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SCREENING OF SODIUM BISULFITE ON SHRIMP: A MODIFIED MONIER-WILLIAMS APPROACH

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BACKGROUND

Sodium bisulfite has been used by the seafood industry for many years to control blackspot in shrimp. First recommended in the 1950's, the correct on board method of application usually requires a 1.25% dip for one minute, although a common malpractice is by direct application of the powder form on the shrimp. Used in accordance with good manufacturing practices, bisufite was recognized as GRAS by the FDA. However, bisulfite, along with several other sulfiting agents, has recently become suspect in causing allergic type reactions when present in 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 and has prompted legislation and/or regulations limiting or eliminating its use in many foods including shrimp. The Food and Drug Administration responded to the concern with an initial informal action level of 40 ppm sulfite residue in shrimp. This level was increased to 100 ppm after a petition presented by the National Fisheries Institute and Texas A & M University demonstrated that shrimp treated with bisulfite in accordance with good manufacturing practices resulted in sulfite residues greater than 40 ppm. FDA label regulations now establish that shrimp with sulfite residues of less than 10 ppm require no label declaration, 10 to 100 ppm must declare sulfite presence on the label, and shrimp with residues greater than 100 ppm are considered adulterated and subject to seizure.

With the advent of such a nationwide concern, many research institutions have initiated research for sulfite alternatives and for new methodologies for sulfite analysis. Currently, there are three AOAC quantitative methods. There is also an AOAC final action qualitative method. In addition to the AOAC methods, other methodologies such as quick test strip methods and disposable titration cells have become available in the marketplace. All the current methods have limitations, especially in analysis time, reproducibility and sensitivity, and some can be expensive to employ. To circumvent these limitations, the National Shrimp Breaders Association and the National Marine Fisheries Service have established a need for a quick screening method. In response, the National Seafood Inspection

Laboratory (NSIL) has developed a test based on the Monier-Williams final action procedure. It is less time consuming and less expensive to run than the Monier-Williams method and more sensitive and accurate than test strip methods. This screening method is devised to definitively detect shrimp with sulfite residues greater than 100 ppm.

PRINCIPLE

When refluxed, an acidified shrimp sample that contains sodium bisulfite ($NaHSO_3$) will produce sulfur dioxide (SO_2). A calculated quantity of potassium permanganate ($KMnO_4$) is subsequently reduced by the SO_2 according to the following equation:

$$2MnO_4^- + 5SO_2 + 2H_2O \rightarrow 2Mn^{+2} + 5SO_4^{-2} + 4H^+$$

If 100 ppm or more of $NaHSO_3$ is in the sample, the $KMnO_4$ will gradually change from a purple color to a clear solution.

MATERIALS AND METHODS

Place a two-neck (24/40) 500 ml distillation flask into a heating mantle which is attached to a powerstat. Connect the flask with a 400 mm Liebig water-cooled condenser in a reflux position. Into the top joint of the condensor place a right angle inner joint adapter that is connected to a piece of silicon tubing approximately 20" long. At the end of the tubing insert a thin glass tube or a small pipet. This rod is placed into a 50 ml test tube so that the the tip of the rod is 1" from the bottom of the tube. Adjust the silicon tubing accordingly.

Into the second neck of the flask attach a gas inlet tube that reaches nearly to the bottom of the flask. This gas inlet tube should be connected to nitrogen gas with a flow rate of 150 ml/min. Allow the entire system to flush with nitrogen for a least 2 minutes prior to analyzing each sample.

Prepare a stock solution of potassium permanganate by weighing exactly 4.50 g into a 1 liter flask. Dilute with 0.5N sulfuric acid, being sure to dissolve all the permanganate. This stock solution can be stored for approximately one month in an amber bottle placed out of direct light. From the stock solution prepare a potassium permanganate working solution by diluting 10 mls to 100 mls with 0.5N sulfuric acid. This solution must be prepared daily. Pipet 10 mls of this working solution into the 50 ml test tube.

Weigh 50 grams of thawed peeled shrimp into a mortar. Shrimp can be thawed at room temperature or in a refrigerate and can be peeled by hand. Grind the shrimp sample with 50 mls of 0.1N potassium hydroxide in order to prevent loss of SO_2 during maceration. Wash the macerated sample into the 500 ml flask with a minimum of water

and 50 mls of 2N sulfuric acid. Check to see that the glass rod is in the tube containing the 10 mls of permanganate solution and that the condenser is water-cooled. Turn the powerstat on to 65 and heat the sample gently. Reflux for 35 minutes. If permangante color changes to clear, the sample has more then 100 ppm SO_2.

RESULTS

Initial observations of the screening test as described prompted an informal collaborative study with the participation of Texas A & M University, Applied Microbiological Services, Inc., and the University of Florida.

Fresh, brown shrimp were obtained in the Pascagoula, Mississippi area. A sample of these shrimp were analyzed for sodium bisulfite using the AOAC Monier-Williams method to determine that they were not treated with sulfites. After this confirmation, the shrimp were dipped in a 1.25% sodium bisulfite solution for one minute, drained, rinsed well with tap water, and placed on ice in a refrigerator. Once a day sub samples were taken and a portion analyzed by the Monier-Williams method in order to establish a range of bisulfite values for the study. The levels of sulfur dioxide used in the study are listed in Table 1.

Table 1. Sulfur dioxide (ppm) by Monier-Williams method.

Sample	ppm
P1	113
P2	106
P3	85
P4	61
P5	242
P6	155

Two bags of shrimp of each level were forwarded to the participating laboratories. Each laboratory was requested to have two analysts work on the study. Each analyst was to thaw, peel and analyze each sample level once by the Monier-Williams method and twice by the screening test. The results of this study are listed in Table 2. Note that Laboratory 3 did not follow the experimental design; one analyst ran two Monier-Williams method and another analyst ran two screening tests. Because of this inconsistency, Laboratory 3's results were not included in the summary of the study which is found in Table 3.

CONCLUSIONS

By reviewing the summary in Table 3, it is shown that the data indicates no support for concluding that the screening procedure will yield false positive results. Also, the data indicates no support for concluding that the screening procedure will yield false negative results. Thus, the screening procedure merits further consideration and will be applied to a formal AOAC collaborative study for AOAC method approval.

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

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Table 2. Results of informal collaborative study of National Seafood Inspection Laboratory screening test.

Laboratory	1				2				3			
Method	Monier-Williams		Screening Test		Monier-Williams		Screening Test		Monier-Williams		Screening Test	
Analyst	A	B	A	B	C	D	C	D	E	E	F	F
P1	84.0	97.4	- -	- -	42.9	63.3	- -	- -	89	83	+	+
P2	51.2	45.1	- -	- -	72.5	58.5	- -	- -	121	115	-	-
P3	72.1	71.4	- -	- -	67.7	74.2	- -	- -	38	45	-	-
P4	48.5	45.5	- -	- -	31.5	68.8	- -	- -	102	102	-	-
P5	177.8	*	+ +	+ +	136.4	159.2	+ +	+ +	178	172	+	+
P6	132.3	118.7	+ +	+ +	90.0	123.6	+ +	+ *	102	102	+	+

* = Not reported
- = Less than 100 ppm
+ = More than 100 ppm

Table 3. Summary of the results of informal collaborative study of National Seafood Inspection Laboratory screening test.

	Laboratory 1			Laboratory 2			Total		
	Average Monier-Williams	# -	# +	Average Monier-Williams	# -	# 1	Average Monier-Williams	# -	# +
P1	<100	4	0	<100	4	0	<100	8	0
P2	<100	4	0	<100	4	0	<100	8	0
P3	<100	4	0	<100	4	0	<100	8	0
P4	<100	4	0	<100	4	0	<100	8	0
P5	>100	0	4	>100	0	4	>100	0	8
P6	>100	0	4	>100	0	3	>100	0	7

BIOCHEMICAL BASIS FOR ACCELERATED MELANOSIS IN THE FLORIDA SPINY LOBSTER (*Panulirus argus*)

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INTRODUCTION

One of the important quality changes limiting shelf-life of thawed tails from Florida spiny lobsters is melanosis. Melanosis, also known as "black spot", is a discoloration due to the oxidation of phenolic compounds by phenoloxidase. These enzymes are present as inert proenzymes that require activation. Coralase and trypsin have been shown to activate phenoloxidase in deep sea crab (2,3). Bavagaon and Sreenivasan (4), using gel filtration techniques reported a latent phenoloxidase from shrimp and lobster that was activated by trypsin. However, the exact mechanism of activation of phenoloxidase in shrimp and lobsters is still not well understood. Therefore, to better understand this activation process, a study was initiated to examine phenoloxidase from Florida spiny lobster.

MATERIALS AND METHODS

Frozen Florida and South African lobsters tails were stored on ice at 4°C in a refrigerator for up to 12 days for the melanosis study. Soluble tyrosine was determine by the method of Hull (1).

Crude extracts of phenoloxidase were prepared by homogenizing one part lobster shell with two parts 0.05 M potassium phosphate buffer (pH 7.2) in a Waring blender for one minute at 4°C. The homogenate was centrifuge at 10,000xg for 10 minutes at 4°C and phenoloxidase activity determined. The crude preparation was then partially purified by precipitation with ammonium sulfate (30 - 50% cut). Phenoloxidase activity was determined with and without trypsin added. 2.8 ml of 0.05 M DL-*B*-3,4-dihydroxy-phenylalanine (DL-DOPA) in 0.05 M phosphate buffer (pH 6.5), 0.1 ml of 1% trypsin solution and 0.1 ml of the enzymatic preparation at 25°C was the reaction mixture when trypsin was used. In the absence of trypsin 0.1 ml of buffer replaced the 1% trypsin solution. An LKB Model 4050 Ultrospec spectrophotometer linked to an Apple computer was used to determine the rate of increase in absorbance at 475 nm. Phenoloxidase activity was expressed as the change in absorbance per minute at 475 nm, pH 6.5 and 25°C.

Non-denatured polyacrylamide gel electrophoresis was carried out on the enzymatic extracts with and without

trypsin added. DL-DOPA was used to develop the phenoloxidase bands. Fast protein liquid chromatography (FPLC) was also used to separate multiple forms of phenoloxidase using a Superose 12 column from Pharmacia. A fraction collector was used to collect the fractions eluting from the column. The fractions were tested for phenoloxidase activity as described earlier.

RESULTS AND DISCUSSION

A comparison of Florida spiny lobster and South African lobster revealed melanosis development occurred mainly in the Florida species. Melanosis in Florida spiny lobster developed between the second and fifth day, resulting in intense blackening of the hypodermis. Levels of tyrosine in Florida spiny lobster were 6 times higher when compared to the South African species and the levels of total bisulfite were lower in South African species compared to the Florida lobster (Table 1). However, South African lobsters did not discolor when dipped in a saturated tyrosine solution for 5 minutes and stored on ice in a refrigerator; indicating substrate limitation was not controlling pigment formation. Melanosis could be controlled in the Florida lobster by dipping in a 1.25% sodium metabisulfite solution for 5 minutes.

Preliminary isolation and characterization of phenoloxidase (PO) showed a 10-fold increase in PO activity from the Florida versus the South African species (Table 1). When trypsin was added to the extract from Florida and South African lobsters, a 9 and 1.5-fold increase in the phenoloxidase activity occurred respectively (Table 1). Phenoloxidase activity from Florida spiny lobster was proportional to initial trypsin concentrations but eventually leveled off at 1000 µg/ml (Figure 1). Phenoloxidase activity at this point was 320 I.U.

Table 1. Tyrosine content and phenoloxidase activity of South African and Florida lobsters

		PHENOLOXIDASE ACTIVITY, IU	
	FREE TYROSINE, mg/g	NO TRYPSIN	TRYPSIN
SOUTH AFRICAN	1.05	1.8	2.5
FLORIDA	6.70	19.8	172.0

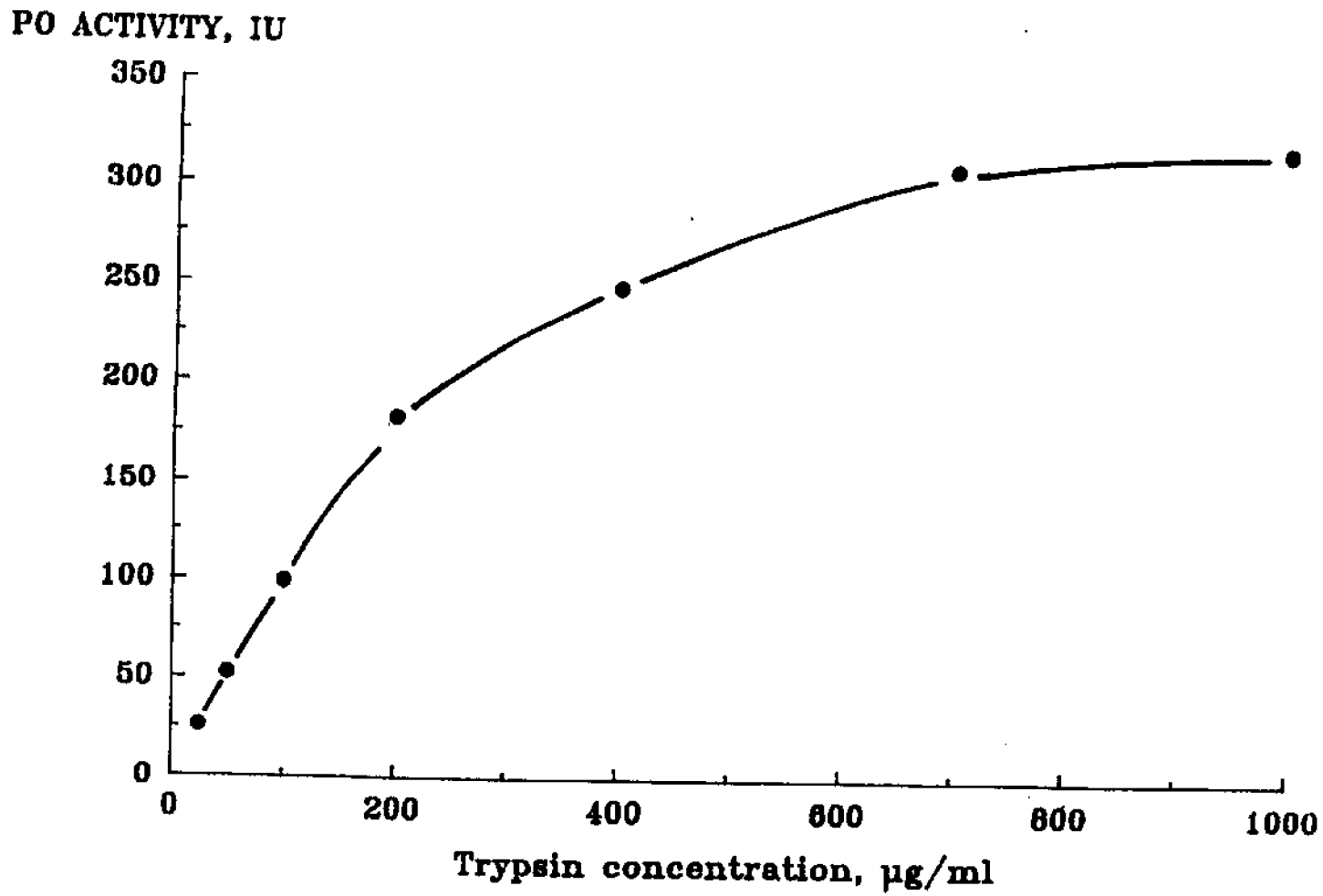

Figure 1. Effect of trypsin concentration on phenoloxidase activity.

Non-denatured polyacrylamide gel electrophoresis showed the occurrence of at least four forms of phenoloxidase when the gels were stained with DOPA. One of the active forms was a very large protein that did not migrate even at low gel concentrations (3%). Only this band was markedly darkened, while the other three bands were slightly darkened (Figure 2a). When a DOPA solution containing trypsin was used, additional bands could be developed (Figure 2b). This suggested the possibility of more than one inactive form of phenoloxidase, which was activated by trypsin.

When the enzymatic extract was treated with trypsin before electrophoresis, the results were markedly different different. Two very dark bands and a very light band at the top of the gel were observed when staining in 0.05 M DOPA solution (Figure 2c). It appears trypsin hydrolyzed the larger molecule, into at least two very active lower molecular weight forms of phenoloxidase. The molecular weight of these two forms was 225,000 and 64,000, as determined by non-denatured polyacrylamide gel electrophoresis (5). Development of the gels with a 0.05 M DOPA solution containing trypsin did not affect the results obtained. In all three cases a phenoloxidase band was seen at the top of the gel (Figure 2).

Non-denatured polyacrylamide gel electrophoresis was conducted on 50 µl of partially purified phenoloxidase extract without adding trypsin. The results were similar to those shown in Figure 2a. The upper band was separated from the gel by cutting off approximately 2 mm of the top of the gel and then homogenated with 100 µl of 0.05M phosphate buffer pH 7.2 and tested for activity without trypsin. The activity was very high, over 100 I.U. This suggests that the electrophoretic process may be causing the activation of this phenoloxidase form. A similar experiment was conducted but the extract was incubated with trypsin before electrophoresis. When the upper band was separated from the gel and assayed for phenoloxidase activity, this was only 20 I.U.

Fast protein liquid chromatography assays of the enzymatic extract with and without trypsin added confirmed the possibility of hydrolysis of the latent form of phenoloxidase into at least two smaller active forms of the enzyme (Figure 3). Without any trypsin added, most of the phenoloxidase activity was present in the void volume, whereas with trypsin added, the phenoloxidase activity in the void volume decreased and two new peaks with phenoloxidase activity emerged (Figure 4).

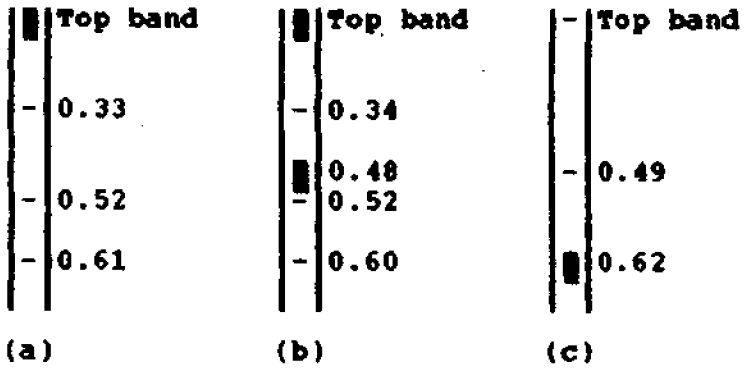

Figure 2. Non-denatured PAGE of a partially purified phenoloxidase extract using DOPA as staining agent. (a) Staining agent is a DOPA solution, (b) Staining agent is a DOPA solution containing trypsin, (c) Extract incubated with trypsin before electrophoresis. Staining agent was DOPA solution.

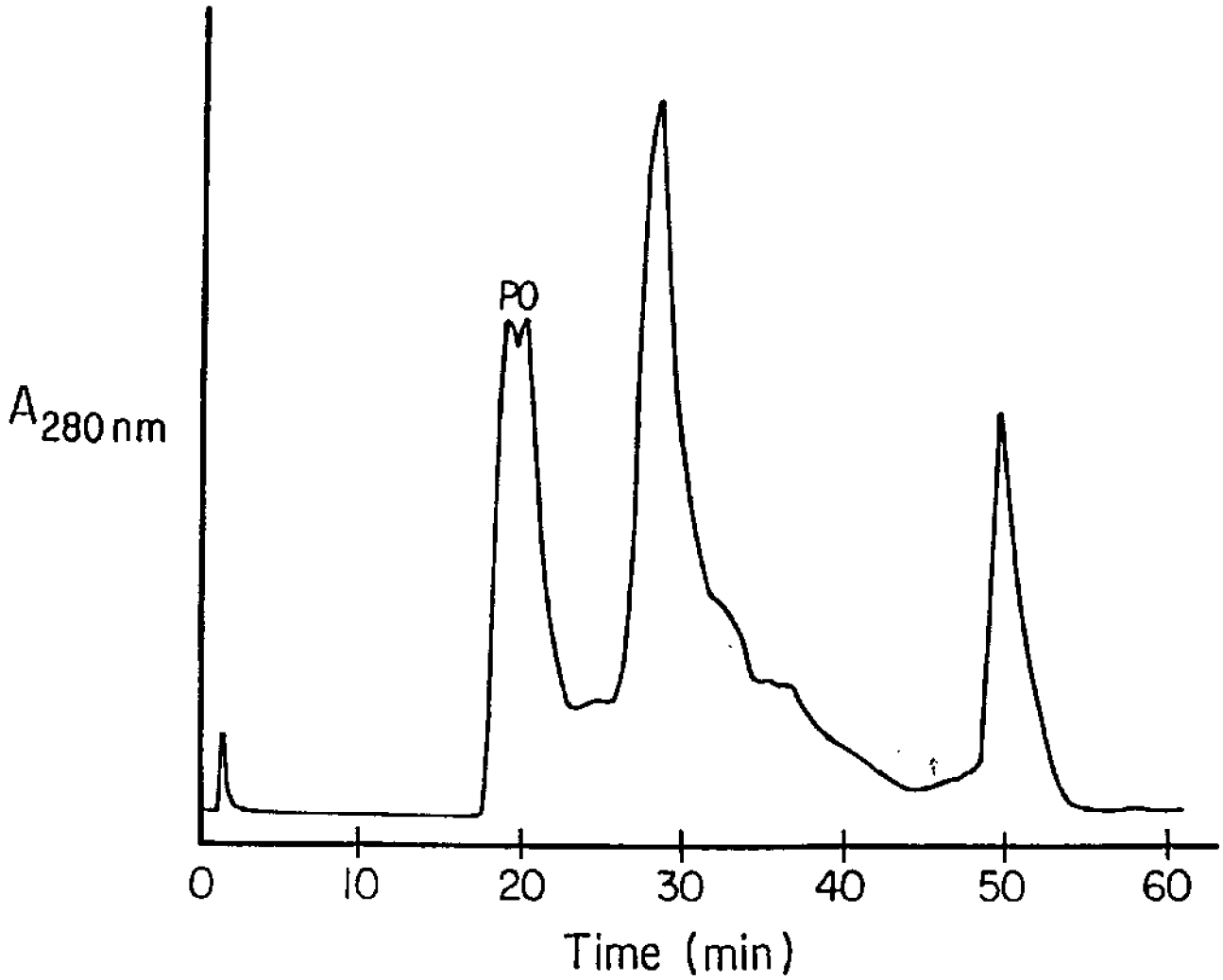

Figure 3. FPLC chromatogram of a phenoloxidase extract without trypsin added.

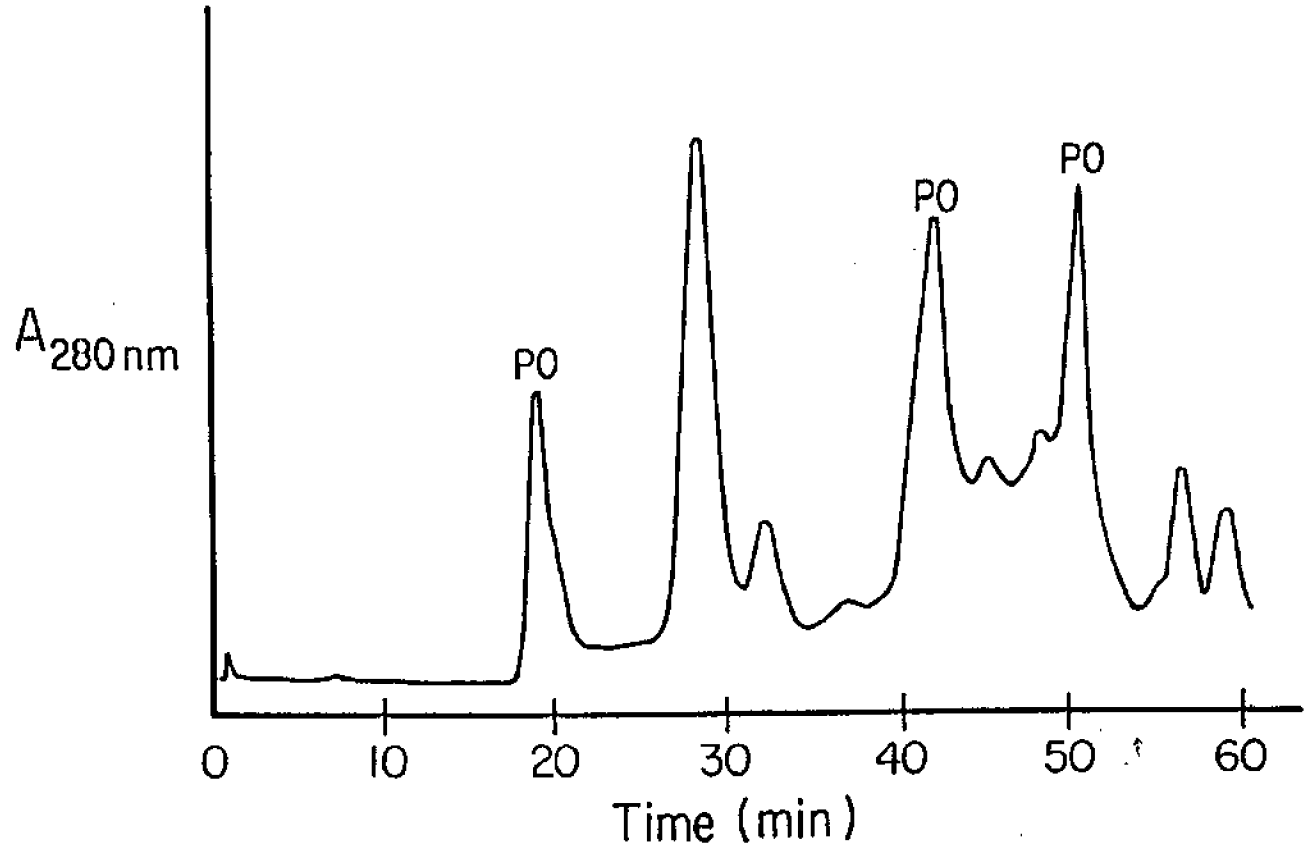

Figure 4. FPLC chromatogram of a phenoloxidase extract after addition of trypsin

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INFLUENCE OF WASHING AND COOKING ON SULFITE RESIDUALS ON TREATED SHRIMP

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INTRODUCTION

Sulfiting agents as food additives have come under close scrutiny due to possible adverse health problems, most common amongst certain asmatics, such as nausea, diarrhea, anaphylactic shock, loss of consciousness, and possible death (Hecht and Willis, 1983). This has caused various federal, state and local food regulatory agencies to propose limiting the residual sulfite on food products. The FDA has placed an acceptable residual sulfite level on shrimp at 100 ppm as SO_2. Thus, shrimp containing residual sulfite greater than the 100 ppm level would be considered adulterated (CFR. 1985).

Processor's concerns that shrimp (either domestically produced and/or imported) meet FDA guidelines, have prompted interest in the possibility of reclaiming adulterated product. Processors, consumers, scientists, and regulatory agencies have inquired about the effect of various cooking methods on the residual sulfite of shrimp. The Codex Alimentarius Commission Standards are 100 ppm (SO_2) residual on raw edible product and 30 ppm on cooked product (FAO/WHO, 1984; CFR. 1984). This international recommendation lacks analytical verification. Therefore, the objective of this work was to examine the effect of cooking on residual sulfite levels and to compare the effectiveness of various reclamation (washing) treatments on lowering excessive sulfite residuals.

MATERIALS AND METHODS

COOKING STUDY

Headless, shell-on white shrimp (Penaeus setiferus), medium size were obtained immediately post-harvest, transported to the Food Science and Human Nutrition Dept. and stored on ice for 1 day. The fresh shrimp were treated with various bisulfite dips (0.5, 1.25, and 2.0% $Na_2S_2O_5$, for 1 min), drained (30 sec), and all samples were stored frozen

(-30°C). A portion of the shrimp was commercially breaded with "Golden Dip + DCA" batter. Cooking treatments included: boiling, shell-on and -off; broiling, shell-on; sauté, shell-off; and frying, shell-off/breaded.

Shrimp (400-500 g) were thawed overnight at room temperature, mixed and drained for 1 min and then divided into two groups of approximate equal weights. Group 1 (control) were raw shrimp, shell-off, which were then chopped, combined and four samples (40-50 g) were weighed, to determine residual sulfite levels. Group 2 (cooking treatments) were shrimp which would be cooked to an internal temperature in excess of 170°C using the following cooking protocol:

Boiling - Shell-on or -off: Place 200-250 g shrimp in 2 l of vigorously boiling tap water for 1.5 min. After cooking, drain and cool to room temperature.

Broiling - Shell-on: Preheat oven 10 min on broiler setting, place 200-250 g shrimp on flat pan and place on rack set at second division, 6 inches from the heating coil (approximately 213°C). Cook for 2.5 min and then turn shrimp over and cook another 2.0 min. Drain and cool to room temperature.

Sauté - Shell-off: Place 15 g of vegetable oil in a teflon pan, heat on a setting of 7 (approximately 199-204°C), and spread shrimp (200-250 g) in pan making sure shrimp are always in contact with the surface. Cook for 2.5-3.0 min with constant stirring and making sure shrimp are turned at least once. Drain and cool to room temperature.

Frying - Breaded, Shell-off: Preheat oil in deep-fat fryer until temperature reaches 149°C (use fresh vegetable oil each time). Place shrimp (200-250 g) in fryer and cook for 2-3 min. Remove shrimp and place on paper towel to drain and cool to room temperature.

Shrimp cooked with shell-on had the shell removed prior to analysis. The edible portion of shrimp for each cooking treatment was chopped, combined, and four samples (40-50 g) analyzed for residual sulfite according to standard AOAC Monier-Williams (M-W) method (AOAC, 1980). The breaded shrimp (frying) were analyzed with breading included as part of the edible portion. An additional experiment was performed as above, however, for the frying treatment, the breading was removed before M-W analysis.

RECLAMATION STUDIES

Two sizes of frozen shrimp (26/30 and 51/60 individual count/lb) having adulterated levels (>100 ppm) of sulfite were obtained from a commercial processor. Three boxes or 15 lb from each size remained frozen as a control. The remaining shrimp were subjected to various reclamation treatments (trt.) using 2 boxes (10 lb) per size per treatment. The frozen shrimp were thawed in flowing water with in-line chlorine (less than 10 ppm) and re-frozen (Thawed trt.), while more shrimp were thawed as above and then commercially peeled and re-frozen (Thawed/Peeled trt.). The final treatment was thawing more of the same shrimp as above, commercially peeling and then washing in flowing cold water (less than 4.4°C) with in-line chlorine (less than 10 ppm) for 30 min and re-freezing (Thawed/Peeled/Washed trt.). Samples from the controls and three treatments were brought to the Food Science and Human Nutrition Dept., Gainesville, FL for sulfite analysis (M-W method).

Pink headed shrimp (Penaeus duorarum), medium size were obtained immediately post-harvest and transported on ice to the Food Science and Human Nutrition Dept., Gainesville, FL. Fresh shrimp were dipped in 1.25% and 2.5% $Na_2S_2O_5$ for 1 min, and a portion of the shrimp from each sulfite dip were frozen for a control. A portion of the remaining shrimp were dipped in ozonated water (1 mg ozone/1 water) for 5 min at a ratio of 1 lb per gallon and frozen (-30°C) until analyzed. Ozone was generated using a portable ozone generator, model 25 HF-1000 (OPT Systems, Inc., Arlington, VA). The remaining portion of fresh shrimp was divided into thirds and treated either by dipping in 3% hydrogen peroxide (H_2O_2), soda or seltzer water for 5 min, then drained and frozen (-30°C) until analyzed. Sulfite analysis on edible tail was performed for all reclamation samples using M-W method.

RESULTS AND DISCUSSION

COOKING EFFECTS

Two cooking methods (broil and fry) did not significantly (α=0.05) reduce residual bisulfite on shrimp (Table 1). A significant (α=0.05) reduction in bisulfite levels occurred at the higher dip (2.0%) concentration for boiled shell-on and shell-off when ANOV and multiple comparison (Duncan) analysis were performed. However, this reduction only averaged approximately 23%. High intensity cooking, sauté, caused a significant (α=0.05) reduction in residual bisulfite levels at all dip concentrations (Table 1). Reductions of 52, 51 and 28% resulted during sauté cooking for 0.5, 1.25, and 2.0% dip concentrations, respectively.

The Codex Alimentarius Commission (CAC) standard for raw edible shrimp is 100 ppm as SO_2 and 30 ppm on cooked shrimp (FAO/WHO, 1984; CFR. 1984). This recommendation implies cooking causes a 70% reduction in residual bisulfite. Our results are contradictory to the CAC standard, indicating the residual bisulfite from the raw product is not reduced by most common cooking methods. Because of the potential significance of this finding, a second experiment was performed.

Table 1. Residual bisulfite levels (ppm as SO_2) on shrimp after various cooking methods: Experiment 1.

Cooking trt.	Dip Concentration 0.5% Raw	0.5% Cook	1.25% Raw	1.25% Cook	2.0% Raw	2.0% Cook
Boiled (shell)						
-on	72 ±30	65 ±32	133 ±17	124 ±23	301 ±100	258 ±75*
-off	42 ±2	66 ±30	141 ±16	115 ±21	270 ±18	197 ±21*
Broiled	41 ±8	52 ±5	188 ±9	184 ±6	215 ±13	230 ±10
Fry	44 ±25	46 ±28	72 ±15	63 ±30	112 ±30	89 ±16
Sauté	46 ±6	22 ±3*	150 ±10	73 ±13*	230 ±29	169 ±22*

[1]Mean ± s.d., n=7 replications.
Numbers followed by an (*) are significantly different (α=0.05) from the raw sample (Duncan's Multiple Comparison).

The second ANOV demonstrated that four of the five cooking methods: boiling, shell-on, -off; broiled; fry; again did not cause significant (α=0.05) reductions in residual bisulfite levels at lower dip concentrations (Table 2). A reduction in residual bisulfite on shrimp may result at the 2.0% dip treatment for these four cooking methods, but the reduction again only averaged 21% (Tables 1 and 2). The second experiment confirmed the results of the first and also, contradicts the CAC standard for cooked shrimp. High intense cooking again caused significant reductions in residual bisulfite levels from uncooked product (Table 2).

Table 2. Residual bisulfite levels (ppm SO_2) on shrimp after various cooking methods: Experiment 2.

Cooking trt.	Dip Concentration 0.5% Raw	0.5% Cook	1.25% Raw	1.25% Cook	2.0% Raw	2.0% Cook
Boiled (shell)						
-on	28 ±2	25 ±2	78 ±18	58 ±4	131 ±10	99 ±11*
-off	22 ±2	16 ±2	56 ±10	58 ±6	115 ±13	130 ±25
Broiled	27 ±2	28 ±2	64 ±10	66 ±6	120 ±7	97 ±7*
Sauté	21 ±7	5 ±0*	55 ±6	19 ±2*	110 ±11	63 ±2*

[1]Mean ± s.d., n=4 replications.
Numbers followed by an (*) are significantly different (α=0.05) from the raw sample (Duncan's Multiple Comparison).

Analyzing fried shrimp with (+) and without (-) breading indicates sulfites do not seem to migrate into the breading upon frying and the breading actually "dilutes" the amount of residual bisulfite on the edible portion of shrimp (Table 3).

Reclamation Effects

Thawing, and thawing and peeling resulted in an approximate 14-20% reduction in residual sulfite on this commercial product (Table 4). Thawing, peeling and then washing for 30 min reduced the residual sulfite levels by 40%. The percent reduction per treatment was similar for either size shrimp. Thus reclamation by common procedures (thawing, peeling, and washing) used in commercial shrimp processing can reduce the concentration of residual sulfites, but the percent reduction is limited.

Table 3. The influence of breading on residual bisulfite levels (ppm as SO_2) in fried shrimp.

	1.25% Dipped Treated Shrimp			
	+ Breading[1]		- Breading[1]	
Trials	Raw	Cooked	Raw	Cooked
1	41	46	63	60
2	33	41	64	59
3	36	36	56	79
4	41	50	71	59
$\bar{X}$ ±sd =	38 ±4	43 ±6	64 ±6	64 ±10

[1](±)Breading implies M-W analysis with (+) or without (-) breading present on fried shrimp.

Ozonated water did not reduce the residual bisulfite levels on shrimp at the 1.25% dip but did reduce (16%) the level on the 2.5% dipped shrimp (Table 5). Again a wash treatment was more effective at a higher residual level, but the ozone treatment enhanced subsequent melanosis. Hydrogen peroxide did reduce substantially the levels of sulfite on shrimp at all dip treatments and the reduction was within FDA guidelines (Table 5). However, the shrimp turned severely melanotic after this treatment and were considered an inferior product. Soda and seltzer water reduced sulfite levels on shrimp approximately 60% and resulted in FDA borderline levels on shrimp. The product appeared to remain free of blackspot after this reduction. Since the chemical washes were applied fairly soon (10-15 min) after bisulfite dipping, a water control must be performed to fully evaluate these treatments. However, soda and seltzer water, unlike ozone and H_2O_2, appear to protect the shrimp from further melanosis after washing.

Table 4. Reclamation of a commercially abused shrimp product after thawing, peeling, and washing treatments.

Treatment	M-W Sulfite[1] (ppm as SO_2)		% Reduction	
	LG[2]	SM[2]	LG	SM
Frozen (control)	250	188	-	-
Thawed	216	150	14	20
Thawed and Peeled	216	168	14	11
Thawed and Peeled and Washed	154	111	38	40

[1]Values are averages of two boxes with two reps. per box.
[2]Large (LG) size, 26-30 count/lb; Small (SM) size, 51-60/lb.

Table 5. Reclamation of shrimp dipped in 1.25 and 2.5% $Na_2S_2O_5$ for 1 min and then dipped in ozonated water, H_2O_2, and soda and seltzer water.

	Average M-W Value[1] (ppm as SO_2)			
	1.25%		2.5%	
	control	wash[2]	control	wash
Ozone water	127 ±18	180 ±7	309 ±20	260 ±20 (16)[3]
3% H_2O_2	127 ±18	78 ±6 (38)	309 ±20	86 ±9 (72)
Soda	-	-	267 ±35	105 ±7 (61)
Seltzer	-	-	267 ±35	99 ±17 (63)

[1]Mean ±s.d., n=4.
[2]Shrimp were dipped in bisulfite then re-dipped in the corresponding treatment usually for 5 min.
[3]Values in () are the % reduction from control.

CONCLUSIONS

Most typical cooking methods offer little advantage in reducing sulfite levels on shrimp. If there is a reduction in sulfite, it occurs at the higher dipping concentration (2.0%). Higher dip concentration may yield a higher portion of free (SO_2) residual. High intensity cooking such as sauté dramatically reduced the residual bisulfite levels on shrimp at all dip concentrations. It would appear, the CAC standard of 30 ppm SO_2 on cooked product must be re-evaluated.

Thawing, peeling and washing can reduce residual (SO_2) sulfite levels on adulterated shrimp, but the percent reductions are limited. The reductions observed were similar for small (51/60) or large (26/30) shrimp.

Ozone reduced (16%) residual bisulfite on the 2.0% dipped shrimp but failed to lower residual levels at 1.25% dip. Hydrogen peroxide (3%) treatment did significantly lower the residual bisulfite on shrimp but melanosis resulted producing an inferior product. Soda and seltzer water dips also resulted in a reduction of residual bisulfite on shrimp. Unlike the H_2O_2, these treatments do not seem to promote melanosis.

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EVALUATION OF ALTERNATIVES TO SULFITING AGENTS AS MELANOSIS INHIBITORS IN RAW SHRIMP

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INTRODUCTION

Much attention has been given in recent years to the problems associated with use of sulfiting agents in foods. Because these additives cause severe allergic reactions in persons with asthma, the U.S. Food and Drug Administration (FDA) has been under pressure to re-evaluate the safety of sulfiting agents. Future regulation of sulfites is a strong possibility, and this action is likely to affect the shrimp industry.

At present, the Codex International Standard for residual sulfite is set at not more than 100 p.p.m. sulfur dioxide (SO_2) units in raw shrimp, and 30 p.p.m. SO_2 in cooked product. These same levels have been suggested by the U.S. FDA. If stronger regulations are imposed, alternatives to sulfiting agents may be needed to combat the problem of melanosis in shrimp. A complete substitute for sulfiting agents that possesses all the properties of this group will be virtually impossible to find. However, other additives alone or in combination may reduce the rate of blackspot formation on shrimp, and thus serve as valuable alternatives.

The selection of additives used in this study was based on the series of reactions converting the amino acid tyrosine to melanin, thus forming black spots on shrimp. Polyphenoloxidase, a key enzyme in the process, is dependent on copper ions for activity. Thus, removal or complexing of those ions may inhibit or reduce polyphenoloxidase activity. EDTA and boric acid may act in this manner to bind copper ions. Enzyme activity may also be reduced by lowering the pH in which the reactions occur; addition of citric acid may help to increase acidity. Since oxygen is a required substrate for polyphenoloxidase, removal of oxygen from the reacting environment may help reduce melanosis. Reducing agents such as ascorbates, erythrobates, and reducing sugars will not only utilize the available oxygen, but may also convert quinones back to di-phenols, thus inhibiting the process of melanosis.

METHODS AND MATERIALS

This study was conducted in three separate phases, with each phase evaluating one of the previously mentioned groups of food additives: acidic compounds, complexing agents and reducing agents. Although the procedures followed in each phase were identical, three separate lots of shrimp were used, so comparisons cannot be drawn among the three parts of the experiment.

White shrimp, Penaeus setiferus, were harvested from Corpus Christi Bay, deheaded, and placed on ice overnight. (Bay shrimp, rather than Gulf shrimp were used to insure that no bisulfite had been previously applied to the shrimp. Bay shrimpers bring in their catch daily, and generally do not use bisulfite.) In each of the three phases, shrimp were divided into equal portions, and treated as follows:

1. One portion dipped in 1.25% sodium bisulfite ($NaHSO_3$) solution for one minute, and rinsed for fifteen seconds. (This is considered good manufacturing practice by the U.S. FDA.)
2. One portion left untreated, and
3.a. Equal portions of shrimp were dipped for five minutes in 1%, 2%, and 5% solutions of citric acid, and rinsed for fifteen seconds.
   b. Equal portions were dipped for five minutes in 1% and 2.5% solutions of EDTA and boric acid, then rinsed briefly.
   c. Equal parts of shrimp were dipped for five minutes in 1% and 2.5% solutions of ascorbate, isoascorbate, sodium ascorbate and glucose, then rinsed briefly.

Following treatment, each lot was divided in half, one-half being glaze-frozen in five-pound boxes for later analysis, and one-half placed on ice at a ratio of two parts of ice to one part shrimp. The iced shrimp were analyzed and evaluated daily for up to two weeks. Laboratory analyses included microbial analysis, visual blackspot evaluation, and surface pH measurement.

Aerobic plate counts were taken from shrimp every other day during the storage period using the agar spread plate method. Data was reported as the mean of duplicate samples. Shrimp from each treatment, along with bisulfite-treated controls and untreated controls, were evaluated for melanosis formation. This consisted of an objective assessment of percentage presence of black spot, from 100

arbitrarily chosen shrimp from each group. Surface pH of the treated and control shrimp was monitored with an Orion model 91-35 surface electrode. Five readings were taken at each contact point, and data reported as the mean of these five readings.

RESULTS AND DISCUSSION

Table 1 shows percentage blackspot in shrimp treated with acidic compounds. Analysis of variance (ANOVA) showed no significant difference between treated samples and controls. Aerobic plate counts from shrimp treated with citric acid are shown in Figure 1. All three concentrations had antimicrobial effects, though none were significantly better. Surface pH measurements (Figure 2) showed no significant acidity increases, although 1% and 2% citric acid decreased pH slightly between days 4 and 6.

Table 1. Percentage of blackspot development on shrimp treated with acidic compounds.

			TREATMENTS		
	Untreated	$NaHSO_3$	Citric Acid		
DAYS ON ICE			1%	2%	5%
1	6	1	none	1	3
3	20	5	15	12	19
5	37	19	30	16	22
7	59	28	39	35	42

Compared to untreated shrimp, both EDTA and boric acid had an inhibitory effect on blackspot formation, as shown in Table 2. A dip in 2.5% boric acid solution was especially effective, giving comparable results to shrimp treated with $NaHSO_3$ according to current good manufacturing practices. Figure 3 shows the effect of complexing agents on microbial numbers. Solutions of both 2.5% EDTA and 2.5% boric acid showed the greatest reductions, or lowest counts, although not significantly lower. The effects of EDTA and boric acid on surface pH are presented in Figure 4. Shrimp treated with 1% and 2.5% EDTA show significantly lower pH values throughout the storage period, while shrimp treated with boric acid did not.

As shown in Figure 5, none of the reducing agents proved effective in reducing microbial numbers during ice storage. During the same storage period, none of the treatments came close to the blackspot inhibition resulting from treatment with $NaHSO_3$ (Table 3). Figure 6 shows the effect of reducing agents on surface pH. Although it looks confusing, ANOVA confirms that 1% and 2.5% solutions of both ascorbate and isoascorbate significantly reduce pH, with 2.5% isoascorbate showing the greatest reduction. Combining effects from all three quality evaluations, a 2.5% isoascorbate solution provides the best results. Isoascorbate imparts a yellow color to the shrimp however, and this color defect would probably be rejected by the consumer.

The treatments which show most promise here are 2% citric acid and 2.5% boric acid; boric acid is especially effective in reducing melanosis. Combinations of these additives will be tested in the future to examine any synergistic effects which may occur. This experiment will be repeated using the treated shrimp frozen in five-pound boxes. These will be slacked out, placed on ice, then monitored for microbial numbers, pH, and blackspot formation. Finally, these treatments will be combined with various on-board handling techniques, such as length of trawl time and length of time on-board before icing, to hopefully come up with an effective alternative to sulfite inhibition of melanosis.

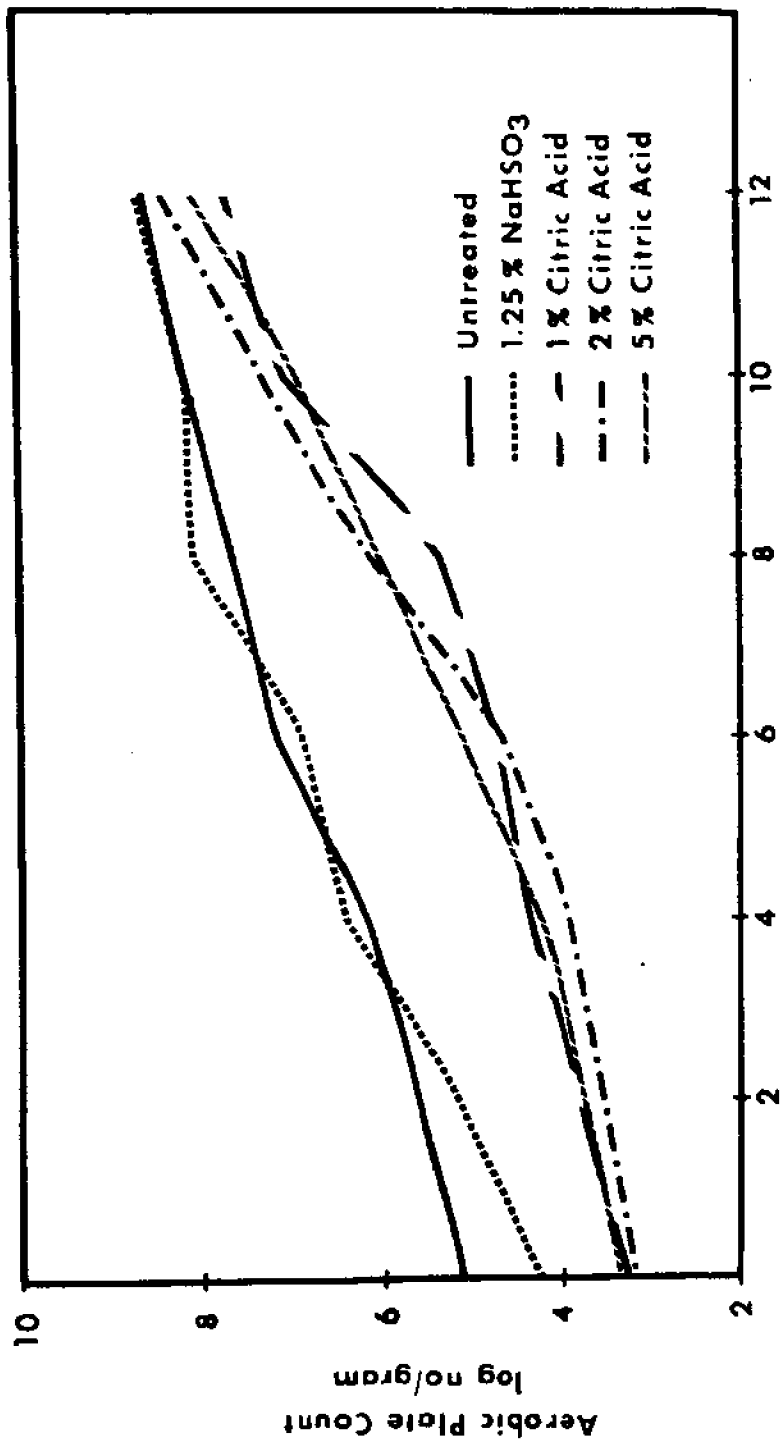

Figure 1. Aerobic plate counts from shrimp treated with acidic compounds.

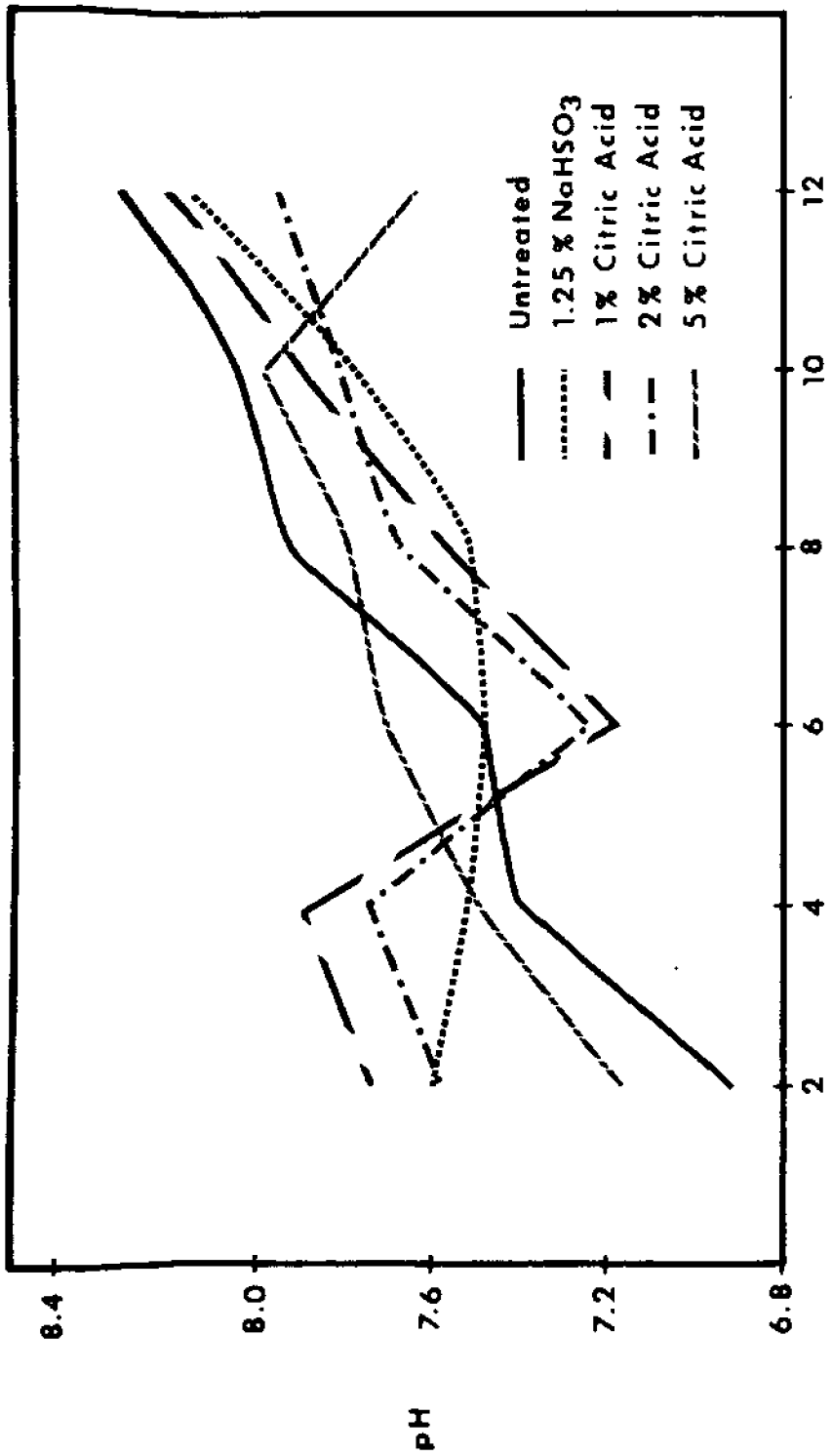

Figure 2. pH values from shrimp treated with acidic compounds.

Table 2. Percentage of blackspot development on shrimp treated with complexing agents.

DAYS ON ICE	Untreated	$NaHSO_3$	TREATMENTS			
			EDTA		Boric Acid	
			1%	2.5%	1%	2.5%
2	7	none	6	2	1	0
4	9	none	11	3	5	0
6	18	none	15	7	6	3
8	24	none	18	14	9	4
10	31	2	23	21	15	4
12	38	4	31	22	17	6

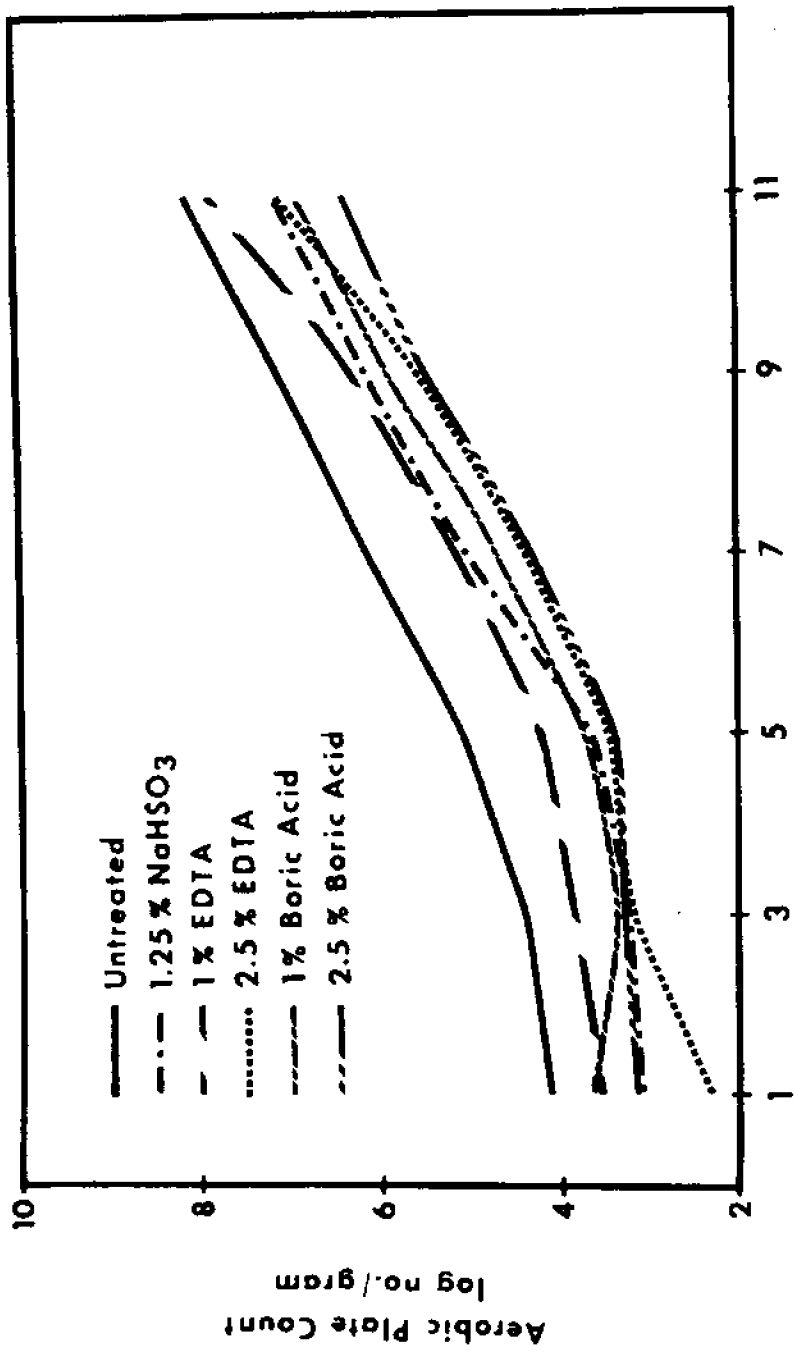

Figure 3. Aerobic plate counts from shrimp treated with complexing agents.

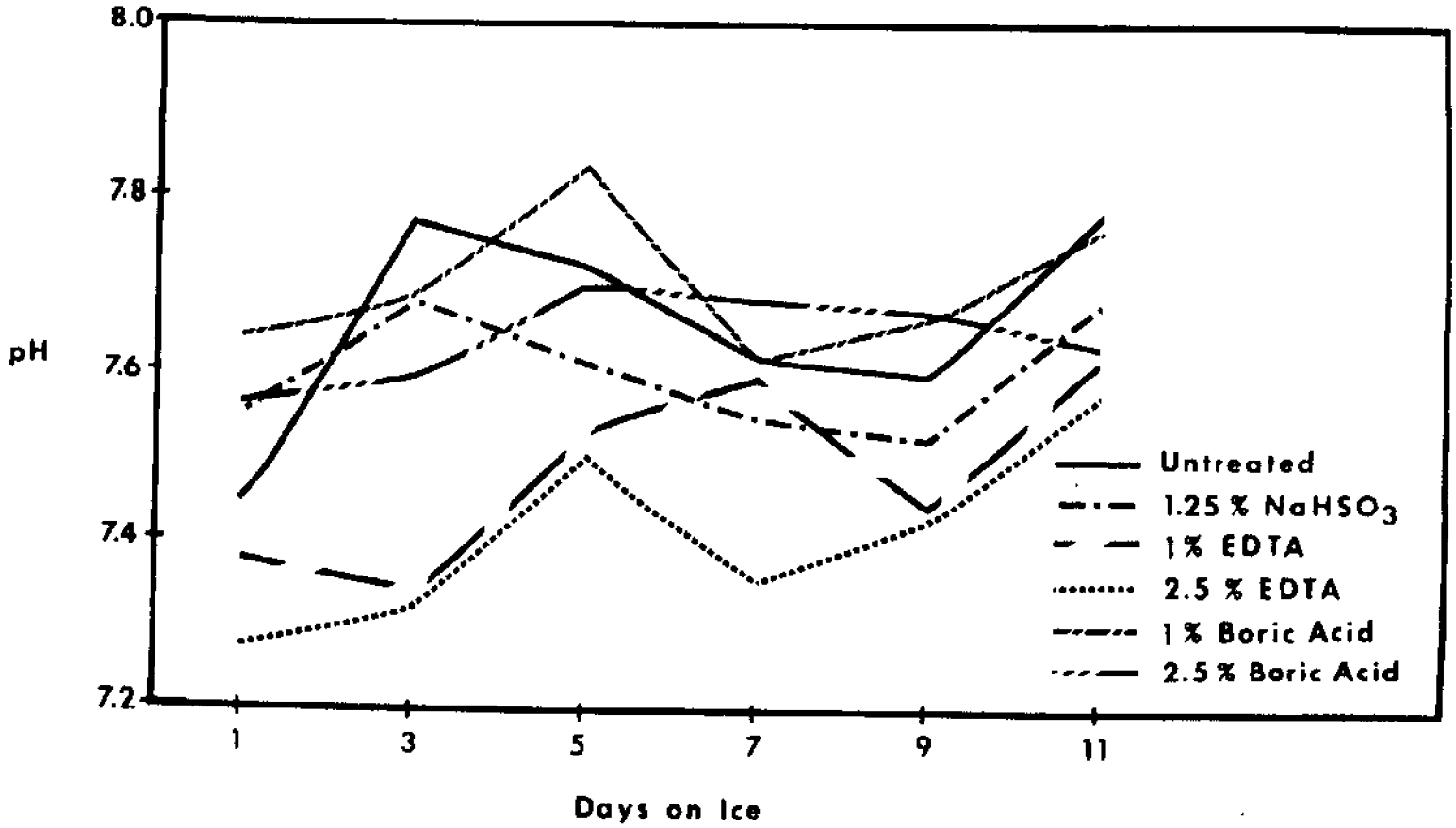

Figure 4. pH values from shrimp treated with complexing agents.

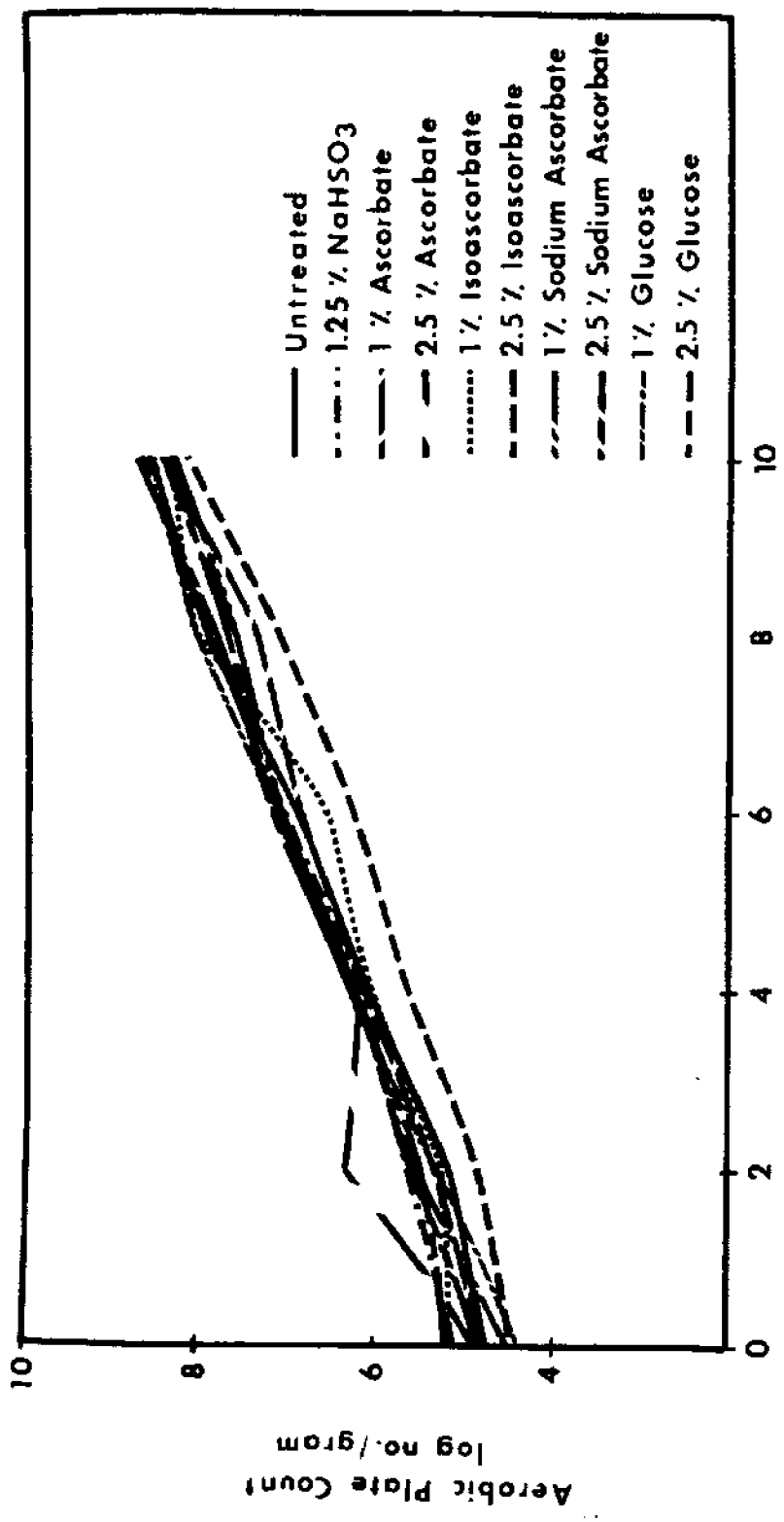

Figure 5. Aerobic plate counts from shrimp treated with reducing agents.

Table 3. Percentage of blackspot development on shrimp treated with reducing agents.

DAYS ON ICE	Untreated	$NaHSO_3$	TREATMENTS							
			Ascorbate		Isoascorbate		Sodium Ascorbate		Glucose	
			1%	2.5%	1%	2.5%	1%	2.5%	1%	2.5%
1	4	2	3	3	7	3	3	3	17	8
3	8	2	5	4	7	3	6	9	23	9
5	14	4	9	6	12	10	17	23	28	17
7	19	6	19	26	25	16	23	41	37	30
9	26	10	31	38	43	33	45	56	47	34
11	30	14	48	59	56	47	50	62	62	46

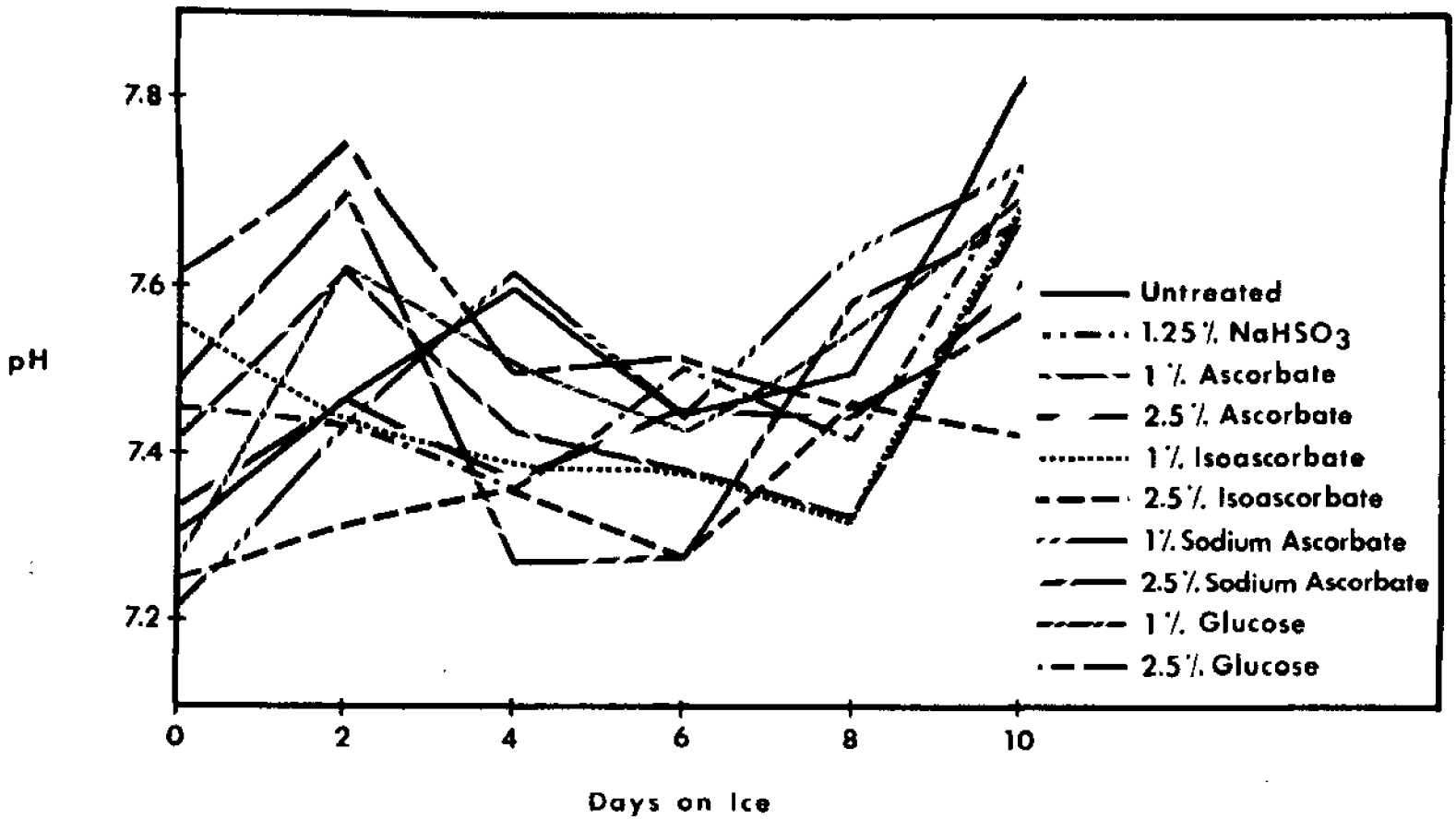

Figure 6. pH values from shrimp treated with reducing agents.

