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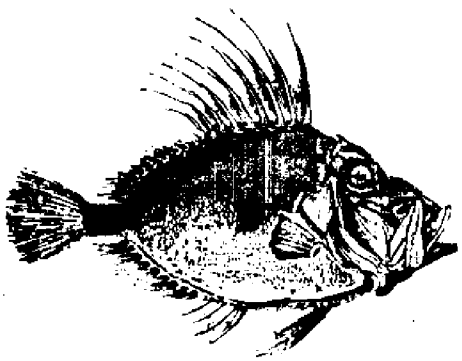
FIFTH ANNUAL TROPICAL AND SUBTROPICAL FISHERIES TECHNOLOGICAL CONFERENCE OF THE AMERICAS

April 27-30, 1980

Charleston, South Carolina



Compiled by

Ranzell Nickelson II

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

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EDITOR'S NOTE

... About that curious looking fish ...

That curious looking fish which appears on the cover sheet of the proceedings of the Tropical and Subtropical Fisheries Conference of the Americas year after year is the John Dory, a stealthy little ambush predator indigenous to the English Channel and the Mediterranean Sea. It is characterized by extremely long dorsal spines that undulate to attract other forms of marine life and jaws that can extend an amazing length to form a circular tube and rapidly suck in unsuspecting prey. The drab yellow-green John Dory rarely exceeds 60 centimeters (less than 24 inches) in length and is marked on each side of its laterally compressed body by a prominant circular black spot, or ocellus, edged with a yellow halo.

In Europe the John Dory (also known at St. Peter's fish) is considered a culinary delicacy. Its fillets resemble those of the flounder, and its flavor, according to fishermen of the English Channel, is unrivaled as the best eating fish. The French insist that the flesh of the Dory is an integral ingredient in bouillabaisse.

An American version of the European variety is found off the east coast of North America from Nova Scotia to Cape Hataras. However, its relatively infrequent occurrence prevents it from being of commercial value to American fishermen.

The John Dory is symbolically present in Blessings of the Fleet throughout the world. Its history stems from the annals of mythology, as suggested by its scientific name, Zeus faber. Historians also theorize that this characteristically placid fish played a part in Biblical chronicles. One popular story is that the ocelli on the Dory's sides appeared after St. Peter cast out his fishing line at Christ's request to retrieve a coin from the mouth of the first fish he caught (Matt. 17:26-27). Allegedly, Peter caught the John Dory and held it so tightly that his fingers made a permanent imprint upon the fish's sides.

And so, this curious little fish with the accordian mouth who has been nominally considered the Patron Saint of fishermen, is now the official symbol of the Tropical and Subtropical Fisheries Technological Society of the Americas.

FIFTH ANNUAL TROPICAL AND SUBTROPICAL
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PRODUCTION OF SALTED MINCE FROM MULLET AND FRAMES OF RED DRUM AND GROUPER

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INTRODUCTION

The utilization of surplus fish in a salted mince product (4, 6,7,10) has a number of advantages not present in other forms of processed fish. It enables the use of surplus and salvaged flesh, is a rapid process requiring minimal energy inputs and appears to have an already existing market (9). However, a number of obstacles exist which must be overcome before the process becomes a commercial reality.

Availability of suitable quantities of surplus stock, improved control of chemical, physical and microbial changes in the product, maintaining simplicity of manufacture, packaging considerations and market development are problems still requiring additional investigation. A number of these problems have been considered by other workers (3,10,11) and could probably be resolved with existing technology, whereas, others will require additional research.

This is a report on preliminary studies conducted under commercial, pilot plant and laboratory conditions on the production of salted mince from species found in Florida.

MATERIALS AND METHODS

Commercial plant experiments utilized a Bibum, SDX-16 meat-bone separator with 3 mm drum holes. Mixing of mince and salt was in a 25 gal stainless steel paddle type mixer. Brine removal was by hydraulic pressure applied to mince suspended in a nylon bag. Flesh was obtained from frames of red drum and grouper, and butterflied mullet following roe removal.

Pilot plant experiments utilized a Baader, 694 meat-bone separator with 5 mm drum holes, a Hobart mixer and a Chisholm-Ryder Model B1 screw-finisher for brine removal. Laboratory scale samples were prepared in a small Hobart mixer, and brine removal was accomplished with a Buchner funnel. Salted mince was generally prepared by mixing 3 parts flesh with 1 part salt (10) and mixing at low speed for 1 hour. Brine formation was allowed to develop for an additional 30 minutes and then removed by one of the above methods. Modifications of the basic procedure included acidification of the salted mince to pH 4.5 with 6N HCl and/or heating to 80°C for 5 minutes before brine removal. Various materials were added to the mince following brine removal and are listed in the results section. Samples were placed in glass jars and stored at 30°C.

Moisture, lipid and protein were determined according to standard procedures (2). Water activity was measured using an electric Hygrodynamics Hygrometer following standardization with solutions of known vapor pressure. Malonaldehyde was determined according to the method of Yu and Sinnhuber (12) with some modificatins in sample preparation. Aerobic plate counts were by standard procedures with incubation at 35°C (1).

RESULTS AND DISCUSSION

The maximum removal of brine following salting is the major factor governing physical stability of the product during storage. Any step that results in a more complete release of the brine will greatly add to the overall stability of the product. Within the range of moisture contents of the products formulated, Aw varied only slightly and appeared to be governed by the Aw of a saturated salt solution. Table 1 shows the Aw and moistures obtained in preliminary studies. In that there are arguments against addition of expensive chemical humectants and/or a drying step (10), a final product Aw within the range of 0.70-0.75 was considered the best that could be achieved under commercial conditions. This was with the realization that microbial growth can occur within this range but could be controlled by the addition of 0.3% sorbic acid (8).

Table 2 shows data obtained from laboratory and pilot plant prepared salted mince. In an attempt to bring about greater release of the brine, application of heat and/or acidification of the salted mince was investigated. The data show that both of these steps resulted in a reduced moisture content and that a combination of the two treatments resulted in an even greater reduction of the final moisture. The importance of this relates to drip accumulation in the final package. Our data indicated that the final product must have a moisture content of less than 40% before drip is controlled. In an attempt to bind additional moisture, an edible hydrophilic material was added. Coarsely ground corn grits were tested and found to improve water retention. In a number of trials, samples that were heated or acidified containing added grits did not release brine for over six months, whereas, control

samples did. This addition of low moisture cereal grains is considered an excellent method for stabilizing brine migration in salted mince produced under commercial conditions. The variability encountered in the data is attributed to differences in raw material as well as to subtle differences in preparation of the product.

When the salted mince is first prepared, the characteristic odor of salt fish is lacking. Depending upon storage conditions, the odor develops in 3 to 7 days and is usually quite mild. The addition of BHT retarded oxidation (Table 3). Concerning species characteristics, both grouper and red drum produced acceptable salted mince. This included characteristics of manufacture and final product. Color and odor as determined subjectively were both considered acceptable. The color varied from light grey to dark yellow. However, mullet flesh presented some problems. Brine release was slow (it formed a gel-like structure when salted), color of the final product was grey and rapidly turned yellow during storage when exposed to air. However, the color stabilized, and the odor was acceptable even when exposed to air for 2 months at ambient temperature. Compositional characteristics of some products are listed in Table 5.

Storage studies of the products (Table 4) indicated that the indigenous microbial flow was not a problem (5) but to insure stability 0.3% sorbic acid should be added (10).

When foods were prepared from stored product, they were acceptable to a small informal panel. Chowder, fish cakes and a mixture of mince and vegetables were considered acceptable to the panel members.

While this work would indicate the feasibility of producing salted mince under commercial conditions, a number of questions still remain. Is pH 4.5 the optimum for maximum brine release and are there additional advantages to be gained from having the mince at a low pH (i.e., microbial suppression) and how will this step effect its physical properties over an extended period of storage? Acidified mince rehydrates readily, however, its color is not as stable as that of heated mince. Heated mince has good physical stability, but its functional properties are altered. That is, it does not rehydrate well, however, is this important from the standpoint of the user? In that this product will probably find its greatest use in institutional feedings, this factor may not be important. In addition, the question of lipid oxidation needs to be answered. Does rancidity need to be controlled and for how long? Current technology is available to solve these problems and only requires an extended storage study combined with sensory work to find an answer. What water binding material to use will be dictated by economics and the end-use of the mince. Soybeans, rice or native starches are all viable options. Finally, additional studies need to be conducted to determine species differences and their effect on product quality.

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	Aw	Moisture
Redfish (Commercial)		
Control	.72	44.40
Control + 2% NFDM	.72	42.78
Control + 1% Tragacanth	.72	43.62
Control + 5% glycerol	.71	42.16
Grouper (Pilot Plant)		
Control	.72	45.90
Control + 20% grits	.72	40.29
Control + 10% glycerol	.71	--
Control + 10% LiCl	.66	--
Corn Grits	.51	8.33
20% glycerol solution	.96	--
Saturated salt solution	.75	--

Table 1. Moisture content and Aw of some salted mince products.

	Aw	Moisture
Grouper (Pilot Plant)		
Control	.75	42.56
Control acidified	.73	40.46
Control heated	.72	24.71
Mullet (Pilot Plant)		
Control	.76	47.09
Control + grits	.76	39.57
Acidified	.77	41.31
Acidified + grits	.78	35.22
Grouper (Laboratory)		
Control	.74	50.54
Heated	.73	40.35
Acidified	.74	48.90
Heated + acidified	.73	36.44

Table 2. Effect of heat and/or acidification on moisture contents and Aw of salted mince.

Treatment	TBA Values (week of storage) 0	1	2	4	8	12
Grouper (Commercial)						
Control	12.03	23.78	24.97	23.91	22.38	63.55
Control + 20% grits	12.03	16.86	18.71	16.76	17.09	29.06
Control + 100 ppm BHT	6.51	7.19	7.41	6.32	5.94	16.45
Control + 0.3% Sorbic acid	13.00	23.28	25.70	22.65	23.63	24.38
Acidified	16.49	45.70	41.34	36.23	30.04	53.24
Acidified + 20% grits	13.29	33.43	31.51	29.59	27.23	59.05
Acidified + 100 ppm BHT	12.00	28.61	29.52	29.01	21.02	57.68
Acidified + 0.3% Sorbic acid	15.24	43.33	34.65	34.52	34.47	75.40

Table 3. Effect of various treatments on rancidity development in salted minced grouper.

Sample	% Salt In Medium	weeks of storage 0	1	2	4	8	16
Control	A	114,000	910	160	20	40	0
	B	3,000	100	10	0	0	10
Control + grits	A	58,000	1600	200	10	30	10
	B	800	50	10	0	5	0
Control + grits + sorbic acid	A	33,000	700	100	40	50	5
	B	2,700	30	5	0	0	0
Acidified	A	1,300	70	60	30	30	50
	B	400	10	0	0	10	0
Acidified + grits	A	600	100	70	80	60	55
	B	200	20	10	5	20	0
Acidified + grits + sorbic acid	A	500	90	80	30	30	20
	B	200	10	5	5	10	0

A = 0.5% NaCl added to medium
B = 15% NaCl added to medium

Table 4. Microbial changes in salted minced grouper during storage.

Grouper	Fat	Protein	Moisture
Control	0.55	14.5	50.5
Acidified	0.59	11.3	49.5
Heated	1.31	18.5	39.7
Acidified + heat	1.41	19.5	36.8
Mullet			
Control	1.62	11.14	47.09
Control + grits	1.42	10.19	39.58
Acidified	2.01	10.81	41.31
Acidified + grits	2.76	11.71	35.22

Table 5. Compositional characteristics of some salted minced products.

PILOT PLANT PRODUCTION OF SAUTEED FISH FLAKES (YU-SONE) FOR EXPORT

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BACKGROUND

In recent years, under the major campaign for pursuing the maximum benefit from the extended 200-mile jurisdiction, great effort to develop and promote new seafood products in different directions has been made by many research institutes and seafood industries. However, the progress in the domestic market area has been slow due to the difficulty in improving the consumer's attitude toward seafood. On the other hand, the promising potential of the export market is being gradually recognized. Although trade of fresh or frozen seafood has always been emphasized more than processed seafood trade, the latter category actually has many advantages. Processing can reduce the transportation cost, increase product stability, modify the product taste and appearance, and keep production cost under the control of the domestic seafood industry. The objective of this study is to investigate the feasibility of producing an exportable, processed seafood product, from underutilized fish, that is traditionally accepted by the foreign market yet still profitable to the seafood industry in the U.S.

Yu-sone, a semi-dried, seasoned fish flake product, is produced from a variety of fish. It is a ready-to-serve product and goes well with Chinese breakfast, as a snack, or in a Chinese lunch box. It is a high protein food and resembles a similar product made from pork, called Zo-sone, yet is cheaper. Thus, it remains very popular in recent years. Currently, Yu-sone is primarily produced by small processors in the Orient with labor intensive methods. In this study, we have investigated various methods for mechanizing each step of Yu-sone production so that a competitive production cost can be maintained. Large quantities were produced so that the experimental data would be similar to that of the industrial operation. The samples thus

produced will be used for a market test.

A general survey on the quality of Yu-sone from about 40 commercial varieties (2) showed that their proximate contents ranged from 1 to 19% for moisture, 11 to 35% for fat, 20 to 40% for protein, and 5 to 10% for ash. This indicated that they were dried for various length of time at various temperatures so that some were scortched more than others. Palatability might be their major rule to produce these products. Naturally, these differences in composition imply differences in nutritional value and product stability. However, yu-sone can be basically divided into two categories, one without adding cooking oil, the other with added oil in the amount of about one third of the final weight. The variety without added oil can not be dried too much or the product will be too dry to be palatable. Yet, a moisture content higher than 20% may bring in the spoilage problem (3). Also, without any antioxidants added, the inherited fat from fish may become rancid easily. On the other hand, the product can be dried to 2% moisture content if 20 to 30% of final product weight of oil is added (6). The dryness and the presence of oil keep the product crisp and with good flavor. It has high microbiological and chemical stability because of the low moisture content and the presence of antioxidants from the added oil. The major differences among products produced from different fish are the different fiber textures and fish odors inherited from the various fish. Fish with long and firm fiber, low fat content, and mild flavor would be the prime raw material for Yu-sone (6). The shape and size of the fish in round may affect the difficulty in the deboning process, yet will not directly affect the quality of the product. Among various underutilized fish, sting ray, shark, gulf sea bass, mullet, and lizzardfish were found to be good raw materials for yu-sone products. In this study, three species were used; mullet representing small bony fish, shark and ray representing large meaty fish.

MATERIALS AND METHODS

Northeast Florida mullet was purchased from a Jacksonville seafood wholesaler in the months of January and February, their size ranged from 1 to 2 lbs. They were dressed by the wholesaler and had never been frozen. The shark slabs were from Mako shark bought from the same place, and frozen with skin on. It was imported from Northern Peru. They weighed about 40 lbs. per piece. The ray was Northern sting ray (Dasyatis centrura) caught off the Georgia coast. It weighed about 30 to 40 lbs. per wing. The ingredients were bought from local food wholesalers. Major equipment used in the production included an Yanagiya deboner with a 3mm

perforated drum, a vertical, perforated basket centrifuge; and a 20-gal agitated, heat-jacketed kettle with an oil heater designed in this pilot plant. The "pilot plant" was actually a 65-foot mobile home with about half of the work area converted to a fire and water resistant processing area. The ceiling and walls were lined with Fire-X® fiberglass panel (Kemlite Co.). The floor was sloped to the center, matted, and spread with fiberglass.

The procedure to make Yu-sone (3) was basically to (i) obtain deboned fish flesh from the round, (ii) cook the meat by boiling or steaming, (iii) dewater the cooked meat, (iv) add ingredients which included salad oil, sugar, flour, salt, soy sauce, MSG, and spices, and (v) be mixed and dried till it was dry and crispy (Figure 1). The main steps for mechanization were in the flesh separation, washing, dewatering, and sautéing. The sautéing step was the most time consuming and quality deciding step.

APPROACHES FOR UNITIZING THE PRODUCTION

Fish cleaning. In this study, mullet was scaled, headed, and gutted by the wholesaler. It was discovered that the blood content of the dressed fish could be greatly reduced by washing off the blood and kidney portion thoroughly. The method of water jetting seemed to work very well. The annoying problem of splashing can be prevented with the jet and fish submerged under water during the washing. It took about six man-hours to jet-wash the blood and kidney off 300 lbs of mullet, which was enough for 25 lbs of finished product. Bleeding at this stage by soaking the cleaned fish in running water before deboning could also reduce the blood content. To mechanize these cleaning tasks, different approaches are needed for small bony fish or large fish. There are several automatic header/gutters for the small fish. Yet, the large shark or ray need only a slabbing or cutting machine to do the job.

Skinning, deboning, and washing. Shark slabs and ray wings were cut into smaller blocks. The blocks were scalded in boiling water for about eight minutes to soften connective tissue under the skin. The skin was then peeled off manually. The meat was slightly cooked which was all right, because it was then boiled anyway in the next step. In an industrial situation, a skinning machine may be used for this step. With ray, a filleting step was needed for deboning. A conventional deboner for small fish was not very efficient for these large fish. It was satisfactory for producing minced fish from the mullet, except that the drum perforation should be 3 mm or larger so that shearing of the fish flesh can be minimized. At this

stage, residual blood extracted from minced flesh could be easily washed away by mixing with cold water and then dewatering by a centrifuge (4). A batch or continuous type centrifuge or even a screw press can be used for this purpose in an industrial situation. This washing and dewatering practice is important in reducing the hemoglobin content in the product so that the off-flavor and rancidity problems can be minimized (1,4). It took us about five man-hours to debone and wash the minced flesh recovered from 300 lbs of mullet and another two man-hours to dewater the flesh by centrifugation. About five man-hours were required to skin and debone enough shark slabs or ray wings to produce a 25 lb batch yu-sone. However, the shark was easier than the ray wings.

Boiling and dewatering . The deboned fish flesh was then cooked by either boiling or steaming. 30 to 40 minutes of cooking was needed so that fibers could be separated easily instead of caking together during sautéing. The boiled ray meat, especially, required a dewatering step to remove excess water. Our approach was manually placing the meat in a cloth bag and weighing down the bag with a heavy weight. This extra dewatering step saved drying time and energy, while it also pressed out some unwanted fish odor producers and fat. The manual boiling and dewatering took us another one man-hour of labor. In an industrial scale operation, a centrifuge or a screw press would serve this purpose.

Sautéing. In this step, the boiled and dewatered fish meat was mixed with ingredients, blended, and heated till crisp and dry. Liquid ingredients included salad oil and soy sauce. Dry ingredients included sugar, flour, salt, MSG, and spices. The mixture was wet and heavy in the beginning with a maximum bulk density of 0.704 g/cc or 5.87 lbs/gal. It became heavy and sticky half way through the sautéing, and finally became light and fluffy at the end with a maximum bulk density of 0.636 g/cc or 5.30 lbs/gal. The superior product usually has long fluffy fibers with a good crisp texture. Therefore, the sautéing machine must possess the ability to break up the fiber gently while the meat is being dried. Traditionally, this has been done by sautéing in a wok; the mixture was dried over a medium fire while being broken down by the pressing and shoveling action of a spatula. After several commercially available agitated cookers were compared, an agitated jacketed kettle was selected for our pilot plant operation due to its relatively economic price and its effectiveness. An oil heater system was also designed as shown in Figure 2 so that the system could be heated economically without using a boiler. Heat transfer oil was used as the heating medium. An LP-gas "candy stove" was the heat source for the whole system. Its heat output was regulated

by the oil temperature, by means of a bulb thermostat gas regulator. The hot oil was circulated through the kettle jacket by a hot oil pump. The best setting for the dry bulb temperature of the kettle was found at 150C (302F) while the product temperature stayed around 70C (158F) most of the time and increased to 100 C (212F) before the sauteing was terminated (Figure 3). The temperature was found to be a very dependable indicator for the finish point of sauteing. At this stage, the product was dry and crisp. With the current set-up, it took about five hours of sauteing to produce 25 lbs of finished product. The speed depended largely on the moisture content of input material, the batch size, the humidity in the environment, and the heating medium used. The heat transfer oil has a lower heat transfer coefficient than steam, thus it is less efficient in heating up the meat inside the kettle. The agitator did mix the material well. Modification of the angle of the agitator to have a 22° inclination helped the mixing tremendously. The optimal agitator speed was found to be around 20 to 30 rpm; the boiled fish flesh could be broken down completely at this speed. About one man-hour labor was required for starting up the system, metering the ingredients, loading, and unloading the product. The total ingredient cost was estimated as about $25.00 for producing 100 lbs of yu-sone.

Packaging. Since the product had a moisture content of 1 to 2%, it was very stable even at the room temperature. It may have minimal shelf life of one year provided that it is protected from moisture and insects. Thus, opague polyester cups were chosen as the containers. The cup size was 16 fluid oz, which could hold 8 oz of tightly packed yu-sone. It took us about two man-hours to manually weigh and package 50 cups or 25 lbs of products.

Product quality. The taste of products from all three species was found excellent. No urea-like odor was present in either the shark or ray product. No oily smell was detected from the mullet product, which was probably due to the washing and dewatering approaches as well as the low fat content of the mullet in January. The color was a natural, brownish meat color, though not as dark as the commercial yu-sone. The ray product had a darker color than shark and mullet, probably due to the dark meat from the sting ray. The shark product actually had a very appealing reddish-brown color. The color of the mullet sample was the lightest among the three products. The shark had the best crispiness and fiber length (Table 1). The mullet had a shortest fiber size which might be

due to the deboning practice, and was found the closest to the fiber appearance of a commercial product produced from sea bass. A consumer sensory evaluation will be conducted in an upcoming market test conducted in two China-towns in the U.S. and two cities in Taiwan. The fiber length was compared statistically as shown in Table 1, which indicated that the fiber size depends significantly on the species of the fish and the size of each sautéing batch at a 99% confidence level. The interaction of the two factors was also very significant at 99% confidence level. The effect of batch size was more pronounced for ray than shark or mullet. The fiber length of shark ranged from 2.21 to 2.66 mm, as compared with 1.96 to 2.44 mm for ray and 1.67 to 1.89 mm for mullet. A batch with the largest fiber size was produced from a 8.5 lb-batch of ray. It reached 2.80 ± 0.6 mm. In summary, some of the factors that may determine the fiber size of the product are (i) the sautéing batch size, (ii) the species, (iii) the agitator configuration, (iv) the agitation speed, (v) the agitation time, and (vi) the extent of boiling. The effect of the batch size is actually related to the agitation time which also depends largely on the kettle capacity. The agitation time is also dependent on the design of the kettle system, the jacket temperature, and the heat transfer coefficient of the heating medium. The heat treatment during the sautéing step for a 16 lb batch with three and half hours of sautéing time was equivalent to a F250 value of 0.5 or 65 of F212 value. The product was exposed to 70 C most of the time and 100°C for at least five minutes. Before that, the fish flesh had also been boiled for 30 to 40 minutes in the previous step. Thus, with these heat treatments and the dryness of the product, the sanitary condition of this product was very sound.

CONCLUSION

The treatment of fish before the boiling step, which included heading, gutting, deboning, washing, and dewatering, was similar to the minced fish production process. Thus, various mechanized approaches to Yu-sone production could be adapted from the minced fish industry. A minor difference in the yu-sone process is the requirement of minimal shearing of the fish fibers. Maintaining the meat at low temperature during processing is not critical because protein functionality is not required for the final product. In fact, the drip-loss caused by freezing and thawing did not affect the quality of yu-sone. Frozen storage was not required if the finished product was marketed within one year, although refrigeration is recommended, especially, after the product

was opened by the consumer.

The pilot plant scale operation used in this study, produced yieldsof 7.86%, 26.96%, and 12.90% from mullet, shark slabs, and ray wings, respectively. Thus, the major fraction of the production cost is the fish used to supply raw materials. This fraction was estimated to range from 47% for shark slabs to 64% for mullet (assuming a fish price of 30 cents per pound).

An example of an unitized production system is shown in Figure 4. The treatment of small fish is as previously stated. For large fish, the scalding-peeling method would be set up as shown. However, a skinning machine could replace the hand scalding-peeling operation. The boiling step can be carried our continuously utilizing a special conveyor with separated chambers to control the movement of meat through the boiling tank. The residence time can then be easily adjusted and controlled. The following dewatering step can use different types of continuous extractors such as a screw press or continuous centrifuge. The size of capital investment and degree of automation of the extractors as well as the rest of the processing machinery should be determined based on the cost-benefit analysis and the market volume of the product. Further market data and consumer acceptance will be obtained from an upcoming market test, which may help indicate the feasibility of this product.

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BATCH SIZE	SHARK	RAY	MULLET
11 - 13 lbs.	2.66 ± 0.47^{Aa}	2.44 ± 0.54^{Ba}	1.89 ± 0.30^{Ca}
20 - 22 lbs.	2.30 ± 0.42^{Aab}	2.16 ± 0.62^{Bb}	1.82 ± 0.40^{Ca}
27 - 29 lbs.	2.21 ± 0.39^{Ac}	1.96 ± 0.30^{Bb}	1.67 ± 0.37^{Cb}

^{ABC}Designation for statistical difference among three species produced by similar batch size; values with same letters are not statistically different ($P<0.05$).

^{abc}Designation for statistical difference among three batch sizes used in sautéing of one specific species; values with same letters are not statistically different ($P<0.05$).

TABLE I

AVERAGE FIBER LENGEH (mm) OF SHARK, RAY, AND MULLET YU-SONE PRODUCED FROM DIFFERENT SAUTÉING BATCH SIZES (FINAL WEIGHTS)

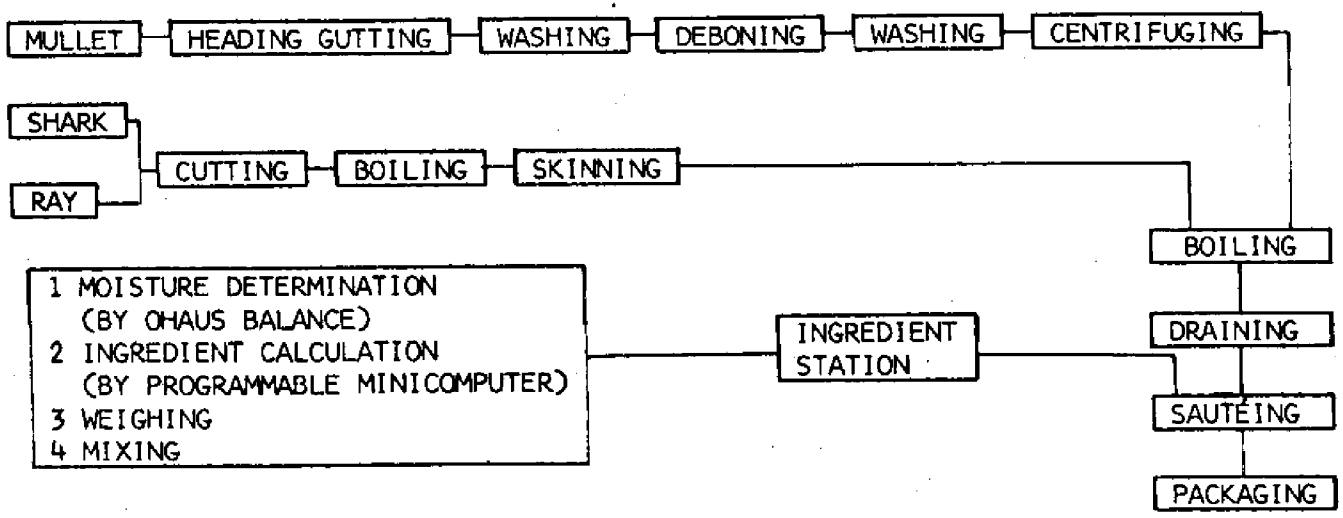

Figure 1. Yu-sone process using either small or large fish.

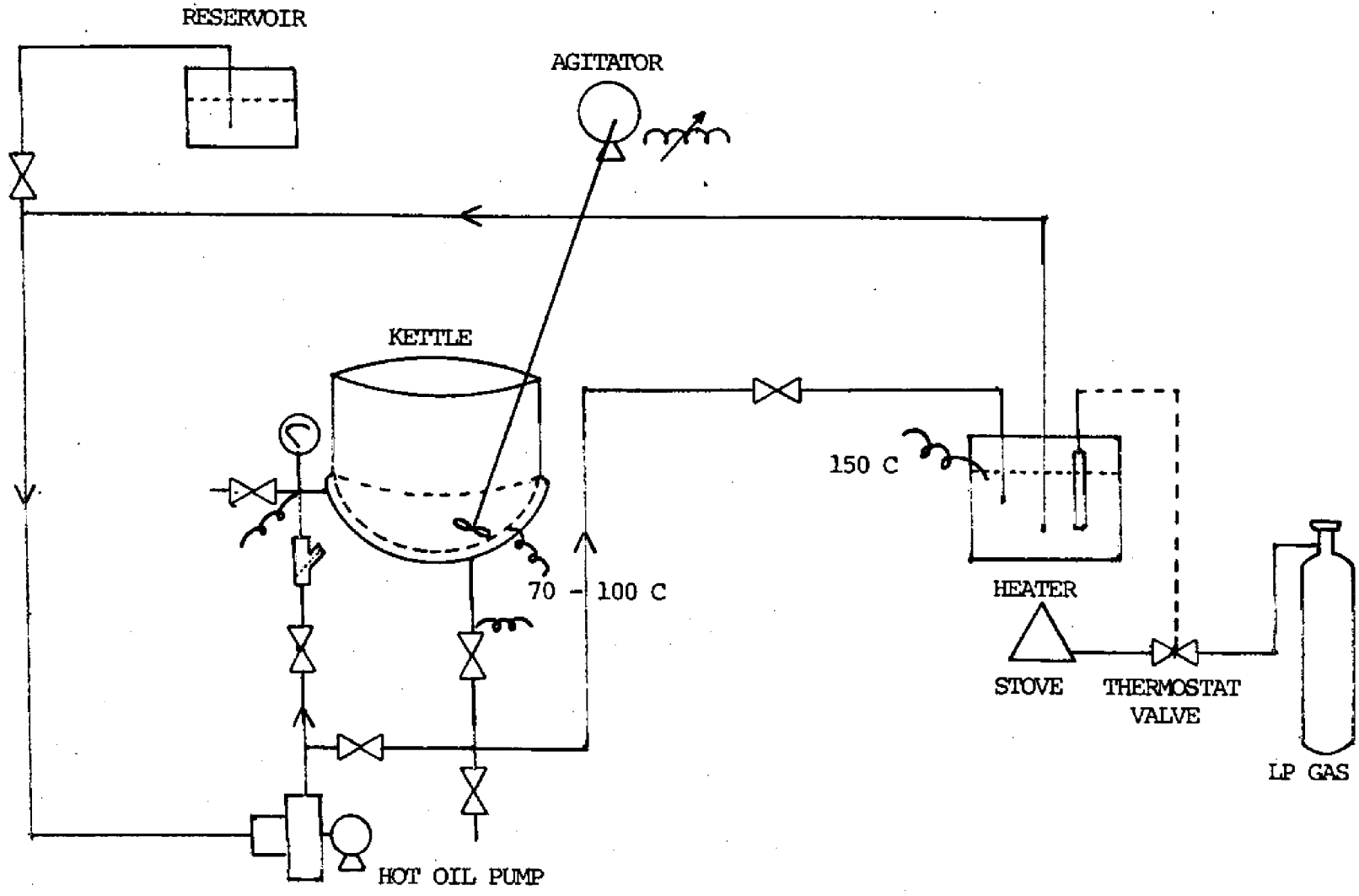

Figure 2. Design of the sautéing system.

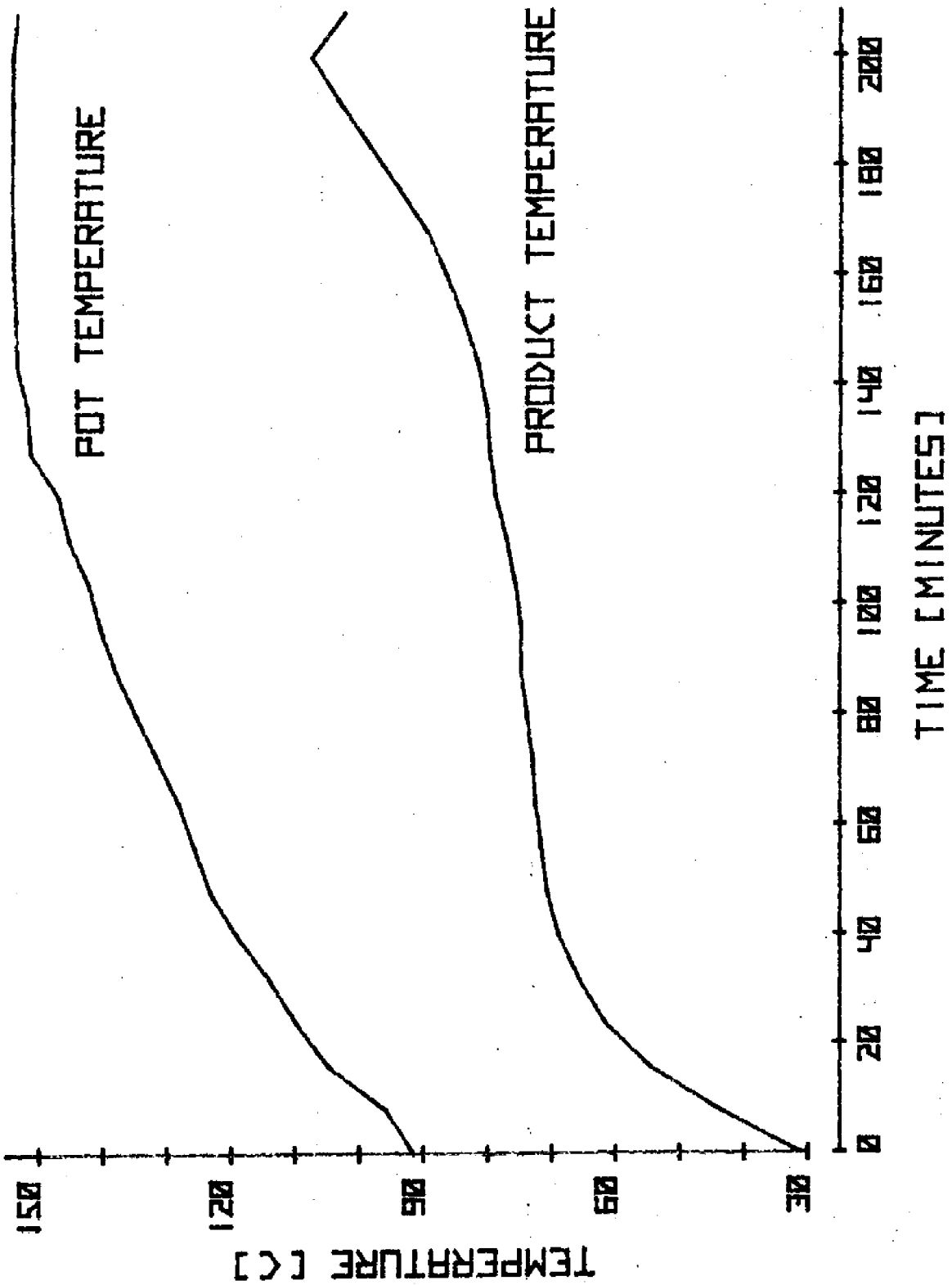

Figure 3. Time-temperature curves of the product and the heating oil during sautéing.

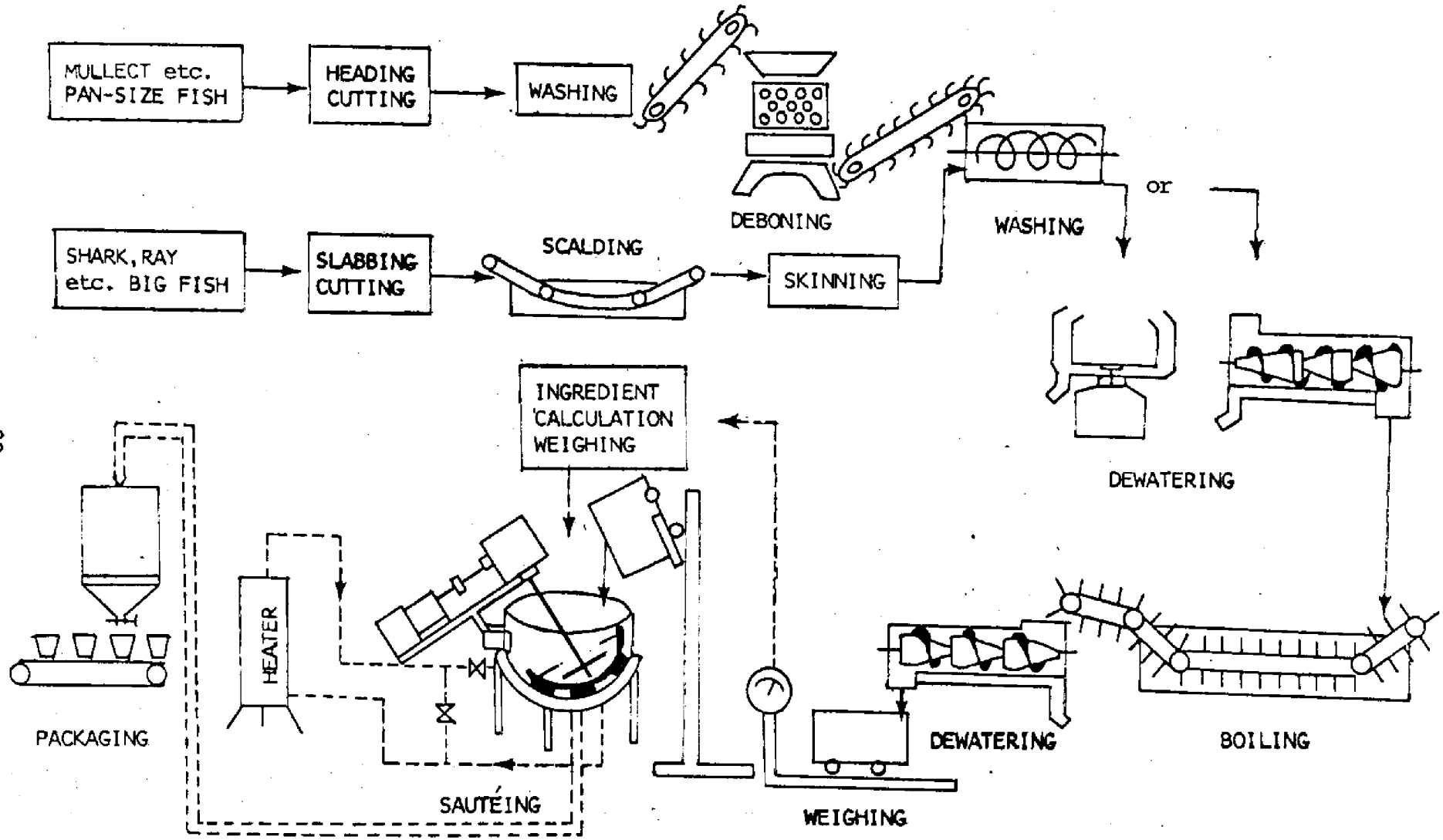

Figure 4. Layout of an unitized system for yu-sone processing.

STEAM UNIT TO AID IN OYSTER SHUCKING

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A shortage of skilled labor and a desire for increased productivity has caused oyster processors to explore new technologies to assist in shucking oysters. One such technology involves the use of heat to relax the adductor muscle of the oyster to facilitate hand-shucking.

The "hot dip" or "shock" method (3, 4) used for this purpose in the 1950-1960's is seldom practiced today. A newer process which is being employed on the east coast uses steam to induce heat shock in oysters. However, the steam tunnels used for this purpose are not standard, usually being designed and constructed by processors to suit their individual plant needs. Further, the technical literature does not provide information on tunnel designs or an evaluation of the effect of a moderate heat treatment on the quality or storage life of oysters.

Oyster processors in Mississippi became interested in the use of steam to facilitate hand-shucking, but were reluctant to invest in the necessary equipment until studies were undertaken to provide an acceptable design for a steam tunnel or to market oysters shucked with the aid of this unit until product quality and shelf life studies were conducted. This project was undertaken to remove these impediments to the use of a steam treatment as an aid to oyster shucking.

MATERIALS AND METHODS

Steam Tunnel:

The plans for the steam tunnel were prepared by engineers with the Mississippi Cooperative Extension Service and are shown in Figure 1. The tunnel used in this study was based on that design and constructed from available materials. The washer was not installed when these studies were conducted.

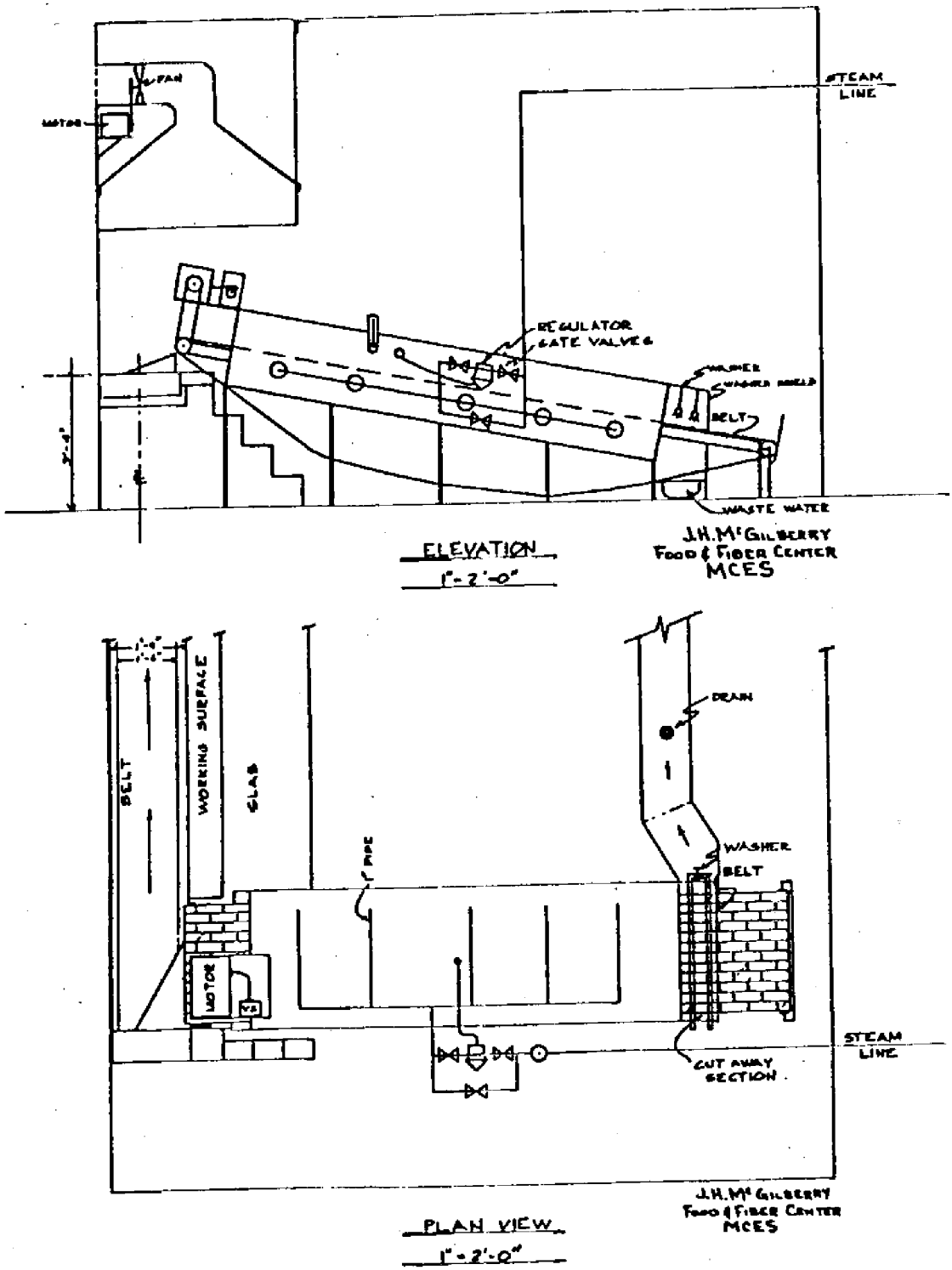


Figure 1. The steam unit design.

Temperature was controlled by a regulator attached to a sensing unit placed near the center of the tunnel. Oysters were moved through the 8-ft. tunnel on a belt in a transit time of 2 3/4 min. The increase in internal temperature of the oysters was approximately linear with travel time through the tunnel.

Oysters:

The shellstock used in each trial was obtained from a single commercial source in a single shipment. Table 1 provides information as to source, harvest date, size and clustering of oysters used in each trial.

Size and clustering analyses were made on a sample of oysters used in each trial from one randomly selected sack. Oysters in each sample were separated into singles, doubles, and clusters containing three or more. The number in each group was divided by the total number of oysters to arrive at the percentage in each group. Each oyster was then measured from bill to hinge to the nearest millimeter to obtain range and average size data.

The internal temperature of the oysters prior to and immediately following heat treatment was measured with a telethermometer (YSI Model 42) equipped with a hypodermic probe. The oysters were opened and the top shell removed. The probe was inserted between the mantle and gills and allowed to equilibrate for 15 seconds before the reading was taken. Temperature measurements on four to six animals were used to obtain an average.

Experimental Design:

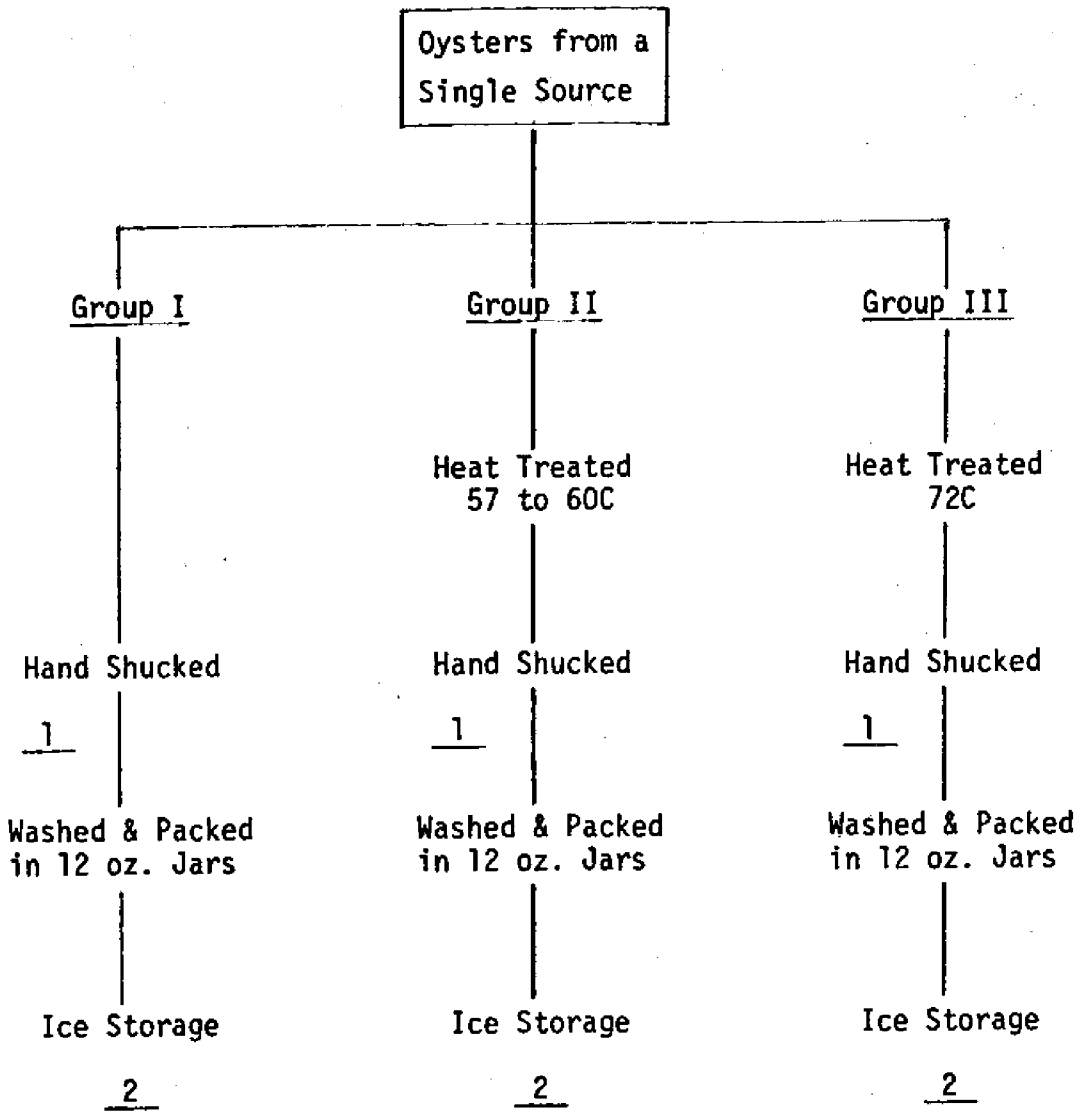
Each lot of oysters was divided into three groups of equal size for processing as shown in Figure 2. Group I oysters represented the control group and were not subjected to the heat treatment. Group II oysters were passed through the steam tunnel regulated to a temperature of 55-60 C. This temperature range, determined by trial and error experimentation, was considered to be the optimum exposure temperature. At this temperature, a few of the oysters gaped and the remaining ones offered little resistance to insertion of the knife between the shells. The oysters generally retained their shell liquor and there was no curling of the mantle. Group III oysters were intentionally overheated at a temperature of approximately 72 C. Overheating was characterized by the oysters gaping widely, a loss of mantle fluid and a curling of the mantle.

Dissection of Group II oysters immediately after they emerged from the steam tunnel revealed that their hearts were still beating. Since the oysters were alive, the heat treatment could not have been severe enough to be considered a cooking process.

Immediately following heat treatment, if any, each group of oysters was processed by plant personnel using their normal

	Trial			
	A	B	C	D
Place Harvested	Motinatit, LA	Belle Fountain, MS Relaying Area	Lake Borgne, LA	Seadrift, TX
Date Harvested	12/3/78	1/20/79 & 1/21/79	2/21/79	3/19/79
Date Processed	12/5/78	1/23/79	2/26/79	3/22/79
Oyster Size:				
Range	60-127mm	70-127mm	63-152mm	63-134mm
Average	89mm	92mm	115mm	98mm
Clustering of Oysters				
Singles	42.5%	48.6%	43.3%	61.3%
Doubles	55.0%	45.7%	53.3%	16.1%
3 or more	2.5%	5.7%	3.3%	6.5%
Temperature of Oysters				
Prior to Steaming	21-22C	20C	22-25C	25-27C
Group II Processing				
Steam Tunnel Temperature	60C	57C	57C	60C
Oyster Temp. after Steaming				
Range	34-42C	30-39C	36-41C	39-44C
Average	39C	35.8C	39.2C	42C
Percent Oysters Gaping	Few	Few	Few	All
Group III Processing				
Steam Tunnel Temperature	72C	72C	72C	72C
Oyster Temp. after Steaming				
Range	45-50C	45-53C	41-52C	52-56C
Average	47.6C	49C	46C	53.5C
Percent Oysters Gaping	All	All	All	All

Table 1. Data on the source, size and clustering of oysters and conditions used in steam processing.



Sampling Scheme:

1 Oysters sampled for bacteriological analysis only.

2 Oysters sampled at intervals and tested for organoleptic acceptability, bacteria count and free-liquid content.

Figure 2. Experimental design and sampling scheme.

commercial procedures. Oysters were handshucked into icewater. As each shucker filled his bucket, it was taken to the packing room, placed on a strainer and washed to remove large shell fragments and other debris. The oysters were then placed in a cascade washer and washed for three to five minutes. Oysters were packed in 12-ounce jars and placed in an insulated chest and iced. Care was taken to see that the jars were covered with ice throughout the storage period. Periodically during the storage period, jars of oysters were removed for testing.

Bacteriological Testing:

The standard plate count technique (2) was used to enumerate bacteria in all samples. In addition, the psychrophilic bacteria which develop during ice storage were numerated by a spread plate technique on Plate Count Agar (Difco) and incubated at 15 C for 72 hours. The latter counting technique was employed since many of the bacteria which cause spoilage in oysters kept on ice will not grow at the 35 C temperature employed with the standard plate count technique. Bacteriological data reported here represents an average of duplicate analyses on each sample.

Free-Liquid Content:

The amount of free liquid in the jars of oysters was measured by the AOAC (1) procedure. Two of three jars from each group were analyzed on each sampling date and results are reported as averages.

Organoleptic Test:

Organoleptic acceptability of the packed oysters was determined by a taste panel consisting of five to seven Gulf Coast Research Laboratory staff members, all of whom frequently ate raw oysters. Attempts were made to maintain the same panel members throughout each of the four trials. Before the tests were conducted, the panel members were briefed on the purpose of the study and instructed as to what the test was to accomplish.

The panel members were asked to rate oysters from each group as compared to a reference standard consisting of Group I oysters. For these tests, a rating scale of 1 to 5 was used with 3 being equal to the control. The score card used in these tests is shown in Figure 3. Group I oysters were included in the blind samples to test the validity of the taste panel scores. Flavor, texture and appearance were evaluated on each group of oysters and an overall rating calculated in which flavor received twice the weight of the other characteristics. Only average results are presented in this report.

Shucking Rate:

Oyster shuckers were asked to open two groups of oysters; one group which had been passed through the steam tunnel and the

OYSTER EVALUATION

Name:_________________
Date:_________________
Trial:__________ Time of Day:_________________

The purpose of this test is to determine if oysters processed by various methods are equal in quality to standard oysters provided to you. Each group of oysters is to be rated on its appearance, flavor, and texture. Please be completely objective in your evaluation.

Directions:

1. Taste each group of oysters and compare with the standard.
2. Mark the appropriate column comparing the test oyster and the standard oyster for each characteristic--appearance, flavor and texture.

SAMPLE:__________

Ratings	Much Better Than Standard 5	Slightly Better Than Standard 4	Equal To Standard 3	Slightly Poorer Than Standard 2	Very Poor In Relation To Standard 1	Comments
APPEARANCE RATING: (Color; liquor color)						
TEXTURE RATING: (Toughness)						
FLAVOR RATING: (How does it taste?)						

SAMPLE:__________

Ratings	Much Better Than Standard 5	Slightly Better Than Standard 4	Equal To Standard 3	Slightly Poorer Than Standard 2	Very Poor In Relation To Standard 1	Comments
APPEARANCE RATING: (Color; liquor color)						
TEXTURE RATING: (Toughness)						
FLAVOR RATING: (How does it taste?)						

SAMPLE:__________

Ratings	Much Better Than Standard 5	Slightly Better Than Standard 4	Equal To Standard 3	Slightly Poorer Than Standard 2	Very Poor In Relation To Standard 1	Comments
APPEARANCE RATING: (Color; liquor color)						
TEXTURE RATING: (Toughness)						
FLAVOR RATING: (How does it taste?)						

Figure 3. Score card used in organoleptic eyaluation of oysters in Trials B, C and D.

other which had not. The length of time required for each shucker to open a given number of oysters in each group was recorded and shucking rates calculated.

RESULTS AND DISCUSSION

Four trials were conducted to assess the effect of the heat treatment on the organoleptic quality, free liquid content, and spoilage rate of oysters. Oysters harvested from different areas during different times of the year were used to gauge the effect physiological conditions of the oysters may have on the characteristics evaluated. Table 1 lists information on each trial.

Bacteriology:

Heat treatment at both temperatures reduced the number of bacteria associated with the oysters. This reduction was reflected in both the 35 C and 15 C counts as shown in Table 2. Group III oysters which were subjected to the highest temperature usually showed the greatest reduction in bacterial numbers.

Oysters were analyzed for bacterial counts at intervals throughout the storage period. Tables 3 and 4 list the standard plate counts and psychrophilic plate counts, respectively. The standard plate counts on each group of oysters at each sampling time were approximately equal, with the exception of Group I in Trial D which started with much higher counts and quickly surged ahead in numbers.

Psychrophilic bacteria in the oysters increased greatly during the storage period. Similar increases were noted in oysters from all groups and though increases in a certain group in one trial were greater, there was no particular pattern among the different trials. It was concluded that the heat treatments employed did not alter the rate at which the bacteria developed on the oysters and therefore did not alter the spoilage rate.

Free Liquid Content:

In many states, the maximum amount of free liquid allowable in a jar of oysters is regulated. Processors attempt to pack oysters to meet these regulations. Oysters frequently bleed (lose fluid) during storage and if packed oysters bleed significantly, a legal pack may become illegal within a few days. Therefore, knowledge of how heat treatment affects the liquid loss rate is important.

Table 5 summarizes the data on free liquid content of the packed oysters in each trial. In that table, results are expressed in terms of percent increase or decrease in the free liquid content relative to the content on the first day it was measured. Also shown are the differences in the percentage of free liquid between Group I oysters and Group II or III oysters on the first day tested. In Trials A and B, there appeared to

Source	Trial A	B	C	D
	35C Standard Plate Count			
Group I Oysters	14.	1.4	6.6	30.
Group II Oysters	3.3	0.54	2.2	4.4
Group III Oysters	0.46	3.9	1.1	>3.0
	15C Spread Plate Count			
Group I Oysters	6.9	45.	78.	300.
Group II Oysters	0.10	3.2	18.	16.
Group III Oysters	0.02	8.0	9.2	5.8

* Numbers x 10^4 per gram of oyster meat.

Table 2. Bacteria* associated with plant shucked oysters prior to washing.

Trial	Day	Group I	Group II	Group III
A	0	4.9*	4.4	0.6
	6	2.9	1.6	2.9
	10	3.2	1.6	2.1
	14	15.	1.5	9.7
B	1	1.3	0.45	0.83
	7	2.5	27.	3.7
	10	10.	93.	4.7
	14	8.5	13.	6.7
C	2	8.9	5.3	1.2
	10	2.1	6.1	4.0
	14	59.	67.	62.
D	1	25.	4.9	>3.0
	6	56.	>3.0	>3.0
	13	210.	17.	26.
	18	>300.	23.	67.

* Numbers x 10^4 per gram of oyster meat.

Table 3. Development of bacteria on oysters during ice storage as measured by the standard plate count (35C) procedure.

Trial	Day	Group I	Group II	Group III
A	0	2.1*	2.8	0.25
	6	7.2	0.77	4.4
	10	22.	1.4	6.6
	14	35.	10.	61.
B	1	4.7	5.0	6.2
	7	83.	130.	35.
	10	28.	130.	37.
	14	130.	740.	560.
C	2	54.	31.	6.4
	10	79.	110.	120.
	14	>3,000.	>3,000.	>3,000.
D	1	16.	9.8	1.2
	6	160.	4.3	7.2
	13	1,100.	1,200.	1,600.
	18	610.	480.	2,200.

* Numbers x 10^4 per gram of oyster meat.

Table 4. Development of psychrophilic bacteria on oysters during ice storage.

