THE EFFECTS OF CRYOPROTECTANTS AND STORAGE TIME ON THE RHEOLOGICAL AND STRUCTURAL PROPERTIES OF SURIMI FROM THREE FRESHWATER SPECIES OF FISH.

by

TWYLLA K. PAWLINSKY

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTERS OF SCIENCE

DEPARTMENT OF FOOD SCIENCE
WINNIPEG, MANITOBA
OCTOBER, 1991
The author has granted an irrevocable non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of his/her thesis by any means and in any form or format, making this thesis available to interested persons.

The author retains ownership of the copyright in his/her thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without his/her permission.

L'auteur a accordé une licence irrévocable et non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de sa thèse de quelque manière et sous quelque forme que ce soit pour mettre des exemplaires de cette thèse à la disposition des personnes intéressées.

L'auteur conserve la propriété du droit d'auteur qui protège sa thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

ISBN 0-315-76882-7
THE EFFECTS OF CRYOPROTECTANTS AND STORAGE TIME
ON THE RHEOLOGICAL AND STRUCTURAL PROPERTIES OF
SURIMI FROM THREE FRESHWATER SPECIES OF FISH

BY

TWYLLA K. PAWLINSKY

A thesis submitted to the Faculty of Graduate Studies of
the University of Manitoba in partial fulfillment of the requirements
of the degree of

MASTER OF SCIENCE

© 1991

Permission has been granted to the LIBRARY OF THE UNIVERSITY OF MANITOBA to lend or sell copies of this thesis, to
the NATIONAL LIBRARY OF CANADA to microfilm this thesis and to lend or sell copies of the film, and UNIVERSITY
MICROFILMS to publish an abstract of this thesis.

The author reserves other publication rights, and neither the
thesis nor extensive extracts from it may be printed or other-
wise reproduced without the author's written permission.
ACKNOWLEDGMENTS

I would like to thank my committee members, Dr. Watts, and Dr. Gallop for taking the time to read through my thesis and for their constructive criticisms. I especially have to thank my advisors Dr. Ismond and Dr. Murray for their encouragement throughout my study -- even when things did not go as planned, and the fish had to be processed yet again. Many people in the Food Science department contributed to this thesis - either by helping on those dreaded "fish processing days", or by lending expertise. I am grateful to Dr. Arntfield for her help in devising an outline for my results and discussion chapters, to Donna Ryland for her assistance with the O.T.M.S., and to Paul Stephen whose computer help was always a phone call away. I must also thank Janine, for assisting my research by providing fish processing skills, research papers, and advice when problems arose.

I must also thank a few people from outside the department for helping me reach this goal. To my friend, Teresa, your assistance in processing fish gels on Sunday mornings, as well as your statistical and typing skills were appreciated. As well I must thank my co-workers at the Canadian Grain Commission, especially Donna and Joan, for continually reminding me I had a thesis to finish.
Finally, I must thank my family - my sisters are probably just as relieved as I am that this feat is finished - I can't use this as an excuse for not visiting anymore. Mom and Dad, thanks for your wisdom, understanding and support during my university years. Thanks for believing enough in me, to give me my graduation present - a year before I finished. I hope this accomplishment makes you both proud.
This study examined the effects of various cryoprotectants and storage time on the texture of surimi produced from three underutilized freshwater fish - whitefish (Coregonus clupeaformis), tullibee (Coregonus artedii) and mullet (Catostomas commersoni). Viscoelasticity was determined with the Bohlin VOR rheometer, set in the oscillatory mode. The storage modulus, $G'$, and the loss modulus $G''$ were monitored over a frequency of 0.1 to 20 Hz; the resultant loss tangent, $\tan \delta$ was subsequently calculated. The rigidity of the gels was evaluated by compression testing using the Ottawa Texture Measuring System. Finally, thin sections of each fish gel were evaluated microscopically to assess the formation of protein networks. Differences were observed in the rigidity, viscoelasticity and protein networks of the three species depending upon the type of cryoprotectant incorporated, length of storage time and specific species involved. Overall the tullibee was the least rigid regardless of cryoprotectants added, whereas mullet was generally the least elastic of the three fish. The whitefish exemplified similar elasticity to tullibee, but ranged between tullibee and mullet when rigidity was compared. The microstructural ratings for whitefish were generally the best. The variation which was observed with the different cryoprotectant combinations and storage conditions for all three testing methods was minimal.
# TABLE OF CONTENTS

ACKNOWLEDGMENTS .................................................. i
ABSTRACT .................................................................. iii
TABLE OF CONTENTS .................................................. iv
LIST OF FIGURES ...................................................... vii
LIST OF TABLES ........................................................ ix

INTRODUCTION ......................................................... 1

REVIEW OF LITERATURE ............................................. 3

2.1. Fish Protein Potential ........................................... 3
2.2. Factors Influencing Surimi Quality ......................... 4
  2.2.1. Species ....................................................... 4
  2.2.2. Cryoprotectants ............................................. 8
  2.2.3. Enzymes ..................................................... 10
  2.2.4. Storage Conditions ....................................... 11
2.3. Gelation .......................................................... 13
2.4. Gel Testing Methods .......................................... 17
  2.4.1. Traditional ............................................... 17
  2.4.2. Modern/Conventional Methods ....................... 19
2.5. Consumer Interest ............................................. 21
2.6. Potential of Freshwater Fish ................................. 24

MATERIALS AND METHODS .......................................... 27

3.1. Preparation of Material ........................................ 27
  3.1.1. Preparation of Minced Fish ............................ 27
  3.1.2. Preparation of Surimi .................................. 28
  3.1.3. Preparation of the Gels ................................ 29
3.2.1. Rheological Assessments using the Bohlin Rheometer ........................................... 32
3.2.2. Preliminary Studies on the Bohlin Rheometer ..................................................... 33
3.2.3. Rheological Assessment using the Ottawa Texture Measuring System ....................... 34
3.2.4. Microscopic Examination of Gel Samples ............................................................. 36
3.3. Statistical Analysis ................................................................................................. 38

RESULTS ......................................................................................................................... 39
4.1. Effects of Frozen Storage and Cryoprotectants on the Dynamic Rheological Properties of Gels Prepared from Mullet, Tullibee and Whitefish .................................................. 39
  4.1.1. Typical Curve ......................................................................................................... 39
  4.1.2. Changes in Tan Delta in Mullet, Tullibee and Whitefish During Storage ................. 40
4.2. Effects of Frozen Storage and Cryoprotectants on the Textural Properties of Gels from Mullet, Tullibee and Whitefish using Large Deformation Compression Testing ........................................ 50
  4.2.1. Typical Curve ......................................................................................................... 50
  4.2.2. Changes in Rigidity in Mullet, Tullibee and Whitefish During Storage ................. 53
4.3. Effects of Frozen Storage and Cryoprotectants on Microstructure .................................. 61
  4.3.1. Preliminary Rating Scheme ..................................................................................... 61

DISCUSSION ..................................................................................................................... 71
5.1. Correlation of Rheology and Microstructure ............................................................. 71
5.2. Effect of Storage Time ............................................................................................. 73
5.3. Relative Effectiveness of Cryoprotectants ............................................................... 83
5.4. Effect of Species ........................................................................................................ 87

CONCLUSION ............................................................................................................... 90

LITERATURE CITED ..................................................................................................... 93

APPENDIX 1 .................................................................................................................... 98
APPENDIX 2 .................................................................................................................... 99
LIST OF FIGURES

3.1. Processing method for freshwater surimi . . . 31

4.1. Typical rheogram for mullet, obtained using the oscillatory mode on a Bohlin rheometer . . . . . . . . . . . . . . . . . . . 41

