Processing and Freezing Methods Influencing the Consistency and Quality of Fresh and Frozen Peeled Crawfish (Procambarus Sp.) Meat.

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Processing and freezing methods influencing the consistency and quality of fresh and frozen peeled crawfish (*Procambarus* sp.) meat

Marshall, Gail Anne, Ph.D.
The Louisiana State University and Agricultural and Mechanical Col., 1988

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PROCESSING AND FREEZING METHODS INFLUENCING THE CONSISTENCY AND QUALITY OF FRESH AND FROZEN PEELED CRAWFISH (Procambarus sp.) MEAT

A Dissertation

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Doctor of Philosophy

in

The Department of Food Science

by

Gail Anne Marshall
B.S. Louisiana State University, 1977
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# TABLE OF CONTENTS

ACKNOWLEDGEMENTS ................................................................. ii
ABSTRACT .................................................................................. xi
INTRODUCTION ............................................................................ xiii
LITERATURE REVIEW ............................................................... 1

- Introduction ............................................................................. 1
- Microbiological Factors ......................................................... 1
  - The role of microorganisms in the quality, shelf life, and safety of fresh and frozen fishery products .......... 1
  - Perishability ........................................................................ 1
  - Spoilage organisms ........................................................... 3
  - Microbiological hazards - foodborne pathogens .................. 4
  - Microbiological criteria ....................................................... 4
- Effects of chill and freezing temperatures on bacterial growth and survival ............................................. 7
- Product quality (safety and utility), as indicated by microbiological analysis ............................................. 9
- Preserving fresh fishery products: delaying microbial spoilage ............................................................... 12
- Microbial quality of fresh and frozen fishery products .......... 14
- Fresh ...................................................................................... 14

iv
INDIVIDUAL RESEARCH CONTRIBUTIONS

1. EFFECT OF BLANCH TIME ON THE DEVELOPMENT OF MUSHINESS IN ICE-STORED CRAWFISH MEAT PACKED WITH ADHERING HEPATOPANCREAS

2. DIFFERENCES IN COLOR, TEXTURE, AND FLAVOR OF PROCESSED MEAT FROM RED SWAMP CRAWFISH (Procambarus clarkii) AND WHITE RIVER CRAWFISH (P. acutus acutus)

3. MICROBIOLOGICAL QUALITY OF FROZEN FRESHWATER CRAWFISH (Procambarus sp.) MEAT: EFFECT OF HEPATOPANCREAS TISSUE, FREEZING METHOD, AND FROZEN STORAGE AT -23°C

4. EFFECT OF FREEZING METHOD, pH, HEPATOPANCREAS, AND FROZEN STORAGE AT -23°C ON THE TEXTURE OF FROZEN
LIST OF TABLES

1. Shear force values (kg/g) of 20-hr ice-stored crawfish meat packed with adhering hepatopancreas...............................................71
2. Mean texture scores from sensory evaluation of 20-hr ice-stored crawfish meat packed with adhering hepatopancreas..................72
3. Hunter "Lab" values of meat from red swamp crawfish (P. clarkii) and white river crawfish (P. acutus acutus)...............................79
4. Hunter "Lab" values of hepatopancreas from red swamp crawfish (P. clarkii) and white river crawfish (P. acutus acutus), freshly processed and after 20 hr storage at 3°C.................................80
5. Incidence of total coliforms (TC), fecal coliforms (FC), and E. coli (EC) in washed (W) and unwashed crawfish meat stored at -23°C........................................91
6. Results of analysis of variance on shear force data for both fresh and frozen crawfish meat......107
7. Correlation of crawfish meat shear force values with storage time.................................................108
8. Results of analysis of variance on data for drip, moisture and pH frozen crawfish meat..............131
LIST OF FIGURES

1. Aerobic plate counts (APC) of crawfish meat at -23°C .......................................................92
2. Effect of freezing method (F) on shear force of crawfish meat ............................................109
3. Effect of hepatopancreas on shear force of crawfish meat ....................................................110
4. Sensory texture scores for washed and unwashed crawfish meat: weeks 1 - 72 .......................111
5. The pH of fresh (time 0) and frozen crawfish meat stored at -23°C for 72 weeks ..................112
6. Sensory texture scores for washed and unwashed crawfish meat: weeks 1 - 24 .....................113
7. Sensory texture scores for washed and unwashed crawfish meat: weeks 30 - 48 ...................114
8. Sensory texture scores for washed and unwashed crawfish meat: weeks 52 - 72 ....................115
9. Effect of freezing method (F) on drip loss of crawfish meat ................................................132
10. Effect of freezing method (F) on moisture of crawfish meat ............................................133
11. Effect of hepatopancreas on drip loss of crawfish meat ...................................................134
12. Effect of hepatopancreas on moisture of crawfish meat..................................................135

13. Sensory moisture scores for crawfish meat frozen by different methods (F): weeks 1 - 72..............136

14. Sensory moisture scores for washed and unwashed crawfish meat: weeks 1 - 72......................137
ABSTRACT

The production and processing of freshwater crawfish (Procambarus sp.) is an important seafood industry in Louisiana. Year round availability of a consistently high quality product will help maximize marketability of peeled crawfish meat. This study addressed several quality problems associated with fresh and frozen crawfish meat.

Fresh, peeled crawfish meat is frequently packaged with hepatopancreas for added flavor in prepared dishes. Sporadic development of mushiness in crawfish meat packed with hepatopancreas was an unpredictable problem for crawfish processors. Hepatopancreatic enzymes were thought to be causing proteolysis of ice-stored meat. Study results showed significant effects (p<0.01) of cooking time on the texture of ice-stored meat. Gelatin degradation by hepatopancreas proved to be a good indicator of enzyme activity and adequacy of blanch treatment.

A popular preference for red swamp crawfish (P. clarkii) over white river crawfish (P. acutus acutus) is maintained by many processors and consumers. Both species are harvested simultaneously in mixed proportions. Easily recognizable exterior physical characteristics distinguish the two species. Sensory differences of processed tail meat had not been determined. Results showed insignificant differences (p>0.05) in texture and flavor between the two
species. Significant differences (p<0.0005) in color were determined. The reddish color of the meat and hepatopancreas could be a source of preference for red swamp crawfish.

Freezing fresh seafood can provide year round supplies of high quality products. Freezing and frozen storage have been associated with quality deterioration in seafoods. Toughness, excessive drip loss, and moisture loss can be significant quality defects in frozen seafoods, such as crawfish meat. Four freezing and two packaging methods were studied to determine effects on initial meat quality and during -23°C storage. Freezing method had little effect on microbiological quality indicators, although frozen storage and packaging with hepatopancreas did. Freezing method did not significantly affect meat texture. Frozen storage significantly (p<0.0001) increased toughness. Cryogenic freezing significantly reduced meat drip loss (p<0.0001) and increased (p<0.05) moisture content. Packaging with hepatopancreas significantly increased drip loss (p<0.0001) and moisture (p<0.05), and reduced toughness (p<0.0001). Results indicate that cryogenic freezing produces better and longer lasting quality in frozen crawfish meat.
INTRODUCTION

The following dissertation is based on a number of integrated research projects involving the processing, packaging, and storage of fresh or frozen crawfish meat. The literature review presents information that will provide the reader with an understanding of the meaning and significance of the research material that follows. The author's research is presented in five sections under different headings. Each section is the basis of a manuscript for journal publication.
LITERATURE REVIEW

Introduction

Food preservation has been used for thousands of years. One of the earliest methods recognized was chilled and frozen storage. Early man discovered that animal carcasses could be stored in winter ice and snow, and consumed for months thereafter (Hallowell, 1980). Obviously the reasons for temperature preservation were unknown at that time. Only in recent history has man applied proven scientific principles to food preservation methods that produce the quality, quantity and variety of preserved foods available today. Two critical factors used to help maintain a supply of safe and wholesome food are temperature manipulation and control (ICMSF, 1980a). Consequently, temperature is used to preserve considerable amounts of fresh and frozen seafoods, including crawfish.

Microbiological Factors

The role of microorganisms in the quality, shelf life, and safety of fresh and frozen fishery products

Perishability

There is little doubt that temperature is the most important variable influencing the spoilage of seafood
Raw finfish and shellfish have long been recognized as being more susceptible to spoilage, even at chill temperatures (0° - 7°C), than red meat and poultry products. This is due in part to the presence of endogenous enzymes and bacterial populations associated with cold blooded animals, that remain quite active, even at chill temperatures (Shaw and Shewan, 1968; Liston, 1982; Hobbs, 1983).

The presence of high levels of trimethylamine oxide (TMAO) in marine fish is considered one reason for their more rapid spoilage than other animal flesh foods, including freshwater fish (Hobbs, 1983). It has been shown by Easter et al. (1982) that many spoilage bacteria, such as Aeromonas \textit{putrefaciens} (Laycock and Regier, 1971; van Spreekens, 1977; Easter et al. 1982) and \textit{Alteromonas} (Gibson et al. 1977), use TMAO as a terminal hydrogen acceptor when oxygen is depleted, reducing it directly to trimethylamine (TMA). Marine vibrios have also been shown to produce TMA (Shaw and Shewan, 1968; Makarios-Laham and Levin, 1984). This process allows rapid growth of nonfermentative bacteria under microaerophilic and anaerobic conditions of spoilage. Thus, the association of TMA with spoilage.

In raw, chilled fishery products, deterioration is initially a result of autolytic enzyme activity. Undesirable odors and flavors associated with fish spoilage are the result of bacterial growth. Bacteria initially metabolize soluble nonprotein nitrogen material, followed by
proteolysis in later stages of spoilage (ICMSF, 1980b; Liston, 1982; Hobbs, 1983). Without bacterial activity, the characteristic symptoms of spoilage do not occur (Liston, 1982).

Spoilage organisms

Fish and shellfish from temperate waters have a dominantly psychrotrophic bacterial population, whereas those from tropical waters have a typically mesophilic population (ICMSF 1980b). The optimum and minimum growth temperatures for these groups are 25° to 30°C and -5° to +5°C, and 30° to 45°C and 5° to 15°C, respectively (ICMSF, 1980a). While bacterial populations will be altered by handling and processing, psychrotrophic bacteria, such as those of the genera *Pseudomonas* and *Alteromonas*, become dominant as spoilage proceeds at chill temperatures (Liston, 1982; Hobbs, 1983). Since these psychrotrophic spoilage bacteria comprise a dominant portion of the initial microflora on fish, less time is required for the onset of spoilage.

Due to their high susceptibility to spoilage, fresh fishery products are most commonly preserved by iced storage. Freezing, traditionally used to preserve fishery products for further processing, is being used increasingly for retail distribution (Jensen, 1982). These preservation methods minimize the growth and number of microorganisms present.
Microbiological hazards - foodborne pathogens

Pathogenic organisms can be introduced into fishery products from the aquatic environment from which they were harvested, or from fishing vessels, processing plants, storage facilities, and human handlers. Waterborne pathogenic bacteria of public health significance include *Vibrio*, *Pseudomonas*, *Erysipelothrix*, *Leptospira*, *Pasteurella*, *Mycobacterium*, and *Aeromonas* (Brown and Dorn, 1977). Pathogens that can contaminate fishery products through water polluted by sewage or diseased animals include *Escherichia coli*, *Salmonella* sp., *Shigella* sp., *Staphylococcus* sp., and *Clostridium botulinum* (Ghittino, 1972). Two of these pathogenic species, *C. botulinum* type E and non proteolytic types B and F, and *Vibrio parahaemolyticus*, are considered a normal part of the microflora of fish (ICMSF, 1980b). Harvesting from unpolluted water, and proper handling, sanitation, and processing methods can minimize the potential of foodborne disease transmission from fishery products (NRC, 1985; ICMSF, 1986).

Microbiological criteria

Seafood supplies 18-20% of the animal protein consumed today. As the world population grows, the need for seafood is increasing worldwide. However, pollution and local overfishing may limit increase in total catches (Persson, 1981). To help meet demands for fishery products, inland and
coastal aquaculture is expanding, while at the same time, growing human populations increasingly pollute many of these aquatic environments (ICMSF, 1986). The FAO (1979) lists over 800 species of fish and shellfish harvested for human consumption, a large portion of which enters international trade (ICMSF, 1986). Fishery products may be obtained from numerous types of environments, and then handled under a variety of conditions. This necessitates an awareness for the potential of microbiological problems in seafoods.

To help insure safety and utility of fishery products, microbiological criteria on bacterial indicator organisms, pathogens, and total numbers, have been established for different classes of raw and processed seafood. The International Commission on Microbiological Specifications for Foods (ICMSF, 1986) has established criteria for six categories of seafoods. These consider existing national and international criteria as well as influences from a variety of geographical regions, handling practices, processing methods, and storage facilities. Certain fishery commodities and their products have not been included in these categories because of lack of microbiological data. Freshwater crawfish and processed crawfish products are among the uncategorized seafoods. Grodner and Novak (1974), however, proposed guidelines following a survey of the bacterial quality of live crawfish and commercially processed meat. Nevertheless, product specifications by buyers vary considerably in numbers and types of
microorganisms tolerated. Federal or state microbiological criteria for fresh crab meat (Cockey, 1983) have been used in Louisiana as a guide in establishing criteria for fresh or frozen, processed crawfish products, including whole crawfish and peeled meat.

In the United States, the majority of finfish, molluscs, and crustaceans are consumed as cooked products. Consequently, most problems of a bacterial nature involve economic losses due to spoilage rather than the occurrences of foodborne disease outbreaks. Bryan (1980) reported that from the years 1970 – 1978, fish, molluscs, and crustaceans accounted for 7.4%, 1.9% and 1.4%, respectively, of foodborne disease outbreaks in the United States. In most instances of known etiology, the primary source of toxigenic or causative agent was naturally present in the aquatic habitat. Considerably less than half of these were attributable to sewage pollution of aquatic habitat, or to food handlers and the processing environment.

Abeyta (1983) conducted a survey of bacteriological quality of 287 fresh fish, molluscs, and crustaceans in retail Seattle markets, and found only 2.1% exceeding the maximum limit for acceptability as suggested by the ICMSF (1974). Such high marks are not, however, standard throughout the U.S. or the world. Continued surveillance of the microbiological quality of seafood products, and factors that influence numbers and types of microorganisms present, is needed. Information provided through such efforts should
help the seafood industry provide products that meet microbiological criteria and likewise minimize the risk of foodborne disease.

Effects of chill and freezing temperatures on bacterial growth and survival

Chilling a fishery product to just above its freezing point will have dissimilar effects on the microflora present. As environmental temperatures fall below optimal levels for an organism, growth slows and eventually stops. Rapid chilling of mesophilic bacteria from optimal growth temperatures (30°-45°C) to near 0°C can result in cold shock, and death of a portion of the population. Psychrophlytic organisms are less sensitive to similar drops in temperature (ICMSF, 1980a). Death due to cold shock is thought to be due to a sudden release of cell constituents through membrane "holes" caused by freezing of certain membrane lipids. Psychrotrophic organisms, with membrane lipids of a lower melting point, are less susceptible to development of these "holes" (Rose, 1968).

Proper ice-chilled storage of fishery products will select for the growth of psychrotrophic organisms in a population mixed with mesophiles. At chill temperatures, psychrotrophic organisms will cause spoilage of fishery products; and while growth rates may be slow, spoilage can occur within commonly used storage periods (ICMSF, 1980a).
Consequently, the ICMSF (1986) recommends the use of psychrotrophic counts as an indicator of incipient spoilage in raw and processed seafoods. Mesophilic counts give numbers on the order of 10% that of psychrotrophic counts, and are not considered to be the best quality indicators in chilled seafood.

Freezing has variable effects on microorganisms. Spores and some vegetative cells survive essentially uninjured, while other microorganisms experience nonlethal injury or death. The growth of many microorganisms ceases at temperatures well above freezing (i.e., minimal growth temperature for thermophiles is 40° to 45°C), however, -8°C is considered the minimum growth temperature in frozen foods (Hall, 1979). The growth of all microorganisms ceases when the medium surrounding them freezes (ICMSF, 1980a). Destruction of bacteria is reported to be most rapid in the range of -1° to -5°C (Haines, 1938). Lethal effects of these temperatures are possibly due to denaturation of cellular proteins, as a consequence of solute concentration and pH change within the bacterial cell. In frozen storage, higher temperatures, in the range of -2° to -10°C, are reported to be more lethal than temperatures of -15° and -30°C (Haines, 1938).

Thawing frozen foods also influences survival of bacteria. In four species studied, Gebre-Egziabher et al. (1982) observed slow thawing to be significantly more destructive to microbial cells than rapid thawing. This
phenomenon could be a result of cell bursting, that occurs when high ionic concentrations within cells (increased during formation of relatively pure intercellular ice crystals in the freezing process) absorb excessive water upon thawing (Rogers and Binsted, 1972).

Product quality (safety and utility), as indicated by microbiological analysis

One indication of product safety is the presence or absence of bacterial indicators of contamination from human (e.g. *Staphylococcus aureus*) or fecal (e.g. fecal coliforms or *E. coli*) origin. When these indicator organisms are found in excessive numbers, or in conjunction with high aerobic plate counts, a potential health hazard exists. This is caused by the possible presence of pathogenic organisms (or their toxins) associated with the indicator bacteria.

Susceptibility to injury and death during freezing, frozen storage, and thawing varies with the organism and conditions applied (ICMSF, 1980a). Haines (1938) observed mortality rates of different bacteria and spores, quick frozen at -70°C, to range from 80% in some bacteria, to none in spores. Temperature (-70°C to -5°C) and rate of freezing had little effect on survival within a species. Differential sensitivity among organisms to frozen storage temperatures of -1°C, -2°C, -5°C, -10°C, and -20°C also was observed as indicated by mortality rates. Relative survival rates among
organisms always followed the same pattern, indicating a similar resistance in an organism to freezing or frozen storage.

The distribution of viable organisms in a thawed product could differ from those in the fresh product because of differential sensitivity to effects of freezing and thawing. Consequently, microbiological analysis of a frozen product may not be indicative of original product quality. Microbial "survivors" will include the most freeze resistant components of the initial population. Gram-negative organisms, such as Escherichia, Pseudomonas, Salmonella, and Vibrio, are more sensitive to freezing than are gram-positive organisms, such as Bacillus, Clostridium, Staphylococcus, and Streptococcus. Some foods, such as prepared meals, have been shown to provide a level of protection from the harmful effects of freezing on enteric pathogens and certain gram-negative organisms (ICMSF, 1980a).

Freezing food does not eliminate the potential transmission of all foodborne pathogens when thawed products are consumed. Greater sensitivity to freezing of an indicator organism, such as Escherichia coli, could potentially result in a health hazard in some frozen foods. Varga and Doucet (1984) found that freezing fresh fish at \(-30^\circ C\) for ca. 24 hours resulted in a 75% reduction in fecal coliforms. Williams et al. (1980) observed a significant reduction of coliforms and E. coli in ground beef after 4
and 11 days of frozen storage, while the aerobic plate count (35°C) remained essentially unchanged. The absence of detectable *E. coli* in a frozen product does not necessarily mean that enteric pathogens are not present (Mossel, 1967; Silliker and Gabis, 1976). On the other hand, the presence of non-*E. coli* fecal coliforms does not necessarily indicate that fecal contamination has occurred, but *E. coli* has not survived frozen storage (Splittstoesser et al., 1982).

