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Changes in the functional properties of two species of salted marine fish, namely, pink perch (*Nemipterus japonicus*) and oil sardine (*Sardinella longiceps*) during storage at ambient temperature revealed significant decrease ($P < 0.05$) in the functional properties of salted fish meat. A reduction in the solubility of proteins during storage profoundly influenced the emulsifying capacity, cook loss, water

binding capacity and relative viscosity of fish proteins. The decrease in the functional properties was significantly greater in the pink perch than in the oil sardine.

KEYWORDS. Pink perch, oil sardine, dry salting, functional properties

A questionnaire on quality management and measurement issues was conducted in the Danish seafood industry. The aim of the survey was to investigate to what extent the industry uses quality management systems, and which measurement methods are used. The results showed that the mandatory Own Check Systems, EU directive no. 91/439, were very well established. However, the application of more comprehensive systems was only found in 4% of the participating companies. Characteristics common to these companies were a high export rate, over 100 employees, and high turnover. The survey demonstrated that the measuring and control methods utilized by companies were mainly directed at controlling temperature, weight, sensory aspects, and microbes.

KEYWORDS. Quality management, quality assurance, ISO 9000, HACCP, Own Check Systems

The objective of this study was to determine the composition and the physicochemical properties of paddlefish meat under refrigerated (0°C) and frozen (−29°C) storage. Paddlefish meat contained 79.0% moisture, 17.5% protein, 3.1% fat, and 1.0% ash. Under refrigerated storage, protein solubility and shear force of the meat decreased after 3 days, whereas TBARS increased from 4.5 to 8.0 nmole/g meat after 7 days. During frozen storage, protein solubility decreased by 20% at the beginning and continued to decline during the first 5 months, while shear force declined after 3 months and TBARS increased from 5.0 to 7.8 nmole/g meat only after 5 months ($p < 0.05$). There was no significant degradation of myosin during refrigerated storage. In addition, neither refrigerated nor frozen storage significantly altered the thermal stability of the muscle proteins.

KEYWORDS. Paddlefish, storage stability, lipid oxidation, protein solubility, texture

A new approach is used to investigate one definite aspect of frozen ocean fish (including scad, mackerel, gray/humped rock cod, red/black sea bream, belted bonito-filleted) after different terms of storage at −35°C. We intended to prove whether the season, place and depth of catch influence the amino acid content of the frozen fish meat. The analysis of the bound amino acids in the proteins of the fish muscle in intervals of 3, 6, 9 and 12 months after draught has shown that even to the end of the storage the irreplaceable amino acids remain unchanged and slight deviations are observed in the amount of the others. The appearance of diaminopimelic acid (DAP) shows a microbiological contamination, especially after long storage, but this very low temperature does not allow a drastic increase of the psychrophyles. The methionine sulfoxide (Ms) which appeared also, shows only an oxidation of the methionine which is common in long storage of samples.

KEYWORDS. Amino acid variations, frozen ocean fish, extended storage

The solubility characteristics of proteins are of interest because of their relation to many functional properties. The solubility of the proteins of washed cod muscle mince increased dramatically between pH 8.9 and 9.2 at an ionic strength of 10 mM, whereas a high but constant solubility was observed at 430 mM sodium chloride over the pH range from 7 to 9.5. At pH 9.2, the proteins of the washed cod muscle were greater than 60% soluble at a sodium chloride concentration of 6.6 mM but were salted out at slightly higher concentrations. Above about 100 mM to about 900 mM salt, the proteins were salted in, and greater than 90% solubility was achieved. Maximal extractability was observed with a ratio of 36 volumes of extracting solution to 1 weight of minced muscle at pH 9.2. At pH 8.5, the extractability of the protein increased from 11% to 24% over a range in extraction volumes of 12:1 to 144:1 of volume of extracting solution to muscle tissue weight.

KEYWORDS. Alkaline solubilization of muscle proteins, cod proteins, muscle proteins, myofibrillar proteins, protein solubility, solubility of muscle proteins

Shrimp texture changes during thermal processing based on its temperature history. Texture measurements and sensory tests were performed on large, medium, and

small tiger shrimp. In isothermal experiments, shrimp were cooked in water at 55, 65, 75, 85 and 95°C for two time periods: for their slowest heating point to reach within 0.5°C of water temperature, and 50% longer than this. Transient experiments were also conducted at 75, 85, 95°C, and in boiling water for a time necessary to achieve a certain microbial lethality. Cooling after heat treatment was done in ice slush and in Ziploc bags in ice slush. Texture Profile Analysis and shear test were performed with a Instron Universal testing machine. Tenderness, juiciness, rubberiness, and overall acceptability were determined by sensory panels and correlated with instrumental results. Temperature significantly affected all textural properties and sensory attributes. High correlation between textural properties and sensory attributes was obtained. This correlation may help in predicting sensory attributes from instrumentally measured texture parameters.

KEYWORDS. Shrimp, thermal processing, sensory analysis, textural properties

The degradation of actomyosin in fillets from pre- and post-spawning hake under frozen storage is studied by electron microscopy and by analysis of changes in protein solubility. The ratio of salt soluble protein did not present significant changes during 240 days of frozen storage for post-spawning hake. Meanwhile the same ratio for pre-spawning hake presented a steady decrease.

In post-spawning hake the proteins retain some of the characteristics of the native structure with some aggregate formation up to 60 days of storage. In pre-spawning hake the formation of aggregates is already extensive after only 15 days of storage.

The solubility of proteins from pre-spawning hake decreased continuously reflecting the changes in the ultrastructure of actomyosin complex. For post-spawning hake, only the formation of soluble aggregates was observed after 240 days of frozen storage.

KEYWORDS. Hake, actomyosin ultrastructure, frozen storage, reproductive cycle, fish fillet

In this study, the masking or reduction of off-flavor in tilapia due to various preservation methods such as salting, drying, frying, smoking, microwave heating, marinat-

ing and fermentation with carbohydrate mixture *(som fak* preparation*)* was investigated by subjecting the processed tilapia to sensory evaluation and analyzing the concentrations of geosmin (1,10-*trans*-dimethyl-*trans*-9-decaol) in the processed samples. Dry salting or brining muddy-flavored fish and then drying either by hot air at 50°C or sun-drying resulted to only a slight reduction in the geosmin content of the product. Deep-frying reduced the muddy flavor intensity and geosmin content in salted-dried tilapia. Pretreatment of tilapia fillets with acidified brine before smoking reduced geosmin content and masked the muddy flavor in the smoked product. Microwave cooking of fresh muddy-flavored tilapia showed no effect on its geosmin content nor its off-flavor. Marinating tilapia in acetic acid solution resulted in decreased muddy flavor, and longer marinating period led to lower geosmin content in the product. The geosmin content of *som fak* made from muddy-flavor and non-muddy-flavor tilapia differed significantly, although sensory evaluation yielded no significant differences between the two types of *som fak*, and the taste panelists preferred the product fermented for 3 days.

KEYWORDS. Off-flavor, geosmin, tilapia, salting, drying, smoking, frying, microwave heating, marinating, fermentation, *som fak*

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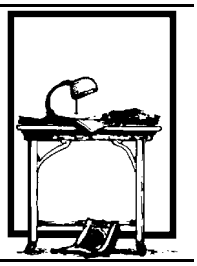

Small Pelagics and Changing Fisheries

I recently had the opportunity to visit Peru and Chile, two giants in fisheries production. Over the past decade, they have consistently been ranked either 2nd or 3rd in terms of total production. For marine capture fisheries, they often represent 20% of the world's capture. They have been making inroads in aquaculture as well. Chile is well known for its salmon aquaculture harvesting around 200,000 metric tons per year while Peru has been involved in shrimp aquaculture in the northern sections of the country that have warmer waters. Each country's fisheries is an important cog in the economic wheel with a large percentage being exported. If history serves me correct, these two countries were also the first ones to impose a 200 mile zone (Exclusive Economic Zone) off their coasts to protect their valuable fisheries and wrestle control from foreign fisheries.

A potential downside to this very productive area is the boom or bust production levels that occur with major climatic shifts. Over 90% of the fisheries in Peru is small pelagics that are usually designated for

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fish meal production. We are all familiar with the havoc that El Niño, a warming of the equatorial currents with the results of warmer waters, less upwelling and poor productivity on the coast, can bring. The Peruvian anchovy harvest can be as high as 10-12 million metric tons (mmt) one year and then cut in half with a strong El Niño. The same scenario, as well, occurs with Chile and their Jack mackerel fisheries. In 1996, the Jack mackerel harvests were over 4 mmt while in 1999, it is reported that they were below 1.5 mmt. This has caused major disruptions in the fisheries and the industry as several companies have gone bankrupt due to these major shifts.

Before my travels to these countries I was given a very interesting paper to read by our university marine librarian. It is in *Fisheries Research* 37:115-125 and entitled ''Long-term climatic change and main fish production in the Atlantic and Pacific'' by Leonard B. Klyashtorin. The paper shows the correlation between commercial catches of the large volume fisheries (Chilean Jack mackerel, Peruvian anchovy, Alaska Pollock, Pacific salmon, and others) and the atmospheric climatic index (ACI). The ACI usually runs in 50-70 year cycles and many of the major species biomass highs and lows run parallel. It appears that we are just coming out of a major regime shift into a new cycle where many of the world's fisheries will change. The bad news, according to the paper, is that certain fisheries, ones that countries have depended on, are predicted to decrease in biomass such as Chilean jack mackerel and Pacific salmon. The good news is that other fisheries will increase such as many of the north Atlantic stocks and Peruvian anchoveta.

Although these shifts will occur over years (the next decade), one may ask how can a seafood scientists help in the adjustment. The key word certainly is flexibility and seafood industries will have to adjust to survive these shifts. New processes that maximize the yields of what is allowed to be harvested will be important to countries. One could even argue for a change in thinking, for the small pelagic industry of Chile and Peru, to utilize more of the pelagic fisheries for human consumption. There have been efforts in the past with fish protein concentrate and marine beef production. These have failed for various reasons including poor functionality of the final product as well as the economics of the processing. New processes looking at

the solubility of proteins to maximize extraction and functionality may open new doors to small pelagic utilization for human consumption.

Michael T. Morrissey
Editor

Changes in the Functional Properties of Dry Salted Pink Perch and Oil Sardine Meat During Storage

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ABSTRACT. Changes in the functional properties of two species of salted marine fish, namely, pink perch (*Nemipterus japonicus*) and oil sardine (*Sardinella longiceps*) during storage at ambient temperature revealed significant decrease ($P < 0.05$) in the functional properties of salted fish meat. A reduction in the solubility of proteins during storage profoundly influenced the emulsifying capacity, cook loss, water binding capacity and relative viscosity of fish proteins. The decrease in the functional properties was significantly greater in the pink perch than in the oil sardine.

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KEYWORDS. Pink perch, oil sardine, dry salting, functional properties

INTRODUCTION

Among the various methods of fish preservation, salting is one of the oldest and most wide spread preservation techniques being practiced by man due to its simplicity and low cost. Salted fish in different forms is very popular in many parts of the developing countries of the world. According to Roberts (1986), salting is a treatment used either to provide taste or to impart storage stability by decreasing water activity to the point where microbial and enzymatic activity are retarded, as well as having a bactericidal effect.

The quality characteristics of salt dried fish have been investigated by Vega Diez (1980), Kalaimani et al. (1988), and Muraleedharan et al. (1989). These studies focus on the types of salt, methods of salting employed in curing, biochemical and microbial aspects of fish during salting and storage, and sanitary measures. However, there is a paucity of literature with regard to the effect of salting and storage on the functional properties of fish meat. Therefore, the present study is an attempt to explore and understand how certain functional properties such as protein solubility (PS), emulsifying capacity (EC), cook loss (CL), water binding capacity (WBC) and relative viscosity (RV) change during salting and storage of two commercially important species of marine fish. The two types of fish are pink perch or the threadfin bream (*Nemipterus japonicus*), a demersal lean fish, and Indian oil sardine (*Sardinella longiceps*), a pelagic shoaling fish, which constitute, on an average, 3.85% and 10% respectively, of the total marine landings of India.

MATERIALS AND METHODS

Fresh pink perch and Indian oil sardine caught off Mangalore coast were iced on board the fishing vessel and brought to the processing hall in an insulated box, within 30 minutes of landing. Fish were then washed in chilled water and re-iced in the ratio of 1:1 ice to fish until further processing (i.e., dressing to butterfly style stage). About 10 kg

of each fish were washed, eviscerated and dressed to butterfly style. The fish were dry-salted by rubbing commercially available crystalline salt on the body of dressed fish using a salt to fish ratio of 1:4. Salted fish were stacked in plastic troughs in layers interspersed with salt (i.e., the amount of salt remaining after rubbing the dressed fish body with salt); weights were kept on the top to prevent fish from floating. The troughs were covered with polyethene sheets. The ratio of salt to fish (1:4) was maintained during the salting process and throughout the storage period at ambient temperature ($26.4 \pm 3.6°C$) by adding dry salt at weekly intervals, equivalent to the amount of salt lost along with the exudate releases due to osmosis. Samples of fish were drawn randomly, initially before salting (fresh) and later at the end of the first, third, fifth, seventh, and ninth week of storage after salting. The handpicked meat was minced by a manually operated mincer and used for analyses.

Analyses

The moisture content was determined by the AOAC (1975) procedure, and salt content by the procedure described by FAO (1981). Total lipids were extracted with chloroform (Bligh and Dyer, 1959). Extraction and determination of water-soluble proteins (WSP) and salt soluble proteins (SSP) in the samples were as described by Vidya Sagar Reddy and Srikar (1991). The sum of WSP and SSP were expressed as protein solubility (PS), calculated as percent of total protein (total nitrogen $\times$ 6.25). Total nitrogen in the salted fish was determined according to Srikar and Chandru (1983). Emulsifying capacity (EC) was measured by the method of Swift et al. (1961). EC was expressed as milliliters of oil emulsified per 1.25 grams of meat. Relative viscosity (RV) of WSP and SSP extracts was determined according to Spinelli et al. (1973). Water binding capacity (WBC) in terms of absorbed moisture in water (AM_w), absorbed moisture in brine (AM_b), retained moisture in water (RM_w) and retained moisture in brine (RM_b) and cook loss (CL) were determined by the methods of Li-Chan et al. (1986) and Kondaiah et al. (1985). The total volatile base nitrogen (TVBN) and trimethyl amine nitrogen (TMAN) content was determined by the method of Beatty and Gibbons (1937). The peroxide value (PV) and the content of free fatty acids (FFA) were determined according to Jacobs (1958) and Takagi et al. (1984) using the chloroform extract.

ANOVA and Students 't' test were employed to determine significant differences in the functional properties during the storage period. Correlation coefficients were also established among the functional properties.

RESULTS AND DISCUSSION

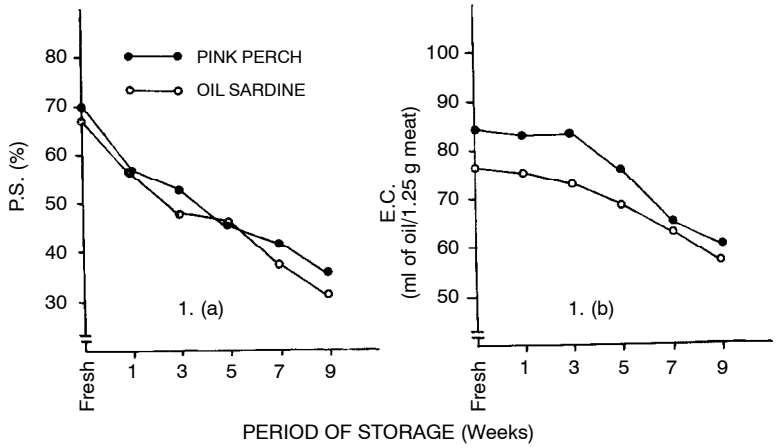
The proximate composition and freshness parameters of freshly caught and salted pink perch and oil sardine are shown in Table 1. The changes in the PS of salted pink perch and oil sardine are depicted in Figure 1a. The PS decreased significantly ($P < 0.05$) from an initial value of 69.70 and 66.69% in fresh samples to 35.40 and 31.45% at the end of nine weeks storage in pink perch and oil sardine, respective-

TABLE 1. The Proximate composition and freshness parameters of freshly caught and salted pink perch and oil sardine meat.

PARAMETERS	PINK PERCH		OIL SARDINE	
	Fresh #	Salted ##	Fresh #	Salted ##
Moisture (%)	80.15 (0.74)	51.50 (0.58)	74.43 (0.42)	51.10 (0.37)
Crude protein (%)	17.08 (0.14)	20.79 (0.18)	19.29 (0.20)	24.44 (0.51)
Total lipid (%)	2.15 (0.01)	3.38 (0.06)	3.99 (0.10)	4.50 (0.04)
Salt (%)	0.91 (0.02)	21.93 (0.20)	0.46 (0.02)	17.89 (0.20)
Total volatile base nitrogen (mg/100 g meat)	5.26 (0.00)	130.38 (2.13)	7.87 (2.40)	112.54 (1.25)
Trimethyl amine (mg/100 g meat)	N.D.	22.60 (1.23)	N.D.	25.25 (1.33)
Free fatty acids (% of total lipid as oleic acid)	4.34 (0.22)	15.41 (0.17)	3.11 (0.13)	33.63 (0.00)
Peroxide value (millimoles of O_2/Kg of fat)	6.16 (1.24)	45.03 (1.90)	6.56 (0.71)	122.07 (1.37)

* : Values presented are mean of minimum three estimates; Standard deviations are given in parenthesis.
ND : Not detectable
: Dressed fish before (prior to) salting
: Salted fish at the end of 63 days (9 weeks) storage period

FIGURE 1. Changes in the protein solubility (PS) and emulsifying capacity (EC) in dry salted pink perch and oil sardine meat during storage.



ly. Fish proteins are denatured by salt (Linko and Nikkila, 1961) and the decrease has been attributed to the loss of soluble proteins along with exudate from the tissue (Leonova, 1970). During the corresponding period, both WSP and SSP contents exhibited a significant ($P < 0.05$) decreasing trend in the salted fish (see Table 2). The changes observed in proteins during salting is a complex process, since both salt and water diffuse from the zone of stronger concentration to the weaker. This diffusion continues until the concentration of the diffusing substances becomes uniform (Zaitsev et al., 1969). It is obvious that along with the liquid that exudes from the fish flesh, some soluble proteins are lost. The migration of these soluble proteins into brine is significant during the initial hours of salting and also when the level of salt in fish flesh does not exceed 7%. When the salt concentration in the flesh is increased further, cell proteins get salted out and their solubility decreases (Daun, 1975). Zaitsev et al. (1969) explained that at a fairly high salt concentration of 12% or more, proteins become denatured and lose their capacity to dissolve. In the present study the average salt content in the pink perch and oil sardine ranged from 20-22% and 17-18%, respectively.

The EC of the salted fish decreased significantly from an initial value of 84.02 ml and 76.43 ml per 1.25 g of meat to 60.70 ml and 57.23 ml per 1.25 g of meat at the end of nine weeks storage in pink

TABLE 2. Changes in the water soluble protein and salt soluble protein* in dry salted pink perch (PP) and oil sardine (OS) meat during storage

STORAGE PERIOD (Week)	WATER SOLUBLE PROTEIN**		SALT SOLUBLE PROTEIN**	
	PP	OS	PP	OS
Fresh #	2.85[a] (0.19)	3.26[a] (0.16)	9.06[a] (0.20)	9.57[a] (0.18)
1	2.73[ab] (0.16)	3.12[a] (0.17)	8.86[a] (0.19)	9.29[a] (0.18)
3	2.54[b] (0.16)	2.83[b] (0.36)	8.57[b] (0.17)	8.83[b] (0.18)
5	2.21[c] (0.12)	2.92[b] (0.18)	7.52[c] (0.27)	8.22[c] (0.16)
7	1.90[d] (0.16)	2.45[c] (0.17)	6.78[d] (0.19)	6.56[d] (0.18)
9	1.64[e] (0.18)	1.96[d] (0.17)	5.75[e] (0.27)	5.69[e] (0.15)

* : Values presented are mean of 3 estimates; Standard deviations are given in parenthesis.
** : Expressed as g/100 g fish meat on wet weight basis.
# : Dressed fish before salting.
a,b,c,d : Means followed by the same superscript within a column do not differ significantly (P > 0.05).

perch and oil sardine, respectively (see Figure 1b). The capacity of muscle protein to emulsify fat is not a static one, since several factors such as pH, protein concentration, ionic strength and nature of neutral salts have been found to effect the EC of the protein (Saffle, 1968). Factors such as physiological conditions of fish, post mortem handling, and processing conditions also exert a great influence on this functional attribute.

Though it is generally acknowledged that SSP possess superior emulsifying properties to that of WSP (Saffle, 1968), the decrease in EC observed in the present study has been attributed to the decrease in both SSP and WSP fractions, since a high positive correlation ($P < 0.01$) existed in both the cases (see Table 3). Throughout the storage period a decrease in both SSP and WSP was seen as a consequence of protein denaturation induced by high concentration of salt (see Table 2). Hence, the change in EC to a great extent could be due to the loss of PS as suggested by the fact that storage affects both parameters similarly (Colmenero and Borderias, 1983). Further, the existence of posi-

TABLE 3. Correlation coefficients (r) among the various functional properties analyzed in dry salted pink perch and oil sardine meat

PARAMETERS COMPARED	'r'	
	PINK PERCH	OIL SARDINE
PS vs. EC	0.8830*	0.9314**
WSP vs. EC	0.9720**	0.9628**
SSP vs. EC	0.9815***	0.9928***
PS vs. CL	– 0.8909*	– 0.9038*
PS vs. RV of WSP	0.9897***	0.9763**
PS vs. RV of SSP	0.9949***	0.9749**
PS vs. Am_w	0.9479**	0.9637**
PS vs. Am_b	0.9008*	0.8976*
PS vs. RM_w	0.8239*	0.9826***
PS vs. RM_b	0.8778*	0.9688**

* P < 0.05
** P < 0.01
*** P < 0.001

PS : Protein solubility
EC : Emulsifying capacity
WSP : Water soluble proteins
SSP : Salt soluble proteins
CL : Cook loss

RV : Relative viscosity
AM_w : Absorbed moisture in water
AM_b : Absorbed moisture in brine
RM_w : Retained moisture in water
RM_b : Retained moisture in brine

tive correlation between PS and EC demonstrated by several researchers supports the present findings (Borederias et al., 1982; Srikar and Vidya Sagar Reddy, 1991).

The relative viscosity (RV) of WSP and SSP extracts decreased significantly ($P < 0.05$) in both the salted samples during storage (see Table 4). Although RV is influenced by a number of factors such as pH, ionic strength, and temperature, the primary factor affecting the viscosity is the protein concentration (Colmenero and Borderias, 1983). The higher the level of soluble protein in the extract, the higher will be the RV. Hence, the decrease in the RV of the protein extracts observed in the present study could be due to the decreased PS throughout the corresponding storage period. The above findings are in total agreement with those of Colmenero and Borderias (1983), Borderias et al. (1985) and Colmenero et al. (1988), who demonstrated existence of a positive correlation ($P < 0.05$) between the PS and viscosity in fish muscle.