SCREENING ALTERNATIVES TO SULFITING AGENTS TO CONTROL SHRIMP MELANOSIS

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INTRODUCTION

Shrimp melanosis, commonly known as 'blackspot' is a harmless but objectionable surface dicoloration caused by polyphenoloxidase enzyme systems which remain active during refrigeration or ice storage. In the early 1950's sulfiting agents, particularily sodium bisulfite was first introduced to prevent or inhibit melanosis, thus yielding a more valuable harvest (1). Such use of sulfites was 'prior sanctioned' by the U.S. Food and Drug Administration (FDA) in 1956 (2). More recent FDA decisions reaffirmed this practice (3), but continuing regulatory scrutiny could restrict or eliminate the application of sulfite on shrimp. The regulatory action is prompted by an increasing concern for adverse 'allergic' reactions most common amongst hyper-(sulfite) sensitive asthmatics. Thus work was initiated to find alternatives to replace or reduce the amount of sulfites required to inhibit shrimp melanosis. This work would screen for possible alternatives which would require subsequent verification with field tests and statistical evaluations.

MATERIAL AND METHODS

Preliminary investigations were necessary to describe the rate and extent of shrimp melanosis. Samples of fresh, untreated white shrimp (Penaeus setiferus) and pink shrimp (P. duorarum) were observed in refrigeration. The occurrence of melanosis was recorded in photographs to establish a subjective scale for comparisons. The white shrimp (harvested off Jacksonville and Apalachicola, FL) did not develop melanosis in a consistent or predictable fashion. Attempts to induce melanosis in white shrimp exposed to elevated oxygen levels in sealed containers or ultraviolet lighting were unsuccessful. The pink shrimp (harveted near Key West, FL) developed melanosis in a predictable fashion usually first evident within 2 days on ice and becoming progressively more prominent during subsequent storage for 14 days. Thus pink shrimp was the choice species for further tests relative to the scale developed to describe melanosis (Table 1). This choice was consistent with the original work by Camber et. al (4) which introduced the use of sulfites through field tests with Key West, pink shrimp.

TABLE 1. Scale used to describe and rate the occurrence of melanosis (black-spot) on pink shrimp.

Melanosis Scale	
0	Absent
2	Slight, noticeable on some shrimp
4	Slight, noticeable on most shrimp
6	Moderate, noticeable on most shrimp
8	Heavy, noticeable on most shrimp
10	Heavy, totally unacceptable

TABLE 2. Compounds used individually and in mixtures to prepare dips for treating fresh pink shrimp to control melanosis.

Compound	Comments
Sodium Bisulfite	Reducing agent
Sodium Bicarbonate	Baking soda
Potassium Bromate	Oxidizing agent; interact sulphydial transport bonds
Calcium Chloride	Geling agent; interfer oxygen
Erythrobate	Acidulant, chelator, reducing agent
Ascorbic Acid	Acidulant, antioxidant
Boric Acid	Acidulant
Citric Acid	Acidulant, antioxidant, chelator
Phosphoric Acid	Acidulant
Sodium Tripolyphosphate	Water control, sequestrant
Disodium phosphate	Water control, buffer
Sodium Hexametaphosphate	Water control, sequestrant
Ehtylene Diamine Tetra Acetate	Chelator
Glycine	Complex with quinones
Taurine	Bond sulfonic acid
Formaldehyde	Complex with proteins
Hydrogen Peroxide	Oxidizing and bleaching
BL7*	Sulfite (67%) + phosphate + erythrobate + phosphates + citrate + tartrate + glutamate + tryptophan (descending order)

*Composition of BL7 provided by letter (1978) from Food Chemistry Division, Environmental Sanitation Bureau, Ministry of Health and Welfare, Japanese Government.

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The melanosis scale can be related to existing recommendations developed by the National Marine Fisheries Service for grading raw shrimp (5). A scale rating of 4 or greater represents a measurable defect in product quality. A rating of 8 or greater would represent a severe defect, approaching unacceptable product.

Harvests were arranged such that the investigators obtained fresh, heads-on pink shrimp while working on the vessel or within less than 12 hours post-harvest at the dock. All shrimp were routinely washed on-board and temporarily stored in ice. The basic experimental procedure was to rinse 400-600 grams of shrimp in 2.5 liters of variable dip compositions and concentrations for 1 minute, then drain and package in plastic bags to be stored in ice. The bags were considered necessary to eliminate the variable influence of melting ice. Iced containers with packaged shrimp were stored in 35°F (1.7°C) refrigeration, and reicing every other day.

Development of melanosis was scored and photographed routinely during 2 weeks storage. The bags of shrimp had been numbered such that the investigator could not distinquish amongst the various treatments. One experienced investigator did all scoring relative to the aforementined scale (Table 1). The scale was accompanied by pre-developed color prints depicting common examples of the advancing stages for melanosis. The intent was to screen for obvious differences between treatments, thus selecting the best treatments for subsequent tests with statistical evaluations.

The various dips or chemical treatments included controls (no treatment), customary sodium bisulfite used in varying concentrations, and a variety of single compounds and/or mixtures prepared in varying concentrations (Table 2). The dip solution was fresh tap water.

Two field trials (I and II) were necessary to accomodate all the variable treatments. Trial I was for shrimp harvested 6/26/85 and Trial II commenced 12/13/85. Water temperatures and atmospheric conditions were clear and similar in Key West during both harvests. The common practice for pink shrimp is night harvest, thus avoiding influence of sunlight. One set of controls (no treatment) and bisulfite treatments were included for each trial to account for any variations amongst shrimp per harvest. Trial II included an additional series of treatments using 3.5% saltwater as the dip solution. The saltwater was made from the same source of fresh tapwater plus 3.5% commerical marine (aquarium) salts.

RESULTS AND DISCUSSION

Preliminary experience in developing a rating scale with accompanying photographs depicting the degrees for melanosis

proved successful. Rating for controls and bisulfite treatments were similar for both trials (compare Table 3 and 4). Melanosis on pink shrimp seem to progress in a linear manner. In controls, melanosis was obvious within 3 days, becoming a defect within 5 days, and approaching a severe defect (unacceptable) on day 7. Thus pink shrimp was a practical test species as opposed to white shrimp which in some instances did not display melanosis.

All bisulfite treatments (0.25 to 2.50% dips) inhibited the onset of melanosis (Talbe 3 and 4). The most effective concentration was 2.50%, thus demonstrating the encouragement for employing treatments in excess of the legally recognized 1.25% dip for 1 minute. The 1.25% bisulfite dip inhibited melanosis until blackening was only slightly noticable on some shrimp after 12 days storage. Melanosis increased to a measureable defect on day 12 after treating with 0.25 and 0.50% dip concentrations.

No treatments in Trial I were as effective as 1.25% sodium bisulfite. The next effective treatment was the commercial preparation, BL7. The inhibitor influence of BL7 at a dip strength of 1.0% was similar to sodium bisulfite at 0.50%. This is expected relative to the formulation for BL7 which is 67.2% sodium hydrogen sulfite. Thus a 1.0% BL7 dip contains the equivalent of 0.67% sodium bisulfite.

A variety of chemical combinations (treatments no. 4-8) provided initial inhibition still evident on the 7th day of storage (Table 3). All of these mixtures contained some level of bisulfite (0.25 or 0.50%). After 12 days storage, shrimp from all these treatments exceeded a score of 6 and some were judged unacceptable. Thus the influence of the other constituents (Asc, DSP, EDTA, SHP, or STP) did not enhance the influence of bisulfite over that recorded for similar, individual bisulfite treatments (0.25 and 0.50%). This suggests the bisulfite provided the dominant influence in these mixtures. The mixture which included ascorbate (treatment no. 4) appeared to have an objectionable yellow tint obvious on day 3.

All remaining dips in Trial I (treatment nos. 9-17) resulted in melanotic shrimp scored within the 3rd day of storage (Table 3). Despite the early onset of melanosis after dips with STP (4.0 and 8.0%) and Ery/EDTA (1.0/0.1%), the final melanosis rating on day 12 did not exceed 6, suggesting some partial control. The adverse results after sodium bicarbonate dips dispell some fishermen's common belief that baking soda can prevent melanosis. Treatments with calcium chloride, hydrogen peroxide and potassium bromate promoted melanosis.

Results from Trial II reaffirm the distinct influence of bisulfite dips (Table 4). Again, the mixtures which were less effective, but approximating the influence of bisulfite dips,

TABLE 3. Trial I. Ratings for the occurrence of melanosis on pink shrimp in refrigerated storage (per day) after treatment in a variety of dips for 1 minute. The dip solution was fresh tapwater. After controls the treatments are numbered and placed in a general order for decreasing effectiveness.

Trt. No.	Dips %	Day Storage 3	7	12
1.	Control (No dip)	2-3	7-9	10
2.	Sodium Bisulfite			
	0.25	2	3	6
	0.50	0	0	3
	1.25	0	0	2
	2.50	0	0	0
3.	BL 7 (Commercial)			
	0.25	0	3	6
	0.50	0	3	6
	1.00	0	0	5
4.	Bis/EDTA/Asc			
	0.5/0.1/1.0	2	3	6
	0.25/0.1/1.0(y)	0	4	6
5.	Bis/STP			
	0.5/2.0	0	2	8
	0.5/5.0	0	3	6
	0.25/2.0	2	4	7
	0.25/5.0	0	3	9
6.	Bis/EDTA/DSP			
	0.5/0.1/1.0	0	4	7
	0.5/0.1/2.0	0	4	6
	0.5/0.1/4.0	0	5	8
7.	Bis/EDTA/STP			
	0.25/0.1/2.0	0	5	8
	0.25/0.2/2.0	2	5	9
	0.25/0.2/5.0	0	4	8
	0.25/0.1/5.0	0	4	7
	0.50/0.1/2.0	0	4	7
	0.50/0.2/5.0	0	4	9
8.	Bis/EDTA/SHP			
	0.5/0.1/1.0	0	4	9
	0.5/0.1/4.0	2	6	10
9.	Ery/EDTA			
	1.0/0.1	2	4	6
10.	STP			
	2.0	3	6	10
	4.0	2	4	6
	8.0	2	4	6
11.	Phosphoric Acid			
	0.5	3	5	7
	1.0	3	6	10
12.	STP/EDTA			
	2.0/0.1	0	3	10
	2.0/0.2	5	8	10
	4.0/0.1	3	6	10
	4.0/0.2	3	6	10
13.	Sodium Bicarbonate			
	2.0	3	8	8
	4.0	3	8	8
14.	Asc/EDTA			
	1.0/0.1(y)	3	8	10
15.	Calcium Chloride			
	1.0	8	8	10
	2.0	4	6	7
	5.0	6	8	10
16.	Hydrogen Peroxide			
	0.1	6	7	10
	0.5	8	10	10
	1.0	8	10	10
17.	Potassium Bromate			
	0.1	10	10	10
	0.5	10	10	10
	1.0	10	10	10

KEY
Asc = Ascorbic Acid
Bis = Sodium Bisulfite
Cit = Citric Acid
DSP = Disodium Phosphate
EDTA = Ethylene Diamine Tetra Acetate
Ery = Erythrobate
SHP = Sodium Hexameta phosphate
STP = Sodium Tripolyphosphate
BL7 = Commercial melanosis inhibitor
(y) = yellowing

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TABLE 4. Trial II. Ratings for the occurrence of melanosis on pink shrimp in refrigerated storage (per day) after treatment in a variety of dips for 1 minute. The dip solution was fresh tapwater. Ratings within parenthesis are for shrimp treated when the dip solution was 3.5% saltwater (commercial marine salts). After controls, the treatments are numbered and placed in a general order for decreasing effectiveness.

	DIP %'s	Days Storage 3	5	7	12
1.	**Control (no dip)**				
	freshwater rinse	2-4	5-6	7-9	10
	saltwater rinse	(4-5)	(5-7)	(9-10)	(10)
2.	**Sodium Bisulfite**				
	0.25	0(0)	0(0)	6(2)	6(5)
	0.50	0(0)	1(0)	2(4)	6(5)
	1.25	0(0)	0(0)	0(2)	2(4)
	2.50	0(0)	0(0)	0(0)	0(2)
3.	**Bis/EDTA/Cit.**				
	0.5/0.1/0.5	0(0)	0(1)	2(4)	5(3)
	0.5/0.2/0.5	0(0)	0(0)	2(3)	3(5)
	0.25/0.1/0.5	0(0)	0(1)	3(5)	3(5)
	0.25/0.2/1.0	0(0)	2(2)	3(5)	4(6)
4.	**Boric Acid**				
	0.5	0(0)	0(0)	5(5)	6(7)
	1.0	0(0)	0(0)	1(3)	4(2)
5.	**Bis/Cit**				
	0.5/0.5	0(0)	1(0)	4(5)	4(4)
	0.25/1.0	0(0)	3(2)	5(6)	7(5)
	0.25/0.5	0(0)	2(1)	4(5)	8(4)
6.	**Bis/Ery**				
	0.5/0.5	0(0)	0(0)	1(2)	2(4)
	0.5/0.1	0(0)	1(2)	4(7)	4(6)
	0.25/0.5	0(0)	2(5)	3(10)	6(10)
	0.25/0.1	0(0)	2(3)	7(4)	6(10)
7.	**Bis/EDTA**				
	0.5/0.5	0(0)	2(3)	5(7)	5(4)
	0.5/0.2	0(0)	1(3)	5(6)	5(5)
	0.25/0.1	0(0)	1(2)	4(7)	5(5)
	0.25/0.2	0(0)	3(5)	6(6)	5(7)

TABLE 4 continued

8.	**Asc/Cit**				
	1.0/1.0(Y)	0(0)	1(1)	5(4)	9(2)
	1.0/0.5(Y)	1(1)	5(4)	5(5)	7(5)
	0.5/1.0(Y)	0(0)	1(1)	5(7)	1(1)
	3.0/1.0(Y)	1(0)	1(1)	1(3)	1(1)
9.	**Formaldehyde**				
	0.5	0(0)	2(4)	3(7)	10(7)
	1.0	0(0)	2(1)	4(1)	7(7)
10.	**BIS/EDTA/ERY**				
	0.5/0.1/0.5	0(0)	2(2)	6(5)	7(7)
	0.25/0.1/0.5	0(0)	2(2)	6(7)	7(7)
	0.25/0.2/1.0	0(0)	1(2)	7(7)	8(7)
11.	**EDTA**				
	0.1	2(2)	3(2)	5(5)	5(4)
	0.2	2(2)	5(3)	6(5)	5(5)
	0.4	2(2)	3(2)	5(5)	5(5)
12.	**ERY/EDTA/CIT**				
	0.5/0.1/0.5	0(0)	3(5)	9(7)	10(10)
	0.1/0.2/0.5	0(0)	5(5)	8(8)	10(9)
13.	**CITRIC ACID**				
	0.5	1(1)	4(4)	9(8)	10(10)
	1.0	1(2)	4(4)	7(6)	10(10)
14.	**GLYCINE**				
	0.5	1(1)	4(4)	8(7)	10(10)
	1.0	1(1)	4(7)	9(9)	10(10)
15.	**ERYTHROBATE**				
	0.1	3(3)	5(5)	10(9)	10(10)
	0.5	4(3)	6(5)	8(7)	10(10)
	1.0	3(3)	5(5)	5(9)	10(10)
16.	**TAURINE**				
	0.5	3(3)	7(6)	9(10)	10(10)
	1.0	3(3)	7(7)	9(10)	10(10)

ASC = Ascorbic Acid
Bis = Sodium Bisulfite
Cit = Citric Acid
Ery = Erythrobate
EDTA = Ethyl Diamine Tetra Acetate
(Y) = Noticeable yellowing

SO/ts/3.22

all included a portion of bisulfite (treatments nos. 3 and 5-7). The most effective mixtures amongst these treatments were essentially equivalent to a 0.50% bisulfite dip and not better than a 1.25% bisulfite dip (Figure 1). The most effective mixture was Bis/Ery (0.5/0.5%), but this effect was not substantiated by similar dips including EDTA (treatments no. 10). All of these moderately effective mixtures contained a portion of bisulfite (0.25 or 0.50%). The mixtures with 0.50% bisulfite appeared superior to similar mixtures with less bisulfite (0.25%). For example, the Bis/Cit dip at 0.5/0.5% provided more prolonged control of melanosis than did the mixtures of 0.25/0.5% or 0.25/1.0%. These results again suggest the dominant influence of bisulfite.

Although boric acid and formaldehyde are not included on the U.S. Food and Drug Administration's 'GRAS' list (generally recoginized as safe), these dips provided some inhibition, thus demonstrating the influence of acidulants and protein binding (Table 4). The Asc/Cit dip retarded melanosis, yet produced a distinct yellowish tint obvious from day 3 through 7. Additional dips (treatments no. 11-16) were least effective, some yielding unacceptable shrimp within 7 days storage.

In Trial II the melanosis rating in parenthesis per treatment and day of storage are results for shrimp rinsed in dips made with 3.5% saltwater (Table 4). General comparisons with the complementary tapwater dips indicate a more favorable response, or less melanosis after freshwater dips. This observation is preliminary and restricted to interpretation relative to the use of a marine (aquarium) grade salt mixture. Further field work with statistical designs and actual seawater (as may be used by the fishermen) would be required before concluding recommendations.

SUMMARY

1. The choice of shrimp species can influence the occurrence of melanosis and the interpretation of tests to develop alternatives to sulfites. The results from this study are relative to the use of pink shrimp (Penaeus duorarum).

2. Raw, untreated pink shrimp develop melanosis in a linear manner, initially obvious on some shrimp within 3 days refrigerated storage and progressing as a severe product defect after 7 days. Thus pink shrimp require some measures to prevent melanosis to assure marketability.

3. A 2.50% bisulfite dip (1 minute) was more effective in preventing melanosis than was the legally recognized 1.25% bisulfite dip.

4. The 1.25% bisulfite dip (1 minute) was superior in preventing melanosis than was any treatment, single

Figure 1. Ratings for the degree of melanosis on pink shrimp following treatment in a variety of alternative dips (% composition) and sodium bisulfite dips (0.50 and 1.25%).

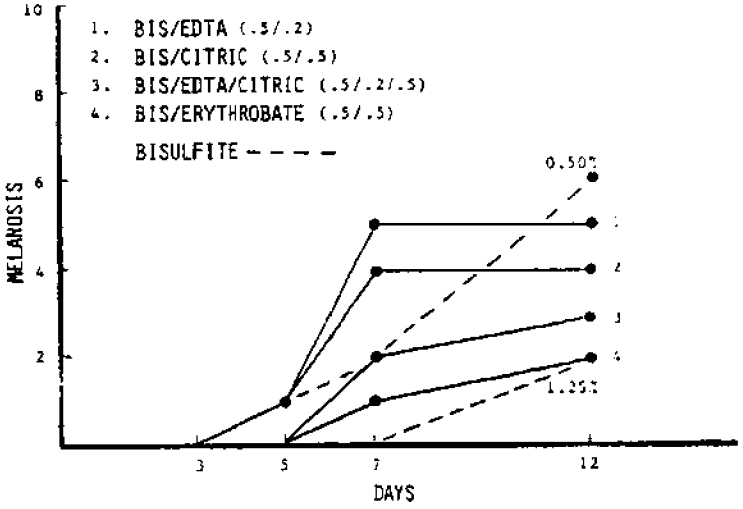
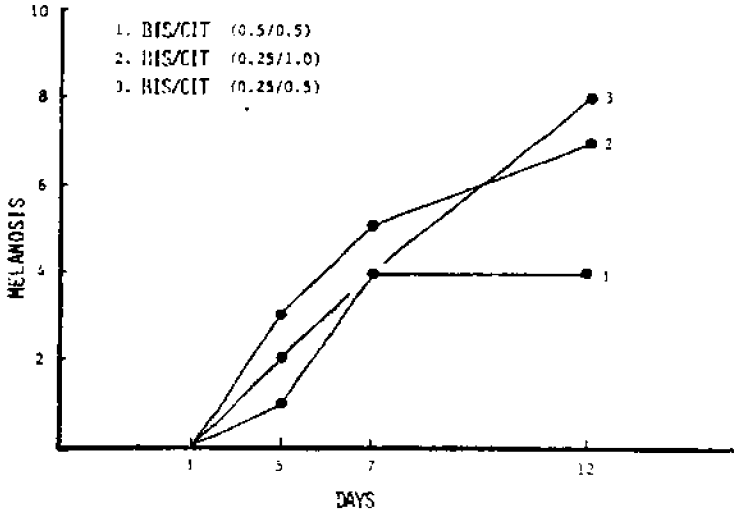


Figure 2. Ratings for the degree of melanosis on pink shrimp following treatment in dips with varying mixtures (% composition) of sodium bisulfite (Bis) and citric acid (Cit).



compounds or mixtures, used in this study.

5. Comparative results suggest dips containing mixtures of bisulfite plus citric acid, erythrobate, and/or EDTA could offer moderate prevention of melanosis. These mixtures are more effective at higher bisulfite concentrations. The bisulfite appears to impart a dominant influence.

6. Further field trials approximating actual fishing practices and employing statistical evaluations are necessary to verify the effectiveness of mixtures including bisulfites, citric acid, erythrobate and/or EDTA This work could also evaluate the influence of freshwater vs. seawater as the dip solution.

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FLUCTUATIONS IN CALICO SCALLOP PRODUCTION (ARGOPECTEN GIBBUS)

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INTRODUCTION

The commercial scallop industry on Florida's eastern coast is a relatively young industry. Although some commercial fishing began as early as 1967 it would be almost a dozen years before the calico scallop industry located in Port Canaveral would become firmly established. It was estimated in 1971 that as much as 20 million pounds of scallop meats could be produced annually from the calico scallop grounds located around the Cape using 40 vessels (Allen & Costello, 1972). In 1984 one of the major commercial scallop companies located in Port Canaveral processed more the 14.5 million pounds of scallop meat using approximately 20 vessels. The processing plant operated around the clock seven days a week.

Unfortunately, in 1985 the numbers of scallops located throughout the Cape Canaveral scallop grounds dropped dramatically. The average number of gallons per trip dropped from 475 gallons in May of 1984 to an average of only 125 gallons in May of 1985 and decreased even further as the summer continued. Eventually this resulted in a cutback in the number of hours the production facility operated and finally in the fall of 1985 the plant was forced to shut down completely for approximately one month.

Since October of 1983 we have been studying the reproduction of the calico scallop, Argopecten gibbus. This has provided us with an excellent base of 26 consecutive months of data. Information collected has included shell morphology, reproductive state, histological examination, body component indices and meat count calculations. By combining this data with production data graciously supplied by one of the scallop companies operating out of Port Canaveral, we have been able to note certain trends and characteristics which should be of particular value to members of the calico scallop industry. Preliminary analysis of some of the data on the reproductive state of the scallops coupled with other measurements which we have made suggests a possible explanation for the decrease in the numbers of scallops seen in 1985.

METHODS

Upon return to the laboratory the shell height and length of each scallop was measured using vernier calipers accurate to 0.1 millimeter.

Shell height is a straight line measurement from the umbo to the ventral edge of the shell while shell length is the maximum straight line measurement from the posterior to the anterior edge of the scallop shell.

The scallops were then divided into two groups. The first group consisted of a minimum of at least 20 scallops from each distinct size class present in the catch. These scallops were then dissected into their component parts (gonads, digestive gland and kidneys, adductor muscle, and the remaining mantle and gills). After visual observations were recorded each component was weighted to 0.0001g on a Mettler balance. This was done both before and after the tissue was dried at 50° C for 7 days. Wet and dry body component indices were then calculated for each component based upon the percentage by weight of each type of tissue (Giese, 1959).

The second group of scallops consisted of at least 16 scallops from each size class present in the catch. These were used for histological studies of the reproductive state of the calico scallop. The scallops were removed from their shells and visual observations recorded. The tissue was then placed in Zenker - Formalin (Helly's) solution (Luna, 1968) for fixation. After 2-3 hours the scallops were bisected to increase penetration of the fixative and returned to the fixative for a total fixation time of approximately 20 hours. Excess tissue was then trimmed away and the samples rinsed in water to remove excess fixative. The tissue was then embedded in Paraplast (MP 56° C) using standard histological procedures, sectioned and stained with hemotoxylin and eosin (Yevich & Barszcz, 1977).

RESULTS

Previous researchers have found that the catch rates off of the eastern coast of Florida are normally highest in the late summer and fall with the catch rate decreasing during the winter and then slowly increasing during the spring (Roe, Cummins & Bullis, 1971). Figure 1 shows weekly production totals in one company for 1984 and the first five months of 1985. The first 8 to 9 months of this graph depict a normal increase in the yield. With the onset of winter, yields decrease and fluctuate widely as a result of weather conditions. The rougher seas of the winter months and the arrival of storms and hurricanes at other times of the year forces temporary reductions in the number of trips. The vessels being used can operate effectively in maximum seas of 6-8 feet with the efficiency of the fishing gear decreasing rapidly in rougher weather.

A more accurate view of the abundance of the scallops is provided by looking at the average gallons of scallop meat per trip for each week. This information for the same time period as the total production

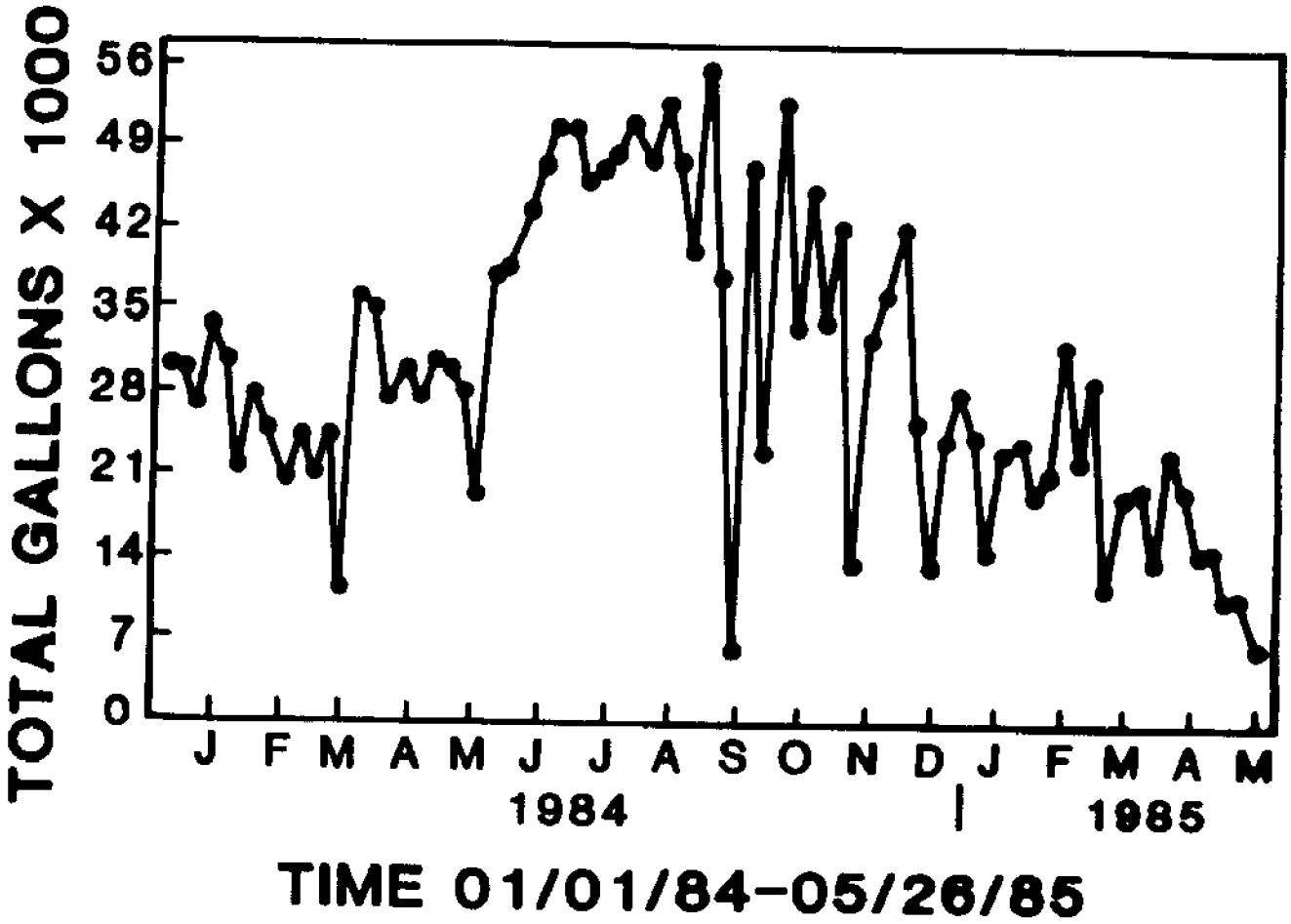

Figure 1. Representative weekly production totals in gallons of scallop meats from January 1984 through May 1985.

data is shown in Figure 2. Most of the large fluctuations are removed in this manner and actual trends are more easily discerned. It can clearly be seen that there was a steady decline in the gallons per trip through the first five months of 1985. This is contrary to the normal increase expected for that time of the year. The average gallons per trip continued to decline during the summer and early fall. By the end of the year the average had risen slightly to approximately the same levels as were observed in April and May of 1985.

The shell height is the logical choice for showing the relative size of the calico scallop. Figure 3 shows the average shell height for samples collected from June of 1982 through 1985. The data for each year is presented separately in order to point out seasonal variations in the scallop size. Each line represents the size class of the majority of the scallops present with outlying points representing other size classes composing at least 20% of the sample. In both 1983 and 1985 there was a drop in the average size of the scallops of between 10 and 15 millimeters during the fall months. The drop in size occurs approximately 3 months later in the year in 1985 as compared to 1983. The most likely explanation for the delay in 1985 is the unusually high temperatures that were present in the fall of that year. In 1984 this decrease is completely absent with the average shell height never dropping below 42.5 millimeters. The data for 1982 also does not show a decrease in the fall but there is not enough data to attempt an interpretation for that time period.

The change in the count per pound (number of scallop meats per pound) versus season (Figure 4) shows the unusual nature of 1984 more clearly. As in the previous figure, outlying points represent the values for size classes present in the catch but in minor amounts. The line follows the meat count of the majority of scallops present at any one point. In both 1983 and 1985 the meat count rose rapidly in the fall indicating an increase in small scallops. In both instances larger scallops with lower meat counts were still present but in much smaller quantities. The bulk of the catch at that point consisted of younger, high count scallops. Throughout 1984, however, the count of the majority of the scallops collected in each sample never rose above a count of 180. The small scallops which should have been present and raised the count did not appear.

Attempts have been made in the past to follow reproduction through the use of ovarian color changes (Roe et al., 1971; Miller et al., 1979). This qualitative measurement of the reproductive state of the scallop can be misleading. Using the dry weight percentages of the reproductive organs is much more accurate and provides a quantitative look at the reproductive state of the scallop. The calico scallop is concurrently hermaphroditic with the male testes and the female ovaries present simultaneously. Figure 5 shows the change in the dry weight percentage of the calico scallop gonads from October of 1983 through 1985. The large peaks in April of 1984 and May of 1985, respectively,

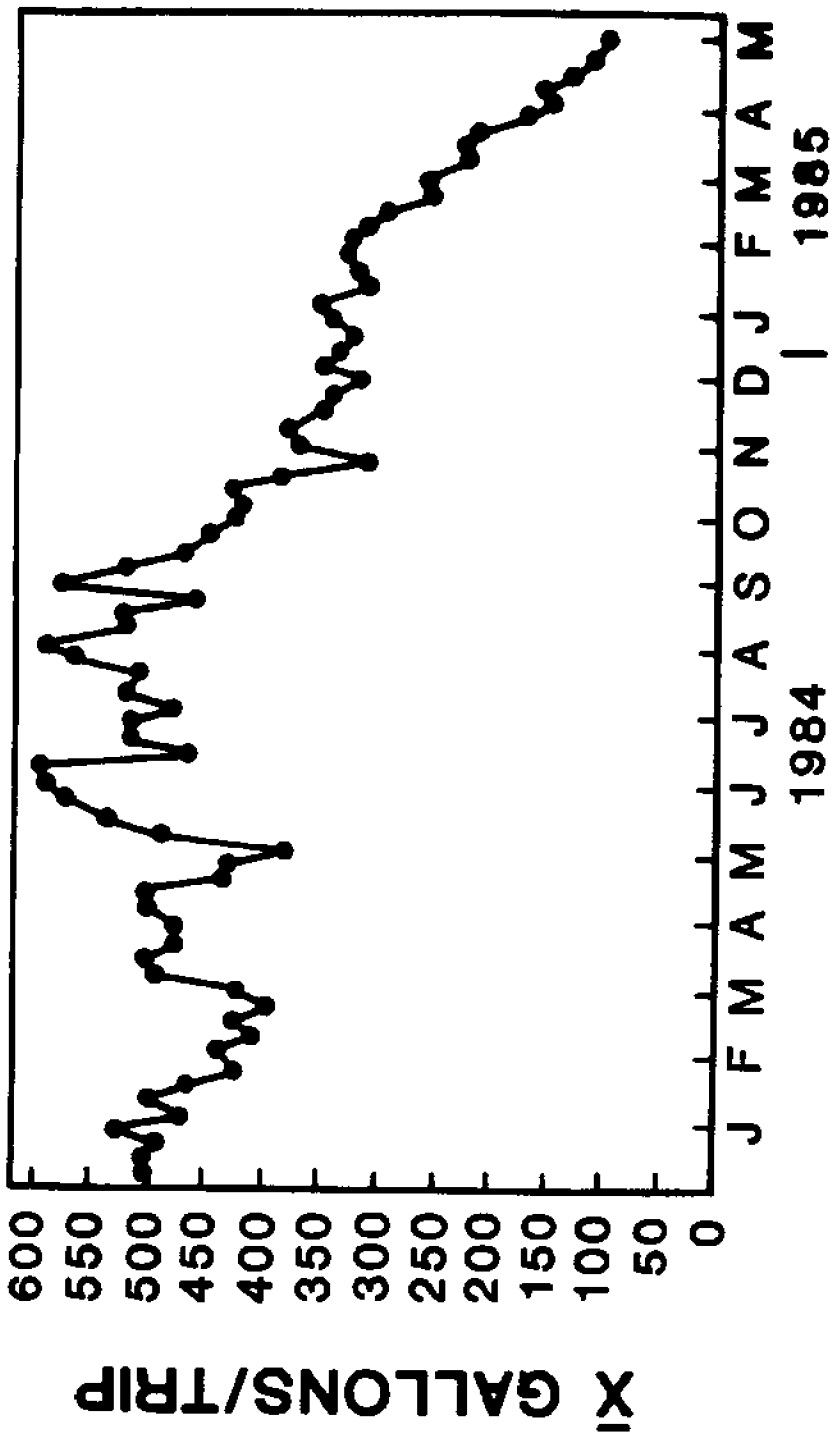

Figure 2. Representative weekly average production per trip in gallons of scallop meat from January 1984 through May 1985.

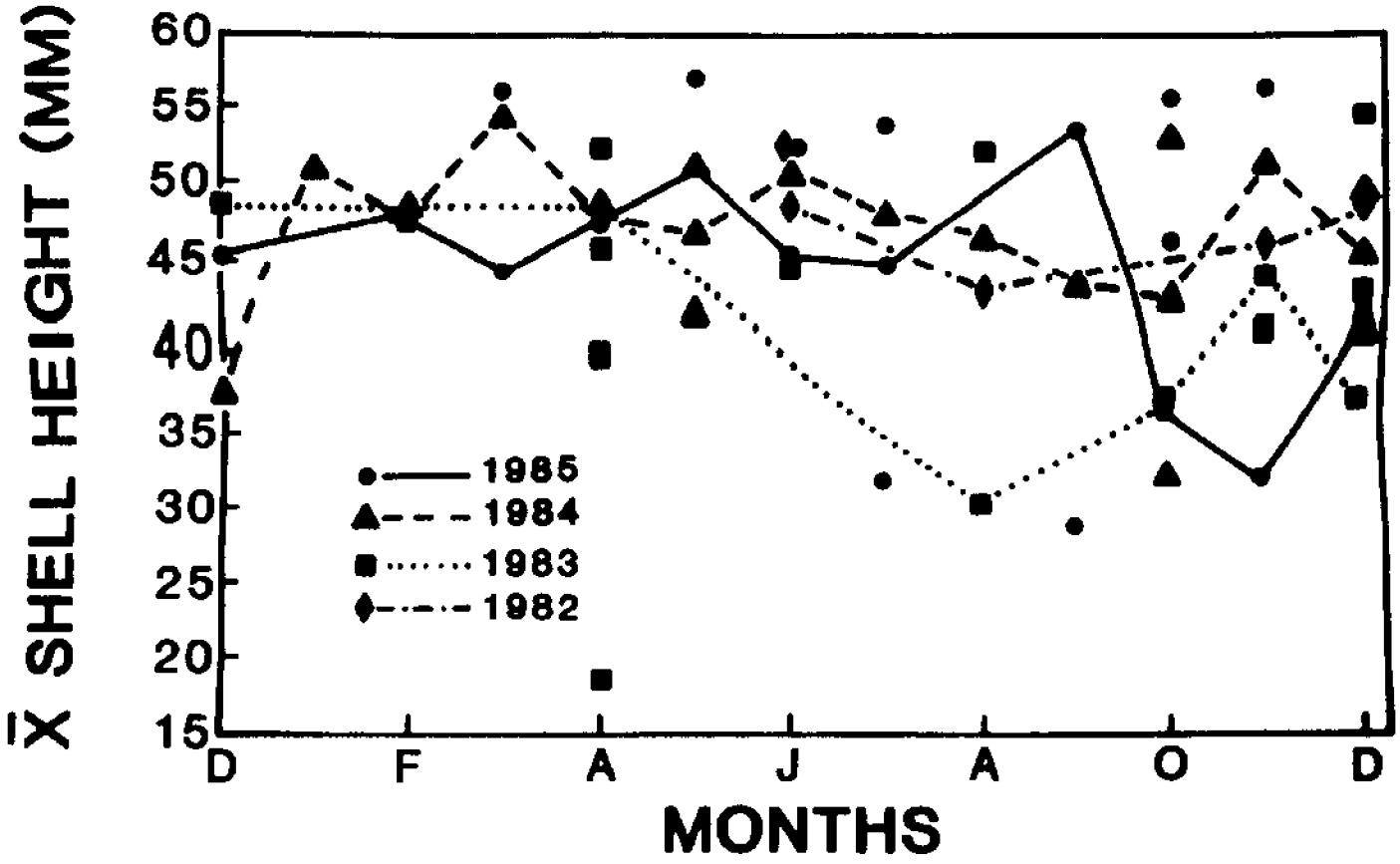

Figure 3. Average shell height of shell stock collected between June of 1982 and December 1985.

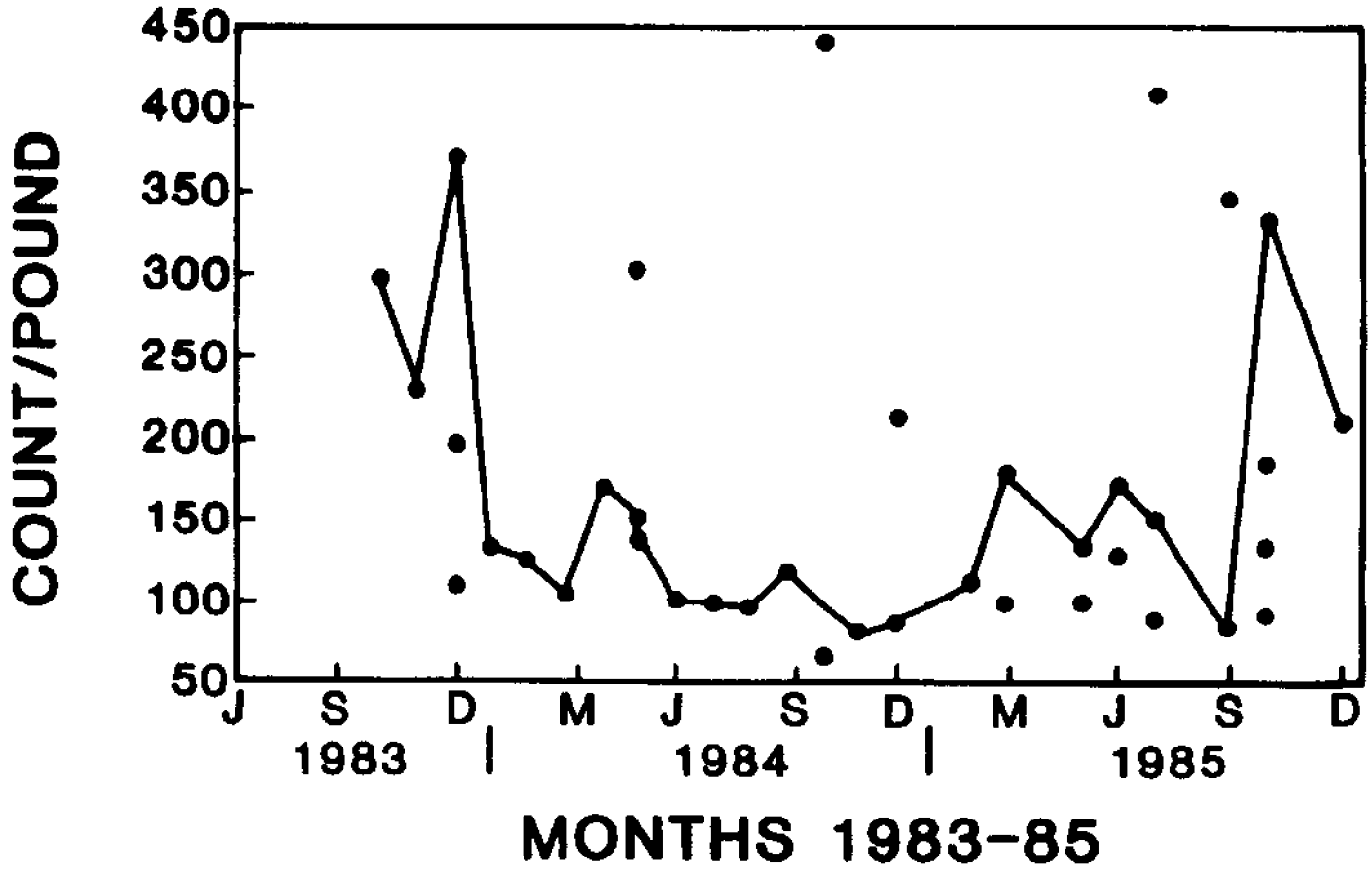

Figure 4. Average count for scallops collected between October of 1983 and December of 1985.

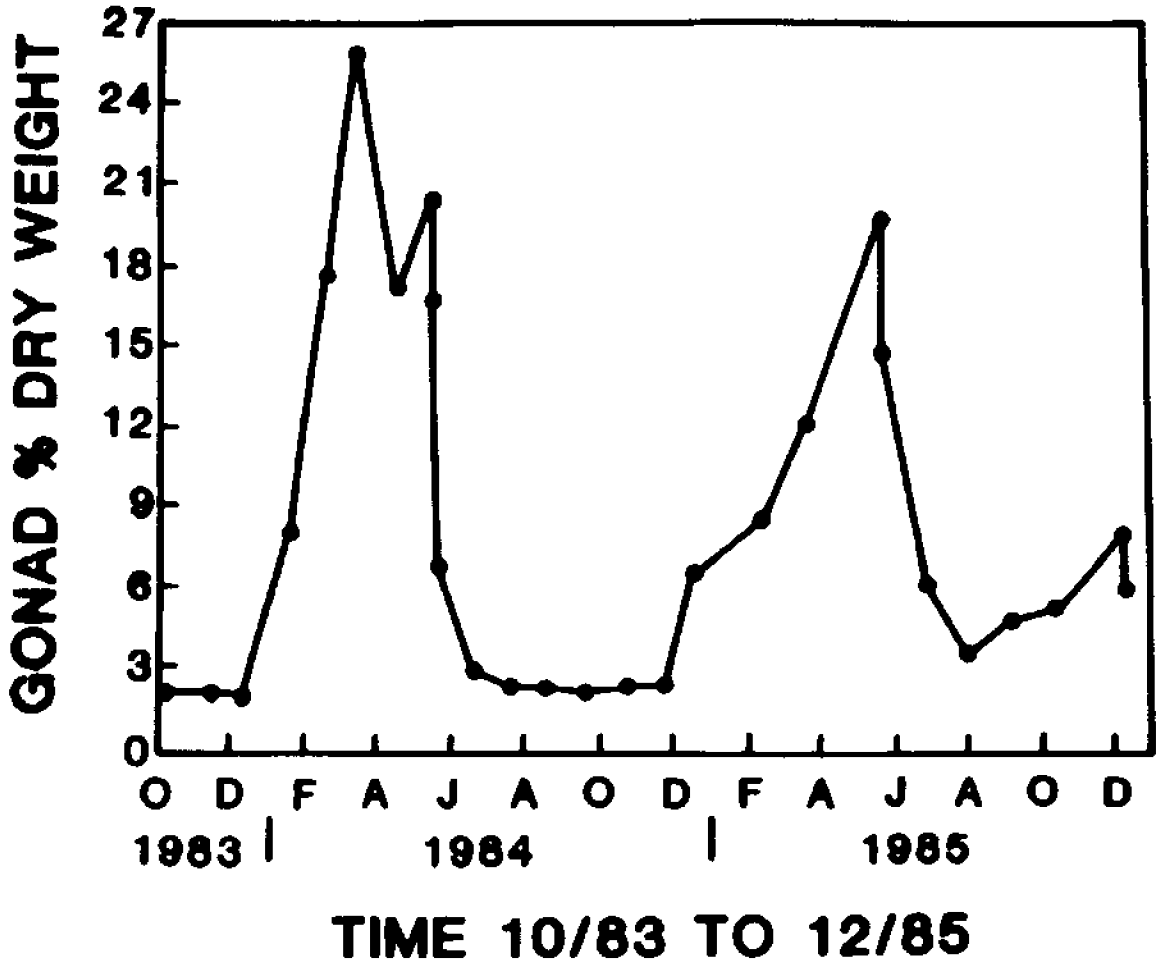

Figure 5. Percentage of the total dry weight consisting of gonadal material for scallops collected between October of 1983 and December of 1985.

mark the major spawning events for the calico scallop in those years. The scallops also normally experience a secondary spawning event in the fall as can be clearly seen in 1985. In 1984, however, the fall spawning never occurred. The graph clearly shows that there was no change in the reproductive state of the scallops from June through December of 1984.

Plotting the meat count per pound of each sample against the mean shell height for that sample (Figure 6) clearly forms a curve with an asymptote of approximately 100/lb as the scallop shell increases in size. When the scallop obtains a shell height of between 40 and 42 millimeters the meat count drops below 200/lb. Although counts of up to 300 can be processed, the scallop is most profitably harvested when the count drops below 200. By the time the scallop reached 50 millimeters in shell height, it has reached the minimum count and further increases in shell size do not result in concurrent increases in the size of the scallop meat.

Figure 7 shows the relationship between the average gallons per trip and the average meat count for those trips. When the meat count is above 160 scallop meats per pound, there is an inverse correlation of -0.816 between the count and the number of gallons obtained. In this region a lower count results in a higher number of gallons caught on each trip. When the count drops below 160/lb, however, there is no longer any correlation (0.032) between the two parameters. Although the count continues to drop, there is no increase in the gallons obtained on each trip and there is a decrease in approximately 50% of the samples.

DISCUSSION

Figure 6 and 7 provide valuable information to the scallop industry. When scallops reach 40 millimeters in size (below 200 count) the meats have become large enough to provide an acceptable profit margin. This gives the fisherman at sea a fast and easy means of determining if the scallops from a particular bed are large enough to be profitably fished. Fishing scallops below this size is ill advised due to both the decrease in the gallons obtained per unit effort and the potential limitation of future yields from these beds.

At the same time the largest catch per unit effort is obtained from scallops of 160 to 180 count as shown in Figure 7. This corresponds to a shell size of no more than 45 millimeters. When the count drops below 160/lb the gallons/trip begins to fluctuate greatly. The scallops of this size are reaching the end of their life span and muscle growth is either reduced or stops completely. This often results in flacid, watery meats which lowers the quality of the product. Senility and parasitism also reduces the size of the scallop meat relative to the size of the scallop.

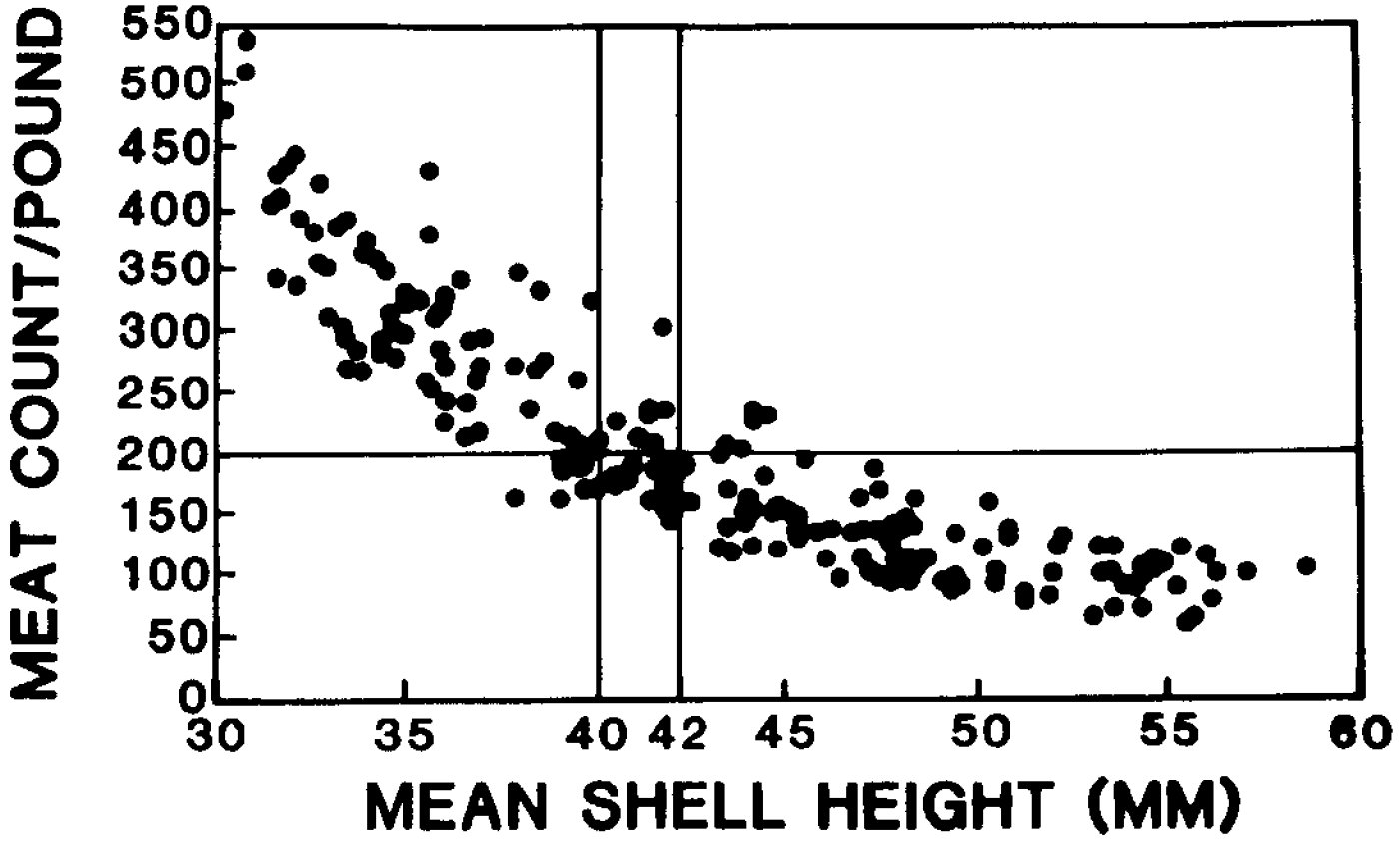

Figure 6. Average meat count versus average shell height for scallop samples consisting of at least twenty individuals of the same size class.

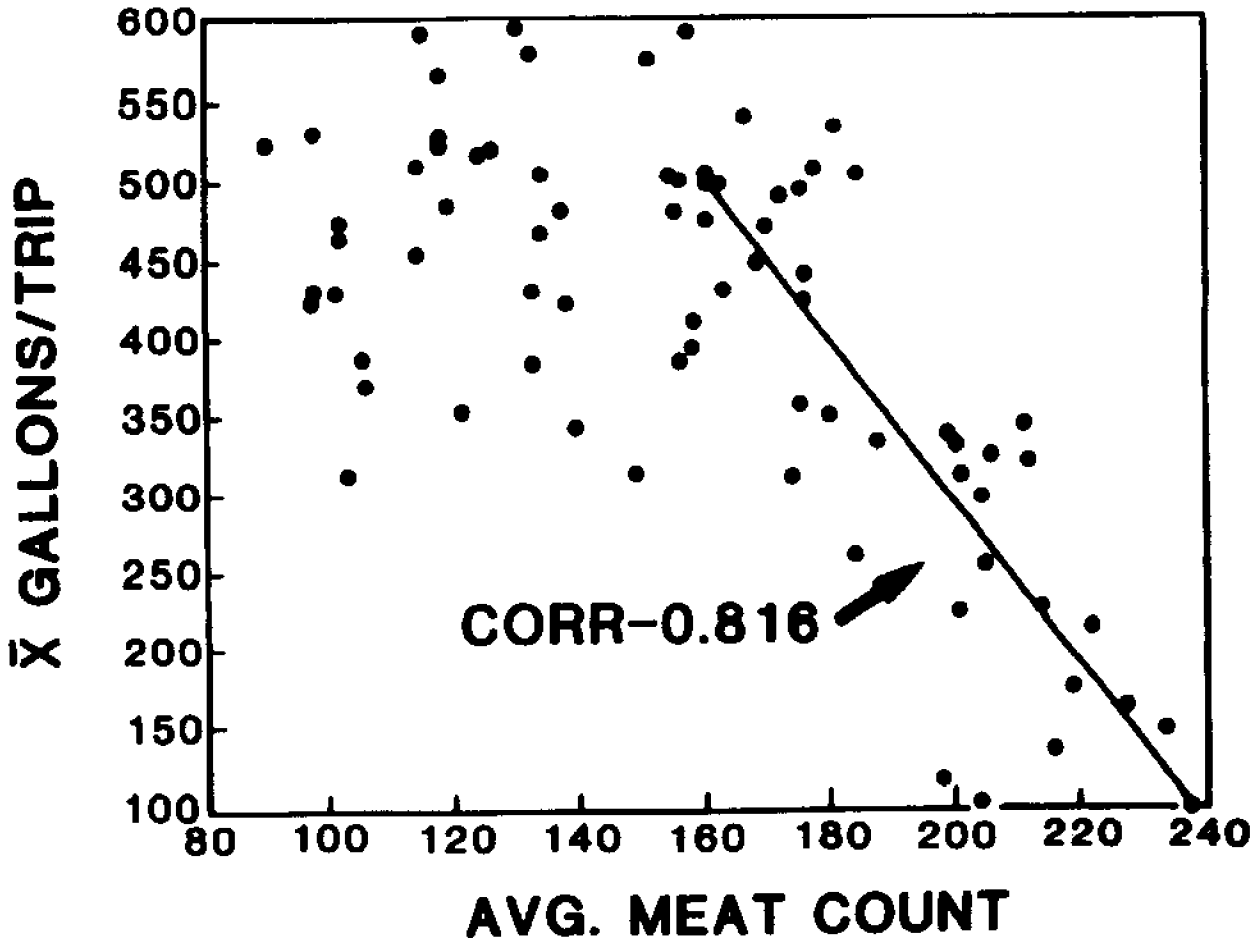

Figure 7. Average weekly production per trip versus average weekly meat count depicting the correlation between the size of the scallop meat and the catch per unit effort.

A further factor which decreases the gallons per trip of the larger scallops is the volume of the scallop. As the scallop increases in size its volume increases at a greater rate than the size of the meat. Since this is exacerbated as the scallop gets larger and the growth rate of the meat decreases, the larger volume results in the loading of fewer scallops on to the vessel which in turn reduces the gallons per trip.

Combining the information from Figure 6 and 7 it appears that the ideal scallop size for commercial harvesting is between 40 and 45 millimeters. Below this size the profit drops greatly and there is a potential for damaging future yields. Above this size there is no increase in yield and often a decrease in both yield and product quality.

It is obvious from the production figures that 1985 saw a drastic decrease in the abundance of the calico scallop off of the eastern coast of Florida and in turn the landings dropped markedly. An analysis of our data clearly points to the absence of a reproductive event in the fall of 1984 as a possible key to the decrease in the scallop population seen in 1985. Instead of the larger scallops spawning in the fall and then expiring as might be expected, they failed to initiate reproduction. Because there was no marked spawning in the fall there were almost no young scallops in the spring. The large scallops that did exist in the spring were decimated by senility. These large scallops had already obtained their maximum size as can be seen by the meat count data. Normally the death of the older scallops is not important due to the presence of the younger scallops. Without the young scallops only those few scallops that overwintered were available to reproduce in the spring.

Since spawning usually accelerates the death of older scallops the lack of reproduction in the fall of 1984 postponed their death and resulted in an excellent yield of scallops for the fall and early winter of 1984. Both the meat count and the gallons per trip remained elevated until the colder temperatures began killing the older scallops. In the long run, however, without the young scallops from the fall spawning, there were insufficient scallops present in the spring to maintain the industry at its normal production level. The smaller fall spawn which has not been previously identified by researchers appears to be very important in maintaining a large scallop population.

The reproductive strategy of the calico scallop is quite different from the reproductive strategies exhibited by other members of the Pectinidae family. Why this should be the case is not yet known. Further work is being conducted on the viability of scallops spawned at different times of the year at a variety of temperatures and nutritive states in an attempt to identify and describe these differences. Why the calico scallop needs to spawn in the fall as well as the spring in order to maintain itself is unknown at this point in time but will be addressed by research currently underway.

Abundance of the calico scallops in the beds off of Cape Canaveral will continue to be highly variable from season to season and from year to year. Years in which production levels are exceptionally high may be followed by years in which the production level is very low. It is apparent that long and short term changes in the local water currents and weather patterns will continue to impact the population levels. The effects of these environmental factors upon population size are not presently understood. Without determining their influence it will remain impossible to develop a predictive model capable of accurately forecasting production levels from one season to the next as well as from year to year.

ACKNOWLEDGEMENTS

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CRAWFISH PRODUCT ECONOMICS AND MARKETS

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The growing of crawfish in ponds in Louisiana is the nation's largest industry devoted to the aquaculture of crustaceans. In 1985 there were approximately 120,000 acres producing an average of 500 to 600 pounds per acre. There is also natural production from areas such as the Atchafalaya basin.

Louisiana currently accounts for the vast majority of production, but the industry is spreading. Texas, Arkansas, Mississippi, and South Carolina have crawfish ponds in production. However, Louisiana is the only state that has specialized crawfish processing plants. There were approximately 80 firms in 1985.

Various factors have brought about an increasing interest in the economics of the state's crawfish industry. Producer prices for crawfish have been relatively low in the last few years. There has been an increase in interest nationwide in cajun cuisine. This is evident from publication of cajun cookbooks, and articles in magazines as varied as Time, Venture, and Seafood Leader. Large seafood restaurant chains have begun to purchase crawfish for the first time. The crawfish industry can capitalize on this interest. However, the marketing practices of Louisiana's processing companies were poorly documented for out-of-state buyers. This research project focused on providing benchmark marketing and processing information to better convey the economic nature of the crawfish industry. Of the 80 crawfish plants in the state, 38 (48 percent) were selected for a personal interview. Data collected covered the 1983-84 crawfish production season. This paper covers the results of the analysis of products and markets for crawfish.

Total original capital investment in the entire Louisiana crawfish processing industry was estimated to be approximately $9 million. An additional $5 million of capital improvements were made by plant owners. The average initial investment per plant was approximately $115,600. Capital improvements to plants averaged $88,000. There was significant variation in the amount of original capital investment by location. In the eastern portion, that area just east of the Atchafalaya basin closest to New Orleans, average original capital investment was $67,200. The

This research was supported by a grant from the Louisiana Board of Regents and the Louisiana Sea Grant College Program.

western area, that area west of the Atchafalaya basin where crawfish was double cropped with rice, had an average original investment of $107,800. The firms in the central region, those of the Atchafalaya basin, had the highest capital investment, $161,700. The central area processed more of their crawfish. The eastern firms sold more live crawfish due to their access to the New Orleans market. The western firms handled smaller volumes of crawfish since less were available. Other regional differences will be described in other sections of this report.

The typical crawfish processing plant is a hand labor operation using a generic type processing floor arrangement. Surveyed plants average 5,188 square feet. Over 40 percent of this space was for processing, offices, and dry storage. The remaining space was divided among freezers, coolers, kitchens, and packing areas. There was no complicated equipment such as peelers or graders that one would find in a shrimp processing operation. Crawfish peeling was done by laborers working on a piece rate basis. Their pay rate was approximately $.90 per pound of peeled meat.

Crawfish production is highly seasonal. As can be seen in Table 1, production begins to increase as temperatures begin to rise in February. March, April, May, and June accounted for over 75 percent of production. Crawfish production fell quickly after the end of the season.

Table 1. Percent of crawfish handled by month, Louisiana, 1984.

MONTH	PERCENT OF SUPPLY
January	4.8
February	8.9
March	19.2
April	19.8
May	20.2
June	17.5
July	2.7
August	0.7
September	0.0
October	0.1
November	2.4
December	3.8

The total amount of crawfish purchased by the surveyed plants was 29,951,489 pounds on a live weight basis. State-wide, approximately 67 million pounds were purchased by the 80 crawfish plants operating in Louisiana. All of the east area plants purchased 27.9 million pounds, the central area purchased 25.5 million pounds, and the west 13.6 million pounds. The average pounds of crawfish purchased by firm also varied by

location (Table 2). These figures do not include any crawfish that were sold directly to restaurants and retailers by crawfish jobbers, farmers, and fishermen. The poundage sold in this manner was estimated to be between 10 to 20 million pounds.

Table 2. Average pounds and value of sales of crawfish purchased by processing plant, by area, Louisiana, 1984.

AREA	POUNDS	VALUE
East	1,450,286	$482,000
Central	688,352	$525,000
West	680,839	$527,000
State	847,015	$517,000

Even though the east area processors sold, on the average, more than twice the poundage of crawfish than the other two areas, it lagged behind the central and west processors in dollar value of sales. This occurred since the central and west firms processed more of their volume of crawfish into meat products (Table 3). Also, the production of firms in the east area tended to be later in the season when prices are lower.

Table 3. Percent of crawfish purchased by processing plants, by type of product sold, in percent, Louisiana, 1984.

AREA	WHOLE	MEAT
East	74.7	25.3
Central	44.6	55.4
West	55.9	44.1
State	59.2	40.8

Whole product form was mostly live crawfish, but includes live purged (crawfish held in tanks without feeding for 36 hours to starve them so no "vein" is evident in the tail), frozen whole, and frozen whole cooked crawfish. Meat products included frozen and fresh peeled meat, peeled and washed meat, and all crawfish that were ingredients in processors' finished products such as crawfish etouffee. On the average, 40 percent of all the crawfish purchased by processors was processed into crawfish meat or meat products. Table 4 contains a further breakdown of products by percent of volume and value of sales.

Table 4. Volume and value of crawfish products, in percent of poundage and percent of total sales, Louisiana, 1984.

PRODUCT	POUNDS (%)	VALUE (%)
Live, unpurged	59.0	32.3
Fresh tail meat	30.5	34.9
Frozen tail meat	7.4	9.6
Live, purged	1.2	5.5
Prepared products	0.6	1.0
Frozen whole cooked	0.6	1.1
Frozen whole raw	0.6	1.1
Other	0.1	14.6

Even though over half of the poundage of crawfish purchased by processors was sold in live form, only 30 percent of total sales were accounted for by these product forms. Little value is added to these crawfish since there is no processing. Value is added through transportation and refrigeration only. The reverse was evident for meat products. Through processing, value was added to fresh tail meat, prepared products, and processed whole products. They all account for a relatively larger proportion of value than volume of sales. Since crawfish are picked by hand, processing creates employment for unskilled labor within the state. On the average, firms picking crawfish hired 27.6 pickers when they were processing. Wages paid to pickers was the third largest expense to crawfish processing plants (Dellenbarger and Roberts).

As was pointed out previously, the supply of crawfish can come from two distinct sources; from natural areas such as the Atchafalaya basin, or from man-made ponds. There was no difference in the marketing of crawfish based on this factor. Processors did not use crawfish from a certain source to produce a certain product. In fact, no attempt was made to identify the source of production. From a marketing standpoint, aquacultured and naturally produced crawfish were equivalent. An occasional preference exists for red swamp crawfish over white river crawfish in sales of live crawfish for boiling.

Market channels for crawfish products were investigated. Processors were asked to identify the areas (Figure 1) in which their products were sold. Louisiana was selected as a market area so that the volume of crawfish consumed in the state could be investigated. The neighboring six states represent roughly a 500 mile radius, which was estimated to be a one day shipment by truck. Another reason that these states were selected as a market area is that they are close to Louisiana and are exposed to cajun cooking. This "cajun belt" of influence was evident from the developing crawfish industries in three of these states. The

remaining market areas, the southeast, northeast, central, west, and export were selected to correspond to market areas in a shrimp marketing study so that the market channels for these Louisiana shellfish products could be compared (Roberts and Pawlyk). Table 5 depicts the market channels for four of the major crawfish products. These figures were based upon the area in which the crawfish processors sold their products.

Table 5. Market channels for four Louisiana crawfish products, in percent, 1984.

AREA	LIVE UNPURGED (%)	FRESH MEAT (%)	FROZEN MEAT (%)	PREPARED (%)
Louisiana	85	83	62	19
Cajun Belt	10	12	18	64
Southeast	2	1	12	13
Northeast	1	2	7	1
Central	1	1	0	2
West	1	1	1	1
Export	0	0	0	0

May not add to 100 due to rounding.

The vast majority of crawfish produced in Louisiana was sold within the state. This, coupled with the short production season, accounted for the relatively low prices of recent years. The Louisiana tradition of backyard crawfish boils has not seemed to have kept pace with expanding production from aquaculture. The percentage of live unpurged crawfish sold within Louisiana would have been greater if it were possible to account for direct marketing by crawfish farmer/fisherman and for sales within the state by jobbers. The estimates in this paper refer only to crawfish moving through processing plants.