The microbiological quality of a fresh or frozen fishery product is generally a useful indicator of its utility and safety. High aerobic plate counts (APC's) are commonly indicative of poor handling practices, inadequate sanitation procedures, or temperature abuse. This minimizes product utility, because the fresh shelf life is reduced. When high plate counts are a result of spoilage organisms growing in the product and producing high levels of enzymes, such as proteases and lipases produced by many species of *Pseudomonas*, frozen shelf life is also reduced. Hall and Blanchard (1983 and 1984) and Hall and Alcock (1985) demonstrated that simply inoculating large numbers of bacteria into foods prior to freezing was not adequate to demonstrate activity of bacterial enzymes at low temperatures (-10°C and -15°C). Growth of the organisms in the product was required to bring about quality changes associated with enzymatic activity during frozen storage.
Preserving fresh fishery products:
delaying microbial spoilage

The condition of seafood at the time of freezing has a major influence on the quality and shelf life of the frozen product. A number of preservation methods have been tried with various fishery products. Their functional aspects, alone or in combination, should be considered for use in a number of products. Potential for application will vary in different geographic regions, depending on technical, economic, and social factors.

Fresh fish are generally marketed raw, iced, and in a variety of processed forms (e.g. gutted, filleted), and packaging styles. Studies to find ways of extending shelf life have evaluated factors such as: effects of delayed icing (Barile et al. 1985a); the use of antioxidants (Sweet, 1973), antibiotics (Neal et al., 1977), and chemically treated ice or ice-water slushes (Fieger et al., 1956; Haraguchi et al. 1969; Porter, 1984); storage temperatures (i.e. iced at 0°C vs. chilled sea water at >0°C) (Barile et al., 1985b); UV irradiation (Huang and Toledo, 1982) or gamma irradiation (Licciardello and Ronsivalli, 1982); and vacuum (Jensen et al., 1980; Clingman and Hooper, 1986), or modified atmosphere packaging (Jensen et al., 1980). In general, lower temperatures, combined with one or more of these other factors, can increase the shelf life of fresh fish. The use of these methods, however, has been limited
because of inadequate information concerning effectiveness and safety, available technology, or legal and/or consumer acceptance.

Most crustaceans are highly perishable and are best marketed live or cooked (whole or peeled meat). The exception to this is shrimp, which not only die rapidly upon removal from water, but many species (particularly marine forms) are more stable in raw, iced storage than other crustaceans such as crabs, crawfish, and lobsters. Chemical treatments have been suggested to help preserve fresh iced shrimp (Fieger et al., 1956), while treatments such as irradiation are being investigated for use in shellfish shelf life extension (Licciardello and Ronsivalli, 1982) and decontamination (Van Cleemput et al., 1980; Wills, 1981). Precooked crustacean products that may not be further cooked prior to consumption introduce microbiological problems other than spoilage.

Heat processes such as steaming or boiling are intended to enhance peeling, as well as bring about desirable "crustacean" texture and flavors (Hanson and Aagaard, 1969; Dagbjartsson and Solberg, 1972). These heat processes should also result in a 100-fold reduction of bacterial counts (Greenwood et al., 1985), and have been shown to significantly reduce plate counts, fecal indicator organisms, and pathogenic organisms in crustaceans (Lovell, 1968; Harrison and Lee, 1969; Lovell and Barkate, 1969; Grodner and Novak, 1974; Wentz et al., 1983; Marshall-
Moertle and Moody, 1985). When adequate heat treatments have been employed, the presence of microorganisms of public health concern and high numbers of bacteria arise principally due to recontamination and temperature abuse during further handling and processing. Ampola and Learson (1971) observed that minimal cooking times of blue crabs resulted in improved sensory quality of frozen crab meat, but posed potential public health problems due to higher bacterial numbers. To counter this problem, they recommended a 1/2 hour depuration in 200 ppm chlorine, which was found to reduce the bacterial population by 95 - 99%.

Considering potential health hazards in precooked fisheries products that could be consumed without further heat treatments, microbiological criteria specify limits for indicator organisms (e.g., E. coli), certain pathogens (e.g., V. parahaemolyticus, C. perfringens, and coagulase-positive S. aureus), and total plate counts (ICMSF, 1974; Cockey, 1983; NRC, 1985; ICMSF, 1986). Zero tolerances are established for some pathogens, such as Salmonella (Cockey, 1983) and Shigella (NRC, 1985).

Microbiological quality of fresh and frozen fishery products

Fresh

The microbiological quality of live crawfish and commercially peeled meat has been evaluated by Lovell (1968)
and Grodner and Novak (1974). The incidence of several pathogenic organisms of public health significance was low (Salmonella and coagulase-positive S. aureus), or not detected (Shigella, C. botulinum type E). Indicators of fecal contamination were high, however, with fecal streptococci and high numbers of E. coli (30% >1100/g and 66% >100/g) being present in 75% and 91%, respectively, of 67 samples. Scalding crawfish in 212°F water was shown to reduce initial plate counts of 2.0 x 10^8 CFU/g to 9.6 x 10^3 CFU/g in five minutes. Lovell (1968) suggested five minutes to be an adequate scald time, relative to initial cooked meat bacterial quality and subsequent ice-chilled shelf life. The latter was found to maintain excellent organoleptic quality through 21 days of storage. Bacterial plate counts were not valid at 21 days (due to contamination), however 9 day and 30 day counts were 3.5 x 10^3 and 8.7 x 10^8, respectively. Since all plate counts were done at 35°C, these results are not indicative of numbers of psychrotrophic bacteria, which are the predominant spoilage organisms in ice chilled seafood products. While psychrotrophic counts are generally negligible on fishery products from of tropical waters, as observed in scampi by Bremner (1985), these organisms become dominant during cold storage (Liston, 1982; Hobbs, 1983). Wentz et al. (1983) observed substantially higher aerobic plate counts (APC's) at 30°C than at 35°C in chilled blue crab meat, clams, and oysters at the retail level. Furthermore, Makarios-Laham and
Levin (1984) found psychrophilic organisms in haddock tissue with maximum growth temperatures of <20°C. The involvement of psychrophiles in spoilage is uncertain, but the ICMSF (1980a) has indicated that their contribution is insignificant. Numbers of psychrotrophic bacteria are considered a more accurate reflection of incipient spoilage in refrigerated or ice chilled fishery products (ICMSF, 1986).

Lovell (1968) also conducted a survey of bacterial quality of crawfish meat from processing plants. Twenty three samples from 1966 and nine from 1967 indicated a need for considerable improvement in reduction of plate counts, as well as E. coli, fecal streptococci, and coagulase positive staphylococci. Plate counts ranged from $2.7 \times 10^2$ to $7.0 \times 10^7$ and $3.1 \times 10^4$ to $5.3 \times 10^6$ in 1966 and 1967 samples, respectively. Of these, 35% and 56%, respectively, were over $1.0 \times 10^6$ (rejection level in many microbiological criteria). In these same years, coagulase-positive Staphylococcus was found in 27% and 100% of samples, and E. coli was present in 50% and 89% of samples (where 15% and 44%, respectively, contained >100/g). Since the crawfish heating process would have destroyed most naturally occurring vegetative organisms of this type, high numbers of bacteria of public health significance, in this case, indicated a high degree of human contamination.
Frozen

Due to the high perishability of fishery products and a growing consumer demand for high quality frozen foods, there is increasing interest in the effects of freezing and frozen storage on the microbiological quality of seafoods. Although Lovell (1968) conducted some frozen storage studies of crawfish meat, no microbiological analyses were made. However, pertinent freezing and frozen storage studies have been done with other crustaceans.

Greenwood et al. (1985) observed that freezing crustaceans may result in a 10-fold decrease in total microbial counts. Effects of different freezing methods on the survival of psychrotrophic and mesophilic bacteria in shrimp were observed by Aurell et al. (1976). Shrimp were frozen by means of a plate freezer, air-blast tunnel, or liquid freon freezant (LFF) process. Mesophilic bacterial counts (37°C), initially $1.4 \times 10^4/g$ were reduced by about 40% in all freezing treatments. Psychrotrophic bacterial counts (22°C) were reduced by approximately 50% with use of the plate freezer or air blast tunnel, but remained practically unchanged when using the LFF-process. Chattopadhyay et al. (1983) found decreased bacterial survival in prawns when frozen with liquid nitrogen ($1N_2$), compared to plate freezers. Although the types of bacteria present may have contributed to the conflicting results of these two studies, more information is needed on the effects of freezing on bacterial survival in foods.
Several studies have evaluated effects of frozen storage on survival of bacteria. Mijayama and Cobb (1978) evaluated frozen storage stability, at -20°C, of pond raised freshwater prawns, *M. rosenbergii*, prepared in six different ways (vacuum packed or glazed; raw tails, whole, or whole-hepatopancreas removed). Significant decreases in counts were observed as a result of storage time, but not as a result of treatment. Initial bacterial counts of $3.2 \times 10^5$ to $1.0 \times 10^6$, were reduced to $3.2 \times 10^4$ to $3.2 \times 10^5$, after 9 months of storage. While vacuum and glazed products of different treatments had similar initial counts with respect to treatment, after 9 months of frozen storage, all vacuum packed treatments maintained higher bacterial counts than did their corresponding glazed treatments. Hale and Waters (1981) also evaluated frozen storage stability of pond raised freshwater prawns, *M. rosenbergii*. Samples of raw or cooked whole prawns, and raw tails, were blast frozen, glazed, packed in waxed cartons, sealed in freezer bags, and stored at -20°C. Analyses were performed at zero time, and after 1, 3, 6, and 9 months. Microbial analyses included aerobic plate count (APC) at 35°C (48 hours) and 22°C (5 days), coliform and *E. coli* most probable number (MPN), and *Salmonella* (presence or absence). Bacterial plate counts and coliform counts decreased steadily throughout storage. *E. coli* and *Salmonella* were not detected. Maxwell-Miller et al. (1982) found that APC's were essentially unchanged in scallops stored 5 months at -18°C. Gates et al. (1985)
observed decreases in aerobic plate counts/g in the order of 1 log throughout 13 months of frozen storage of breaded shrimp in one wholesale and two retail freezers. Temperatures of the retail freezers reached a minimum of \(-20^\circ\text{C}\), with 12-18\(^\circ\text{C}\) daily fluctuations. The wholesale freezer temperature was \(-20^\circ\text{C}\), with maximum daily temperature fluctuations of 2-3\(^{\circ}\text{C}\). Total coliform (MPN) and coagulase-positive staphylococci (MPN) counts also were monitored. Although mean freezer temperatures and amount of temperature fluctuations varied, no consistent microbiological differences could be attributed to either of the storage conditions.

**Enzymatic factors**

Enzymatic spoilage of fishery products

Spoilage in fisheries products, initially caused by endogenous enzymes, is a major quality problem in a number of finfish and shellfish species. Enzymes contribute to problems such as melanosis ("black spot") in crustaceans, and lipolysis (hydrolysis of triacylglycerols and phospholipids) in finfish and crustaceans. Proteolysis, observed as the softening of muscle tissue, also is a problem in finfish and crustaceans. Enzymes, thought to be responsible for these tissue changes, have been demonstrated to occur endogenously in finfish tissues (Su et al., 1981)
and in the digestive fluids of crustaceans (Vonk, 1960). Certain bacteria, such as _Clostridium_ and _Pseudomonas_, can produce significant amounts of proteases (Bernal and Stanley, 1968), thus excessive growth of such bacteria in fishery products could have adverse effects on texture.

Many enzymes which contribute to spoilage of fishery products are not adequately identified or characterized, thus complicating efforts to minimize their negative effects. Enzyme activity in fresh, raw, or blanched fishery products is affected by temperature, as well as processing methods such as washing, cleaning, heading, and gutting. Endogenous enzymes become inactive after sufficient heating of the product. Residual enzyme activity, however, may contribute to eventual spoilage in fishery products held at temperatures commercially employed for chilled and frozen storage.

Proteolytic enzymes

**Intramuscular**

Proteolytic enzymes, such as cathepsins, associated with muscle tissue have been demonstrated to contribute to spoilage of fish (Siebert, 1962). Ting et al. (1968) partially purified what was apparently two cathepsins from salmon muscle tissue. These enzymes, which hydrolysed and denatured hemoglobin, had optimal activity at pH 3.7, and two minor optimal pH's at 7.0 and 8.5.
Fish muscle tissue enzymes with different properties than cathepsins have been implicated in textural problems in minced fish gels. Makinodan et al. (1963) suggested that an alkaline protease was causing softening of fish muscle paste when processed around 60°C, but not at 30°C to 40°C or 70°C. In further research, Makinodan and Ikeda (1969) determined optimum activity of the enzyme to be at pH 8.0 and 65°C. Cheng et al. (1979) observed thermal effects (heating rate and final temperature) on fish gels made from Gray trout (*Cynoscion regalis*) and Atlantic croaker (*Micropogon undulatus*). Changes in muscle tropomyosin and myosin were thought to be caused by proteolytic factors in the sarcoplasm. Indeed, a calcium-activated proteolytic fraction was isolated with optimum activity at 60°C, pH 8.0 – 8.5, and a calcium ion concentration of 1 mM. Deng (1981) reported tenderizing of mullet muscle during heating, with optimum activity occurring at 65°C and pH 8.0. It was suggested that this softening could be attributed to alkaline protease activity (hydrolysis). Lin and Lanier (1980) reported an alkaline protease from the skeletal muscle of Atlantic croaker. The enzyme was heat stable, degraded fish actomyosin at 50°C – 60°C in vitro, and was not calcium activated. Lanier et al. (1981) correlated the strength of a gel made from Atlantic croaker with activity of an alkaline protease at temperatures around 60°C. Su et al. (1981), looking for sources of alkaline protease in Atlantic croaker, found it present in the sarcoplasmic
fraction, skin, and internal organs. Similar properties were observed in protease isolated from all points of origin, while that from the internal organs demonstrated the highest activity.

**Digestive tract**

Enzymes associated with the digestive tract also have been associated with spoilage of fishery products. Proteolytic digestive enzymes in the guts of finfish have been associated with the spoilage and breakdown of surrounding muscle tissue. The resulting condition is generally referred to as "belly burn" and "belly bursting." Muscle tissue of capelin was shown by Hjelmeland and Raa (1980) to resist hydrolysis caused by digestive enzymes, such as trypsin, that can exude from the belly cavity. This protective action was attributed, in part, by the presence of enzyme inhibitors in the sarcoplasmic fraction of the muscle tissue.

Proteolytic enzymes from the digestive tract also have been associated with spoilage problems involving softening of muscle tissue in crustaceans. Frequently implicated as a source of these enzymes is the hepatopancreas, so called because to a great extent it fulfills the role of a liver in vertebrates, while it's enzyme secreting function is comparable to that of a vertebrate pancreas (Huxley, 1880; Vonk, 1960). In addition, it carries out a significant portion of primary food absorption (Vonk, 1960), an activity
performed by the small intestine in vertebrates. Almost all crustaceans have a hepatopancreas, consisting of one or more pairs of glandular appendages, located in the mid gut (Vonk, 1960). Huxley (1880) described the appearance and function of this gland in crawfish in great detail.

At least two types of digestive enzymes, trypsin and collagenase, have been implicated in the occurrence of textural problems in *Macrobrachium rosenbergii*. The presence of trypsin was determined by Lee et al. (1980), in a quantitative analysis of digestive enzymes in the hepatopancreas of pond raised freshwater prawns, *M. rosenbergii*. Their goal was to determine if the omnivorous prawns could effectively utilize a commercial ration. Activity of ten enzymes was demonstrated, including amylase, trypsin, chymotrypsin, pepsin, carboxypeptidase A and B, leucine aminopeptidase, and lipolytic enzymes. It was observed that *M. rosenbergii* is appropriately classed as an omnivore.

The presence of collagenase, an enzyme that breaks down the native structure of collagen, a constituent protein of animal tissues, was absent from the list of Lee et al. (1980). This would not be expected of an omnivore. Indeed, other researchers have isolated collagenolytic enzymes from the hepatopancreas of the prawn, *M. rosenbergii* (Baranowski et al., 1984; Nip et al., 1985a) and a crab, *Uca pugilator* (Eisen and Jeffrey, 1969).
Muscle tissue textural problems in crustaceans

**Influence and characteristics of endogenous enzymes.**

*M. rosenbergii*, a tropical freshwater prawn, has considerable potential for intensive aquaculture. Postmortem texture deterioration of fresh-iced, or previously frozen-iced prawns, however, has affected value and marketability. This texture problem, which develops within 48 hours of iced storage, is described as a mushy, soft, or chalky texture of the cooked product (Rowland et al., 1982). A similar situation with beef, in which it disintegrates on cooking, has been associated with too few crosslinks in collagen (Bailey, 1979).

Several researchers have investigated textural problems encountered in iced or frozen storage of *M. rosenbergii*. Mijayama and Cobb (1977) evaluated preparation methods (i.e. headed or whole; vacuum or glazed), and -20°C frozen storage stability of *M. rosenbergii*. Informal taste panels noted significant decreases in taste and moisture of samples within 6 months of storage, while texture remained relatively unchanged for 9 months. Nip and Moy (1979) observed a significant decrease in firmness of fresh or frozen-thawed *M. rosenbergii* when stored on ice for 48 hours. Hale and Waters (1981) also evaluated frozen storage stability of *M. rosenbergii* at -20°C for 9 months. Firmness of raw tails and whole *M. rosenbergii* increased considerably at 3 months, relative to the standard reference
(raw tails stored at $-40^\circ$C), then became softer at 6 months, while increasing in firmness again at 9 months. The reference became somewhat firmer throughout the study. The whole raw or whole cooked M. rosenbergii were softer than the tails. This difference was attributed to muscle tissue contact with hepatopancreatic enzymes in the whole prawns.

A histological study of 48 hour iced M. rosenbergii tail muscle tissue was done by Rowland et al. (1982) to find evidence of proteolysis, thought to be responsible for development of texture problems. They suspected involvement of digestive enzymes, migrating from the hepatopancreas and the vein, which extends the length of the tail on the dorsal surface and is also involved in digestion. In one treatment, whole M. rosenbergii were stored after the hepatopancreas had been ruptured with a knife. Proteolytic activity, as indicated by loss of z-line structure and gapping in the sarcoplasm between myofibers, was observed in muscle tissue samples from this treatment. In another treatment, M. rosenbergii heads were removed and all hepatopancreas tissue was washed off, while veins were left intact. Tissue samples with this treatment away from the vein showed an intact z-line structure and little separation of myofibers. There appeared to be slightly increased proteolytic activity in tissue near the vein, observed as slight separation between myofibers. Another treatment was of headed washed and deveined M. rosenbergii, injected with trypsin. Samples of muscle tissue had complete loss of z-lines, and
disorganization of myofibers similar to samples stored with heads on and ruptured hepatopancreases, suggesting that a proteolytic enzyme system was responsible for softening in *M. rosenbergii*.

Baranowski et al. (1984) investigated the nature of proteases from *M. rosenbergii* in a crude hepatopancreas extract, in effort to determine what enzymes were responsible for structural and textural changes occurring in prawn muscle tissue in ice-chilled storage. Activities of several types of enzymes were found in the extract, including collagenolytic and trypsinolytic. When testing commercial enzymes, only collagenase was found to significantly degrade prawn tissue, suggesting that the collagenolytic activity demonstrated in the hepatopancreas extract may be a cause of textural changes observed. Nip et al. (1985a) later characterized this collagenolytic fraction, demonstrating optimum activity at 37°C and pH 6.5 to 7.5. Activity was also present at 0°C, suggesting the possibility that this enzyme could be responsible for texture changes during ice-chilled storage. Nip et al. (1985b) tried purging prawns for 18 hours to minimize observed ice-chilled textural problems. No significant relief was obtained.

