample (Cans)	Defective Cans in First Sample For: Legal Action	Defective Cans in First Sample For: Resample	Second Sample (cans)	Total Sample (cans)	Defective Cans in Total Sample For: Legal Action
1000-1499*	12	3	1-2	32	44	5

Note: a) Legal action numbers include both Class II and Class III cans.
b) Two or more Class III cans supports legal action.

Table II (6) Probabilities of Acceptance (Percent).

		Percent Class II Cans in Lot							
		0	1	5	10	15	25	35	45
Percent Class III Cans in Lot	0	100	100	94.92	64.72	30.37	4.06	0.58	0.08
	1	96.50	95.94	86.76	54.12	24.06	3.26	0.48	0.06
	5	60.67	57.47	43.35	22.66	9.46	1.46	0.22	0.02
	10	29.53	26.52	16.69	8.00	3.44	0.57	0.08	0.00
	15	14.38	12.56	7.16	3.28	1.41	0.22	0.02	0.00
	25	3.17	2.70	1.38	0.57	0.22	0.02	0.00	0.00
	35	0.57	0.47	0.22	0.08	0.02	0.00	0.00	0.00
	45	0.08	0.06	0.02	0.01	0.00	0.00	0.00	0.00

* Calculations based on 17988 cans (1499 cases of 12 cans each).

FDA SALMON DECOMPOSITION SAMPLING PLANS
COMPLIANCE POLICY GUIDE 7108.10, CHAPTER 8 - FISH AND SEAFOOD
TABLE II

(over 1 pound through 4 pound cans)

PLAN 7

Lot Size (Cases)	First Sample (Cans)	Defective Cans in First Sample For: Legal Action	Defective Cans in First Sample For: Resample	Second Sample (cans)	Total Sample (cans)	Defective Cans in Total Sample For: Legal Action
>1500*	16	3	1-2	38	54	6

Note: a) Legal action numbers include both Class II and Class III cans.
b) Two or more Class III cans supports legal action.

Table II (7) Probabilities of Acceptance (Percent).

		Percent Class II Cans in Lot							
		0	1	5	10	15	25	35	45
	0	100	99.95	93.66	59.00	22.37	1.44	0.10	0.01
	1	94.54	93.69	82.96	46.54	16.23	1.06	0.08	0.01
Percent	5	49.29	45.29	31.05	13.75	4.35	0.36	0.03	0.00
Class III	10	19.13	16.38	8.64	3.32	1.10	0.10	0.01	0.00
Cans in	15	7.47	6.21	2.90	1.03	0.34	0.03	0.00	0.00
Lot	25	1.00	0.81	0.33	0.10	0.03	0.00	0.00	0.00
	35	0.10	0.08	0.03	0.01	0.00	0.00	0.00	0.00
	45	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00

* Probabilities Stable for Lot Sizes Greater Than 1500 Cases of 12 Cans Each.

C. Guide 7108.11 Shrimp Fresh or Frozen Raw Headless, Peeled or Breaded Adulteration Involving Decomposition

1. Criteria for Decomposed Subs: A sub shall be classified as decomposed: If five percent (5%) or more of the shrimp are class 3; or if twenty percent (20%) or more of the shrimp are class 2; or if the percentage of class 2 shrimp plus 4 times the percentage of class 3 shrimp equals or exceeds twenty percent (20%).

2. The product is defined by three classes of decomposition. The probabilities of acceptance were determined using the simulation procedure described in A. 2 above to estimate the percent decomposed for specified percentages of class 1, class 2, and class 3 using the above criteria for decomposition. The estimated percentages were then used to determine the probabilities of acceptance based on the binomial distribution. The sampling plans are as follows:

NO. OF SUBS EXAMINED (N)	ACCEPTABLE NO. DECOMPOSED (C)
6	1
12	2
18	3

3. Presented in the following tables are the (a) simulated percent decomposed subsamples, (b) probability of accepting a lot using a N=6, C=1 plan, (c) probability of accepting a lot using a N=12, C=2 plan, and (d) probability of accepting a lot using a N=18, C=3 plan.

a.) Simulated Percent decomposed based on criteria for decomposition and assuming 100 shrimp per sub.

		Percent Class_3					
		0	1	3	5	10	15
Percent Class_2	0	0.0	0.4	19.8	55.6	97.8	99.8
	1	0.0	0.6	21.2	59.6	98.2	100.0
	3	0.0	0.8	23.2	59.8	99.2	100.0
	5	0.0	2.4	34.2	70.8	99.4	100.0
	10	0.2	15.8	52.0	86.0	99.6	100.0
	15	8.2	44.2	84.0	95.4	99.8	100.0
	20	55.2	77.2	96.8	99.6	100.0	100.0

b.) Probability of Accepting (%) A Lot Using A n=6, c=1 Sampling Plan

		Percent Class_3				
		0	1	3	5	10
Percent Class_2	0	100.00	99.98	66.03	6.52	0.00
	1	100.00	99.95	62.59	4.52	0.00
	3	100.00	99.91	57.71	4.19	0.00
	5	100.00	99.19	33.43	0.96	0.00
	10	99.99	75.76	9.17	0.03	0.00
	15	91.92	17.37	0.05	0.00	0.00
	20	6.79	0.30	0.00	0.00	0.00

c.) Probability of Accepting (%) A Lot Using A n=12, c=2 Sampling Plan

		Percent Class_3				
		0	1	3	5	10
Percent Class_2	0	100.00	100.00	56.54	0.70	0.00
	1	100.00	100.00	51.62	0.31	0.00
	3	100.00	99.99	44.83	0.29	0.00
	5	100.00	99.74	16.51	0.02	0.00
	10	100.00	70.80	1.37	0.00	0.00
	15	93.08	4.73	0.00	0.00	0.00
	20	0.76	0.00	0.00	0.00	0.00

d.) Probability of Accepting (%) A Lot Using A n=18, c=3 Sampling Plan

		Percent Class_3				
		0	1	3	5	10
Percent Class_2	0	100.00	100.00	50.97	0.08	0.00
	1	100.00	100.00	45.02	0.02	0.00
	3	100.00	100.00	37.06	0.02	0.00
	5	100.00	99.92	8.89	0.00	0.00
	10	100.00	68.58	0.23	0.00	0.00
	15	95.54	1.42	0.00	0.00	0.00
	20	0.09	0.00	0.00	0.00	0.00

D. Guide 7108.24 Decomposition and Histamine in Canned Albacore, Skipjack and Yellowfin Tuna

1. Criteria For Recommending Legal Action to the Division of Regulatory Guidance (DRG)

a.) Danger to health: The histamine content of any sample equals or exceeds 50 mg per 100 grams in both the original and check analysis; or referral to DRG is authorized where there is documented evidence of an illness associated with tuna containing levels of histamine lower than above.

b.) Decomposition: A minimum of two subsamples (cans) per samples equals or exceeds 20 mg histamine per 100 grams in both the original and check analysis.

c.) Referral: Referral to DRG is authorized where a minimum of two subsamples (cans) per samples contain between 10 and 20 mg histamine per 100 grams (both original and check analysis) and either odor or is present and confirmed by a qualified organoleptic analyst.

d.) For the purposes of this guide, 24 subsample of canned tuna will be examined organoleptically and for histamine.

2. Class Designations for Histamine Levels (mg/100 grams)

Class _1 - ≤ 9
Class _2 - ≥ 10 to 19 and either odor or honeycombing confirmed to be present as above.

Class _3 - ≥ 20 to 49
Class _4 - ≥ 50

3. Decision Rule For Four Class Sampling Plan

a.) The lot is accepted if the original analysis indicates lot acceptance (i.e., In a sample of size 24 cans, there are no more than one can with histamine levels in Class_2, and no more than one can with histamine level in Class_3, and no can with histamine levels in Class_4); or, the lot is accepted if the original analysis does not indicate lot acceptance and the check analysis indicates lot acceptance based on the above criteria for number of cans sampled and the acceptance numbers associated with Class_2, Class_3, and Class_4.

4. The probabilities of acceptance P(A) were determined by using a multinomial expression involving four class designations.

   P(A)=(N!/K1!K2!K3!K4!)(P1**K1)(P2**K2)(P3**K3)(P4**K4)
   where P1-P4 are the proportions of units having histamine levels in Class 1 through Class 4, respectively; and K1-K4 are the number of subsamples in N=24 that are permitted to have histamine levels within the class range levels.

5. Tables 1-7 present the probabilities (%) of acceptance based on the decision rule 3.a. above. In these tables, P2, P3, and P4 are the percentages of subsamples in the lot that fall in Class 2, Class 3, and Class 4, respectively.

Table 1.

P_2	P_3/P_4	0	1	3	5	10	15
	0	100	95.4	73.1	49.9	15.4	4.0
	5	88.5	76.6	52.5	33.7	9.6	2.4
	10	49.9	40.1	24.8	4.8	3.8	0.8
0	15	20.1	15.5	9.0	5.1	1.2	0.2
	20	6.5	4.9	2.8	1.4	0.4	0.0
	25	1.8	1.4	0.8	0.4	0.0	0.0
	30	0.4	0.4	0.2	0.0	0.0	0.0
	40	0.0	0.0	0.0	0.0	0.0	0.0

Table 2.

P_2	P_3/P_4	0	1	3	5	10	15
	0	99.9	94.6	71.8	48.7	14.8	4.0
	5	87.3	75.2	51.4	32.9	9.2	2.2
	10	48.7	39.0	24.1	14.4	3.6	0.8
	15	19.5	15.0	8.8	4.9	1.2	0.2
1	20	6.3	4.7	2.6	1.4	0.2	0.0
	25	1.8	1.2	0.6	0.4	0.0	0.0
	30	0.4	0.2	0.2	0.0	0.0	0.0
	35	0.0	0.0	0.0	0.0	0.0	0.0

Table 3.