While the volume of fresh meat production was smaller than that of live unpurged crawfish, it had very similar distribution. Frozen meat was more widely distributed than fresh meat. This was due to the perishability of the fresh product. More than 10 percent of production was shipped to the southeast. Prepared crawfish products, etouffee, bisque, and other finished products, were the most widely distributed of these four products, with more than 75 percent sold outside of Louisiana. This product form is the easiest to prepare for a consumer or restaurant that has not been previously exposed to cajun cooking techniques. It was felt that products of this type could be useful in attempts to introduce new consumers to crawfish.

In order to assess the concerns of the crawfish processing industry, a question on what the processors believed would affect their firms

future growth was included in the survey. Each firm was allowed to chose one or two factors. A total of 68 responses were recorded and are presented in Table 6.

Table 6. Factors identified by processors as most likely to affect future firm growth, in percent, Louisiana, 1984.

FACTOR	PERCENT OF RESPONSES
Lack of quality control	14.7
Crawfish too small	13.2
Lack of capital	13.2
Out of state markets	11.8
Prices	8.8
Interest rates	8.8
Waste disposal	7.4
Unstable crawfish supply	5.9
Louisiana market	5.9
Foreign supplies of crawfish	5.9
Present health standards	1.5
Other	2.9

Quality control on crawfish products was identified as the greatest single problem facing the industry. Marketing was identified as an important factor affecting future growth. When both factors which address marketing are added together, they account for almost 30 percent of the replies. The unstable crawfish supply was relatively unimportant, with less than six percent of the total replies. Problems with lack of capital, prices, and interest rates would occur in any industry which was made of many small firms.

Louisiana's crawfish processing industry is expanding due to the growing production from aquaculture within the state. Pond raised and natural crawfish were found to be equivalent from a marketing standpoint. If crawfish is to capitalize on the current popularity of cajun cuisine, consumers outside of Louisiana must be introduced to crawfish products. Prepared products, etouffee, bisques, etc, had the widest acceptance outside of the state due to there ease of preparation for consumers not previously exposed to cajun cooking. Yet relatively little of these products were produced when compared to sales of live crawfish, which was sold in the largest volume.

Crawfish were peeled by hand. Cost of picking labor was, on the average, the third largest expense of the processors. It will be difficult to develop markets outside of Louisiana due to these high costs since hand picked crawfish will have to compete with other seafood

including lower priced, machine-peeled shrimp. A machine to remove the tough crawfish shell would lower the cost of producing prepared products.

Much of the crawfish business in Louisiana is on a cash basis. If processors are to develop markets out of state it will be necessary for them to move away from this basis to one that has accounts payable in 30 or more days. It may not be possible for some of the processors to slow down their cash flow in this manner. Many of the processors lack the capital for new packaging, product development, advertising, and inventory that would be required to market their crawfish outside Louisiana to large institutional accounts.

Two separate groups have been set up in order to help the crawfish industry. The Crawfish Marketing and Promotion Board works exclusively with crawfish and the Louisiana Seafood Promotion and Marketing Board works with all seafood. Both of these groups are less than two years old, and market development is a long-term undertaking. The affects of these boards on crawfish markets must be evaluated over a long period of time. With time and work, the Louisiana crawfish industry can capitalize on the growing interest in cajun cuisine.

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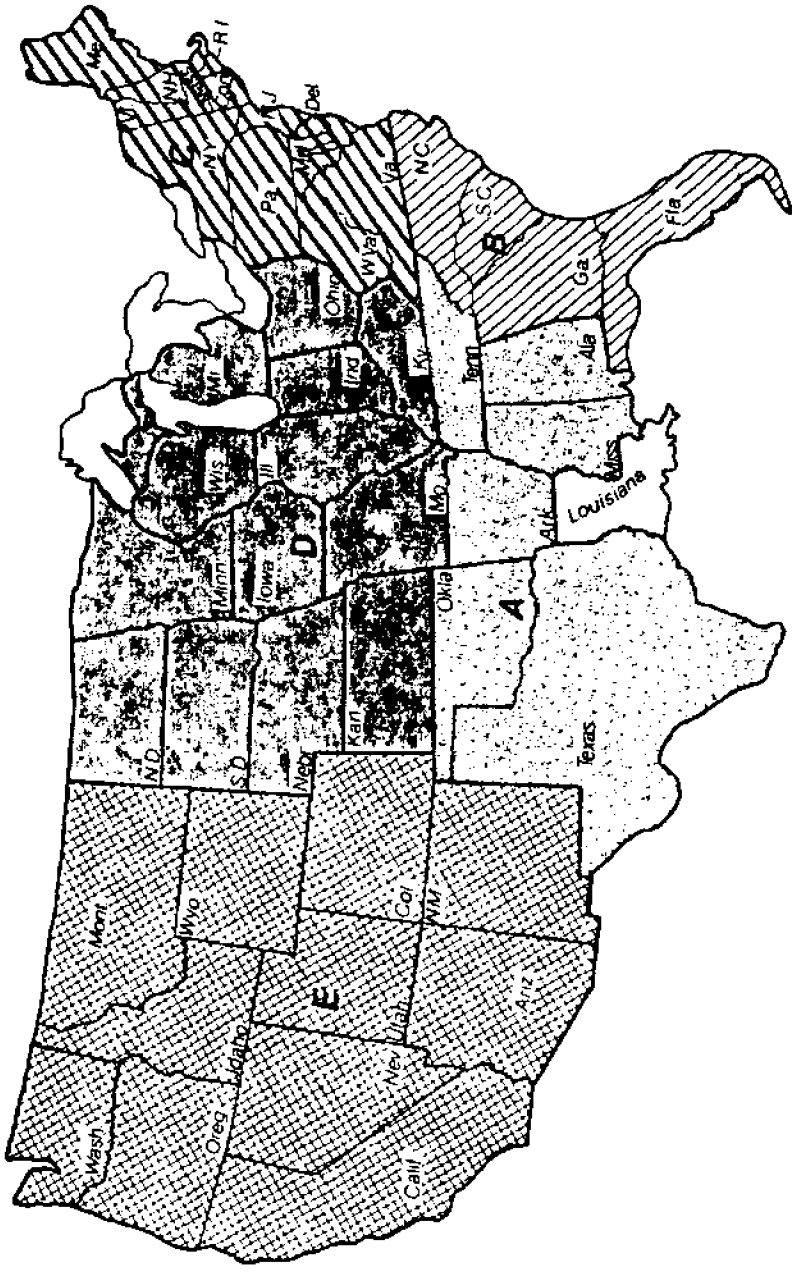

Figure 1. Market areas for Louisiana crawfish in U.S.

FACTORS INFLUENCING CRAWFISH MARKET DEVELOPMENT

by

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and
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INTRODUCTION

Louisiana farmers are responding to the current national agriculture crisis in a variety of ways. Those with access to the state's abundant water resources are considering aquaculture production as an alternative to maintain income levels and improve their cash flow situation. Crawfish production is an emerging and significant Louisiana industry. Currently, Louisiana has approximately 120,000 acres devoted to the production of crawfish with projections of up to 300,000 acres being used.

As the production of crawfish increases a better understanding of the potential markets and processing capacity is needed. Interest by the seafood wholesaling sector for carrying crawfish on a nationwide basis is also important. The capacity and growth of Louisiana's crawfish processing facilities will be indirectly influenced by yields and by market demand for crawfish on a nationwide basis. This paper looks at factors influencing the development of new markets for crawfish outside Louisiana.

A mail survey of wholesale and retail food outlets nationwide was conducted during the fall of 1985. Characteristics such as size and price of product desired, promotional material needed to help market the product, type of payment and other factors influencing the growth in market development of crawfish were obtained.

CHARACTERISTICS OF THE SURVEYED DISTRIBUTORS

Surveys were mailed to 550 distributors, located in major metropolitan areas of the country. A total of 236 usable surveys were returned resulting in a 43 percent response rate.

Respondents to the survey included 103 wholesalers, 2 restaurants, 11 retail outlets, 19 supermarkets and 97 a combination of the above. Of the respondents 134 carried both fresh and frozen seafood. Participants obtained seafood from a variety of sources including processors (76), local wholesalers (26), regional distributors (8) and 61 were a combination of the above. A total of 60 respondents did not answer the

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question. Of the 63 supermarkets responding 5 had a self-serve area for fresh seafood, 34 had a full service counter and 24 had both.

Survey participants were asked if they would like a directory of the Louisiana crawfish processors. The question was designed to determine how many of the survey respondents were interested in carrying crawfish as one of their offerings. Of the 236 respondents, 55% (129) indicated that they would like a copy of the directory. Table 1 contains a list of cities surveyed, number of surveys sent per city, number requesting a directory per city and the percent requesting a directory by city. Figure 1 provides a list by percentages for regions requesting a copy of the processors directory.

Figure 1 shows that only the western region did not have at least 50% requesting a directory. As would be expected the south central region immediately surrounding Louisiana had the largest request rate at 74%.

Table 1. Cities of Wholesalers, Which Participated in Crawfish Marketing Study

City	State	Number of Surveys Mailed	Number of Surveys Received	Number Requesting Directory	Percent Requesting Directory
Birmingham	AL	11	7	4	57
Montgomery	AL	4	2	2	100
Phoenix	AZ	4	2	2	100
Little Rock	AR	4	3	3	100
Los Angeles	CA	37	19	4	21
San Diego	CA	15	6	2	33
San Francisco	CA	21	11	4	40
Boulder	CO	2	2	2	100
Denver	CO	5	2	1	50
Jacksonville	FL	13	8	8	100
Miami	FL	19	7	3	43
Tampa	FL	2	1	1	100
Atlanta	GA	20	4	2	50
Savannah	GA	9	6	3	50
Chicago	IL	32	13	8	53
Indianapolis	IN	4	0	0	0
Lexington	KY	3	2	2	100
Louisville	KY	8	1	0	0
Baltimore	MD	17	6	1	17
Bethesda	MD	1	0	0	0
Jessup	MD	5	1	1	100
Boston	MA	44	15	6	40

(Continued)

Table 1. (Continued)

City	State	Number of Surveys Mailed	Number of Surveys Received	Number Requesting Directory	Percent Requesting Directory
Detroit	MI	2	1	1	100
Minneapolis	MN	9	4	2	50
St. Paul	MN	2	1	1	100
Biloxi	MS	10	6	4	67
Jackson	MS	5	2	1	50
Kansas City	MO	10	6	2	33
St. Louis	MO	8	3	2	67
Omaha	NE	5	1	1	100
Reno	NV	1	1	1	100
Bronx	NY	4	0	0	0
Brooklyn	NY	4	2	0	0
New York	NY	31	10	4	40
Beaufort	NC	7	5	1	20
Raleigh	NC	4	4	1	25
Cincinnati	OH	5	2	1	50
Cleveland	OH	5	4	1	25
Columbus	OH	6	4	3	75
Norman	OK	1	1	0	0
Oklahoma City	OK	2	0	0	0
Shawnee	OK	1	0	0	0
Tulsa	OK	5	4	3	75
Portland	OR	2	1	1	100
Philadelphia	PA	27	11	8	73
Pittsburg	PA	3	2	2	100
Charleston	SC	9	4	3	75
Memphis	TN	7	5	5	100
Nashville	TN	5	3	2	67
Dallas	TX	9	4	2	50
Fort Worth	TX	3	1	1	100
Galveston	TX	5	1	1	100
Houston	TX	13	3	3	100
San Antonio	TX	4	1	1	100
Salt Lake City	UT	2	1	0	0
Newport News	VA	1	0	0	0
Norfolk	VA	3	2	2	100
Richmond	VA	10	4	2	50
Roanoke	VA	1	1	1	100
Seattle	WA	34	9	5	56
Spokane	WA	5	4	2	50
Total		550	236	129	55

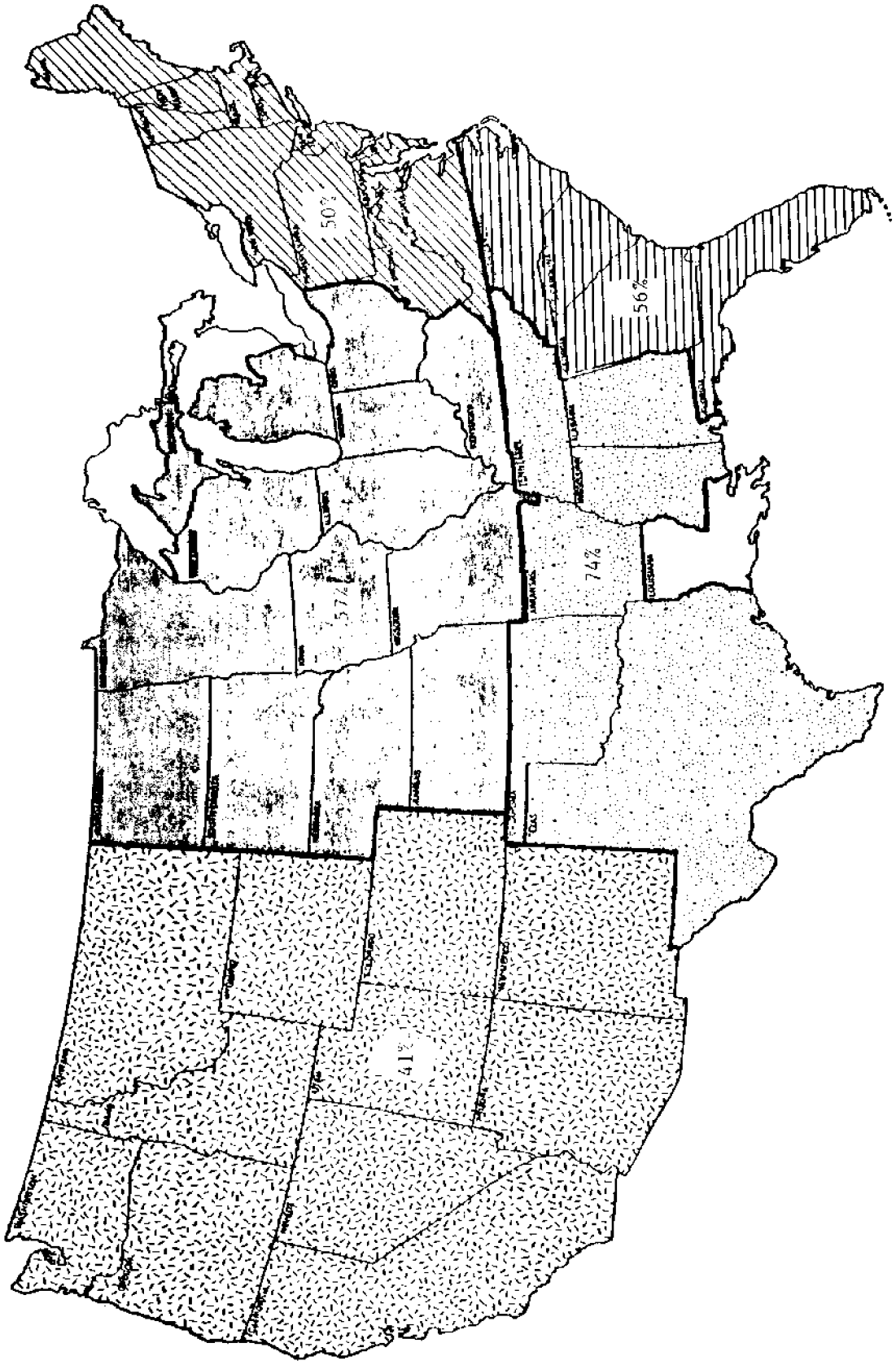

Figure 1. Regional Markets for Crawfish in Percentages

Three questions were designed to determine if the distributors had previously carried crawfish, currently carried crawfish, or had considered carrying crawfish. The response to the three questions are contained in Table 2.

Table 2. Number of Distributors Which Have Previously Carried, Presently Carry, or Have Considered Carrying Crawfish, Results of a Nation Survey Of Wholesale Distributors, 1985

Question	Yes	(%)	No	(%)	No Response	(%)
Have you ever carried crawfish?	92	(39)	86	(36)	57	(25)
Do you presently carry crawfish?	54	(23)	121	(51)	60	(26)
Have you considered carrying crawfish	56	(24)	57	(24)	122	(52)

From the above results it can be seen that some of the wholesalers have previously carried crawfish and then discontinued selling them. Problems associated with carrying crawfish and factors which would improve their marketability was of interest. Of the 236 respondents 154 answered the question concerned with identifying problems associated with carrying crawfish. Forty-three percent felt the major problem associated with selling crawfish was a lack of consumer awareness. The two major problems identified were lack of consumer awareness and lack of available sources. (See Table 3)

To aide in the sale of crawfish the wholesalers offered suggestions for the type of promotional material needed. The respondent was given four choices to choose from. A total of 140 responses were received for this question. The results are contained in Table 4. Seventeen percent felt that all four promotional aids were needed. This compares to 14 percent that felt advertising art or information for employees were major needs.

Table 3. Problems Associated With Carrying Crawfish, Survey Of Distributors, 1985

Responses	Number of Responses	Percent
Lack of consumer awareness	66	43
Lack of consumer awareness and lack of available sources	22	14
Lack of available sources	20	13
Seasonality	10	7
Seasonality, lack of consumer awareness and lack of available sources	8	5
Other	8	5
Seasonality and lack of available sources	6	4
Seasonality and lack of consumer awareness	4	3
Lack of consumer awareness and other	4	3
Lack of consumer awareness, lack of available sources, and other	3	2
Seasonality and other	2	1
Lack of available sources and other	1	1

Also of importance is the need to determine the price for crawfish which the wholesalers are willing to pay, the type of transportation desired, terms of payment which could be expected and whether the wholesalers wished to carry crawfish on a monthly, seasonal or annual basis. Respondents to the survey were given three categories of prices to choose from. Of the respondents 68 percent chose the $4.00-$5.50 category in which they would be willing to carry peeled, frozen crawfish tail meat. The next category having the highest percentage of responses was the $5.50-$6.50 category with 14 percent. The category having a price range of $6.50-$8.00 contained 2 percent of the responses. A choice of both the $4.00-$5.50 and the $5.50-$6.50 categories accounted for 6 percent of the responses. The remaining responses, 11 percent, were split between other variations of the three alternatives. The results indicate that the wholesalers would prefer a lower priced item to sell in the range of $4.00-$5.50.

Respondents were asked which type of transportation would be used by the company. Of 137 responses 95 responded to using a truck, 70 percent. Fifteen percent responded that they use air transport and another fifteen percent that they use both air and ground transportation. Representatives of the wholesalers were also asked what types of terms of payment that the companies use. The results of the survey question are contained in Table 5. Results indicate the longer the time frame for payment the more respondents that were in the category. Of the respondents 45 percent dealt with a monthly payment schedule.

Table 4. Promotional Needs to Help Sell Crawfish, Survey of Distributors, 1985

Responses	Number of Respondents	Percent
Menu clip-ons, in-store taste test, advertising art, and information for employees	24	17
Information for employees	21	14
Advertising art	19	14
Advertising art and information for employees	14	10
In-store taste test and advertising art	9	6
Menu clip-ons and advertising art	9	6
Menu clip-ons and advertising art, and information for employees	8	6
In-store taste test	6	4
Menu clip-ons, in-store taste test, and information for employees	6	4
Menu clip-ons, in-store taste test, and advertising art	5	4
In-store taste test and advertising art	4	3
Menu clip-ons	3	2
Menu clip-ons and information for employees	3	2
Menu clip-ons and in-store taste test	2	1

Table 5. Terms of Payment, Survey of Distributors, 1985

Type	Number of Responses	Percent
C.O.D.	11	7
One week	18	12
Two weeks	24	15
A month	70	45
Combination of the above	33	21

A majority of the respondents would be willing to carry crawfish on an annual basis. A total of 57 respondents indicated that they would be willing to carry crawfish on an annual basis, 48 percent. Twenty-six percent indicated that they would consider carrying crawfish on a

monthly basis and 22 percent indicated carrying crawfish on a seasonal basis. The remaining four percent indicated a combination of the above.

CONCLUSION

This study presents results which show some of the factors influencing crawfish market development. Markets outside Louisiana are interested in carrying crawfish. Survey results showed that 54 percent of the respondents in the nationwide mail survey desired a directory. Major problems associated with carrying crawfish were lack of consumer awareness, and lack of available sources. Respondents suggested promotional needs to sell crawfish in their market areas. Forty-eight percent of the respondents indicated they would prefer to carry crawfish on an annual basis with terms of payment most often listed as one month. The information contained in this report should aid crawfish processing plant management gain insight for potential markets outside Louisiana.

SOCIOECONOMIC DETERMINANTS OF AT-HOME SEAFOOD CONSUMPTION

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INTRODUCTION

The purpose of this study was to provide an understanding of current socioeconomic and demographic factors hypothesized to influence at-home consumption of total seafoods and specific seafood products. An increased understanding of the factors determining at-home consumption of seafoods also provides information which can be used in examining the away-from-home seafood market, seafood import demand, and ultimately the total demand for seafood in the United States.

Tobit procedures were used to analyze seafood consumption by the 14,930 households interviewed in the 1977-78 USDA Nationwide Food Consumption Survey. Households with incomplete data (4,241) were omitted from the analyses. Estimates presented in the present paper are generally limited to overall basic conclusions with respect to weekly household expenditures on total, fresh, frozen and canned seafood products (additional estimates are presented in Keithly, 1985). The set of explanatory variables included in the analyses are grouped into four major categories for purposes of presentation: (1) demographic and seasonal, (2) social and ethnic, (3) family size and composition, and (4) economic demand factors. The analyses of the partial income effects in the last category are further examined in terms of (1) consumers versus nonconsumers, (2) three consumption dimensions: expenditures, quantities and quality income elasticities and (3) the general categories of finfish and shellfish. In all cases results are partial effects, that is, the estimates show the effect of specific variables after all other variables have been accounted for.

DEMOGRAPHIC AND SEASONAL

Demographic and seasonal explanatory variables were included in the analyses of seafood consumption for two basic reasons. First, prices vary by region, urbanization and seasons due to differences in supply. Second, consumer tastes and preferences are likely to vary in these dimensions which cause variations in demand. Price considerations are generally not explicitly considered within cross-sectional data for two reasons. Within any region-urbanization location during a given period in time (season) consumers theoretically all face the same price alternatives and thus price is not a variable in the sense that supply is a variable. This is not necessarily so between regions, urbanizations, and seasons and thus the explicit

inclusion of these variables is intended to account for possible price variations due to supply shifts (other variables discussed later may also serve this purpose). The second reason is that food categories reported are usually not homogeneous in the sense that different quality seafood products with varying amounts of added marketing services are generally reported in aggregate categories. These differences are estimated and accounted for with respect to income changes in the section reporting income elasticities.

Northeast household seafood expenditures for total seafood purchases were significantly more ($.45 per household per week) than those in the base region which was defined to be the West in the analyses (Table 1). Greater expenditures were also found for fresh and canned products. Given other variables equal, households in the South and the North Central regions spent less on all seafoods than did Western households except frozen.

It should be re-emphasized that these are partial effects of region on consumption. The estimates show the regional effect on households after all other variables in the model have been accounted for. Thus with respect to all other included variables, the households are identical except for regional location. Simple unadjusted mean values may provide different interpretations. For example, consumers in the South have the largest average weekly expenditures of all regions when simple means are compared. However, the partial effects in Table 1 indicate Southern households spend less on fresh seafoods than those in the Northeastern households. It is thus the accumulated effect of all variables that show the South as leading area in fresh seafood expenditures. Similar interpretations are also applicable to the remainder of the estimates presented in this paper.

Statistically significant variations exist among household seafood expenditures because of the location with respect to central city, suburban and nonmetro (Table 1). Weekly expenditures are highest for central city households and lowest for nonmetro household in all categories except frozen seafoods. No significant differences were found among households for consumption of frozen products.

Limited variation in seafood expenditures was found with respect to season. Using the winter months as the base season, the exceptions were (1) lower expenditures for frozen seafoods in the summer, (2) higher expenditures for fresh seafoods in the summer and spring, and (3) lower expenditures for canned products in the spring and fall.

FAMILY SIZE AND COMPOSITION

Changes in family size were entered into the analyses in polynomial form in order that the rate of change in expenditures could vary with family size. In addition, family size was interacted with family income. Estimates presented in Table 2 are for mean

Table 1.--Demographic and seasonal factors affecting at-home expenditures[a].

Independent variable	Seafood class			
	Total	Fresh	Frozen	Canned
Region[b]:				
Northeast	.445	.278	-.063	.212
	(4.90)	(3.04)	(-1.91)	(6.55)
Northcentral	-.381	-.335	.051	-.114
	(-5.41)	(-6.20)	(1.25)	(-5.26)
South	-.162	-.040	-.023	-.110
	(-2.15)	(-.52)	(-.65)	(-5.17)
Urbanization[b]:				
Central City	.564	.438	.010	.176
	(7.51)	(5.72)	(.33)	(6.87)
Suburban	.231	.133	.012	.101
	(3.63)	(2.18)	(.42)	(4.70)
Season[b]:				
Spring	.007	.116	.003	-.038
	(.10)	(1.64)	(.09)	(-1.57)
Summer	.070	.280	(-.062	-.019
	(.94)	(3.58)	(-2.05)	(.78)
Fall	-.066	.034	-.012	-.059
	(-.95)	(.52)	(-.36	(-2.60)

[a]Coefficients are partial effects and show household weekly dollar expenditure per unit change in independent variable. Numbers in parentheses are asymptotic t values associated with original parameter estimates from which expected total changes were estimated.

[b]Base variables for 0-1 variables are west, nonmetro, and winter for region, urbanization and season, respectively.

Table 2.-- Family size and composition affecting at-home seafood expenditures[a].

Independent variable	Seafood class			
	Total	Fresh	Frozen	Canned
Family size (No.)[b]	.206	-.026	.064	.082
	(2.72)	(-1.02)	(2.69)	(3.86)
	(-1.04)	(1.71)	(-1.80)	(-2.51)
Family composition[c]				
Young, single w/o children	-.291	-.443	-.085	.156
	(-1.80)	(-3.71)	(-1.21)	(2.45)
Young, married w/o children	-.314	-.317	-.079	.024
	(-2.29)	(-2.51)	(-1.31)	(.51)
Young single w/ children	-.504	-.440	-.117	.044
	(-3.24)	(-3.52)	(-1.69)	(.73)
Young, married w/children	-.437	-.426	-.050	.008
	(-3.85)	(-4.72)	(-.91)	(.20)
Middle-aged,single w/o children	-.255	-.286	-.052	.072
	(-1.76)	(-2.16)	(-.76)	(1.38)
Middle aged, married w/o children	.089	-.039	.030	.053
	(.70)	(-.31)	(.49)	(1.31)
Middle aged, single w/children	-.029	-.246	-.017	.171
	(-.19)	(-1.77)	(-.23)	(3.02)
Middle aged, married w/children	-.157	-.236	-.044	.058
	(-1.44)	(-2.02)	(-.77)	(1.34)
Elderly, single	-.264	-.172	-.047	-.032
	(-1.98)	(-1.28)	(-.73)	(-.76)

[a]Coefficients are partial effects and show weekly household dollar expenditure per unit change in independent variable. Numbers in parentheses are asymptotic t values associated with original parameter estimates from which expected total changes were estimated. Values in the second set of parentheses are of squared terms for family size.

[b]Family size was interacted with income and estimated in polynomial form. These effects are included in the estimates presented.

[c]Elderly married were the base. Young households include those with with household heads less than 35 years of age while elderly refer to those who are 65 years or older.

values of these variables. Increases in family size were statistically significant and positive for total seafood expenditures and for expenditures on frozen and canned seafood products. Expenditures increased as family size increased at a decreasing rate for these product categories and were positively related to family income levels.

A set of ten family composition categories analyzed suggests considerable variation among household seafood expenditures due to the makeup of the household for given household size, income etc. (Table 2). Composition variables were defined on the basis of three characteristics; age, presence of children and marital status. Overall conclusions are as follows: (1) younger families generally spent less than middle-age and elderly households for total seafoods and for fresh and frozen seafood products; (2) young-single household without children made significantly higher weekly expenditures for canned products than other groups of young household; (3) all classes of middle aged households spent less in total and less on fresh and frozen seafoods than elderly married couples but they generally spent more than younger households; (4) with respect to the overall effect of children, the analyses show younger households with children tended to purchase less seafoods than young households without children while presence of children did not have a clear effect among middle aged households, and; (5) Elderly households consumed the least amount of canned products while the middle-aged households generally consumed significantly more canned products.

SOCIAL AND ETHNIC CONSIDERATIONS

Seafood expenditures were analyzed with respect to social and ethnic factors through the use of seven variables. The estimated effects were statistically significant for 25 of the 32 estimates given in Table 3.

Race of the household was concluded to be a factor significantly affecting the taste and preferences of households for seafood products. Expenditures by white households were significantly less than those made by black households for all categories except canned seafoods where the reverse was the case. The largest difference was noted in the fresh seafood category where black households spent $1.06 more per week than white households and $.67 more than households of other races.

The fact that households caught fish for their own use was included mainly as a correction factor in the data set. A price representing the market price for a similar product in a given region was assigned to these catches in the data base. As would be expected, this fact showed a highly significant impact on seafood expenditures (other than canned).

Characteristics of the meal planner were analyzed with respect to employment status, sex and education. Households where the meal

Table 3.-- Social and ethnic factors affecting at-home seafood expenditures[a].

Independent variable	Seafood class			
	Total	Fresh	Frozen	Canned
Race [b]:				
White	-.855	-1.062	-.009	.088
	(-4.64)	(-8.98)	(-1.44)	(1.85)
Other	-.207	-.667	-.088	.133
	(-.99)	(-3.33)	(-1.25)	(2.25)
Caught fish for own use	.772	.856	.182	.008
	(10.72)	(10.56)	(5.48)	(.37)
Meal planner[b]:				
Employed	-.057	-.117	-.005	-.011
	(-.96)	(-2.15)	(-.19)	(.54)
Female	.272	.160	.020	.152
	(2.43)	(1.48)	(.36)	(3.92)
Education (yrs.)	.041	.009	.017	.014
	(3.98)	(.99)	(3.53)	(4.09)
Guest meals (no.)	.079	.049	.013	.016
	(7.81)	(5.42)	(2.91)	(4.72)
Meals away from home (dol.)	-.006	-.003	-.003	-.001
	(-3.60)	(-2.04)	(-3.47)	(-2.31)

[a]Coefficients are partial effects and show weekly household expenditure per unit change in independent variable. Numbers in parentheses are asymptotic t values associated with original parameter estimates from which expected total changes were estimated.

[b]Black race is the base variable for the 0-1 variables estimated for effect of race on seafocd expenditures. Unemployed and male were the alternatives for the 0-1 variables estimated for employed and female variables.

planner was employed outside of the home spent significantly less on fresh seafoods for at-home consumption than did households whose meal planner was not employed outside the household. The fact that the meal planner was a female had a highly significant positive effect on total seafood expenditures and on expenditures for canned products ($.27 and $.15, respectively, more per household per week than for households with male planners). The final meal planner category also had significant positive effects on seafood consumption (Table 3). As education level increased, expenditures significantly increased for all seafood categories other than fresh seafoods. These three measures describing the meal planner and the estimated partial effects tend to support two beliefs with respect to seafood consumption: increased opportunity cost of the meal planners time (being employed outside the home) reduced the demand for at-home seafood consumption while higher levels of education suggest increased knowledge of the nutritional value of seafoods and thus increased demand for at-home consumption.

Entertainment of household guests during the survey week had a highly significant statistical effect on at-home seafood expenditures in all categories (Table 3). This positive impact on seafood sales could represent two effects. Households with guests require a greater volume of food than households without guests, given household size has been accounted for in the analyses. Second, since many seafoods are considered luxury items, it is possible that seafoods were served because of the special occasion of having guests.

The final category in this section is dollar expenditures on meals consumed away from home. As hypothesized, this factor decreased the demand for seafoods in all categories. Away-from-home consumption serves as a substitute for seafoods consumed in the home. This conclusion is based on the fact that for many categories of individual seafood products the away-from-home market represents the largest market outlet.

ECONOMIC DEMAND FACTORS

Income and food stamps were the main economic factors considered in the analyses although the variables discussed above were directly related to expenditures through their price and quantity effects and through their implications for the shape of household preference functions. Prices were not directly included for reasons discussed in the above section on demographic and seasonal variables.

The partial effect of households receiving food stamps did not have a significant effect on any seafood category presented in Table 4. The lack of a significant effect was somewhat unexpected and therefore requires a digression from discussing only expenditures. Interviews with operators of fresh seafood markets in Florida have indicated the food stamp program was benefical in terms of seafood sales. Comparison of simple mean values of expenditures by recipients

Table 4.--Economic factors affecting at-home seafood expenditures.[a]

Independent variable	Seafood class			
	Total	Fresh	Frozen	Canned
Food stamp recipient	.103	.074	-.001	-.018
	(.87)	(.66)	(-.02)	(-.45)
Income before taxes[b]	.025	.023	.007	.006
(1000 dols.)	(3.57)	(3.96)	(2.65)	(-.16)
	(-2.62)	(-1.96)	(-2.00)	(-.55)

[a]Coefficients are partial effects and show weekly household dollar expenditure per unit change in independent variable. Numbers in parentheses are asymptotic t values associated with original parameter estimates from which expected total changes were estimated.

[b]The effects of interaction and/or squared terms have been accounted for in the construction of the associated linear terms.

of food stamps and nonrecipients reveals greater quantities consumed in all categories except shellfish where the quantities were essentially equal (1.99 verses 2.07 pounds per week). Partial quantity effects (Keithly, 1985) however, were mixed between positive and negative coefficients and generally were statistically insignificant. Simple mean values for expenditures show food stamp recipients with higher expenditures for canned products and for finfish as a general category. It must therefore be concluded that as a group, recipients consume more seafood but overall prices paid are approximately equal to those paid by nonrecipients, however, the estimated partial effects suggest it is the other characteristics of food stamp recipients such as race, family size, age, etc. that account for this difference in consumption rather than the existence of a food stamp program.

Income was interacted with race and family size in the models estimated. The overall general conclusions are as follows. Before tax household income had an overall significant positive effect on seafood expenditures. However, the marginal propensity to consume was declining (consumption increased at a decreasing rate). White households had a lower marginal propensity to consume seafood than did non-white households while the interaction with family size showed a general tendency for the marginal propensity to consume to increase as family size increased.

Additional understanding of the effect of changes in household income on weekly at-home consumption of seafood is gained from examination of income elasticities. Income elasticities represent the percentage change in consumption due to a one percent change in income. Consumption is measured in terms of expenditures, pounds and prices for the set of income elasticities presented in Table 5.

All of the expenditure-income and quantity-income elasticities were positive and less than one, that is, a one percent change in before tax income results in less than a one percent change in seafood expenditures and quantities purchased. Shellfish income elasticities for expenditures and quantities are considerably higher than those estimated for the general category of finfish. Among specific product forms consumption of fresh seafood products had the highest elasticities while canned seafood products had the lowest. This difference in income elasticities probably is part of the reason why the growth in canned seafood consumption has been about one fourth of the growth in fresh and frozen seafood consumption since 1960 (NMFS, 1984). Although estimates of income elasticities from other studies are not directly comparable due to differences in model structure, data bases, etc., the present estimates of total elasticities generally fall within the range of previous estimates (Capps, 1982; Salathe,1979; Perry, 1981 and; Haidacher et. al., 1982).

The decomposition of total income elasticities into separate estimates for consumers and nonconsumers provides additional market information. In all cases both expenditure and quantity elasticity

Table 5.-- Expenditure, quantity, and quality income elasticities of demand for seafood consumers and non-seafood consumers.

Category	Consumers	Nonconsumers	Total	Nonconsumers as % of total
Expenditures:				
Fresh	.0899	.3771	.4620	81
Frozen	.0629	.2403	.3032	79
Canned	.0561	.1361	.1922	71
Finfish	.0558	.0927	.1485	62
Shellfish	.0958	.4476	.5434	82
Total	.0953	.1436	.2389	60
Quantities:				
Fresh	.0795	.3334	.4129	81
Frozen	.0516	.1994	.2510	79
Canned	.0310	.0667	.0977	68
Finfish	.0419	.0744	.1163	64
Shellfish	.2059	.7228	.9287	78
Total	.0759	.1055	.1814	58
Qualities:				
Fresh	.0104	.0437	.0541	81
Frozen	.0113	.0409	.0522	78
Canned	.0251	.0694	.0945	73
Finfish	.0139	.0183	.0322	57
Shellfish	-.1101	-.2752	-.3933	70
Total	.0194	.0381	.0575	66

estimates were larger for nonconsumers than for consumers. This indicates that entry (exit) into the seafood market resulting from increases (decreases) in income have a greater percentage consumption effect than that resulting from existing consumers varying consumption as their income changes. These differences suggest differential market impacts from increased incomes, for example, a given percentage increase in income will impact shellfish expenditures over four times the predicted impact on finfish expenditures. In addition, the fact that the number of nonconsumers of seafood at home in any one week is quite large and possess larger elasticities suggest market promotions may be more rewarding if directed to nonconsumers. This conclusion is further supported when the overall results of the study are considered. For total seafood consumption models, it was estimated that approximately 65 percent of the change in at-home seafood consumption resulted from changes in variables that caused increased (decreased) consumption by consumers entering (or leaving) the market (Keithly, 1985).

The last category of elasticities is referred to as quality elasticities and is estimated as the difference between expenditure and quantity elasticities. Positive quality elasticities show households paying a higher price per pound as incomes increase. Within these broad categories of seafoods, higher prices reflect improved grades, higher priced species and/or more associated marketing services given that normal price variations due to supply changes have been accounted for in the model. These changes are referred to by economists as changes in "quality".

The quality elasticities are positive for all products except shellfish for both consumer categories. This suggests consumers generally "upgrade" their purchases as incomes increase. However, quality improvements are less than increased quantities consumed for given changes in income (smaller quality elasticity estimates). The unexpected negative quality elasticity for shellfish might be explained for nonconsumers if entry into the seafood market is accomplished through purchases of different types of shellfish which vary widely in price (blue crab verses lobster as an example). This line of reasoning, however, is inconsistant with the other seafood categories where quality elasticities were generally larger for nonconsumers compared to those estimated for consumers. The negative shellfish quality elasticity for consumers appears unexceptable. The relatively high quality elasticity for canned seafood products probably reflects a substitution in type of canned product, such as, canned salmon for canned tuna, substitution of canned smoked oysters for canned fish fillets, etc.

SUMMARY AND CONCLUSIONS

A wide variety of socioeconomic and demographic variables have impacts on at-home seafood consumption. The effects of these variables

on seafood consumption vary considerably among individual seafood categories. Futhermore, conclusions drawn from the estimated partial effects, in many cases differ from conclusions based on comparisons of simple mean consumption levels for different categories of existing and potential seafood consumers.

Expansion and contraction of the at-home seafood market appear to depend more on entry and exit from the market when demand parameters change than on increased or decreased consumption by households who consume seafood on a regular basis. Approximately 65 percent of the change in consumption is due to entry and exit from the market.

Quality of seafoods purchased varies with changes in demand parameters. These changes reflect differences in grades, species and marketing services.

The differential impacts of individual socioeconomic and demographic variables on at-home seafood consumption suggest forecasts of future consumption must consider a variety of trends in individual variables such as family structure, income levels, etc. Changes in the demand for quality seafood products and the amount of market services along with the potential for increased consumption from nonconsumers should be carefully considered in seafood market promotion programs.

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EDIBILITY CHARACTERISTICS OF 40 SOUTHEASTERN FINFISH SPECIES

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INTRODUCTION

Many finfish species are common along the United States coastline, but only a few are harvested for food. About 200 finfish species inhabit waters near the Atlantic coast; and throughout the total U.S. continental waters there are about 1000 such species (Kapsalis and Maller, 1980). However, only 10-12 of these are currently utilized in any substantial quantities for human consumption. In addition, many of these species are being heavily fished, depleting them as a food source.

A potential exists for both the U.S. fishing industry and the consumer if both the supply and demand for underutilized species could be expanded. This potential includes the opening and/or expansion of domestic markets, lengthening of harvest seasons, and improving the U.S. trade deficit by creating new export products. In addition, consumers would benefit from the opportunity to purchase lower-priced underutilized fish that often have quality characteristics similar to more familiar market species. The consumer's reluctance to try new species is in large part due to a combination of unappealing common names such as ratfish or grunt, lack of consumer education, and the scarcity of data on the edibility characteristics (flavor and texture) of fish. A prime example is the goosefish, whose common name is monkfish, now gaining in popularity because it has become known as "poor man's lobster." The fish has a grotesque appearance and color; however, when baked, its flesh is white, the texture is very flaky, and it has a shellfish flavor similar to lobster.

Part of the research program at the National Marine Fisheries Service (NMFS) Charleston Laboratory has focused on determining the edibility characteristics and chemical compositions of regional finfish species. Natick Laboratories, under a NMFS contract, developed a standard protocol that we use for the evaluation of species edibilities, applying both sensory and instrumental methods (Kapsalis and Maller, 1980). These standard procedures are also being used by the Gloucester, Massachusetts and Seattle, Washington Laboratories of NMFS to evaluate the edibility characteristics of their own regional species.

In accordance with Natick protocol, at least three different seasonal samples were evaluated by a trained sensory panel in order to define the edibility characteristics for a given species. Instrumental texture and color measurements were made on each sample using the standard procedures as specified in the protocol. We have also determined proximate chemical compositions and fatty acid profiles for both raw

samples and samples that had been cooked according to the standard procedure. However, for the sake of brevity, proximate and fatty acid results are not included in this paper. They will be reported in a future publication.

MATERIALS AND METHODS

Only very fresh fish were used in this study. They were carefully identified as to species before being served to the sensory panel. Skinless fillets were prepared with the belly flaps, nape, and tail sections trimmed off. Fillet portions were placed in boil-in-bag pouches with drainage pockets and suspended in an agitated water bath at 159 to 160°F (71°C). Thermocouples were centered in several fillets and the fish were cooked to an internal temperature of 70°C (158°F).

Freshly cooked samples were placed on pre-heated, coded dishes with covers and served to the sensory panel. The sensory panel consisted of 10 Laboratory staff volunteers, who underwent training during 1982 and early 1983. Score sheets, utensils, water, and descriptive literature on procedures were supplied to each panelist. The sensory texture attributes that were evaluated and their definitions are listed in Table 1. Sensory flavor terms and definitions are listed in Table 2. After rating each sample for texture and flavor, individual panel members reported their scores using a discrete integer scale from 0 to 7, with 0 representing the absence and 7 representing the intense presence of a particular characteristic. The results were then tabulated on a large easel pad and discussed.

Instrumental Texture - Instrumental texture measurements were made with a punch and die shear cell attached to an Instron Model 1000 Universal Testing Instrument,[1] using a procedure similar to that described by Segars et al. (1975). Whenever possible, individual flakes were tested to eliminate the effects of variable flake orientation normally found in a fillet. Layers and crisscross patterns of flakes generated higher instrumental values. Three instrumental texture parameters were calculated: (1) the maximum shear stress, γ m, which is a function of the peak force and the shear area as calculated from the punch diameter and the sample thickness; (2) the maximum strain, ε m, measuring the distance of punch travel from the surface of the sample to the point of peak force, divided by the sample thickness; and (3) the stiffness, S, which is a function of the slope of the curve measured at the longest linear segment between 5% and 40% of the peak force. This calculation requires the chart recording of the force-deformation curve.

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1 The use of trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

Instrumental Color - Color analyses were made on cooked samples, homogenized in a food processor. Where there was concentrated dark tissue along the lateral line, it was removed before the sample was homogenized in a food processor. A Gardner XL20 colorimeter was used to measure L (lightness), a (redness), and b (yellowness) values of the homogenized samples packed into optical glass cups.

RESULTS AND DISCUSSION

The common and scientific names (American Fisheries Society, 1980) of the 40 species described in this paper are listed in Table 3. Most of the finfish were purchased from Charleston seafood markets. Some were landed locally while some species were harvested off the North Carolina coast, but all were examined for freshness before purchase and evaluation. The shark samples were obtained from research survey cruises of the South Carolina Wildlife and Marine Resources Department or from a local charter boat captain. The sharks were properly identified, bled, and chilled onboard the vessel.

Edibility Characteristics - The species were split into 6 groups, as shown in Table 4, based upon their habitat and the area where they are commercially harvested. Both coastal and ocean pelagic species were included in one group; the sharks are listed in a separate group because of their unique physiology, even though most of them are ocean pelagics. The four small tiger shark specimens ranged in weight from approximately 100 to 400 lbs. They were labeled "small" to distinguish them from two large tiger sharks we evaluated (estimated weights of 600 lbs. and 1000 lbs.). The larger specimens were much harder and more chewy than the smaller ones.

The average ratings of the sensory panel for seven texture and appearance attributes and nine flavor attributes are listed in Tables 5-10. The panel had the most difficulty rating sample flakiness. A total of 25 of 40 species had average standard deviations ranging from 1.00 to 1.71 for this attribute. Six species had standard deviations greater than 1.00 for four of the attributes (moistness, oily mouth-coating, sour, and earthy); darkness and total flavor intensity (TIF) showed no standard deviations greater than 1.00 for any of the 40 species.

Pearson's Correlation Coefficients were calculated among the 16 texture and flavor attributes using the panel's average for each attribute on each of the 40 species. The Minitab computer program (Ryan et al., 1981) was employed to analyze the data. Selected correlations that were greater than 0.40 (either plus or minus) are listed in Table 11. Bear in mind that "correlation tells how two variables relate to each other statistically, but it does not imply causality" (Moskowitz, 1981). For

example, if stiffness, S, and subjective fibrousness were significantly correlated, it would be possible to say that this physical measurement could underlie the perception of fibrousness.

The bar graph in Figure 1 shows profiles of selected texture attributes for five of the species groupings. Average values of each attribute for all the species in each group are compared. The freshwater benthic category is not shown because it contains only one species, the channel catfish. The pelagics exhibited the darkest cooked flesh, and reef fish were the most flaky, while sharks were the least flaky. Selected flavor attributes for the 5 species groupings are diagrammed in Figure 2. The sharks were characterized by a high sourness rating. Overall, pelagics and estuarine fish were rated highest for gaminess and earthiness.

Instrumental Measurements - Average values of instrumental textures and color parameters (lightness, redness, and yellowness) for the evaluated species are shown in Tables 12 and 13. The means and standard deviations of the Instron measurements were calculated and are included in Table 12. The Instron data show high standard deviations and obvious inconsistencies between instrumental texture (especially maximum shear stress, γ m, values) and sensory texture ratings. Borderias et al. (1983) attempted to correlate results obtained by a taste panel with physical analyses of texture on raw and cooked fish fillets. In conclusion, they stated that "no significant correlation was found between any of the indices obtained from the instrumental analyses and the results of sensory tests." Pearson's Correlation Coefficients for the stiffness, S, and γ m versus the sensory ratings of hardness, chewiness, and fibrousness were calculated using the Minitab program. These results are shown in Table 14. The highest r value was 0.522 for sensory-objective correlations. Generally, a value of 0.70 is considered significant for correlation coefficients relating subjective and objective data (Segars et al., 1975). Concerning sensory-objective correlations, Powers (1982) observed, "there may not be any relation between those factors which permit prediction of the sensory quality of a food from objective measurements." In addition, all mechanical measurements do not necessarily relate to a textural property.

A good correlation, however, was found between the instrumental "darkness" parameter, 100-$\bar{L}$ (where $\bar{L}$ corresponds to lightness or light reflectance), and the sensory darkness rating. Figure 3 shows a plot of 100-$\bar{L}$ versus sensory darkness. The crevalle jack, king mackerel, and bluefish exhibited the darkest cooked flesh, while the gag grouper and black sea bass had the lightest cooked flesh.

CONCLUSIONS

The edibility characteristics as well as instrumental texture and color parameters have been described for 40 finfish species of the southeast region. Average values for selected edibility characteristics were presented for groupings of fish based on general habitat and resulted in characteristic profiles. Further statistical analysis of the sensory panel data will be performed for more detailed grouping of individual species by edibility characteristics. Once this is completed, the groupings according to habitat may need to be modified because grouping according to edibility characteristics will produce not only certain distinctive groups but also groups with a large amount of overlap. It may even be necessary to "weight" various attributes for relative importance.

It is also planned to combine the data developed by the three regional laboratories and to group the species by cluster analysis and multidimensional scaling. These groupings could then form the basis for a national marketing system based on edibility characteristics that could expand the harvest and consumption of some currently unfamiliar and underutilized species.

Table 1. Definitions of Sensory Texture Terms as Applied to Cooked Fish Samples

Term	Definition
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.
Oily Mouthcoating	The perceived degree of oil left on the teeth, tongue, and palate after swallowing.

Table 2. Definitions of Sensory Terms for Description of Fish Flavor

Term	Definition
Total Flavor Intensity	The initial or early total impact of flavors.
Salty-briny	A combination of the taste sensations of sodium chloride and the other salt compounds found in ocean water.
Sour	The taste sensation produced by acids. The taste of vinegar or lemon are typical examples.
Shellfish	The flavor associated with any cooked shellfish, such as lobster, clam, crab, or scallop.
Gamey*	The flavor associated with the heavy, gamey characteristics of some cooked fish such as Atlantic mackerel, as opposed to a delicate flavor such as sole; analogous to the relationship of the heavy, gamey characteristics of fresh cooked venison compared to fresh cooked beef, or duck to chicken.
Fish Oil	The flavor associated with fish oil, such as found in mackerel, and canned sardines or cod liver oil.
Sweet	The basic taste sensation of which the taste of sucrose is typical.
Earthy	The flavor associated with slightly undercooked boiled potato, soil, or muddy fish.
Mouth Drying	The sensation of dry skin surfaces of the oral cavity; dry feeling in the mouth after swallowing; astringency.

* The correct spelling is "gamy". However, the word was "gamey" in the protocol so we continued this usage.

Table 3. Southeastern Finfish Evaluated for Edibility Characteristics.

Species	Scientific Name	Number of Evaluations
Barracuda, Great	Sphyraena barracuda	3
Bass, Black Sea	Centropristis striata	3
Bluefish	Pomatomus saltatrix	5
Catfish, Channel	Ictalurus punctatus	3
Croaker, Atlantic	Micropogonias undulatus	3
Dolphin	Coryphaena hippurus	4
Drum, Red	Sciaenops ocellatus	5
Flounder, Southern	Paralichthys lethostigma	3
Flounder, Summer	Paralichthys dentatus	3
Goosefish (Monkfish)	Lophius americanus	4
Grouper, Gag	Mycteroperca microlepis	4
Grouper, Scamp	Mycteroperca phenax	3
Grouper, Snowy	Epinephelus niveatus	3
Grouper, Yellowedge	Epinephelus flavolimbatus	3
Grunt, White	Haemulon plumieri	3
Hind, Speckled	Epinephelus drummondhayi	4
Jack, Crevalle	Caranx hippos	3
Kingfish, Southern (Whiting)	Menticirrhus americanus	3
Ladyfish	Elops saurus	3
Mackerel, King	Scomberomorus cavalla	4
Mackerel, Spanish	Scomberomorus maculatus	3
Mullet, Striped	Mugil cephalus	4
Porgy, Longspine	Stenotomus caprinus	3
Porgy, Red	Pagrus pagrus	3
Seatrout, Spotted	Cynoscion nebulosus	3
Shad, American	Alosa sapidissima	3
Shark, Atlantic Sharpnose	Rhizoprionodon terraenovae	3
Shark, Lemon	Negaprion brevirostris	3
Shark, Sandbar	Carcharhinus plumbeus	4
Shark, Scalloped Hammerhead	Sphyrna lewini	3
Shark, Tiger (Small)	Galeocerdo cuvieri	4
Sheepshead	Archosargus probatocephalus	3
Snapper, Red	Lutjanus campechanus	4
Snapper, Vermilion	Rhomboplites aurorubens	3
Spot	Leiostomus xanthurus	3
Swordfish	Xiphias gladius	3
Tilefish	Lopholatilus chamaeleonticeps	3
Tilefish, Blueline	Caulolatilus microps	3
Triggerfish, Gray	Balistes capriscus	4
Weakfish	Cynoscion regalis	3

Table 4. Groupings of Finfish Based on the Locality of Commercial Harvest.

Estuarine

Croaker, Atlantic
Drum, Red
Flounder, Southern
Flounder, Summer
Kingfish, Southern
Ladyfish
Mullet, Striped
Seatrout, Spotted
Shad, American
Spot
Weakfish

Pelagic

Barracuda, Great
Bluefish
Dolphin
Jack, Crevalle
Mackerel, King
Mackerel, Spanish
Swordfish

Ocean Benthic

Goosefish
Tilefish
Tilefish, Blueline

Reef

Bass, Black Sea
Grouper, Gag
Grouper, Scamp
Grouper, Snowy
Grouper, Yellowedge
Grunt, White
Hind, Speckled
Porgy, Longspine
Porgy, Red
Sheepshead
Snapper, Red
Snapper, Vermilion
Triggerfish, Gray

Sharks

Shark, Atlantic Sharpnose
Shark, Lemon
Shark, Sandbar
Shark, Scalloped Hammerhead
Shark, Tiger (Small)

Fresh-Water Benthic

Catfish, Channel

Table 5. Sensory Panel Ratings of Texture and Flavor Attributes of 11 Estuarine Finfish Species (Mean; Scale of 0-7. 0 = not present and 7 = strongly present).

Fish Species:	Atlantic Croaker	Red Drum	Southern Flounder	Summer Flounder
Texture Profile:				
Darkness	3.60	3.03	2.37	1.58
Hardness	2.04	2.35	1.88	2.01
Flakiness	3.02	2.63	1.30	1.18
Chewiness	2.33	2.72	2.17	2.23
Fibrousness	2.07	2.40	2.57	1.85
Moistness	3.26	3.01	2.98	2.40
Oily Mouthcoating	1.42	1.33	0.32	0.21
Flavor Profile:				
Total Flavor Intensity	3.65	3.67	2.32	2.25
Salty	1.91	1.67	1.39	1.19
Sour	0.62	1.44	0.98	0.48
Shellfish	0.62	0.39	0.67	0.50
Gamey	1.10	0.99	0.13	0.00
Fish Oil	0.61	0.80	0.05	0.00
Sweet	1.20	0.78	0.56	0.68
Earthy	1.52	0.86	0.46	0.62
Mouth Drying	0.87	1.76	1.98	1.99

Table 5. (continued)

Fish Species:	Southern Kingfish	Ladyfish	Striped Mullet	Spotted Seatrout
Texture Profile:				
Darkness	2.86	3.00	3.78	2.62
Hardness	1.91	2.43	2.35	1.70
Flakiness	3.64	0.54	2.44	3.07
Chewiness	1.85	2.77	3.00	1.77
Fibrousness	2.08	2.60	2.70	2.29
Moistness	3.51	2.11	3.11	2.38
Oily Mouthcoating	1.26	0.35	1.89	0.50

Table 5. (continued)

Fish Species:	Southern Kingfish	Ladyfish	Striped Mullet	Spotted Seatrout
Flavor Profile:				
Total Flavor Intensity	3.20	3.45	4.31	3.22
Salty	1.78	1.44	1.50	1.46
Sour	0.83	2.07	1.56	0.89
Shellfish	1.05	0.06	0.18	0.63
Gamey	0.69	0.82	1.62	0.40
Fish Oil	0.65	0.18	1.07	0.13
Sweet	0.95	0.40	0.80	0.73
Earthy	1.11	1.07	1.37	1.43
Mouth Drying	0.88	2.33	0.95	1.24

Table 5. (continued)

Fish Species:	American Shad	Spot	Weakfish
Texture Profile:			
Darkness	3.69	3.28	3.17
Hardness	1.35	1.83	1.88
Flakiness	0.87	2.83	3.84
Chewiness	1.82	2.28	2.42
Fibrousness	2.06	2.28	2.88
Moistness	3.70	3.33	3.17
Oily Mouthcoating	2.27	1.50	1.00
Flavor Profile:			
Total Flavor Intensity	4.27	3.72	3.05
Salty	2.08	1.89	1.58
Sour	1.30	0.78	0.67
Shellfish	0.40	0.39	0.88
Gamey	1.40	1.11	0.71
Fish Oil	1.43	0.95	0.25
Sweet	1.00	1.05	0.92
Earthy	0.88	0.72	0.79
Mouth Drying	0.71	0.72	1.13

Table 6. Sensory Panel Ratings of Texture and Flavor Attributes of 13 Reef Fish (Mean; Scale of 0-7).

Fish Species:	Black Sea Bass	Gag Grouper	Scamp Grouper	Snowy Grouper
Texture Profile:				
Darkness	1.80	1.90	2.02	1.94
Hardness	2.60	3.96	2.53	3.21
Flakiness	4.98	4.55	6.15	4.83
Chewiness	3.06	4.04	2.77	3.55
Fibrousness	2.38	3.49	3.14	2.84
Moistness	2.84	2.58	3.37	3.33
Oily Mouthcoating	0.76	0.73	0.78	0.63
Flavor Profile:				
Total Flavor Intensity	2.34	2.75	2.82	2.52
Salty	1.31	1.20	1.17	1.49
Sour	0.66	1.04	0.61	0.63
Shellfish	0.92	0.68	1.13	0.75
Gamey	0.15	0.49	0.00	0.11
Fish Oil	0.15	0.22	0.16	0.05
Sweet	1.11	1.04	1.49	1.23
Earthy	0.24	0.41	0.28	0.23
Mouth Drying	1.01	1.82	0.50	1.09

Table. 6 (continued)

Fish Species:	Yellowedge Grouper	White Grunt	Speckled Hind	Longspine Porgy
Texture Profile:				
Darkness	1.64	2.82	2.43	3.25
Hardness	3.52	1.89	3.74	1.63
Flakiness	4.76	3.07	4.68	1.82
Chewiness	3.61	2.38	4.09	1.96
Fibrousness	3.27	2.60	3.15	2.39
Moistness	3.21	3.51	3.19	3.11
Oily Mouthcoating	0.63	0.68	0.98	0.97

Table 6. (continued)

Fish Species:	Yellowedge Grouper	White Grunt	Speckled Hind	Longspine Porgy
Flavor Profile:				
Total Flavor Intensity	2.68	2.73	2.76	4.00
Salty	0.98	1.43	1.47	1.26
Sour	0.52	0.98	0.60	0.82
Shellfish	0.98	0.49	0.71	0.30
Gamey	0.23	0.59	0.03	1.28
Fish Oil	0.09	0.38	0.33	0.99
Sweet	0.95	1.13	1.07	1.08
Earthy	0.42	0.79	0.24	1.08
Mouth Drying	0.82	1.14	0.92	1.12

Table 6. (continued)

Fish Species:	Red Porgy	Sheeps-head	Red Snapper	Vermilion Snapper	Gray Trigger-fish
Texture Profile:					
Darkness	2.55	3.16	2.01	1.82	2.40
Hardness	3.15	2.56	2.20	2.45	2.77
Flakiness	4.00	3.40	3.86	2.86	4.31
Chewiness	4.05	2.81	2.60	2.82	2.85
Fibrousness	2.90	3.29	2.54	3.18	2.48
Moistness	2.50	3.07	2.92	2.92	2.72
Oily Mouthcoating	0.76	0.72	0.53	0.38	0.46
Flavor Profile:					
Total Flavor Intensity	3.00	2.80	2.75	2.63	3.13
Salty	1.56	1.27	1.74	1.55	1.29
Sour	2.03	0.81	0.88	1.37	1.25
Shellfish	0.94	0.17	0.53	0.33	0.59
Gamey	0.63	0.41	0.10	0.41	0.39
Fish Oil	0.20	0.33	0.20	0.27	0.24
Sweet	0.83	1.05	1.08	0.51	0.90
Earthy	0.93	0.62	0.47	0.44	1.03
Mouth Drying	2.18	1.23	1.90	1.84	2.11

Table 7. Sensory Panel Ratings of Texture and Flavor Attributes of 7 Pelagic Finfish Species (Mean; Scale of 0-7).

Fish Species:	Great Barracuda	Bluefish	Dolphin	Crevalle Jack
Texture Profile:				
Darkness	2.51	3.89	3.11	5.06
Hardness	3.54	2.30	2.93	3.11
Flakiness	3.20	2.16	2.26	2.67
Chewiness	4.37	2.96	3.16	3.33
Fibrousness	3.87	2.86	3.27	3.17
Moistness	2.47	3.14	2.95	2.83
Oily Mouthcoating	0.83	1.32	0.78	0.61
Flavor Profile:				
Total Flavor Intensity	2.96	3.63	3.17	3.44
Salty	1.88	1.76	1.74	1.78
Sour	2.09	1.35	1.48	1.50
Shellfish	0.10	0.22	0.12	0.06
Gamey	0.60	1.68	0.86	1.56
Fish Oil	0.05	0.81	0.36	0.50
Sweet	0.79	0.83	0.91	0.95
Earthy	0.47	0.68	0.49	0.39
Mouth Drying	1.34	1.29	1.55	0.95

Table 7. (continued)

Fish Species:	King Mackerel	Spanish Mackerel	Swordfish
Texture Profile:			
Darkness	4.55	2.99	2.98
Hardness	3.09	1.73	2.10
Flakiness	3.34	1.32	1.89
Chewiness	3.58	1.98	2.56
Fibrousness	3.84	2.18	3.14
Moistness	2.61	2.83	3.31
Oily Mouthcoating	1.60	1.21	1.04

Table 7. (continued)

Fish Species:	King Mackerel	Spanish Mackerel	Swordfish
Flavor Profile:			
Total Flavor Intensity	3.88	3.58	3.42
Salty	1.69	1.52	1.71
Sour	1.78	1.20	2.84
Shellfish	0.20	0.21	0.13
Gamey	2.15	1.04	0.88
Fish Oil	0.76	0.95	0.60
Sweet	0.84	1.17	0.79
Earthy	1.01	0.96	0.79
Mouth Drying	1.24	1.07	1.86

Table 8. Sensory Panel Ratings of Texture and Flavor Attributes of 5 Shark Species (Mean; Scale of 0-7).

Fish Species:	Atlantic Sharpnose	Lemon	Sandbar
Texture Profile:			
Darkness	2.17	2.08	2.51
Hardness	2.62	3.37	3.03
Flakiness	1.68	0.61	0.56
Chewiness	2.61	3.58	3.07
Fibrousness	2.54	3.44	2.35
Moistness	2.78	3.01	2.77
Oily Mouthcoating	0.44	0.62	0.48
Flavor Profile:			
Total Flavor Intensity	3.42	3.45	3.21
Salty	1.29	1.61	1.47
Sour	2.83	2.94	2.46
Shellfish	0.24	0.08	0.41
Gamey	0.66	0.44	0.43
Fish Oil	0.12	0.00	0.11
Sweet	0.19	0.39	0.46
Earthy	0.59	0.77	0.48
Mouth Drying	1.69	1.87	1.20

Table 8. (continued)

Fish Species:	Scalloped Hammerhead	Tiger (Small)
Texture Profile:		
Darkness	2.93	2.43
Hardness	4.02	1.85
Flakiness	0.85	0.85
Chewiness	4.09	2.29
Fibrousness	3.17	2.29
Moistness	2.26	3.94
Oily Mouthcoating	0.55	0.51
Flavor Profile:		
Total Flavor Intensity	3.80	3.60
Salty	1.83	1.49
Sour	3.88	2.58
Shellfish	0.06	0.17
Gamey	0.79	0.52
Fish Oil	0.00	0.11
Sweet	0.19	0.56
Earthy	0.76	0.47
Mouth Drying	2.27	0.62

Table 9. Sensory Panel Ratings of Texture and Flavor Attributes of 3 Ocean Benthic Fish Species (Mean; Scale of 0-7).

Fish Species:	Goosefish	Tilefish	Blueline Tilefish
Texture Profile:			
Darkness	2.17	2.54	2.63
Hardness	2.64	3.00	3.21
Flakiness	3.40	4.15	3.26
Chewiness	2.86	3.42	3.94
Fibrousness	3.36	3.37	3.29
Moistness	3.33	3.05	2.51
Oily Mouthcoating	0.39	1.22	0.59

Table 9. (continued)

Fish Species:	Goosefish	Tilefish	Blueline Tilefish
Flavor Profile:			
Total Flavor Intensity	3.24	3.19	2.97
Salty	1.44	1.10	1.10
Sour	0.65	0.84	0.93
Shellfish	2.52	1.02	0.99
Gamey	0.21	0.17	0.26
Fish Oil	0.00	0.18	0.11
Sweet	1.47	1.10	0.92
Earthy	0.55	1.17	0.75
Mouth Drying	0.87	1.26	1.11

Table 10. Sensory Panel Ratings of Texture and Flavor Attributes of a Freshwater Benthic Finfish Species (Mean; Scale of 0-7).

Fish Species:	Channel Catfish
Texture Profile:	
Darkness	2.59
Hardness	1.60
Flakiness	2.73
Chewiness	1.86
Fibrousness	1.74
Moistness	3.65
Oily Mouthcoating	0.53
Flavor Profile:	
Total Flavor Intensity	2.52
Salty	1.20
Sour	0.54
Shellfish	0.26
Gamey	0.44
Fish Oil	0.11
Sweet	1.32
Earthy	1.09
Mouth Drying	0.63

Table 11. Pearson's Correlation Coefficients Among Texture and Flavor Attributes. Significance level (P) = 0.01.

	Hardness	Chewiness	Darkness	Flakiness
Chewiness	0.953	1.000		
Fibrousness	0.738	0.781		
Moistness	-0.453	-0.434		
Oily M.C.			0.595	
TIF			0.682	-0.455
Sweet				0.651
Salty			0.542	
Sour				-0.595
Gamey			0.883	
Fish Oil	-0.457		0.668	
Shellfish				0.544
Earthy	-0.413		0.412	

Table 11. (continued)

	Moistness	Oily M.C.	TIF	Sweet
TIF		0.659	1.000	
Sweet	0.465			1.000
Salty		0.494	0.515	
Sour			0.429	-0.768
Gamey		0.670	0.795	
Fish Oil		0.865	0.701	
Shellfish				0.523
Earthy		0.411	0.528	
Mouth Drying	-0.678	-0.401		-0.612

Table 11. (continued)

	Salty	Sour	Gamey	Fish Oil
Gamey	0.557		1.000	
Fish Oil	0.478		0.769	1.000
Shellfish		-0.511	-0.470	
Earthy			0.431	0.403
Mouth Drying		0.469		

Table 12. Instrumental Texture Measurements for 40 Southeastern Finfish Species.

	Instron Data		
Fish Species	γ_m, N/cm^2	S, N/cm^2	ϵ_m
Barracuda, Great	26.52 ± 6.61	3.97 ± 1.27	0.91 ± 0.03
Bass, Black Sea	14.91 ± 3.26	4.56 ± 0.74	1.02 ± 0.03
Bluefish	23.82 ± 5.47	3.45 ± 1.32	1.00 ± 0.08
Catfish, Channel*	1.73 ± 0.48	1.55 ± 0.42	0.97 ± 0.08
Croaker, Atlantic	10.84 ± 3.67	4.40 ± 3.00	1.12 ± 0.20
Dolphin	22.35 ± 4.55	12.33 ± 2.60	1.11 ± 0.12
Drum, Red	11.88 ± 3.05	5.11 ± 1.20	0.93 ± 0.10
Flounder, Southern	21.46 ± 6.82	7.16 ± 2.51	1.05 ± 0.03
Flounder, Summer*	8.62 ± 2.33	1.38 ± 0.39	1.08 ± 0.08
Goosefish	7.48 ± 2.67	3.33 ± 1.38	1.05 ± 0.06
Grouper, Gag	20.16 ± 3.73	10.15 ± 2.92	0.99 ± 0.03
Grouper, Scamp	7.58 ± 1.49	2.91 ± 0.84	1.04 ± 0.12
Grouper, Snowy	11.18 ± 1.97	7.00 ± 1.92	0.94 ± 0.05
Grouper, Yellowedge	12.23 ± 1.75	6.98 ± 1.76	0.97 ± 0.04
Grunt, White	11.25 ± 4.10	3.73 ± 1.51	1.14 ± 0.17
Hind, Speckled	16.05 ± 3.64	3.52 ± 1.18	0.95 ± 0.05
Jack, Crevalle	16.12 ± 5.02	6.38 ± 2.68	1.02 ± 0.04
Kingfish, Southern	8.04 ± 2.95	3.36 ± 1.63	1.15 ± 0.13
Ladyfish	18.37 ± 7.73	5.34 ± 2.62	1.44 ± 0.35
Mackerel, King	17.84 ± 4.00	4.49 ± 1.42	1.00 ± 0.02

* Boneless fillets used because individual flakes could not be separated.