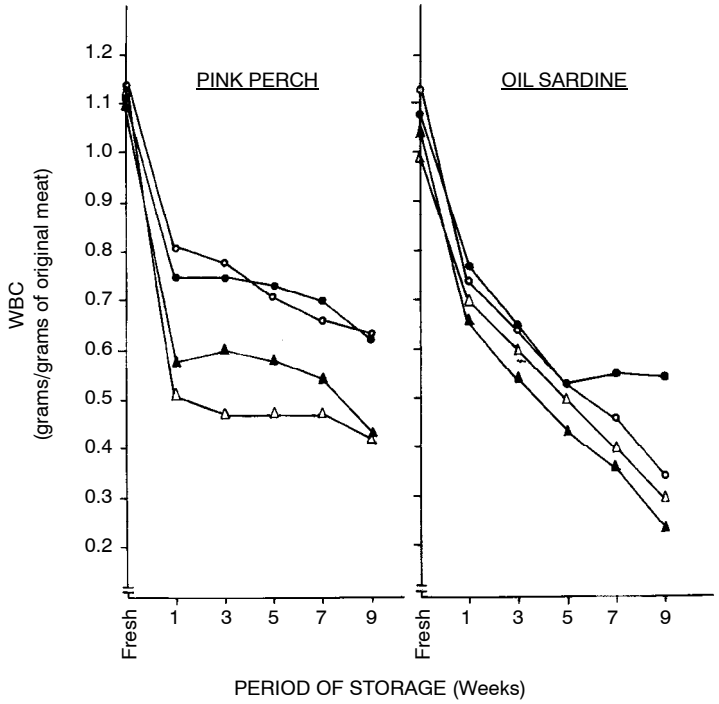
TABLE 4. Changes in the relative viscosity and cook loss in dry salted pink perch (PP) and oil sardine (OS) meat during storage

PARAMETERS / STORAGE PERIOD (Weeks)	* RELATIVE VISCOSITY (Centipoises)				* COOK LOSS (%)	
	PP		OS			
	WSP	SSP	WSP	SSP	PP	OS
Fresh #	141.1[a] (1.4)	163.4[a] (0.3)	134.9[a] (0.3)	158.8[a] (0.3)	14.62[a] (0.45)	16.19[a] (0.09)
1	128.0[b] (0.3)	145.3[b] (0.3)	127.1[b] (0.4)	141.7[b] (0.5)	15.08[ab] (0.69)	19.62[b] (0.10)
3	122.9[c] (0.4)	136.8[c] (0.3)	122.2[c] (0.5)	134.4[c] (0.25)	15.69[b] (0.33)	8.38[b] (1.61)
5	117.3[d] (0.4)	126.0[d] (0.3)	118.7[d] (0.2)	126.6[d] (0.5)	17.85[c] (0.58)	20.36[bc] (0.74)
7	114.3[de] (0.3)	115.5[e] (1.1)	117.3[de] (0.2)	120.2[e] (0.4)	21.39[d] (0.81)	22.77[c] (1.40)
9	111.2[e] (1.4)	111.9[e] (1.0)	114.1[e] (0.9)	116.3[e] (0.2)	25.53[e] (2.23)	22.39[c] (0.37)

* : Values are a mean of three estimates; Standard deviations are given in parenthesis
\# : Dressed fish before salting
a,b,c,d,e : Means followed by the same superscript within a column do not differ significantly (P > 0.05)

WBC decreased steadily in both the species of salted fish during storage (Figure 2). The decrease could be attributed to the decrease in muscle protein solubility, since both these parameters exhibited a high positive correlation (Table 4). Water present in the muscle foods exists either in the form of bound water or free water. Ninety percent of water holding capacity of meat is ascribed to the fluid (free water) that is trapped in the lattice spacings between the protein filaments of the myofibrils, the remainder being the bound water. The free water is mechanically immobilized by the network of the cellular protein membrane and protein filaments (Haurowitz, 1950) as well as by the cross linking and electrostatic forces between the peptide chains (Hamm, 1960). According to Hamm (1960), changes in the water holding capacity of meat during storage and processing affect this tightly bound free water only under conditions of high temperature, high concentrations of salt, or any other drying procedures. Hence, the decrease in WBC observed in the salted species of fish could obviously be attributed to the denaturation of proteins, since only soluble proteins play a

FIGURE 2. Changes in the water binding capacity (WBC) in terms of absorbed moisture in water (AM_w), absorbed moisture in brine (AM_b), retained moisture in water (RM_w) and retained moisture in brine (RM_b) in dry salted pink perch and oil sardine meat during storage.



significant role in the water holding phenomenon. The present findings agree with those of Akande et al. (1988), who found that fish muscle proteins exposed to salt above a critical concentration (> 12%) result in loss of water holding capacity.

The impurities present in common salt such as chlorides of calcium and magnesium and traces of copper and iron may also decrease the hydration of proteins due to cross-linking (Haurowitz, 1950), consequently causing tightening of the protein structure. Toughening of the network of proteins decreases the bound water and easily increases the free moisture, whereas loosening of protein structure has the opposite effect.

CL percent showed an overall increasing trend in both the species

of salted fish (Table 3). The significant ($P < 0.05$) increase observed is the direct outcome of the decrease in PS due to denaturation of proteins during storage, thus resulting in reduced water holding capacity which was later released during the cooking process.

Significant correlations between various functional properties indicate their interdependence on changes in proteins (see Table 4).

From the above studies it is clear that proteins with high solubility exhibit superior functionality. Denaturation of proteins caused by salt leads to insolubilization of proteins (denatured) resulting in poor functional qualities. Further work is needed to correlate the changes in functional properties of salted fish with the sensory attributes to assess the quality of salted fishery products.

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Quality Issues in the Danish Seafood Industry: Results from a Survey

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ABSTRACT. A questionnaire on quality management and measurement issues was conducted in the Danish seafood industry. The aim of the survey was to investigate to what extent the industry uses quality management systems, and which measurement methods are used. The results showed that the mandatory Own Check Systems, EU directive no. 91/439, were very well established. However, the application of more comprehensive systems was only found in 4% of the participating companies. Characteristics common to these companies were a high export rate, over 100 employees, and high turnover. The survey demonstrated that the measuring and control methods utilized by companies were mainly directed at controlling temperature, weight, sensory aspects, and microbes. *[Article copies available for a fee from The Haworth Document Delivery Service: 1-800-342-9678. E-mail address: <getinfo@haworthpressinc.com> Website: <http://www.HaworthPress.com>]*

KEYWORDS. Quality management, quality assurance, ISO 9000, HACCP, Own Check Systems

INTRODUCTION

The seafood processing industry plays an important role in the Danish food industry. Seafood products account for 24% of the total

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value of exported food (Statistical Yearbook, 1997). The seafood industry, like other food industries, is under increasing pressure to react to the demands and expectations of a highly competitive and changing markets where there has been an increasing emphasis on food safety and health. Changes in European legislation have required seafood companies to become responsible for measurement and documentation of processes and product quality in accordance with the Own Check System (EU regulation 91/493). Quality management systems and different quality control methods are increasingly applied. As a result there has been increasing integration of food safety programs, such as the integration of HACCP (Hazard Analysis Critical Control Point) into quality management systems (Early, 1995; Huss, 1995). However, despite the increased use of quality management and HACCP systems, only 2% of the industry had actually ISO certified quality management systems by 1996 (BVQI, 1996). The latest figures show that no new certifications have been registered since (BVQI, 1998). The number of certifications according to ISO 9000 series is the only official measure of the quality management in the seafood industry. In 1997, a new Danish Standard, DS 3027 for HACCP systems made it possible for seafood companies to get their HACCP systems certified. This standard is based on international HACCP guidelines including the Codex Alimentarius. Compared to Own Check Systems, this standard is more comprehensive as companies must also include customer requirements. The present study sought answers to the following questions:

- To what extent are quality management and assurance applied in the industry?
- And when applied, what are the results (quality data) used for?
- Which measurements are utilized on raw material, material in processing, and end products?
- How are the results from these measurements used in the company?

In addition, information concerning the main characteristics of Danish seafood processors as regards size, export, products, etc., were sought in order to correlate internal and external conditions relevant to quality assurance and management.

A questionnaire was employed to find answers to the above ques-

tions. In this paper, the aim is to outline and discuss important results from the survey.

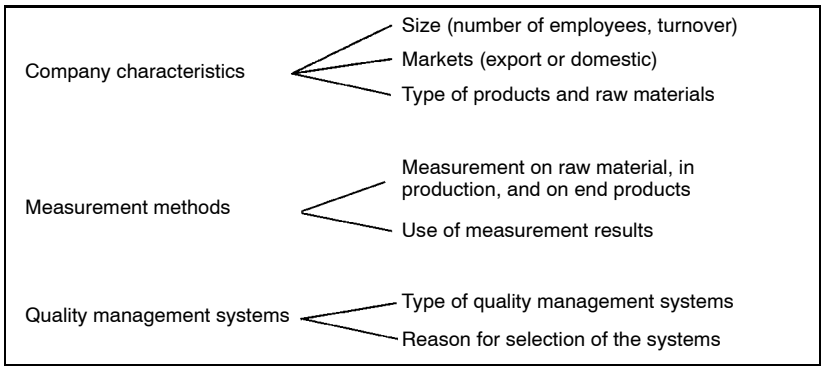
THE QUESTIONNAIRE

The questionnaire was sent to 260 Danish seafood processors, which were selected from a list of companies authorized for processing of fish and seafood (Danish Ministry of Food and Agriculture, 1996). The results are based on the response from 52% of the addressed participants.

The latest official statistics on the size of the Danish seafood processing industry says there were 257 companies in 1995 (Statistical Yearbook, 1997). Although this survey from 1996 was based on a list showing 260 active companies it is hereafter assumed that the distribution of company size according to small, medium, and large is practically unaltered between the two sets of data. Results from this survey indicated 38% were small companies having 10 or fewer employed, about 70% were medium sized companies with 11-100 employees, and 46% were large companies with more than 100 employed. This relatively unequal distribution as regards company size affects our ability to make general conclusions for the small and large companies. The basic content of the questionnaire is shown in Figure 1.

In order to overview possible correlation within the large matrix of data obtained by the survey a multivariate data analysis (Principal Component Analysis–PCA) was applied to the data.

FIGURE 1. The main content of the questionnaire.



RESULTS AND DISCUSSIONS

Characteristics of the Participating Companies

Danish seafood companies are, with a few exceptions, small and medium in terms of number of employees. Ninety percent of the participating companies had less than 100 employees (see Table 1).

The raw material used in the industry was generally based on imported raw material, as only 24% of the total value of raw material used in 1996 was Danish catch (DFE, 1997). The respondents ranked salmon, cod, shellfish, herring and mackerel as the most commonly used species (see Table 2).

Some 62% of the companies produced more than one type of product. Fresh fish was the product of 55%, frozen fish of 37%, and smoked fish of 32% of the participating companies. The distribution of the most common product types is shown in Table 2.

According to export statistics (DFE, 1997) frozen cod fillet ac-

TABLE 1. Distribution of companies as regards number of employees.

No. of employees	≤ 10	11-100	101-200	201-500	> 50	Total
% of participating companies	40	50	6	3	1	100
No. of companies	55	67	8	4	1	135

TABLE 2. Distribution (in %) of seafood raw material types applied, products made and export rate in the participating companies.

Distribution of raw material used*	Herring mackerel	Cod, saithe,	Salmon and trout	Shell fish	Other				
	41	61	68	45	36				
Distribution of products*	Fresh fish	Frozen fish	Breaded fish	Ready meals	Canned	Lightly preserved	Smoked fish	Shell fish	Other
	55	37	10	7	6	19	32	26	15
Distribution of export of products	None	0-25	25-50	50-75	75-100				
	19	8	12	13	48				

* Percentages do not add up to 100 since some companies use more than one type of raw material and/or produce more than one type of product.

counted for the greatest part of the exported products. However, frozen fillet only accounted for 21% of exported value, while shrimp and lobster represented approximately 25% in value.

More than the half (61%) of the participating companies exported over half of their products, while only 19% solely produced for the local market (see Table 2). Germany, France, Italy, Great Britain, Sweden, and Japan were the most important export markets (DFE, 1997). No correlation was found between export rate and number of employees.

Current Use of Quality Assurance and Quality Management Systems

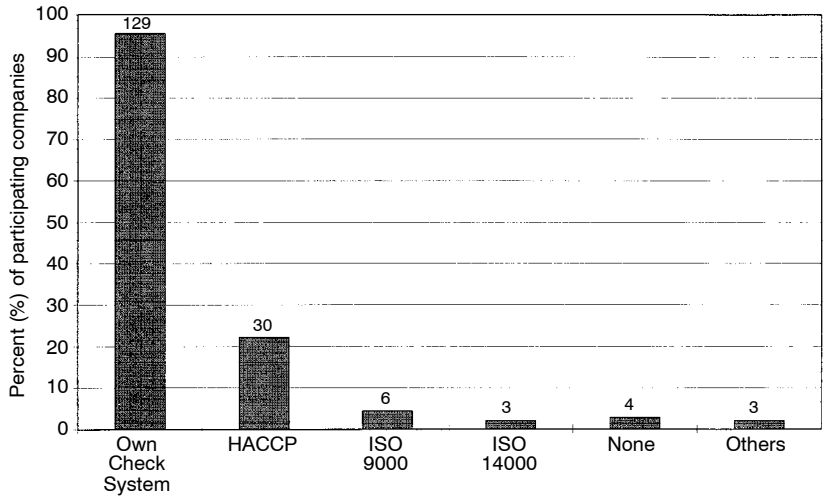
The mandatory Own Check System was the most common system employed by the companies in the survey. The principle behind this system is that the responsibility for checking and insuring quality is placed on the manufacturer and it is recommended that principles from HACCP be included in the system. A full HACCP system, however, can be much more extensive than required in the Own Check System. Therefore, the questionnaire was designed to differentiate between the Own Check System and HACCP. The distribution of quality management systems in the industry is shown in Figure 2. Since the various quality management systems are usually not fully integrated, some companies have more than one system.

The results showed that 96% of the companies had established Own Check systems. Three percent responded that they had only HACCP.

Quality management systems designed according to the ISO 9000-serie were only implemented in 4% of the companies surveyed. Of these, only four had certificates, which in all cases was according to ISO 9002.

A broader use of quality management systems was expected. Quality issues and documentation of processes and products has been on the agenda for some years. In addition, the industry exports a large proportion of their products and should, therefore, need documented quality systems that meet the customers' requirements. Only 2% of the participating companies had environmental management according to ISO 14001 or BS 7750. We had expected more extensive use of environmental management systems, as the seafood industry generally has relatively extensive water consumption and waste production.

FIGURE 2. Quality systems. The columns indicate the percentage (%) of the participating companies, while the number written on the columns reflects the number of companies having the current system. The percentages may not be added up to 100 because more than one system may be applied in the companies.



Comparing Quality Management Systems and Company Characteristics

The selection of quality systems was compared with the company characteristics by use of a multivariate data analysis (PCA–Principal Component Analysis). The PCA model explained 74% of the variance. The analysis of the companies that had ISO 9000 showed that:

- Except for one, all had the HACCP system.
- Except for one, all had a high number (more than 100) of employees.
- All had high export rate (more than 74%, and 3/4 had more than 80%).
- All had high annual turn over.

Generally the percentage of exports had less correlation with the choice of ISO, HACCP and environmental control than the annual turnover and the number of employees. As regards export rate, we had

expected a close correlation between high export rate and presence of ISO 9000 related quality systems.

Reason for Choice of System

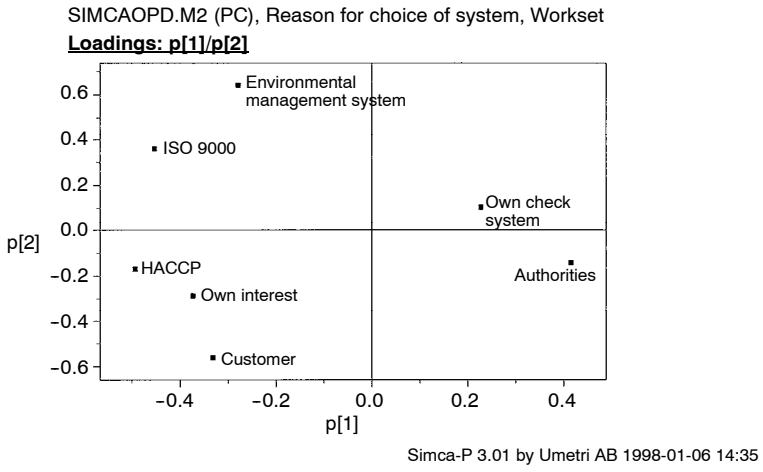
Most of the companies had chosen systems for more than one reason. Eighty percent indicated that authority demand played a significant role in selecting their systems. Customer demand and own interest also influenced the choice of system. A PCA analysis of chosen system and reason for the specific selection is illustrated in Figure 3 where the model explained 68% of the variance.

As expected, the analysis showed that the demand from authorities and the presence of the Own Check System were closely related. The use of HACCP was related to both Own Interests and to Customers' Demand, however no correlation could be found for why companies implemented ISO and Environmental management systems.

Usage of Measurement Methods

Quality assurance in seafood companies covers, to a great extent, use of measuring methods for control and documentation of the quali-

FIGURE 3. First two principal components of a PCA analysis of chosen quality management systems and reason for the selection (p[1] 0.339, p[2] 0.182, p[3] 0.159, sum 0.680).



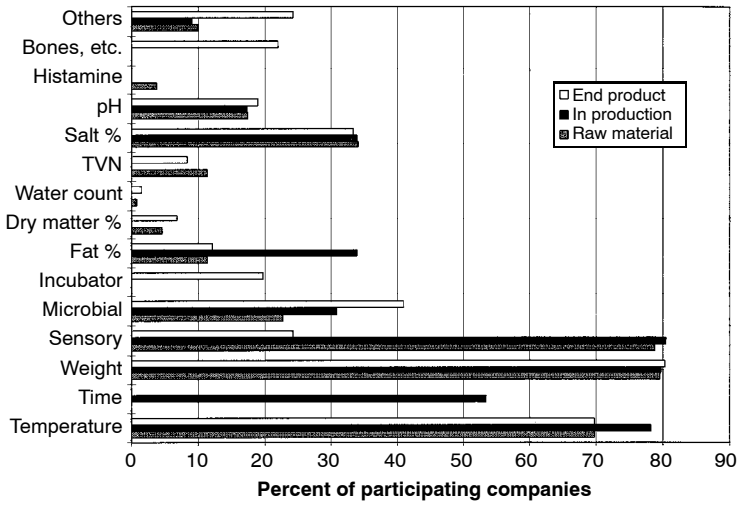
ty of the seafood products. These methods are applied for measurement of the raw material (before processing), during production and of the end product. The most commonly used methods are shown in Figure 4.

Sensory analysis, weight, temperature and time measurements are the most commonly used measurements. These were generally used for controlling quality in raw material, products during processing and end products. Due to a failure in the questionnaire scheme the percentage of companies using sensory analysis for end product control may be inaccurate.

All companies used more than one type of measurement to assure the quality of their product. An analysis of the relation between measuring method and type of product showed no correlation between these. One reason for this could be that 62% of the companies produce more than one type of product.

In 35% of the companies, staffs both from production and laboratory were involved in measuring activities. While 57% solely involved staff from production, 20% involved solely staff from the laboratory when carrying out the measuring methods. Of the participating companies, approximately 70% had their own laboratory facilities while

FIGURE 4. The distribution of measuring methods utilized for quality control given in percentages of participating companies that use the method.



63% made use of external laboratories for different measurements of raw material and on end products. Surprisingly, 30% of the companies had neither their own facilities nor did they use external laboratories. No correlation was found between size of company and laboratories. An analysis of these showed that their measurements were primarily of temperature, weight and sensory attributes that naturally can be performed without laboratory facilities. No correlation was found between quality management systems and the measuring methods used. However, more information about different measurement methods and their usage and integration in quality management systems is needed.

Usage of Results from Measurement Methods

Usage of the results from the quality measurements obtained in 35% of the participating companies was for multiple purposes. Seventy percent took measurements to meet authorities' requirements and 60% measured in order to document processes and product according to the customers' product specifications. Finally, in 60% of the companies the measurements were used for production control. We had expected a more extensive use of measurements, particularly for production control. The relation between these figures for use of measurement was compared to quality systems in a PCA analysis. The analysis of quality management system and use of the measurement results showed a correlation between Own Check Systems and usage for documentation towards the authorities. Surprisingly, the presence of a HACCP system was only slightly related to the measurements for production control, while ISO 9000 systems and environmental systems were related to documentation of the customer demands.

CONCLUSION

A survey about quality issues in the Danish seafood industry demonstrated that the mandatory Own Check System (according to EU directive 91/493) was generally well established. Measurements of temperature, sensory attributes, and microbes were the most common for quality assurance. In spite of extensive measuring activities, the results were mainly used as documentation for authorities and customers.

Very few companies had comprehensive quality systems. Companies that had ISO 9000 related systems were characterized as having

high export rates, more than 100 employees, and high turnover. Therefore, other companies with similar characteristics can be regarded as potential candidates for future ISO certification. From the survey statistics this could mean double the certificates.

In addition, the new Danish Standard (DS 3027) requires HACCP certification for seafood companies. This should help companies to meet not only authorities' but customer demands as well, thus improving quality management and strengthening communication with customers and authorities.

Further research on quality management in the seafood industry should focus more extensively on how to tailor quality systems to relatively small companies and keep these systems low in bureaucracy. The most important future aspect of improving quality management systems in the food industry is to increase the utilization of the measured data. They should be used for more than documentation purposes. It is evident that further considerations should be made into how data collection can be structured for better use in production control, quality management, and long term production planning.

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*The references are available from the authors

Physicochemical Stability of Paddlefish (*Polyodon spathula*) Meat Under Refrigerated and Frozen Storage

X. Lou
C. Wang
Y. L. Xiong
B. Wang
G. Liu
S. D. Mims

ABSTRACT. The objective of this study was to determine the composition and the physicochemical properties of paddlefish meat under refrigerated (0°C) and frozen (−29°C) storage. Paddlefish meat contained 79.0% moisture, 17.5% protein, 3.1% fat, and 1.0% ash. Under refrigerated storage, protein solubility and shear force of the meat decreased after 3 days, whereas TBARS increased from 4.5 to 8.0 nmole/g meat after 7 days. During frozen storage, protein solubility decreased by 20% at the beginning and continued to decline during the first 5 months, while shear force declined after 3 months and TBARS increased from 5.0 to 7.8 nmole/g meat only after 5 months ($p < 0.05$). There was no significant degradation of myosin during refrigerated storage. In addition, neither refrigerated nor frozen storage significantly altered the thermal stability of the muscle proteins. *[Article copies available for a fee from The Haworth Document Delivery Service: 1-800-342-9678. E-mail address: <getinfo@haworthpressinc.com> Website: <http://www.HaworthPress.com>]*

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KEYWORDS. Paddlefish, storage stability, lipid oxidation, protein solubility, texture

INTRODUCTION

American paddlefish (*Polyodon spathula*) is a native species. It grows rapidly, at a rate of 5 kg/year with an average size of 18 kg commonly found in Kentucky (Mims, 1991). There is tremendous potential for large scale production of paddlefish through reservoir ranching or polyculture with other species (Semmens and Shelton, 1986; Mims, 1991).

The success of paddlefish production requires proper post-harvest storage of the fish meat. However, storage at low temperatures, especially below the freezing point, may cause undesirable changes resulting in quality loss of the fish products. For instance, lipid oxidation may adversely affect the color, flavor, and nutritional value of the meat (Erickson, 1997; Kelleher et al., 1992), whereas some biochemical and functional changes in fish muscular proteins may elicit deterioration in the texture of the frozen fish meat (Xiong, 1997). Considering that these changes vary substantially depending on the species, data collected from one species may not be applicable to other species (Davies et al., 1994; Sikorski et al., 1976). As far as paddlefish meat is concerned, we have found only one study (Decker et al., 1991) in which very small paddlefish (about 0.5 kg) were used so that the results may not apply to larger fish. Therefore, the objectives of this study were to determine the composition of the meat from commercial-sized paddlefish (5-10 kg) and to investigate its physicochemical stability under either refrigerated (0°C) or frozen storage (-29°C) conditions using instrumental techniques.

MATERIALS AND METHODS

Preparation of Fish Fillets

Paddlefish, captured from Lake Cumberland, Kentucky in September, were manually headed, gutted, skinned, and filleted using a fillet knife. The thin layer of the red muscle was removed manually to

improve the taste and color of the fillets. Twenty-five fish were used for proximate analysis and six fish were used for storage study. For proximate analysis, the fillets were put into Cryovac® polyethylene plastic bags (Cryovac® Division, W.R. Grace & Co., Duncan, SC), sealed, frozen at −70°C, and used within seven days. For storage observation, each fillet from the 6 fish was cut into seven sections. The sections from the left-side fillet were put into Cryovac® polyethylene bags, put on ice, and placed in a refrigerator (0°C) with one section from each fish taken randomly for analysis on day 0, 1, 2, 3, 5, 7, and 10. The sections from the right-side fillet were put into Cryovac® polyethylene bags, sealed, and frozen at −29°C with one section from each fish taken randomly after 0, 1, 2, 3, 5, 7, and 9 months for analysis. After thawing at 4°C, the fish meat was minced with a food grinder (Kitchen Aid Inc., Model KSM90, St. Joseph, MI) with the exuded liquid from thawing incorporated.

Proximate Analysis

The moisture content was analyzed by drying the meat mince in a 100°C oven for 24 h (AOAC, 1990). Then, the dried samples were ashed at 550°C for 24 h to determine its ash content (AOAC, 1990). The dried samples were also used for protein determination on an automatic nitrogen analyzer (Model FP-228, Leco Corp., St. Joseph, MI) and a conversion factor of 6.25 g protein per gram nitrogen was used to calculate the percent protein content based on the wet basis of the meat. Total lipids were determined according to Bligh and Dyer (1959).

Protein Solubility

The fish meat mince was suspended in a sodium chloride buffer (0.6 M NaCl, 0.05 M NaH_2PO_4, pH 6.0) to give a final protein concentration of 5.0 mg/mL. After setting at 2°C overnight, the suspension was centrifuged at 5000 × *g* for 15 minutes at 2°C. With the protein concentration assayed using the Biuret method (Gornall et al., 1949), protein solubility was expressed as the protein concentration of the supernatant divided by that of the original suspension, then multiplying by 100.

Texture Analysis

Each fillet section was cut into pieces measuring 20 × 15 × 15 mm^3 (L × W × H). Shear tests were performed using a Warner-Bratzler shearing device mounted to an Instron universal testing instrument (Model 4301, Instron Corp., Canton, MA). The cross-head speed of the Instron was 20 mm/min. Each sample was measured 4 times and the mean value was used to represent the shear force required to rupture the fish meat.