4.2. Effects of cryoprotectants on the tan delta of mullet, tullibee and whitefish at week 0. . 42

4.3. Effects of cryoprotectants on the tan delta of mullet, tullibee and whitefish at week 2. . 44

4.4. Effects of cryoprotectants on the tan delta of mullet, tullibee and whitefish at week 4. . 45

4.5. Effects of cryoprotectants on the tan delta of mullet, tullibee and whitefish at week 6. . 46

4.6. Effects of cryoprotectants on the tan delta of mullet, tullibee and whitefish at week 8. . 47

4.7. Effects of cryoprotectants on the tan delta of mullet, tullibee and whitefish at week 12. . 48

4.8. Effects of cryoprotectants on the tan delta of mullet, tullibee and whitefish at week 16. . 49

4.9. Typical curve for whitefish, obtained using the Ottawa Texture Measuring System . . . . . 51

4.10. Effects of cryoprotectants on rigidity of mullet, tullibee and whitefish at week 0. . . 54

4.11. Effects of cryoprotectants on rigidity of mullet, tullibee and whitefish at week 2. . . 55

4.12. Effects of cryoprotectants on rigidity of mullet, tullibee and whitefish at week 4. . . 57

4.13. Effects of cryoprotectants on rigidity of mullet, tullibee and whitefish at week 6. . . 58

4.14. Effects of cryoprotectants on rigidity of mullet, tullibee and whitefish at week 8. . . 59

4.15. Effects of cryoprotectants on rigidity of mullet, tullibee and whitefish at week 12 . . 60
4.16. Effects of cryoprotectants on rigidity of mullet, tullibee and whitefish at week 16 . . 62
4.17. Ratings of 1 and 2 for microstructural evaluation of gels. . . . . . . . . . . . . . . . . 64
4.18. Ratings of 3 and 4 for microstructural evaluation of gels. . . . . . . . . . . . . . . . 65
4.19. Rating of 5 for microstructural evaluation of gels . . . . . . . . . . . . . . . . . . . . . 66
5.1. Effect of storage on tan delta of mullet. . . 74
5.2. Effect of storage on rigidity of mullet . . . 76
5.3. Effect of storage on tan delta of tullibee. . 77
5.4. Effect of storage on rigidity of tullibee . . 79
5.5. Effect of storage on tan delta of whitefish. . 80
5.6 Effect of storage on rigidity of whitefish. . 81
<table>
<thead>
<tr>
<th>Table</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1.</td>
<td>Preliminary Results from Bohlin Rheometer</td>
<td>35</td>
</tr>
<tr>
<td>4.1.</td>
<td>Microscopic Ratings for Mullet Gels Over 16 Weeks</td>
<td>67</td>
</tr>
<tr>
<td>4.2.</td>
<td>Microscopic Ratings for Tullibee Gels Over 16 weeks</td>
<td>69</td>
</tr>
<tr>
<td>4.3.</td>
<td>Microscopic ratings for Whitefish over 16 Weeks</td>
<td>70</td>
</tr>
</tbody>
</table>
INTRODUCTION

Surimi is a Japanese term for the wet frozen concentrate of the myofibrillar proteins of fish muscle. It is prepared by repeatedly washing mechanically separated fish flesh with chilled water until it becomes odorless and colorless or technically until most of the water soluble protein is removed (Lee, 1984). It is used as the base ingredient for a wide variety of seafood analogues, since manipulation with other ingredients can result in products with very different textures. At 40 °C, this product is a fine translucent, elastic gel, but it is transformed into an opaque rigid gel upon further thermal processing (Lanier, 1986). Research done to determine the chemical changes which occur during this transformation has revealed the interaction of hydrogen, hydrophobic and disulfide bonds.

This fish base has been used in the formulation of a variety of products by the Japanese for centuries. However, it was not until the late 1950’s, prompted by the over-fishing of near-shore areas, that technologies were developed which enabled the stockpiling of surimi (Pigott, 1986). This technology included the development and availability of modern freezing and cold-storage facilities for large and small plants and on-board ships. As well, it was discovered that frozen surimi could be stabilized by the use of cryoprotectants (Matsumoto, 1980). Aided with this new technology, and the development of factory-ships,
the Japanese were able to commercialize and process off-shore fish (Lee, 1984). Alaskan pollock, which was previously underutilized, was found to have good quality flesh and was ideal for large scale harvesting (Pigott, 1986).

Today, many countries are investigating the different techniques required to make surimi from a particular, locally available species. The University of Manitoba Food Science Department in cooperation with the Federal Department of Fisheries and Oceans have been investigating the potential for using freshwater fish in surimi production. For this study, whitefish (*Coregonus clupeaformis*), tullibee, (*Coregonus artedii*) and mullet (*Catostomus commersoni*) were examined. The marketability of these species has been difficult in the past as a result of parasite infestation, and thus the potential for utilizing them in a minced form was great.

The objectives of this study were;

1) To determine the effects of various cryoprotectants or cryoprotectant combinations on the gel forming capacity of surimi from the three freshwater species. This was achieved by correlating rheological results as measured by the Bohlin rheometer and the Ottawa Texture Measuring System, with actual structural characteristics as observed by microscopy.

2) To monitor textural changes in the surimi from the three species over a four month storage period at -40°C. This was done from a rheological and microscopic perspective.
REVIEW OF LITERATURE

2.1. Fish Protein Potential

Fish is an excellent source of protein. The protein itself possesses many functional properties and is known in part for its gel forming capacity. This ability makes fish protein competitive with the more traditional animal and vegetable proteins. The development of surimi technology, the semi-processed wet fish protein, is still in its infancy on this continent. In contrast, the Japanese have been improving and experimenting with fish proteins for hundreds of years. Much of what is known relies on Japanese research and deals mainly with saltwater species (Martin, 1986). The numerous underutilized freshwater species found in Manitoba provides an extensive supply of starting material, about which very little is known with respect to surimi processing.

The main product of surimi technology is kamaboko which is the generic term for the elastic or rubbery Japanese-style fish cakes. It is made from minced fish to which starch has been added as a thickening agent, and sugar and monosodium glutamate added for flavoring. Salt is an essential ingredient; its role is to solubilize the
protein, enabling the formation of a gel upon heating. The fish cakes are marketed in a variety of forms, textures, flavors and colors. The Japanese refer to the fish cakes differently depending upon their shape and the method of cooking, (Suzuki, 1981). For example, kamaboko is used for the elastic fish cakes cooked mainly by steaming, whereas chikuwa are tubular shaped fish cakes cooked by broiling and satsumaage are deep fried fish cakes of various shapes.

2.2. Factors Influencing Surimi Quality

2.2.1. Species

Fresh surimi can be made from more than 60 different fish species, including tuna, mackerel, croaker, and shark, with each species requiring slightly different processing techniques (Lee, 1984). Alaskan pollock is the predominant species which is commercially processed into surimi and sold in North America; however, a number of species are processed and sold in Japan.

Kudo et al. (1973), tested a variety of Pacific coast species of fish to determine their gel-forming capacity. The gel forming capacity was evaluated in terms of elasticity and flexibility by the traditional Japanese folding test and by sensory analysis to evaluate firmness and cohesiveness. In this study both washed and unwashed flesh were monitored. This group found that
lingcod, pacific cod, rockfish and some sharks possessed good gel forming properties, while flounder, hake and dogfish did not.

In another study, Holmquist et al. (1984), looked at the suitability of red hake, caught in the Atlantic, for kamaboko production. Samples of fillets, minced fish and surimi stored at -5°C, were the starting materials for this study. Tests evaluating the gel forming ability were similar to those conducted by Kudo’s group and included the fold test, moisture content, expressible water as well as the quantitation of salt-extractable protein. At this elevated temperature, the group found that acceptable kamaboko could only be manufactured from fillets if stored less than two weeks, from minced fish up to two weeks and from surimi up to 7.5 weeks of storage.

Cheng et al. (1979) experimented with four species - Atlantic croaker, mullet, ribbon and sand trout, which were harvested from the Gulf of Mexico. In order to investigate the species effect on the properties of raw fish and processed gels, a number of techniques were used. These included SDS polyacrylamide gel electrophoresis (SDS-PAGE), water holding capacity and textural properties as determined by a sensory panel and an Instron Universal testing machine. They found that the concentration of soluble myofibrillar proteins, particularly myosin, in raw fish tissue was not closely related to the texture of
gelled products among species. However, with regard to water holding capacity, they found it to be closely related to gel structure. This varied significantly with species. In those gels with a higher percentage of weight loss, the texture of the gel was firmer and springier. Inter-species differences in texture were also found in terms of cohesiveness and soluble proteins of cooked gels.

A study conducted by Shimizu et al. (1981) investigated 49 species, including four species of freshwater fish, four species of cartilaginous fish and one species each of squid, prawn, chicken and rabbit. These investigators studied the temperature gelation curves of each species as well as the gel strength as measured by a tensile test on a Shimizu tensile tester. Based on these assessments, the fish were divided into four categories, depending on the ease or difficulty of setting and disintegrating. For example, Alaskan pollock, croaker and other cold water fish were classified into the easy setting/ easy disintegrating group whereas sharks, chicken and rabbit were part of the other extreme, difficulty in setting and disintegration. The third category, easy setting and difficult disintegration consisted of fish like barracuda, grub fish and flying fish whereas the red meat fish like mackerel and tuna made up the final category of difficult setting and easy disintegration.
Investigations reveal the limited potential for surimi processing, despite the great availability of underutilized fish species. This is understandable as different fish species have very different muscle tissue - even within a species there is considerable textural variation.

Some of the causes for this variation are thought to be due to seasonal effects, nutritional status and location of fishing grounds (Howgate, 1977). In addition, Dunajski (1979), has stated that size, maturity, sex and activity of the species also affects texture.

The seasonal effect is interrelated with several other factors. For example, from spring until fall there exists an abundance of food and the fish are able to build up their lipid and protein reserves which they rely on for the remainder of the year when food is scarce. Dyer and Dingle (1961) found that lean fish such as lizard fish, cod and haddock with a fat content of less than 1% were less stable and showed a fairly rapid decrease in protein extractability in comparison to fatty fish. This led Dyer to hypothesize that moderate levels of lipids may protect the fish proteins or increase their resistance to denaturation during frozen storage.