Table 12. (continued)

Mackerel, Spanish	11.73 ± 2.50	5.08 ± 2.45	1.39 ± 0.19
Mullet, Striped	28.41 ± 7.25	5.61 ± 3.46	1.09 ± 0.08
Porgy, Longspine	13.29 ± 4.69	4.31 ± 1.99	1.03 ± 0.07
Porgy, Red	25.16 ± 7.77	9.26 ± 2.74	1.06 ± 0.19
Seatrout, Spotted	9.62 ± 2.96	3.13 ± 1.23	1.05 ± 0.04
Shad, American	10.28 ± 2.18	3.47 ± 0.74	1.08 ± 0.04
Shark, Atlantic Sharpnose	11.15 ± 2.67	4.49 ± 1.29	0.95 ± 0.03
Shark, Lemon	12.28 ± 2.28	6.22 ± 2.95	1.00 ± 0.17
Shark, Sandbar	11.95 ± 4.41	7.45 ± 2.48	1.09 ± 0.15
Shark, Scalloped Hammerhead	15.52 ± 4.66	6.98 ± 2.46	1.00 ± 0.17
Shark, Tiger (Small)	12.91 ± 3.51	8.47 ± 2.95	1.03 ± 0.05
Sheepshead	9.10 ± 1.94	1.73 ± 0.60	1.00 ± 0.04
Snapper, Red	14.33 ± 3.86	4.63 ± 1.15	1.03 ± 0.08
Snapper, Vermilion	13.36 ± 3.13	3.71 ± 2.25	1.02 ± 0.08
Spot	12.44 ± 2.96	4.27 ± 1.36	1.21 ± 0.17
Swordfish	11.93 ± 1.60	5.43 ± 1.90	1.05 ± 0.04
Tilefish	9.74 ± 1.80	4.27 ± 1.14	1.01 ± 0.05
Tilefish, Blueline	15.86 ± 2.85	4.44 ± 1.05	0.99 ± 0.02
Triggerfish, Gray	8.38 ± 1.37	6.26 ± 1.98	0.87 ± 0.08
Weakfish	8.32 ± 1.03	3.22 ± 0.61	1.00 ± 0.08

Table 13. Instrumental Color Measurements for 40 Southeastern Finfish Species.

	Color Measurements		
Fish Species	L	a	b
Barracuda, Great	79.23	0.43	8.93
Bass, Black Sea	83.80	0.27	9.85
Bluefish	69.86	1.26	8.35
Catfish, Channel	75.88	2.43	11.02
Croaker, Atlantic	69.75	1.45	10.05
Dolphin	77.80	2.38	11.28
Drum, Red	73.56	1.85	10.21
Flounder, Southern	75.78	0.50	9.40
Flounder, Summer	77.66	0.09	7.08
Goosefish	81.85	0.37	9.77
Grouper, Gag	84.12	-0.70	9.84
Grouper, Scamp	75.84	1.43	9.12

Table 13. (continued)

Grouper, Snowy	83.70	0.70	10.55
Grouper, Yellowedge	82.36	0.85	9.40
Grunt, White	76.09	2.33	10.80
Hind, Speckled	82.13	1.52	9.40
Jack, Crevalle	61.43	6.12	11.90
Kingfish, Southern	71.57	0.42	8.75
Ladyfish	75.48	3.33	11.36
Mackerel, King	66.68	1.43	8.33
Mackerel, Spanish	73.43	2.06	11.10
Mullet, Striped	71.88	4.07	11.20
Porgy, Longspine	70.50	3.80	12.60
Porgy, Red	83.23	0.73	10.48
Seatrout, Spotted	75.59	1.42	10.27
Shad, American	72.25	3.04	12.22
Shark, Atlantic Sharpnose	82.45	1.20	11.16
Shark, Lemon	81.52	1.46	10.97
Shark, Sandbar	79.85	1.05	11.20
Shark, Scalloped Hammerhead	77.68	2.08	10.97
Shark, Tiger (Small)	78.05	2.15	11.93
Sheepshead	74.01	1.31	10.20
Snapper, Red	81.50	1.93	10.81
Snapper, Vermilion	83.55	0.88	11.16
Spot	73.83	1.65	9.92
Swordfish	76.88	3.50	11.58
Tilefish	76.98	2.73	11.73
Tilefish, Blueline	77.82	1.63	11.44
Triggerfish, Gray	82.76	1.02	10.35
Weakfish	71.35	0.70	8.49

Table 14. Correlation Coefficients Between Instrumental Measurements and Sensory Ratings for 40 Southeastern Finfish Species.

Instrumental Parameter	Sensory Attribute	Correlation Coefficient
S, Stiffness	Hardness	0.441*
Stiffness	Chewiness	0.407*
Stiffness	Fibrousness	0.249***
γ_m, Shear Stress	Hardness	0.376**
Shear Stress	Chewiness	0.522*
Shear Stress	Fibrousness	0.405*
100-L Value	Darkness	0.883*

* P = 0.01 ** P = 0.05 *** P = 0.20

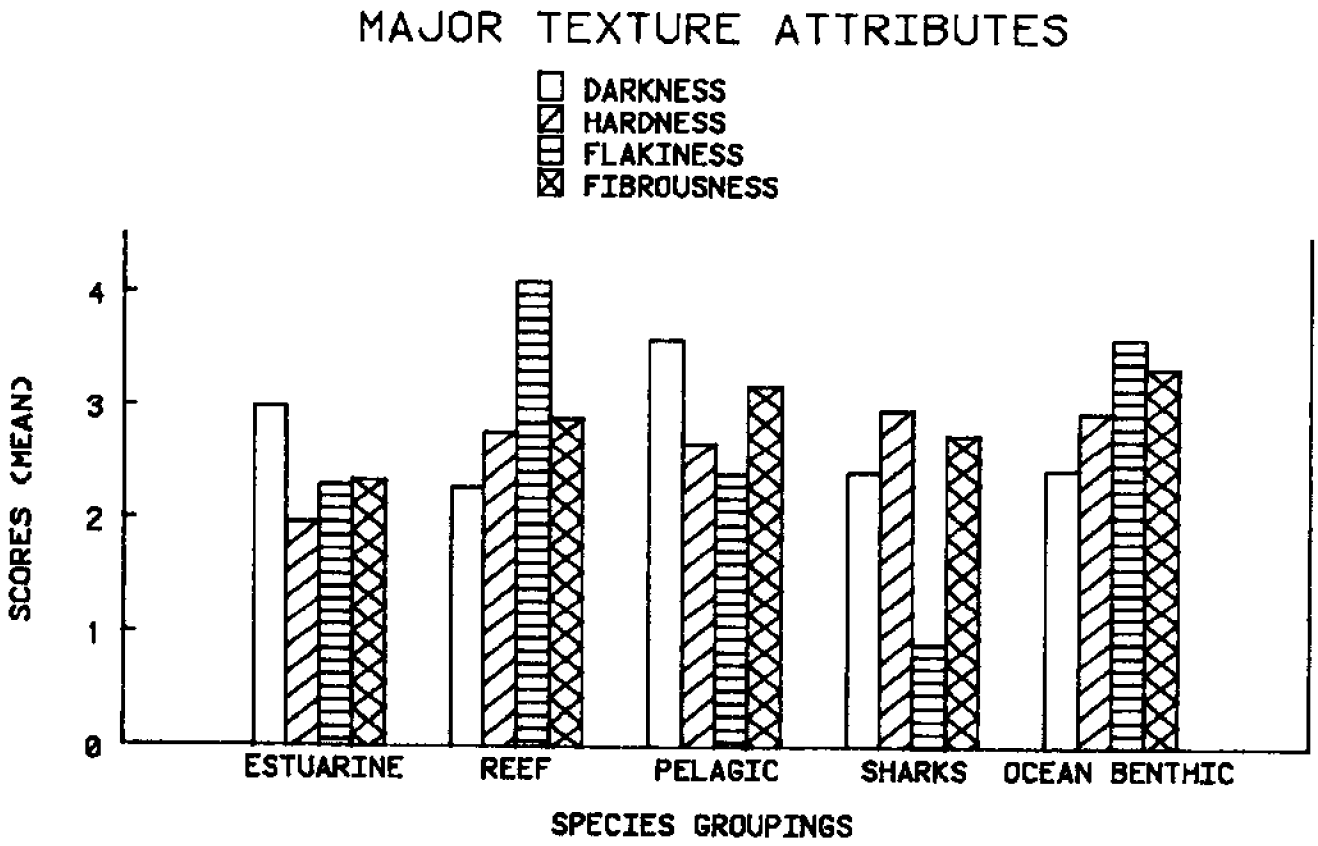

Fig. 1. Average Profiles of Major Texture and Appearance Attributes for 5 Species Groupings of Finfish.

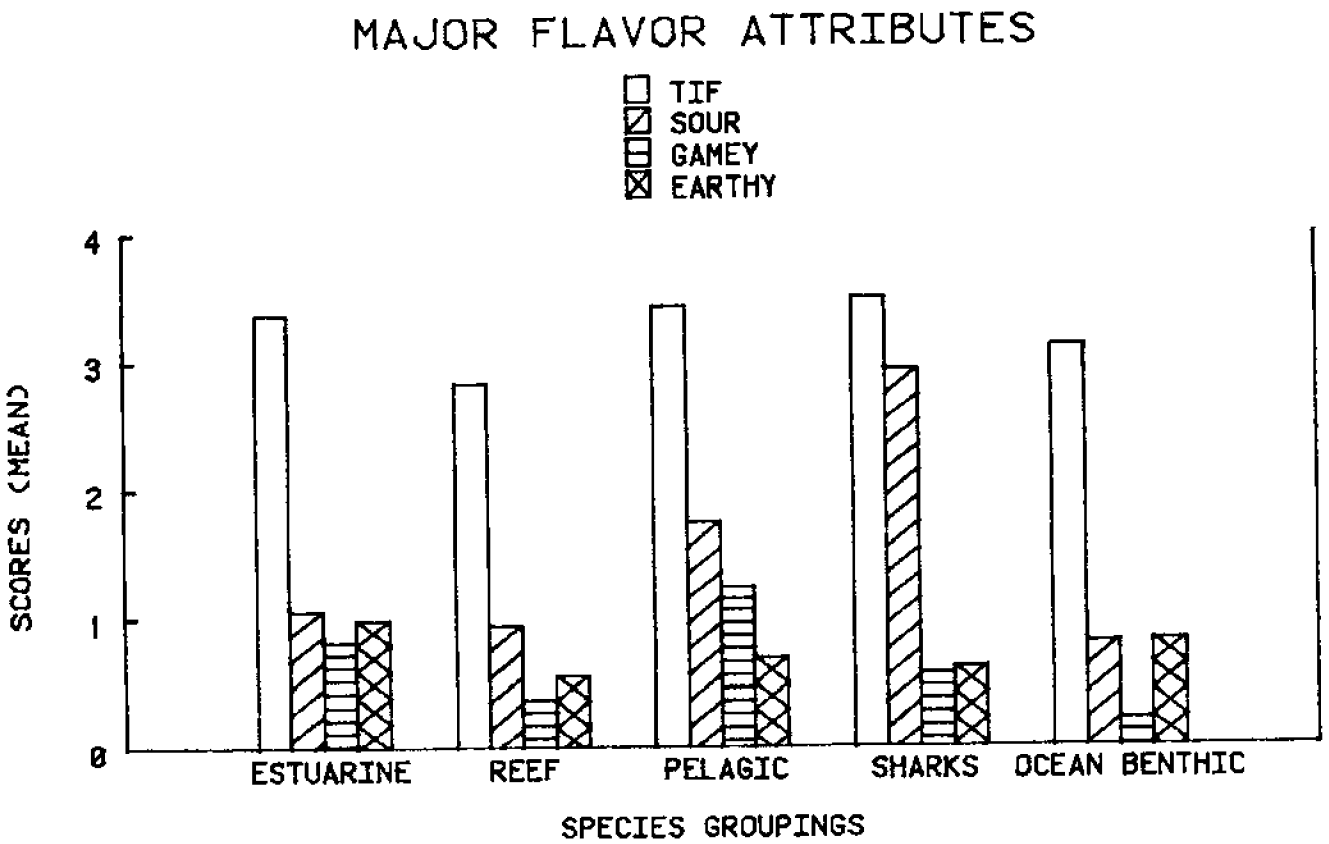

Fig. 2. Average Profiles of Major Flavor Attributes for 5 Species Groupings of Finfish.

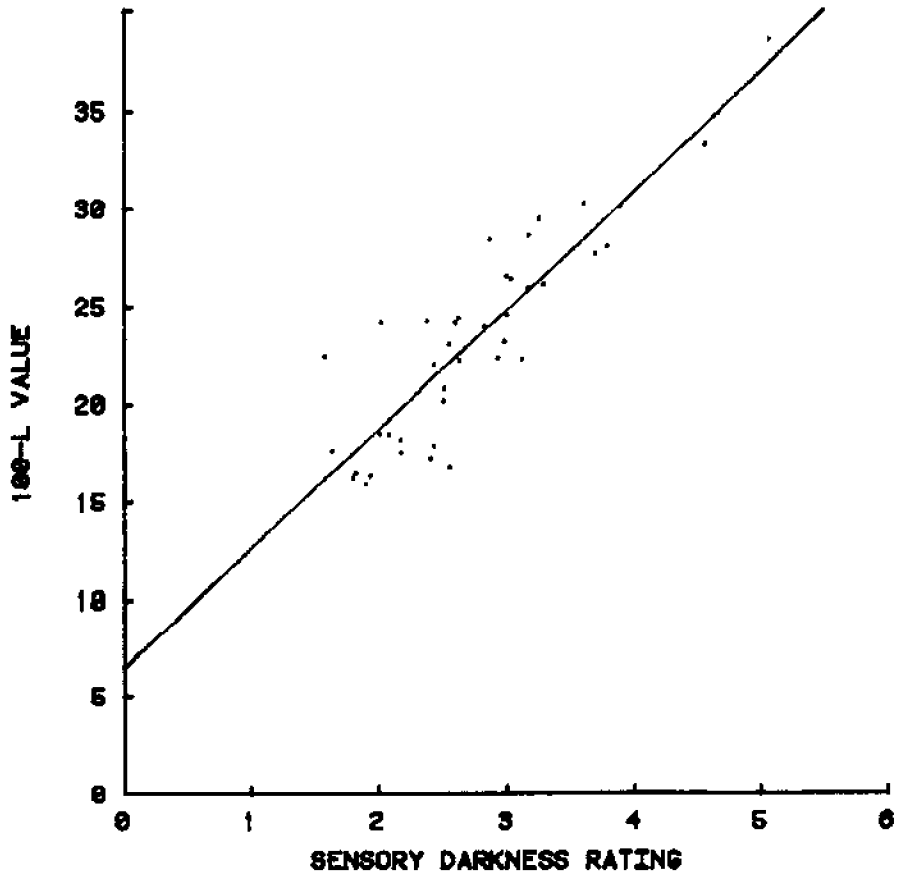

Fig. 3. Instrumental Color (100 - Lightness) Versus Sensory Darkness Rating for 40 S.E. Finfish Species. r = 0.88.

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ALTERNATE SPECIES - FACT OR FICTION

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INTRODUCTION

As traditional marine resources become less available due to fishing pressure, management practice or other factors, the producing segment of the industry is challenged to identify alternate resources to maintain access to market opportunities. Varieties which were ignored a decade ago are being harvested or examined to fill voids in the supply of product.

Worldwide these include a wide variety, including squids, krill, lanternfish, deepwater crustacea, sea mount resources and a variety of pelagic fishes. In the Southeast and adjacent area, significant increases are notable from aquaculture production; molluscs (scallops and clams), specialty products for export (such as mullet roe); sharks and a variety of other products which have increased their market share.

Examination of other resources is underway. These included deepwater crabs and other crustacea, small tunas, butterfish, squids and octopus and other products. Additional opportunities await identification--some of these are suggested along with a discussion of known impediments.

RECENT EXPERIENCE

For more than a decade, fisheries scientists have been looking to new opportunities to increase the resource base of productive and profitable fisheries (14, 15, 19). There are numerous examples of successful developments which have evolved from harvesting innovation, processing and handling adaptation, market development. Most often it is not possible to identify a specific pattern of development but rather the progress is attributable to a combination of factors and/or events. A quotation attributed to Julius H. Comroe suggests an important element-- "Serendipity is looking in a haystack for a needle and discovering the farmer's daughter." Some examples of recently successful fisheries include:

During 1983 record production was established for menhaden, American lobsters and flounder; in 1984 record totals for sablefish, Alaska pollock, clam and scallop meats were indicated. Per capita consumption of

fish and shellfish reached record levels during 1984 with an increase from 13.1 to 13.6 pounds which is significant and represents cause for optimism (20, 21). In the Southeast dramatic increases in production have been noted in numerous fisheries including scallops and hard clams, swordfish, mullet, small tunas, rock shrimp and other varieties. Culture assisted development has included spectacular increases in production of catfish and crawfish. Other opportunities exist and these will be briefly reviewed.

AREAS OF OPPORTUNITY

Cephalopods

Cephalopods, including four or more species of squids (figures 1 and 2) and octopus are resources which appear to be available in substantial amounts but industry motivation not to mention adequate knowledge of the resources, fishing/handling and processing techniques are lacking. Without regular production it is difficult to impossible to generate market opportunities. There exists anecdotal and factual evidence that these resources represent very real opportunities but indifference, high cost of resource investigation and questionable marketability impact negatively on development progress.

Contintental Shelf and Slope Fish Resources

Indications of coastal pelagic fishes such as butterfish, round herring, driftfish and other varieties including several species of tunas are in various levels of development. Resource access, harvesting, handling, processing and product forms all represent challenging problems. Substantial tonnage may be accomplished with resolution of outstanding problems.

Exotic Crustacea

The outer portion of the continental shelf and the adjacent upper slope area (50 - 350 fathoms) is an area known to be inhabited by a wide variety of interesting crustacea.

On the inner edge are rock shrimp which are beginning to be sought out during off season periods by the traditional shrimp fleet. Intermittant but regular effort has been evident on stocks of "Royal Red Shrimp," both on the East Florida coast and at several locations in the Gulf of Mexico. Other varieties of shrimp as well as "Lobsterette" type crustaceans are potential targets for imaginative and innovative producers. Jonah crabs (figure 3) and bulldozer lobsters may offer developmental opportunities given adequate information on harvest and market opportunities.

Culture

Progress in the culture of crustacea such as shrimp and crawfish open the imagination to other varieties which could include high value crustacea (crabs, lobster) as well as pompano and other finfish.

Molluscs including clams, scallops, whelks and ocotpus could take a place alongside the traditional oyster as objects of aquaculture. Promising opportunities await the resolution of social and legal obstacles to coastal area use.

IMPEDIMENTS

Most of the deterrents to more use of these resources relate to resource access and to a myriad of levels required through marketing. Rigorous and disciplined application of unique technology is called for. The use of satellite derived productivity information, instantaneous reference to shifts of thermal fronts, undersea submersible reconnaissance, and new harvest techniques employing behaviour and attraction are called for. There is decreasing opportunity to consider these goals as competition for available research funding increases.

POSITIVE ASPECTS

A great opportunity which is only now becoming clearer is relating to the probable beneficial aspects of seafood use being heralded by medical researchers. While much of the preliminary support points to coronary related medical benefits, there are indications that positive dietary aspects of seafood may be broader based. Thus, with traditional resource supplies becoming more fully utilized or as in some cases over utilized, the public and the industry are put into a mode of looking for alternative supplies which for one reason or another have not been attractive up to this point. New management approaches to organization, i.e. vertical integration, joint ventures, cooperative harvesting/processing relationships, etc., can provide opportunities for broader resource use. Processing at sea is another approach which may be considered to alleviate some problem areas.

New approaches to harvesting such as the use of aggregating devices, i.e. rafts, lights and traps (figure 4), may provide economically practical harvest levels where other methods have not. Better detection through electronics and the use of environmental intelligence, such as water temperature or clarity can enhance the fisherman's ability to accomplish profitable catch rates.

Ultimately better and more efficient ways to preserve, process and market products raise the economic attractiveness of products.

CONCLUSION

The foregoing discussion suggests that with somewhat wider vision and innovations there may be cause for some optimism in establishing a broader base for marine resource use. For consideration we offer a list indicating species groups which are possible candidates for utilization on a more intensive basis (Table 1).

Ultimately, the ability to accomplish gains will require a more integrated and imaginative approach than has been pursued in the past.

Remember Serendipity.

ACKNOWLEDGEMENT

This work was supported through a grant from the Gulf and South Atlantic Fisheries Development Foundation of Tampa, Florida. Appreciation is also expressed to numerous colleagues and members of the fishing industry for their supportive collaboration and ideas.

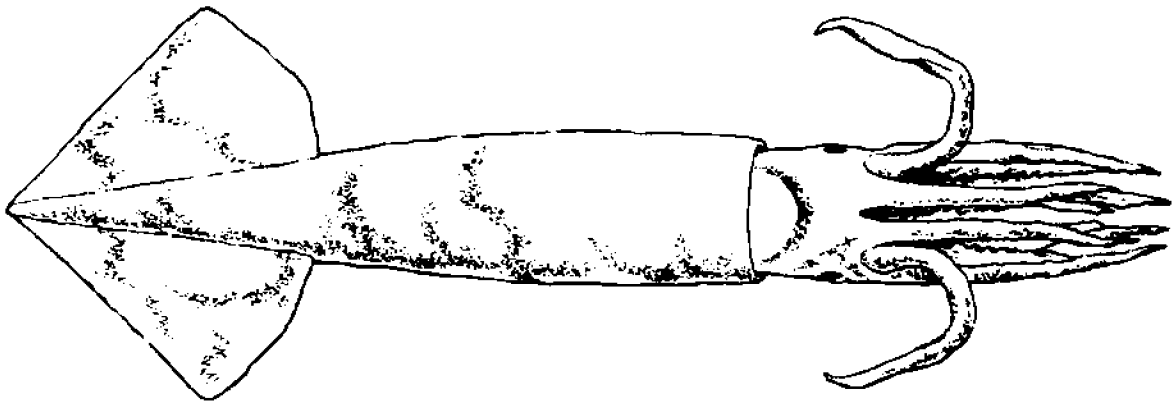

FIGURE 1. The short-finned squid Illex is a candidate for possible development off the Southeast. Resource information is sketchy but some encouraging indications have been noted.

FIGURE 2. Squid offers the processor and consumer some unique preparation opportunities shown above is a Loligo squid which has been prepared in several forms, whole mantles, split mantles, rings and strips. The product in this and other modes offers an almost infinite selection of culinary procedures.

FIGURE 3. The "Jonah" crab Cancer borealis widely distributed and sometimes abundant along the edge of the continental shelf off the Southeastern United States.

FIGURE 4. A selection of experimental crustacea traps. Some of these may be useful in harvesting latent crab resources of the region.

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TABLE 1. An Arbitrary List
Indicating some Species Candidates for further Development

SPECIES		MAJOR PROBLEMS [A]	REFERENCE
Shrimps			
Royal Red	Hymenopenaeus	M	7,9
Scarlet Prawn	Plesiopenaeus	A	9,13
Rock Shrimp	Eusicyonia	R,M	9
Sea Bobs	Xiphopenaeus	M	7,9
Megalops	Penaeopsis	A	7,9,11
Other		--	
Crabs			
Golden	Geryon	H,M	2,12
Red	Geryon		2,26
Jonah	Cancer	A	15,26
Rock	Cancer		15,26
Lithodes	Lithodes	A	26
Galathea	Munida	R,H,P	26
	Eumunida		
Lobsters			
Lobsterettes	Nephrops/Eunephrops	R,H,P	1,10,17
Shovel-Nose	Scyllarus	R,H	26
Squids			
Longfinned	Loligo (2)	R,H	3,13,15,16,23
Shortfinned	Illex (2 or more)	R,H	13,15,16,23
Octopus			
Common	Octopus	P,M	24,25
Fishes			
Butterfish	Peprilus	H,P,M	3,22
Tunas	Thynnus/Euthynnus	R,H	5
Herring Like	Etrumes/Sardinella	R,H,P	19,27 [B]
Menhaden	Brevortia	P,M	19,27
Other			
Seaweed	Gracilaria, other	A	6
Processing Waste	Scallop/crab/fish	P,M	4,19

[A] Major Problems

R = Resource Comprehension
H = Harvest Techniques
P = Processing/Handling
M = Marketing
A = All of the above

[B] Personal communication with staff of SEFC/NMFS laboratory, Pascagoula, MS.

DEVELOPMENT OF A SALTED DRIED PRODUCT FROM SELECTED UNDERUTILIZED FISH FOR INTERNATIONAL MARKETS

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INTRODUCTION

Along the southeastern and Gulf coasts of the U. S., many species of finfish are abundant and easily caught, but because there is little domestic demand for them, they are of low value. Although marketing efforts have increased general seafood consumption in the U. S., the abundance of underutilized fish such as mullet far exceeds present or foreseeable domestic demand.

In developing countries, dried fish products comprise the largest volume of processed seafood consumed because they are stable without refrigeration and can be packaged, stored, and shipped economically. According to Waterman (1976), 20 to 25% of the total world fishery production is processed in dried, salted, smoked, and other cured forms. In 1981 total dried, salted, and smoked fish imports to South American and Asian countries exceeded 70,000 metric tons (FAO, 1983). Capturing even a fraction of these markets would be of tremendous benefit to the U. S. fishing industry. However, top quality products must be developed in order to compete in these markets (Rose, 1983).

In this collaborative study supported by the Georgia Sea Grant Program and the National Marine Fisheries Service, the optimal drying process for each of several species was developed and prototype products

were prepared in a form as similar as possible to products obtained from several potential foreign markets.

The rationale behind this approach was to provide the U. S. seafood industry with the technical information required to produce products for foreign markets which are familiar to local consumers and eliminate the necessity to "promote" the consumption of seafood among people who normally prefer beef, pork, or poultry. The only promotion required would be to demonstrate the quality and quality control of foreign seafood products made with U. S. fish.

In order to modify or change the prototype products to meet consumer preferences in the specific country for which the product is targeted, our prototype products were sent to target countries by NMFS marketing specialists through foreign trade missions and international food shows.

MATERIALS AND METHODS

Striped mullet (Mugil cephalus) and black drum (Pogonias cromis) were obtained from Florida and shipped to the National Marine Fisheries Service's Charleston Laboratory. Striped mullet was butterfly cut and gutted with and without scales. Black drum were split, gutted, and made into different forms: fillet with bone and without bone, fillet with and without scales, and fillet without skin. Menhaden (Brevoortia tyrannus) and thread herring (Opisthonema oglinum) were obtained from a bait dealer in Florida and transported to the pilot plant at The University of Georgia Marine Extension Service, Brunswick Station. Both of them were gutted with heads on. Menhaden was either cooked or uncooked before drying.

Striped mullet and black drum were used to produce heavily salted products which were soaked in a saturated salt solution with 1% sodium erythorbate at 2°C. The ratio of fish to brine solution was 1:2. Total brining time was 5 days. Thread herring and menhaden were soaked in a 10% salt solution for 7 hours. In order to eliminate the strong odor, part of the menhaden was boiled in a 10% salt solution for 30 minutes.

A closed system dryer with controlled temperature, relative humidity, and air velocity was used to dry the fish (Huang and Stephens, 1984).

In order to match our products with traditional products, samples from markets of Singapore and Hong Kong were obtained and used as reference. The product profile consisted of moisture, salt and fat contents, TBA and TMA values, water activity, and rehydrability. All the analyses used the same methods which were described in Huang and Stephens (1984, 1985).

RESULTS AND DISCUSSION

Production of Salted Dried Fish

Results of the brining process showed that five days of brining is necessary to get an equilibrium of salt and water content in the flesh of butterfly-cut mullet (about 2.0 lbs. each) and black drum fillet (about 2.0 lbs. each).

The optimal drying condition for heavily-salted mullet was using 30°C and 20% Rh for the first two hours, then changing to 24°C, 45% Rh for the remaining drying time. Figure 1 shows that it takes 115 hours to produce salted dried mullet having a final moisture content of 30.5% without backbone and 44.0% with backbone.

Figure 2 shows that a two-stage drying method using 35°C, 20% Rh for the first two hours then changing to 30°C, 45% Rh for the remaining drying time to dry the black drum fillet. The fillet without skin required much less drying time than the fillet with scales and backbone.

For producing lightly-salted dried products, small thread herring and menhaden took much less time to lose their moisture contents. Figure 3 shows the drying curve of thread herring at the condition of 24°C and 45% Rh. It took 100 hours to get a final product with a moisture content of 26.5%. Figure 4 shows that cooked menhaden had greater weight loss than that of uncooked fish. After 100 hours of drying time at 24°C and 45% Rh, the former had a final moisture content of 32.5% and the latter had that of 38.3%.

Product Profiles of Prototypes and Commercial Products

The moisture contents of prototype salted dried black drum were higher than that of mullet (Tables 1 and 2). This was due to the thickness of black drum being greater than that of mullet. TBA values of mullet were higher than that of black drum because mullet has a higher fat content. Salted dried black drum from Singapore was similar to our prototype black drum (fillet without bone). However, our prototype had a lower moisture content but higher salt content (Tables 2 and 3). Interestingly, the TBA value of prototype black drum was higher than that of the product from Singapore, while the TMA-N value was much lower.

The only sample obtained from Hong Kong was Kabeljou. The result of analysis shows that moisture content was 40.70%, salt content 18.59%, fat content 2.32%, TBA value 2.99, and TMA-N value 165.47. The species of salted dried fish from Singapore and Hong Kong may not be the same as that found on the coasts of the South Atlantic and the Gulf of Mexico. However, in general, the product profile of our prototypes was similar to that of commercial products (Huang and Stephens, 1984).

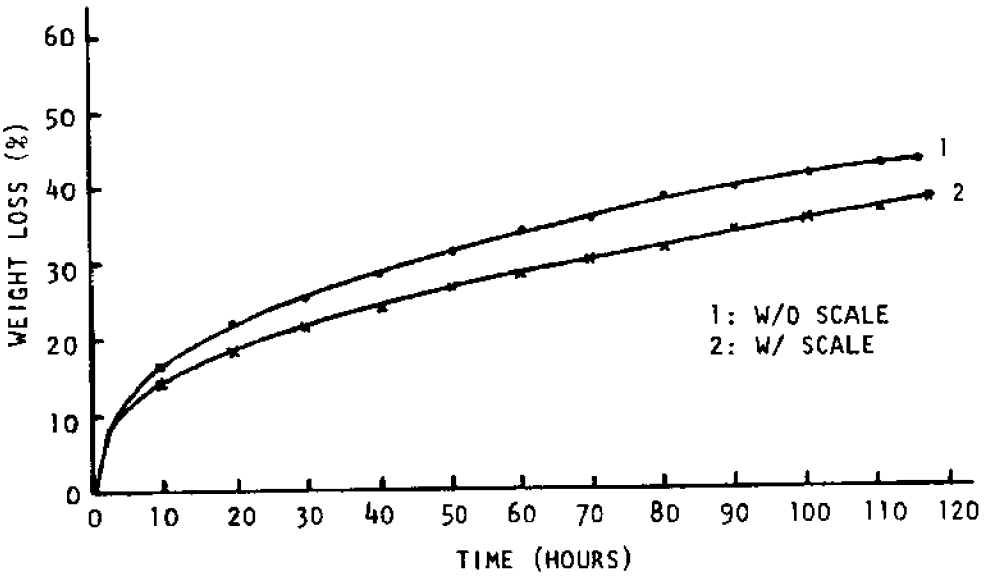

Figure 1. The drying curve of salted mullet

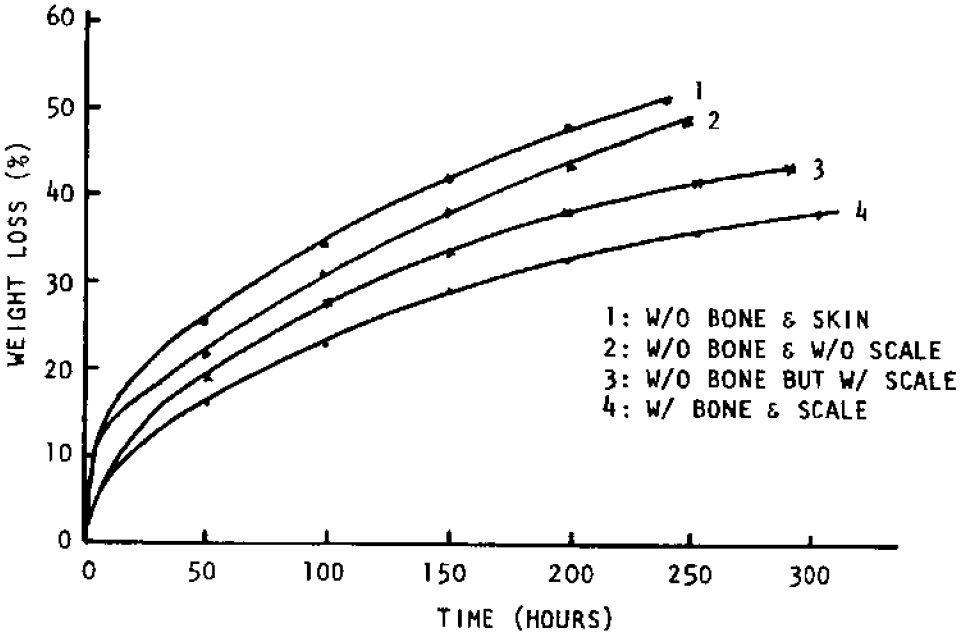

Figure 2. The drying curve of salted black drum

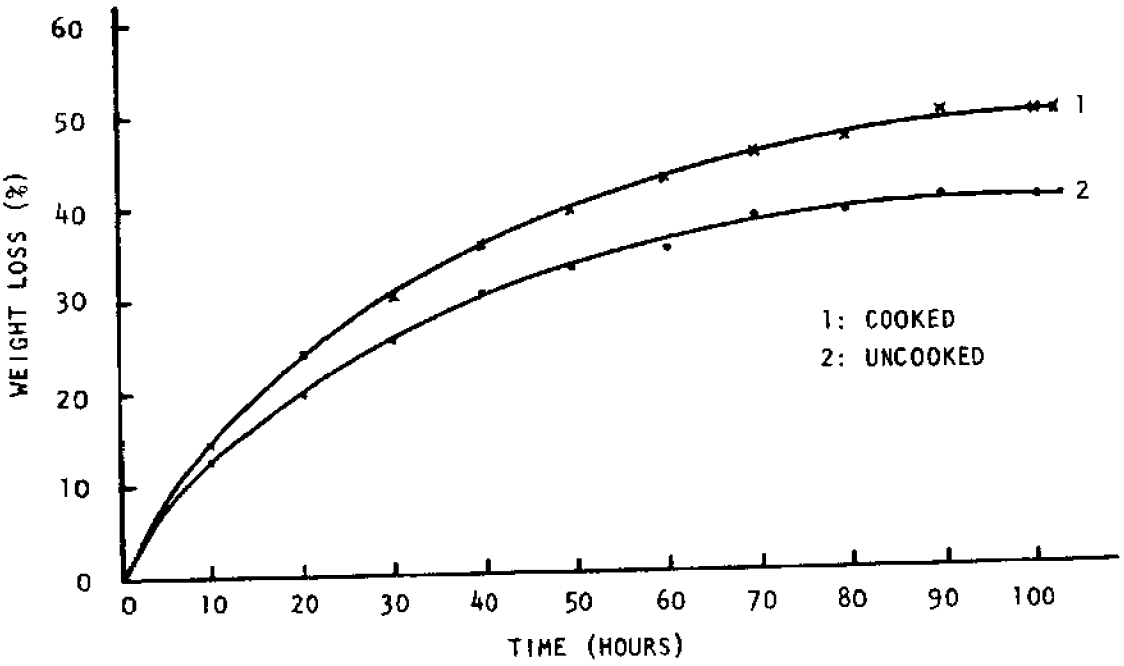

Figure 3. THe drying curve of salted menhaden

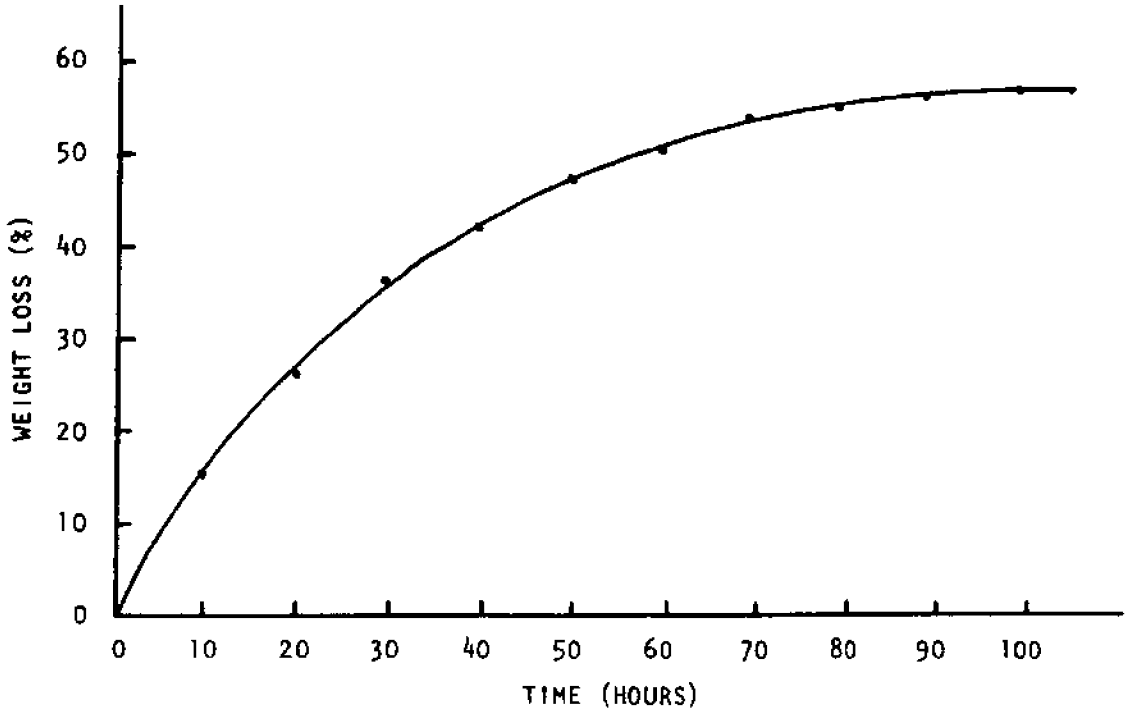

Figure 4. The drying curve of salted thread herring

Table 1. Product profile of prototype salted dried mullet (butterfly cut)

Product Form	Moisture (%)	Salt (%)	TBA (μm MA/100g)	TMA-N (mg/100g)	Water Activity (A_w)	Rehydrability (%)
w/bone	44.04[a]	21.13	73.42	8.41	0.708	131.46
w/o bone	30.53	26.84	133.69	7.92	0.620	149.43

[a] Means of three samples

Table 2. Produce profile of prototype salted dried black drum (fillet)

Product Form	Moisture (%)	Salt (%)	TBA (μm MA/100g)	TMA-N (mg/100g)	Water Activity (A_w)	Rehydrability (%)
w/bone	40.46[a]	21.53	40.59	7.58	0.725	135.96
w/o bone	37.01	24.51	39.00	6.10	0.678	139.57
w/o bone and w/o skin	34.41	33.24	28.47	25.92	0.629	157.37

[a] Means of three samples

Table 3. Product profile of salted dried seafoods from Singapore

Product	Moisture	Salt	Fat	TBA	TMA-N	Water activity	Rehydrability
	(%)	(%)	(%)	(um MA/100g)	(mg/100g)	(A_w)	(%)
Black drum	40.82[a]	18.9	0.16	1.59	155.32	0.683	137.24
Mackerel	46.42[b]	20.8	2.39	32.47	57.61	0.718	142.98
Croaker	46.07[b]	18.3	8.40	10.21	25.71	0.751	143.82
Jack	37.36[b]	19.8	3.61	18.98	32.62	0.735	138.48
Cutlassfish	47.58[b]	19.4	3.04	3.93	58.96	0.755	128.24
Black pomfret	56.71[c]	12.4	5.09	3.15	42.69	0.825	N/A
Squid	17.20[a]	7.2	3.70	11.26	34.33	0.472	217.02
Sea Cucumber	15.60[c]	11.1	0.26	4.45	22.66	0.628	179.98

[a] Means of two samples
[b] Means of three samples
[c] Data of one sample

Cost Estimates of Producing Salted Dried Products

Based on the work that has been done at the NMFS's Charleston Laboratory and The University of Georgia Marine Extension Service, Table 4 shows cost estimates of salt drying roe mullet carcasses. The costs are based on a four-month season for a plant handling 10,000 pounds of round weight fish per day for 20 days per month. The output of the plant would be 3,060 pounds of final product per working day. It is also assumed that the plant would have adequate work-room space and cold-storage facilities available. The costs do not include administrative overhead, sales costs, or profit, and they are broken into two parts - the cutting and salting operation and the drying and packaging operation. The cost per pound of dried mullet output is $0.802 when the cost of raw materials is $0.14 per pound.

When producing salted dried fish from black drum, cost estimates show two major differences in the components of the costs (Table 5). First is the difference in raw product costs. The reasons for this are: (1) the difference in the overall process yield of the salt mullet process; we could get about 41% final product from the carcasses, and in the black drum we could only get about 10.4% final product from the whole fish, and (2) the black drum is a more expensive fish; the mullet carcasses ran $0.07-$0.14/lb. while the black drum was $0.15-$0.25/lb. Second is the labor costs. Because of (1) the higher costs of filleting instead of butterflying, (2) the different shape of the drum which makes cutting more difficult, and (3) the presence of worms which will have to be removed by hand, the cost of labor is, therefore, set at $0.30/lb. These costs are for a plant already in existence, and do not include any of the normal indirect costs: administration, building and indirect costs, sales costs, or a profit margin. As an artifact of using the mullet figure, the plant as envisioned would operate intensively over a short season, 30,000 pounds per day and four months per year. However, since the equipment costs component of $0.10/lb. is small, no great error is introduced by the use of a short season.

Market Testing of Prototype Products

Prototype salted dried thread herring and menhaden were displayed with other seafoods from coasts of the South Atlantic and the Gulf of Mexico in food shows of a trade mission in Taiwan, R.O.C. and Hong Kong, B.B.C. during April, 1983 (Smith and Lacey, 1983). Results show that there was no interest in either of these products in any market form - raw, dried, or smoked - in these two markets. The result led us to concentrate on mullet and black drum.

Salted dried mullet was tested for market acceptability in the 3rd International Food and Drink Exhibition held in London Olympia, England, February 28 - March 4, 1983. Our samples were highly favored by buyers from different countries (Youngberg *et al.*, 1983).

Salted dried black drum and mullet fillets were also tested for market acceptability in San Juan, Puerto Rico, during the 11th Food and

Table 4. Cost estimates of producing salted dried mullet

Item	At $0.07/lb. of whole fish	At $0.14/lb. of whole fish
A. Cutting and Salting		
Raw materials	$0.172	$0.343
Labor	0.157	0.157
Salt and Anti-oxidant	0.083	0.083
Equipment (5 years straight line)	0.016	0.016
Utilities	0.005	0.005
Subtotals	$0.433	$0.604
B. Drying and Packaging		
Equipment	$0.083	$0.083
Utilities	0.021	0.021
Labor	0.039	0.039
Packaging	0.055	0.055
Subtotals	$0.198	$0.198
Totals	$0.631/lb.	$0.802/lb.

Table 5. Cost estimates of producing salted dried black drum

Item	At $0.15/lb. of whole fish	At $0.25/lb. of whole fish
A. Cutting and Salting		
Raw materials	$1.44	$2.40
Labor	0.30	0.30
Salt and Anti-oxidant	0.08	0.08
Equipment	0.02	0.02
Utilities	0.01	0.01
Subtotals	$1.85	$2.81
B. Drying and Packaging		
Equipment	$0.08	$0.08
Utilities	0.02	0.02
Labor	0.06	0.06
Packaging	0.06	0.06
Subtotals	$0.22	$0.22
Totals	$2.07/lb	$3.03/lb.

Equipment Trade Exposition, April 9-11, 1983. Results show that our prototypes are a possible lower priced substitute for bacalao (dry-salted cod) (Antozzi, 1983). Since large drum (10 pounds and up) are the most difficult to market and are, therefore, the best candidates for salt-drying. However, the product form should be a fillet without skin. Mullet, unfortunately, suffers from an image problem in Puerto Rico and was not as highly regarded (Antozzi, 1983). Compared to our prototype salted dried mullet, Venezuelan product has a higher moisture content and the fish head is left on.

A sample of salted dried mullet was also sent to a company in France for evaluation on August 17, 1984. Responses show that prototypes can't substitute for salted dried cod, because mullet has a darker color than that of cod (Antozzi, 1985).

Limited marketing efforts, unfortunately, couldn't send prototype salted dried mullet and black drum to Asian markets such as Hong Kong and Singapore for market testing. Since these prototypes are closer to their products, it might be a higher potential exporting market.

CONCLUSION

Matching the prototype product to the potential market is of primary importance in exporting. However, a major problem in this project is that without foreign consumer studies, which are outside the scope of this Sea Grant - NMFS effort, it is impossible to make the refinements necessary to produce a competitive product.

Underutilized species from coasts of the South Atlantic and the Gulf of Mexico could be processed into a salted dried product for exporting as long as the marketing studies can be well matched.

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THE PROCESSING OF CANNONBALL JELLYFISH (*Stomolophus meleagris*) AND ITS UTILIZATION

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INTRODUCTION

The shrimp industry is one of the most important fisheries in the United States. The Gulf of Mexico and south Atlantic regions play a significant role in the nations shrimp industry, accounting for approximately 70% and 8.6%, respectively (NMFS, 1985). The primary gear used in shrimp fisheries is the otter trawl, a non-selective bottom net that incidentally catches numerous fish and other invertebrates. Except for a few larger fish which are landed and sold by crews of the shrimp boats, the remaining by-catch, which comprises the bulk of the catch, is thrown overboard.

Among the species in the by-catch are cannonball jellyfish or jellyballs, *Stomolophus meleagris*. These are a hindrance at times to shrimp trawling. Large numbers of jellyballs clog and damage nets, increase the sorting time of the catch, and shorten the length of time trawls can be set. Shrimpers are not the only group to have difficulty with jellyfish. During September 1984, a nuclear power plant in Fort Pierce, Florida shut down two of its reactors when billions of jellyballs clogged intake filters leading to the reactor's ocean-fed cooling system (Anonymous, 1984).

Cannonball jellyfish have been reported from southern New England to Venezuela, and occur in large numbers along the coasts from the mouth of the Chesapeake Bay to Texas. They also occur in the eastern Pacific from Panama to San Diego (Mayer, 1910; Kramp, 1961). One swarm observed at Port Aransas, Texas was estimated drifting through the channel at a rate of 2 million per hour (Meinkoth, 1981).

While these large jellyfish are abundant in U. S. waters and are a nuisance to American fishermen, jellyfish are a popular and highly valued item in Chinese and other Asian cookery. China was the first country to produce jellyfish for human consumption, and several countries in Southeast Asia soon followed. World harvest of jellyfish in 1982 was over 116,000 tons and valued over $12.5 million in U. S. currency. Most of the catch was made in the western Pacific and eastern Indian Oceans. About 85% of it was landed in Thailand (SEAFDEC, 1984).

Japan is one of the leading consumers of jellyfish. Because its domestic production remains low, a substantial quantity is imported annually. According to Morikawa (1984), demand for salted dried jellyfish in Japan is as much as 7,000 tons per annum, and about 95% of it is imported.

Taiwan is another significant importing country. In the past four years, the quantity of imported jellyfish products ranged from 1,100 to 1,700 tons (Anonymous, 1985). This demand is met entirely by imports. Singapore and Hong Kong are also significant importing areas.

While there are no statistical reports on the consumption of jellyfish in the United States, demand in domestic markets is entirely met by imports. To date, little attention has been given to producing salted dried jellyfish using local species (Huang, 1985).

The objective of this study was to evaluate the possibility of using cannonball jellyfish as raw materials to produce a salted dried product which is compatible to existing commercial products. The proximate composition of imported commercial products obtained from domestic Oriental markets were also analyzed for comparison.

MATERIALS AND METHODS

Cannonball jellyfish or jellyballs were caught as by-catch from shrimp trawling on the Georgia coast, placed on ice, and delivered to our pilot plant within six hours. The fresh jellyfish were then dressed and cleaned using sea water.

The umbrella and manubrium of jellyfish were separated and treated with different mixtures of salt and alum in a four-phase salting process. In the first phase, 7.5% of salt and 2.5% of alum were used to make a brine solution. The fresh materials were soaked in the solution at a ratio of 1:1 for three days, then transferred to the second brine solution. In the second phase, 12.5% of salt and 1% of alum were used, and the jellyfish were soaked for three additional days. The salted jellyfish were then drained. In the third phase, a saturated brine solution was used to treat the jellyfish for seven days. In the final phase of processing, the salted jellyfish were piled up to 20 cm high, one above the other, and kept for three days. Seven kg of pressure were applied to the top. The finished products were packed in poly bags.

The salt content of jellyfish products was measured by using a Corning pH meter with an Orion chloride electrode (model 94-17B). Proximate compositions of raw and processed jellyfish, including moisture, fat, protein, and ash contents, were determined according to AOAC (1984) methods.

RESULTS AND DISCUSSION

The brining method is a slow process in which salt is drawn up through the body of the jellyfish by gradual diffusion of body fluid through its exterior. In the four-phase salting process, salt contents in the brining solution gradually increased. The weight loss of jellyfish during the second phase of brining was larger than that of other phases (Figure 1). Alum was used as a disinfectant and to maintain the firmness of the tissue. The weight loss of the salted umbrella finally reached 85%, while that of the manubrium was 79%.

Figure 1. The weight loss of cannonball jellyfish during the four phases of the salting process

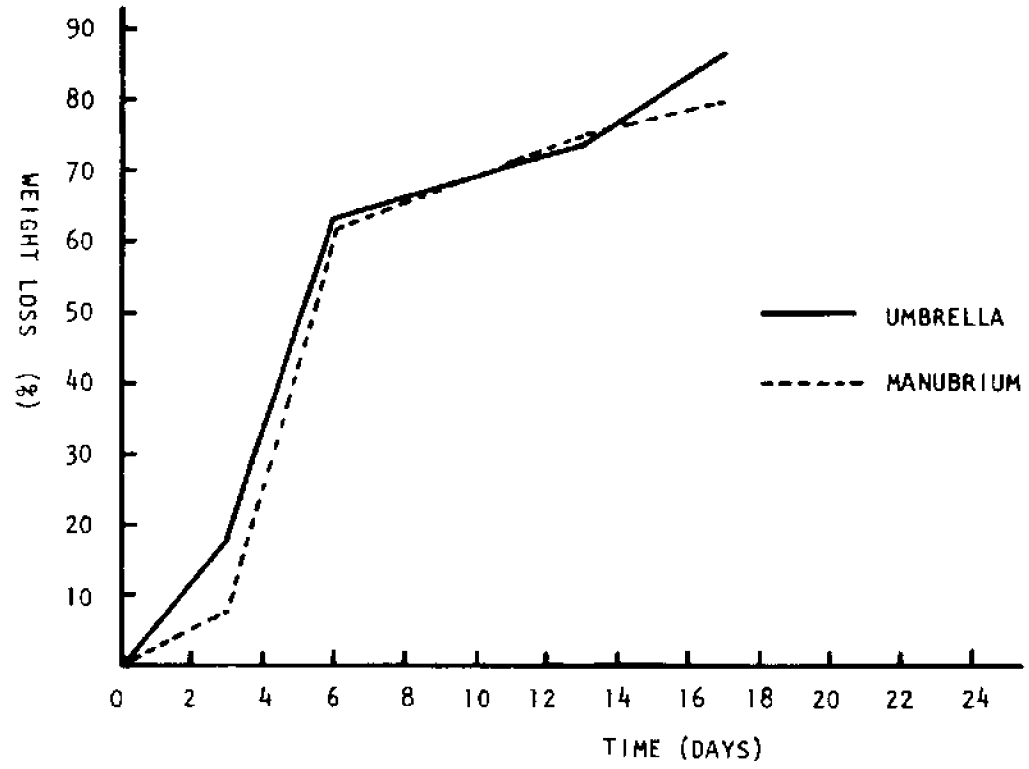

Table 1 shows the proximate composition of cannonball jellyfish before and after processing. The moisture content of processed jellyfish in the umbrella portion was lower than that of the manubrium, while it was the reverse in the raw materials.

Table 1. Proximate composition of fresh and processed cannonball jellyfish

	Moisture %	Protein %	Salt %	Ash %	Aw %
Fresh (umbrella)	96.10[a]	1.07	-	2.30	-
Fresh (manubrium)	95.85	1.02	-	2.81	-
Salted (umbrella)	65.88	5.77	27.00	27.28	0.74
Salted (manubrium)	67.72	3.94	27.29	28.63	0.77

[a] Means of three samples

In comparison to the imported jellyfish (Table 2), results show that the composition of salted dried cannonball jellyfish is very similar to that of commercial products except that the salt contents of our products are higher than that of imported ones.

Table 2. Proximate composition of imported salted jellyfish

	Moisture %	Protein %	Salt %	Ash %	Aw %
Chinese (umbrella)	67.36[a]	6.81	22.18	22.90	0.77
Malaysian (umbrella)	66.98	5.87	25.43	25.76	0.74
Malaysian (manubrium)	68.84	5.12	24.71	24.88	0.75

[a] Means of three samples

The proximate composition of the cannonball jellyfish product is also similar to that of Australian jellyfish (*Catostylus spp.*) in both fresh and processed jellyfish (Davis, 1982). However, the protein content of Australian jellyfish is higher. This may be due to the species difference.

The finished product of the cannonball jellyfish has a bland flavor and a light yellow color. When preparing for a dish, the salted dried jellyfish are soaked in water for several hours, then cut into strips and scaled. The curled strip has a unique texture which has been described as a combination of tender, elastic, and crunchy. The jellyfish curls are usually served as a cold plate in a dressing composed of soy sauce,

vinegar, sugar, and sesame oil. They are also cooked with other meat and vegetabes in Chinese cuisine.

The market value of jellyfish is primarily determined by its texture. However, when only the umbrella portion is purchased, the size of 12 inches or more in diameter is preferred. In general, according to the informal tasting panel, our cannonball jellyfish product was acceptable as food and tasted even better than existing commercial products.

CONCLUSION

Using locally abundant cannonball jellyfish, a shrimp by-catch, to produce salted, dried products which are readily acceptable in domestic ethnic markets and foreign markets could diversify the fisheries of the south Atlantic and Gulf of Mexico. Preliminary results show that cannonball jellyfish can be processed into a commercial product which is competitive to commercial products of species now on the market. However, a time and energy saving processing method needs to be developed to reduce the total processing time; the texture profiles and quality requirements are need to be identified before the new product can become an export item.

ACKNOWLEDGEMENTS

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RECENT PROGRESS IN THE PRODUCTION OF THE SOFT SHELL BLUE CRAB, CALLINECTES SAPIDUS.

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INTRODUCTION

The soft shell blue crab industry currently forms a small but growing part of the total catch and sales of the blue crab, Callinectes sapidus, in the Gulf of Mexico, Atlantic, and Chesapeake Bay regions. Although it is a minor component of the fishery, the price per crab is higher for the soft shell crab than for the hard shell crab (Otwell and Cato, 1982). Moreover, the market for soft shell crabs appears to be far greater than is currently exploited and expansion of both the domestic and foreign markets is possible.

Major problems affecting the growth and viability of the soft crab fishery include the continuing decline in the quality of coastal waters and the limited supply of peeler (premolt, shedding) crabs. Perry et al. (1982) reviewed methods for recognizing, harvesting and shedding peeler crabs and discussed the theory of operation and design for a closed, recirculating seawater system to hold and shed crabs. Recognizing the potential value of and tremendous interest in this fishery, the Sea Grant Programs of Mississippi, Alabama and Louisiana began a multi-agency, multidisciplinary project to establish production levels and operating parameters for closed systems currently in use in the fishery and to investigate design changes to increase filter efficiency and carrying capacity. As a result of the 1982-84 effort, management guidelines for operating closed systems were developed and engineering design changes increased filter efficiency with a subsequent increase in carrying capacity (Manthe et al. 1983, 1984).

The development of these closed, commercial-scale, recirculating seawater systems to hold and shed peeler crabs has allowed for expansion of the industry independent of coastal water quality. Thus, the supply of peeler crabs now becomes the major limiting factor in the growth of the fishery in the Gulf (Perry et al. 1982). Continued expansion of the industry will depend on improved fishing techniques for peelers, the development of a closed, recirculating seawater system to hold intermolt-stage blue crabs until they show visible signs of molting, or the use of techniques to initiate pro-ecdysis hormonally.

The introduction and adaptation of traditional peeler gear (fykes, jimmy pots, bush lines) to states with no directed fishery for premolt crabs has, to date, been largely unsuccessful. Bishop et al. (1983) tested bush lines, peeler pots (jimmy pots), crab fykes (peeler pounds), experimental habitat pots and hard crab pots in South Carolina and concluded that shedding operations would have to pursue a multifarious

approach to obtaining peelers if they were to maintain production. The problem of getting fishermen to try new gear remains unsolved.

To design a facility to hold intermolt blue crabs requires a knowledge of basic physiological data relating metabolic activity to environmental factors and behavioral and physiological information related to blue crabs held in confinement. Ogle et al. (1982), in their review of available literature, noted that while some useful information for design purposes exists, much of the data necessary to develop a system for holding intermolt crabs is lacking.

The third approach to the problem of source of supply, the use of hormones to initiate premolt conditions, may be a more viable alternative. Smith (1973) investigated the effects of ecdysterone and inokosterone on molting in juvenile blue crabs. He noted that ecdysis was a sequential event governed by the above-mentioned hormones. Inokosterone was found to be responsible for the initiation of apolysis and, if injected in low doses, induced a nearly normal molt sequence. Plant-derived ecdysones are now available and the application of these phytoecdysones in a commercially acceptable manner merits investigation.

The molt cycle of all crustaceans is controlled by molting hormones. The hormone that stimulates the molt cycle and induces the entrance into premolt is 20-hydroxyecdysone (ecdysterone). This hormone increases in quantity during the premolt period. Thus, it may be possible to artificially stimulate entrance into premolt in green crabs (in intermolt) by treating them with 20-hydroxyecdysone. Once in premolt, the crab is committed to molt and, by using already established criteria to identify premolt integumental changes, the crab shedder can easily predict how long it would take for the next ecdysis to occur. One potential drawback to such a procedure is a lack of knowledge of the dose of hormone to be used. An overdose will hyperstimulate the molt cycle and eventually kill the crab. An underdose, on the other hand, will have little effect on the crab and be a waste of hormone. The dose employed will depend directly on the length of the time remaining in the intermolt period; that is, how long until premolt. The only way of determining such an interval is to have a means of breaking down the intermolt phase into substages.

In 1985, a project to develop a commercially applicable technique using hormones to initiate pro-ecdysis in intermolt blue crabs was begun. Support was provided by the Mississippi-Alabama Sea Grant Consortium, the University of South Alabama, and the Gulf Coast Research Laboratory. Data presented in this paper provide the initial information necessary for development of a technique to stimulate onset of pro-ecdysis hormonally: the morphological criteria used to divide the intermolt phase into recognizable substages. Preliminary data on dosage and application of the hormone, 20-hydroxyecdysone, are also reported.

MATERIALS AND METHODS

Blue crabs (40-120 mm carapace width) were maintained individually in plastic containers in artificial sea water (7 ppt, 25°C). The artificial sea water consisted of RILA® seasalts dissolved in well water with crushed oyster shell added. The crabs were fed four times per week on fish and beef liver and the water changed daily. Premolt crabs generally did not feed and so feeding was reduced or eliminated after a crab entered premolt. The molt cycle stage was determined 3-4 times per week by viewing the edge of the paddle under a dissecting microscope at 10-30X magnification. Other particular methods are mentioned in the results section.

RESULTS AND DISCUSSION

Stages of the Intermolt Cycle

To determine the stage of the molt cycle, criteria similar to those that have been used to determine the molt cycle stages of many different species of crustaceans (Drach, 1939; Drach, 1944; Drach and Tchernigovtzeff, 1967; Freeman and Bartell, 1975; Mangum, 1985) were employed. These criteria are based on the change in appearance of the epidermis and the overlying cuticle during the molt cycle. In particular, the thickness and lamellar condition of the cuticle was observed. This was done with a dissecting microscope. Since the crabber would not have access to a microscope, or may not be able to afford to buy one, we have designed a reasonably inexpensive, portable, and easy to use "crab stager" which carries out all the functions of the microscope for determining the morphology of the paddle cuticle. The stager consists of a box open on the bottom with a glass plate in the center of the top surface, a light source below, and a 20X magnification pocket microscope which is mounted on a brace, is semimovable, and has a fixed focal distance (Figure 1A). We anticipate that the crab can be held so that the paddle is in position on the glass plate under the microscope and the stage determined quickly. Thus, handling of the crab should be minimal.

The intermolt period is the phase of the molt cycle between the postmolt (A-B) and premolt (D) stages. In the blue crab it begins after the shell has hardened and synthesis of the endocuticle has begun. The postmolt period usually lasts one day, although, in larger crabs, it may last two days (Figure 2). The crab usually passes through the soft and leathery phases of the postmolt period and is in the paper shell condition. The cuticle at this time appears to have a homogeneous structure without lamellae. After the secretion of the endocuticle begins, the lamellae of both the exocuticle and the endocuticle can be clearly defined. In addition, the exocuticle is slightly pigmented while the endocuticle is not. Thus, within two to three days after the molt the two primary sections of the cuticle can be delineated using low magnification microscopy (Figure 1B).

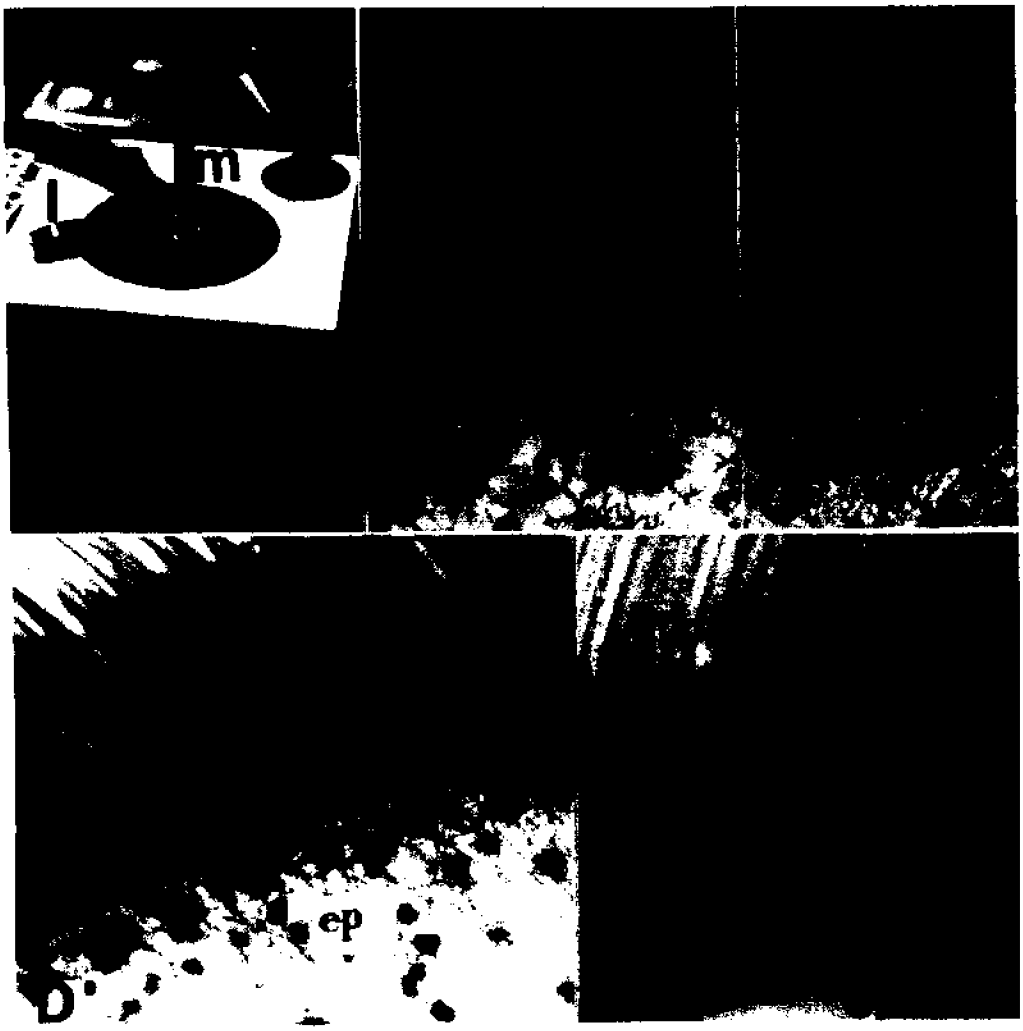

Figure 1. Molt cycle staging device and methods. A. Stager consisting of box (b), hand held microscope (m) and light sources (l). B-E. Appearance of the edge of the paddle of the swimming leg at stages C_1 (B), C_2 (C), C_3 (D), and D_0 (E). Abbreviations: en, endocuticle; ep, epidermis; ex, exocuticle. Magnifications: B-D, 240X; E, 1400X.

INTERMOLT STAGING METHOD

STAGE	CUTICULAR CHARACTERISTICS	DURATION	TIME TO PREMOLT
A-B	Soft	2-3 Hr	
	Leathery	2-3 Hr	24-26 Days
	Paper Shell	4-18 Hr	
C_1	Exocuticle>Endocuticle	3 Days	21-23 Days
C_2	Exocuticle<Endocuticle	4-5 Days	17-20 Days
C_3	Membranous Layer Present	18 Days	2-22 Days
D_0	Apolysis (Hair Line)		
D_1	White Line (6-12 Days to Shedding)		
D_2	Pink Line (3-6 Days to Shedding) 12 Days		
D_3	Red Line (1-3 Days to Shedding)		

Average Total Molt Cycle= 38-40 Days

Average % Increase in CW= 17%

Figure 2. The molt cycle stages, criteria, and durations for the blue crab, Callinectes sapidus.

The first stage of the intermolt period is called C1 and is defined as the condition where the thickness of the exocuticle is greater than the thickness of the endocuticle, as determined by viewing the cuticlar layers at the edge of the paddle (Figure 1B, 2). The endocuticle is growing during this time and so it will be very faint at the beginning of the stage C1 and lamellar, but equal in size to the exocuticle, at the end of the stage. Stage C1 usually lasts three days (Figure 2).

The next stage is stage C2 and is defined as the condition where the endocuticle is thicker than the exocuticle (Figure 1C, 2). The endocuticle is still being secreted and calcified during this period and the appearance of the lamellae is easily seen at a gross level. This phase lasts four to five days and ends when the membranous layer is formed. The membranous layer marks the end of the period of cuticular growth and calcification. Because of the thin and non-lamellar nature of this layer, it appears as a dark line at the innermost limit of the cuticle and separates the lamellar cuticle from the epidermal cells. At this point the crab is said to be in stage C3 (Figure 1D).