P_2	P_3/P_4	0	1	3	5	10	15
	0	97.4	88.2	64.0	42.4	12.6	3.2
	5	79.8	67.2	44.6	28.1	7.6	1.8
	10	42.2	33.4	20.4	12.0	3.0	0.6
3	15	16.3	12.4	7.1	4.0	0.8	0.2
	20	5.1	3.8	2.2	1.2	0.2	0.0
	25	1.4	1.0	0.6	0.2	0.0	0.0
	30	0.4	0.2	0.2	0.0	0.0	0.0

Table 4.

P_2	P_3/P_4	0	1	3	5	10	15
	0	88.5	76.6	52.5	33.7	9.6	2.4
	5	67.3	55.4	35.5	21.9	5.7	1.4
	10	33.3	26.0	15.5	9.0	2.2	0.4
5	15	12.4	9.4	5.3	3.0	0.6	0.2
	20	3.8	2.8	1.6	0.8	0.2	0.0
	25	1.0	0.8	0.4	0.2	0.0	0.0
	30	0.2	0.2	0.0	0.0	0.0	0.0

Table 5.

P_2	P_3/P_4	0	1	3	5	10	15
	0	49.9	40.1	24.8	14.8	3.8	0.8
	5	33.3	26.0	15.5	9.0	2.2	0.4
	10	14.3	10.9	6.1	3.4	0.8	0.2
10	15	4.7	3.6	2.0	1.0	0.2	0.0
	20	1.2	1.0	0.4	0.2	0.0	0.0
	25	0.4	0.2	0.0	0.0	0.0	0.0
	30	0.0	0.0	0.0	0.0	0.0	0.0

Table 6.

P_2	P_3/P_4	0	1	3	5	10	15
	0	20.1	15.5	9.0	5.1	1.2	0.2
	5	12.4	9.4	5.3	3.0	0.6	0.2
15	10	4.7	3.6	2.0	1.0	0.2	0.0
	15	1.4	1.0	0.6	0.2	0.0	0.0
	20	0.2	0.2	0.2	0.0	0.0	0.0
	25	0.0	0.0	0.0	0.0	0.0	0.0

Table 7.

P_2	P_3/P_4	0	1	3	5	10	15
	0	6.5	4.9	2.8	1.4	0.4	0.0
	5	3.8	2.8	1.6	0.8	0.2	0.0
20	10	1.2	1.0	0.4	0.2	0.0	0.0
	15	0.4	0.2	0.2	0.0	0.0	0.0
	20	0.0	0.0	0.0	0.0	0.0	0.0

MODEL SEAFOOD SURVEILLANCE PROGRAM

E. Spencer Garrett and Martha A. Hudak-Roos
NOAA/NMFS/National Seafood Inspection Laboratory
P. O. Drawer 1207, Pascagoula, MS 39568-1207

INTRODUCTION

Seafood inspection is one of the most discussed topics in the area of food inspection today. Attention from the media, consumer groups, and Congress has brought seafood inspection to the forefront of food protection discussions.

MODEL SEAFOOD SURVEILLANCE PROGRAM

Because of the attention drawn to seafood inspection by such consumer groups as "Public Voice" (Public Voice 1986, 1987), TV exposes, and legislative proposals (U.S. Congress H.R. 1483; U.S. Congress S. 1813) requesting the mandatory federal inspection of fish and fishery products, Congress appropriated $350,000 to NOAA (Report to Accompany H.R. 5161) for a two (2) year study designing a program of certification and seafood surveillance.

The Congressional mandate requires NOAA to base the program on the HACCP system and consult and coordinate with FDA, USDA, the fish and seafood industry and the States. Initial work on the project began in Fiscal Year (FY) 87.

The objective of the Model Seafood Surveillance Program (MSSP) is to design a fishery products surveillance system which will provide consumers reasonable protection in the consumption of fishery products. This system, based upon the HACCP concept, will provide for equitable treatment among domestic and imported products.

In order to systematically follow a HACCP approach, the MSSP has opted to follow the recommendations made in a report published by the National Academy of Sciences (NAS) in 1985 (NRC 1985). This report defined the recommended procedures for utilizing HACCP in food processing plants. In the 1985 publication, the NAS emphasized that while regulatory authorities should provide the broad outline of a HACCP system for each commodity, the specific details of applying HACCP should be the prerogative of each industry. The NAS recommends that industry commodity groups should take the lead to define each operational step of a processing operation, indicate the hazard and relative importance in each step, identify the critical control points for the significant hazards, define preventative measures to minimize the hazard, and detail the monitoring procedures either by observation or measurement which can be used for compliance procedures. After such an industry driven system is developed, the regulatory authorities should then have the opportunity to assess the appropriateness of selected critical control points and monitoring procedures. Further,

the NAS recommended that regulatory authorities should have the prerogative to review the action taken when in-plant monitoring results indicated the need for corrective action.

Having made the decision to follow the recommendations of this NAS publication, NMFS built on this basis for the conduct of the study by classifying potential consumer hazards in the consumption of foods, including seafoods, into three categories, i.e., (1) product safety, (2) plant/food hygiene, (3) economic fraud (see Figure 1).

Our initial efforts in this study to address public health hazards have focused on separating perception from fact. Causative agents of public hazards in seafood are either environmental (natural or man-made), or process or distribution-chain induced. In reality, a review of the Center for Disease Controls' (CDC) seafood-borne illness outbreaks published data (CDC 1979-1985) indicate that the problem is manageable. Specifically:

- eighty-seven percent of seafood related illnesses are attributed to very few species--those that are ciguatoxic, scombrotoxic, or illness commonly associated with raw molluscan shellfish consumption (Ciguatoxin and scombrotoxin are primarily found in fishes located in tropical waters. Specific species sometimes related to ciguatoxic or scombrotoxic illnesses include red snapper, grouper, barracuda, jacks, mackerel, tuna, and mahi-mahi);

- eighty-one percent of all seafood related illnesses are reported from only nine (9) states or territories: Hawaii, Puerto Rico, Virgin Islands, Guam, New York, California, Washington, Connecticut and Florida;

- nearly one-half of all seafood related illnesses (49%) are reported from just four (4) states or territories: Hawaii, Puerto Rico, Virgin Islands, and Guam;

- thirty-five percent of all seafood related illnesses are reported from Hawaii with ciguatoxin and scombrotoxin as the principal causative agents.

Public safety issues in the consumption of seafood are compounded, however, with the fact that 65% of total U.S. seafood consumption is from imported product. These issues can be resolved, and it is the intention of the MSSP to have the NAS address, through a two-year contract, product safety issues associated with seafood. From this we hope to gain insight into problems, the relevancy of current programs established to handle these problems, and options to further improve seafood safety.

Food hygiene and economic fraud issues will be addressed through industry workshops.

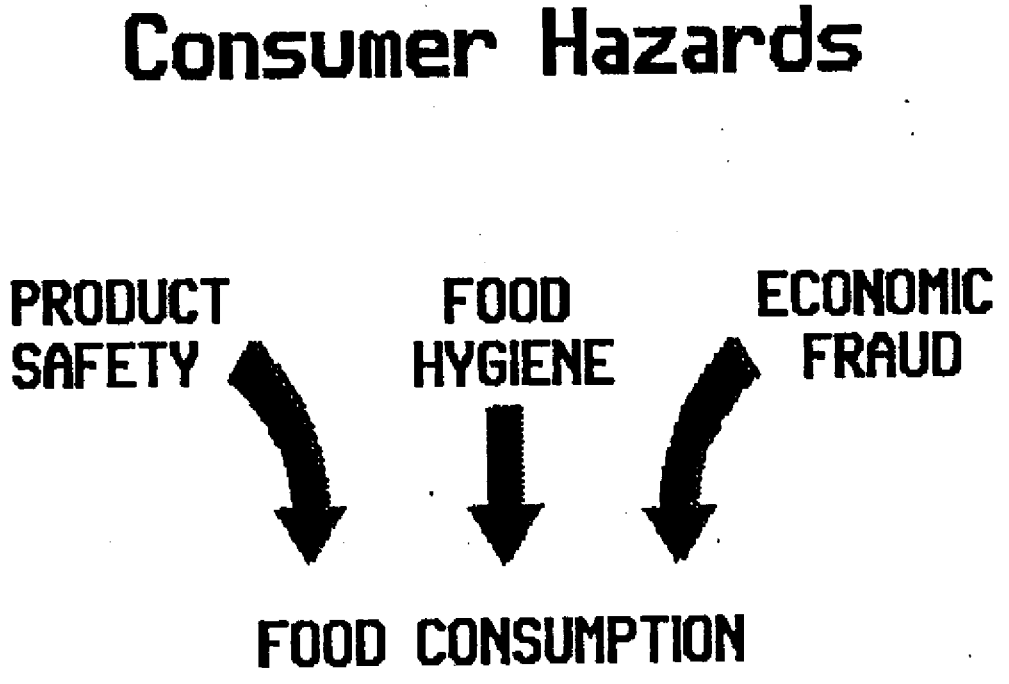


Figure 1. Potential Consumer Hazards in the Consumption of Seafoods.

Following the recommendations made by NAS in their previously mentioned publication, the MSSP is designed to have industry take the lead in defining the criteria by which they should be inspected. In operating under a Saltonstall-Kennedy grant to industry, the Program plans to conduct workshops on a commodity by commodity (industry by industry) basis. The workshop attendees will examine the hazards associated with the end use of their product and develop a HACCP model applicable to their industry. The model will cover plant hygiene (sanitation), food hygiene (wholesomeness), and economic malpractices (fraud). The attendees will then nominate a steering committee to follow the testing of the model in a statistically representative number of plants in their industry. The testing will be done by representatives of the MSSP who have previously signed an oath protecting confidential plant information.

The conduct of this program described above has been placed under the direction of E. Spencer Garrett, Laboratory Director, National Seafood Inspection Laboratory with a core team of senior NMFS staff. The study conduct is detailed in a Plans of Operation (PLANOPS) manual. The team is in contact with FDA, USDA, CDC, other NOAA components and NAS. By request,they are appraising state agencies, interstate associations, and industry associations of the program and progress.