Trial	Day	Group I	Group II	Group III
		%	%	%
A	0	-	- (1.0)*	- (0.6)
	6	-3.7	-6.8	-3.1
	10	-2.6	0.7	3.9
	14	-6.2	-3.3	-4.4
B	1	-	- (-2.2)	- (-0.3)
	7	3.3	-1.8	-2.0
	10	2.2	0.2	0.0
	14	2.4	3.6	-1.0
C	2	-	- (3.3)	- (10.5)
	8	0.1	2.4	-3.0
	14	4.9	11.7	-0.1
	23	4.2	11.7	7.7
D	1	-	- (1.6)	- (5.2)
	6	8.7	6.2	6.6
	13	11.2	8.9	3.7
	18	9.6	12.6	0.0

* Difference between free-liquid content in Group I oysters and Group II or III oysters.

Table 5. Change in free-liquid content in jars of oysters during storage on ice.

be little difference in the free liquid content among the three groups of oysters even at the end of the storage period. However, in Trials C and D, all groups of oysters bled significantly with Group II oysters showing the greatest loss.

Throughout the four trials, no clear picture developed on the effect of heat treatment on the liquid loss of oysters during storage. These results could have been affected by the low precision of the test as well as differences in the oysters taken during different times of the year.

Organoleptic Test:

Organoleptic evaluation of oysters from Trial A was attempted without using a reference standard. This procedure proved unsatisfactory because the quality of all groups deteriorated with time. Results from this trial are not reported.

In Trials B, C, and D, the test panel was provided with a reference standard (oysters from Group I) with which the test groups were to be compared. Test groups were identified only by random numbers and one of the test group was the same as the identified control. In this type of comparison, Group III oysters generally received the lowest ratings on all characteristics in all trials. As shown in Table 6, Group I oysters generally received the highest ratings but did not always score a perfect 3.0 as it should. In all characteristics, Group II oysters rated close to 3.0 indicating that oysters receiving a moderate heat exposure cannot be distinguished organoleptically from oysters that do not receive a heat treatment.

Group III oysters received the lowest scores on the appearance characteristic. Group II oysters always received lower scores than Group I oysters on the appearance characteristic although it frequently received higher ratings than Group I on other characteristics. It was concluded that appearance is the most heat sensitive of the organoleptic characteristics in oysters.

The test panel noted that the liquid in the Group III oyster jars had a cloudy or milky appearance which caused the oysters to be rated lower. The milky color apparently results from a bleeding of glycogen from within the oyster. Heat accelerates this process and thus the oysters which received the largest amount of heat developed a more cloudy appearance.

Shucking Rate:

The average rate at which shuckers opened oysters which had not been heat treated was 6 per minute. This rate increased to 8 or 9 per minute when shuckers were presented with oysters that had been passed through the steam tunnel. This limited study indicated a shucking rate increase of 33% is attainable using the steam treatment.

	Trial B*				Trial C		Trial D			
	Day				Day		Day			
	1	7	10	14	2	8	1	6	13	15
Appearance:										
Group I	2.7	3.0	3.0	3.2	3.0	3.2	2.8	2.8	3.0	3.2
Group II	2.7	2.8	2.8	3.0	2.0	2.7	2.7	2.7	2.8	3.0
Group III	2.5	2.5	2.8	2.5	2.2	2.2	2.2	2.2	2.0	2.0
Flavor:										
Group I	3.0	2.3	3.0	2.8	2.8	3.3	2.5	2.7	2.7	3.5
Group II	3.2	3.0	3.2	3.0	2.0	2.8	3.2	3.0	2.7	2.8
Group III	3.0	3.2	2.8	2.5	2.0	2.2	2.7	2.8	2.5	2.3
Texture:										
Group I	2.8	2.8	3.2	3.0	3.0	3.2	3.0	3.0	3.0	2.8
Group II	3.2	3.2	2.8	2.8	3.0	2.8	2.8	3.0	3.2	2.8
Group III	2.5	2.7	2.7	2.7	2.8	2.3	2.5	2.7	2.7	2.8
Overall Rating**:										
Group I	2.9	2.6	3.1	3.0	2.9	3.3	2.7	2.8	2.8	3.3
Group II	3.0	3.0	3.0	3.0	2.3	2.8	3.0	2.9	2.9	2.9
Group III	2.8	2.9	2.8	2.6	2.3	2.2	2.5	2.7	2.5	2.4

* In Trials B, C and D, a standard of comparison was used and evaluation being based on a scale of 1 to 5 with 3 being equal to the standard. Figures represent an average for the entire taste panel.

** Overall ratings were calculated from appearance, flavor and texture scores by giving flavor twice the weight of texture and appearance and then averaging the values.

Table 6. Results of organoleptic test.

SUMMARY

When changing any processing step within an industry, both the advantages and disadvantages must be weighed. The addition of a step to the processing of raw oysters in which the oysters are given a heat treatment to relax their adductor muscle, thus making the oysters easier to open, must likewise be approached with caution.

In the steam treatment of raw oysters, care must be taken to use the smallest amount of heat required to relax the oysters for easy opening. Excess heat will deteriorate the quality of the oyster by bringing about changes characteristic of cooking.

Size, shell thickness and initial temperature of the oyster are important factors in determining the increase in internal temperature of individual oysters when exposed to a given temperature. Since grading of oysters is impractical and since it is not always practical to equilibrate the temperature of the oysters prior to applying heat, experiments with oysters of mixed sizes and initial temperatures of 20 to 25 C were conducted to determine the optimum temperature exposure. For this study, an exposure time of 2 3/4 minutes was set since that was the time required for the oysters to pass through the tunnel. A temperature of 60 C in the steam tunnel appeared optimum, providing sufficient relaxation of most oysters without producing any immediate visible change in the oyster. This exposure time and temperature corresponded to an internal temperature in the oysters of approximately 39 C.

The selected exposure temperature was tested with oysters from several states along the northern Gulf of Mexico and proved adequate. However, this temperature may need to be altered for oysters grown in other areas of the country.

Heat treatment of oysters at 60 C did not alter the shelf life or bleeding rate of oysters as compared to a control group of oysters. Organoleptic ratings placed the heat treated oysters equal to or only slightly lower than the control. From these results, it has been concluded that the heat relaxing process can be successfully utilized in the raw oyster industry provided the internal temperature of the oysters does not exceed 40 C.

In experiments with oysters exposed to an excess amount of heat, the organoleptic quality of the oysters was substantially reduced and this quality loss was closely tied to the appearance of the oysters. The excess heat appears to damage the oyster membranes and accelerate the leakage of glycogen into the liquid surrounding the oyster causing it to become milky or cloudy. This problem appears to be more acute during the time of the year when the oysters have the highest glycogen content.

Users of the heat treatment for relaxing oysters should be aware that excessive heating of the oysters can cause a change in

the appearance of the oyster liquor which may adversely affect the sale of the product. This problem can be minimized by adequate temperature control of the steam tunnel.

ADDENDUM

After this project had been completed, we became aware of research being conducted by Dr. Frank Huang of Virginia Polytechnic Institute. Results of his research were presented in a paper entitled, "Oyster Steam Shucking Process" to the Interstate Seafood Seminar held at Danvers, Massachusetts, in October, 1979.

ACKNOWLEDGMENTS

The steam tunnel and oyster processing facilities used in this project are owned by C. F. Gollott and Sons, Inc., Biloxi, Mississippi. The use of those facilities was graciously provided by Arny and Linda Gollott. Financial support was provided in part by the Mississippi Department of Wildlife Conservation, Bureau of Marine Resources, Long Beach, Mississippi. Further, we wish to acknowledge the technical assistance of Alan Criss and thank the GCRL staff members who participated in the organoleptic evaluation of the oysters.

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TEXTURE IMPROVEMENT IN FABRICATED SHRIMP SHAPES BY ADDITION OF SURIMI (WASHED MINCED FISH)

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In recent years the rising cost and lower availability of shrimp supplies has presented a growing problem for seafood processors. This situation has meant that not only must broken pieces and undersized shrimp be utilized as food, but that substitutes for whole breaded shrimp be developed for those consumers unable to pay the price of this premium product. At the current exorbitant prices of whole 30-50 count shrimp, this group of consumers obviously represents the majority of our population. Thus was born the fabricated shrimp shape, which consists of shrimp minced or flaked mechanically to which so-called "binding" agents such as gums, soy proteins or starch might be added along with appropriate flavorings, and the homogenous mixture extruded into a shrimp-like shape. The resulting product, when breaded and fried, is fairly reminiscent of whole breaded shrimp in appearance and flavor.

The resemblance ends here, however, for upon biting into most of the fabricated products now being marketed one encounters a texture more like a shrimp patty than an intact shrimp. The resulting letdown in consumer expectations has undoubtedly led to a reduction in repeat sales of this product, as well as a reluctance on the part of the food service industry to include it on their menus, even in fast-food type restaurants. It would appear that processors have decided that good appearance and flavor alone are sufficient to insure consumer acceptance of seafood and have therefore chosen to neglect texture, possibly out of ignorance or necessity, as an important quality attribute of their products. Let me refer, however, to a paper published by Wesson et al in the Journal of Food Science (1979) in which they evaluated the ability of consumers to distinguish between what would be considered good and poor quality seafoods by experts. They reported that while "flavor characteristics were major determinants of preference when distinctly oxidized flavors were present, texture was an extremely influential determinant of preference when samples exhibited moderate to low intensities of fishy and/or oxidized flavors."

It is precisely this lack of texture which has plagued a myriad of products which have been developed from mechanically-deboned or "minced" fish in the past. Besides the often bloody appearance and sometimes stronger flavor of minced fish which technologists have contended with in seeking applications for this material, the mushy texture

of products containing minced fish at economically significant levels has often resulted in the abandonment of many product concepts based on the incorporation of this cheap commodity.

SURIMI AS A SOLUTION TO THE PROBLEM

The conversion of plain minced fish into surimi, a term coined by the Japanese who most highly developed this process, opens many exciting avenues for product development, however. The process for surimi manufacture (Figure 1) basically entails the washing of minced fish immediately after deboning followed by dewatering and straining of the material to remove extraneous skin, scales, etc, and to restore the fish to approximately its original water content. The surimi is then chopped with polyphosphate, sugars or other desired ingredients depending on the intended end-use and block-frozen for storage or shipment to another location for further processing.

The washing process results in material which, aside from being desirably white and much blander in flavor, is a "concentrate" of the highly functional salt-soluble proteins such as actin and myosin. Upon chopping with 1-2% salt to extract these proteins followed by heat processing, extremely firm-textured products with a bite and resiliancy similar to freshly cooked shellfish meats may be obtained. The light color and bland flavor characteristics lend this material to the addition of food colorants and added flavors so that excellent fabricated products such as shrimp, clam, lobster and crab-like substitutes may be prepared which have textural properties more like their natural counterparts than fabricated products prepared without the incorporation of surimi. Table 1 reveals the scores of a highly trained texture profile panel for shrimp shapes obtained commercially or prepared on an AutoProd extruder in the laboratory using similar formulations and varying the makeup of the meat portion. Note the surimi-containing product scored closer to the standard breaded shrimp in all textural characteristics than either the all-shrimp fabricated prodcut or two of the leading brands of fabricated shrimp currently being marketed. Consumer response to these same products and one additional formulation was evaluated by RJR Foods in Winston-Salem, N. C. Two fifty-member consumer panels, comprised of individuals chosen for their high consumption of prepared and convenience foods, rated each of the products in monadic testing for their appearance, texture and flavor. As revealed in Figure 2 both the machine formed (as above) and the molded shrimp shapes containing surimi as 50 percent of the meat portion rated much closer to the whole breaded shrimp in textural characteristics. The molded shrimp shape contained an improved flavoring, coloring and breading system in comparison with the earlier shrimp shapes prepared with the AutoProd extruder.

LABORATORY EXPERIMENTAL DATA

Recent work in our laboratory has centered on studying the effects of various processing variables on the textural quality of products prepared with surimi. Specifically, to date, we have determined the effects of

chopping time, water content, cooking schedule and setting on the firmness of gels as determined by compression testing on an Instron universal testing machine, using the texture-profile analysis of the resulting curves according to Bourne (1968). The effects of applying pressure to the gel during cooking on gel strength have also been determined but must remain proprietary at this time due to a pending patent application. Additional studies relating to the effects of various washing procedures, species, muscle pH and other variables are currently in progress.

Figure 3 shows the effects of chopping time on gel strength, using a Hobart lab-scale silent cutter to comminute the fish muscle prior to stuffing into stainless steel tubes and cooking for 20 min at 80°C. Chopping time was measured beginning at the moment when the tempered chunks of fish took on the appearance of a fine powder just before melting into a paste. Note that the firmness of surimi gels far exceeded those prepared with unwashed tissue and that increased chopping resulted in increased firmness in the gels. Greater than 12 min chopping under these conditions was not judged practical due to an abrupt rise in temperature and the onset of gel formation or setting, which is undesirable as will be demonstrated later in this paper.

Figure 4 depicts the resulting gel strengths as affected by varying the processing temperature at a cooktime of 20 min. Aside from the apparent difference between washed and unwashed fish noted in the gel strengths, note that a much higher gel strength can be obtained with surimi when the gel is set at lower temperatures. The effect of alkaline protease and other protease activity in the 50-70° range which was reported on last year at this conference (Su et al., 1979) is also apparent. Figure 5 again demonstrates this same "setting" effect at lower temperatures in increasing the gel strength. Varying the cooking time and temperature appears to have had only small to moderate effects on the strength of gels. However, setting of the gel at 40° prior to cooking at 80° for 10 additional min drastically increased the textural firmness of the surimi gels. Such a "preset" at 40° could thus be used to greatly increase the textural firmness of extruded products provided it posed no health problem due to increases in bacterial numbers. Recent bacteriological studies in our laboratory (data not yet available) indicate that while a preset at 40°C does result in an increased bacterial load, further processing by deep frying would eliminate any potential health or storage problems with the product.

We also verified reports from Japan that holding of a chopped surimi-containing product at refrigerated temperature has a texture-increasing effect. Figure 6 reveals that the effect is most pronounced when the product is molded or formed prior to cooking. The mechanism of such setting would thus appear to entail more than just additional extraction of myosin and likely involves the formation of intermolecular bonds which are disrupted to some extent by extrusion or forming prior to cooking. This phenomenon is currently being further studied in our laboratory.

The textural qualities of the product may also be greatly altered by slight adjustment of the water content, as shown in Figure 7. Small

increases in the moisture content result in a rapid decrease in the textural firmness of gels prepared from both washed and unwashed fish. It is obvious that, in the case of surimi, higher water contents can be used and acceptable texture still be obtained than with plain minced fish.

The use of the Instron texture profile may also be extended to a routine test for evaluating the textural potential of production lots of surimi, shrimp or other fish meats for input into least-cost linear programs to maintain uniform product quality and minimize costs. The texture of products prepared from a mixture of raw seafoods may be derived by assuming a linear gradation of textural firmness or springiness values in the mixed product when combining seafood of good and poor textural quality. Thus, for the method to have value a 50:50 mixture of two materials having high and low firmness/springiness values should have firmness/springiness values midway between that of either ingredient alone. To test the hypothesis, predicted and actual values for firmness and springiness were calculated and measured for products prepared by blending seafoods having high and low textural values when measured by the Instron texture profile. Figure 8 shows that a reasonably good fit is obtained using either firmness ($R = 0.98$) or springiness ($R = 0.73$) values of fish samples obtained on the Instron to predict the texture of various mixtures.

CONCLUSIONS

The development of a market for minced fish, as for any other product, depends upon the ultimate cost/advantage ratio of that product to the industry and the consumer. Conversion of minced fish to surimi yields a material of superior functional properties for producing fabricated shellfish meats at a considerable cost savings with respect to products prepared with 100% shellfish. The desirable textural characteristics which surimi imparts to restructured seafoods result from the concentration of the highly functional salt-soluble proteins of fish muscle by the washing process. As such, we can only echo the words of Robert Young of Unilever Research Laboratory who in his presentation two years ago to the Atlantic Fisheries Technology Conference, entitled "The Potential of Food Production from the Sea," concluded that surimi as an ingredient commodity represents the "fish protein concentrate of the future."

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Profile Panel Comparison of Breaded Shrimp Shape Texture* - products were prefried, frozen and oven heated.

Commercial Products	Firmness	Springiness	Cohesiveness
Brand A	6	2	6
Brand B	7	2	2
Test Products			
Whole breaded shrimp	11	10	9
All-shrimp fabricated product	5	1	2
Half-shrimp/Half surimi product	12	8	12

*evaluated on an absolute 14 point scale

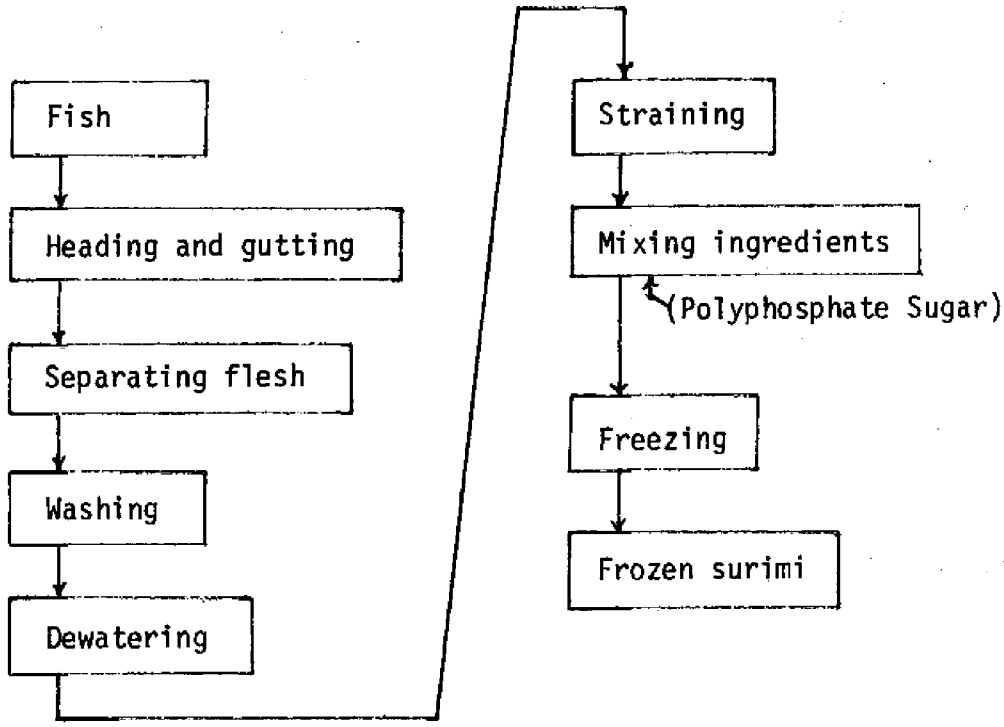

Figure 1. Steps in processing of surimi.

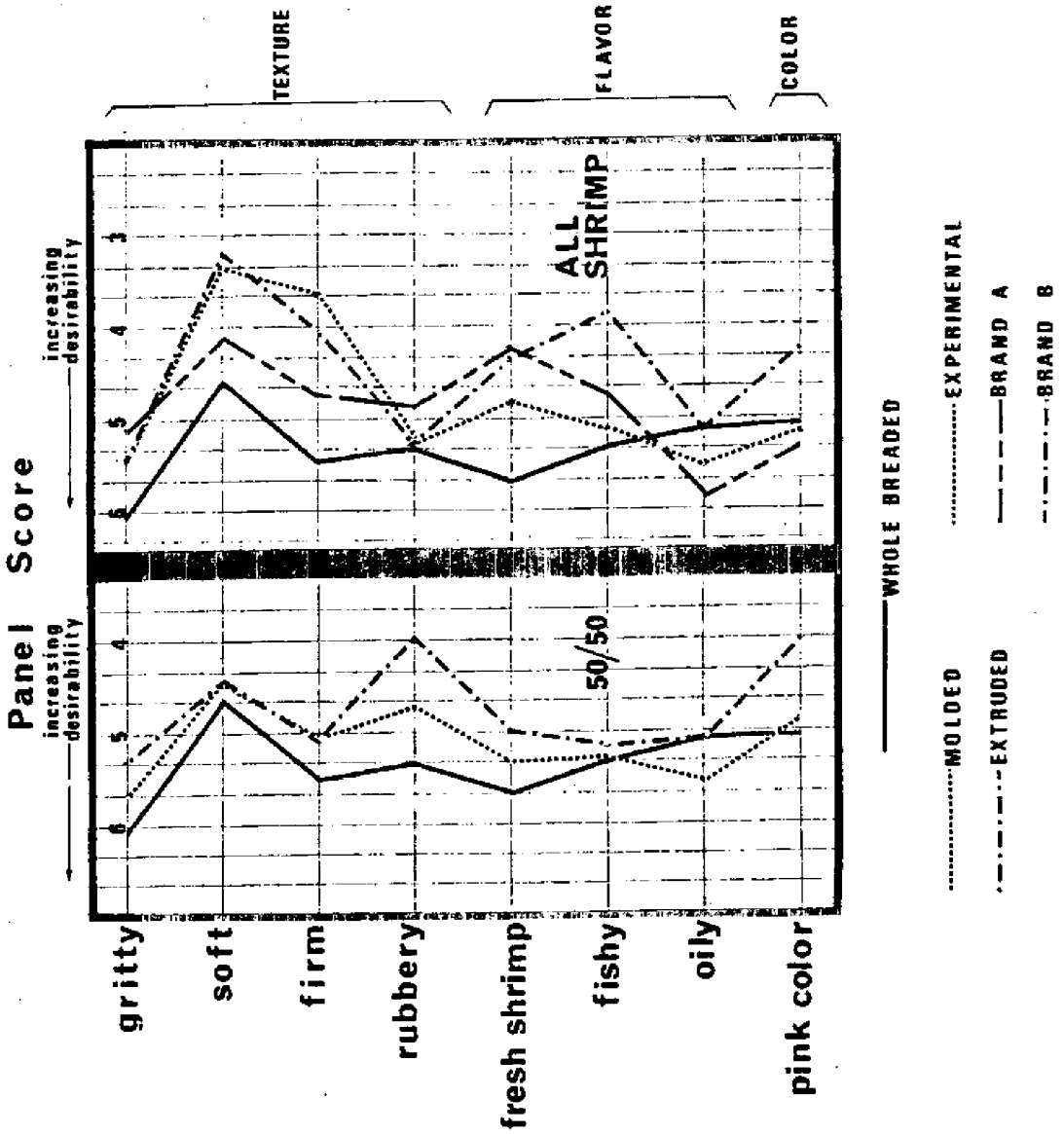

Figure 2. Consumer panel comparisons of whole breaded shrimp with surimi-containing (50/50) and all-shrimp prepared and commercial shrimp shapes.

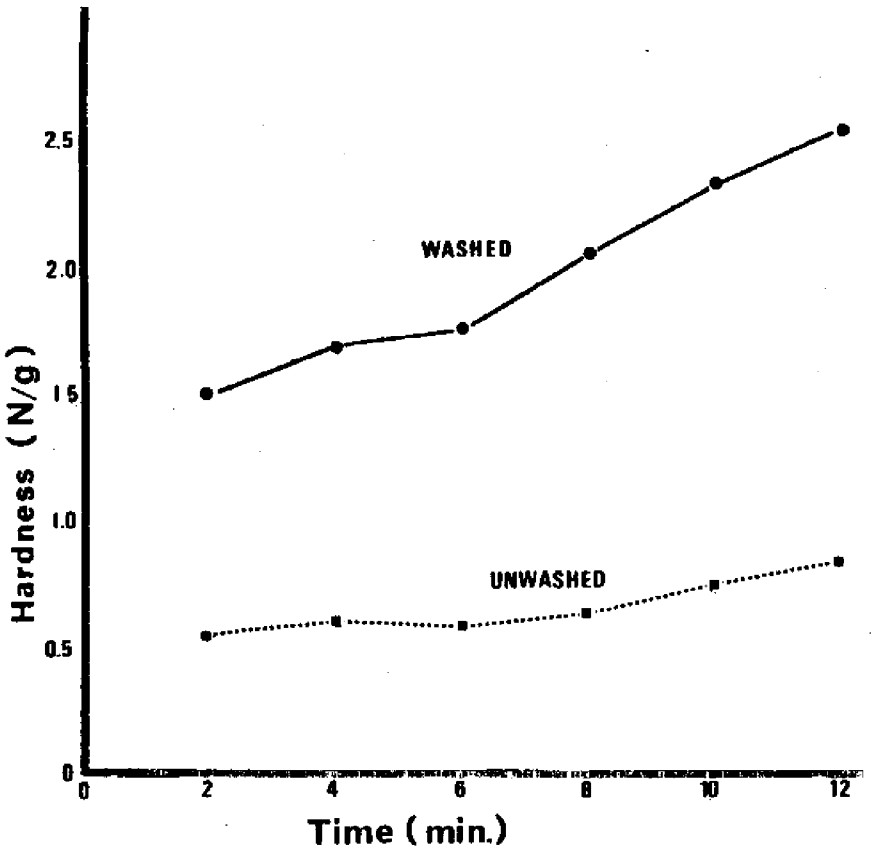

Figure 3. Effect of chopping time on textural firmness of gels prepared from washed and unwashed minced croaker.

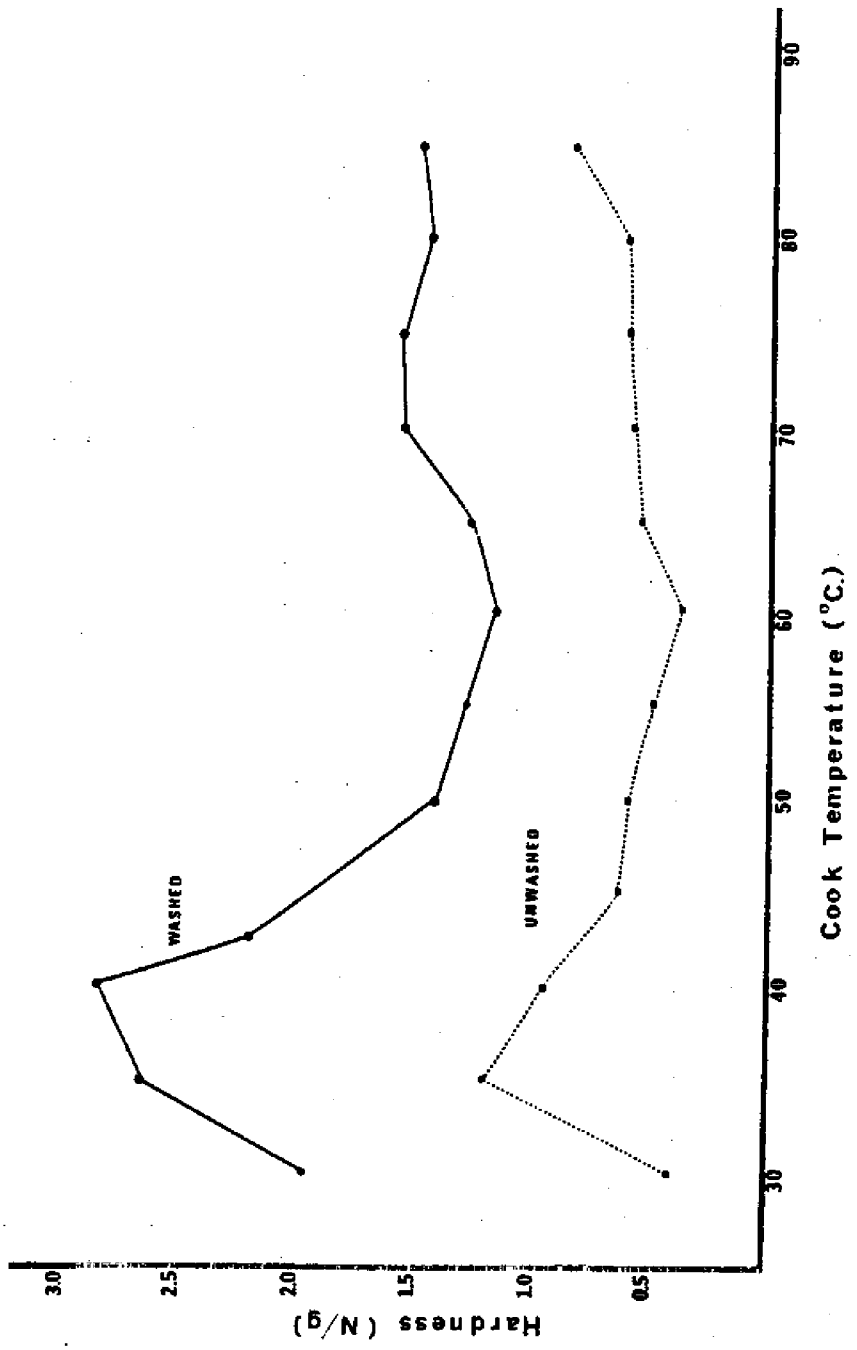

Figure 4. Effect of cook temperature on textural firmness of gels prepared from washed and unwashed minced croaker.

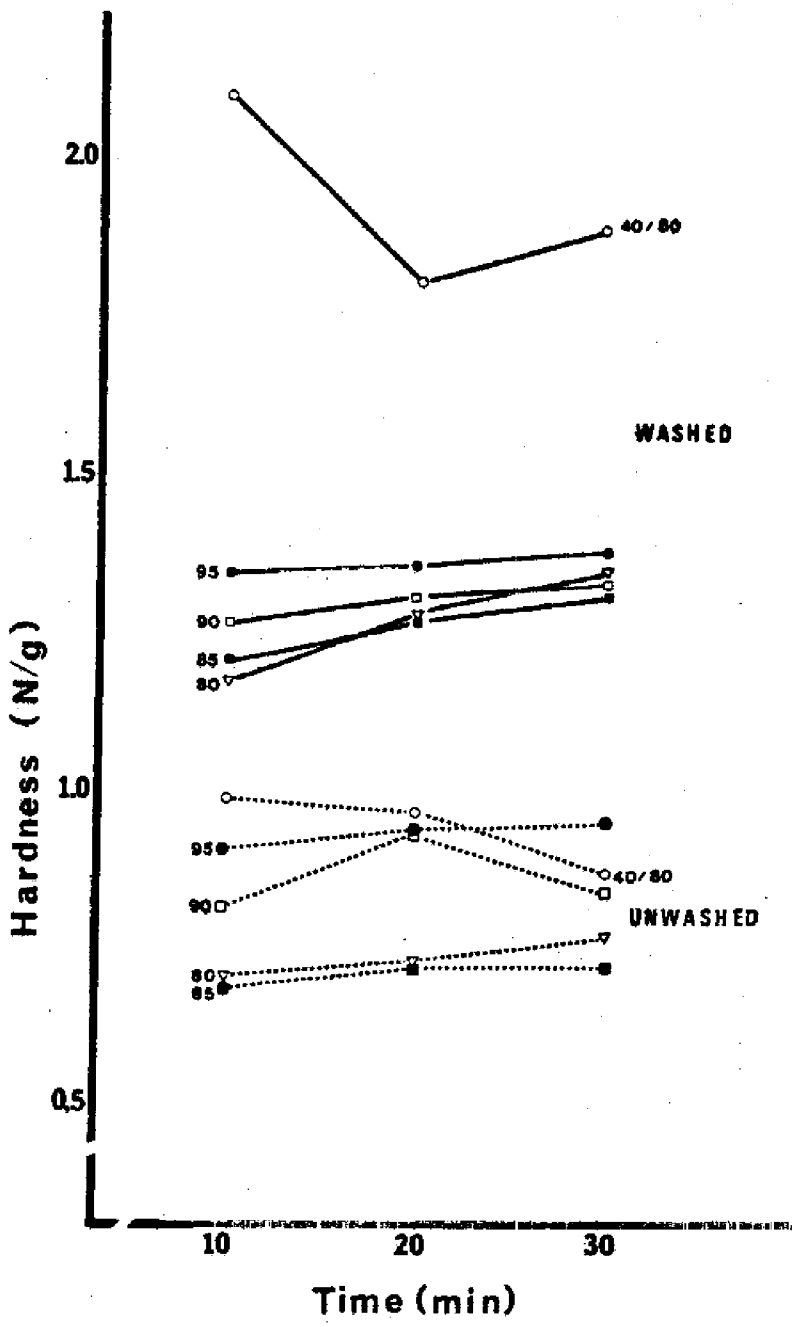

Figure 5. Effect of cook time at various temperatures on textural firmness of gels prepared from washed and unwashed minced croaker.

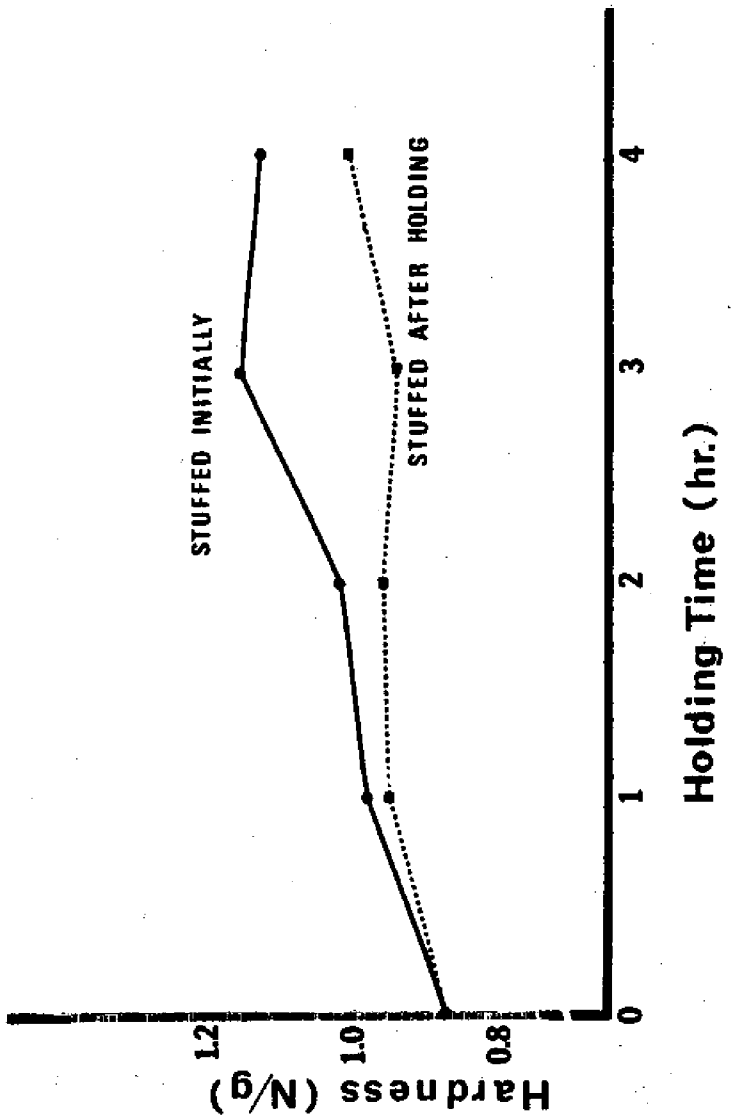

Figure 6. Effect of time of stuffing and holding at 0°C prior to cooking on textural hardness of gels prepared with washed minced croaker.

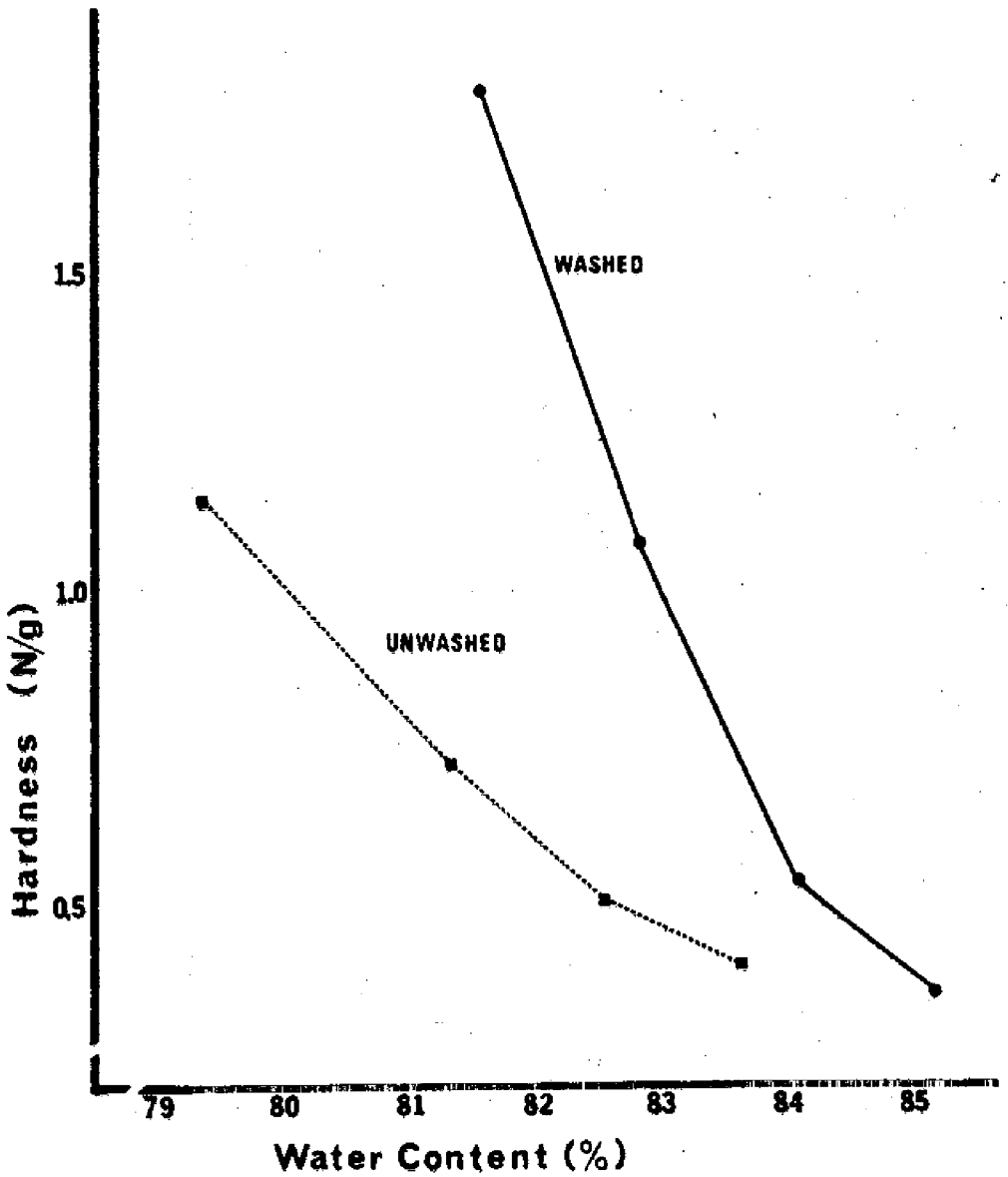

Figure 7. Effect of water content on textural firmness of gels prepared from washed and unwashed minced croaker.

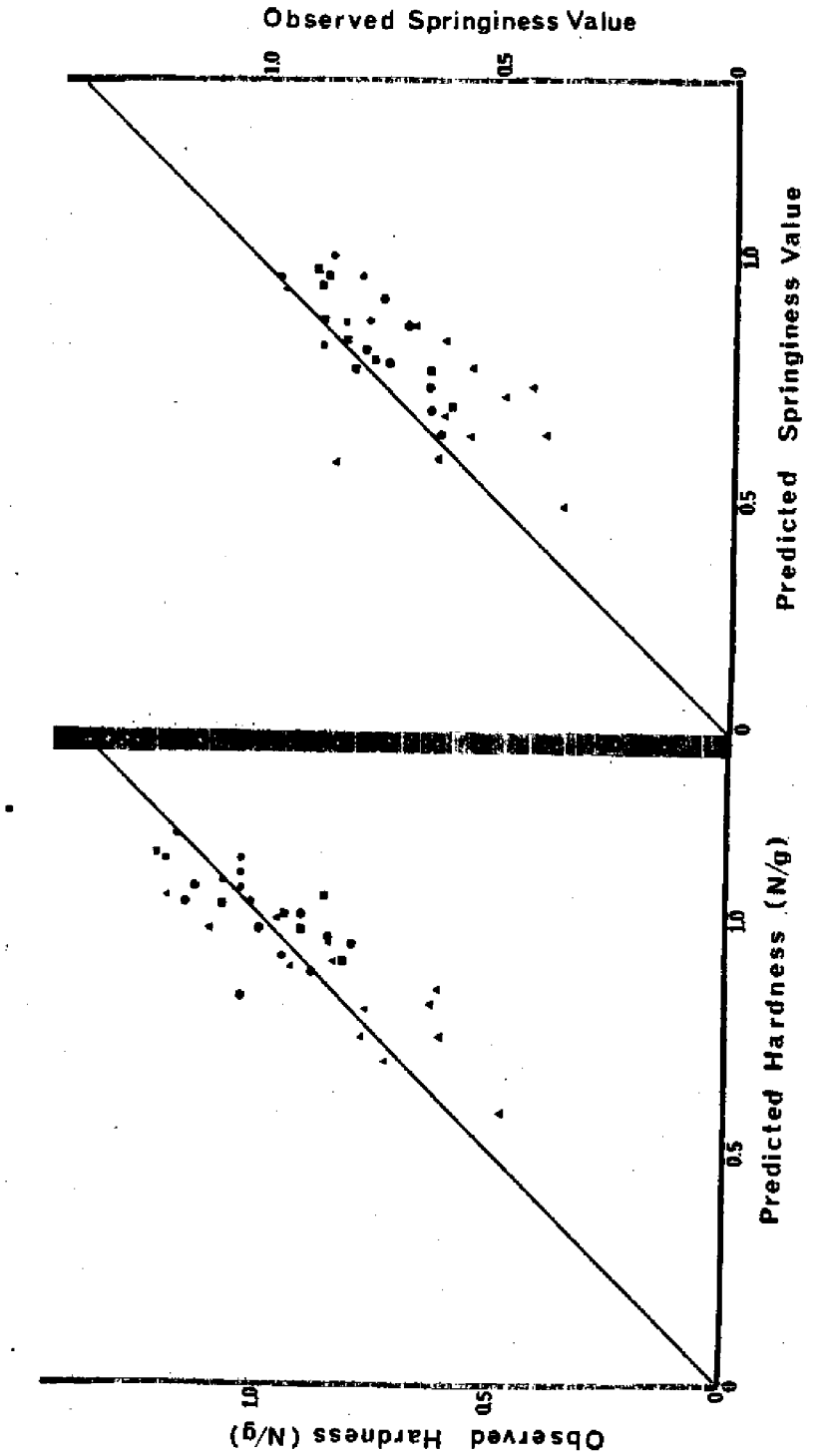

Figure 8. Predicted vs observed values for textural firmness and springiness for gels prepared from mixtures of poor and good textural quality minced fish.

FLORIDA SMOKIES: A FRIED COLD SMOKED FILLET PRODUCED FROM ROE MULLET

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Effective utilization of spent mullet following the removal of roe is a pressing problem to the seafood industry in Florida. As demand increases and experience is gained in recovery of roe for the export market, the problem of utilizing the carcass will be increasing in magnitude.

Problems in handling, processing, storage and marketing of fresh mullet are well recognized (1). Presently, smoking of mullet produces a product that is locally acceptable and markedly increases the economic return in relationship to other processed forms. Hot smoked butterflied mullet is the most popular product form. In an attempt to expand the utilization of mullet, particularly roe mullet, we have explored the acceptance of fillets prepared as a cold smoked product. The choice of cold smoking was based on previous reports (2,3) and the desire to explore new product forms for mullet.

MATERIALS AND METHODS

Mullet (Mugil cephalus) of about 2 lbs were obtained from various locations on both coasts of Florida. They were brought to the laboratory in Gainesville, packed in ice. Fish not needed for immediate processing were packed in plastic bags and frozen in the round at -30°F. Only boneless and skinless fillets were used for smoking. If frozen, the mullet were thawed in flowing tap water, filleted and packed in ice the day before smoking

On the morning of smoking, the fillets were brined for 30 min in a ratio of 1.5 parts brine to 1 part fish, drained for 30 min and smoked at 120°F for the desired time. Smoking was done in a Koch Grandprise Smokehouse using hickory sawdust. Smoke vents were open during the first 30 min of smoking. The effect of brine concentration (0, 2, 4, 6% sodium chloride w/w) was studied based on panel evaluations of preference for mullet cold smoked for 1.5 hr, cooled, and fried for 2 min at 350°F. The effect of smoking times (no smoking, 1 1/2, 3 hr) was studied on similar panel evaluations of mullet presoaked in 4% brine. A 4% brine soak and 1 1/2 hr smoke time was used to prepare mullet for similar panel evaluations of subsequent cooking procedures. Cold smoked mullet were fried (2 min at 350°F), broiled (6 min in electirc oven), and baked in an aluminum foil wrap (20 min at 450°F).

For the storage studies the fish were brought to the laboratory and divided into two lots. One lot was processed and smoked fresh. Half of the fresh smoked fillets were used the following day for sensory evaluation, the remainder were frozen for two weeks at -30°F prior to panel evaluations. The other lot was fresh frozen in the round for six weeks, thawed, processed then smoked as with the first lot. Both lots were prepared with a 4% brine soak and 1 1/2 hr smoke time. Frying was the subsequent cook method.

Sensory evaluation was by a 20 member consumer panel. Evaluation sheets were designed according to attributes being tested (4).

RESULTS AND DISCUSSION

A previous study (2) had indicated that acceptance of cold smoked butterflied mullet was quite good. However, the nature of the product did not lend itself to an easy method of reheating. In addition there was a need to develop products from spent roe mullet. Therefore a study was conducted using fillets obtained from roe mullet. Emphasis was placed on the brining and smoking requirements necessary for an acceptable product.

In Table 1 are the data in which various brine concentrations were evaluated in order to determine the most desirable conditions. Over half of the panelists indicated they preferred the fish prepared in 4% brine. On the basis of these results a brine concentration of 4% was selected for use in further studies.

It was observed during preliminary studies that cooking of the cold smoked fish intensified both the smoked flavor and color. Evaluations in Table II indicate the panelists preferred some smoke time, but there was no significant difference in preference for product smoked 1.5 or 3.0 hours. The longer smoke time produced a slightly darker product. Based on the highest overall rating the 1.5 hour smoke time was selected for further studies.

To determine if there was one preferred method of final preparation, we prepared the cold smoked fillets by baking, frying and broiling (Table III). The combination of first cold smoking to impart flavor, then cooking results in a final product that differs from a hot smoked fish. Cooking adds in good color development and the flesh is moist and flavorful. The data indicates that all three methods are acceptable. There was no significant difference in overall preference for any final product preparation, but the fried product was rated the highest preference for color and flavor.

Two weeks frozen storage (-30°F) of the smoked fillets did not appear to have any detrimental affects on preference for the final product. Average product ratings in all sensory catagories were higher for the frozen product, but there was no significant difference in overall preference for day one or frozen product (Table IV). Similarily, six weeks frozen storage prior to smoking did not have any apparent detrimental affect on final product preference (Table IV).

This approach to the utilization of spent roe mullet appears to be quite feasible. Based on the current price for typical hot smoked butterflied mullet, the cold smoked mullet should prove a favored economic return. In addition consumer acceptance of the product is very high and it would appear that the product should find its best use at the restaurant level. Modification of the process based on individual smokehouse differences may have to be made to compensate for such factors as smoke density and temperature.

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Table I. EFFECT OF BRINE CONCENTRATION ON PANEL PREFERENCE FOR FRIED COLD SMOKED MULLET FILLETS PREVIOUSLY SMOKED FOR 1.5 HOURS. (Average ratings for three studies)

Brine Conc. %	Liked best 1	2	%* 3	Liked least 4
0	0	2	13	85
2	12	30	52	7
4	54	40	5	3
6	35	30	30	5

*Percent preference is the total percent of panelists selecting different preference levels per brine concentration.

Table II. EFFECT OF SMOKING TIME ON PANEL PREFERENCE FOR FRIED COLD SMOKED MULLET FILLETS PREVIOUSLY SOAKED IN 4% BRINE. (Average ratings for two studies)

	Time of Smoking		
	3 hrs	1 1/2 hrs	None
Odor	7.05	7.05	6.68
Color	6.45	7.30	6.36
Texture	6.80	7.30	7.00
Flavor	7.25	7.00	6.25
Overall acceptance	7.20a	7.45a	6.30b

Scale: 9 = excellent; 1 = extremely poor

Means followed by the same letter are not different at the 0.05 level of significance.

Table III. EFFECT OF COOKING PROCEDURE ON PANEL PREFERENCE FOR COLD SMOKED MULLET FILLETS. (Average ratings for two studies)

	Fried	Broiled	Baked
Odor	7.1	6.5	7.0
Color	7.2	6.3	6.4
Texture	6.6	7.0	6.7
Flavor	7.4	6.7	7.0
Overall acceptance	7.1a	6.7a	7.0a

Scale: 9 = excellent; 1 = extremely poor
Means followed by the same letter are not different at the 0.05 level of significance.

Table IV. EFFECT OF FROZEN STORAGE ON PANEL PREFERENCE FOR FRIED COLD SMOKED MULLET FILLETS. (Average ratings for two studies)

	Fresh		Whole Fish Frozen 6 Weeks	
	Day 1	Frozen 2 Weeks	Day 1	Frozen 2 Weeks
Odor	7.7	8.3	7.8	7.9
Color	7.6	8.4	8.3	8.2
Texture	8.0	8.1	7.9	7.6
Flavor	7.8	8.4	8.1	7.9
Overall Acceptance	8.0a	8.3a	8.2a	8.1a

Scale: 9 = excellent; 1 = extremely poor

Means followed by the same letter are not different at the 0.05 level of significance.

OVERVIEW OF CIGUATERA SEAFOOD POISONING

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INTRODUCTION

Ciguatera is the name applied to the human illness resulting from the ingestion of certain tropical and subtropical reef-associated fish; such fish are normally edible but can become poisonous as a result of their habitation.

Of the 400 species of fish cited by Halstead (10) that have been implicated in this illness, 91 species may be found in the Caribbean (6). Many of these fish are regarded as highly prized seafood and therefore are of significant commercial value.

The toxin responsible for ciguatera, first termed ciguatoxin by Scheuer et al. (14), enters the food chain at the level of a coral dwelling microorganism and is transferred to herbivorous fish which graze upon benthic algae and coral. Predatory fish, such as snapper and grouper, may haphazardly consume reasonable quantities of ciguatoxic herbivorous fish and concentrate the toxin in their body tissues without observable ill effects. Unfortunately, when the heat stable toxin is introduced to man via a ciguatoxic seafood meal, the pharmacological effects of the poison are manifested. In light of this "food web" hypothesis (13), any one of the coral reef associated fish has the potential to become ciguatoxic, although the most common species associated with the illness are red snapper, grouper, barracuda and amberjack. One of the noted characteristics of the illness is its ecology. Not all fish of a given species are ciguatoxic. Fish that are highly toxic in the waters of one island may be edible in the waters of a neighboring island. A report by Banner (4) suggested that certain islands having no prior episodes of seafood poisoning may also experience a sudden incidence of ciguatera. Thus, ciguatera is dependent on geographic location and time.

The actual incidence of ciguatera remains unknown even on islands where the illness is considered endemic. Many of the early symptoms mimic other forms of "seafood poisonings" and no readily available diagnostic test exists to confirm ciguatera. Since the disease is short-lived and self-limiting in the majority of cases, many patients avoid seeking medical attention. As a result, most researchers can only estimate the true incidence of the illness. Very recently, a team of public health epidemiologists from the

Center for Disease Control conducted an intensive, on-site investigation of the incidence of ciguatera at St. Thomas, U.S.V.I., and documented an annual morbidity rate of 7 per 1000 population (Dr. J. Glenn Morris, personal communication), this incidence level approximates that reported by McMillan (12) for the same location.

Paresthesia (tingling or burning of the skin) is considered the clinical hallmark of ciguatera, and is commonly used to differentiate this poisoning from other forms of food poisoning or mild cases of gastroenteritis (3). Ciguatera is not significantly more prevalent in any particular age group or in either sex. Symptoms almost always develop within 24 hours and are primarily neurological, although gastrointestinal symptoms, including nausea and diarrhea may be the first to appear. Early symptoms characteristically include the tingling of the lips, mouth and tongue and may extend over time to an intense itching on the extremities. Other neurological symptoms may include sensory disturbances such as cold objects feeling hot or painful. Exhaustion, pain, and muscle weakness, particularly in the legs, are frequent symptoms. The duration of the acute phase is usually one or two days, but residual weakness and sensory changes may persist for months. No specific clinical treatment is available for the intoxication beyond supportive and symptomatic relief. Contrary to earlier reports (1, 7, 9), the case fatality rate is low.

COMMERCIAL FISHING INDUSTRY'S CONCERN

In addition to the obvious public health issues, ciguatera has affected tropical and subtropical commercial fisheries in three general areas:

(a) Impact on the Development of Tropical Fisheries. For the populace living near extensive tropical coral reef habitats, the avoidance of potentially ciguatoxic fish can deny them a substantial portion of their natural legacy. In addition, the likelihood of biological hazards of marine toxins increases with man's use of the oceans for food purposes. As reported by Dammann (8), the danger of ciguatera poisoning remains one of the major deterrents to efficient and wide-spread marketing of most tropical species of shallow-water food fish. Until the health hazards of ciguatera can be resolved, there appears to be little possibility of developing or improving the present in-shore fishery in ciguatera-endemic tropical areas. The problem is not restricted to the shallow-water fishery since species previously considered non-toxic because of their normal depth range ($>$ 100 fathoms) have also been implicated in ciguatera cases (6). Unquestionably, ciguatera has adversely affected the development of domestic fisheries in certain tropical areas. The economic loss attributable to ciguatera remains conjecture.

(b) Litigation. Individuals employed in the harvesting, processing, distribution or retailing of seafood have potential liability for the sale of contaminated fish or fish products at the federal and probably at the state level as well. The Federal Food, Drug and Cosmetic Act of 1906 established both civil and criminal penalties

for the sale and distribution of adulterated food. An adulterated food is defined as a food which "bears or contains any poisonous or deleterious substance which may render it injurious to health." In addition, the Act provides that a food shall be deemed "misbranded" if "its labeling is false or misleading in any particular." Not only may a ciguatoxic fish product be considered "adulterated," but if no warning of a potentially harmful toxin appears on the label, the product labelling may be argued as "false or misleading."

Within the last several years, a substantial number of court cases have evolved against restaurateurs and wholesalers alleging ciguatera poisoning to an injured party. Although most of these cases have been filed in Dade County, Florida, several new cases have emerged in states with more temperate climates, presumably due to the exportation of ciguatoxic fish from tropical waters. It is difficult to assess the economic impact of these cases. There is no doubt, however, that the litigation involving ciguatera has triggered a state of apprehension in the South Florida seafood industry at the present time.

(c) Public Apprehension. Public concern for food safety has been growing rapidly in light of increased consumer awareness through the news media. In the case of ciguatera, the striking clinical manifestations of the illness, the occasional atypical psychotic symptoms associated with this seafood poison, and the current litigation involving seafood wholesalers and restaurateurs often are reported, unfortunately, with melodramatic flair. Undoubtedly, the negative publicity has stimulated the public to avoid seafoods they fear may be contaminated.

CURRENT RESEARCH ENDEAVORS

Despite more than 25 years of active research in ciguatera and the voluminous literature addressing this problem, the most basic of questions remain unanswered: What microorganism(s) synthesizes ciguatoxin? What is the chemical structure of ciguatoxin? What is the mechanism of biogenesis of ciguatoxin (or its related compounds)? What environmental factors stimulate (or trigger) ciguatoxin production? How is ciguatoxin transmitted through the food chain? During transmission, are chemical and pharmacological properties of ciguatoxin altered? Is there only one ciguatoxin entity or a family of related toxins? Is ciguatoxin from toxic fish in the Pacific chemically identical to ciguatoxin from Caribbean waters? Can ciguatoxin be inactivated, particularly by a method convenient to food processing? Is the turnover rate of ciguatoxin in particular fish flesh so low as to permit the accumulation of toxin over time?

Answers to these questions have only partially and indirectly been advanced. Perhaps the single biggest impediment in attempts to resolve these questions has been the lack of a sensitive, accurate and quantitative assay for ciguatoxin. Currently, the animal bioassay, particularly the intraperitoneal injection of fish extracts into laboratory mice, appears to be universally accepted. Several

deficiencies make the test less than ideal. Symptoms in toxin-injected mice do not necessarily reflect the symptoms observed in ciguatera-afflicted individuals, and the mouse appears to respond poorly when administered an extract from an equivalent amount of toxic fish known to elicit a severe human response.

Two avenues of research are being pursued with funding from the Food and Drug Administration and the National Marine Fisheries Service under a cooperative, interagency agreement. The first involves the identification of the causative microorganism. Preliminary evidence suggests that the dinoflagellate, Gambierdiscus toxicus, may be responsible for the biogenesis of ciguatoxin (2, 15). Current attempts to isolate G. toxicus in axenic culture and to extract ciguatoxin from mass cultures of the dinoflagellate are underway, utilizing organisms isolated from the South Pacific as well as from Caribbean waters.

The second avenue involves the development of a specific and sensitive assay for ciguatoxin. Recently, Hokama et al. (11) at the University of Hawaii reported on the potential applicability of the radioimmunoassay for the detection of ciguatoxic fish. This assay, while promising, has failed to provide convincing evidence of its reliability (5), a shortcoming currently being addressed by Hokama. Recently, a team of faculty members from the Medical University of South Carolina and the College of the Virgin Islands, and the staff of the Charleston Laboratory of NMFS aided by the Caribbean Fishery Management Council, have initiated a series of studies designed to assess the efficiency of different extraction procedures for the toxin as well as the feasibility of obtaining anti-ciguatoxin antibodies from immunized animals utilizing the extracted material as the immunogen. If specific anti-ciguatoxin antibodies can be obtained, the development of an accurate and sensitive laboratory test may be possible.

Until recently, most of the literature dealing with this problem was descriptive, and limited in scope and content. The available references were concerned with frank reporting of clinical cases, the species of fish involved, and the region affected. Only theories were advance and our basic understanding was fragmentary. Hopefully, an interagency task force representing NMFS, FDA, NIH, CDC and NSF as well as appropriate state agencies will be established to provide a cohesive definition of the problem. Scientists should be challanged to explore new research avenues directed to the difficult task of alleviating an important problem facing not only the fishing industry in tropical and subtropical regions, but the consumer as well, whose attitude is generally predicated on the belief that anything that swims in the ocean must be (or should be) wholesome.

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THE MARKETING OPPORTUNITY FOR FRESHWATER SHRIMP IN SOUTH CAROLINA: A PRELIMINARY SURVEY

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INTRODUCTION

Commercial farming of freshwater shrimp, Macrobrachium rosenbergii has been investigated in South Carolina since 1972. Currently the biological and technical aspects of the production systems are well developed, but information on the marketing and acceptability of this product is lacking. Thus, a study was set up to examine the marketability for freshwater shrimp in local seafood retail markets since such outlets would probably be the initial marketing channel for this farm-reared product. A preliminary market survey was conducted at the seafood retailer and consumer levels. This paper provides some initial results of the survey.

SURVEY METHOD

Four seafood retail stores in Charleston were selected as representative of a wide range of sales environments. About 100 pounds of freshwater shrimp (both fresh and frozen) were provided to each retailer. These retailers were asked to simply place the product in their display case with a small sign to indicate the product name and to set a price at least as high as that for saltwater shrimp. Detailed information on retailers' attitude to the product was solicited by personal interview after sales were completed.