In addition the season of the year may also affect the species activity, such as its reproductive cycle. During
spawning, the ratio of moisture to protein in the flesh is high, resulting in a watery mince of poorer quality.

2.2.2. Cryoprotectants

Denaturation of the myofibrillar proteins plays a dominant role in the functional quality changes of frozen stored meats (Matsumoto, 1980). In order to inhibit or retard the amount of denaturation, a number of researchers have experimented with various chemicals. In the early 1960's, Japanese researchers proposed and experimented with sucrose and polyphosphates. They found that these additives exerted a cryoprotective effect on the proteins. These findings led others to investigate possible cryoprotectants and their mode of action.

The cryoprotective effects of 150 organic compounds were evaluated by Noguchi and Matsumoto (1975). The assessment of these compounds was based on their solubility, viscosity, ATPase activity and kamaboko forming capacity. Of the 150 compounds tested, 30 were found to have a marked effect; these included aspartate, cysteine, dicarboxylic acids, hydrocarboxylic acids, poly alcohols, carbohydrates and polyphosphates. Twenty of the compounds tested showed only a moderate effect and these included lysine, histidine, ornithine and serine. The remaining compounds had little cryoprotective effect.

These findings led Matsumoto (1980) to propose
criteria for good cryoprotectants. These were as follows:

1/ The molecule has to contain one essential group, such as -OH, -COOH, -OPO$_3$H$_2$ and one supplementary group such as -OH, -COOH, -NH$_2$, -SH, -SO$_3$H or -OPO$_3$H$_2$. 

2/ The functional groups must be suitably spaced and properly oriented with respect to each other.

3/ The size of the molecule must be comparatively small.

In a related study, Park et al. (1987) investigated the effects of cryoprotectants on minimizing the physicochemical changes in bovine actomyosin during frozen storage. In this study, sorbitol, a polyhydric alcohol and polydextrose, a polymer of dextrose containing minor amounts of sorbitol and citric acid, were evaluated by the decrease in soluble actomyosin. This parameter was considered to be an acceptable index for the activity of the cryoprotectants. Park and co-workers (1987), found that the control samples experienced a 35% loss in solubility, whereas, the polydextrose samples only decreased by 22% and the sucrose/sorbitol by 18%. These findings support Matsumoto (1980) who regards a decrease in the amount of soluble actomyosin as a primary criterion of freeze denaturation.
Based on these findings Park et al. (1987) stated the five properties which are common to effective cryoprotectants:

1/ Low volatility
2/ Ability to readily penetrate the cell membrane
3/ Considerable solubility in H₂O, with each molecule having the ability to form multiple hydrogen bonds.
4/ At least some capacity to dissolve electrolytes.
5/ Possession of at least one essential functional group i.e. COOH or OH.

2.2.3 Enzymes

The role of destructive enzymes in textural loss of surimi is an important issue which has been investigated. Lanier et al. (1981) found that the presence of heat-stable protease significantly decreased the gel strength of Atlantic croaker, (as evaluated as hardness) with the Instron Universal Testing Machine. This decrease in gel strength was particularly evident when the processing temperature was in the 50° to 70° C range. In this range, the enzymes disrupt the textural strength and hardness of the gels. This softening phenomenon has been termed "modori" and occurs most often in unwashed fish tissue, where much of the water soluble protein and protease still remain (Suzuki, 1981). Lanier and his associates concluded that proteolytic enzymes play an important role in the
ability of different fish samples to bind water and to produce a firm texture when heat processed.

In another study, Cheng et al. (1979) found that alkaline protease exhibited maximum effects around 60°C, the temperature of optimum proteolytic activity. As this resulted in a decrease in firmness, they concluded that this loss of texture was due mainly to proteolytic degradation of the fish muscle proteins.

The enzyme trimethyl amine oxidase has also been studied with regard to minced fish (DaPonte et al., 1985). This enzyme has been found to be involved in the production of formaldehyde and dimethylamine during frozen storage. It is believed that formaldehyde affects gel formation by contributing to textural toughening and the loss of water holding capacity of the fish muscle. Dimethyl amine may also cause flavour impairment. It was found that heat treatments of up to 80°C or more for short periods of time significantly reduced the production of both formaldehyde and dimethylamine during frozen storage and led to improved elasticity and flavour.

2.2.4 Storage Conditions

Suzuki (1981) showed a decrease in gel-forming abilities of fish proteins which were subjected to fluctuating temperatures, known as freeze-thaw cycles,
during frozen storage. If fish, stored over a long period of time was thawed, quality deterioration resulted. This led to flavour loss, and softening of texture. The major cause of such changes was believed to be the freeze denaturation of myofibrillar proteins. Dyer and Dingle (1961) define denaturation in frozen fish muscle as a change in the protein, such that it is no longer soluble or extractable by salt solutions under conditions in which the native protein is soluble or extractable. This decrease in solubility is due to alterations in the myofibrillar fraction, and results in reduced emulsifying capacity, lipid-binding capacity, water-holding capacity, and gel-forming capacity compared to the fresh form of the fish.

Kim et al. (1986) found that gel strengths were largely dependent upon both processing temperature and the number of freeze-thaw cycles. Some of the negative effects of freezing and thawing on gel strength could be reduced by pretreatment (setting) at either 4°C or 40°C prior to the final cook at 90°C. Such a pretreatment increased the strength and formability of the protein gels.

Several guidelines have been proposed to help maintain the quality of frozen fish (Suzuki, 1981). These include: rapid freezing to the appropriate storage temperature, storage at the lowest possible temperature, keeping the storage period to a minimum, avoiding freeze-thaw cycles and using fresh fish where possible.
2.3 Gelation

Aggregation refers to protein-protein interactions, whereas coagulation is defined as random aggregation which includes heat denaturation of protein molecules. Gelation is defined as aggregation during denaturation with the formation of a continuous protein structure (Acton et al., 1981).

Fish muscle protein is of three types: sarcoplasmic, connective tissue and myofibrillar (Suzuki, 1981). The sarcoplasmic proteins are found in the cytoplasm and are made up primarily of the water soluble myogens. The second type of proteins are found in the connective tissue and consist of elastin and collagen and make up 1.3% to 1.6% of the total protein. The third category, myofibrillar proteins, include myosin, actin and the regulating proteins tropomyosin, troponin and actinin. As a group, these myofibrillar proteins make up 66% to 77% of the protein in fish and as such, they play an important role in gel formation.

The washing step of the surimi process is necessary to remove blood, pigments, lipids and enzymes from the minced fish. In addition, it also serves to remove the sarcoplasmic proteins. This is important, as researchers have found that they adhere to the myofibrillar proteins when the fish meat is heated, severely impeding the
formation of gels (Suzuki, 1981).

The grinding of surimi with salt, usually between 2.5 and 4 %, is a crucial step in gel formation. In a study conducted by Lanier et al. (1980), a number of machines were evaluated with respect to their ability to maximize protein solubilization and thus gel strength. Several of the mixers and microcutters were found to be acceptable. However, the time of comminution was found to vary greatly, ranging from five minutes to almost an hour. Salt serves to extract the salt-soluble natural actomyosins, which are essentially the actin, actomyosin, tropomyosin and troponin. The extent to which each of these proteins contributes to gel formation has been studied by a number of researchers in both pure systems and in actual foods. A number of conflicting results exist.

Fukazawa et al. (1961) postulate that actin and tropomyosin do not interfere with the binding quality of sausage. However, Nakayama and Sato (1971) found an increase in heat gelling ability of meat minces when F-actin and tropomyosin were present. The interaction of F-actin and myosin occurs through the head portion of myosin and the resulting complex molecule is then surrounded by the tail portion of the myosin molecule. The optimum conditions for this interaction have been found to be a ratio of 1.5 moles myosin to 2 moles of actin, a temperature near 40° C, a pH of approximately 6.0 and a
salt concentration of 0.6 Molar (Acton et al. 1981).

Fish meat paste made by grinding meat at low temperatures with salt results in a slightly elastic transparent gel which the Japanese have called "suwari". Samejima and Hashimoto (1969) and Samejima et al. (1980) proposed that it was the myosin tail portion that was primarily involved in this network formation. The tail partly unfolds as temperature increases, going from an orderly helical conformation to a random coil. They also proposed that gelation was first initiated by disulfide cross-linking of the myosin head portion prior to unfolding. However Niwa et al. (1982, 1983), concluded that such setting results from localized exposure of hydrophobic amino acid residues leading to hydrophobic interactions.