To date two workshops have been completed, one for breaded shrimp (Tampa, Fl, October 6-8, 1987) and one for cooked shrimp (Chicago, IL, November 3-5, 1987). Of the thirty steps identified by the industry in producing breaded shrimp, nine were considered critical. Much the same was true for cooked shrimp processing. In-plant testing of the HACCP model developed by the breaded shrimp industry is scheduled to begin the last week in January 1988. The in-plant testing for the HACCP Cooked Shrimp Model will be conducted concurrently with the Breaded Shrimp Model. A raw shrimp (headless and peeled meat) HACCP Workshop is scheduled to be held at the New Orleans Airport Sheraton Hotel, January 19-21, 1988. Additional workshops which are being considered include: raw, whole, dressed, steaked, and filleted fish; molluscan shellfish; imports; vessels; blue crab; king, snow, and dungeness crab; special product hazards with Pacific and Caribbean species; smoked and cured products; breaded fish and specialty items; scallops; and lobsters.

Upon completion of all the workshops and the NAS contract, NOAA intends to deliver to Congress a surveillance system for seafood products which will contain options for possible implementation with an economic and feasibility analysis of each option. During the conduct of the workshops information for plant quality assurance manuals will have been developed for each industry. These manuals have applications for industry Quality Assurance and management personnel as well as government regulatory officials.

The model seafood surveillance system then, as delivered to Congress, will be based on the HACCP principle, separate the nice from the necessary, have intensive industry input, have been built on existing state, federal, and industry programs, address major problem areas, and offer a basis from which innovative consumer education techniques might be drawn.

REFERENCES

1. Public Voice for Food and Health Policy. 1986. "The Great American Fish Scandal: Health Risks Unchecked."

2. Public Voice for Food and Health Policy. 1987. "Hazardous Fish: The Raw Facts."

3. U.S. Congress. House. Joint Committees on Merchant Marine and Fisheries and Agricultural. "Mandatory Fish Inspection Act of 1987." H.R. 1483. (100th Congress, 1st Session).

4. U.S. Congress. Senate. Committee on Agriculture. "Safe Foods Standards Act." S. 1813. (100th Congress, 1st Session).

5. U.S. Congress. Senate. Committee on Appropriations. "Report to Accompany H.R. 5161." Report 99-425. (99th Congress, 2nd Session).

6. National Research Council (NRC). Subcommittee on Microbiological Criteria, Committee on Food Protection, Food and Nutrition Board. An Evaluation of the Role of Microbiological Criteria for Foods and Food Ingredients. National Academy Press, 1985.

7. Centers for Disease Control. 1979. Annual Summary of Foodborne Diseases, 1978. U.S. Department of Health and Human Services. 55 pp.

8. Centers for Disease Control. 1981. Annual Summary of Foodborne Disease, 1979. U.S. Department of Health and Human Services. 40 pp.

9. Centers for Disease Control. 1983. Annual Summary of Foodborne Diseases, 1980. U.S. Department of Health and Human Services. 41 pp.

10. Centers for Disease Control. 1983. Annual Summary of Foodborne Disease, 1981. U.S. Department of Health and Human Services. 41 pp.

11. Centers for Disease Control. 1985. Annual Summary of Foodborne Disease, 1982. U.S. Department of Health and Human Services. 38 pp.

CONTRIBUTION OF CATHEPSIN C TO THE FERMENTATION OF FISH SAUCE PREPARED FROM CAPELIN (MALLOTUS VILLOSUS) AND SQUID HEPATOPANCREAS

Nongnuch Raksakulthai and Norman Haard
Institute of Marine Resources
Department of Food Science and Technology
University of California
Davis, CA 95616

INTRODUCTION

Capelin (Mallotus villosus) is the most abundant fish in the North Atlantic and one of the top ten species landed in the world (Anon., 1981). More than 99% of the world capelin catch is used for the production of meal and oil (Jangaard, 1974). In Canada, male capelin are a by-catch in the fishery for roe-laden female fish. A problem with producing fish sauce from male capelin was reported earlier (Raksakulthai, Lee and Haard, 1986). It was observed that the fermentation rate using capelin: salt (4:1 w/w) was very slow and the sauce obtained after more than 1 year contained a relatively low content of soluble nitrogen compounds, e.g. less than half the free amino acid content of commercial products from Thailand or Philippines. Addition of either pronase, trypsin, chymotrypsin, or fungal protease 31,000 (Miles Laboratories, Elkart, IN) to salted capelin was effective in accelerating the rate of fermentation and yielded products containing a relatively high content of soluble nitrogen. However, the sensory evaluation of fish sauces prepared with proteolytic fermentation aids indicated they were less acceptable than commercial products.

Earlier we found that supplementation of the fermentation of squid or herring with hepatopancreas tissue from Atlantic short finned squid (Illex illecebrosus) helped to develop the desirable taste of these products (Lee, Simpson and Haard, 1982). Squid hepatopancreas also accelerates the rate of fermentation for capelin fish sauce and, as well, yields a product rated very high by sensory evaluation (Raksakulthai et al., 1986). The purpose of this study was to understand why squid hepatopancreas tissue acts to both accelerate the rate of fish sauce fermentation and to yield an excellent tasting product.

MATERIALS AND METHODS

Preparation of fish sauce-

Inshore capelin were harvested by hand net near St. John's, Newfoundland. Females were removed and the male fish were washed with tap water and ground to pass a 6 mm plate fitted to a Hobart 7 horsepower meat grinder. The mince was mixed with salt at a ratio of fish to salt, 4:1 (w/w). The mixture was left at 4 C for 16 h to allow equilibration of the brine. Duplicate 1 Kg samples were packed in glass jars. Squid hepatopancreas tissue, normally 2.5% w/w of brined mince, was added to jars to

aid the fermentation. The jars were incubated at ambient temperature (20-24 C) for up to 6 months. The liquid (fish sauce) was recovered by filtration through Whatman No. 1 paper.

Squid hepatopancreas tissue which had been heated 30 min. at 100 C to inactivate proteolytic enzyme activity was also added to fish-brine to determine if it affected the fermentation. A set of experiments was designed to test the contribution of capelin digestive enzymes to the fermentation, i.e. fish were eviscerated prior to mincing. Another set of experiments was designed to test the contribution of bacteria to the fermentation, i.e 100 mg gentamycin sulfate was added to each Kg fish-salt at the start of the fermentation. Also, the influence of pH on the rate of fermentation was evaluated by adjusting the pH of fish-brine using 6 N HCl or 50% NaOH.

Analytical Methods-

The pH of fish sauce was measured directly with a pH meter. The degree of protein hydrolysis was measured by formol titration as described by Beddows, Isamail and Steinkraus (1976). Free amino acids were determined by diluting one volume of fish sauce in 99 volumes water, de-proteinizing with 12% (w/v) sulfosalicylic acid and appropriate dilution with 0.2 M Lithium citrate, pH 2.2 prior to analysis in a Beckman Model 121MB analyzer (Beckman bulletin 121MTB-013). Soluble protein was estimated by the Biuret method (Cooper, 1977) and total nitrogen by micro-kjeldahl (Lang, 1958). Sodium chloride was determined according to the volumetric method in AOAC (1980). Total viable bacteria was estimated using trypticase soy agar and trypticase soy agar with 10% NaCl and reported as described by Gilliland, Busta, Brinda and Campbell (1976). Sensory evaluation was by a panel of 5 Vietnamese-Canadians using a nine point hedonic scale and analyzed for statistical significance (Larmond, 1977). The molecular size distribution of hydrolyzed protein in fish sauce was evaluated by gel filtration or Bio-Gel P-2, 80x1.5 cm, and eluted with 0.05 N HCl containing 0.1 N NaCl at 20 ml/h. The absorbance of eluant at 214 nm was monitored with a Waters Model 441 ultraviolet detector.

Isolation of Proteolytic Enzymes-

The enzymes in the fermentation brine were recovered from the fish sauce by ultrafiltration. A Millipore Ultrafiltration Pellicon Cassette system with polysulfone filter of 10,000 dalton nominal exclusion limit was used to remove salt and other low molecular weight components from the fish sauce. The retentate was dialyzed against about 100 volumes (x3) of distilled water at 4 C for 72h. Cathepsin C was purified from squid hepatopancreas as described by Hameed & Haard (1985).

Assay of Protease Activity-

Protease activity was measured with azocasein substrate, i.e. the assay mixture contained 1.0 ml 1.5% azocasein, 0.9 ml 0.2 M sodium acetate-HCl, pH

6.0 and 0.1 ml enzyme. After incubation at 30 C for different time intervals, the reaction was stopped by the addition of 0.5 ml 20% trichloroacetic acid. Thirty minutes later the mixtures were filtered through Whatman No. 1 paper and the filtrate Absorbance at 366 nm was read. The pH of the assay solution was varied from 2.2 to 8 by use of 0.1 M citric acid-0.2 M Na_2HPO_4 (McIlvaine, 1921). Cathepsin C was assayed for dipetidylhydrolase and for transferase activity as described by Hameed & Haard (1985).

RESULTS AND DISCUSSION

Analyses of Fish Sauce-

Fish sauce prepared with 2.5% squid hepatopancreas (SHP) received significantly higher sensory scores than product prepared with heated-SHP, SHP added to fish-brine which had been adjusted to pH 4.5, or with no added SHP (Table 1). Sauce prepared with SHP at the natural fermentation pH had a golden brown hue and was significantly darker than the other samples (Table 1). Sauce prepared with SHP at about pH 6 contained significantly more total nitrogen, formol N (titratable amine groups), and free amino acids than the other samples (Table 2). Free amino acids were 78% and 57% of total amino acids (recovered after acid hydrolysis) respectively for SHP supplemented and control fish sauce. Gel permeation chromatography revealed similar fractionation patterns for the control and SHP supplemented sauces; however, the void volume peak (protein) was larger and the lower molecular weight fractions were smaller for the control sample (Fig. 1). These data show that the fish sauce prepared with SHP contained more protein hydrolysis products, and also contained products which underwent more hydrolysis than occurred for the other samples.