Consumer questionnaires were provided to those who purchased freshwater shrimp. A total of 80 questionnaires was distributed by the retailers and 23 completed questionnaires were returned to us. To increase sample size, we also provided 1 pound samples of the product without charge to 100 individuals and asked them to return consumer questionnaires after they had tested the product. About 80 usable questionnaires were received from this group.

SEAFOOD RETAILER'S EVALUATION OF FRESHWATER SHRIMP

The four retailers indicated that freshwater shrimp demonstrated high saleability in their outlets. About 90% of the 450 pounds provided were sold in a few days with the remainder withheld by the retailers for home consumption. About 315 pounds were sold as ungraded tails at an average price of $4.35 per pound (range $3.99 to $5.00). These retail prices were comparable to those for saltwater shrimp during the product sales period.

All retailers used "Freshwater Shrimp" as the product brand name. This name suggests the product as a saltwater shrimp substitute and saltwater shrimp are premium priced species in South Carolina. In Hawaii, the product is called "Hawaiian prawn" which is expected to emphasize that the product is locally grown and not to be considered as a saltwater shrimp substitute.

Seafood retailers were asked to evaluate the product in terms of freshness, appearance, texture, and saleability. About 75 percent of retailers rated the product as excellent in freshness and appearance and 100 percent of them said that texture and saleability were very good or excellent. When seafood retailers were asked whether they would market the product, all retailers indicated that they would.

When asked whether they would pay a higher or lower price for freshwater shrimp than penaeid shrimp, they responded as follows: 50% said they would pay the same price for both products; 25% would pay $1.00 per pound higher; and 25% would pay 10% lower than penaeid shrimp prices. The majority of retailers indicated a preference for the product in the 36-50 tail count category which typically represents a "medium" market class.

Seafood retailers were asked to provide a summary evaluation of the product from a business man's viewpoint and their responses are listed below:

a. "I feel there is a good market for freshwater shrimp. I found the customer reaction to the product was good."

b. "Product very good - needs development. Public not sufficiently aware of its value."

c. "I have seen there would be no problem in marketing the product heads-off. They have excellent taste and eye appeal. Heads-on may be a little more difficult to market."

d. "I think we have a definite market for this product."

CONSUMER'S EVALUATION OF FRESHWATER SHRIMP

The consumers were asked to indicate the manner in which they prepared freshwater shrimp at home. From the survey, it was determined that 30% of consumers prepared the product by frying, 17% by boiling, 15% by broiling, and 11% sauteed the shrimp in butter. Further, 20% used more than one method in preparing the product. It is interesting to note that 11 percent of the consumers gave a fair or poor evaluation on taste and 13 percent indicated a fair or poor rating on the texture of the product. Many of these respondents prepared their freshwater shrimp either boiled or sauteed in butter. Fifty percent of consumers rated the quality of freshwater shrimp as similar to saltwater shrimp while 19 percent reported that freshwater shrimp were superior to saltwater shrimp.

Approximately 89% of consumers indicated that they would buy freshwater shrimp from the seafood retail store and the majority of these consumers also indicated that they would be willing to pay prices comparable to those paid for saltwater shrimp. Consumers product form preferences were explored and 89 percent of the consumers said they would prefer freshwater shrimp in the heads-off form.

SUMMARY

This paper presents the results of a preliminary survey of seafood retailers and consumers conducted in Charleston, October - December, 1979. The main objective of the study was to obtain preliminary information on the marketing opportunity for freshwater shrimp at local seafood retail stores. Data were gathered from 4 seafood retailers and 103 consumers. The following results were obtained:

1. Freshwater shrimp demonstrated high saleability in seafood retail outlets and all retailes were anxious to add freshwater shrimp to their existing product lines.

2. The product was sold by the retailers as ungraded tails at prices ranging from $3.99 to $5.00/lb.

3. The majority of retailers indicated a preference for fresh shrimp in the 36-50 tail count category.

4. The majority of consumers evaluated freshwater shrimp as similar to saltwater shrimp.

5. About 89% of seafood consumers surveyed were willing to purchase freshwater shrimp from seafood stores.

6. The majority of consumers were willing to pay prices comparable to those charged for saltwater shrimp.

7. Additional market research is needed to assess the marketing opportunities for freshwater shrimp in outlets such as supermarkets, restaurants, hotels, etc.

PRELIMINARY DATA ON THE INFLUENCE OF ENVIRONMENTAL MICROFLORA ON THE SAFETY AND UTILIZATION OF BLUE CRABS AND OYSTERS

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The fishing industry is occasionally the victim of unfavorable publicity arising from reports of bacterial food poisoning attributable to fishery products. Examples are the cases of Vibrio parahaemolyticus food poisonings in Maryland in 1971 and the V. cholerae outbreak in Louisiana in 1978, both of which were attributed to crab meat. In these cases, a predominant feature was the lack of solid data on the occurrence of these bacteria in the environment, such as how frequently they were encounterted, their numbers, and the source of the organisms. Knowledge of the level and types of bacteria that are present in the marine environment can enable the industry to assess the extent and magnitude of actual or potential problems, should they arise with these organisms. Moreover, information of this kind should provide a basis for public health agencies to act in a rational and equitable fashion without causing undue hardship to processors or distributors of fishery products.

The Charleston Laboratory of the National Marine Fisheries Service, Southeast Fisheries Center, has initiated seasonal surveys in urban and pristine estuaries to determine the impact that the levels and types of bacteria found in oysters (Crassostrea virginica), blue crabs (Callinectus sapidus) and their habitat have on the quality and safety of these important seafoods. The main goal of the study is to identify the predominant microflora and also any possible transient or indigenous pathogen, primarily of the genus Vibrio. Through the use of immunofluorescence microscopy, we hope to define the ecological niche of potential pathogens and determine their biomass, respiration and growth rates in relationship to physical and chemical environmental parameters. The

data should also provide information on organisms that can cause disease in the crabs and oysters themselves. This project is part of a larger program at the Charleston Laboratory that includes seasonal surveys of oysters, crabs, sediments, and growing waters for trace metals, bacteria and viruses.

MATERIALS AND METHODS

Sampling sites. The two sampling areas are Charleston Harbor,S.C., considered heavily polluted by both domestic and industrial wastes and St. Helena Sound, a pristine estuary 35 miles south of Charleston, which is relatively free of pollutants. Two sites, representing salinities of approximately 10% and 25%, are sampled in each area on a quarterly basis to coincide with the highest and lowest temperatures in the water column and during the two transitional periods in water temperatures. This sampling regimen provides for examining the various effects that pollution, temperature and salinity may have on bacterial microflora.

Collection of samples. Bottom sediments are collected with a Peterson dredge. The top centimeter of sediment is removed with a sterile tongue depressor and placed in a sterile container. Surface water samples (1 m below the surface) are collected with a sterile Niskin bag sampler (General Oceanics[1]; Miami, FL). Water samples are divided into a 500 ml sample, from which total viable aerobic heterotrophic bacteria and fecal coliforms are enumerated, and a 100 ml sample is fixed with filtered (0.2 um) Formalin (final concentration, 2% formaldehyde) and is used to determine direct microscopic bacterial counts. Intertidal oysters are collected manually at low tide from all sites except the low salinity site in St. Helena Sound, where subtidial oysters are collected using an oyster dredge. Blue crabs are harvested using commercial-type crab pots. All sediment, water and oyster samples are immediately cooled with ice. Crabs are maintained at their in situ temperature by placing them in a cooler. All samples are analyzed within 12 h. During a survey, three representative samples of blue crabs and oysters and two each of surface water and sediment are collected and analyzed at each sampling site. A total of 40 samples are analyzed each survey which is completed within four days.

Physical and chemical parameters. Dissolved oxygen of surface water is determined according to the azide modification of the Winkler method (2) and titrated using phenylarsine oxide in place of sodium thiosulphate because it is very stable in storage. Temperature and salinity of the surface water were measured using a YSI Model 33 Salinity-Conductivity-Temperature meter.

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[1]The use of trade names does not imply endorsement by the National Marine Fisheries Services, NOAA; it is merely to facilitate descriptions.

Bacteriological analyses. Preparation of oysters and water samples for analyses follows standard procedures (3, 1). Blue crabs are cooled to reduce their activity and their backs are split with a crack or oyster knife. The carapace is then removed by pulling up on the lateral spines and the gills are aseptically picked with forceps and placed in sterile containers. The crab gills are then homogenized and further prepared following the standard procedure for oysters (3). Oysters and crab gills are sampled on a wet-weight basis, but sediments are analyzed on a volume to volume basis because of the great differences in sediment densities found in the environment. The initial dilution is made by volume displacement of the diluent by the sediment in a calibrated container as previously described (4). All dilutions are made using a sterile 0.1% peptone (Difco) saline solution (1.5% NaCl)

Total viable aerobic heterotrophic bacterial counts are determined using the spread-plate technique and a modified low-nutrient, artificial seawater plating medium (ASWLN) of Litchfield et al. (10) containing the following ingredients per liter of half strength artificial seawater (Rila Marine Mix, Teaneck, NJ): 0.5 g peptone (Difco), 0.5 g yeast extract (BioQuest), 0.1 g sodium glycerophosphate (MC/B) and 20 g agar (BioQuest). Three replicates of each dilution are plated, and the inoculated plates are incubated at 20°C for 14 days. Representative colonial types, as observed with oblique illumination through a stereomicroscope are picked from the plates, purified and maintained in tubed ASWLN medium (0.3% agar) at room temperature. The isolates are then identified to genera by examining for biochemical, cultural, morphogical, nutritional and physiological characteristics according to basic diagnostic schemes (5,12). Fecal coliform counts in all samples are estimated by the three-tube most-probable-number (MPN) procedure prescribed for seawater and tissues (1,3). Lauryl sulfate tryptose broth (BioQuest) is used in the presumptive test, with confirmation in E.C. broth (BioQuest) incubated at 44.5°C in a circulating water bath. Selective fecal coliforms are further confirmed by IMViC testing (2) or using the API 20E system (Analytab Products, Inc., Plainview, NY).

Enrichments of Salmonella spp. are obtained by using an ambient temperature, primary, nonselective procedure (8). Surface water samples are filtered through 0.45 um membrane filters using 100 ml volumes. The filter or 5 gm samples of sediment, oyster or crab gills are each inoculated into 50 ml volumes of dulcitol broth, which is incubated at ambient temperature for 4 h then 18-20 h at 35°C. After this nonselective enrichment, 1 ml is transferred to a selective enrichment of selenite cystine broth (BioQuest), which, after incubation, is streaked onto brilliant green agar (Difco) or Xylose lysine deoxycholate agar (XLD; Difco). Isolated colonies are screened, and presumptive Salmonella cultures are biochemically characterized by using the API 20E system.

Vibrio-like organisms were enumerated on thiosulfate citrate bile salts agar (TCBS;BioQuest) for all samples. Representative

colonial types are picked only from crab gill inoculated TCBS plates, and these cultures are purified and then characterized biochemically using the API 20E system.

V. cholerae. The same sample sizes used for Salmonella enrichments are inoculated into a simple alkaline enrichment broth containing peptone (Difco, 10 g/liter) and NaCl (10 g/liter) at pH 8.5 (9). After inoculation, the enrichment flasks are incubated for 3 days at 20°C, after which they are streaked onto TCBS agar plates. Isolated colonies are screened (9) and the presumptive V. cholerae cultures are characterized using the API 20E system. Cultures confirmed in this procedure are then tested for enterotoxin production using Y1 mouse adrenal cells (11).

Epifluorescence microscopy. Total direct bacterial counts are made on surface water and crab gill samples using the epifluorescence technique described by Hobbie et al. (7). Acridine orange (0.1% w/v), a fluorescent dye, is used 1:1 with the sample and the counts are made on Nuclepore membrane filters (Nuclepore Corp., Pleasanton, CA), which have been dyed 24-48 h in a solution of Hydrolan Black BGL (Empire Dyestuff Corp, New York, NY) at 2 g/l using 2% (v/v) acetic acid. The acridine orange attaches to the bacterial DNA and under blue excitation fluoresces green, which permits total bacterial count determinations. It is also possible to simultaneously differentiate between respiring and apparently nonrespiring bacteria (13). The electron transport system of respiring organisms reduces 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium chloride (INT) to INT-formazan, which accumulates intracellularly as dark red spots that are visible when examined by light microscopy. When this technique is combined with epifluorescence microscopy determinations of the total number of bacteria, the proportion thereof involved in respiration can be directly obtained in the same microscopic image. To determine biomass, volumes of bacteria are calculated from cell measurements obtained from photographs and transparencies made of the bacteria during the counting procedure. Observations of the frequency of dividing cells provide an indirect measure of the mean growth rate of the bacterial community (6). In the future, we hope to combine immunofluorescence microscopy with the techniques just described to define the environmental niche and the environmental parameters effecting the growth rates of specific pathogens.

RESULTS AND DISCUSSION

Although the surveys were initiated only last August, some interesting data have been collected on the bacterial flora found on the gills of blue crabs. During the first survey, samples of crab digestive tracts and gills were examined both together and separately. The meat was not examined because, unless the crab has been injured or the meat contaminated during picking, it is considered free of bacteria. When the gills and digestive tract were

examined together, the bacterial counts varied considerably from sample to sample. The coliform counts were also high. Hurricane David interrupted the collection of crabs from St. Helena Sound during this first survey, so that the previous data on combined gill and digestive tract samples were available before crabs were collected from the high salinity (25%) site in St. Helena Sound. We thought that the coliforms might have been coming primarily from the digestive tract and were indicators that the crabs had recently eaten contaminated material. However, when the gills from the crabs caught after the hurricane were examined separately from their digestive tracts, the level of coliforms and bacterial counts was highest in the gills. Therefore, we decided to concentrate on examining crab gills for types and levels of bacteria during future surveys and to separate the dark gray-brown colored gills, observed during this initial survey, from the normal light colored gills and analyze them separately.

The analyses to date have established that the gills of blue crab harbor high levels of bacteria (10^6 - 10^8/g) particularly in those gills that are dark brown. (Figure 1). The dark gills have bacterial levels that are approximately ten times those of the light gills, both with the coliform count and the total bacterial count and 2-5 times the total Vibrio-like organism counts (Tables 1 and 2). The percentage of healthy crabs with these dark gills averaged 50% in warm water samplings and 25% during the winter survey and was not related to the area sampled (urban vs. pristine). Crab gills have also yielded high fecal coliform counts (43000 /g) in pristine areas where oysters sampled concurrently were relatively free of contamination (Table 3).

We hope with these data to be able to predict, to some degree, the conditions and location of crab harvesting areas that might present problems for crabbers and processors and to define the meaning of the levels and types of environmental bacterial flora, so that the impact on the fishing industry of occasional problems due to these bacteria will be lessened.

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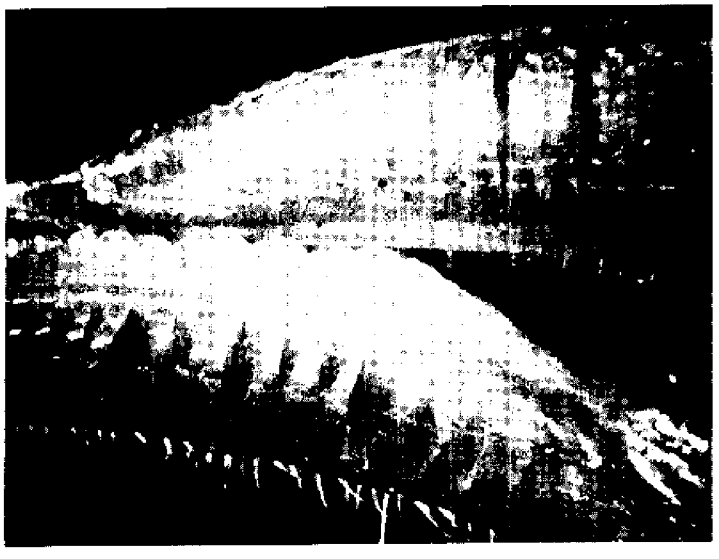
FIGURE 1

Dark Brown Gills



Light Colored Gills

TABLE 1. AVERAGE BACTERIAL COUNT OF CRAB GILLS

	CLEAN AREA[a]	
BACTERIA/g	LIGHT GILLS (6)[b]	DARK GILLS (4)[b]
Coliforms	2×10^2	2×10^3
Total Count	1×10^7	7×10^7
Vibrio-like	4×10^5	2×10^6

[a]Within acceptable bacterial limits for oyster growing waters according to National Shellfish Sanitation guidelines.

[b]Number of samples analyzed.

TABLE 2. AVERAGE BACTERIAL COUNT OF CRAB GILLS

	CONTAMINATED AREA[a]	
BACTERIA/g	LIGHT GILLS(3)[b]	DARK GILLS(3)[b]
Coliforms	3×10^3	3×10^4
Total Count	4×10^6	3×10^7
Vibrio-like	3×10^4	6×10^4

[a]Bacterial limits above thos acceptable for oyster growing waters according to National Shellfish Sanitation guidelines.

[b]Number of samples analyzed.

TABLE 3. FECAL COLIFORMS IN SUBTIDAL OYSTERS

AREA	Number of Samples	Fecal coliforms/100g Oyster: Arithmetic Mean	Fecal coliforms/100g Oyster: Range
Charleston Harbor			
Site A (25‰ Salinity)	9	5161	36 - 24000
Site B (10‰ Salinity)	9	1595	<23[a] - 4300
St. Helena Sound			
Site A (25‰ Salinity)	9	36[b]	<23[b] - 36

[a] Three oyster samples <23 fecal coliforms/100g (February survey)

[b] Only one oyster sample positive for fecal coliform, 8/9 <23 fecal coliform/100g

BACTERIOLOGICAL SURVEY OF CRAWFISH PROCESSING IN LOUISIANA

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Crayfish commonly also referred to as crawfish, crawcrab, crab, stonecrab, crawdad, creek crab, yabbie, mudbug, ecrivisses and other local names, inhabit freshwaters on all continents except Africa (1,6). Of the 29 known species inhabiting Louisiana, only 2 are sufficiently abundant and posses adequate tailmeat to warrant commercial practices, namely the red swamp crawfish, Procambarus clarkii Girard and the white river crayfish, Procamborus acutus acutus (3,6).

Louisiana crayfish are trapped in habitats which include swamps, creeks, rice fields, natural flood basins and control - flooded ponds. Daily catches are delivered live to wholesale and retail outlets or processed in peeling plants and sold to outlets as fresh or frozen peeled tailmeat. During 1977-78, 40 million pounds valued at $25 million dollars were recorded. The demand for crayfish has increased in recent years, therefore the industry has grown rapidly. In any rapidly expanding food industry, sanitation and quality control measures become significantly important and therefore the bacterial quality of the product at various stages of the processing operation is of prime concern.

This investigation was undertaken to study the following relationships: (1) To determine the bacterial quality of crayfish tailmeat utilizing Standard Plate Counts, Total Coliforms, Escherichia coli and Coagulase Positive Staphylococcus aureus, (2) To determine during the crayfish tailmeat processing, the site of possible bacterial contamination and the degree of contamination if occurring, and (3) To determine if there exists a monthly variation in the bacterial levels in the processed crayfish tailmeat.

MATERIALS AND METHODS

General Handling Procedures Currently Employed in Crayfish Processing

In Louisiana, the crayfish processing industry consists mainly of small scale processors with a relatively small numer of processing plants designed specifically with good sanitation and processing efficiency as a requirement.

The general plan of handling procedures during processing of crayfish is seen in Figure 1 and is rather uniform throughout the industry. In Figure I the stages of processing are numbered and identified.

Initially crayfish are washed then scalded in hot water, the temperature varying from 80° C to 100° C for a period of from 6 to 15 minutes in the plants surveyed. The crayfish are then cooled and the peeling personnel then remove the tail shells and heads by hand. The tailmeat is placed in stainless steel colanders holding 3 to 5 pounds and then transferred to designated weighing vessels and weighed and the total weight recorded.

The tailmeat is packaged as either 1, 2, or 5 pounds in plastic bags which then are heat sealed. Usually, the packaged peeled tails are refrigerated, however the time elapsed from scalding can vary from 45 minutes to 2½ hours. Most processors icepack the packages while some are stored in refrigerators varying between temperatures of 1.7° to 7.2° C. The processors market this tailmeat rapidly (less than 7 days maximum) due to possible spoilage losses.

METHOD OF SAMPLINS

From the beginning of March to the end of May 1979, four commercial crayfish plants in Louisiana were sampled once every 4 weeks. This is considered the peak of the crayfish processing season. Statistical analysis was performed on the 89 samples collected from the colanders of peelers (Figure 1, stage 3) through samples stored 4 days at commercial refrigeration temperatures (Figure I, stage 6). The same number of samples were obtained from each plant, except plant D as plant D's processing eliminated the transfer of peeled tailmeat to a designated weighing vessel (Figure I, stage 4) and therefore fewer samples.

Two 100-gram random samples were taken from each of the following stages in Figure I in the processing of crayfish: live washed crayfish (WLC); scalded whole crayfish (SWC); peeled tailmeat taken at random from colanders of individual peelers (PTM); a weighed crayfish sample (WTM); packaged crayfish tailmeat (PCM;)and a 4-day refrigerated tailmeat sample (RTM).

Sterile procedures were followed in the sampling procedure at all the sampling stages. Sterile plastic Whirlpak bags were used

to store the tailmeat samples. Forceps, which had been flamed three times with alcohol, were used to place the samples in the Whirl-pak bags. A sterile stainless steel microbiological sampling can was used to collect the live crayfish samples. All samples were put on ice for transportation to the laboratory in the Food Science Department, L.S.U., Baton Rouge, Louisiana where the samples were subsequently run within a few hours after collection.

Preparation of Sample Homogenate

Fifty-gram samples of the washed whole live crayfish and scalded whole crayfish were placed into separate sterile Waring blendors, diluted 1:10 with 450 ml of sterile phosphate buffer and blended for two minutes. Twenty-five grams of each of the remaining samples were placed into separate sterile Waring blendors, diluted 1:10 with 225 ml of sterile phosphate buffer and blended for two minutes. These mixtures were used to make the appropriate dilution (i.e. 10^{-2} to 10^{-6}) in the analysis for the selected microorganism. Eleven milliters of each of the mixtures was pipetted into 99ml of diluent (phosphate buffer) to make the appropriate dilutions. All dilution water and media used were autoclaved for 15 minutes at 15 psi to assure sterile media, dilution water, and glassware. Methods recommended by the Bacteriological Analytical Manual for Foods (2) were utilized in determining the Standard Plate Count (SPC, Total Coliforms (TC), E. coli and coagulase - positive Staphylococcus aureus numbers.

RESULTS AND DISCUSSION

Variation in Bacterial Levels Among Crayfish Processing Plants

The comparison of each plant showed that the mean standard plate counts (mSPC) were significantly different ($p<0.01$), indicating that each plant followed different sanitation and handling practices. From Table 1, Plant B had the lowest mean standard plate count of 1.04×10^5 org/g. Plant D had the highest mean standard plate count of 6.52×10^5 org/g.

The comparison of each plant in regard to mean E. coli levels showed the crayfish plants were significantly different ($p<0.05$). Plant D had the highest overall mean E. coli count of 2.21×10^2 MPN/g. Plant B had the lowest of 0.20 MPN per gram.

The mean total coliform counts and mean coagulase-positive S. aureus counts were not significant ($p<0.05$). The total coliform levels were relatively low during the three month period. Even though the coagulase-positive S. aureus counts were not significantly different among the plants, the counts were sufficiently high to warrant some concern by the crayfish industry.

Variation in Bacterial Levels Among Sampling Stages

Results showed there is a significant difference ($p<0.05$) in mean standard plate counts among the samples (3-6) collected from the processing line of each plant (see Table 2). Live washed crayfish (Stage 1) had a mSPC of 2.83×10^7 org/g.

Whole scalded crayfish (Stage 2) had a mSPC of 1.22×10^5 org/g. This mean was higher than the mean that Kergosien (M.S. Thesis, University of Southwestern Louisiana, Lafayette, La. 1969) reported of 1.3×10^4 org/g., but it was within the upper limits he reported. Although not all the organisms were destroyed by the scalding process employed by the plants, the scalding procedure did reduce the initial bacterial load by approximately 99%. Kergosien (MS) stated that if commercial scalding conditions were adequate, 98% of the autochthonous flora of crayfish would be destroyed.

Crayfish tailmeat taken from the colanders of individual peeling personnel (Stage 3) had a mSPC of 1.15×10^5 org/g. Kergosien (MS) reported a mean TPC of 1.48×10^5 org/g.

Weighed crayfish tailmeat (Stage 4) had an average of 1.76×10^5 org/g.

The packaged crayfish tailmeat (Stage 5) from the four plants had a mSPC of 4.18×10^5 org/g. It has been pointed out that fresh crayfish tailmeat with bacterial counts approaching 10^6 per gram would not be expected to maintain an acceptable shelf-life and quality for any length of time (4,7). The mean SPC ranged from 2.0×10^4 to 2.18×10^6 org/g (Table 4).

The four-day refrigerated tailmeat samples (Stage 6) showed that overall mean SPC's in the tailmeat increased after being refrigerated. The mean SPC was 7.77×10^5 org/g.

Statistical analysis of the total coliform counts revealed that there was a significant difference ($p<0.05$) among sample Stages 3-6. Table 2 shows that mean total coliform numbers at each individual stage increased as further processing occurred, excluding Stages 1 and 6. The mean total coliform count (TC) of washed live crayfish (Stage 1) was 2.06×10^4 MPN/g. This count is actually much higher, but the 3-tube MPN method limited the count $\geq 2.4 \times 10^4$ MPN/g when all tubes were positive.

Scalded whole crayfish had a mean total coliform count of 1.4×10^1 MPN/g. It appears that the scalding procedure employed reduced the number of coliforms significantly.

The mean total coliform count of crayfish tailmeat taken from colanders of individual plant personnel (Stage 3) showed an average of 6.4×10^1 MPN/g. Likely sources of coliform contamination during

peeling are the colanders, the peelers' hand and the table surfaces.

The mean total coliform count of weighed crayfish tailmeat (Stage 4) was 2.90 X 10^2 MPN/g. This increases can be attributed to the improper cleaning and sanitizing of the weighing scales and/or the multiplication of these bacteria during the processing of the crayfish tailmeat.

The packaged crayfish tailmeat had a mean total coliform count of 4.72 X 10^2 MPN/g. This number was higher than the counts reported by Kergosien. The counts ranged from 7.0 to $\geq$2.4 X 10^3 MPN/g. However, the mean reported is within the guidelines recommended by Grodner and Novak (4). Only 16.67% of the samples were above 1.1 X 10^3 MPN/g (Table 4) compared to the 30% reported by Grodner and Novak (4).

The mean total coliform count of the 4-day refrigerated tailmeat samples (Stage 6) decreased to 3.78 X 10^2 MPN/g (Table 2). This reduction could be due to a die-off.

Statistical analysis of the data showed that mean E. coli counts among samples Stages 3-6 were significantly different ($p<0.01$), Table 3. The results show that during the processing of crayfish tailmeat, the mean E. coli counts increased.

The packaged crayfish tailmeat showed a mean E. coli count of 8.8 X 10^1 MPN/g, with counts ranging from 0.0 to greater than 1.1 X 10^3 MPN/g (Table 4). Kergosien (MS) reported an average of 0.06 MPN of E. coli/g, and a range of 0.03 to 0.12 per gram. Sixty-two and one-half percent of the samples had no detectable E. coli (Table 4). Thirty-seven and one-half percent of the samples had greater than 3.6 org/g. This percentage is higher than that for E. coli recommended by Grodner and Novak (4).

Of interest, the mean E. coli counts increased in the 4-day refrigerated tailmeat samples (Table 3). This is evidence that the commercial refrigeration employed is not sufficient enough to retard the growth of E. coli effectively.

Analysis of the mean coagulase-positive S. aureus counts showed that there was no significant difference between sample Stages 3-4, but was significant between 1-6 (Table 3). This is because washed live crayfish and scalded whole crayfish have been found to be relatively free of S. aureus as this study found and others have reported (4, 7, 8).

The mean coagulase-positive S. aureus counts of the packaged crayfish tailmeat was 2.12 X 10^3 MPN/g. (Table 4). The range was 0.0 to 1.1 X 10^4 MPN/g. Over 79% of the crayfish samples had greater than 1.0 X 10^2 MPN per gram. This mean is higher than that recommended by Grodner and Novak (4). These high coagulase-positive S. aureus counts revealed that the crayfish tailmeat is contaminated and it

could possibly present a severe health problem. The increase in coagulase-positive S. aureus counts at each stage of processing supports the findings of others reported in the literature (8). Contamination of the crayfish tailmeat probably occurred from handling by plant personnel and their utensils. Contamination could also have occurred from improperly sanitized equipment and table surfaces. Table 4 shows the bacteriological quality of packaged crayfish tailmeat from the four plants.

Season Variation in Bacterial Levels

Table 5 shows the monthly variation in bacterial levels in the four crayfish processing plants. Averaging the four plants together, mean SPC were significantly different ($p<0.01$) during the three month period (March, April, and May). As Table 5 demonstrated, the mSPC's show an overall increase as the crayfish season progressed.

The mean E. coli counts were significantly different ($p<0.05$) during the three month study period.

The mean total coliform counts were significantly different ($p<0.05$) during the three month period. The counts increased during the season in a manner similar to the SPC.

The mean coagulase-positive S. aureus counts did not change significantly during the three month period. This indicates that from the beginning of the processing season through the end of the season, the peelers' hands and fingers remained cut and sore and were a possible abundant source of coagulase-positive S. aureus.

CONCLUSIONS

These results lead to the conclusion that the bacteriological quality of processed crayfish tailmeat is of significant concern. During processing the tailmeat becomes contaminated by the peeling personnel, colanders, utensils, weighing scale, and table surfaces. The implementation of handling procedures which conform to recommended federal good manufacturing practices (G.M.P.'s) is advisable, and standard bacteriological quidelines for processed crayfish tailmeat should result in lower bacterial levels. Finally, the consumer should continue to enjoy the opportunity to purchase fresh, wholesome, and safe crayfish tailmeat.

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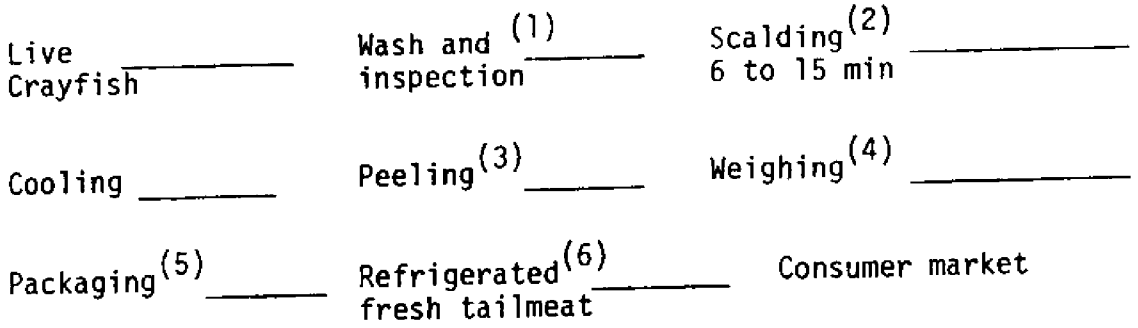
Figure 1.

Stages in the Processing of Crayfish



Identification of sampling stages:

(1) WLC - washed live crayfish

(2) SWC - scalded whole crayfish

(3) PTM - peeled tailmeat taken from individual colanders of the peelers

(4) WTM - weighed crayfish tailmeat

(5) PCM - packaged crayfish tailmeat

(6) RTM - 4-day refrigerated crayfish tailmeat.

Table 1

Average of Mean bacteriological values of four plants[1].

Plant	N[1]	SPC** (A)	S. aureus (B)	E. coli* (C)	TC (D)
A	24	5.34×10^5	2.54×10^3	1.10×10^2	4.83×10^2
B	19	1.04×10^5	1.10×10^{3a}	0.2^a	1.03×10^{2b}
C	24	2.72×10^5	2.93×10^3	6.4^a	3.30×10^2
D	18	6.52×10^5	5.93×10^2	2.21×10^2	2.60×10^2

N[1] - Number of observations.
(A)-SPC, Standard Plate Count, reported as org/g.
(B) (C) and (D) - Reported as MPN/g
a - Average of 23 observations.
b - Average of 22 observations.
* - $p<0.05$, significant at the 5% level.
** - $p>0.01$, significant at the 1% level.

1. - Averaged for samples at stage 3 through stage 6 (Peeling through 4 day refrigerated tailmeat)

Table 2

Mean standard plate count and total coliform levels at each stage of processing; average of four crayfish plants.

Sample Stage	N^1	*SPC(A)	*Total Coliforms(B)
1-WLC	22	2.83×10^7	2.06×10^4
2-SWC	23	1.22×10^5	1.40×10^{1a}
3-PTM	24	1.15×10^5	6.40×10^1
4-WTM	15	1.76×10^5	2.90×10^{2b}
5-PCM	22	4.18×10^5	4.72×10^{2a}
6-RTM	24	7.77×10^5	3.78×10^{2c}

N^1 - Number of observations.
(A)- SPC, Standard Plate Count, reported as org/g.
(B)- Reported as MPN/g.
a - Average of 24 observations.
b - Average of 17 observations.
c - Average of 23 observations
* - $p<0.05$, significant at the 5% level analyzing Stages 3-6 only.
1-WLC, washed, live crayfish.
2-SWC, scalded, whole crayfish.
3-PTM, peeled crayfish tailmeat.
4-WTM, weighed crayfish tailmeat.
5-PCM, packaged crayfish tailmeat.
6-RTM, 4-day refrigerated crayfish tailmeat.

Table 3

Mean *E. coli* and coagulase-positive *S. aureus* values at each stage of processing; average of four crayfish plants.

Sample Stage	N[1]	(A) *E. coli***	(B) *S. aureus*
1-WLC	22	5.4×10^{2}	0.5
2-SWC	24	1.7	0.2
3-PTM	24	1.8	1.51×10^{3}
4-WTM	17	2.9×10^{1}	2.76×10^{3}
5-PCM	24	8.8×10^{1}	2.12×10^{3}
6-RTM	24	1.8×10^{2}[a]	1.38×10^{3}

N[1] - Number of observations.
(A) and (B) - Reported as MPN/g.
a - Average of 23 observations.
** - p 0.01, significant at the 1% level analyzing Stages 3-6 only.
1-WLC, washed, live crayfish.
2-SWC, scalded, whole crayfish.
3-PTM, peeled crayfish tailmeat.
4-WTM, weighed crayfish tailmeat.
5-PCM, packaged crayfish tailmeat.
6-RTM, 4-day refrigerated crayfish tailmeat.

Table 4

Bacteriological quality of packaged crayfish tailmeat from four crayfish processing plants.

Mean Standard Plate Count - 1.18×10^6 org/g

Range: 2.00×10 to 2.18×10^6 org/g
9.09% of crayfish samples $>1.0 \times 10_5^6$ org/g
31.80% of crayfish samples $\geq 5.0 \times 10_5$ org/g
68.20% of crayfish samples $\leq 5.0 \times 10_5$ org/g
63.60% of crayfish samples $\geq 1.0 \times 10_5$ org/g
41.00% of crayfish samples $\leq 1.0 \times 10_4$ org/g
31.80% of crayfish samples $\leq 5.0 \times 10$ org/g

Mean Total Coliform Count - 4.72×10^2 MPN/g

Range: 7.0 to $> 2.4 \times 10^3$ MPN/g
16.7% of crayfish samples $> 1.1 \times 10_2^3$ MPN/g
41.7% of crayfish samples $\geq 1.0 \times 10_2$ MPN/g
58.3% of crayfish samples $\leq 1.0 \times 10$ MPN/g

Mean *E. coli* Count - 8.8×10^1 MPN/g

Range: 0.0 to 1.1×10 MPN/g
37.5% of crayfish samples> 3.6 MPN/g
62.5% of crayfish samples= 0.0 MPN/g

Mean Coagulase-positive *S. aureus* Count - 2.12×10^3 MPN/g

Range: 0.0 to 1.1×10^4 MPN/g
79.2% of crayfish samples $>1.0 \times 10^2$ MPN/g

Table 5

Monthly mean bacteriological counts[1] associated with four crayfish plants.

Time	N[2]	(A)$_{SPC}$**	(B)$_{TC}$ *	(C)$_{E.\ coli}$*	(D) S. aureus
March	26	1.73×10^{5}	1.73×10^{2}[a]	2.4[a]	1.84×10^{3}[a]
April	30	3.52×10^{5}	2.20×10^{2}[b]	1.34×10^{2}	7.38×10^{2}
May	29	6.16×10^{5}	5.14×10^{2}	9.60×10^{1}[c]	3.10×10^{3}

Counts[1] - Only the crayfish tailmeat from the peeling stages through the refrigerated samples were used for the analysis.
N[2] - Number of observations.
(A) - SPC, Standard Plate Count, reported as org/g.
(B), (C) and (D) - Reported as MPN/g
a - Average of 30 observations.
b - Average of 29 observations.
c - Average of 28 observations.
* - $p<0.05$, significant at the 5% level.
** - $p<0.01$, significant at the 1% level.

INTERACTION OF SELECTED ANTIBIOTICS ON FOUR COMMON BACTERIA ASSOCIATED WITH FISH[1]

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INTRODUCTION

Many antibiotics and disinfectants have been used to treat fish and fish habitats for conditions attributed to bacteria (Trust, 1972), although documentation of the utility of these compounds is limited. The four compounds used in this study were chloramphenicol, erythromycin, fupyrinol, and oxytetracycline. These compounds were selected because of their popularity among aquaculturists and hobbyists.

Chloramphenicol is a broad spectrum bacteriostatic antibiotic antagonistic to Gram negative bacteria which is absorbed well via the gastrointestinal tract of mammals over a pH range of 2 to 9 (Aronson & Kirk, 1974). Erythromycin, a broad spectrum bacteriostatic antibiotic is often used in human medicine in place of penicillin and has an activity which is very limited by a pH of less than 7.5 (Aronson & Kirk, 1974). Oxytetracycline, one of two drugs approved for aquaculture use, has been extensively studied and is a proven compound for aquatic antimicrobial therapy (Herman, 1969). This compound has been noted to enhance production of plasmid mediated resistance in aquatic bacteria (Shotts et al, 1976). Furpyrinol, a highly conjugated nitrofuran, is unique and was developed specifically for marine and aquatic antibacterial therapy (Anon., 1970). This compound is a broad spectrum bacteriocidal agent effective against most aquatic bacteria except Pseudomonas.

The purpose of this research was to determine the minimal inhibitory concentration (MIC) and minimal bacteriocidal concentration (MBC) for the above compounds with regard to the growth of A. hydrophila complex, A. salmonicida, Flexibacter columnaris like organisms and Pseudomonas fluorscens.

MATERIALS AND METHODS

Bacteria. The bacteria used in this investigation were from the stock collections of the Department of Medical Microbiology, University of Georgia and the National Fish Health Research Laboratory, Fish and Wildlife Service, USDI, Kearneysville, W. Va. The strains of A. hydrophila complex (24 isolates), A. salmonicida (12 isolates), Flexibacter columnaris like organisms (14 isolates) and Pseudomonas fluorscens (14 isolates) were recovered from clinical situations using standard methodology (Shotts and Bullock, 1976).

Antibiotics. The antibiotics used were prepared as follows:

1. 0.1 gms of chloramphenicol (Rochelle Laboratories, Long Beach, CA.) was dissolved in distilled water (Ph 6.4 - 6.6) to make a 1000 gm/ml solution.
2. 0.1 gms of erythromycin, carrier free (Sigma Chemicals, St. Louis, Missouri) was dissolved in a minimal amount of acetone and distilled water added to make a 1000 mcg/ml solution.
3. 0.1 gms of a 10% preparation of furpyrinol (Furnace^{R}, Abbott Pharmaceutical, North Chicago, Illinois) with distilled water added to make a 1000 mcg/ml solution.
4. 0.1 gms of oxytetracycline powder (Pfizer & Co., Inc. Brooklyn, NY) was dissolved in distilled water to make a 1000 mcg/ml solution.

Determination of Minimum Inhibitory Concentration (MIC). Determinations of MIC of the respective antibiotic was done by serial two-fold dilutions of the stock solution of the antibiotic in Pennassay broth (Difco) to achieve mcg/ml concentrations of 500 through 1. Tryptone water (0.5%) was used rather than Pennassay broth for studies related to F. columnaris like organisms. Bacteria for inoculation of these series were grown in 10 ml. of either Pennassay or 0.5% Tryptone broths and adjusted to a population of 3×10^8 to 6×10^8 organisms per ml (McFarland turbidity of 1 to 2). The organisms were further diluted so that a 0.1 ml inocula contained approximately 1×10^6 organisms. This suspension was added to each of the test and control tubes in a given series. The procedure used varies in this regard from the method of Collins and Lynne (1970) who used 24 hour cultures. This modification was necessary since 37°C incubation retards the growth of Ps. fluorscens, F. columnaris like organisms, and A. salmonicida. The incubation time and tempreature used to prepare cultures were 24 hours at 37°C for A. hydrophila complex, 48 hours at 22°C for A. salmonicida, 72 hrs. at 22°C for F. columnaris like organisms and 24 hrs at 22°C for Ps. fluorscens. The minimum inhibitory concentration (MIC) was recorded on replicate trials as the concentration of antibiotic in the first tube of a given series without visible growth.

Determination of minimum bacteriocidal concentration (MBC). Determination of a minimum bacteriocidal concentration was done by transferring a loop of broth from each of the MIC tubes of a

given series not showing visible growth onto either trypticase soy agar or to 0.5% tryptone agar in the case of F. columnaris like organisms. The lowest antibiotic concentration with no growth after 24 to 72 hrs at the appropriate incubation temperature was designated as the MBC. Colonies from A. hydrophila complex and A. salmonicida cultures with erratic MBC patterns were further studied for the presence of antibiotic resistance plasmids (Shotts et al, 1976).

RESULTS

Mean and modal values for MIC and MBC were calculated for each antibiotic and the bacterial interaction graphed (Figures I - IV).

Comparison of the data would indicate that furpyrinol was the most effective antibiotic studied. This compound is not recommended for use against the genus Pseudomonas and is borne out by this study. Inhibitory concentrations of furpyrinol (Figure 1) against the other genera studies were in a magnitude of $\leq$1.0 mcg/ml or approcimately 1 part per million (ppm). Next in general effectiveness was oxytetracycline (Figure IV) where the MIC was achieved at levels of $\leq$ 8 mcg/ml except in instances where antibiotic resistant plasmids were present. Chloramphenicol studies (Figure II) were not carried out for Ps. fluorscens, however, with the other organisms studied erratic MIC were encountered that were not plasmid associated and levels of $\leq$ 62 mcg/ml were necessary for inhibition. Carrier free erythromycin provided an even more erratic pattern than chloramphenicol, and in two cases Ps. fluorscens and A. salmonicida levels of $\leq$ 500 mcg/ml were necessary for establishment of inhibition.

The MBC for the respective bacteria was usually found to be several dilutions higher as would be expected since all but furpyrinol are bacteriostatic drugs rather than bacteriocidal drugs. In instances where plasmids were involved, marked increases were noted in MBC. Strains of A. hydrophila complex studied for presence of plasmids (5/5) showed increases in MBC averaging 30 mcg/ml while strains of A. salmonicida studied (3/3) had increases of approximately 20 mcg/ml.

DISCUSSION

Generalization of these findings is somewhat difficult but can be best summarized by saying that furpyrinol and oxytetracycline base upon MIC data appear to be very useful against A. hydrophila complex, A. salmonicida and F. columnaris-like organisms. The potential usefulness of oxytetracycline extends also to Ps. fluorscens. It does not appear, based upon these data that chloramphenicol is particularly useful except under special circumstances. Carrier free erythromycin is of very little or no use against the genera of bacteria studied.

It has been documented that the inherent error in the tube dilution method used is plus or minus one dilution or from two to four fold (Collins and Lynne, 1970, Parry, 1977). Since the data presented represent a mean of replicate trials the values presented should reflect stable reference values for these drugs under the parameters of this study. The inherent error would be of negligible importance when considering the action of oxytetracycline against all groups of bacteria studied, but not in the case of furpyrinol where the compound is known to not be a suitable therapy in Pseudomonas infections. The concentration requirements and general inefficacy of chloramphenicol and erythromycin raise doubts as to their general use.

The bacteriostatic character of the drugs examined may be noted by the approximately sixteen fold differences seen between MIC and MBC values. Bacteriocidal effects were observed in the action of furpyrinol against A. salmonicida and F. columnaris like organisms. However, this data would indicate that, although this drug is considered bacteriocidal, its action against A. hydrophila appears to be bacteriostatic. This suggestion of stasis remains despite the consideration of inherent errors in the assay system.

Distinct biomodal distribution of MBC were observed in A. hydrophila complex and A. salmonicida when oxytetracycline was examined. In all of the A. hydrophila and A. salmonicida isolates having high MBC, resistance plasmids to oxytetracycline were demonstrated. These ratios (approximately 20% and 33% respectively) closely parallel other workers' observations of aquatic bacterial populations subjected to antibiotic pressure (Aoki, 1974). The plasmid resistance observed was not noticeable in the MIC studies and possibly suggest that the plasmid is distributed throughout the population at a low frequency.

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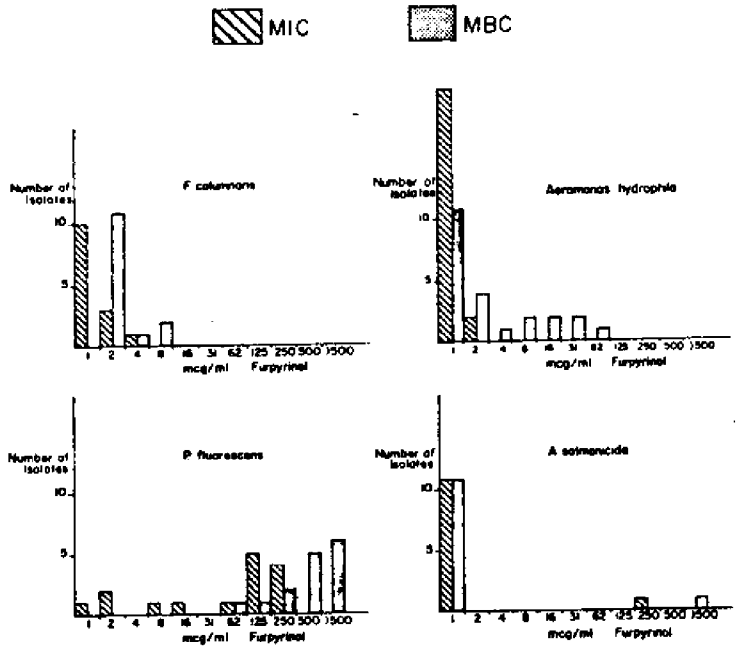

FIGURE I

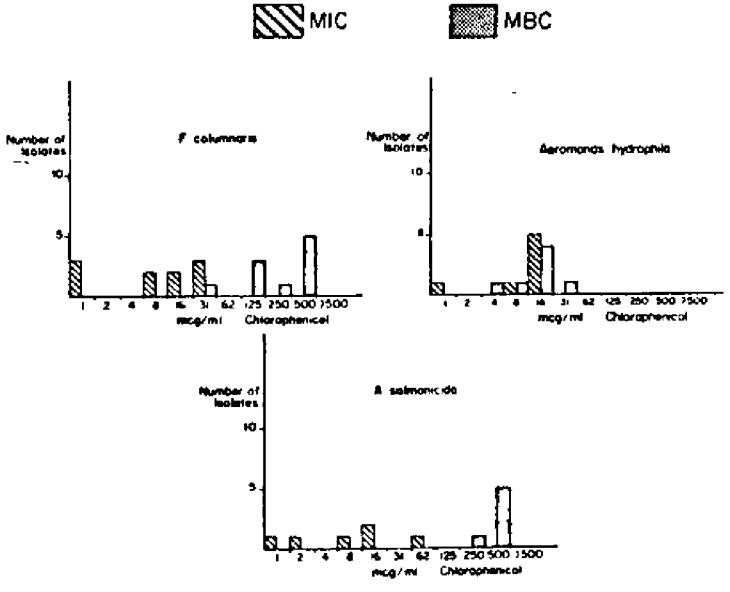

FIGURE II

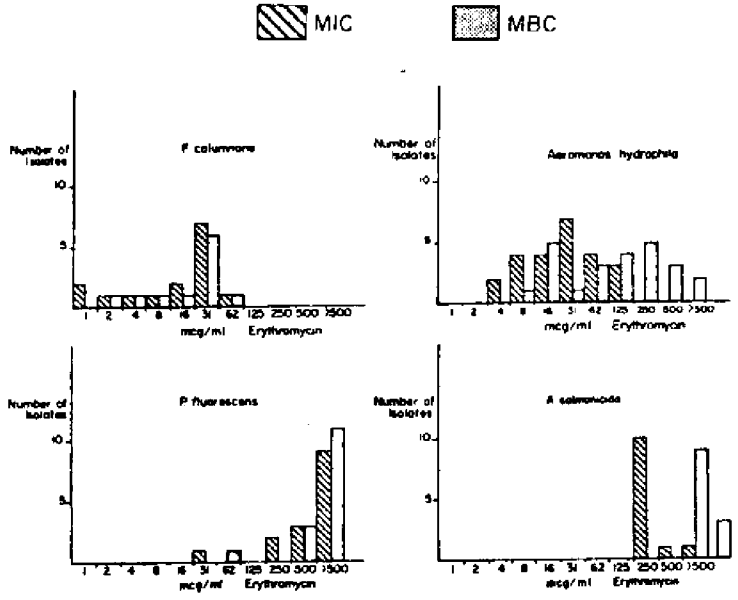

FIGURE III

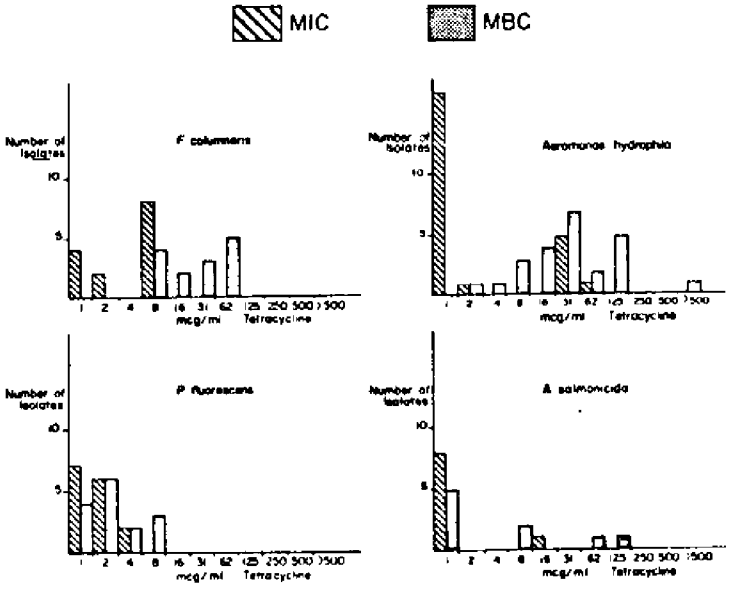

FIGURE IV

DEVELOPMENT OF PACKAGING FOR INLAND MARKETING OF FRESH AND FREEZE/THAW FRESH FISH

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INTRODUCTION

This study to date has been conducted in three phases: (1) a consumer market survey of packaging types, (2) a market test, (3) laboratory shelf life studies. Through these devices, we have attempted to initiate development of what we hope will be the best system for prepackaging and retail sales of North Carolina fish. To accomplish this objective, we had to consider three things: (a) the desires of the consumer; what he/she considers to be the most desirable package, (b) the desires of the packer; what packaging system enables him to sell more fish at a profit, (c) the demands of the product; what packaging/handling system best maintains the quality of the product. We realized from the start that each of these requirements might be quite different; that the final selection will most likely be a compromise. However, we will transmit information that we obtain to packaging manufacturers; what final packaging systems are developed will obviously be determined by the economics and capabilities of each company.

PACKAGE SURVEY

In trying to determine what type of package the consumer wants for fish, we examined several types of meat packages currently in use. The film-overwrap tray is the most popular for meats/poultry; therefore, it was chosen as the point of comparison. Vacuum packaging is being used or tested to some degree throughout the country for all types of flesh foods; therefore, we selected the Cryovac system to test this package type. Rigid or semi-rigid poly trays with a heat-seal printed film cover are now used to some extent for poultry livers and gizzards, and a larger version (10-20 lb.) is now the leading fresh-pak method for handling fish in Canada and the U.S. Therefore, the Gen Pak Corporation lent us the equipment to seal a 10-pound tray so that the concept of a 1-2 pound tray could be evaluated.

These three packages were chosen to conduct a survey concerning fish packaging at a large shopping mall in Raleigh, N. C. Approximately 400 people participated in the survey over a two-week period. The three packages of pan trout were presented in an ice chest and several questions were asked: (1) Looking at each of these

packages of fish. which would you say contains the highest quality fish? (2) Which of the three package types is most appealing? At this point, each package type was explained so that the judge clearly understood the features of each package. It was explained that the large poly tray (Gen Pak) should be evaluated as a large version of a package which would actually hold only 1-2 pounds of fish. Then a final question was asked concerning the overall best package type. (3) Which package would you rather purchase fresh fish in?

Table 1 contains the percentage responses for the above three questions. The data is divided into week 1 and week 2 for the following reason: it was observed that the initial vacuum packages (week 1), while of normal commercial quality, were perhaps not the best that could be achieved; purge was quite prevalent, giving a somewhat wet or bloody appearance around the fish within the package. Also, an air space existed between the fish and the top film in the poly tray of the Gen Pak package, such that the fish were somewhat hard to see through the film. Both these deficiencies were eliminated in the packages used the following week (week 2); the vacuum package was very dry with no apparent purge and the top film was pressed tightly against the fish in the poly tray. Note that while the change in the poly tray only slightly affected the response to this package, elimination of the purge from the vacuum package drastically improved its acceptability. The consumers questioned seemed to particularly like the idea of vacuum packaging fish, as this equated with better fish quality in many of their comments, yet the package still did not rate as highly overall as the poly tray. The reasons given for selection of a package included many of the following: reuse container, neater, easier, fresher, appearance, air tight, good seal, not compressed, no blood, no dripping, something different, etc. A final report on this phase of the project will include a listing of reasons given for selection of each package type.

Thus, from this survey we can tentatively draw the following conclusions: (a) a rigid poly tray appears to be very attractive to consumers as a package for fresh fish; (b) additionally, a vacuum-package with a dry appearance also seems to have reasonably good appeal. From experience, we know that while definite preferences were expressed in the survey, any of the package types would probably be acceptable to consumers of fish.

MARKET TESTING

Initially, the emphasis of this project was to develop a packaging/handling system for strictly fresh, unfrozen fish. Such a goal was based on recent surveys of Midwestern markets which revealed that consumers prefer to buy fish in an unfrozen form (4). However, discussions with North Carolina fish dealers and processors revealed that their past experience had shown that supplying fresh fish in good selection, at predictable prices, and in sufficient quantity to meet the needs of supermarket chains was extremely

difficult due to seasonal gluts and unavailability of each fish species with accompanying wide fluctuations in price from day to day. Thus, it was determined that to meet the demands of the market, i.e. good selection year round, predictable prices, and large inventory, while satisfying the consumer's demand for unfrozen, high-quality fish, the best solution would be a freeze/thaw marketing scheme. The end-product of such a scheme must, however, meet the following criteria: (a) The product must be presented in a manner which, while not attempting to fool the consumer by concealing the fact that the fish have been previously frozen, does not imply lower quality due to having been previously frozen. (b) The fish should be of equal or greater quality and attractiveness as "fresh" fish currently available in inland markets at present. Previous research has shown that, while most non-fatty species hold their quality well after freezing, higher fat containing species tend to become rancid fairly quickly upon thawing and storage at cooler temperatures. Therefore, the elimination of oxygen from the package to reduce oxidative deterioration would be desirable. Additionally, freezer burn (drying) and excessive purge upon thawing must be dealt with in an effective manner to insure a desirable appearance to the packaged fish.

To meet the requirements of (b) above, a vacuum package was chosen to minimize both oxidative damage and freezer burn. Rapid freezing and thawing via a liquid-nitrogen or effective air, blast freeze, and thawing in water was adopted to minimize the purge problems, although other methods for dealing with this problem will be investigated in the future.

Therefore, the second phase of this study consisted of a market test of two packages similar in appearance, being both conventional foam tray packs of fish with an absorbent paper pad underneath the fish, differing only in that one package was over-wrapped with an air-permeable meat-type plastic film, while the other package type was contained in a Cryovac-E-type vacuum bag with a heat seal and heat shrunk to eliminate air pockets from the package. These are identical to the two foam-tray packages mentioned previously. To evaluate the consumer's response to being informed on the package label that the fish had been previously frozen, two labels, one with and one without such a legally-correct statement, were developed to be displayed on top of each package. Additionally, another small label was added to each vacuum package to draw attention to this package type. The testing of the four resulting packages (two package types x two labels) was conducted in a six-week market sales test in six chain supermarkets with a pan-ready sea-trout as the test species. The four package types were rotated between stores from week to week; thus, there were six paired combinations possible, requiring six weeks to be rotated to each store. The two package types being compared in a store were displayed in two full rows in the meat counter, being separated by a plastic divider. Tabulation of the data for the six-week, six-store study is shown in Table 2. A significantly different preference was shown for the traditional overwrap package compared to the vacuum tray pack. Several comments

from shoppers and market managers indicated that excessive purge in the vacuum packages was the primary factor for this preference and not the package type per se. Overwrapped packages were prepared by repackaging the fish previously frozen and held in vacuum packages; thus, thaw drip was easily eliminated at the time of packaging. Such was obviously not possible with the vacuum packages. Additionally, it was observed that two factors contributed to the purge problem in the vacuum package: (a) the absorbent pad did not seem to be as effective under the pressure of the vacuumized fish (b) even small amounts of purge spread throughout the package, especially around the sides and bottom of the package, in a thin film. Purge did not seem to accumulate between the package film and the fish itself in the middle of the package top. Thus, it can be recommended from these observations that purge should be dealt with more effectively to increase the desirability of the vacuum package. Possible solutions to this might include: (a) methods to decrease thaw drip (ex: use of phosphates, cryogenic freezing, etc.), (b) redesign of the absorbent pad to increase its efficiency under vacuum, (redesign of the package to effectively hide the purge around the sides and bottom of the package while allowing full view of the fish in the middle top portion of the package. This could be accomplished by either a bacon-type box or opaque printing of the bag.

The presence of the "previously-frozen" statement on the label did not significantly deter the sales of fish so-labeled, although a slight preference for fish not carrying this statement is evident in Table 2. This result reinforced comments from shoppers and market managers that the selection of fish by consumers was due more to a fresh, dry appearance of fish than the labeling information for the package type per se. Thus, previously-frozen fish would be expected to compete equally well with fresh fish if they are of high quality and packaged attractively.