**Influence and characteristics of collagen**

The possibility that *M. rosenbergii* collagen could enhance enzymatic breakdown of prawn collagen and subsequent
textural problems was investigated by Nip et al. (1981). Their work showed some characteristics of *M. rosenbergii* collagen that were atypical of other crustaceans, such as is found in white shrimp, *Penaeus setiferous*, (Thompson and Thompson, 1968), lobsters, and other crustaceans (Kimura et al., 1969). Nip et al. (1981) studied the amino acid profile of insoluble collagen of *M. rosenbergii* and observed an absence of hydroxylysine and a low glycine:imino acid ratio. They observed that these characteristics could have an effect on the textural property of muscle tissue through decreased stability in the triple helical structure. Kimura and Tanaka (1986), used different methods to characterize prawn collagen, and found its amino acid content to be similar to other crustaceans. They concluded that development of mushiness in cooked prawns, following cold storage, was not caused by unusual collagen characteristics.

Preservation of optimum texture quality relative to proteolytic activity

Fresh and frozen preservation of crustaceans other than freshwater prawns also has been hampered by textural problems associated with digestive enzymes. A number of proteases have been demonstrated in the gastric juices of the American lobster, *Homerus americanus* (Brockeroff et al., 1970), and in a hepatopancreas extract of the rock lobster, *Jasus lalandii* (Olley et al., 1973). Digestive fluids of a number of decapod crustaceans have all demonstrated
proteolytic activity (Vonk, 1960).

The freezing of raw crustaceans, such as crabs and lobsters, is in some cases desirable. Such products can develop textural problems in muscle tissue near the hepatopancreas, however, and it is difficult to remove the meat from the shell after cooking. Consequently, some cooking before freezing is preferable (Altenburg, 1950; Hanson and Aagaard, 1969). A minimal blanch time is often necessary to avoid weight losses of up to 25% that can occur in fully cooked lobsters and crabs (Reay and House, 1951; Gillespie et al., 1983). This also has been shown to produce better textural quality in frozen lobsters (Getchell and Highlands, 1957; Dagbjartsson and Solberg, 1972; Badonia, 1981) and crabs (Cook and Lofton, 1979; Gillespie et al., 1983).

Several processing factors, in addition to blanching, have been shown to influence textural quality of minimally heat-processed crustaceans. These include overnight starvation prior to the killing of lobsters (Wessels and Olley, 1973) and crabs (Gillespie et al., 1983) and use of killing methods that avoid disruption of internal organs which release digestive enzymes into the body cavity (Gillespie et al., 1983). After blanching, disturbance of viscera (by carapace removal and vacuum evisceration) and delays prior to freezing, adversely affect body meat texture (Gillespie et al., 1983).
Basis of texture in muscle foods

The texture of muscle tissue is considered to be a primary quality attribute, and is largely dependent on the condition of its major components, muscle fibers (i.e., actin and myosin) and connective tissue (i.e., collagen). Muscle tissues of land animals and aquatic animals differ with respect to these components, due in part, to requirements for body support and motility.

Land animals require an extensive and strong network of connective tissue to support the complex muscle system. The collagenous connective tissue forms crosslinks to provide strength. In land animals, the turnover time of collagen is very long, thus, in older animals collagen becomes tougher due to the accumulation of crosslinks with increasing stability. This also increases the thermal stability and decreases solubility of the collagen. On the other hand, the contractile elements actin and myosin have about a 12 day turnover, and do not become tough due to "old" age (Bailey, 1972). However, they do toughen as a result of post mortem conditions, due to contraction during rigor mortis.

The dominant factors affecting the tenderness of meat from land animals are thought to be the age of the animal and degree of cross linking in collagen, as well as the state of contraction of muscle fibers resulting from post
mortem handling. Consequently, these factors are the ones most extensively researched regarding meat texture. Red meat is quite stable to the effects of freezing, and may even become more tender as a result. Since the effects of freezing meat are not considered to seriously impact textural quality, the subject has not been researched as extensively as has the effects of rigor on texture.

The texture of fish and crustacean muscle tissue is dependent on different factors than that of red meat. Because of their aqueous environment, aquatic animals do not require the extensive, strong network of connective tissue of land animals. Fish muscle contains less connective tissue than does muscle from land animals, along with different structural characteristics. The collagen in aquatic animals is significantly less crosslinked than that of land animals, due in part to a lower hydroxyproline and proline content. The decreased level of crosslinking in collagen gives it a greater solubility and lower temperature stability. Fish collagen is readily soluble by normal cooking procedures and is generally an insignificant factor in the texture of cooked products (Dunajski, 1979; Hultin, 1985). Consequently, it is the texture of muscle fibers that contribute most significantly to the texture of fish and crustaceans (Dunajski, 1979).
Factors that modify the texture of muscle tissue

Innate factors

Since tougher fish have been observed to have a shorter cold-storage life than tender ones (Kelly, 1969), the following factors affecting the textural qualities of fishery products should be of interest to processors. Factors reported to affect the texture of fish and crustaceans include species, age, size (within a species), nutritional state, and post mortem factors, such as glycolysis, rigor mortis, pH, and temperature of storage and cooking (Dunajski, 1979).

Muscle fibers in fish are relatively short, not exceeding about 12 mm, even in large fish (Dunajski, 1979). This is more impressive considering that muscle fibers 34 cm in length have been observed in an adult human (Bailey, 1972). Diameter of muscle fibers in fish is mainly dependent on species, age, and muscle function. Again, considerable differences exist between aquatic and land species. The diameter of a large muscle fiber in an adult fish, such as a 95 cm cod, range from approximately 150 - 300 μm (Love, 1958). The numbers of muscle fibers do not change in postlarval crustaceans, so muscle growth is entirely a factor of individual fibers increasing greatly in size. Indeed, the diameter of muscle fibers in crustaceans can become comparatively huge, such as the 4 mm fiber observed in Alaskan king crab (Atwood, 1972). The size of fish has
been shown to affect texture, where in general, larger fish are tougher than smaller fish (Love et al., 1974). This is presumably due in part to increased length and diameter of muscle fibers (Love, 1958), which results in an increased coarseness in muscles (Dunajski, 1979). Again, differences between land animals and fish are seen in the aging process relative to collagen. As fish age, collagen content increases, but becomes less cross linked, contrary to that reported for land animals (Hultin, 1985).

Nutritional status of aquatic species affects texture in several ways (Love et al., 1974; Dunajski, 1979). Reproductive cycles and food availability affect the amount of water, protein, and fat in muscle tissue. During spawning or lack of food, depletion of muscle tissue causes increased water content and decreased protein and fat. These changes vary with fishing grounds and seasons, and affect texture and mouth feel (Love, 1964 and 1975). If pH of the tissue is not altered by the depletion process, texture is not necessarily affected (Love et al., 1974); thus, pH appears to be one of the dominant factors affecting muscle texture. Feeding and nutritional status of the animal can also affect pH of muscle tissue during rigor through its effect on glycogen reserves.

Other researchers have found pH to influence fish muscle texture. Lower pH levels were associated with tougher fish muscle (Cowie and Little, 1966; Kelley et al., 1966; Connell and Howgate, 1968; Love et al. 1974; Dunajski,
Natural pH levels of live species and the subsequent alterations that occur during post mortem rigor have been reported to affect texture of muscle tissue. Dunajski (1979) observed that an increased toughness occurs in fish muscle tissue with a decrease in pH, particularly below 6.7. Above this value, a gradual decrease in the effect of pH on texture occurred.

The pH of fish flesh is usually neutral at death, but proceeds to drop post mortem as lactic acid is produced from anaerobic glycogen degradation. As the isoelectric point of the myofibrillar proteins is approached, ionized negative groups become neutralized. The decrease in number of unipolar charged groups results in a decrease in repelling forces and a tightening of the protein structure. Consequently increased cooking loss occurs with concentration of myofibrillar proteins. The results are reflected in a dryer, tougher product (Dunajski, 1979). It was postulated that dryness or juiciness would probably change in the same manner as affected by pH. Love et al. (1974) demonstrated with correlation coefficients and regression analysis that water content had a variable influence on texture of cod, but its impact was always much less that that of pH.

**Processing factors**

Handling. When aquatic species are transferred to the processing plant, several factors will affect texture of the
processed product. Lower storage temperatures result in a slower development of rigor. Increased shortening and a tougher texture of raw and cooked cod muscle was observed when stored at 18°C rather than at 0°C (Dunajski, 1979). In general, fish stored on ice becomes toughest within 1-2 days post mortem. This coincides with the lowest pH levels and maximum contraction of muscle fibers during rigor. The tissue becomes more tender as rigor resolves. In addition, fish muscle removed from its skeletal support before rigor is tougher when cooked following resolution of rigor, than that which remained attached to the skeleton throughout rigor. This differential effect diminishes with storage time.

**Heating or cooking.** The texture of cooked tissue is greatly affected by the precise time during this post mortem period that it is cooked. Cooking pre-rigor tissue causes rigor to develop so rapidly that contraction cannot be avoided. Delaying cooking until rigor is in progress will result in a more tender fish, due to a partial resolution of rigor; cooking after resolution of rigor will produce the most tender fish (Dunajski, 1979).

Maximum muscle contraction, that can occur during the cooking of pre-rigor fish muscle, does not always take place. Dunajski (1979) observed this phenomenon, concluding that more extensive contraction of the pre-rigor tissue increased toughness up to a point; further contraction resulted in structural damage and a more tender muscle
Textural changes associated with heating or cooking of muscle tissue is due to a combination of factors. A general pattern of toughening followed by tenderizing occurs, resulting from toughening of myofibrillar tissue, followed by softening of collagenous connective tissue. Texture (i.e., shear force) - temperature relationships have been observed in red meat (Bouton et al., 1981) as well as in fishery products (Dunajski, 1979). Dunajski and co-workers (1979) reported this relationship to be pH-dependent in cod. The influence of temperature was observed to be greater at lower pH values and negligible at neutral or slightly alkaline values.

In red meats, collagen becomes more highly crosslinked (i.e. tougher) and more thermally stable with age. Cooking and the subsequent melting of collagen is an important factor in meat tenderness. Specifically, tenderness is enhanced by breakdown of collagen, a process that begins at about 50°-60°C, depending on the animal's age (Hultin, 1985). Collagen is not considered a significant textural factor in cooked fish muscle tissue, because of its lower thermal stability (Love et al., 1974). Fish collagen is readily broken down under normal minimal cooking procedures. Fish collagen becomes solubilized at approximately 60°C, therefore, textural changes occurring above this temperature are thought to be associated with myofibrillar changes. Collagen breakdown occurs at a lower temperature in cold
water than in warm water species (Dunajski, 1979).

Denaturation of muscle tissue, such as during cooking, also decreases water holding capacity, causing free water to be expelled (Hultin, 1985). The free water increases the perception of juiciness, which is closely related to tenderness. Prolonged heating causes a loss of this water and eventual drying of the product.

Freezing. Freezing has long been considered an excellent means of preserving meat. The effectiveness of freezing preservation stems from a dehydration of tissue as water is converted to ice, and from the decrease in storage temperature (Hultin, 1985). Freezing can cause changes in muscle tissue that affect the frozen shelf life as well as the quality of the thawed product. Main factors thought to be involved in observed changes include size and location of ice crystals formed, concentration of salts and other components of the water phase, and the concomitant change in pH (Hultin, 1985).

Freezing rate is considered to be the most important factor influencing changes incurred during the freezing process. Freezing rates for foods were categorized by Ciobanu (1976) as: slow freezing, <0.2 cm/hr; deep freezing, 0.5-3 cm/hr; rapid freezing, 5-10 cm/hr and ultrarapid (cryogenic) freezing as >10 cm/hr.

Rasmussen (1972) noted that of the muscle foods, fish and seafoods are the most sensitive to freezing rate. It has been generally considered that faster freezing always
results in a higher quality thawed product. This is based on histological studies of cellular damage resulting from ice crystal formation and drip exuded from the thawed product. These changes are not always recognized in the product's sensory quality, e.g., color, texture, flavor, and moistness. In addition, products such as red meats, fish, fruits, and vegetables respond differently to freezing rates.

Several changes often observed in frozen or thawed foods are initiated during the freezing process. The extent of these changes can be influenced by the freezing rate. The occurrence of drip has long been associated with frozen foods. In meats, a direct correlation was assumed to exist between the drip and textural changes, e.g., toughness and stringiness. The development of rapid, i.e., cryogenic, freezing methods has demonstrated that a higher drip loss does not necessarily result in a noticeably tougher cooked product (Jul, 1984).

Jul (1984) noted three factors associated with drip, including internal pressure, freezing rate, and crystal formation. Theories involving internal pressure suggest that as a shell freezes on a product, e.g., a large section of meat, pressure builds up inside when expansion from continued freezing is restrained. Rupture in the product releases the pressure but causes drip loss in the thawed product. Jul (1984) considered this factor generally to be of little significance, noting that water freezes out
gradually and tissue remains elastic for some time.

Freezing rate has been associated with quality of frozen food, perhaps originating with the work of Plank et al. (1916). They conducted studies on the quality of fresh fish frozen by the "Ottesen" method of quick freezing in rapidly circulating \(-17^\circ C\) brine, vs. traditionally frozen fish, still-frozen at \(-7^\circ C\). A better quality frozen fish was obtained with the quick freezing process. Jul (1984) commented that the quality of the raw products used probably influenced differences in quality of the end product more than acknowledged. Typically, in that day, fish that did not sell as fresh were "conventionally" frozen for later sale. Not only had they already undergone autolytic or microbiological deterioration, but the freezing process used was often very slow, i.e., boxes of fish stacked in a room, allowing for further product deterioration during the lengthy freezing process. The work leading to the theory on high quality obtained from quick freezing had some mitigating factors which complicated interpretation of results.

The association of ice crystal formation during the freezing process with product quality and drip loss also was noted by Plank et al. (1916). They conducted histological studies on fish tissue frozen by the Ottesen method and other faster or slower methods. The tissue samples studied were fixed in the frozen state, sliced, stained, and examined in a conventional way. Holes in the tissue were
assumed (correctly so, as indicated by later research) to be the location of ice crystals. Rapid freezing was observed to cause formation of small intra- and intercellular ice crystals. However, when compared to rapid freezing, slow freezing caused a greater amount of disorganization, dissociation, and rupture of cells. Structural differences due to different freezing rates also were observed by Nusbaum (1979), Piskarev et al. (1971), and Coleman et al. (1986). Reuter (Plank et al., 1916) concluded that less structural damage would result in reduced change in taste and texture, due to freezing (Jul, 1984). This was observed by Sebranek et al. (1978) and Nusbaum (1979), who related improvements in palatability of more rapidly frozen ground beef patties to less structural damage. Similar structural improvements were observed in the tissue of crab and shrimp, by Giddings and Hill (1978), and blue crab, by Coleman et al., (1986). It was thought that frozen products, with decreased alteration in physical appearance, would have correspondingly less change in sensory quality of any prepared ready-to-eat product.

Observers of the investigations of Plank et al. (1916) concluded that less drip loss would occur in rapidly frozen products. However, ruptured cells were observed in the interiors of larger pieces of rapidly frozen (i.e., brine) product, but not in slow frozen products, in which crystal formation was mostly intercellular. It was suggested that ruptured cells (rapid freezing) may lead to greater drip...
loss than distorted cells (slow freezing), that could potentially allow water to be reabsorbed during thawing. Later studies demonstrated that ruptured cells have actually decreased drip loss in frozen muscle tissues, due to release of myofibrillar proteins which serve as effective water binders (Jul, 1984).

In spite of the fact that Plank et al. (1916) stated that the majority of changes in a frozen product develop during frozen storage, and are not due to the freezing process, the idea persists that changes in microstructure are reflected in palatability of thawed tissue (Jul, 1984). The belief that there is an intimate connection between quick freezing, small intracellular ice crystals, and high quality frozen products is still maintained by individuals in government, industry, and scientific circles. Many studies fail to show a difference between rapid and moderate freezing rates (Jul, 1984). A connection between ice crystals and product quality does exist. However, the superiority of rapid freezing has not always been demonstrated in the thawed product, particularly for the reasons given, e.g., texture, moisture and flavor.

There does not appear to be a substantiated association between ice crystal size and location and resultant thaw drip and loss of quality in muscle tissues. However, protein denaturation has been clearly associated with the aforementioned quality factors (Shenouda, 1980). Protein denaturation is indicated by excessive drip loss, and is
often associated with texture changes. Protein denaturation may occur during freezing and is enhanced by slow freezing (Hultin, 1985). When muscle tissue is frozen slowly, ice crystals form first in extracellular spaces. Because of a lower concentration of solutes this area has a higher freezing point. As the material is chilled further, super cooled intracellular water migrates to these ice crystals, where vapor pressure is lower. This results in forming large ice crystals and concentration of solutes in the cells. The resulting high salt concentration and altered pH can denature proteins and reduce water holding capacity. When the tissue is maintained longer at higher subfreezing temperatures, denaturation is enhanced. Deterioration characteristic of frozen storage actually occurs more rapidly at \(-3^\circ\) to \(-5^\circ\)C, where approximately 60-80% of the water is frozen, than at \(-1^\circ\) to \(-2^\circ\)C, where up to about 50% of the water is frozen (Connell, 1975). The more rapid the product is moved from \(0^\circ\) to \(-5^\circ\)C, the better will be the resulting quality. Connell (1975) suggested that when freezing fishery products, the \(0^\circ\) to \(-5^\circ\) change should occur in < 5 to 10 hours. Hultin (1985) observed that loss of water holding capacity caused by protein denaturation, and mechanical damage to cells due to formation of large ice crystals, are largely responsible for excessive thaw exudate.

It is important to note that while the freezing process can directly affect the quality of many products, the
temperature and duration of frozen storage have a significant effect on the quality of a thawed product. Fennema (1968) ranked the freezing process as the third most important factor influencing quality of frozen food, behind frozen storage conditions and thawing methods. When fish is frozen properly, quality and yield advantages for different types of freezing equipment are usually marginal (Connell, 1975). Little if any loss in quality results from the freezing process itself.

**Frozen storage**

In general, fishery products do not suffer much quality loss as a consequence of the freezing process if it is done rapidly. However, significant deterioration has frequently been observed during frozen storage. Detrimental changes include development of off-odors and off-flavors as well as changes in texture, moisture, and appearance (Connell, 1975; Shenouda, 1980). These deteriorative changes are due in part to the presence of enzymes. Enzyme activity is only partially arrested by the reduced temperatures of frozen storage. Changes in texture and related quality characteristics, such as thaw drip and moisture content, are the most notable quality defects in frozen fishery products. Consequently, there has been considerable research into the mechanisms of these changes reviewed by Matsumoto (1979) and Shenouda (1980).

Deteriorative changes in color, flavor, and
particularly texture, during frozen storage are, for the most part, unsolved problems for fishery products. A major consideration in judging the quality of frozen seafood is texture. As a consequence of protein denaturation during frozen storage, frozen seafood can change considerably from the fresh product (Dyer, 1951; Shenouda, 1980; Hultin, 1985). Shenouda (1980) described these texture changes as: "extra firmness, toughness, springiness, sponginess, stringiness, dryness, rubbery texture, lack of succulence, loss of water holding properties, and loss of juiciness."

Connell (1975) observed that frozen storage of fish may cause changes in the texture of the cooked product. He described the textural difference as changing from "the usual soft, springly, moist succulence of fresh or recently frozen fish" to a texture that is "unacceptably firm, hard, fibrous, woody, spongy or dry." Connell (1975) also noted that "whereas little fluid can be expressed from raw fish, copious amounts exude or can be pressed out after frozen storage under poor conditions." These changes occur even in well-protected products. Poor packaging and/or glazing adds to texture deterioration due to the drying action of "freezer burn." The latter leaves the product irreversibly dry and porous, typically described like "balsa wood" (Connell, 1975).

Under typical commercial conditions of frozen storage, some protein denaturation in frozen fish is unavoidable. Numerous factors are involved in protein denaturation and
can be grouped as those related to moisture, lipids, or trimethylamine oxide (TMAO) and the enzyme TMAOase (Shenouda, 1980).