Influence of SHP on Rate of Protein Hydrolysis-

Protein hydrolysis proceeded at a faster rate when SHP was added to brined fish (Fig. 2). Samples containing SHP and which were made to pH 4.5, showed a faster hydrolysis rate than the control during the first week; but, there was less hydrolysis during the remainder of the 6 month fermentation. The formation of free amino acids by all samples was more or less parallel to that of formol nitrogen (data not shown). The mole percentage of free and peptide amino acids in the fermentation brine were not significantly different for the three lots of fish sauce prepared at the natural fermentation, about pH 6. However, the free amino acid composition of sauce prepared at pH 4.5 was different; the major free amino acid was leucine as opposed to alanine, glutamic and lysine for the sauces prepared at pH 6. These data indicate that the differences in protein hydrolysis for samples fermented at pH 6 were mostly quantitative; whereas protein hydrolysis at pH 4.5 also gave different products. The qualitative differences may explain why fermentation with SHP at pH 4.5 gave a less acceptable product even though protein hydrolysis proceeded faster than the control (Table 1).

Table 1. Properties of Fish Sauce Prepared With and Without the Aid of Squid Hepatopancreas[1]

Treatment	Preference Score[2]	pH	Lightness (L)	NaCl(%)
SHP	8.3[b]	5.92	18.8[b]	27.0[a]
Heated SHP	5.3[a]	5.97	26.2[a]	27.4[a]
Control	5.3[a]	5.99	31.4[a]	27.2[a]
SHP, Acidified	4.8[a]	4.59	30.5[a]	28.8[a]

[1]All samples prepared with fresh male, inshore capelin and salt (4:1 w/w) at 20-24 C for 6 months. SHP = 2.5% squid hepatopancreas. Values in the same column followed by the same letter are not significantly different ($P < 0.05$).

[2]Preference score based on 9 point hedonic scale; commercial product had a score of 5.8. Data for SHP and control representative of fish sauces prepared from capelin in three different harvest seasons.

Table 2. Nitrogenous Components in Fish Sauce Prepared With and Without the Aid of Squid Hepatopancreas[1]

Treatment	Total-N	Formol-N	Protein-N	NH_3-N	Free Amino Acid-N
		mg N/ml			
SHP	23.85[b]	13.72[b]	3.42[b]	4.78	8.94
Heated SHP	12.82[a]	6.23[a]	4.09[c]	2.55	3.68
Control	13.94[a]	7.00[a]	3.74[a]	2.52	4.48
SHP, Acidified	14.43[a]	9.80[c]	3.32[b]	3.66	6.14

[1]See Footnote to Table 1. Values are average of duplicate determinations for two lots of fish sauce. Soluble protein-N was determined by dividing Biuret protein by 6.25. NH_3-N is Formol-N less free amino acid -N and is probably somewhat overestimated because it includes other sources of titratable -N.

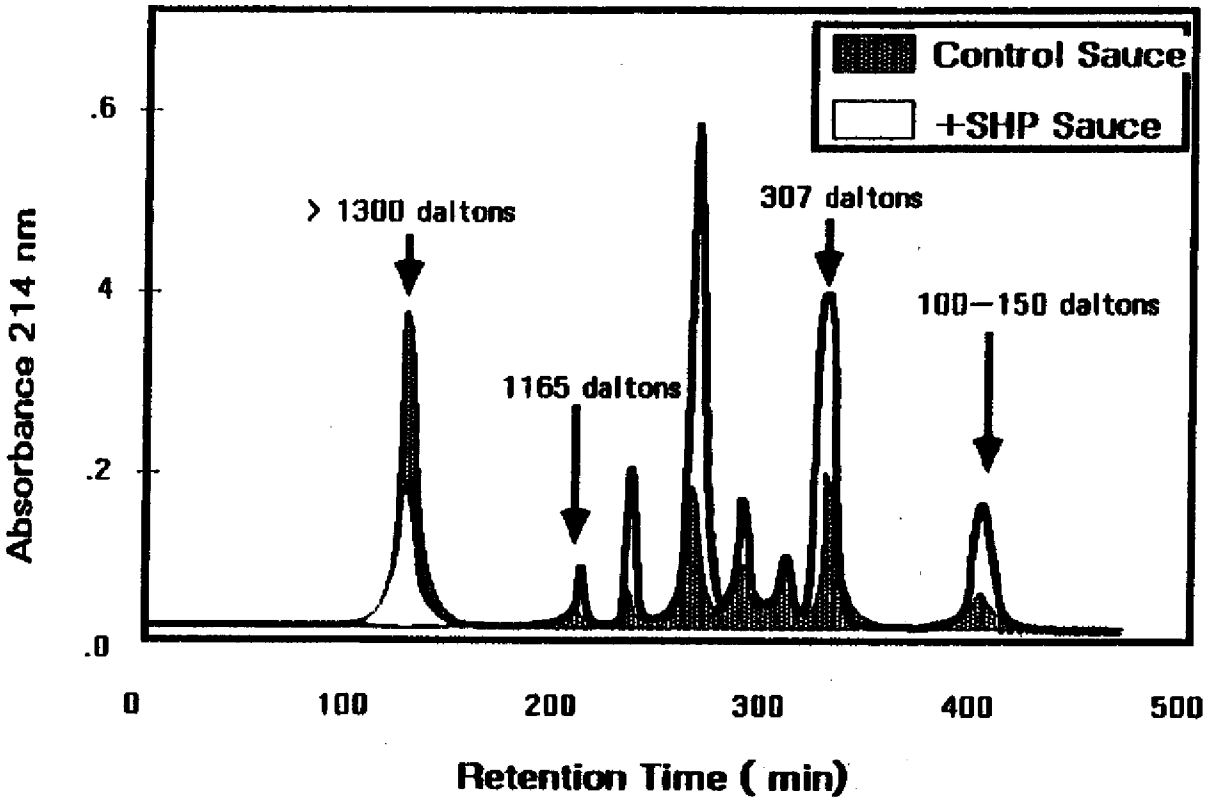


Figure 1— Bio—gel P2 chromatography of sauce after 6 months fermentation. Initial peak is at void volume of column, >1300 Collected fractions showed that other peaks were mixtures of free amino acids and small peptides. Heavy line is for fish sauce prepared with SHP, and hatched area is for sauce prepared without added SHP.

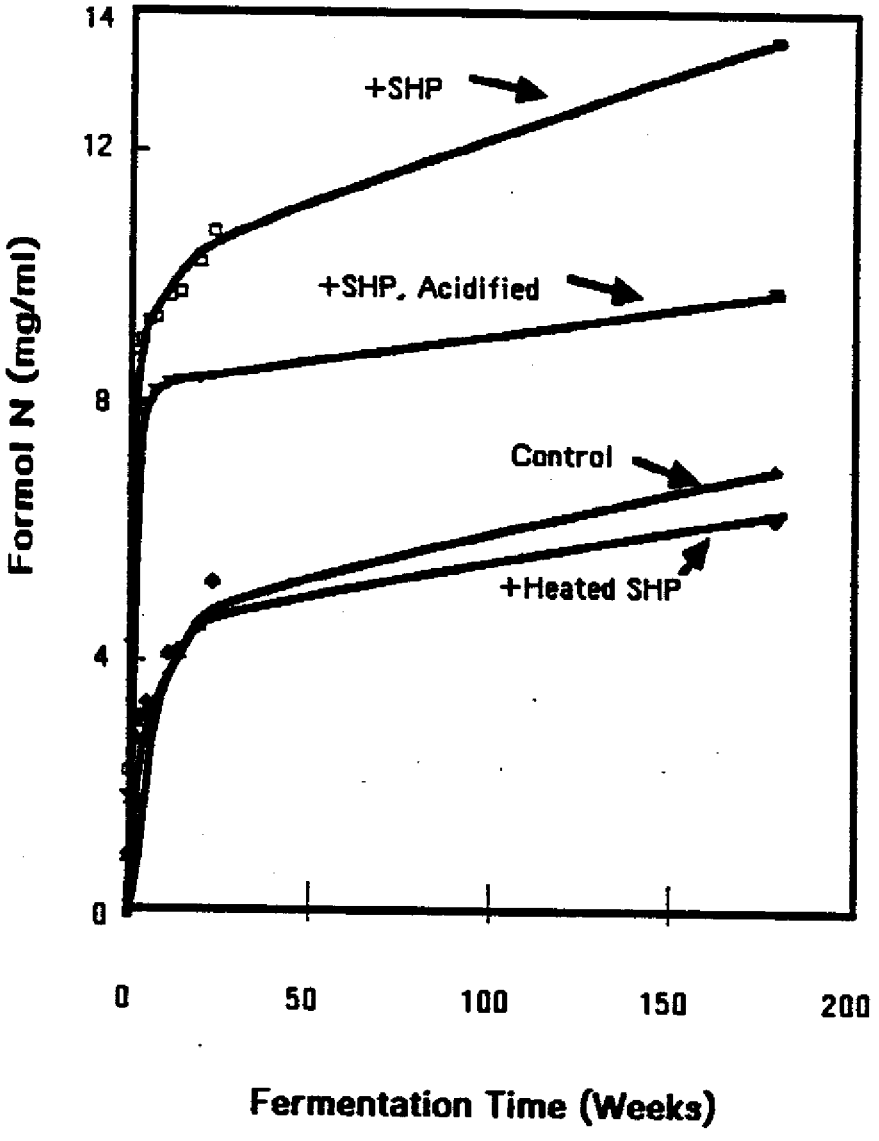


Figure 2— Progress of protein hydrolysis during fermentation of fish sauce at 20°C. SHP=2.5% squid hepatopancreas, heated SHP= proteolytic enzymes inactivated by heating; acidified= initial brine adjusted to pH 4.5 with HCl. All samples were prepared from 4 parts fresh male, inshore capelin and 1 part NaCl(w/w). Formol N is a measure of titratable amine groups

Enzymes contributing to the fermentation-

Fish sauces prepared from eviscerated capelin underwent a slightly, but statistically significant ($P<0.05$) lower rate of protein hydrolysis than was observed for round fish (Figure 3-A). Using a triangle test for product identification, judges could not distinguish between fish sauce prepared with and without evisceration; i.e. the contribution of digestive proteases to fermentation was minor.