SHELF LIFE STUDIES

The final phase of this study has consisted of a laboratory study to evaluate the vacuum package for its ability to maintain fish quality during storage at cooler temperatures (retail meat case; 0-2°C) after thawing from frozen storage. Figure 1 schematically presents the experimental design. A previous study had indicated no advantage in vacuum packaging bluefish in the relatively oxygen permeable Cryovac E bag versus packaging fish in an aerobic, film-overwrap tray during cooler storage. Therefore, in this study we compared the storage life of fish (grey trout) vacuum packaged in the E bag versus the more anaerobic Barrier bag manufactured by Cryovac. With reduced amounts of oxygen available to the product in the more anaerobic bag it was hoped that rancidity development would be curtailed in the previously-frozen product during cooler storage. Rancidity development was monitored by the 2-thiobarbituric acid (TBA) method (3) and subjectively evaluated by a trained profile panel on a 14 point absolute scale (5). Other tests used to monitor the fish quality were total volatile nitrogen (TVN) determinations (1); aerobic plate count at 25°C incubation with the inclusion of 1.5% salt in the media (6), drip loss upon

thawing and/or cooler storage, and a subjective quality scoring of the raw fish based on odor and appearance criteria (2).

The results for the TVN assessment and aerobic plate counts are presented in Figure 2. These reveal that the rate of bacterial spoilage during fresh holding or thawed storage after 100 or 200 days of frozen storage was unaffected by the type of package used. More surprisingly, in terms of bacterial-associated spoilage, previously-thawed fish appear to have a similar shelf-life to fresh, unfrozen fish. These same conclusions are substantiated by the subjective quality (sensory) scores (Figure 3) except that the quality of fish frozen 200 days was not as high initially upon thawing and deteriorated somewhat more rapidly upon holding in the cooler.

TBA levels (Figure 4) and taste panel scores for rancidity (Figure 5) would indicate that the Barrier bag was relatively successful in minimizing rancidity development in previously-frozen fish. Taste panel data for the 200 day samples is not yet available and data for the fresh samples past day 8 was unavailable due to a freezer malfunction. The high fishiness value for day 4 fish in the Barrier bag was not consistent with other data and is likely in error.

A comparison of the mean drip loss for all packages following 0, 100 or 200 days of frozen storage during holding in a commercial cooler is given in Figure 6. Drip-loss data was found to be rather erratic when comparing the raw data for each bag type, and no trend was evident other than a gradual accumulation of drip upon holding at cooler temperatures. Among the three storage treatments the fresh fish obviously had the least drip loss.

CONCLUSIONS

To date the results of this study do not suggest any serious obstacles to the development and implementation of a freeze/thaw handling regime for fresh fish. Consumers appear to be very receptive to such a product as long as the packaging is attractive and the product has a fresh appearance. Such a handling scheme should be considered as complementary to the marketing of strictly fresh or frozen fish; our recommendation would be that processors make available fresh, frozen and previously-frozen product to insure the maximum marketability of the total available catch, at predictable prices year round. Thawing of fish at the retail level should be discouraged, as this not only defeats the benefits of prepackaging to the retailer but also entails the danger that partially-frozen product might be presented to consumers in the fresh meat case. Work is presently underway in this laboratory to further reduce the problems of rancidity and drip-loss development in thawed fish previously held frozen for extended periods. It is expected that newer rapid freezing technology, possibly in combination with dips of phosphate, antioxidant, etc. solutions should prove adequate for this purpose.

Although true vacuum packaging of the thawed, previously-frozen fish seems desirable in terms of minimizing rancidity, recent work by other researchers suggests that for fresh (and therefore, logically, previously-frozen) fish a CO_2-containing modified atmosphere might further extend the refrigerated shelf-life of these products. Additionally, it remains to be determined whether an oxygen-free package poses any risk of botulinal toxin poisoning through temperature abuse of the packaged fish. Thus, while vacuum packaging in bulk is certainly recommended for holding fish during frozen storage, further work is now underway to determine what packaging methods should be recommended for retailing the consumer unit of fresh or thawed product. These include both vacuum and gas-flush packages of the bag and tray variety or of the semi-rigid, heat-sealed top-film type. The results of the survey portion of this study would indicate a high consumer preference for the latter type of package and this hopefully will be confirmed in future marketing trials now planned.

Table 1. Consumer response to three package types, by percent. Wrap = film overwrap; Vac = vacuum bagged tray; Tray = rigid tray with heat seal top film.

	Week 1			Week 2		
	Wrap	Vac	Tray	Wrap	Vac	Tray
Quality	35	18	41	21	24	38
Appearance	39	11	50	21	22	57
OVERALL	15	22	63	11	37	52

Table 2. Retail sales of packaged pan-ready sea trout as affected by package type and label information.

TOTAL SALES - INDIVIDUAL TREATMENTS

0- (Overwrap - no statement)	0+ (Overwrap + statement)	V- (Vacuum - no statement)	V+ (Vacuum + statement)
320	289	264	230

TOTAL SALES - PAIRED TREATMENTS

0- vs 0+	0- vs V-	0- vs V+	0+ vs V-	0+ vs V+	V- vs V+
102, 89	125, 95	93, 82	107, 82	93, 75	87, 73

TOTALS - INDIVIDUAL : Total number of packages sold per treatment at all six participating stores in six weeks

TOTALS - PAIRED : Total number of packages sold per treatment when paired with one another

LABEL STATEMENT : Previously Frozen for your Protection - Safe to Refreeze or Refrigerate

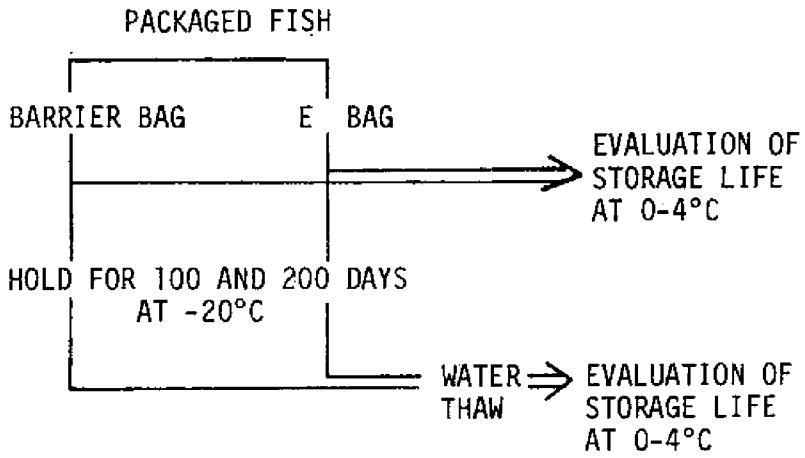

Figure 1. Evaluation of freeze-thaw marketing scheme utilizing vacuum packaging.

Sampling scheme for quality tests began on Day 0 or day of thawing through Day 14.

Tests for quality included: aerobic plate counts (25°C), with 1.5% salt added; TVN : total volatile nitrogen; subjective quality (sensory) score; drip loss, and profile taste panel.

Oxygen Transmission at 73°F (m2, 24 hrs., 1 atm.)	Barrier Bag	E Bag
	30-40 cc.	4000 cc.

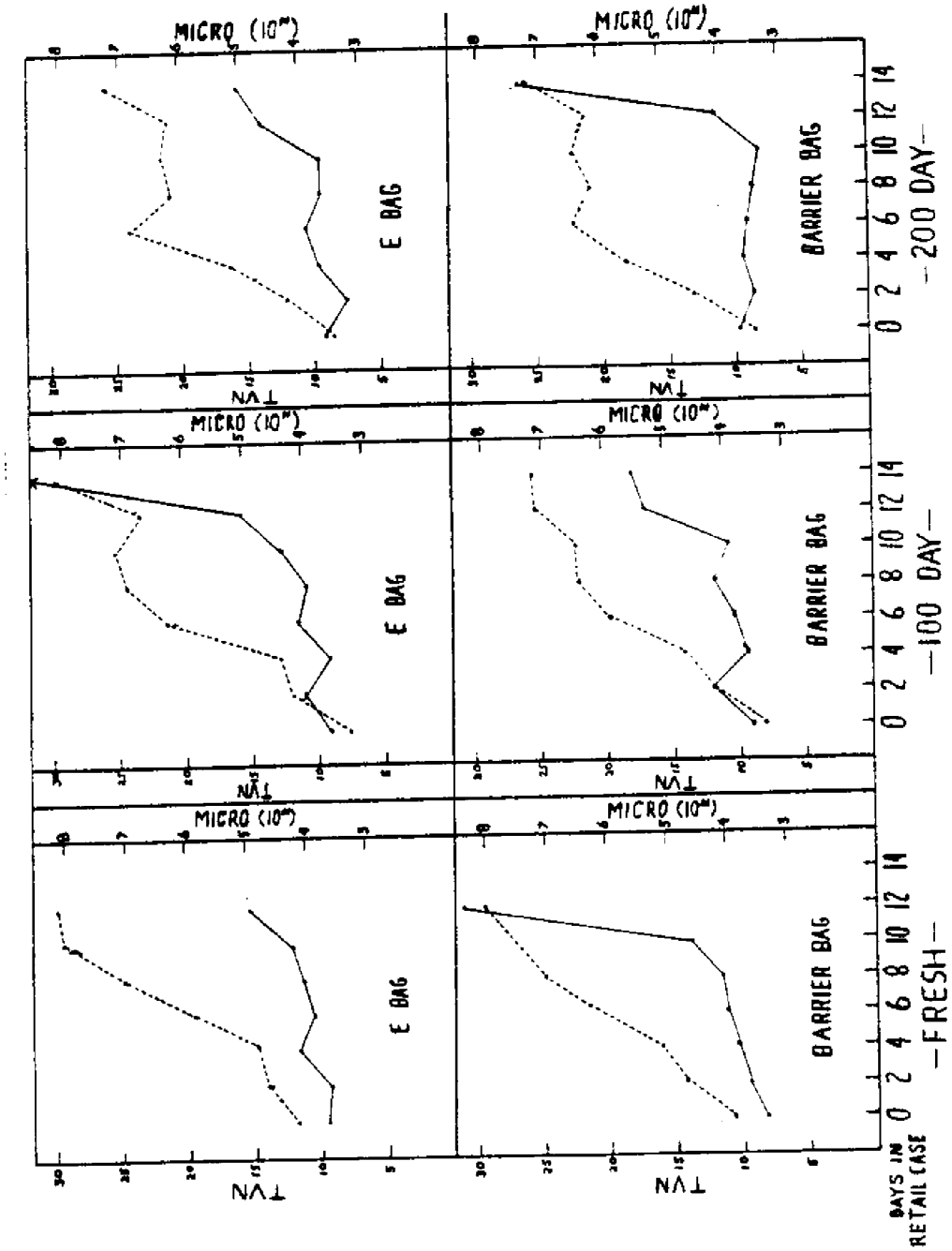

Figure 2. TVN values and plate counts for vacuum packaged trout fillets in Barrer and E bags held fresh and frozen for 100 and 200 days. _____:TVN -----:MICRO

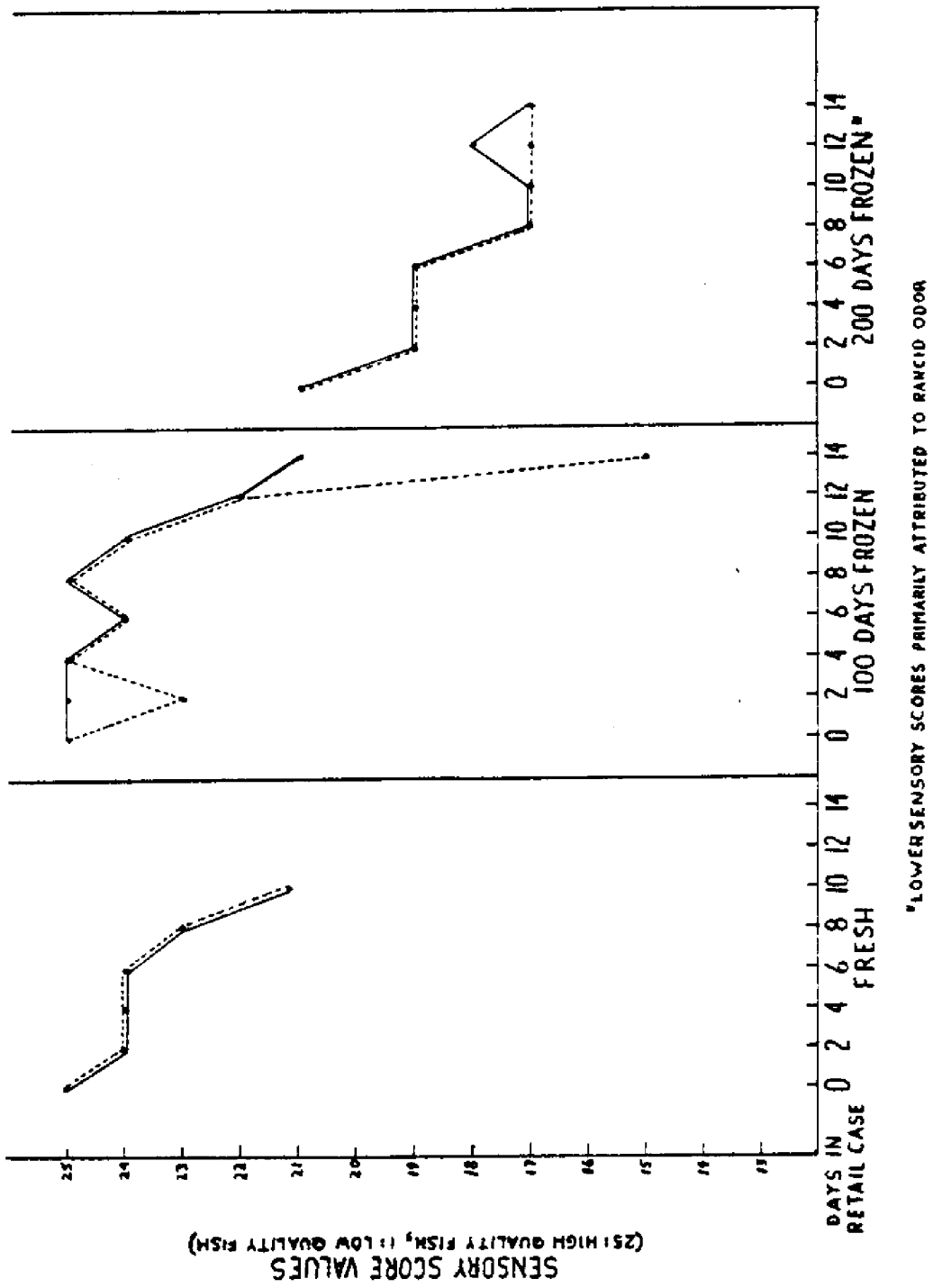

Figure 3. Sensory scores for vacuum packaged trout fillets in Barrier and E bags held fresh and frozen for 100 and 200 days.
_____:Barrier Bag -----: E Bag

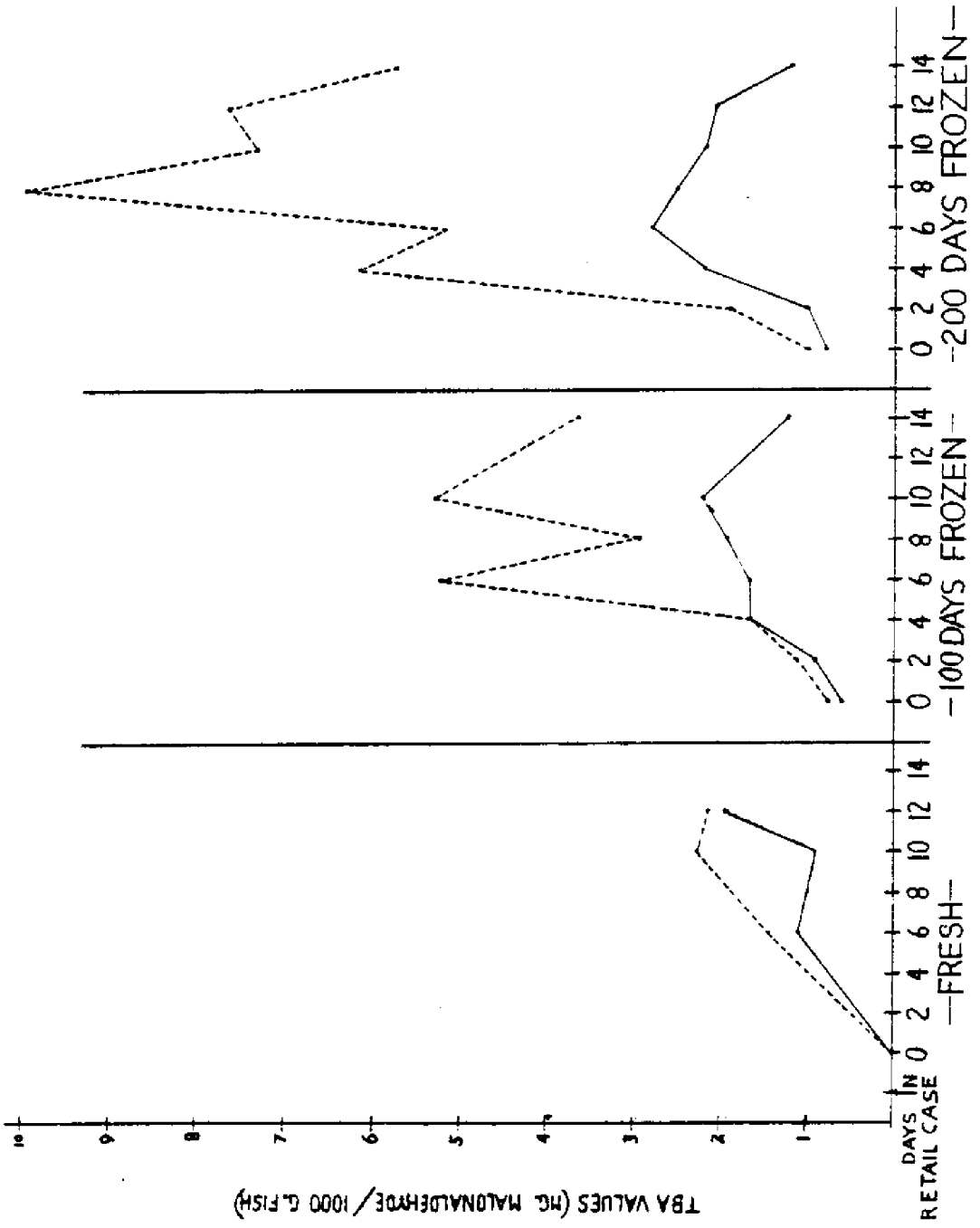

Figure 4. TBA values for vacuum packaged trout fillets in Barrier and E bags held fresh and frozen for 100 and 200 days.
_____:Barrier Bag -----:E Bag

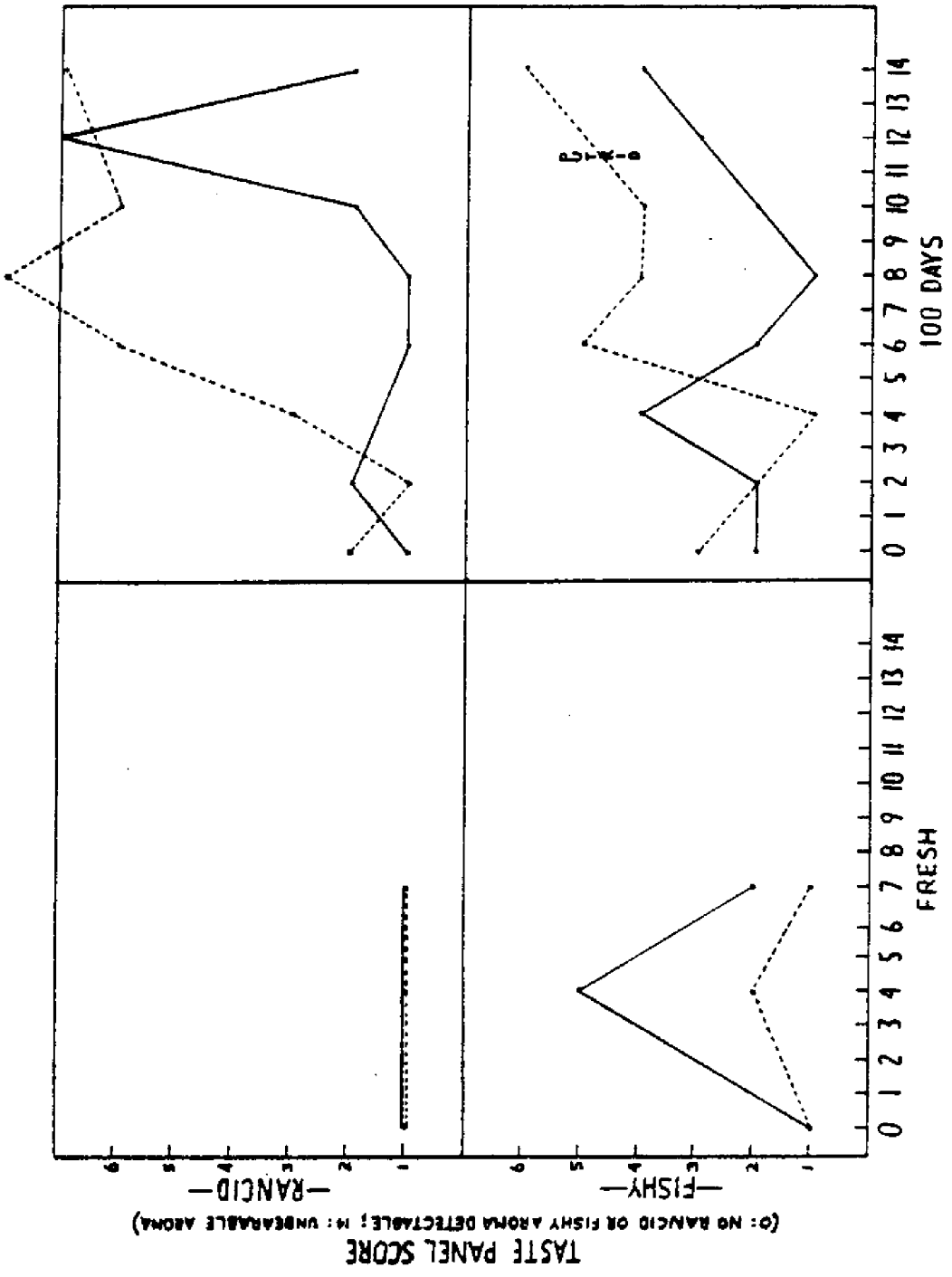

Figure 5. Taste panel scores for vacuum packaged trout fillets in Barrier and E bags held fresh and frozen for 100 days.
_____:Barrier Bag ----:E Bag

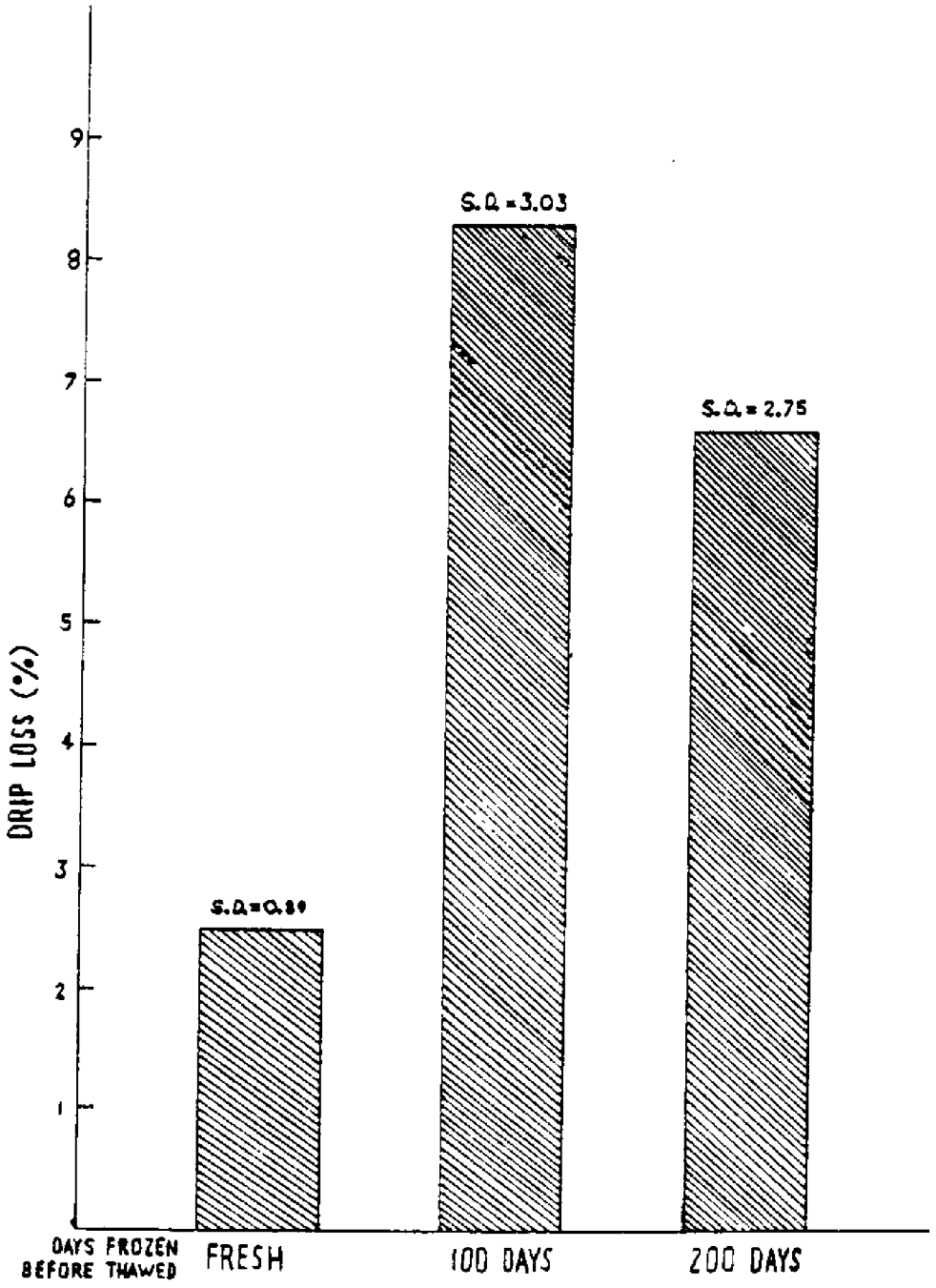

Figure 6. Combined mean drip loss percentages for vacuum packaged trout fillets held fresh and frozen for 100 and 200 days.

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USE OF ALASKA KING CRAB BOARD METHOD FOR DETERMINATION OF PERCENT STATED NET WEIGHT FOR SPLIT SNOW OR KING CRAB LEGS

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Problems are encountered in determining net weight using conventional methods in split crab legs due to excessive thawing of crab meat while deglazing the entire contents of the package and because of the difficulty of draining the contents of a package in a sieve without altering net weight results.

Because of the increasing volume of this underutilized product being produced recently and the absence of information regarding determinations for split legs by processors, it becomes necessary for the development and implementation of specific methods for this product.

It would be valuable, therefore, to have an official method which is rapid, reliable, repeatable and sufficiently simple for many different people with varying qualifications, experience and training to perform successfully and still provide acceptable, dependable, objective results which could be used to make decisions concerning the lots tested.

The purpose of the study presented in this paper is to recommend two methods (end product and on line product method) to resolve the problem encountered when using conventional methods on king and snow crab split legs.

MATERIALS AND METHODS

Source and Sample Preparation

Commercially prepared glazed crab split legs in institutional 3½ and 4 pound bags were randomly sampled in a crab split leg processing plant during normal production. Sampling was in accordance with USDC statistical sampling plan contained in "Regulations Governing Processed Fishery Products."

Seventy-three sample units of 4 pound bags and thirty-seven sample units of 3½ pound bags were randomly sampled and used to draw the subsamples for determination of percent stated net weight. Each

4 pound subsample was further subdivided into four specimens of not less than one pound, and the 3½ pound subsample was further subdivided into three specimens of not less than one pound. Acceptance and rejection criteria is based on Section 105.01C of Alaska King Crab Marketing and Quality Control Board for percent of stated net weight (90% minimum).

Procedure

Method one, hereinafter referred to in this paper as the "subsample or end product method", utilized the following procedure.

1. Weigh each subsample on a scale, determining the weight of the sample to the nearest 0.1 gram or 0.001 ounce.

2. Weigh not less than sixteen ounces. The remaining part of the subsample is stored in a freezer unit while working with the first specimen.

3. Record the weight of the first specimen.

4. Place the first specimen on the U.S. standard ASTM No.8 mesh sieve, 12 inches, opening in inches .0937; single layered with exposed meat facing down and widest part towards the same direction.

5. Place the sieve with the first specimen in a container filled with water to a predetermined level (water should come in contact with the exposed meat).

6. Using a soft spray of water, 65-80^{o} (water temperature) for ten to fifteen seconds, deglaze the specimen.

7. Drain the specimen for two minutes in the inclined sieve, (approximately 45 degree angle), with widest part of legs toward the highest part of the inclined sieve.

8. Weigh the drained specimen and record results.

9. Repeat steps two through eight for the remaining subsample.

10. Add specimen's results for each subsample and calculate the percent stated net weight for the subsample as follows:

$$\text{Percent stated net weight} = \frac{\text{drained weight}}{\text{declared net weight}} \times 100$$

Specimen's Drained Weight for a 64.00 ounce declared net weight subsample.

Example: 1st 14.3750 ounce + 2nd 14.2500 ounce + 3rd 14.5000 ounce + 4th 16.5000 ounce = 59.6250 ounce

$$\text{2nd } \frac{59.6250}{64.00} \times 100 = 93.1641\% \text{ stated net weight}$$

Method two, hereinafter referred to in this paper as the "specimen or on-line product method", utilized the following procedure.

1. Steps one through eight are the same as for end product method, using only one specimen from each subsample.

2. The specimen result is calculated for the whole subsample as follows:

a. Percent glaze of specimen.

(1) Weight of glazed specimen - drained weight = glaze weight.

(2) (Glaze weight/weight of glazed specimen) X 100 = percent glaze.

b. (Weight of subsample with glaze) X (percent glaze of specimen) = estimated glazed weight of subsample.

c. (Weight of subsample with glaze) - (estimated glazed weight of subsample) = estimated drained weight of subsample.

d. (Estimated drained weight of subsample)/(declared net weight of subsample) X 100 = percent stated net weight.

Example: a. Percent glaze of specimen.

(1) (16.375 ounce) - (14.375 ounce) = 2.00 ounce.

(2) (2.00 ounce)/(16.375 ounce) X 100 = 12.2137%.

b. (67.8750 ounce) X (12.2137%) = 8.2900 ounce.

c. (67.8750 ounce) - (8.29 ounce) = 59.5850 ounce.

d. (59.5850)/(64.00 ounce) X 100 = 93.1016% stated net weight.

RESULTS AND DISCUSSION

Comparison of Means

For the 4 pound samples the means of the subsample or end product method was 92.9805 and the means of the specimen or on-line product method was 93.4810 showing a difference of .5005 for 73 observations. Table 1 shown below summarizes the results and the statistical data used in the computations.

TABLE 1

N	$\bar{X}_1$	$\bar{X}_2$	Var. 1	Var. 2	SD_1	SD_2
73	92.9805	93.4810	7.78	7.51	2.79	2.74

STATISTICAL COMPARISON OF TWO MEANS

For the 3½ pound samples the means of the subsample or end product method was 92.5847 and the means or end product method was 92.5847 and the means of the specimen or on-line product method was 93.1549 showing a difference of .5702 for 37 observations. Table 2 shown below summarizes the results and the statistical data used in the computations.

TABLE 2

N	$\bar{X}_1$	$\bar{X}_2$	Var. 1	Var.2	SD_1	SD_2
37	92.5847	93.1549	2.34	2.92	1.53	1.71

STATISTICAL COMPARISON OF TWO MEANS

The Z score test was used to compare the two sample means of the percent stated net weight obtained by the two test methods. A Z score between -1.96 and +1.96 accepts the typothesis that there is no difference at the 5% level of significance between the results of the two methods.

The null hypothesis with an alternative hypothesis was established as follows:

$$H_0: \bar{X}_1 = \bar{X}_2$$

$$H_1: \bar{X}_1 \neq \bar{X}_2$$

Results were computed as follows:

$$\text{(4 pound) } Z = \frac{(\bar{X}_1 - \bar{X}_2)}{\sqrt{\frac{S_1^2}{N_1} + \frac{S_2^2}{N_2}}}$$

$$Z = \frac{92.9805 - 93.4810}{\sqrt{\frac{7.78}{73} + \frac{7.51}{73}}}$$

$$Z = -1.09$$

(3½ pound) $Z = \frac{92.5847 - 93.1549}{\sqrt{\frac{2.341}{37} + \frac{2.924}{37}}}$

$Z = -1.50$

Since Z falls in the acceptance region, (which for this test extends from -1.96 to +1.96), we accept the hypothesis that there is no difference at the 5% level of significance between the means of the results of the two methods, that the population is the same, and that the two methods give statistically equivalent results for determining percent stated net weight on glazed split legs.

The means of the specimen or on-line product method were observed to be slightly higher than the means of the subsample or end product method.

This suggests that ice crystals are formed in the bag due to water added during the glazing operation when using the subsample or end product method.

Comparison of Proportions

The proportion of the subsample or end product method for the 4 pound samples was .863 and the proportion of the specimen or on-line product method was .890 showing a difference of .027 for 73 observations. Table 3 shown below summarizes the results and the statistical data used in the computations.

TABLE 3 (4 POUND)

N	X_1	X_2	$\hat{P}_1$	$\hat{P}_2$	$\hat{P}$
73	63	65	.863	.890	.877

STATISTICAL COMPARISON OF TWO PROPORTIONS

For the 3½ pound samples the proportion of the subsample or end product method was .9730 and the proportion of the specimen or on-line product method was 1.0000 showing a difference of .0270 for 37 observations. Table 4 shown below summarizes the results and the statistical data used in the computations.

TABLE 4 (3½ POUND)

N	X_1	X_2	$\hat{P}_1$	$\hat{P}_2$	$\hat{P}$
37	36	37	.9730	1.0000	.9865

STATISTICAL COMPARISON OF TWO PROPORTIONS

The Z score test was used to compare the two sample proportions of the percent stated net weight obtained by the two test methods. A Z score between -1.96 and +1.96 accepts the hypothesis that there is no difference at the 5% level of significance between the results of the two methods. A null hypothesis with an alternative hypothesis was established as follows:

$$H_0: \tilde{11}_1 = \tilde{11}_2$$

$$H_1: \tilde{11}_1 \neq \tilde{11}_1$$

Results were computed as follows:

$$P_1 = \frac{X_1}{N_1}, \quad \hat{P}_2 = \frac{X_2}{N_2} \quad \hat{P} = \frac{X_1+X_2}{N_1+N_2}$$

$$Z = \frac{(\hat{P}_1-\hat{P}_2) - (0)}{\sqrt{\frac{\hat{P}(1-\hat{P})}{N_1} + \frac{\hat{P}(1-\hat{P})}{N_2}}}$$

(4 Pound) $$Z = \frac{(.863 - .890) - (0)}{\sqrt{\frac{(.877)\ (.123)}{73} + \frac{(.877)\ (.123)}{73}}}$$

$$Z = -.500$$

(3½ Pound) $$Z = \frac{(.9730 - 1) - (0)}{\sqrt{\frac{(.9865)\ (.0135)}{37} + \frac{(.9865)\ (.0135)}{37}}}$$

$$Z = -1.01$$

Since Z falls in the acceptance range, (which for this test extends from -1.96 to +1.96), we accept the hypothesis that there is no difference at the 5% level of significance between the proportion of successes of the results of the two methods. The two methods gives statistically equivalent results for determining percent stated net weight of glazed split legs.

Table Number 5 and Number 6 summarizes the results between the subsample and the specimen methods.

TABLE 5 (4 POUND)

	Subsample	Specimen
Mean	92.9805	93.4810
Median	93.4571	93.8982
Mode	93.50 - 94.49	93.50 - 94.49 & 94.50 - 95.49
Range	13.8671	13.7596
Standard Deviation	2.79	2.74
Fraction Defective	.137	.110
Confidence Interval	UCL .4011, LCL - 1.4021 for the means	
Confidence Interval	UCL .0821, LCL - .1361 for the proportion	

TABLE 6 (3½ POUND)

	Subsample	Specimen
Mean	92.5847	93.1549
Median	92.7456	93.6889
Mode	93.50 - 94.49	93.50 - 94.49
Range	6.6959	6.3617
Standard Deviation	1.53	1.71
Fraction Defective	.027	-0-
Confidence Interval	UCL .1746, LCL - 1.3150 for the means	
Confidence Interval	UCL .0253, LCL - .0793 for the proportion	

CONCLUSIONS

Numerical results of method 1 for determining percent of stated net weight, by using a whole subsample, compared closely with numerical results of method 2, by using one specimen from each subsample.

The latter method which is less time consuming would be adequate for a processing plant during production where time is of primary concern in order to make adjustments so that the end product will conform to specific requirements or specifications and still be accurate enough for the product to meet those specifications for the final inspection.

Method 1 which is more time consuming would be adequate for final inspection (accept/reject) where time is of secondary importance and final decision upon acceptance or rejection of the lot is taken.

By using either method, excessive thawing of crabmeat split legs, while deglazing is kept to a minimum and the draining of the contents is performed with no difficulty.

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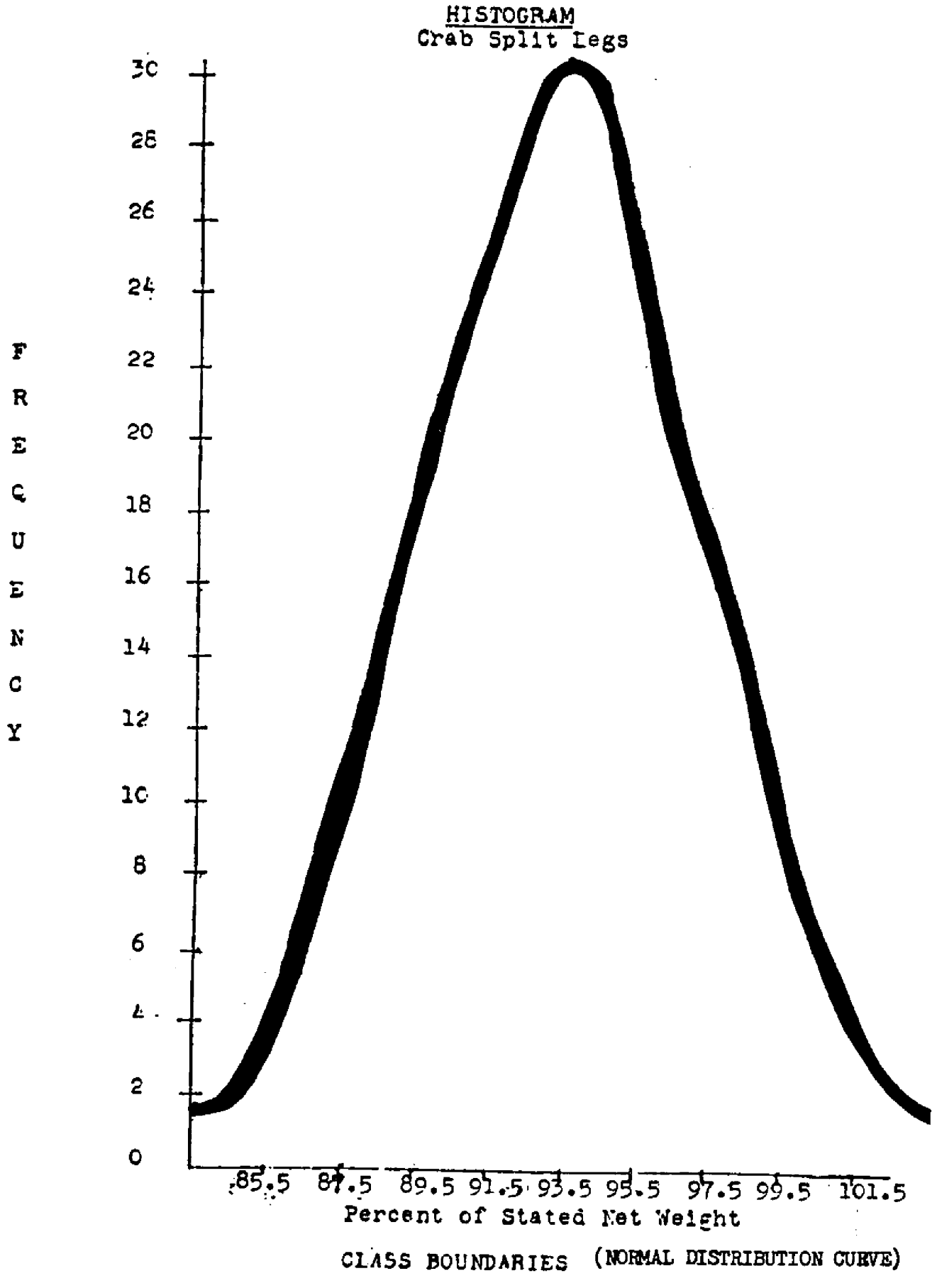
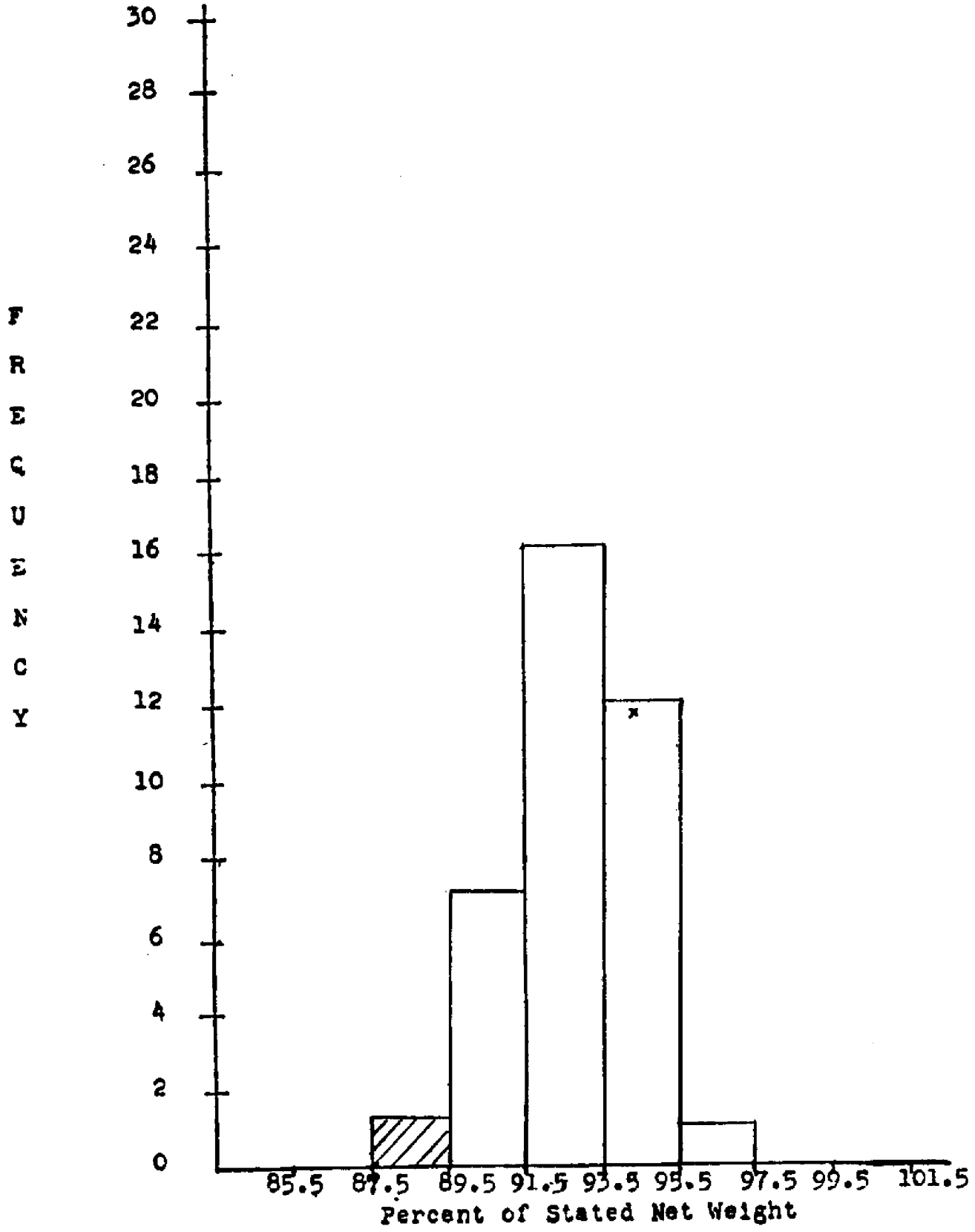


///// - less than 90% stated net weight

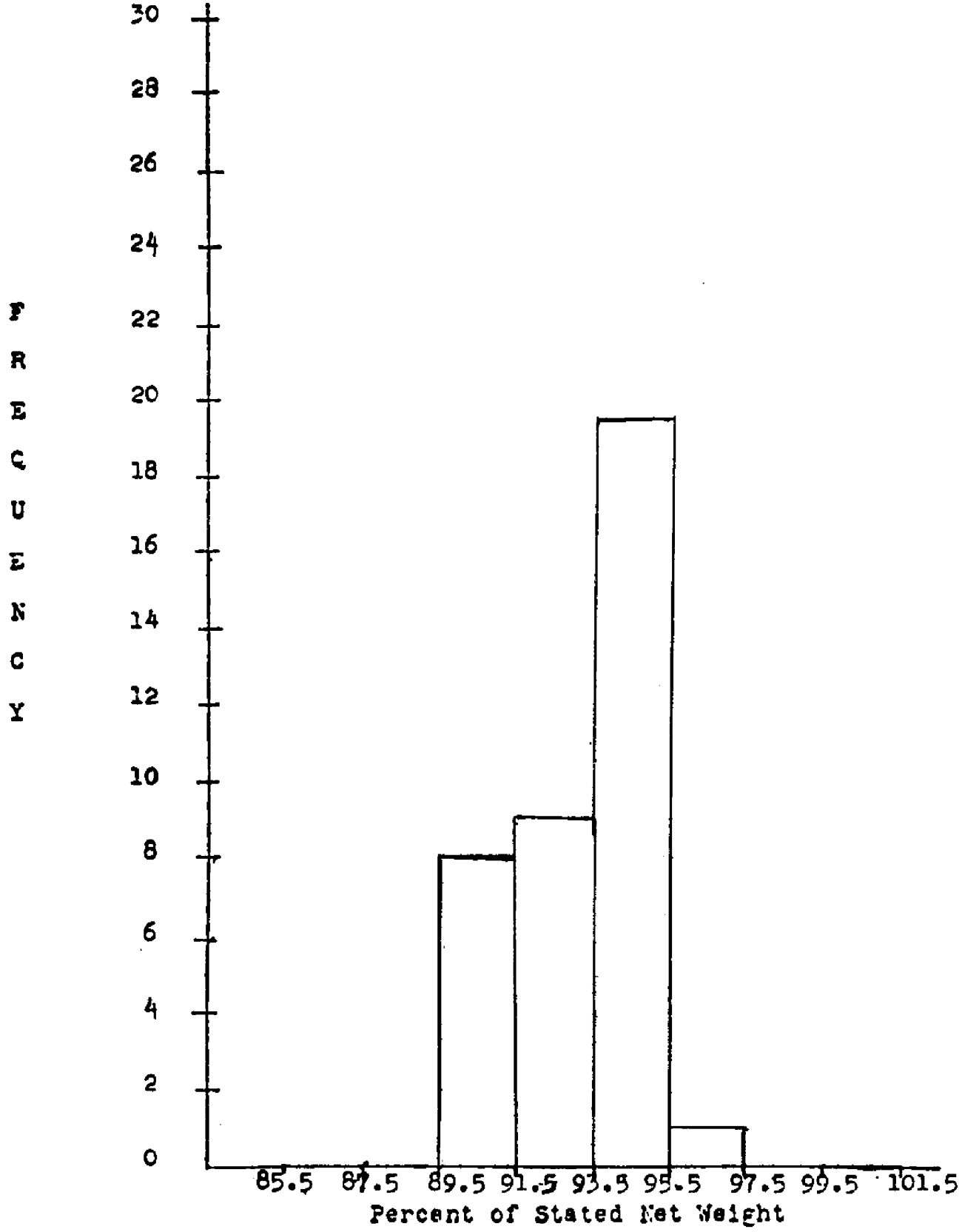
HISTOGRAM

Crab Split Legs (3½ LB)



///// - less than 90% stated net weight

HISTOGRAM
Crab Split Legs (3½ LB)

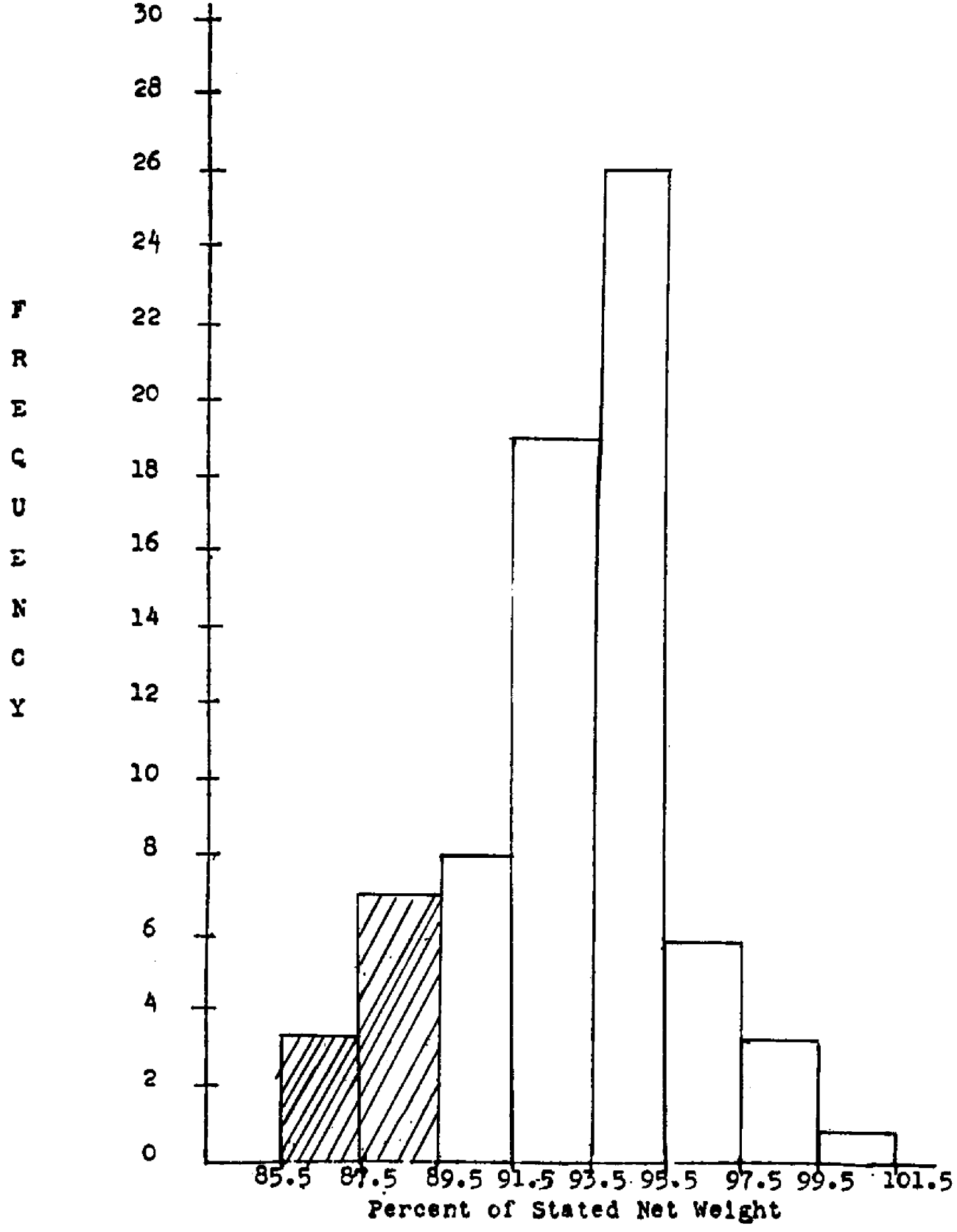


CLASS BOUNDARIES (SPECIMENS)

///// - less than 90% stated net weight

HISTOGRAM

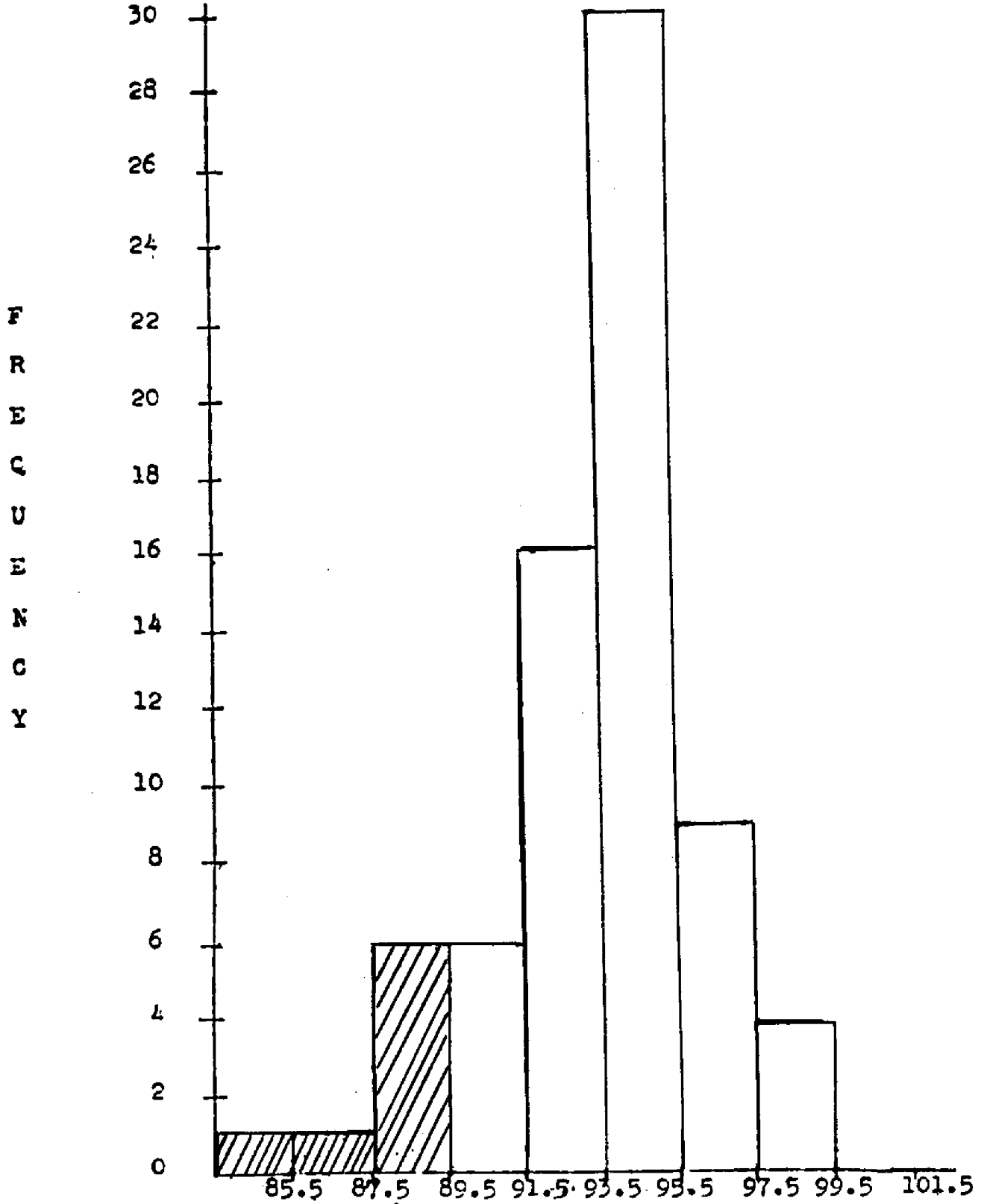
Crab Split Legs (4 LB)



CLASS BOUNDARIES (SUBSAMPLES)

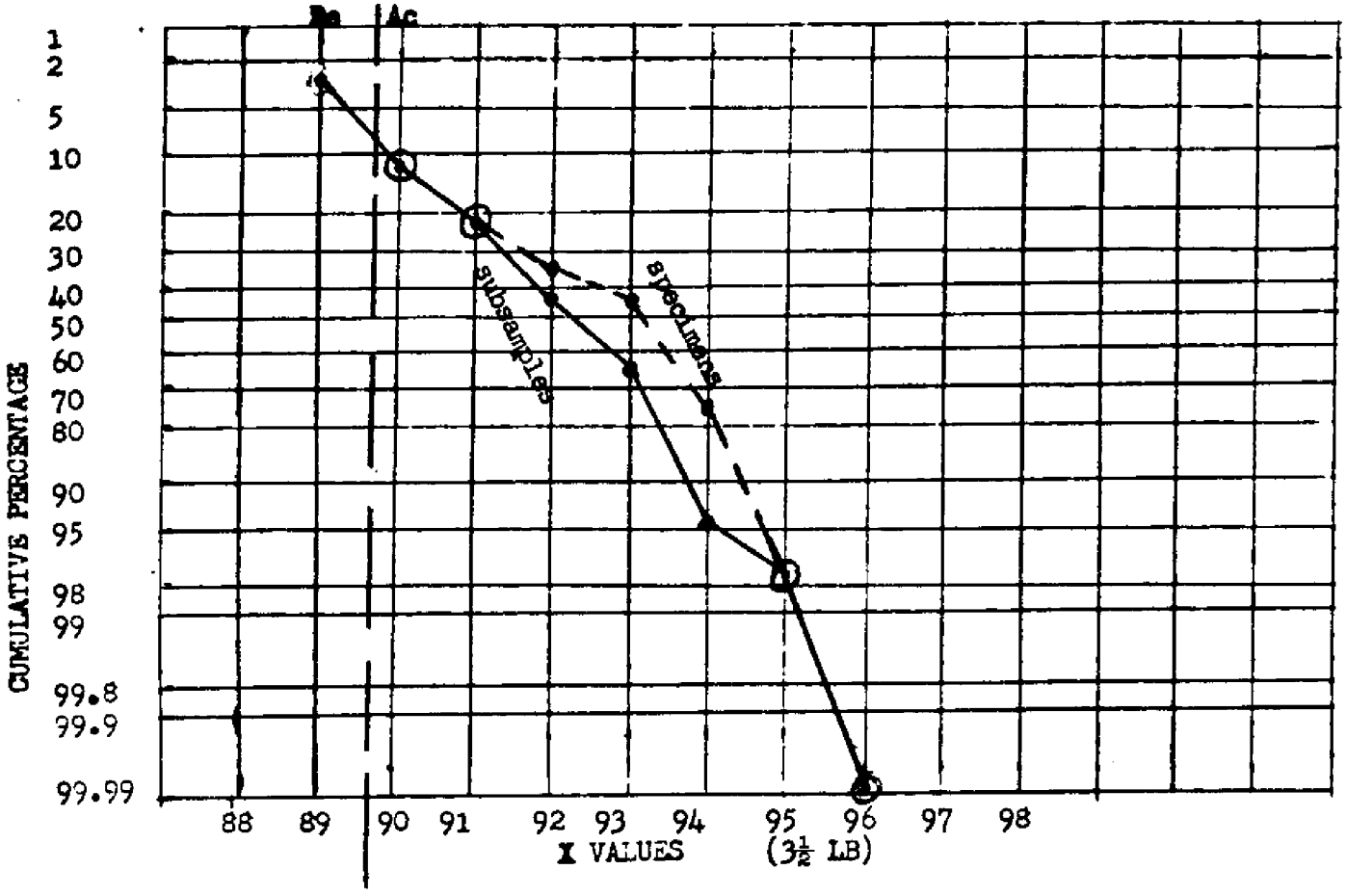
///// - less than 90% stated net weight

HISTOGRAM
Crab Split Legs (4 LB)



CLASS BOUNDARIES (SPECIMENS)

///// - less than 90% stated net weight


Re
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CUMULATIVE PERCENTAGE
subsamples
specimens
88
89
90
91
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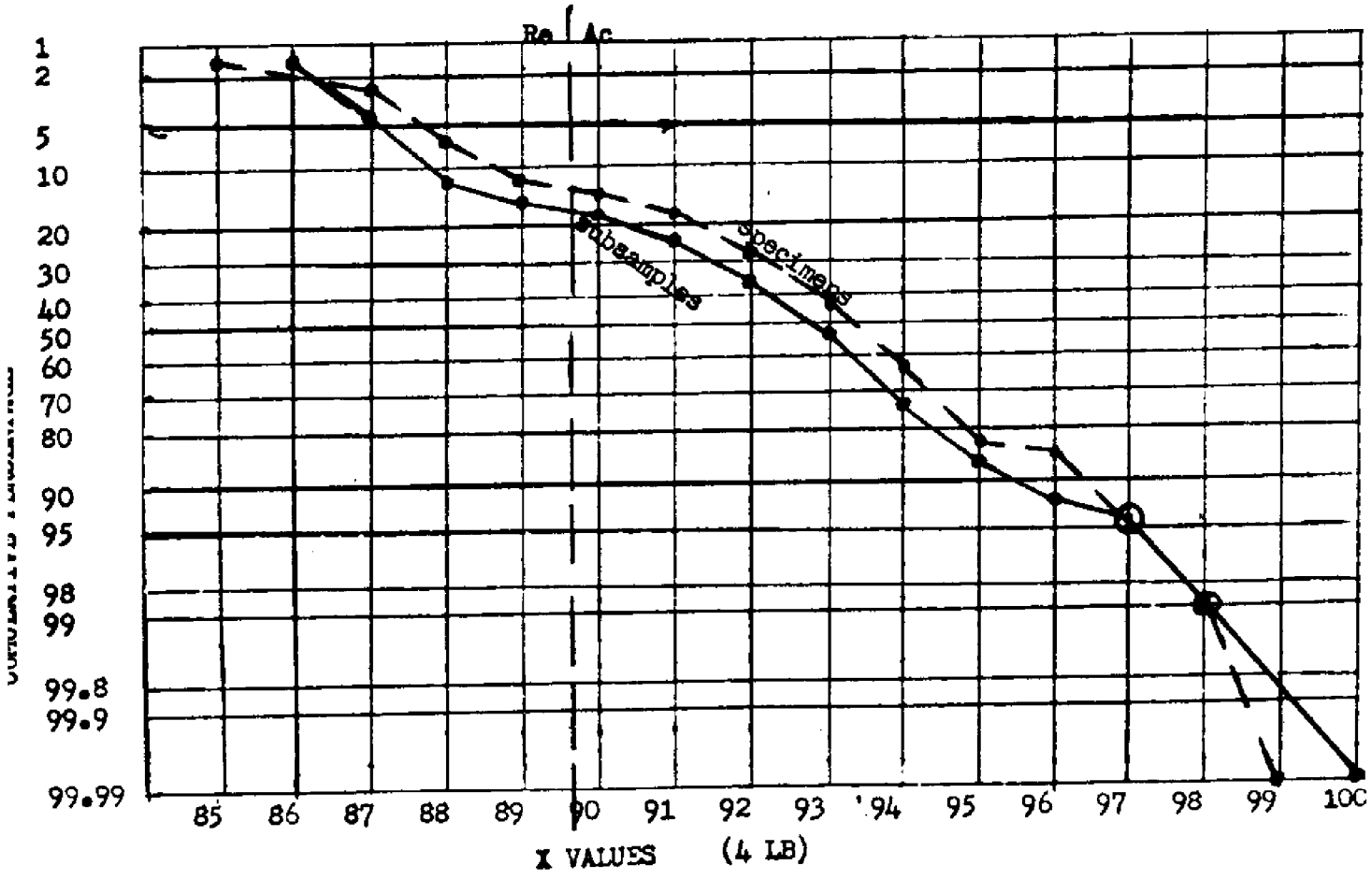
96
97
98
X VALUES
(3½ LB)

SPLIT LEGS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99.99
subsamples
specimens
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98
99
100
X VALUES
(4 LB)

ECONOMICAL FACTORS ON SNOW AND KING CRAB

PROCESSED WEIGHT 5,218 pounds -A LOT
RECOVERED WEIGHT 4,000 pounds -A DAY'S PRODUCTION(AVG)

SNOW CRAB		KING CRAB	
delivered price/lb	selling price/lb	delivered price	selling price
LOW $1.50	$2.80	$2.35	$2.90
HIGH $1.60	$3.00	$3.30	$3.53

PERCENT RECOVERY FROM CLUSTERS:
a) LOW 72.61% b) HIGH 80.35% c) AVERAGE 76.66%

LABOR COST PER POUND RECOVERED:
a) LOW .1220¢=$488.00 b) HIGH .1575¢=$630.00 c) AVERAGE .1435¢=$574.00

DIFFERENCES ON PACKAGING 90% vs 100% STATED NET WEIGHT

stated net weight	declared net weight	
percent	64 oz	56 oz
90	1,111 pack	1,269 pack
100	1,000 pack	1,142 pack
difference	111 pack	127 pack

PRICE DIFFERENCES

	SNOW CRAB	KING CRAB
	64 ounces	
90% LOW	$12,887.60	$12,443.20
100% LOW	$11,600.00	$11,200.00
difference	$ 1,287.60	$ 1,243.20
90% HIGH	$15,687.32	$13,332.00
100% HIGH	$14,120.00	$12,000.00
difference	$ 1,567.32	$ 1,332.00
	56 ounces	
90% LOW	$12,880.30	$12,436.20
100% LOW	$11,591.30	$11,191.60
difference	$ 1,289.05	$ 1,244.60
90% HIGH	$15,678.50	$13,324.50
100% HIGH	$14,109.41	$11,991.00
difference	$ 1,569.09	$ 1,333.50

PRELIMINARY REPORT ON THE USE OF A FLUORESCAMINE FLUORESCENT TECHNIQUE TO EVALUATE SHRIMP QUALITY CHANGES DURING STORAGE

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With a catch of approximately 477 million pounds and a dollar value of $358 million in 1978, the shrimping industry represents an important seafood industry in the United States (2). This seafood product, however, has a relatively high retail cost partially because of keeping quality problems related to bacterial growth and enzymatic degradation.