Protein denaturation factors related to changes in moisture include formation of ice crystals, dehydration of protein molecules, and an increased salt concentration in the remaining unfrozen water (Shenouda, 1980). These denaturing conditions are initiated during the freezing process and continue to effect changes in muscle tissue during frozen storage.

Formation and growth of ice crystals causes pressure on the ultrastructure of tissue and serve to disrupt and compact muscle fibrils. It has been postulated that this promotes denaturation through formation of cross bridges. Dehydration of protein is thought to denature the three dimensional configuration of proteins by removing water molecules that mediate the stabilizing hydrogen bond network. Salt concentrations in unfrozen water of fish muscle tissue are increased roughly 10-fold at commercial storage temperatures (-10°C to -20°C). At these temperatures, only about 90% of the water is converted to ice. Increased salt concentrations have been shown to denature protein through aggregation, or dissociation, caused by disruption of tertiary and quaternary macromolecule configuration. The stability of these structures depends on ionic, van der Waals, hydrogen and hydrophobic forces, which become altered by increased salt concentration. All of these moisture-
related factors continue to denature fish muscle proteins during frozen storage (Shenouda, 1980).

Most of the lipid material in fish muscle cells is associated with membranes. Membrane lipids are typically rich in phospholipids (Hultin, 1985), used for structural and metabolic purposes (Opstvedt, 1984). The neutral lipids, principally triacylglycerols and cholesterol (Hultin, 1985), are located elsewhere. Triacylglycerols ("triglycerides") are used mainly for storage of fat. This is done predominantly in separate organs, i.e., the liver, in lean fish such as cod, but stored throughout the body in fatty fish such as mackerel (Opstvedt, 1984). While the amount of neutral lipids in fish varies considerably with species and nutritional status, the concentration of phospholipid is not as variable.

Lipids in fish have variable effects on muscle proteins during frozen storage, depending on the type of lipid (triacylglycerol or phospholipid) and state (intact vs. hydrolysed or oxidized). Shenouda (1980) cited evidence that intact neutral lipids, such as triacylglycerols, found in fatty fish seem to protect proteins from denaturation by free fatty acids (FFA), possibly by dissolving the FFA and neutralizing or diluting their capability to bond hydrophobically to proteins. Phospholipids, such as lecithin, also have been demonstrated to have protective effects. There is also evidence that intact lipids can have a detrimental effect on frozen fish tissue. This is thought
to occur when cellular structures, i.e., membranes, are broken down, allowing liberated lipids to come in contact with previously unencountered proteins and form insoluble lipoprotein complexes (Shenouda, 1980).

Free fatty acids (FFA) produced by enzymic and nonenzymic hydrolysis of fish lipids, particularly phospholipids, have been demonstrated to attach hydrophobically or hydrophilically to the appropriate site on protein surfaces. Primarily myofibrilar proteins are attacked. By surrounding proteins with this hydrophobic environment, protein solubility is decreased. A correlation has been documented repeatedly between increased FFA, decreased protein solubility, and increased toughness of frozen fish. The same effects have been demonstrated in model systems treated with antioxidants and in a nitrogen atmosphere, implicating FFA products of lipid hydrolysis rather than oxidative products. Other work has demonstrated different protein aggregates formed as a consequence of interaction with products of lipid hydrolysis and oxidation (Shenouda, 1980).

Lipid oxidation shortens the shelf life of frozen fish not only by the initiation of rancidity, but also by interaction with proteins. Lipid oxidation has been demonstrated to cause frozen stored fish tissue to become firmer and more elastic and to form insoluble complexes. The interactions are not entirely understood, but two possible mechanisms have been hypothesized. One proposed that
denaturation is a consequence of polymerization with unstable free radical intermediates of lipid peroxidation. Another suggested that the mechanism is of covalent bonding with stable oxidation products such as carbonyl compounds (Shenouda, 1980).

TMAO, and its enzymatic breakdown by TMAOase to dimethylamine (DMA) and formaldehyde (FA) during frozen storage, repeatedly has been implicated in textural deterioration of some fishery products. The resulting tough and spongy tissue is reported to be related to formation and accumulation of FA, rather than DMA. Protein alteration is thought to occur through covalent bonding with FA to cause deformation, accompanied by cross linking between protein peptide chains (Shenouda, 1980). Mathews et al. (1980) found increased cross linking to correlate with accumulations of FA, but not FFA.

If TMAO and TMAOase are to be implicated in protein denaturation and texture changes in frozen fishery products, the presence of TMAO and TMAOase activity must be demonstrated. The presence of TMAO in freshwater species is negligible or nonexistent (Shenouda, 1980; Hebard et al., 1982; Regenstein et al., 1982; Hultin, 1985). Furthermore, crustaceans such as lobsters and shrimp apparently lack TMAOase (Castell et al., 1970), even though they contain small amounts of TMAO (Shenouda, 1980). TMAOase has been reported to be stable up to 60°C, while heat treatments of 80°C have arrested its activity (Shenouda, 1980). Based on
all of these facts, it would seem quite unlikely, for example, for FA accumulation to be implicated in textural changes of cooked freshwater crustacean muscle during frozen storage.

Textural changes occurring in frozen fishery products are a major factor in determining quality and shelf life. Depending on the species, as well as pre-freezing handling and processing conditions, any combination of factors involving moisture, lipids, and TMAO could be involved in protein denaturation. In addition, a number of hypothetical interactions also could have detrimental effects on textural qualities (Shenouda, 1980). While these processes have detrimental effects on texture, flavor will also be negatively affected by development of rancidity in the lipid fractions, as well as loss of flavor with increased drip losses due to reduction in water holding capacity of denatured proteins.

**Effects of Freezing Method, Packaging, Frozen Storage, and Thawing on the Quality of Fishery Products**

**Research results**

**Freezing**

Finfish and shellfish frequently have been reported to benefit from quick freezing (Tressler, 1932; Rogers and Binsted, 1972; Hultin, 1985). The muscle tissue of fish is
more sensitive to protein denaturation than is that of birds and mammals (Hultin, 1985). The degree of protein denaturation that occurs during freezing fish is affected by freezing rate (Matsumoto, 1979) and minimized by quick freezing (Hultin, 1985).

Quick freezing, with cryogenic (LN2 or CO2) or immersion (LFF or DDM) processes, has sometimes been shown to produce quality advantages over slower methods, i.e., sharp or still, and blast, when freezing crab (Gangal and Magar, 1963; FMC Corp., 1969; Ampola and Learson, 1971; Strasser et al., 1971; Webb et al., 1976; Cook and Lofton, 1979; Coleman et al., 1986) and rock lobsters (Simmonds et al., 1984). While these workers have found improvements in factors such as sensory quality, appearance, drip, and texture, others have been unable to demonstrate similar advantages when quick freezing several finfish and shellfish (Anonymous, 1965; Bucholz and Pigott, 1972; Aurell et al., 1976; Houwing, 1984). Another advantage attributed to quick freezing is less dehydration during freezing (Bucholz and Pigott, 1972; Aurell et al., 1976; Anonymous, 1982; Sebranek, 1982; Nusbaum et al., 1983).

In addition to direct benefits of quality and yield, quick freezing has been shown to be a factor in increasing high quality life (HQL) of frozen fishery products (Ampola and Learson, 1971; Sebranek, 1982) given proper storage conditions, while slower freezing resulted in poorer sensory quality after frozen storage (USDC, 1970). Lindeløev (1978)
and Winger (1982) have shown that the end temperature of freezing also is an important factor in frozen shelf life, by demonstrating that shorter shelf life is associated with freezing to a temperature less than the storage temperature.

Packaging

Deterioration of frozen fishery products in commercial cold stores and retail cabinets can be minimized by protecting them from oxidation and desiccation. Packaging and/or glazing have been shown to be effective barriers between the product and the atmosphere.

Packing in oxygen impermeable films has been shown to be effective protection, but this requires oxygen removal by vacuum or displacement with an inert gas such as nitrogen (Connell, 1975). As early as 1916, Plank et al. (1916) reported "anaerobic" packaging to be the second most important factor affecting quality of frozen foods. Minimizing exposure to oxygen is particularly important for fatty fish (Jul, 1984).

Vacuum packaging has been shown to improve frozen shelf life (Dassow et al., 1962; FMC Corp., 1969; Strasser et al., 1971; Mijayama and Cobb, 1977; Badonia, 1981; Reddy et al., 1981), while other workers found no advantage to vacuum packaging (Webb et al., 1976).

Desiccation, caused by sublimation of ice crystals during frozen storage (Hultin, 1985), is a problem in frozen fishery products (Faulkner and Watts, 1955; Gangal and
Magar, 1963; Pawar and Magar, 1966; Rogers and Binsted, 1972; Nagle and Finne, 1980; Hallowell, 1980; Shenouda, 1980; Gates et al. 1985). The aforementioned can be reduced by glazing (Pawar and Magar, 1966; Nagle and Finne, 1980). For best results, glazes on frozen products must be periodically replenished as they evaporate or become damaged (Rogers and Binsted, 1972). Glazes of 0.5 - 2 mm, or about 5 - 15% by weight, usually are sufficient to protect individually quick frozen (IQF) or block frozen products. Glazing is inexpensive and effective, but not always practical (Connell, 1975). Mijayama and Cobb (1977) observed little dehydration in vacuum packaged prawns, compared to standard glazed products, as long as the package itself remained undamaged. Vacuum packing can be more practical, but is frequently only applied to smaller more expensive products (Connell, 1975). Keeping quality of seafood has been enhanced by combining glazing with vacuum packing in oxygen impermeable membranes (Jul, 1984).

Frozen storage

Once fishery products are properly frozen and packaged, further deterioration is affected primarily by temperature and duration of frozen storage (Dagbjartsson and Solberg, 1972; Rogers and Binsted, 1972; Connell, 1975; Ronsivalli and Baker, 1981; Jarman, 1982; Jul, 1984; Summers, 1984; Gates et al., 1985; Hultin, 1985). Because fish and shellfish are reported to be more sensitive to the negative
effects of frozen storage than other muscle foods (Rogers and Binsted, 1972; Ronsivalli and Baker, 1981; Hultin, 1985), it is considered especially important that storage temperature be kept low. A temperature of $-18^\circ$C has been suggested as a maximum acceptable storage temperature (Strasser et al., 1971; Hallowell, 1980; Jul, 1984; Hultin, 1985).

Connell (1975) notes that even at $-18^\circ$C, frozen fresh fish will only keep in good condition for 2 to 4 months. Numerous researchers have demonstrated increasing quality and shelf life associated with decreasing storage temperatures, and have advocated temperatures such as $-20^\circ$C (Dassow et al., 1962; Webb et al., 1975; Hale and Waters, 1981; Gates et al., 1985), $-23^\circ$C (Botta et al., 1982), $-25^\circ$C (Houwing, 1984), $-26^\circ$C (Crawford et al., 1979), $-27^\circ$C (Dagbjartsson and Solberg, 1972), $-29^\circ$C (Rogers and Binsted, 1972), $-30^\circ$C (Connell, 1975; Webb et al., 1976; Clucas and Sutcliff, 1981), $-40^\circ$C (Rasekh et al., 1977; Ronsivalli and Baker, 1981), and $-108^\circ$F (Strasser et al., 1971). Hansen and Aagaard (1969) recommended that crab meat not be stored at a temperature above $-20.5^\circ$C for three months, and that this temperature be reduced $2.8^\circ$C for each additional three month storage period needed. The economic benefits obtained from storing fishery products at temperatures below $-18^\circ$C is questionable (Jarman, 1982; Houwing, 1984). Persson (1982) recommended against raising storage temperatures in order to reduce expenses. Relative to total costs, this expense was
usually considerably less than other factors, such as transportation and distribution.

Another aspect of frozen storage is detrimental effects of temperature fluctuations, i.e., moisture migration and ice crystal growth (Connell, 1975; Bevilacqua and Zaritsky, 1982; Jul, 1984). Maintenance of a lower temperature is an effective way to prevent ice crystal growth in frozen products, shown to occur at storage temperatures above approximately -10°C (Moran, 1932; Fennema et al., 1973; Bevilacqua and Zaritsky, 1982). Moran (1932) demonstrated lack of ice crystal growth at -20°C. Jul (1984) recommended frozen storage temperatures of ≤-18°C to avoid reduction in quality due to recrystallization.

**Thawing**

When freezing or thawing fishery products, excessively slow rates enhance the amount of drip produced. Connell (1975) suggested application of the same general temperature change rate for freezing and thawing, i.e., time spent between 0°C and -5°C should not be over 5 to 10 hours. Jul (1984) observed that the effect of thawing on drip is minimal, but pointed out considerable variation between experiments. Several studies were noted that reported little impact of thawing rate on drip. Reay (1933) demonstrated that haddock frozen at -21°C exuded approximately twice as much drip when the time interval from -5°C to 0°C increased from 60 min to 400 min. Tanaka and Tanaka (1956)
demonstrated greatly reduced drip in large pieces of whale meat with dielectric heating rather than with slow thawing in air. On the other hand, just as slow freezing has sometimes been shown to increase tenderness and reduce drip in beef, Calvelo (1981) also found slow thawing to reduce drip in beef.

Evaluation of seafood quality

pH

Measurement of the pH of seafoods has been used as an indicator of spoilage during iced storage. The pH of fish and shrimp increases with iced storage due to production of ammonia and amines by microbial and enzymatic activity (NRC, 1985). The pH of molluscs decreases, due to acids produced from utilization of stored glycogen. The initial microbial flora, handling and processing, and packaging methods will influence pH changes, but physical factors are still needed to assess quality. The pH of cooked crustacean products such as crabmeat (Webb, 1976) has been shown to be of minimal value as an indicator of quality. Measurement of trimethylyamine (TMA) and total volatile nitrogen (TVN), the production of which increases pH, has been used to assess quality of marine fish and crustaceans. Guidelines and specifications for acceptance of seafoods in several countries have included TMA and TVN levels (NRC, 1985).

Researchers have demonstrated a correlation between pH
and fish texture (Cowie and Little, 1966; Kelley et al., 1966; Connell and Howgate, 1968; Love et al., 1974; Dunajski, 1979). This effect has been shown to be more consistent within a species (Feinstein and Buck, 1984), and more pronounced below pH 6.7 (Dunajski, 1979). Considering the combined toughening effects of freezing and a lower pH on fish muscle tissue, it has been suggested that fish of a "favorable" pH (i.e., higher) be used for frozen storage (Bosund and Beckeman, 1972; Love et al., 1974).

A number of researchers have monitored the pH of fishery products during frozen storage to evaluate its relationship to quality deterioration. Often, little or no pH change has been observed in muscle tissue as related to storage time or temperature of fish (Bosund and Beckeman, 1972), shrimp (Webb, et al., 1975), crab (Webb et al., 1976), prawns (Hale and Waters, 1981), or scallops (Maxwell-Miller et al., 1982). Gangal and Magar (1963) found a marked increase in the pH of thaw exudate from crabmeat during frozen storage. This rise was attributed to increased concentrations of nitrogenous compounds, which were of negligible value as a specific quality indicator, due to inconsistent levels.

Methods used to evaluate tissue pH vary with the fishery commodity being evaluated as well as with available equipment. Slurries of 1:1 tissue:water have been used for fish (Bosund and Beckeman, 1972), crabmeat (Webb et al., 1976), and shrimp (Webb et al., 1975). Feinstein and Buck
(1984) used 3g of fish to 30 ml of water, while others used lower or unspecified amounts (Wilaichon et al., 1978; Maxwell-Miller et al., 1982; Wang and Brown, 1983). Direct contact with a probe has been used to measure the pH of minced fish (Rasekh et al., 1977) and macerated prawns. Nip et al., (1985b) used a surface pH electrode (Orion) to measure surface pH of prawns, when monitoring pH changes related to muscle degradation.

**Drip**

The measurement of thaw drip is a well established indicator of quality of frozen seafoods (Bucholz and Pigott, 1972; Gibbard, 1978; Shenouda, 1980; Jarman, 1982; Jul, 1984; Hultin, 1985). It is quite useful as a simple, well correlated, indicator of protein denaturation, as affected by freezing and frozen storage (Shenouda, 1980; Jul, 1984; Hultin, 1985). Protein denaturation has been associated with undesirable texture changes in many frozen fishery products (Connell, 1975). Drip also has been associated with loss of nutrients from muscle tissue and development of off odors and off flavors (Gangal and Magar, 1963).

Thawing rate has been observed to affect the amount of thaw drip produced (Reay, 1933; Tanaka and Tanaka, 1956; Connell, 1975; Jul, 1984). Consequently, consistent thawing methods are important if meaningful results are to be obtained. Jul (1984) suggested that considerable variation in reported effects of freezing method and frozen storage on
thaw drip in products could be explained by variations in thawing. A single optimal method for thawing all fishery products has not been suggested or used.

A variety of procedures have been used in studies on freezing methods and frozen storage. Thawing has been done at room temperature (Gangal and Magar, 1963; Pawar and Magar, 1966; Ampola and Learson, 1971; Bucholz and Pigott, 1972), under refrigeration (Dagbjartsson and Solberg, 1972; Webb et al., 1975; Webb et al., 1976; Maxwell-Miller et al., 1982; Samson et al., 1983), and in 16°C water (Aurell et al., 1976) or 80°C water (Faulkner and Watts, 1955). Unfortunately, many authors do not indicate thawing methods used.

Thaw drip has been calculated by measuring weight loss of the thawed muscle tissue (Bucholz and Pigott, 1972; Aurell et al., 1976; Webb et al., 1976;), or by collecting and weighing the drip as the product thawed (Gangal and Magar, 1963; Pawar and Magar, 1966). Bucholz and Pigott (1972) commented on the importance of wrapping the product when thawing at room temperature to prevent condensation of moisture from the atmosphere onto the product surface in order to prevent erroneous results.

Cooking drip, rather than thaw drip, also has been evaluated as a quality index of frozen seafood (Rasekh et al., 1977; Borderias et al., 1981).
Moisture

Moistness, or succulence, is an important sensory quality in seafood (Connell, 1975). Desiccation during frozen storage and thaw drip may have the effect of depleting muscle tissue of moisture. While analysis of drip loss is a useful method of monitoring changes in tissue and texture (Shenouda, 1980), change in moisture content often reflects and coincides with quality deterioration.

Objective measurements of moisture content are useful as they provide a consistent determination of this important parameter. Moisture content is calculated by weight difference after the product has been dried. AOAC methods were used by Pawar and Magar (1966), Peplow et al. (1977), and Findlay and Stanley (1984) for drying finfish, shrimp, and scallops, respectively. Peplow et al. (1977) described the AOAC method as "overnight" drying at 100°C of a mixture of 10g ground tissue, 2g asbestos fibers, and 5ml water. Ablett and Gould (1986) dried ground mussels for 24 hours at 105°C. Some researchers do not describe the objective methods they used to determine moisture content.

Sensory evaluation is considered to be the most important determinant of quality in seafood (Shenouda, 1980), and has been used to monitor moisture content in studies of iced or frozen stored fishery products. A sensory panel was used to evaluate moisture in fish (Johnson et al., 1981) and in prawns (Mijayama and Cobb, 1977). Visual observations of desiccation have been used to assess
moisture changes in crabmeat (Gangal and Magar, 1963).

Sensory assessments

The importance of subjective evaluation of seafood quality should not be underestimated. Improved methods of processing, packaging, and storage may yield measurable improvements in quality and/or shelf life. However, if differences are not detectable by sensory methods, extra efforts and cost may not be justified for the processor.