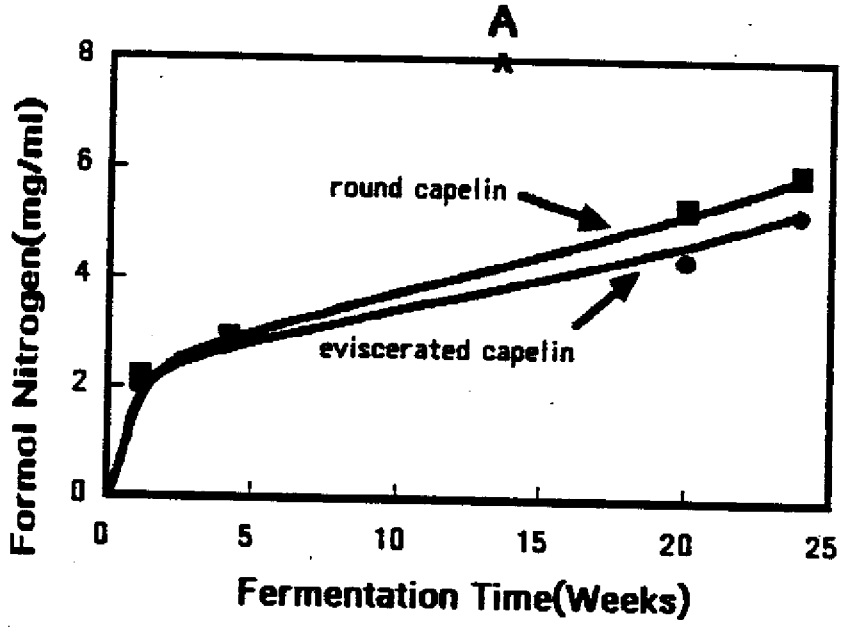
Addition of a broad spectrum antibiotic (gentamycin sulfate) to the fermentation brine was effective against the growth of microorganisms. No viable cells were detected in antibiotic treated fermentation brine for the duration of the experiment while the control, initially containing 5.3×10^2 CFU/g in growth media with 10% NaCl, contained detectable bacteria during the first few weeks of the fermentation (Figure 3-B). However, the antibiotic treatment did not influence the rate of protein hydrolysis (Figure 3-B).

Ultrafiltration of fish sauce samples, using a 10,000 dalton exclusion membrane, was effective in recovering all of the residual proteolytic activity in the retentate fraction. The pH optimum for hydrolysis of azocasein with enzyme isolates from either control or SHP-sauce, was 6.0. Assay of these samples revealed the sauce supplemented with SHP contained 3 times as much general protease activity at pH 6. Both samples contained aminopeptidase activity, however, the rate of this reaction was not different in the samples. Moreover, the transferase activity with Gly-Phe-NH_2, a reaction specific for cathepsin C (Fruton, 1982), was 7 times as active in enzyme isolate from SHP-sauce compared to that from the control-sauce.

The pH optima for rate of protein hydrolysis during fermentation (judged by formol titration), for hydrolysis of azocasein by the enzyme(s) recovered from sauce by ultrafiltration, and for dipetidylaminopeptidase activity of Gly-Phe-NA (a cathepsin C substrate) by enzyme(s) recovered from the sauce were each 6.0 (Figure 4-A,B,C). Indeed the pH-activity profile for azocasein hydrolysis and cathepsin C activity were very similar (Figs. 4B,C). These data indicate that cathepsin C may be a key enzyme in the fermentation of fish sauce from capelin. The dipeptidylaminopeptidase reaction of this enzyme results in formation of dipeptide products, which may subsequently be hydrolyzed to free amino acids by amino peptidases. Since SHP did not contribute aminopeptidase activity to the fermentation and since free amino acid accumulation paralleled overall fermentation rate in both samples, it appears that contribution of cathepsin C rather than aminopeptidase explains the utility of SHP as a fermentation aid.

Additional evidence for the involvement of cathepsin C-

Three additional lines of experimentation support the notion that cathepsin C contributes to this fermentation. First, the dipeptidylaminopeptidase activity of purified cathepsin C from SHP is dependent on Cl^- and is activated by low levels of NaCl (Fig. 5). Secondly, when the residual enzyme activity from fish sauce is assayed at increasing



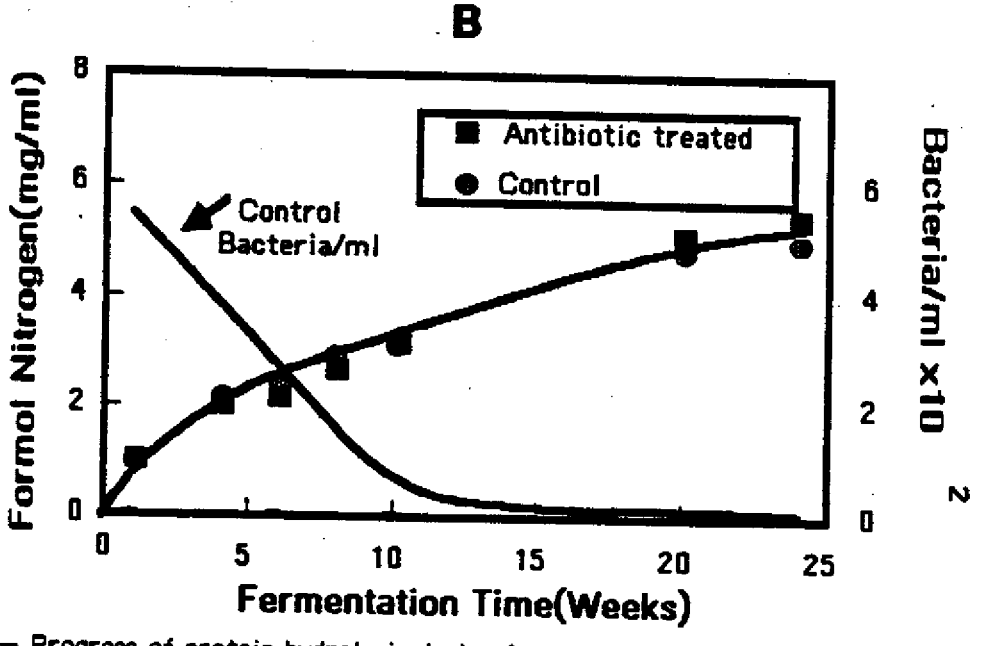
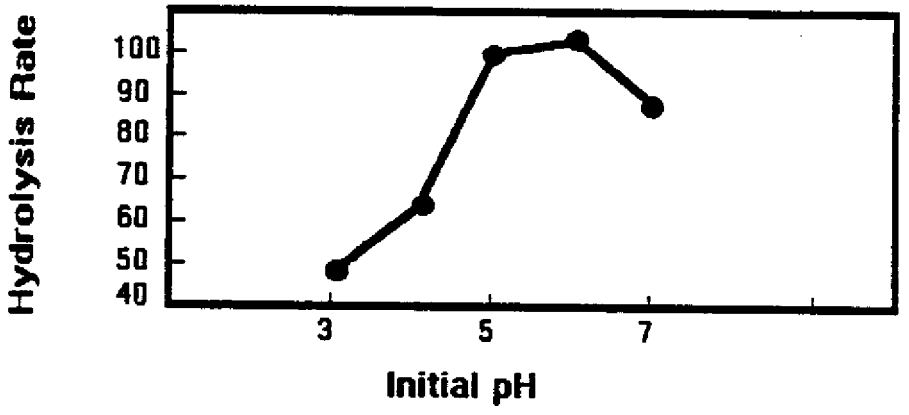


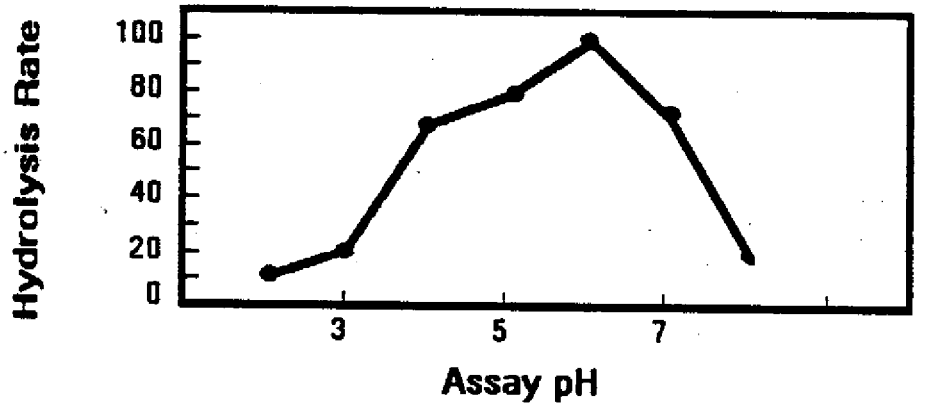
Figure 3— Progress of protein hydrolysis during fermentation of fish sauce at 20°C.
A. Eviscerated capelin— fish were eviscerated to remove digestive enzymes prior to addition of salt;
B. antibiotic treated— addition of 0.1% gentamycin sulfate to the brine at the start of the fermentation. Line without data points is bacterial colony forming units for control. Antibiotic treated sample contained no detectable bacteria during course of fermentation.