Based on differential scanning calorimetry, Wu et al. (1985) have found transitions at 43°, 54° and 71° C in fish gels. The first two transitions were attributed to myosin denaturation. It has been found by other researchers (Montejano et al., 1984) that these temperatures (43° and 54°) correspond to the helix-coil transition of the myosin rod. The low temperature is thought to be the point at which aggregation of both the myosin head and the tail portion occurs. The 71° C transition temperature is thought to be the point of actin denaturation.
Investigators have experimented with setting gels at two different temperatures (Lanier, 1986). The first near 40°C, known as the high temperature setting and the other near 0°C known as the low temperature setting. Kim et al. (1986), using these different setting times, produced gels with different degrees of gel strength following processing at 90°C. Many chemical changes occurred with heating. Between 10°C and 30°C it is believed that disruption of hydrogen bonds among and between water and protein molecules leads to partial unfolding. Pontekhin et al. (1979) postulated that the high temperature setting (40°C) caused thermal denaturation (unfolding) of the myosin, with subsequent formation of a network due to the aggregation of unfolded molecules. Setting of fish can thus be viewed as a process in which the solubilized proteins partly unfold as temperature increases and then interact to form a fine elastic translucent gel network. At high temperatures (80°C to 90°C), the gel transforms from being fine, translucent and elastic to opaque and rigid, indicating the occurrence of coagulation. This phenomenon has been attributed to the fibrous myofibrillar proteins undergoing a conformational change and groups on the molecular surface interacting to form a 3-dimensional network.
2.4. GEL TESTING METHODS

2.4.1. Traditional

The gel-forming capacity of the fish proteins was traditionally measured by the quality of the kamaboko which was prepared. Kamaboko with strong ashi is recognized by its glossiness, elasticity and palatability. It does not easily break under pressure or from stretching and it has a strong binding potential.

A. Fold Test

The simplest test used to evaluate kamaboko is the Fold Test. Suzuki (1981) describes this test by folding a 375mm thick piece in half. If no cracking occurs along the fold it is again folded perpendicular to the first fold. If cracking is still not evident, a rating of AA is given to the sample. However, if cracking is evident the rating is reduced to an A. If cracking shows on part of the folded edge when it was initially folded, the rating given is a B. If the crack runs through the folded edge the sample is designated as a C. A rating of D is given if the gel completely breaks apart.
B. Expressible Water

Another simple test used to evaluate the gel forming capacity has been outlined by Kudo et al. (1973) and is termed expressible water. The water holding capacity was determined as follows: a slice of kamaboko (2 cm in diameter by 0.3 cm thick) was placed between two filter papers and pressed by a small oil compressor under a fixed pressure (10Kg/cm²) for 20 seconds. The weight difference, before and after pressing, reported as a percentage reflects the amount of expressed water.

C. Sensory Evaluation

Another test which is usually carried out is sensory evaluation. For this, trained panelists rate the kamaboko with respect to cohesiveness, firmness and moistness. A ten point scale is used with 9 - 10 being excellent, 7 - 8 being good, 5 - 6 standard, 3 - 4 substandard and 1 - 2 as poor.

D. Instrumental Methods

Two devices have been used extensively by the Japanese to evaluate the gel structure of surimi. The first is known as an Okado gelometer, (Suzuki, 1981). For this device, a test piece of about 25 mm thickness is placed under a plunger with a round top. By applying pressure with a plunger, the testing material is gradually deformed
and broken. The stress/strain curve produced is recorded by a kymograph and allows for the calculation of ashi strength, as 1/2 the product of load and degree of strain.

The second instrument is one which evaluates gel forming capacity based on the measurement of tension (Suzuki, 1981). In this test a piece of kamaboko is cut in a ring and attached to two hooks and stretched a rate of 0.3 mm/sec until it breaks. It is then possible to calculate tension according to this relationship:

\[ E = \frac{F}{A} \times \frac{1}{\Delta l} \]

where:
- \( E \) = tension (dynes/cm\(^2\))
- \( F \) = stretching power (dynes)
- \( A \) = cross section area (cm) of sample
- \( l \) = length (cm) of sample before stretching
- \( \Delta l \) = elongation length (cm) of sample

2.4.2. Modern/Conventional Methods

Aside from these traditional methods, there also exists more sophisticated instrumental methods which make use primarily of the Instron Universal tester.

One method involves placing a 2.54 cm thick piece of specimen on a compression table and lowering a compression anvil twice. The two stroke method is used to simulate
chewing. The first compression stroke reduces the specimen to 70% of its original height, whereas the second stroke compacts it to 50%. This test, known as textural profile analysis, enables a number of parameters to be calculated. These include hardness, cohesiveness, springiness, gumminess, and chewiness (Szczesniak, 1963).

Another device used to analyze the gel strength, is called a thermal scanning rigidity monitor (Montejano et al., 1984). As the name implies, this device monitors the rigidity modulus of a fish paste as it undergoes thermal processing. In this device, the fish paste is packed into two cells, the unit is then attached to a bath which circulates water through the walls of the cell. At given intervals, usually every two minutes, a force is applied to the cells and the rigidity modulus is subsequently calculated.

Lee and Toledo, (1976) measured the textural properties of gels using attachments which were compatible with the Instron. One of these methods involved using the Warner Bratzler Shear device, which measures shear strength. This device consists of a blade having a triangular opening to hold the specimen and two shear bars. Another device which was used was a puncture apparatus. For this test, the puncture force (force required for an incisor shaped punch to break through a specimen,) was measured.
Another instrument, which may be used for the assessment of fish gels is the Bohlin rheometer. This instrument is used for the continuous measurement of the viscoelastic properties of gels. Dynamic testing by this method, subjects the sample to a harmonic shear strain of a fixed frequency. The varying deformation in the sample yields corresponding shear forces in the sample, and the resulting shear stress will be in harmony with the same frequency as the applied shear strain. In an elastic sample the stress will be in phase with the strain, but in a viscous liquid the stress will be out of phase. By monitoring the phase shift, the relative contribution from elastic and viscous flow in a gel can be measured. The ratio $G''/G' = \tan \delta$, gives a direct measure of the relative importance of viscous and elastic effects in the sample (Bohlin et al. 1984).

2.5 Consumer Interest

Traditionally, surimi was prepared from fresh fish and immediately processed into kamaboko products. However in 1959, a group of scientists at the Hokkaido Fisheries laboratory discovered that the surimi could be stored if cryoprotectants were added prior to freezing (Matsumoto, 1988). This discovery, plus advanced technology in equipment design, opened the door for surimi advancement into the world marketplace.
Okado et al. (1973) have stated the following reasons as factors for the growth of kamaboko production in Japan, but they can also be applied to North American culture:

1/ With the recent rise in incomes, changes in dietary patterns resulted in a greater consumption of proteinaceous and ready to eat types of foods.

2/ Appearance and flavor of kamaboko can be easily altered to meet consumer demands by adding various ingredients to the minced flesh.

3/ Underutilized species and fish having low acceptance in the fresh state can be used successfully as raw materials.

4/ Recent development in processing machines permit large-scale production.

5/ Keeping quality has been improved with recent advances in packaging and processing.

6/ Basic studies on fish muscle protein has hastened the development of a technology that improves quality and the economics of production.

The largest surimi producers today are Japan, the United States and Canada (Pigott, 1986). The main fish utilized are white fleshed species particularly Alaskan pollock. Several European countries are expected to become significant surimi producers in the future. As well, in New Zealand, species such as blue whiting and hoki show promise as raw materials. Furthermore, efforts are currently being made to adapt the surimi process for fatty
fish species such as mackerel, sardines and herring. The plants which are in operation in the United States and Canada produce a variety of seafood products which includes formed crab legs, scallops, lobster and shrimp as well as a flaked form of crab. The manufacturers suggest that the analogues can replace the real seafood in a variety of recipes. In addition, many restaurants have made the substitution where feasible.

Consumer interest and awareness in surimi-based products is steadily increasing. Ten years ago you would have had a problem trying to buy a surimi-based product in the supermarket, but today there exists a wide variety of products. In 1985, 60 million pounds of surimi was consumed in North America in one form or another and it is predicted that this figure could rise to 1 billion by 1990 (Roche, 1985). There has to be a reason for this surge of interest in surimi. J A C Creative Foods, producers of "Krab Legs", claim their product is healthier for consumers than real crab. This is substantiated by the fact that real crab has thirty times as much cholesterol as the new product (Havighorst, 1984). Dr. Ho, of Terra Nova Fisheries in Newfoundland (Roche, 1985), feels that his product, a simulated crab leg, has capitalized on two North American trends - a shift away from high caloric, high cholesterol foods and the growing popularity of pre-cooked snacks, the so called finger foods.
2.6. **Potential of Freshwater Fish**

Lately, many investigations have been made regarding kamaboko forming abilities of various deep sea fishes. Although several of these researchers have referred to the potential for freshwater fish species, almost no papers exist in the literature.