Stage C3 is the last stage of the intermolt period in molting crabs. An attempt is being made to further subdivide this stage into early and late phases. Crabs that have entered the terminal molt, such as a mature female, are said to be in stage CT. The duration of stage C3 is highly variable and, although it averages 18 days, may range from 10 to 25 days. This variability was not found to be correlated with the size of the crabs in the laboratory. Since the cuticle is completely formed at this time, we feel that this stage is physiologically distinct and further subdivision may not have any importance for consideration of the action of molting hormone.

The end of the intermolt period is signalled by the beginning of the premolt period. This is defined by the separation of the epidermis from the cuticle, a process called apolysis, and is seen as a very light region between the membranous layer and the epidermal cells (Figure 1E). In our laboratory tests, we have observed apolysis before the "white line" condition, and this may be the "hair line" stage that some crabbers have observed. Shortly after apolysis, the while line stage is seen and the progression of the crab through the white, pink, and red line conditions (Figure 2) follows the schedule previously described by others (Perry et al, 1982).

In the laboratory the mean duration of the molt cycle was approximately 38-40 days with the larger crabs having a slightly longer molt cycle. These data are very close to that obtained by Tagatz (1968) with crabs of various sizes held in the field. Thus, we feel that the data reported here for the lengths of the individual stages of the molt cycle can be used to accurately predict the stage lengths of animals caught in the field. Since laboratory molt cycle times have been considered to be slightly longer in animals held in the laboratory than durations of molt cycles of animals in nature, it is possible that the length of stages C2 and C3 may actually be shorter.

Stimulating the Onset of Premolt

The molting hormone 20-hydroxyecdysone (20-HE) has been shown to be the molt-stimulating hormone in all crustaceans (Kleinholz and Keller 1979). It is present in low quantities during the intermolt period and increases markedly during premolt where it stimulates and coordinates the integumental events leading to the next molt (Soumoff and Skinner 1983). The crustacean hormone, 20-hydroxyecdysone, and an analog, makisterone A, were selected for testing. These hormones have both been found to be present in Callinectes and increase in concentration during the premolt period (Faux et al, 1969). Preliminary tests, carried out by injecting 20-hydroxyecdysone in crabs in stages C2 and C3, demonstrated that premolt was stimulated in over 50% of crabs in stage C3 with doses of 500 ng/crab. Higher doses and other treatment techniques will be tested in the coming year. The success of such low doses, and no mortality, shows that this technique is an economicaly feasible means of initiating the premolt condition. Moreover, some analogs of the molting hormone may actually be less expensive and more effective, thus further decreasing the cost of stimulating apolysis. We have also tested another technique of inducing premolt (forced apolysis, O'Brien and Skinner, 1985) by placing the crabs in ice water for one to two hours. Several attempts with this technique resulted in no apolysis in any of the crabs.

Use of Low Calcium Seawater

In a related study we have begun holding crabs in tanks in artificial seasalts and well water but without crushed oyster shell added. This technique results in a greatly reduced level of calcium in the water. Preliminary results (in press, Northeastern Gulf Science) show that the use of this water retards the calcification process and results in a greater increase in carapace width at ecdysis. With certain restrictions, the use of such seawater should enable the crab shedder to reduce the number of times per night that he must check the tanks for soft crabs, thus making the process less labor intensive. We are continuing to examine the use of this technique in both laboratory and field settings (filters composed of non-calcareous materials) to determine the applicability of the low calcium water to commercial shedding operations.

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TEXTURE OF *Macrobrachium rosenbergii* DURING ICED AND FROZEN STORAGE

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INTRODUCTION

With the extensive research conducted in aquaculture in the past few years, the technology now exists to produce the freshwater prawn, *Macrobrachium rosenbergii*, at maximal rates through improved culture techniques. To accommodate the increased production, markets for this product must be developed. No established widespread market currently exists for *M. rosenbergii* in the United States.

Two marketing strategies have been attempted in order to establish a market for these prawns. The first strategy involves marketing the prawns as "tails" in direct competition with penaeid (marine) shrimp. In taste panel studies comparing *M. rosenbergii* with penaeid shrimp (*Penaeus aztecus*, *P. setiferus* and *P. vannamei*), Ellis and Rowland (unpublished data) found significant differences in flavor and texture; *M. rosenbergii* tended to be less flavorful and generally mushier than the penaeids. As such, they recommended that freshwater prawns should not be marketed as a substitute for penaeid shrimp due to these texture and flavor differences.

A second marketing strategy involves marketing the prawns whole, as a specialty or gourmet item. This marketing stategy may prove unsuccessful since prawns stored whole have a shorter shelf-life (Passey et al., 1983) and a more pronounced loss of overall texture (Papadopoulos and Finne, 1985) than deheaded prawns during iced storage. Passey et al. (1983) reported shelf-life of whole prawns to be 25% shorter than that of deheaded prawns due to elevated microbial counts. In a 10-day iced storage study, Papadopoulos and Finne (1985) found that chill-killed prawns deheaded immediately post-harvest were significantly firmer and springier than prawns stored whole. Conversely, whole prawns were significantly mushier and had a greater degree of adhesion of tissues to the shell upon peeling. During storage, firmness, springiness and hardness of membrane decreased while mushiness and adhesion of tissues increased in both deheaded and whole prawns. These changes, however, were minimal in deheaded prawns, particularly with respect to the mushiness attribute.

The majority of changes that occurred during the 10-day iced storage period occurred within the first 4 days of storage. As there was generally no loss of texture from day 4 to day 10, it is possible

that if prawns were acceptable on day 4 they should also be acceptable on day 10, provided there was no deterioration in flavor (Papadopoulos and Finne, 1985). The next step would therefore be to use a consumer panel to evaluate differences in tail muscle texture during iced storage and to determine consumer preferences for those samples.

An alternative to storing prawns on ice would be to market them in a frozen state. In preliminary studies using freshwater prawns, Miyajima and Cobb (1977) showed that frozen prawns had a shelf-life of 6 months. There was, however, significant loss in organoleptic quality after this period. In studies using various freezing methods, Nip and Moy (1979) found that although prawns stored for one month showed a loss of muscle elasticity, no significant loss of sensory quality was found. Hale and Waters (1981) reported a general decline in acceptability of prawns during 9 months of frozen storage.

If successful marketing of M. rosenbergii is to be achieved, major obstacles such as the mushiness problem must be alleviated or at least minimized. The purposes of this study were first to determine if consumer panelists could detect differences in texture of deheaded and whole prawns that were found to be significantly different in previous iced storage studies (Papadopoulos and Finne, 1985), and second to determine the effects of precooking and deheading on prawn texture during frozen storage using a trained panel.

MATERIALS AND METHODS

Experimental Animals

Two different sources of experimental animals were used for evaluation by the consumer and trained panelists. For the consumer panel, live prawns, M. rosenbergii (15-35 g), were harvested from aquaculture ponds at Mississippi State University, Starkville, Mississippi and quick-killed in an ice slurry. The prawns were divided into two lots, deheaded and whole, and held on ice for 5 days. Four days later, a third lot (controls) was harvested, quick-killed and immediately deheaded. All three lots were then transported to the Texas A&M University Seafood Technology Laboratory for sensory and instrumental analysis.

For the trained panel, live freshly harvested prawns (15-35 g) were obtained from aquaculture ponds at Texas Southmost College, Brownsville, Texas. The prawns were quick-killed in an ice slurry and divided into lots for storage as either whole or deheaded, and precooked or raw samples. For both panels, prawns to be stored deheaded were immediately deheaded with a sterile knife and rinsed carefully to remove any remaining hepatopancreatic tissues thereby eliminating any possible proteolytic activity from hepatopancreatic enzymes. Further

subdivision of the lots by size (large and small) was done to minimize the large variation in texture found in preliminary trials. All prawns were then placed on ice and transported to the Texas A&M University Seafood Technology Laboratory where they were vacuum packaged for frozen storage at -25°C. Samples were taken once a month for 6 months and evaluated by both sensory and instrumental analysis.

Sample Preparation

Frozen prawns were thawed under cold running water. Due to the large variation in size, samples were cooked in boiling tap water according to prawn size and treatment: small deheaded prawns for 3 min; large deheaded and small whole prawns for 4 min; and large whole prawns for 5 1/2 min. Cook-times were determined in preliminary trials and are defined as the times required to reach an internal temperature of 94-98°C as measured by a Leeds and Northrop temperature recorder.

Immediately after cooking, prawns were placed in an ice bath (two parts ice to one part water) for approximately 3 min for rapid cooling. Prawns were then held on ice until evaluation. Whole prawns were deheaded prior to serving.

Sensory Evaluation

Consumer Panel

Fifty-one untrained volunteers evaluated samples from the three treatments for tail muscle firmness and preference. Firmness was evaluated on an 8-point scale (1=extremely soft, 8=extremely hard) while preference was evaluated on a 9-point hedonic scale (1=dislike extremely, 9=like extremely).

Three samples were evaluated by each panelist, with no individual replication of treatments being performed. Samples were presented unpeeled in petri dishes coded with three-digit random numbers. Order of sample presentation was determined by partially balanced random block design.

Trained Panel

A 9-member descriptive attribute panel was selected to evaluate prawn texture. Selection and training were based on the methods of Cross et al. (1978) and Civille and Szczesniak (1973), respectively. Panelists were asked to peel each sample and evaluate it according to the procedures outlined in Figure 1. Shell hardness was evaluated on a scale of 1 (extremely soft) to 6 (extremely hard) while the remaining attributes were evaluated on a scale of 1 (not adhesive, springy, mushy or gummy; extremely soft or dry) to 8 (extremely adhesive, hard, springy, moist, firm, mushy, gummy). The 6-point scale was preferred

over the 8-point scale for use in evaluating shell hardness as a smaller scale with fewer categories from which to choose proved to be easier for the panelists to use. The 8-point scale was selected for use in evaluating the remaining attributes as more categories were needed to properly evaluate the intensity of each attribute. Texture attributes, intensity scales, definitions and methods of evaluation were determined by the panelists during the first two training sessions.

Eight prawns were evaluated in each session, with four replicates per treatment. Prawns were presented individually in shallow plastic cups coded with three-digit random numbers. Order of sample presentation was determined by a balanced random block design.

Testing for both sensory panels was conducted in the Meats and Muscle Biology Sensory Testing Facility at Texas A&M University.

Instrumental Analysis

Shear force was measured using an Instron Universal Testing Machine Model 1122 equipped with a 10-blade Kramer shear compression cell. Peeled samples were placed laterally in the compression cell with the sample oriented parallel to the blades so as to maximize surface area to be sheared. Full load, crosshead speed and chart speed were 500 N, 50 mm/min and 100 mm/min, respectively. Samples were evaluated in quadruplicate.

Data Analysis

Texture panel data were analyzed by analysis of variance while the Instron data were analyzed by analysis of covariance and least squares means analysis using sample weight as the covariate (SAS Institute, 1982).

RESULTS AND DISCUSSION

Iced Storage

Sensory Evaluation

As can be seen in Table 1, consumer panelists were able to differentiate ($P<0.0001$) between fresh prawns (deheaded, 1 day on ice) and those which had been held on ice for 5 days, both deheaded and whole. Deheaded prawns were significantly firmer than whole prawns, and fresh prawns were significantly firmer than those which had been stored on ice for 5 days. In the 5-day post-harvest samples, deheaded prawns were significantly firmer than whole prawns.

Although deheaded fresh prawns were firmer than deheaded 5-day old prawns, panelists slightly preferred the latter over the former (Table 1); difference in preference between the two however were not significant. The most common comment by the panelists on texture was that the fresh prawn was too tough. The differences in preference between deheaded and whole prawns was significant with panelists preferring deheaded prawns over whole prawns.

Instrumental Analysis

Storage time and heading had significant effects on shear force. As can be seen in Table 1, greater force ($P<0.05$) was required to shear the tail muscles of fresh as compared to 5-day post-harvest prawns, and to shear deheaded as compared to whole prawns. These results are consistent with results of sensory evaluation and of instrumental analysis of prawns presented to the trained panel previously described by Papadopoulos and Finne (1985). Scores for prawns presented to the consumer panel however were consistently higher than those for the trained panel. Data from both the trained and consumer panels showed the same general trend of texture loss with time on ice.

Frozen Storage

Sensory Evaluation

Unlike during iced storage (Papadopoulos and Finne, 1985), storage time had only a minimal effect on prawn texture through six months of frozen storage (Figures 2-9). Only hardness of membrane and adhesion of tissues to the shell changed significantly during storage: hardness of membrane decreased ($P<0.034$), especially in whole prawns while adhesion of tissues increased ($P<0.016$), especially in deheaded raw prawns. Scores remained relatively constant during storage in the remaining characteristics, particularly in the raw deheaded samples. Whole prawns, both precooked and raw, exhibited the greatest amount of variability during storage with raw prawns exhibiting more variability than precooked prawns. Texture was somewhat more variable in precooked deheaded prawns than in raw deheaded prawns.

Since the effect of storage time was minimal, sample means of precooked and raw prawns, as well as for deheaded and whole prawns were pooled to determine the effects of deheading and precooking, respectively (Table 2). In precooked and raw prawns, deheaded prawns were significantly firmer, springier, more moist and had a harder external membrane. Conversely, whole prawns were significantly mushier, gummier and had a greater degree of adhesion of tissues to the shell than deheaded prawns.

The effect of precooking was not as apparent as the effect of heading (Table 2). Raw prawns were only slightly firmer and springier

than precooked prawns. Precooked prawns were slightly more moist but also mushier and gummier than raw prawns. While the degree of adhesion of tissues to the shell was significantly greater in raw than in deheaded prawns, the membrane surrounding the tail muscle was slightly harder to penetrate in precooked than in raw samples.

As was the case in the iced storage study (Papadopoulos and Finne, 1985), prawn size had no significant effect on the various texture attributes, with the exception of shell hardness (Table 2). Again small prawns generally had softer shells than large prawns. This difference was due to the increased frequency of molting in smaller prawns than in the larger prawns.

Shell hardness was evaluated primarily to determine the relationship between shell hardness (stage of molt) and prawn texture, the premise being that a harder shell could minimize bruising of the tail muscle during harvesting and processing. Tissue bruising results in loss of overall texture and increased tissue breakdown, primarily through microbial or enzymatic processes. Furthermore, there are certain enzymes associated with molting that could perhaps have an effect on tail muscle texture. According to statistical analysis, there was a positive correlation between shell hardness and tail muscle firmness. Although this correlation was significant ($P<0.0001$), it was not very strong ($r=0.11$). Shell hardness ($P<0.005$, $r=0.10$) and springiness ($P<0.016$, $r=0.08$) were also significantly correlated to hardness of membrane but these correlations were also not very strong. It therefore appears that shell hardness, or stage of molt, does have an effect on tail muscle texture as perceived through sensory evaluation, but that this effect is minimal.

Instrumental Analysis

There was no apparent pattern of textural changes during storage as measured by the Kramer shear compression cell (Figure 10). Since no significant increases or decreases in shear force occurred during the six months of frozen storage ($P<0.263$), measurements were pooled over time. The overall effect of heading was not significant ($P<0.327$) with deheaded prawns having slightly greater shear force than whole prawns (199.8 N and 194.3 N for deheaded and whole prawns, respectively). Shear force was significantly greater ($P<0.0086$) in precooked than in raw prawns: 203.6 N and 190.4 N for precooked and raw prawns, respectively.

Data from instrumental analysis are somewhat contradictory to sensory data. Both means of analyses showed the same general trend of loss of texture during storage but the effect of deheading was more pronouced than that of precooking as measured by sensory analysis; the converse was true as measured by instrumental analysis.

CONCLUSIONS

Consumer panelists were able to significantly differentiate between fresh (deheaded, 1 day post-harvest) and 5 days post-harvest samples, both deheaded and whole. These sensory observations were consistent with results of instrumental analysis. Although there was no significant difference in preference between fresh and 5-day deheaded samples, panelists actually preferred the latter over the former. Panelists significantly preferred deheaded prawns over prawns stored whole.

Since prawns were still acceptable on day 5 and only minimal changes occurred in the various texture parameters from day 4 to day 10 during a 10-day iced storage period using a trained sensory panel, sensory shelf-life of ice stored prawns should be extended past the 3-4 day maximum previously reported in the literature. To produce the most desirable texture, however, prawns should be deheaded soon after chill-killing. Furthermore, excessive bruising and mishandling should be avoided during harvesting, processing and storage.

Texture of frozen prawns was generally stable through six months of frozen storage. Heading had the greatest effect on prawn texture: deheaded prawns were significantly firmer, springier, more moist and had a harder external membrane. Conversely, whole prawns were significantly mushier, gummier and had a greater degree of adhesion of tissues to the shell than deheaded prawns.

Precooking the samples had a lesser effect on prawn texture with only springiness, mushiness and adhesion of tissues to the shell showing significant differences. Prawns stored raw were significantly springier and less mushy but also had a greater amount of adhesion of tissues to the shell than those stored precooked.

It must be stressed that these studies were conducted under ideal conditions with prawns chill-killed immediately post-harvest. Internal temperatures were quickly dropped and maintained with adequate icing (2 parts ice to 1 part prawns). At no time were the prawns subjected to temperature abuse. Good harvesting and processing procedures should be practiced to achieve the most desirable product.

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Table 1. Mean values for firmness, preference and shear force of ice-stored prawns as evaluated by the consumer panel.

Treatment	Texture Attributes		Kramer Shear Force (N)
	Firmness**	Preference***	
Deheaded, 1 day on ice	6.88[a]	6.77[a]	362.21[a]
Deheaded, 5 days on ice	5.44[b]	7.06[a]	280.22[b]
Whole, 5 days on ice	4.96[c]	6.10[b]	182.48[c]

* Means within each column having the same letter are not significantly different ($P>0.05$).

** Firmness was evaluated on a scale of 1 (extremely soft) to 8 (extremely firm).

*** Preference was evaluated on a scale of 1 (dislike extremely) to 9 (like extremely).

Table 2. Mean sensory scores for frozen prawns as evaluated by trained panelists in which: 1) precooked and raw samples, both large and small, were pooled in order to determine the effect of heading on prawn texture; 2) deheaded and whole prawns, both large and small, were pooled to determine the effect of precooking on prawn texture; 3) precooked and raw, and deheaded and whole samples were pooled to determine the effect of prawn size on texture; 4) large and small samples were pooled to determine the additive effects of heading and precooking on prawn texture.

	Texture Attributes							
	Firmness	Springiness	Hardness of membrane	Adhesion of Tissue	Moistness	Mushiness	Gumminess	Shell Hardness
I.								
deheaded	5.27	4.57	5.16	2.45	4.59	2.33	2.49	3.38
whole	4.75	3.83	4.24	3.16	4.37	3.22	2.73	3.63
p-value	0.0001	0.0001	0.0001	0.0001	0.017	0.0001	0.0009	0.0001
II.								
precooked	4.96	4.06	4.78	2.37	4.52	2.88	2.63	3.56
raw	5.06	4.34	4.63	3.24	4.44	2.67	2.59	3.42
p-value	0.24	0.0056	0.13	0.0001	0.31	0.049	0.58	0.049
III.								
large	5.02	4.22	4.73	2.80	4.42	2.83	2.65	3.90
small	5.00	4.19	4.67	2.81	4.54	2.72	2.57	3.07
p-value	0.85	0.83	0.56	0.90	0.17	0.24	0.29	0.0001
IV.								
precooked, deheaded	5.06	4.37	5.04	2.10	4.78	2.60	2.50	3.53
precooked, whole	4.85	3.75	4.52	2.64	4.27	3.15	2.75	3.41
raw, deheaded	5.48	4.78	5.29	2.80	4.40	2.07	2.48	3.16
raw, whole	4.65	3.91	3.97	3.69	4.47	3.28	2.70	3.68
p-value	0.0024	0.21	0.0001	0.065	0.0011	0.0015	0.89	0.0011

PRAWN TEXTURE PANEL

Turn the prawn laterally, with the tail turned either to the left or the right, and bite into the dorsal aspect of the first three segments. Evaluate the prawns for:

CHARACTERISTIC	DEFINITION	STAGE OF MASTICATION
Shell Hardness*	Shell hardness; resistance to breakage	Press empty shell between thumb and index finger
Adhesion of tissues*	Degree to which tissues adhere to the shell	Amount of tissues remaining in shell after peeling
Hardness of membrane	Force required to penetrate the external membrance surrounding the tail muscle tissues	Taken at the first bite, using the front teeth
Springiness	Rate at which the deformed tissues regain original shape; bounce	Taken at the first bite (front teeth) and the first chew (molars)
Mushiness	Combination of adhesiveness and fracturability; mealy, lack of consistency	Taken from the first chew (molars) to just prior to swallowing
Firmness	Force required to rupture the prawn tissues	Taken from the first chew (molars) to just prior to swallowing
Gumminess	Degree to which the tissues adhere to the teeth	Taken from the second chew (molars) to just prior to swallowing
Moistness	Amount of moisture present in the prawn tissues	Taken from the first bite (front teech) to just prior to swallowing

*Evaluate before first bite

Figure 1. Procedures used to evaluate prawn texture.

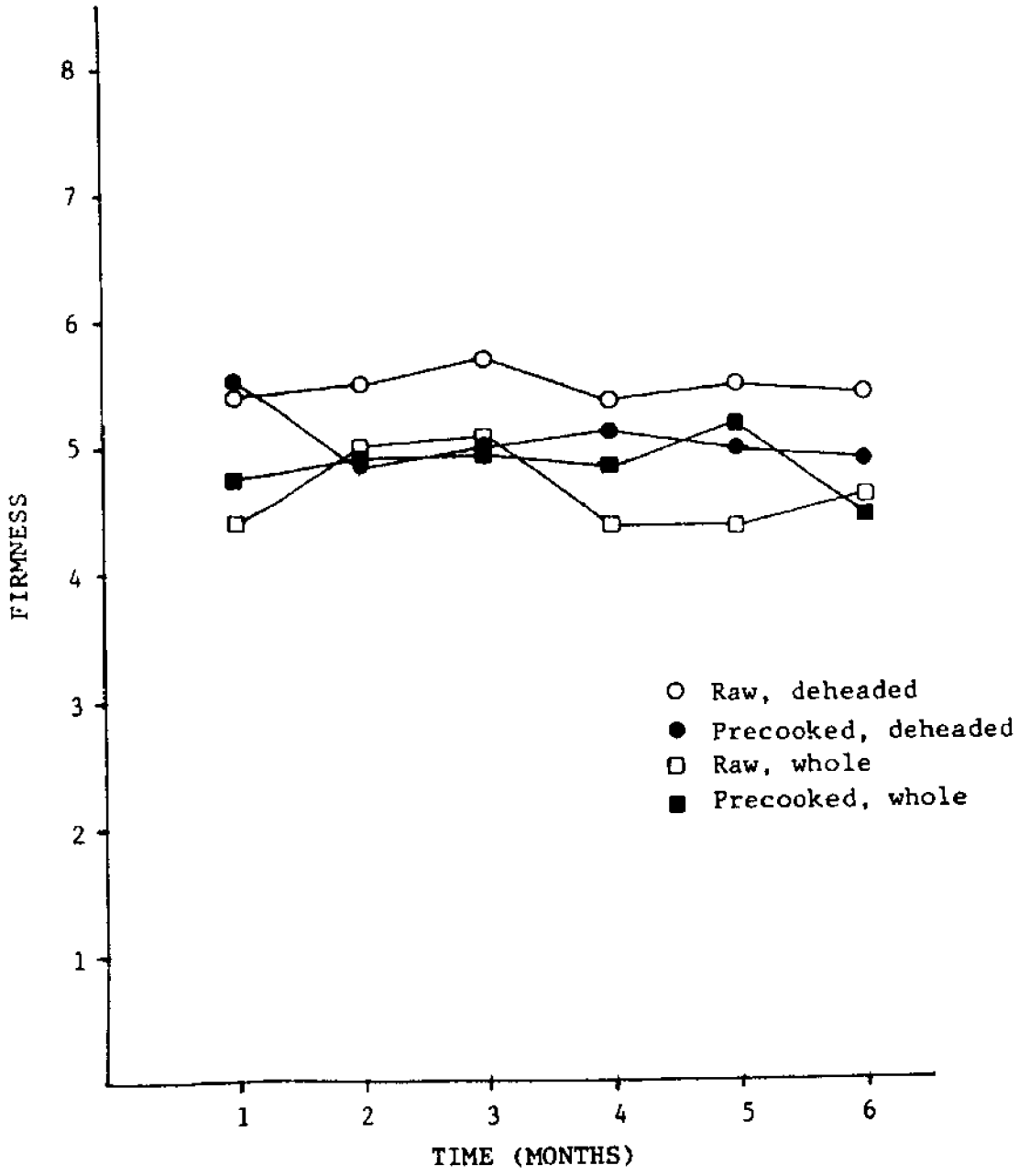

Figure 2. Tail muscle firmness in freshwater prawns during frozen storage.

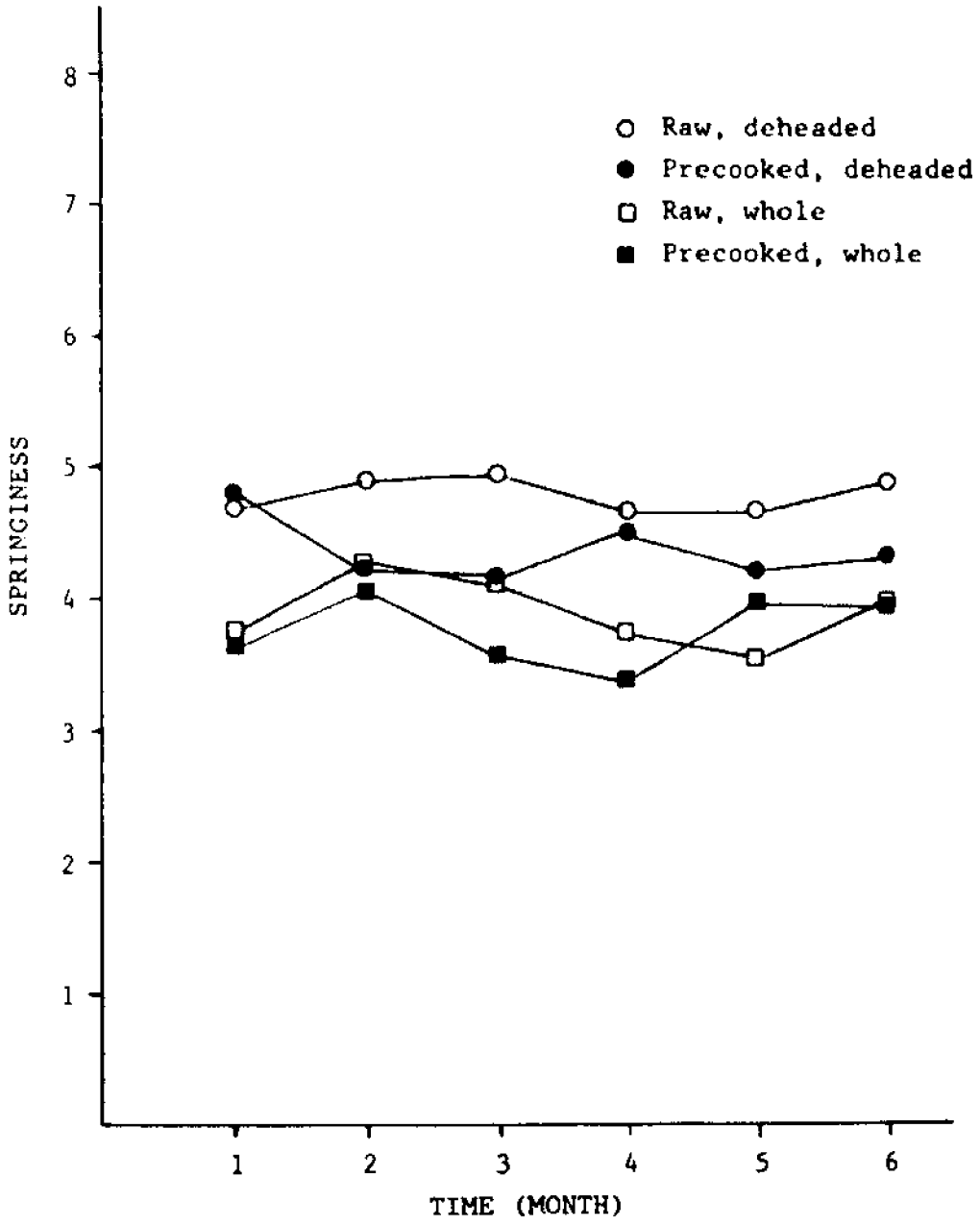

Figure 3. Tail muscle springiness in freshwater prawns during frozen storage.

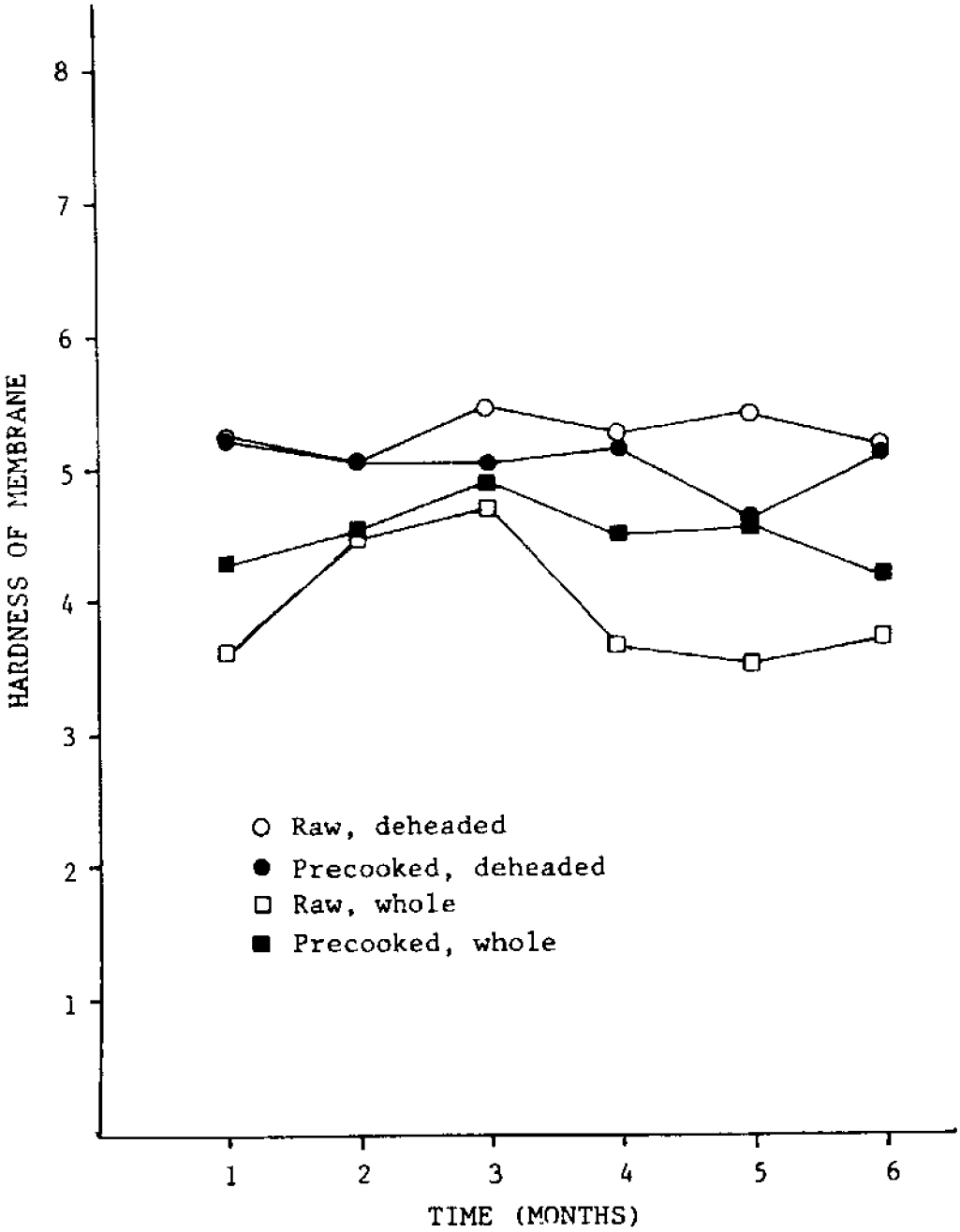

Figure 4. Hardness of membrane in prawn tail muscle during frozen storage.

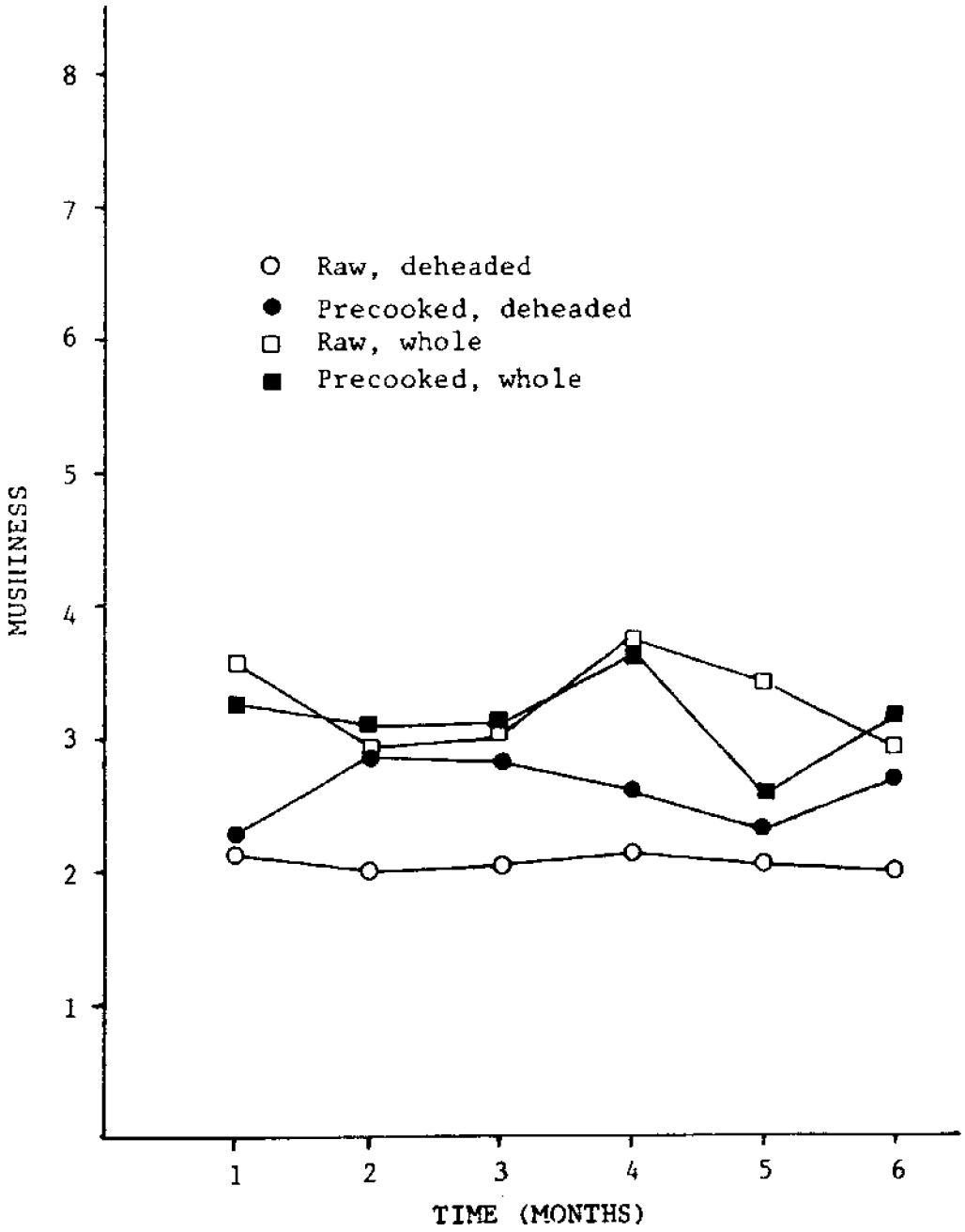

Figure 5. Mushiness in prawn tail muscle during frozen storage.

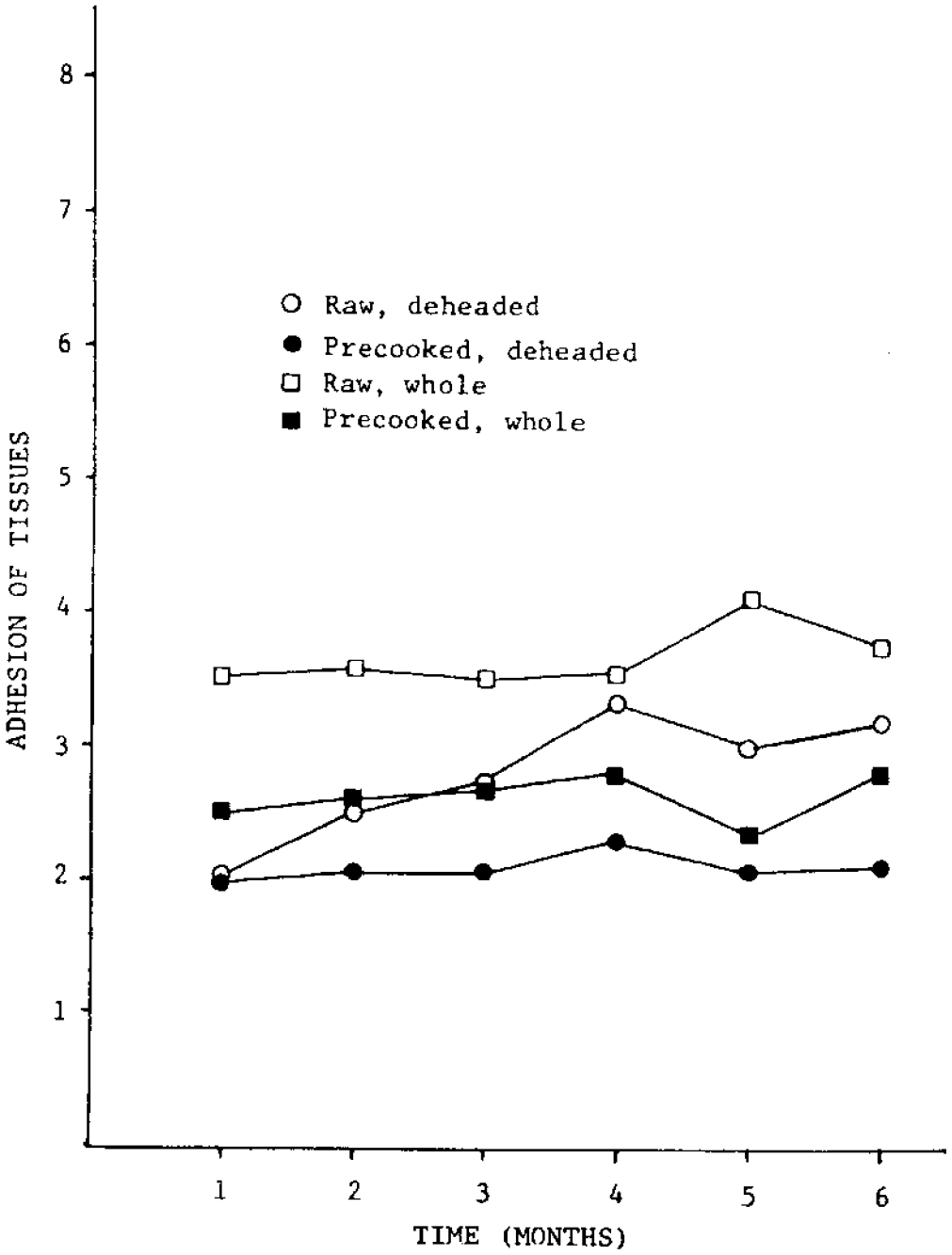

Figure 6. Adhesion of tissues in prawns tail muscle during frozen storage.

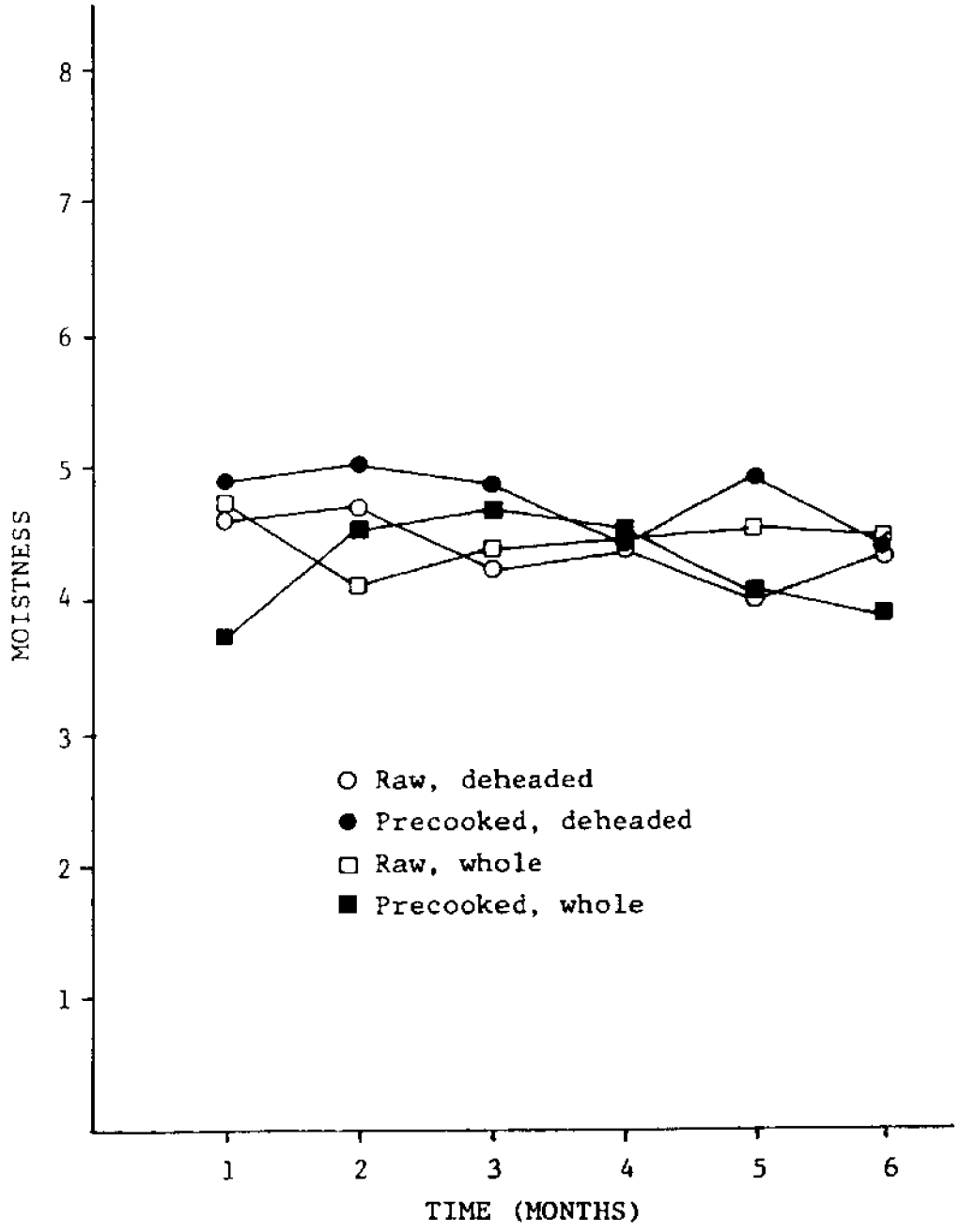

Figure 7. Moistness in prawn tail muscle during frozen storage.

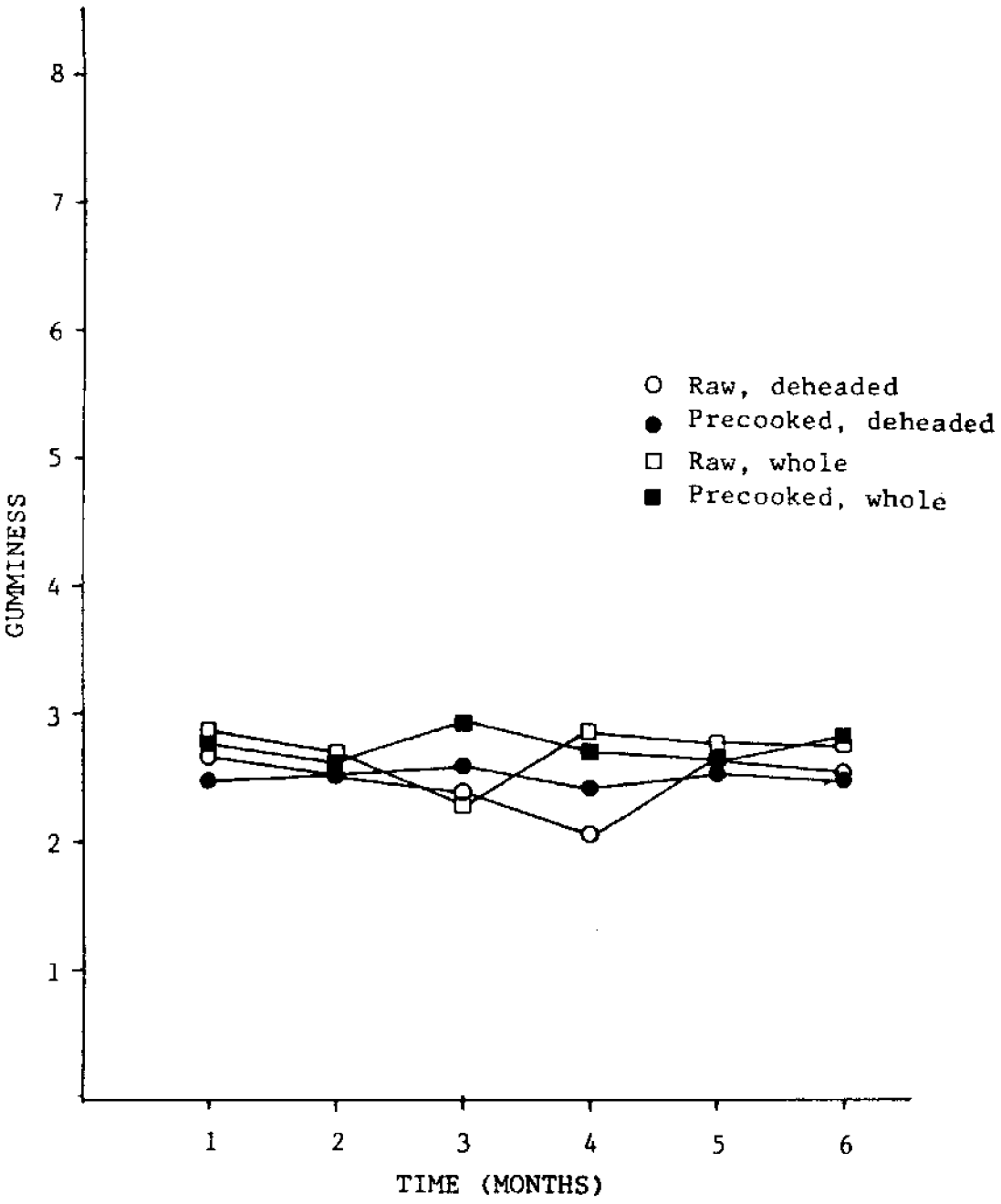

Figure 8. Gumminess in prawn tail muscle during frozen storage.

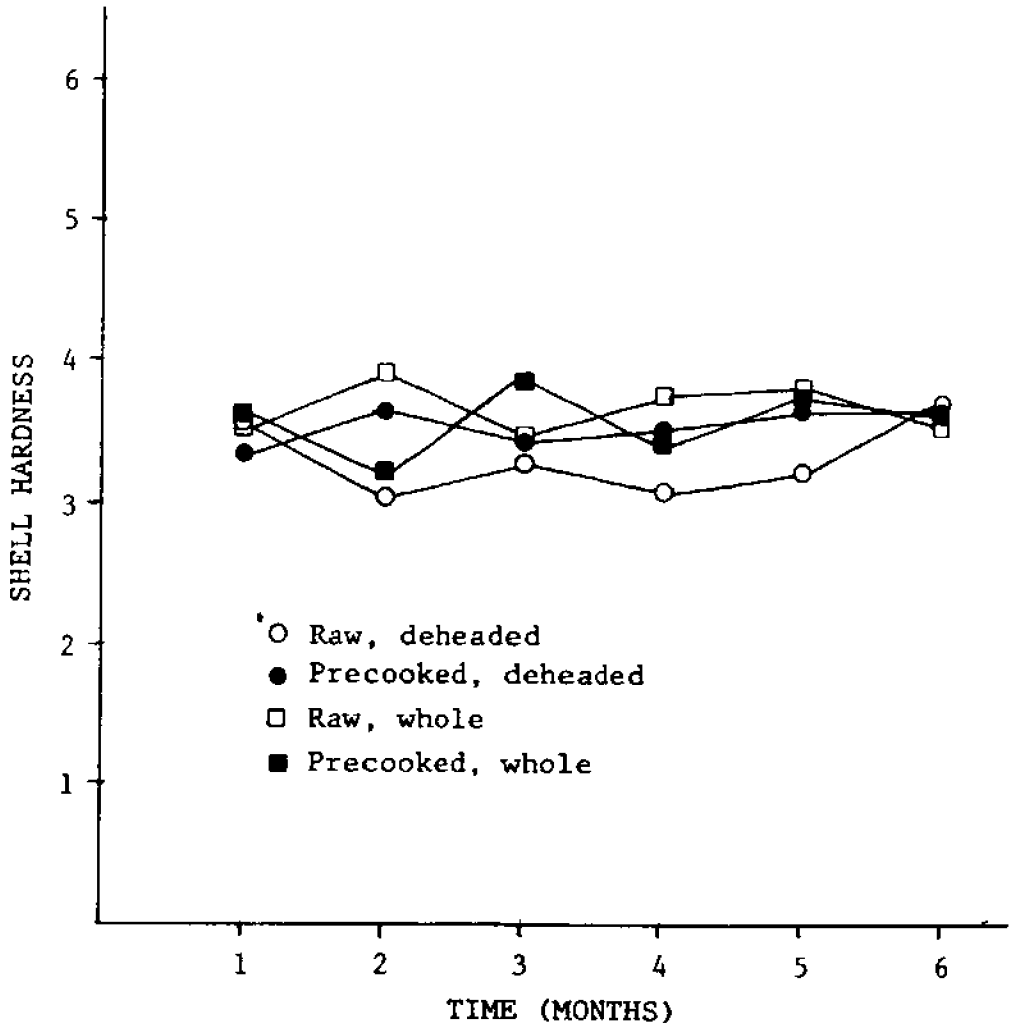

Figure 9. Mean shell hardness of freshwater prawns during frozen storage.

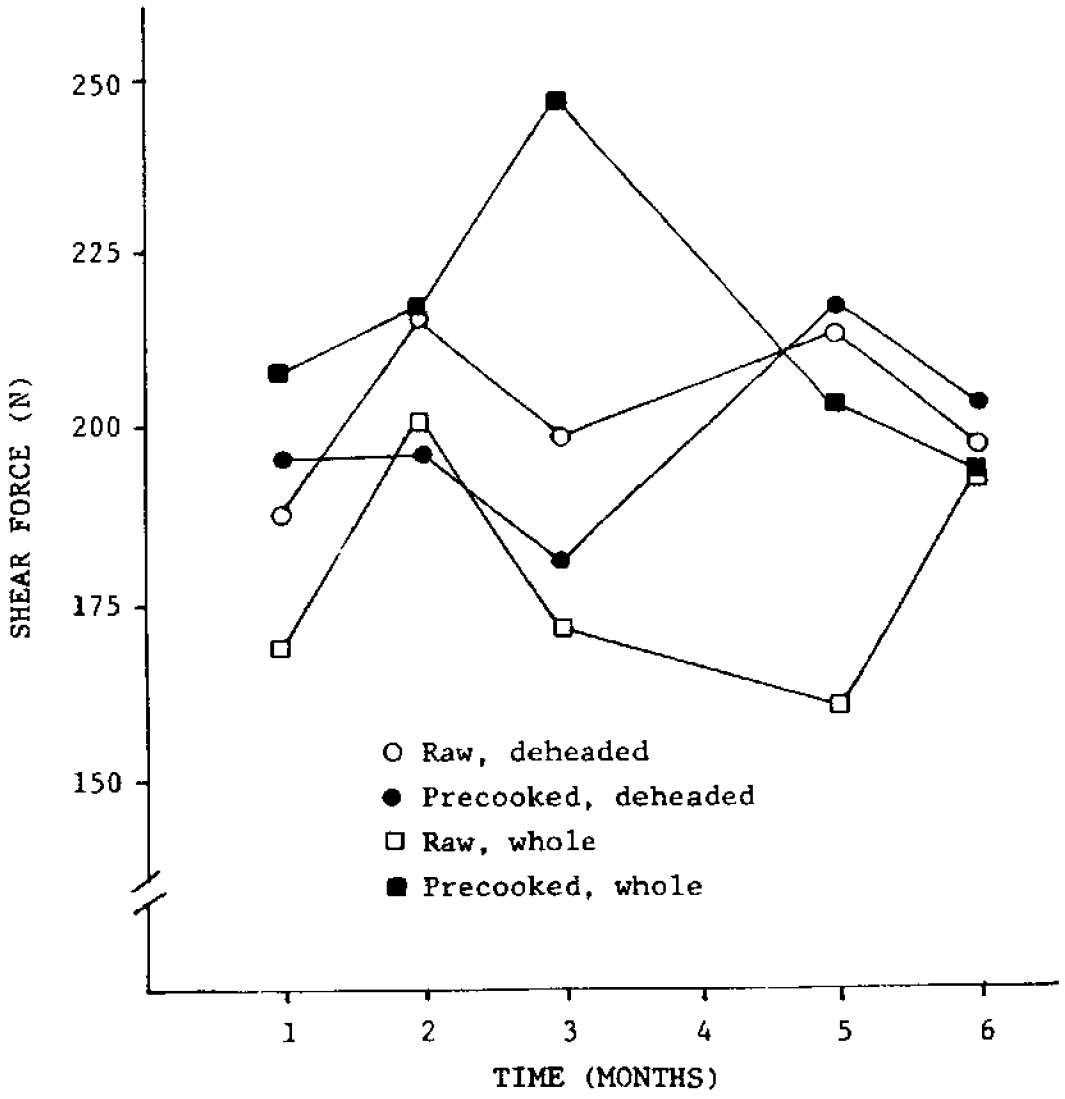

Figure 10. Mean shear force in prawn tail muscle during frozen storage.

A PROCEDURE TO MAINTAIN QUALITY OF STONE CRAB (MENIPPE MERCENARIA) CLAWS ICED PRIOR TO COOKING

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The fishery for the Florida stone crab, Menippe mercenaria (Say), is based on removal of legal-size claws from living crabs which must then be returned immediately to the water (Florida Statute 370.13c). This unique management practice permits some surviving crabs to continue contributing to the spawning stock and, by regeneration, to contribute additional harvestable claws to fishery landings. Florida Department of Natural Resources (FDNR) studies indicate this release practice may increase total harvest by about 10% (Savage et al., 1975).

The Florida Seafood Quality Control Code requires that fresh seafood be iced or refrigerated while onboard a vessel [Chapter 16N-27.22 (5), Florida Administrative Code]. However, unpublished experiments by Waters and Haines (manuscript) confirm reports by fishermen and processors that icing freshly harvested (raw) claws causes the meat to stick firmly to the inside of the shell after cooking, substantially reducing product value. Most fishermen circumvent the icing requirement and maximize fishing efficiency by placing crabs in boxes upon capture and retaining them for up to 8 hours while remaining traps are pulled. Claws are usually removed from crabs while vessels are enroute to port, often within 1-2 h before landing (Figure 1), and

Figure 1. Declawing stone crabs aboard a commercial vessel. Boxes on deck contain whole crabs from previously pulled traps.

then cooked immediately after docking. The Gulf of Mexico Fishery Management Council (1981) concurred with industry spokesmen that keeping stone crabs aboard vessels prior to declawing maintains product quality. Although this procedure eliminates the need to ice claws (Waters and Haines, manuscript), FDNR studies have shown that prolonged desiccation during holding causes stress and increases crab mortality (Schlieder, 1980; Simonson and Hochberg, manuscript). Exposure has also been demonstrated to cause mortality among Florida spiny lobsters similarly maintained in holding boxes (Lyons and Kennedy, 1981). Additionally, Schlieder (1980) demonstrated that even a relatively short 2 h exposure period reduces hatching success of egg-bearing stone crabs due to desiccation.

The effect on product quality of chilling claws prior to cooking has not been previously documented in published literature, so present experiments were initiated in 1981 to reaffirm the relationship between icing and claw quality and to assess a technique which could allow immediate claw removal and crab release, reducing crab mortality.

MATERIALS AND METHODS

Both experiments tested for differences in taste, texture and sticking. Claws of stone crabs trapped during 1981-1983 in Tampa Bay, Florida, were removed after capture and allotted equally to control or experimental categories. Claws of the control half were removed within 2 h of cooking and kept without ice. Claws in the experimental half of each sample were iced immediately aboard ship; some claws were iced only, whereas others were first iced and then freezer-cooled for 6 h before cooking. Cooking entailed placing claws in boiling tap water, allowing the water to return to a boil (5-10 min), cooking an additional 3 min and refrigerating overnight, in simulation of present fishery practice. Waters and Haines (manuscript) reported that commercial processors cook claws in 500 lb. lots for 7 min after water returns to a boil, but claws in this study were fully cooked 3 min after boiling recommenced. Differences may be related to quantities of claws cooked. All claws were marked with randomly-chosen numbers for identification during testing.

A second experiment tested the effectiveness of a procedure to allow icing of raw claws without causing the meat to stick. All claws were removed and controls were treated as in the first experiment. Experimental claws were iced 6 h, cooked as above, rapidly cooled in icewater, drained, double-wrapped in plastic bags, placed in the freezer compartment of a standard refrigerator for 8, 10, or 16 h, and then allowed to thaw at room temperature. Claws were marked as above and reheated in boiling water or served without reheating for nine tests conducted as follows: 8 h freezing vs. controls; 10 h freezing vs. controls; 16 h freezing vs. controls.

Volunteers from the St. Petersburg FDNR Bureau of Marine Research were each given two claws to rate during each test. Claws were rated in

various combinations involving control/control, control/experimental, or experimental/experimental; combinations were not revealed to raters. Ratings were based on three categories for each claw, each category having four conditions. Taste and texture categories were each judged subjectively as excellent, good, fair, or poor. The sticking category (meat fibers sticking inside shell) was recorded as none, light (a few fibers), moderate (less than 1/3 of fibers in contact with shell), or heavy (more than 1/3 of fibers, extremely difficult to extract meat). Results were analyzed with a goodness of fit chi-square for multiple data categories (Zar 1974) with 3 d.f. at $P<0.05$. Paired values (observed = experimental, expected = control) for each of the four conditions were compared within each of the three testing categories. Data were analyzed such that each of the four rating conditions was weighted independently. For graphic purposes only, rating conditions were combined into a "good" condition for both figures as follows: excellent + good + fair for taste and texture categories; none + light for the sticking category.

RESULTS

In the first experiment, claws iced 6 h prior to cooking differed significantly from controls (N=29 claw pairs) in both texture ($x^2=17.75$*) and sticking ($x^2=113.29$*), but not in taste ($x^2=5.11$ n.s.). Claws freezer-cooled for 6 h prior to cooking differed significantly from controls (N=22 claw pairs) in taste ($x^2=10.70$*), texture ($x^2=19.51$*) and sticking ($x^2=113.01$*) (Tables 1, 2; Figure 2A).

In the second experiment (claws iced, cooked, and then freezer-cooled), meat fibers of experimental claws still stuck to shells significantly more after freezing for 8 h than did meat fibers of controls in either test. However, sticking of fibers among experimental claws frozen 10 or 16 h was not significantly different than that of controls. Texture of experimental claws was judged inferior to that of controls in one of two tests of claws frozen 8 h and in one of five tests of claws freezer-cooled 16 h, but no differences between experimental claws and controls were noted in two tests of claws frozen 10 h. Taste of experimental claws was judged superior to that of controls in one test of claws frozen 10 h. There was no significant difference in taste between control and experimental claws in any other test (Tables 3, 4; Figure 2B).

DISCUSSION

Icing or freezing prior to cooking each reduced stone crab claw quality. Tests indicated a small difference between control and experimental claws in taste, a moderate difference in texture, and a large difference in the tendency of meats to stick to shells. Taste was significantly poorer in claws freezer-cooled prior to cooking vs.

Table 1. Numbers of control (C) and experimental (E) stone crab claws[1] receiving various ratings for taste, texture and sticking.

Rating		6 h iced		6 h freezer	
Category	Condition	N = 29		N = 22	
		C	E	C	E
Taste:	Excellent	8	5	13	5
	Good	13	13	7	12
	Fair	8	9	2	5
	Poor	0	2	0	0
Texture:	Excellent	9	1	11	6
	Good	14	15	9	8
	Fair	6	10	2	4
	Poor	0	3	0	4
Sticking:	None	12	5	12	1
	Light	14	7	9	3
	Moderate	3	8	0	6
	Heavy	0	9	1	12

[1]C = cooked fresh, then refrigerated; E = iced or freezer-cooled 6 h prior to cooking.
N = number of claw pairs per test.

Table 2. Goodness of fit chi-square values[1] derived from four rating conditions within taste, texture and sticking categories of control vs. experimental stone crab claws[2].

Treatment	N (claw pairs)	Taste	Texture	Sticking
6 h iced	29	5.11 n.s.	17.75*	113.29*
6 h frozen	22	10.70*	19.51*	113.01*

[1]Data coded by adding "1".
[2]C = cooked fresh, then refrigerated; E = iced or freezer-cooled 6 h prior to cooking.
n.s. = no significant difference.
* = difference significant ($P<0.05$, 3 d.f.).

Table 3. Number of control and experimental stone crab claws[1] rated for taste, texture and sticking.

Rating		8 h freezer				10 h freezer				16 h freezer			
		Reheated N=29		Cold N=23		Reheated N=29		Cold N=27		Reheated N=48		Cold N=62	
Category	Condition	E	C	E	C	E	C	E	C	E	C	E	C
Taste:	Excellent	15	15	8	10	7	13	11	10	15	12	19	26
	Good	11	8	12	10	15	11	10	15	23	25	32	29
	Fair	3	6	3	3	5	5	6	2	9	9	10	7
	Poor	0	0	0	0	2	0	0	0	1	2	1	0
Texture:	Excellent	10	9	6	6	10	10	8	5	15	16	25	25
	Good	17	13	17	9	14	14	16	20	23	20	27	28
	Fair	2	6	0	7	5	3	3	2	8	8	7	8
	Poor	0	1	0	1	0	2	0	0	2	3	3	1
Sticking:	None	15	16	21	10	18	23	17	20	32	29	42	41
	Light	14	8	2	7	9	3	8	6	11	11	11	14
	Moderate	0	1	0	5	2	2	2	1	3	4	7	5
	Heavy	0	4	0	1	0	1	0	0	2	4	2	2

[1]C = cooked fresh, then refrigerated; E = iced 6 h, freezer-cooled 8, 10, or 16 h, then thawed.

N = number of claw pairs tested.

Table 4. Goodness of fit chi-square values[1] derived from four rating conditions within taste, texture and sticking categories of control and experimental stone crab claws[2].

Treatment	Serving Method	N (claw pairs)	Taste	Texture	Sticking
8 h	Reheated	29	3.82 n.s.	6.51 n.s.	19.46*1
	Cold	23	0.73 n.s.	53.56*1	155.33*1
10 h	Reheated	29	8.21*2	4.67 n.s.	5.92 n.s.
	Cold	27	5.26 n.s.	2.46 n.s.	1.53 n.s.
16 h	Reheated	29	1.92 n.s.	11.51*1	4.89 n.s.
	Reheated	19	6.37 n.s.	7.50 n.s.	1.60 n.s.
Σ	Reheated	48	1.77 n.s.	0.96 n.s.	2.61 n.s.
	Cold	20	3.17 n.s.	2.47 n.s.	3.75 n.s.
	Cold	12	0.00 n.s.	1.50 n.s.	1.78 n.s.
	Cold	30	2.61 n.s.	1.17 n.s.	2.48 n.s.
Σ	Cold	62	4.76 n.s.	1.51 n.s.	1.41 n.s.

[1]Underlined values indicate data coded by adding "1".
[2]C = cooked fresh, then refrigerated; E = iced 6 h, cooked, freezer-cooled 8, 10, or 16 h, thawed.
n.s. = no significant difference ($P<0.05$, 3 d.f.).
*1 = controls significantly better than experimental claws.
*2 = experimental claws significantly better than controls.
Σ = both reheated or all cold combined in 16 h treatment.

control claws, but not in claws iced prior to cooking vs. controls. Despite extreme sticking of meat in iced claws, volunteers recorded little difference in taste between those and controls. This seems to indicate that volunteers attempted to judge objectively in all categories. Results support the findings of Waters and Haines (manuscript) and verify contentions of fishermen and processors that icing or freezing stone crab claws prior to cooking reduces product value. The industry's need for a quality product must be reconciled with management's need to return crabs to the water as soon as possible to reduce mortality and increase fishery yield. A procedure should be adopted to serve both needs.

Freezing previously iced stone crab claws for brief periods (at least 10h) after cooking seems to negate deliterious effects caused by icing. Although sticking was significantly greater in claws freezer-cooled for only 8 h after cooking, there was no significant difference between controls and claws frozen for either 10 or 16 h. Further testing under actual fishing and processing conditions may prove icing to be a viable option for holding claws on vessels during harvest operations, allowing immediate release of crabs without reducing product value, and thereby improving crab survival. The freezing technique would also be applicable when claws exposed aboard vessels during winter harvest are chilled as if iced, causing sticking and reducing product value.

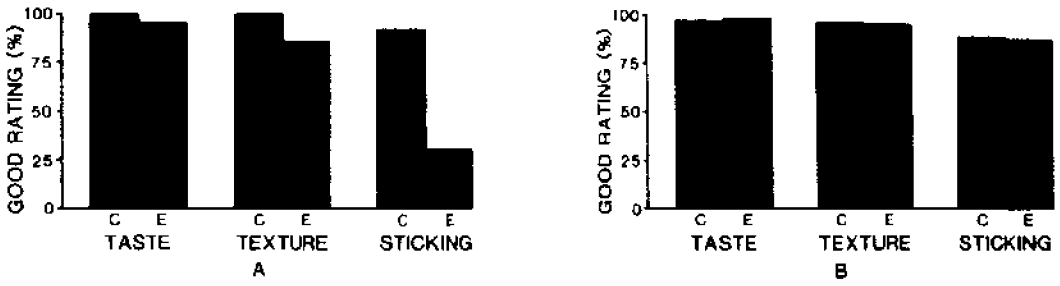

Figure 2. Percent combined "good" ratings in taste (excellent + good + fair), texture (excellent + good + fair) and sticking (none + light) received by control (C) and experimental (E) claws. A: C = cooked fresh, then refrigerated; E = iced or freezer-cooled 6 h prior to cooking; N = 51 claw pairs. B: C = cooked fresh, then refrigerated; E = iced 6 h, cooked, freezer-cooled 10 or 16 h, thawed; N = 166 claws pairs.

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THE USE OF PHOSPHATES IN THE SOUTHERN SHRIMP PROCESSING INDUSTRY

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Seafood processors who pack shrimp experience significant finished product weight losses under traditional processing methods. Weight losses experienced during packing result from the nature of the finished product - peeled shrimp - and the process that must be followed to produce the product.