Rate of of protein hydrolysis in sauce as a function of fermentation pH



Azocasein in 4 N NaCl—

Enzymes recovered from fish sauce after 6 months



Hydrolysis of Gly—Phe—naphylamide by purified cathepsin C

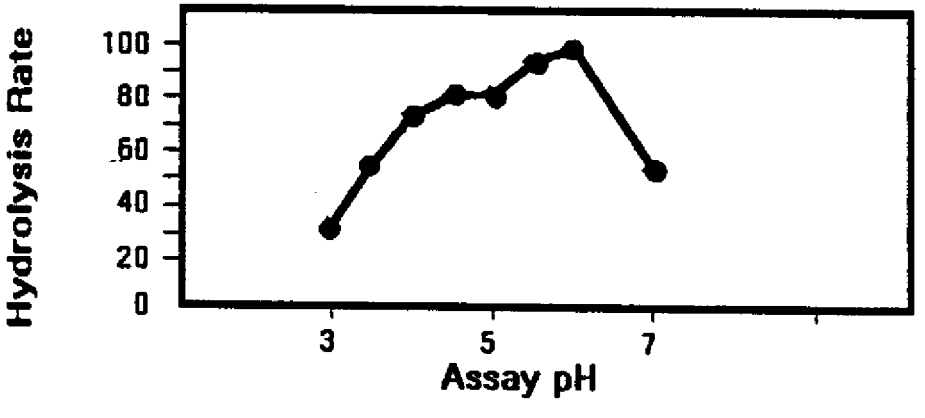


Figure 4— Influence of pH on rate of fermentation and key enzymes involved with fermentation.

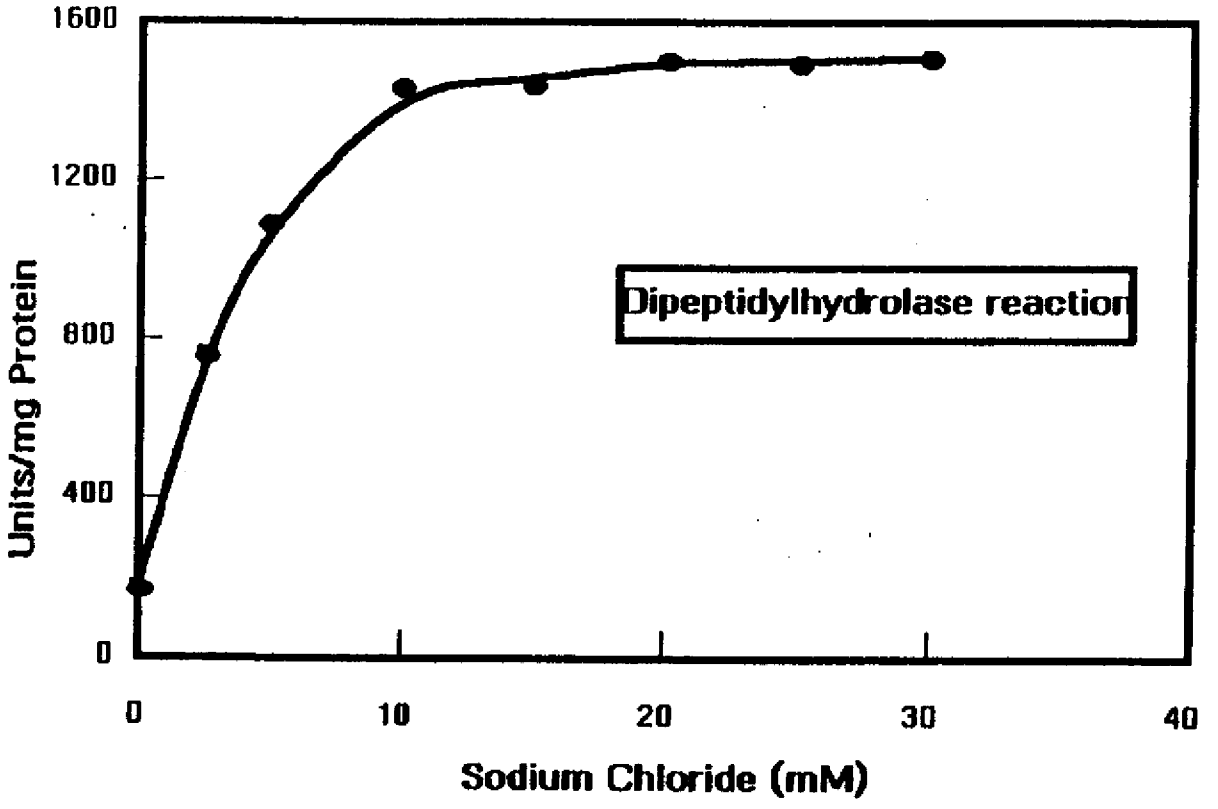


Figure 5— Sodium chloride activation of cathepsin C catalyzed hydrolysis of Gly—Phe—amide at pH 6.0, 30°C. Other chloride salts also activate this enzyme; cathepsin C was purified from squid hepatopancreas as described by Hameed & Haard(1985).

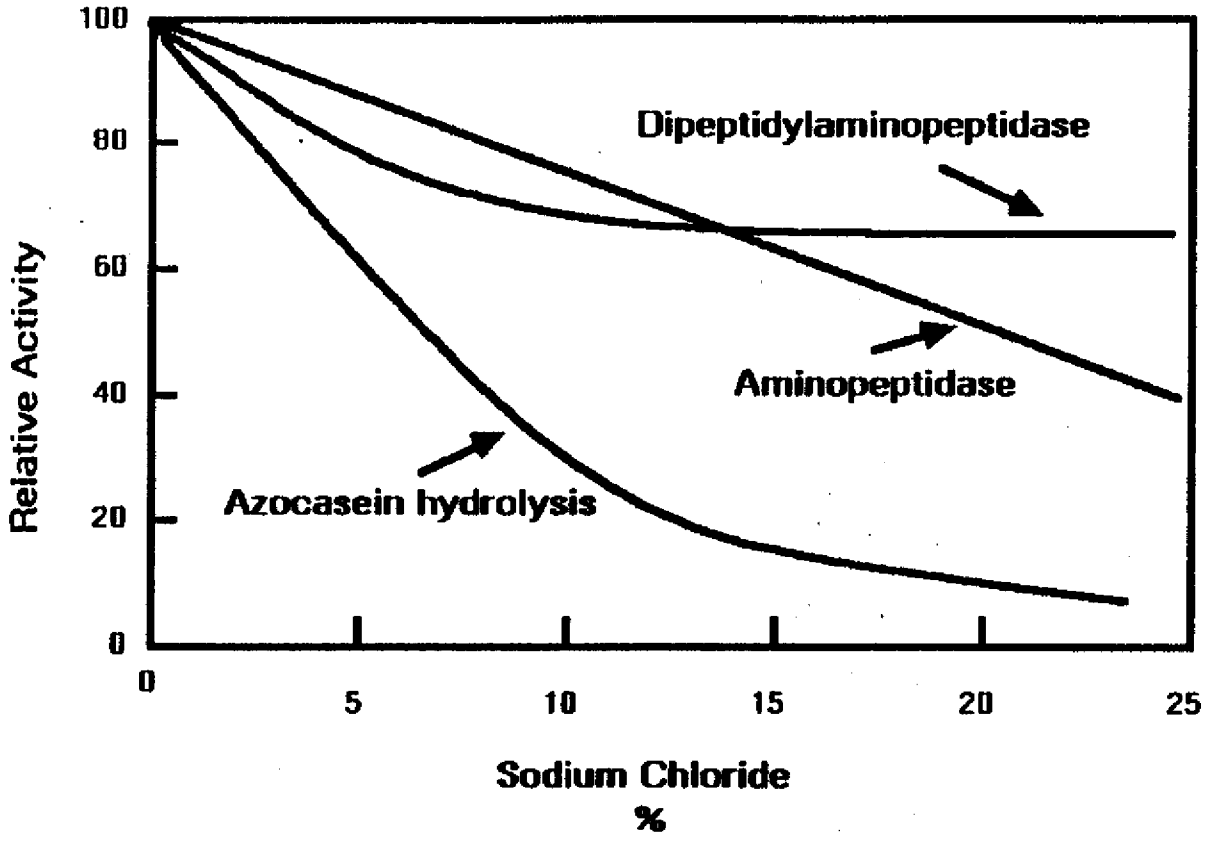


Figure 6— Influence of sodium chloride on protease activity isolated from fish sauce after 6 months fermentation. Note that the activities of cathepsin C and aminopeptidase are less sensitive to inhibition by elevated NaCl than is general protease activity. The transferase activity of cathepsin C is also retained at high NaCl copncentrations.

NaCl concentrations, general protease activity is progressively inhibited, i.e. at 4 M NaCl the rate of azocasein hydrolysis was only 5% of that at 25 mM NaCl (Fig. 6). However, the dipeptidylaminopeptidase activity retained about 70% of the optimal activity observed in 25 mM NaCl. Substantial aminopeptidase activity was also sustained when the salt concentration of the assay medium was like that in the fermentation brine (Fig. 6). Thirdly, when the residual proteases isolated from fish sauce were assayed at low NaCl concentration, part of the azocasein hydrolyzing activity was inhibited by trypsin inhibitor (35% inhibition at .25 mg 0.1 ml enzyme), part by EDTA (5% inhibition at 1 mM) and part by pCMB (30% inhibition at 1 mM) indicating the retention of several types of protease in sauce. However, when the same enzyme(s) were assayed in a reaction medium containing 4 M NaCl, only sulfhydryl protease inhibitors (pCMB or $HgCl_2$) inhibited the reaction. Cathepsin C is a sulfhydryl protease and is sensitive to pCMB or $HgCl_2$ (Fruton, 1982; Hameed & Haard, 1985); thus the data for protease inhibitor studies are also consistent with the retention and active expression of cathepsin C activity in the fermentation brine.

CONCLUSIONS

An excellent quality fish sauce can be obtained from male, inshore capelin when the fermentation is aided by squid hepatopancreas tissue. The best conditions for fermentation were 2.5% hepatopancreas tissue, 25% NaCl, natural pH (about 6.0), temperature of 20-25 C, and a time of about 6 months. Additional aging of the product for 6 months to one year did not have a significant affect on the sensory properties nor on the chemical composition of the product.