There exists several differences between the two fish categories. Anatomically, freshwater fish and marine fish vary in their gill and kidney systems (Harder, 1975). Body fluids of freshwater fish are hypertonic to their milieu, (i.e. they have a higher osmotic pressure than the surrounding water), whereas, marine fish are hypotonic. Since different osmotic pressures strive to become identical, freshwater fish are exposed to the danger of a steady influx of water into their bodies. Marine fish are subjected to the opposite danger, namely that of drying out physiologically, since water tends to be drawn from their bodies. Since the respiratory function of the gills require that they always remain in contact with the aqueous milieu - they also act as the sites of osmotic exchanges. The kidney system of freshwater fish has glomeruli which are responsible primarily for the retention and excretion of excess water.
Biologically freshwater fish and marine fish have another difference. Marine species contain an endogenous enzyme that is responsible for the breakdown of trimethylamine oxide, to dimethylamine and formaldehyde. It has been suggested that formaldehyde causes cross-linking of muscle proteins, rendering them insoluble and causing flesh to be tough (Hultin, 1976).

Knowledge of the amino acid composition of freshwater fish is limited (March et al., 1967). A study conducted by Mai et al. (1980) looked at six species of freshwater fish, to determine if variation existed in protein content and amino acid composition. The fish studied included white sucker, burbot, black crappie, rainbow trout, walleye pike and yellow perch. The protein content was found to range from 15.83 to 18.29 g per 100 g, with white suckers having the lowest value and rainbow trout the highest. The study also revealed no significant differences among species with respect to amino acid analysis. The researchers concluded that no major difference existed between the amino acid composition of freshwater fish compared to those of marine (Mai et al., 1980).

Manitoba lakes contain numerous species of freshwater fish that are underutilized for one reason or another. For many, the fish are infected with the tapeworm Triaenophorus crassus, which although harmless to man, are unsightly and destroy their market value. The three fish
chosen for this study, parasitized whitefish, tullibee and mullet, are just a sampling of the species to be caught. From the little information found in the literature, there is no evidence against the use of freshwater fish in the expanding surimi industry. The aim of this study is three-fold; to study the potential of gel formation from freshwater fish, to monitor changes during frozen storage, and to record the impact of cryoprotectants on these changes.
3.1 Preparation of Materials

Whitefish, (Coregonus clupeaformis), tullibee (Coregonus artedii) and mullet (Catostomus commersoni) obtained from the Freshwater Fish Marketing corporation served as the raw materials for this research project. The fish caught in various locations, were gutted by the Marketing Corporation. The fish were then packed on ice and shipped to the Freshwater Institute for further processing.

3.1.1 Preparation of Minced Fish

The whole dressed fish were beheaded (i.e. if not previously done) and then scraped thoroughly to remove the viscera. The fish were then washed and subsequently split along the backbone. The fillets were deboned by placing them scale side down onto the conveyor belt of a Baader 694 Flesh Separator, equipped with a 5mm perforated drum.

After deboning, the minced fish was weighed and separated into two portions. One portion was destined to have no cryoprotectants added during its processing; as a result, this fraction was designated as "fresh". The second portion was destined to have specific levels of tripolyphosphate, sucrose, and sorbitol added to the mince after the final washing. The minced fish was washed with water, 4 times its weight (w/w) for 5 wash cycles. Each
water-fish mixture was gently stirred for two minutes with an aluminum paddle. The purpose of the washing step was to remove the blood, pigments and water soluble components as well as lipid materials from the fish flesh. Following stirring, the mixture was allowed to settle for a further two minutes, after which the water was decanted. Prior to the final stir, 0.1% (by weight) food grade NaCl was added to the appropriate fractions.

The washed flesh was then poured into fine mesh polyester bags, and these were allowed to drain for one hour. The bags were kneaded to assist in water removal. After draining, the flesh was again subjected to the Baader Flesh Separator, in order to remove excess moisture and other undesirable materials. For this process, a 3 mm diameter perforated drum was used.

3.1.2. Preparation of Surimi

The minced, dewatered flesh was now ready to be processed further into surimi by adding various cryoprotectants. The "fresh" portion or the control, was immediately packed into a metal tray and placed into a plate freezer. The remaining minced fish, to which sodium chloride had been added in the final washing step, was divided into 4 equal portions. Each portion was weighed and placed into chilled mixing bowls which were encased in ice. To each of these four samples, 0.1% sodium tripolyphosphate (TPP) was added. In addition, 1% sucrose
was added to sample number two, 5% sucrose was added to sample number three and 4% sucrose and 4% sorbitol were added to sample number four, (Figure 3.1). The last sample was considered to be equivalent to a "traditional" surimi mixture. (Suzuki 1981, describes typical surimi made from Alaskan Pollock as containing 4% sucrose, 4% sorbitol, 0.35% polyphosphate and 0.3% sugar alcohol.) The samples were mixed for 15 minutes at low speed using a Kitchen Aid mixer. Following mixing the blended surimi was packed into metal trays and frozen in a double plate Dole Freeze-Cel contact freezer for four hours.

After freezing, the samples were cut using an electric saw into appropriate sized blocks. These were then covered with two layers of cellophane wrap and placed in freezer bags. The labelled bags were stored at -40° C, until required for experimentation.

3.1.3. Preparation of the Gels

At two week intervals, samples of each of the fish containing the various types of cryoprotectants were thawed. The labelled samples were allowed to thaw at room temperature for four hours and then refrigerated overnight. Sodium chloride (1.5% by weight) was added to approximately 200 g of each of the samples. Each sample was then mixed for 15 minutes, using a Kitchen Aid mixer at -18° C. This was accomplished by placing the mixer in the freezing compartment of a refrigerator.
FIGURE 3.1  Processing method for freshwater surimi
WHOLE DRESSED FISH

BEHEADED AND SPLIT INTO FILLETS

DEBONED USING BAADER 694 FLESH SEPARATOR

MINCE - WASHED 5 TIMES

MINCE - SALTED AND WASHED

MINCE - DEWATERED

FRESH 0.1% TPP 0.1% TPP 0.1% TPP 0.1% TPP
1% SUCROSE 5% SUCROSE 4% SUCROSE 4% SORBITOL

PACKED INTO BLOCK FORM

FROZEN IN PLATE CONTACT FREEZER

CUT INTO PORTIONS AND WRAPPED

HELD AT - 40°C
The fish gels were then firmly packed, to minimize the number of air bubbles, into glass jars which had been sprayed with PAM (a nonstick aerosol). The jars were placed in water and cooked for 25 minutes at 90°C. The cooked gels were chilled in a pan of cold water, removed from the jars and placed into labelled freezer bags and refrigerated overnight.

3.2.1. Rheological Assessments using the Bohlin Rheometer

Samples for the Bohlin VOR rheometer were prepared by thinly slicing the cylindrical fish gels on a Hobart meat slicer. The meat slicer width setting was set at 8 which gave slices of approximately 1.75 mm in thickness. The slices were cut into 35 mm diameter discs using a steel cookie cutter. The set-up used for the sample was two parallel plates with a torque element of 93.2 cm.g. All tests were performed at room temperature (25°C), in triplicate. By programming the Bohlin rheometer in the oscillatory mode, the storage modulus, G', and the loss modulus, G'', were monitored over a shear strain frequency sweep of 0.1 to 20 Hz at an amplitude of 5%.

To calculate the loss tangent, tan delta, which is the ratio of energy lost to energy stored during deformation, the following equation was used:

\[
\text{Tan Delta} = \frac{G''}{G'}
\]
3.2.2 Preliminary Studies on the Bohlin Rheometer

In order to optimize the conditions for the samples required for the Bohlin Rheometer, two preliminary experiments were conducted. The first involved determination of the influence of sample thickness on final results. For this, mullet samples containing only 0.1% sodium tripolyphosphate (TPP) and tullibee samples with the traditional blend (i.e. 0.1% TPP, 4% sucrose, and 4% sorbitol) were studied. These samples were cut on a Hobart meat slicer to various thicknesses (1.75, 2.0, 2.5 mm), and tested in duplicate on the rheometer. As can be seen from table 3.1 B and C, very little difference was noted between the thicknesses of 1.75, 2.0 and 2.5 mm at the lower amplitudes. To see if this trend held for thinner samples, gaps of 1.0 and 1.5 were also tried. Results for these thinner samples proved to be quite variable, (Table 3.1 A). Therefore a thickness of 1.75 mm was chosen for this experiment.

A second study was conducted to determine the influence of amplitude on experimental results. The same samples were used as in the thickness study, however the amplitude was changed from 2 to 50 % at varying intervals. For the mullet very little change in tan delta was found to exist before the amplitude reached 30% (Table 3.1 B). However in the tullibee sample dramatic changes became evident at amplitudes of 15% (Table 3.1C). To ensure
that amplitude was not impacting on the experimental results an amplitude of 5% was chosen.

3.2.3. Rheological Assessment using the Ottawa Texture Measuring System

A steel cookie cutter was used to bore out central core samples from the gels prepared in 3.1.3. The length of the gel sample was adjusted to 2.5 cm. Each sample was then vertically oriented in the bottom of the compression cell. The cell consisted of a circular table, mounted on a plate, which slid into the base. This formed a flat surface to support the test sample. An identical table mounted on a shaft, which fitted into the crosshead chuck, served as the compression cell plunger.