The majority of the shrimp entering a seafood packing plant will be packed out as a peeled product in a 5-pound box and then frozen. The peeling machines use water and mechanical pressure to remove the heads and shells from the shrimp. On a weight basis, between 45% to 50% of the losses experienced in a shrimp plant is attributable to removing the head and body shell. There is further loss due to breaking and leaching of tissue fluids caused by prolonged contact with water. There appears to be little opportunity for reducing the processing-caused weight losses with the present machinery in use.

A second category of weight loss which could lend itself to corrective measures is that which is experienced during frozen storage. When frozen shrimp are thawed, there is a demonstrable loss of weight from that which was originally packed. This weight loss or thaw drip results from fluids which pass out of the tissues as the ice within the cells themselves melts. If a large ice crystal has punctured the cell wall, then the protoplasm will run out through the break (Nagle,et.al.,1980).

Under rapid freezing conditions, such as the IQF process, the cell is frozen before the intracellular ice crystals can grow very large, and thus thaw drip should be reduced. Nevertheless, blast freezing is the most common method used by the industry due to its affordability even though it is considered a slow method.

The seafood industry as a whole has not effectively utilized the vast array of food-grade chemicals available to it (Redmayne, 1982; McCormik,1983). There is a group of compounds, the phosphates, that has been used in the food processing industry for many years. Phosphates in several forms are used in the dairy, poultry, soft drink, baking, and red meat segments of the industry (Anonymous; Shimp, 1981).

The major value of phosphates for the shrimp industry would be to make use of their ability to decrease thaw drip. At present it is an industry nesessity to over-pack the 5-pound boxes of shrimp by several ounces so that when the frozen box is thawed at the next step down the distribution chain, it will weigh at or very near the net weight declaration on the label.

Studies indicate that phosphates increase water binding capacity in meat products through increases in pH and ionic strength, chelation of divalent metal ions, a physical binding to the myofibrillar proteins, protein extractibility, and dissociation of actomyosin (Hamm, 1970; Prusa & Bowers, 1984). The dissociation of actomyosin and increased protein extractibility has been found to increase the ability of myosin to form gel systems (Siegel & Schmidt, 1979). This gelatinized protein-phosphate complex forms a layer on the surface of treated shrimp (Tenhet, et. al.,1980; Aitken, 1975) which would appear to inhibit thaw drip from passing out of the product as thawing takes place.

Commercial manufacturers of phosphates often claim that their products have antimicrobial properties. However, studies (Molins, et.al.,1985[A],1985[B]) would indicate that phosphates do not have a significant effect on the growth of mesophilic or psychrotrophic bacteria in refrigerated cooked and uncooked processed meats. Nevertheless, in temperature abused meat products, sodium acid pyrophosphate, sodium tripolyphosphate, and tetra- sodium pyrophosphate can have noticable inhibitory effects on growth of these type organisms (Molins, 1985[B]). A study of the bacterium *Aeromonas hydrophilia* isolated from Indian mackerel (Venugopal, 1984) showed that STP inhibited the secretion of extracellular protease enzymes but it had only a slight effect on the growth of the organism.

METHODS

Dejean Packing Company of Biloxi, Mississippi provided the shrimp used in this study. The shrimp were collected at the plant while it was in production. The majority of the experiments were conducted using peeled shrimp; therefore, the shrimp were taken off the line after they had their shells removed by the peeling machines. This was done to keep the results reflective of what would be encountered by a plant operator rather than peeling the shrimp in the lab where more careful handling could effect the results. The temperature of the shrimp was kept at ambient levels rather than icing for transport since the Lab was only two blocks away and plant conditions were being adhered to in the lab.

The shrimp were divided into approximately 454 gram (1 lb) portions. The dip solutions were mixed using tap water on a weight/weight basis. Distilled water was not used in any part of the experiment due to its observed inhibitory effects on water uptake in raw shrimp. Furthermore, a Gulf seafood plant would not be expected to have distillation equipment in it. Each solution volume was three liters. FMC Corporation, Industrial Chemical Group, provided the phosphates used in the study and consultation on their use. Dip duration was one minute. It was evident from visits to seafood plants in Mississippi and Alabama that a lengthy dip period would be disruptive to the product flow along the processing line. Plant owners said that they would not want a soaking step in their operations. The shrimp were drained for two minutes at an inclined angle of 30°. This is following the method advocated by the American Shrimp Processors Association in an effort to standardize the thaw process for block frozen shrimp.

All shrimp were frozen. Each sample was placed in a plastic bag with 100 ml water added for glaze protection. The freezer was held at -21°C (-6°F). Thawing took place in a sink containing flowing water at ambient temperatures. The step sequence was : weigh the raw sample, dip in solution, drain, reweigh, freeze, thaw, drain, weigh.

Sodium tripolyphosphate is the most commonly encountered phosphate in the meat industry and for that reason it was used as the basis for this study. A phosphate mixture consisting of 75% STP and 25%

SAP was tested because of its purported advantages in suppressing the clearing phenomenon in shrimp tail meat (Shimp & Steinhauer, 1983).

In an effort to provide the plant workers with a simple means for testing the strengths of mixed solutions, two hydrometers were chosen to evaluate. One was scaled for percentage of salt (Fisher catalog #11-605) and the other was percentage of salt by weight (Fisher catalog #11-606).

Experiments were conducted on cooked meat yields of shrimp treated with phosphates. After the shrimp had been frozen and thawed, 100 grams were cooked in boiling water for 8 minutes then drained and cooled for 4 minutes before weighing. After 4 minutes steam had stopped rising off the shrimp and the weight was stabilized. This was a modification of a method used by Thomas (1978) on dipped scallops.

RESULTS and DISCUSSION

The results of tests using STP at ambient temperatures are shown in Figure 1. The temperature during the tests ranged from 23°C (74°F) to 27°C (80.5°F). The pH of the solutions was higher than the control. The tap water itself was rather high - 8.6 to 8.7. The solution pH's did not show any association with increasing phosphate strengths. The range was a narrow 9.0 to 9.2. Final weight gain generally increased with increasing solution strengths. The initial weight gain as measured immediately after dipping did not follow a similar pattern. Indeed it could be said that the initial weight gained was essentially the same, irrespective of the solution concentration. At all concentrations there was a straight line increase in weight gain through both steps of the experiment. At concentrations of 2% and above, there was a marked increase in weight. In this experiment and all others the control gained and retained weight. This could have been augmented by the high pH of the local water. Why the 0.5% and 1% shrimp would gain less weight than the control is not known.

As the Clearing Index shows, pronounced weight gain(or water uptake) above 2% was detrimental to the appearance of the thawed shrimp. The high weight gains corresponded to a marked clearing of the outer tissues and at the tip of the tail such that the shrimp would probably be rejected by a buyer in the raw state. Once the shrimp had been cooked, however, there was no difference in the outward appearance of shrimp from one solution concentration to another. It was odd to note

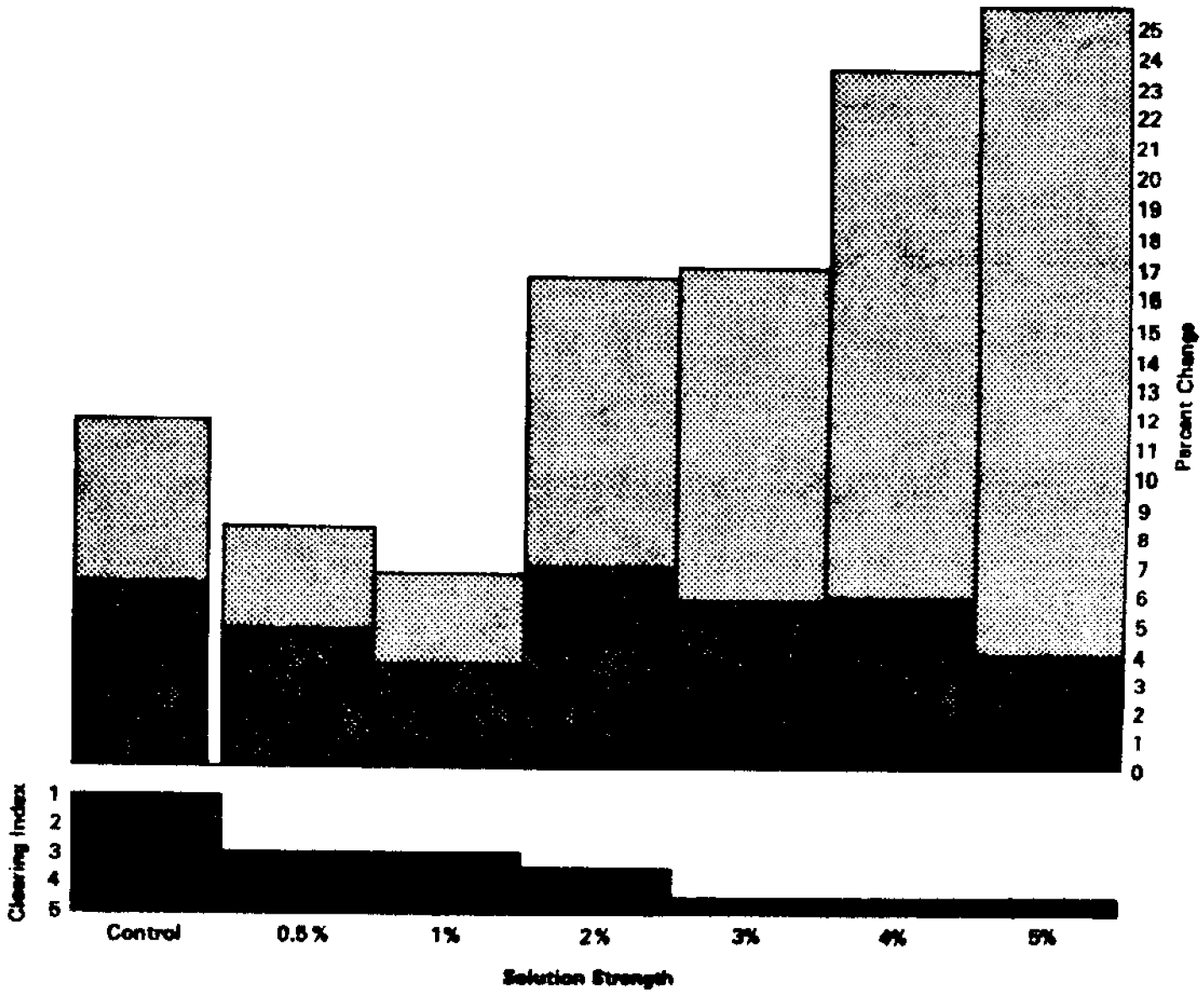

Figure 1. STP ambient temperature: percentage gain (▨ after dip, ▩ after freezing) and clearing index (1 normal, 2 slight, 3 moderate, 4 pronounced, 5 excessive).

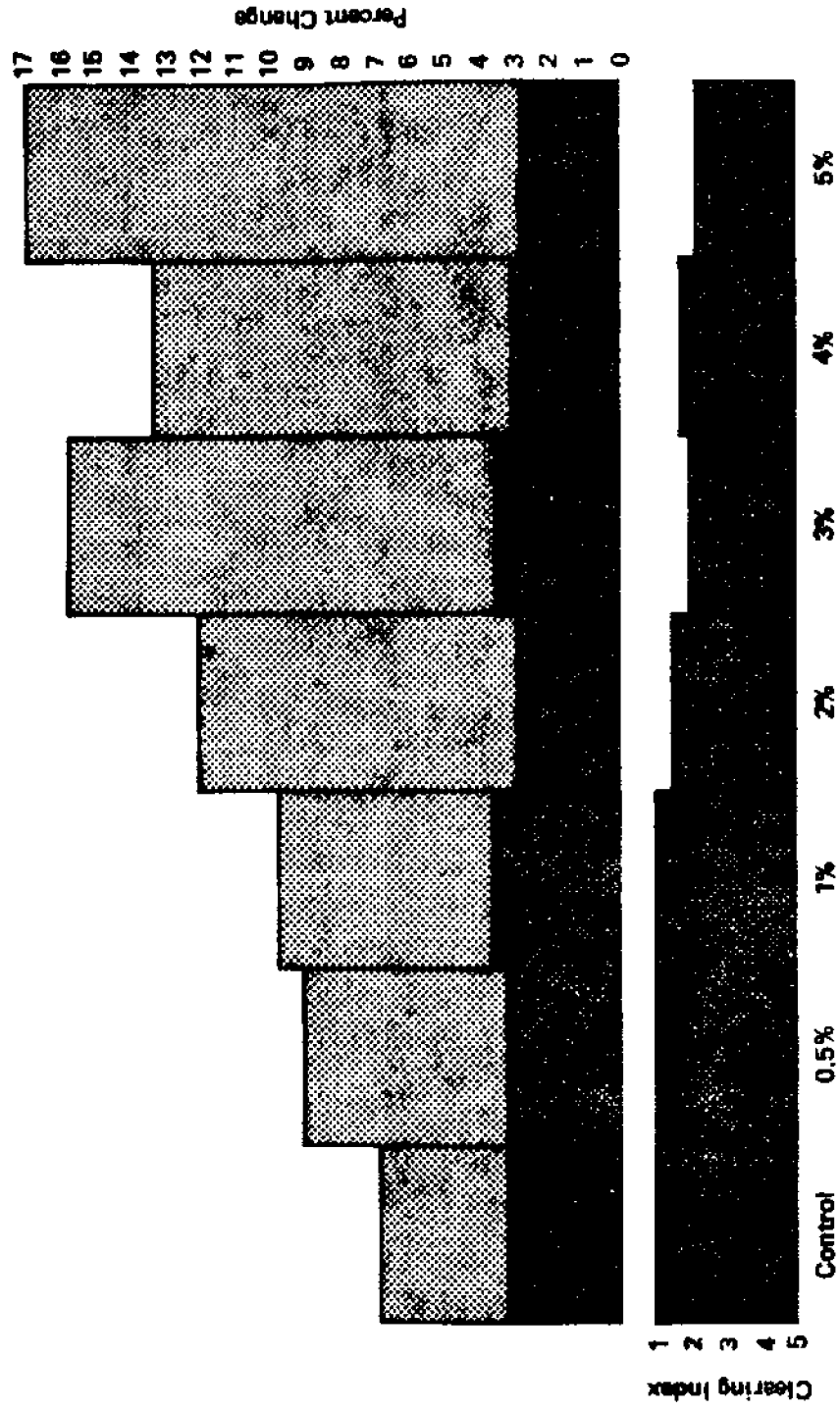

Figure 2. STP/LAP ambient temperature: percentage gain (■ after dip, ⊠ after freezing) and clearing index (1 normal, 2 slight, 3 moderate, 4 pronounced, 5 excessive).

that even though the control gained more weight than the 0.5% and 1% shrimp, it did not show any clearing of the tissues.

The results of tests using a mixture of 75%STP/25%SAP are shown in Figure 2. The ambient solution temperatures ranged from 21.5°C (71°F) to 28°C (82°). The STP/SAP mixture decreased the pH of the solutions from that of the control. This time the pH's did respond to changes in solution strengths. The control was 8.6. The 0.5% solution was 7.9 and the readings gradually dropped as the concentration increased so that it was 7.25 at the 5% solution.

Final weight gains tended to increase with increased solution strengths. However the initial weight gain resulting from dipping in the solution was not influenced by the strengths of the various solutions. It remained very near the same level of increase no matter what the concentration of the solutions. There was a straight line increase in weight shown at both steps of the experiment. The increase in weight was gradual rather than abrupt as the dip concentrations changed to higher values. The greatest weight gains were experienced during the time frame after the shrimp had been dipped and placed in the freezer. The shrimp probably continued to absorb fluids until they were frozen, but more likely most weight gain took place during the thawing step. This was a slow process carried out underwater. The Clearing Index shows that all shrimp were of acceptable appearance.

Cold phosphate solutions were tested for their effects on clearing and weight gain. The resulting data for cold STP is shown in Figure 3. The 0.5%, 1%, and 2% shrimp lost weight during the second step of the experiment after they had gained weight from the dip step. At 3% and above, weight was gained during the freeze/thaw step. Once again the initial weight gain from the dip was essentially the same no matter what the solution concentration. The average solution temperature was 4.4°C (40°F). Maximum weight gains were less than that experienced by shrimp dipped in ambient STP. However, the Clearing Index shows a higher level of acceptability until the 4% level is reached. There was less clearing of the tissues even though the pH (at 9.2) was the same as the ambient dips.

Limited work with cold STP/SAP indicated that final weight gain was much less than at ambient temperatures and the degree of clearing was the same.

Figure 3. STP cold dip: percentage gain (■ after dip, ▣ after freezing) and clearing index (1 normal 2 slight, 3 moderate, 4 pronounced, 5 excessive).

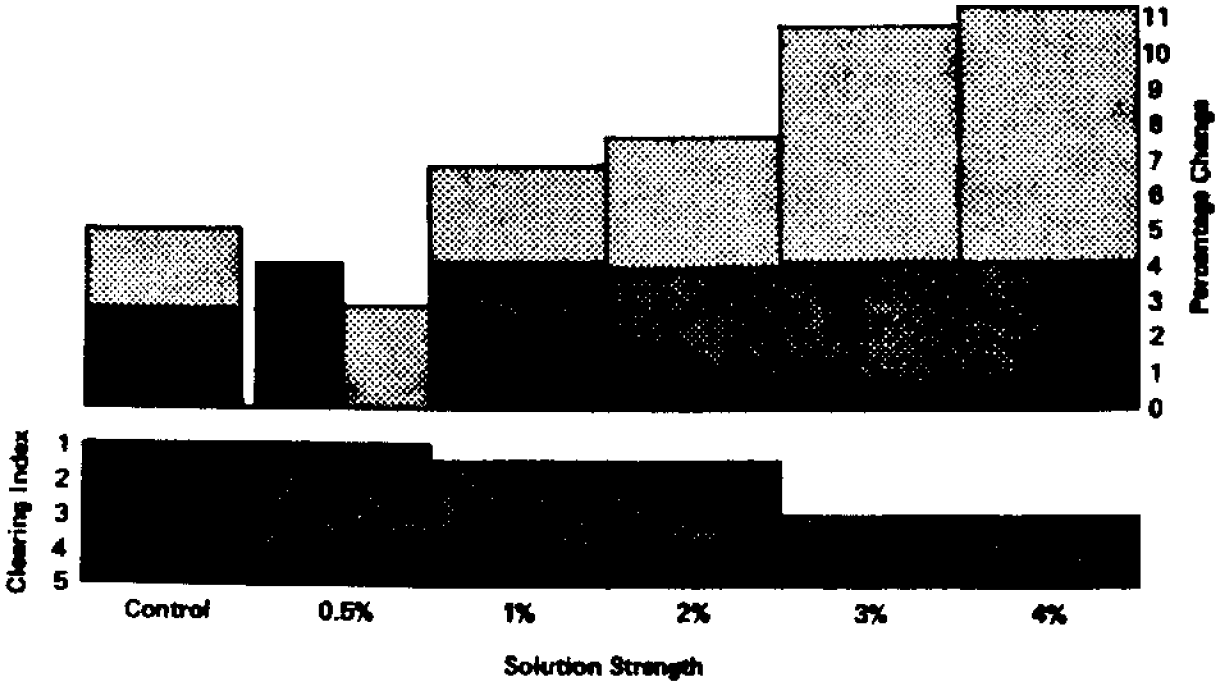

Figure 4. STP with 3% NACl ambient temperature: percentage gain (■ after dip, ▣ after freezing) and clearing index (1 normal, 2 slight, 3 moderate, 4 pronounced, 5 excessive).

Experiments with added sodium chloride were run. Data from tests with STP+3% NaCl are shown in Figure 4. The solution temperatures ranged from 22°C (72°F) to 25°C (77°F). Starting with the 1% solution, final weight gains increased with increasing solution strengths. Initial weight gains were exactly the same for all dip solution strengths. The addition of NaCl depressed the pH somewhat in relation to a STP solution without the salt. The pH range was from 8.6 to 8.7. The amount of clearing was practically nonexistent until the 3% and 4% solutions were used, and they created a moderate clearing which would be an acceptable product. The control in this case was water with 3% NaCl added.

Shell-on shrimp which had been block frozen and in storage from six to nine months were tested with low concentrations of phosphates. The phosphate solutions were kept at low levels on the assumption that previously frozen meat would be more susceptible to water adsorption than a firm, fresh shrimp. The shrimp were thawed and hand peeled before being dipped. A phosphate blend, STP/SAP, was used rather than the single compound, STP. Four dip solutions were used - 0.25%, 0.5%, 1%, and 1.5%. The initial gain was essentially equal for all solutions, but it was not a large gain: 1.95%, 1.55%, 1.35%, and 2% respectively.

The 0.25% solution shrimp lost weight after being frozen and thawed while the three higher percentage solutions gained weight. There was an overall gain for all four solutions - 1.75%, 3.3%, 3.3%, and 2.6% respectively. The control gained 1.8% with the dip but lost 0.9% upon thawing to give an overall gain of 0.9%. The thawed appearance was normal for all solution strengths. The pH decreased with increasing percentages of the STP/SAP mixture. They ranged from 8.2 to 7.5. The control was 8.7.

Limited work was done with frozen/thawed shrimp using STP solutions to which 3% w/w NaCl had been added. Eight and nine month old frozen shell-on shrimp were thawed and hand peeled for use in the experiments. Seven solution strengths were used. In addition to the four mentioned above, 2%, 3%, and 4% were mixed. Once again the initial gain was very similar. The final gain (thawed weight) for concentrations of 1.5% and lower did not show a relationship with the solution strength, but the final weights for the 2%, 3%, and 4% shrimp increased stepwise along with the increasing concentrations of the dip solutions. Thawed weight

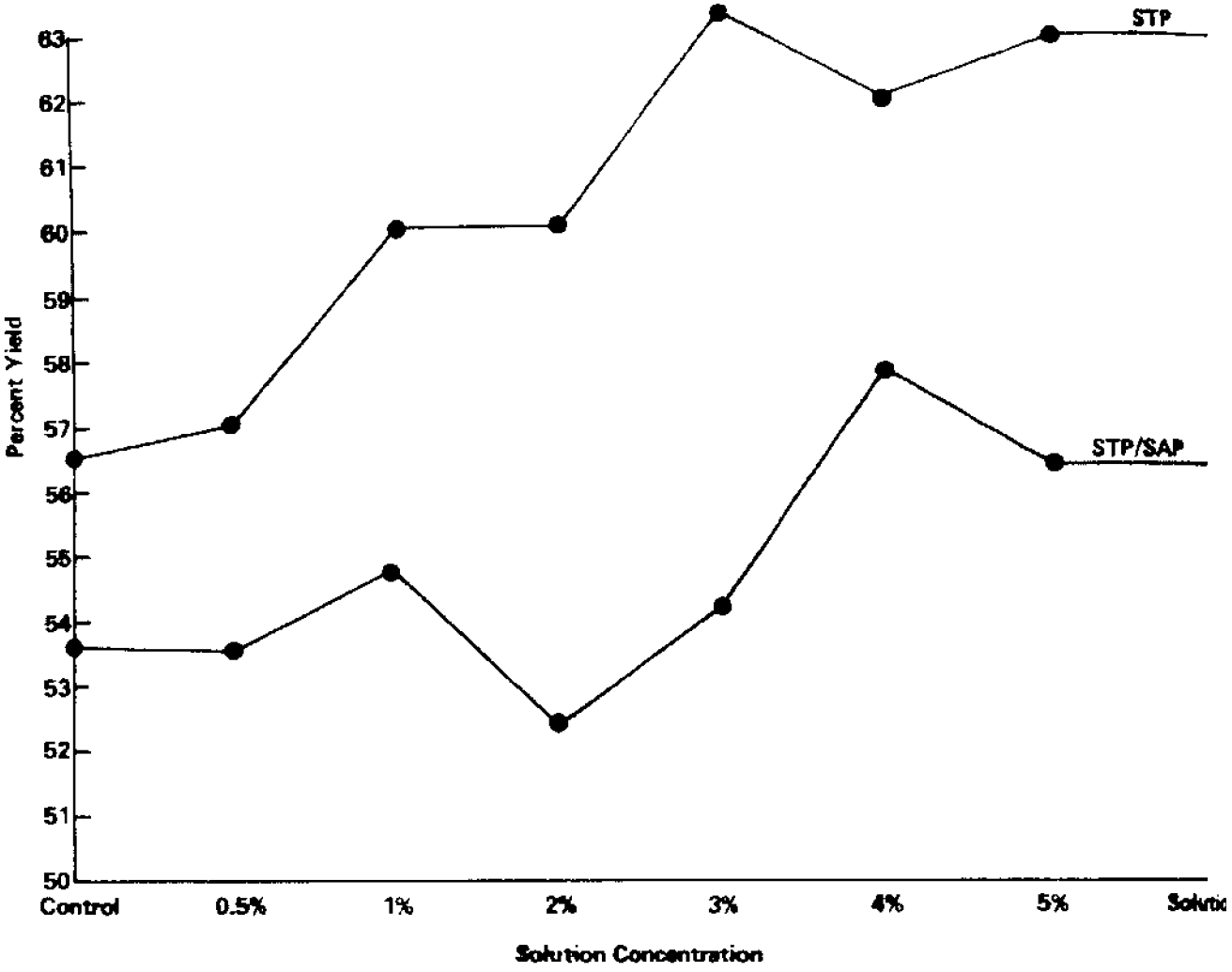

Figure 5. Cooked Meat Yield

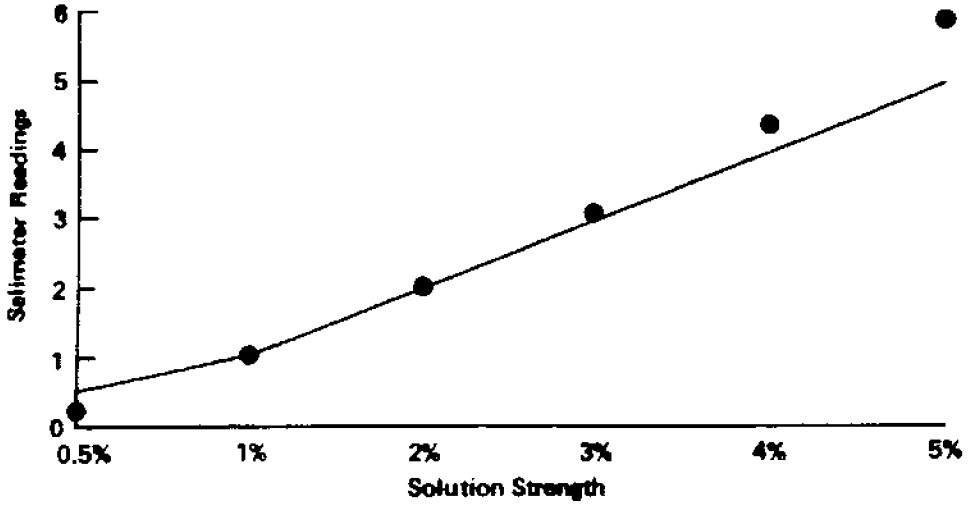

Figure 6. Relationship of Salimeter to Tripoly Concentration

gains ranged from 4.4% at 0.25% to 6.1% at 4%. The pH increased slightly above the control by 0.2 to 0.6 of a standard unit. There was no clearing in any of the shrimp.

Data on cooked meat yields are given in Figure 5 for two solutions. Except for the 2% STP/SAP, meat yields generally increased with increasing concentrations of phosphates in the solutions. Therefore, the weight picked up in the dipping process was not lost in cooking. As might be expected, the yields for STP shrimp were highest. However, the appearance may have been good, but the characteristic shrimp taste was missing, probably as a result of the water pickup.

The method for checking solution strengths for plant personnel found that best results were obtained with a salimeter which measures the percentage NaCl by weight. It has a scale of 0 to 24.6 in 1/2 percent increments. Figure 6 shows the relationship of the readings to the actual strength of the solutions. It was quite accurate until the 5% level where it tended to read closer to 6 than to 5. All readings were taken at ambient temperatures. In solutions with added NaCl, the readings were increased by the percentage of salt added.

DISCUSSION

Sodium tripolyphosphate may be the choice compound for stopping thaw drip or adding weight based on price, but at ambient temperatures it can produce the least acceptable raw product due to appearance changes brought about by high water pick-up. The tip of the tail and the ragged tissue at the head break become clear. The main objective of phosphate use is to prevent having to over-pack the wholesale packages. Phosphates prevent the loss of tissue fluids upon thawing, but if not used judiciously they will increase the weight of shrimp through water absorption. STP proved to be very reactive with dipped shrimp such that solution concentrations above 2% caused the product to take up water to the detriment of its appearance. Low dip solution strengths work best with this compound.

On the other hand, if a cooked product is to be produced, then the clearing which would be quite evident in the raw shrimp is not found in the cooked and the weight gain advantage can be utilized. It was noted,

however, that there was a flavor loss in shrimp with a high percentage of weight gain.

A plant owner could use STP along with table grade salt. This work found that by adding salt, only modest weight gains were experienced and thawed appearance was good. But there is increased labor involved with the double mixing. FMC recommended adding the salt after the STP had been dissolved first.

Another alternative for STP use would be to dip shrimp in cold solutions. This proved to give a lot better raw appearance than ambient temperature STP, and weight gains were negligible. This would be useful for packing 5 pounds in a box and expecting to get 5 pounds out when thawed. The one drawback is that tripoly dissolves quite slowly at lower water temperatures. An alternative would be to dissolve the STP in a portion of the needed water at ambient temperatures and add the rest as ice.

For those companies who buy block frozen, headless shrimp which will be thawed, peeled, and refrozen; indications are that STP w/ NaCl will provide weight stabilization with no clearing defects upon thawing for the second time.

The blend of STP/SAP in a 75%/25% ratio was chosen as best among several ratios tested by Dr. Larry Shimp while he was with FMC Corporation. The mixture was formulated in the laboratory since it is not produced commercially. At one time there was a 70%/30% blend available from Stauffer Chemical Company under the trade name of Seafos. In Shimp's work (Shimp & Steinhauer, 1983) such a blend had essentially equal weight gains as a 75%/25% mix and a 3% lower cooked meat yield.

A blend in the 75%/25% range would appear to be the best choice for working with raw shrimp because weight gains were not excessive and clearing was not a problem. The tripoly and acid phosphate can be purchased separately and mixed at the plant for testing; however, chemical company representatives said that they might consider blending an order to specifications.

As can be noted, all control samples gained, rather than lost, weight. The basic premise of this experiment was that untreated shrimp lose

fluids after being frozen and thawed. In fact they do when packed under commercial conditions using boxes containing five pounds of product. The present anomalous experimental results must be related to the greater efficiencies of the laboratory environment.

In dealing with an annual resource that is being fished at its maximum yield, increases in profits cannot come from increases in domestic landings anymore; so, the product must be protected from in-plant losses that were acceptable in past years. If utilized within the confines of approved FDA regulations and good sense, phosphates should make shrimp processing more efficient and carry no negative consumer perceptions.

ACKNOWLEDGEMENT

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HYDROLYTIC AND ENZYMATIC BREAKDOWN OF FOOD GRADE CONDENSED PHOSPHATES IN WHITE SHRIMP (*Penaeus setiferus*) HELD AT DIFFERENT TEMPERATURES

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INTRODUCTION

The use of polyphosphates is quite widespread in the food industry. The ability of phosphates to perform many useful functions is related to their reactions and interactions with the different food systems. Stabilizing proteins against denaturation, an increase in the water binding capacity, improving emulsification and buffering capacity (acid-base relationships), nutrient contributions, chelation of metal ions and antioxidant functions are among the contributions of polyphosphates to a variety of foods and food products. Protein reactions and water binding are perhaps the most important reasons for considering the use of phosphates in seafoods. Condensed food phosphates such as sodium tripolyphosphate (STPP) and sodium hexametaphosphate (SHMP) are effective on fish and shellfish in preventing 'drip-loss' when frozen products are thawed, and in enhancing tenderness by restricting protein denaturation during freezing and frozen storage (Akiba et al., 1967; Boyd and Southcott, 1965; Halliday, 1978; Mahon, 1962).

When added to seafoods, STPP and SHMP are not very stable but tend to hydrolyze in the muscle to monophosphates during prolonged storage (Gibson and Murray, 1973; Sutton, 1973). The decomposition products do

not possess the same properties as its parent compounds (Crowther and Westman, 1953; Sutton, 1973). There are indications that in addition to undergoing hydrolytic decomposition, condensed phosphates may also be subject to hydrolysis by endogenous tissue enzymes (Harold, 1966; Roche, 1950). Due to lack of reliable analytical technique, the information on the studies of these effects in shrimp muscle is limited.

In order to determine the extent of hydrolysis, an improved thin-layer chromatographic method was used for detection and an accurate determination of phosphate components at any stage of the reaction. The objective of this study was to determine the stability of linear condensed phosphates in shrimp held at 5° and 10°C by measuring the rates of hydrolysis of these phosphates under both enzymatic and non-enzymatic conditions.

MATERIALS AND METHODS

I. Preparation of shrimp extract for thin-layer chromatography (TLC)

Fresh white shrimp (Penaeus setiferus), (30-40 tails per pound) were used in this study. Deheaded and peeled shrimp were homogenized with distilled water (1:2) and centrifuged at 14,000 rpm for one-half hour. Appropriate amount of supernatant was added to 10 mL of 0.25% STPP and SHMP and monitored for hydrolysis by determining phosphate species distribution at regular intervals.

II. TLC - Qualitative and quantitative analysis

A. Qualitative analysis:

Using a micropipet, samples and standard solutions in 50 mL volumes were applied at the lower edge of TLC plates which were placed

in the developing tanks containing appropriate mobile phase mixtures. Plates were removed from tanks when the solvent front had ascended 10 cm from the origin of the sample spot and dried in an oven at 60°C. Dried plates were first sprayed lightly with the developing reagent, dried and sprayed again with the reducing agent in order to detect phosphate containing areas on the plate. Migration distances of phosphate spots in the sample were compared with those from standard solutions which were run simultaneously.

B. Quantitative analysis:

When the separation and identification technique for condensed phosphates was completed, the stationary phase containing phosphates was transferred into a 50 mL Erlenmeyer flask. A dilute solution of ammonium hydroxide was employed as the elution medium. In order to obtain a complete hydrolysis of SHMP, STPP and pyrophosphate to monophosphate, eluted phosphates were heated in the presence of sulfuric acid in a water bath at 100°C for 30 min. Ammonium molybdate and hydrazine hydrochloride solutions were added to the cooled flasks which were placed again in the water bath for about 10 min. The second heating period was necessary for maximum color development. The resultant color intensity was measured on the spectrophotometer and the amount of phosphorus calculated from appropriate standard curves.

III. Preparation of enzyme solution

Shrimp tissue was homogenized with deionized distilled water (1:3) and centrifuged at 14,000 rpm for 30 min at 0°C. The supernatant was brought to 100% saturation with solid ammonium sulfate. The forming precipitate was collected by filtration and dialyzed overnight against 0.05 M Tris-HCl buffer, pH 7.8. After centrifugation, the supernatant

was used as enzyme solution.

IV. Enzyme assay

Phosphatase activity was determined using p-nitrophenyl phosphate as the substrate. An incubation mixture containing 3.33 mM p-nitrophenyl phosphate, 1.6 mM magnesium sulfate, 55.5 mM potassium chloride, and 36.7 mM Tris-HCl buffer (pH adjusted to 7.8) was prepared in order to determine enzymatic activity in the shrimp extracts. Liberated p-nitrophenol was followed spectrophotometrically at 405 nm.

RESULTS AND DISCUSSION

Tables 1 and 2 show the percent distribution of breakdown products of sodium tripolyphosphate at 5° and 10°C respectively, at various time intervals. The hydrolysis data for sodium hexametaphosphate are presented in Tables 3 and 4. Phosphates were separated on glass plates and each component satisfactorily recovered by using TLC system that we previously described (Reddy and Finne, 1985). Because naturally present inorganic phosphorus and the orthophosphorus component resulting from breakdown of STPP or SHMP collectively migrated on TLC plate, the endogenous phosphorus content (equivalent to 141±14 mg P/100 g shrimp) was subtracted before reporting data for orthophosphate species. Results were normalized to 100% in order to account for the large variation in the endogenous phosphorus content of shrimp.

A complete breakdown of tripolyphosphate to orthophosphate in the presence of phosphatase enzyme occurred within 12 days at 5°C and in 15 days at 10°C, whereas the overall rate of degradation of hexametaphosphate was slightly lower (Tables 3 and 4). No such

Table 1. Hydrolysis of sodium tripolyphosphate in shrimp muscle at 5°C.

REACTION TIME (DAYS)	% DISTRIBUTION OF PHOSPHORUS						k_1 FIRST ORDER RATE CONSTANT (DAY^{-1})	
	TRI PHOSPHATE		PYRO PHOSPHATE		ORTHO PHOSPHATE			
	-ENZ	+ENZ	-ENZ	+ENZ	-ENZ	+ENZ	-ENZ	+ENZ
0	96.1		3.6		0.3			
2	94.4	83.6	4.1	14.0	1.5	2.4	.009	.069
4	91.8	74.3	5.7	18.3	2.5	7.4	.011	.064
6	89.7	64.8	6.9	22.6	3.4	12.6	.011	.066
8	87.8	53.9	8.0	25.8	4.2	20.3	.011	.072
10	85.7	38.0	6.4	34.2	7.9	27.8	.011	.093
11	83.1	00.0	5.2	26.8	11.7	73.2	.013	----
12	81.4	00.0	5.0	00.0	13.6	100.0	.014	----

Table 2. Hydrolysis of sodium tripolyphosphate in shrimp muscle at 10°C.

REACTION TIME (DAYS)	% DISTRIBUTION OF PHOSPHORUS						k_1 FIRST ORDER RATE CONSTANT (DAY^{-1})	
	TRI PHOSPHATE		PYRO PHOSPHATE		ORTHO PHOSPHATE			
	-ENZ	+ENZ	-ENZ	+ENZ	-ENZ	+ENZ	-ENZ	+ENZ
0	96.1		3.6		0.3			
2	90.2	81.7	6.0	15.1	3.8	3.2	.032	.081
4	86.9	71.6	7.3	20.0	5.8	8.4	.025	.074
6	81.0	56.9	11.0	28.8	8.0	14.3	.028	.087
8	80.0	44.0	5.6	25.5	14.4	30.5	.023	.098
11	76.1	24.9	4.8	22.0	19.1	53.1	.021	.123
14	75.2	00.0	4.6	6.2	20.2	93.8	.018	----
15	72.4	00.0	4.1	0.0	23.5	100.0	.019	----

decomposition was seen in non-enzymatic environment. Because of the stability of polyphosphates in aqueous solutions and the fact that phosphatase was inactivated in the shrimp extract by heat prior to treatment, the small degree of breakdown of polyphosphates found under non-enzymatic conditions is not understood at the present time. During enzyme-catalyzed breakdown of condensed phosphates, phosphatase did not lose its activity either at 5^{o} or $10^{o}C$.

The variation in concentration of STPP or SHMP with time followed a characteristic sequential reaction of the type $A \xrightarrow{k_1} B \xrightarrow{k_2} C$. The polyphosphates decomposed first to pyrophosphate which then hydrolyzed into orthophosphate. Each step of the reaction is governed by the first order rate constants, k_1 and k_2 respectively. The reported rate constant, k_1, was determined from the ratio of two concentrations determined at two times by using the following equation:

$$\ln[a/(a-x)] = kt$$

where a = initial concentration at time equal to 0

(a-x) = new concentration after a time, a quantity x has been tranformed from A into B

t = time, in days

Stoichiometric degradation of sodium tripolyphosphate to one mole each of ortho-, and pyrophosphate was found to follow a first-order reaction (Reddy and Finne, 1986). The distribution of phosphate species at various time intervals during enzyme-catalyzed hydrolysis of sodium tripolyphosphate at $10^{o}C$ is shown in Fig. 1. It can be seen that during hydrolysis, the concentration of orthophosphate steadily increased until all phosphate was present as that species. The amount of pyrophosphate present increased to pass through a maximum at which point the rate of

Table 3. Hydrolysis of sodium hexametaphosphate in shrimp muscle at 5°C.

REACTION TIME (DAYS)	% DISTRIBUTION OF PHOSPHORUS						k_1 FIRST ORDER RATE CONSTANT (DAY^{-1})	
	HEXAMETA PHOSPHATE		PYRO PHOSPHATE		ORTHO PHOSPHATE			
	-ENZ	+ENZ	-ENZ	+ENZ	-ENZ	+ENZ	-ENZ	+ENZ
0	94.4		4.2		1.4			
2	92.3	77.1	5.6	16.2	2.1	6.7	.011	.101
4	89.3	53.3	3.8	34.6	6.9	12.1	.014	.143
6	88.1	43.9	4.2	40.2	7.7	15.9	.012	.128
8	85.5	36.9	5.8	28.7	8.7	34.4	.012	.117
10	83.0	28.1	4.3	22.1	12.7	49.8	.013	.121
11	81.6	00.0	3.6	20.0	14.8	80.0	.013	----
13	80.8	00.0	3.2	00.0	16.0	100.0	.012	----

Table 4. Hydrolysis of sodium hexametaphosphate in shrimp muscle at 10°C.

REACTION TIME (DAYS)	% DISTRIBUTION OF PHOSPHORUS						k_1 FIRST ORDER RATE CONSTANT (DAY^{-1})	
	HEXAMETA PHOSPHATE		PYRO PHOSPHATE		ORTHO PHOSPHATE			
	-ENZ	+ENZ	-ENZ	+ENZ	-ENZ	+ENZ	-ENZ	+ENZ
0	94.4		4.2		1.4			
2	91.4	76.3	6.1	15.8	2.5	7.9	.016	.106
4	88.0	51.6	5.8	28.0	6.2	20.4	.017	.151
6	86.0	40.4	6.4	22.6	7.5	37.0	.016	.141
8	82.9	33.6	8.0	14.1	9.1	52.3	.016	.129
11	76.0	20.7	11.7	17.0	12.3	62.3	.020	.138
14	71.0	00.0	5.0	3.9	24.0	96.1	.020	----
16	70.1	00.0	4.8	0.0	25.1	100.0	.019	----

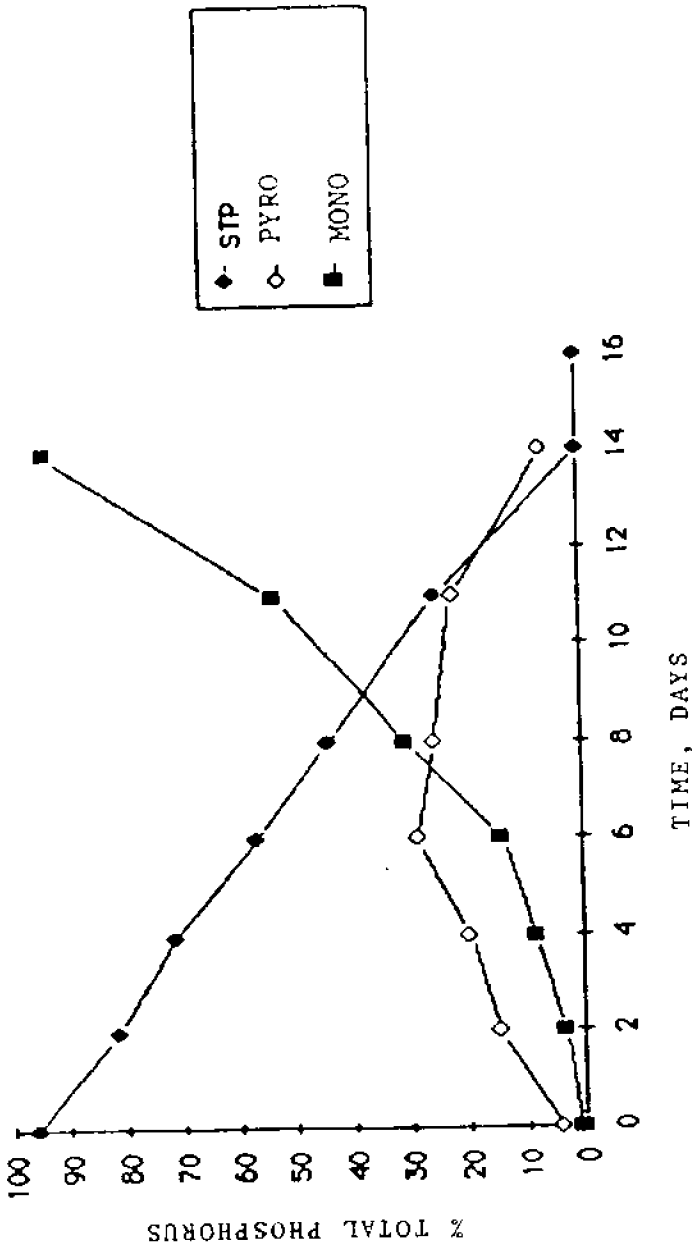

Figure 1. Enzyme catalyzed hydrolysis curves for sodium tripoly-phosphate in shrimp muscle at 10°C.

breakdown of pyro- into orthophosphate had begun to exceed its rate of formation from the residual tripolyphosphate. Similar type of curves were obtained during enyme-catalyzed decomposition when the phosphate distribution data from Tables 1, 3 and 4 were plotted.

CONCLUSION

In shrimp muscle, the decomposition of sodium tripolyphosphate or sodium hexametaphosphate occurred more rapidly in the presence of phosphatase. A six-fold increase at 5°C and a four-fold increase at 10°C were found in the rates of hydrolysis of STPP. During the hydrolysis of SHMP, the rates were as much as 10X faster at 5°C and 8X faster at 10°C than those found for the uncatalyzed hydrolysis reactions. Therefore, it can be assumed on the basis of these findings that a rapid breakdown of linear condensed phosphates in shrimp muscle is enzyme-induced.

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MICROBIOLOGY OF MULLET HARVESTED FROM A BRACKISH-WATER SITE

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Mullet is the number one finfish harvested in Florida and is second in total poundage only to shrimp (5). This trend in production undoubtedly will continue in the future.

In spite of the important role that mullet plays in the overall landings in Florida, little is known regarding the microbial flora of this fish. As recently as 1976, Florida Sea Grant published a series of articles on mullet, and under the section discussing its microbiology, acknowledged that little or no work has been done with this species. In fact, the microbiology of this species was discussed in terms of a "hypothesis" (6).

The only report available, to our knowledge, that discribes the microbiology of fresh mullet was published on the bacterial flora of mullet harvested in the Queensland, Australia area (3). These workers found, in order of decreasing recovery, the following genera: Micrococcus (49%), Pseudomonas (18%), coryneforms (12%), Moraxella (8%), Flavobacterium-Cytophaga (8%), Staphylococcus (2%), Bacillus (2%), Acinetobacter (1%), with other genera accounting for less than one percent of the total flora found.

In discussing the normal flora of any marine product, it must be recognized that many factors affect the numbers and kinds of microorganisms that will be recovered. These factors include location of harvest, location of sample on fish, environment on fish skin (kinds and availability of substrates), temperature, pH, salinity and method of enumeration (medium, temperature of incubation, etc.). While many of these factors represent fairly constant conditions, as shown by the relatively select group of genera found on many fish (7), it is the variable factors, i.e., environment, which probably account for the differences in the microbial flora noted in the literature.

The purpose of this work was to develop data on the normal flora of Florida mullet. The site of harvest was selected to insure maximum diversity of microbial species which was accomplished by harvesting the fish from brackish water canals that open to the Gulf of Mexico via the Suwannee River. In addition, sampling and plating was conducted within one hour of harvest. This was to insure the least possible opportunity for a change in population to occur due to such factors as handling, icing and storage. Sample dates were also selected to account for some seasonal variation that might occur in the flora due to water temperature and other extrinsic changes.

MATERIALS AND METHODS

Mullet were obtained from the canal system in Suwannee, Florida during December of 1982 and April and August of 1983 by hook and line. The samples were immediately placed in sterile plastic bags and brought

to the laboratory for analysis. Fish ranged in weight from about 400 to 500 g. Skin-on fillets were removed aseptically from the fish and used for analyses which included total and fecal coliforms, aerobic plate counts (APC), identifiction of APC colonies and Salmonella (8). The gut and gills were also removed and analyzed only for Salmonella. The fish were sampled and plated within one hour of capture.

Plate Count agar with an added 0.5% sodium chloride was used for aerobic plate counts. The fillets were blended for 2 minutes in a 1:10 dilution of Butterfield's phosphate buffer using a Hamilton Beach blender. Decimal dilutions of the homogenate were prepared and surface inoculated onto the prepared plates. Plates were incubated at 25C for 5 days. Following incubation, the plates were counted, and 50 randomly selected colonies were picked for identification at each sampling time. Salmonella and coliform counts were by standard methods (8). Identification of the isolates was by accepted procedures (2). All analyses were run in duplicate. All media were Difco products, Difco Laboratories, Detroit, MI.

RESULTS AND DISCUSSION

A previous report (6) suggested that the decreasing order of predominance of bacterial species on mullet would be coryneform bacteri: Moraxella-Acinetobacter, Micrococcus, Flavobacterium, Bacillus and the Vibrio-Aeromonas-Pseudomonas group. This order being affected by catch anatomical location of sample, etc. This suggested order is not different than one would expect based on studies of other species harvested from similar areas (1,4,7,9) and is, in fact, supported by actual studies of Australian mullet (3). This study listed Micrococcus (49%), Pseudomonas (18%), coryneforms (12%), and Moraxella (8%) as the four predominant species recovered from mullet. Our studies (Table 1)

Table 1: Ten most common genera of bacteria isolated from fresh mull

	Number of isolates			% of all
	December	April	August	Isolates
Corynebacterium	12	10	11	22
Bacillus	5		21	17
Staphylococcus	10	4		9
Pseudomonas	5	7		8
Flavobacterium		11		7
Moraxella		4	3	5
Aerococcus	8			5
Micrococcus			6	4
Aeromonas		4		3
Arthrobacter			3	2
% of 50 isolates	80	80	88	
Number of species isolated	15	27	12	

found similar genera in a somewhat different order. This is to be expected and points out the consistency of bacterial species that can be found on various seafoods even when harvested from widely differing geographical areas (7). It should be noted, as with the Australian study (3), and a study of iced rock shrimp (4), that gram positive bacteria can also be present on fresh seafoods and stored products as well, with 6 of the 10 most frequently isolated genera in this study being gram positive. It is not possible, due to the limited number of samples, to discuss in detail seasonal effects.

Corynebacterium was the only genus which was isolated at all sampling times which is probably related to a number of factors. The coryneforms are predominantly soil organisms (2) of limited physiological capabilities, able to grow over a wide range of temperatures and well adapted to a saprophytic existence. This is borne out by their frequent isolation from fresh seafoods (7); particularly those harvested from locations adjacent to soil (ponds, canals, and shallow brackish water areas). Bacillus, Staphylococcus, Pseudomonas, and Moraxella all were found at two sampling periods and are also commonly isolated from marine foods. The recovery of pseudomonads from fresh fish should be noted as possibly contributing to the ultimate spoilage of these products during iced storage, and their presence in spoiled samples should not be attributed solely to post harvest contaminations. All fillet samples analyzed for Salmonella were negative. Salmonella was isolated from only one sample of "guts and gills" during the December sampling period. In addition, coliform counts (Table 2) were low considering the nature of the harvest sites. The sites are located in canals within a housing development serviced by septic tanks. Aerobic plate counts were also low considering the harvest location and were rather consistent over the time span.

Table 2: Aerobic plate count and coliform profile for mullet samples

	December	April	August
Total Coliforms (MPN/g)	35[a]	12	10
Fecal Coliforms (MPN/g)	<2	<2	6.5
Aerobic Plate Count (/g)	3.4×10^4	3.8×10^4	1.3×10^4

[a]All data are the average of 2 samples.

While the data points out nothing unusual regarding the normal flora of mullet, it does reinforce the concept of commonality of the microbial flora of many species of seafoods. This commonality appears to be independent of the area of harvest as seen by comparison of our results to the Australian mullet data. While this data is limited, it probably accurately reflects the nature of the natural flora of Gulf mullet in general.

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RESEARCH IN SEAFOOD TECHNOLOGY

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From our perspective as the largest trade association representing the many and varied facets of the seafood industry let me outline for you the extent of our involvement in research and development.

(1) Irradiation
(2) Bisulfite Substitutes and Cooking Effects on
(3) Minced and Meat Combinations
(4) Surimi Nutritional Equivalency
(5) Drip Loss in Oysters
(6) CO_2 Extraction and Purification of Fish Oil
(7) Fractionation and Concentration - Fish Acids From Fish Oil
(8) Cholesterol in Shrimp
(9) Surimi from Menhaden
(10) Dairy, Pig and Beef Cattle Feeding Trails - Fish Meal
(11) Methods Development For:
    (a) Ciguatera
    (b) PSP Fly Bio-Assay
    (c) Bisulfite
    (d) Bone Detection
(12) Health Effects of Polyunsaturated Fatty Acids From Fish
(13) Depuration of Shellfish
(14) Surimi Quality Parameters
(15) Bisulfite Patient Hypersensitivity
(16) Controlled and Modified Atmosphere Packaging
(17) Fishery Product Standards:
    (a) Shrimp
    (b) Catfish
    (c) Fish Sticks and Portions
    (d) Minced Fish
    (e) Fish Steaks
    (f) Fish Blocks
(18) Allergies to Consumption of Crustaceans
(19) Containerization for Air Transport For Live Crabs
(20) Shelf Life vs. Oxidative Rancidity of Frozen Minced Fish
(21) Low Temperature Chilling vs. Regular Refrigerated Temperatures on Storage & Shelf Life
(22) Indicies - Total Volatile Base vs. Trimethylamine Analysis
(23) Prostaglandins Yields From the Marine Environment
(24) Glazing Technology and Practices
(25) Physical and Chemical Basis of the Mechanisms in Batter-Fried and Tempura-Type Fish Product Production
(26) Effect of Methyl-Mercury on the Human Fetus
(27) Parasite Data Bank

(28) Flexible Retort Pouch Acceptance for Seafoods
(29) Selenium in Seafood and Its Protective Effects Against Breast Cancer
(30) Effect of Vacuum Packing on Texture

Other: (1) Video Tape Teaching Tools
(a) Blue Crab Meat Pasteurization
(b) Can Seam Inspection
Under development: Sanitation
(2) 26 Chapter Correspondence Course

In summary, NFI is deeply committed to the advancement of technology, information transfer and the commercial development of products and processes that will enable our industry to keep pace with the ever changing face of the U.S. food picture.

To meet the challenges of the 1990's should be a hallmark for all of us.

LOW DOSE GAMMA IRRADIATION OF VIBRIO CHOLERAE IN CRABMEAT (CALLINECTES SAPIDUS)

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INTRODUCTION

Pasteurization doses of irradiation may be used to increase the refrigerated shelf life of whole crabs and crabmeat. When stored at 1°C the shelf life of precooked jumbo crabs is extended to 72, 96, and 111 days after being treated with 93, 230, and 450 krad, respectively. The unirradiated crabs stored under the same conditions spoiled in 16 days (9). Unirradiated crabs stored at 7°C spoiled in only two days (10).

Crabmeat treated with 250 krad and stored 3.3°C was acceptable after four weeks, while treated with 500 krad extended the shelf life to five weeks. The unirradiated crabmeat spoiled in one week (9).

Irradiation increases the crabmeat's shelf life by reducing the number of spoilage bacteria present. Pasteurization levels of irradiation can destroy up to 95% of the spoilage organisms in the microflora (2).

Besides the non-pathogenic organisms which occur as part of the crab's natural microflora, pathogens may be present if the water from which the crabs were taken is contaminated. Contaminated crabs were responsible for 11 cases of Vibrio cholerae infections in Louisiana in 1978 (5).

The purpose of this study was to determine the effect of pasteurization levels of gamma irradiation of V. cholerae in crab meat and the effect of cold storage on the survival of the organism in the crab meat.

MATERIALS AND METHODS

Preparation of crabmeat homogenate. Fresh crabmeat was purchased from local seafood markets. The unsterile crabmeat homogenate was prepared by blending two parts crabmeat with one part sterile saline in a Waring blendor to form a smooth paste. The sterile crabmeat homogenate was prepared in the same manner, except that the crabmeat was sterilized at 121°C for 15 minutes before blending with saline.

The V. cholerae 01 inoculum was prepared and added to the homogenate as described by Grodner and Hinton (3,4) to contain a final concentration of 10^7 V. cholerae/g.

Irradiation and storage of the samples. Homogenate samples were placed in 250 ml Nalgene bottles and treated with 0, 25, 50, or 100 krad as previously described (3,4).

After irradiation the homogenate samples were stored at 4°, 0°, or -8°C for 21 days. The number of V. cholerae surviving the irradiation treatment and the cold storage was enumerated at 0, 7, 14, and 21 days (3).

Irradiation of V. cholerae in sterile and unsterile crabmeat homogenates and storage of the homogenates were performed in triplicate. The number of V. cholerae surviving was calculated by determining the average number of V. cholerae recovered from three samples subjected to the same irradiation treatments and cold storage.

RESULTS AND DISCUSSION

Effect of irradiation. Pasteurization levels of irradiation were effective for destroying approximately 10^7 V. cholerae/g. In the unsterile homogenates, the original population of 5.18×10^6 V. cholerae/g was reduced to 1.43×10^3/g with 25 krad (Figure 1). No V. cholerae were recovered from the homogenates treated with 50 krad or 100 krad.

In the sterile homogenates, the number of V. cholerae recovered was reduced from 3.07×10^6/g to 5.0×10^0/g with 25 krad. No V. cholerae were recovered from the sterile homogenates treated with 50 krad or 100 krad.

Effect of time. Results in the unsterile homogenates treated with 0 krad and 25 krad were similar (Figure 2). In the unirradiated unsterile homogenates stored at 4°C, the number of V. cholerae recovered decreased from 5.18×10^6/g on day 0 to 8.87×10^5/g on day 7 (Figure 2). The V. cholerae population decreased further to 1.07×10^3/g on day 14, and no V. cholerae were recovered on day 21. In the homogenates treated with 25 krad, 1.43×10^3/g were recovered on day 7. No V. cholerae could be recovered on day 14 or day 21.

Vibrio cholerae were able to survive for a longer period of time in the sterile homogenate stored at 4°C (Figure 3). The number of V. cholerae recovered from the unirradiated sterile samples on day 0 decreased from 3.07×10^6/g to 1.82×10^5/g on day 21.

The number of V. cholerae recovered from the sterile homogenates treated with 25 krad and stored at 4°C also decreased with time. There were 5.0×10^0 V. cholerae/g isolated from the homogenates on day 0. The population decreased to 1.33×10^0 V. cholerae/g on day 14. No V. cholerae were recovered from the homogenates on day 21.

Although there was a constant decline in the V. cholerae population, the organism was recovered from the unsterile unirradiated homogenates stored at 0°C for 21 days (Figure 4). The V. cholerae population decreased steadily from 5.18×10^6/g on day 0 to 9.13×10^3/g on day 21 in the unirradiated homogenates. In the unsterile homogenate treated with 25 krad, the number of V. cholerae decreased from 1.43×10^3/g on day 0 to 8.0×10^2/g on day 14. No V. cholerae were recovered from the homogenates on day 21.

The unirradiated sterile crabmeat stored at 0°C showed only a small decrease in its V. cholerae population during the 21 day storage period (Figure 5). On day 0, 3.07×10^6 V. cholerae/g were isolated from the crabmeat. On day 7 and day 14 approximately 4.5×10^5 V. cholerae/g were recovered. The number of V cholerae decreased slightly to 1.69×10^5/g on day 21. In the sterile homogenates treated with 25 krad, the original V. cholerae population was reduced from 5.0×10^6/g on day 0 to 4.0×10^0/g on day 7. No V. cholerae were recovered from the homogenates on day 14 or day 21.

Vibrio cholerae were also able to survive for 21 days in the unirradiated unsterile homogenates stored at -8°C (Figure 6). The initial population of 5.18×10^6 V. cholerae/g decreased to 1.15×10^4/g on day 21. In the unsterile homogenate treated with 25 krad a small increase in the V. cholerae population was noted between day 7 and day 14. After 21 days no V. cholerae were recovered from the homogenates.

In the unirradiated sterile homogenates stored at -8°C there was a large decrease in the initial V. cholerae population of 3.07×10^6/g on day 0 to 3.35×10^2/g on day 7 (Figure 7). The number of V. cholerae recovered decreased further to 1.93×10^1/g on day 21. On day 0 there were 5.0×10^0 V. cholerae /g isolated from the homogenates treated with 25 krad. Only 1.33×10^0 V. cholerae/g were recovered on day 7, and none were recovered on day 14 or day 21.

Effect of temperature. In the sterile homogenates stored at 0°C or 4°C, there was very little difference in the number of V. cholerae recovered from homogenates receiving the same irradiation treatments. In all cases there was less than a 1 log difference in the number of organisms recovered from the sterile homogenates exposed to equal doses of radiation and stored at 0°C or 4°C. There were fewer V. cholerae recovered from the sterile homogenates stored at -8°C, however. While the V. cholerae population in the homogenates stored at 4°C or 0°C remained relatively constant (Figures 3 and 5), there was a large decrease in the V. cholerae population of the homogenates stored at -8°C. After 21 days the number of V. cholerae recovered had decreased over 5 log cycles. This indicates that V. cholerae is adversely affected by freezing temperatures under certain conditions. It has been reported that no V. cholerae were recovered from sterile crabmeat homogenates inoculated with 10^6 organisms/g after 21 days of storage at -20°C (7).

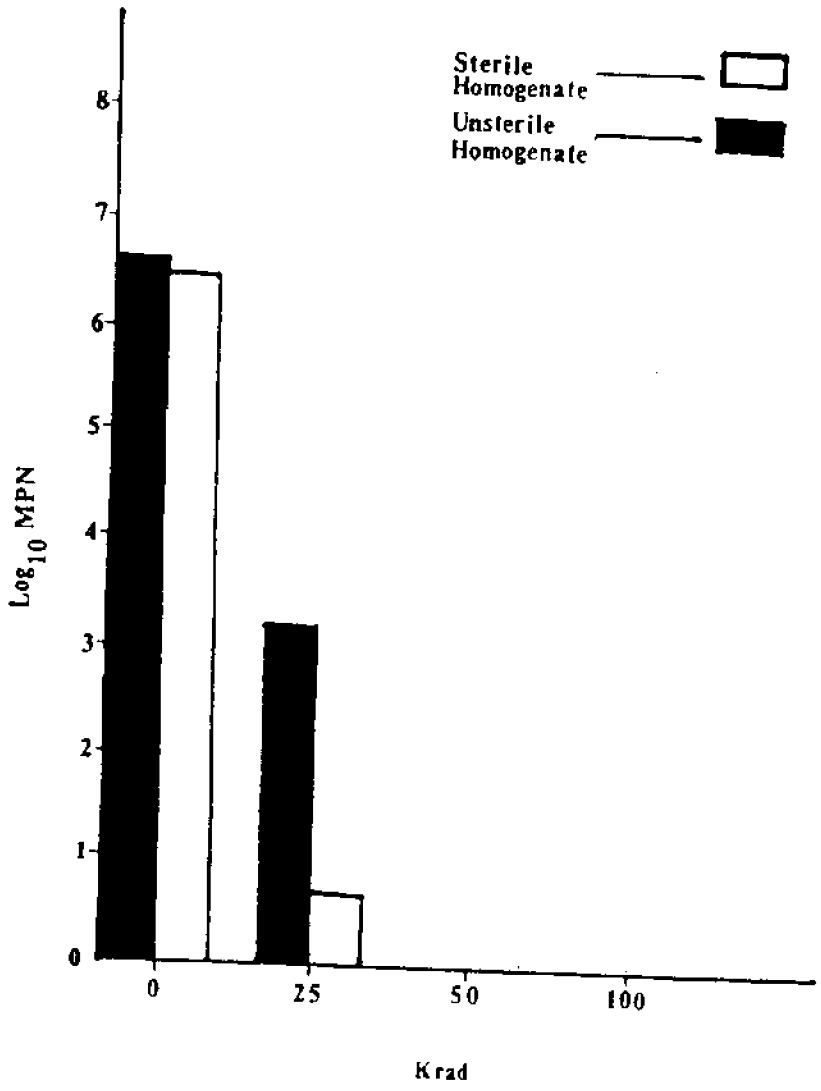

Figure 1. Effect of low dose gamma radiation on Vibrio cholerae #5875 in crabmeat homogenates. MPN values are averaged of 3 replications.

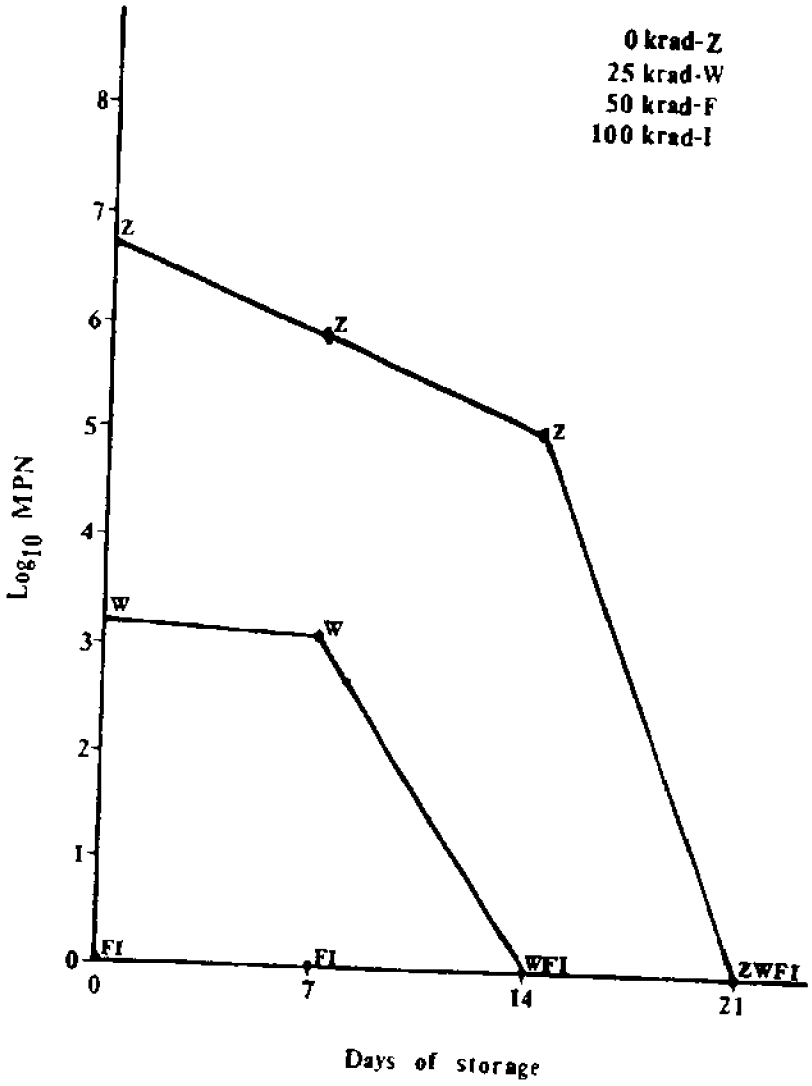

Figure 2. Survival of Vibrio cholerae #5875 in unirradiated and irradiated unsterile crabmeat homogenates stored at 4C. MPN values are averages of 3 replications.