Squid hepatopancreas contains proteolytic enzyme(s) which aid the fermentation by virtue of component(s) which have optimum catalytic activity at pH 6, are not inactivated by very high salt concentrations, and retain catalytic activity in media containing very high salt content, and catalyze nearly complete hydrolysis of protein. The enzyme(s) which persist and remain active during the fermentation are sulfhydryl proteases and include cathepsin C.

Both dipeptidylhydrolase and transferase activities of cathepsin C probably contribute to the high degree of hydrolysis observed during fermentation of capelin; i.e. approximately 80% of the protein amino acids are hydrolyzed to free amino acids in 6 months. The dipeptidylhydrolase reaction produces dipeptides which appear to be hydrolyzed to free amino acids by aminopeptidase(s). The transferase reaction can "shuffle" the sequence of those peptides which are not amenable to hydrolysis by the hydrolase reaction, thus creating new peptides which have the appropriate N-terminus for subsequent hydrolysis by the dipetidylhydrolase reaction.

REFERENCES

1. Anon. (1981). Fishing News International. June 1981, p. 60.
2. AOAC. (1980). Official Methods of Analysis, 13th ed. p. 289-290.

3. Association of Official Agricultural Chemists, Washington, D.C.
4. Beddows, C.G., Isamail, M., and Steinkraus, K.H. (1976). The use of bromelain in the hydrolysis of mackerel and the investigation of fermented fish aroma. J. Food Technol. 11: 379-388.
5. Cooper, T.G. (1977). The Tool of Biochemistry, Wiley-Interscience Publication, New York, p. 51-53.
6. Fruton, J.S. (1982). Proteinase-catalyzed synthesis of peptide bonds. Advances in Enzymology 53: 209-307.
7. Gilliland, S.E., Busta, F.F., Brinda, J.J. and Campbell, J.E. (1976). Aeorbic plate count. In: Compendium of Methods for the Microbiological Examination of Foods, M.L. Speck, Ed., APHA Intersociety Agency Committee on Microbiological Methods for Foods, p. 107-130.
8. Hameed, K.S. and Haard, N.F. (1985). Isolation and characterization of dipeptidyl aminopeptidase 1 (cathepsin C) from Atlantic short-finned squid Illex illecebrosus. Comp. Biochem. Physiol. 82B: 241-246.
9. Jangaard, P.M. (1974) Prospects for utilizing capelin (Mallotus villosus) for human consumption. In: Fishery Products, R. Kreuzer, ed. Fishing News Books Ltd., London, p. 203-206.
10. Lang, C.A. (1958). Simple microdetermination of Kjeldahl nitrogen in biological material. Anal. Chem. 30: 1692-1694.
11. Larmond, E. (1977). Laboratory Methods for Sensory Evaluation of Food. Publication 1637, Canada Department of Agriculture, Ottawa.
12. Lee, Y.Z., Simpson, B.K. and Haard, N.F. (1982). Supplementation of squid fermentation with proteolytic enzymes. J. Food Biochem. 6: 127-134.
13. McIlvaine, T.C. (1921). A buffer solution for colorimetric comparison. J. Biol. Chem. 49: 183-186.
14. Raksakulthai, N., Lee, Y.Z. and Haard, N.F. (1986). Effect of enzyme supplements on the production of fish sauce from male inshore capelin Mallotus villosus. Can. Inst. Food Sci. Technol. 19: 28-33.

ABSTRACTS

FRACTIONATION OF FATTY ACID ETHYL ESTERS USING SUPERCRITICAL FLUID CARBON DIOXIDE

William B. Nilsson, Ph.D.
Research Chemist
Northwest and Alaska Fisheries Center
Utilization Research Division
2725 Montlake Boulevard East
Seattle, WA 98112

Preliminary evidence from several clinical studies has created an increasing demand for concentrates of omega-3 fatty acids, particularly eicosapentaenoic (EPA) and docasahexaenoic (DHA) acids. Fractionation of fatty acide ethyl esters derived from menhaden oil using supercritical fluid CO_2 has been found capable of producing EPA and DHA in purities as high as 95%. Factors affecting yield and production rates will be discussed.

SPATIAL PATTERNS OF ABUNDANCE FOR DEEP-WATER GOLDEN CRAB (*GERYON FENNERI*) AND RED CRAB (*G. QUINQUEDENS*) IN THE EASTERN GULF OF MEXICO

William J. Lindberg
Department of Fisheries & Aquaculture
University of Florida
7922 N.W. 71st Street
Gainesville, Florida 32606

Frank Lockhart
Univeristy of Florida

Harriet Perry and Richard Waller
Gulf Coast Research Laboratory

Submersible transects and trap sampling confirmed concentrations of golden crabs (1) along intermediate depth contours, associated with rock outcroppings, and (2) off peninsular Florida rather than the northern Gulf. Red crabs were deeper and at northern as well as southern stations. Both species showed sex differences in distribution.

FEASIBILITY OF USING STRESS/STRAIN MEASUREMENTS FOR LEAST-COST FORMULATION OF SURIMI-BASED FOODS

Tyre C. Lanier
North Carolina State University
Food Science Department
North Carolina State University
Raleigh, NC 27695-7624

Measurement of stress and strain at failure, determined most appropriately for surimi-containing gels by a torsion test, is the quality control test for gel-forming ability recently recommended for industry adoption by the Technical Subcommittee of the Surimi Committee, National Fisheries Institute. The present work was conducted to determine the utility of such measurements for purposes of least-cost formulation. It was found that, although the effect of water addition alone on gel texture was somewhat unpredicatable, mixtures of surimis at the same moisture content formed gels in a fairly predictable (linear) manner. Stress/strain measurements were also shown to be more sensitive indicators of gelling quality than a least-concentration endpoint test.

SURIMI STUDIES ON WHITE HAKE (UROPHYCIS TENUIS)

E.G. Bligh and K. Spencer
Canadian Institute of Fisheries Technology
Technical University of Nova Scotia
Halifax, Canada B3J 2X4

A series of studies underway at the Canadian Institute of Fisheries Technology (funded by Fisheries and Oceans Canada and N.S.E.R.C.) is examining the processing parameters and product performance associated with the use of selected Canadian Atlantic fish in the manufacturing of surimi. White hake is one of the targeted species and data will be presented on the effects of post-mortem age, use of frozen material, water washing regime, etc., on surimi quality.

EFFECT OF WASHING ON SOLUBLE PROTEIN CONTENT IN DARK AND ORDINARY MUSCLE TISSUES OBTAINED FROM ATLANTIC MENHADEN

D.P. Green and T.C. Lanier
Food Science Department
North Carolina State University
Raleigh, NC 27695-7624

Proximate composition and protein distribution for excised Atlantic menhaden dark and ordinary muscle tissues were determined. Washing studies suggest high content of dark muscle in mince prior to leaching decreases removal efficiency of water soluble and will result in surimi of darker color and higher lipid and connective tissue content.

THE EFFECTS OF VARIOUS SALTS ON THE TEXTURAL AND CHEMICAL CHANGES IN FROZEN GADOID AND NON-GADOID FISH MINCES

Karl Ragnarsson and Joe M. Regenstein
Department of Poultry and Avian Sciences
Institute of Food Sciences
112 Rice Hall
Cornell University
Ithaca, NY 14853-5601

Freezing and frozen storage of gadoid fish minces causes textural changes. The effect of various salts (NaCl, NaI, NaSCN, NaAc, MgCL2 and CaC12) added to the fish prior to freezing were studied with respect to the texture and the water retention properties of fish muscle. When NaAc and NaCl were added to ocean perch and cod, the rate of textural toughness and expressible moisture decreased during frozen storage. On the other hand, NaI, NaSCN, CaC12, and MgC12 accelerated the textural toughness developed and increased the expressible moisture during frozen storage. SDS-PAGE showed that a crosslinked protein of 280,00 da occurs only with gadoid fish. For cod, 20 days at -7C are needed, whereas for whiting only 3 days are needed. Cod samples treated with NaSCN did not show this band. Ascorbic acid accelerated the time of appearance of the band, while fresh storage of cod for 10 days prior to freezing eliminated the band. Instron hardness seemed to increase in proportion to the development of the SDS-PAGE band.

RECENT PROGRESS IN THE DEVELOPMENT OF A RECEPTOR BASED BIOSENSOR FOR DECTECTION OF MAINE TOXINS

L.V. Sick and P.B. Travis
NMFS-Charleston Labortory
Charleston, S.C. 29412

One of the most innovative approaches for developing a detection method to screen seafood for the presence of natural toxins concerns engineering membrane channel protein receptors into field applicable detection devices. Such devices have the advantage of detecting several toxins with a single chip and are inexpensive. Maitotoxin, a putative agent in ciguatoxin disease, was investigated for physiological properties involving its calcium ionophoric characterists and its binding affinity to cortical synaptosomes. Resulting information is being applied to development of a capacitance, receptor based biosenor.

EXTENDING STORAGE LIFE OF FRESH TROPICAL FISH BY BLANCHING COMBINED WITH STORAGE IN ICE/SALT MIXTURES

L.J. Toledo-Flores and R.R. Zall
Department of Food Science
Cornell University
Ithaca, NY 14853

Fresh queen snapper (Etelis oculatus) and cardinal snapper (Pristipomoides marcrophtalmus) were treated by blanching in hot water at 90°C for 5 sec combined with storage in an ice/salt mixture at -3°C. Storage life of the two species in plain ice: 16.0 days for queen snapper and 15.8 days for cardinal snapper was extended to 30.8 and 35.5 days, respectively, in the treated fish. The combination of treatments also retarded the formation of spoilage compounds and microbial growth.
778

EFFECTS OF PHOSPHATES, NACl, WATER CONTENT, AND PROCESS TEMPERATURES ON TEXTURE OF SURIMI GELS

Vivian M. Higgins, Tyre C. Lanier, Jae W. Park and Jose Montejano
Food Science Dept. N.C. State University, Raleigh, NC 27695-7624

Selected phosphates were evaluated for their effect in altering the texture of gels prepared either primarily of surimi (Alaskan pollack, Theregra chalcogramma) or of surimi in combination with starch and other ingredients (commercial crab analog formulation). The latter formulations were systematically varied in NaCl and moisture content. Measurement techniques included differential scanning calorimetry, press moisture, torsional failure testing, and thermal scanning of rheological properties. Results indicate that phosphates had little effect on the textural properties of either pure surimi gels or those prepared by a commercial crabstick formulation at 3% or 2% NaCl contenct was reduced to 1%, phosphate had a significant effect in improving textural properites. Results also showed that water content could be appreciably increased without detriment to product texture by use of a pre-"set" incubation at 4°C for 16 hours prior to cooking.