Objective measurement of sensory characteristics, or related factors, can provide a consistent point of reference for comparison. Consequently, considerable effort has been made to determine physical and chemical methods of quality evaluation to be correlated with sensory data. Chemical quality measures have been utilized, but their usefulness as indices of sensory quality has been questioned (Mijayama and Cobb, 1977; Nakayama and Yamamoto, 1977; Hale and Waters, 1981; Botta et al., 1982). Physical measurements of quality characteristics, such as texture, often have been used, but they are limited in scope. Human perception of texture, for example, encompasses a great number of influencing factors (Connell, 1975), whereas most instrumental methods of texture evaluation involve three or fewer characteristics (Voissey, 1972).

The consistency of sensory evaluation is limited by the variability and fatigue inherent in use of human subjects (Dassow et al., 1962; Connell, 1975; Jul, 1984).
Nevertheless, sensory evaluations are considered to be the ultimate criteria for determining differences in seafood quality (Shenouda, 1980; Houwing, 1984).

The nine point hedonic scale is the most commonly used form of preference testing (Larmond, 1977). However, it should be limited to evaluations of liking or disliking. A nine point multiple comparisons test is often used when the subject of evaluation is the effect of processing, packaging, or storage on a product. The test allows efficient comparison of several test samples with a reference/control, and enables detection of small differences (Larmond, 1977). Verbally ranked ratings are converted to numbers for the purpose of statistical analysis.

Many seafood researchers have used multiple comparison tests to detect differences in quality parameters, e.g., texture, flavor, color, aroma, moisture, and overall acceptability, of seafoods frozen in different ways and/or during frozen storage. Nine point scales have often been used (Ampola and Learson, 1971; Ahmed et al., 1973; Alvarez and Koburger, 1979; Crawford et al., 1979; Nip and Moy, 1979; Hales and Waters, 1981; Houwing, 1984). Others have modified the point scale to five (Rasekh et al., 1977; Botta et al., 1982; Maxwell-Miller et al., 1982; Gates et al., 1985), seven (Ablett and Gould, 1986), or ten (Faulkner and Watts, 1955; Gangal and Magar, 1963).

Interval scales, such as the nine point hedonic scale,
may bias sensory evaluation by assuming equal intervals on the rating scale (Stone and Sidel, 1985). This bias has been avoided by using unstructured or minimally structured lines to evaluate differences in seafoods (Nakayama and Yamamoto, 1977; Gill et al., 1979; and Wang and Brown, 1983). This method typically uses a horizontal line marked with verbal anchors of low and high intensity characteristics. Subjects place a vertical line across the horizontal line at the position that best reflects the intensity of the characteristic being evaluated.

References used for the control/comparison varied, due to seasonal changes in availability and quality of the species being evaluated. Frequently, the same species, processed and stored under optimum conditions, e.g., headed shrimp stored at $-40^\circ$C, was used as a reference (Hale and Waters, 1981). In other situations, fresh samples of the same or similar species have been used (Mijayama and Cobb, 1977), as have "hidden blanks" of a treatment being evaluated (Maxwell-Miller et al., 1982). Lacking a reference fish muscle that would be a consistent texture reference throughout their study, Gill et al. (1979) used rehydrated soy protein as a reference, designated as 7.5 on a 10cm horizontal line.

Descriptive profiling (Amerine et al., 1965; Larmond, 1977; Stone and Sidel, 1985) often has been used to evaluate multiple flavor and odor notes, textural characteristics, and assess differences in acceptability between species,
processing methods or over time (Friedman et al., 1963; Webb et al., 1975; Edmunds and Lillard, 1979; Weddle, 1980; Johnson et al., 1981; Samson et al., 1985).

Triangle tests (Larmond, 1977) have been used to evaluate differences in flavor, texture, appearance, and palatability (Cook and Lofton, 1979). Dagbjartsson and Solberg (1972) used the "chew count" method with a trained texture panel to evaluate differences in texture of lobster. This method involves rating texture by the number of "chews" required to masticate a sample.

Texture evaluation

Texture is one of the most important quality characteristics of muscle tissue. Consequently, considerable research has been done on factors that influence texture, particularly those causing mushiness or toughness. Methods of evaluating texture also have received considerable attention.

Evaluation of the texture of fishery products most commonly includes both instrumental and sensory approaches. It is desirable to have good objective methods that can closely duplicate human perception of texture yet not be subject to the fatigue and variability inherent in sensory evaluation. There is, however, no substitute for sensory evaluation (Dassow et al., 1962). Several researchers have attempted to correlate various aspects of instrumental measurements, e.g., shear slopes and peaks, with different
sensory characteristics such as chewiness, fibrousness, hardness, toughness, compression, and cohesiveness (Dassow et al., 1962; Friedman et al., 1963; Bosund and Beckeman, 1972; Voisey, 1972; Johnson et al., 1981). When evaluating the importance of small but significant quality changes determined by a well-trained sensory panel, it should be stressed that consumers would not be likely to be as sensitive to such changes (Houwing, 1984).

Studies on sensory analysis of texture have most commonly used point scales to rank textural parameters of mussels (Ablett and Gould, 1979), scallops (Maxwell-Miller et al., 1982), crabmeat (Gangal and Magar, 1963; Ampola and Learson, 1971; Webb et al., 1976), shrimp (Faulkner and Watts, 1955; Ahmed et al., 1973; Gates et al., 1985), prawns (Mijayama and Cobb, 1977; Nip and Moy, 1979; Hale and Waters, 1981), crayfish (Wang and Brown, 1983), fish (Botta et al., 1982; Houwing, 1984) and minced fish (Rasekh et al., 1977; Laird et al., 1981). Texture profile panels were used to evaluate texture of shrimp (Webb et al., 1975), fish (Johnson et al., 1981), squid (Otwell and Hamann, 1979), and various products (Friedman et al., 1963). Dagbjartsson and Solberg (1972) used "chew counts" to evaluate the texture of lobster. Gill et al., (1979) used the unstructured line method of rating texture of fish.

The most common instrumental measurement of muscle tissue is shear force. This is considered by many to be the most appropriate measurement of intact seafood muscles,
since it is a measure of myofibril strength (Findlay and Stanley, 1984), and not connective tissue. The latter makes up a relatively small portion of the tissue, and is readily gelatinized by cooking (Love et al., 1974). Force required to break through tissue (peak shear force) is evaluated. This measurement has been found to be highly correlated with sensory panel evaluations of toughness (Bosund and Beckeman, 1972), hardness (Borderías et al., 1981; Johnson et al., 1981), and chewiness and fibrousness (Johnson et al., 1981).

Single or multi-blade devices, wires, and punch and die equipment have been used to measure shear force of shrimp (Ahmed et al., 1973; Webb et al., 1975; Wilaichon et al., 1978), prawns (Nip and Moy, 1979; Hale and Waters, 1981; Nip et al., 1985b), lobster (Dagbjartsson and Solberg, 1972), scallops (Maxwell-Miller et al., 1982; Findlay and Stanley, 1984), squid (Otwell and Hamann, 1979), fish (Bosund and Beckeman, 1972; Voisey, 1972; Gill et al., 1979; Borderías et al., 1981; Johnson et al., 1981; Houwing, 1984;), minced fish (Rasekh et al., 1977; Borderías et al., 1981), and various products (Dassow et al., 1962; Friedman et al., 1963).

Force required for 50% compression was used to evaluate the texture of fish and scallops (Feinstein and Buck, 1984), and minced fish (Nakayama and Yamamoto, 1977; Borderías et al., 1981). Samson et al. (1985) monitored the relaxation time following a 50% compression of minced fish.
January 25, 1988

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The effect of blanch time on the development of mushiness in ice-stored crawfish meat packed with adhering hepatopancreas

ABSTRACT

Loss of a desirable firm texture in fresh crawfish meat packed with adhering hepatopancreas was influenced by blanch time. Analysis of texture by Instron Kramer shear force and sensory panel showed that after 20 hours of iced storage, crawfish meat cooked < 7 minutes was significantly (p<0.01) softer than crawfish meat cooked 7 to 13 minutes. Hepatopancreas tissue obtained from crawfish cooked less than seven minutes prevented firm gelation of a 12% aqueous gelatin solution. Results indicated that heat labile proteolytic enzymes were involved in the development of mushiness in fresh crawfish meat.

INTRODUCTION

Processing freshwater crawfish, Procamburus clarkii and P. acutus acutus, for meat is an important developing seafood industry. In Louisiana, which produces 90% of the crawfish consumed in the U.S., processing typically involves blanching whole live crawfish in boiling water to enhance peeling of meat by hand. Meat for fresh sale is usually packed with adhering hepatopancreas, an important flavor ingredient in many prepared dishes. In recent years,
development of mushiness in such fresh meat products has been a sporadic and unpredictable problem.

The presence of hepatopancreas tissue in other crustaceans has been implicated in similar textural problems (Papadopoulos and Finne, 1985; Lightner, 1973). Collagenolytic enzymes from crustacean hepatopancreas tissue, partially characterized by Nip et al. (1985), were found to degrade tail meat and collagen gels (Eisen and Jeffrey, 1969; Baranowski et al., 1984). The presence of similar enzymes in crawfish hepatopancreas could be expected to degrade the texture of crawfish meat as well as gelatin (amorphous collagen). Presently there are no established crawfish processing procedures, therefore blanching times vary considerably. If hepatopancreatic enzymes are not inactivated by blanching, the presence of this tissue in packages of fresh crawfish meat could cause mushiness.

The objectives of this work were to evaluate the effect of blanch time on the texture of ice-stored crawfish meat packed with adhering hepatopancreas, and the use of gelatin degradation as a simple in-plant test for adequacy of blanch process.

MATERIALS AND METHODS

The entire study was performed in triplicate. Each time, 12 kg live crawfish, P. clarkii, were obtained from a local seafood market. Using procedures simulating a typical processor, washed crawfish were placed in boiling water and
heat was adjusted to achieve a return to boil in approximately 7 minutes. Samples of whole crawfish (1.1 - 1.4 kg) for all analyses were removed every two minutes, beginning one minute after crawfish were added to the water and ending at 13 minutes. Blanched crawfish were chilled 2 minutes in an ice-water slush. An uncooked portion of crawfish was retained as a control.

Tail meat with adhering hepatopancreas was peeled and deveined by hand and placed in Whirl-pak bags. Hepatopancreas tissue did not adhere to raw meat, therefore none was packed with it. Packaged meat was left at room temperature (23°C-25°C) for 1 hour, as would typically occur in a commercial operation, prior to being stored in crushed ice for 20 hours.

Crawfish meat texture was determined instrumentally using an Instron Universal Testing Machine (Model 1122) fitted with a 10-blade Kramer food testing attachment. Prior to analysis, the meat was brought to room temperature. Five replicate samples of meat (approximately 30 g) from each blanch time and the control were tested in a completely randomized block design. The number of tails in each sample was noted to determine their average weight.

Sensory evaluation of crawfish meat texture was by a nine-member panel with previous experience in sensory work with seafoods, including crawfish. A multiple comparisons test, as described by Larmond (1977), was used to evaluate samples in a completely randomized block design. Duplicate
sets of samples of 20 hour ice-stored meat from crawfish blanched 1, 3, 7, 11, or 13 minutes were compared to the reference meat which was blanched 7 minutes. Prior to serving, samples were steamed five minutes and cooled to room temperature (23°-25°C). Panelists were served in partitioned booths under red lights, and received approximately 6 g samples of each treatment.

Gelatin degradation by hepatopancreas tissue was monitored in tissue samples removed from crawfish sampled at each blanch time and the control. For each treatment, approximately 5 g of tissue was pooled from several crawfish. The tissue was macerated and four 0.2 g samples were placed in 20 mm pyrex tubes, to which 5.0 ml of a cooled 12% aqueous gelatin solution was added and mixed. Gelatin solution mixed with raw or no hepatopancreas tissue served as viscosity references. After capping the tubes, samples were left at room temperature (23°-25°C) 1 hour, then stored an additional 23 hours at 3°C. Viscosities of the gelatin-hepatopancreas mixtures were evaluated subjectively after 24 hours.

Statistical analysis of Instron and sensory data was by analysis of variance, using a split plot with a randomized block on the main plot (Snedecor and Cochran, 1967). Tukey's studentized range test (Ott, 1977) was used to analyze critical differences between means.
RESULTS AND DISCUSSION

Cooking time of whole crawfish significantly affected the texture of ice-stored peeled tail meat, packed with adhering hepatopancreas. Statistical analysis of Instron shear force values showed highly significant (p<0.01) differences in the texture of crawfish meat blanched for different times (Table 1). Meat from crawfish cooked one or three minutes was significantly (p<0.01) softer than that cooked five minutes, or uncooked (to which no hepatopancreas adhered). Meat blanched seven minutes or more was significantly (p<0.01) firmer than that blanched less. Significant differences in meat texture (p<0.01) between replicate studies was attributed to differences in average tail weights (Table 1). Statistical analysis by ANOVA of sensory data showed highly significant (p<0.01) differences in texture scores between treatments. Analysis of treatment means by Tukey's test showed meat from crawfish cooked one or three minutes was judged to be significantly (p<0.01) softer than that cooked 7, 11, or 13 minutes (Table 2).

Whole crawfish that produced the firmest meat samples also yielded hepatopancreas samples without apparent proteolytic enzyme activity, based on gelatin degradation. No gel formation occurred in hepatopancreas-gelatin mixtures prepared with tissue from crawfish blanched three minutes or less, while preparations from those blanched five minutes resulted in soft gels. Only samples removed from crawfish blanched seven or more minutes resulted in firm gelatin
mixtures after 24 hours. Thus, gelatin seemed to be a suitable substrate for monitoring activity of enzymes thought to cause mushiness in crawfish meat.

In conclusion, this study demonstrated that undercooking of whole crawfish can result in mushiness of peeled meat packed with adhering hepatopancreas, and that gelatin degradation could be used by processors to conduct a simple in-plant test of the adequacy of their heat process.
Table 1. Shear force values (kg/g) of 20-hour ice-stored crawfish meat packed with adhering hepatopancreas.

<table>
<thead>
<tr>
<th>Blanch time (min.)</th>
<th>A&lt;sup&gt;a&lt;/sup&gt;</th>
<th>B</th>
<th>C</th>
<th>X&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.81 ± 0.07</td>
<td>0.87 ± 0.04</td>
<td>1.12 ± 0.05</td>
<td>1.27&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>1</td>
<td>1.65 ± 0.07</td>
<td>0.73 ± 0.07</td>
<td>0.98 ± 0.09</td>
<td>1.12&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>1.44 ± 0.16</td>
<td>0.77 ± 0.09</td>
<td>0.92 ± 0.08</td>
<td>1.04&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>2.02 ± 0.14</td>
<td>1.19 ± 0.06</td>
<td>1.21 ± 0.03</td>
<td>1.47&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>7</td>
<td>2.17 ± 0.12</td>
<td>1.44 ± 0.18</td>
<td>1.46 ± 0.03</td>
<td>1.69&lt;sup&gt;de&lt;/sup&gt;</td>
</tr>
<tr>
<td>9</td>
<td>2.17 ± 0.13</td>
<td>1.33 ± 0.08</td>
<td>1.48 ± 0.08</td>
<td>1.66&lt;sup&gt;de&lt;/sup&gt;</td>
</tr>
<tr>
<td>11</td>
<td>2.15 ± 0.12</td>
<td>1.34 ± 0.05</td>
<td>1.54 ± 0.03</td>
<td>1.68&lt;sup&gt;de&lt;/sup&gt;</td>
</tr>
<tr>
<td>13</td>
<td>2.05 ± 0.04</td>
<td>1.44 ± 0.07</td>
<td>1.56 ± 0.08</td>
<td>1.68&lt;sup&gt;de&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mean ± S.D. of five replications; average tail weights for replicate studies A, B, and C are 6.8 g, 2.5 g, and 3.0 g, respectively.

<sup>b</sup> Mean value for blanch time. Means with different superscripts are significantly different (p<0.05).
Table 2. Mean texture scores\textsuperscript{a} from sensory evaluation of 20-hr ice-stored crawfish meat packed with adhering hepatopancreas.

<table>
<thead>
<tr>
<th>Blanch time (min)</th>
<th>Column A\textsuperscript{b}</th>
<th>Column B\textsuperscript{b}</th>
<th>Column C\textsuperscript{b}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1\textsuperscript{c}</td>
<td>2\textsuperscript{c}</td>
<td>1\textsuperscript{c}</td>
</tr>
<tr>
<td>1</td>
<td>1.22\textsuperscript{d}</td>
<td>1.00\textsuperscript{d}</td>
<td>2.22\textsuperscript{d}</td>
</tr>
<tr>
<td>3</td>
<td>2.44\textsuperscript{e}</td>
<td>2.00\textsuperscript{e}</td>
<td>2.20\textsuperscript{e}</td>
</tr>
<tr>
<td>7</td>
<td>5.67\textsuperscript{e}</td>
<td>5.67\textsuperscript{e}</td>
<td>5.56\textsuperscript{e}</td>
</tr>
<tr>
<td>11</td>
<td>4.67\textsuperscript{e}</td>
<td>5.56\textsuperscript{e}</td>
<td>5.00\textsuperscript{e}</td>
</tr>
<tr>
<td>13</td>
<td>5.33\textsuperscript{e}</td>
<td>4.78\textsuperscript{e}</td>
<td>5.00\textsuperscript{e}</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Scores based on a nine point verbally ranked scale, where 1 = extremely softer and 9 = extremely firmer.
\textsuperscript{b} Columns 1 and 2 represent duplicate sets of panel evaluations; values are means of nine evaluations.
\textsuperscript{c} Means in the same column with different superscripts are significantly different (p<0.05).
Differences in color, texture and flavor of processed meat from red swamp crawfish \((\text{Procambarus clarkii})\) and white river crawfish \((\text{P. acutus acutus})\)

**ABSTRACT**

Red swamp crawfish \((\text{Procambarus clarkii})\) and white river crawfish \((\text{P. acutus acutus})\) were evaluated for differences in texture, color and flavor of meat and hepatopancreas. A significant difference between species was not detected by sensory evaluation using triangle tests. Analysis of Instron Kramer shear force values showed no significant difference in meat texture. Color analysis of meat and hepatopancreas by Hunterlab colorimeter showed highly significant differences \((p<0.0005)\) in L-values and a-values between the species.

**INTRODUCTION**

Freshwater crawfish processing has become a commercially important part of the U.S. seafood industry. In Louisiana, where 90% of the crawfish consumed in the U.S. is produced, two commercially important species, red swamp crawfish \((\text{Procambarus clarkii})\) and white river crawfish \((\text{P. acutus acutus})\), are harvested simultaneously in varying proportions. A popular preference for red swamp crawfish is maintained by many processors and consumers. Although
easily recognizable exterior physical characteristics distinguish the two species, sensory differences of processed tail meat have not been determined. The purpose of this study was to evaluate differences in color, texture and flavor of processed meat from these two species of crawfish.

MATERIALS AND METHODS

Source and cooking of crawfish

Live red swamp and white river crawfish were obtained from a local seafood market on three occasions. The two species of crawfish were cooked separately in boiling water using methods described by Marshall et al. (1987). Crawfish for instrumental analysis were removed after seven minutes, when the water began to boil, typical of commercial blanch procedures. Crawfish for sensory evaluation were boiled an additional six minutes to achieve a full cook.

Preparation of hepatopancreas and meat samples

As crawfish meat was peeled, hepatopancreas tissue representative of the color range naturally present in each species was set aside. Eight samples of hepatopancreas tissue from several crawfish were macerated. Samples were placed in petri dishes for color analysis, then covered with lids and stored in plastic bags at 3°C until final analysis, 20 hours later.
Deveined crawfish meat was washed of adhering hepatopancreas before analysis of its color and texture. Four samples of meat from each species were pureed with deionized water (1:1) for color analysis. Crawfish meat for sensory evaluation was deveined, and adhering hepatopancreas that could affect sensory characteristics was washed from half of the meat of each species. Individual tails within each category (i.e. washed or unwashed from each species) were cut into three or four pieces, then mixed to provide uniform samples for panelists.