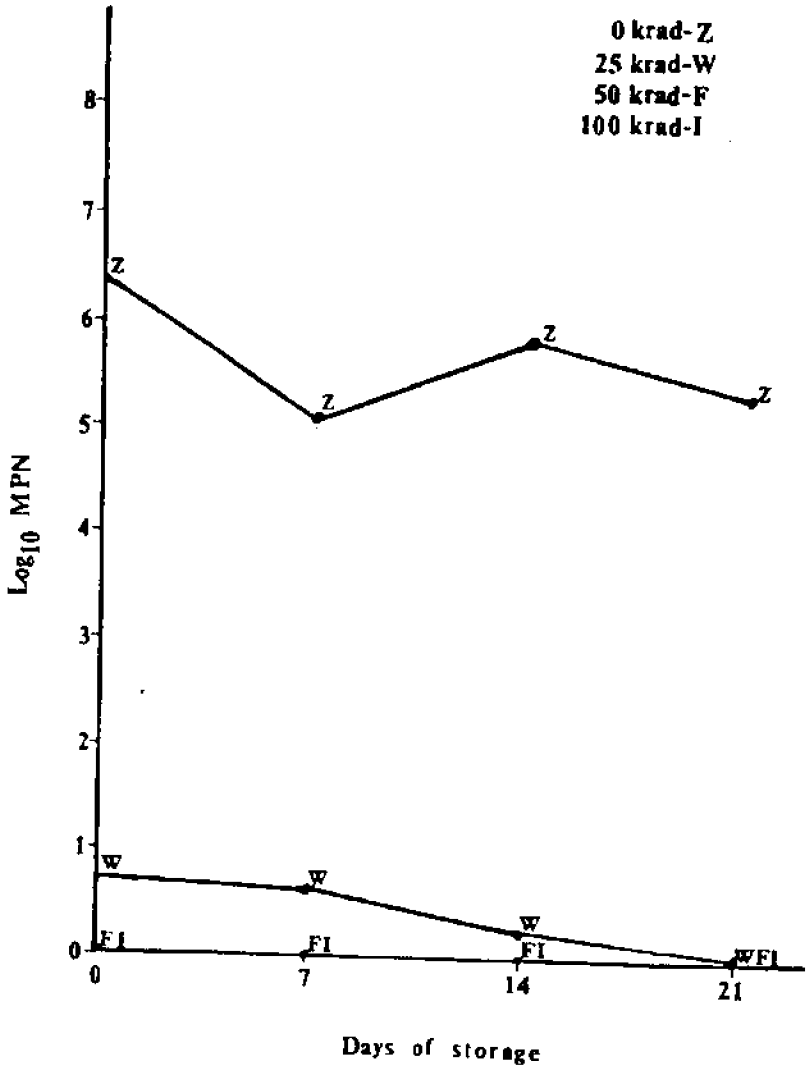

Figure 3. Survival of Vibrio cholerae #5875 in unirradiated and irradiated sterile crabmeat homogenates stored at 4C.MPN values are averages of 3 replications.

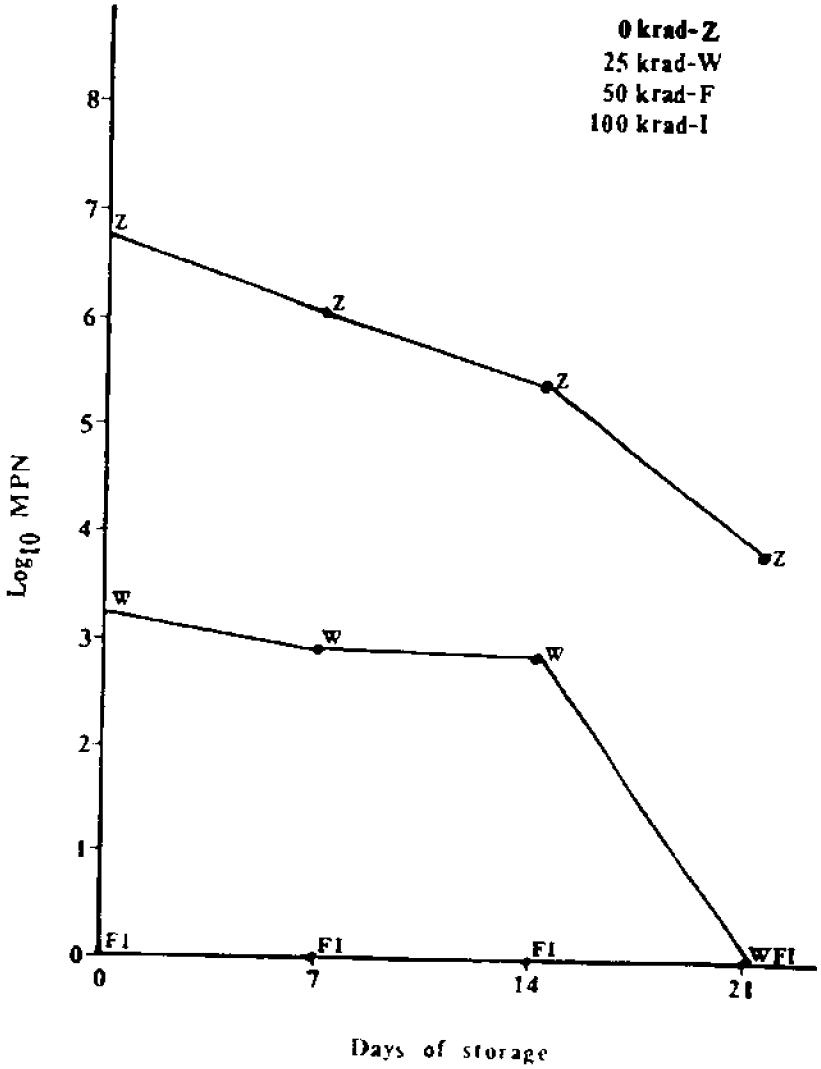

Figure 4. Survival of Vibrio cholerae #5825 in unirradiated and irradiated unsterile crabmeat homogenates stored at 0C. MPN values are averages of 3 replications

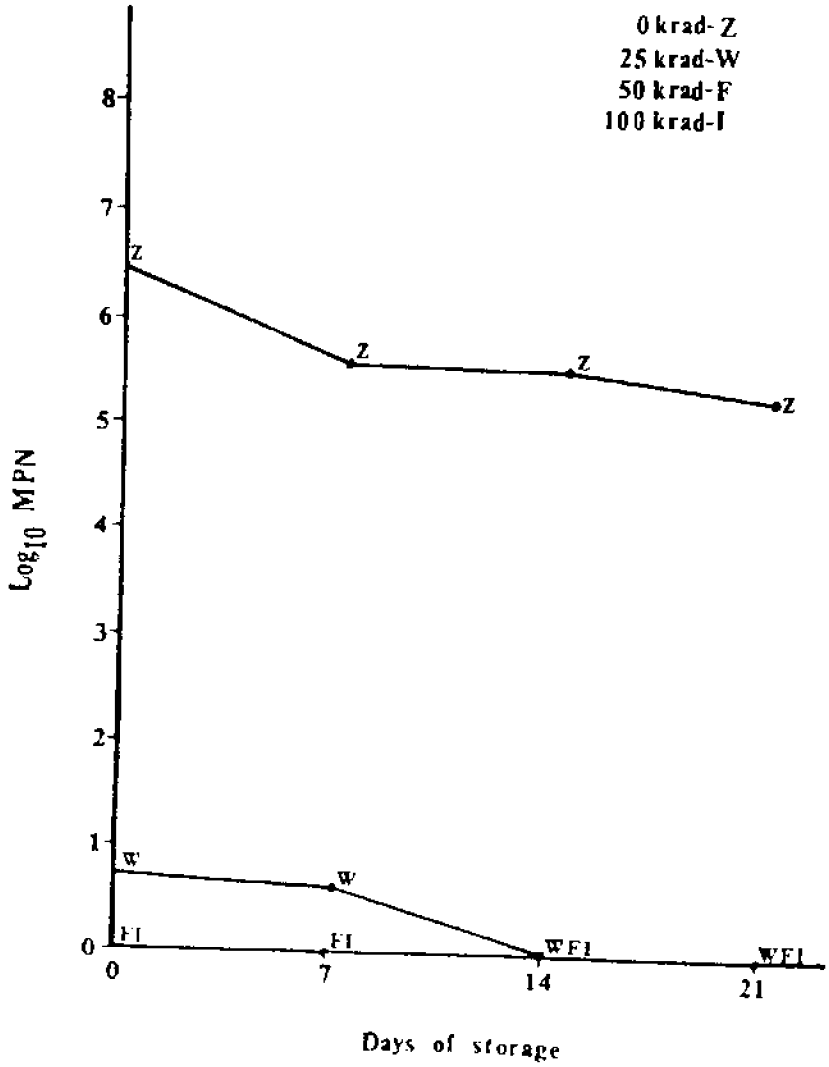

Figure 5. Survival of Vibrio cholerae #5875 in unirradiated and irradiated sterile crabmeat homogenates stored at 0°C. MPN values are averages of 3 replications.

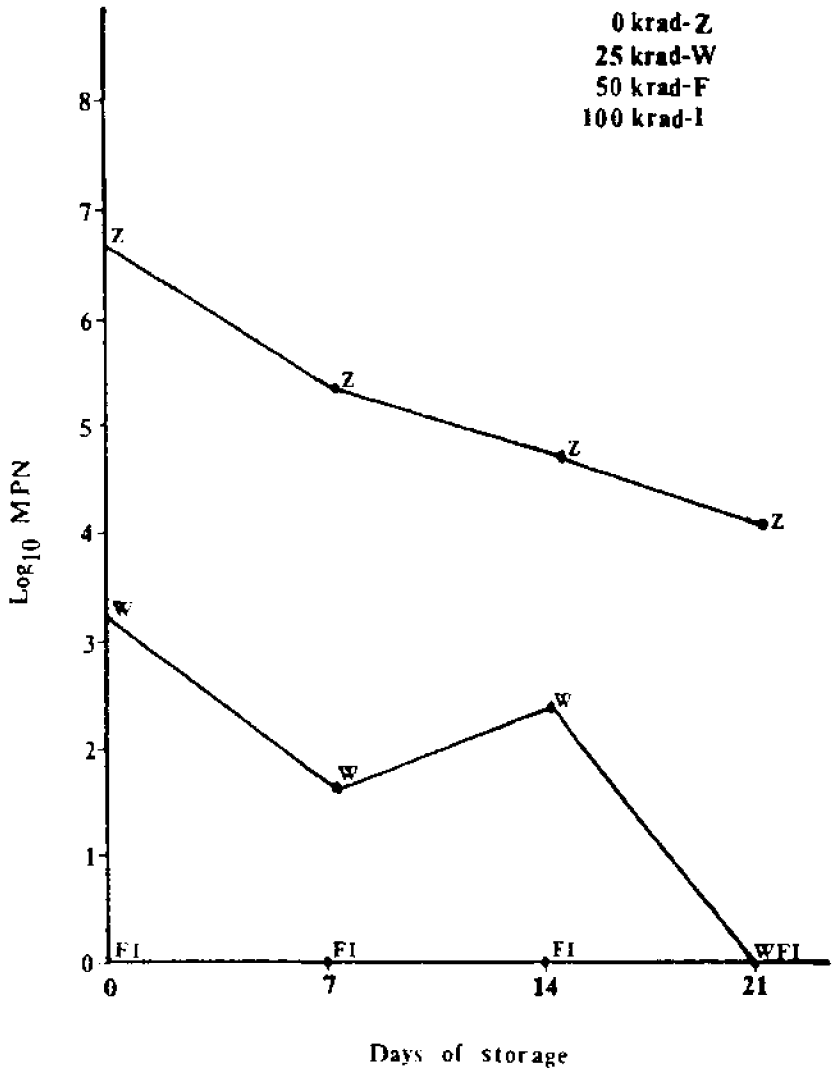

Figure 6. Survival of Vibrio cholerae #5875 in unirradiated and irradiated unsterile crabmeat homogenates stored at -80. MPN values are averages of 3 replications.

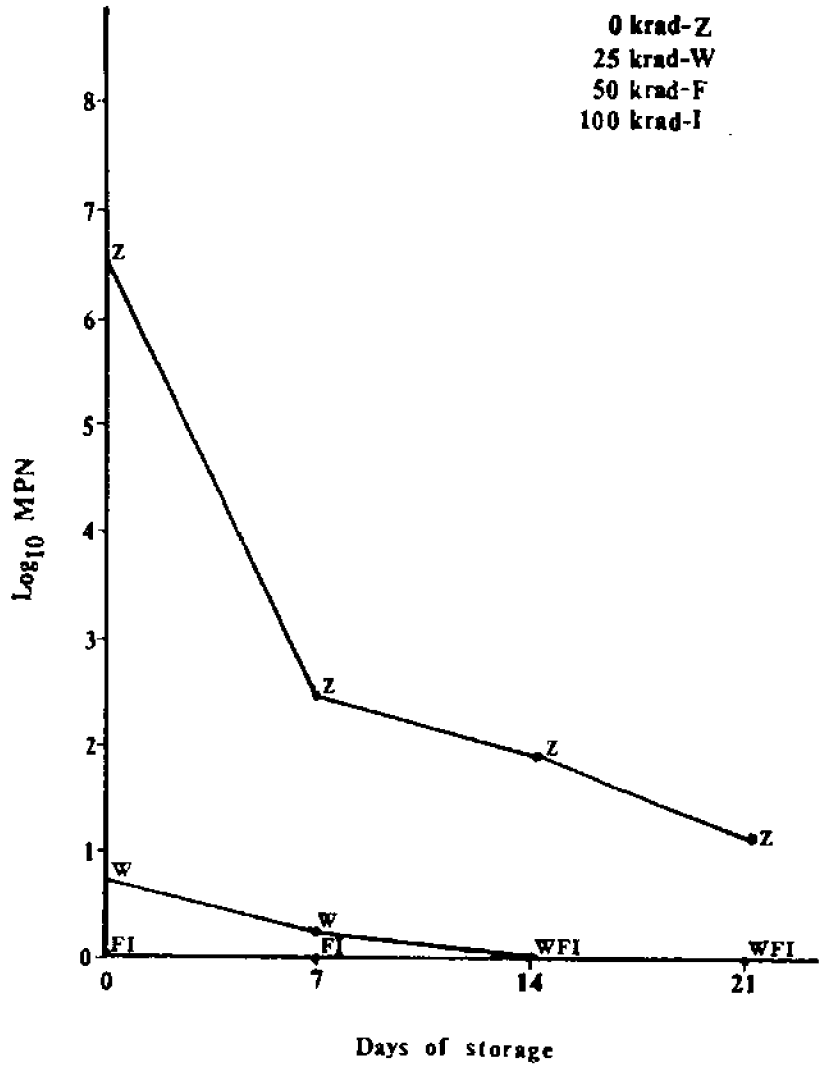

Figure 7. Survival of Vibrio cholerae #5875 in unirradiated and irradiated sterile crabmeat homogenates stored at -8C. MPN values are averages of 3 replications.

Up to day 14 there were few differences in the number of V. cholerae recovered from the unsterile homogenates stored at 4°, 0°, or -8°C, though fewer organisms were recovered from the homogenates stored at -8°C than at the other temperatures. On day 21 however, more V. cholerae were recovered from the homogenates stored at -8°C than at the other temperatures. On day 21 however, more V. cholerae were recovered from the homogenates stored at -8°C than from the homogenates stored at 4° or 0°C. In fact, no V. cholerae were recovered from the unsterile homogenates stored at 4°C for 21 days. The probable reason for this is discussed below.

Survival in sterile and unsterile homogenates. Because commercially prepared crabmeat is picked from the shells after the crab has been boiled for approximately 20 minutes, the microflora is similar to that of a semiprocessed food (10). Fresh crabmeat contains an average of 4.0×10^5 microorganisms/g (1). When the crabmeat is stored at 2° to 4°C for 12 and 15 days, the natural microflora increases to between 2.0×10^9/g and 1.2×10^{10}/g. During storage the dominant microflora also changes. Some researchers have reported that Moraxella, Pseudomonas, and Acinetobacter are the predominant bacteria present in crabmeat (6). Shiflett (10) reported that in crabmeat treated with up to 100 krad, 99% of the surviving organisms were Achromobacter, and that Achromobacter was able to survive refrigerated storage. Members of the Achromobacter group have now been renamed as either Moraxella or Acinetobacter. The V. cholerae in the unsterile homogenates had to compete with the natural flora of the crabmeat to survive.

The effect of competition was most evident in the unsterile homogenates stored at the warmest temperature (4°C). In the unirradiated unsterile samples stored at 4°C, no V. cholerae could be recovered on day 21. In the homogenates treated with 25 krad, no V. cholerae were recovered on day 14 or day 21. In the sterile homogenates however, V. cholerae were recovered from the unirradiated homogenates on day 21, and from the homogenates treated with 25 krad on day 14.

More V. cholerae were also recovered from the unirradiated sterile homogenates stored at 0°C than from the unirradiated unsterile homogenates stored at 0°C.

No V. cholerae were recovered from the sterile homogenates treated with 25 krad and stored at 0°C for 14 days, and no V. cholerae were recovered from the unsterile homogenates treated with 25 krad and stored at 0°C for 21 days. There were fewer V. cholerae in the sterile homogenates on day 0, however.

More V. cholerae were recovered from the unsterile homogenates stored at -8°C than from any of the other unsterile homogenates. The lower temperature may have retarded the growth of the natural microflora of the crabmeat. Less competition allowed the V. cholerae to survive for longer periods of time.

Fewer V. cholerae were recovered from the sterile homogenates than from the unsterile homogenates stored at -8°C. The sterilization treatment to which the sterilized homogenates were subjected may have destroyed substances (e.g. proteins) in the fresh crabmeat which protected the V. cholerae from the harsh effects of the freezing.

The native proteins in the unsterilized homogenates have a greater capacity for binding water than the denatured proteins in the sterilized homogenates (7). Native proteins may therefore effect the rate of crystallization during freezing and allow the bacteria to survive for a longer period of time.

The results indicate that V. cholerae can survive for some time in crabmeat under certain conditions. The organism can be destroyed in crabmeat by using pasteurization levels of irradiation.

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Transfer of a Dinoflagellate Produced Toxin to Tissues of the Black Sea Bass, Centropristis striata

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ABSTRACT

Black sea bass, Centropristis striata, were exposed to the toxic benthic dinoflagellate Gambierdiscus toxicus in an effort to demonstrate the food chain hypothesis for ciguatoxin accumulation in fish. Among selected tissues examined, following intraperitoneal injection of dinoflagellate cells, visceral tissue generally had the greatest toxicity. However, toxicity was undetectable in visceral and muscle tissue after 160 hours from initial exposure but was detectable in liver tissue for up to 336 hr. After 24 hours from initial exposure, higher concentrations of toxin were detected in whole fish homogenates when fish were exposed to whole algal cells, either gavaged or injected intraperitoneally, rather than when toxin was presented as an algal extract. However, after 48 hr, only fish that had been injected intraperitoneally with whole cells had detectable concentrations of toxin.

Chromatographic separations of either algal or fish extracts, using HPLC-fluorescence, indicated the presence of only a single toxin, presumably the algal toxin, maitotoxin. Although toxin was transferred from a dinoflagellate origin to fish tissue, no indication of metabolic or other biochemical conversion of algal-produced toxin to other toxins, including ciguatoxin, was found in fish tissue.

INTRODUCTION

It has been hypothesized that ciguatoxic fish result from a food chain transfer of toxin or toxin progenitor produced by one or more species of benthic marine dinoflagellates. This general concept was first proposed by Randall (1958) and later reiterated after several observations of toxic dinoflagellate consumption by various species of fish (Helfrich and Banner, 1963; Banner et al., 1966; Yasumoto et al., 1971). These observations resulted in hypothetical, but unvalidated, evidence that herbivorous fish consume toxic dinoflagellates and either store or biochemically convert the toxin to ciguatoxin (Helfrich and Banner, 1963).

Several mechanisms that could explain transfer of ciguatoxin, or its progenitor, from a benthic dinoflagellate population to herbivorous fish have been proposed. Specifically, there are three possible mechanisms that could explain the trophic transfer of toxin. These are, (1) the

direct transfer and storage of ciguatoxin from marine dinoflagellates to fish (2) storage of ciguatoxin in fish tissue following biochemical alteration by fish of a progenitor produced by dinoflagellates, and (3) storage of dinoflagellate-produced ciguatoxin or fish-produced ciguatoxin as relatively metabolically inert chemical entities in fish tissue. The storage of toxins infers that transfer occurs as a physiologically inert toxin to higher trophic levels within the marine food web, becoming actively toxic only when ingested by a mammalian consumer.

The objective of the present study was to document the possible transfer of dinoflagellate-produced toxin(s) (tentatively identified as maitotoxin and ciguatoxin) to fish tissue under laboratory controlled conditions. Substantiation of toxin transfer between dinoflagellates and fish would allow for further examination of physiological mechanisms involved in toxin accumulation in fish tissues as well as suggest methods for routinely producing ciguatoxin within a controlled laboratory environment. Ciguatoxin production is necessary in order to have sufficient material for identifying its chemical structure as well as developing additional techniques for chemical detection of the toxin.

MATERIALS AND METHODS

Black sea bass, Centropristis striata were caught approximately 10 miles seaward of Charleston, S.C. and transported to the laboratory using a continuous flow sea water live tank. All fish were acclimated for approximately 6 weeks in a closed re-circulating system having Instant Ocean seawater maintained at a salinity of 27 ppt and a temperature of 21° ± 1.0°C. The system consisted of elliptical fiberglass, 1000 liter raceways having 100 liter biological filters (Millikin, 1982).

Toxic algal extract of the benthic dinoflagellate Gambierdiscus toxicus was prepared by concentrating algal cultures via filtration and drying cells under nitrogen. A known weight of dried cells was extracted with 80% methanol for 48 hr. using a wrist arm shaker. Extract was weighed and brought to volume with 80% methanol. Culture conditions for growing G. toxicus were as described by Babinchak et al., (1986).

Whole cells being prepared for gavaging fish were made into a slurry by gently mixing with isotonic salt solution and pipetted into empty gelatin capsules (No. 00, Eli Lilly and Co., Indianapolis, IN). Each capsule contained a cell mass equivalent to ≈ 100 mouse units (MU) (a mouse unit was defined as the amount of extracted toxin necessary to produce a LD_{50} value when toxin was intraperitoneally injected into ≈ 20 g mouse) of toxicity. The capsule was forced into the stomach cavity of fish with a polished glass rod.

In addition to gavaging with encapsulated algal cells, algal cells having a known toxicity were incorporated into formula feed. The feed

consisted of 25% fish meal, 5.5% linseed oil, 4.2% vitamin-mineral mix, 31% cellulose, and 33% gelatin. The diet was hand mixed and allowed to dry into a pliable gel and then was cut into 10 mm cubes. Ten representative cubes were homogenized with 80% methanol in a blender (Waring, Commercial) for 1 hr and the sediment further extracted with 80% methanol at room temperature for 48 hr with a wrist arm shaker (Burrell, Model 75). Extract plus filtrate from the homogenation were combined, dried under nitrogen gas and resuspended in 1% Tween 20 (Fisher Scientific) for mouse bioassay. Each 10 mm cube was found to contain 13.6 $\pm$ 3.2 MU of toxicity. Pellets were introduced to fish in the raceways. Since fish ingested each pellet on command, a daily ration of $\approx$ 50 MU of toxicity per fish per day could be assured by visual observation during the daily feeding.

Following the given incubation times (Figures 1 and 2), including a zero time control (i.e. fish that were either gavaged or injected with toxic material and then sacrificed), fish were frozen. Frozen animals were then homogenized in a whole animal blender (Waring, Commercial) or if individual organs were monitored the organs were dissected from semi-frozen animals and homogenized in a blender. The homogenized slurry was then extracted sequentially with acetone-methanol-chloroform, (2:1 v/w in each case), and filtered through silicic acid at room temperature. The sample was then dried by roto-evaporation, split and extracted with methanol and chloroform. The methanol fraction was dried under nitrogen gas and suspended in 1% Tween 20 (Fisher Scientific) for injection into mice. Mouse assay results were based on time-to-death analysis and body temperature depression as described by Sawyer *et al.*, (1984). The time-to-death analysis was done using four mice for each dose of toxic extract tested and then constructing a dose response curve. Using this dose response curve and computed LD_{50} values, a mouse unit (MU) was defined as the amount of extracted toxin necessary to produce a computed LD_{50} when intraperitoneally injected into $\approx$ 20 g mouse. Based on results from the dose response curve, the formula for estimating algal toxicity was determined to be: mouse units (MU) = $[K_1]\,[(TD)^{K_2}]^{-1}$ where K_1 = 80.07, K_2 = 1.141, and TD = time to death.

A stock solution of *G. toxicus* toxin extract used to construct a calibration curve, had a concentration of 1.12 mouse units (MU) $(\mu l)^{-1}$. The calibration curve for algal toxin was linear in a range of 15 to 200 MU, had a slope of 0.428, a Y intercept of 13.08, and a regression coefficient of 0.987. Purified saxitoxin used to standardize analytical response was obtained from the U.S. Food and Drug Administration. *G. toxicus* extract was chromatographically separated from the crude, methanol extract of *G. toxicus* using a DuPont model 820 high performance liquid chromatograph (HPLC). An Alltech 25 cm column packed with 10 μ, CN (cyano) substrate was used. Methods for establishing a calibration curve, analytical standardization and subsequent conversion to units of toxicity were as previously reported (Sick, *et al.*, 1986).

FIGURE 1. Comparison of toxicity among selected fish tissues following an initial i.p. injection. Injections consisted of whole algal cells equivalent to 100 MU of toxicity injected per fish. Values are averages and standard deviations based on three replicate fish per treatment.

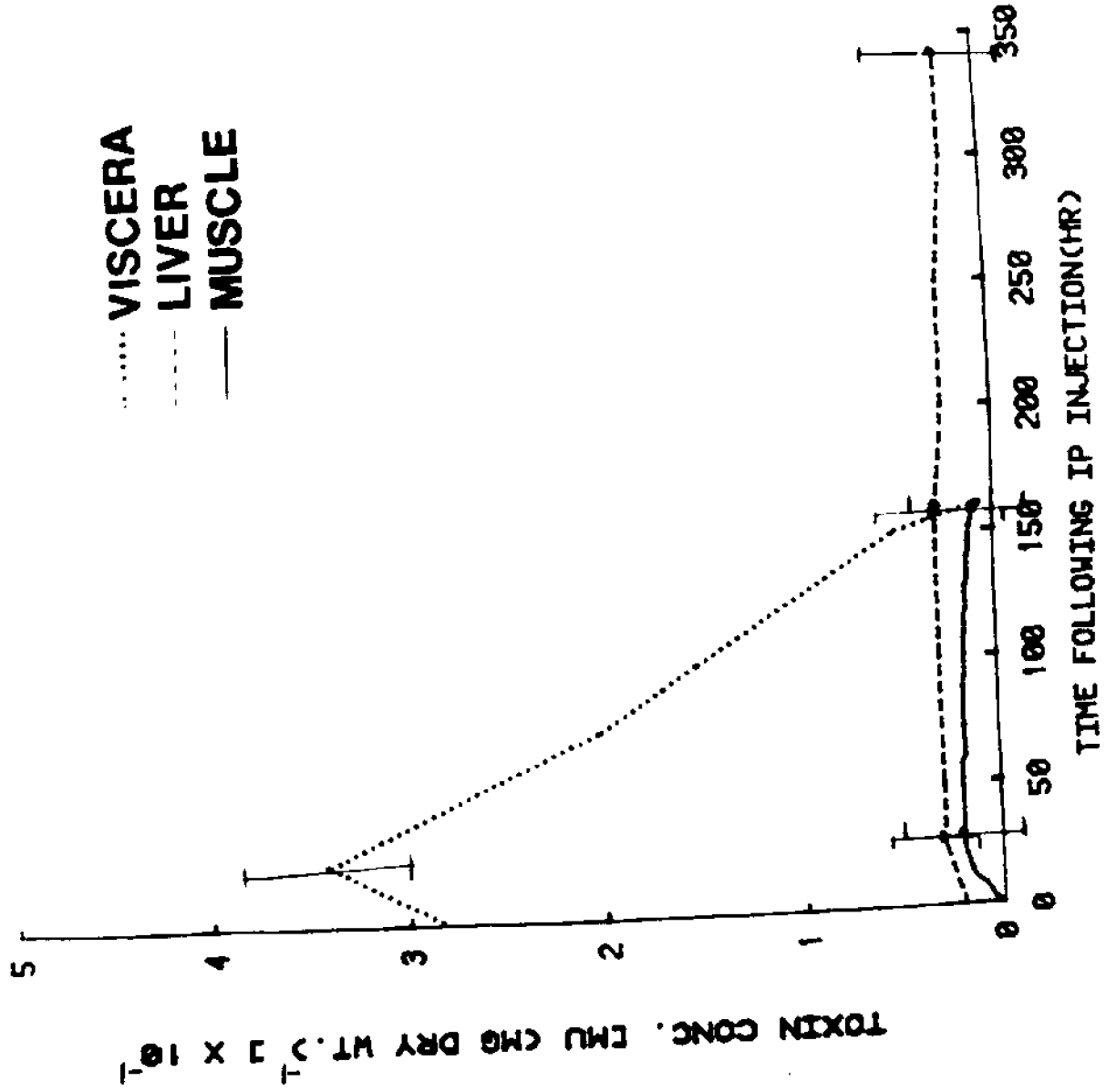
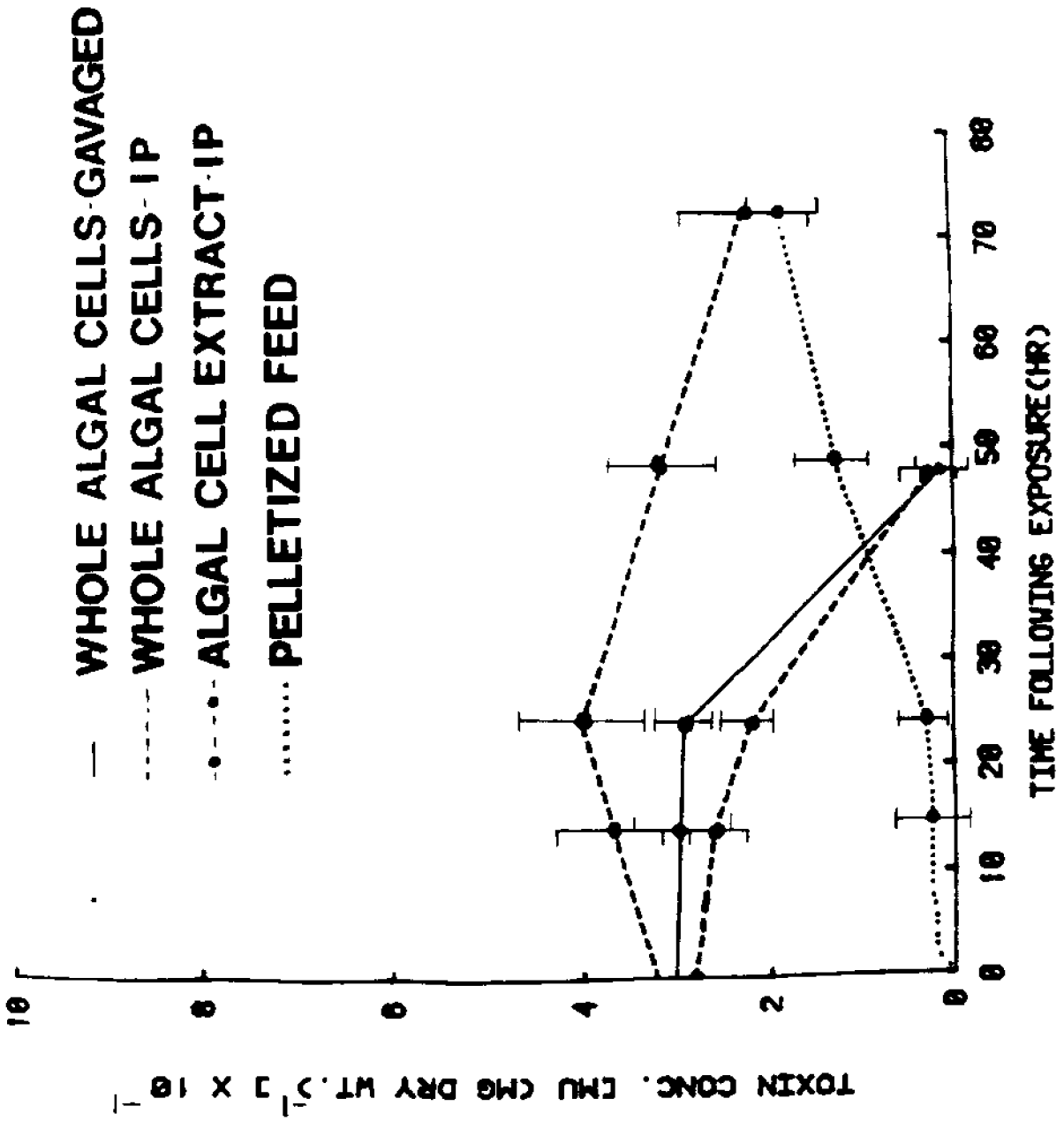


FIGURE 2. Comparison of toxicity, in whole animal homogenates among fish exposed to toxicity via selected routes of exposure. Each fish, except those fed pelletized feed, were exposed to an initial dose equivalent to 100 MU of toxicity. Values are averages and standard deviations based on three replicate fish per treatment.

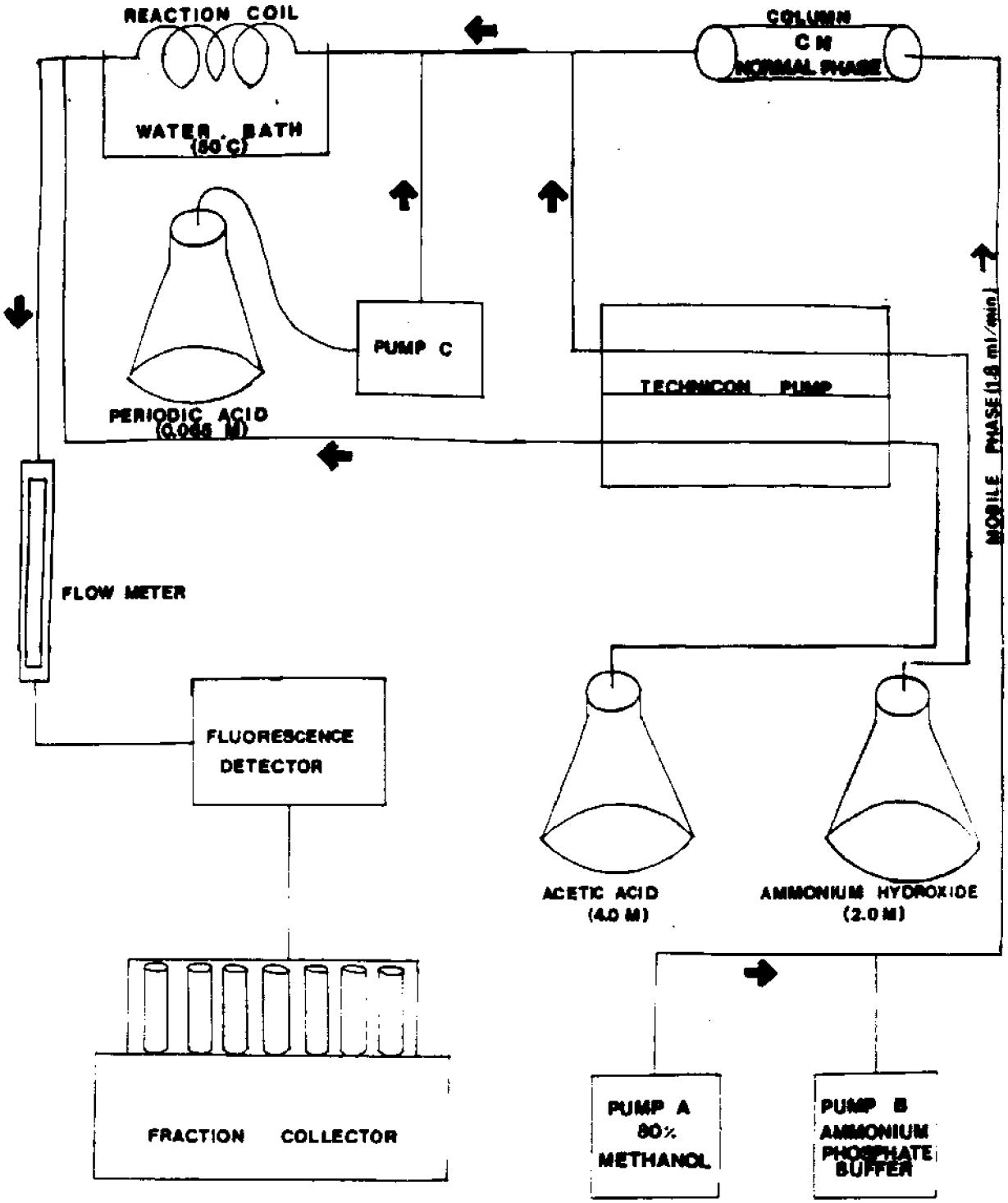


Both algal and fish extracts were monitored for the possible presence of multiple toxins using HPLC chromatographic separation. Direct detection of toxin was possible by forming alkaline oxidized derivatives of the respective toxin and using fluorescence detection. The system consisted of three separate reagent reservoirs tieing into the mobile phase line after the column (Figure 3). The three reagent reservoirs contained periodic acid, ammonium hydroxide, and acetic acid at concentrations of 0.065 M, 2.0 M, and 4.0 M respectively. Reagents were pumped into the mobile phase manifold via a single Technicon Auto Analyzer peristaltic pump. Flow rates of each reagent were regulated by a series of check valves and clamps to insure a pH of 9.5 to 9.8 in an oxidizing atmosphere in the reaction coil (Figure 3) and the acetic acid flow rate regulated to insure a pH of 5.5 to 5.7 before the mobile phase entered the fluorometer. The mobile phase consisted of either water or ammonium phosphate buffer (pH 7.1) and 80% methanol run at an elution gradient from 50 to 100% and a flow rate of 1.3 ml min^{-1}. Residence time in the reaction coil was approximately 90 sec. The fluorometer was set at an excitation wavelength of 340 nm and emission wavelength of 410 nm.

Although parameters for eluting toxin from the LC column were set up for G. toxicus toxin and no standards for ciguatoxin were available, these parameters probably were adequate for ciguatoxin, based on published information. Specifically, Higerd (1986) and Tachibana (1980) both reported separation of ciguatoxin (from a fish extract) using a methanol-water gradient similar to that employed in this investigation.

Recovery efficiency of toxin from minced fish tissue was estimated using non-toxic fish visera that had been minced and spiked with semi-purified G. toxicus toxic extract. The extract was semi-purified by successively eluting an aliquot of crude toxin from an HPLC, CN column, as described above, and collecting toxic fractions three separate times. Spiked tissue samples for recovery estimates were prepared by using a single fish, having a wet weight of ≈ 250 g, for each treatment tested (i.e. each solvent combination, Table 1). Each fish was homogenized in a blender, the slurry divided into three equal portions by volume, each portion spiked with 100 MU of semi-purified G. toxicus toxic extract, and each portion mixed with a micro-homogenizer (Virtis, Model 23). The first solvent combination examined consisted of extracting with acetone (2:1, v/w) for 1 hr while blending at room temperature followed by extraction with methanol for 1 hr (2:1, v/w) also using a blender. A second treatment consisted of extraction with acetone-methanol, as described above, but conducting all extractions at 110°C for 30 min. In the third treatment, extraction of tissue was done as described for acetone-methanol only using methanol for both parts of the extraction. Similarly the acetone-methanol-chloroform extraction was conducted at room temperature, each extraction done while blending, and 2:1, v/w proportions used for each solvent. Following extraction by each of these four treatments, the sample was filtered through silicic acid, extracted

FIGURE 3. Schematic diagram of the HPLC-fluorescence system including the post column derivatization apparatus.



with methanol-chloroform (1:1 v/v in each case), and the methanol fraction bioassayed as described above.

Data resulting from experiments concerning toxin distribution among tissues and those involving various routes of toxin exposure were subjected to analysis of variance for each time period sampled. Test of probability of a significant difference (95% confidence level) among treatments were applied to treatments having a significant variance (Duncan, 1955).

RESULTS

Recovery of G. toxicus toxin from spiked fish tissue preparations using a acetone-methanol-silicic acid extraction was ≈ 1% (Table 1). An attempt to eliminate the possibility of enzyme degradation of toxin was made by heating the spiked tissue homogenate to 110°C for 60 min. Recovery of algal toxin from the heated homogenate, using the acetone-methanol preparation also yielded an average recovery of only 1 to 2%. Replacing acetone with methanol and extracting using a methanol-methanol partitioning also failed to significantly increase extraction efficiency. However, using a series of acetone-methanol-chloroform extractions, prior to elution over silicic acid, improved recovery efficiency from an average of 1% to an average of 38%.

Twenty-four hours following exposure, sea bass that had been gavaged with encapsulated whole algal cells, having a total toxicity of ≈ 100 MU per fish, had significantly ($P < 0.05$) higher visceral tissue toxicity than found in other tissues (Figure 1). However, the toxicity measured in visceral tissue decreased to less than measurable toxicity after 160 hr. Relatively low concentrations of toxicity was measured in muscle tissue, between 0 and 160 hr, and in liver tissue excised from fish samples at all time periods following initial gavaging.

Among several selected routes for introducing toxin to fish, whole algal cells introduced via intraperitoneal (i.p.) injection resulted in significantly higher ($P < 0.05$) tissue concentrations of toxin (at 24 and 72 hr after exposure) than when toxin was introduced via whole cells gavaged or i.p. injection of algal cell toxin extract (Figure 2). In addition, toxicity in fish injected i.p. with algal cells could be detected up to 72 hr after injection in contrast to only 48 hr for fish gavaged or injected with cell extract. Although not directly comparable to gavaging or extract exposures, fish fed pellets equivalent to a daily ration of 50 MU were found to progressively accumulate toxin over a 72 hr period. Between initial exposure and 24 hr, concentration of toxin in tissues of fish exposed to 50 MU of algal toxin per day via pellets were significantly lower ($P < 0.05$) than fish exposed to only a single, initial dose of 100 MU by exposure to treatments using whole cells or an algal cell extract. From 48 to 72 hr, accumulation due to periodic

feeding was significantly higher ($P < 0.05$) than when fish were exposed to toxin via whole cells, i.p. or gavaged.

TABLE 1. Extraction efficiencies for toxin extracted from fish tissue with selected solvent systems. Concentrations are averages and standard deviations based on three replicate extractions.

TREATMENT	CONC DETECTED [MU $(G)^{-1}$]	EFF. (%)
ACETONE - METHANOL	1.0 ± 0.05	1.0
ACETONE - METHANOL - HEAT	1.2 ± 0.07	1.2
METHANOL - METHANOL	1.5 ± 0.8	1.5
ACETONE - METHANOL - CHLOROFORM	8.0 ± 6.7	38.0

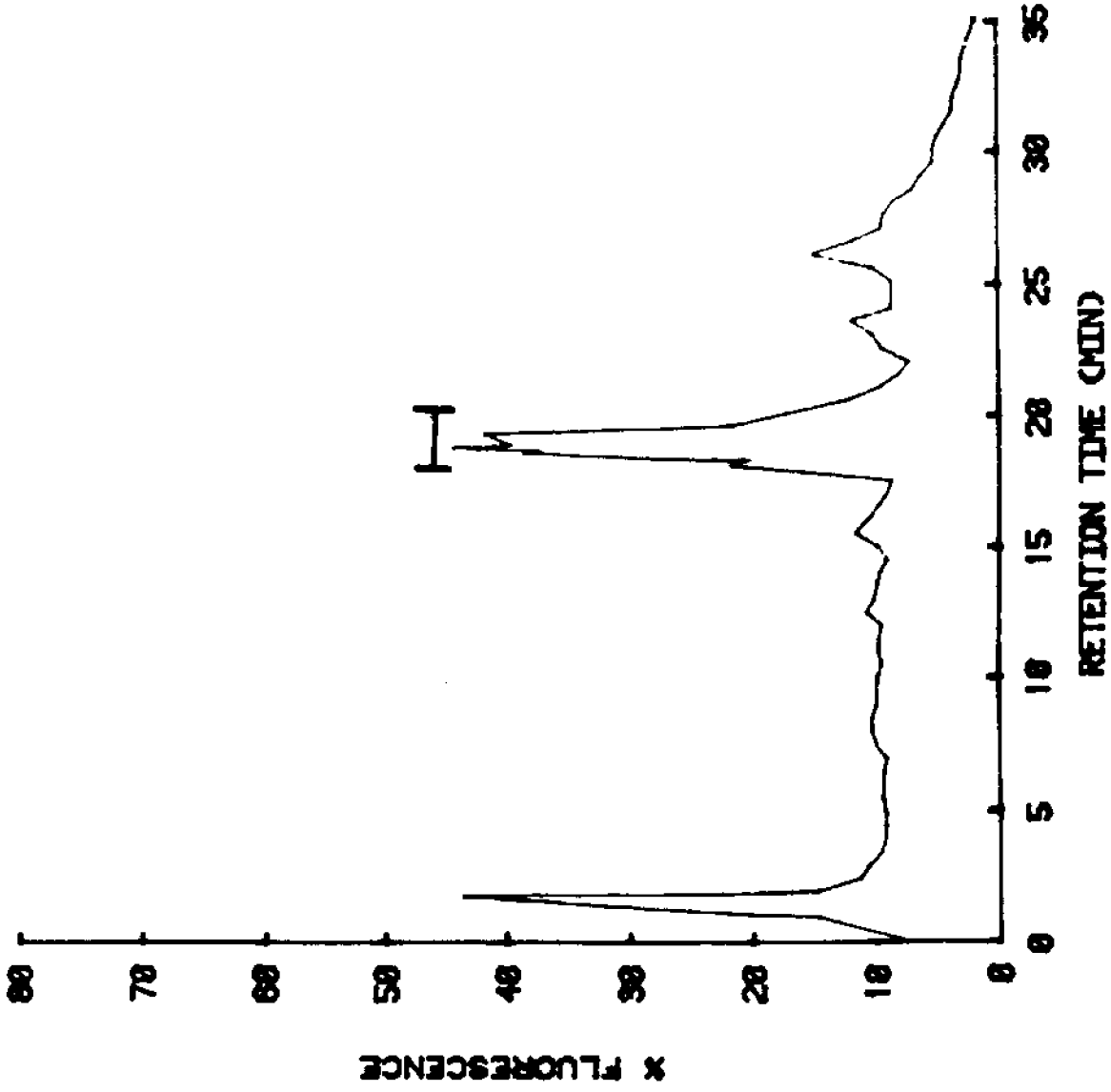
Liquid chromatographic analysis of toxin extracted from fish tissue indicated a single toxic fraction that eluted with the same retention time as algal toxin (Figure 4). Fish extract was from viscera of fish that had been exposed for 48 hr to 100 MU via whole algal cells injected i.p.

DISCUSSION

Toxicity observed among any of the fish tissues analyzed in this study probably represented accumulation and storage of algal toxin rather than any bioconversion endogenously in fish tissue. For example, toxicity in visceral tissue declined after 24 hr following initial injection and no corresponding increase in toxicity in muscle tissue was observed in succeeding hours, as may be indicative of the metabolism of toxin (Figure 1). The visceral toxicity was probably indicative of algal cellular residue from i.p. injection. The relatively low concentrations of toxicity measured in both liver and muscle tissues may have represented contamination of tissues from cellular debris being enzymatically degraded in the peritoneal cavity or dispersion of toxin from the peritoneal cavity to liver and muscle via the circulatory system. The fact that chromatographic separations identified only a single toxin eluting at the same time as algal toxin in extracts of visceral tissue (Figure 4) also suggested that no bioconversion of algal produced toxin had occurred in the black sea bass.

Although the food chain hypothesis for the biogenesis of ciguatoxin (Randall, 1958) assumed that either ciguatoxin is produced by a benthic dinoflagellate and transferred to fish or a progenitor produced by dinoflagellates is bioconverted to ciguatoxin by fish, there is no scien-

FIGURE 4. Chromatographic results of HPLC-fluorescence analysis of fish visceral tissue extract using continuous flow, post-column derivatization. The chromatogram represents results from an homogenate pool of three fish each injected i.p. with 100 MU of toxin and allowed to incubate for 48 hr. The only area identified by mouse bioassay of chromatographic fractions as being toxic was a single peak which eluted with the same RT as the toxic fraction from dinoflagellate extracts (see Sick et al., 1986).



tific evidence for either assumption. In cases concerning accumulation of natural toxins other than ciguatoxin in finfish, the mitigating toxin such as saxitoxin, paralytic shellfish poison, and probably tetrodotoxin, is thought to be transferred from algae to fish through the trophic process (Mosher and Fuhrman, 1984). Helfrich and Banner (1963) observed that herbivorous fish continuously feeding on semi-wild stocks of the benthic dinoflagellate, G. toxicus, in laboratory controlled conditions became ciguatoxic, as ascertained via mouse bioassay. Since there is tentative evidence that G. toxicus can produce both maitotoxin and ciguatoxin (e.g. Withers, 1981; Tindall et al., 1984), the occurrence of ciguatoxic fish in the Helfrich and Banner (1963) experiment could have simply been transfers of ciguatoxin from algae to fish rather than bioconversion by fish. Given that most known marine biotoxins in fish are thought to result from trophic transfer rather than produced endogenously, an exogenous rather than endogenous source of ciguatoxin in ciguatoxic fish is probable.

Although the hypothesis regarding trophic transfer of ciguatoxin from a dinoflagellate source to herbivorous and, subsequently, to carnivorous fish assumes that ciguatoxin can be produced by selected species of dinoflagellates, there is only circumstantial evidence that the toxin can indeed be produced by an algal source. One of the dinoflagellates implicated in the production of ciguatoxin, G. toxicus isolated from populations of benthic dinoflagellates in the Pacific Ocean, has been reported to produce both maitotoxin and ciguatoxin (Yasumoto et al., 1976; Withers, 1981). Similarly, Tindall et al. (1984) reported that G. toxicus, isolated from several sites in the Virgin Islands, produced ciguatoxin plus at least one other toxin. However, all of the above reports were based on liquid-liquid partitioning between various solvents and aqueous phases. Such partitioning does not preclude the possibility of a single toxin partitioning among two or more phases of differing polarities. In contrast, Sick et al. (1986) and Babinchak et al. (1986) using solvent extraction coupled with ion exchange chromatography were able to detect only a single toxin in extracts of laboratory cultured G. toxicus. In addition to chemical separations employed, the differences in number and types of toxins reported for analysis of G. toxicus may reflect differences in culture conditions, both in the field and among laboratory conditions, as well as among different geographic strains of G. toxicus.

The biochemical and physiological mechanisms associated with the occurrence of ciguatoxin in fish tissues haven't been investigated. Physiological effects of ciguatoxin at the cellular and tissue levels, however, have been examined in rodents. The principal physiological effect of ciguatoxin is in depolarization of the cellular membrane by directly causing increased sodium permeability (Rayner et al., 1969). Because changes in membrane permeability may affect more than just sodium channels, it is probable that intracellular and intercellular ionic

balance may be altered by both an algal produced toxin called maitotoxin (Yasumoto et al., 1976) and ciguatoxin. Calcium is known to be antagonistic to cellular alterations in membrane permeability caused by ciguatoxin (Rayner et al., 1969). Algal extract, presumably maitotoxin, has also been reported to cause physiological effects indistinguishable from those caused by ciguatoxin (Sawyer et al., 1984). Although the physiological mechanisms aren't known, fish may physiologically mobilize ingested ciguatoxin to inert lipid deposits rather than metabolize the ingested toxin. Mammals, on the other hand, may metabolize ingested toxin through the processes for intermediary metabolism. This hypothesis would explain how ciguatoxin may be transferred through the marine web without imparting toxicity until ingested by a mammal.

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Seafoods: New Considerations for Coronary Artery Disease and Health

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INTRODUCTION

Coronary artery disease, (CAD), is the number one cause of death in the United States, accounting for 600,000 lives a year. Over the past 20 years increasing emphasis has focused on ways to prevent this disease. A recent trend is the evaluation of seafood in the diet and its role in prevention of coronary artery disease. This article attempts to review what is currently known about the effects of seafood on the development of CAD, and to also put into perspective the other nutritional and health aspects of seafoods in the diet.

SEAFOOD AND CAD

Several epidemiologic studies since the early 1970's have pointed to the decreased incidence of CAD in populations that consume large amounts of fish and marine mammals. Eskimos in Greenland, who consume 400 gram (about 12 oz.) of fish per day, have a substantially lower incidence of CAD compared with Westerners (1). The CAD death rate in Japan is very

low, where average fish consumption is 100 gm/day. Within Japan, CAD is lowest in Okinawa, where fish consumption is twice as high as the rest of Japan (2). Also within Japan, a comparison study of a farming district to a fishing district, where fish consumption averages 90-250 gm/day, showed much lower CAD in the fishing village.

A long term study was recently reported, involving 852 middle-aged, urban Dutch men (age 40-59) without evidence of CAD in 1960, who were followed prospectively for 20 years. One-hundred percent follow-up was obtained (3). Careful dietary histories were secured by trained dietitians interviewing both patient and spouse regarding weekday and weekend food consumption, estimates of diet intake away from home, and estimates of food purchasing. In-home measurements of food validated the recall data. Seventy-eight men in the study group died of CAD. Risk ratios of death from CAD to fish consumption were made over the 20 year period. The ratios were adjusted for confounding variables such as cigarette smoking, blood pressure, serum total cholesterol, subscapular skinfold thickness, physical activity, energy intake, dietary cholesterol, prescribed diet, and occupation. Results showed a greater than sixty percent reduction in death from CAD for men eating as little as 7 oz of fish per week. Considering a standard portion of fish is 3.5 ounces, (and Americans tend to eat larger portions) this would suggest a substantial benefit from eating a small portion of fish twice weekly.

PROTECTIVE EFFECT

Part of the explanation for this protective effect is thought to be found in the unique lipid compounds found in the seafood. Fish oil contains EICOSAPENTAENOIC ACID (C20:5, omega-3), also called EPA, and DOCOSAHEXAENOIC ACID (C16:5, omega-3), also called DHA. These fatty acids differ from others in two ways: 1) they tend to be very unsaturated, and 2) the initial point of unsaturation begins at the third carbon from the methyl end of the fatty acid (Fig. 1). Other major families of unsaturated fatty acids are oleic acid (C18:1 omega-9), linoleic acid (C18:2 omega-6) and linolenic acids (C18:3 omega-3). All of these fatty acids are derived largely from vegetable and nut oils. Members of a particular omega-3 or n-3 family may be metabolically converted to more proximally unsaturated (towards the carboxyl end) or chain-elongated fatty acids, but no conversion from one family to another occurs in humans (4).

These unusual fatty acids seem to exert an effect on human lipid homeostatis. Serum triglycerides can be dramatically reduced by reasonable amounts of fish in the diet. This has been noted in studies on normal human volunteers, hypertriglyceridemic patients and diabetics with hypertriglyceridemia (6, 7). The mechanism of the effect of omega-3 fatty acids on the levels of VLDL (the major carrier of triglycerides) is uncertain, but many studies suggest a depression of hepatic VLDL synthesis (8, 9). However, since triglycerides are not well documented as a risk factor for CAD, it is unlikely that fish protect against CAD through alterations in triglyceride only.

The more critical question to address is what effect fish lipids have on serum lipid profiles of those with hypercholesterolemia, especially elevated LDL cholesterol. There are beneficial effects on serum total cholesterol and variable effects on serum HDL reported in the literature (8, 10, 11). A recent study reported on the effects of feeding fish oil, vegetable oil (safflower oil and corn oils) or control diets to Type IIb and Type V hyperlipoproteinemic patients. Fish oil (20 gm EPA/day) uniformly reduced VLDL and LDL cholesterol in both groups. In addition, vegetable oil actually raised VLDL levels, illustrating that polyunsaturates of marine and vegetable sources have dissimilar metabolic effects (5).

In contrast, plasma cholesterol was not lowered in Type IIa and IIb patients when fed 6 gm/day of n-3 fatty acids as a capsule of MaxEPA$*$. When 16 gm/day of n-3 fatty acids were fed to Type V patients, plasma triglycerides decreased by 58% and 35%, respectively. LDL cholesterol, however, elevated by 7%. Additionally, in the previously mentioned Dutch study, the protection was present even after controlling for total serum cholesterol (3). Current studies are equivocal and it appears that further work is needed before there can be clear cut understanding of the role of omega-3 fatty acids in relation to hypercholesterolemia (12-14).

OMEGA-3'S AND PROSTANOIDS

EPA is the prescursor to platelet thromboxane A_3 and prostaglandin I_3 made in vessel walls. EPA competes successfully with arachidonic acid for

cyclooxygenase to make the 3-series prostaglandins rather than the 2-series. Thromboxane A_2, made in platelets from plant oil n-6 fatty acids, has pro-aggregating properties. Prostaglandin I_2 made in vessel walls from plant source arachidonic acid has potent anti-aggregating effects. Prostaglandin I_3 prevents platelet aggregation, however, and thromboxane A_3 has no effect on aggregation. Therefore, when n-3 fatty acids predominate in the diet, blood coagulation occurs less readily. Studies have shown that people who eat more than 4 gm of EPA per day (standard 3.5 ounce portion of fish would contain 2-3 gm EPA) have elevated levels of thromboxane A_3 and prostaglandin I_3. It is also known that there is a reduction in the plasma concentration of thromboxane A_2 and prostaglandin I_2 after eating of n-3 fatty acids (15, 16, 17). In support of this concept, as early as 1940 it was reported that Japanese fishermen and Eskimos had increased bleeding times and decreased platelet aggregation (18).

A recent study (19) also evaluated the effects of fish oil on the function of polymorphoneutrophils and monocytes. The release of arachidonic acid (a stimulator of platelet aggregation) and its leukotrine metabolites was inhibited in both cell types, and the leukocyte response to these metabolites was also diminished (decreased chemotaxis and endothelial cell adherence). In addition, fish oil ingestion seems to directly decrease monocyte adherence to the arterial endothelium. The initial phases of the atherosclerotic process require monocytes to adhere to arterial endothelium. This is followed by migration through the vessel

wall, with the subsequent conversion of monocytes to macrophages. Macrophages stimulate cholesterol accumulation within the blood vessel intima, and also release growth factors that stimulate proliferaton of arterial smooth muscle cells. Therefore, fish oil effects on monocytes represent yet another pathway by which artherosclerosis is prevented.

A recent study (20) tried to compare the effects of EPA against another proposed intervention for prevention of CAD. Volunteers with known atherosclerosis were fed 10 grams of EPA per day to achieve comparable blood effects to those reported in Eskimos, as measured by red blood cell membrane lipid omega-3 fatty acid levels. Initially, elevated levels of thromboxane A2 in these volunteers were reduced by 58%, but did not achieve a return to normal levels, and were not equivalent to the reduction seen from taking a single dose of 325 mg of aspirin. Many modalitoes affect the multiple pathophysiologic mechanisms in the development of atherosclerosis: for example, exercise, aspirin and other platelet inhibitors, fish oils, monounsaturated oils in olive oils, and dietary fiber. The relative value of each, and the possible synergy among various modalities remains an intriguing question.

THE FAT COMPOSITION OF FISH

Americans are consuming fish in record amounts. Average per capita consumption of seafood in 1985 was 13.6 lb. This is 1/2 lb higher than 1984 (21). Seafood is classified as fish and shellfish. In simplified terms, fish may be subdivided into round fishes such as salmon, tuna and

striped bass and flat fishes such as soles, flounders, and halibut. Shellfish are characterized by an exoskeleton and have no backbone. There are three catagories, two of which are commmonly consumed. Crustaceans are exemplified by crab, shrimp, lobster, and crayfish. There are three types of mollusks: 1) bivalves such as clams, oysters, scallops, and mussels, 2) univalves such as abalone and conch, and 3) cephalopods such as squid, octopus and cuttlefish (22).

In general, the darker the meat of the fish, the fattier the meat will be. It is commonly accepted that fish with greater than 5% fat in raw muscle are high fat fish. Species with less than 5% fat in raw muscle are considered low fat. Fat content of fish varies within species due to time or season of year, spawning status, diet, age, location of muscle, geographic location and degree of cultivation. For example, the dark muscle of cod, a lean fish, contains 3 times as much lipid as does the light muscle. Table 1 shows the fat content of selected fin and shellfish. Food composition data on fish are inexact. These data represent composites and should be used as approximations, not exact figures.

There is no definite linear relationship between the total fat content or omega-3 fatty acid content and possible health benefit. Table 2 shows ranges of the omega-3 fatty acid content of selected species. It is not implicit in this table that salmon imparts greater health benefits than, say, clams due to its higher omega-3 fatty acid content. The optimum level of total omega-3 fatty acids as well as the ratio of omega-3

to omega-6 fatty acids is unknown and further study is readily needed in this area. In addition, cooking has been shown to cause appreciable fat losses from high fat species (23, 24). It is premature to recommend selected species based on approximate unsaturated fatty acid content, because there is no known correlation to health.

THE GREAT CHOLESTEROL-SHELLFISH CONTROVERSY

In the past it was thought that shellfish were very rich sources of cholesterol and were typically avoided by patients on low cholesterol diets. New methods of cholesterol determination utilizing gas liquid chromatography have established that, with few exceptions, shellfish are not excessively high in cholesterol. Table 3 shows cholesterol content of selected cooked shellfish species. Shrimp have approximately 100 mg cholesterol per 3.5 oz. raw serving, but the range between species extends from 58 to 182 mg/100 gm raw. Lobster contains about 100 mg/3.5 oz. serving, blue crab contains about 102 mg/3.5 oz., clams, oysters and mussels range from about 55-190 mg/3.5 oz. raw serving. Finfish are somewhat lower in cholesterol, content ranging from about 21-58 mg/3.5 oz raw product in low fat fish such as herring, halibut and pollack and 60-9 mg/3.5 oz. in (raw) higher fat species such as salmon, mackerel, and bigeye tuna. It is well understood that total cholesterol content of a food is not a clear indicator of the effect on plasma cholesterol of eating that food. Non-cholesterol steroids in shellfish may in fact compete with cholesterol for gastrointestinal absorption. Furthermore

shellfish contain negligible amounts of saturated fats. Therefore, because so little is known about bioavailability of cholesterol and food-food interactions, it is not yet possible to say whether shellfish or finfish consumption would be better as protection against CAD.

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Table 1: Total Fat Content of Selected Raw Finfish and Shellfish (raw)*

Finfish	Approximate range
	Percentage
High fat	
•Mackerel	1.4 - 9.6
•Salmon	1.8 - 11.6
•Albacore tuna	3.0 - 6.3
Lowfat	
•Snapper	0.3 - 1.8
•Grouper	1.3 - 7.5
•Halibut, Altlantic	0.4 - 3.3
Mollusks	
•Abalone	0.4 - 0.6
•Clam	0.5 - 2.0
•Scallop	0.4 - 1.1

Sidwell, V.: Chemical and nutritional composition of finfishes, whales, crustaceans, mollusks, and their products. NOAA Technical Memorandum NMFS F/SEC-11, U. S. Dept. of Commerce, NOAA, Nat'l Marine Fisheries Service, 1981.

Table 2: Unsaturated Fatty Acids of Selected Species of Finfish and Shellfish

	Range(gm/100gm raw portion)
Finfish*	
High fat	
•albacore tuna •sockeye salmon •Atlantic mackerel	3.30 - 6.25
Low fat	
•channel catfish •yellow flounder •ocean perch	1.06 - 2.46
Crustaceans	
•blue crab •Alaska king crab •spiny lobster •shrimp (different species)	0.14 - 0.23
Mollusks**	
•scallop •squid •oysters •clam •albalone	0.02-0.72

*Exler, J, Kinsella, J. E., and Watt, B. K.: Lipids and fatty acids of important finfish. New data for nutrient table s J. Am. Oil Chem. Soc 52:154, 1975.

**Exler, J. and Wihrauch, J. L. Comprehensive evaluation of fatty acid in foods. J.A.D.A. 71: 518.

Table 3: Approximate Total Cholesterol Content of Shellfish, mg/3.5 oz. Raw Serving

Mollusks	
•Abalone	111
•Razor clams	107
•Mussels	55
•Oysters	190
•Scallops all species	116
Crustaceans	
•Blue crab	102
•Lobster	48
•Shrimp	100
Finfish	
•Herring	21
•Halibut	47
•Pollack	58
•Salmon	66
•Mackerel	60
•Bigeye tuna	92

Sidwell, V.: Chemical and nutritional composition of finfishes, whales, crustaceans, mollusks, and their products. NOAA Technical Memorandum NMFS F/SEC-11, U. S. Dept. of Commerce, NOAA, Nat'l Marine Fisheries Service, 1981.

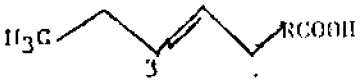
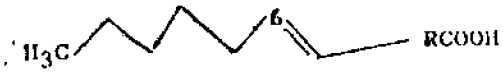
Family	Fatty Acid	Structure	Dietary Sources
omega-3	Eicosapentaenoic Acid (C20:5ω3)		Marine oils, fish
omega-6	Linoleic Acid (C18:2 ω3)		Vegetable oils
omega 9	Oleic-Acid (C18:1 ω9)		Vegetable oils animal fats

Figure 1. The Major Families of Polyunsaturated Fatty Acids. NEJM 1985, 312:(19) 1210,

From: Phillipson, B. et al.(5).

FISH OIL RESEARCH AIDS FISHING INDUSTRY AND CONSUMERS

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INTRODUCTION

Since 1978, the Charleston Laboratory of the Southeast Fisheries Center, National Marine Fisheries Service (NMFS), has had an active program in the chemistry, biochemistry and analysis of marine fats and oils. Our investigations have, in the past, included studies in oxidation of marine oils, rancidity development in refrigerator- and freezer-stored fish, fatty acid composition of endangered sea turtle oils, seasonal and geographic differences in fatty acid composition of commercial menhaden oils, and lipid and fatty acid compositions of certain underutilized species. In addition, staff of the Charleston Laboratory have worked actively with the National Fish Meal and Oil Association in development of a petition for submission to the U.S. Food and Drug Administration (FDA), requesting GRAS (Generally Recognized As Safe) status for refined and partially hydrogenated menhaden oil (PHMO). Our current and future activities fall into one of three major categories: (a) lipids of latent resources; (b) menhaden oil research; and (c) development of large-scale fish oil fractionation procedures for production of biomedical test materials. This paper describes some of these activities that we believe will aid the U.S. fishing industry and consumers, alike.

For those who are not familiar with the structure or analysis of marine lipids and fatty acids, a brief overview of their structures, occurrence, and analysis as carried out at the Charleston laboratory follows.