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Changes in the protein profiles of the fish meat during refrigerated storage were monitored by SDS-PAGE according to Laemmli (1970) with the separating gel containing 10% acrylamide and the stacking gel containing 3% acrylamide. A SE 250 Mighty Small II slab gel electrophoresis unit (Hoefer Scientific Instruments, San Francisco, CA) was used. With the protein concentration adjusted to 1 mg/mL, an aliquot of 25-μL sample was loaded into each gel slot. The separated protein bands were visualized by staining with Coomassie Brilliant Blue R-25.

Differential Scanning Calorimetry (DSC)

Both refrigerated and frozen stored fish meat was analyzed by DSC following the procedure of Srinivasan et al. (1997) using a Model 2920 modulated DSC instrument (TA Instruments, New Castle, DE). The instrument was calibrated for temperature and baseline using indium as the standard. Accurately weighed meat samples (14-17 mg) were placed in hermetically sealed polymer-coated aluminum pans (TA Instruments, New Castle, DE). The samples were thermally scanned from 10 to 100°C at a heating rate of 10°C/min with a sealed empty pan as the reference. Temperature at the maximum of heat flow (T_m), i.e., temperature at the highest peak of the endotherm, was recorded. The total enthalpy change (ΔH) associated with protein denaturation was estimated by integrating the area above the DSC transition curve with a straight baseline constructed from the start to the end of the endotherm.

Thiobarbituric Acid Reactive Substances (TBARS)

The TBARS in the fish meat was measured according to the method of McDonald and Hultin (1987). Five grams of meat mince was homogenized with 10 mL distilled water for about 1 min. An aliquot of 1.0 mL of the resulting slurry was mixed with 2.0 mL 7.5% trichloroacetic acid solution containing 0.1% propyl gallate and 0.1% EDTA. After centrifugation, the supernatant was used for reaction with thiobarbituric acid in a 100°C water bath for 30 min. The absorbency was read at 533 nm and the TBARS content was calculated using a molar extinction coefficient of 15,600 $M^{-1}cm^{-1}$.

Statistical Analysis

Data were analyzed using the GLM procedure of SAS program (SAS Institute, 1990). Each fish was a replicate, for proximate analysis, the total number of replicates was twenty-five and for storage observation, the total number of replicates was six. Each sample was analyzed at least in duplicate; where the coefficient of variance was > 5%, the data were rejected and the analysis was repeated. For composite data, regression analysis was run to examine the relationship of body weight with fat, and moisture content of the fillet. The storage studies used a randomized complete block design with each fish as the block. Therefore, fish (block) and storage time were the independent variables in the model. When the overall F test was significant, means were compared with the Tukey's test. Significant differences were declared at $p \leq 0.05$.

RESULTS AND DISCUSSION

Proximate Composition

On average, the body weight of the fish used in this study was 7.7 kg and their meat contained 79.0% moisture, 17.5% protein, 3.1% fat, and 1.0% ash. The average fillet yield was 27.0%, which fell within the upper range of 18-35% commonly found for other finfish (Pigott and Tucker, 1990). Note that the fat content of the paddlefish meat was 3.1%, which was higher than the 0.27% reported by Decker et al. (1991). The discrepancy could be explained by the differences in the

size of the fish used in the two studies, i.e., they used small fish with an average body weight of 0.5 kg, which was 10 times less than the fish used in the present study. This explanation was supported by our regression analysis indicating that the fat content of paddlefish meat was positively correlated and the moisture content was negatively correlated with the fish body weight ($p < 0.05$) (Figure 1). When the fish weighed more than 8 kg, their fillet had a fat content as high as 4.6%. Nevertheless, based on the data of this study, paddlefish could be classified as a low-fat fish according to Pigott and Tucker (1990).

Protein Solubility

During refrigerated storage, protein solubility only experienced a slight decrease after 4 days (Figure 2A). In contrast, freezing caused an immediate reduction in protein solubility from 82% to 66% and continued storage caused further decline in protein solubility (Figure 2B). It has been known that a drastic decline in protein solubility is characteristic of many fish species during frozen storage (Sikorski et al., 1976). However, the underlying mechanisms are not well under-

FIGURE 1. The fat and moisture contents of paddlefish meat in relation to their body weight.

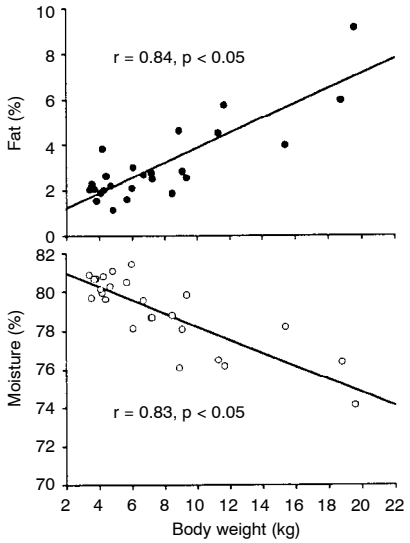
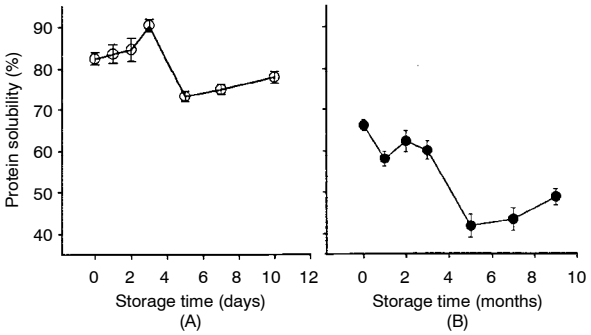


FIGURE 2. Protein solubility of paddlefish meat under 0°C refrigerated (A) and −29°C frozen storage (B). Each point represents the mean ± standard error from six replicates.

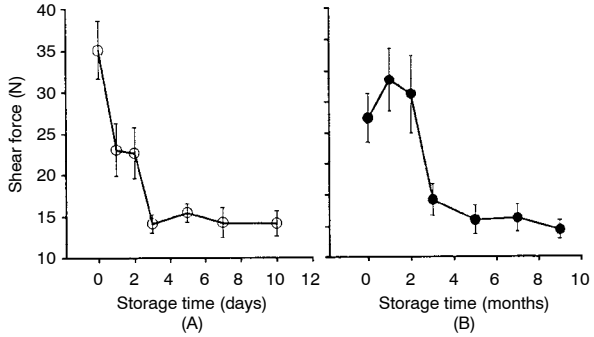


stood. It is suggested that some detrimental physicochemical changes are responsible for the decreased protein solubility. As the freezing temperature decreases, ice crystals are formed and bring about some mechanical damage to cellular components; concurrently, a more hydrophobic environment is created in the frozen meat as its aqueous phase is transformed from a liquid state to a solid state (Xiong, 1997). The above changes might make the embedded internal hydrophobic amino acid residues extend to the surface so that myofibrillar proteins interact with each other hydrophobically to form high molecular protein aggregates as observed by Buttkus (1970) in trout myosin solutions. In addition, freezing might also break the disulfide bonds and cause denaturation of the proteins (Castrillón et al., 1996). The combination of these factors brings about the decline in protein solubility of frozen stored fish meat. Although freezing significantly reduced the protein solubility of paddlefish meat, overall, the decline in protein solubility was relatively small compared to other fish species. For example, a 70-80% reduction in protein solubility was reported for frozen cod (Connell, 1962). Hence, paddlefish meat proteins seemed comparatively resistant to freezing damage.

Texture

Under refrigerated storage, the shear force decreased steadily during the first 3 days and remained at the reduced level during further storage (Figure 3A). Under frozen storage, shear force showed no

FIGURE 3. Shear force of paddlefish meat under 0°C refrigerated (A) and −29°C frozen storage (B). Each point represents the mean ± standard error from six replicates.



appreciable changes within the first 2 months, then dropped to 15 N and stayed at that level for the rest of the frozen storage period (Figure 3B). Generally speaking, during storage, the texture of fish meat may become tough (Gill et al., 1979) or soft and crumble (An et al., 1994) depending on the species. There is evidence indicating that the texture of high fat fish tends to become tough during frozen storage because these fish usually undergo high level of lipid oxidation; the resulting derivative products facilitate the formation of cross-links between myofibrillar proteins so that their water-holding capacity is also reduced (Gill et al., 1979; Sikorski et al., 1976). However, this should not be a major concern for paddlefish meat because it is relatively low in fat content and its shear force did not increase during the entire period of frozen storage. Compared to the shear force of hybrid striped bass (Xiong et al., 1996), which has a shear force below 13 N in terms of the same cross area, paddlefish had relatively high shear force of 15 N even after refrigerated and frozen storage. Degradation of both stromal and myofibrillar proteins can lead to decreases in shear force of fish meat from different species (An et al., 1994; Porter et al., 1996; Sikorski et al., 1984). For myofibrillar proteins, proteolytic degradation during storage can be seen from the following electrophoretic analysis.

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis

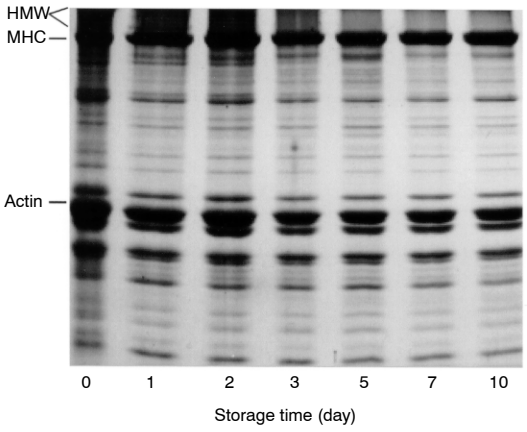
The SDS-PAGE showed that the intensity of the protein bands above myosin heavy chain (MHC) decreased for paddlefish meat

stored under the refrigerated condition (Figure 4). These changes corresponded to the decline in shear force, suggesting that the degradation of the high molecular weight proteins, which include nebulin and titin (Wang et al., 1979), might be responsible for the lowering of shear force during storage. In contrast, there were no apparent changes in myosin and actin (Figure 4). This is different from the meat of other fish species, such as Atlantic herring and mackerel, which undergoes significant proteolytic changes in both myofibrillar and stromal proteins during postmortem storage (Haard et al., 1979; Sun-Pan et al., 1986). Since the integrity of myofibrillar proteins is critical to the functionality of fish meat, degradation of myofibrillar proteins, especially myosin, results in very poor gel-forming ability of some fish products, such as Pacific whiting (An et al., 1994). Hence, the lack of myosin degradation was an indication that paddlefish meat could maintain its functionality during storage.

Differential Scanning Calorimetry

Thermal scan of the fish meat paste with a differential scanning calorimeter showed two major endothermic transitions, which were ascribed to denaturation of myosin (55°-57°C) and actin (77°-79°C), respectively (Stabursvik and Martens, 1980; Srinivasan et al., 1997).

FIGURE 4. SDS-PAGE pattern of paddlefish meat proteins during refrigerated storage. HMW = high molecular weight proteins and MHC = myosin heavy chain.



There were no significant changes in the T_m (temperature at maximum heat flow) and ΔH (change in total enthalpy) for myosin (transition I) and actin (transition II) during either refrigerated or frozen storage (Figure 5). In contrast, Davies et al. (1994) observed marked changes in the thermograms of herring and snapper meat during frozen storage, reflecting the instability of the conformation of their myofibrillar proteins. The DSC results from the present study further indicated that myofibrillar proteins of paddlefish meat was relatively stable under the storage conditions examined.

Thiobarbituric Acid Reactive Substances

During refrigerated storage, TBARS content did not vary significantly until day 10 (Figure 6A), suggesting minimal lipid oxidation during that period. During frozen storage, TBARS increased only after 7 months (Figure 6B). Other studies showed that lipid oxidation of fish meat might facilitate the cross-links between peptides, rendering the fish meat tough (Gill et al., 1979; Sikorski et al., 1976). Our results suggested that paddlefish meat was stable oxidatively for up to 7 days under refrigerated storage. The TBARS in fish meat frozen for 9 months was essentially the same as that in the fish meat refrigerated

FIGURE 5. Thermograms of paddlefish meat under 0°C refrigerated (A) and −29°C frozen storage (B).

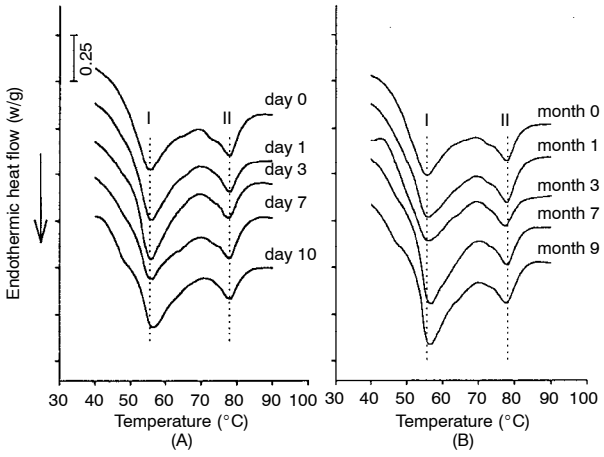
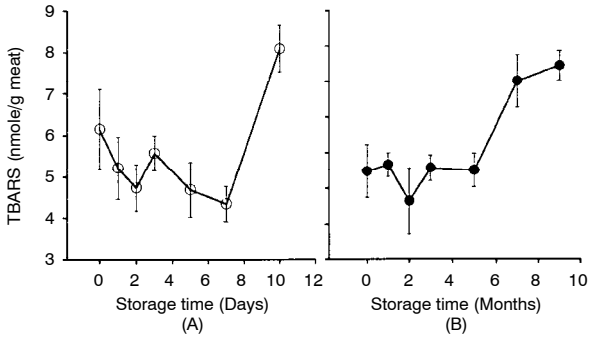


FIGURE 6. TBARS content of paddlefish meat under 0°C refrigerated (A) and −29°C frozen storage (B). Each point represents the mean ± standard error from six replicates.



for 10 days, indicating that frozen storage effectively extended the oxidative stability of lipids in paddlefish meat.

CONCLUSION

Paddlefish meat contained 79.0% moisture, 17.5% protein, and 3.1% fat. Its protein solubility and shear force maintained a relatively high value after the initial decline under both storage conditions. There was about an 80% increase in TBARS toward the end of both refrigerated and frozen storage. Although some high molecular weight proteins were broken down, the major myosin and actin of paddlefish meat did not change significantly during refrigerated storage. Therefore, paddlefish meat was relatively stable under both refrigerated and frozen conditions.

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Amino Acid Composition of Fish Meat After Different Frozen Storage Periods

Diana Wesselinova

ABSTRACT. A new approach is used to investigate one definite aspect of frozen ocean fish (including scad, mackerel, gray/humped rock cod, red/black sea bream, belted bonito-filleted) after different terms of storage at −35°C. We intended to prove whether the season, place and depth of catch influence the amino acid content of the frozen fish meat. The analysis of the bound amino acids in the proteins of the fish muscle in intervals of 3, 6, 9 and 12 months after draught has shown that even to the end of the storage the irreplaceable amino acids remain unchanged and slight deviations are observed in the amount of the others. The appearance of diaminopimelic acid (DAP) shows a microbiological contamination, especially after long storage, but this very low temperature does not allow a drastic increase of the psychrophyles. The methionine sulfoxide (Ms) which appeared also, shows only an oxidation of the methionine which is common in long storage of samples. *[Article copies available for a fee from The Haworth Document Delivery Service: 1-800-342-9678. E-mail address: <getinfo@haworthpressinc.com> Website: <http://www.HaworthPress.com>]*

KEYWORDS. Amino acid variations, frozen ocean fish, extended storage

INTRODUCTION

There are very scarce data (if any at all) about certain amino acid investigations of fish meat. Spotte (1992) points out that after disin-

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tegration of live water communities the resulting products (including amino acids) have two origins: from the dead bacterial cells and from the organic tissue. The author gives an example for the content of some common (essential) amino acids in the raw muscle tissue for several seawater fish species in g/100 g total protein (e.g., Atlantic and Pacific herring, Atlantic cod, Atlantic/chub mackerel, etc.). But we could not find an examination of the amino acid content value of the fish meat after long storage in frozen state. Such examinations would be desirable because of the necessity to know how the frozen product would affect the consumer.

The micro- and macro-elements in fish do not undergo remarkable deviations, depending on the fish, but it is known that some constituents change and influence the quality and fitness of fish meat. For example, it is known that the uric acid in the fish meat does not change the nutrients, but the trimethylamminoxide (TMO), which is increasing in adult fish, breaks down with storage. Together with other disintegration products such as indole, ammonium, mercaptan and others promote an undesirable taste and smell. The decarboxylation of histidine leads to histamine, which is a poison. The possibility to investigate a great number of ocean fish species, frozen immediately after draught, provided an opportunity to make an amino acid analysis of their meat protein after different terms of storage.

MATERIALS AND METHODS

Fish. The investigated fish species we divided in 10 groups depending on species, area of catch and season.

- 1st group–Mackerel icefish (*Champsocephalus gunnari*): φ–(latitude) 61°11′ OS; λ–(longitute) 36°24′ W; H-(depth) 320 m; t°C = 2; time of catch: 5:50 p.m.-9 p.m., 15th of February (summer); place: Southern Atlantic.
- 2nd group–Scad (*Trachurus trachurus*): φ–18°06′OS; λ–11° 31′OE; H-420 m; t°C = 19.5; time of catch: 10:40 a.m.-1:30 p.m., 13th of February (summer); place: Southern Atlantic.
- 3th group–Mackerel (*Scomber scombrus*): φ–42°59′ ON; λ–62° 09′ SW; H-166 m; t°C = 8; time of catch: 10 a.m.-1 p.m., 14th of February (winter); place: Northern Atlantic.

- 4th group–Mackerel: φ–58°12′ ON; λ–5°35′ OW; H-93 m; t°C = 6; time of catch: 2:30 p.m.-3:40 p.m., 14th of February (winter); place: Northern Atlantic.
- 5th group–Mackerel: φ–18°34′ S; λ–11°46′ E; H-150 m; t°C = 17; time of catch: 1:55 a.m.-4 a.m., 14th of February (summer); Southern Atlantic.
- 6th group–Gray rock cod (*Lepidonozothen squamiformis*): φ–60° 37′ OS; λ–47°00′OW; H-300 m; t°C = 1; time of catch: 8:20 a.m.-10 a.m., 10th of February (summer); place: Southern Atlantic.
- 7th group–Humped rock cod (*Gobinotothen gibberifrons*): φ–55° 12′ OS; λ–36°15′ W; H-280 m; t°C = 1; time of catch: 9:20 a.m.-11:30 a.m., 10th of February (summer); place: Southern Atlantic.
- 8th group–Red sea bream (*Pagrus major*): φ–18°06′ OS; λ–11° 31′ OE; H-245 m; t°C = 19.5; time of catch: 6:20 a.m.-9 a.m., 13th of February (summer); place: Southern Atlantic.
- 9th group–Black sea bream (*Spondyliosoma cantharus*)–the same parameters as group 8.
- 10th group–Belted bonito (*Palamic sarda*): φ–20°30′ OS; λ–17° 35′ OW; H-78 m; t°C = 18; time of catch: 4 p.m.-6:10 p.m., 4th of February (summer); place: Southern Atlantic.

Estimation of protein content: The crude protein was estimated by the method of Bradford (1976).

Material for the aminoacid investigation: From each fish group several samples of fish muscle were taken and stored for different periods of time (3, 6, 9 and 12 months) at −35°C. Every 3 months, 10 samples from each group were thawed and prepared for aminoacid investigation.

Amino acid analysis: 250 mg of fish muscles (from each 10 samples separately) in ampules were overloaded with 10 ml 6N HCl each. They were put for 24 h in 110°C, washed from the HCl and dissolved in citrate sample buffer pH 2.2. Afterwards, the amino acid analysis was carried out in aminoanalysor AAA 881 (‘‘Microtechna,’’ Praha) by the instructions for this analysor (1973).

RESULTS

As shown in Tables 1-4, the quantitative variations of the amino acids, depending on the time of storage are slight. More interesting is the deviation by the irreplaceable amino acids (Lys, Thr, Met, Leu, Val, Phe, Iso and Try). A slight decline of the Met-values on the 12th month of storage in groups 4, 5, 9 and 10 was observed. Methionine sulfoxide (Ms) was found also. The Lys values in the same groups did not change to the end of the 12th month and the Thr values showed significant variations by the 12th month only in groups 4 and 6. Val, Leu and Phe did not change at all up to the end of storage and Iso was reduced on the 12th month in the groups 8, 9 and 10. On the 12th

TABLE 1. Amount of bound amino acids (μM/mg protein) in samples from different fish species stored frozen for 3 months after catch.

Amino acids	Groups									
	1	2	3	4	5	6	7	8	9	10
Lys	3.75	5.00	4.59	4.47	3.53	4.36	4.75	5.13	3.54	3.92
His	1.00	2.50	1.62	2.11	2.74	0.77	1.25	2.05	1.46	2.94
Arg	2.00	2.75	2.70	2.57	2.54	1.79	2.00	3.08	2.29	2.35
Asp	4.25	5.75	5.41	4.47	5.88	4.10	5.50	6.15	4.17	5.29
Thr	2.50	3.00	3.51	3.68	3.14	2.82	4.50	2.82	2.50	2.73
Ser	2.25	3.00	3.24	3.16	2.94	3.08	4.50	3.08	2.50	2.54
Glu	5.50	9.25	8.92	8.42	8.24	7.69	8.00	9.74	7.08	7.45
Pro	1.75	2.00	1.89	1.58	2.35	1.54	2.00	2.31	1.88	2.16
Gly	3.00	4.00	4.32	4.47	3.92	3.33	4.00	3.85	3.54	4.31
Ala	4.00	4.00	4.59	4.21	5.10	3.85	6.00	5.13	4.58	4.31
Val	2.00	2.75	2.70	2.37	2.73	2.95	2.25	2.56	2.08	1.96
Met	1.00	1.50	1.35	1.32	1.37	1.03	1.50	1.28	1.04	1.36
Iso	1.75	2.50	2.43	2.11	2.54	1.79	2.25	2.56	2.08	2.35
Leu	3.50	4.50	4.05	3.95	4.31	3.85	4.50	4.28	3.75	3.92
Tyr	1.25	1.25	1.35	1.32	1.37	0.77	1.25	1.56	1.25	1.18
Phe	1.50	1.75	1.89	1.58	1.76	1.28	1.75	1.79	1.66	1.57
Other	-	DAP[1]	DAP[1]	DAP[1]	-	DAP[1]	DAP[1]	-	DAP[1]	DAP[1]

[1] 2,6-diaminopimelic acid, not counted

TABLE 2. Amount of bound amino acids (μM/mg protein) in samples from different fish species stored frozen for 6 months after catch.

Amino acids	Groups									
	1	2	3	4	5	6	7	8	9	10
Lys	3.25	4.25	4.74	3.24	4.12	3.08	3.00	4.00	4.06	3.92
His	1.00	1.75	2.11	1.89	4.12	1.03	1.25	1.00	1.22	2.94
Arg	1.75	2.50	2.63	2.16	2.16	1.79	2.00	2.50	2.38	2.35
Asp	5.25	6.50	6.58	4.86	5.59	5.13	4.75	5.75	5.51	6.08
Thr	2.25	2.75	2.63	2.16	2.35	2.05	2.75	2.50	1.63	2.54
Ser	2.50	3.25	3.68	2.43	2.75	2.56	2.50	2.25	2.24	2.94
Glu	6.00	7.75	7.37	5.79	7.06	6.15	6.75	6.50	4.90	6.66
Pro	2.25	3.50	4.74	2.97	2.94	2.30	2.25	3.25	2.24	3.53
Gly	3.50	4.75	5.00	4.05	3.72	3.08	3.50	4.00	2.86	4.90
Ala	4.50	5.50	4.74	4.06	4.70	4.70	2.75	4.75	3.67	4.90
Val	2.00	2.75	2.89	2.16	1.76	2.05	2.25	2.00	1.63	2.35
Met	1.25	1.75	1.58	1.35	1.37	1.28	1.25	1.50	1.02	1.37
Iso	1.25	1.75	2.11	1.35	1.76	1.03	1.50	1.75	1.02	1.76
Leu	3.75	4.50	4.74	3.78	4.31	3.59	4.00	4.00	3.47	4.70
Tyr	1.00	1.50	1.58	1.08	1.31	1.03	1.00	1.50	1.02	1.37
Phe	1.25	1.75	1.58	1.35	1.57	1.03	1.75	1.29	1.43	1.76
Other	MS[1]	-	-	-	-	-	-	-	-	-

[1] Methionine sulfoxide, trace

month of storage (Table 4) an increased amount of all amino acids was observed (by mg protein).

At the beginning of storage DAP (diaminopimelic acid) was not observed, but by the end of 12th month it appeared in almost all the samples.

DISCUSSION

In the literature we did not find similar investigations. Orlova and Kuranova (1977) described differences in amino acid analysis on different fish-parts. Their results are not compatible to ours because the

TABLE 3. Amount of bound amino acids (μM/mg protein) in samples from different fish species stored frozen for 9 months after catch.