The transducer capacity was achieved by using an 11.36 kg (25 lb) load cell. The expected maximum force was set at 10 N, and the test time was set at 30 seconds. The crosshead down speed was timed and set at 10.90 cm/min which corresponded to a setting of 40 on the dial. A computer program (version April, 1986) from the Engineering and Statistical Research Institute in Ottawa was used in conjunction with an Apple IIe computer to record resultant curves. The samples were compressed to 70% of their original height (i.e. the crosshead stops were adjusted to give clearance of .75 cm between the plates at maximum compression). Each sample was tested in triplicate and the rigidity (N/mm) was monitored.
### Table 3.1 Preliminary Results for Bohlin Rheometer

#### A. Tan Delta Value at 1.00 Hz for Mullet with 0.1% TPP Over a Variety of Amplitudes at Two Different Thicknesses (Gap)

<table>
<thead>
<tr>
<th>Amplitude</th>
<th>2%</th>
<th>5%</th>
<th>10%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gap</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.00</td>
<td>.432</td>
<td>.385</td>
<td>1.17</td>
</tr>
<tr>
<td>1.50</td>
<td>.066</td>
<td>.103</td>
<td>.531</td>
</tr>
</tbody>
</table>

#### B. Tan Delta Value at 1.00 Hz for Mullet with 0.1% TPP Over a Variety of Amplitude Ranges and at Three Different Gel Thicknesses (Gap)

<table>
<thead>
<tr>
<th>Amplitude</th>
<th>2%</th>
<th>5%</th>
<th>10%</th>
<th>15%</th>
<th>20%</th>
<th>30%</th>
<th>50%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gap</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.75</td>
<td>.224</td>
<td>.225</td>
<td>.221</td>
<td>.224</td>
<td>.234</td>
<td>.288</td>
<td>.609</td>
</tr>
<tr>
<td>2.00</td>
<td>.248</td>
<td>.241</td>
<td>.229</td>
<td>.225</td>
<td>.228</td>
<td>.235</td>
<td>.242</td>
</tr>
<tr>
<td>2.50</td>
<td>.248</td>
<td>.246</td>
<td>.238</td>
<td>.236</td>
<td>.238</td>
<td>.266</td>
<td>.343</td>
</tr>
</tbody>
</table>

#### C. Tan Delta Value at 1.00Hz for Tullibee "Traditional" Over a Variety of Amplitudes and at Three Different Gel Thicknesses (Gap)

<table>
<thead>
<tr>
<th>Amplitude</th>
<th>2%</th>
<th>5%</th>
<th>10%</th>
<th>15%</th>
<th>20%</th>
<th>30%</th>
<th>50%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gap</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.75</td>
<td>.180</td>
<td>.180</td>
<td>.186</td>
<td>.235</td>
<td>.298</td>
<td>.546</td>
<td>1.14</td>
</tr>
<tr>
<td>2.00</td>
<td>.183</td>
<td>.184</td>
<td>.194</td>
<td>.272</td>
<td>.423</td>
<td>.614</td>
<td>1.03</td>
</tr>
<tr>
<td>2.50</td>
<td>.190</td>
<td>.193</td>
<td>.220</td>
<td>.330</td>
<td>.693</td>
<td>.860</td>
<td>1.13</td>
</tr>
</tbody>
</table>
According to Voisey et al, (1972), the slope of the linear portion of a compression curve is related to the elasticity properties of the sample. Within the elastic limit, where stress is directly proportional to strain, Young’s modulus of elasticity can be determined. This modulus is defined as the ratio of stress to strain, and is the slope of the stress - strain curve. Tung, (1987), has stated that different methods of stress application provide various moduli that describe a material. The modulus of elasticity or Young’s modulus, E, relates tensile stress to tensile strain; the shear modulus or modulus of rigidity, G, relates shear stress to shear strain; and the bulk modulus, K, relates hydrostatic stress to volumetric strain. For the compression testing in this study it is the modulus of rigidity as measured by the slope which will be studied.

3.2.5. Microscopic Examination of Gel samples

For this procedure, gel cubes (1 cm) were cut from the fish gels and embedded in Tissue-Tek on a metal sample holder. The holder was then placed at the bottom of an American Optical Cryocut II Microtome and allowed to freeze for 15 minutes. The temperature of the cryotome was maintained between -30° C and -35° C for the entire procedure. The samples, when completely frozen, were cut
into thicknesses of approximately 5 microns. For each sample a total of 9 sections were cut and attached to 3 labelled glass slides.

The microscopic samples were subsequently stained with the protein stain, Eosin Y (Fisher Lot 852148A). This was accomplished by exposing the slides to a series of solutions as follows:

1. water
2. 50 % Ethanol
3. 70 % Ethanol
4. 1 % Eosin in 70 % Ethanol

The slides were dipped into each solution for 10 seconds and then moved along the series. After reaching the staining solution, they were dipped through a descending series returning in the end to water, using the same protocol. The slides were then heated on a modified slide warmer, consisting of an aluminum plate and water bath.

The samples were subsequently examined using a Zeiss Universal Research microscope. Any notable structures were photographed using a model C35M Carl Zeiss automatic exposure 35 mm camera equipped with Kodak Ektachrome 160 ASA film.
3.3. **Statistical Analysis and Methodology**

All tests were done in at least triplicate and recorded. Analysis of variance was performed on the different treatments and time periods. Duncan’s multiple range test was used to compare the means.
RESULTS

4.1 Effects of Frozen Storage and Cryoprotectants on the Dynamic Rheological Properties of Gels Prepared from Mullet, Tullibee and Whitefish

The viscoelastic properties of various fish gels were studied using a dynamic testing technique. In dynamic tests, the specimen is subjected to a small sinusoidal deformation while the amplitude and frequency of the imposed deformation are controlled. Since the stress and strain are not in phase in viscoelastic materials the phase lag can provide an indication of the rheological characteristics of the tested material. Ideally an elastic solid will have no lag between stress and strain, whereas the phase angle for Newtonian fluids is 90° (Peleg, 1987). It is possible to calculate the elastic or storage modulus and the complex or loss modulus from the results of such tests. It is these moduli which approximately represent the contributions of the elastic and viscous components of the viscoelastic material, thus providing us with rheological information related to the rigidity of the material.

4.11 Typical Curve

Figure 4.1 illustrates a typical curve for mullet, over a frequency sweep of .1 to 20 Hz, using parallel
plates and a gap of 1.75 mm. In this curve the $G'$ (storage modulus) and $G^*$ (complex modulus) are almost identical, and are linear and parallel to the $G''$ (loss modulus) results. The value which was chosen to be monitored from these curves was the tan delta or loss tangent. As defined previously, it is the loss modulus $G''$ divided by the storage modulus $G'$. The loss tangent is of practical interest in studying rheological changes in food systems. For gel formations, the tan delta will decrease as the gel network is formed and the material becomes more elastic. This experiment generated results over a wide frequency; in order to adequately deal with this information it was necessary to choose a single frequency. The values at 1 Hz were selected, as at this point the two components usually produced parallel lines.

4.12 Changes in Tan Delta in Mullet, Tullibee and Whitefish during Storage and with Different Cryoprotectants

Figure 4.2 illustrates the changing elasticity in each treatment of the various fish at week 0. At this time the whitefish is consistently the most elastic of the three fish and it exhibits little change regardless of cryoprotectants added. Both the tullibee and mullet showed changes in elasticity as the cryoprotectant treatments varied, however, excluding the 0.1% TPP, the mullet was consistently the least elastic of the two.
Figure 4.1 A typical rheogram for mullet, obtained using the oscillatory mode on a Bohlin rheometer
Figure 4.2  Effects of cryoprotectants on the tan delta of mullet, tullibee and whitefish gels at week 0
X-AXIS CRYOPROTECTANT CODES:
1 = NO CRYOPROTECTANTS
2 = 0.1% TRIPOLYPHOSPHATE (TPP)
3 = 0.1% TPP, 1% SUCROSE
4 = 0.1% TPP, 5% SUCROSE
5 = 0.1% TPP, 4% SUCROSE, 4% SORBITOL
At week 2 (Figure 4.3) the whitefish and tullibee were found to be more elastic than the mullet, regardless of the addition of cryoprotectants.

At week 4, (Figure 4.4) the addition of 0.1% TPP and 1% sucrose was found to increase the tan delta values of all three fish. As in the first two weeks of study, the mullet has the highest tan delta values, except for the "traditional" sample, whereas the whitefish shows the lowest tan delta value or greatest elasticity.

Results for week 6, (Figure 4.5) show an overall slight decline in elasticity for all three fish as the sucrose was increased. The mullet continues to have the highest overall tan delta values.

For week 8 (Figure 4.6), the addition of cryoprotectants had a stabilizing effect on all three fish. The values for whitefish and tullibee were similar but consistently lower than those for mullet. Week 12 (Figure 4.7) shows a similar trend. The addition of cryoprotectants caused a decrease in tan delta and then had a subsequent stabilizing effect on the three fish. Both tullibee and whitefish were observed to have lower tan delta values than the mullet.