Sample analysis

Texture of crawfish meat was determined using an Instron Universal Testing Machine (Model 1122) fitted with the 10-blade Kramer food testing attachment, using procedures described by Marshall et al. (1987). For each lot, five samples of meat from each species were run in a randomized block design. Peak values were used to determine kg/g shear force for each sample. Statistical analysis for differences due to species, lot or order of sample evaluation, was by analysis of variance, as described by Snedecor and Cochran (1967).

Sensory evaluation was by a nine member panel of Food Science Dept. faculty, staff, and students experienced in sensory work with crawfish meat. Triangle tests, as described by Larmond (1977), were used to determine differences between species. Approximately 6g samples of
crawfish meat at room temperature were placed in small paper cups, labeled with 3-digit codes, and served on paper plates. A random numbers table was used to determine panelist's order of sample evaluation, and type of odd sample in each set. Panelists were asked to pick the odd sample and specify any distinguishing characteristics based on color, texture, or flavor. On each occasion, panelists evaluated four sets of washed meat. The first two triangles were presented under red light and the second two under white light. This was followed by unwashed meat, presented in a like manner. Larmond's (1977) tables for rapid analysis of triangle data were used to analyze data.

Color of prepared meat and hepatopancreas samples was measured with a Hunterlab Tristimulus Colorimeter (Model D25M-9), using the "Lab" scale. Four measurements were made on a two-inch circular area of each sample, using methods described by Himmelbloom et al. (1983). Statistical analysis for color differences originating from species, lot, time and species x time, was by analysis of variance, as described by Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

Mean Instron shear force values for red swamp and white river crawfish were 1.56kg/g and 1.60kg/g, respectively. Statistical analysis showed no significant difference (p>0.05) between species.
Analysis of sensory panel data showed panelists could not detect a significant difference (p>0.05) in color, texture, or flavor between species when samples were evaluated under red or white light. Differences in color, which were more obvious under white light, were apparently not sufficiently great or consistent enough as to significantly affect correct identification of odd samples.

Statistical analysis of Hunterlab color values (Tables 1 and 2) showed highly significant differences (p<0.0005) in L-values and a-values of meat from the two species. Lower mean L-values and higher mean a-values indicate that meat from red swamp crawfish is less white and more red, respectively, than meat from white river crawfish. There was also a highly significant difference (p<0.0005) in the a-values of hepatopancreas samples from the two species. Lower a-values of hepatopancreas from white river crawfish indicates a greener color. After 20 hr storage at 3°C. L-, a-, and b-values indicated little or no color change in the hepatopancreas tissue of the red swamp crawfish, whereas tissue from the white river crawfish became more greenish/blueish, as indicated by lower a-values and b-values.

Processed crawfish meat is frequently packaged with adhering hepatopancreas tissue, particularly in products intended for fresh sale. Results from this study indicate that, of parameters evaluated, the only significant sensory difference in processed meat from red swamp and white river
crawfish is the color. This could be a source of preference for red swamp crawfish, which has a more reddish color in the meat and hepatopancreas. Considering the less desirable greenish color of the hepatopancreas of white river crawfish, it may be desirable for processors to avoid dominance of this species when packaging meat.
Table 1. Hunter "Lab" values of meat from red swamp crawfish (P. clarkii) and white river crawfish (P. acutus acutus).

<table>
<thead>
<tr>
<th>Species</th>
<th>Meat</th>
<th>0 hours</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L</td>
<td>a</td>
<td>b</td>
<td></td>
</tr>
<tr>
<td>Red swamp</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean&lt;sup&gt;a&lt;/sup&gt;</td>
<td>64.70**</td>
<td>11.53**</td>
<td>9.75</td>
<td></td>
</tr>
<tr>
<td>range&lt;sup&gt;b&lt;/sup&gt;</td>
<td>63.98-65.46</td>
<td>10.06-12.34</td>
<td>8.79-10.52</td>
<td></td>
</tr>
<tr>
<td>Wh. river</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean&lt;sup&gt;a&lt;/sup&gt;</td>
<td>67.62**</td>
<td>8.77**</td>
<td>9.42</td>
<td></td>
</tr>
<tr>
<td>range&lt;sup&gt;b&lt;/sup&gt;</td>
<td>65.64-68.93</td>
<td>7.09-9.73</td>
<td>8.50-10.47</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Each value is a mean of 32 readings on 8 samples.

<sup>b</sup> Each value is a mean of 4 readings on 1 sample.

** Value is significantly different (p<0.0005) by ANOVA from corresponding value of other species. (F<sub>0.0005</sub> = 44.91**, F<sub>L</sub> = 21.1; F<sub>a</sub> = 41.96**, F<sub>b</sub> = 1.63).
Table 2. Hunter "Lab" values of hepatopancreas from red swamp crawfish (*P. clarkii*) and white river crawfish (*P. acutus acutus*), freshly processed and after 20 hours of storage at 3°C.

<table>
<thead>
<tr>
<th>Species</th>
<th>0 hours</th>
<th>20 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L a</td>
<td>b</td>
</tr>
<tr>
<td>Red swamp</td>
<td>41.34</td>
<td>7.21**</td>
</tr>
<tr>
<td>mean^a^</td>
<td>41.34</td>
<td>7.21**</td>
</tr>
<tr>
<td>Wh. river</td>
<td>42.94</td>
<td>2.22**</td>
</tr>
<tr>
<td>mean^a^</td>
<td>42.94</td>
<td>2.22**</td>
</tr>
</tbody>
</table>

^a^ Each value is a mean of 96 readings on 24 samples.

^b^ Each value is a mean of 4 readings on 1 sample.

** Value is significantly different (p<0.01) from corresponding value of other species. (F\_0.05 = 3.95; F\_0.0005 = 13.1; F\_species = 3.56; F\_time = 0.19; F\_species = 145.54**; F\_a time = 2.07; F\_b species = 4.98; F\_b time = 0.65).
Microbiological quality of frozen freshwater crawfish (Procambarus sp.) meat: Effect of hepatopancreas tissue, freezing method, and frozen storage at -23°C

ABSTRACT

Commercially processed crawfish meat was frozen by one of two conventional or two cryogenic methods prior to 72 wks of frozen storage at -23°C. Meat packaged with adhering hepatopancreas had significantly (p < 0.0001) higher aerobic plate counts (APC's) at 7°C and 35°C, and total coliform (p<0.01) numbers (MPN). Only APC 7°C was significantly (p<0.05) affected by freezing method. Time of frozen storage significantly affected APC 7°C (p<0.0001) and 35°C (p<0.05), and total coliform numbers (p<0.01). The number of samples positive for total coliforms, fecal coliforms, and Escherichia coli declined throughout storage. There was no significant effect by any of these factors on E. coli numbers, which had a very high coefficient of variation.

INTRODUCTION

Aerobic plate counts (psychrotrophic and mesophilic), coliform, and Escherichia coli levels in fresh seafood products are useful indicators of hygienic practices employed during processing, and temperature conditions during storage. These same indicators can be used in predicting the shelf life potential of the product.

Psychrotrophic bacteria become dominant in seafoods as
spoilage proceeds at chill temperatures (Liston, 1982; Hobbs, 1983). Mesophilic bacteria include several recognized foodborne pathogens that can contribute to plate counts (ICMSF, 1978), thus, these organisms are useful indicators of the utility and safety of seafood products.

E. coli is indigenous to the enteric tract of man and warm-blooded animals. Its presence in food is consequently considered to be an indicator of the possible presence of enteric pathogens through direct or indirect fecal contamination. The detection of fecal coliforms in food is a more rapid method of determining probable fecal contamination (ICMSF, 1978).

Susceptibility to injury and death during freezing, frozen storage, and thawing varies with the microorganism (ICMSF, 1980a). When quick freezing at -70°C, Haines (1938) observed mortality rates ranging from 80% in some bacteria, to none in spores. Temperature (i.e., -70°C to -5°C) and rate of freezing were found to have little effect on survival within a species. Destruction of bacteria was reported to be most rapid in the range of -1°C to -5°C. Higher mortality rates (1 or 2% up to 90%) have been reported immediately after freezing, followed by a slower die-off in frozen storage (Simmonds and Lamprecht, 1985). Higher temperatures in the range of -2°C to -10°C have been reported more lethal than those below -20°C (Haines, 1938; Christophersen, 1968; Jay, 1978). Slow thawing of frozen foods has been found to be significantly more destructive to microbial cells than
rapid thawing (Speck and Ray, 1977; Gebre-Egziabher et al., 1982). Connell (1975) suggested that time spent between 
\(-5^\circ C\) and \(0^\circ C\) should not exceed 5 to 10 hours when thawing.

Differential sensitivity to effects of freezing could alter the distribution of viable organisms in a thawed product and may not be indicative of original product quality. Greenwood et al. (1985) observed that freezing cooked crustaceans may result in a 10-fold decrease in the APC. Chattopadhyay et al. (1983) found decreased bacterial survival in prawns when frozen with liquid nitrogen \((1N_2)\), as compared to plate freezers. Aurell et al. (1976) observed mesophilic bacterial counts \((37^\circ C)\) in shrimp to be reduced by about 40% when frozen by means of a plate freezer, air blast tunnel, or liquid freon freezant \((LFF)\) process. Psychrotrophic bacterial counts \((22^\circ C)\) were reduced by approximately 50% when using the plate freezer or air blast tunnel, but remained practically unchanged when using the LFF-process.

Gram-negative organisms, such as \(E.\ coli\), are more sensitive to freezing than gram-positive organisms (ICMSF, 1980a; Simmonds and Lamprecht, 1985). While coliforms have been reported to survive long periods in frozen storage (Lamprecht and Elliot, 1971), Varga and Doucet (1984) observed a 75% reduction in the number of fecal coliforms after freezing fresh fish at \(-30^\circ C\) for ca. 24 hr. Some foods, however, have been shown to provide a level of protection from the harmful effects of freezing to enteric
pathogens and certain gram-negative organisms (ICMSF, 1980a). The absence of detectable \textit{E. coli} does not ensure the absence of enteric pathogens in a product (Mossel, 1967; Silliker and Gabis, 1976; ICMSF, 1978), nor does the presence of non-\textit{E. coli} fecal coliforms necessarily indicate the presence of fecal contamination where \textit{E. coli} have not survived frozen storage (Splittstoesser et al., 1982).

The objective of this study was to determine the effects of hepatopancreas tissue, freezing method, and $-23^\circ C$ frozen storage on the numbers of indicator microorganisms in freshwater crawfish meat.

**MATERIALS AND METHODS**

**Product preparation and storage**

Three lots of freshwater crawfish (\textit{Procambarus clarkii} and \textit{P. acutus acutus}) meat were obtained from a commercial processor at two week intervals during May and June. Half of the meat in each lot was washed (W) in water to remove adhering hepatopancreas tissue that was left adhering to the unwashed (U) meat (a common market form, frequently preferred for additional flavor). Peeled and deveined meat was packed 2.27 kg/bag, chilled in an ice-water slush, and packed in ice for transportation.

At the Food Science Department, meat was packed in Koch laminated nylon/polyethylene bags (approximately 225 g/bag), nitrogen flushed and vacuum packed. Individually quick frozen (IQF) meat was packaged in the same manner.
immediately after freezing. Meat was kept packed in ice until frozen by one of the following methods: conventional freezing by 1) still ("sharp") freezing at -23°C, or 2) blast freezing at -40°C; or cryogenic freezing with 1N₂ in a chamber freezer (Air Products Cryo-Test Chamber, Model CT-1818-12F), by 3) freezing packaged meat at -101.1°C (-150°F) or 4) freezing individual tails at -59.4°C (-75°F).

Frozen meat was stored at -23°C for 72 weeks.

Sampling

In order to establish a base number of microorganisms, each lot of processed crawfish meat, with or without hepatopancreas, was sampled (4 samples of each type) before freezing. During frozen storage, 1 sample per treatment per lot was removed and sampled at 1 wk, 6 wks, and every 6 wks thereafter for 72 wks.

Sample preparation

Frozen samples were partially thawed in a 3°C-4°C walk-in cooler for approximately 14 hours. The initial fresh and the subsequent thawed samples (25 g) were blended with 250 mL 3°C saline peptone water (0.1% peptone, 0.85% NaCl, 0.01% antifoam B). Appropriate dilutions were made for plating and coliform enumeration (APHA, 1984; FDA, 1984).

Microbiological analyses

Aerobic plate count (APC). Two sets of duplicate plates
were prepared from dilutions of $10^{-1}$ to $10^{-3}$ by the pour plate method with tryptone glucose extract (TGE) agar. Plates were incubated at $7^\circ C$ for 10 days for psychrotrophic organisms, or $35^\circ C$ for 48 h for mesophilic organisms (APHA, 1984; FDA, 1984).

**Total coliforms, fecal coliforms, and Escherichia coli.** The 3-tube most probable number (MPN) method was used to determine total coliform and fecal coliform numbers, by culturing in lauryl sulfate tryptose broth (LST), incubated at $35^\circ C$, followed by subculturing in EC broth, incubated at $45.5^\circ C$. Positive EC tubes were subcultured on Levine eosin methylene blue (EMB) agar. Typical colonies were confirmed by IMViC patterns (APHA, 1984; FDA, 1984).

**Statistical analyses**

Analysis of data for APC, total coliforms, fecal coliforms, and *E. coli* was by analysis of variance (ANOVA) and regression. Duncan's multiple range test was used to determine significant differences at the $F=0.05$ level between means (Snedecor and Cochran, 1967).

**RESULTS AND DISCUSSION**

**Aerobic plate counts**

**APC $35^\circ C$ (mesophilic counts).** The mean mesophilic count (log 10) per gram of frozen meat (4.08) varied only 0.11 from that of fresh meat (3.97), indicating little overall effect of freezing and frozen storage. Freezing method did
not have a significant effect (p>0.05) on mesophilic counts. These data confirmed similar findings of other researchers (Simmonds and Lamprecht, 1985).

Minor variations (<=0.2 log) in mesophilic counts during frozen storage were significant (p<0.05) by ANOVA, but there was no linear trend (p>0.05) (Fig.1). These findings were not consistent with the ca. 1 log mesophilic APC decreases observed in other crustacean products during frozen storage (Mijayama and Cobb, 1978; Hale and Waters, 1981; Gates et al., 1985). Differences in product type and storage conditions may have contributed to the inconsistent findings. Furthermore, Mijayama and Cobb (1978) observed that vacuum packaged products maintained higher APC's than did corresponding glazed treatments. The nitrogen flush and vacuum pack used in the present study could have helped to preserve microorganisms by minimizing the deleterious effects of oxidation.

Mean APC's of frozen crawfish meat packed with hepatopancreas (4.47) were significantly higher (p<0.0001) than in meat without it (3.70) (Fig.1). These differences were due in part to the washing of the meat to remove hepatopancreas, a procedure that also washes off some bacteria. In addition, the fat in the hepatopancreas may have a protective effect on microorganisms present. The APC differences found in this study were consistent with the findings of Mijayama and Cobb (1978) in frozen prawns.

APC 7°C (psychrotrophic counts). The mean psychrotrophic...
count of fresh crawfish meat (3.18) was higher than that of frozen meat (2.56), indicating a higher mortality of this group of organisms. This may have been the result of a die-off of *Pseudomonas*, a gram-negative species particularly susceptible to freezing (ICMSF, 1980a; Simmonds and Lamprecht, 1985), and likely to represent a major part of the psychrotrophic flora of a blanched, ice-chilled crustacean product.

Differences in psychrotrophic counts attributable to freezing method were minor (ca. 0.2 - 0.3 log), yet significant (p<0.01) by ANOVA. Duncan's analysis showed packaged samples cryogenically frozen at -101.1°C (-150°F) had significantly (p<0.05) lower counts than did the other treatments. Numbers varied significantly (p<0.0001) as a function of storage time, with a significant (p<0.0001) negative linear trend (Fig.1). This drop in numbers is consistent, although not as great, as observed in prawns by Hale and Waters (1981). Psychrotrophic counts were significantly higher in samples containing hepatopancreas (p<0.0001) (Fig.1).

**Coliforms and *E. coli***.

The numbers and incidence of detection (Table 1) of total coliforms (TC), fecal coliforms (FC), and *E. coli* in the crawfish meat during frozen storage were variable, probably due to initial inconsistent numbers present in the fresh, commercially processed product. Consequently,
coefficients of variation (C.V.) for the ANOVA were quite high (262.74, 456.80, and 802.01, respectively). Only data for TC (Table 1) indicated significant effects of time (p<0.01), and the presence of hepatopancreas (p<0.01).

Regression analysis also was limited by high C.V.'s, which were 286.08, 504.07, and 826.04 for TC, FC, and E. coli, respectively. Analysis of TC numbers indicated significant (p<0.05) quadratic trends, and FC numbers indicated significant (p<0.05) cubic trends. Trends for E. coli were not significant, but considering the high C.V., the F values of 0.08, 0.06, and 0.06 for cubic, quadratic, and linear trends were notable.

Results of this study showed a similar mortality rate of coliforms and E. coli to the 95% or more reported in prawns during freezing and frozen storage (Simmonds and Lamprecht, 1985). Duration of survival of fecal coliforms and E. coli was greater in crawfish meat packaged with hepatopancreas (Table 1), which seemed to afford some sort of protection from the lethal effects of freezing and frozen storage.

SUMMARY AND CONCLUSIONS

Results indicate that using good commercial practices for packaging, freezing, storing, and thawing freshwater crawfish meat has a negligible effect on the mortality rate of mesophilic and psychrotrophic bacteria. These organisms were a surprisingly good index of product quality even after
72 wks of frozen storage. The survival of coliforms and *E. coli* up to 72 weeks at -23°C appeared to be enhanced by the presence of hepatopancreas tissue, possibly due to a protective effect afforded by lipids in the tissue.
<table>
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<tr>
<th>Week</th>
<th>TC (MPN/g)</th>
<th>FC (MPN/g)</th>
<th>EC (MPN/g)</th>
</tr>
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<tr>
<td></td>
<td>W  U</td>
<td>W  U</td>
<td>W  U</td>
</tr>
<tr>
<td>0</td>
<td>3.2^a</td>
<td>12.4</td>
<td>0.1</td>
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<tr>
<td></td>
<td>(7)</td>
<td>(12)</td>
<td>(3)</td>
</tr>
<tr>
<td>1</td>
<td>3.0</td>
<td>19.2</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>(11)</td>
<td>(12)</td>
<td>(2)</td>
</tr>
<tr>
<td>6</td>
<td>1.3</td>
<td>3.6</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>(10)</td>
<td>(12)</td>
<td>(5)</td>
</tr>
<tr>
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<td>4.0</td>
<td>10.1</td>
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</tr>
<tr>
<td></td>
<td>(8)</td>
<td>(12)</td>
<td>(4)</td>
</tr>
<tr>
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<tr>
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<td>(3)</td>
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<td>(10)</td>
<td>(0)</td>
</tr>
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</tr>
<tr>
<td></td>
<td>(6)</td>
<td>(11)</td>
<td>(1)</td>
</tr>
</tbody>
</table>

^a Mean MPN of 12 samples (3 each, of 4 freezing methods).
^b MPN's of <0.3/g treated as 0.0 for arithmetic purposes.