Amino acids	Groups									
	1	2	3	4	5	6	7	8	9	10
Lys	ND	ND	ND	ND	4.31	ND	3.75	7.25	ND	ND
His	ND	ND	ND	ND	3.33	ND	1.50	2.75	ND	ND
Arg	ND	ND	ND	ND	3.14	ND	2.75	3.25	ND	ND
Asp	ND	ND	ND	ND	7.45	ND	7.00	8.75	ND	ND
Thr	ND	ND	ND	ND	3.53	ND	2.75	3.50	ND	ND
Ser	ND	ND	ND	ND	3.53	ND	3.50	3.75	ND	ND
Glu	ND	ND	ND	ND	7.84	ND	8.50	9.75	ND	ND
Pro	ND	ND	ND	ND	1.76	ND	2.75	4.50	ND	ND
Gly	ND	ND	ND	ND	5.69	ND	4.25	5.75	ND	ND
Ala	ND	ND	ND	ND	7.25	ND	5.50	6.50	ND	ND
Val	ND	ND	ND	ND	2.94	ND	2.50	3.00	ND	ND
Met	ND	ND	ND	ND	0.98	ND	1.00	1.25	ND	ND
Iso	ND	ND	ND	ND	2.35	ND	2.00	2.50	ND	ND
Leu	ND	ND	ND	ND	5.49	ND	5.00	6.25	ND	ND
Tyr	ND	ND	ND	ND	1.76	ND	1.75	2.25	ND	ND
Phe	ND	ND	ND	ND	2.16	ND	1.75	2.25	ND	ND
Other	ND	ND	ND	ND	DAP[1]	ND	-	DAP[1]	ND	ND

ND = not done
[1] 2,6-diaminopimelic acid, not counted

authors used other fish species and measured the amino acids in different kind raw tissue.

The aim of our study was to state whether the long frozen storage of ocean fish at $-35°C$ (the temperature for storage of meat product by the manufacturer) would influence the amino acid content of the fish protein and thus the nutritious value for the consumer.

It is important that the deviation by the irreplaceable amino acids throughout of the whole storage period was very slight. Only a decline of the Met-values on the 12th month of storage (Table 4) in groups 4, 5, 9 and 10 was stated and we assume that in these groups an oxidation of the Met to methionine sulfoxide (Ms) was carried out (‘‘traces’’ of

TABLE 4. Amount of bound amino acids (μM/mg protein) in samples from different fish species stored frozen for 12 months after catch.

Amino acids	Groups									
	1	2	3	4	5	6	7	8	9	10
Lys	3.00	5.75	3.42	4.59	3.73	5.26	5.75	4.75	4.08	3.92
His	2.52	2.00	1.58	1.89	2.16	1.32	1.50	1.00	1.43	3.14
Arg	2.00	3.00	2.11	2.16	2.16	2.63	2.75	2.50	2.24	1.96
Asp	4.75	9.00	5.53	5.69	5.49	6.58	7.50	7.25	5.30	5.69
Thr	2.25	3.50	2.11	2.43	2.16	2.37	3.00	2.25	2.04	2.16
Ser	2.50	3.75	3.16	3.24	2.75	3.68	4.75	3.25	3.27	3.14
Glu	6.75	9.50	6.58	6.49	6.08	8.16	10.00	7.25	6.33	6.47
Pro	2.50	4.75	2.63	2.70	2.75	2.89	3.75	2.50	3.27	2.55
Gly	3.50	6.25	2.63	3.24	2.55	2.89	4.25	2.75	2.04	2.55
Ala	5.00	7.00	3.42	3.78	3.14	3.95	5.25	3.50	3.06	2.94
Val	1.50	2.25	ND	2.16	1.96	1.58	3.00	2.00	1.84	2.75
Met	1.25	1.75	ND	0.54	0.78	1.32	1.75	1.00	0.84	0.59
Iso	1.50	2.25	ND	1.62	1.18	1.05	2.50	-	1.43	1.18
Leu	4.25	5.75	ND	3.24	3.14	3.16	5.00	3.75	2.86	3.14
Tyr	1.50	1.25	1.05	1.35	1.18	1.58	2.00	1.75	1.01	1.18
Phe	1.75	2.25	1.01	1.35	1.18	2.10	2.25	2.25	1.46	1.37
Other	DAP[1]	DAP[1]	DAP[1]	DAP[1]	DAP[1]	DAP[1]	DAP[1]	DAP[1]	DAP[1]	DAP[1]
	-	-	Ms[2]	Ms[2]	-	MS[2]	-	Ms[2]	-	Ms[2]

[1] 2,6-diaminopimelic acid, not counted
[2] Methionine sulfoxide, trace
ND = not done

it). The increased amount of all amino acids (by mg protein) on the 12th month of storage could be due to transition of one kind of amino acid to another (oxidation, de-amination, etc.). Very important is the fact that this increase does not affect the irreplaceable amino acids.

The appearnace of DAP during the different terms of storage may be explained by the presence of bacterial contamination (*E. coli* or its membranes which contain specifically this amino acid) or with the bacterial increase. The presence of DAP at the start of storage (month 3) (Table 1) means that a bacterial contamination was already present at

the time the fish were caught and processed. At 6 months of storage (Table 2) DAP is absent; thus, most of the initial microorganisms did not survive at this low temperature. The appearance of DAP again at the end of storage (9 and 12 months-Tables 3 and 4) could be explained with a secondary multiplying of psychrophyles in the samples. As microbiological investigations on frozen ocean fish (Bailyozov et al., 1976) show, this multiplying depends not only on the period of storage but on the keeping of constant temperature as well. In our case this storage was at $-35°C$ and the observed variations of the amino acids undoubtedly show a prolonged preservation of the fish protein. Changes in the fish color up to the end of storage were not observed. The variations of some amino acids in the same type of fish could be explained to great extend with the different place, time, season and depth of catch. The season and depth are extremely important because during winter and on a deeper place the contamination of the fish (fish samples) is practically zero; we know that during winter some fish species (especially these with a higher fat content, e.g., *Scomber scombrus*) accumulate more proteins per g body weight (probably compensatory) and this reflects on the higher content of the amino acids per g protein.

In conclusion it could be said that the extended storage of the examined fish species at $-35°C$ does not influence dramatically the amino acid values. The investigation shows as well that the amino acids differ in the same type of fish depending on the time and place of catch. Slight contaminations could be observed and DAP can appear, but neither DAP nor the deviations of the amino acids change the nutritious quality of the fish.

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Solubility of Cod Muscle Myofibrillar Proteins at Alkaline pH

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ABSTRACT. The solubility characteristics of proteins are of interest because of their relation to many functional properties. The solubility of the proteins of washed cod muscle mince increased dramatically between pH 8.9 and 9.2 at an ionic strength of 10 mM, whereas a high but constant solubility was observed at 430 mM sodium chloride over the pH range from 7 to 9.5. At pH 9.2, the proteins of the washed cod muscle were greater than 60% soluble at a sodium chloride concentration of 6.6 mM but were salted out at slightly higher concentrations. Above about 100 mM to about 900 mM salt, the proteins were salted in, and greater than 90% solubility was achieved. Maximal extractability was observed with a ratio of 36 volumes of extracting solution to 1 weight of minced muscle at pH 9.2. At pH 8.5, the extractability of the protein increased from 11% to 24% over a range in extraction volumes of 12:1 to 144:1 of volume of extracting solution to muscle tissue weight. *[Article copies available for a fee from The Haworth Document Delivery Service: 1-800-342-9678. E-mail address: <getinfo@haworthpressinc.com> Website: <http://www.HaworthPress.com>]*

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KEYWORDS. Alkaline solubilization of muscle proteins, cod proteins, muscle proteins, myofibrillar proteins, protein solubility, solubility of muscle proteins

INTRODUCTION

The solubility of proteins is an important property that has both theoretical and practical aspects. Solubilization is a first step in the separation and isolation of proteins. Many functional properties of food proteins have been related to solubility (Hultin et al., 1995). It has been hypothesized that the formation of protein gels for the food and pharmaceutical industries requires solubilization of the principal muscle protein, myosin, to allow gel formation (Suzuki et al., 1981; Lee, 1984, 1986; Shimizu, 1985). Although it is generally believed that myofibrillar proteins require high salt concentrations for solubilization (Hultin et al., 1995), we have demonstrated that cod myofibrillar proteins can be solubilized at low salt concentrations at both neutral and acid pH (Stefansson and Hultin, 1994). The myofibrillar proteins of chicken breast muscle (Krishnamurthy et al., 1996) and mackerel light muscle (Feng and Hultin, 1997) are also soluble at low salt concentrations and neutral pH if certain proteins are first extracted which appear to inhibit the water solubility of the majority of the myofibrillar proteins. Montecalvo et al. (1984) obtained maximal extractability (about 85%) of the total nitrogen from mechanically deboned flounder frame paste at pH 11. Slightly lower values were obtained at pH 10 and about 40% at pH 9. Trevino et al. (1990) reported that the proteins of sardine surimi had solubilities as high as 30 to 50% at pH 9 with no added sodium chloride. The surimi did contain, however, 0.3% of polyphosphates. Presumably the base necessary to raise the pH would contribute some increase in ionic strength as well. Alkaline extraction has been used to recover proteins from beef bones (Jelen et al., 1979), mechanically separated chicken meat (McCurdy et al., 1986) and seal meat and bones (Shahidi and Synowiecki, 1995). Our objective in this work was to evaluate the solubility characteristics of the myofibrillar proteins from washed cod muscle mince at alkaline pH as affected by salt concentrations.

MATERIALS AND METHODS

Fresh, gutted, whole cod (*Gadus morhua)* or fresh cod fillets were purchased from local fish dealers in Gloucester, MA. The muscle was

cut with a sharp knife into cubes about 2 cm in size and placed in 12 times its weight of cold, distilled, deionized water. The muscle was homogenized in a Kinematica Blender (Model PCU-1 Kinematica Gmbh, Switzerland) for 2 min at high speed. The speed of the homogenizer was controlled by supplying it with power through a variable transformer (Adjust-A-VOLT, a product of Standard Electrical Products Co., Dayton, OH). A transformer setting of 80 comprised high speed. The resultant homogenate was then centrifuged at 30,400 × g for 20 min in a Beckman L50 -65B Ultracentrifuge using a Model 19 rotor (Beckman Instruments, Fullerton, CA). The sediment obtained after this centrifugation was considered the washed, minced cod muscle.

L-Histidine, tissue culture grade, was a product of Fisher Scientific Co., Pittsburgh, PA. All other chemicals were reagent grade.

The effect of pH on cod protein solubility was tested by resuspending the washed muscle preparation in 5 mM histidine buffer adjusted to the desired pH. The weight of the buffer used was 5-fold the weight of the supernatant removed during the previous washing step. The second homogenization was performed by blending samples in an homogenizer for 30 sec at a setting of 40 on the variable transformer (low speed). After the various treatments, the homogenates were centrifuged again at 30,400 × g for 20 min. The protein content of the resuspended whole homogenate was determined and compared to the protein content of the supernatant after this second centrifugation. The ratio of the latter to the former was taken to determine the extent of solubility. These effects of pH were determined at two concentrations of sodium chloride. One sample had an effective ionic strength of 10 mM. In preparing this sample, the ionic strength contributed by the histidine at each pH was determined and taken into account. Then sufficient sodium chloride was added to bring the ionic strength to 10 mM. Solubilization as a function of pH was also determined at 430 mM (2.5%) sodium chloride as representative of a high salt treatment. No corrections were made for buffer contributions at this salt concentration.

The effect of salt concentration on solubilization of the proteins of washed, minced cod muscle was determined in a similar way. The resuspended washed, minced muscle was adjusted to pH 9.2 in 5 mM histidine buffer with sodium hydroxide and appropriate concentrations of sodium chloride were added.

The effect of tissue concentration on protein solubility was determined by resuspending the washed, minced cod muscle in different quantities of 5 mM histidine buffer brought up to pH 9.2. The ratio of buffer to muscle was varied from 10-100 times the weight of the original muscle sample. A similar set of experiments was done maintaining the pH of the suspended minced muscle at pH 8.5. In this case, the variation in extracting solution used was 12-144 times the weight of the original muscle. Other conditions were the same as for the effect of pH on solubilization.

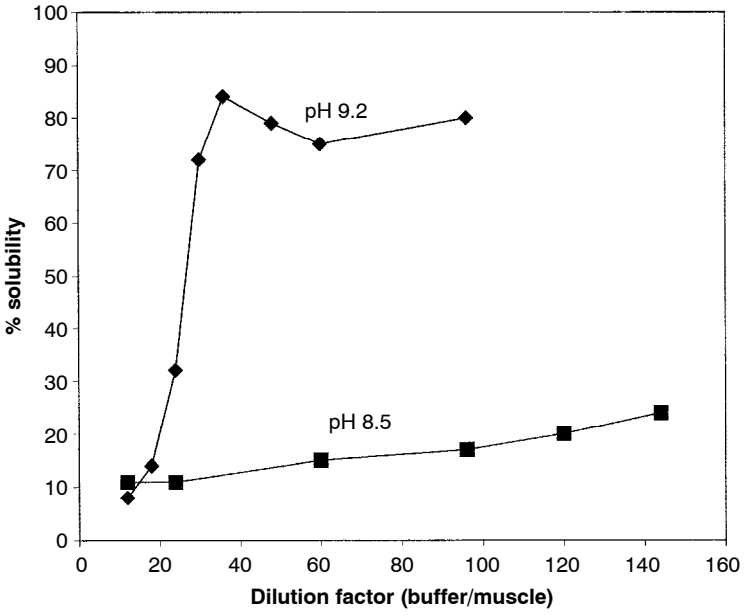
Proteins were measured by the Lowry method as modified by Markwell et al. (1978). The ionic strengths of the various muscle suspensions and protein solutions were estimated using a YSI Model 35 Conductivity Meter equipped with a YSI Conductivity Cell having a cell constant of 1 (YSI Inc., Yellow Springs, OH). Conductivity readings were interpreted using a standard curve prepared with sodium chloride in the concentration range of 0-2 mM. Dilutions were made as necessary to bring the test solutions to the proper concentration range.

All experiments were carried out at least in duplicate with assays done in triplicate.

RESULTS AND DISCUSSION

Results of the experiments to determine the effect of pH on the solubility of proteins of washed, minced cod muscle as a function of pH showed that the solubility of the proteins at 430 mM (2.5%) sodium chloride was almost constant over the pH range from 7 to 9.5 (Figure 1). The extent of the solubility at this high salt concentration was between 82-86%. On the contrary, the effect of pH on the solubility of the cod proteins at low ionic strength (10 mM) was very dependent on pH. There was a great change in solubility between pH 8.9 (20% soluble) and pH 9.2 (93% soluble). Over the range of pH 9.6 to 11.2, 97-98% of the proteins were soluble. The rapid increase in solubility over a relatively small pH change under these alkaline conditions was similar to the results that were observed with washed, minced cod muscle at neutral pH when the ionic strength was less than 1 mM (Stefansson and Hultin, 1994). Thus, the higher salt concentration of approximately 10 mM used in these experiments compared to our earlier ones (Stefansson and Hultin, 1994) caused an upward shift

FIGURE 1. The effect of pH on the solubility of minced, washed cod muscle proteins at 10 and 430 mM sodium chloride.



of about 2 pH units in the solubilization curve of the cod muscle proteins over those observed at an even lower ionic strength (< 1 mM). Sarkar (1950) showed that the isoelectric point of rabbit myosin shifted from about 5.3 in a salt-free solution to just below 7 in the presence of 25 mM potassium chloride. This would indicate that the addition of salt at an ionic strength of 25 mM would shift the point of maximal insolubility by about 2 pH units towards the alkaline side. To introduce enough negative charges to make the proteins soluble again would require raising the pH. Thus, our results are similiar to those of Sarkar (1950) with purified rabbit myosin in that an increase in salt concentration caused a shift in the solubility curve of about 2 pH units towards the alkaline side. Minced cod muscle is made up of many contractile and cytoskeletal proteins, not all of which show the same solubility dependence on pH and ionic strength (Stefansson and Hultin, 1994). Presumably, much of the approximately 15-20% of protein which is soluble at pH 8.9 and below in these studies represent the

more soluble components of cod muscle myofibrils such as tropomyosin and troponins.

Muscle proteins have a net negative charge at neutral pH. There would also be, however, some positive charges. These may generate sufficient attractive forces that the proteins interact and are not soluble. Myosin molecules interact with one another through their hydrophobic tail regions in a staggered fashion. This is caused by four patches each of positive and negative charges on the carboxy terminal half of each myosin tail arranged with a stagger of about 14 nm (Shoffner and De Lozanne, 1996). It has been suggested that myosin molecules have to first dissociate from the thick filaments before they become soluble (Parsons and Knight, 1990). As the pH increases, there is an increase in the negative charge of the molecules as amino groups lose their positive charge and negative charges are produced from other side chain amino acids. Béchet and Ozog (1995) have suggested that ionization of tyrosine residues make the tryptophan region of rabbit myosin less stable at alkaline pH. This change in the tryptophan region can be measured by fluorescence; the change is observed to occur beginning at about pH 8.5. The two tryptophan residues involved occur in the middle of each rod chain and are thought to be located at hydrophobic interfacial sites (Béchet and Ozog, 1995). The two ionizable tyrosines are located near these tryptophan residues. These areas of the tail portion could be very important in maintaining structural integrity of the thick filaments. These authors also showed that myosin maintains its helicity over the pH range of 2 to 11.

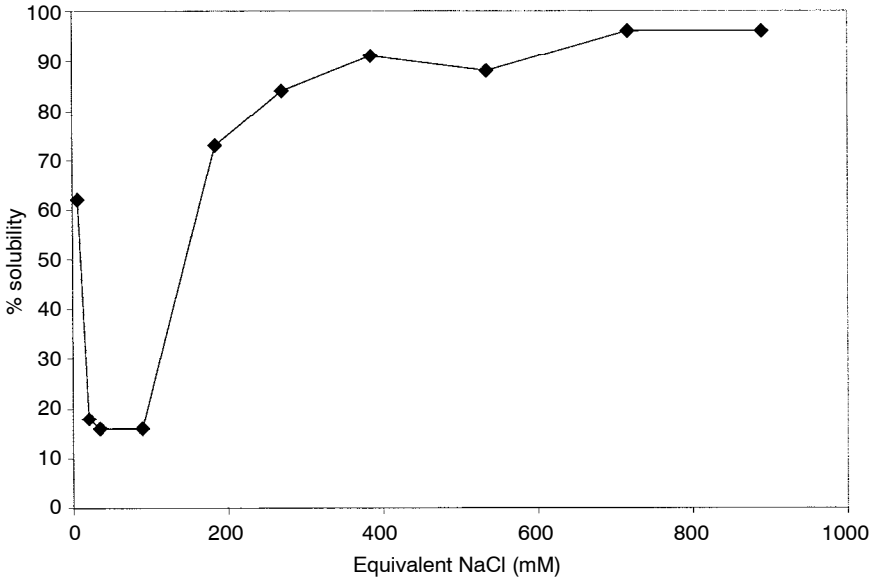
Contrary to the results at low ionic strength (10 mM), there was little change in solubility observed at a sodium chloride concentration of 430 mM from pH 7 to 9.5. At the same concentration of potassium chloride, the isoelectric point of isolated rabbit myosin was shifted down close to 4 (Sarkar, 1950). There should be a large negative charge at pH 7 on myosin under those conditions. The 14-18% protein that remained insoluble under these conditions may have been those that were salted-out at 430 mM sodium chloride. At the high ionic strength, the charges on the proteins may have been reduced by the high concentrations of cations and anions contributed by the salt. The net result was that ionizations in some of the proteins had little effect at these high salt concentrations. Salting-out is generally ascribed to the loss of a stable hydrophilic surface, causing the exposed hydropho-

bic areas of proteins to interact and cause aggregation and precipitation (von Hipple and Schleich, 1969).

Solubility of the proteins of washed, minced cod muscle as a function of increasing sodium chloride concentration at pH 9.2 was determined (Figure 2). Solubility at an ionic strength of 6.6 mM was over 60 percent. This then decreased rapidly to a minimum of 16 to 18 percent solubility over a range of ionic strengths from about 20 to 100 mM. Thereafter, solubility increased rapidly reaching 96 percent at an ionic strength of 717 mM.

It is difficult to describe completely the pathway by which the myofibrillar proteins solubilize as a function of salt concentration. In addition to the protein:protein and protein:solvent interactions, there are probably conformational changes and polymerization:depolymerization shifts. It is likely that the forces that determine whether the protein molecules are stable in solution are those which are involved in protein polymerization and depolymerization (monomer-filament formation). Tonomura et al. (1962) found that a number of physical

FIGURE 2. The effect of sodium chloride concentration on the solubility of the proteins of washed cod muscle mince at pH 9.2.

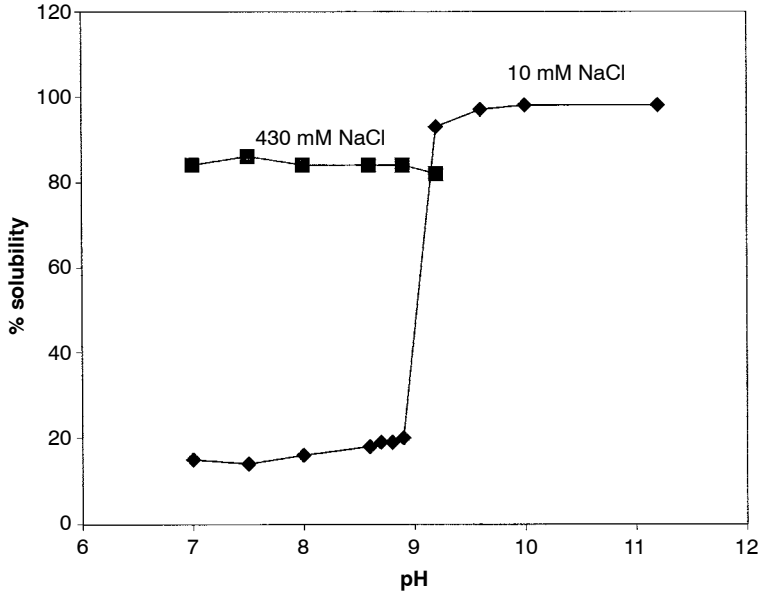


properties of myosin, such as α-helical content and viscosity, changed sharply over a narrow range of salt concentration.

A similar explanation can be given for the ionic strength effect as was given above for the pH effect. At pH 9.2 the muscle proteins have a negative charge. The repulsive forces from these negatively charged side chains are sufficient to keep the individual protein molecules apart. When salt is added, the negative charges are neutralized by the cationic component of the salt. This reduces the overall negative charge of the molecules and allows for interaction through other forces such as hydrophobic. As the ionic strength is increased, the proteins undergo a salting-in. Shen (1981) pointed out that there is a class of proteins where solubility first decreases and then increases as salt concentration increases. The proteins of soy isolates were the only proteins considered by Shen (1981) to behave this way, although he recognized that the polymerization and depolymerization of muscle actin also showed this behavior (Nagy and Jencks, 1965). Later we showed that cod muscle proteins undergo salting-out/salting-in at neutral pH. In addition, there was a further salting-out at very high salt concentrations (> 1 M) (Stefansson and Hultin, 1994). The salting-out/salting-in phenomenon was also seen in this study when the experiment was conducted at pH 9.2 (Figure 2).

Muscle proteins have a great capacity to bind water. In extracting these proteins from tissue, it is necessary to have a sufficient volume of water to prevent the formation of a gel. At neutral pH and low ionic strength, it was shown that the proteins of washed, minced cod muscle gelled at a ratio of mince to water of 1:20 (Stefansson and Hultin, 1994). A 1:50 extraction was required to obtain maximal extraction of the proteins. A similar study here was done at pH 9.2 where the cod muscle proteins showed high solubility, and at pH 8.5 where the solubility was poor (Figure 3). A low ionic strength was maintained in both cases. The ionic strength was that contributed by the 5 mM histidine buffer that was required to adjust the pH to the appropriate value. At pH 9.2, maximal protein solubility was seen at dilutions 36-fold or greater; solubility at these dilutions was ten times the amount of solubilization that was observed at a dilution of 1:12. No greater increase was observed at dilutions up to 96-fold. At pH 8.5, solubility at the lowest dilution (an extraction ratio of 12:1) was about the same as it was in the case of extraction at the higher pH at this dilution. The extractability of the washed cod muscle proteins at pH

FIGURE 3. The effect of the extent of dilution on the solubilization of the proteins of washed cod muscle mince at pH 8.5 and 9.2.



8.5 increased slowly and continuously, giving an approximately two-fold increase in solubility at 144-fold dilution compared to that at a 12-fold dilution. It is clear from these results that pH is a more critical factor in solubilizing cod muscle proteins than is dilution.