In the final trial, week 16 (Figure 4.8) the tan delta for whitefish and tullibee was found to decrease gradually, (thus increasing elasticity) as the quantity of sucrose increased, however very little effect was seen for the mullet.
Figure 4.3 Effects of cryoprotectants on tan delta of mullet, tullibee and whitefish gels at week 2
X-AXIS CRYOPROTECTANT CODES:
1 = NO CRYOPROTECTANTS
2 = 0.1% TRIPOLYPHOSPHATE (TPP)
3 = 0.1% TPP, 1% SUCROSE
4 = 0.1% TPP, 5% SUCROSE
5 = 0.1% TPP, 4% SUCROSE, 4% SORBITOL

TAN δ (LOSS MODULUS/STORAGE MODULUS)

<table>
<thead>
<tr>
<th>CRYOPROTECTANTS</th>
<th>WEEK 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>WHITEFISH</td>
<td></td>
</tr>
<tr>
<td>MULLET</td>
<td></td>
</tr>
<tr>
<td>TULLIBEE</td>
<td></td>
</tr>
</tbody>
</table>

Week 2 CRYOPROTECTANTS
Figure 4.4 Effects of cryoprotectants on tan delta of mullet, tullibee and whitefish gels at week 4
X-AXIS CRYOPROTECTANT CODES:
1=NO CRYOPROTECTANTS
2=0.1% TRIPOLYPHOSPHATE (TPP)
3=0.1% TPP, 1% SUCROSE
4=0.1% TPP, 5% SUCROSE
5=0.1% TPP, 4% SUCROSE, 4% SORBITOL

CRYSOPROTECTANTS

TANδ (LOSS MODULUS/STORAGE MODULUS)

Week 4
Figure 4.5 Effects of cryoprotectants on tan delta of mullet, tullibee and whitefish gels at week 6
X-AXIS CRYOPROTTECTANT CODES:
1 = NO CRYOPROTTECTANTS
2 = 0.1% TRIPOLYPHOSPHATE (TPP)
3 = 0.1% TPP, 1% SUCROSE
4 = 0.1% TPP, 5% SUCROSE
5 = 0.1% TPP, 4% SUCROSE, 4% SORBITOL
Figure 4.6 Effects of cryoprotectants on tan delta of mullet, tullibee and whitefish gels at week 8
X-AXIS CRYOPROTECTANT CODES:
1 = NO CRYOPROTECTANTS
2 = 0.1% TRIPOLYPHOSPHATE (TPP)
3 = 0.1% TPP, 1% SUCROSE
4 = 0.1% TPP, 5% SUCROSE
5 = 0.1% TPP, 4% SUCROSE, 4% SORBITAL

CRYOPROTECTANTS

TANδ (LOSS MODULUS/STORAGE MODULUS)

Week 8

0.0
0.1
0.2
0.3
0.4

1 2 3 4 5
Figure 4.7 Effects of cryoprotectants on tan delta of mullet, tullibee and whitefish gels at week 12
X-AXIS CRYOPROTECTANT CODES:
1 = NO CRYOPROTECTANTS
2 = 0.1% TRIPOLYPHOSPHATE (TPP)
3 = 0.1% TPP, 1% SUCROSE
4 = 0.1% TPP, 5% SUCROSE
5 = 0.1% TPP, 4% SUCROSE, 4% SORBITOL
Figure 4.8 Effects of cryoprotectants on tan delta of mullet, tullibee and whitefish gels at week 16
X-AXIS CRYOPROTECTANT CODES:
1 = NO CRYOPROTECTANTS
2 = 0.1% TRIPOLYPHOSPHATE (TPP)
3 = 0.1% TPP. 1% SUCROSE
4 = 0.1% TPP. 5% SUCROSE
5 = 0.1% TPP, 4% SUCROSE, 4% SORBITOL

CRYOPROTECTANTS

Week 16
Overall, the mullet was generally the least elastic of the three fish, whereas little difference appeared between the tullibee and whitefish. The addition of cryoprotectants in almost all cases markedly decreased the tan delta for these two species, but much smaller effects were seen for the mullet.

4.2. **Effect of Frozen Storage and Cryoprotectants on the Textural Properties of Gels of Mullet, Tullibee and Whitefish Using Large Deformation Compression Testing**

4.2.1 **Typical Curve**

Figure 4.9 depicts a curve for a fish sample which has undergone compression testing using the Ottawa Texture Measuring system. The initial linear portion of the curve (A) represents compression by the cell. The slope of this line is related to the apparent elastic properties of the sample. A change in slope occurs when the sample commences rupturing (B), at this point there is sufficient stress to overcome the forces holding the sample together. The peak of this curve reveals the maximum force during deformation. The drop of the force following the peak indicates that the sample was compressed until a catastrophic failure of the stressed area occurred.

The initial slope of the force-deformation curve gives an indication of the elasticity of the sample. In this linear region, the slope can also be referred to as Young's modulus which is defined as a ratio of stress to strain.
Figure 4.9  A typical curve for whitefish, obtained using the Ottawa Texture Measuring System
GENERAL DATA ANALYSIS
AND GRAPH FOR WTPP71
TEST DATE: 19 JAN 1987

04 AUGUST 1987
The parameter, rigidity, was chosen so that it could be compared to data obtained using the Bohlin rheometer.

In order to correlate the curves obtained from compression testing, a number of factors must be considered. Voisey et al (1972), stated that the shape and magnitude of curves are influenced by:

a) elasticity of the material
b) viscoelasticity of the material
c) viscosity of the material
d) rupture behaviour of the material
e) sample size
f) deformation rate
g) sample temperature
h) sample preparation method
i) type of test cell
j) sample particle size
k) homogeneity of the sample

With the exception of the first four, an attempt to keep the other factors constant was made during this study. Since the linear portion of the curve may vary greatly, the slope was taken between two specific time intervals for the experiment.
4.2.2 Changes in Rigidity in Mullet, Tullibee and Whitefish during Storage with Different Cryoprotectants

Figure 4.10 shows the changing rigidity in the various fish for each of the treatments at week 0. Overall, the tullibee was consistently the least rigid whereas whitefish, in combination with all the cryoprotectants, except the "traditional" mixture and the 0.1% TPP, was more rigid than mullet.

At week 2 (Figure 4.11), the tullibee sample with no cryoprotectants was the most rigid initially, but decreased as cryoprotectants were added. The rigidity of the mullet samples increased progressively as the TPP and sucrose were added but decreased with the traditional blend. During this time period, the rigidity of whitefish was found to be intermediate between the other two fish.

Results for week 4 (Figure 4.12) are quite erratic. All three fish increased substantially in rigidity with the addition of TPP and with TPP/1% sucrose combination. However the addition of 5% sucrose and the traditional blend seemed to cause a decrease in rigidity. The only exception to this was the increase in whitefish rigidity with the traditional cryoprotectant mixture.
Figure 4.10 Effects of cryoprotectants on rigidity of mullet, tullibee and whitefish gels at week 0
X-AXIS CRYOPROTECTANT CODES:
1=NO CRYOPROTECTANTS
2=0.1% TRIPOLYPHOSPHATE (TPP)
3=0.1% TPP, 1% SUCROSE
4=0.1% TPP, 5% SUCROSE
5=0.1% TPP, 4% SUCROSE, 4% SORBITOL

Week 0

CRYOPROTECTANTS

RIGIDITY (N/mm)
Figure 4.11 Effects of cryoprotectants on rigidity of mullet, tullibee and whitefish gels at week 2
Results for week 6, (Figure 4.13) show the more expected trend. Each subsequent addition of TPP or sucrose caused a progressive increase in rigidity. As before, the mullet and whitefish had the higher rigidity values and tullibee was consistently lower except for the fresh sample.

For week 8, (Figure 4.14), cryoprotectants had very little effect on the rigidity of the tullibee. In whitefish a decrease in rigidity occurred after the addition of sucrose. The mullet was variable, and showed an increase in rigidity with the exception of the 5% sucrose addition.

Week 12 (Figure 4.15) was similar to other weeks in that the TPP was found to produce more rigid samples compared to those which had no cryoprotectants added. The sucrose addition was found to stabilize the rigidity in all three fish. For all cryoprotectants (except the 5% sucrose combination,) tullibee was found to be distinctly less rigid than the other two species.