Number of positive samples (out of a possible 12).
Figure 1. Aerobic plate counts (APC at 35°C and 7°C) of washed (W) and unwashed (U) crawfish meat stored at -23°C.
Effect of freezing method, pH, hepatopancreas, and frozen storage at -23°C on the texture of frozen freshwater crawfish (Procambarus sp.) meat

ABSTRACT

Commercially processed crawfish meat, with or without adhering hepatopancreas, was frozen by one of two conventional or two cryogenic methods prior to 72 weeks of storage at -23°C. Mean shear force of thawed meat (2.246 kg/g) was 25% higher than that of fresh meat (1.795 kg/g). Freezing method did not significantly affect texture (p>0.05), as indicated by Instron shear force (0.037 kg/g maximum difference), or sensory evaluation. The pH of meat (mean 7.28) was not significantly (p>0.05) correlated with shear force. Shear force of stored meat packed with hepatopancreas was slightly but significantly (p<0.001) (0.104 kg/g) softer than washed meat. Highly significant (p<0.0001) increases in shear force were observed during storage, with minimum values (2.005 kg/g) observed at 24 wks and a maximum (2.755 kg/g) at 54 weeks.

INTRODUCTION

The texture of muscle tissue is considered to be one of its most important quality attributes. In seafoods, freezing and frozen storage have long been recognized as factors which cause development of undesirable texture characteristics, particularly toughening. In addition,
muscle tissue pH and enzymes have been observed to contribute to undesirable textural changes during frozen storage.

Because of seafood seasonality, perishability, and distance to markets, freezing maximizes the use of fishery resources. Seafood products respond differently to the effects of freezing methods and frozen storage conditions. Consequently, an evaluation of available options identifies those factors that maximize quality, shelf life, and profitability.

Finfish and shellfish frequently have been reported to benefit from quick freezing (Tressler, 1932; Rogers and Binsted, 1972; Hultin, 1985), while intermediate freezing rates have sometimes been shown to be more detrimental to muscle tissue than slow freezing (Hultin, 1985). Jul (1984) observed that many studies have failed to show differences in quality between intermediate and fast (i.e., cryogenic) freezing rates.

Quick freezing, with cryogenic processes (i.e., liquid nitrogen [LN₂] or liquid carbon dioxide [CO₂]) or immersion processes (i.e., liquid freon freezant [LFF] or dichlorodifluoromethane [DDM]) sometimes produce quality advantages over slower methods (i.e., sharp or still, and blast), when freezing crab (Gangal and Magar, 1963; FMC Corp., 1969; Ampola and Learson, 1971; Strasser et al., 1971; Cook and Lofton, 1979; Coleman et al., 1986), and rock lobsters (Simmonds et al., 1984). While the previous workers found
improvements in factors such as sensory quality, appearance, drip, and texture, others have been unable to demonstrate similar advantages when quick freezing several finfish and shellfish (Anonymous, 1965; Bucholz and Pigott, 1972; Aurell et al., 1976; Houwing, 1984).

Quick freezing has also been shown to be a factor in increasing the high quality shelf life of frozen seafood products (Ampola and Learson, 1971; Sebranek, 1982) given proper storage conditions, while slower freezing resulted in poorer sensory quality after frozen storage (USDC, 1970).

Deterioration of frozen seafood products can be minimized by protecting them from oxidation and desiccation. Packing in oxygen impermeable films has been shown to be effective protection, but requires oxygen removal by vacuum or displacement with an inert gas such as nitrogen (Connell, 1975). Minimizing exposure to atmospheric oxygen is particularly important for fatty fish (Jul, 1984). Vacuum packaging has been shown by some to improve frozen shelf life (Dassow et al., 1962; FMC Corp., 1969; Strasser et al., 1971; Mijayama and Cobb, 1977; Badonia, 1981; Reddy et al., 1981), while others found no similar advantage (Webb et al., 1976).

Several researchers have demonstrated a correlation between pH and fish texture. Lower pH levels are associated with tougher fish muscle (Cowie and Little, 1966; Kelley et al., 1966; Connell and Howgate, 1968; Love et al., 1974; Dunajski, 1979). This effect has been demonstrated to be
more consistent within a species (Feinstein and Buck, 1984), and more pronounced below pH 6.7 (Dunajski, 1979). Little pH change has been observed in muscle tissue as related to storage time or temperature of fish (Bosund and Beckeman, 1972) or shellfish (Webb et al., 1975; Webb et al., 1976; Hale and Waters, 1981; Maxwell-Miller et al., 1982).

Histological studies have linked enzymes in crustacean hepatopancreas tissue with proteolysis of tail muscle (Rowland et al., 1982). The presence of this tissue has been associated with softening of fresh or thawed, ice-stored tail meat (Nip and Moy, 1979; Nip et al., 1985a; Marshall et al., 1987) and frozen tail meat (Hale and Waters, 1981). Conversely, Miyayama and Cobb (1977) did not observe similar tissue softening in freshwater prawns. Breakdown of hepatopancreatic lipids into unsaturated fatty acids, that can denature and toughen myofibrils (Hultin, 1985), could have mediated textural changes in crustacean tail meat.

Once seafood products have been frozen and packaged, further deterioration is affected primarily by temperature and duration of frozen storage (Dagbjartsson and Solberg, 1972; Rogers and Binsted, 1972; Connell, 1975; Ronsivalli and Baker, 1981; Jarman, 1982; Jul, 1984; Summers, 1984; Gates et al., 1985; Hultin, 1985). Because fish and shellfish have been reported to be more sensitive to the effects of frozen storage than other muscle tissues (Rogers and Binsted, 1972; Ronsivalli and Baker, 1981; Hultin, 1985), it is considered especially important that storage
temperature be kept low. A temperature of $-18^\circ C$ has been suggested as a maximum acceptable storage temperature (Strasser et al., 1971; Hallowell, 1980; Jul, 1984; Hultin, 1985). Maintaining frozen products at low temperatures is also an effective way of preventing ice crystal growth, shown to occur at storage temperatures above approximately $-10^\circ C$ (Moran, 1932; Fennema et al., 1973; Bevilacqua and Zaritsky, 1982), but not below $-20^\circ C$ (Moran, 1932). It has been questioned whether the benefits obtained from storing fishery products at temperatures $<-18^\circ C$ warrant the additional energy costs (Jarman, 1982; Houwing, 1984). Connell (1975) observed that even at $-18^\circ C$, fish that was frozen fresh will keep in good condition for only 2 to 4 months.

The objective of this study was to determine the effects of freezing method, pH, hepatopancreas, and $-33^\circ C$ frozen storage on the texture of freshwater crawfish meat.

MATERIALS AND METHODS

Product preparation and storage

Three lots of freshwater crawfish (Procambarus clarkii and P. acutus acutus) tail meat were obtained at two week intervals from a commercial processor in Louisiana. Meat was hand peeled and deveined from crawfish that had been blanched by placing live crawfish in boiling water until a return to boil was reached (7 min). Half of the meat in each
lot was washed (W) of hepatopancreas tissue, that was left adhering to the unwashed (U) meat (a common market form, frequently preferred for additional flavor). Freshly peeled and deveined meat was packed 2.27 kg/bag, chilled in an ice-water slush, and packed in ice for transportation.

At the Food Science Department, meat was packed in Koch laminated nylon/polyethylene bags (approximately 225 g/bag), nitrogen flushed and vacuum packed. Individually quick frozen (IQF) meat was packaged in the same manner immediately after freezing. Meat was kept packed in ice until frozen by one of the following methods: conventional freezing by 1) still ("sharp") freezing at -23°C, or 2) blast freezing at -40°C; or cryogenic freezing with LN₂ in a chamber freezer (Air Products Cryo-Test Chamber, Model CT-1818-12F), by 3) freezing packaged meat at -101.1°C (-150°F) or 4) freezing individual tails ("IQF") at -59.4°C (-75°F).

Frozen meat was stored at -23°C for 72 weeks.

Sampling and sample preparation

For each lot, sample packages of crawfish meat from the eight treatments (4 freeze X 2 wash) were randomly selected at 1 wk, 6 wks, and every 6 wks thereafter for the duration of the study. Packaged meat was left to thaw overnight (approximately 18 hours) in a 3-4°C walk in cooler, and brought to room temperature (23°C-25°C) just prior to use for Instron analysis or sensory evaluation.
Texture analysis by Instron shear force

Prior to analysis, adhering hepatopancreas was washed from crawfish meat samples and then drained 10 minutes.

Six replicate samples of meat (approximately 25 g) from each treatment were analyzed in a completely randomized block design. Texture was determined with an Instron Universal Testing Machine (Model 1122), fitted with a 10-blade Kramer food testing attachment. Methods used were like those described by Marshall et al. (1987).

Peak height (mm) was used for determining shear force (kg/g) of samples.

Statistical analysis of data to determine differences due to lot, treatment, and frozen storage time was by analysis of variance. Duncan’s multiple range test was used to determine differences between treatment and time period means. Regression was used to determine trends throughout storage. Pearson correlation coefficients were used to determine relationships between shear force and sample size, mean tail weight and time (Snedecor and Cochran, 1967; Ott, 1977).

Texture evaluation by sensory panel

Sensory evaluation of crawfish meat texture was by a trained 10 member panel of Food Science Dept. faculty, staff, and students. A multiple comparisons test, as described by Larmond (1977) was used to evaluate samples in a completely randomized block design. Samples were presented
to panelists in partitioned booths under red lights, to minimize bias that could be imparted by meat color. Water and apples were available for clearing the palate between sample evaluation. Approximately 12 g samples of crawfish meat from each treatment were placed in small paper cups labeled with 3-digit codes, and placed on paper plates. Meat packaged without adhering hepatopancreas tissue was evaluated first, separately from that with the tissue. In both evaluations, samples of all four freezing treatments were compared to the reference, a blind sample of freezing treatment 4 (IQF). Panelists were instructed to chew all crawfish tails in each sample before evaluating texture. Panelists rated sample texture, using verbally ranked 9 point scales.

Analysis of data was by Chi-square analysis of frequency distribution, for differences due to treatments and time of storage.

Measurement of pH

Crawfish meat pH was measured by direct contact with an Orion surface pH electrode (Serial No. 816300), a method used by Nip et al. (1985b) to monitor pH changes related to muscle degradation. Measurements were made on 5 randomly selected thawed tails from each treatment during each sampling period.
RESULTS AND DISCUSSION

Freezing method

Freezing crawfish meat resulted in toughening, as indicated by increased shear force values in samples evaluated at one week (Fig. 1). Mean shear force of fresh meat was 1.795 kg/g, while that of samples frozen one week was 2.028 kg/g.

The texture of meat frozen by different methods was not significantly different (p>0.05), as determined by ANOVA of shear force data (Table 1), or Chi-square analysis of sensory data (p>0.05). Meat frozen by methods 1 through 4 had 1 wk shear force values of 2.054, 2.065, 2.005, and 1.987 kg/g, respectively. The greater shear force of blast frozen meat could reflect more extensive denaturation of protein, sometimes observed at intermediate freezing rates (Hultin, 1985). The lower shear force of cryogenically frozen meat indicated some benefit associated with quick freezing, as observed by others (Tressler, 1932; Rogers and Binsted, 1972; Hultin, 1985).

Hepatopancreas

The texture of washed (W) fresh meat was not significantly different (p>0.05) than that of the fresh unwashed (U) meat, with adhering hepatopancreas. The difference in shear force between W (mean 2.298 kg/g) and U (mean 2.194 kg/g) frozen stored samples was small (Fig. 2),
but highly significant as determined by ANOVA (Table 1), and Duncan's multiple range test (p<0.05). Chi-square analysis of sensory data also indicated a highly significant difference (p<0.0001) in texture rating distributions between W and U meat (Fig. 3).

The softer texture of meat stored with hepatopancreas tissue indicated that proteolytic enzyme activity remained in the tissue after the blanching process. Activity was not great enough to affect the texture of fresh tissue held on ice for approximately 24 hr. Proteolysis was not prevented by frozen storage at -23°C, as indicated by the softening of U meat during storage. Results concurred with findings of Hale and Waters (1981).

**pH**

The difference in pH of fresh (7.286) and frozen (7.281) crawfish meat was negligible. Although the change in pH during storage was small (Fig. 4), it was significant by ANOVA (Table 1). Freezing method did not have a significant effect on pH of meat (Table 1). The difference in pH between W (7.316) and U (7.252) meat (Fig. 4) was marginally significant by ANOVA (Table 1), and significant (p<0.05) by Duncan's.

The effect of pH on fish texture has been reported to be much less pronounced at pH levels above 6.7 (Dunajski, 1979). This could explain why pH differences in the range found in this study (approximately 7.25 - 7.35) did not
correlate with differences in texture. The negligible change in pH during storage concurred with findings of others (Webb et al., 1975; Webb et al., 1976; Hale and Waters, 1981; Maxwell-Miller et al., 1982).

Lower pH levels have been associated with tougher fish (Love et al., 1974). In the present study, the meat (U) with the lower pH was softer. This suggested that the presence of proteolytic enzymes in hepatopancreas tissue had a stronger influence on meat texture than did the toughening effect of pH.

Frozen storage

The shear force values of crawfish meat increased significantly during frozen storage, as determined by ANOVA (Table 1), and had a significant (p<0.05) positive linear trend. Highly significant correlations were observed between shear force and time (Table 2). Duncan's analysis confirmed significant differences (p<0.05) in shear force over time, with lowest values (mean 2.005 kg/g) observed at 24 wks, and highest values (mean 2.755 kg/g) at 54 weeks.

Results show that texture was affected to a greater extent by frozen storage than by freezing or freezing method. This substantiated Connell's (1975) observation that of properly frozen fish, little loss in quality results from the freezing process. Plank et al. (1916) stated that the majority of changes in frozen products develop during frozen storage, and Fennema (1968) ranked frozen storage first.
among factors that affect the quality of frozen food.

The presence of hepatopancreas on U meat was associated with lower shear force values throughout the study (Fig. 2). Chi-square analysis of sensory data showed frequency patterns of texture ratings of W meat were significantly different from those of U meat in different storage periods (Figs. 5, 6, and 7). As storage time increased, the U meat was more frequently rated as softer than the reference, while the W meat was more frequently rated as firmer. The shifting patterns indicated that panelists were able to detect textural changes such as proteolytic softening effects of hepatopancreas tissue, and toughening effects of frozen storage.

There was a significant linear trend (p<0.05) in pH of cryogenically frozen W meat (treatments 3 and 4), and a significant quadratic trend (p<0.05) in cryogenically frozen unwashed meat (treatments 7 and 8). These findings did not help explain textural changes, however, as pH and shear force were not significantly (p>0.05) correlated, and freezing method did not significantly (p>0.05) affect the texture.

SUMMARY AND CONCLUSIONS

The texture of fishery products, such as crawfish meat, is one of the most important sensory qualities. Maintaining a desirable texture after freezing and frozen storage is important to the value of the product. Several factors known
to have significant detrimental effects on the textural quality of frozen fishery products have been evaluated in this study using freshwater crawfish meat.

Frozen storage had the greatest effect on texture. Sensory panelists detected undesirable changes, but at no time was the product considered rejectable based on texture. Should it be necessary to maintain crawfish meat in commercial cold stores for periods considerable longer than 72 wks, lower temperatures recommended by a number of researchers might be considered.

Proteolytic enzymes in the hepatopancreas tissue had a softening effect on the texture of crawfish meat. Some panelists observed that this tenderizing effect seemed to counteract toughening effects of frozen storage. If breakdown of lipid material in the fatty hepatopancreas tissue contributed to the denaturation and toughening of myofibrils in the muscle tissue, the effects were masked by proteolysis.

The pH of crawfish meat was not found to have any significant bearing on texture, and was not a useful indicator of significant textural changes that occurred during frozen storage.

All of the freezing methods used in this study were considered highly acceptable relative to the textural quality produced. Cryogenic freezing methods, in some instances, have been reported to produce better products initially, as well as enhance frozen shelf life. In this
study, small differences in texture due to freezing method were not significant initially or throughout storage. Furthermore, there was no significant interaction between the freezing method and storage time.
Table 1. Results of analysis of variance on shear force data for both fresh and frozen crawfish meat.

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<th>Shear force</th>
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<tr>
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<sup>a</sup> Not applicable  
<sup>b</sup> Probability  
<sup>c</sup> F value  
<sup>d</sup> Degrees of freedom
Table 2. Correlation of crawfish meat shear force values with storage time.

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<th>Wks 42-72</th>
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<td>0.0001</td>
</tr>
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<td>0.4681</td>
</tr>
<tr>
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<td>0.0144</td>
<td>0.0008</td>
</tr>
<tr>
<td>Lot 3</td>
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</tr>
<tr>
<td></td>
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<td>0.0011</td>
</tr>
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</table>

<sup>a</sup> Pearson correlation coefficient
<sup>b</sup> probability
Figure 1. Effect of freezing method (F) on shear force of crawfish meat. Methods F1 - F4 are still, blast, \( \text{LN}_2 \) in-pack, and \( \text{LN}_2 \)-IQF, respectively.
Figure 2. Effect of hepatopancreas on shear force of crawfish meat.
Figure 3. Sensory texture scores for washed and unwashed crawfish meat: weeks 1 - 72.
Figure 8. The pH of fresh (time 0) and frozen crawfish meat stored at -23°C for 72 weeks.

Figure 4. The pH of fresh (time 0) and frozen crawfish meat stored at -23°C for 72 weeks.
Figure 5. Sensory texture scores for washed and unwashed crawfish meat: weeks 1 - 24.
Figure 6. Sensory texture scores for washed and unwashed crawfish meat: weeks 30 - 48.
Figure 7. Sensory texture scores for washed and unwashed crawfish meat: weeks 54 - 72.
Factors affecting the drip loss and moisture content of frozen freshwater crawfish (*Procambarus* sp.) meat

ABSTRACT

Commercially processed crawfish meat was frozen by one of two conventional or two cryogenic methods, prior to 72 weeks of storage at -23°C. Freezing method had a significant effect on thaw drip loss (*p*<0.0001) and moisture content (*p*<0.05) of meat. Frozen storage was associated with significant (*p*<0.0001) changes in drip loss and moisture. There was a significantly higher (*p*<0.001) drip loss from meat packed with adhering hepatopancreas tissue (7.80%), than in meat packed without it (6.86%). There was a small, but significant (*p*<0.001) correlation between pH and drip (-0.295), and drip and moisture (-0.285) in meat packed without hepatopancreas. Meat frozen by cryogenic methods had a significantly (*p*<0.05) higher moisture content and lower drip loss than conventionally frozen meat.

INTRODUCTION

Moistness, or succulence, is an important sensory quality in seafood (Connell, 1975). Desiccation during frozen storage and thaw drip losses may deplete muscle tissue of moisture.

The measurement of thaw drip loss is a well established indicator of quality for frozen seafoods (Bucholz and
Pigott, 1972; Gibbard, 1978; Shenouda, 1980; Jarman, 1982; Jul, 1984; Hultin, 1985). Thaw drip loss is also a simple, well correlated indicator of protein denaturation, as affected by freezing and frozen storage (Shenouda, 1980; Jul, 1984; Hultin, 1985). Protein denaturation has been associated with undesirable texture changes in many frozen fishery products (Connell, 1975).

Many researchers have demonstrated a significant relationship between freezing rate and the amount of drip exuded from thawed muscle tissue. Findings vary considerably. Structural damage, caused by formation of large ice crystals, has been demonstrated to increase as freezing rate decreases (Piskarev et al., 1971; Nusbaum, 1979; Coleman et al., 1986). Tissue damage has been implicated in both higher (Plank et al., 1916) and lower drip losses (Jul, 1984); explanations for these occurrences have included "leakage" from cells, and water binding capacity of released myofibrillar proteins, respectively (Jul, 1984). The relationship between ice crystal formation and drip loss is not clear.