SUMMARY

The solubility profile of cod muscle proteins at an ionic strength of 10 mM was similar in form to that previously observed at neutral pH, i.e., there was a sharp increase in solubility over a narrow pH range. At 10 mM sodium chloride this increase occurred between pH 8.9 and 9.2 to almost 100%. The solubility of the cod muscle proteins at an ionic strength of 10 mM at neutral pH was less than 10%. Thus solubilizing the proteins at pH 9.2 permits greater quantities of salt to be used while still maintaining solubility than can be tolerated at pH 7. Cod muscle proteins at pH 9.2 showed the salting-out and salting-in effects previously observed at neutral pH. In extracting muscle proteins at pH

9.2 and an ionic strength of 10 mM, a 36:1 ratio of extracting solution to muscle tissue gave optimal extraction. At pH 8.5 where solubility was poor, there was a continual but small increase in extractability at least up to a 140-fold ratio of extracting solution to muscle tissue. The extractability of the proteins of washed, minced cod muscle at high ionic strength (430 mM) was greater at pH 9.2 than it was in our previous study at neutral pH.

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Thermal Processing Effects on the Textural Attributes of Previously Frozen Shrimp

Ferruh Erdoğdu
Murat O. Balaban

ABSTRACT. Shrimp texture changes during thermal processing based on its temperature history. Texture measurements and sensory tests were performed on large, medium, and small tiger shrimp. In isothermal experiments, shrimp were cooked in water at 55, 65, 75, 85 and 95°C for two time periods: for their slowest heating point to reach within 0.5°C of water temperature, and 50% longer than this. Transient experiments were also conducted at 75, 85, 95°C, and in boiling water for a time necessary to achieve a certain microbial lethality. Cooling after heat treatment was done in ice slush and in Ziploc bags in ice slush. Texture Profile Analysis and shear test were performed with a Instron Universal testing machine. Tenderness, juiciness, rubberiness, and overall acceptability were determined by sensory panels and correlated with instrumental results. Temperature significantly affected all textural properties and sensory attributes. High correlation between textural properties and sensory attributes was obtained. This correlation may help in predicting sensory attributes from instrumentally measured texture parameters. *[Article copies available for a fee from The Haworth Document Delivery Service: 1-800-342-9678. E-mail address: <getinfo@haworthpressinc.com> Website: <http://www.HaworthPress.com>]*

KEYWORDS. Shrimp, thermal processing, sensory analysis, textural properties

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INTRODUCTION

The shrimp industry in the United States is very important, especially in the Gulf and South Atlantic states. In 1997, 71% of the total United States landings were produced in this region. Eighty-two percent of the shrimp consumed in the U.S. was imported, worth $2.9 billion, accounting for 37% of total edible imports (NOAA, 1998). The quality and safety of both raw and cooked shrimp are important. Thermal processing can be used to assure the safety of shrimp; however, it causes yield loss due to changes in moisture content and affects the texture and sensory attributes.

The sensory quality of shrimp is defined as a complex set of characteristics including appearance, aroma, taste and texture (Shahidi and Botta, 1994). Thermal processing changes the sensory and textural properties of shrimp partly due to the denaturation of proteins. Texture is considered to be the most important sensory quality since it may change dramatically during extended cooking, while the characteristic shrimp flavor develops relatively early during the process and does not change substantially after prolonged heating (Ma et al., 1983). Texture is a multi-point property of foods (Bourne, 1978), and is defined as the properties arising from the structural elements and physiological senses (Szczesniak, 1963; Brady and Hunecke, 1985). Toughness, or hardness is probably the most critical texture attribute in meat or seafood products, and can be defined as a property which depends on the connective tissue, consisting of mainly collagen (responsible for tensile strength) and the myofibrils, consisting of myosin and actin (Martens et al., 1982).

Texture can be measured by two ways: instrumental or sensory methods. Since no instrument can detect, analyze and integrate a large number of textural sensations at the same time, sensory evaluation is more valuable in the measurement of food texture (Larmond, 1976). However, it may not be repeatable since it has methodological, physiological, and psychological variations, and it is costly and time consuming. Poor sensory analysis methods can be another source of problems in testing. Therefore, correlation of repeatable instrumental methods to sensory data is useful. The correlation between sensory and instrumentally obtained texture values was reported in the literature (Ahmed et al., 1973; Beilken et al., 1991; Brady et al., 1985; Chen and Trout, 1991; Chung and Merritt, 1991; Edmunds and Lillard, 1979; Ma et al.,

1983; Meullenett et al., 1994; Reid and Durance, 1992; Soo et al., 1977; Szczesniak, 1963, 1987; Troutt et al., 1992; Webb et al., 1975).

Texture Profile Analysis (TPA) and shear tests are two common instrumental tests to measure textural characteristics. TPA is a two cycle compression test (Bourne, 1978), and leads to the evaluation of seven textural parameters: five measured (hardness, kg; fracturability, kg; cohesiveness, dimensionless; springiness, mm; and adhesiveness, kg-mm) and two calculated from the measured parameters (gumminess, kg, and chewiness, kg-mm) (Bourne, 1978; Breene, 1975; Peleg, 1976). Definitions of these parameters were given by Bhattacharya et al. (1993), Bourne (1978), Correia et al. (1991), Erdoğdu (1996), Peleg (1976) and Szczesniak (1963), and are shown in Table 1. A typical TPA curve is given in Figure 1 (Bourne, 1978). Breene (1975) gives the important variables during TPA measurement as the following:

- Sample size and shape.
- Relative size of compressing unit (probe) to the sample: For larger probes, the recorded forces are compression. For smaller probes, the forces are a combination of compression and shear.
- Percent deformation: Researchers used a wide range of deformations (20%-80%). The choice of most workers has been 75-80% deformation level.
- Cross head speed.
- Number of replicates: Plant and animal tissue vary in their physical properties and homogeneity. A sufficient number of samples must be examined to produce statistically meaningful data.

Another test that can be used for the determination of food texture is the shear test, in which a piece of material is cut, and the force required for the blade to do the work is determined. Several researchers related shear force with hardness and chewiness (Ahmed et al., 1972 and 1973; Ma et al., 1983; Webb et al., 1975).

Ahmed et al. (1972) determined the effects of pH, cooking time, length of storage, and method of thawing on the texture of cocktail shrimp (280-540 count/kg). An Instron Universal Testing machine was used to determine the force required to shear the shrimp muscles through a distance of 5 mm at the thickest portion, and this force was used as an index of toughness. They concluded that texture was influenced by pH, cooking time, and length of storage, but not by thawing method. Ahmed et al. (1973) observed that soaking shrimp in

TABLE 1. Definitions and determination methods of Texture Profile Analysis (TPA) parameters.*

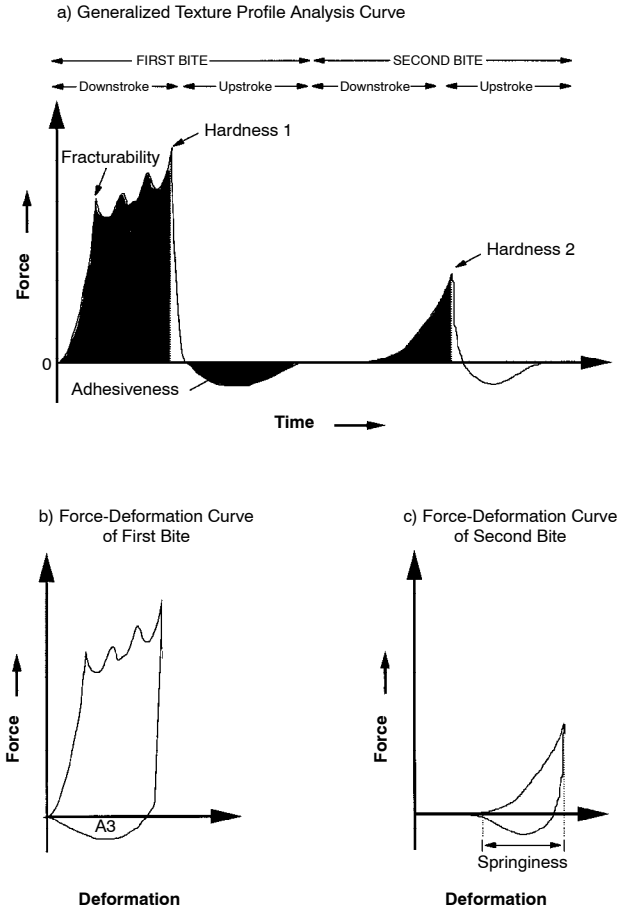
TPA parameter	Definition	Determination
Hardness	the force required to compress the food between teeth (or between tongue and palate for soft foods) to a given deformation	the peak force during the first compression cycle ("first bite")
Fracturability	the force at which food crumbles or cracks	the force at the first significant break in the force-deformation curve
Cohesiveness	the extent to which food can be deformed before it ruptures	the ratio of the positive area during the second compression to that during the first compression
Springiness	the rate at which a deformed material recovers to its undeformed condition after the deforming force is removed	the height that food recovers during the time that elapses between the end of the first bite and the start of the second bite
Gumminess	the amount of energy required to disintegrate a semi-solid food product to a state ready for swallowing	the product of hardness and cohesiveness
Chewiness	the length of time (or number of chews) required to masticate a solid food	the product of gumminess and springiness
Adhesiveness	the force required to remove food that adheres to the mouth	the negative area for the first bite, representing the work necessary to pull the compressing plunger away from the sample

* Adapted from Bhattacharya et al. (1993), Bourne (1978), Correia et al. (1991), Szczesniak (1962), Peleg (1976).

water or polyphosphate prior to cooking resulted in their tenderization compared to controls. It has been concluded that polyphosphate resulted in the weakening of muscle fiber structure and the swelling of its protein gel systems, thus increasing water holding capacity. Sensory panelists described the texture of the polyphosphate treated shrimp as juicy or similar to fresh shrimp. Webb et al. (1975) analyzed the effect of additives, processing techniques and frozen storage on the texture of peeled and deveined shrimp. Shrimp was tested for maximum shear force using an Instron. They found that phosphate presoaked raw shrimp had significantly lower shear values than untreated raw shrimp.

Soo et al. (1977) developed a technique to measure the texture of extruded shrimp shapes and cooked patties by determining hardness, elasticity and cohesiveness using an Instron. They used 80% compres-

FIGURE 1. (a) Generalized Texture Profile Analysis curve, (b) Force-Deformation curve of first bite, and (c) Force-Deformation curve of second bite. Adapted from Bourne (1978).



sion TPA and sensory evaluation by trained panelists, and correlated the sensory scores with instrumental results. The correlation coefficients were found to be higher than 0.8. Lerchenfeld (1981) investigated the effects of various cooking times and temperatures on texture. Shrimp were de-headed, shelled but not deveined, and cooked at temperatures from 35 to 100°C from 1.5 to 60 min. Texture measurements were run by two different methods with an Instron and Warner-Brat-

zler shear apparatus using the method of Ahmed et al. (1972). The maximum shear force value was used as an indicator of the degree of toughness of shrimp. The author concluded that cooking resulted in initial toughening at all temperatures. Interestingly, heating at 65°C produced the most tender shrimp, except for 35°C, which was close to the optimum temperature of neutral proteinase and similar to the optimum temperature of alkaline proteinase. The shear method of Ahmed et al. (1972) was found to be superior to the Warner-Bratzler shear apparatus when there are uniform sample sizes. Ma et al. (1983) determined the textural changes in canned shrimp by sensory and instrumental methods. They found a direct relationship between sensory perception of toughness and instrumental shear force measurements in canned shrimp processed at 124°C. Shrimp muscle toughened during the initial stages of heating and softened during the later stages.

Texture problems in cooked or frozen shrimp were described as toughness, attributed to the degree of cooking, pH (Ma et al., 1983), and length and conditions of frozen storage (Ahmed et al., 1972; Webb et al., 1975). It is generally accepted that the major components in muscle that can affect meat tenderness are muscle fibers and connective tissues. Therefore, heat denaturation of the myofibrillar proteins and shrinkage of collagen experienced in the early stages of heat treatment result in a tightening and stiffening of the structure. The softening during prolonged cooking is caused by the conversion of collagen to gelatin and dissociation of muscle proteins (Ma et al., 1983; Webb et al., 1975). All of these studies showed that the cooking time, temperature and their combination affect the textural properties of foods. However, there is little information about the sensory and textural changes of shrimp of different sizes with respect to temperature, time and their combination during thermal processing. Therefore, the overall objective of this study was to measure the changes in textural attributes of shrimp of different sizes by cooking at isothermal and transient temperatures, and to find a correlation between instrumental texture measurements and sensory properties.

MATERIALS AND METHODS

Raw Material

Tiger shrimp (*Penaeus monodon*) obtained from Singleton Seafood Company, Tampa, FL, in sizes of large (35-44/kg), medium (90-110/kg),

and small (110-132/kg), in frozen blocks was used in this study. No additives were used during pre-shipping and freezing processes. Each block was placed in a Ziploc bag, flushed with nitrogen, and stored in a freezer at −15°C. The blocks needed for a set of experiments were thawed under a stream of tap water at 21°C (thawing time around 45 min) and re-frozen in water in Ziploc bags (10 shrimp/bag) at −15°C. This assured that one freeze-thaw cycle was applied to a block during the study. Before cooking, the shrimp in the bag were thawed in 20-25 minutes under cold tap water while still in the bag, and then hand-peeled.

Cooking Temperatures for Isothermal and Transient Experiments

For isothermal experiments, five temperatures (55, 65, 75, 85 and 95°C) and two cooking times were chosen to study the effect of time, temperature and their combination on the sensory attributes and textural changes. The first cooking time was selected for the coldest point of shrimp to reach within 0.5°C of the water temperature. This time depended on the size of shrimp and water temperature. The second cooking time was chosen as 1.5 times the first one. When the slowest heating point reached the cooking water temperature, there was a uniform temperature distribution throughout the shrimp. The second cooking time was chosen as 1.5 times of the first to see if prolonged time at a given constant temperature had a significant effect on the change of sensory and textural attributes. Cooling of the cook products was done in Ziploc bags immersed in ice-slush to prevent shrimp from absorbing water.

In transient temperature studies, each size of tiger shrimp was cooked in water at 75, 85 and 95°C, and in boiling water (industrial practice). The minimum cooking time for each size of shrimp to reduce the numbers of *Vibrio cholera* by 6 log cycles were calculated using the mathematical model previously developed by Erdoğdu et al. (1998). This model simulated heat transfer in shrimp and took into account changes in the dimensions and thermal properties during treatment. For the current study, this model was expanded to predict the sterilization values using inactivation kinetics (D- and z-values at a reference temperature) of a target organism (Erdoğdu, 1996). All calculations were carried out using a computer program written in Visual Basic V. 6.0 (Microsoft, 1998).

After cooking, cooling was performed in ice slush and in Ziploc bags immersed in ice slush to see if absorption of water by cooked shrimp caused significant changes in textural parameters. During cooking and cooling, a type T, 36 gauge thermocouple (Ecklund, Fort Myers, FL) was inserted into the slowest heating point of shrimp to record the temperature.

Determination of TPA Parameters and Shear Force

The Instron Universal Testing Machine (Model 4411, Instron Corp., Canton, MA) was used. Shrimp was placed on its side and a spherical indenter, 1 cm in diameter (flat surface) was used for compression at the center of the shrimp's 2nd segment (the thickest portion). The TPA parameters were:

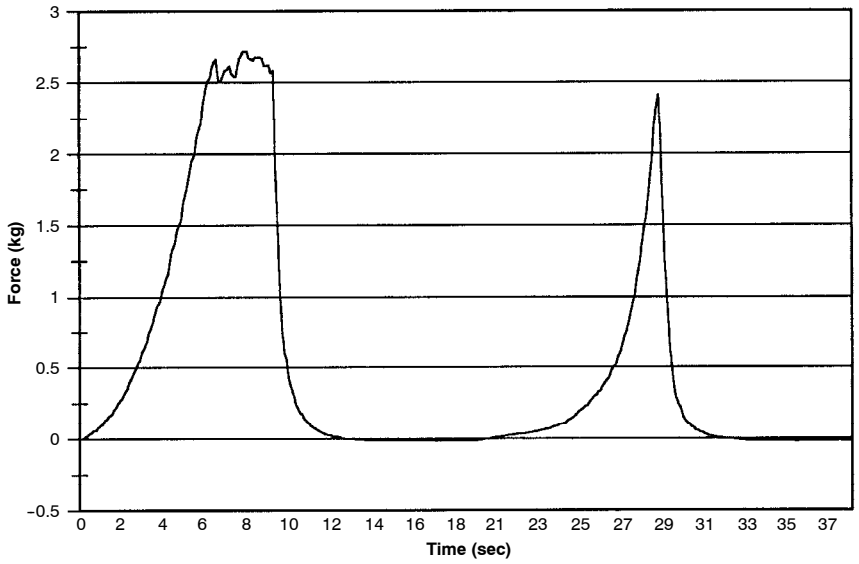
- 80% compression with two cycles,
- F = 50 kg load cell, and 50 mm/min load cell speed.

Figure 2 shows a typical TPA curve for shrimp. All texture measurements were done at room temperature (20-22°C), and five shrimp were used for each time and temperature combination.

For the shear force tests, the shrimp was placed on its side in the Instron, and cut at the center of 2nd segment with a custom designed shear blade (1 mm in thickness). The shear force parameters were F = 50 kg load cell and 50 mm/min load cell speed. The maximum shear forces, determined from the force-deformation curves, were used as an indicator of toughness.

For both TPA and shear tests, force-deformation data were gathered by the software package Instron Series XII Software (Instron Corp., Canton, MA), saved in ASCII files, and the TPA parameters were calculated using a Visual Basic V. 3.0 computer program developed by Luzuriaga (1995) using the methodology given by Bourne (1978). Five shrimp were used for each time-temperature combination and each cooling method for isothermal and transient texture measurements. Statistica for Windows (Statsoft, 1994) was used for Analysis of Variance (ANOVA) to analyze the significant effects of temperature, time, and their combination. In all statistical analyses, the significance level of $\alpha = 0.05$ was used.

FIGURE 2. Texture Profile Analysis curve for medium shrimp cooked at 85°C for 7.5 min.



Sensory Analysis

A quantitative descriptive analysis for the texture profile was used. Taste panel participants with previous experience working with shrimp were screened and selected. Fifteen to twenty panelists, consisting of the graduate students and faculty members of the departments of Food Science and Human Nutrition and Agricultural and Biological Engineering at the University of Florida, Gainesville, Florida, were presented 3 shrimp/person in the case of small and medium, and 2 shrimp/person for large shrimp, and were asked to use a 15 cm unstructured line scale to grade the samples. The tests were accomplished in a sensory panel room with partitioned booths under white light. Four parameters (tenderness, juiciness, rubberiness and overall acceptability) were evaluated. Samples treated at 55 and 65°C were not included into the sensory experimental design due to the microbial safety concerns. Two replicates were used for each time-temperature combination. Before the sensory tests, two training sessions were performed. During the first session, small shrimp cooked at 75°C for

the shorter cooking time (most tender shrimp), and at 95°C for the longer cooking time (toughest shrimp) were presented to the panelists as the extreme cases. After they graded the samples for the attributes of tenderness, juiciness, rubberiness and overall acceptability, the results were discussed with them. For the second training session, the same samples and one cooked at 85°C for the shorter cooking time (for sensory properties between the other two samples) were presented to the panelists to evaluate their discriminating ability. For the training session and sensory experiments, three-digit random numbers were assigned to each cooking time-temperature combination. Each day, one size of shrimp with six time-temperature combinations (3 temperatures × 2 cooking times) was presented to the panelists. The ballots were evaluated using a ruler, and the numerical data were used for further statistical analysis. The definitions of these parameters, according to the discussions with the panelists during the training sessions are given as follows: Tenderness is the indication of the force required to compress the shrimp between the teeth, it is the opposite of hardness. Juiciness is the feeling of moistness during the disintegration and mastication process. Rubberiness is the length of time required to disintegrate and masticate the shrimp to a state ready for swallowing. Overall acceptability is the combination of all the sensory attributes.

The data were analyzed using ANOVA to see if the temperature, time or their combined effects have a significant effect on the sensory attributes. The significant effects due to panelists, and the correlation between sensory attributes and TPA parameters and shear force were also evaluated.

RESULTS AND DISCUSSION

Cooking Times of Shrimp at Isothermal and Transient Experiments

Tables 2 and 3 summarize the cooking times and temperatures for different sizes of tiger shrimp used in the isothermal and transient experiments.

Texture Profile Analysis and Shear Tests

The average and standard deviations for TPA parameters and shear force for large, medium, and small tiger shrimp treated at different

TABLE 2. The heating temperatures and times for large (35-44/kg), medium (90-100/kg), and small (110-120/kg) tiger shrimp.

Size of Tiger Shrimp	Cooking Temperature (°C)	Cooking Time 1 (min)	Cooking Time 2 (min)
Large	55	8.00	12.00
	65	11.00	16.50
	75	13.00	19.50
	85	15.00	22.50
	95	16.00	24.00
Medium	55	5.00	7.50
	65	5.50	8.25
	75	6.50	9.75
	85	7.50	11.25
	95	8.00	12.00
Small	55	4.00	6.00
	65	5.00	7.50
	75	5.20	7.80
	85	5.50	8.25
	95	6.00	9.00

TABLE 3. The calculated heating times for 6 log cycles reduction of *V. cholera* for large (35-44/kg), medium (90-100/kg), and small (110-120/kg) tiger shrimp at different cooking temperatures

Size of Shrimp	Cooking Temperature (°C)			
	75	85	95	100
Large	5.6 min	3.7 min	2.9 min	2.6 min
Medium	3.5 min	2.2 min	1.7 min	1.5 min
Small	3.4 min	2.1 min	1.6 min	1.4 min

time-temperature conditions are given in Tables 4 to 6. Effects of time and temperature on machine measured textural attributes were investigated using ANOVA. Temperature was significant for cohesiveness, hardness, fracturability, gumminess, chewiness and adhesiveness. In large tiger shrimp, the springiness was not affected either by temperature ($p = 0.228$), time ($p = 0.092$) or their combination ($p = 0.638$). Temperature and the combination of time and temperature ($p < 0.05$)

TABLE 4. Texture Profile Analysis (TPA) parameters and shear force data for large tiger shrimp (35-44/kg).

Temperature (°C) and Time (min)	Cohesiveness	Hardness (kg)	Fracturability (kg)	Springiness (mm)	Gumminess (kg)	Chewiness (kg-mm)	Adhesiveness (kg-mm)	Shear Force (kg)
Raw	0.06 ± 0.06	3.84 ± 0.36	3.54 ± 0.70	4.61 ± 0.65	0.24 ± 0.25	1.24 ± 1.37	0.11 ± 0.02	5.00 ± 0.45
55-8	0.15 ± 0.04	4.77 ± 0.30	4.61 ± 0.38	6.35 ± 2.04	0.58 ± 0.36	4.29 ± 3.80	0.11 ± 0.03	3.59 ± 0.81
55-12	0.18 ± 0.04	4.38 ± 0.50	4.35 ± 0.50	6.28 ± 1.54	0.82 ± 0.26	6.20 ± 2.66	0.12 ± 0.05	4.24 ± 0.81
65-11	0.12 ± 0.10	4.40 ± 0.53	4.15 ± 0.54	6.86 ± 1.66	0.73 ± 0.40	5.54 ± 3.83	0.13 ± 0.04	3.03 ± 0.23
65-16.5	0.17 ± 0.03	4.11 ± 0.16	4.01 ± 0.19	7.00 ± 0.68	0.69 ± 0.14	4.86 ± 1.62	0.10 ± 0.03	2.99 ± 0.43
75-13	0.15 ± 0.08	4.38 ± 0.58	4.04 ± 0.40	6.05 ± 1.22	0.65 ± 0.34	4.29 ± 2.41	0.13 ± 0.02	3.20 ± 0.34
75-19.5	0.20 ± 0.02	4.35 ± 0.66	4.36 ± 0.50	5.55 ± 1.15	0.87 ± 0.10	6.31 ± 1.36	0.09 ± 0.01	2.52 ± 0.65
85-15	0.21 ± 0.02	4.36 ± 0.50	3.92 ± 0.37	7.15 ± 0.84	1.03 ± 0.29	7.40 ± 2.29	0.08 ± 0.01	2.99 ± 0.38
85-22.5	0.35 ± 0.05	4.77 ± 0.31	4.73 ± 0.36	6.25 ± 0.44	1.29 ± 0.21	10.5 ± 2.85	0.09 ± 0.05	2.70 ± 0.14
95-16	0.41 ± 0.04	4.90 ± 0.92	4.67 ± 1.08	8.44 ± 0.76	2.07 ± 0.50	17.3 ± 3.68	0.06 ± 0.03	2.90 ± 0.39
95-24	0.37 ± 0.08	4.78 ± 0.46	4.67 ± 0.46	5.54 ± 0.68	1.86 ± 0.41	15.2 ± 4.45	0.05 ± 0.07	2.64 ± 0.10

TABLE 5. Texture Profile Analysis (TPA) parameters and shear force data for medium tiger shrimp (90-110/kg).