Considerable research effort has been directed towards the development of a simple reliable technique to assess the quality of shrimp. However, in most techniques, variation can result from such factors as age, size and species of shrimp, geographic area of catch, and handling conditions. Many of the presently available quality tests provide limited information about reactions occurring prior to spoilage. Moreover, lack of good correlation between subjective sensory quality and objective quality tests has been demonstrated (9,10).

Several methods for determining storage effects on non-protein nitrogeneous compounds in shrimp have been previously discussed in the literature. These techniques are of limited use for routine analysis because of their complexity, time consumption and inconsistencies. The sensitivity, rapidity and simplicity of a fluorescamine technique may make it more applicable to routine analyses. Fluorescamine was introduced as a novel reagent for the fluorometric quantitation of primary amines. It reacts with primary amines to form highly fluorescent pyriolinones. This reaction proceeds efficiently and very rapidly at room temperature and allows the assay of minute concentrations of amines (15).

The objective of this investigation is to compare a fluorescamine fluorescent technique to several traditional techniques (pH, aerobic plate count (APC), total volatile nitrogen/amino acid nitrogen (TVN/AA-N) ratio, trimethyl amine-nitrogen (TMN) and percent total extractable protein (% TEP)) for shrimp quality assessment.

MATERIALS AND METHODS

Samples: Approximately 25 lb of fresh Penaeus sp. shrimp were obtained from Mayport Beach, Florida, for each study conducted. The shrimp were transported on ice to the laboratory at Gainesville, Florida. After heading and washing, the shrimp were

divided into lots and placed in sterile Whirl-Pak® Bags (20 to a bag). Biochemical and microbial studies were conducted on different samples after 0, 4, 8, 12 and 16 days of storage at 5°C. All analyses were done in triplicate.

Microbiological Analyses: Standard Plate Count agar (SPC, Difco) with 0.5% NaCl added was used for total aerobic plate counts (APC) with incubation at 20°C for 5 days. The methods outlined in the Compendium of Methods for the Microbiological Examination of Foods (1) were followed.

Shrimp Extracts: Trichloroacetic acid (TCA) extracts were prepared by blending at least five shrimp at a ratio of 1 g of shrimp to 2 ml 7% TCA. Blending was in a Waring blender for 2 min at low speed. The mixture was then filtered (Whatman #4) and the filtrate stored at 5°C until used. For amino acid analyses, the filtrate was centrifuged after storage to remove additional precipitated protein.

Fluorescamine Technique: The Fluorescamine reagent used was 4-phenylspiro-(furan-2 (3H),1'-phthalan)-3,3-dione (Fluoram®, Roche Diagnostics, LaRoche, Inc., Nutley, NJ) according to Udenfriend et al. (15). An aliquot (10 to 250 µl) of the 7% TCA shrimp filtrate (adjusted to pH 8.0) was transferred to a test tube, and the total volume brought to 1.5 ml with 0.05 M sodium phosphate buffer, pH 8.0. While the tube was vigorously shaken, 0.5 ml of Fluoram dissolved in dioxane (30 mg/100 ml) was rapidly added to the buffered TCA filtrates. Fluorescence intensity (F) was determined with a Perkin Elmer 204-A Fluorescence Spectrophotometer at 390 and 490 nm excitation and fluorescence spectra, respectively (Figure 1). The amount of fluorescence is indicative of extent of proteolysis in the shrimp (or shrimp quality degradation) due to bacterial and/or endogenous enzymes.

Total Nitrogen and Trimethylamine: A modified Conway (6) microdiffusion dish technique (11) was utilized for analysis of total volatile nitrogen(TVN). The procedure outlined by Cobb et al. (3) was followed using Na_3PO_4 · KOH as releasing agent to prevent production of extraneous NH_3 during analyses. For trimethyl amine-nitrogen (TMN) analyses, 0.5 ml 40% formaldehyde (HCHO) was added to the sample prior to reaction with the releasing agent. Values were multiplied by 1.3 to correct for incomplete distillation (3,5). Results are expressed as mgN/100 g of shrimp.

Amino Acid Nitrogen: Amino acid nitrogen (AA-N) analysis was done by a modification of the copper procedure of Spies and Chamber (14) as modified by Cobb et al. (3). Solutions were prepared according to Pope and Stevens (13). Results are expressed as millimoles N/100 g shrimp (mmN/100 g shrimp).

pH: The pH was measured electrometrically using a Corning pH meter model 130. A homogenate containing 2 parts distilled water to 1 part shrimp tissue was used.

Total Extractable Protein: The percent extractable nitrogen attributable to protein was calculated by subtracting the percent non-protein nitrogen (NPN), determined according to Kahn (8), from the percent total extractable nitrogen, determined by a modification of the method described by Dyer (7). (The percent total extractable protein (%TEP) was calculated by multiplying percent total extractable protein nitrogen (wet basis) by a factor of 6.061).

Statistical Analyses: The data was analyzed by computer using the Statistical Analysis System (SAS) program package for the two-way analyses of variance calculations, correlation coefficients and multiple regression analyses.

RESULTS AND DISCUSSION

A general increase in fluorescamine fluorescence, TVN/AA-N and TMN data was observed with increase storage at 5°C. These data trends were supported by an increase in aerobic plate count (APC) and a slight increase in pH. Increased storage time decreased % TEP data. A dramatic TEP decrease was observed with storage from 4 to 12 days at 5°C (Table 1).

Table 2 shows the statistical correlation between the fluorometric analyses, APC, pH, TMN, % TEP and TNV/AA-N ration of Peanaeus shrimp stored at 5°C for 16 days. The Fluorescence data were positively correlated ($\alpha = 0.05$ level) with APC, pH, TMN and were negatively correlated with % TEP data. A highly significant correlation ($\alpha = 0.01$ level) occurred between TVN/AA-N ratio and fluorescence data. In 1973, Cobb et al. (3) reported a high correlation between TNV/AA-N ratio and quality of shrimp. Later work (4) suggested that the TVN/AA-N ratio and the logarithm of bacterial count increase at approximately the same rate after the initial lag phase of bacterial growth and a ratio of 1.3 indicated a short shelf life of the shrimp. The highly significant correlation between the TVN/AA-N ratio and the fluorometric data as evidenced by this study suggests that an increase in fluorescence indicates a decrease in overall shrimp quality.

To further investigate the correlation between TVN/AA-N ratio and the fluorescamine fluorescent technique, the data were subjected to multiple regression analysis. Figure 2 shows the regression line and the regression coefficient ($r^2=0.996$) of the TVN/AA-N ratio and fluorescamine data. A TVN/AA-N ratio of 1.3 extrapolates to approximately 81 fluorescence intensity (Figure 2). Additional research will be required to determine if a fluorescence intensity of approximately 80 or above indicates shrimp of low shelf life. However, as illustrated in Table 1, a fluorescence intensity of approximately 80 and a TVN/AA-N ratio of 1.3 was achieved after 12 days of storage at 5°C. These data suggest that the fluorescamine technique may assess shrimp quality changes during storage as effectively as the TVN/AA-N ratio.

The fluorometric technique is advantageous to traditional methods in terms of simplicity and rapidity and appears to be comparable to traditional techniques in assessing changes in shrimp quality. Additional research is in progress to further demonstrate the use of the fluorescamine fluorescent technique in evaluating storage effects on shrimp quality. However, since fluorescent intensity seems to correlate with more traditional ways of expressing shrimp quality (APC, pH, TMN, % TEP, TVN/AA-N ratio), this method may be useful to the shrimp industry for the evaluation of raw shrimp quality.

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Storage Time[b]	F[c]	APC	pH	TMN	TEP	TVN/AA-N
0	25.9	6.76	7.20	0.070	5.70	0.51
4	49.0	7.85	7.50	0.140	5.25	0.83
8	61.0	8.90	7.82	0.187	3.30	1.07
12	78.0	9.08	7.93	0.240	2.18	1.32
16	127.3	9.90	8.08	0.271	1.72	2.05

[a]Data are means of nine observations

[b]5°C for 16 days

[c]Fluorescamine reaction of 7.0% TCA filtrates

Table 1. Changes in fluorescence (F), aerobic plate count (APC), pH, tri-methyl amine nitrogen (TMN), percent total extractable protein (TEP) and total volatile nitrogen/amino acid nitrogen (TVN/AA-N) ratio of Penaeus shrimp stored at 5°C for 16 days[a].

	Storage Time[a]	F	APC	pH	TMN	TEP	TVN/AA-N
Storage Time	1.00						
F	0.96*	1.00					
APC	0.98*	0.92*	1.00				
pH	0.98*	0.90*	0.99**	1.00			
TMN	0.99*	0.92*	0.98*	0.99*	1.00		
TEP	-0.98*	-0.88*	-0.96*	-0.97*	-0.97*	1.00	
TVN/AA-N	0.97*	0.99**	0.94*	0.91*	0.94*	-0.91*	1.00

*Significant at the $\alpha = 0.05$ level

**Significant at the $\alpha = 0.01$ level

[a]5°C for 16 days

Table 2. Statistical correlation between fluorometric analyses (F), aerobic plate count (APC), pH, tri-methyl amine nitrogen (TMN), % total extractable protein (TEP) and total volatile nitrogen/ amino acid nitrogen (TVN/AA-N) ratio of Penaeus shrimp stored at 5°C for 16 days.

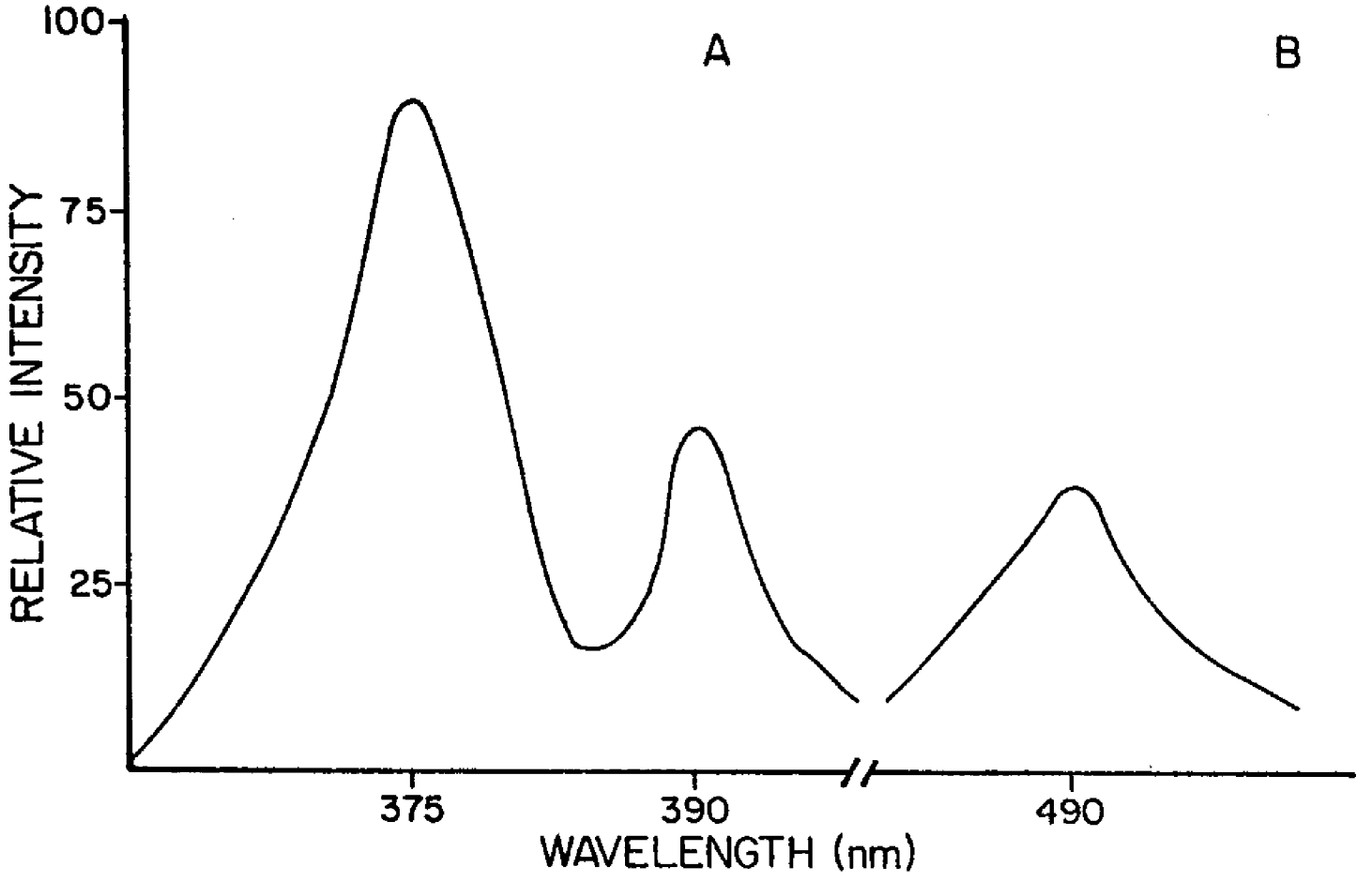

FIGURE 1: EXCITATION (CURVE A) AND FLUORESCENCE (CURVE B) SPECTRA FOR SHRIMP SAMPLES (pH 8)

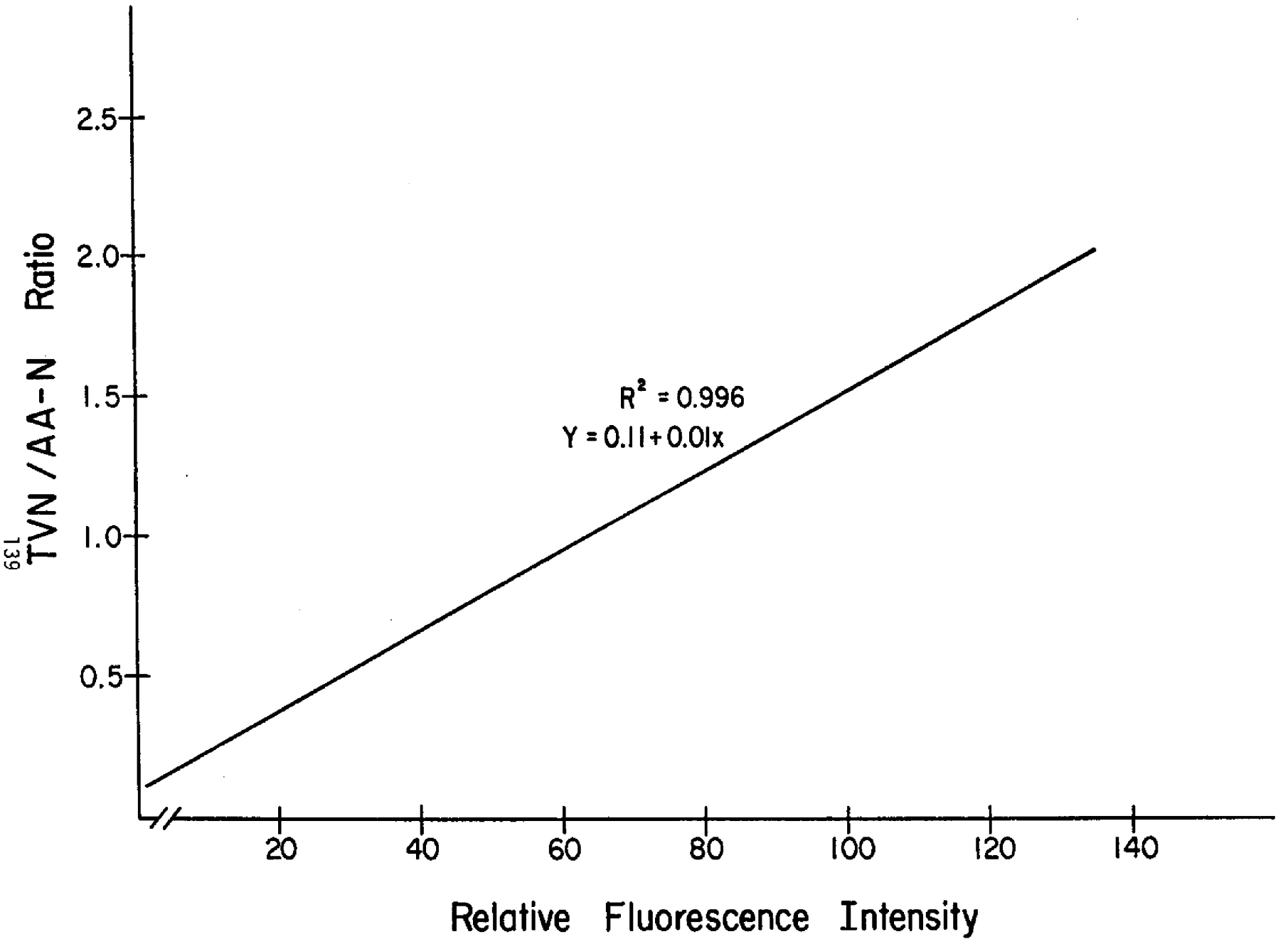

FIGURE 2: RELATIONSHIP BETWEEN TOTAL VOLATILE NITROGEN/AMINO ACID-NITROGEN (TVN/AA-N) RATIO AND THE FLUORESCAMINE FLUORESCENT TECHNIQUE

PROCESSING FOR BLUE CRAB (*callinectes sapidus*) MEAT STABILITY IN STORAGE: ABSENCE OF PEROXIDASE ENZYME ACTIVITY AND POSSIBLE ROLE OF ACID-SOLUBLE NITROGEN CONSTITUENTS

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INTRODUCTION

In the seafood industry of South Carolina, the blue crab sector represents a substantial economic value when both fresh and processed products are considered. In 1978, 9.4 billion pounds of blue crabs were landed, representing a total value of approximately $1.8 billion (commodity value). Approximate dollar value of retail products (which includes processing) was near $3.4 billion. The economic value of the total industry to the state would be about double the $3.4 billion figure because of the sale of boats, fuel, and other gear.

Due to the short usable shelf life of fresh, picked crab meat, a considerable portion is processed to the pasteurized product. This extends the shelf life from 7-10 days in the fresh product, to approximately 6 months in the pasteurized, canned product.

In 1977, Burnette and Flick (1) suggested that the presence, activity, and heat resistance of the peroxidase enzyme in crab meat tissue might be related to off-flavor development in pasteurized and cooked crab meat, following prolonged storage. They compared three common peroxidase assays for detecting peroxidase activity in extracts of crab tissue. In 1978, they (2) applied the most sensitive method, a modified Nagle and Haard (4) procedure, to follow the thermal inactivation of peroxidase using raw backfin extracts. When tested on pasteurized crab meat, prepared following commercial times and temperatures, an almost 100% regeneration of peroxidase activity was found, two weeks after refrigerated storage.

We were initially interested in examining other time-temperature combinations for pasteurization which could also be used to achieve pasteurization and peroxidase inactivation. However, we were never able to detect or to measure peroxidase activity or presence in crab tissue extracts in any of our attempts, as the title of this presentation indicates.

MATERIALS AND METHODS

For our studies, the backfin tissue, which is commercially known as "lump meat", was removed from blue crabs at three stages:

i. live crabs as received at the dock (in some cases, live crabs were transported to Clemson for study);

ii. steamed crabs which were in the chill room about 4 to 6 hours after receiving the steam treatment; and

iii. commercially pasteurized lump meat which was stored under refrigeration anywhere from a few days to eleven months.

On two occasions, a single delivery of crabs from one trapper was tagged and followed through the plant. Thus, in replicate, all tissues came from crabs of that particular trapper's territory. Other crabs or products came from random selection. Tissue for extraction was generally a composite mixed sample from 6 to 8 crabs.

The peroxidase assay was that reported by Burnette and Flick (2), using 0.01M phosphate buffer at pH 6.0, H_2O_2 as substrate, and o-dianisidine as the hydrogen donor. The crab meat extraction procedure of Burnette and Flick (1) was used in initial extractions without success. We were never able to obtain a filtrate consistently.

For this study, the extraction method of Haard (3) was utilized, which is a two-step extraction procedure, yielding "soluble" and "bound" crude fractions of peroxidase. Modifications of the extraction method were:

i. using a lower G force; and

ii. omitting the casein dispersion which acts as a tannin scavenger for plant materials.

RESULTS AND DISCUSSION

For crab tissue extracts, no evidence of peroxidase activity was detected by any extraction procedure utilized.

To test our modified Haard (3) extraction procedure, bananas (peroxidase present) of yellow peel color were extracted and the peroxidase activity compared to Haard's reported results. We also compared Burnette and Flick's (1) extraction procedure using the banana tissue.

The results (Table 1) were compatible with those reported initially by Haard, when expressed in the same manner as in the original reference (O.D./min of entire extract).

With no results from crab tissue extracts, we examined the possibility of an endogenous inhibitor in crab tissue. Backfin tissue was spiked with horseradish peroxidase and held refrigerated overnight. We then followed the two-step extraction procedure.

Results from three samples reported in Table 2 were obtained from:

i. a standard solution of horseradish peroxidase at 5×10^{-5}mg in the assay mixture (of 3 ml);

ii. crab meat control sample extracted for soluble and bound fractions; and

iii. a spiked crab meat sample, where the "expected" concentration on extraction is also 5×10^{-5}mg of horseradish peroxidase.

No activity was found in the control crab meat samples.

The activity in the soluble fraction of the spiked crab meat had 91.5% of the standard, and the bound fraction had 6.8% of the standard's activity. Thus, 98% of the "expected" activity was found, indicating that other components of the crab tissue do not inhibit the enzyme's function.

We then concentrated the soluble and bound fraction extracts from fresh raw tissue for use as antigenic material in Ouchterlony's gel immunodiffusion technique (Table 3). An anti-peroxidase antibody was obtained from Miles Laboratories. (The antibody was produced in the goat).

In the antigen wells, 0.2 ml samples were placed, with 0.1 ml of phosphate buffer, and 0.1 ml of peroxidase antibody was placed in the center well.

Protein concentrations (mg/ml) for the soluble and bound fractions of the crab tissue were the maximum concentrations tested, at which no precipitin reaction was noted. (Ca++ ions were dialyzed from the bound fraction).

For the horseradish peroxidase standard, 25 ug/ml was the lowest concentration used which still yielded a detectable, faint precipitin band.

The soluble extract of yellow peel banana, concentration unknown, also yielded a positive reaction.

To summarize relative to the peroxidase activity testing at this point:

i. Our laboratory has not been able to find evidence of peroxidase activity in crude extracts of crab meat tissue using the described extraction and assay procedures;

ii. We found no evidence of inhibition of a standard peroxidase preparation when incubated with and recovered from crab meat;

iii. We found no evidence of peroxidase in the crude soluble or bound fraction extracts of crab tissue when tested by a gel immunodiffusion procedure.

If peroxidase cannot positively be established in relation to quality changes that occur on storage, what factor(s) might be eventually correlated to quality?

Preliminary results (Table 4) from our laboratory point to shifts in the acid soluble (perchloric acid) nitrogen tissue constituents as having a possible role in explaining deteriorative changes in the flavor of pasteurized crab meat during long term refrigerated storage. There is a significant decrease in the total acid-soluble N constituents and the alpha-amino nitrogen (free amino acids and peptides) fraction between 7 and 11 months of storage at 32-34°F. Following the same pattern, there is a significant increase in the free ammonia content between 7 and 11 months of storage. The inosine content appears fairly constant, whereas the hypoxanthine content numerically declines in value but was so variable, there was not a significant change.

The whole pattern makes logical sense, in that the NH_3 increase is derived from the degradation of adenosine monophosphate (AMP) and adenosine, an enzymatic deamination. The total soluble fraction increase can be almost accounted for by the NH_3 and α-amino nitrogen fractions -- both derived from enzymatic activities. This means that the microbial population, after long term storage, has probably begun to impact on the quality of the product. We are now just beginning to examine these activities in relation to the findings of Table 4.

ACKNOWLEDGMENTS

The authors wish to express their appreciation to the National Fisheries Institute for financial assistance with this study.

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TABLE 1. Peroxidase activity of yellow peel banana of several extraction methods

Peroxidase fraction	ΔO.D. 420 nm/min of Entire Extract		
	Haard (1973)	Present Study	Burnette & Flick (1977)
Soluble	17	16	21
Bound	14	11	--

TABLE 2. Peroxidase activity of crab tissue spiked with standard horseradish peroxidase (HRP), compared to standard activity and control tissue.

	ΔO.D. 460nm/min	
	Soluble Fraction	Bound Fraction
Standard Solution[a]	0.59	--
Crab Tissue-control	0.00	0.00
Crab Tissue-spiked[b]	0.54	0.04

[a]Based on 5×10^{-5} mg HRP in assay.

[b]"Expected" extraction of 5×10^{-5} mg HRP for assay.

TABLE 3. Evidence of Precipitin formation in testing of extracts against anti-peroxidase antibody: 2.1 mg/ml of antibody.

Antigenic Test Substance	mg Protein/ ml	Precipitin Reaction
Buffer Control		--
Crab Tissue-Soluble Extract	6.62	--
Crab Tissue-Bound Extract	1.99	--
HRP Standard	0.025	+
Banana-Soluble	--	+

TABLE 4. Distribution of acid-soluble nitrogen constituents from pasteurized, canned crab backfin (lump) meat.

Months of Storage	Fraction mg N/100 g wet tissue				
	Total Soluble	α-NH_2	NH_3	Inosine	Hypoxanthine
One	490[a]	3.4[a]	42.5[a]	179.7[a]	78.4[a]
Seven	440[a]	3.8[a]	47.1[ab]	194.7[a]	51.6[a]
Eleven	630[b]	7.4[b]	64.3[b]	186.2[a]	47.7[a]

[ab]Any two column means having the same letter are not significantly different (P>0.05).

A PRELIMINARY STUDY OF THE EDIBILITY CHARACTERISTICS OF SOUTHEASTERN FINFISH

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A major problem in the marketing of seafood products is the large number of fish species with widely varying edibility characteristics. To better disseminate information on foodfish to consumers, therefore, Brand Group Inc. (under NMFS contract) developed a Model Identification Plan (2) for finfish relative to eight sensory attributes: odor, flakiness, coarseness, flavor, moisture, fat, firmness and color. This system was designed to inform the consumer about a fish species in regard to its edibility characteristics. The less-familiar species would be better incorporated into the marketplace through descriptions of edibility which could be compared with characteristics of more familiar species. We have attempted to evaluate the usefulness of the Brand Group system and to apply it to familiar and unfamiliar species of the Southeast region.

In this study, a previously trained taste panel evaluated the edibility characteristics of 16 different species of local (South Carolina) fresh finfish. Major objectives included: (i) determination of the precision and reproducibility of taste panel evaluation methods for each characteristic; (ii) determination of which of the eight proposed characteristics were relevant for our use; and (iii) determination of a grouping system for the species tested.

MATERIALS AND METHODS

Taste panel

Thirteen employees of the Charleston Laboratory were selected as panelists and trained for sensory evaluations. Each panelist was briefed by the panel leader as to the general nature of the study and the panelists were trained in order to familiarize them with test procedures and to improve their ability to identify and compare sensory attributes. Methods, scales, score sheets, and terminology to be used in the tests were discussed; training was through individual and group sessions in which various samples of products were evaluated and discussed. Principles of quantitative descriptive analysis as described by Stone, et al. (3) were employed.

Sample preparation

Samples, purchased locally, were examined for freshness. Sample selection was based on the availability of fresh local finfish at the time of the tests and many of the samples were reef-fish from the snapper-grouper fishery. The less familiar species were identified by an experienced marine biologist.

Two skinless fillets from each fish were placed skinned sides together in seal-a-meal boiling bags to be weighed, coded and refrigerated for at least 1-1/2 hours. Thermocouples were placed between pairs of unseasoned fillets and the open end of the bag was folded and clamped around the thermocouple lead. Temperatures were recorded before and during cooking using a Honeywell multipoint recorder. The bag was immersed in boiling water until the internal temperature reached 160^{o}F. The bag was removed and drained of free liquid and the samples were divided into approximately 1 oz. portions and placed into 4 oz. styrofoam cups and sealed with coded lids.

Sample presentation

The samples were given to panelists seated at booths in our special palatibility room. They were asked to evaluate each sample according to the guidelines posted in each booth.

Two characteristics described by Brand Group were eliminated from our panel evaluation form -- coarseness and fat content. It was found during panel training that coarseness was a difficult concept for the taste panel to relate to finfish and that fat content, which can be measured chemically, was not a clearly percieved attribute. Therefore, these two characteristics were eliminated from the sensory evaluation form.

All evaluations were made on the cooked flesh (the Brand Group Plan recommends a raw-odor evaluation). It was assumed that a consumers' evaluation of raw fish flesh would be misleading at a fish market where a mixture of strong odors is prevalent.

The edibility characteristics were rated by marking the appropriate boxes numbered 1 through 5 (integers only) on the basis of mild to strong odor, light to dark color, not flaky to flaky, soft to firm, mild to strong flavor and dry to very moist.

Statistical analyses

The Wilcoxon signed rank test and Kendall's correlation coefficient were used to statistically assess the edibility characteristics (3). Non-parametric statistics were used because the characteristic evaluations were discrete numbers and the data cannot be assumed to follow a normal distribution. The BMDP (1) cluster analysis program and a multidimensional scaling (MDS) program were also used to group the different species on the basis of similar edibility characteristics.

RESULTS AND DISCUSSION

Panel reliability

For a majority of the species reported in this paper, only one evaluation was made by the thirteen taste panelists. For red porgy (Pagrus sedecim) there were seven different taste tests and for black sea bass (Centropristis striata) five tests. The data were segregated into three groups: (i) sea bass tests, (ii) red porgy tests and (iii) the data for all other tests with the remaining 14 species. The standard deviation of each variable of each group was evaluated for each taste panelist. The median standard deviations for each characteristic for groups I and II were compared with those of group III using the Wilcoxon signed rank test of the BMDP3S non-parametric statistics package (1). The statistical results as tabulated in Table 1 show that the panelists were discriminating in their ratings. When $P \leq 0.05$ we accept the fact that the taste panel can discriminate one species from the others for that edibility characteristic. Only the odor evaluations and the flakiness evaluation for red porgy resulted in P greater than 0.05. In general, the P values are quite low.

Correlations between characteristics

The mean values of the six edibility characteristics- odor, color, flakiness, firmness, flavor and moisture were evaluated for the 16 species of local finfish. Sensory characteristic profiles for four of the species are shown in Figure 1. The profiles are drawn as connected lines for better visual comprehension; no serial relationship is intended. The red porgy and vermilion snapper (Rhomboplites aurorubens) have similar profiles except for the lighter color of the red porgy. The longspine squirrelfish (Holocentrus rufus) was firm but scored low in flakiness. The American shad (Alosa sapidissima) shows a dark color and a soft texture (low firmness). The Kendall's correlation coefficients between the means of the six variables for all species were calculated and are shown in Table 2. These correlation coefficients were used to test if the variables were independent. At the 0.05 level of significance, the independent hypothesis is rejected if the absolute value of the correlation coefficient is larger than 0.34375. Based on this test, odor, color. flakiness and flavor failed to be independent from each other. Firmness and moisture were (negatively) correlated with each other but no other pairs of variables failed the test for independence.

Grouping of species

The mean values for each species were standardized such that each variable has a mean of zero and a standard deviation of one. The Euclidean distances between species, calculated by the standardized means of the six variables (dimensions), were analyzed with a cluster analysis computer program to determine logical groupings of the species (Figure 2). Dissimilarities between groups of fish are indicated by the distance at which they are joined. Red porgy and vermilion snapper form the first similar group at a distance of 1.13 units.

Grouping of species by the results of cluster analysis is based upon the analyst's judgment. Based on our judgment, the fish were divided into four groups. Group 1 consists of knobbed porgy, gag, vermilion snapper, red porgy, gray triggerfish, short bigeye, scamp, black sea bass and blackfin snapper. Group 2 consists of American shad, blue angelfish, black drum and white grunt. Group 3 consists of longspine porgy and longspine squirrelfish. Group 4 consists solely of red drum, but this species should be eliminated since the sample had been frozen and it was a large fish. Smaller red drum have been reported to have much better edibility characteristics and the smaller size would be more likely encountered commercially.

A multidimensional scaling (MDS) program developed by Bell Laboratories was used to measure the dissimilarities between the 16 species. Based on the standardized means the procedure provides each species with a point in an Euclidean space. We used MDS to study our dissimilarity data with 1-, 2-, 3- and 4-dimensional spaces. Figure 3 is the final configuration plot of the 16 points on the two-dimensional space. Each letter on the MDS plot represents a species. If two letters are relatively close together, then the corresponding species are relatively similar.

Based on our study of the two-dimensional MDS plot, and with some considerations of the other dimensions, we divided the fish into four groups. Comparing these groups with the previous grouping based on cluster analysis, we find that the present group 1 contains black drum and white grunt which previously were members of group 2.

CONCLUSION

There was a relatively large variability in panel ratings of odor and some difficulty with flakiness for the repeated samples of red porgy but valid differences in profiles between species were defined. Odor was eliminated as a characteristic for future work because of its variability and negative character. Grouping of the species on the basis of edibility characteristics was accomplished through computer programs. A majority of the species fell into one group with desirable edibility characteristics (mild flavor, light color, moderate firmness and flakiness).

Although there is inherent variability in taste panel results and variations within some fish species due to size and season, the concept of evaluating and recording edibility characteristics promises to benefit consumers of seafoods and to improve the marketability of underutilized species.

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	GROUP I Sea Bass	GROUP II Red Porgy
Odor	0.068	0.079
Color	0.008	0.017
Flakiness	0.006	0.136
Firmness	0.014	0.003
Flavor	0.002	0.003
Moisture	0.021	0.042

Table 1. P- Values from Wilcoxon signed rank test for differences between medians of standard deviations. Groups I and II versus Group III consisting of all other species.

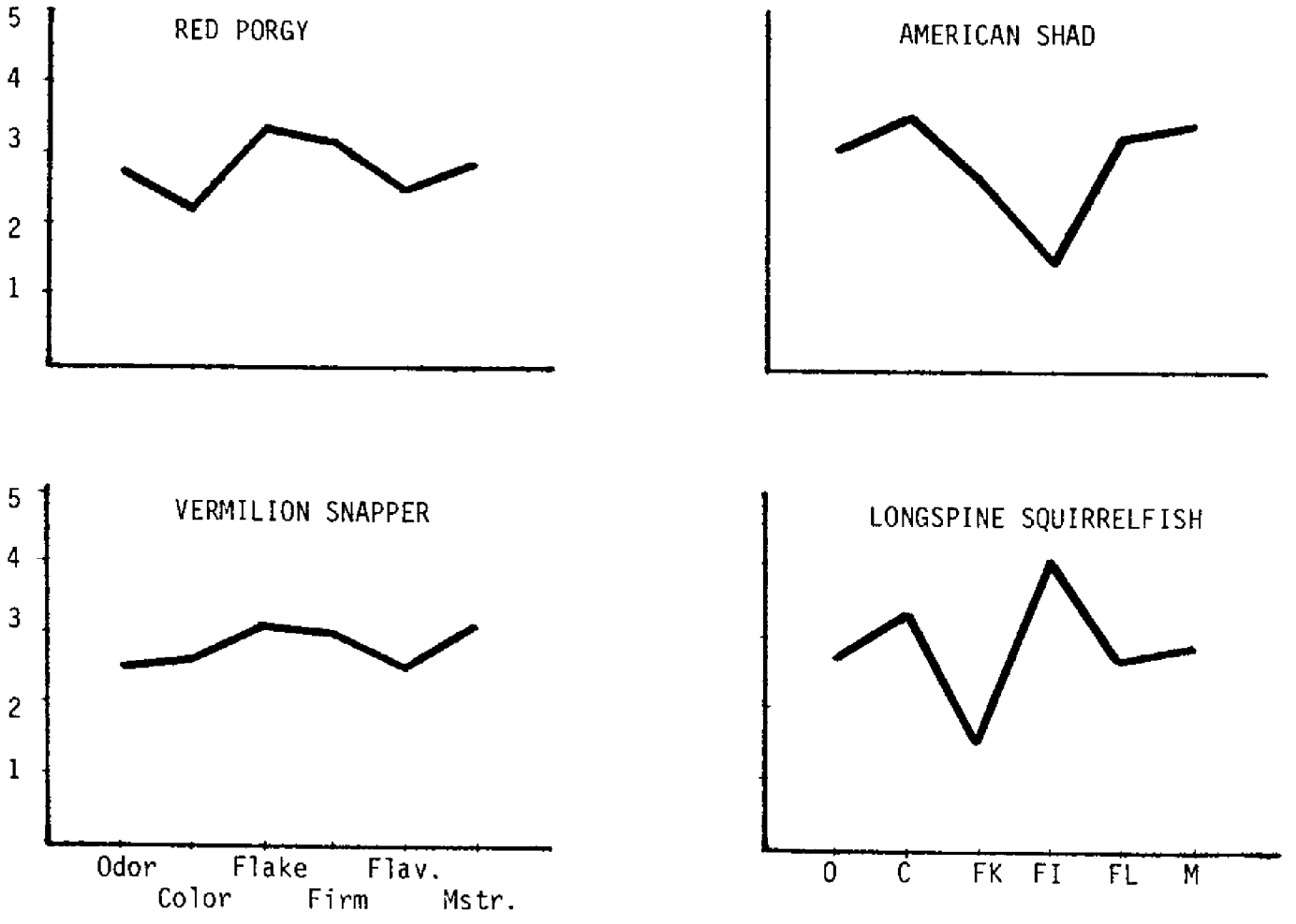


Figure 1. Edibility profiles for selected species.

	Odor	Color	Flakiness	Firmness	Flavor	Moisture
Odor	1					
Color	0.4603	1				
Flakiness	-0.3933	-0.4874	1			
Firmness	0.1097	0.3390	-0.1186	1		
Flavor	0.5378	0.5317	-0.5317	0.1277	1	
Moisture	0.1667	-0.251	-0.0921	-0.3798	0.1849	1

Table 2. Kendall's Correlation Coefficients Determined by the Mean Values.

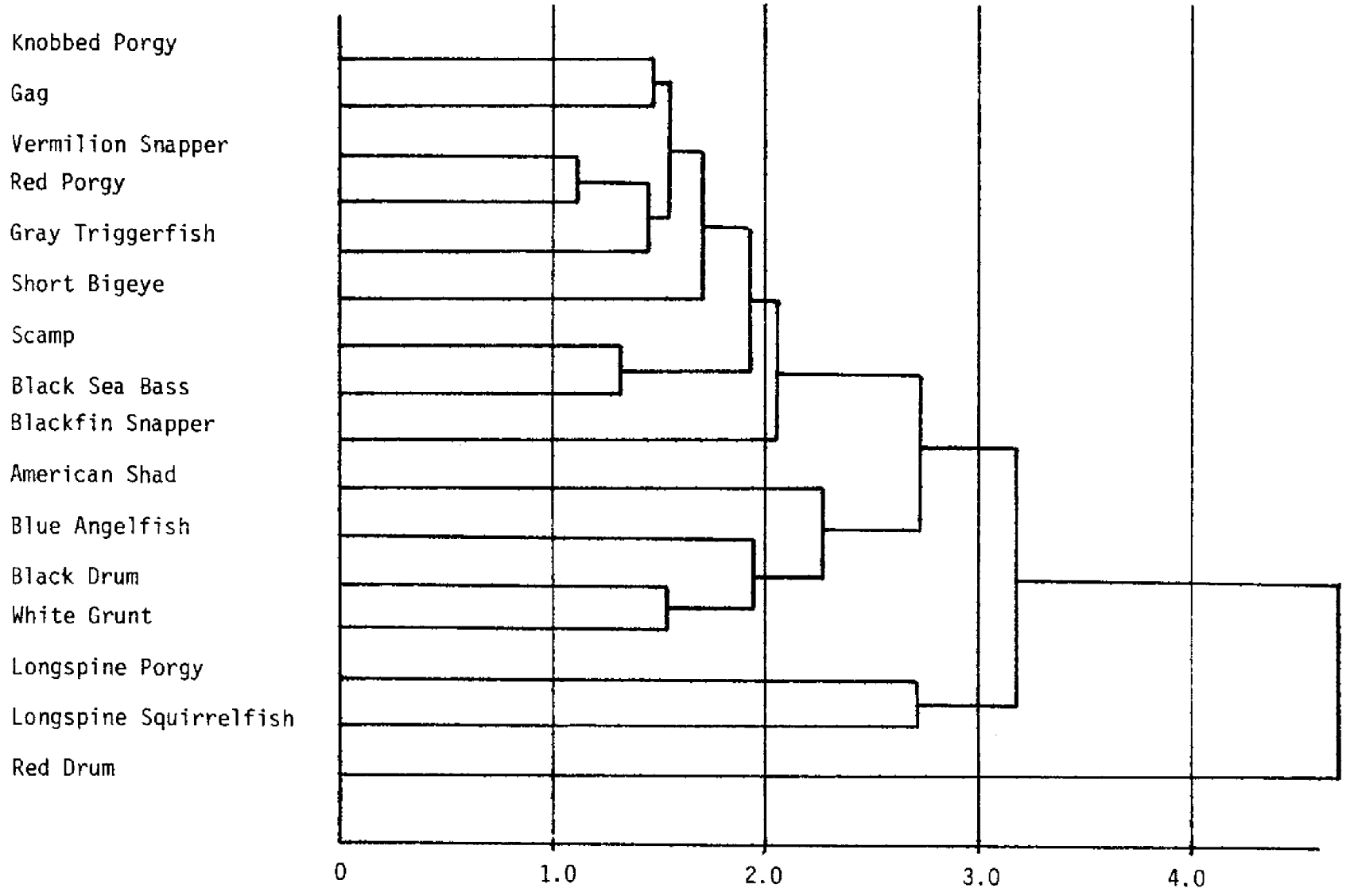
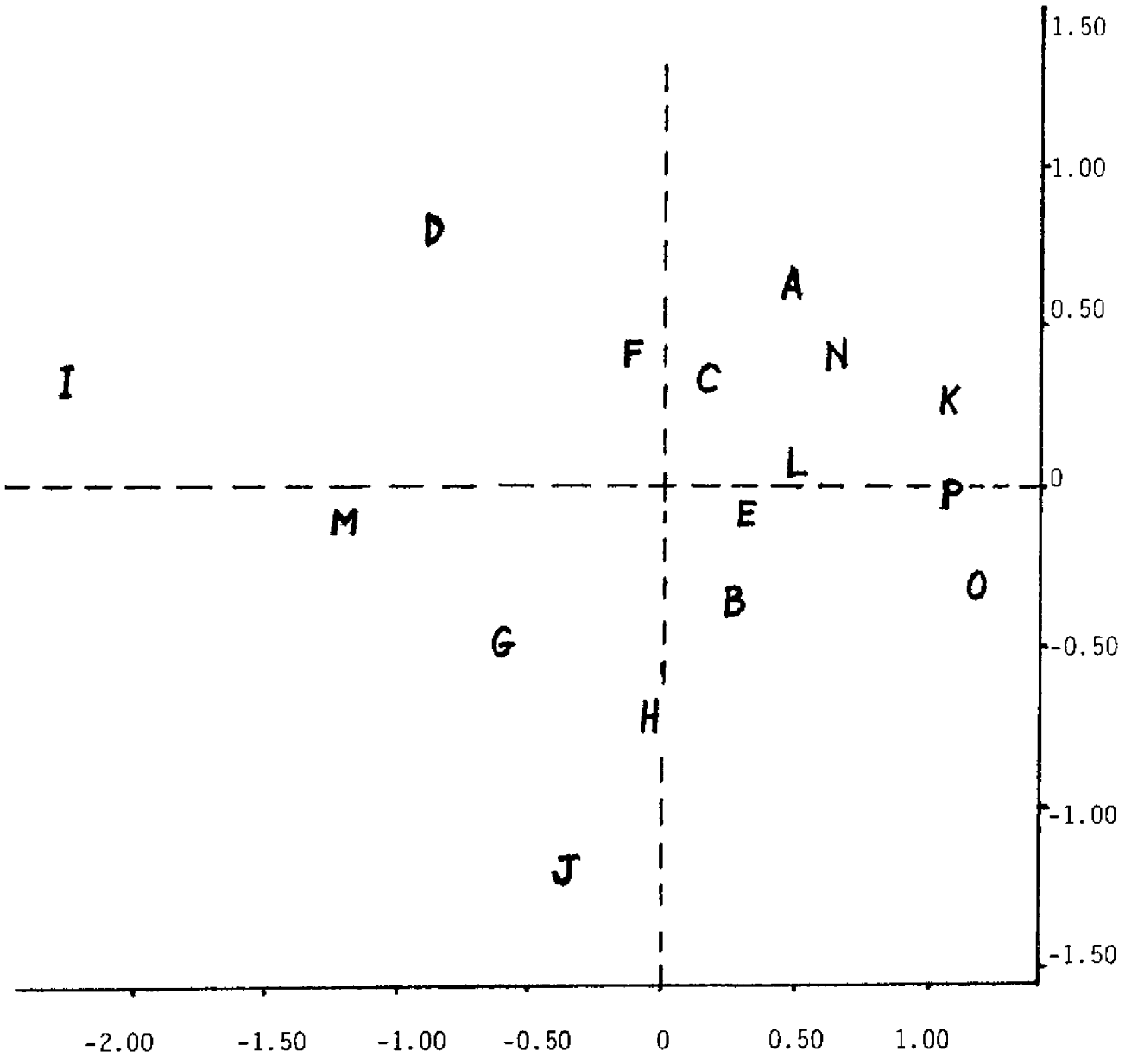


Figure 2. Cluster Analysis of 16 Southeastern Species.



A - Knobbed Porgy
B - White Grunt
C - Gray Triggerfish
D - Long Spine Squirrelfish
E - Vermilion Snapper
F - Short Bigeye
G - Blue Angelfish
H - Black Drum
I - Red Drum
J - American Shad
K - Black Sea Bass
L - Red Porgy
M - Longspine Porgy
N - Gag
O - Scamp
P - Blackfin Snapper

Figure 3. Grouping of Fish Species by Multidimensional Scaling (MDS) - 2 - Dimensional Plot.

BRINE FREEZING SHRIMP

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One of the biggest quality problems in the shrimp industry is the production of a uniformly high quality product. This is due, in part, to the methods used to hold the shrimp while aboard the vessel. The more traditional method utilizes ice to maintain the freshness of the shrimp. The shrimp are caught, headed on deck and mixed with crushed ice in large bins below deck. The quality of the shrimp when they are finally unloaded varies greatly depending on handling, amount of ice and time of storage.

A more recent method to preserve the freshness is to freeze the shrimp on board the vessel. Refrigerated tanks hold a brine solution and the headed shrimp are placed in 40 to 50 pound open meshed sacks and dipped into the brine until they are frozen. Then they are placed in freezers and held in a frozen state until they are unloaded, thawed and graded at the dock. Freezing is considered the best method to preserve the quality of fresh shrimp because it inhibits or greatly slows down enzymatic reactions and bacterial deterioration. (Anonymous, 1977).

There are several advantages to the brine freezing method over the ice boat method. A great economic advantage is that freezer boats can stay out in the gulf longer because they do not worry about their ice melting and their product deteriorating; the product has a longer shelf life. The boats can shrimp until their holds are full and fuel is not wasted by making unnecessary trips to port to replinish ice stores. Also, ice is inconvenient, becoming increasingly expensive and can contribute to high bacterial loads on shrimp.

Another advantage of brine frozen shrimp is that the boat can deliver a consistantly high quality product to port since freezing preserves the quality of fresh shrimp better than other methods.

One of the problems encountered with ice boat shrimp is that breakage occurs during unloading. Shrimp are either suctioned or shoveled with the ice bins. Either way, shrimp are damaged by the excess handling. The brine freezing method produces fewer pieces since shrimp are transported from ship to deck in sacks.

A source of many of the difficulties encountered by freezer boat operators is improper use of the brine tank. Many of the problems that occur are due to the fact that the basic principles of freezing are either ignored or poorly understood. If the brine in the tanks has the correct salt concentration and is adequately cooled, the shrimp will freeze quickly.

If shrimp are allowed to freeze slowly, large ice crystals will grow within each individual muscle cell and eventually puncture the cell walls (Figure 1.).

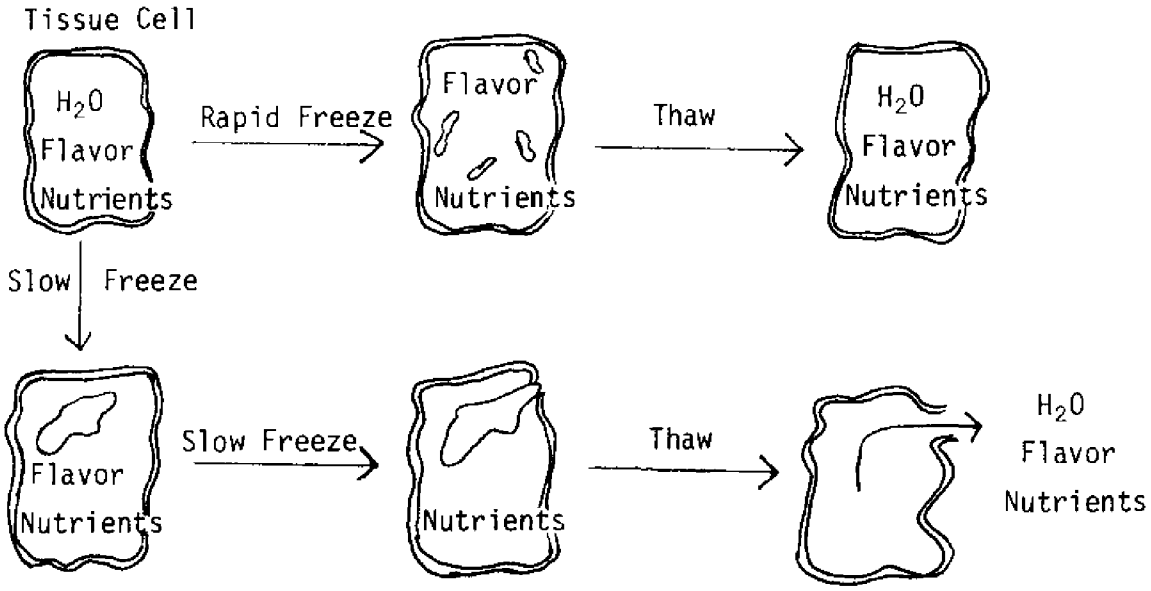

Figure 1. Affects of slow and rapid freezing on tissue cells.

Subsequent thawing at dock side or at the processor allows the materials within the cells to escape. These materials are often refered to as "thaw drip" and contain much of the moisture and flavor of the product.

Loss of moisture means loss of weight from shrimp and less profit. In addition, some chemicals lost during "thaw drip" are directly involved with promoting "black spot".

Rapid freezing will allow only small ice crystals to develop within cells. The small crystals will not puncture as many cell walls and moisture loss will be minemal when shrimp are thawed. Also, "black spot" will not be as serious.

The concentration of salt in the brine tank is critical for the proper freezing of shrimp. Brine tanks will only refrigerate to their mazimum capacity if the correct amount of salt is added. A given concentration of salt will lower the freezing point of water by a predictable degree (Figure 2).

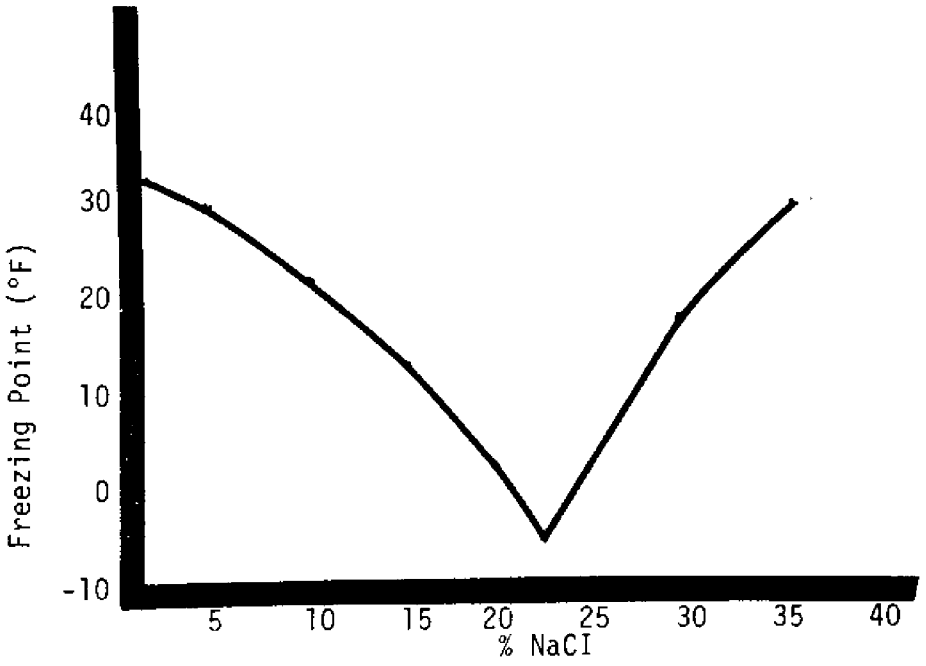

Figure 2. Affect of salt concentration on water freezing point.