EFFECT OF REDFEED CONTENT UPON THE FREQUENCY OF DEGRADATION OF SPAWNING FEMALE CAPLIN DURING STORAGE PRIOR TO FREEZING, DURING FROZEN STORAGE DURING THAWING

David R.L. White
Dir, Inspection Services Branch
P.O. Box 5667
St. Johns, Newfoundland AIC 5X1

Controlled experiments were conducted on commercially-caught caplin to determine the relationship between the level of redfeed content and the frequency of degradation of female caplin. Caplin were graded, following four different iced storage times, and three different frozen storage (-26°C)/thawing (+15°C) time combinations. Results of these experiments showed that the frequency of degradation was more affected by handling than by redfeed level.

PRODUCTION OF FISH HYDROLYSATES FOR AGRICULTURAL APPLICATIONS

Robert J. Learson, Vincent G. Ampola, Burton L. Tinker
and Robert VanTwuyver
Northeast Fisheries Center
Gloucester Laboratory
Emerson Avenue
Gloucester Laboratory, MA 01930

and

Ronald P. Athanas
University of Massachusetts

A process for fish hydrolysate production for fish processing wastes was developed using both formic acid and phosphoric acid as acidulants. A pilot scale production line was designed and installed in Gloucester, MA for low technology hydrolysate production from fish frames. Chemical analysis of the hydrolysates indicated that the resulting liquid fertilizer material using phosphoric acid had 1.6 - 2.4% nitrogen, 4 - 5% phophorous and 0.8 - 1.0% potassium. Additional micronutrients of interest included significant levels of magnesium, manganese, boron and zinc. Problems relating to production and stabilization are discussed.

EFFECTS OF FISH HYDROLYSATE ON GREENHOUSE GROWN CAPSICUM FRUTESCENS "JALAPENO"

Amul Purohit and Michael Potter
Essex Agricultural & Technical Institute
Hathore, MA 01937

Ronald P. Athanas
University of Massachusetts

and

Robert J. Learson
National Marine Fisheries Service, Gloucester

Fish hydrolysate (FH) made from Atlantic cod processing wastes, with an analysis of 1.6-4.2-0.8 (NPK) was compared to Peters 20-20-20 fertilizer on greenhouse grown "Jalapeno" peppers (Capsicum frutescens). These peppers were grown in Pro-Mix "BX" (PX), Metro-Mix #350 (MX) and top soil (TS) at 0, 80 and 160 ppm Nitrogen applied in weekly and bi-weekly intervals during June 9 to August 21, 1987. Compared to the Peters fertilizer applications, FH increased yeilds by 48 and 76% at 80 and 160 ppm respectively when used at a weekly rate. FH at 160 ppm applied bi-weekly, consistently increased yields by 123, 97 and 20% in PX, MX and TS respectively as compared to the Peters fertilizer applications. Overall, FH at 160 ppm increased yield by 50% compared to the Peters fertilizer applications.

PRELIMINARY REPORT ON APPLICABILITY OF COMMERCIAL MICRO KITS IN DETECTING FECAL COLIFORMS IN SEAFOODS

Bohdan M. Slabyj, Susan A. Ismail and Stefanie O. Slabyj
Department of Food Science, University of Maine
Orono, ME 04469

Several seafoods were inoculated at two levels with 4 different Escherichia coli isolates and enumrated using standard MPN procedure as well as inoculating Petrifilm, Redigel, and Violet Red Bile (VRB) Agar which were incubate at 44.5°C. Discrepancies were detected between estimated level of inoculum and the MPN count. Petrifilm, Redigel, and VRB were observed to have limited accuracy at the non-acceptable contaimination level. VRB counts at 45°C tend to include coliforms, while Petrifilm can identify gas producing colonies and therefore provide a more reliable fecal coliform count.

MICROFLORA CHANGES IN POST-HARVEST SHELLSTOCK OYSTERS

Angela D. Ruple and David W. Cook
Microbiology Section
Gulf Coast Research Laboratory
P. O. Box 7000
Ocean Springs, MS 39564

Changes in microflora were monitored during both commercial handling and controlled temperature storage of shellstock oysters. The effects of temperature and salinity of the harvest area and shell stock storage temperatures on fecal coliform and potentially pathogenic bacteria levels were investigated. Multiplication of fecal coliforms, including *E. coli*, was observed at temperatures above 10°C, but not at 10°C. Multiplication of some vibrios and *Aeromonas hydrophila* was also observed in shellstock oysters.

SKATE (*RAJA* SPP.) HANDLING, PROCESSING AND STORAGE CHARACTERISTICS

Kurt A. Wilhelm and Barbara L. Jobe
National Marine Fisheries Service
Gloucester Lab
30 Emerson Avenue
Gloucester, MA 01930

Experiments in skate handling and processing included: (1) the relationship of weight of skate wings to size (weight, length and width) of whole skate; (2) fillet yields from skate wings; (3) processing methods and equipment; (4) iced storage time of skate wings, (5) various product forms; and (6), a frozen storage study which is still in progress. Discoloration of the wings was also investigated, but a satisfactory method of preventing this quality defect was not identified.

CALORIMETRIC DENATURATION KINETICS OF SURIMI AS AFFECTED BY SUGAR AND/OR SALT

Jae W. Park and Tyre C. Lanier
Department of Food Science, North Carolina State University
Raleigh, NC 27695-7624

The kinetics of heat-induced denaturation and aggregation of surimi proteins, as affected by additional of sugar and/or salt, were investigated by differential scanning calorimetry (DSC). Net enthalpic changes were always endothermic in nature, and of a greater magnitude at lower heating rates led to a gel structure in which potential bondings were more completely accomplished; that is, a more energetically favorable structure was attained with slow heating. The stablization of proteins by sugar, and destabilization of proteins by salt, were also measured calorimetrically.

BIOMEDICAL TEST MATERIALS FROM MENHADEN OIL

Jeanne D. Joseph, Sylvia B. Galloway, and Robert R. Kifer
U.S. Department of Commerce
National Marine Fisheries Service, Charleston Laboratory
P.O. Box 12607
Charleston, S.C. 29412

Using chemical and physical separation techniques, the Charleston Laboratory is producing quality-assured biomedical test materials from menhaden oil for use in NIH-approved research. Over time, these materials will included not only refined oil and n-3 ester concentrates, but also n-3 enriched glycerides, purified EPA and DHA.

FISHFAX: A COMPUTERIZED INFORMATION SYSTEM

Martha Hudak-Roos
National Seafood Inspection Laboratory
P.O. Drawer 1207
Pascagoula, MS 39568-1207

With the coming of age of computerized information systems, vast amounts of information and data on almost any topic can be examined, read, and printed using a personal computer in almost any location. The National Seafood Inspection Laboratory (NSIL) has developed one such system - FISHFAX. FISHFAX contains information on a species specific basis, covering such topics as biological profiles, processing methods, nutritional values, economic considerations, and public health information. All information and data contained within FISHFAX has been previously published. This information is verified, summarized, and quality assured before being entered into the information base. A poster presentation demonstrates FISHFAX using a personal computer.

PROGRESS IN THE DEVELOPMENT OF MONOCLONAL ANTIBODIES TO SEMI-PURIFIED MAITOTOXIN EXTRACTS FROM *GAMBIERDISCUS TOXICUS*

Ronald C. Lundstrom, Christopher Martin, Debra A. Adams,
Debra J. Rhoades (N.M.F.S., Gloucester, MA), and
Lowell V. Sick (N.M.F.S., Charleston, S.C.)

Maitotoxin, a water soluble toxin produced by the dinoflagellate *Gambierdiscus toxicus*, is thought to be associated with the development of ciguatoxicity in tropical marine reef fishes. Spleen cells from BALC/C mice immunized with semi-purified maitotoxin extracts were fused with myeloma cells to produce hybridoma cells using standard somatic cell fusion technology. Five separate cell fusion experiments have resulted in the development of 31 hybridoma cells lines which secrete monoclonal antibodies reactive with various components in the maitotoxin extracts. Specificity experiments using an indirect ELISA assay have identified certain monoclonal antibodies which appear to be reactive with various pigments present in the maitotoxin extracts. Other monoclonal antibodies react in direct proportion to measured toxicity regardless of maitotoxin extract pigmentation, suggesting that they are reacting with the actual toxin molecule or a closely related component. Several monoclonal antibodies have shown detectable reaction with maitotoxin extract at toxicity levels as low as 0.002 mouse units. Further characterization experiments still need to be carried out to determine the antigenic determinants recognized by these monoclonal antibodies.

ILLUMINATION STUDIES FOR IMPROVED INSPECTION SYSTEMS FOR DETECTION OF PHOCANEMA

J.H. MacNeil, J.C. Wambold and H.J. Sommer
Department of Food Science and Mechanical Engineering
The Pennsylvania State University
University Park, PA 16802

Many segments of the food industry are currently using or investigating the uses of various automated inspection systems to measure quality characteristics or for the detection of defects. The problem of Phocanema within the fishing industry has long been a serious concern and this study deals with the investigation of a machine vision system for the detection of this parasite. Very early in this investigation it was found that the choice of the lighting scheme to illuminate the cod worm is still a critical aspect of the total machine vision system. Details of the lighting systems which result in the highest contrast between fish tissue and the cod worm are presented as well as quality considerations of some systems.