Temperature (°C) and Time (min)	Cohesiveness	Hardness (kg)	Fracturability (kg)	Springiness (mm)	Gumminess (kg)	Chewiness (kg-mm)	Adhesiveness (kg-mm)	Shear Force (kg)
Raw	0.16 ± 0.03	2.87 ± 0.17	2.36 ± 0.24	6.07 ± 0.64	0.46 ± 0.06	3.10 ± 0.90	0.03 ± 0.01	2.4 ± 0.43
55-5	0.21 ± 0.05	3.31 ± 0.40	3.05 ± 0.54	7.00 ± 0.41	0.70 ± 0.23	4.90 ± 1.72	0.02 ± 0.006	1.72 ± 0.20
55-7.5	0.21 ± 0.03	3.47 ± 0.39	2.77 ± 0.24	5.24 ± 0.22	0.71 ± 0.06	3.72 ± 0.36	0.02 ± 0.02	1.76 ± 0.18
65-5.5	0.20 ± 0.03	3.63 ± 0.32	3.28 ± 0.34	6.61 ± 0.83	0.69 ± 0.10	4.52 ± 0.54	0.03 ± 0.01	1.61 ± 0.30
65-8.25	0.26 ± 0.02	3.25 ± 0.12	3.12 ± 0.32	5.29 ± 0.25	0.85 ± 0.07	4.49 ± 0.23	0.01 ± 0.005	1.32 ± 0.08
75-6.5	0.21 ± 0.05	3.13 ± 0.63	2.83 ± 0.53	7.15 ± 1.27	0.67 ± 0.23	4.89 ± 2.14	0.02 ± 0.004	1.38 ± 0.20
75-9.75	0.29 ± 0.02	2.60 ± 0.48	2.48 ± 0.43	5.85 ± 0.32	0.75 ± 0.12	4.38 ± 0.88	0.01 ± 0.006	1.28 ± 0.12
85-7.5	0.25 ± 0.06	3.80 ± 0.62	3.46 ± 0.57	6.46 ± 0.78	0.92 ± 0.20	6.04 ± 1.83	0.01 ± 0.009	1.37 ± 0.19
85-11.25	0.31 ± 0.04	3.54 ± 0.47	3.26 ± 0.45	5.84 ± 0.67	1.10 ± 0.28	6.25 ± 1.18	0.03 ± 0.03	1.24 ± 0.18
95-8	0.36 ± 0.04	4.18 ± 0.98	4.13 ± 0.91	6.06 ± 0.36	1.53 ± 0.48	9.23 ± 2.78	0.01 ± 0.003	0.95 ± 0.54
95-12	0.38 ± 0.02	4.50 ± 0.56	4.40 ± 0.57	5.62 ± 0.22	1.71 ± 0.21	9.63 ± 1.44	0.02 ± 0.02	1.33 ± 0.19

TABLE 6. Texture Profile Analysis (TPA) parameters and shear force data for small tiger shrimp (110-120/kg).

Temperature (°C) and Time (min)	Cohesiveness	Hardness (kg)	Fracturability (kg)	Springiness (mm)	Gumminess (kg)	Chewiness (kg-mm)	Adhesiveness (kg-mm)	Shear Force (kg)
Raw	0.17 ± 0.02	2.38 ± 0.34	2.13 ± 0.13	4.67 ± 0.31	0.40 ± 0.07	0.73 ± 0.71	1.00 ± 0.79	2.30 ± 0.40
55-4	0.23 ± 0.04	4.38 ± 0.62	3.32 ± 0.35	5.88 ± 0.70	0.99 ± 0.16	5.91 ± 1.51	0.02 ± 0.003	2.20 ± 0.40
55-6	0.24 ± 0.07	4.79 ± 0.15	3.97 ± 0.53	5.80 ± 0.43	1.13 ± 0.34	6.64 ± 2.40	0.02 ± 0.02	2.69 ± 0.55
65-5	0.11 ± 0.07	3.19 ± 0.81	2.81 ± 0.78	5.03 ± 0.88	0.39 ± 0.27	2.16 ± 1.62	0.03 ± 0.02	2.14 ± 0.40
65-7.5	0.25 ± 0.06	2.79 ± 0.29	2.49 ± 0.31	5.20 ± 0.81	0.70 ± 0.21	3.76 ± 1.48	0.03 ± 0.02	1.77 ± 0.63
75-5.2	0.32 ± 0.04	2.84 ± 0.23	2.69 ± 0.25	4.45 ± 0.32	0.92 ± 0.19	4.07 ± 0.75	0.03 ± 0.01	2.01 ± 0.38
75-7.8	0.27 ± 0.04	2.75 ± 0.56	2.65 ± 0.61	5.15 ± 0.28	0.75 ± 0.21	3.89 ± 1.23	0.01 ± 0.001	1.71 ± 0.50
85-5.5	0.32 ± 0.02	4.47 ± 0.73	4.12 ± 0.68	4.59 ± 0.13	1.42 ± 0.17	6.53 ± 0.76	0.04 ± 0.02	1.95 ± 0.27
85-8.25	0.31 ± 0.02	3.74 ± 0.64	3.59 ± 0.63	5.62 ± 0.41	1.17 ± 0.26	6.52 ± 1.43	0.03 ± 0.04	2.05 ± 0.53
95-6	0.33 ± 0.02	5.33 ± 1.45	4.92 ± 1.48	6.04 ± 0.61	1.83 ± 1.61	11.1 ± 3.51	0.03 ± 0.02	2.42 ± 0.28
95-9	0.38 ± 0.04	4.50 ± 0.55	4.23 ± 0.67	5.69 ± 0.36	1.69 ± 0.27	9.57 ± 1.53	0.02 ± 0.02	2.10 ± 0.08

affected shear force. In medium shrimp, time was significant for springiness while temperature ($p = 0.29$) and their combination ($p = 0.218$) were not. Adhesiveness was found to be independent of temperature ($p = 0.618$), time ($p = 0.652$) and their combination ($p = 0.097$). However, time had a significant effect on cohesiveness and springiness ($p < 0.05$). For small tiger shrimp, adhesiveness was independent of any effect. Springiness depended on temperature. Both temperature and time-temperature combination was significant for cohesiveness.

Least Significant Difference (LSD) test was also applied to the data (Table 7). In large, medium and small tiger shrimp, the change in cohesiveness versus temperature was similar. In LSD tests, the results for 55 and 65°C, and those for 75 and 85°C overlapped. However, the change in cohesiveness at 95°C was significantly different from the rest. Only in small tiger shrimp was the change at 85 and 95°C found

TABLE 7. LSD (Least Significant Difference) test results for the changes of texture profile analysis (TPA) parameters and shear force for large tiger (35-44/kg), medium tiger (90-110/kg), and small tiger (110-132/kg) shrimp on the basis of temperature effect (C: cohesiveness, H: hardness, F: fracturability, G: gumminess, Ch: chewiness, A: adhesiveness, S.F.: shear force).

Size	T (°C)	C	H	F	S	G	Ch	A	S.F.
Large	55	a	ac	a	a	ab	ab	a	a
	65	a	abc	abc	a	a	b	a	b
	75	ab	b	b	a	a	b	a	b
	85	b	c	bc	a	ab	a	a	b
	95	c	cd	c	a	c	c	b	b
Medium	55	a	ab	ab	a	a	a	a	a
	65	ab	a	a	a	a	a	a	b
	75	bc	b	b	a	a	ab	a	c
	85	c	a	a	a	b	b	a	b
	95	d	c	c	a	c	c	a	bc
Small	55	a	ac	a	a	ac	a	a	a
	65	b	b	b	b	b	b	a	bc
	75	c	b	b	b	bc	b	a	b
	85	cd	a	a	b	a	a	a	bc
	95	d	c	c	a	d	c	a	ac

* Same letters in a column within a shrimp size are not significantly different.

to be similar. Small and large shrimp gave the lowest hardness around 65°C. Medium shrimp gave a lower hardness value at 75°C. The change in hardness at 95°C was significantly different than the rest. The changes in fracturability values for all sizes of shrimp showed a similar trend. Springiness did not change with temperature for large and medium shrimp. However, in small shrimp, changes at 55 and 95°C, and those at 65, 75 and 85°C were not significantly different. The changes in gumminess versus temperature were similar to those in hardness and cohesiveness since gumminess is the product of cohesiveness and hardness. The changes in chewiness also followed a similar trend. Only in large shrimp was adhesiveness affected by cooking temperature, and changes were not significantly different at any temperature except at 95°C.

The shear force was also affected by cooking temperature. However, the results were different than those of hardness or fracturability. In large tiger shrimp, only the changes at 55°C were significantly different than the rest (Table 7), with a similar trend in medium shrimp. In small shrimp, largest changes occurred at 55 and 95°C (Table 6). Temperature was the significant parameter, and time was not significant in any experiment.

Sensory Analysis Results

The sensory data for all sizes are given in Table 8. ANOVA was applied to see the effects of temperature and time on sensory attributes for each size of shrimp. Temperature significantly affected all sensory attributes. There was also a significant difference between the panelists. Therefore, each panelist in replicate experiments was treated as a different person. Time and the combination of time and temperature were not significant for tenderness, juiciness, rubberiness and overall acceptability. Time had a marginally significant effect on the overall acceptability of large size shrimp ($p < 0.05$), and time and temperature combination had a significant effect on that of medium size shrimp ($p < 0.05$).

Since temperature was the only significant factor on the sensory attributes, the LSD test was used for the grouping effect of temperature. In large tiger shrimp, cooking at 95°C resulted in the toughest, driest and most rubbery shrimp. In overall acceptability, the panelists could not find a significant difference between shrimp cooked at 75

TABLE 8. Sensory Analysis data for large (35-44/kg), medium (90-110/kg), and small (110-120/kg) tiger shrimp.

T (°C)	Large Tiger				Medium Tiger				Small Tiger			
	Tenderness	Juiciness	Rubberiness	Overall	Tenderness	Juiciness	Rubberiness	Overall	Tenderness	Juiciness	Rubberiness	Overall
75^1t1	5.3 ± 3.5	9.3 ± 2.8	4.4 ± 3.4	9.0 ± 3.1	3.5 ± 2.4	9.5 ± 3.2	3.2 ± 3.0	9.6 ± 6.2	2.4 ± 1.6	11.0 ± 1.9	2.5 ± 2.1	8.7 ± 4.0
75^2t1	4.6 ± 3.4	9.3 ± 3.9	6.7 ± 4.2	9.0 ± 3.6	1.8 ± 1.3	12.4 ± 1.2	2.1 ± 2.7	7.7 ± 5.0	3.7 ± 3.6	10.5 ± 3.9	6.4 ± 4.8	9.4 ± 4.4
75^1t2	4.4 ± 3.2	9.6 ± 2.8	4.7 ± 3.7	8.5 ± 3.8	2.0 ± 1.3	10.9 ± 2.6	2.4 ± 2.5	9.7 ± 4.2	2.7 ± 1.8	10.2 ± 2.7	3.2 ± 2.9	6.9 ± 4.0
75^2t2	4.0 ± 2.7	8.7 ± 3.2	4.9 ± 3.8	8.4 ± 3.5	2.1 ± 1.1	11.6 ± 1.8	2.4 ± 2.5	8.8 ± 4.5	2.0 ± 0.7	11.7 ± 2.1	3.1 ± 3.1	8.4 ± 4.4
85^1t1	9.3 ± 2.2	4.4 ± 2.5	6.8 ± 3.4	8.1 ± 2.2	6.9 ± 2.3	7.2 ± 2.5	4.5 ± 2.9	9.8 ± 2.3	6.4 ± 3.2	7.9 ± 3.3	5.8 ± 3.8	9.1 ± 3.2
85^2t1	6.6 ± 3.3	7.1 ± 3.4	5.8 ± 3.5	10.0 ± 2.6	6.2 ± 3.4	8.5 ± 3.0	5.0 ± 3.1	10.2 ± 3.1	7.2 ± 3.4	7.0 ± 3.3	6.5 ± 3.9	9.3 ± 3.8
85^1t2	8.9 ± 2.9	5.7 ± 2.7	7.0 ± 3.7	7.0 ± 2.4	7.8 ± 2.8	5.4 ± 3.1	5.9 ± 2.9	8.8 ± 2.4	8.1 ± 3.3	5.8 ± 2.8	7.5 ± 3.8	8.0 ± 2.7
85^2t2	7.6 ± 3.5	7.1 ± 3.0	6.4 ± 3.1	8.7 ± 2.6	7.5 ± 3.5	7.2 ± 3.2	5.9 ± 3.5	8.8 ± 2.3	7.5 ± 3.1	7.0 ± 3.5	7.0 ± 4.1	9.1 ± 2.9
95^1t1	11.4 ± 1.9	2.9 ± 1.5	8.2 ± 4.1	5.3 ± 2.5	8.9 ± 3.0	4.5 ± 3.7	7.6 ± 3.3	6.4 ± 2.5	10.3 ± 2.7	4.3 ± 2.4	8.9 ± 3.2	6.8 ± 3.3
95^2t1	9.6 ± 3.1	4.5 ± 3.0	7.3 ± 3.8	7.3 ± 3.2	9.5 ± 3.1	5.0 ± 3.2	8.0 ± 3.0	7.7 ± 2.3	9.9 ± 2.9	3.6 ± 2.5	8.6 ± 3.1	6.6 ± 2.8
95^1t2	11.5 ± 1.9	2.5 ± 1.5	8.0 ± 3.8	5.3 ± 2.6	10.6 ± 2.5	3.8 ± 2.3	7.9 ± 3.3	5.9 ± 2.8	10.5 ± 2.5	4.2 ± 2.7	8.3 ± 3.4	6.6 ± 3.3
95^2t2	10.8 ± 2.8	3.1 ± 2.5	7.7 ± 4.1	6.2 ± 3.3	8.7 ± 3.6	5.6 ± 3.7	5.7 ± 3.5	8.3 ± 2.8	11.0 ± 1.9	3.2 ± 2.1	8.4 ± 3.5	6.4 ± 2.7

*The superscripts 1 and 2 show the first and second replicates for that time-temperature combination. ''t1'' and ''t2'' show the first and second cooking times for that temperature. For any attribute, 0 means a low value, and 15 means a high value.

and 85°C. Similar results were obtained in medium and small tiger shrimp.

The correlation between instrumental and sensory analysis results was calculated. Even though there were significant differences within the panelists, strong correlation was found between juiciness and adhesiveness, chewiness, cohesiveness and gumminess; between rubberiness and chewiness and cohesiveness; between tenderness and chewiness, cohesiveness, gumminess and hardness in tiger shrimp (Tables 9 to 11). Predictive equations between sensory attributes and textural parameters for tiger shrimp were also determined (Table 12). These correlations may help in predicting sensory attributes by measuring instrumental texture parameters. A more detailed sensory analysis, concentrating on the screening of the panelists and better training may be needed for these equations to be more representative.

Transient Texture Measurement Results

The TPA results for the transient texture measurements for each size

TABLE 9. The correlation between sensory and instrumental textural parameters in large tiger shrimp (35-44/kg). (T_s: tenderness, J_s: juiciness, R_s: rubberiness, O_s: overall acceptability, C: cohesiveness, H: hardness, F: fracturability, G: gumminess, Ch: chewiness, A: adhesiveness, S.F.: shear force).*

Correlation Coefficients												
	T_s	J_s	R_s	O_s	C	H	F	S	G	Ch	A	S.F.
T_s	1.00	**−0.99**	**0.99**	**−0.83**	**0.88**	**0.86**	0.75	0.77	**0.92**	**0.91**	−0.81	−0.11
J_s	**−0.99**	1.00	**−0.96**	**0.84**	**−0.89**	−0.81	−0.67	−0.77	**−0.92**	**−0.91**	**0.88**	0.17
R_s	**0.99**	**−0.96**	1.00	**−0.84**	**0.88**	**0.87**	0.79	0.73	**0.92**	**0.92**	−0.72	−0.03
O_s	**−0.83**	**0.84**	**−0.84**	1.00	**−0.95**	**−0.87**	**−0.83**	−0.78	**−0.94**	**−0.94**	0.78	0.41

TABLE 10. The correlation between sensory and instrumental textural parameters in medium tiger shrimp (90-110/kg). (T_s: tenderness, J_s: juiciness, R_s: rubberiness, O_s: overall acceptability, C: cohesiveness, H: hardness, F: fracturability, G: gumminess, Ch: chewiness, A: adhesiveness, S.F.: shear force).*

Correlation Coefficients												
	T_s	J_s	R_s	O_s	C	H	F	S	G	Ch	A	S.F.
T_s	1.00	**−0.99**	**0.96**	**−0.81**	0.74	**0.97**	**0.96**	−0.47	**0.91**	**0.92**	−0.01	0.03
J_s	**−0.99**	1.00	**−0.98**	**0.83**	−0.75	**−0.94**	**−0.93**	0.47	**−0.91**	**−0.92**	−0.03	0.02
R_s	**0.96**	**−0.98**	1.00	**−0.92**	**0.83**	**0.93**	**0.96**	−0.50	**0.96**	**0.97**	−0.08	−0.17
O_s	**−0.81**	**0.83**	**−0.92**	1.00	**−0.91**	**−0.82**	**−0.89**	0.54	**−0.97**	**−0.96**	0.22	0.33
C	0.74	−0.75	**0.83**	**−0.91**	1.00	0.67	0.76	**−0.84**	0.93	0.85	−0.21	−0.30
H	**0.97**	**−0.94**	**0.93**	**−0.82**	0.67	1.00	**0.99**	−0.32	**0.89**	**0.94**	−0.17	−0.07
F	**0.96**	**−0.93**	**0.96**	**−0.89**	0.76	**0.99**	1.00	−0.40	**0.95**	**0.98**	−0.25	−0.19
S	−0.47	0.47	−0.50	0.54	**−0.84**	−0.32	−0.40	1.00	−0.63	−0.48	0.01	0.01
G	**0.91**	**−0.91**	**0.96**	**−0.97**	**0.93**	**0.89**	**0.95**	−0.63	1.00	**0.98**	−0.20	−0.23

shrimp are given in Table 13. The shear force was not measured since there was no correlation between the sensory textural attributes and shear force. ANOVA was applied to see if temperature and the cooling procedure had a significant effect on the changes in TPA parameters. The results showed that temperature had a significant effect ($p < 0.05$) on adhesiveness in large tiger shrimp; cohesiveness, gumminess and chewiness in medium tiger shrimp; and all TPA parameters in small tiger shrimp. The smaller the shrimp size, the more significant the effect of temperature on the TPA parameters. In small shrimp, proportionally more tissue is subjected to the effects of high outside temperature compared with larger sizes. Therefore, the changes in the TPA parameters with respect to cooking temperatures in larger sizes were not significant. In isothermal texture studies, the effect of temperature was significant due to the longer cooking times. Cooling method did not have a significant effect. This showed that the water absorption during in-ice-water cooling did not affect the TPA parameters.

TABLE 11. The correlation between sensory and instrumental textural parameters in small tiger shrimp (110-132/kg). (T_S: tenderness, J_S: juiciness, R_S: rubberiness, O_S: overall acceptability, C: cohesiveness, H: hardness, F: fracturability, G: gumminess, Ch: chewiness, A: adhesiveness, S.F.: shear force).*

Correlation Coefficients												
	T_S	J_S	R_S	O_S	C	H	F	S	G	Ch	A	S.F.
T_S	1.00	**−0.99**	**0.99**	−0.58	0.79	**0.90**	**0.92**	0.73	**0.94**	**0.95**	0.16	0.77
J_S	**−0.99**	1.00	**−0.99**	0.63	−0.76	**−0.90**	**−0.92**	−0.77	**−0.94**	**−0.96**	−0.11	−0.78
R_S	**0.99**	**−0.99**	1.00	−0.56	0.68	**0.91**	**0.93**	0.78	**0.91**	**0.94**	0.16	0.76
O_S	−0.58	0.63	−0.56	1.00	−0.39	−0.48	−0.50	−0.82	−0.56	−0.71	0.66	−0.45
C	0.79	−0.76	0.68	−0.39	1.00	0.64	0.65	0.31	0.79	0.72	0.28	0.65
H	**0.90**	**−0.90**	**0.91**	−0.48	0.64	1.00	**0.99**	0.59	**0.97**	**0.94**	0.30	0.80
F	**0.92**	**−0.92**	**0.93**	−0.50	0.65	**0.99**	1.00	0.62	**0.98**	**0.95**	0.28	0.81
S	0.73	−0.77	0.78	−0.82	0.31	0.59	0.62	1.00	0.60	0.77	0.41	0.61
G	**0.94**	**−0.94**	**0.91**	−0.56	0.79	**0.97**	**0.98**	0.60	1.00	**0.97**	0.25	**0.83**
Ch	**0.95**	**−0.96**	**0.94**	−0.71	0.72	**0.94**	**0.95**	0.77	**0.97**	1.00	0.06	**0.86**

CONCLUSIONS AND RECOMMENDATIONS

In both isothermal and transient temperature experiments, ANOVA showed that temperature was the significant factor affecting changes in textural attributes, and there was high correlation among the textural properties and sensory attributes. The shrimp size was also a significant factor. Using the effect of time-temperature relationships, the kinetics of texture changes can be found and correlated to sensory attributes with respect to the shrimp sizes. These can also be used in mathematical models to predict the textural properties of shrimp during thermal processing. A similar study for yield loss prediction for shrimp during thermal processing was conducted by Erdoğdu et al. (1999), and good results were obtained. Predictions of the changes in textural and sensory attributes, yield losses, and microbial lethalities should facilitate the optimization of thermal processing of shrimp.

TABLE 12. The predictive equations between sensory and instrumental texture parameters in large (35-44/kg), medium (90-110/kg), and small (110-120/kg) tiger shrimp. Only correlations with higher absolute values than 0.8 were calculated.

Predictive Equations		Large Tiger			Medium Tiger			Small Tiger		
		a	b	r	a	b	r	a	b	r
Tenderness =	a + b*Cohesiveness	−0.33	33.33	0.88	-	-	-	-	-	-
	a + b*Hardness	−48.75	12.50	0.86	−10.76	4.76	0.97	−8.31	3.85	0.90
	a + b*Fracturability	-	-	-	−9.09	4.55	0.96	−8.75	4.17	0.92
	a + b*Gumminess	0.03	0.18	0.92	−2.67	8.33	0.91	−4.73	9.09	0.94
	a + b*Chewiness	15.72	5.56	0.91	−4.17	1.59	0.92	−2.10	1.28	0.95
Juiciness =	a + b*Cohesiveness	15.67	−33.33	−0.89	-	-	-	-	-	-
	a + b*Hardness	-	-	-	22.57	−4.35	−0.94	20.76	−3.45	−0.90
	a + b*Fracturability	-	-	-	21.63	−4.17	−0.94	21.50	−3.85	−0.92
	a + b*Gumminess	12.50	−5.00	−0.92	14.57	−7.14	−0.91	17.00	−7.69	−0.94
	a + b*Chewiness	12.40	−0.60	−0.91	16.34	−1.41	0.92	15.00	−1.14	−0.96
	a + b*Adhesiveness	−2.00	100.00	0.88	-	-	-	-	-	-
Rubberiness =	a + b*Cohesiveness	2.88	12.50	−0.88	−8.50	50.00	0.83	-	-	-
	a + b*Hardness	−16.50	5.00	−0.87	−7.34	3.45	0.93	−4.83	2.78	0.91
	a + b*Fracturability	−10.80	4.00	−0.83	−5.81	3.23	0.96	−5.18	3.03	0.93
	a + b*Gumminess	3.53	2.33	−0.94	−0.94	5.56	0.96	−5.07	6.67	0.91
	a + b*Chewiness	3.90	0.26	−0.94	−1.93	1.06	0.96	−0.47	0.94	0.93
Overall =	a + b*Cohesiveness	−11.43	−14.29	−0.95	19.33	−33.33	−0.91	-	-	-
	a + b*Hardness	34.59	−5.88	−0.87	19.81	−3.12	−0.82	-	-	-
	a + b*Fracturability	26.78	−4.35	−0.83	17.32	−2.63	−0.97	-	-	-
	a + b*Gumminess	11.24	−2.63	−0.94	12.83	−4.17	−0.97	-	-	-
	a + b*Chewiness	10.60	−0.30	−0.94	14.08	−0.83	−0.96	-	-	-
	a + b*Springiness	-	-	-	-	-	-	19.69	−2.22	−0.82

TABLE 13. Texture Profile Analysis (TPA) results for the transient experiments for large (35-44/kg), medium (90-110/kg), and small (110-120/kg) tiger shrimp.