The graph in Figure 2 illustrates that the lowest brine temperature that can be reached is -6 degrees F at 23.3% salt concentration (by weight). This point is technically refered to as the eutectic point and any concentration of salt above or below this point will result in a brine temperature above -6 degrees F. (Hildebrand, 1973).

In order to calculate the correct amount of salt to add to the water to cause optimum chilling, one must first know the volume of the tank. The calculation is simple and either fresh water or sea water may be used.

$$\text{Volume} = \text{Length X Width X Height (ft.) X 7.5 gal/ft}^3$$

Then add 2.53 pounds of salt per gallon of fresh water or 2.21 pounds of salt per gallon of sea water. The primary concern is the concentration of salt since this dictates the freezing point depression of the brine. Another consideration is that the brine is depleted with increasing numbers of shrimp that are dipped. This seems like an easy problem to solve, just add more salt; but how much and when?

A series of chemical analyses were performed on brine samples taken at various stages of the freezing operation, initially and after every ten boxes (1,000 pounds) of shrimp. These analyses indicated that there was a gradual decrease in brine concentration due to absorption of salt by the shrimp and loss of water from the shrimp to the brine. The rate of decrease of salt concentration and the increase in temperature of the brine after fifty boxes of shrimp (5,000 pounds) were frozen in four different vessels are shown in Figure 3.

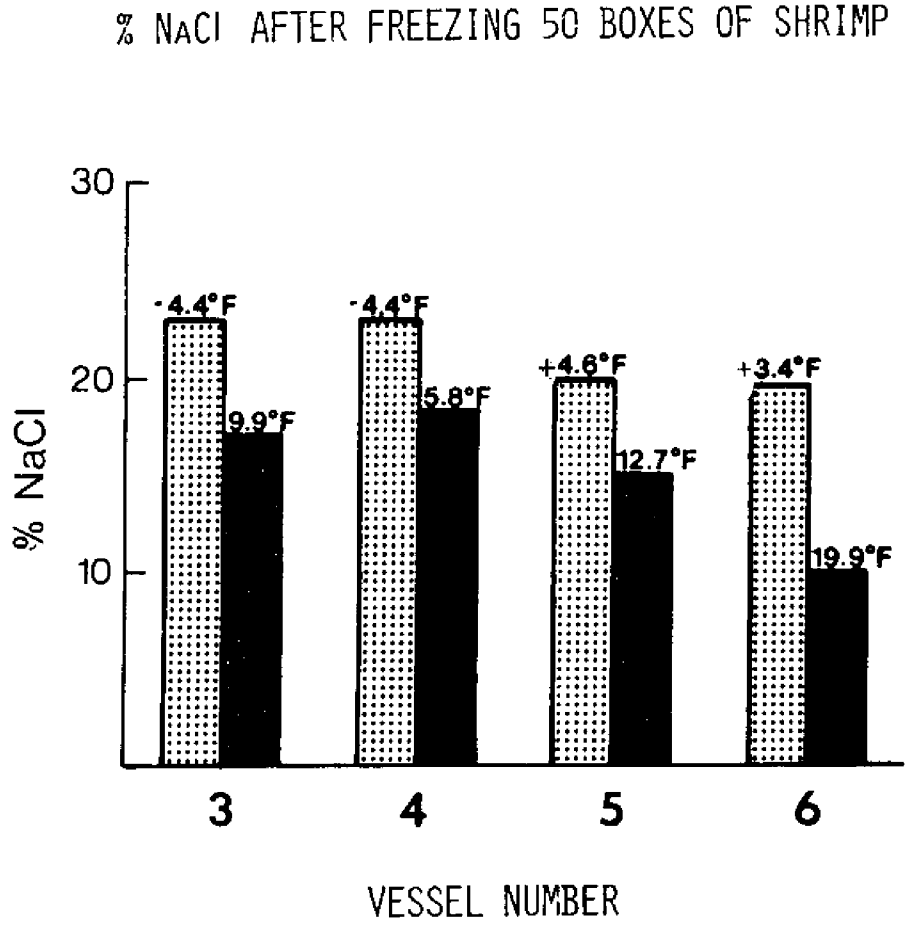

Figure 3. The salt concentration and temperatures of four brine tanks after 5,000 lbs of shrimp were frozen.

Notice that the rate of decrease is greater in smaller brine tanks. The pounds of salt lost from another four brine tanks after 10 boxes (1,000 pounds) of shrimp had been frozen are shown in Table 1. Again the size of the tank made a difference in the rate of depletion of salt. The smaller tank lost salt at a greater rate with the same amount of shrimp.

Pounds NaCl DEPLETED/10 boxes

Tank Size	NaCl/10 Boxes
146	29.12
180	26.14
270	29.36
332	30.72
$\bar{x}$	28.80

Table 1. The pounds of NaCL lost from four brine tanks as ten 1,000 pound boxes of shrimp were frozen.

Fishermen usually leave bags of shrimp in brine tanks until they appear frozen. If the brine tank is chilled to the maximum, -6 degrees F, shrimp will freeze within 12 to 15 minutes. The freezing profile of shrimp in a brine tank chilled to -4 degrees F is shown in figure 4. The graph shows that there is a rapid period of chilling and then a long plateau in the curve before shrimp are completely frozen. It takes at least 12 to 14 minutes to freeze shrimp at this brine temperature. If the brine tank is not properly chilled, the shrimp will be left in the brine for extended periods of time before freezing. The shrimp lose moisture to the brine and take up salt causing them to be salty and tough.

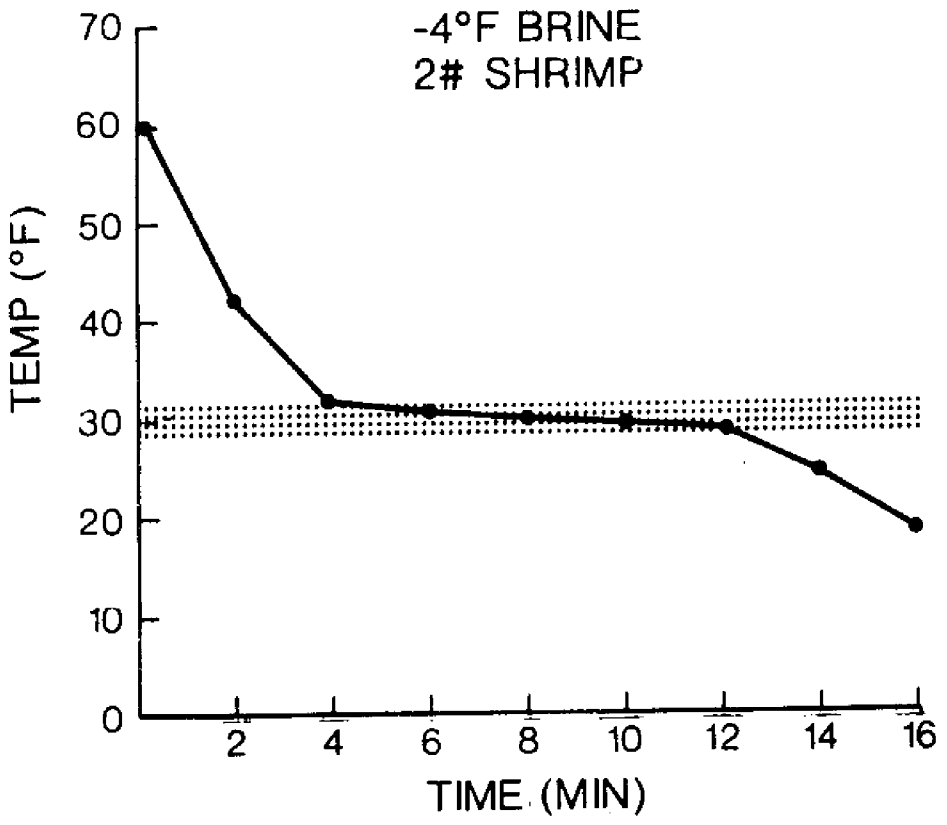

Figure 4. Freezing profile of 2 pounds of shrimp in a brine tank chilled to -4° F.

A recent study found that when brine tanks were below deck, the crew tended to leave shrimp in the brine longer than if the tanks were above deck. This was because the crews disliked having to stay below in a refrigerated atmosphere to wait for the shrimp to freeze. There was a greater chance of shrimp becoming salty and tough. Aldrin (1967) used a method that prevents salty tough shrimp, a cold (near 0 degree C) fresh water rinse is applied just before and after freezing.

The second rinse removes salt deposited by the brine and coats the prawns with an adherent fresh water glaze.

The length of time of storage conditions of frozen shrimp can be a factor in creating salty, tough shrimp. Normally the storage freezers are held at -20 degrees C (Montgomery et al., 1970). Prolonged storage or large fluctuations in the temperatures will cause dehydration and loss of quality. The results of holding frozen shrimp in storage fro 42 days are shown in Figure 5. There is a rapid increase in the % salt due to the moisture loss of the shrimp during freezer storage.

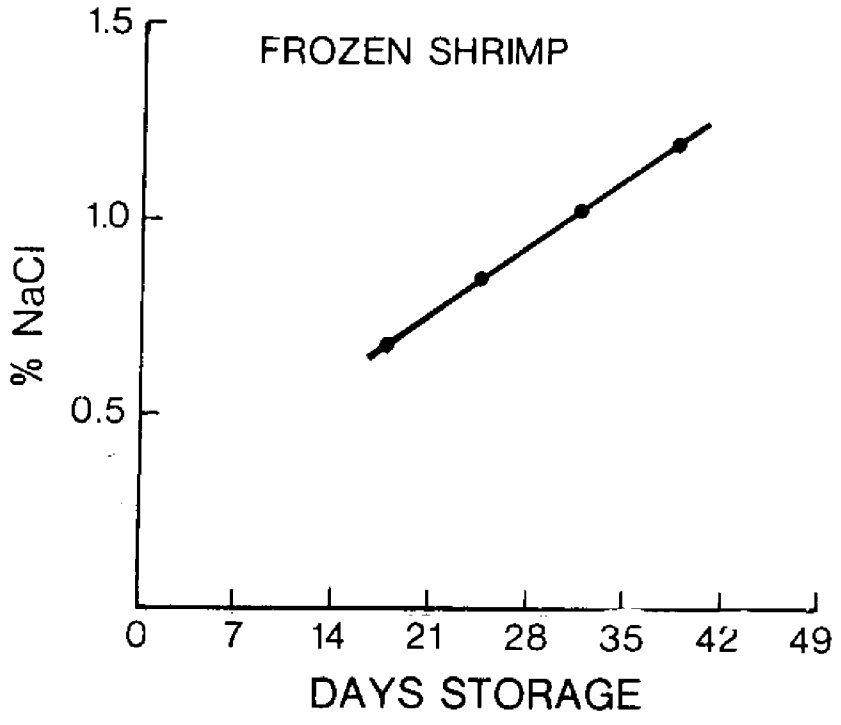

Figure 5. The affect of length of storage on the salt concentration in frozen shrimp.

A quality defect in ice boat and freezer boat shrimp that shrimpers try to avoid is black spot. Black spot is a discoloration of the shell caused by an enzymatic reaction which takes place in the shell and sometimes on the surface of the meat of the shrimp tail. The black appearance results from the oxidation of the amino acid tyrosine to the black pigment dopamine. The reaction requires oxygen. Syrup glazes are used to coat shrimp tails to inhibit the black spot reaciton and prevent excessive dehydration during storage. They work by out-competing the black spot enzyme for oxygen. Syrup, in essence, serves the same function as "shrimp dip" (sodium bisulfite). Corn syrup, molases and sugar are used as glazes though sugar is not recommended because it will sometimes form a brittle coat and crack in storage.

Freezer boat shrimp are notorious for developing black spot after thawing. Black spot chemicals which are normally dilluted on ice boats by melting ice are still concentrated on freezer boat shrimp. The black spot reaction does not occur during normal freezer storage, (a constant temperature of -20 C) but once shrimp are thawed, it will proceed at an accelerated rate.

For this reason slow thawing of freezer shrimp is not recommended. Fast thawing under cool running water is highly recommended. The freezer drip loss will be lessened and the chemicals that promote black spot which are released during thawing will be diluted.

Quality freezing and successful processing of freezer boat shrimp are the result of close attention to proper procedure and a good understanding of the theories involved in the freezing process. Freezer boat shrimp can be a superior product and for shrimp boat owners, profitable.

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PENETRATION MECHANISM AND DISTRIBUTION GRADIENTS OF SODIUM TRIPOLYPHOSPHATE IN PEELED AND DEVEINED SHRIMP

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While much research has been done on the effects of sodium tripolyphosphate (STP) treatment on food products such as meats and seafoods, the actual mechanism of action of STP on proteinaceous foods is not well understood. Working with fish fillets, Love and Abel (1966) stated that STP interacted with proteins to produce a surface film on the treated fillets. They theorized that such a film would seal in fluids and thus not only reduce thaw drip but also minimize moisture loss during frozen storage. Scheurer (1968) used radioactive STP (P^{32} isotope) to show that fillets dipped in a 12.1% STP solution for 10 minutes had only a slight increase in STP as compared to fillets dipped for only 10 seconds. The penetration of STP to the center of the fillet was also shown to be slight as compared to other salts evaluated. Finally, although the concentration near the surface increased with an increase in dip time, the concentration at the center remained fairly constant. The data implies a rapid formation of a protein-phosphate barrier complex which inhibits further STP uptake and further raises many interesting questions concerning the STP penetration mechanism.

This study was designed to investigate, using P^{32} labled STP, the penetration of STP into shrimp muscle. The specific objectives were to determine the effect of STP dip concentration and dip time on the penetration of STP into peeled and deveined (P & D) shrimp muscle.

MATERIALS AND METHODS

Preparation of Isotope

Irradiation of commercially available purified granular STP (Fisher Scientific Co.) yielded the radioactive isotope. The prepared isotope was allowed to decay for 14 days, virtually eliminating the activity of Na^{24} (half-life of 15 hrs.) as compared to the activity of P^{32} (half-life of 14.3 days).

Treatment of White Shrimp (Penaeus setiferus)

A 10% stock STP solution was prepared using the four grams of irradiated STP, 62g of "cold" STP and 660 ml of water. Appropriate

dilutions of the stock solution to 5%, 1% and 0.5% were prepared and used for treatments.

Fresh white shrimp (Penaeus setiferus) of size 26-30 tails per pound were used for the first part of the experiment. The shrimp were headed, then divided into two equal lots. One lot was peeled, deveined and treated immediately, while the other was frozen in 5 pound boxes (green headless) and kept frozen at -26°C for one week. Before being treated, the frozen shrimp samples were thawed in cold running water, peeled and deveined.

The shrimp were treated with the four different concentrations of STP for four different time intervals (20 sec, 1 min, 5 min and 20 min) each, resulting in 16 different treatments per lot. Upon completion of each treatment, the shrimp were given a single dip in distilled water to remove unbound surface STP, drained, and placed in a moist chamber until analyzed.

Determination of STP Uptake

The second abdominal carapace segment was removed from each sample, mounted on a specimen holder and frozen by immersion in liquid nitrogen. After freezing, the frozen sections were accurately trimmed to 1 cm^3 and placed in a cryostat. Slices approximately 16 μm in thickness were collected from each shrimp. One ml of quarternary ammonium hydroxide tissue solubilizer (ICN) was immediately added to each vial. After the shrimp tissue had been dissolved (normally overnight), 10 ml of a high efficiency liquid scintillation cocktail (ICN) was added. The fractions were counted using a Packard Tri-Carb Liquid Scintillation Spectrometer Model 3330.

Treatment of Brown Shrimp (Penaeus aztecus)

Fresh brown shrimp (Penaeus aztecus) of the same size range as the whites were used for this portion of the study. Sample preparation, preliminary treatment and all other procedures were identical except that for each vial the weight of the sliced fraction was accurately determined before the addition of tissue solubilizer.

RESULTS AND DISCUSSION

Penetration of STP in White Shrimp

Activity of treatment solutions:

After fourteen days of decay the active STP was diluted with 62 grams "cold" STP and the mixture was dissolved in 660 ml of distilled water. The resulting solution had an activity of 0.58 μC of P^{32}/ml. Counts for fresh and frozen shrimp samples were adjusted to allow for decay by using the following equation:

$$A_2 = A_1 e^{-\lambda t}$$

where: A_1 and A_2 are activities at the beginning and end of time period t measured in days. Lambda (P^{32}) is a constant with value 0.0485.

STP distribution in white shrimp:

The white shrimp fractions were counted for 10 minutes and results were recorded as total counts per fraction per 10 minutes. The counts obtained for fresh and prefrozen white shrimp revealed high data fluctuation with some specific trends evident.

As expected, the activity in the surface fractions was shown to be proportional to time of treatment. However, at lower concentrations, independent of treatment time, there was a distinct concentration gradient set up between the surface and the center of the shrimp (Figure 1). This was not the case at the 10% STP level where STP was evenly distributed throughout the shrimp muscle.

The high data fluctuation was presumed to be due to variation in slice thickness during the microtome slicing. In order to overcome this, a second experiment using brown shrimp where the weight of each fraction was determined was undertaken.

Penetration of STP in Brown Shrimp

Activity of treatment solution:

The isotope and the treatment solutions used in this phase of the experiment was prepared in exactly the same manner as that used for white shrimp.

STP distribution in brown shrimp:

Each fraction was counted for 10 minutes, and the counts are reported as counts per minute per mg of shrimp muscle. Counts for fresh and prefrozen were adjusted as before to allow comparison. Figures 2 through 3 show examples of the data obtained for distribution of STP in fresh brown shrimp after treatment. The most interesting aspect of the data is that while the surface concentration is dependent on both treatment concentration and time, this is not uniformly true for the interior fractions. Even though, as indicated in Figures 2 and 3, the surface layers when treated with 0.5% and 1% STP show large differences, the interior fractions were of about equal activity. This is in agreement with Scheurer's (1968) observations on treated haddock fillets.

Figures 4 and 5 show the data for prefrozen brown shrimp treated with STP after thawing. With exception of the 0.5% treatment levels where fresh shrimp absorbed more STP than prefrozen, there was no apparent difference between STP penetration into fresh as compared to prefrozen shrimp. Since some protein damage (denaturation) must have occurred during the freezing process, it was initially expected that such damage would be reflected by the STP penetration data. However, no such difference was evident.

As was the case with treated fresh shrimp, the drop in counts from the outside to the inside fractions at low treatment levels was also shown to be true for treated prefrozen shrimp. Even after treatment at 5% STP for 20 minutes a distinct concentration gradient was observed in

the shrimp muscle. At the 10% treatment level (Figure 5) STP seemed to become uniformly distributed through the shrimp. The higher counts of some underlying fractions as compared to surface fractions of shrimp treated in the concentrated solutions were most probably due to the washing out of unbound surface STP during the distilled water dip.

CONCLUSION

Using isotope labeling, this study has shown that at low treatment concentrations an apparent surface reaction forms a concentration equilibrium or barrier-type restriction to further STP uptake. The data is in agreement with Nikkila et al. (1967) who showed restricted STP penetration into haddock fillets after an initial rapid absorption. The physical characteristics of such a surface phenomena have been described by Spinelli et al. (1967) who reported treated fillets to be slippery and translucent. The barrier is most likely a layer of gelitenized protein which forms at the surface of the treated shrimp. This layer is not, however, impermeable to STP as was evidenced by the high counts in the interior fractions of those shrimp treated with more concentrated STP solutions. The counts on each of the four samples treated at 0.5% STP for both fresh and frozen shrimp were essentially the same except for the outermost layers. Prolonged treatment of shrimp at the 0.5% level does not markedly increase the total STP content of the shrimp. Treatment at the 5% and 10% levels caused substantial uptake of STP, even in the interior fractions of the shrimp.

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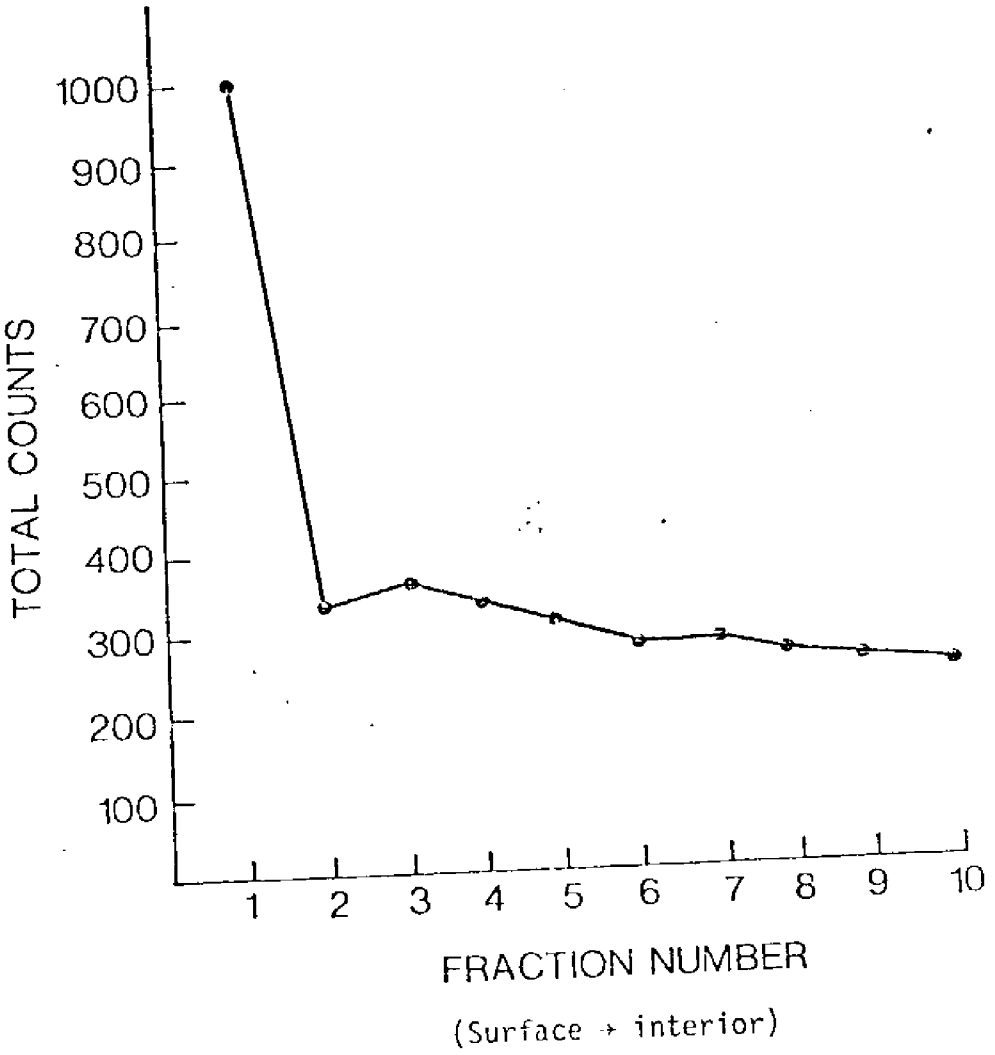

Fig. 1 - Activity (counts/10 min) of Fractions from Fresh White Shrimp (Penaeus setiferus) Treated in the 0.5% STP Solution for 20 Seconds.

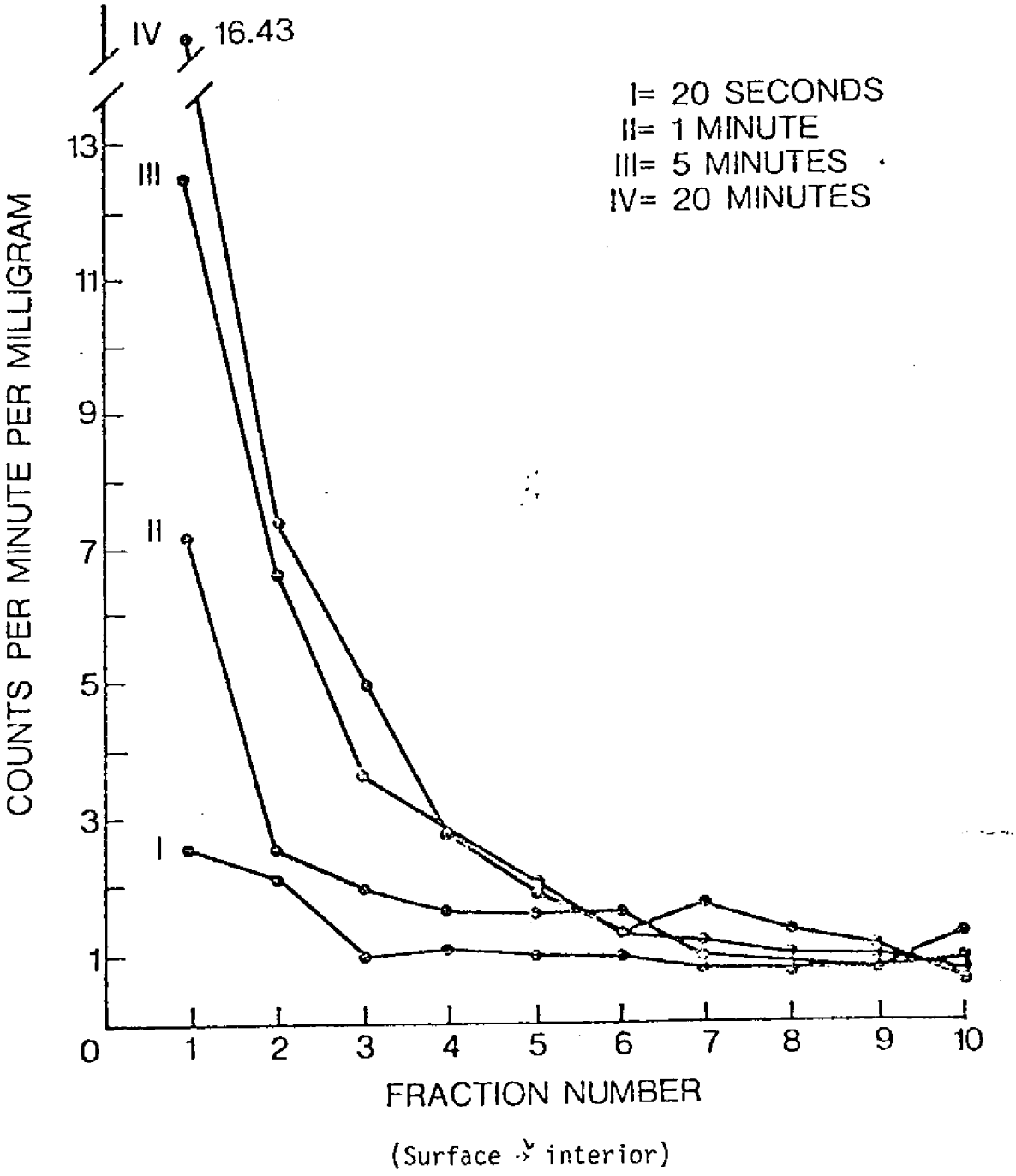

Fig. 2 - Counts on Fresh Brown Shrimp (Penaeus aztecus) Treated in the 0.5% STP Solution.

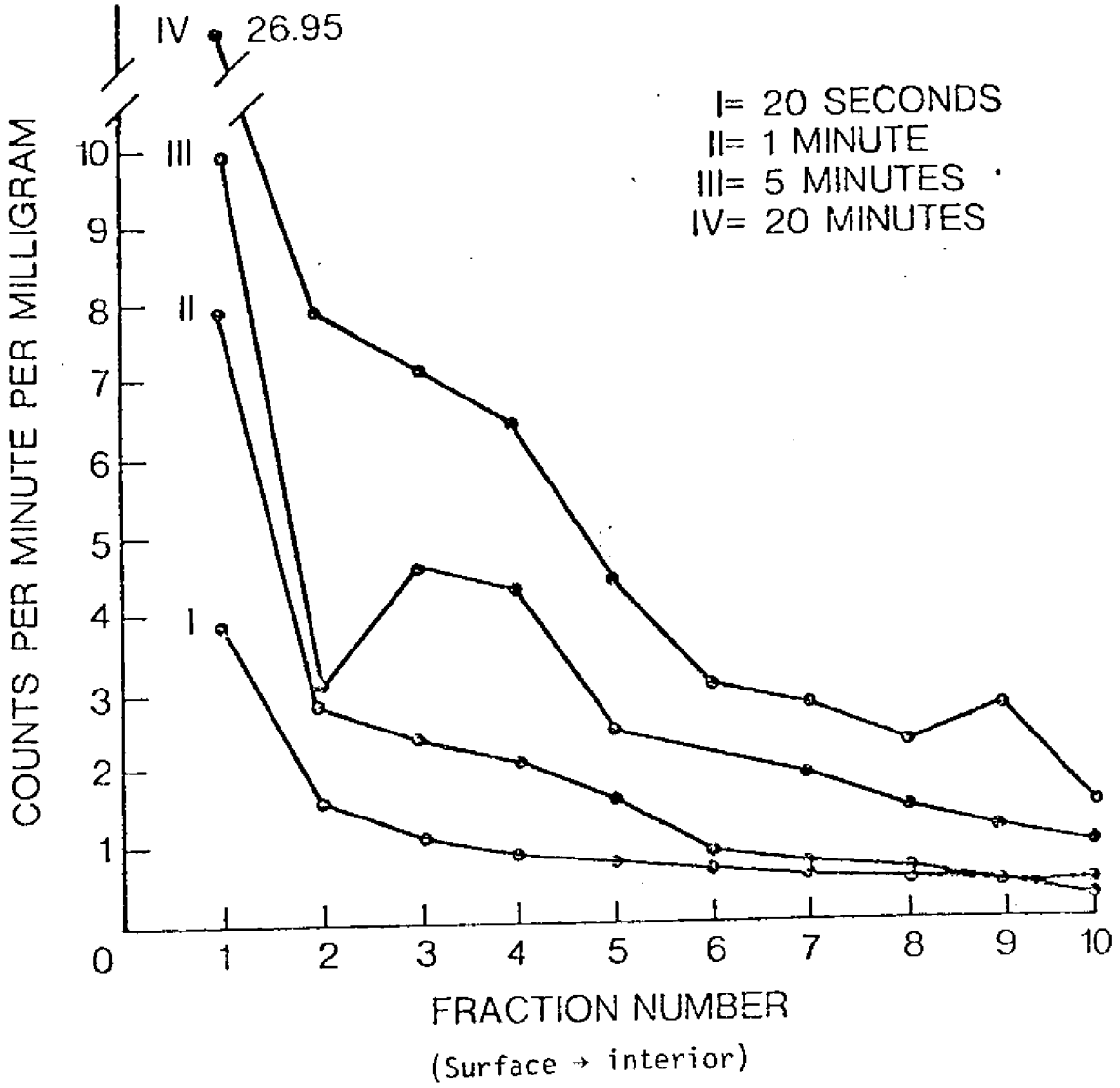

Fig. 3 - Counts on Fresh Brown Shrimp (Penaeus aztecus) Treated in the 1% STP Solution.

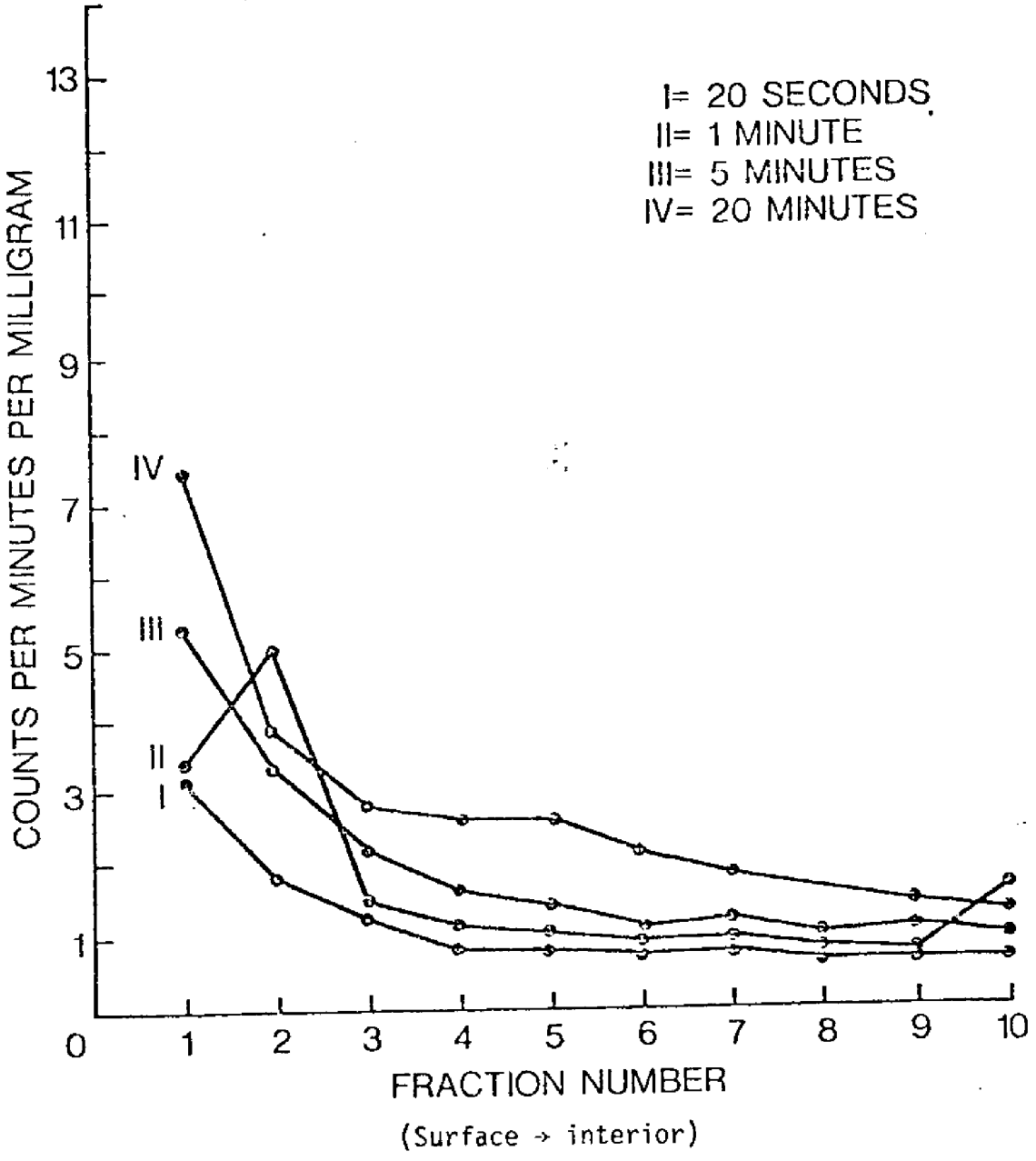

Fig. 4 - Counts on Frozen Brown Shrimp (Penaeus aztecus) Treated in the 0.5% STP Solution.

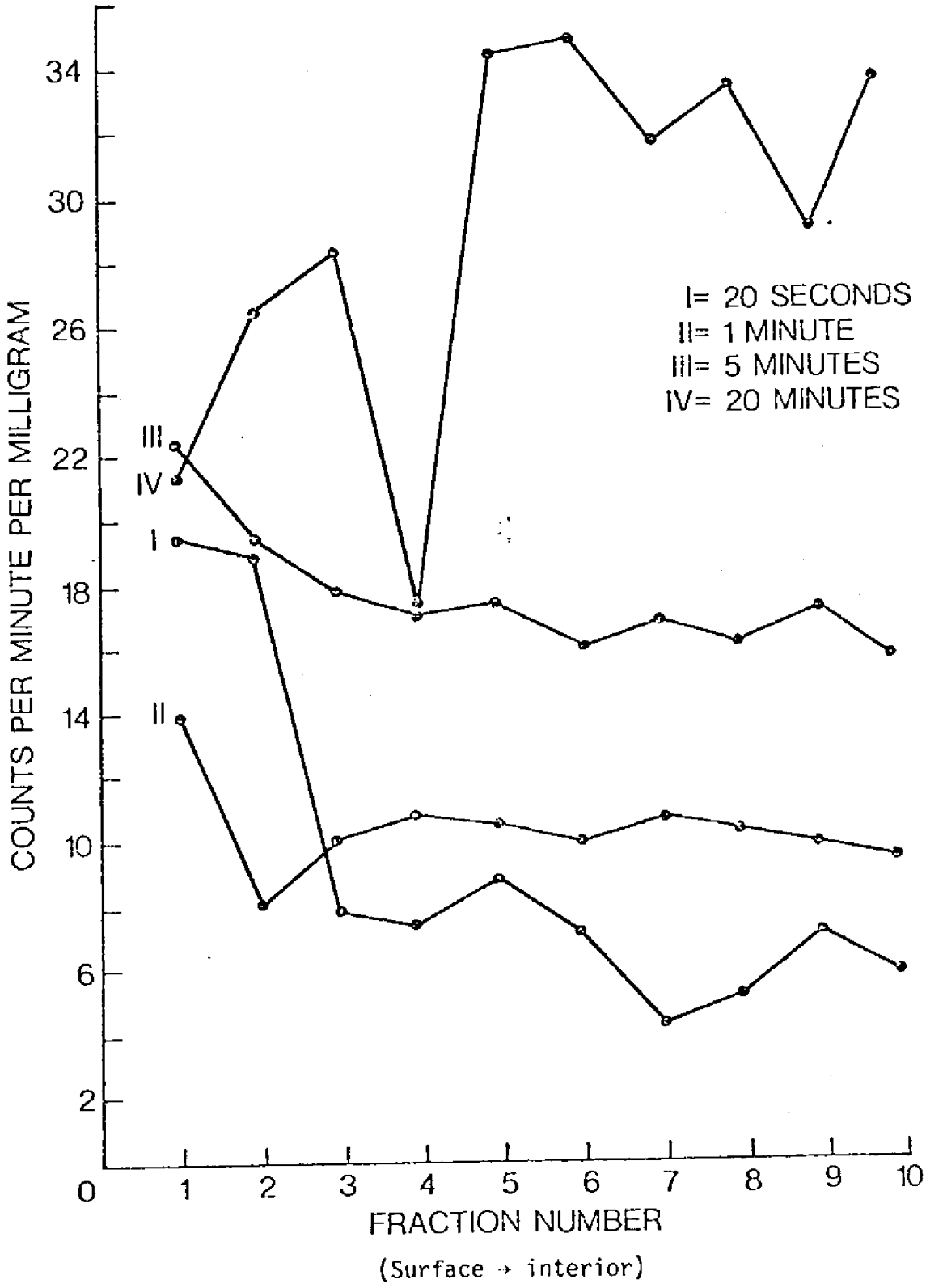

Fig. 5 - Counts on Frozen Brown Shrimp (Penaeus aztecus) Treated in the 10% STP Solution.

THE QUALITY AND SAFETY OF SHRIMP SOLD BY ROADSIDE VENDORS IN TEXAS

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In 1978, over eighty-four million pounds of shrimp, valued at $140,980,840 were landed off the Texas coast. A large percentage of this shrimp is processed to some degree and moved inland, a small percentage is sold as fresh shrimp to retail merchants. Some of the product sold to merchants is eventually sold at roadside shrimp stands. Health agencies and domestic consumers had repeatedly questioned the public health and freshness quality of these stands. Their location, often just outside city limits, clears them from municipal health regulations.

The purpose of this project was to ascertain information useful to health agencies and the domestic consumer on the quality of the product sold by these merchants.

Bacterial Quality

The initial quality of the shrimp depends on the method of their capture, on deck handling, and of treatment before storage. Initial bacterial counts of Gulf shrimp range from about 2×10^4 to 2×10^6/g[7], The dominant flora present consists usually of coryneforms, Achromobacter, Flavobacterium and Bacillus [3,7].

Bacterial reductions occur in washings, deck treatments and deheading of the raw shrimp. As the shrimp are handled and kept in cold storage the flora changes. An increase in Gram-negative, psychrophilic, spoilage bacteria occurs. The bacterial flora of human handling may become evident with the increased levels of coliforms, Staphylococci and possible Salmonellae due to improper handling and inadequate storage may be present [3,7].

A human pathogen frequently isolated from freshly caught seafood and part of the normal flora on shrimp is Vibrio parahaemolyticus. However, the strain is fragile and numbers are reduced by refrigerated storage [7].

The bacterial flora present, if allowed to flourish, will produce off-products that will affect the flavor and texture of the shrimp.

Therefore, by initially lowering the bacterial population and given proper refrigreation, the storage life and the quality of the shrimp can be increased.

Chemical Quality

The primary and most measured parameter of chemical quality of shrimp are the levels of ammonia and/or other short chain volatile nitrogens [1,],2.5,11].

The production of ammonia and/or other short chain volatile nitrogens are formed from three different areas; (i) bacterial growth, (ii) free amino acid degradation, and (iii) nucleotile breakdown [1]. The initial levels of these volatile nitrogen waste products are reduced by washings and on-deck treatments. Refrigerated storage slows the process, but does not halt it. If the shrimp are properly kept in crushed ice the drip from the melting ice serves as an effective wash to rinse the shrimp of any buildup of volatile nitrogens.

Ward [11] in his correlation between TPC, TVN and NH_3 (specific ion electrode), determined a value of 12 mg NH_3/100 g as a guideline for fresh shrimp. TVN values of 19 mg TVN/100 g shrimp on freshly caught shrimp are accepted by other investigators [1,5].

Physical Analysis

A variety of physical parameters can be measured on shrimp. The net weight of the product sold should be slightly more than the weight bought - 2-3%, in fact, to account for water gain. The count or number of shrimp per pound should conform to the various ranges. The shrimp should have a fresh smell, firm texture and no slimy surface film.

The most noticable physical defect of fresh shrimp is black spot or melanosis. The condition is caused by a buildup of black pigment under the shell and is similar to sun tanning in people. On-deck treatments can inhibit the formation of black spot provided the shrimp is handled and stored properly after treatment. The condition does not render the shrimp inedible, but is a sign of age and is considered a defect [6].

MATERIALS AND METHODS

In order to establish baseline data related to quality, one pound samples of shrimp were purchased from 50 roadside shrimp stands and 20 established retail supermarkets. The purchaser appeared to be an average consumer to avoid special treatment.

The general appearance of the facilities and the manner in which the shrimp were handled and stored was noted. The temperature of the sample was taken after purchase by insertion of a sterile thermometer into the center of individual shrimp. Samples were then iced and transported to the Food Quality and Safety Laboratory, Texas A&M University, for testing. Samples were aseptically weighed, and the count and species of shrimp were determined. Data was compared to the information quoted by the merchant. The percent of shrimp with melanosis (black spot) was also recorded. A 50-g sample was homogenized with 450 ml of 0.1% Tryptone Broth (Difco) and decimal dilutions made to enumerate Total Aerobic Plate Count (TPC) (Standard Methods Agar, BBL), total and fecal coliforms (MPN Procedure), Staphylococcus aureus (Baird Parket Agar BBL) for pre-enrichment followed by an enrichment for Salmonella isolation. A second 25-g sample was homogenized in 0.1% Tryptone Broth (Difco) with 2% NaCl added for isolation of Vibrio parachaemolyticus on TCBS Agar (Difco). The temperature of incubation was 25°C, rather than 35°C, for the TPC test. This was done to fully enumerate the psychrophilic flora. All other bacteriological methods were performed according to the "Bacteriological Analytical Manual for Foods" [10].

The ammonia concentration was determined by means of a specific-ion electrode from the 50-g homogenized dilution according to Ward et al. [12]. The final 25-g sample was homogenized with 50 ml of a 7% Trichloroacetic Acid solution. This solution was used to determine the Total Volatile Nitrogen (TVN) concentration using the micro-diffusion method of Cobb et al. [2].

RESULTS AND DISCUSSION

The objective of this project was to gather public health and freshness information about the quality of shrimp sold by roadside merchants throughout the state. A variety of laboratory tests and physical observations were used to determine the quality of the shrimp being sold. The laboratory tests used to measure the public health and freshness quality of shrimp are tests that are commonly applied in both business and research.

Chemical Analysis

The main chemical tests used to measure freshness of raw shrimp are the measure of Total Volatile Nitrogen (TVN) using the micro-diffusion method [2] and the measure of ammonia concentration using a specific-ion electrode [12]. Results of both roadside and retail samples are tabulated in the Appendix in Tables 1 and 2.

Fresh shrimp is considered to have a TVN concentration of 19 mg TVN/100 g or less, spoiled shrimp over 30 mg TVN/100 g [1,5]. Ammonia levels of fresh shrimp are considered to be less than 12 mg NH_3/100 g and 42% exceeded 19 mg TVN/100 g. These results would indicate that shrimp from the roadside dealers was of a fresher quality.

The lack of smoothness in the relative frequency diagram (Figure 2) for ammonia concentration is likely due to the fact that some of the retail shrimp samples had previously been frozen and thawed for display.

In Ward's [11] paper on the ammonia probe, he expressed values for ammonia concentrations in molarity (M). This author felt that an easier figure to work with and compare values would be an expression of mg NH_3/100 g sampled.

Bacterial Analysis

The Total Aerobic Plate Count (TPC) is a measure of the number of mesophilic aerobes present on the product. The number gives an indication of the bacterial quality of the shrimp, the product past history, and how long a shelf life it will have. The bacterial results are tabulated in Tables 1 and 2 of the Appendix.

The relative frequency diagram of the TPC values for roadside and store bought shrimp are shown in Figure 3. The roadside shrimp show a two-log lower bacterial count than the shrimp purchased in stores. The TPC's shown in this study were incubated 48 hours at 25°C rather than the 35°C commonly used in the BAM Manual [10]. This usually gives a count of one to two logs higher than the standard 35°C count.

To compare the relative quality of samples a value of 1×10^6 TPC/g was used. From Figure 3, 49% of the roadside shrimp samples had a TPC of greater than 1×10^6/g while 88% of the shrimp purchased in stores exceeded this value. Probably the shrimp in the stores was held in storage longer, handled more and/or inadequately stored longer than the roadside shrimp.

Fecal coliforms determined by Most Probable Number (MPN) techniques were reported by 36% of the shrimp from roadside stands and 45% of the established retail markets.

The presence of coagulase-positive *Staphylococcus aureus* on fresh shrimp is usually a sign of improper handling [7]. Coagulase-positive *Staphylococcus aureus* was isolated in 36% of the roadside facilities and 45% of the retail stores. The most found on any sample were 50 per gram with the majority being less than 10 per gram. These numbers are not sufficient to cause toxin and staphylococcal food-borne illness. It is generally assumed that numbers of approximately 5×10^5/g must be present to cause illness. All samples tested in this study were less than 10 per gram for *Vibrio parahaemolyticus* and negative for *Salmonella* in 25 g.

The bacteriological results from these samples seem to indicate that the shrimp purchased from roadside merchants had lower bacterial counts, thus were of better quality than those purchased from established retail markets.

Physical Analysis

One pound samples were weighed and counted to see how they compared to the amount claimed at purchase. The weight of shrimp purchased was less than one pound 28% of the time for roadside and 30% of the time for retail stores.

The count of shrimp per pound was less than quoted 22% of the time in roadside stands. No retail stores had lower counts than quoted.

A noticeable physical defect in fresh shrimp is black discoloration under the shell. Texas industry guidelines specify that not more than 10% and 3% black spot by count on the shell and in the meat repectively (R. Nickelson, personal communication) is allowed. Sixty-eight percent of the roadside sales had 10% or less shrimp with black spots and 40% of the retail shrimp had 10% or less shrimp with black spots. In this study any blackening of the shell no matter what size was counted as black spot. The Texas guidelines require the spot on the shell to be greater than 1/3 of the circumference of a segment. If this criterial had been used the results would fall inside the industry guidelines. The majority of Texas shrimp harvested are bay shrimp which are caught during the day. Since sunlight accelerates black spot formation, these shrimp could have a higher percentage of black spots.

Physical Observations

At each stand observations were made as to the general appearance, storage of the shrimp and method of handling the product. These observations were made at each roadside stand:

1. A wide variety of vehicles were used. 64% were trucks, 32% were trailers and 4% were cars.
2. 76% of the vehicles were covered or had shade.
3. 96% of the vendors did not drain the melt out of their ice chests.
4. 98% of the vendors used bare metal scales for weighing product.
5. 96% of the vendors used their bare hands to handle the shrimp.

A general observation of each stand was made and is tabulated in the results (Table 3 in Appendix). To determine if the appearance of the area had any bearing on the quality of the shrimp, the geometric mean of the TPC and the arithmatic mean of the TVN data was taken for each group designated Good, Adequate and Poor.

	Good	Adequate	Poor
Geometric Mean of TPC	6.4×10^5/g	9.8×10^5/g	1.71×10^6/g
Arithmatic Mean of TVN	18.4mg TVN/100g	14.3mg TVN/100g	14.6mg TVN/100g

The bacterial counts indicate a trend in lower numbers as the stand's appearance improves. This would lead one to believe that a stand with overall good appearance would tend to sell a shrimp with a lower TPC. The TVN results appear inconclusive, probably because it is not directly related to sanitation and cleanliness.

CONCLUSIONS

Data from this report show that roadside shrimp vendors offer a fresh and wholesome product. This does not necessarily imply a better original product than market shrimp, but rather a fresher (more recently caught) product held for a shorter period of time and handled less.

Areas that could help merchants provide a fresher, safer product with a longer shelf life would be to improve the methods of icing, storage and handling.

Adequate icing is probably the most important aspect in the storage of a fresh, perishable product like shrimp. Proper icing can significantly lower the number of bacteria and volatile nitrogen waste products. The most effective icing technique is alternating layers of ice and shrimp, and letting the melting ice escape by means of a "false bottom" and/or drain.

Handling, weighing and packaging are also important factors in determining the quality of shrimp. Most merchants handled the shrimp with bare hands, placed them on a bare metal scale, and bagged them. Between sales there was little evidence of hand cleaning or cleaning of the scales.

Better methods in handling, storage and sanitation will improve the public health and freshness quality of both roadside and store-bought shrimp.

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AN ENZYMATIC ASSAY FOR THE MEASUREMENT OF AMMONIA IN SEAFOOD PRODUCTS

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Organoleptic examination by trained inspectors is the most common method for detecting decomposed fish and fishery products. Although this method is a rapid, inexpensive technique to quickly evaluate seafood quality, it is only semi-quantitative, and hence only estimates the degree of spoilage which has taken place. Since organoleptic examination relies on human sensory organs, such tests are subjective and can vary from one inspector to another.

As early as 1937, Beatty and Gibbons (3) recognized the need for a quantitative method to quantitate decomposition in seafood. These investigators developed a technique to quantitate volatile amines and demonstrated that the concentration of these amines could be correlated to decomposition in seafood. Since the work of Beatty and Gibbons, numerous investigators have developed methodologies for quantitating various nitrogen containing components and have shown that concentrations of these compounds in fishery products can be correlated to the extent of decomposition (2).

During postmortem ice storage of panaeid shrimp, it has been shown that ammonia is produced at the rate of approximately one mg/100g/day (8). This enzymic production of ammonia results in a pH change of from about seven to eight in shrimp muscle (6), which in turn causes an increased production of ammonia, since the pH optimum of the ammonia-producing enzymes is approximately 8.5 (5). In addition, urease activity has also been detected in commercially caught shrimp (7). This enzyme is believed to be of bacterial origin and is probably important in the production of ammonia during the latter stages of ice storage when urea concentrations (another decomposition product) increase (5).

Although much of the ammonia formed is leached from the shrimp, the quantitation of free ammonia has been used as a measure of decomposition, and numerous countries have established limits for ammonia in shrimp (15). This use of ammonia as a measure of decomposition is not limited to shrimp, but rather is widely used as a means to detect and quantitate decomposition in a variety of seafood products ranging from shellfish (4) to dogfish (24).

Various methods for determining ammonia, and hence decomposition in fishery products, have been proposed including Conway's microdiffusion technique coupled with Nesslerization, the Okaloff

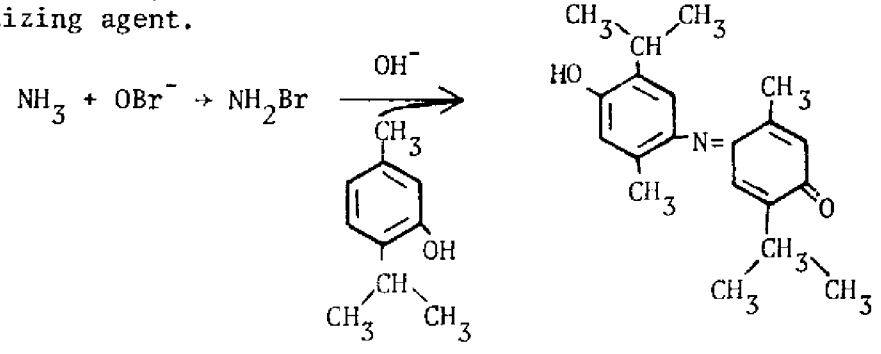
magnesium oxide distillation volumetric method and the Berthalot reaction. Nessler's reagent (potassium mercuric iodide) readily reacts with NH_3 in alkaline conditions to form colloidal dimercuric ammonium iodide. This product is yellow to orange-brown depending on concentration, and hence the amount of ammonia can readily be calculated spectrophotometrically.

$$NH_4^+OH + 2(KI)_2HgI_2 + KOH^- \rightarrow NH_2HgI_3 + 5\ KI + 2\ H_2O$$

However, prior to reaction with Nessler's reagent, free ammonia must first be removed from other amines in the fishery product. A number of techniques have been suggested, all of which rely on a Conway diffusion technique (10) or a similar method for volatilizing and trapping ammonia in the sample (9,24). Hence, the major disadvantage of the method is the time-consuming volatilization of ammonia prior to the Nesslerization reaction.

The Okaloff magnesium oxide distillation volumetric method is recommended by many food standards authorities for the determination of ammonia (15). As the name implies, this procedure relies on the separation of ammonia from interferring substances by distillation of an alcohol/aqueous extract in the presence of magnesium oxide. Titration of the distillate following the addition of formaldehyde completes the quantitation for ammonia (26). Not only is the procedure lengthy (the extraction step itself requires 24 hours) but the multiple titrations in addition to the distillation step each add potential sources of error, resulting in a method of low precision.

The Berthelot analysis for ammonia involves the formation of a blue chromagen (indophenol) when ammonia and aromatic amines react under alkaline conditions with phenol and hypochlorite (14,17). A number of variations using alternate oxidizing agents and various aromatic alcohols have been suggested as preferred modifications of the technique. One such modification was proposed by Burnett (4) for use as an indicator of decomposition in crabmeat. This modification, which subsequently was adopted by the AOAC as the official method for the analysis of ammonia in crabmeat (21), uses thymol as the alcohol and bromine as the oxidizing agent.



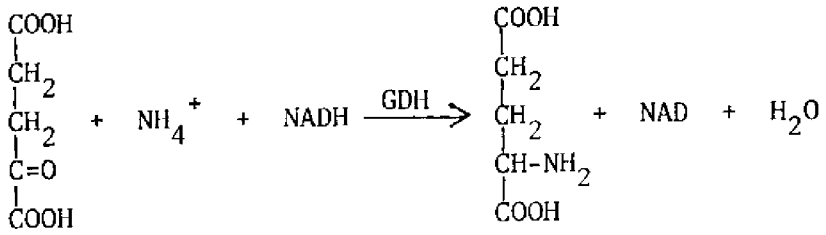
Thymol (Blue)

Due to the lengthy extractions required throughout this procedure,

the requirement for the addition of reagents in small aliquots with mixing between additions and the overall length of the procedure, this method has found only limited use in routine, quality control situations.

The use of enzymes as reagents is an area receiving considerable attention in quality control laboratories associated with both the halth care and food industries (11,22). Unlike the chemical methods which must utilize distillations, extractions, and/or chromatography steps to make a method specific for a specific compound, enzymatic analyses utilize the specificity of selected enzymes to react only with the substance of interest.

The enzyme glutamate dehydrogenase (GDH) has been used to measure ammonia in a number of biological samples (18-20,23). An aliquot of sample to be analyzed is added to a solution of the substrate α-ketoglutarate, the coenzyme NADH and the enzyme GDH. Reaction of the sample ammonia with substrate forms glutamic acid with the simultaneous oxidation of NADH to NAD. The extent of reaction (which is dependent on the amount of ammonia added) is readily measured by following the decrease in absorbance at 340 nm due to the oxidation of NADH.



Although this technique is widely utilized for the analysis of ammonia in both clinical samples and waste materials (18,20), no studies on the potential use of this method for ammonia determinations in food products have been undertaken. It is the purpose of this report to evaluate the potential of this technique as a quality control method for quantitating ammonia in seafood samples.

MATERIALS AND METHODS

For the enzymatic assay, a ten to twelve gram portion of shrimp muscle was weighed to the nearest 0.1 gram, placed in a blender, and homogenized for two minutes with 200 ml of phosphate buffer (0.1 M, pH 7.3). The homogenate was then certrifuged for five minutes at 1000 X G and the supernate used directly as the test sample.

Substrate (2.4 ml), consisting of 3×10^{-3} M α-ketoglutaric acid, 1×10^{-4} M ADP and 1×10^{-4} M EDTA in 0.1 M phosphate, pH 7.3, was added to a 1 cm disposable cuvette and mixed with 0.3 ml of NADH (1×10^{-3} M) and 0.2 ml of supernate to be assayed. The absorbance of the mixture was recorded, and the reaction initiated by the addition of 0.1 ml glutamate dehydrogenase

(110-150 IU/ml). After 15 minutes, the absorbance was once again read and the difference between initial and final readings calculated. A reference solution in which 0.2 ml of buffer replaced the shrimp supernate was run with each set of cuvettes. Standards consiting of 0.05, 0.1, 0.2, 0.3, and 0.4 μg NH_3/0.2 ml were run with each assay, and a standard curve was constructed. Ammonia concentrations of each sample were read from the standard curve and divided by the weight of shrimp to express results as μg NH_3/g shrimp.

The AOAC procedure for analyzing ammonia in crabmeat was followed (1). Twenty grams of shrimp were homogenized in 180 ml of 2.5% phosphotungstic acid and the homogenate filtered through Whatman #1 filter paper. To separatory funnels, 2 ml of sample filtrate, 8.0 ml of deionized/distilled water, 1 ml 2.5 N NaOH, 2 ml thymol and 5 ml bromine solution were added. After thorough mixing, and following a 20 minute reaction time, 20 ml of n-butanol was added, the flasks swirled, and the phases allowed to separate. The aqueous layer was then removed and discarded, and the alcohol layer was passed through anhydrous Na_2SO_4, the absorbance read at 680 nm, and the concentration of NH_3 determined from a standard curve in which ammonia standards had been treated in the same manner.