The major classes of marine lipids are cholesterol (a simple lipid), triacylglycerols, phospholipids, and, somewhat less frequently, wax esters (Fig. 1). The latter three classes are complex lipids in which constituent fatty acids form part of the molecular structure. Phospholipids are generally considered to be structural or functional lipids and, along with cholesterol, are incorporated, to a large extent, into cell membranes. These cellular lipids normally account for a minimum of about 0.6% of wet weight of light muscle tissue (3) and, in very lean fish, may comprise nearly the total lipid content. In more fatty fish, the bulk of the remaining fat, usually triacylglycerols, occurs as depot fat. However, the depot fats of numerous species of epipelagic zooplankton and some species of fish, the castor oil fish (Ruvettus pretiosus)

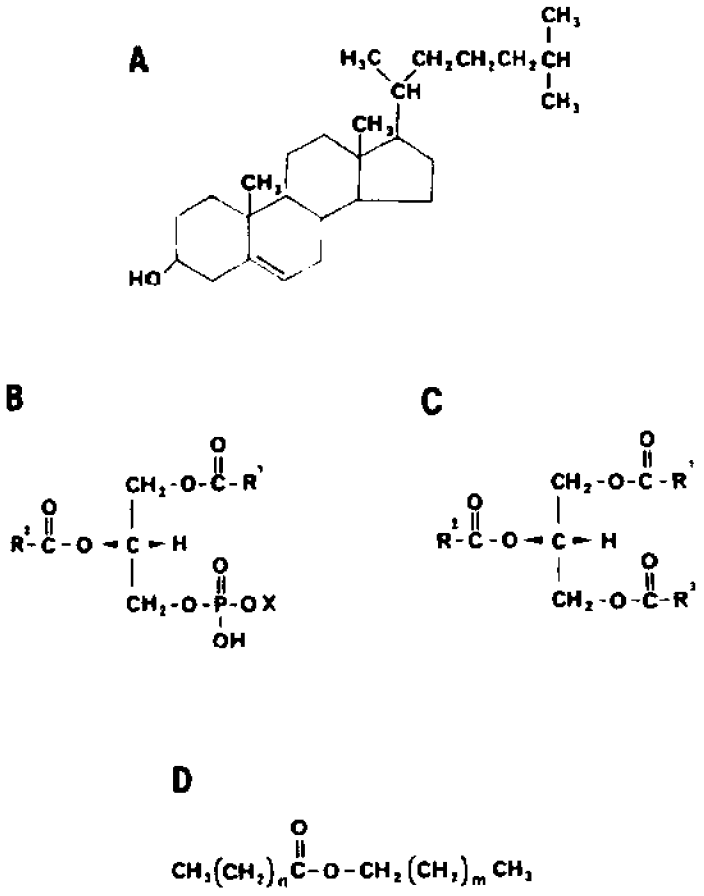
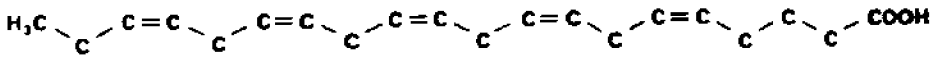


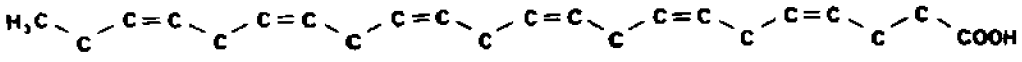
Fig. 1 Molecular structures of some common lipids. A, cholesterol; B, phosphatidylcholine (R^1 and R^2 are fatty acid residues, X is choline residue); C, triacylglycerol (R^1-R^3 are fatty acid residues); D, wax ester (n and m are the number of methylene groups in the fatty acid and fatty alcohol moieties, respectively).

and the South African butterfish (Lepidocybium flavobrunneum) for example, consist primarily of wax esters (19, 21, 22). The fatty acids found in the depot fats largely reflect the diet of the fish and serve as an energy source or a reserve from which fatty acids may be selected for incorporation into the phospholipids. Although fatty acids occur in very large amounts as constituents of the complex lipids, only traces occur in non-esterified form in cells and tissues (3).

Marine fatty acids generally contain 12-24 carbon atoms in the molecule and these carbon chains may be saturated or contain one to six double (ethylenic) bonds. It is the fatty acids with five and six double bonds that give marine lipids their unique characteristics. These fatty acids enter the food chain from phytoplankton or seaweeds that are ingested by marine herbivores (1). The two major polyunsaturated fatty acids (PUFA) found in marine lipids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are shown in Fig. 2.



20:5ω3 - EPA



22:6ω3 - DHA

Fig. 2. Molecular structures of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

Both EPA and DHA are members of the "omega-3" family of fatty acids that became the focus of much attention when the beneficial effect of a seafood diet on the human cardiovascular system was recognized in Greenland Eskimos (6). The term "omega-3" (also denoted as "n-3") is based on the shorthand notation that may be used to describe fatty acids, denoting the number of carbon atoms in the chain, the number of double bonds, and the position of the first ethylenic bond relative to the methyl (omega) end of the molecule (Fig. 2). Species such as cod, menhaden, pilchard, herring, and anchovies, that provide the world's major supply of commercial fish oil, are all rich in omega-3 fatty acids.

Substantial amounts of arachidonic acid (AA), an omega-6 PUFA biochemically important in land animals, have been reported in lipids of fish taken from waters of Australia and Malaysia (7, 8). Limited reports on the fatty acid composition of seaweeds and benthic algae

collected from temperate waters off the southern coast of Australia (14, 20) suggest that the omega-6 fatty acids of these fish, like the omega-3 fatty acids of colder water fish, may be of exogenous origin.

For practical purposes, the composition of most marine fish oils can be described on the basis of 8 to 10 fatty acids; 14, 16, and 18 carbon saturated fatty acids, the 16, 18, 20, and 22 carbon monoenes, and the PUFA, 20:5ω3 and 22:6ω3 (16). Although these fatty acids usually total 80-90% of all the fatty acids in the oils, it is not unusual to detect as many as 70-80 fatty acids in marine lipids using wall-coated open-tubular (capillary) gas-liquid-chromatography (GLC).

Most modern gas chromatographs are equipped with computers that greatly facilitate the handling of the large amount of data that can be derived using capillary columns. Since we are using older equipment (Hewlett-Packard 5840)[1] we have "updated" by interfacing two chromatographs with an Apple IIe micro-computer. Data (retention times, area counts, and area percentages) are transferred directly from the chromatograph by means of an RS-232C interface to the Apple IIe for disk storage and later transferred to a Model 4 Radio Shack micro-computer using the commercial communications program, Videotex Plus (Tandy Corp.). Fatty acids are identified using a BASIC program that calculates equivalent chain length (ECL) values from their retention times (13), compares the ECLs with those of authentic primary and secondary standards and reports probable identities. Before any additional data processing is attempted, these identifications are inspected and corrections made, when necessary, by means of the Model 4 commercial word processor program, Superscripsit (Tandy Corp.). Additional BASIC programs compile data and produce tabulated reports.

To determine quantitative lipid class composition, we are employing a relatively new technique that combines thin layer chromatography (TLC), long an important and convenient analytical tool in lipid analysis, with flame ionization detection (FID), a very popular detection device for GLC because of its high sensitivity and linearity. This technology was developed in Japan and, to date, the only commercially available instrument, the Iatroscan TH-10, is marketed by Iatron Laboratories of Japan. The Charleston Laboratory was one of the first in the United States to use this technique for marine lipid analysis.

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1 Mention of commercial products or companies does not constitute endorsement by the National Marine Fisheries Service, NOAA.

LATENT RESOURCES

Gooch and Hale have described the ongoing latent species project at the Charleston Laboratory (9). In addition to determining the edibility characteristics of some 40 species, data have been obtained on fatty acid and proximate composition of the species in both raw and cooked forms. Sampling technique, one of the major causes of inconsistency in fatty acids data reported in the literature, was carefully standardized. Fatty acid composition was determined and data processed and stored using the GLC/computer system described above. When published, these data will be a valuable source of information for nutritionists, dieticians, and consumers.

The nutritional value of seafood has been recognized for many years, but only recently has attention been focused on specific components of marine lipids, the long-chained omega-3 PUFA and their relative effects on the cardiovascular and immune systems. Although EPA is found almost exclusively in marine lipids, large amounts of DHA have been detected in brain and nervous system of man (2), suggesting that omega-3 fatty acids may be essential components of the diet. Figure 3 shows EPA and DHA

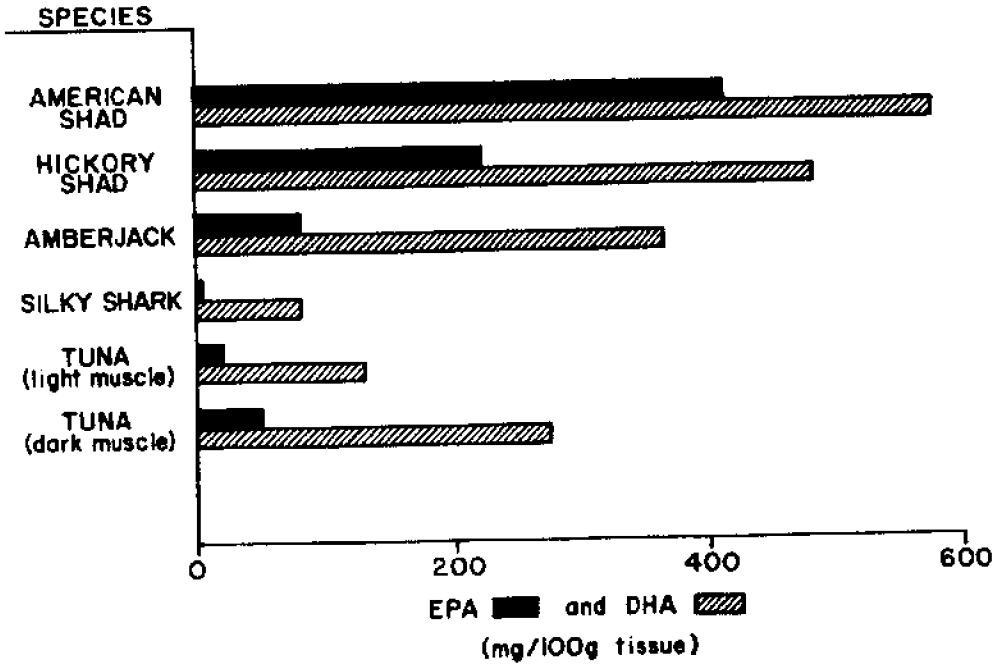

Fig. 3. Amounts of EPA and DHA in a one-quarter pound serving of five southeast underutilized species.

content of several species obtained from research cruises of the South Carolina Marine Resources Research Institute, Charleston, SC. For the species listed, we have calculated the amount of EPA and DHA available from each of the different species, in mg/100 g of tissue, or actual amount of EPA and DHA that would be ingested in a serving of approximately 1/4 lb.

Many underutilized species are not favored by consumers because they are fatty fish and may have a short shelflife unless handled properly. However, as this figure shows clearly, a fatty fish like the American shad contains far more EPA and DHA per edible portion than a low-fat fish like yellowfin tuna.

MENHADEN OIL RESEARCH

Domestic use of menhaden oil is currently limited to industrial products (protective coatings, lubricants, and as an animal feed ingredient), because menhaden oil is not approved by the FDA as a human food. Most menhaden oil produced in this country is sold in Europe where it has a long history of use in margarine and shortening products. Due to current increased economic stress on the menhaden fishing industry, coupled with increased market demands for traditional food fish, and emphasis on optimal utilization of our marine resources, serious attention is being directed towards higher-value utilization of the menhaden resource. Fish oils typically sell at lower prices than other oils marketed as edible fats and oils. Approval of menhaden fish oil as a human food by FDA would not only increase its value by offering a domestic market but would also strengthen world prices for fish oils in general because of the high regard other countries have for the actions of the FDA. The menhaden industry is taking action to upgrade the use of menhaden for human food and has requested assistance and cooperative efforts from both the private and public sectors.

A Special Menhaden Task Force was established in 1977 with members from both industry and NMFS. The role of the Task Force was to define the information needs and the research strategy that could ultimately lead to the submission, to FDA, by industry, of a menhaden oil food additive petition including both refined and partially hydrogenated menhaden oils. It was determined that long-term animal toxicological studies would be required as part of the petition. Saltonstall-Kennedy funds were requested and granted to conduct three animal feeding studies using PHMO. The studies were designed to establish the safety of menhaden oil when fed at different levels to several species of mammals. All feeding studies were conducted under contract and monitored by staff of the Charleston Laboratory. The three feeding studies included a rat lifespan study with an *in utero* phase, a rat multigeneration reproductive study with teratology, and a 12-month dog feeding study. These studies have been successfully completed and the results will be incorporated into the petition.

As part of the petition initiative, the Charleston Laboratory began collecting published articles dealing with fish oils. This collection has grown rapidly and now contains over 6,000 articles dating back to 1878. Topics covered by the collection include the history, production, analysis, chemistry, uses, toxicology, and therapeutic value of fish oils. A Selected Bibliography on Fish Oils (5) listing the references has been published and will soon be available for distribution. Also, a computerized catalog software package called the Online Reprint Accession Library System (ORALS) was developed to provide computer retrievable topic oriented searches of the fish oil bibliography.

The Charleston Laboratory has also been involved in studies to develop detailed information on both the physical and chemical characteristics of menhaden oils. One such study undertaken by our laboratory was designed to determine the extent of seasonal and geographic differences in the fatty acid composition of commercially produced menhaden oils. Composite menhaden oil samples, representing one month's plant production, were provided by selected menhaden plants on both the Atlantic and

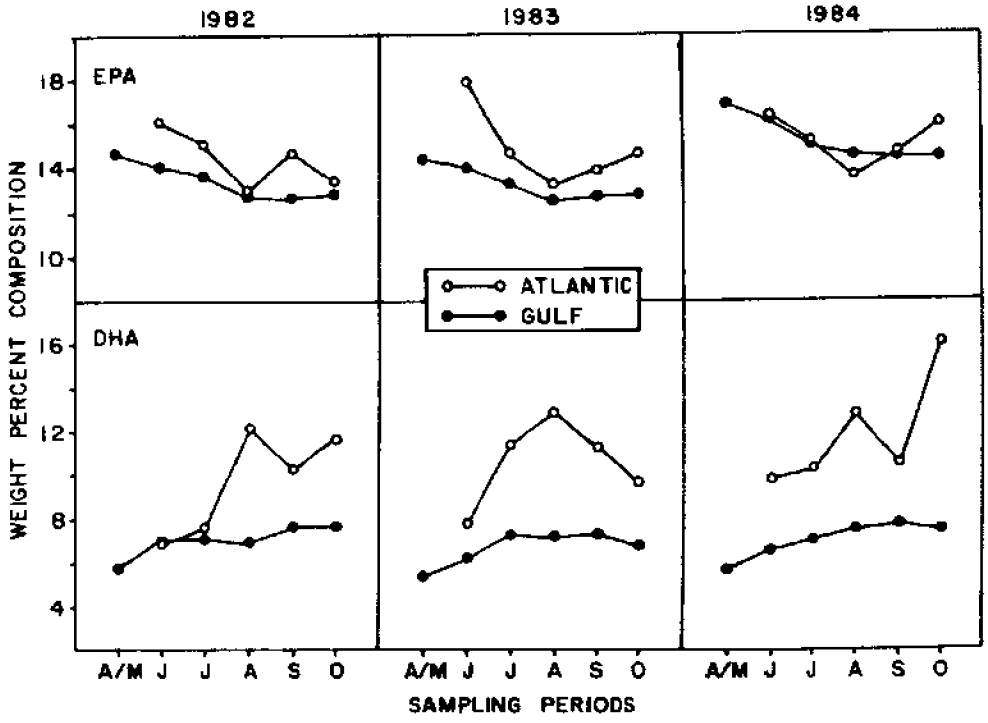

Fig. 4. Seasonal and geographic variations in EPA and DHA of commercial menhaden oils during 1982-1984.

Gulf of Mexico coasts for each month of the 1982-1984 fishing seasons. A total of 65 samples for 1982, 63 samples for 1983, and 55 samples for 1984 were analyzed in duplicate.

Thirty-six fatty acids were selected for calculation of geographic (Atlantic and Gulf) means for each year. Percentages of 10 biochemically important fatty acids were averaged for oils from plants within each geographic area, for each month of the fishing season, to obtain seasonal mean values. The levels and changes in seasonal mean values of the two major omega-3 fatty acids are illustrated in Fig. 4. The percentages of EPA and DHA, as well as the basic pattern of seasonal changes, were similar for the three years with the exception of the level of EPA in the 1984 Gulf oils, which was higher than had been observed in the previous two years. A manuscript reporting data from the first two years of the study is in press (15). Data for the third year are available in the form of an annual report.

This and other information generated by our research is being utilized by the industry in drafting the menhaden oil petition. The menhaden industry has reported that they plan to submit the petition to FDA early in 1986.

PRODUCTION OF BIOMEDICAL TEST MATERIALS

Data from research on the effects of omega-3 PUFA derived from seafoods were reviewed at the Conference on the Health Effects of Polyunsaturated Fatty Acids in Seafoods held June 24-26, 1985, in Washington, DC, sponsored by the Nutrition Coordinating Committee of the National Institutes of Health (NIH), the National Fisheries Institute (NFI), and the NMFS. After reviewing the available data, areas needing additional research were defined. At the "Seafood and Health '85" conference held in Seattle, WA, on November 16-17, 1985, Dr. Artemis P. Simopoulos, Chairman of the Nutrition Coordinating Committee of the NIH, reported that the NIH would soon issue a program announcement, calling for proposals for research on "Biological Mechanisms of Omega-3 Fatty Acids in Health and Disease States". This announcement appeared in the December 6, 1985 issue of NIH Guide for Grants and Contracts (4).

For these NIH-funded studies, participating investigators will require a variety of test materials: refined, deodorized fish oil; PUFA concentrates; purified EPA and DHA; and deuterated PUFA. Clearly, uniformity and guaranteed availability of test materials, free of toxic substances, and of known fatty acid composition, will be crucial for the successful interpretation and correlation of results from the various studies. The NMFS, already experienced in the field of marine lipids, has proposed to serve as the producer and supplier of all test materials for NIH-funded researchers to insure this essential availability and uniformity.

Menhaden oil has been selected as the source for all test materials since it is produced in the largest volume (U.S. production in 1984, almost 366 million pounds (24)) and has been the most thoroughly analyzed. Additionally, the menhaden industry has long been concerned with upgrading the value of its oil and has contributed large quantities of oil for scientific and biomedical research. All three NMFS utilization laboratories, in Charleston, SC, Gloucester, MA, and Seattle, WA, will have roles to play in this major endeavor. The Charleston Laboratory will be responsible for establishing a pilot plant to produce deodorized fish oil and fractions containing concentrated percentages of EPA and DHA by conventional methods (molecular distillation and urea complexing). The technology of supercritical fluid CO_2 purification and fractionation of fish oils is being investigated by the Seattle Laboratory. The Gloucester Laboratory will explore the suitability of preparative high performance liquid chromatography (HPLC) as a tool for isolation of purified EPA and DHA. If improved purification and fractionation techniques are developed, they will be scaled up and installed in the Charleston Laboratory pilot plant for production of EPA and DHA concentrates and purified fatty acids. All fractions produced in the Charleston Laboratory will be subjected to stringent in-house analyses for quality and composition.

Urea complexing to concentrate PUFA of mixed non-esterified fatty acids or esters is a technique that has long been used as an analytic tool in structural identification (12, 17, 23) and for bench-scale isolation of limited quantities of purified PUFA (10, 11, 18). However, to satisfy the needs of the NIH-funded investigators, large-scale procedures will be required. Currently we are investigating the feasibility of scaling-up urea complexing techniques using a 72-liter all-glass reactor to carry out the necessary saponification, urea complexing and esterification reactions. These procedures can reasonably be expected to increase the EPA of menhaden oil from about 15% to 35-40% and DHA, from 10% to about 20%. We expect to be able to produce PUFA concentrates containing 70-75% omega-3 acids or esters (including the minor components, 18:4ω3 and 22:5ω3) in a yield of about one ton by the end of the first year of plant operation. If necessary, low temperature crystallization can also be used to increase omega-3 concentrations. Centrifugal molecular distillation will be used to deodorize refined menhaden oil.

Until additional information is available, investigators are not ready to recommend ingestion of large amounts of EPA/DHA concentrates for the general population. Most do, however, suggest that increased consumption of fish and seafood products would be beneficial in establishing a balance of omega-3 and omega-6 fatty acids in the average American diet. Information currently being compiled by the NMFS on composition of latent species and supportive work for the menhaden oil petition will be of immediate benefit to the consumer and to the fishing industry. Our

responsibilities in the joint NIH/NMFS initiative will be critical to the success of this initiative which should demonstrate the specific benefits from inclusion of fish and fish oils in the human diet.

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OMEGA-3 FATTY ACIDS AND FISH OILS: IS THE NEWS ALL GOOD?

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The report of low death rate from coronary heart disease associated with the high consumption of fish has been well publicized (1). Dietary fish oils, rich in omega-3 polyunsaturated fatty acids (omega-3 PUFAs), have promoted the following physiological changes in clinical trials: lowering of plasma total triglycerides, total cholesterol and very low density lipoproteins (2); reduction in platelet aggregability (3); prolonged bleeding time (4); and lowering of blood pressure (5). The beneficial effects of dietary fish oils rich in omega-3 PUFAs and their mechanisms of action are the subject of reviews in these Proceedings and elsewhere (6). Many of the physiological effects resulting from increased fish and fish oil consumption are regarded as beneficial in relation to the established risk factors for coronary heart disease.

While research and epidemiological surveys continue to investigate and define the health benefits of fish and fish lipids in the diet, the potential negative aspects of increased usage of fish oil products also should be considered. In the present report, we suggest some medical, nutritional and toxicological concerns regarding dietary supplementation with fish oils. Certainly, we have not identified all possible areas of concern, and others may become apparent from ongoing research.

I. MEDICAL CONCERNS

Fish oils presently are being marketed in concentrated capsule and emulsion forms throughout the U.S. Capsule forms usually provide 1 g marine fish oil per capsule and contain 180 mg eicosapentaenoic acid (EPA) and 120 mg docosahexaenoic acid (DHA), with vitamin E (1-2 I.U.) included as an antioxidant. Recommended dosages range from two capsules per day to two capsules with each meal. Associated health claims include control of high blood pressure and levels of triglycerides and cholesterol. At least one commercial manufacturer has suggested that his product may provide a remedy for cardiovascular disease and eliminate the need for drugs along with their undesirable side effects.

Medical concerns regarding ingestion of fish oil are centered around the self-treatment aspect. Most persons are aware of the fact that major cardiovascular diseases are the leading cause of death in the U.S. (410.5 deaths per 100,000 people in 1985). Persons who are likely to supplement their diets with fish oil products are those who are concerned, or possibly overconcerned, about cardiovascular disease.

Presently there is no evidence that certain cardiovascular disease processes, such as atherosclerosis, are reversed by fish oils. Although dietary fish oil may reduce any further atherosclerotic plaque buildup, surgical and/or drug intervention may be more prudent therapy for some persons. The use of fish oils in any therapeutic regimen should follow the advice of a physician.

Fish oils are known to delay blood-clotting time. In one extreme case, a subject who had consumed a strict marine animal diet, rich in omega-3 fatty acids, had a tenfold increase in bleeding time with an approximately 50% decrease in platelet count (7). Typically, more moderate yet significant increases (i.e., 25-50%) in bleeding time brought about by increased fish and/or fish oil consumption have been noted in clinical trials (4,8,9). The prolongation in bleeding time caused by dietary omega-3 PUFAs appears to be time- and dose-dependent. A 12% increase in bleeding time occurred after 4 weeks of administration of 3 g omega-3 PUFAs per day (10), while a 100% increase was observed after 12 months of ingestion of 20 g fish oil (3.6 g EPA) per day (11).

In patients who have suffered a recent heart attack, a delay in blood-clotting time is sought through medication with anticoagulative drugs. The optimum dose of the active ingredient in fish oil that delays clotting is presently unknown. A delayed clotting time may be undesirable in an individual who has been involved in an accident or who is undergoing emergency surgery. Clearly, the intake of omega-3 PUFAs should be controlled in persons who already suffer from a bleeding disorder. The pharmacologic interactions of fish oils and other drugs such as aspirin also must be considered. Aspirin, like fish oils, is known to reduce platelet aggregation and prolong bleeding time. This action of aspirin has been explained by its ability to inhibit prostaglandin biosynthesis. One study demonstrated that ingestion of aspirin shortens bleeding time in persons whose diet is rich in omega-3 fatty acids (12), while another indicated a synergistic (additive) effect in prolonging bleeding time (13).

II. NUTRITIONAL CONCERNS

The nutritional concerns relating to increased consumption of fish oil products are varied. The daily intake would certainly be a factor in defining any nutritional consequences. Doses of fish oils used in clinical trials have been as high as 60-90 ml/day (4), although doses of 10-40 ml/day have been more common.

One area of concern may be the influence of dietary fish oil on the intake and requirement of certain fat-soluble vitamins. Fish oil capsules, which are sold as omega-3 PUFA supplements, are produced from fish body lipids and do not contain high levels of vitamins A and D. However, commercially available fish liver oils (e.g., cod and shark liver oils) may be rich sources of these vitamins and are being sold for supplementation purposes. Some contain as much as 2½-5 times the U.S.

recommended daily allowance (RDA) for these two vitamins per capsule. With the predicted rise in the popularity of fish oil products, the possibility exists of fish liver oils being purchased as omega-3 PUFA supplements by the uninformed consumer. If the fish liver oils are ingested at the levels used in most clinical trials of fish body oils, the intake of these vitamins would be excessive and potentially toxic. The same individuals may already be supplementing their diets with these vitamins. The difference between these two fish oil products should be stressed.

For animals, the requirement for vitamin E is influenced by the level of PUFAs in the diet. In general, as the intake of PUFAs increases, so does the requirement for vitamin E. A major role of vitamin E is to act as an in vivo antioxidant by protecting biological membranes from oxidative lipid reactions. Fish oils, which are rich in PUFAs, are more prone to autoxidation in vitro than are fats or oils that contain more saturated fatty acids. Increased consumption of fish oils will alter the fatty acid composition of biological membranes by favoring the more peroxidation-prone polyunsaturated types (14).

As early as 1941, it was recognized that dietary supplementation with fish oil exacerbates the signs of vitamin E deficiency in animals (15). In 1949, an increased requirement for vitamin E was demonstrated in rats fed fish oil compared with those fed lard at similar levels in the diet (16). Later, in 1957, it was cautioned that the prolonged use of highly unsaturated oils for the purpose of lowering serum cholesterol levels in man may precipitate deficiencies in certain vitamins, such as vitamins A and E (17). Thus, even in these earlier studies, a relationship between polyunsaturated fatty acids (from fish oils) in the diet and the requirement for vitamin E was recognized.

If recommendations are to be made to shift our intake of lipids to include more unsaturated omega-3 fatty acids, reevaluation of the current RDA for vitamin E may be necessary. Research needs in these and other areas regarding dietary omega-3 PUFAs (e.g., dosage, omega-3/omega-6 ratio) have been identified (6).

III. TOXICOLOGICAL CONCERNS

A. Lipid Oxidation

As mentioned above, fish oils are rich in highly unsaturated fatty acids and thus are very susceptible to autoxidation and rancidity. PUFAs can undergo autoxidation at ambient temperatures quite readily, forming hydroperoxides and various secondary decomposition products (e.g., malonaldehyde). Several methods are available to detect the products of lipid autoxidation in vitro, such as measurement of the peroxide value (POV) and the thiobarbituric acid (TBA) test for malonaldehyde. The TBA test is often used to detect in vivo peroxidation of lipids as well.

One implication of this oxidative process is that the intended nutritional quality of fish oils will be decreased because of the decomposition of the PUFAs. While one function of vitamin E is to prevent lipid oxidation, it becomes oxidized itself in the process. Additionally, both lipid hydroperoxides and secondary autoxidation products are recognized to be toxic in biological systems (18). The likelihood of the occurrence of autoxidation in fish oil products will depend on several factors, such as the content and form of antioxidants, the degree of unsaturation of fatty acids, presence of prooxidants and storage conditions. These factors must be monitored and controlled to assure full benefit of fish oil supplementation to the diet.

B. Environmental Contaminants

There are two classes of environmental contaminants which may enter fishery products: organic chemicals and metals. Such contaminants are regulated under the Federal Food, Drug and Cosmetic Act. Environmental contaminants may inadvertently enter the human food supply (19), either directly or indirectly as a consequence of human activities (e.g., agriculture, mining, energy production). The Food and Drug Administration (FDA) has authority to set limits (i.e., action and tolerance levels) for the amounts of unavoidable contaminants that are permissible in food. The distinction between action levels and tolerances has been summarized as follows (19):

> "Regulatory procedures employed to control environmental contaminants in food include the establishment of action levels or tolerances. A formal tolerance is a regulation having the force of law. Tolerances are adopted through formal rulemaking procedures and specify the level of a contaminant that will render a food adulterated. If supported by substantial evidence in the rulemaking record, FDA's tolerance cannot be questioned by any court. An action level is an informal judgement about the level of a food contaminant to which consumers may safely be exposed. It is a statement of FDA's professional judgment and represents a commitment to initiate regulatory enforcement action against any lots of food discovered containing excess levels."

Most environmental contaminants are regulated under action rather than tolerance guidelines. The levels have occasionally been raised or lowered on the basis of new information. The FDA action levels for certain contaminants in fish and shellfish are given in Table 1.

Table 1. Action Levels of Contaminants for Fish and Shellfish

CONTAMINANTS	ACTION LEVEL (ppm)
Aldrin/Dieldrin	0.3
Chlordane (fish only)	0.3
DDT/DDE/TDE (fish only)	5.0
Endrin	0.3
Heptachlor/Heptachlor epoxide	0.3
Kepone	0.3
Methyl mercury	1.0
Mirex (fish only)	0.1
Toxaphene (fish only)	5.0
PCBs (tolerance level)	2.0

More specific information on chemical contaminants in fish and fish oils would be desirable. One concern is that since most of these chemical contaminants are lipophilic, they are likely to be greatly concentrated in fish oils. There are cleanup procedures available to remove these contaminants from fish oils, but the extent to which the procedures are being applied is uncertain. These procedures increase production costs and may not be employed by low-budget operations. Recently the issue of chemical contaminants in fish oils has been raised (20):

> "Many chemical residues can be absorbed by animals but are not easily excreted so that they accumulate in body tissues. Most organic compounds are distributed in fat tissues, liver and other organs that are usually not consumed so that even contaminated fish may have negligible levels of toxic chemicals in the edible flesh. The caution is raised about fish oil supplements that may contain high levels of noxious substances because these supplements may be derived from fish livers and waste tissues."

Contaminants have been found in a variety of aquatic species, regardless of the site of collection or whether from fresh or saltwater. In 1981, the Council on Environmental Quality published information on the yearly trends of contaminants found in fish collected in the U.S. (21). Detectable levels of DDT, toxaphene, dieldrin, PCBs and mercury were reported. Most samples do not exceed the FDA maximum allowable levels of chemical contaminants in raw fish for human consumption.

No information is presently available on chemical contaminants in fish oil capsules. However, analyses of other fish oil samples have been conducted by FDA laboratories. While results from only four samples appear in Table 2, they illustrate the range of chemical contaminants which may be encountered. Three of the four oils contained chlordane, DDE and/or PCBs. In addition to these contaminants, one sample also contained the herbicide trifluralin.

Table 2. Chemical Contaminants in Fish Oils*

SOURCE	CONTAMINANT	CONC. (ppm)
Cod liver	Chlordane	0.26
	Dieldrin	0.14
	DDE	0.29
	PCBs	2.1
Menhaden	DDE	0.18
	Heptachlor epoxide	0.10
	Toxaphene	1.2
Herring	Chlordane	0.04
	DDE	0.15
	Dieldrin	0.05
	Hexachloro-benzene	0.03
	Lindane	0.11
	PCBs	1.69
	Trifluralin	0.05
Unspecified	Chlordane	0.21
	Dieldrin	0.13
	Lindane	0.08
	PCBs	2.45

*Surveillance or compliance samples collected and/or analyzed under FDA monitoring programs, 1983-1985.

While mercury and methyl mercury are still being found in aquatic products, less attention has been given to lead and tetraalkyl lead compounds (i.e., from gasoline), which also have been reported in a number of fish species. Much of the total lead content found in certain fish samples could be accounted for by the more toxic tetraalkyl lead compounds (22). Both methyl mercury and tetraalkyl (e.g., tetraethyl) lead compounds also are very lipid soluble compared with their inorganic forms.

SUMMARY

In this paper, and in a companion one in these Proceedings, the reader has been provided with an overview of both the beneficial and the potentially adverse effects of the use of fishery and fish oil products. History tells us that with virtually all clinical trials with new agents, the early results are predominantly good. It is only after more widespread use of a substance that the less desirable effects are manifested. The major pharmacologic actions of the omega-3 polyunsaturated fatty acids, such as changes in clotting mechanisms, stem from alterations in prostaglandin metabolic pathways. Such basic biochemical and physiological processes are susceptible to minor perturbations and can lead to unintended effects. Some toxicologic concerns also have been raised in this paper. These involve both exogenous contaminants such as PCBs and pesticides, as well as endogenously generated substances such as peroxides. Although there are procedures available that can reduce or minimize the levels of hazardous substances in fish oils, the extent to which they are being applied by all manufacturers is unknown. Nonetheless, the good news/bad news ratio presently appears favorable.

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HELMINTHS AND HUMAN HEALTH: AN UPDATE ON LARVAL ASCARIDOID NEMATODES IN SEAFOOD PRODUCTS

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Serious human disease may be the result of ingesting seafood products infected with certain parasites; therefore, it has become increasingly more important to study the parasites of marine animals. Researchers have implicated at least fifty species of helminths as producing zoonotic infections resulting from eating raw seafoods. Most of these zoonotic infections do not occur in the U.S. This can be attributed to a lack of specific intermediate hosts required to complete the parasite's life cycle, improved sanitation, refined foodhandling procedures, and our traditional "meat and potatoes" diet.

Some parasitic diseases, however, do occur in the U.S. and the number of new case reports, as well as previously unrecognized parasitic diseases, continues to increase. One reason for this increase may be attributed to the changing dietary habits of our cosmopolitan society---a change that may permit new types of parasitic infection to be acquired. For example, one of the fastest growing types of restaurants in recent years is the Japanese sushi bar whose specialities are raw seafoods.

Recently, we began to study the parasites of finfish and shellfish in the Gulf of Mexico at the Fishery Research Branch (FRB). A few potential parasite problems associated with larval ascaridoid nematodes will be discussed. Because of current interest in the nematodes that cause the zoonotic disease anisakiasis, this report addresses this disease in more detail.

Humans may become infected with anisakid nematodes by consuming raw or inadequately prepared seafoods. When a third-stage anisakid larva is ingested, it may penetrate into or through the gastrointestinal tract of a host. The most commonly implicated agent of anisakiasis is the third-stage larva of Anisakis simplex. The greatest number of human cases occur in areas where seafood constitutes a major portion of the protein intake. Most cases of human anisakiasis have been reported from Japan and The Netherlands, but since 1958, cases of human anisakiasis have been reported in the U.S. In 1985, I have become aware of 12 new cases in the U.S.

Much controversy exists among researchers concerning the life cycles of anisakid nematodes. Not all anisakid nematodes have the same life cycle and the rate of development of a larva may differ. A generalized life cycle for this group of worms is as follows: eggs are expelled by a mature female nematode into the gastrointestinal tract of the host (e.g., marine mammals, fishes, birds, turtles) and passed out with the feces into the water. Development to the first-stage larva occurs within the egg with the second stage larva released a few days later. This free-swimming larva is ingested by an acceptable intermediate host (invertebrates and fishes in which the parasite develops but not to maturity) and migrates to the hemocoel or mesentaries where it develops into a third-stage larva. The third-stage larva, the infective stage for the definitive host, will molt to a fourth-stage larva and then to its adult form in the acceptable definitive host. Both transport hosts, often copepods, and paratenic hosts (invertebrates or fishes in which the parasite survives but does not develop) may be involved in this life cycle. Humans may become infected by interrupting the cycle by eating the intermediate host. Humans, however, have not been shown to serve as a definitive host for these parasites.

Hundreds of different species of finfish and shellfish are infected with larval anisakid worms and, thus, represent the intermediate host for many of the life cycles of anisakid nematodes. The FDA has sponsored surveys of marine fishes caught along the Atlantic, Gulf, and Pacific coasts and in the waters near the Hawaiian Islands to determine the prevalence of larval anisakid nematodes. The number of worms infecting a host appears to correlate with the occurrence of the parasite's definitive host. For example, the definitive hosts of larval Anisakis simplex are marine mammals (e.g., whales, dolphins, seals). In offshore areas with more marine mammals, the numbers of larval Anisakis per fish and the numbers of infected fishes increase. Thus, the West coast fish are more infected than fish along the East or Gulf coasts. Deardorff et al. (1982) discussed this topic in more detail.

We all need to become more aware of potential problems and risks involved in eating foods so that we can make informed decisions. In the Southeast, the apparent absence of human infections with anisakid nematodes may not be a true reflection of the inherent consumer hazards. Unquestionably, this disease existed for many centuries before we recognized it in the 1950s. The apparent absence of disease in the South may be attributed to the fact that the trend of eating raw foods, so prevalent in other areas of the U.S, has not yet caught on here. Fried seafood seems to be preferred in the South. Another reason may be a lack of awareness on the part of the local medical community. Cases may have occurred here, but may have been misdiagnosed. This latter reason was apparently the case in Hawaii. Prior to 1984, no cases had been reported from these islands. Since the medical community was made more aware of this parasitic disease, three cases of human infection, one in 1984 (see Deardorff et al., 1986) and two in 1985 (M. Kliks, personal communication) have been confirmed.

Our research interests with these worms are not only limited to their invasive potential. We had previously observed that the third-stage larvae of an anisakine worm caused destruction of gastric tissue in excess of what would be expected by mechanical damage from the boring tooth of the invading worm. This tissue reaction was not confined to the immediate area of penetration, and the cells that were affected appeared abnormal.

Excretory and/or secretory (ES) products, which apparently are being released by the larva, may aid the larva in penetrating the host and may play a role in the etiology of the associated gastrointestinal lesion. ES products also may have an effect on the immune system. Table 1 shows that the larvae of some anisakid nematodes produce a bioactive substance(s) that exerts a potent inhibitory effect on mitogen-stimulated lymphocyte blastogenesis, as well as lymphoid (P3/X63-Ag8) and epithelioid (HeLa) cell lines. The decreased transformation of the cells was observed by a decrease in ^{3}H-thymidine incorporation in the cell cycle during the labeling period. Additional information and methodology are provided by Raybourne et al. (1983; in press).

Table 1. Inhibition of Cells by High Molecular Weight Excretory-Secretory Materials from Anisakis simplex Third-Stage Larvae

CELL LINE	ORIGIN OF CELL	% INHIBITION
Rodent lymphocyte*	Rodent splenocyte	99
P3/X63-Ag8	Mammalian lymphoid	99
HeLa-S3	Human epithelioid	71

*Concanavalin A-induced blastogenesis
Data from Raybourne et al., 1983; Raybourne et al., in press

The materials responsible for suppression are greater than 10,000 molecular weight and are heat labile. Inhibition of blast transformation was a result of cytostatic rather than cytotoxic effects on proliferating lymphoid cells. The inhibition was reversible following removal of the ES materials. The degree of inhibition by Anisakis simplex ES material on splenocytes cultured in the presence of various concentrations of ES material and concanavalin A is shown on Table 2. An increase in inhibition was seen between 10 and 40 ug/ml. Raybourne et al. (1983) calculated that one worm produces sufficient ES material in one day to exert a significant inhibitory effect on cultures of cells.

Table 2. Inhibition of Anisakis simplex FS Material on Splenocytes Cultured in the Presence of Various Concentrations of Excretory-Secretory Material*

PROTEIN CONCENTRATION (ug/ml)	INHIBITION (%)
0	0
10	79
20	94
40	97

*Concanavalin A-induced blastogenesis
Data from Raybourne et al., 1983

It has generally been assumed that removing the worms from the edible musculature of a fish would eliminate the health risks. The finding of bioactive substances eliminated by these parasites suggests that this may not be the case. The worm's products, released while encysted, may remain in the tissues. Also, the ES materials may be carried by the circulatory system of infected fish. If so, the presence of worms in nonedible areas of fish (e.g., viscera) may allow for contamination of the whole fish. We are currently examining this possiblity.

While molecular weight fractions greater than 10,000 daltons have not been found to be toxic, the fractions less than 10,000 daltons are heat stable and have shown indications of being toxic under certain conditions. ES materials exhibited a positive response in the Saccharomyces cerevisiae (D6) yeast test. This test correlates with tumor promoter activity.

In the Southeast region, third-stage larvae of Anisakis simplex are not the only potential health problem for humans. The third-stage larvae of a species belonging to the genus Hysterothylacium, commonly found in numerous fish and invertebrates (Deardorff and Overstreet, 1981), was shown to penetrate into the stomach wall of laboratory animals (Ebert, 1976; Overstreet and Meyers, 1981). Species belonging to this genus mature in fish---not marine mammals. Consequently, using the presence or absence of marine mammals as a barometer to indicate the presence of invasive nematodes in fish is not always accurate. Our laboratory is continuing to study these worms.

We also are studying two other larval nematodes found in commercially important species from the Gulf of Mexico and adjacent waters. One is the third-stage larvae of Contracaecum multipapillatum,

previously called Contracaecum robustum (see Deardorff and Overstreet, 1980). The larval stage is found in the liver and kidney of the striped mullet. When mullet are eviscerated, the worms in the kidney often remain with the edible portion. If the fish is to be fried, the worms represent no health risk because they will die when exposed to extreme heat. However, if the mullet is destined to be smoked, and an insufficient amount of heat is used, the worm will survive. This larval type does not appear to be invasive to laboratory animals; however, more experiments should be done to confirm these findings. The ES products of this third-stage larva are being tested. Birds serve as the definitive host for Contracaecum multipapillatum. In areas where pelicans, cormorants, and herons are commonly found, mullet generally harbor larger worm burdens.

The other nematode we are studying is presumably the larval stage of Sulcascaris sulcata. The third- and fourth-stage larvae are often found encysted in the adductor muscle of scallops. The definitive hosts for this nematode are sea turtles. Researchers have shown a lack of invasiveness for both these larval types. Scientists at FRB are currently studying the ES products of these worms. Preliminary findings indicate bioactivity similar to that seen with the ES products of Anisakis simplex larvae. Also, these ES materials have been detected by immunofluorescence, not only at the immediate area of worm encystment, but throughout the flesh of infected scallops (Raybourne and Bier, personal communication).

Preventive measures to render seafoods safe from these parasites are under study. Temperature extremes appear to be most effective. The heat from thoroughly cooking seafoods kills the parasites. However, heating the seafood products is not always desirable. Freezing is currently regarded as the most promising preventive measure (e.g., cost effective, ease of regulation) against infection with anisakid larvae. The safe freezing period appears to vary, based on the product and type of larvae being tested. Deardorff et al. (1984) reviewed the effects of cold temperatures on the larvae of ascaridoid nematodes and concluded that -20° C for at least five days would be effective in killing all the worms in whole fish. We are currently investigating the feasibility of using irradiation as a means of killing worms in fish fillets designated for consumption in a raw condition.

While thorough cooking or adequate freezing of seafoods are good preventive measures against anisakiasis and other parasitic diseases, these practices will not always be followed and are difficult to enforce. Prevention of this disease is probably best accomplished by educating the public to the health risks of eating raw seafoods. The consumer should know the risks and evaluate the potential consequences. He is more than likely aware that raw beef (i.e., steak tartare) may be the vector for the beef tapeworm or be the cause of toxoplasmosis and that raw pork may transmit the pork tapeworm or be the cause of trichinosis. When he chooses to eat these foods, he has considered the risks.

As with beef and pork, the vast majority of seafood products are safe to eat; however, the importance of consumer awareness of possible hazards of eating raw seafoods cannot be over emphasized. The consumer should be aware that merely cooking or freezing seafoods does not necessarily ensure that the product is safe to consume. Various bacteria, toxins, and/or heavy metals, which may naturally be present in the seafood or are the result of improper handling of seafood following capture, may not be rendered harmless by the narrow range of temperature extremes commonly used by consumers and, therefore, may still represent a potential health hazard. In addition to the risk of encountering invasive foodborne parasites, their soluble ES products may be a possible source of toxic materials. Awareness of these potential problems, along with proper handling and cooking techniques, is advised.

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PROCESSING MENHADEN FOR CONVENTIONAL FOOD PRODUCTS, MINCED INTERMEDIATES, AND SURIMI

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INTRODUCTION

About 45% of the U.S. commercial fisheries catch in 1984 was menhaden (Brevoortia sp.), but this accounted for only about 5 percent of the landed value (National Marine Fisheries Service, 1985). A majority of U.S. consumers have never heard of menhaden, and most of those who have are of the opinion that menhaden are inedible or at least unsuitable for human food. Because they are small, bony, and oily, menhaden are unsuitable for use as fresh or frozen fillets, but with proper handling and processing into appropriate product forms they can be used for good human food products.

Appropriate product forms for menhaden will generally require either separation and removal of bones, as in minced products, or softening of bones, as in canned products. We have investigated a number of menhaden products, and studies of conventional food products from menhaden have been made recently at Virginia Polytechnic Institute under a Sea Grant project. These products,however, have not yet been described in the literature. We have described the canning of other coastal herring species and the stability of the polyunsaturated fatty acids to the canning process (Hale and Brown, 1983). The seasonal chemical compositions of both Atlantic menhaden (Brevoortia tyrannus) and gulf menhaden (B. patronus), and the protein quality of fish protein concentrates prepared from both species were reported by Dubrow et al. (1976).

Only recently have menhaden been seriously considered for surimi production. Based on pilot plant and semi-commercial trials, Lanier et al. (1983) have reported very good gel strengths for menhaden surimi. A proteolytic enzyme that is active at 60°C was reported to be present in menhaden flesh, and it could inhibit gel formation in slowly cooked products.

CONVENTIONAL PRODUCTS

Canned menhaden products with either brine or vegetable oil as the packing medium have been prepared and evaluated by the staff of the Charleston Laboratory, Southeast Fisheries Center. Smoked menhaden fillets have a desirable flavor and we softened the bones by canning and also by heat processing on open racks in the steam retort. We have also done some preliminary work with menhaden sausage products. The proximate chemical compositions of raw and processed products from one lot of

Atlantic menhaden are listed in Table 1. Fat was determined by a chloroform-methanol extraction (Smith et al., 1964).

Table 1. Proximate composition of Atlantic menhaden and products.

Product Form	Moisture %	Protein %	Fat %	Ash %	Salt %
Fillet, Raw	73.25	19.14	6.63	1.77	0.20
Headed & Gutted (H&G), Raw	72.26	17.91	8.02	2.35	0.19
Canned H&G (in brine)	70.15	18.37	7.71	2.87	1.45
Fillet, Smoked	64.29	23.87	8.41	3.85	2.58

Smoked Menhaden. Whole fish were passed through a mechanical scaler and finished by hand scaling. Because menhaden are difficult to scale, an alternate procedure was to smoke fillets with scales attached and then remove the skin before canning. Menhaden fillets were soaked overnight in refrigerated brine (5% NaCl at a 1:1 ratio), rinsed and dried before smoking. The fillets were smoked on racks in an AFOS smoking kiln (AFOS, Ltd.)[1]. A 3-hour smoking cycle, with auxiliary heat applied during the final 2 hours, is suitable. Internal temperatures and yields for some Gulf and Atlantic menhaden fillets are shown in Table 2.

A process we developed for shad has also been applied to menhaden. The smoked fillets are heat processed on racks in a steam retort to a sterilization value equivalent to 3 minutes at 250°F (internal temperature). This eliminates the bone edibility problem. The processed fillets can be either vacuum packed or tray wrapped and stored as a refrigerated or frozen product.

Canned Product. Several product forms and packing media have been tested with menhaden. A very acceptable product was prepared from lightly smoked fillets canned with sunflower oil. The fillets were trimmed and packed into 307 x 113 (1/2 lb. tuna) cans. Needle-type thermocouples were centered in several cans and the temperatures and sterilization values were recorded by a Kaye data logger during processing. The cans were heat processed to a sterilization value equivalent to about 12 minutes internal temperature at 250°F.

1 Mention of trade names or products does not imply endorsement by the National Marine Fisheries Service, NOAA.

Table 2. Internal temperatures and yields for smoked menhaden fillets.

Species	Gulf Menhaden		Atlantic Menhaden	
Smoking period, hr.	2	4	2	3
Avg. round weight, gm	103	103	388	421
Oven temp. setting, F	140	176	176	176
Final temp. internal, F	114	170	126	141
Yield, % of raw fillets	81.4	60.8	79.5	74.0
Yield, % of whole fish	28.5	21.3	27.4	25.4

Table 3. Sensory evaluations of some canned products on a 0 to 10 rating scale.

Species	Gulf Menhaden	Atlantic Menhaden			Atlantic Herring
Description	Smoked	Smoked	Smoked	Smoke Flavor	Commercial
Packing Medium	Oil	Oil	Brine	Mustard Sauce	Mustard Sauce
Appearance	7.4	8.2	7.4	3.9	5.3
Texture	5.5	5.3	4.7	3.5	5.7
Smoke Flavor	4.3	5.5	4.7	4.7	2.0
Overall Flavor	4.3	5.1	4.8	4.9	5.0
Overall Acceptance	7.8	8.2	7.9	6.1	6.6

Canned products were rated by a sensory panel of staff members, trained for the evaluation of edibility characteristics. An acceptability rating was included on the evaluation forms to obtain an indication of possible consumer preferences. We recognize that the opinions of a small expert panel do not necessarily correspond to those of larger consumer panels or the general public.

Sensory ratings for several canned menhaden products and a commercial herring product are shown in Table 3. Ratings of 0 to 10 corresponded to: appearance (poor to good); texture (soft to firm); smoke flavor (no smoke to too much); overall flavor (mild to strong); overall acceptability (poor to good). The smoked menhaden compared favorably with the commercial herring product. The canned but not smoked menhaden was softer and had a poorer appearance, largely because the fillets stuck together.

MINCED INTERMEDIATES AND SURIMI

In 1982 the Charleston Laboratory initiated a project aimed at defining the holding and processing requirements to produce a washed, minced intermediate product from menhaden. Our earlier experience had been with the preparation of minced fish products from several underutilized species, primarily small bottomfish such as spot and Atlantic croaker. We have used a Bibun model meat-bone separator for a number of years to prepare minced fish. Our experimental processing laboratory now also includes a 60-gallon wash tank, a rotary screen, a screw press, and a Bibun strainer. Our basic process for conversion of menhaden into washed mince or surimi is depicted in the flowsheet of Figure 1.

Refrigerated Holding. In our initial studies we evaluated the holding of whole menhaden in three different systems: refrigerated seawater (RSW); chilled seawater (CSW); and on ice. Extended storage studies were carried out with both Atlantic menhaden and Gulf menhaden. Fish were inspected and rated for periods of up to 14 days, but storage periods in excess of 4 days would not be recommended.

Fish can be held satisfactorily on ice, in CSW, or in RSW, but there are important design and application factors that need to be considered. Iced fish must not be packed too deeply and adequate drainage is required. The RSW and CSW systems are especially applicable to bulk handling and can be used with large volumes of fish in a single tank. The most rapid chilling can usually be achieved in a well designed and operated CSW system with good agitation to prevent temperature stratification. Fresh fish should be chilled rapidly, held at near freezing temperature, and not held very long.

Heading and Gutting Menhaden. The first requirement for mechanical processing is proper sizing of the fish. Although particular schools of

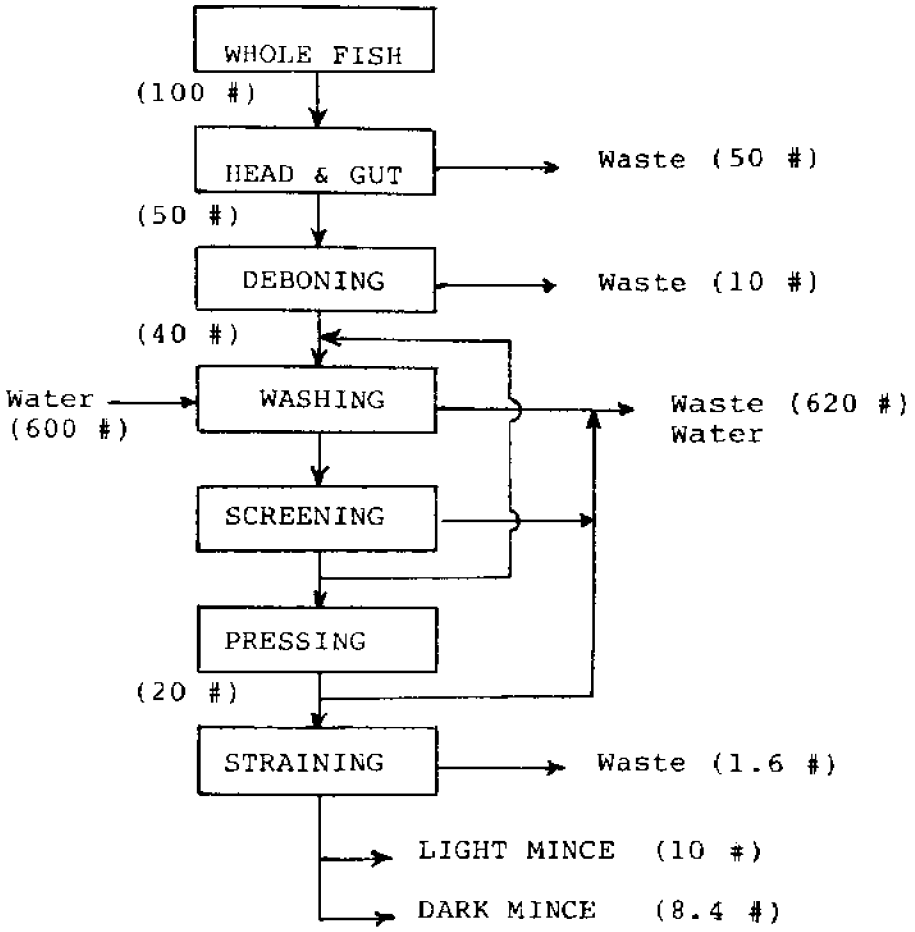

Figure 1. Washed, minced menhaden/surimi processing flowsheet.

menhaden usually contain fish of approximately the same size, a day's catch will generally be from a number of schools. Several types of sorting machines are available for the sizing of herring, sardines, and other species and should work for menhaden as well.

Several companies have tested menhaden on their gutting or filleting machines and state that their machines will work, but we have not seen actual demonstrations. Machines we have had available for testing at our laboratory have not been satisfactory for processing menhaden. Menhaden should be horizontally cut across the belly because of the sharp, bony keel. An attempt at a vertical cut is deflected to one side, interfering with a clean central cut into the belly cavity.

Since menhaden are generally soft fleshed and very difficult to scale, it appears better not to scale the fish before deboning. Scales help to hold the skin together during deboning, whereas scaling tends to damage the fish. The mechanical strainer removes scales that get through the deboner.

Deboning. The only deboner we have used is the Bibun, which operates on the principle of pressing the fillet or dressed fish against a perforated drum by means of a flexible belt. Several manufacturers make this type. Other types of deboners may operate equally as well, but we do not have sufficient information to evaluate them. Since intact skin and bones and discrete flesh particles are desirable, a deboner requiring pregrinding of the fish may be less satisfactory.

We have used both 5 mm and 3 mm perforated drums. The smaller perforations result in fewer bone, skin, and scale particles in the minced fish. Bone analyses indicate both size perforations pass a relatively large amount of bone pieces, but most of these are removed by the mechanical strainer.

Washing Minced Fish. When washing minced fish with water, the solids and liquid must not be mixed too vigorously. The best results are obtained by a gentle mixing which will keep the meat particles intact but will break up agglomerates of particles. We mix the slurry manually and gently for about 3 minutes, then allow the mince to settle. We have experienced poor settling when washing with a 3 to 1 water to fish ratio, but have had relatively little trouble at a 5 to 1 ratio.

Within 10 to 15 minutes the solids will generally settle well enough to permit decanting 1/3 to 2/3 of the liquid, which contains floating fat particles. Additional water can be separated by screening. A rotating screen is quite satisfactory. Counter current washing is applicable to continuous processing and would significantly reduce water usage.

Dewatering. A screw press is preferred for the removal of excess water from the final wet screened solids after the washing of minced menhaden. We made a limited number of trials with an available centrifugal decanter as the dewatering device. Acceptable solids recoveries and moisture contents could only be obtained at unacceptably low feed rates. However, we understand that good results have been obtained at the surimi demonstration plant in Kodiak, Alaska using a centrifugal decanter for decanting and dewatering in a single step.

Straining. We use a Bibun strainer to remove residual fine bones and scales from the washed, minced menhaden. Multiple passes thru a mechanical strainer can result in several fractions of washed mince having different shades of darkness. The lighter meat passes through the perforations of the strainer drum more rapidly and easily than does the darker meat. Most of our work on straining has been with washed minced fish. The color difference of unwashed meat is not as great when strained, largely because of the soluble blood pigments remaining.

Table 4. Typical yields in the processing of menhaden to produce washed mince.

	Yield, Percent		
Stage	Overall	Stagewise	Stagewise Range
Whole fish	100	100%	
Headed & gutted	51	51	46-55
Deboned	38	75	69-80
Washed mince	20	52	40-65
Strained Washed Mince[1]			
Light fraction	11	55	45-60
Dark fraction	7	35	15-70
Residue	2	10	2-18

[1] Stagewise yields as a percent of washed mince

Processing Yields. Some typical processing yields for the conversion of menhaden into washed, minced products are presented in Table 4. Yields vary depending on size and condition of the fish, deboning conditions, degree of washing and strainer operation. The ranges for incremental yields encountered at each stage and for fractions recovered from the straining operation are included.

Higher yields must be balanced against product quality and/or washing requirements. If production is adjacent to an existing menhaden plant, all solid wastes from heading and gutting can be utilized in fish meal production. It must be remembered, however, that food grade menhaden for surimi production will be more expensive than the fish harvested and transported conventionally for meal and oil production.

Mincing or deboning yields of light meat can be adjusted by the way in which the dressed fish is presented to the deboner and by the amount of belt pressure. If the fish is split and opened after cleaning (butterfly) the cut meat side can be presented to the drum and much of the dark meat can be left on the skin by using a low belt pressure. This will also reduce the amount of fat in the minced meat by not including the fat layer found next to the skin. This will reduce the yield but will produce a lighter meat with a lower fat content.

A moisture content of 78 to 80% appears to be desirable in a washed mince intermediate product but the moisture content can be higher if dry solids are to be added to prepare surimi. The moisture content of unwashed menhaden meat will be low if fat content is high. Therefore, it is not reasonable to have the same concentration of moisture in the washed mince as in the raw fish if the initial fat content is high.

The amount of moisture in the washed mince can vary quite a bit depending on the condition of the fish and the dewatering efficiency. The apparent yield, based on total weight of material, can be distorted by the moisture content of the washed mince as is illustrated in Figure 2. For example, the yield at 80% moisture (20% solids) would appear to be doubled if the washed mince contained 90% moisture (10% solids). The apparent fat concentration will decrease linearly as the moisture content increases (Fig. 2).

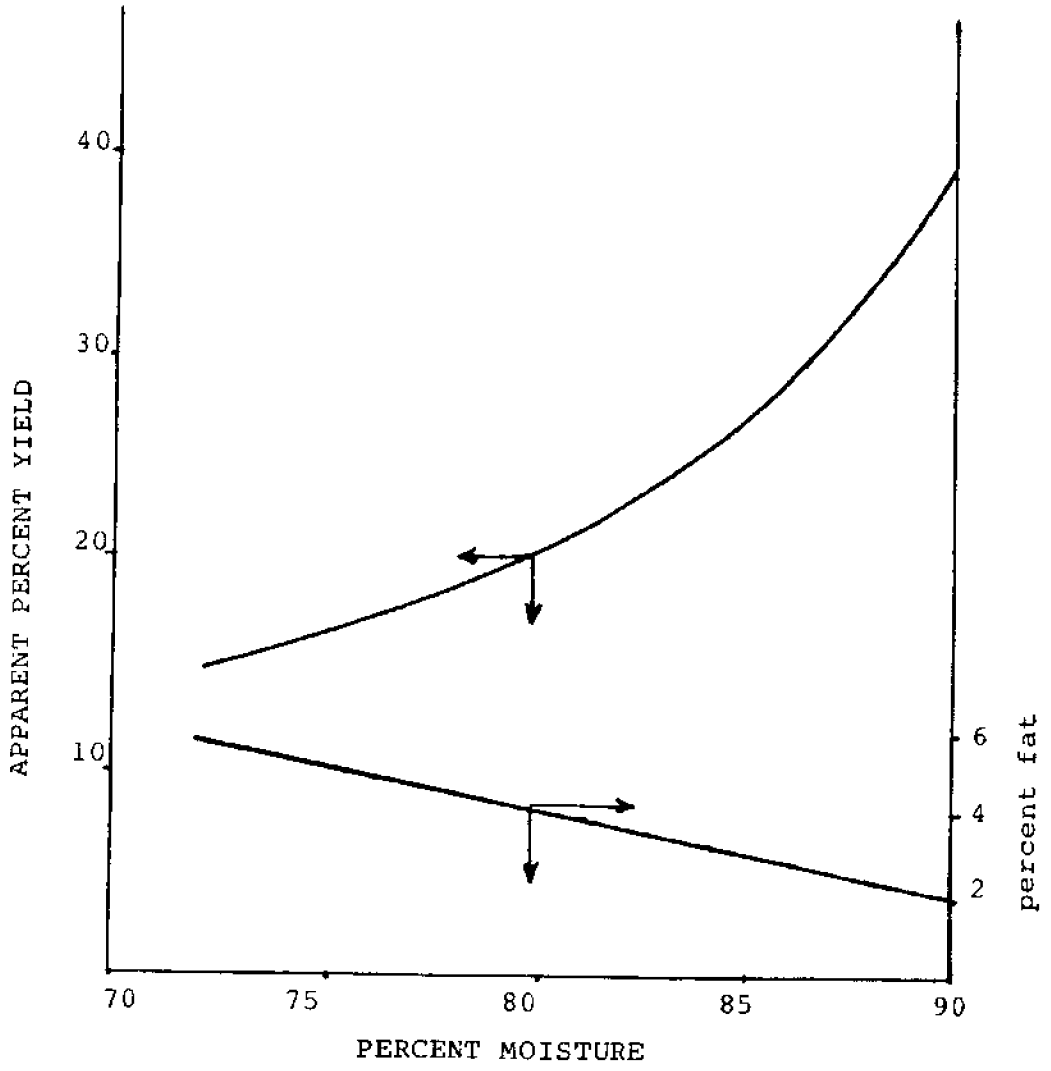

Figure 2. Effect of varying the moisture content of washed, strained menhaden mince on the apparent yield and percent fat.

Another effect of moisture content is shown in Figure 3. The moisture content of a washed, strained mince (light fraction) was adjusted by increments and instrumental color readings (L, a, b values) were recorded. Increases in moisture content have a pronounced positive effect on the lightness (L value) while the redness (a value) decreases. A higher yield and a lighter color can be obtained if the final moisture content is high, but this will be at the expense of the gel strength ("ashi") of the surimi.

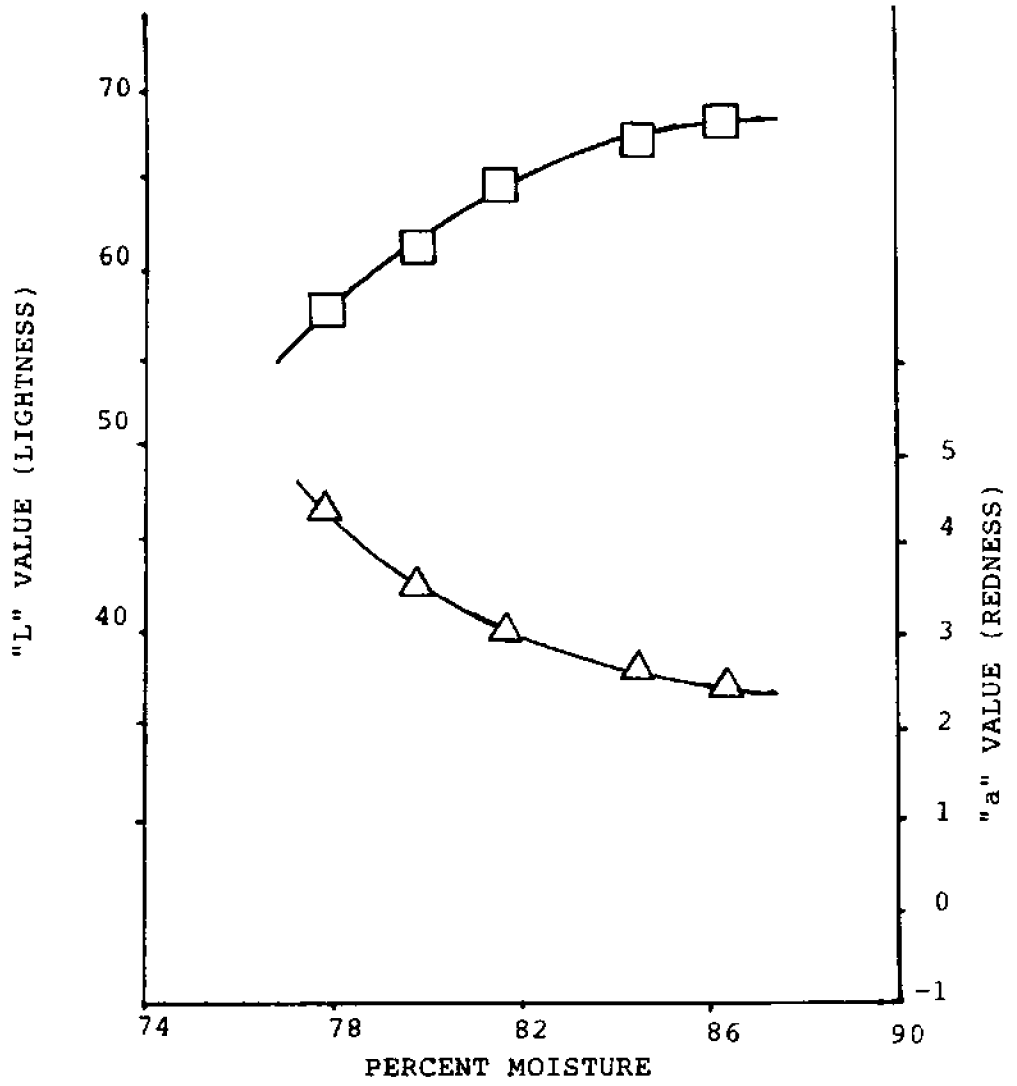

Figure 3. Effect of moisture content of a washed, strained, minced menhaden on the "L" value and "a" value (instrumental lightness and redness).

The method of operating the strainer will affect yields. By controlling the back pressure on the reject (bone and scales) stream from the strainer to a low level, only light meat is allowed to pass through the strainer screen. Restraining the reject solids can then permit recovery of most of the remaining meat as a dark fraction. Several color fractions can be obtained if desired.

Surimi formulations will determine the final yield and moisture content. If 4% sorbitol and 4% sugar are to be added and a final moisture content of 78% is desired, then a moisture content of the washed, strained, minced meat can be 84.2%. The addition of 8% by weight of cryoprotectants to a light washed mince at 11% yield would produce a surimi yield of 11.9%. This yield would be further increased if the moisture level is adjusted by adding water.

Table 5. Instrumental color values for raw minced Atlantic menhaden, washed mince, and surimi prepared from light and dark fractions.

Sample	% Sorbitol	% Sucrose	Lightness L	Red, a	Yellow, b
Minced Fish	--	--	44.2	8.9	11.3
Washed Mince (light)	--	--	61.6	5.3	12.3
Surimi (light)	4	4	58.4	4.9	12.7
Surimi (light)	0	5	60.3	4.5	11.9
Surimi (dark)	4	4	56.9	5.2	12.5
Surimi (dark)	0	5	58.1	4.9	12.2

The instrumental color values for minced Atlantic menhaden, the washed mince light fraction, and two surimi formulations each for both light and dark meat fractions are listed in Table 5. The a value (redness) is decreased by washing. The minced fish is much lighter after washing and is somewhat lighter than the surimi formulations. This, and the fact that the 5% formulations are lighter than the 8% formulations, is apparently due to the moisture levels in the materials. Cooking generally lightens the color significantly.

We have described some of our experiences with the processing steps required to prepare a washed, minced intermediate product from menhaden, an oily and relatively dark fleshed species.

During the next two years we will be looking at the information needed to obtain approval for menhaden minces and surimi in sausage type products as well as providing technical support to help assure the suc-

cess of the menhaden surimi demonstration plant that will be constructed and operated under a government contract. Minced fish and surimi samples will be made available for product research when the plant is fully operational, probably in the fall of 1986.

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