Shrimp size	Cooling Method	Temperature (°C)	Cohesiveness	Hardness (kg)	Fracturability (kg)	Springiness (mm)	Gumminess (kg)	Chewiness (kg-mm)	Adhesiveness (kg-mm)
Large Tiger	In ice-water	75	0.06 ± 0.07	3.8 ± 0.4	3.7 ± 0.5	5.3 ± 1.3	0.2 ± 0.3	1.5 ± 1.7	0.1 ± 0.01
		85	0.04 ± 0.07	4.4 ± 0.3	3.9 ± 0.3	4.8 ± 1.5	0.2 ± 0.3	1.5 ± 1.7	0.3 ± 0.2
		95	0.07 ± 0.07	4.0 ± 0.8	3.6 ± 0.4	4.7 ± 0.9	0.3 ± 0.3	1.7 ± 1.9	0.4 ± 0.1
		100	0.08 ± 0.09	3.9 ± 0.2	3.4 ± 0.2	6.0 ± 1.6	0.3 ± 0.4	2.5 ± 3.0	0.3 ± 0.02
	In ziploc bags in ice-water	75	0.08 ± 0.08	3.4 ± 0.5	3.4 ± 0.5	5.3 ± 1.0	0.2 ± 0.2	1.4 ± 1.5	0.2 ± 0.2
		85	0.11 ± 0.09	3.9 ± 0.3	3.6 ± 0.5	6.0 ± 1.7	0.5 ± 0.3	3.3 ± 2.6	0.3 ± 0.2
		95	0.11 ± 0.08	3.7 ± 0.2	3.6 ± 0.1	6.0 ± 1.0	0.4 ± 0.3	2.8 ± 2.3	0.2 ± 0.1
		100	0.06 ± 0.07	4.1 ± 0.1	3.5 ± 0.4	5.3 ± 1.5	0.3 ± 0.3	1.7 ± 2.1	0.2 ± 0.02
Medium Tiger	In ice-water	75	0.15 ± 0.04	3.0 ± 0.3	2.9 ± 0.4	6.5 ± 1.4	0.4 ± 0.1	3.1 ± 1.6	0.04 ± 0.06
		85	0.25 ± 0.07	3.1 ± 0.2	3.0 ± 0.2	6.5 ± 0.7	0.8 ± 0.2	5.2 ± 1.7	0.05 ± 0.02
		95	0.25 ± 0.05	3.1 ± 0.2	2.9 ± 0.4	6.7 ± 0.4	0.8 ± 0.2	5.1 ± 1.2	0.02 ± 0.001
		100	0.21 ± 0.04	2.7 ± 0.2	2.6 ± 0.1	6.4 ± 0.7	0.6 ± 0.1	3.7 ± 0.9	0.01 ± 0.01
	In ziploc bags in ice-water	75	0.16 ± 0.06	3.1 ± 0.2	2.8 ± 0.1	6.2 ± 0.9	0.5 ± 0.2	3.1 ± 1.6	0.07 ± 0.06
		85	0.22 ± 0.06	2.8 ± 0.3	2.7 ± 0.3	6.4 ± 1.1	0.6 ± 0.2	4.3 ± 2.8	0.03 ± 0.04
		95	0.29 ± 0.10	3.1 ± 0.3	2.9 ± 0.5	7.0 ± 0.8	0.9 ± 0.3	6.5 ± 2.8	0.03 ± 0.04
		100	0.22 ± 0.03	3.2 ± 0.3	2.8 ± 0.3	6.7 ± 1.1	0.3 ± 0.1	4.9 ± 1.6	0.02 ± 0.01
Small Tiger	In ice-water	75	0.2 ± 0.02	2.1 ± 0.5	1.8 ± 0.2	5.3 ± 0.5	0.4 ± 0.1	2.2 ± 0.7	0.01 ± 0.02
		85	0.3 ± 0.02	2.9 ± 0.2	2.6 ± 0.3	6.5 ± 0.5	0.9 ± 0.1	5.9 ± 0.8	0.001 ± 0.001
		95	0.3 ± 0.02	2.9 ± 0.2	2.8 ± 0.3	5.4 ± 0.4	0.7 ± 0.1	4.0 ± 0.7	0.05 ± 0.03
		100	0.3 ± 0.04	3.3 ± 0.2	3.1 ± 0.1	5.7 ± 0.2	1.0 ± 0.2	5.7 ± 0.9	0.004 ± 0.003
	In ziploc bags iin ice-water	75	0.17 ± 0.02	2.5 ± 0.4	2.3 ± 0.4	5.7 ± 0.6	0.4 ± 0.1	2.5 ± 0.4	0.01 ± 0.02
		85	0.27 ± 0.06	3.3 ± 0.4	3.0 ± 0.5	6.6 ± 0.3	0.9 ± 0.2	5.9 ± 1.7	0.002 ± 0.002
		95	0.28 ± 0.01	3.3 ± 0.3	3.0 ± 0.5	6.0 ± 0.5	0.9 ± 0.1	5.6 ± 0.5	0.01 ± 0.01
		100	0.26 ± 0.09	2.9 ± 0.3	2.5 ± 0.2	5.8 ± 0.9	0.8 ± 0.3	4.7 ± 2.2	0.02 ± 0.01

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Ultrastructure of Actomyosin in Pre- and Post-Spawning Hake (*Merluccius hubbsi* Marini) During Frozen Storage

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M. Crupkin

ABSTRACT. The degradation of actomyosin in fillets from pre- and post-spawning hake under frozen storage is studied by electron microscopy and by analysis of changes in protein solubility. The ratio of salt soluble protein did not present significant changes during 240 days of frozen storage for post-spawning hake. Meanwhile the same ratio for pre-spawning hake presented a steady decrease.

In post-spawning hake the proteins retain some of the characteristics of the native structure with some aggregate formation up to 60 days of

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storage. In pre-spawning hake the formation of aggregates is already extensive after only 15 days of storage.

The solubility of proteins from pre-spawning hake decreased continuously reflecting the changes in the ultrastructure of actomyosin complex. For post-spawning hake, only the formation of soluble aggregates was observed after 240 days of frozen storage.

KEYWORDS. Hake, actomyosin ultrastructure, frozen storage, reproductive cycle, fish fillet

INTRODUCTION

Changes in quality of fish muscle during frozen storage is attributed to the denaturation of actomyosin. Changes in the actomyosin composition of mature hake are influenced by the metabolic state of the fish and are attributed to its reproductive cycle (Crupkin et al., 1988). The adverse effect of the pre-spawning condition of hake on the chemical, physicochemical and functional properties of actomyosin during frozen storage was reported in previous work (Montecchia et al., 1997). The fact that fillets from pre-spawning hake deteriorate faster than fillets from post-spawning hake during storage at $-20°C$ was indicated by the rate of protein insolubilization, the reduced viscosity and the hydrodynamic properties (Montecchia et al., 1997).

Analysis of changes in protein solubility is the simple and common method for estimating the rate of protein denaturation and its significance for assessing the denaturation mechanism. Electron microscopic observations are frequently used together with the solubility changes of protein to check the protein denaturation during frozen storage (Ohnishi et al., 1978).

In this work, the degradation of actomyosin in fillets from pre- and post-spawning hake under frozen storage is studied by electron microscope observation and by analysis of changes in solubility.

MATERIALS AND METHODS

Fish Samples

Female specimens of hake (*Merluccius hubbsi*) caught in the southwestern Atlantic Ocean between 36°S and 53°S were kept on ice for

48-72 hrs, until they reached the laboratory in an early post-rigor condition. Specimens were 35 to 45 cm long. Quality of the raw material was assessed after the fish had been washed and classified. To determine the maturation stage of each fish the gonadosomatic index (Crupkin et al., 1988) was calculated as follows:

$$\text{GSI (gonadosomatic index)} = \frac{\text{Wet weight of gonads}}{\text{Wet weight (fish} - \text{gonads)}} \times 100$$

Fish were manually filleted and the fillets were interweaved into blocks of approximately 2 kg each. The blocks, wrapped in polyethylene film, were frozen in a commercial plate freezer during 180 min at $-30°C$ and stored in laboratory cabinets at $-20°C$ until they were analyzed. Samples for analysis were made up with three fillets from three different fish. Analyses of protein solubility was performed on the fresh raw material (day 0= assumed fresh + unfrozen sample) and on the frozen samples at 45, 120 and 240 days of storage. Frozen samples were thawed for 8 hr at 5°C. Electron microscopy was performed on samples at 0, 15 and 60 days.

Actomyosin Preparation

Purified natural actomyosin (NAM) was obtained from fillets by the method of Tsuchiya et al. (1975) with the modifications introduced by Crupkin et al. (1982). The myofibrillar protein extract in 0.6 M KCl–0.003 M $NaHCO_3$ (pH 7.0) was centrifuged at 7500 × g. The supernatant was diluted with cold water to a final concentration of 0.2 M KCl. The resulting precipitate was solubilized in 0.6 M KCl. This dilution-solubilization cycle was repeated three times to obtain the purified natural actomyosin.

Protein Solubility

The total myofibrillar extract was obtained by homogenizing 8 g of muscle in 160 mL of a 0.6 M KCl–0.003 M $NaHCO_3$ (pH 7.0) solution for 1 min in a Sorvall Omni-Mixer 17106 (Du Pont Instruments, Newtown, CT). The homogenate was centrifuged for 20 min at 5000 × g in a refrigerated centrifuge Sorwall RC-5B (Du Pont Instruments, Newtown, CT) at 3-5°C. The collected supernatant was de-

fined as the salt-soluble fraction. Results were expressed as percentages of salt-soluble protein to total protein ($S/S_0 \times 100$).

Protein Determination

The Lowry method (Lowry et al., 1951), with bovine serum albumin as standard, was used to determine protein concentration of salt-soluble extracts, total myofibrillar extract and actomyosin solution.

Electron Microscopy

Purified actomyosin was supported on carbon-coated formvar membranes mounted on regular copper grids (300 mesh). Samples were negatively stained applying a 3% uranylacetate solution for 30-60 seconds, followed by several washes with distilled water (Roura et al., 1992). Microscope observations were performed in a JEOL J.S.M. 35 CF microscope, at high magnification, with an accelerator voltage of 80 kV.

Statistical Analysis

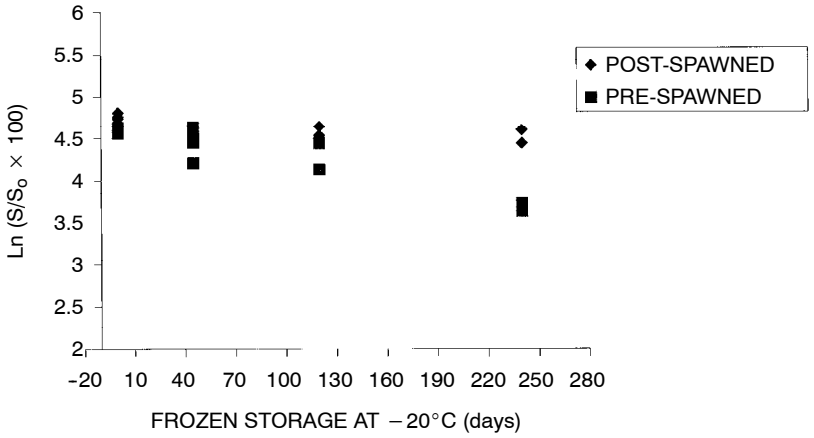
Trends were calculated by the least-square method. The slope for post-spawning solubility values was calculated by the null slope test (Volk, 1969).

RESULTS AND DISCUSSION

The ratio of soluble to total protein during frozen storage is plotted in log scale in Figure 1 for both pre- and post-spawning hake. For post-spawning hake the log of the ratio remained around 4.61 throughout the 240 days storage. The best straight line fit did not explain the results better than the mean value, as tested by the null slope test (Volk, 1969). For pre-spawning hake, however, the ratio decreased continuously and in a semilog plot it can be represented by the straight line

$$\text{Ln}\,(S/S_0 \times 100) = -0.0035\,t + 4.5904 \quad (R_2 = 0.8414),$$

FIGURE 1. Ratio of salt-soluble to total protein in hake during frozen storage at −20°C



Where S/S_0 is the ratio of salt-soluble protein to total protein and t is the storage time.

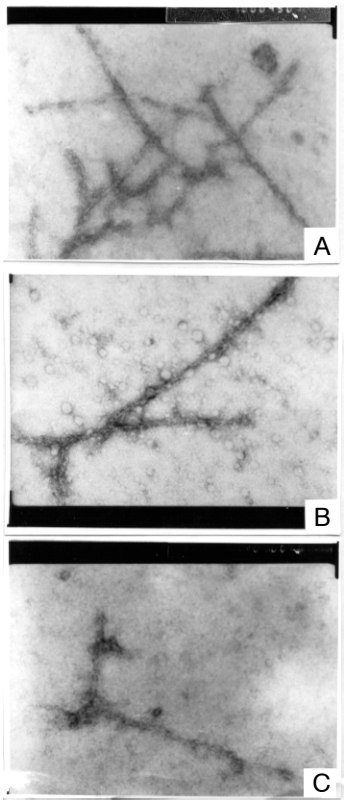
The final soluble protein content in pre-spawning hake is much lower than in post-spawning hake, indicating that the gonadal condition of fish affects the solubility of proteins. These results are in agreement with results reported in a previous work (Montecchia et al., 1997). Castell and Bishop (1973) and Ironside and Love (1958) also reported changes in the salt-extractable proteins related to the physiological condition of fish and to spawning and feeding cycles. Ohnishi et al. (1978) proposed two stages in the solubility of carp actomyosin during frozen storage.

Electron microphotographs of the actomyosin in post-spawning hake are presented in Figure 2 for Day 0 and for frozen samples after 15 and 60 days storage. The corresponding microphotographs of the actomyosin in pre-spawning hake are presented in Figure 3.

The actomyosin of fresh post-spawning hake presented the characteristic arrowhead structure (Figure 2A). After 15 days of frozen storage the actomyosin filaments present little deformation and the arrowhead structure was still discernible (Figure 2B). At day 60 of frozen storage, the arrowhead structure was no longer discernible, filaments were entangled and some aggregate formation was observed (Figure 2C).

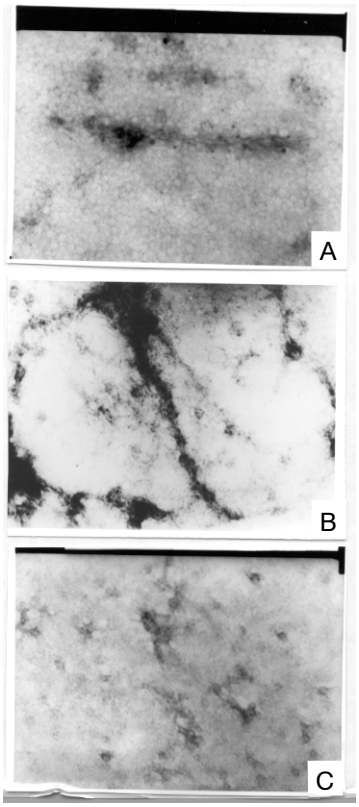
The actomyosin of fresh pre-spawning hake showed a partial loss of

FIGURE 2. Electron micrographs of actomyosin from post-spawning hake muscle during fillets frozen storage at −20°C. A–Fresh actomyosin (magnification: 100,000×) B–After freezing (15 days), (magnification: 100,000×) C–After 60 days, (magnification: 140,000×).



the filament structure; the arrowheads were not discernible and the filaments were shorter, thicker and entangled, with the presence of aggregates (Figure 3A). This loss of the native structure of actomyosin in fresh pre-spawning hake had already been observed in a previous work. It was attributed to a decreased affinity between myosin and actin, probably due to an *in vivo* increase of the proteolitic activity that selectively degrades the heavy myosin chain (Roura et al., 1992). After 15 days of frozen storage, the actomyosin of pre-spawning hake was extensively deformed; the filaments were thick and entangled and

FIGURE 3. Electron micrographs of actomyosin from pre-spawning hake muscle during fillets frozen storage at −20°C. A–Fresh actomyosin (magnification: 100,000×) B–After freezing (15 days), (magnification: 100,000×) C–After 60 days, (magnification: 140,000×).



some aggregates and granules were observed (Figure 3B). At day 60 of frozen storage the actomyosin filaments could not be singled out and were incorporated in aggregates (Figure 3C).

Ohnishi et al. (1978) proposed three types of deformation in the actomyosin filaments from carp during frozen storage: filaments with little or no deformation, filaments deformed to moderate extent, and filaments or objects extensively deformed. Following this classification the actomyosin filaments from post-spawning hake after 60 days storage would be deformed to moderate extent; whereas the actomyo-

sin filaments from pre-spawning hake would be extensively deformed after only 15 days of frozen storage.

The gonadal condition of fish affects the chemical, physicochemical and functional properties of the myofibrillar proteins (Crupkin et al., 1988; Roura et al., 1990; Roura and Crupkin, 1995). The differences in these properties cause different rates of degradation between pre- and post-spawning fillets stored at $-20°C$, as reflected by protein insolubilization, reduced viscosity and hydrodynamic properties (Montecchia et al., 1997). By analyzing the hydrodynamic properties of actomyosin from pre- and post-spawning hake during frozen storage, Montecchia et al. (1997) concluded that after 45 days of storage the actomyosin from pre-spawning hake was more susceptible to aggregation, and its deterioration included loss of extractability and formation of insoluble aggregates. However, in fillets from post-spawning hake, insoluble aggregates were not observed after 240 days of storage.

The solubility of proteins from pre- and post-spawning hake presented different values at time 0. During frozen storage the evolution of the ultrastructure of actomyosin and the solubility of proteins in both samples followed different patterns of degradation. While the actomyosin of post-spawning hake lost its characteristic arrowhead structure only gradually, that of pre-spawning hake presented severe degradation after only 15 days of storage and by day 60 only protein aggregates could be observed. Ohnishi et al. (1978) found that changes in the solubility of proteins do not coincide with ultrastructural changes detected by electron microscopy and, therefore, they proposed that solubility of proteins alone should not be used as an indicator of the status of proteins. According to Sikorski (1978) the loss of protein extractability during frozen storage is caused by aggregate formation. However, Wagner (1986), working with bovine muscle, suggested that during the first stage of protein denaturation soluble aggregates are formed and, only in a second stage, the aggregates become insoluble. Moreover, when working with carp muscle, Oguni et al. (1975) and Tsuchiya et al. (1975) found aggregates of actomyosin filaments in the supernatant actomyosin fraction that was defined as soluble.

Referring to Wagner's suggestion and to the microscopic observations, the different behaviors between pre- and post-spawning hake would indicate that in post-spawning hake the degradation of the

ultrastructure of the actomyosin complex, after 240 days of frozen storage, proceeds only to the point of formation of some soluble aggregates. On the other hand, although some soluble aggregates may form in the actomyosin from pre-spawned hake, they would evolve into insoluble aggregates and the solubility of the protein from pre-spawning hake would decrease continuously (Figure 1).

The changes observed in the microstructure of actomyosin and the results of the solubility of proteins during frozen storage of fillets from pre- and post-spawning hake would constitute further evidence that the gonadal condition of fish affects the properties of myofibrillar proteins.

CONCLUSIONS

The freezing of hake muscle affects the ultrastructure of proteins, both for pre- and post-spawning fish. However these changes are different for both gonadal conditions. In post-spawning hake the proteins retain some of the characteristics of the native structure with some aggregate formation up to 60 days of storage. In pre-spawning hake the formation of aggregates is extensive, even after only 15 days of storage. The solubility of protein from pre-spawning hake decreased continuously reflecting the changes in the ultrastructure of actomyosin from the characteristic arrowhead structure to the formation of insoluble aggregates. For post-spawning hake, only soluble aggregates were observed after 240 days of frozen storage.

Since the actomyosin of fresh post-spawning hake is in its native state and partially retains this structure, resisting the freezing process, it is concluded that this gonadal condition is the best suited for the preparation of frozen products.

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Effects of Preservation Methods On Geosmin Content and Off-Flavor in Nile Tilapia (*Oreochromis niloticus*)

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ABSTRACT. In this study, the masking or reduction of off-flavor in tilapia due to various preservation methods such as salting, drying, frying, smoking, microwave heating, marinating and fermentation with carbohydrate mixture *(som fak* preparation*)* was investigated by subjecting the processed tilapia to sensory evaluation and analyzing the concentrations of geosmin (1,10-*trans*-dimethyl-*trans*-9-decaol) in the processed samples. Dry salting or brining muddy-flavored fish and then drying either by hot air at 50°C or sun-drying resulted to only a slight reduction in the geosmin content of the product. Deep-frying reduced the muddy flavor intensity and geosmin content in salted-dried tilapia. Pretreatment of tilapia fillets with acidified brine before smoking reduced geosmin content and masked the muddy flavor in the smoked product. Microwave cooking of fresh muddy-flavored tilapia showed no effect on its geosmin content nor its off-flavor. Marinating tilapia in acetic acid solution resulted in decreased muddy flavor, and longer marinating period led to lower geosmin content in the product. The geosmin content of *som fak* made from muddy-flavor and non-muddy-flavor tilapia differed significantly, although sensory evaluation yielded no significant differences between the two types of *som fak*, and the taste panelists preferred the product fermented for 3 days. *[Article copies available for a fee from The Haworth Document Delivery Service: 1-800-342-9678. E-mail address: <getinfo@haworthpressinc.com> Website: <http://www.HaworthPress.com>]*

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KEYWORDS. Off-flavor, geosmin, tilapia, salting, drying, smoking, frying, microwave heating, marinating, fermentation, *som fak*

INTRODUCTION

Tilapia has tremendous economic potential in Thailand where most tilapia culture systems are integrated with pigs and/or chicken-raising rather than using either feed or fertilizer alone (Tungthongpairoj, 1993). However, not all cultured fish are acceptable to consumers due to the presence of musty and muddy flavors in a substantial percentage of the produce. The geosmin compound (1,10-*trans*-dimethyl-*trans*-9-decaol) synthesized and excreted by some species of blue green algae and actinomycetes (Ploeg and Boyd, 1992) has been found to be one of the main causes of off-flavors in fish (Lovell, 1983; Yurkowski and Tabachek, 1980). Since a considerable proportion of cultured tilapia production is tainted by off-flavors, effective and economical ways should be explored to increase the acceptability and utilization of this muddy-flavored tilapia. Alternatives include modification of the culture system to prevent occurrence of off-flavors, removal of off-flavors from the harvested fish, or reduction or masking of off-flavors to undetectable levels in fish products subjected to common processing and preservation treatments (Lovell, 1976). Of these three options, most farmers have difficulty in adopting the first two alternatives effectively and economically, thus an investigation into the third option is needed to determine the processing methods which can effect the desired results.

In many countries of the world, salting is a traditional method of processing and preserving fish, often used in combination with drying. The salting process can impart specific desirable flavors on the product (Wheaton and Lawson, 1985). Smoking is another method of preserving fish which combines three effects, namely: preservation, drying and cooking (Clucas and Ward, 1996). Iredale and Rigby (1972) found out that smoke treatment significantly masked the muddy odors and taste in rainbow trout (*Salmo gairdneri*). In addition, acidified brine treatment of the samples prior to smoking may have beneficial effects on geosmin content compared to conventional brining since it was found that the muddy odor substance, geosmin, reacts with acid and is transformed into an odorless substance, argosmin (Gerber and Lechevalier, 1965).

Most salted dried fish are fried before consumption since flavors are developed during frying and fat imparts characteristic flavors to fried foods (Clucas and Ward, 1996). Likewise, present consumption trends gave rise to dramatic increase in the number of microwaveable seafood products sold commercially (Mudgett, 1989). Microwave heating causes moisture migration in foods and may affect the flavor of the product. Another common fish preservation method is the use of acetic acid and salt marinade mixtures. Marinated fish products have extended shelf life and distinctive flavor (Clucas and Ward, 1996). In inland areas of Thailand, a popular traditional fish product called *som fak* is prepared by fermenting a mixture of fish flesh, salt, garlic and cooked rice, the latter serving as substrate for lactic-producing bacteria during fermentation (Clucas and Ward, 1996). Literature concerning methodology, nutritive values and microorganisms involved in fish fermentation had been reported (Adams et al., 1985; Saisithi et al., 1986) but no work had been done on processing *som fak* from muddy-flavored tilapia.

In this study, the reduction and/or masking of muddy flavor in tilapia due to application of different preservation methods such as salting and drying combination, smoking, frying, microwave heating, marinade treatment and carbohydrate fermentation was investigated.

MATERIALS AND METHODS

Raw Materials

Live tilapia (5-6 fish/kg) were caught from farms at the Asian Institute of Technology and transported to the laboratory. The fish were transferred to an aerated tank with clean water and depurated for two weeks during which period water was changed daily and pellet feed was provided to the fish. The fish were divided into two lots, one used as raw material for control or non-muddy-flavor fish while the second lot was used as muddy-flavored fish. Muddy-flavored fish were produced by holding the fish in water containing 5 μg/L geosmin for 12 hours. Each lot was further divided into 6 lots, then packed in polypropylene bags and kept in a freezer at $-20^{\circ}C$ for further studies. Where applicable, all experiments, measurements and analyses were done in triplicate.

Salting and Drying Treatments

The fish were descaled, headed, eviscerated and washed in clean water. For wet salting, muddy-flavor and non-muddy-flavor tilapia were soaked separately in saturated brine solution (26% w/v) for 2 hours at fish to brine ratio of 2:1. Another lot was arranged in layers with salt in between for 4 hours at fish to salt ratio of 5:1. After the desired duration, all brined and dry-salted fish were rinsed, drained, then dried either by sun-drying or oven-drying at 50°C. The drying time was pre-determined in preliminary experiments for about 50-55% final moisture content (AOAC, 1984) of the finished products. The final products were analyzed for geosmin and subjected to sensory evaluation.

Pre-Smoking Treatments

Muddy-flavor and non-muddy-flavor fish were descaled, headed, eviscerated, filleted, washed and subjected to pre-smoke processing treatments as shown in Table 1. All fish samples were smoked in a smoking oven for 3 hours at 40°C, then at 60°C for another hour. All smoked products were kept in cold storage prior to chemical and sensory analysis.