NADH, α-ketoglutaric acid, ADP, GDH (ammonium sulfate free), EDTA, spermine, spermidine, trimethyl amine and putrescine were all purchased from Sigma Chemical Company, Saint Louis, MO. Ammonium chloride, sodium sulfate, sodium bisulfite, sodium hypochlorite, phosphotungstic acid, butanol, bromine, and thymol were all the products of J. T. Baker.

RESULTS AND DISCUSSION

The selection of an optimum time for incubation of fish extract with substrate and enzyme is critical for an analysis such as this. An incubation time too short to permit complete reaction will not only result in an inaccurate calculation of ammonia concentration, but the precision will also suffer unless all test vials are sampled at the exact time. On the other hand, excessive incubation times severely limit the number of samples which can be processed. As shown in Figure 1, the enzymatic conversion of ammonia to glutamic acid under the assay conditions employed is rapid. Within two minutes, 50% of the ammonia has reacted, while the reaction is complete within eight minutes. A reaction time of 15 minutes was thus selected for all subsequent assays. Not only is this time short enough that numerous assays can be run in a workday, but the seven minute interval between completion of reaction and time of analysis is more than adequate to compensate for day to day variations in enzymatic activity and minor fluctuations in substrate concentrations.

As can be seen in Figure 2, the assay, as described, is linear for ammonia concentrations ranging from 0 to 600 μg/g of sample. Since permissible levels of ammonia if fishery products are between 400 and 500 μg/g, this test will readily differentiate

acceptable from unacceptable products. For those unacceptable samples where ammonia concentrations are calculated to be more than 600 µg/g, a simple 1:2 or 1:4 dilution of sample extract followed by a second enzymatic determination will readily yield a total ammonia concentration.

In addition to ammonia, decomposing seafood also contains elevated levels of other nitrogen containing compounds. These include volatile, primary and secondary amines, as well as numerour amines arising from the decarboxylation of amino acids. Thus, it is important that any assay for ammonia in seafood not only be specific for ammonia, but also not be inhibited by these other nitrogen containing compounds. As shown in Figures 3 and 4, a number of nitrogen containing compounds typically found in decomposing seafood were added to spiked shrimp extracts prior to analysis. Although concentrations far in excess of naturally occurring concentrations of these compounds were used, none of the added amines produced results significantly different from the spiked ammonia samples. Thus, the assay is specific for ammonia, and other amines present in the sample do not interfere with the analysis.

It is common for shrimpers to add sodium bisulfite to their catch to retard black spotting (melanosis) (25). Thus, any assay for ammonia in shrimp must be insensitive to this additive. While the Codex International Standard for quick frozen shrimp has recommended a tolerance of not more than .003% total SO_2 in shrimp (12), a one minute dip in a 1.25% $NaHSO_3$ solution is the recommended application technique (13). As shown in Figure 5, extracts containing 0.15% $NaHSO_3$ did not interfere with the assay at any ammonia concentration. However, higher concentrations of $NaHSO_3$ (1.25% and 5%) did partially inhibit the enzyme, resulting in calculated ammonia values approximately 10% below controls. Although instances of shrimp containing such high $NaHSO_3$ levels are extremely rare, this inhibition can be overcome by the addition of 0.05% hypochlorite to the sample extract as shown in Figure 5. It should be pointed out that excessive treatment of shrimp with $NaHSO_3$ severely affects the appearance of the shrimp. Hence, the addition of hypochlorite to the shrimp extract would be suggested by their physical appearance prior to processing.

The expected precision of the assay is presented in Table 1. As can be seen from the table, multiple analysis of a homogeneous extract of an acceptable shrimp extract results in a coefficient of variation of under 7%. Analysis of five different, clearly decomposed samples still produced a coefficient of variation of well under 15%.

TABLE 1

Precision of Assay

	NH_3 Concentration (µg/g shrimp)		
	Mean	Standard Deviation	CV %
Single Sample (n = 10)	165	11	6.7
Multiple Samples (n = 5)	1163	152	13.0

In order to compare the enzymatic method to the present AOAC method, shrimp containing various levels of ammonia were required. To obtain such samples, shrimp were placed on ice and samples removed and assayed by both the AOAC and enzymatic methods every two days for a month. As shown in Figure 6, a correlation coefficient of 0.86 was obtained between the two methods. However, as the figure illustrates, ammonia concentrations were consistently 15-25% lower with the enzymatic assay. This was not unexpected, however, since other investigators have demonstrated that, unlike the enzymatic assay, the AOAC method is not specific for ammonia. Thus, the enzymatic method would appear from this study to be the more accurate of the two methods.

CONCLUSIONS

Use of the enzyme glutamate dehydrogenase for the quantitation of ammonia in seafood products appears to overcome many of the limitations of techniques presently used for such analyses. The method is fast, reproducible and does not require sophisticated instrumentation or techniques. In addition, unlike other methods, the enzymatic technique is specific for ammonia and other decomposition products and food additives do not interfere with the assay.

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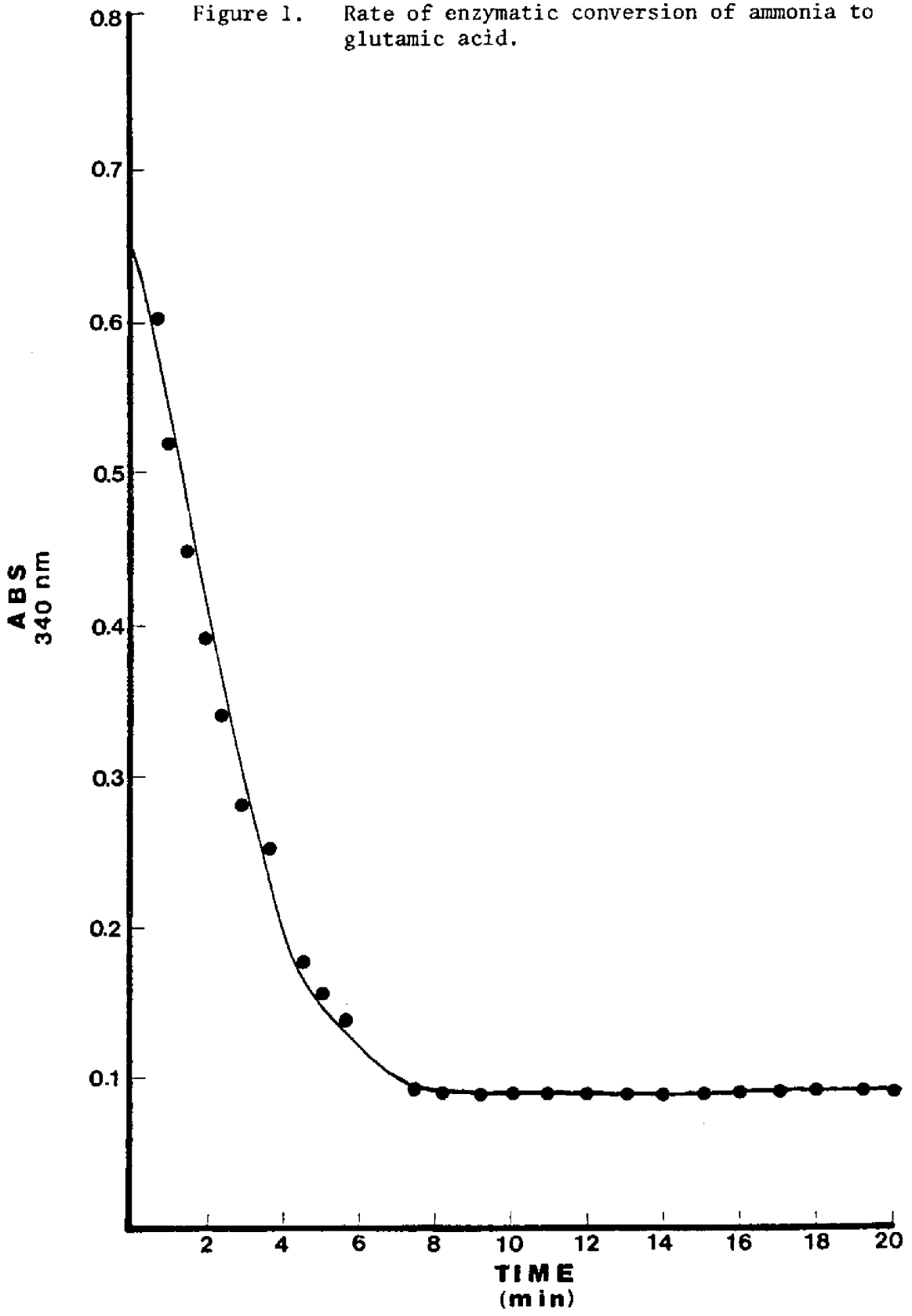
Figure 1. Rate of enzymatic conversion of ammonia to glutamic acid.



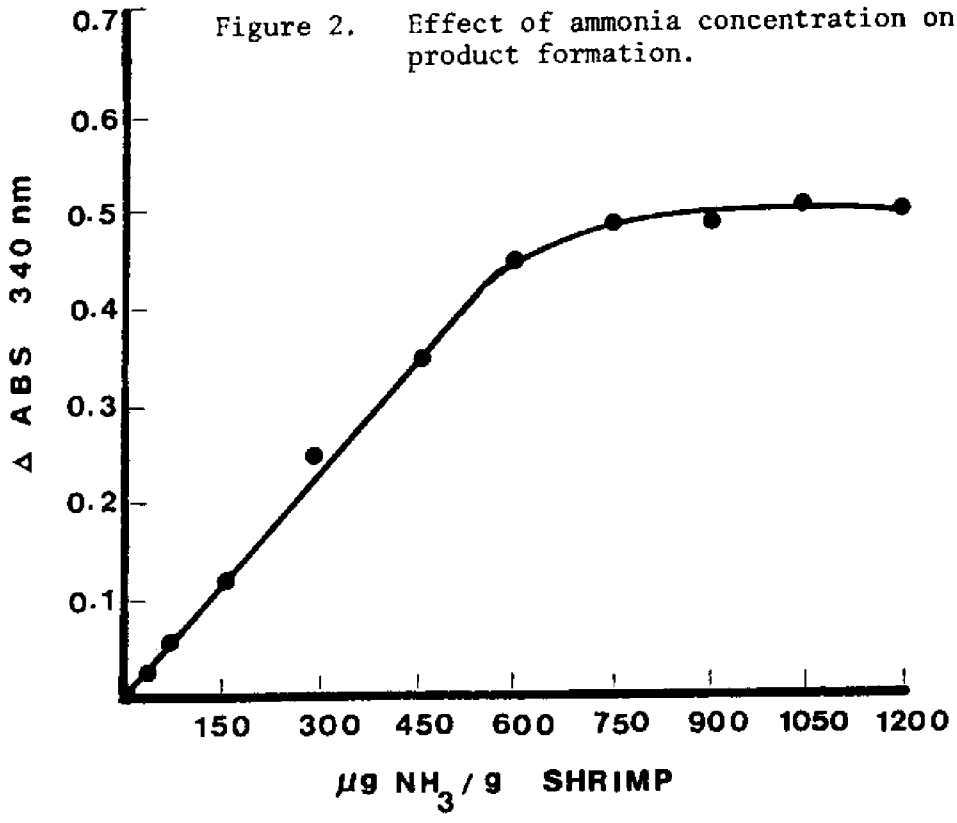
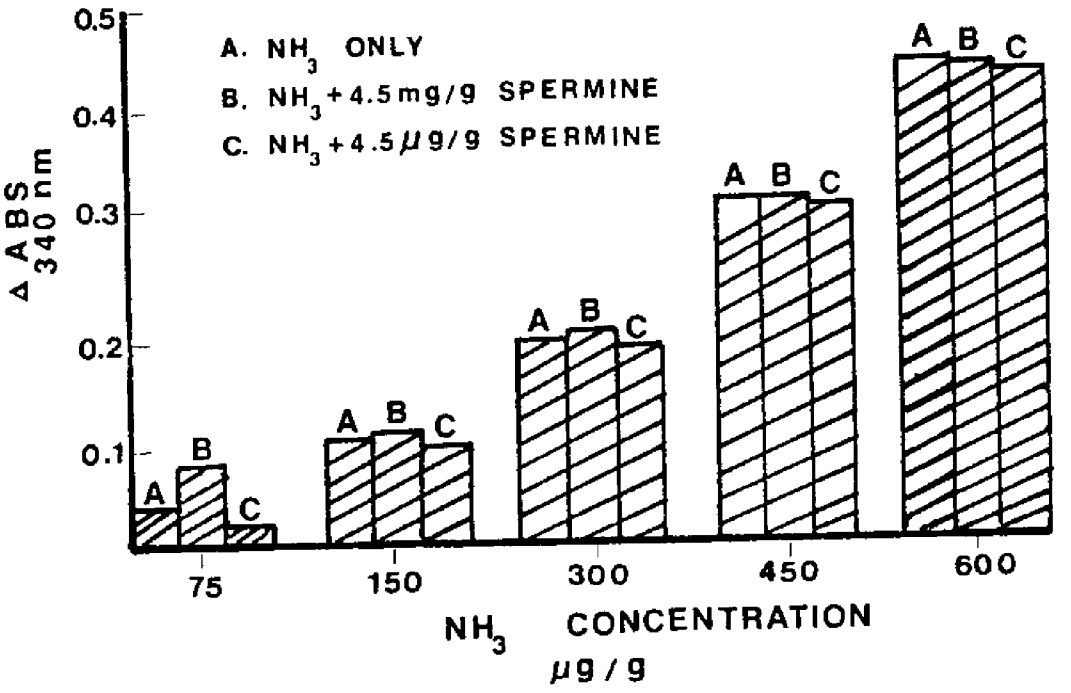


Figure 2. Effect of ammonia concentration on product formation.

Figure 3. Effect of naturally occuring amines on enzymatic determination of ammonia.



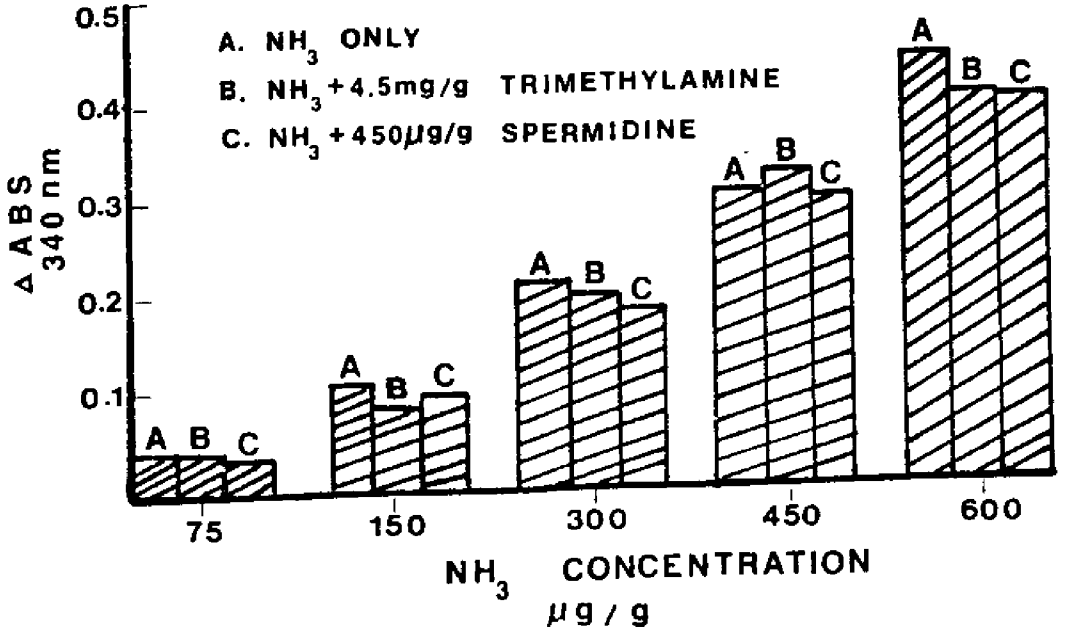


Figure 4. Effect of putrescine on enzymatic determination of ammonia.

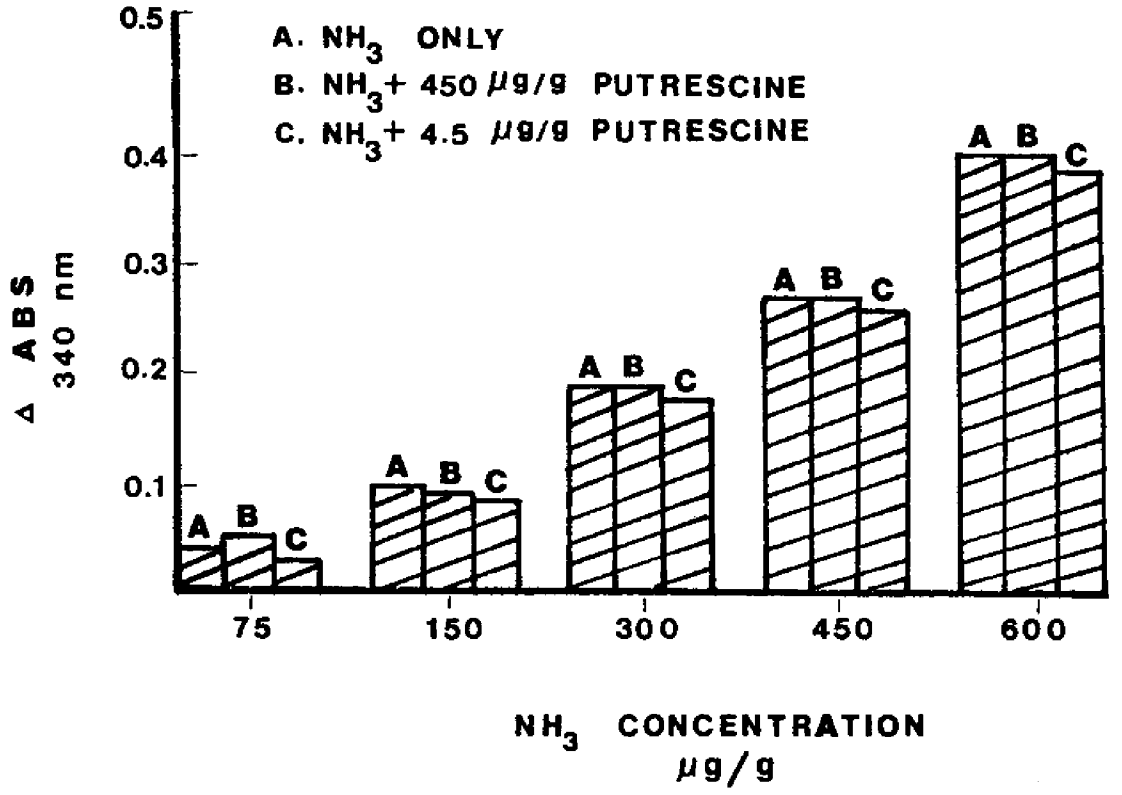
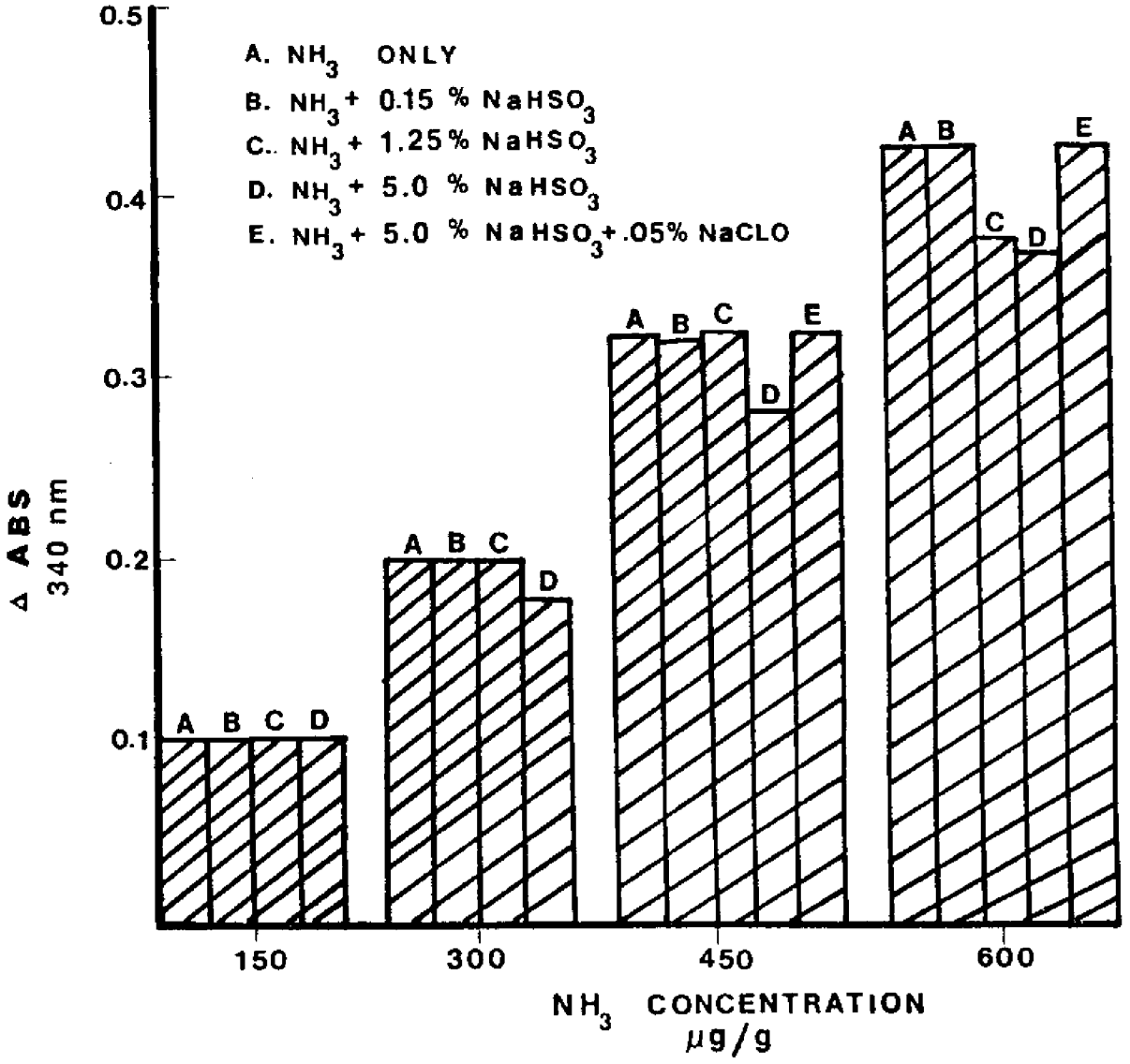


Figure 5. Effect of added sodium bisulfite on enzymatic determination of ammonia.



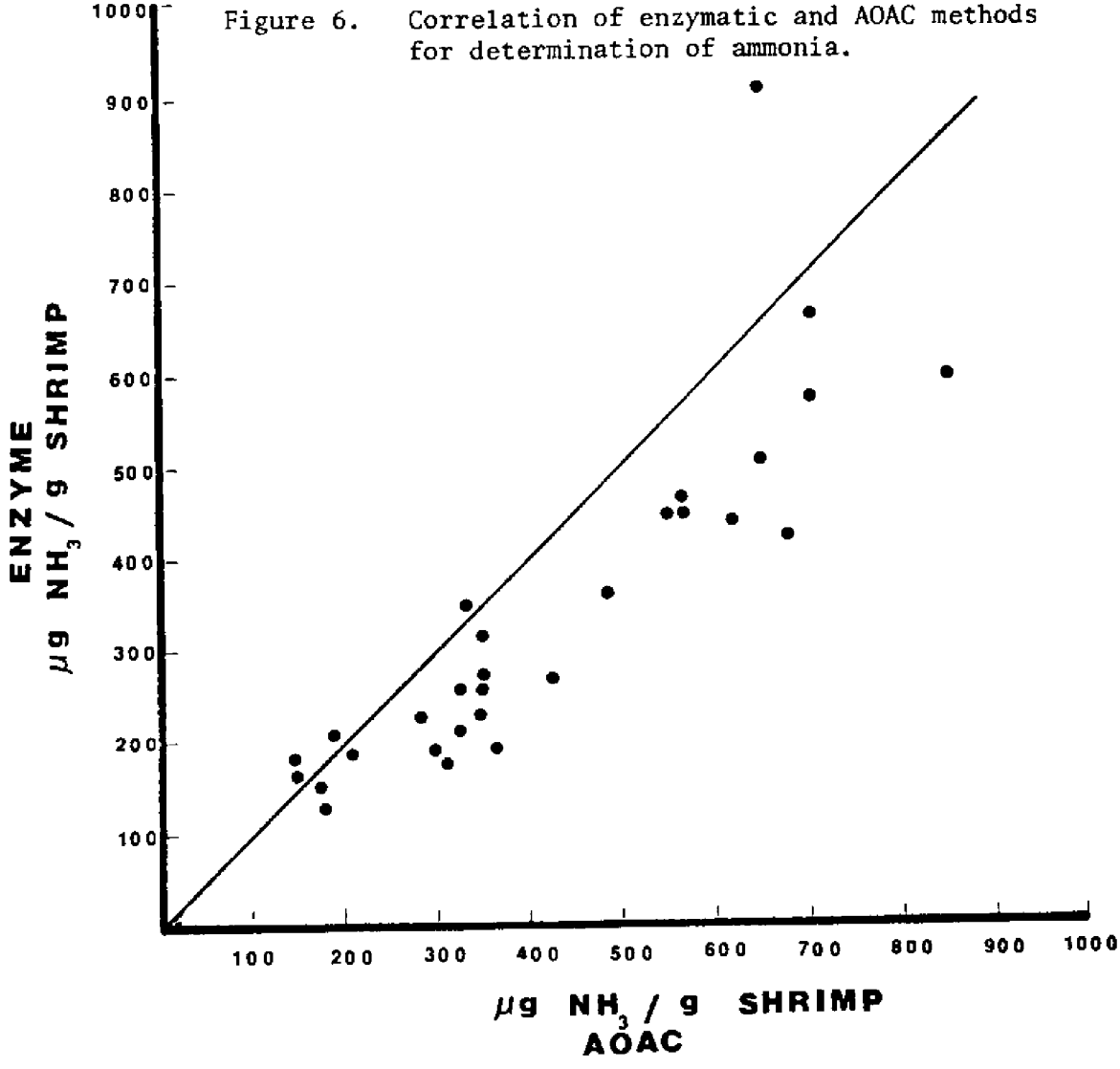


Figure 6. Correlation of enzymatic and AOAC methods for determination of ammonia.

DETERMINATION OF PHOSPHOROUS IN SHRIMP TREATED WITH SODIUM TRIPOLYPHOSPHATE

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Polyphosphates are being used extensively by the seafood industry for both fresh and frozen products. Among many advantages claimed, the most important reasons for using polyphosphates include: limited weight loss during frozen storage, less drip loss, less toughening of the product when stored in the frozen state and less loss in weight in cooking (Ellinger, 1972). Since polyphosphates, according to FDA, (Food and Drug Administration) have GRAS (Generally Recognized As Safe) status, it is up to the individual processor to formulate treatment mixtures and application techniques.

To determine the mechanism of action of phosphates on foods, it is essential that added phosphates be accurately determined at a later stage. For many proteinacious foods such as meats, poultry, processed cheese, and seafoods this is difficult because of the high and variable endogenous phosphate levels. These foods contain phosphorous in the form of nucleotides, phospholipids, etc. together with naturally occuring orthophosphates. Recommended methods for the analysis of phosphorous in foods involve a nitric acid digestion which will convert total phosphorous into orthophosphate which subsequently is determined. With varying background levels, added phosphates are thus difficult to determine accurately.

The objectives of this study were to: (a) determine the phosphorous content in peeled and deveined shrimp after different STP treatments, (b) determine the stability of STP during treatments and subsequent frozen storage using a P^{32} labeled STP isotope.

METHODS AND MATERIALS

Phosphorous Determination

Alkalimetric. Brown shrimp, (Penaeus aztecus), frozen as shell-on tails (green-headless) in 5 pound boxes, were used for this portion of the study. The shrimp were thawed, peeled and deveined prior to

polyphosphate treatment. Aqueous sodium tripolyphosphate (STP) solutions were prepared using purified granular STP (Fisher Scientific Company). Solutions of 0.5%, 1.0%, 5.0% and 12.0% were employed and dip times at each concentration were 20 sec, 1 min, 5 min and 20 min. Shrimp from each of the 16 different treatments were digested in nitric acid and analyzed for phosphorous according to the alkalimetric ammonium molybdophosphate method (AOAC, 1970). Untreated shrimp were analyzed as controlled samples.

Spectrophotometric. Shrimp used in this phase of the study were of the same species and size as those above. In this case, however, shrimp were obtained fresh from shrimp trawlers, put on ice and transported to the laboratory in College Station. The shrimp were divided into two lots of which one was peeled, deveined and polyphosphate treated immediately while the other was frozen as green-headless in 5 pound boxes. After two weeks of storage at -26°C, the shrimp were thawed in running cold water, peeled, deveined and polyphosphate treated. The phosphate treatments were the same as described for the alkalimetric method. After treatment, shrimp were digested in concentrated nitric acid and the digests determined for phosphate according to the method of Halmann (1972) using a Perkin-Elmer Coleman 124 double beam spectrophotometer with untreated shrimp as reference cell blank.

Hydrolysis of Sodium Tripolyphosphate

To determine the stability of STP in frozen shrimp during storage, peeled and deveined fresh brown shrimp were soaked in a 10% P^{32} labeled STP isotope solution for 20 minutes according to the method of Tenhet et al. (1980). After treatment, the shrimp were frozen in plastic bags and held at -26°C. After two weeks of storage, two 50 g portions were removed from the freezer and thawed at room temperature. One portion was blended in a Waring blender with 100 ml of a 7% trichloroacetic acid solution while the other was blended with 100 ml of distilled water. The slurries were centrifuged at 5000 x g for 20 min. at 4°C and the supernatants analyzed for phosphates using thin layer chromotography (TLC). Ascending chromatography was carried out on 20 x 20 cm glass plates coated with cellulose MN 300 HR (Machery Nagel Co.) using a mixture containing 150 ml isopropanol, 125 ml H_2O, 100 ml ethanol, 75 ml n-propanol, 50 ml n-butanol, 10 g trichloroacetic acid, and 0.5 ml concentrated ammonia (0.88) as irrigant. This is a modified compositon of one reported by Gibson and Murray (1973) and another suggested by Stahl (1969). After 10 min heat activation at 110°C the cooled plates were prepared by spotting a band of extract across the entire plate. To determine correct Rf values, two standard plates each spotted with 5ul of a 2% STP solution, a 2% sodium pyrophosphate solution and a 2% sodium orthophosphate solution were run concurrently with the active phosphate analysis. One of the two standard plates was developed using the ammonium molybdate-stannous chloride method described by Gibson and Murray (1973) while the other was sprayed with the phosphate stain suggested by Kates (1972). The two plates containing the isotope extracts were dried but not color developed. One centimeter bands of coating, beginning at the solvent front and working downwards, were

scraped off across the entire plate and collected in scintillation vials. Thirteen one centimeter fractions were collected from each plate and Rf values are reported as the mid-point of each fraction. After additon of scintillation fluid and thorough mixing, each fraction was counted for ten min using a Packard-Tri Carb Liquid Scintillation Spectrometer Model 3330.

All subsequent TLC analyses related to frozen storage stability of STP were performed on water extracted samples. These samples were analyzed after five, eight and ten weeks of frozen storage of the treated shrimp.

RESULTS AND DISCUSSION

Phosphorous Determination

The phosphorous concentration, reported as P_2O_5, in the first set of shrimp treated with STP determined by the alkalimetric AOAC method are shown in Table 1. The data indicates that when treating shrimp with diluted STP concentrations (0.5% and 1.0% STP dips) the phosphate uptake is very low. In only two of eight treatments at these concentration levels did the phosphorous concentrations in treated shrimp exceed the range found in untreated samples. The wide phosphorous range found in the control samples is in agreement with the data by Sidwell et al. (1977). In a review article they reported an average phosphorous level of 239 mg P/100 g shrimp and prawns (mixed species) with a range from 127 mg P/100 g to 912 mg P/100 g shrimp based on 44 analyses. Reported as percent P_2O_5 these values correspond to an average of 0.55% P_2O_5 with a range of 0.29% to 2.1% P_2O_5. Bailey et al. (1956) showed that acid soluble orthophosphate dropped during storage on ice from approximately 650 mg $PO_4^{\equiv}$/100g shrimp after two days to 400 mg $PO_4^{\equiv}$/100g shrimp after 14 days. Reported as P_2O_5 the data indicate a drop from 0.49% P_2O_5 to 0.30% P_2O_5 during storage on ice.

The low polyphosphate uptake on low treatment concentrations is in agreement with the findings of Tenhet et al. (1980; paper in press) who showed low polyphosphate penetration into shrimp muscle during sodium tripolyphosphate treatments at 0.5% and 1.0% STP. Low polyphosphate uptake after treatment of fish fillets was also demonstrated by Gibson and Murray (1973).

The phosphorous concentrations in the second set of shrimp treated both fresh and prefrozen with STP are shown in Tables 2 and 3. A sample of untreated shrimp was in this case used as instrument blank achieving automatic corrections for natural background phosphorous levels. Even though the polyphosphate uptake in the second set of shrimp was higher than in the first set, it was again evident that polyphosphate uptake at weak dip concentrations was bery low. No significant difference in polyphosphate uptake between fresh and prefrozen shrimp was determined.

Thin layer chromatography of phosphates

Shrimp treated with a P^{32} labeled STP isotope were kept in frozen storage at -26°C and a first sample was removed after two weeks. To determine the effect of extraction media on the polyphosphates present in the shrimp, the sample was divided into two equal parts. One part was extracted with 7% TCA and the other with distilled water. Immediately after extraction and centrifugation, the samples were analyzed by thin layer chromotography. Standard phosphate solutions were analyzed simultaneously with the shrimp extracts.

The thin layer chromatography (TLC) system separated the phosphates well. Rf values obtained from the developed standard plates were: 0.32 for tripolyphosphate, 0.47 for pyrophosphate and 0.71 for orthophosphates. For the pyrophosphate standard, a faint spot could be seen at the Rf value of orthophosphate and for the tripolyphosphate faint pyrophosphate and orthophosphate spots were evident. This indicates that some hydrolysis of the condensed phosphates will occur during the TLC analysis.

Although color development of the phosphates worked well at the high concentrations used on the standard plates, the method was not sensitive enough for analyzing added phosphates in treated shrimp. At treatment levels used in these experiments only faint spots could be seen after extraction and TLC analysis. Using a STP isotope makes the TLC method sensitive and low levels of phosphates could be detected. Figures 1 and 2 show the radioactive distribution of TCA and water extracted samples respectively. For the TCA extracted samples two distinct Rf values of 0.47 and 0.75 indicate complete hydrolysis of STP to pyro and orthophosphate. This was not unexpected since polyphosphates undergo hydrolysis under acid conditions. In the water extracted sample on the other hand, three high activity Rf values could be detected on the TLC plates (Figure 2). These three peaks represented according to Rf values from the standard plates: STP, pyrophosphate and orthophosphate.

The total plate activity of the TCA extracted samples was 962 counts/min as compared to a total plate activity of 1074 for the distilled water extracted sample. Since sample size, extraction and spotting values were the same for the two samples, there was apparently little difference in the phosphate extraction efficiency between the two solvents used.

To determine the stability of STP in frozen treated shrimp over a prolonged time interval, samples were analyzed after five, eight, and ten weeks of frozen storage. The samples were extracted with distilled water and the extracts analyzed by TLC and scintillation counted as described above. Figure 3 shows the distribution of active phosphates after different storage periods. As indicated, there was an initial drop in STP down to approximately 12% of total activity during treatment and the first two weeks of storage. During the same time, pyrophosphate increased to 23% and orthophosphate to 27%. During

continued frozen storage STP appeared to remain fairly constant at around 12% while pyrophosphate dropped down to 10% after 5 weeks and 2% after 10 weeks. Orthophosphate increased gradually and showed 45% of total activity after 10 weeks of frozen storage.

Activity observed on the plates at unidentifiable Rf values most likely represents phosphates tied up with compounds present in shrimp. This was specifically evident after 10 weeks of frozen storage where 15% of total activity was found to be completely immobile at Rf = 0. This immobile fraction could well represent phosphates complexed with high molecular water soluble proteins.

CONCLUSION

Because of the natural variation in the phosphorous level in shrimp and the breakdown of STP during treatment and frozen storage, it is not possible to determine STP added to shrimp. The study has shown however, that at high treatment concentrations there is enough of an increase in phosphorous so that potential overtreatments can be detected.

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Table 1.

Phosphorous in treated shrimp as determined by alkalimetric method.

% STP in treatment solution	% phosphorus (reported as P_2O_5) in shrimp muscle			
	Time of Treatment			
	20 sec	1 min	5 min	20 min
0.5	0.78	0.80	0.59	0.59
1	0.66	0.67	0.57	0.49
5	0.57	0.72	1.42	1.70
12	0.91	1.28	1.49	1.61

Note: Untreated shrimp gave a value of 0.57% P_2O_5 with a range for five samples of 0.41% to 0.76%.

Table 2.

Phosphorous in treated fresh shrimp as determined by spectrophotometric method.

% STP in treatment solution	% phosphorus (reported as P_2O_5) in shrimp muscle			
	Time of Treatment			
	20 sec	1 min	5 min	20 min
0.5	0.27	0.32	0.53	0.42
1	0.47	0.52	0.27	0.23
5	0.56	0.65	1.10	1.37
12	1.03	1.35	2.43	2.37

Table 3.

Phosphorous in treated prefrozen shrimp as determined by spectrophotometric method.

% STP in treatment solution	% phosphorus (reported as P_2O_5) in shrimp muscle			
	Time of Treatment			
	20 sec	1 min	5 min	20 min
0.5	0.56	0.64	0.37	0.10
1	0.52	0.47	0.53	0.45
5	0.79	1.09	1.40	1.80
12	1.19	1.75	1.76	2.70

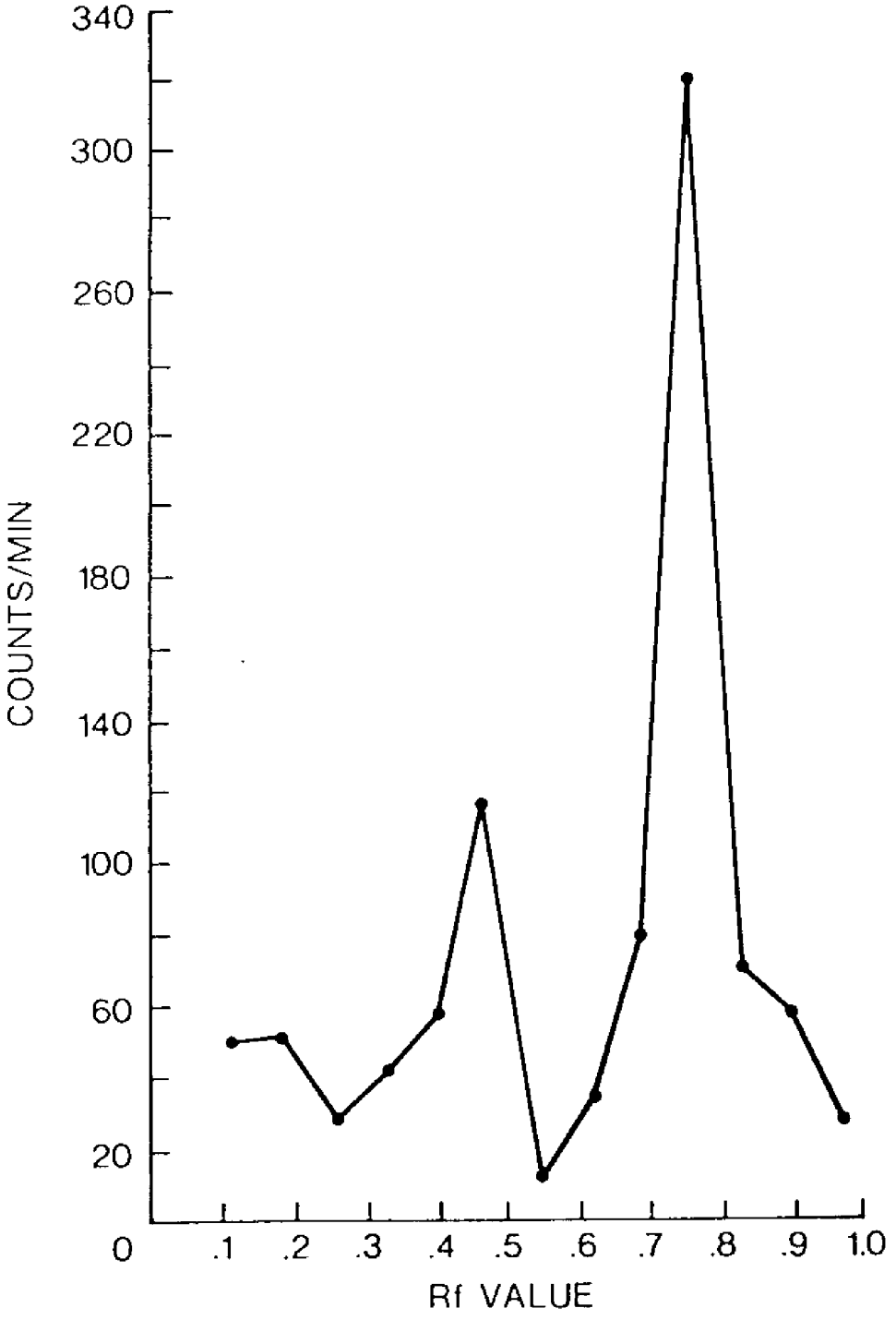

Figure 1. Distribution of activity on TLC plate for TCA extracted sample.

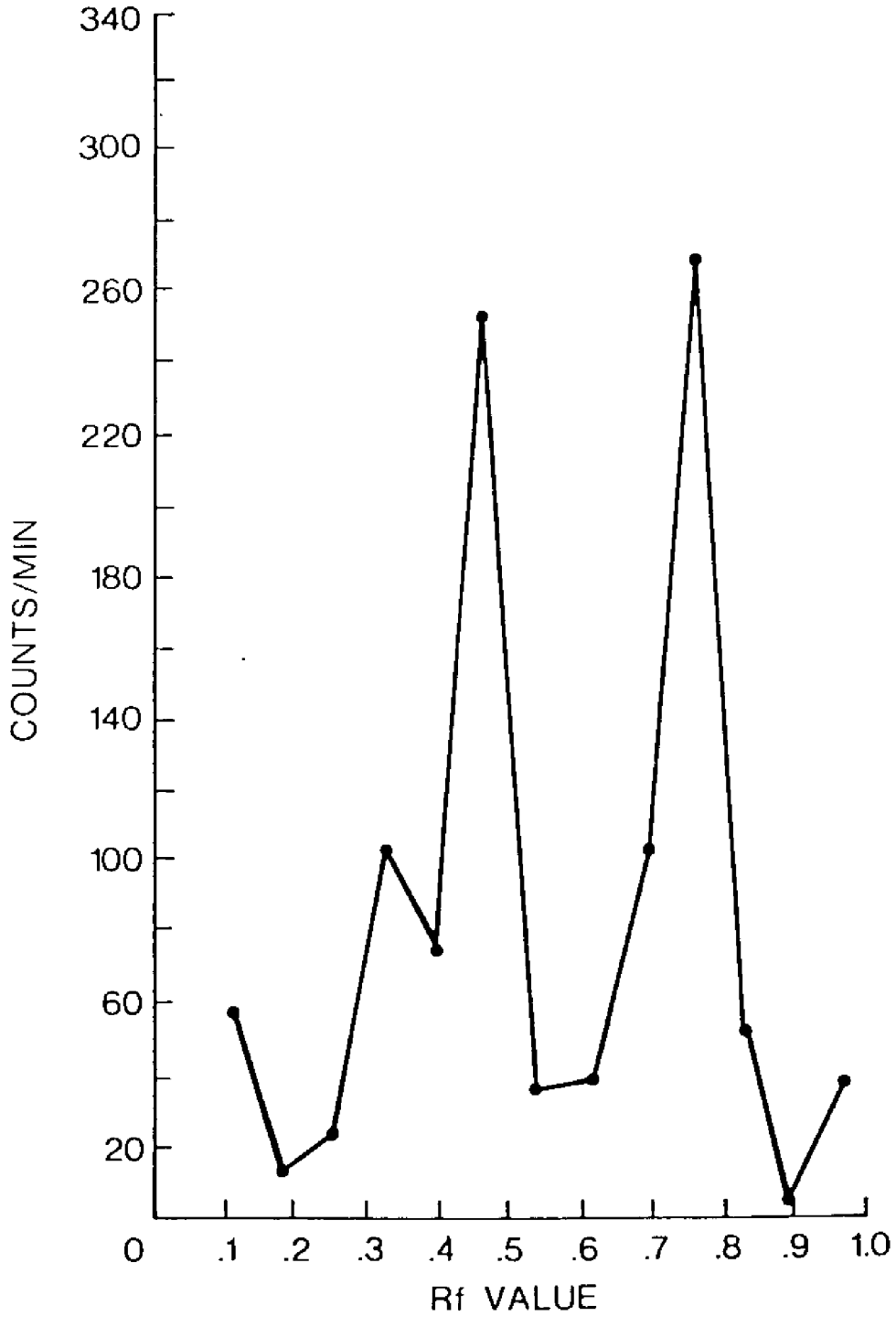

Figure 2. Distribution of activity on TLC plate for distilled water extracted sample.

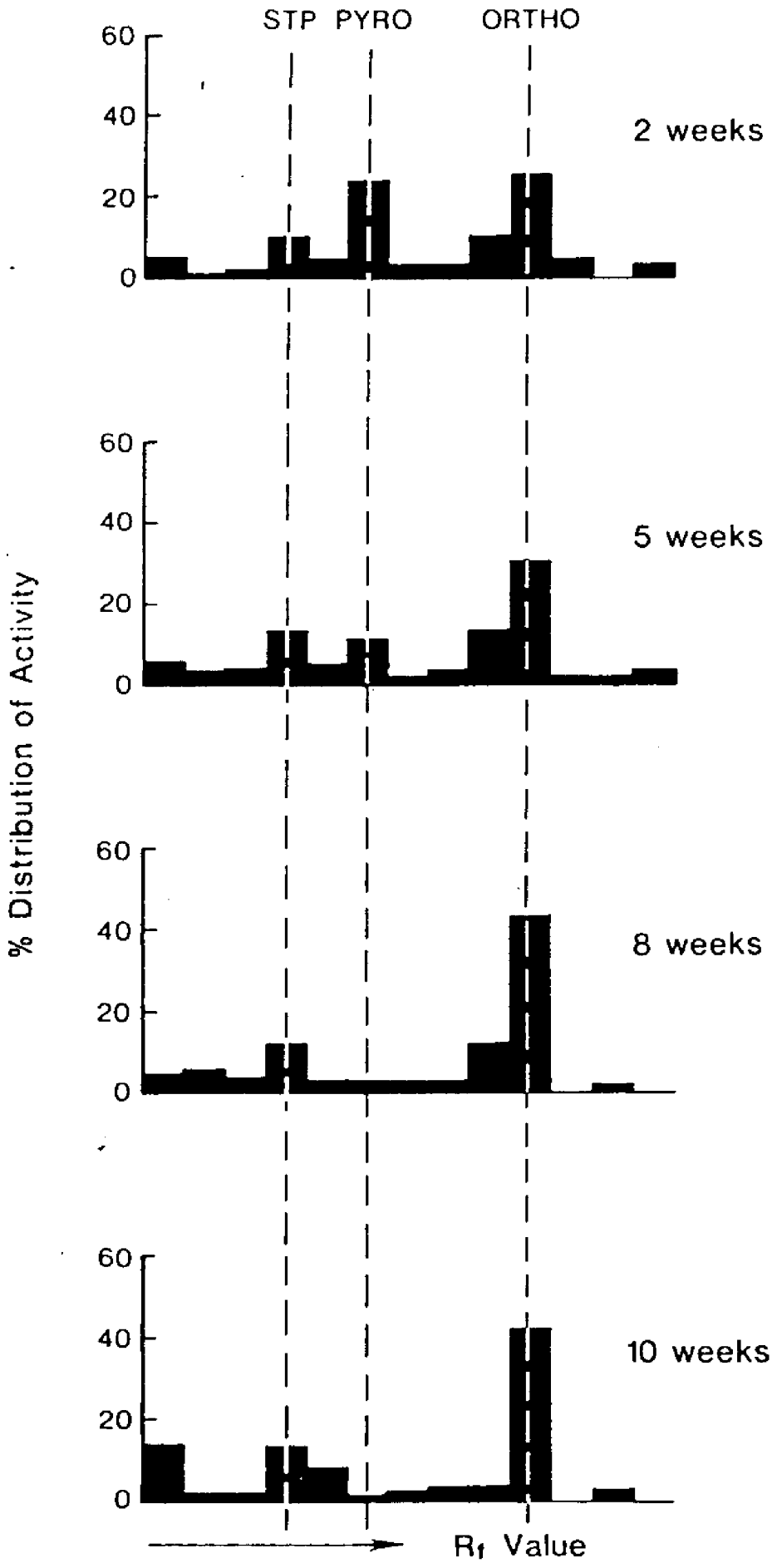

Figure 3. Distribution of activity on TLC plates as a function of frozen storage time.

TEXTURAL VARIABILITY IN FISH FILLETS

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INTRODUCTION

The growing cost of marine food products has created potential markets for fish species that have been underutilized. The U.S. Department of Commerce has initiated studies that will create the data basis for establishing policies and priorities regarding the introduction of new species to the domestic market. One such study focuses on the quality and consumer acceptability of such "new" fishes as compared with traditional and popular species. One of the more specific objectives of this project has been to monitor and evaluate the textural components of fish quality. This has been done through organoleptic assessment by a trained tasting panel and through objective mechanical analysis based on compression tests. The latter has introduced some unusual procedural and interpretation problems. The reason is that obtaining proper specimens for mechanical analysis is frequently very difficult. Even when tests can be performed,at acceptable degree of accuracy, there are still interpretation problems that are associated with the possibly inherent error of the test vis a vis the natural textural variability within and among the different fillets. In this report, this problem of variability is addressed and its implications assessed.

MATERIALS AND METHODS

Refrigerated fillets of a variety of fish species were brought to the laboratory within 24 hours after commercial filleting. Some of the fillets were cooked in plastic bags, submerged in a hot water bath, with circulating water at a temperature of 69°C. The process was stopped when the center temperature reached 65°C. The required cooking time was 6 to 35 minutes

depending on the thickness of the fillet. The samples were then removed from the bath and left to cool at ambient temperature.

Specimens of both raw and cooked fillets were carefully cut using an electric carving knife. During cutting the surrounding tissues were held in place by a device constructed of a dense array of needles which guided the knife and at the same time prevented the disintegration of the outer wall of the specimen. The specimens were always cut in a direction perpendicular to the fillet plane regardless of the local orientation of the flakes.

The cross-sectional area of each specimen was 4x4 cm and the height varied between 0.8 to 3 cm depending on the species.

The compressive deformation tests were performed by an Instron Universal Testing Machine equipped to provide force deformation output in a digital form. The data were converted to true stress-strain relationships from which the deformability modulus was calculated. The calculation procedure is described below.

Calculation of the Modulus

The apparent modulus of deformability M (Fig. 1) is defined as:

$$M_{APP} = \frac{\sigma_T}{\varepsilon_T} \qquad (1)$$

where σ_T and ε_T are the true stress and strain respectively.

For an incompressible material and with the assumption that the specimen retains its general shape during deformation:

$$\sigma_T = \frac{F(t)}{A(t)} = \frac{F(t)[H_o - \Delta H]}{A_o H_o} \qquad (2)$$

where F(t) is the force, A(t) the actual cross-sectional area, A_o and H_o are the original cross-sectional area and height of the undeformed specimen and ΔH the absolute deformation.

The true compressive strain under these conditions is:

$$\varepsilon_T = \ln \left[\frac{H_o}{H_o - \Delta H} \right] \qquad (3)$$

Since the specimens, for obvious reasons, were rather flat in shape the apparent moduli, as calculated by Eq. 1, were supposed to depend not only on the tissue properties but also on the dimensions of the specimens. This phenomenon, well recognized in mechanics, makes it necessary to correct the calculated values for the characteristic shape effects. This was done by adapting the method described by Lindley (1) which was originally developed for rubbery materials. According to this method, the corrected or independent modulus (M_{CORR}) is calculated from:

$$M_{CORR} = \frac{M_{APP}}{1+2kS^2} \qquad (4)$$

where k is a constant that is related to the hardness of the specimen and S is the specimen shape factor. The magnitude of S is determined from the ratio between the loaded area and the free (lateral) area. Since all the reported specimens had a 4x4 cm loaded area, the shape factor was:

$$S = \frac{4x4}{2(4+4)H_o} = \frac{1}{H_o} \qquad (5)$$

where H_o is the initial thickness of the specimen.

RESULTS AND DISCUSSION

The Shape of the Stress-Strain Curve

Schematic representation of prefailure stress relationships of raw and cooked fish fillets are shown in Figure 1. The figure demonstrates that up to a certain strain, usually in the range of 20 to 40%, the relationship was linear, supported by correlation coefficients of 0.95 to 0.99 as reported by Johnson et al. (in press). This enabled the calculation of the apparent deformability modulus from the slope of the straight line for each individual specimen.

The correction of the modulus magnitude by Eq. 4 requires knowledge of the hardness of the specimen. (Hardness in this context is the resistance to penetration which is not identical to the deformability modulus). Since this parameter was not measured, and it is still an open question whether it can meaningfully be determined in materials like fish flesh, the correction procedure was applied using three levels of k covering the whole hardness range as reported in Ref. 1.

Typical values of the apparent and corrected moduli for fillets of three fish species are demonstrated in Tables 1 to 3. (Compiled results of a variety of species have been shown during the oral presentation of this paper).

Analysis of the data revealed that the variability of the moduli among and between fillets is real and not a result of experimental artifacts resulting from specimen shape differences and imperfections. Although these certainly do contribute to the variability to some extent they cannot account for such large differences, especially in the corrected moduli. Similarly, cooking of the fillets did not produce consistent results with regard to the magnitude of the deformability moduli. In some cases, considerable softening has been observed while in other cases the texture has become firmer. Again, only part of the phenomenon can be attributed to variations in the time-temperature histories of the cooked fillets that resulted from their dimensional variability. The irregularity with regard to the overall trend is an indication that the individual fish were in different states from a biochemical standpoint (3,4).

It is interesting to note, however, that from a mechanical point of view all the cooked specimens demonstrated the same deformation pattern which was clearly distinct from that of the raw specimen. This is clearly expressed in the shape of the true stress-strain curve continuation after the end of the linear region (Fig. 1).

The concave downwards shape in the case of cooked fillets is an indication of a progressive disintegration of the tissue as a result of fibers and flakes separation and breakdown. In the raw fillets the concave upwards continuation is most likely the result of hydrostatic pressure buildup as well as some compressibility of the tissue itself.

CONCLUSIONS

A method for monitoring and evaluating textural variability in fish fillets has been demonstrated. Its application revealed that natural mechanical variations in commercially available fillets is considerably large within and among specimens. Mainly this is due to biochemical factors resulting from seasonal, and feeding differences. It is also most likely, however, that the age and postmortem history of the fish are also major factors. The latter is also true with regard to the filleting operation itself that introduces differences in the mechanical history of the various regions of the fillet.

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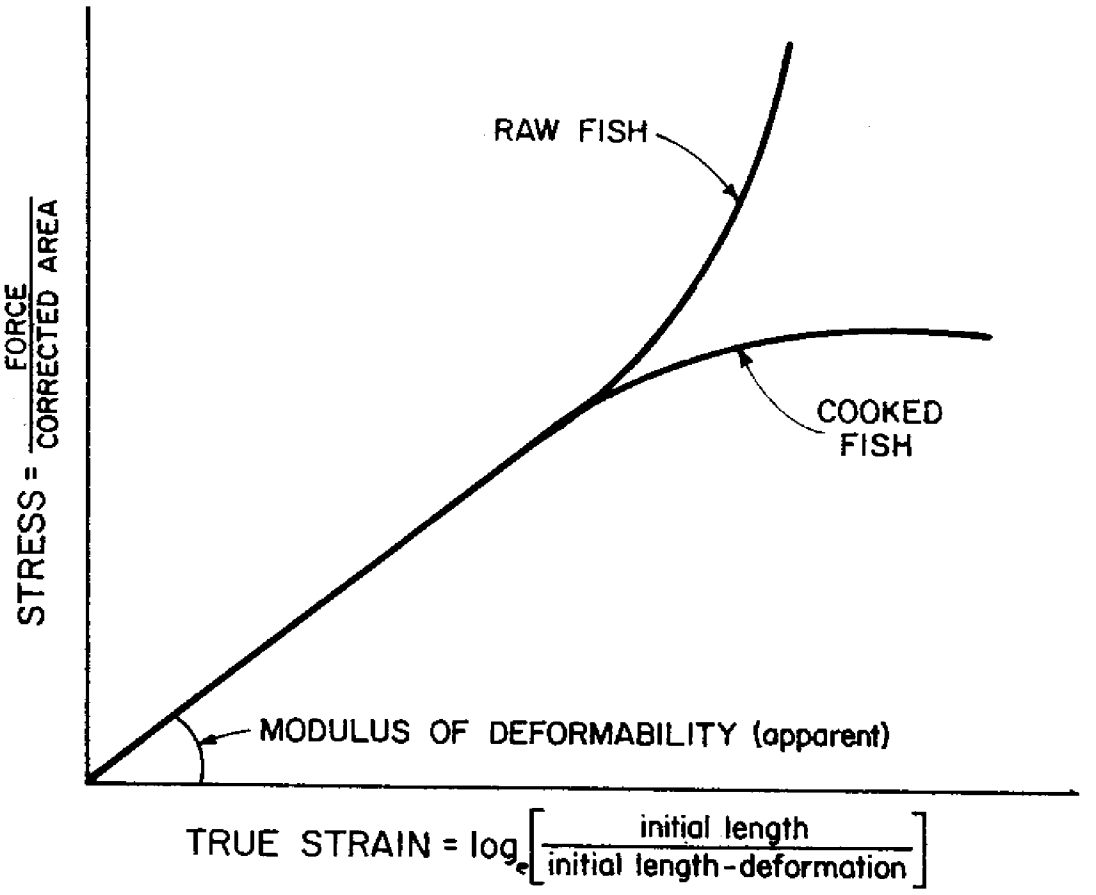

Fig. 1 Compressive stress-strain relationships of fresh and cooked fish fillets (schematic view).

State	Specimen Location	Apparent Modulus ($N.cm^{-2}$)	Shape Factor S	Corrected Modulus ($N.cm^{-2}$)		
				k=0.5	k=0.7	k=0.9
Raw	front	0.86	0.35	0.77	0.73	0.70
Raw	front	1.08	0.39	0.94	0.89	0.84
Raw	front	0.66	0.45	0.55	0.51	0.48
Cooked	front	1.59	0.63	1.14	1.02	0.92
Cooked	middle	1.11	0.55	0.85	0.78	0.72
Cooked	front	0.97	0.56	0.73	0.67	0.62

Table 1. Compressive deformability moduli of fresh and cooked bluefish fillets.