Protein denaturation can occur during freezing and has been clearly associated with drip (Shenouda, 1980). Slow freezing enhances protein denaturation caused by the concentration of solutes and altered pH (Hultin, 1985). This type of denaturation occurs most rapidly at $-3^\circ$ to $-5^\circ$C (Connell, 1975). Consequently, the faster the product is moved from $0^\circ$ to $-5^\circ$C, the better.
Quality deterioration is associated with changes in moisture content. Desiccation caused by sublimation of ice crystals during frozen storage (Hultin, 1985) has been shown to be a problem in frozen fishery products (Faulkner and Watts, 1955; Gangal and Magar, 1963; Pawar and Magar, 1966; Rogers and Binsted, 1972; Nagle and Finne, 1980; Hallowell, 1980; Shenouda, 1980; Gates et al. 1985). This problem can be reduced by glazing (Pawar and Magar, 1966; Nagle and Finne, 1980). Glazing is cheap and effective, but not always practical, due to physical limitations, proportion control, and evaporation (Connell, 1975). Vacuum packing can be more efficient, but is frequently only applied to smaller more expensive products (Connell, 1975). Oxygen impermeable films enhance the keeping quality of seafood when combined with glazing, vacuum packing (Jul, 1984), or displacement of oxygen with an inert gas such as nitrogen (Connell, 1975). Provided packaging material remained undamaged, Mijayama and Cobb (1977) observed little dehydration in vacuum packaged prawns, compared to the standard glazed product.

A lower pH of fish muscle is known to influence the denaturation of myofibrils and reduce water holding capacity, resulting in a tougher, dryer product (Dunajski, 1979). The correlation between lower pH levels and tougher fish muscle has been demonstrated (Cowie and Little, 1966; Kelley et al., 1966; Connell and Howgate, 1968; Love et al., 1974; Dunajski, 1979). The pH of fishery products has been monitored during frozen storage to evaluate its relationship
to quality deterioration (e.g., texture and moisture changes). Often, little or no pH change has been observed in muscle tissue as related to storage time or temperature of fish (Bosund and Beckeman, 1972), shrimp (Webb, et al., 1975), crab (Webb et al., 1976), prawns (Hale and Waters, 1981), or scallops (Maxwell-Miller et al., 1982). A relationship between pH and drip loss or moisture content in frozen crawfish meat has not been demonstrated.

Digestive enzymes from the hepatopancreas of the freshwater prawn have been associated with softening of muscle tissue during iced (Nip and Moy, 1979) and frozen (Hale and Waters, 1981) storage. Histological studies have demonstrated the role of digestive enzymes in the breakdown of prawn muscle structure (Rowland et al., 1982). At least two of the enzymes thought to be involved have been isolated: trypsin (Lee et al., 1980) and collagenase (Eisen and Jeffrey, 1969; Baranowski et al., 1984; Nip et al., 1985a). Only collagenase was found to significantly degrade prawn tissue (Baranowski et al., 1984). This enzyme fraction demonstrated activity at 0°C (Nip et al., 1985a), and consequently may cause tissue breakdown during frozen storage. Such breakdown could affect moisture and drip losses in frozen crustacean products stored with hepatopancreas tissue.

The objective of this study was to determine the effects of freezing method, pH, hepatopancreas, and -23°C frozen storage on the drip loss and moisture content of
freshwater crawfish meat.

MATERIALS AND METHODS

Product preparation and storage

Three lots of freshwater crawfish (Procambarus clarkii and P. acutus acutus) tail meat were obtained at two week intervals from a commercial processor in Louisiana. Meat was hand peeled and deveined from crawfish that had been blanched by placing live crawfish in boiling water until a return to boil was reached (7 min). Half of the meat in each lot was washed (W) to remove hepatopancreas tissue, that was left adhering to the unwashed (U) meat (a common market form, frequently preferred for additional flavor). Freshly peeled and deveined meat was packed 2.27 kg/bag, chilled in an ice-water slush, and packed in ice for transportation.

At the Food Science Department, meat (approximately 225 g/bag) was packed in Koch laminated nylon/polyethylene bags (moisture and vapor proof), nitrogen flushed and vacuum packed. Bags were labeled, and the meat in each bag was weighed and recorded for later use. Individually quick frozen (IQF) meat was packaged in the same manner immediately after freezing.

Meat was kept packed in ice until frozen by one of the following methods: conventional freezing by 1) still ("sharp") freezing at -23°C, or 2) blast freezing at -40°C; or cryogenic freezing with 1N₂ in a chamber freezer (Air Products Cryo-Test Chamber, Model CT-1818-12F), by 3)
freezing packaged meat at \(-101.1^\circ \text{C} (-150^\circ \text{F})\), or 4) freezing individual tails ("IQF") at \(-59.4^\circ \text{C} (-75^\circ \text{F})\).

Frozen meat was stored at \(-23^\circ \text{C}\) for 72 weeks.

**Sampling and sample preparation**

For each lot, sample packages of crawfish meat from the eight treatments (4 freeze X 2 wash) were randomly selected at 1 wk, 6 wks, and every 6 wks thereafter for 72 wks. Packaged meat was thawed overnight (approximately 18 hours) in a 3-4°C walk in cooler, and brought to room temperature (23°C-25°C) just prior to use.

**Measurement of pH**

To monitor pH changes related to muscle degradation, crawfish meat pH was measured by direct contact with an Orion surface electrode (Serial No. 816300), a method used by Nip et al. (1985b). Measurements were made on 5 randomly selected thawed tails from each treatment during each sampling period.

**Determination of thaw drip and moisture content**

Crawfish meat in each bag was emptied into a weighed colander, that was placed on absorbant paper toweling. The meat was drained 10 min before it was weighed in the colander. Weight of the meat was calculated by difference. Drip loss (\%) of thawed crawfish meat was calculated as:

\[
\text{Drip loss} = \left( \frac{\text{packed weight} - \text{thawed weight}}{\text{packed weight}} \right) \times 100
\]
Samples of W meat for moisture determination were placed in Whirl Pak bags. The U meat was washed to remove adhering hepatopancreas, and drained as before, prior to taking samples for determination of moisture in the meat. The W meat had been given the same type of washing and draining when it was freshly processed. Triplicate samples of meat (approximately 7g) from each treatment were weighed to 4 decimal places in tared aluminum drying pans. Samples were dried (Precision Gravity Convection Oven, Model 17) 24 hours at 115°C, cooled in a desiccator, and weighed. Wet and dry weights of meat were determined by difference from pan weight. Moisture (%) was calculated as: \[\left(\frac{\text{wet weight} - \text{dry weight}}{\text{wet weight}}\right) \times 100\].

Statistical analysis of data to determine differences due to lot, freezing method, the presence of hepatopancreas, and frozen storage (time) was by analysis of variance. Duncan's multiple range test was used to determine differences between treatment and time period means. Regression was used to determine trends throughout storage. Pearson correlation coefficients were used to determine the significance of relationships between pH, drip, and moisture (Snedecor and Cochran, 1967; Ott, 1977).

Moisture evaluation by sensory panel

Sensory evaluation of moisture in crawfish meat was by a trained 10 member panel of Food Science Dept. faculty,
staff, and students. A multiple comparisons test, as described by Larmond (1977) was used to evaluate samples in a completely randomized block design. Samples were presented to panelists in partitioned booths under red lights. Water and apples were available for clearing the palate between sample evaluations. Approximately 12g samples of crawfish meat from each treatment were placed in small paper cups labeled with 3-digit codes, and placed on paper plates. Meat packaged without adhering hepatopancreas tissue was evaluated first. In both evaluations, samples of all four freezing treatments were compared to the reference, which was freezing treatment 4 (IQF). Panelists were instructed to chew all crawfish tails in each sample, before evaluating moisture, using verbally ranked 9 point scales.

Analysis of data was by Chi-square analysis of frequency distribution, for differences due to freezing method, wash, and storage time.

RESULTS AND DISCUSSION

Freezing method

The effect of freezing method on thaw drip (Figure 1) of crawfish meat was highly significant (p<0.0001) as determined by ANOVA (Table 1). Mean drip losses from meat frozen by methods 1 (8.854%) and 2 (8.974%) were not significantly different (p>0.05) from each other. Mean drip losses from meat frozen by methods 3 (6.893%) and 4 (4.593%) were significantly different (p<0.05) from each other, and
from meat frozen by methods 1 and 2.

Freezing method significantly (p<0.05) affected moisture (Figure 2) in crawfish meat as determined by ANOVA (Table 1). Mean moisture content in meat frozen by methods 1 (80.200%), 2 (80.209%), and 3 (80.459%) were not significantly different (p>0.05). Moisture content of meat frozen by method 4 (80.478%) was not significantly different than that frozen by method 3.

Analysis of sensory data indicated a highly significant difference (p<0.0001) in the frequency patterns of moisture ratings (Figure 5). Meat frozen by methods 1 and 2 were rated less moist than that frozen by methods 3 and 4.

Slow freezing can greatly increase thaw drip (Rogers and Binsted, 1972; Jul, 1984; Hultin, 1985), while very fast freezing (i.e. cryogenic) has been shown to be particularly helpful in reducing drip (Jul, 1984). Advantages in factors such as appearance, sensory quality, drip, and texture have been demonstrated when quick freezing crustaceans such as crabs (Gangal and Magar, 1963; FMC Corp., 1969; Ampola and Learson, 1971; Strasser et al., 1971; Webb et al., 1976; Cook and Lofton, 1979; Coleman et al., 1986) and rock lobsters (Simmonds et al., 1984). Results of this study indicate that freshwater crawfish also benefit from quick freezing, not only in measurable quantities of drip loss and moisture, but also in an increased moistness perceived by sensory methods.
Frozen storage

Time of frozen storage had a highly significant effect on thaw drip (p<0.0001) and moisture (p<0.01) as determined by ANOVA (Table 1). There was a highly significant (p<0.001) linear trend in drip loss (Figures 1 and 3). There were no significant trends (p>0.05) in moisture content (Figures 2 and 4).

Chi-square analysis of sensory data showed significant differences in frequency of moisture ratings throughout storage for W meat frozen by method 3 (p<0.05) and method 4 (p<0.01), and U meat frozen by method 4 (p<0.0001). In all instances this involved an increased frequency in "slightly less moist" scores (#4) and a decrease in "slightly more moist" scores (#6). These differences detected were unanticipated since the reference was also crawfish meat frozen by method 4. Furthermore, the data presented in Figures 2 and 4 suggest that W meat frozen by method 1 would more likely be perceived as less moist. It was thought that moisture migration from the surface of the unglazed IQF crawfish meat could have influenced these results.

Fennema (1968) ranked frozen storage as the most important factor affecting the quality of frozen food. In the present study, highly recommended methods were used to freeze and package crawfish meat. Even though the frozen storage temperature was within recommended levels, there were highly significant effects of frozen storage time on the thaw drip and moisture content of crawfish meat.
Freezing method was ranked by Fennema (1968) as the third most important factor affecting the quality of frozen food. Others have found quick freezing to be a significant factor in increasing high quality shelf life of frozen fishery products (Ampola and Learson, 1971; Sebranek, 1982) given proper storage conditions. Similar findings are shown in Figure 1. Drip losses in conventionally frozen crawfish meat increased rapidly until 18 wks of storage. A much slower increase was observed in cryogenically frozen meat. Within 18 weeks, drip losses in conventionally frozen meat exceeded those observed in cryogenically frozen meat at any time during the study. This trend suggested a more rapid and extensive deterioration in quality of more slowly frozen meat.

Hepatopancreas

The presence of hepatopancreas tissue had a significant effect (p<0.01) on the drip loss from crawfish meat (Figure 3), as determined by ANOVA (Table 1). Mean drip losses of W meat (6.861%) and U meat (7.796%) were significantly different (p<0.05) by Duncan's test. The hepatopancreas tissue may have contributed directly to the higher drip. The fresh product contained approximately 7% (by weight) hepatopancreas. Unfortunately, determining an accurate weight of residual hepatopancreas on the product after thawing was difficult. Hepatopancreatic enzymes caused breakdown of the muscle tissue. Consequently, washing the
meat to remove hepatopancreas also caused a loss of muscle fibers.

Hepatopancreas also had a significant effect (p<0.05) on the moisture of crawfish meat (Figure 4), as determined by ANOVA (Table 1). The mean moisture of U meat (80.450%) was significantly greater (p<0.05) than that of W meat (80.223%), as determined by Duncan’s test.

It was not anticipated that the U meat, with a higher drip loss, would also have a higher moisture content. This may be explained by proteolysis of muscle tissue exposed to hepatopancreatic enzymes. Rowland et al. (1982) conducted a histological study of freshwater prawn tail muscle tissue that was exposed to hepatopancreas tissue. They found proteolytic activity indicated by a loss of z-line structure and gapping in the sarcoplasm between myofibers. Breakdown of beef muscle tissue during slow freezing has been demonstrated to reduce drip loss. This is a result of released myofibrillar proteins that have a high water binding capability (Jul, 1984). Increased exposure of myofibrillar proteins in crawfish muscle, resulting from proteolysis, may increase the water binding capacity of crawfish muscle in a manner comparable to that observed in beef. If so, the moisture content of U meat may have increased by absorbing moisture from the hepatopancreas tissue, or during the washing process. The freshly peeled W meat in the study was given a similar wash, however it occurred before extended contact with the hepatopancreas.
There were small, but highly significant (p<0.001) correlations between pH and drip (-0.29539) and drip and moisture (-0.28483) in W crawfish meat. There were no significant correlations between pH, drip, and moisture in U meat. This indicated that factors associated with the hepatopancreas tissue (i.e., enzymes) may have dominated other interactions.

Chi-square analysis of sensory moisture data showed highly significant differences (p<0.0001) in frequency patterns of ratings given to W and U meat (Figure 6). Panelists were able to perceive small moisture differences between freezing treatments. As seen in Figure 6, U meat was more frequently evaluated as the same, or more moist than the reference. The moisture content in meat frozen by methods 2 and 3 (80.478% and 80.551%, respectively) were both higher than the reference meat (80.387%), frozen by method 4, and meat frozen by method 1 (80.383%). It was interesting to note that even though sensory evaluation of U meat was done with hepatopancreas intact, the samples were ranked in relatively the same order as indicated by moisture determination (that was done without hepatopancreas). The panel also accurately evaluated the moisture of W meat. Figure 6 shows a greater frequency of sample evaluations as being the same or less moist than the reference. Moisture measurement indicated that moisture was highest in the reference (80.568%). Moisture in meat frozen by methods 3, 1, and 2 followed, with moisture contents of 80.368%,
Results of the sensory evaluation indicated that small differences in the moisture of crawfish meat may be perceived by consumers.

**pH**

The pH of crawfish meat was not significantly (p>0.05) affected by freezing method, and was slightly (p=0.0551) affected by the presence of hepatopancreas tissue, as determined by ANOVA (Table 1). A significant (p<0.05) difference in the pH of W meat (7.316) and U meat (7.252) was indicated by Duncan's test, however.

There were no significant correlations (p>0.05) between pH and drip loss or moisture, overall (i.e., both U and W samples). Samples of U meat had a lower pH, higher drip loss, and higher moisture content (7.252, 7.796%, and 80.450%, respectively) than W meat (7.316, 6.861%, and 80.223%, respectively), but there was no significant correlation (p>0.05) among these values. When analyzing data for U and W meat separately, no significant correlations (p>0.05) were found in U meat. In W meat there was a significant (p<0.001), but small correlation (-0.295) between pH and drip. This could reflect denaturation of myofibrils and a reduced water holding capacity (Dunajski, 1979). The lack of correlation associated with the low pH and high drip in U meat indicated that the higher drip may have been influenced by enzyme activity, rather than pH.
SUMMARY AND CONCLUSIONS

Retaining the moisture in frozen fishery products frozen is an important part of preserving their quality and value. Moistness, one of the most important sensory characteristics in seafoods, can be readily lost by using poor freezing methods or storage conditions.

In this study with crawfish meat, quick freezing resulted in immediate and long term improvements in quality of meat. Drip loss of cryogenically frozen meat was significantly less initially. The lower drip loss indicated less tissue alteration had occurred in the quick frozen meat. The increase in drip loss occurred less rapidly in cryogenically frozen meat than in conventionally frozen meat. These results support the findings of others, who determined that the high quality of fishery products can be maintained longer in frozen storage when the products have been quick frozen.

The crawfish meat frozen with hepatopancreas tissue had a significantly higher drip loss. Although breakdown of the hepatopancreas itself may have contributed to this loss, it is considered a loss of product nevertheless.

Quick freezing resulted in significantly moister crawfish meat. This difference was not only measurable, but was also significantly detected by sensory evaluation. This is important, because the consumer ultimately will determine the acceptability and marketability of frozen fishery products.
Table 1. Results of analysis of variance on data for drip, moisture and pH of frozen crawfish meat.

<table>
<thead>
<tr>
<th></th>
<th>% Drip</th>
<th>% Moisture</th>
<th>pH</th>
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<tr>
<td><strong>Time</strong></td>
<td></td>
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<tr>
<td></td>
<td>0.0001&lt;sup&gt;a&lt;/sup&gt;</td>
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<td></td>
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<td></td>
<td>12.36&lt;sup&gt;c&lt;/sup&gt;</td>
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<td><strong>Wash</strong></td>
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<td>15.82</td>
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<tr>
<td><strong>C.V.</strong></td>
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<td>0.729</td>
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<td><strong>Mean</strong></td>
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<td>80.336</td>
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<sup>a</sup> Probability
<sup>b</sup> F value
<sup>c</sup> Degrees of freedom
Figure 1. Effect of freezing method (F) on drip loss of crawfish meat. Methods F1 - F4 are still, blast, 1N2-in-pack, and 1N2-IQF, respectively.
Figure 2. Effect of freezing method (F) on moisture of crawfish meat. Methods F1 - F4 are still, blast, 1N2-in-pack, and 1N2-IQF, respectively.
Figure 3. Effect of hepatopancreas on drip loss of crawfish meat.
Figure 4. Effect of hepatopancreas on moisture of crawfish meat.
Figure 5. Sensory moisture scores for crawfish meat frozen by different methods (F): weeks 1 - 72. Methods F1 - F4 are still, blast, LN2-in-pack, and LN2-IQF, respectively.
Figure 6. Sensory moisture scores for washed and unwashed crawfish meat: weeks 1 - 72.
SUMMARY AND CONCLUSIONS

The quality of post mortem fishery products is invariably changed to some extent by preservation processes. A number of desirable textures, flavors, and colors are associated with freshly killed, high quality seafood. A goal of food science researchers working with seafood preservation is to develop processing and storage methods that best maintain those fresh-like qualities. Studying the effects of processing and handling methods on seafood quality will help establish those procedures that are essential in order to produce the best quality product.

The research presented herein has addressed several quality problems associated with crawfish meat. Based on results obtained, scientifically proven recommendations can be made to the crawfish processing industry regarding optimum methods for blanching whole crawfish, and packaging and freezing fresh peeled meat.

Although this research answered a number of questions, it also raised many others. The author suggests further investigation into the microbiology of fresh and frozen crawfish products, and the effects of processing, packaging (e.g., modified atmospheres and the presence of hepatopancreas), and storage on the numbers and survival of various types of microorganisms.

Further investigation into the enzymes present in the crawfish hepatopancreas and tail meat would be useful. This information would be valuable from a biological standpoint,
and would allow better recommendations to processors for improving processing, packaging, and storage methods. This work might include isolation and characterization of proteolytic enzymes, as well as consideration of commercial uses of hepatopancreatic proteases or collagenases.

A more in-depth study on texture changes in fresh and frozen crawfish meat is needed to help understand the roles of endogenous enzymes and those of microbial origin. It would also be useful to study protein denaturation associated with freezing rate and frozen storage temperature. Literature suggests that TMAO would not likely be a significant factor in toughening during frozen storage of freshwater crustacean, but it would be of interest to determine its presence and involvement.
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Major Professor and Chairman
Dean of the Graduate School

EXAMINING COMMITTEE:

[Signatures]

Date of Examination:

December 17, 1987