Microwave Heating Treatments

Muddy-flavor and control fish were descaled, deheaded and eviscerated. Prior to washing with clean water, cuts were made on both

TABLE 1. Pre-treatments for muddy-flavor and non-muddy-flavor (control) tilapia prior to smoke processing.

Treatment No.	Description of fish	Pre-smoking ingredients			Soaking period
		Salt	Vinegar	Sugar and spice*	
1	Muddy	6%	–	8%	24 hrs
2	Control	6%	–	8%	24 hrs
3	Muddy	10%	0.5%	–	5 mins
4	Control	10%	0.5%	–	5 mins
5	Muddy	10%	–	–	5 mins
6	Control	10%	–	–	5 mins

* Monosodium glutamate 1% of brine, mixed spices 0.5% of brine (pepper: 5 parts, nutmeg: 2 parts, all-spice: 1 part).

sides of the samples. For one lot, a single cut was made while for the rest, two cutting slices were made on each side to determine whether the number of slices on the flesh have any influence on the effects of microwave heating on the fish flavor. The samples were subjected to microwave heating at 100% microwave generating power (750 watt) for 8 minutes, then the products were analyzed for geosmin content.

Marinade Treatments

Three types of marinating procedures were applied to both muddy- and non-muddy-flavor fish.

Cold marinade: The fish samples were prepared by filleting, rolling tightly, secured with toothpicks and immersed in 7% acetic acid and 1.5% salt solution in a glass container. After 2 weeks, the fillets were transferred into another glass container and covered with solution containing 2% acetic acid and 4% salt.

Cooked marinade: Rolled fish fillets prepared using the same procedure used for the cold marinades were placed in a hot solution (85°C) of 2% acetic acid and 3% salt for 15 minutes. The cooked fish were washed with clean cold water then immersed in 2% acetic acid and 3% salt in a glass container.

Fried marinade: The fish samples were cut into 1-inch thick slices (with backbone), breaded, then deep-fried in oil at 160-165°C for 10 minutes. The fried fish were immersed in brine with 2% acetic acid and 3% salt in a glass container.

Carbohydrate Fermentation Treatments

A traditional Thai procedure was used for making *som fak* in the laboratory. The fish were prepared by filleting (skin-off) and minced with the mincer. Then, a mixture of minced fish flesh combined with boiled rice (15%), salt (4%) and minced garlic was thoroughly mixed and kneaded into an elastic firm mass, then packed in banana leaves with about 30-40 g per portion and allowed to ferment. From the first day (day 0), the packages were incubated at ambient temperature (28-30°C). For the first 5 days of fermentation, two samples were withdrawn daily for analysis, afterwards sampling was done at longer intervals. Since traditionally-prepared *som fak* is normally considered suitable for consumption after 3 days of fermentation, the samples

were divided into two batches after this duration. One batch was stored at 3-5°C and the other was stored at ambient conditions until considered spoiled by the sensory panel.

Geosmin and Muddy Flavor Intensity Analysis

The concentration of geosmin in all raw and processed tilapia was analyzed by applying high-vacuum distillation, extraction and gas chromatography techniques (Yurskowski and Tabachek, 1974). The seven-point scale by Krasner (1988) which ranged from threshold (0), very slight (0.5), slight (1), slight to moderate (1.5), moderate (2), moderate to strong (2.5) and strong level (3) was used to evaluate the flavor intensity of the processed products. Ten panelists trained and familiarized with muddy odors evaluated the samples for each session.

RESULTS AND DISCUSSION

Effect of Salting and Drying on Geosmin Content and Muddy Flavor Intensity

Salted-dried non-muddy fish had geosmin concentration ranging from 4.33-5.44 μg/kg while the muddy-flavor products had geosmin content ranging from 17.4-19.8 μg/kg after salting and drying. The initial level of geosmin in raw muddy-flavor tilapia was 21.9 μg/kg. Statistical analysis showed no significant difference in geosmin content of dried fish subjected to either brining or dry salting. The drying method, sun-drying or oven-drying, has also no effect on the geosmin content of the products.

Effect of Deep-Frying on Geosmin Content and Flavor Intensity in Salted Dried Product

Generally, salted-dried fish is consumed after deep-frying in oil. Deep-frying was found to be effective in reducing the geosmin content of salted-dried products at an average of about 26%, which could be attributed to the fact that geosmin is a neutral oil (with boiling point of approximately 270°C) and could have been vaporized during frying (Gerber, 1983). Panelists detected very slight muddy flavor in deep-fried salted-dried fish produced from muddy-flavor tilapia with geosmin content ranging from 10.45-18.33 μg/kg after frying (Table 2).

Geosmin Content and Muddy Intensity Score on Smoked Tilapia

There were significant differences at 5% level between the three pre-smoking treatments as shown in Table 3. The geosmin content of all the smoked muddy-flavored products was reduced from an initial level of 21.9 μg/kg prior to smoking. The smoked product pre-treated

TABLE 2. Geosmin content and muddy intensity score of raw salted dried fish and fried salted dried fish.

Condition	Geosmin content (μg/kg)		Muddy intensity score*
	Raw	Fried	
Non-off flavor, brine and sun dry	4.33 ± 0.06	2.13 ± 0.05	0
Non-off flavor, brine and oven	5.44 ± 0.15	4.2 ± 0.105	0
Non-off flavor, dry salt and sun dry	4.45 ± 0.05	3.73 ± 0.27	0
Non-off flavor, dry salt and oven	4.93 ± 0.12	3.74 ± 0.06	0
Muddy flavor, brine and sun dry	17.56 ± 0.22	10.45 ± 0.62	0.5 ± 0.1
Muddy flavor, brine and oven	19.5 ± 0.75	18.33 ± 1.10	0.5 ± 0.26
Muddy flavor, dry salt and sun dry	19.83 ± 0.51	15.3 ± 0.5	0.5 ± 0.1
Muddy flavor, dry salt and oven	17.46 ± 0.9	12.8 ± 0.3	0.5 ± 0.1

* 0 (threshold), 0.5 (very slight), 1 (slight), 1.5 (slight to moderate), 2 (moderate), 2.5 (moderate to strong), 3 (strong)

TABLE 3. Geosmin content and muddy intensity score of smoked tilapia.

Sample	Geosmin (μg/kg)	Muddy intensity score*
Non-off flavor, brine and spice	7.1 ± 0.04	0.9 ± 0.65
Non-off flavor and brine	5.58 ± 0.2	0.833 ± 0.28
Non-off flavor, brine and acetic acid	4.74 ± 0.07	1.125 ± 0.25
Muddy flavor, brine and spice	15.16 ± 0.35	0.9 ± 0.65
Muddy flavor and brine	15.5 ± 0.55	1.18 ± 0.65
Muddy flavor, brine and acetic acid	14.13 ± 0.305	1.2 ± 0.83

* 0 (threshold), 0.5 (very slight), 1 (slight), 1.5 (slight to moderate), 2 (moderate), 2.5 (moderate to strong), 3 (strong)

with acidified brine had the lowest geosmin (14.13 μg/kg), which could be attributed to the reaction of acetic acid with geosmin which converted the compound into an odorless substance (Gerber and Lechevalier, 1965). The sensory panelists detected only slight muddy flavor intensity in the smoked products made from muddy-flavored tilapia (scores: 0.9-1.2), almost in the same range as the non-muddy-flavored smoked tilapia products (score: 0.8-1.125), although the geosmin contents were significantly different for the two product groups (non-muddy: 4.7-7.1 μg/kg, muddy: 14.1-15.5 μg/kg). This indicated that the smoking process effectively masked the muddy flavor in the products since the actual geosmin content was still high but sensory perception of the muddy flavor was significantly reduced. Some of the 200 compounds identified by Gilbert and Knowles (1975) in smoke may have masked the muddy off-flavor, resulting to non-detection of muddy flavor by panelists.

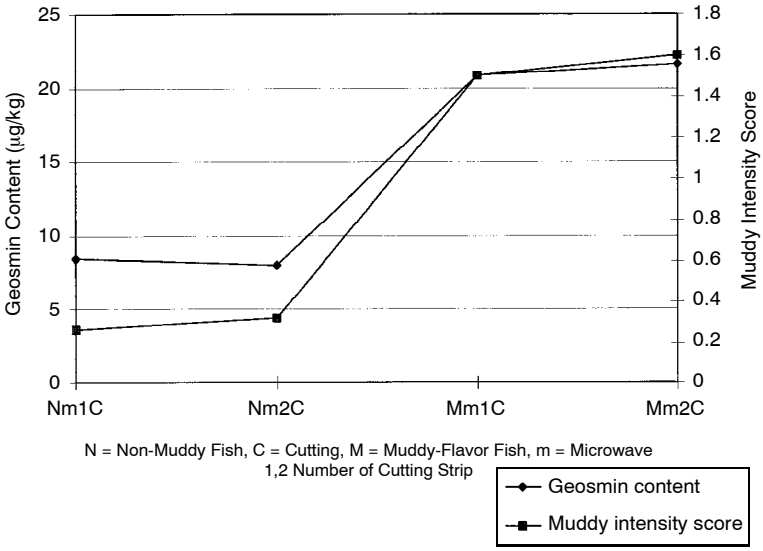
Effect of Microwave Heating

Figure 1 shows the relationship between geosmin content and muddy intensity score of samples subjected to microwave heating. Statistical analysis showed that the number of cuts did not influence the flavor of microwaved muddy and non-muddy fish products. In fact, after cooking with microwave, the geosmin content in both muddy and non-muddy fish were not affected (20.89-21.66 μg/kg and 8.49-8.0 μg/kg, respectively) and the panelists detected very slight to slight to moderate muddy flavor from the microwaved products (score: 0.26-1.60). Although microwave heating causes moisture migration to the surface of foods due to heating of the interior of the food (Shiffman, 1990), apparently geosmin does not evaporate with the water vapor produced during the process.

Effect of Marinating Process

Figure 2 shows the geosmin content of the three kinds of marinated products made from both non-muddy and muddy-flavor tilapia. After 14 days under marinade, it was found that geosmin content in the muddy-flavor products decreased from an initial value of 21 μg/kg to 15.4 μg/kg for the cooked-marinated samples and to 8 μg/kg for the fried-marinated samples. However, very slight change in geosmin

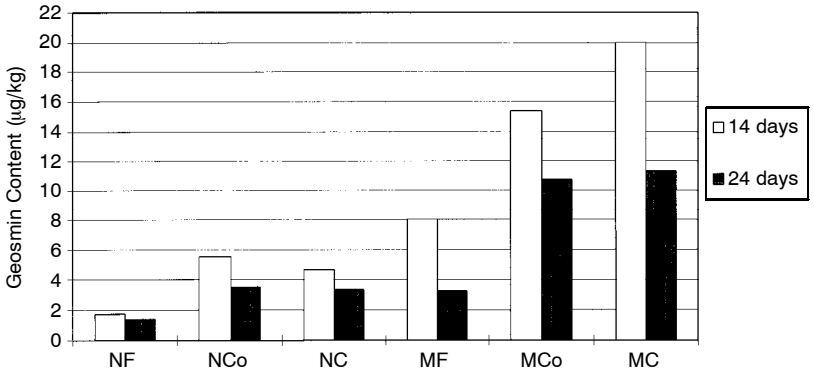
FIGURE 1. Geosmin content and muddy flavor intensity score of microwave tilapia



content was observed for the cold (uncooked) marinated sample (20 µg/kg). The reduction in geosmin content of the fried-marinated product could be attributed to the loss of water and geosmin compound during frying (Clucas and Ward, 1996). In addition, the pickling process with acetic acid and brine solution resulted in substantial extraction of fresh fish volatile carbonyls into the brine (Josephson et al., 1987) and degradation of geosmin into odorless argosmin compound (Gerber and Lechevalier, 1965; Gerber, 1983). These trends were in agreement with the results from the sensory panel which detected no muddy flavor in fried-marinated samples while slight and very slight muddy flavor were detected in the cold and cooked marinated product respectively as shown in Table 4.

After 24 days under marinade at 3 ± 2°C, the geosmin concentrations in the fried-, cooked- and cold-marinated products were found to be 3.19, 10.70 and 11.23 µg/kg, respectively (Figure 2), indicating that longer periods in the marinade (while under refrigeration) resulted in further reduction of geosmin concentration in the products. This may be due to deeper penetration of acetic acid into the tissues, thus de-

FIGURE 2. Geosmin content in marinade tilapia stored for 14 and 24 days



N = Non-Muddy-Flavor Tilapia, F = Fry, Co = Cook C = Cold, M = Muddy-Flavor Tilapia

TABLE 4. Muddy intensity score of marinade tilapia stored for 14 and 24 days

Sample	Muddy intensity score*	
	14 days	24 days
Non-off flavor and fry	0	0
Non-off flavor and cook	0	0
Non-off flavor and cold	0	0
Muddy flavor and fry	0.2 ± 0.05	0
Muddy flavor and cook	0.5 ± 0.09	0.3 ± 0.05
Muddy flavor and cold	1 ± 0.01	0.8 ± 0.07

* 0 (threshold), 0.5 (very slight), 1 (slight), 1.5 (slight to moderate), 2 (moderate), 2.5 (moderate to strong), 3 (strong)

grading the geosmin compound further. Muddy flavor was not detected in the fried-marinated samples after 14 and 24 days. In addition, cold- and cooked-marinated products also had lower muddy flavor intensity score after longer marination time (Table 4). Clucas and Ward (1996) reported that the shelf life of cold, cook and fried marinade can be up to 1, 6 and 12 months, respectively.

Effect of Carbohydrate Fermentation

The initial 8.6 μg/kg geosmin concentration of *som fak* (at day 0) made from muddy-flavored fish flesh decreased to 1.45 μg/kg after 20 days of storage (until spoilage) under refrigeration (Figure 3). The reduction in geosmin was due to the action of lactic acid produced during fermentation which degraded geosmin into an odorless argosmin compound (Gerber and Lechevalier, 1965; Gerber, 1983). The geosmin content of control *som fak* (from non-muddy-flavored fish flesh) was about the same level at 1.2-1.6 μg/kg after the same storage period. However, there was significant difference in the geosmin content between the *som fak* produced from non-off-flavor and from muddy-flavor tilapia during the initial stages of fermentation.

Som fak can be consumed after 3 to 4 days of fermentation depending on the degree of acidity developed in the product (Figure 4). *Som fak* is characterized by a sour, garlic, lightly-salted taste. However, variations in acid content was found to result in differences in acceptability. Good quality *som fak* should have more than 2% lactic acid, with pH of less than 4.5, and about 4.5% salt content (Saisithi et al., 1986). Without distinction whether the product was made from non-muddy or muddy-flavor tilapia, the taste panel preferred the *som fak* fermented for 4 days, with 2.2-2.4 % lactic acid content and the products had no muddy flavor.

FIGURE 3. Geosmin content of *som fak* from non-muddy and muddy-flavor tilapia

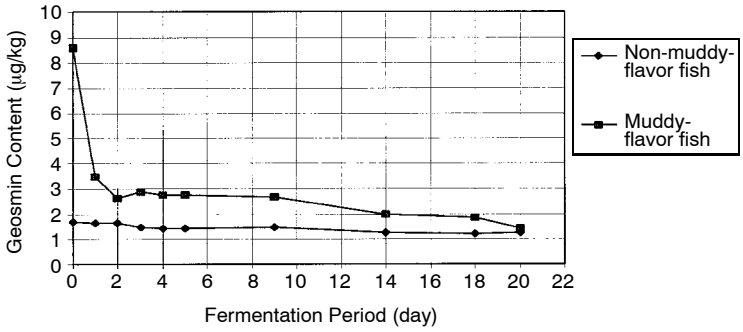
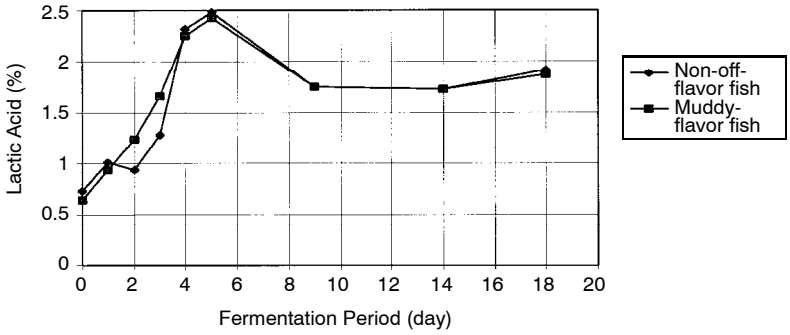


FIGURE 4. Lactic acid (% acidity) of *som fak* from non-muddy and muddy-flavor tilapia



CONCLUSION

The utilization of cultured tilapia tainted with muddy flavor due to geosmin can be promoted by applying certain preservation and processing techniques commonly employed in households and in the fish-processing industry. Deep-frying, smoking after certain pre-treatments, and marinating in acetic acid solutions at certain durations were found to be effective in reducing and/or masking the muddy flavor in tilapia. Likewise, a traditional Thai preparation called *som fak* was found to be a potential means for increasing utilization of muddy-flavored tilapia since the fermentation process involved was found to result to masking the off-flavor in the raw material. On the other hand, combined salting and drying can reduce the geosmin content in muddy-flavored tilapia only slightly and microwave heating produced no beneficial effect on the level of geosmin in tilapia.

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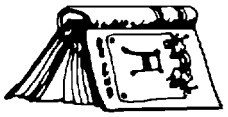
BOOK REVIEWS



FOOD PRODUCT DESIGN, A COMPUTER-AIDED STATISTICAL APPROACH. R. Hu. *Lancaster, PA, USA. Technomic Publishing Company, 1999, 225 pages. $149.95.*

Product optimization to meet consumer needs is a continuing quest for food production professionals. Researchers use modern computer-aided statistical methods to solve food product and process design problems. In the past, the extensive use of statistical methods was hindered by lack of proper training and appropriate computer hardware and software. The situation has changed dramatically with the rapid growth of powerful personal computers and well-designed computer programs. Statistical experimental methodology can be considered a quality control technology that achieves food product development and product excellence at the lowest possible price. Using a significant model, food scientists may examine whether changes made in any food variable have a critical effect. If the model proves to be statistically valid, it can be used to predict the effect of different variable levels on food product characteristics.

The author provides a valuable tool for food engineers, technologists, scientists, and industrial personnel who need to expand their knowledge of computer-aided statistical experimental methods applied to food product design. Practical examples of available statistical programs, designs, and applications are presented. The first chapter introduces common problems in food product design, contrasting traditional trial and error product development with a modern statistical approach. Chapter 2 discusses system identification and definitions. Food quality indices that effectively define the system are first

selected. Variables can usually be separated into those that are important to desired food quality indices and those that produce little or no impact. Important indices should be controlled during product design and the minor ones held constant. The author discusses the concepts of black-box modeling and the classification of independent variables into process and mixture variables. Practical examples are used to explain the difference between factorial experimental design and mixture design.

Chapter 3 presents experimental techniques for food process development. The chapter emphasizes task identification and screening of important variables, selecting the correct model form and experimental design, building a suitable model and testing its significance, analyzing the single and joint effects of variables, and product optimization and prediction. Highly qualified personnel are needed to complete problem identification and proper data gathering and analyses that lead to reliable modeling and process optimization. Various commercial software programs that solve the problems of modeling, optimization, and prediction, are discussed in detail. A completed example of statistical product design, shrimp cracker development and optimization through cooking extrusion, is presented.

Solutions to recipe or formulation modeling using statistical mixture experimental methods are explored in Chapter 4. The raw materials in a mixture system are dependent on each other. The fundamental consideration of recipe modeling is to determine the most suitable mathematical equation that can describe the surface of the simplex region, where the functional relationship between the n component X1, X2, X3, . . . Xn and a food quality index Z are related in a continuous fashion to mixtures comprised of X1, X2, X3, . . . Xn and is considered a response surface. The author derives the Scheffé polynomial from the relationship and then explores several typical mixture experimental designs including the Simplex Lattice Design, mixture designs with two, three, and four components, the Simplex Centroid Design, the Simplex Axial Design, and the constrained Mixture Design. He continues with model building and an analysis of component effects using both graphical and numerical approaches. Specific graphical treatments include a Response Trace Plot, 2D Simplex Plot, and 3D Simplex Response Surface Graphics. Recipe optimization and prediction use both numerical and graphical strategies through sup-

plied BASIC programs to explore numerical optimization of one or more quality objectives and to generate contour plots.

Chapter 5 tackles the most complicated, but one of the most common problems in food product design: modeling and optimizing food systems including recipe and process variables. A detailed practical example designing a nutritious flavorful extruded instant-soluble soy, rice, and wheat product illustrates the technique. The model isolates and optimizes both raw material and extrusion cooking effects. A new aspect of computer applications, an expert system, is presented in Chapter 6. The structure of the expert system is described, consisting of a shell and a knowledge base. The shell incorporates a user interface, an inference engine or control mechanism, and a data interface. Fuzzy logic and a neural network are used to build an expert system for the development of the extruded product described in Chapter 5.

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MICROBIAL FOODBORNE DISEASES: MECHANISMS OF PATHOGENESIS AND TOXIN SYNTHESIS. J. W. Cary, J. E. Linz, and D. Bhatnagar. *Lancaster, PA, USA. Technomic Publishing Co., 2000, 550 pages. $139.95.*

With daily news stories of emerging foodborne pathogens and an increasing emphasis on food safety by both government and industry authorities this volume provides a needed and appropriate reference for the food professional. Possible foodborne hazards that impact our food supply are caused by a wide range of organisms that include pathogenic Gram-positive and Gram-negative bacteria, fungi and algae, parasitic protozoa and viruses, and related agents. The authors rightly contend that knowledge of the molecular basis of pathogenicity will help food professionals develop effective and affordable interventions

for foodborne pathogens. A food safety system based on science also fosters accurate quantitative risk assessment, permitting the most effective deployment of limited resources to provide the greatest potential impact on food safety. Cultivation of efficacious strategies for prevention and treatment of foodborne diseases relies in part on the tools of molecular biology. Molecular and cellular biological techniques have produced many advances in understanding the molecular basis of virulence mechanisms and toxin biosynthesis in pathogens contaminating human and animal foods. *Microbial Foodborne Diseases: Mechanisms of Pathogenicity and Toxin Synthesis* serves as an advanced text for those techniques applied to some of the most important foodborne pathogens.

The main focus of the book is the molecular basis of virulence mechanisms and toxin biosynthesis in organisms that routinely contaminate food, feeds, and water. The volume includes the latest information on pathogen association with particular foods and water, epidemiology, methods of early detection, toxicology, and the economic impacts of pathogens. The authors effectively cover the most important bacterial, fungal, protozoan, and viral pathogens including detailed information on emerging health concerns. Cutting-edge chapters include discussions of prion-based BSE (''Mad Cow'' disease), Norwalk virus associated diseases, and comprehensive reviews on the molecular biology of aflatoxins, Fusarium toxins, and the PSP toxins of marine dinoflagellates.

The work covers selected organisms from five major pathogen groups including Gram-positive and Gram-negative bacteria, toxigenic fungi and marine organisms, parasitic protozoa and viruses, and related infectious agents. The following chapter titles comprise the book: (1) *Molecular and Cellular Biology of Salmonella Pathogenesis,* (2) *Shigella Infections: Epidemiology, Pathogenesis and Host Immune Response,* (3) *The Molecular Pathogenesis of Escherichia coli Infections,* (4) *Virulence Determinants of Yersinia enterocolitica: Mode of Action and Global Regulation,* (5) *Molecular Pathogenesis of Vibrio Infections,* (6) *Molecular Mechanisms Governing Campylobacter Pathogenicity,* (7) *The Genetics, Synthesis, and Action of Clostridium perfringens Enterotoxin,* (8) *Mechanisms of Pathogenesis and Toxin Synthesis in Clostridium botulinum,* (9) *Pathogenesis Determinants of Listeria monocytogenes,* (10) *Aflatoxins: Biological Significance and Regulation of Biosynthesis,* (11) *Fusarium Toxins: Molecu-*

lar Biology of Trichothecene and Fumonisin Biosynthesis, (12) *PSP Toxins: Their Biosynthesis by Marine Dinoflagellates and Molecular Identification, (*13) *Toxoplasma Gondii Strain Variation and Pathogenicity,* (14) *Virulence Associated Factors of Enteric Protozoans Entamoeba histolytica & Cryptosporidium parvum,* (15) *Norwalk and Other Human Caliciviruses: Molecular Characterization, Epidemiology, and Pathogenesis,* and (16) *Molecular Biology of Prion Disease*. Each chapter includes an introduction to the pathogenic group, comprehensive treatment of the pathogens, up-to-date information on the molecular mechanisms of pathogenesis, including virulence factors and toxins which influence adherence, colonization, host-cell entry, and resistance to host immune response and extensive references mechanisms. The chapters on toxigenic fungi and marine dinoflagellates cover advances in the molecular biology of toxin synthesis in detail. The authors introduce new areas of research and consider strategies for the control of the disease process.

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