State	Specimen Location	Apparent Modulus ($N.cm^{-2}$)	Shape Factor S	Corrected Modulus ($N.cm^{-2}$)		
				k=0.5	k=0.7	k=0.9
Raw(I)	front	4.53	0.29	4.18	4.06	3.94
	middle	4.78	0.67	3.31	2.94	2.65
Cooked(I)	front	2.64	0.35	2.36	2.26	2.17
	middle	2.09	0.41	1.79	1.69	1.61
	tail	1.87	0.45	1.56	1.46	1.37
Raw(II)	front	2.08	0.36	1.84	1.76	1.68
	middle	1.69	0.46	1.39	1.30	1.22
Cooked(II)	front	2.80	0.41	2.39	2.26	2.14

Table 2. Compressive deformability moduli of fresh and cooked fillets from same individual codfish.

State	Specimen Location	Apparent Modulus ($N.cm^{-2}$)	Shape Factor S	Corrected Modulus ($N.cm^{-2}$)		
				k=0.5	k=0.7	k=0.9
Raw	front	1.20	0.53	0.94	0.86	0.80
Raw	front	2.24	0.51	1.79	1.65	1.54
Raw	front	2.08	0.28	1.92	1.87	1.82
Raw	middle	1.71	0.42	1.46	1.37	1.30
Cooked	front	2.15	0.60	1.59	1.44	1.31
Cooked	front	1.92	0.60	1.42	1.28	1.17

Table 3. Compressive deformability moduli of fresh and cooked hake fillets.

OXIDATIVE RANCIDITY IN WHOLE-GLAZED FROZEN CRAWFISH

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INTRODUCTION

The increasing demand for crawfish in the European market has resulted in the overseas shipment of crawfish by a number of processors. The crawfish were shipped either live or frozen; however, problems were encountered with both methods. Plane delays, poor handling practices, and lack of refrigeration have resulted in poor survival rates when shipping the product alive. Shipping the product frozen eliminates the survival problem; however, rancidity problems and general overall poor quality have hampered frozen shipments. Thus, the need has risen for a more appropriate method for the long term preservation of crawfish for shipping to distant markets.

Many processors thought that the shell (exoskeleton) protected the product from dehydration and oxidative deterioration; however, this does not appear to be the case. Moreover, a number of workers (3,4,8,9) have indicated that enzymes in the hepatopancreatic tissue of crawfish are responsible for the development of rancidity during storage which minimizes the role of the shell in protecting the product.

The main purpose of the present study is to monitor the development of oxidation rancidity in whole-glazed crawfish during ten months of frozen storage, as indicated by TBA values; this, consequently, is to serve as an index to the maximum length of freeze-storage time without development of oxidative rancidity.

MATERIAL AND METHODS

Samples: Crawfish were obtained from a local seafood market in Baton Rouge, Louisiana, and transported to the Food Science Department at Louisiana State University. Dead animals were discarded and the live ones were divided into three lots. The first lot was frozen alive, whereas the other two lots were cooked for 5 and 10 minutes respectively in boiling water ($100^{o}C$). The samples were placed in separate plastic trays, sealed and frozen at $-18^{o}C$.

Glazing: Samples were glazed one day after freezing by dipping in water at $0^{o}C$ for three seconds; and ice film was formed around the animals instantly after which the trays were drained and re-sealed. This process was repeated every two months throughout the storage period.

2-Thiobarbeturic Acid Test: TBA values of Trichloroacetic acid extracts were determined by the method of Vyncke (16). Determinations were made at two month intervals on the tailmeat alone as well as the tailmeat with the hepatopancreatic tissue for each given cooking time. A 1,1,3,3 - tetraethoxypropane (TEP) standard curve was used to calculate TBA values.
Statistical Analysis: A Randomized Complete Block Design was followed in designing the experiment. Analysis of variance were run on the data using General Linear Model procedure (1). Duncan's Multiple Range Test was run on both time within treatment and cooking time. All samples were run in duplicates and two determinations were made on each duplicate.

RESULTS AND DISCUSSION

The overall means for the various treatment combinations concerning TBA values are shown in Table I. The analysis of variance (Table II) revealed that the first order interactions were all highly significant ($P<.01$). The effect of storage time and the presence or absence of hepatopancreatic tissue (crawfish fat) with regard to TBA values are depicted in Figure I. For the first four months of storage the washed tailmeat obtained from the whole frozen crawfish exhibited lower TBA values than tailmeat analyzed with the hepatopancreatic tissue. However, as the storage period extended, the differences became progressively less thus resulting in the highly significant interaction. Ying (18) determined that the tailmeat of crawfish contains only 2% fat, whereas the hepatopancreatic tissue contains approximately 50% fat on a dry weight basis. Therefore, it is unlikely that the rapid increase in TBA values in the tailmeat is due to oxidation of fat in this area. It is probable that malonaldehydes which are volatile and responsible for the TBA test, permeated from the hepatopancreatic tissue to the tailmeat as storage progressed, resulting in an equilibrium condition being established. Another possible explanation to this phenomena would be the difference in nature of fat between the tailmeat and the hepatopancreatic tissue which resulted in a more stable tailmeat.

The effect of time within treatment (presence or absence of hepatopancreatic tissue) as measured by Duncan's Multiple Range Test is shown in Table III. A rapid increase in TBA values occurs in the first two months of storage followed by a period of several months with little change. In the latter stages of frozen storage, TBA values again increased. This trend was observed for both treatment and cooking time.

The cooking time vs. storage time was also found to be highly significant ($P< .01$). This interactional effect is shown in Figure II. Although the trend observed in Figure II is inherent to the TBA test, and has been reported by a number of workers (11, 5, 2), it seems that a reaction between the malonaldehydes produced and other constituents such as proteins is responsible for the drop in malonaldehydes present at the observed points; if so, this should have an effect on the texture of the tailmeat as well as the solubility of the protein. This point is being investigated in this lab and will be reported on in the near future.

Figure II also shows that cooking resulted in an initial increase in TBA values as opposed to the raw product. This confirms the work of Mai (11), Yamauchi (17), and Lee and Toledo (10) who found that cooking of meat or fish accelerates the oxidative rancidity during short term refrigeration as indicated by TBA values. This was found true only in the first two months of frozen storage (Figure II and Table I) when raw meat had lower TBA values than cooked crawfish tailmeat. TBA values were accelerated, however, at a higher rate in the case of raw meat and became higher than the values in the cooked meat after the second month of storage. In the light of these findings, it seems likely that an oxidative enzyme system similar to the lipoxygenase found in plants is present in crawfish and is inactivated by heat treatment which resulted in the superior keeping quality of the cooked meat. This hypothesis confirms the finding by Tsuduka and Amano (15) that an enzyme similar to lipoxygenase existed in fish and is responsible for the discoloration of the carotenoid pigment of the red fish skin. This hypothesis was dismissed by Eskin (6) and the observed effects were attributed to the hemeprotein present in the blood of fish. It is well established that such a hemeprotein does not exist in the crustaceans.

As seen in Table IV raw tailmeat samples showed a rather steady or consistent increase in TBA values, whereas samples blanched for 5 to 10 minutes showed faster initial increases followed by a lag period, and finally another increase after the sixth month.

TBA values corresponding to rancidity are not fully agreed upon; they range from a value of one (7) to forty (12). These values apparently vary by the type of food system and the method of analysis applied. Sanches (14), using the same method used in this study, did not detect any rancidity in crawfish tailmeat with a TBA value as high as 4.95 when using a taste panel. During the course of this study no rancid flavor was detected in the cooked samples throughout the ten months of storage. On the other hand, a slightly rancid odor was observed in the raw crawfish fat after the sixth month of storage. This prompted the authors to set the level of rancidity at a TBA value of 6.5 for crawfish. The overall high TBA values observed using this procedure as compared to the distillation method(s) is attributed to the fact that other TCA extractable substances as well as the copper ion present in crawfish blood (13) tend to amplify the TBA values.

The absence of apparent oxidative rancidity observed during this study is due to the effect of glazing which helped exclude the oxygen. The main problem in the packaging of whole-glazed frozen crawfish is that there is a considerable void area in the package due to their body configuration. Thus, considerable surface area is exposed, resulting in rapid loss of the glaze. In this study the product was reglazed every two months which would be difficult under commercial conditions.

CONCLUSIONS

Frozen storage of whole-glazed crafish resulted in elevation of TBA values by increase in storage time; the rancidity threshold level was exceeded only in the case of the raw uncooked product at the sixth month of storage. The presence of hepatopancreatic tissue tended to raise the TBA values while the cooking of crawfish helped reduce them, thus making it possible to freeze crawfish for a period of ten months without objectionable rancid odor.

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Table I. Effects of Various Treatment Combinations and Storage Times on TBA Values in Frozen-Whole Crawfish*

STORAGE TIME	TAILMEAT			TAILMEAT + FAT		
(Months)	Raw	5 Min	10 Min	Raw	5 Min	10 Min
0	1.32	0.51	0.61	1.68	2.38	1.53
2	1.97	2.83	2.45	3.09	3.71	3.90
4	3.52	2.81	2.47	3.82	3.64	3.08
6	7.61	3.44	3.36	6.47	3.82	4.36
8	7.50	6.24	3.17	5.28	6.24	5.51
10	8.99	4.29	2.70	5.05	4.59	5.60

* Each reading is an average of four observations.

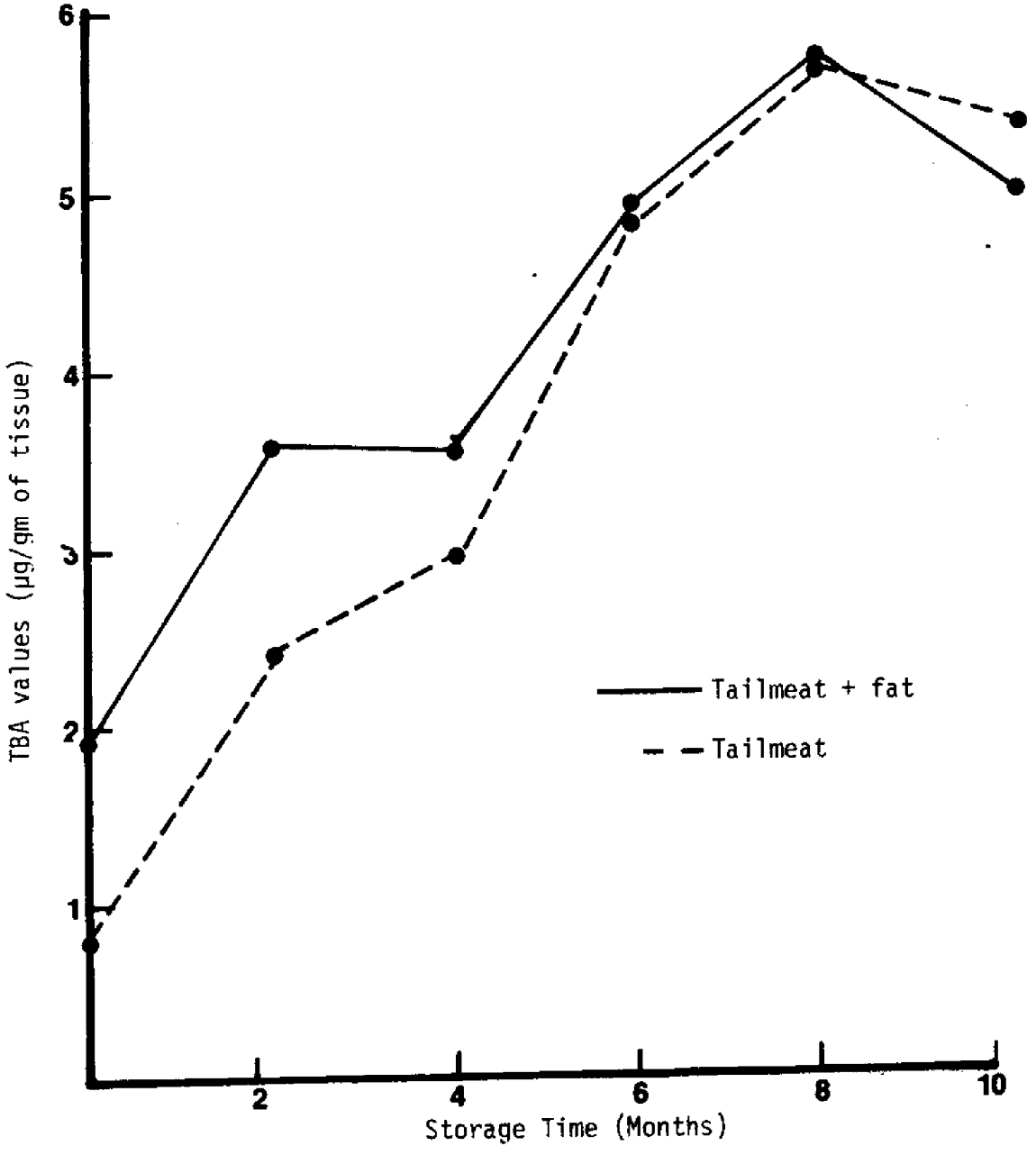

Figure I. Effect of treatment on TBA values

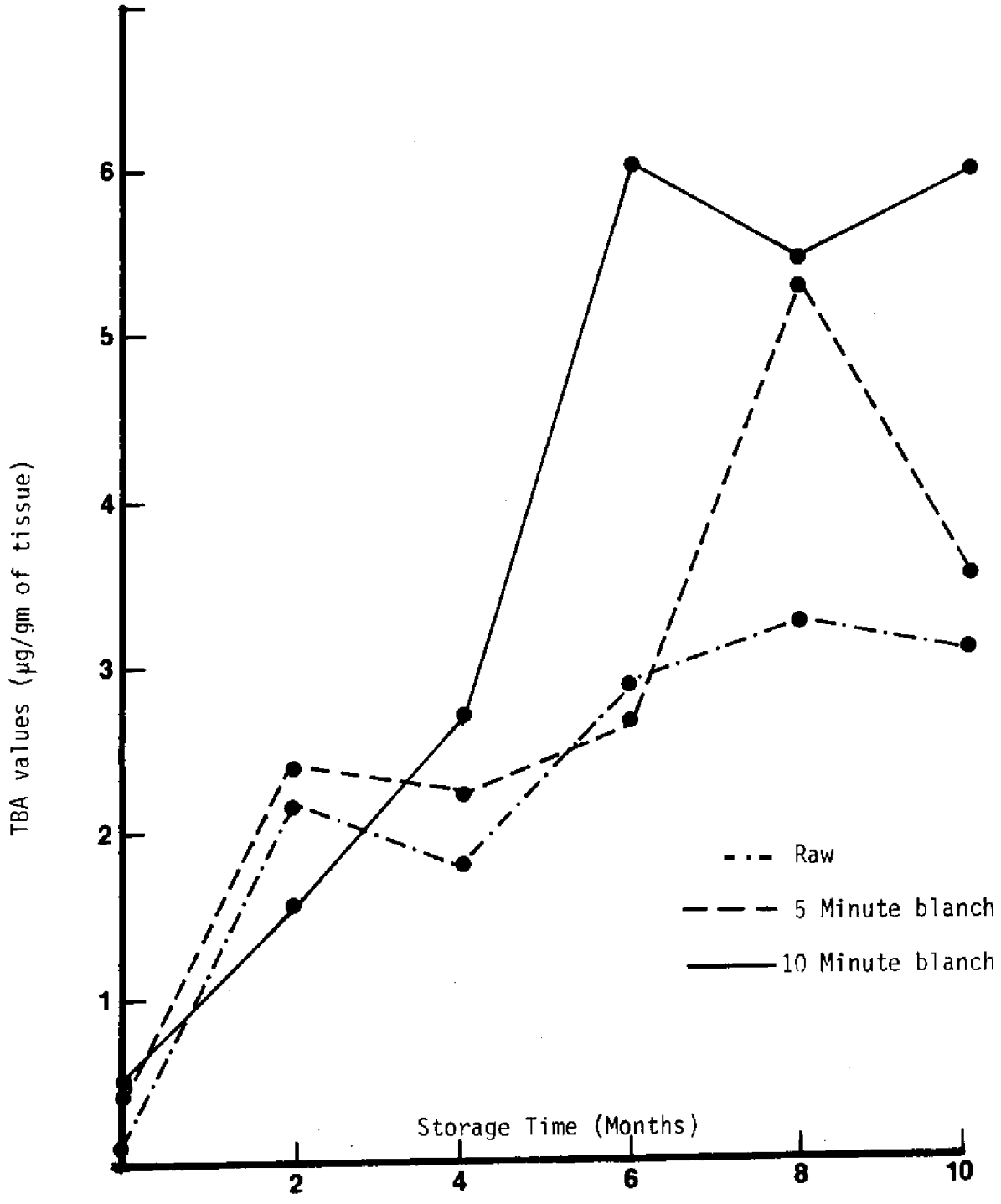

Figure II. Effect of cooking time on TBA values

Table II. Analyses of Variance of TBA Values of Whole-Glazed Crawfish

Source of Variation	D.F.	S.S.	M.S.	F
Storage Time[1]	5	324.63	64.92	441.63**
Treatment	1	7.05	7.05	47.98**
Storage Time x Treatment	5	9.97	1.99	13.54**
Cook Time	2	53.44	26.72	181.76**
Storage Time x Cook Time	10	72.04	7.20	48.98**
Treatment x Cook Time	2	37.41	18.70	127.21**
Storage Time x Cook Time x Treatment	10	38.85	3.88	26.39**
Error (Rep-Storage Time x Treatment x Cook Time)[2]	36	5.29	0.147	
Sampling Error	72	2.70		
TOTAL	143	551.38		

** Significant at 0.01 level.

[1] Storage Time = storage time in months
Cook Time = cooking time in minutes
Treatment = treatment "presence or absence of fat"

[2] Error term used to test variables in the model

Table III. The Effect of Time Within Treatment as Measured by the Duncan's Multiple Range Test on TBA Values in Frozen - Whole Crawfish.

Storage Time (months)	Tailmeat	Tailmeat + Fat
10	A 5.33	B 5.08
8	A 5.64	A 5.68
6	A 4.81	B 4.89
4	B 2.94	C 3.57
2	B 2.42	C 3.51
0	C .81	D 1.86

* Means with the same letter are not significantly different.

Table IV. Duncan's Multiple Range Test With Regard to the Effect of Storage Time Within Cooking Method on TBA Values in Frozen - Whole Crawfish*

Storage Time (months)	Raw	5 Min	10 Min
10	A 7.02	B 4.44	B A 4.15
8	A 6.39	A 6.24	A 4.34
6	A 7.04	C 3.63	B A 3.86
4	B 3.67	C 3.27	B C 3.17
2	C 2.53	C 3.22	C 2.77
0	C 1.50	D 1.44	D 1.06

* Means with the same letter are not significantly different.

A COMPARATIVE STUDY OF ANALYTICAL TECHNIQUES TO QUANTIFY AMMONIA IN DOGFISH

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Many methods for detecting quality losses in seafood have been proposed, and some of these such as the hypoxanthine assay (9) have wide application to fishery products. However, chemical methods that take advantage of unique features of the product being tested are often the most successful indicators of quality. For example, the official method for determining decomposition in tuna is the measurement of histamine (11), the decarboxylation product of free histidine which is present in high concentration (2 percent) in tuna muscle.

Ammonia is one of the most typical of spoilage products since it arises from a variety of sources common to all biological tissues. It is produced by the action of microorganisms as well as endogenous enzymes through deamination of proteins and amino acids and through breakdown of purines and various amines. Even so, a significant increase in ammonia often occurs too late in the course of quality loss for it to be used as a good index of decomposition. In cartilaginous fish such as sharks, skates, and rays, however, both trimethylamine oxide (TMAO) and urea are especially plentiful (8), and these fish develop a strong ammoniacal odor as they deteriorate. Elasmobranchs contain 1.5 to 2.5 percent urea distributed fairly evenly throughout their body tissues (12). Urea is converted to ammonia and carbon dioxide by marine bacteria possessing the enzyme urease (2) according to the equation:

$$\underset{\text{urea}}{H_2N\text{-}CO\text{-}NH_2} + \underset{\text{water}}{H_2O} \xrightarrow{\text{urease}} \underset{\text{carbon dioxide}}{CO_2} + \underset{\text{ammonia}}{(2)\ NH_3}$$

The production of two moles of ammonia from each mole of urea can lead to a rapid build-up of ammonia in shark bodies. Vyncke (14) found that the ammonia content of dogfish (*Squalus acanthias*)

correlated well with organoleptic judgment, and recommended the measurement of ammonia for objective quality assessment.

When dogfish are gutted and stored in ice immediately after being caught, the ammonia concentration increases very slowly for the first few days, then rises to an average level of 28 mg percent by eight days with a range of values from 20 to 40 mg percent (14). After this period, the increase is more rapid. By 14 days, the average content of ammonia is found to be in the range of 60 to 90 mg percent. These results are supported by data collected on other species of shark (15). Suyama et al. (13) have shown that shark held at 35°C developed an ammonia concentration in excess of 100 mg percent in less than one day, whereas at 9°C, the ammonia concentration did not begin to rise for four days. Therefore, ammonia concentration is an indicator of the temperature at which dogfish has been held and the handling it has received during processing.

Standard methods (10) for the determination of ammonia include two colorimetric techniques. One of these depends on the blue end-product of the reaction of ammonia with phenolic compounds and forms the basis for the current Association of Official Analytical Chemists (AOAC) method for determining ammonia in crabmeat. The other colorimetric method is known as nesslerization wherein ammonia forms a reddish-orange complex with mercuric oxide. Techniques for the measurement of ammonia in biological material are generally dependent on an isolation procedure in order to separate ammonia from various interfering substances. Hence, nesslerization is often coupled with the Conway micro-diffusion technique for the quantification of ammonia and other volatile amines (4). A third method involves distillation in the presence of magnesium oxide and titration steps which render it inapplicable to dogfish due to the high content of urea in this fishery product (1). Yet another method which has promise as a rapid test for ammonia in dogfish is a specific ion electrode. An ammonia-sensing electrode has been used to detect quality changes in shrimp (16), but its applicability to dogfish has not been examined. Unlike the chemical methods described above which require an ammonia isolation procedure, an enzymatic assay based on the conversion of α-ketoglutarate to glutamate in the presence of ammonia relies on the inherent specificity of the enzyme system for its substrates. The enzyme glutamate dehydrogenase catalyzes the reaction which is followed spectrophotometrically as the decrease in absorbance of nicotinamide adenine dinucleotide (NADH) due to oxidation. The technique is widely utilized for the analysis of ammonia in clinical samples and waste materials (6). Knight and Toom (5) have described the use of the enzymatic assay to determine ammonia in shrimp as a measure of decomposition.

Although there is at present only a limited market for elasmobranch fish in the United States, dogfish commands a good price in certain foreign countries and is exported when accompanied by documents attesting to the quality of the fish. Heretofore, National Marine Fisheries Service (NMFS) inspectors have been called upon to certify the quality of dogfish shipments based

primarily on organoleptic evaluation. Recently, two European countries have established limits for the amount of ammonia permitted in dogfish as determined by chemical testing, and a number of shipments have been rejected. Belgium denies entry to dogfish if the concentration of ammonia exceeds 55 milligrams per 100 grams of fish. In France, the norm for fresh dogfish is considered to be 50 to 70 mg percent ammonia, and the outer limit is 100 mg percent. All dogfish for export to these countries must now be sampled and tested for ammonia content prior to U.S. Department of Commerce (USDC) certification and the issuance of official export certificates.

The official Belgium method requires special, non-commercially available equipment, and, as a result, private laboratories normally utilized by USDC inspectors will not accept dogfish samples for analysis by the Belgium method. Although there are a number of analytical methods for the determination of ammonia as indicated above, the specificity of these tests for ammonia in dogfish has not been established. A study was conducted to compare the Belgium accelerated micro-diffusion method with two other methods: (i) an enzymatic method which is available in kit form and (ii) a method having the sanction of the AOAC for the determination of ammonia in crabmeat. The objective of the comparative analysis was to identify a method that can be reliably substituted for the Belgium method.

METHODS AND MATERIALS

Dogfish samples were obtained through lot inspection by NMFS inspection personnel. Homogenates used in the accelerated micro-diffusion tests and the enzymatic assays were prepared by blending 10.0 grams fish in 400 mL deionized water for two minutes. Standard ammonia solution preparation is described in the AOAC method (1). All solutions were prepared in deionized water. *Belgium Accelerated Micro-diffusion Method* (14). Nessler reagent was prepared according to Standard Methods (10) with working solutions (1:25) made fresh daily. Micro-diffusion cells were 60 mL glass bottles fitted with plastic screw caps and teflon gaskets, drilled to accept a glass rod with a ground glass tip. Rods were tissue grinding pestles with six millimeter diameter stems. The rotation head was taken from a GM Company fraction collector, and the rotator was a product of U.S. Stoneware. Rod tips were moistened with 1 N sulphuric acid. One (1.0) mL dogfish homogenate was pipetted into a micro-diffusion cell and made alkaline by addition of 1.0 mL saturated potassium carbonate. Cells were quickly sealed and placed in a rotation head. The head was turned such that bottles were oriented horizontally, and 15 cells per run were mechanically rotated at 50 rpm for 20 minutes. Rods were removed and dipped into 5.0 mL Nessler reagent. After 5 minutes, the absorbance was measured at 400 nm in a Bausch and Lomb Spectronic 21. *Enzymatic Method to Determine Ammonia.* Kits were purchased from Sigma Chemical Company, and assays were performed according to the technical bulletin instructions (7). The test solution was 0.2 mL dogfish homogenate described above. *AOAC Method of Determining Ammonia.* The AOAC procedure was followed (1) with the exception

that 5.0 grams of dogfish were homogenized in a Waring blender for one minute with 45 mL 2.5 percent phosphotungstic acid.

RESULTS AND DISCUSSION

In principle, the official Belgium method of accelerated micro-diffusion is similar to the widely used Conway micro-diffusion test (4) since a sealed container is utilized to volatilize low molecular weight amines which then pass via the gas phase from an alkaline solution to an acidic solution where they are trapped and analyzed. Accelerated micro-diffusion represents an improvement over Conway micro-diffusion in that even high concentrations of urea and TMAO do not interfere with ammonia determination. Furthermore, the time required for diffusion is reduced to half an hour. Once the equipment has been assembled, this test works well and is inexpensive; however, only about 30 samples can be run per day, requiring the time of two technicians.

Compared to micro-diffusion, the AOAC method for measuring ammonia in crabmeat is complicated, involving an elaborate series of steps and constant attention on the part of two analysts in order to process 25 to 30 samples per day. The method is, however, quite straightforward, and could be performed routinely in the average laboratory.

The third method used to determine ammonia in dogfish samples was an enzymatic assay available in kit form from a major chemical supply house (7). The test requires the use of commonly available laboratory equipment, including a narrow-bandwidth spectrophotometer, and reagents necessary for the assay are supplied as a part of the kit. The directions are simple and easy to perform. A number of tests can be run simultaneously, and 100 tests per day can be run by one technician.

Figure 1 shows standard ammonia curves for each of the three methods being compared. The relationship of the standard curve to the concentration of ammonia in dogfish samples is shown as well, with sample concentrations expressed in milligrams ammonia per 100 grams fish (mg percent). Each of the three methods produce results linear for ammonia in the range of interest for application to dogfish certification. Figure 1 also indicates that the enzymatic method is approximately three times more sensitive than the Belgium accelerated micro-diffusion method, and that the accelerated micro-diffusion method is ten times more sensitive than the AOAC method. When dogfish samples are homogenized as described herein for each of the methods, accurate results can be obtained for samples containing up to 60 mg percent ammonia. Results of the enzymatic method have been shown to deviate from linearity when the concentration of ammonia in shrimp exceeds 60 mg percent (5). For ammonia determinations in excess of 60 mg percent, appropriate dilutions of sample are required in order to obtain accurate results by means of either the enzymatic test or the accelerated micro-diffusion test. The AOAC test, however, can be used without further dilution on samples containing up to 140 mg percent ammonia.

Duplicate results obtained on 24 different dogfish samples by each of three methods is shown in Table 1. The large standard deviations calculated can be attributed to wide variation in ammonia content of the dogfish samples, some of which were of poorer quality than others. In general, the enzymatic kit method yielded lower results than the micro-diffusion method, reflecting the greater specificity characteristic of enzymic methods. In contrast, AOAC results were usually higher than those obtained by the micro-diffusion method. Although a lengthy extraction process is involved in the AOAC procedure, interfering substance(s) may not be completely removed, making this procedure less specific for ammonia than either of the other methods being compared. The relative specificity of these methods is also manifested in the range of ammonia concentrations measured by each method. The average range of values obtained by both the enzymatic method and the micro-diffusion method compared very favorably: 9 to 35 mg percent for the kit and 7 to 37 mg percent for micro-diffusion. AOAC results were not only shifted to higher values, but also had a much wider range, 15 to 59 mg percent.

Over the 24 samples involved in the comparison, the average values were 19.6 mg percent for the micro-diffusion method, 17.9 mg percent for the kit method, and 25.3 mg percent for the AOAC method. Therefore, there was an average difference of less than 2 mg percent between the results of the micro-diffusion method and the enzymatic method, whereas there was almost a 6 mg percent difference between the results of the micro-diffusion method and the AOAC method.

A graphical comparison of data obtained by the Belgium method against the enzymatic method and the AOAC method is given in Figure 2. Enzymatic kit results show a high positive correlation with micro-diffusion results, as evidenced by a correlation coefficient of 0.84. The equation of the line which can be used to predict results of the Belgium method based on results actually obtained by the enzymatic method is:

$$\hat{y} = 5.93 + 0.76\,(x),$$

where $\hat{y}$ is the predicted Belgium result,

and x is the result obtained using the enzymatic kit.

Figure 2B shows that the AOAC results are also positively correlated with micro-diffusion results. A correlation coefficient of 0.79 was calculated for this relationship. The regression line is described by the equation:

$$\hat{y} = 5.92 + 0.5388\,(x),$$

where $\hat{y}$ is the predicted Belgium result,

and x is the AOAC result.

Of the three methods being compared, the AOAC method yielded the most reproducible results as indicated in Table 2. This precision is especially noteworthy since the analyses were performed in two different laboratories. Results obtained by the micro-diffusion method were the least precise of the three methods examined. The high variation associated with the micro-diffusion assay may have been caused by partial loss of ammonia during the isolation phase of the procedure. One of the disadvantages of using the accelerated micro-diffusion test is that such a loss can occur without the knowledge of the analyst.

CONCLUSIONS

A comparison of results obtained by an enzymatic method and an AOAC method with results obtained by the accelerated micro-diffusion test used in Belgium for measuring ammonia in dogfish indicates that either of these tests could be reliably substituted for the accelerated micro-diffusion test. The method of choice is the enzymatic assay due to the speed and simplicity of sample preparation and analysis. A prediction model enables the estimation of accelerated micro-diffusion results based on experimental results obtained by means of either the enzymatic test or the AOAC test.

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Table 1. Concentration of ammonia in 24 different dogfish samples. Two determinations were made by means of three methods on each dogfish sample.

DOGFISH SAMPLE NO.	CONCENTRATION OF AMMONIA (mg percent)								
	ACCELERATED MICRODIFFUSION METHOD			ENZYMATIC METHOD			AOAC METHOD		
	Trial 1	Trial 2	Mean	Trial 1	Trial 2	Mean	Lab 1	Lab 2	Mean
1	36.40	34.00	35.2	31.40	31.40	31.4	43.50	34.00	38.8
2	16.68	16.80	16.7	14.30	14.30	14.3	20.00	16.50	18.2
3	14.68	15.60	15.1	13.30	6.70	10.0	26.00	22.00	24.0
4	48.84	14.68	31.8	37.10	37.10	37.1	60.00	50.50	55.2
5	37.08	33.20	35.1	36.70	23.30	30.0	43.50	39.50	41.5
6	21.28	44.80	33.0	34.30	34.30	34.3	61.00	56.00	58.5
7	20.04	15.60	17.8	6.70	10.00	8.4	23.50	21.50	22.5
8	16.80	1.60	9.2	14.30	11.40	12.8	22.00	17.50	19.8
9	21.52	15.20	18.4	15.00	16.70	15.8	12.00	18.00	15.0
10	19.40	2.40	10.9	16.70	10.00	13.4	21.00	21.50	21.2
11	21.16	14.40	17.8	16.70	16.70	16.7	19.50	18.50	19.0
12	22.40	13.88	18.1	28.30	21.70	25.0	23.50	22.50	23.0
13	19.16	20.80	20.0	15.00	15.00	15.0	16.00	19.00	17.5
14	21.08	19.60	20.3	13.00	3.30	8.2	23.50	14.50	19.0
15	22.40	22.40	22.4	28.30	10.00	19.2	20.00	21.00	20.5
16	21.72	23.96	22.8	16.70	15.00	15.9	26.00	26.50	26.2
17	15.24	19.49	17.4	28.30	10.00	19.2	16.00	17.00	16.5
18	6.40	11.20	8.8	6.70	10.00	8.4	17.50	18.50	18.0
19	27.40	27.66	27.5	35.00	20.00	27.5	28.50	28.00	28.2
20	14.22	11.76	13.0	20.00	20.00	20.0	23.00	21.00	22.0
21	12.99	17.36	15.2	6.70	16.70	11.7	20.00	22.00	21.0
22	12.66	5.71	9.2	6.70	6.70	6.7	19.50	19.00	19.2
23	21.60	10.40	16.0	23.30	10.00	16.6	25.00	19.50	22.2
24	21.20	14.00	17.6	14.30	11.40	12.8	19.50	20.50	20.0
Mean	21.3	17.8	19.6	20.0	15.9	17.9	26.2	24.4	25.3
Std Dev	8.90	9.89	7.88	10.06	8.68	8.66	12.80	10.52	11.55
Std Err	1.82	2.02	1.61	2.05	1.77	1.77	2.61	2.15	2.36
Range	42.4	43.2	26.4	30.4	33.8	30.4	49.0	41.5	43.5
Min	6.4	1.6	8.8	6.70	3.3	6.7	12.0	14.5	15.0
Max	48.8	44.8	35.2	37.1	37.1	37.1	61.0	56.0	58.5

Table 2. Comparison of precision for three methods of analyzing ammonia concentration in dogfish

	Belgium accelerated micro-diffusion method	Enzymatic kit method	AOAC method
Average of duplicate determinations on 24 dogfish (mg NH_3/100 g dogfish)	19.6	17.9	25.3
Duplicate variance	50.9	27.4	7.8
Standard deviation of duplicate determinations	7.1	5.2	2.8
Coefficient of variation (x 100%)	36%	29%	11%

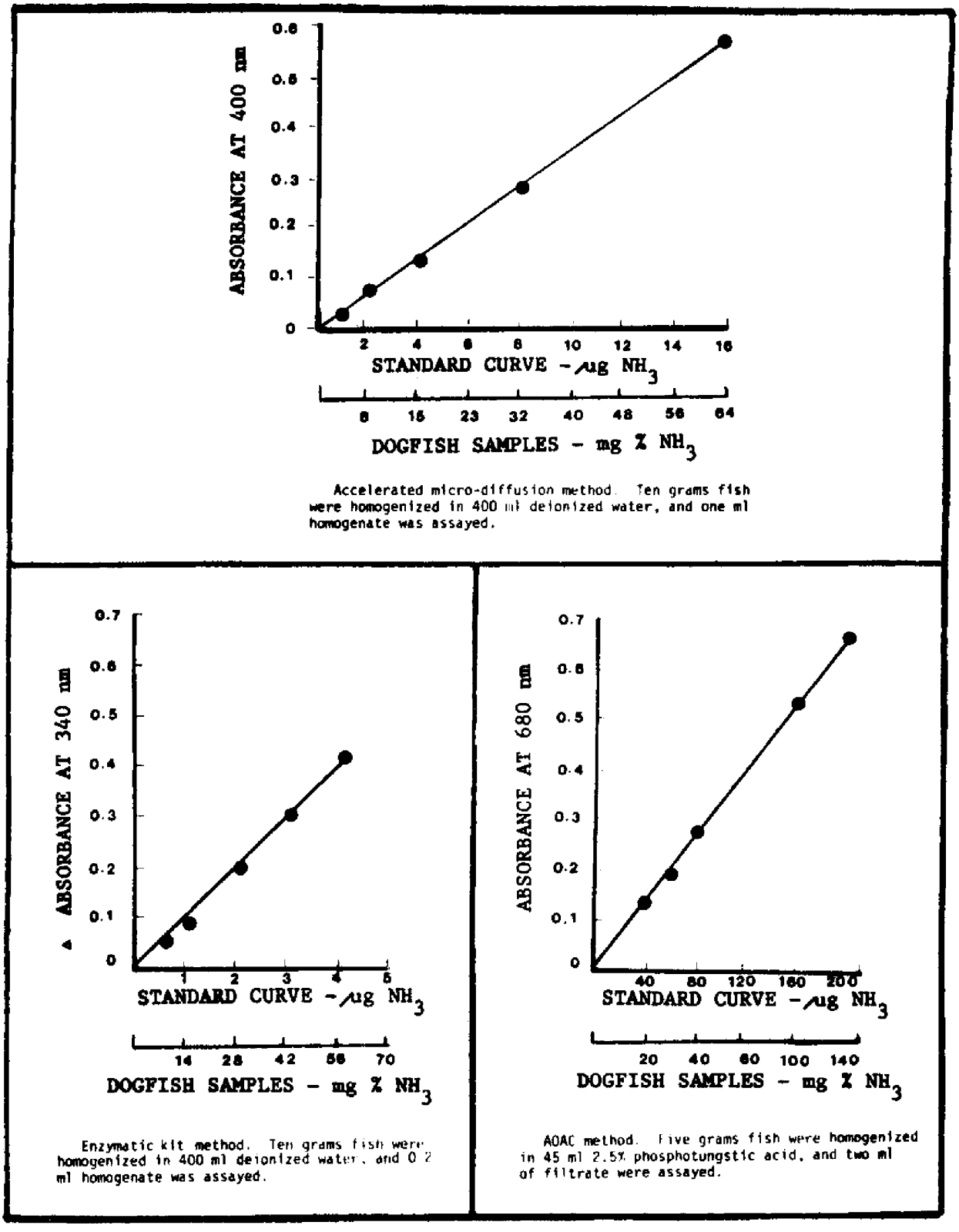
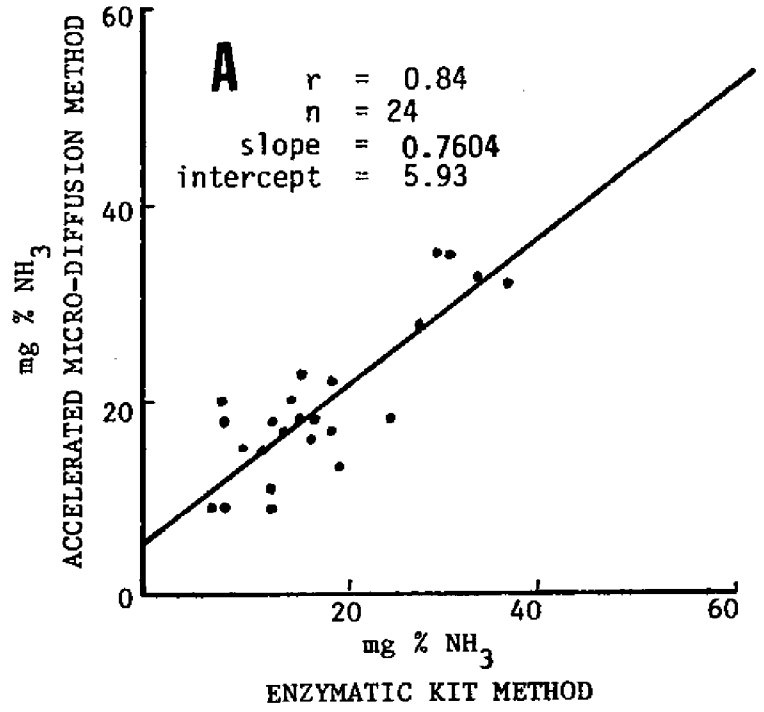


Figure 1. Ammonia standard curves obtained by three different methods as they relate to the concentration of ammonia in dogfish samples.



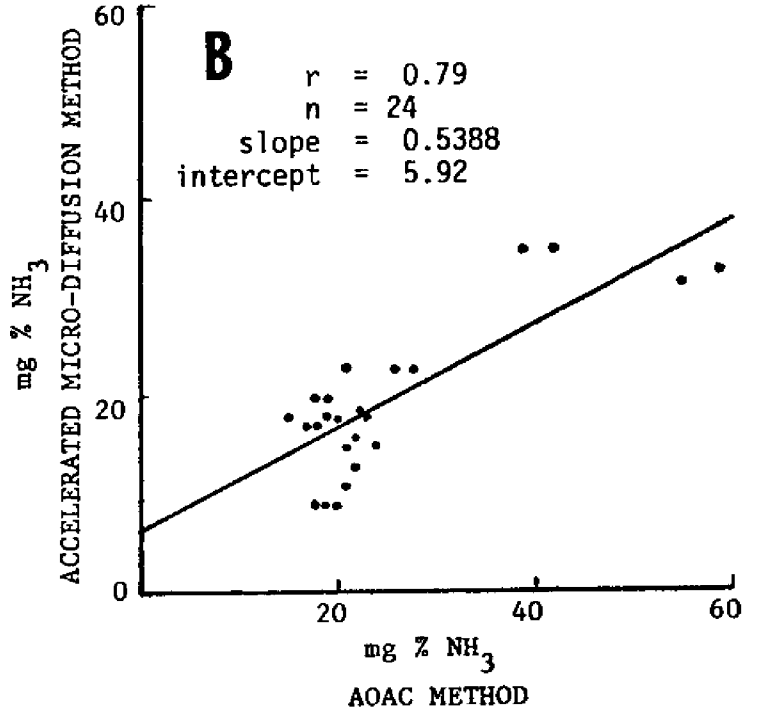


Figure 2. Correlation of results for ammonia concentration in dogfish by the Belgium accelerated micro-diffusion method with (A) enzymatic kit method and (B) AOAC method.

A STUDY OF FISH BONES AS A QUALITY FACTOR IN SEAFOOD PRODUCTS*

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INTRODUCTION

Fish provides about 5.2 percent of the world's supply of food and 12.2 percent of the total supply of protein (25). In the U.S. per capita consumption of fish was as low as 13.4 pounds in 1978 and total consumption of fish and shellfish was 2,756 billion pounds (15). Out of this amount close to one billion pounds were in the form of fish fillets, steaks, sticks, and portions. Four hundred forty and one half million pounds of fish blocks were processed into sticks and portions. The United States imports about 99.5 percent of its blocks and 72.6 percent of fresh or frozen fillets and steaks annually from ten different countries (15). Table 1 shows the total production, consumption, and imports of the different fishery products in the United States in 1978.

Table 1: U.S. production, imports, and consumption of different fishery products, 1978 (15).

Products	Domestic Production	Imports	Consumption
	(Thousands of Pounds)		
Steaks/fillets	158,250	398,110	548,335
Sticks/portions	442,673	577	440,521
Canned	864,000	178,000	992,000
Minced	2,000	387,145	385,138

* This paper was presented at the Fourth Annual Tropical and Subtropical Fisheries Technological Conference of the Americas held in St. Petersburg, Florida in April, 1979.

It is possible that bones in fishery products may affect the U.S. consumption. Bones can cause esthetic and public health problems. Bones exist in almost all major forms of seafood products and affect 50-60 percent of the total U.S. edible seafoods. As technology changes and new manufactured products are developed, the quality control of unexpected bones in fishery products becomes more critical because the processes and the products become more complicated.

SURVEY

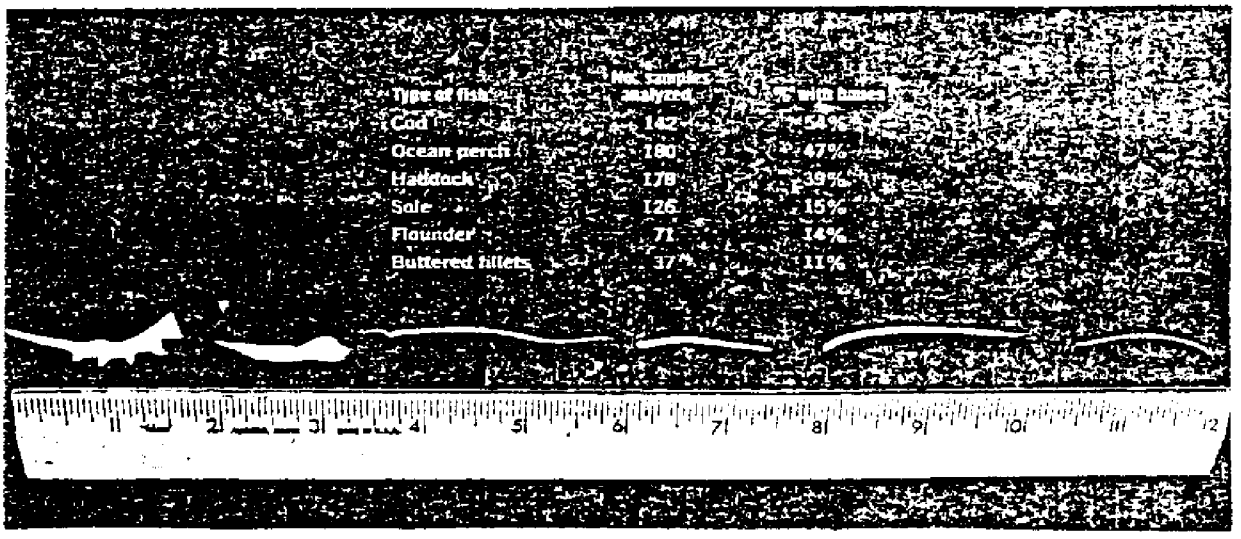
From 1958 to 1962, the Bureau of Commercial Fisheries Technology Laboratory in Boston made several surveys of retail fresh and frozen fishery products. It was found that about 80 percent of 16 different brands of retail frozen haddock fillets had bones and all brands had from 0-4 instances of bones per pound of fish fillets (32). In another study on frozen cod fillets, 87.5 percent of samples tested had 0-9 instances of bones per pound (33). Table 2 shows quality defects including bones found in retail size consumer packages of frozen fried fish portions. Only 3.04 percent of samples had bones. The National Marine Fisheries Service has made no similar surveys since that time. In 1961, the Consumers Union in the United States reported on their survey and the presence of bones in fish fillets (9). They could not find any bones among the samples examined. In 1970, Consumers Union rated 18 brands of fish sticks and considered 20 percent of the samples to be unacceptable, because of bones (10). In 1973, the Consumers Union did another comprehensive survey of frozen fish fillets (11).

Table 2: Quality defects including bones found in 296 samples of institutional and retail size consumer packages of frozen fried fish portions. J. Ryan 1958 (32).

		Percentage of Defects
1.	Coating	86.14
2.	Distortion	83.78
3.	Texture of Coating	52.36
4.	Damaged Portions	54.72
5.	Blemishes	57.77
6.	Condition of Packages	28.71
7.	Uniformity of Size	24.66
8.	Uniformity of Weight	19.93
9.	Texture of Fish Flesh	8.78
10.	Bones	3.04
11.	Color	8.10
12.	Ease of separation	3.71
13.	Broken Portions	1.01

Figure 1 shows the results of this survey. These ground fish fillets were imported from Canada, Iceland, Norway, and Denmark. In this study, 12 brands in 19 major market areas across the country were studied. The results showed about 33 percent of the samples tested had bones 1 1/2 inch long or longer. Cod and ocean perch were the most likely to be bony.

Figure 1: Type, size, and percentage of bones found in frozen fillets from different species. Consumer Reports 1973 (11).



In a separate study in the United Kingdom in 1978, fishery companies recorded consumer complaints on 10 million fish fingers sold during a three-month period. Table 2 shows the results of this analysis, which was presented at a Codex Alimentarius meeting held in Portsmouth, Rhode Island, on September 1978. It shows that 82 percent of the consumer complaints were about bones between 10mm by 3mm and 40mm by 10mm long. They found consumer attention is drawn to small bones when they find larger bones (39).

Table 2: Analyses of bone complaints received from consumers in 10 million fish fingers sold in the United Kingdom. Codex Alimentarius Meeting, Portsmouth, Rhode Island, 1978 (39).

Bone complaints as percentage of total complaints received on fingers	Bone compliants per 10 million fingers sold		Greater than 10mm by 3mm but less than 40mm by 10mm	
		< 40mm by 10mm		> 40mm by 10mm
		(Percentage)		
11 (Range 8-14)	11 (Range 7-19)	12	82	6

Fish bones in certain fishery products are objectionable and discourage consumers from eating more fish. They are also potential health hazards. Flexible bones can lodge in the throat or be inhaled into the lungs. They can sometimes injure the mouth or oesophagus. Children can accidentally ingest fish bones which cause stomach and intestinal injuries (2, 3, 6, 7, 8, 12, 14, 18, 20, 22, 24, 26, 27, 28, 29, 35, 36, 37, 38, and 41). Most reported incidents are from Japan, Poland and Russia. A report from Queen Mary Hospital, Hong Kong, indicated that between 1965 and 1976 a total of 146 patients with fish bones in the oesophagus were treated (26). A search in the U.S. literature shows that no data are available to indicate how many injuries or deaths are due to fish bone.

VIEWS OF DIFFERENT AGENCIES ON FISH BONES

National Marine Fisheries Service - (NMFS):

In NMFS voluntary quality standards for fishery products bones or pieces of bones are considered quality defects. The potential degree of harmfulness of bones is determined by bending the bone between two fingers. This technique has been demonstrated by NMFS to be an inadequate way of determining the degree of harmfulness, because flexible needle-like fish bones that are difficult to detect could be more harmful than flat and rigid bones. Table 3 shows the tolerances for bone in certain U.S. quality standards and International Codex Alimentarius Standards (4).

Table 3: The tolerances of bones for U.S. and Codex Grade Standard of Frozen Fillets of different species. Brooker 1978 (4).

Fillet	U.S. Standard	Codex Standard (minimum quality)
	(Instances of bone/lb.)	
Cod Fillets	U.S. Grade A-6	2-10 1 kg.
	U.S. Grade B-7	1-5 1 lb.
		(depending on size of bone)
Haddock Fillets	U.S. Grade A-4	Same as Cod
	U.S. Grade B-6	Same as Cod
Ocean Perch Fillets		
6 or less fillets/lb.	U.S. Grade A-8	4-16 1 kg.
	U.S. Grade B-9	(depending on size of bone)
7 or more fillets/lb.	U.S. Grade A-7	2-8 1 lb.
	U.S. Grade B-8	
Flounder/Sole Fillets	U.S. Grade A	4 1 kg.
	5 (bones in 1 sq. inch)	(except for pin bones)
	U.S. Grade B	
	10 (bones in 1 sq. inch)	2 1 lb.
Hake Fillets	No Standard	5 1 kg.
		(except for pin bones)
General Fillets	U.S. Grade A	
	4 (bones in 1 sq. inch)	No Standard
	U.S. Grade B	
	4 (bones in 1 sq. inch)	

Codex Alimentarius:

In 1975, the Codex Alimentarius Committee on Fish and Fishery Products held a meeting in Gloucester, Massachusetts (34). Table 4 shows the suggestion made at that meeting by the represenative of South Africa concerning the size and tolerance for bone.

Table 4: South Africa definition of bone by size in the scoring of defects in fish blocks. Codex Alimentarius Meeting, Gloucester, Massachusetts, 1975 (34).

	Serious	Major	Minor
A) Blocks not designated boneless each single bone, other than pin bones, greater than 1/3mm in diameter or each cluster of such bones with an area of 3 cm.	-	2	-
B) Blocks designated boneless each single bone greater than 15mm long or greater than 1/3mm in diameter.	2	-	-

In 1978, at the Codex Alimentarius meeting in Portsmouth, Rhode Island, a represenative from the United Kingdom suggested a definition of fish bones based on fish blocks imports from other European countries - Table 5 (39).

Table 5: A study of distribution of bone size in fish blocks imported from different countries. United Kingdom and Codex Alimentarius Meeting, Portsmouth, Rhode Island, 1978 (39).

Origin	Average 1 kg.	Bone size distribution < 10mm by 3mm	> 10mm by 3mm but < 40mm by 10mm	> 40mm by 10mm
	No.		Percent	
Iceland	5.06	-	-	-
Norway	1.28	4	80	17
Denmark	0.53	4	89	7
United Kingdom	0.17	3	84	13
Singapore	0.12	3	94	3
Argantina	0.11	8	92	0

CHARACTERISTICS AND CHEMICAL COMPOSITION OF FISH BONE

Cod is a typical commercial bony fish with bone problems. During fillet processing, dorsal rib bones are frequently allowed to remain, because their removal involves a five percent loss in yield and results in a fillet with a peculiar shape. Herring have many fine hair-like intramuscular bones that are impossible to remove and still have an attractive fillet.

The mineral composition of bone in fishes varies in the same general way as in land animals. The main difference is in the degree of ossification (hardness). Hard bone has a greater mineral content than soft bone and the presence of calcium salts of phosphoric acid are dominant.

Soft bone has less phosphorus and calcium in the form of calcium carbonate. The degree of ossification changes with species, sex, and age. In general, fish bones have a less complex composition than bones of land vertebrates. Only limited data are available about the trace elements in fish bones. The industry has monitored certain species and/or products for calcium, cadmium, flouride, and lead (19, 38).

EFFECT OF PROCESSING ON THE BONE CONTENT OF FISHERY PRODUCTS

Fish sticks and fish portions are the two important products that are made from fish blocks. The only difference between the two products is the size and shape. When using these products the consumer faces a difficulty of not knowing the history of the products. Therefore, they have no basis for assessing the risk of encountering bones and can only rely on sensory evaluation of the product to detect bone.

In hand processed fish fillets, some bones are left in the product. To remove these bones completely it would require changing the shape and the integrity of the natural fillet.

In mechnical deboning of fish, the various perforation sizes of the drums and the degree of adjustment of the equipment during operation affect the size and the total amount of bones left in the product (42, 43). It was found that as the perforation size became larger and the belt pressure increased, the total number of bone particles left in the product increased. Table 6 shows the number and size of bone fragments in deboned rockfish using a drum with 5mm opening.

Table 6: Number and size of bone fragments in deboned rockfish flesh. Wong, et al., 1975 (43).

Species	Bone fragments found [a] 2-5mm	5-10mm	Over 10mm
Silvergray	0	1	0
Ocean Perch	0	0	0
Bocaccio	0	0	0
Canary	1	0	0
Widow	0	0	0
Red-Banded	5	1	1
Sharpchin	6	2	0
Shortspone Thornyhead	0	0	0

[a] Fine, hair-like bones and fragments less than 2mm long were not counted. Samples weighing 100g were used.

The effect of drum perforation size on the average weight and average number of bone fragments recovered from minced fish is shown in Table 7.

Table 7: Effect of drum perforation size on the average weight and average number of bone fragments recovered from minced fish. Wong, et al., 1974 (43).

	Weight and number of bone fragments recovered							
	2mm Orifices		3mm Orifices		5mm Orifices		7mm Orifices	
Species	Wt. (mg)	No. of bones	Wt. (mg)	No. of bones	Wt. (mg)	No. of bones	Wt. (mg)	No. of bones
Pollack	2	5	6	7	13	14	38	12
Rockfish	4	4	4	4	22	10	29	13
Herring	7	68	9	74	80	389	122	378

The effect of drum perforation size on the average size and average number of bone fragments recovered from minced fish made from different species is shown in Table 8.

Table 8: Effect of drum perforation size on the bone size distribution of minced flesh. Wong, et al., 1974 (41).

		Size distribution of bones			
	Size of bone fragments	2mm Orifices	3mm Orifices	5mm Orifices	7mm Orifices
Species	Range(mm)	Percent			
Pollack	3	100	88	83	43
	3-6	0	6	9	32
	6	0	6	8	25
Rockfish	3	100	100	86	78
	3-6	0	0	7	9
	6	0	0	7	13
Herring	3	74	49	41	15
	3-6	16	25	26	21
	6	10	16	33	64

In certain canned fish, such as canned salmon and sardines, heat processing softens the bones, and their comsumption apparently presents no problem.

METHOD FOR DETECTION OF FISH BONES

Bones in most fishery products are a matter of concern for consumers, industry and Government. A real need exists to develop a reliable, simple and economical method to detect fish bones in the highly processed fishery products including products made from fish blocks. These methods could be of great interest to domestic industry and those countries that export or import fishery products.

To measure the bone content of fishery products, several objective methods have been tried, but none have been satisfactory. These methods can be divided into chemical and physical methods.

Chemical methods

In one chemical method a one molar hot sodium hydroxide solution is used to digest the protein. Then chloroform is added to the sample which makes the bones float on the surface and they are be easily separated (13). In another method which is reported from the University of Mississippi, Department of Food Science, a 10 percent formalin solution is added to the sample which is put in a blender at a very low speed. Then an alizarine solution is used to dye the bones, which are separated on a piece of filter paper (1). The disadvantage of this method is that it is time-consuming. None of these methods mentioned are satisfactory, because they are not accurate, simple, or fast and consequently, cannot be used effectively by inspectors.

Physical methods

Patashnik and his co-workers from the NMFS Technological Laboratory at Seattle, Washington, have reported on a floatation technique for separation of bones (30). This method also is not very accurate because other materials besides bones can float on the surface and be reported as bones. In 1955 and 1957, scientists from the Bureau of Commercial Fisheries made a preliminary report on the use of X-Rays for detecting bones in fish fillets and fish blocks. Their results shows that under these conditions, both radiography and fluoroscopy could reveal bone if the product is not more than 1 1/4 inches thick (16, 31). Similar work has been carried out by Canadian researchers at the Halifax Laboratory. In 1977, researchers from Battelle Columbus Laboratories, Ohio, proposed a method of using automated X-Radiography to detect bones in fishery products (5). This method was suggested as a non-destructive technique and claimed to be fast and simple (i.e., can be used in line operation for quality control purposes). This method has not been investigated.

Another method that possibly might be feasible is the use of ultrasonic techniques similar to those being used in the medical profession. The technique is new and there are not any reports on its application to the detection of bone in fishery products. This method, however, does not work for frozen products.

CONCLUSIONS AND RECOMMENDATIONS

As technology is rapidly changing and new fabricated products are introduced to the market, the quality control of bones in fishery products becomes more difficult. The problem affects almost two-thirds of all fishery products.

The discussion reveals that:

- There is a real need to better define harmful "bone" in fishery products. The definition should consider the size, shape, number of bones, and the degree of rigidity in the cooked stage and the relationship to sensory evaluation in regard to physical hazards. This definition of bone should be agreed upon between industry, consumers, and Government agencies. Also, this definition should be agreed upon internationally for the purpose of export/import and it should be incorporated into the definition of Codex Alimentarius Standards for Fishery Products.

- Based on this definition of bone, the U.S. voluntary standards issued by NMFS should be modified. It seems logical that NMFS by changing provisions for bones in

quality standards could increase the confidence of the consumer in buying a wholesome and practically bone-free fishery product.

- Because of the importance and seriousness of bones in fishery products, I recommend comprehensive research for developing a safe non-destructive and economical method for detecting bones in fishery products be supported. The method should be practical enough to be used by any plant inspector and/or industry quality assurance personnel as a means of quality control during processing.

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