The overall trends during week 16 (Figure 4.16), was for the tullibee to show a slight decrease with successive cryoprotectants. The whitefish peaked with addition of the TPP / 1% sucrose combination and then declined. The mullet, as was the case throughout the study had the highest rigidity values, and it also peaked with the addition of 1% sucrose. Of the three fish, tullibee was consistently the least rigid when cryoprotectants were added, whereas mullet was generally the most rigid.
Figure 4.12  Effects of cryoprotectants on rigidity of mullet, tullibee and whitefish gels at week 4
The graph shows the rigidity (N/mm) of different fish species over five weeks. The species included are Whitefish, Mullet, and Tullibee. Week 4 is highlighted, and the rigidity values are compared among the species.
Figure 4.13 Effects of cryoprotectants on rigidity of mullet, tullibee and whitefish gels at week 6
Figure 4.14 Effects of cryoprotectants on rigidity of mullet, tullibee and whitefish gels at week 8
Week 8

CRYOPROTECTANTS

RIGIDITY (N/mm)

- WHITEFISH
- MULLET
- TULLIBEE

Week 8
Figure 4.15 Effects of cryoprotectants on rigidity of mullet, tullibee and whitefish gels at week 12
4.3. **Effect of Frozen Storage and Cryoprotectants on Microstructure**

4.3.1 Preliminary rating scheme

For the structural assessment, thin sections of approximately 5 microns were prepared using a cryocut microtome, after which they were stained with Eosin Y. In order to semi-quantitate these results, it was necessary to establish a rating scheme. In this regard, various fish gels were produced under different treatment extremes. A five point scale was used to describe the degree of interaction of the fish muscle.

A rating of 1 (Figure 4.17 A) was given if there was no network, i.e. there was no cross-links or bridges between the different fibers. A rating of 2 (Figure 4.17 B) was indicative of a sample which was beginning to produce a network, although it may have thin linkages, with breakage, or only a few main strands. A rating of 3 (Figure 4.18 A) was given to a network, if there were crosslinkages. However these crosslinkages were thin, with a number of open areas. A rating of 4 (Figure 4.18 B) was indicative of a relatively good network which was continuous, with many bridges. The rating of 5 (Figure 4.19) was reserved for a continuous network which had thick strands and crosslinkages, as well as being uniform in appearance.
4.16 Effects of cryoprotectants on rigidity of mullet, tullibee and whitefish gels at week 16
When ratings were assessed for each of the samples over the 16 week period, at least three different sections were examined, before the rating was assigned.

In table 4.1, the ratings are listed for mullet over the 16 weeks. Initially an average network rating of 3 was given to the fresh sample and the one containing TPP. Samples which contained sucrose were somewhat more continuous and were given ratings of 4. Following time zero, the fresh samples seemed to deteriorate and this continued throughout the remainder of the time frame. The samples with TPP, remained fairly stable giving an average rating of 3. The samples with 1% sucrose varied between the open network depicted by a 3 and the more continuous and cross-linked rating of a 4. Overall, the 5% and traditional samples had mainly ratings of 4.

In Table 4.2 the ratings assigned to tullibee are given. Similar to mullet, the samples with no cryoprotectants or only the addition of TPP, had weak networks over the 16 weeks. The addition of sucrose tended to give networks with more structural integrity, and therefore higher ratings of 3 and 4.

The microstructural results for whitefish are shown in Table 4.3. Again the impact of the addition of cryoprotectants is evident. The samples with TPP, and with the addition of 1% sucrose give networks with more cross-
Figure 4.17  A. Rating of 1 for microstructure evaluation  
B. Rating of 2 for microstructure evaluation
Figure 4.18 A. Rating of 3 for microstructure evaluation
Rating of 4 for microstructure evaluation
Figure 4.19 Rating of 5 for microstructure evaluation
<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>12</th>
<th>16</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>WEEKS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>FRESH</strong></td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>0.1% TPP</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>0.1% TPP</td>
<td>4</td>
<td>3</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>1% SUCROSE</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>0.1% TPP</td>
<td>5</td>
<td>4</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>5% SUCROSE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1% TPP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4% SUCROSE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4% SORBITOL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 4.1** Microstructural Ratings for Mullet Gels over 16 weeks
linkages than in the sample with no cryoprotectants. The samples with the increased sucrose i.e. 5 % or the 4 % sucrose/ 4 % sorbitol combination gave consistently good networks, with ratings of 4 or 5.

Overall, in the absence of cryoprotectants, the gels had less structural integrity than those where cryoprotectants were added. Over time, it becomes more evident that the addition of sucrose had a positive influence over the microstructure of all three fish species.

Microstructure of the samples relates to the rheological components of the study quite well. The samples which had no cryoprotectants or only the addition of TPP were the least viscoelastic and the least firm, these were also the samples which usually rated a "2". Microscopy shows that these were the samples which had no network so with no crosslinkages, these gels had no manner in which to exemplify elasticity.

Samples which were assigned a rating of 4 or above, generally contained the higher concentrations of sucrose. These samples had a higher degree of network structure, with many crosslinks between the main branches. This could account for these samples showing the higher elasticity and rigidity.
TABLE 4.2  Microstructural Ratings for Tullibee Gels over 16 weeks

<table>
<thead>
<tr>
<th>WEEKS</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>12</th>
<th>16</th>
</tr>
</thead>
<tbody>
<tr>
<td>FRESH</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>0.1% TPP</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>0.1% TPP 1% SUCROSE</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>0.1% TPP 5% SUCROSE</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>4</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>0.1% TPP 4% SUCROSE 4% SORBITOL</td>
<td>4</td>
<td>3</td>
<td>4</td>
<td>3</td>
<td>4</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>
TABLE 4.3  Microstructural Ratings for Whitefish Gels over 16 weeks

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>12</th>
<th>16</th>
</tr>
</thead>
<tbody>
<tr>
<td>FRESH</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>0.1% TPP</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>0.1% TPP 1% SUCROSE</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>0.1% TPP 5% SUCROSE</td>
<td>5</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>0.1% TPP 4% SUCROSE 4% SORBITOL</td>
<td>5</td>
<td>4</td>
<td>3</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td>4</td>
</tr>
</tbody>
</table>
DISCUSSION

5.1 Correlation of Rheology and Microstructure

Some of the best results of structural variation are evident by following the microstructure. There was structural deterioration in fresh mullet and whitefish after 2 weeks of storage (Table 4.1 and 4.3) and after 4 weeks of storage with fresh tullibee (Table 4.2). In general, cryoprotectants helped to maintain gel structure, especially with the addition of sucrose to the 0.1% TPP. Whitefish, for example, required a 5% sucrose addition to the 0.1% TPP to show an improvement in the gel structure. Ratings progressed from 3 to mainly 4's and 5's (Table 4.3). Some of the best gels were reported for mullet with 0.1% TPP, 4% sucrose and 4% sorbitol after 8 weeks of storage (Table 4.1) and for whitefish with the same mixture after 12 weeks of storage (Table 4.3). According to microstructural analysis, cryoprotectants, in all cases, helped maintain gel structure over the 16 weeks of storage.

In terms of tan delta values, the lower the value, the better the crosslinked networks. Thus you would expect high microstructural values to correspond with low tan delta values. For mullet, the lowest tan delta value occurred at week 6 with 0.1% TPP, 4% sucrose and 4% sorbitol (Appendix 1); this corresponded to a microstructural rating of 4. With this exception, there was little variation in the tan delta values for mullet.
was little variation in the tan delta values for mullet over the 16 weeks with the four levels of cryoprotectants. For tullibee, the tan delta values, generally are lower than those for mullet (Appendix 2). This is not particularly noted in the microstructure; values of 3 and 4 are assigned to the various weeks with 0.1% TPP, 5% sucrose and 0.1% TPP with 4% sucrose and 4% sorbitol (Table 4.2). For tullibee, the lowest tan delta values, 0.173 at week 6 with 0.1% TPP and 5% sucrose plus 0.174 at week 6 with 0.1% TPP, 4% sucrose and 4% sorbitol (Appendix 2) corresponded to microstructural ratings of 3, not 4 or 5 as might be expected. Whereas mullet had two values of 5 assigned to the traditional cryoprotectant mixture corresponding to weeks 0 and 8 (Table 4.1).

In terms of whitefish, the fresh fish values are the highest in terms of tan delta corresponding to the lowest microstructural ratings (Appendix 3, Table 4.3). Lower tan delta values can be observed from weeks 0 to 16 with 0.1% TPP, 1% sucrose, 0.1% TPP, 5% sucrose, and 0.1% TPP, 4% sucrose, 4% sorbitol (Appendix 3). These values corresponded mainly to microstructural readings of 5 and 4. The lowest tan delta value (0.165 at week 6 for 0.1% TPP, 4% sucrose, 4% sorbitol) was accompanied by a microstructural rating of 4 (Appendix 3, Table 4.3).

Compression testing was not as valuable in following microstructural patterns or tan delta values due to the high variability which limited significant differences.
variation than tan delta values. For example, for mullet, the highest rigidity was for a gel at week 4 with 0.1% TPP (Appendix 4). This corresponded to a microstructural value of 3 (Table 4.1) and an intermediate tan delta value, 0.224 (Appendix 1). For mullet, there appeared to be no significant trends with rigidity values in comparison with tan delta and microstructural ratings over the 16 weeks and with the 4 cryoprotectants.

For tullibee, the most rigid sample occurred at week 4 with 0.1% TPP, 1% sucrose (Appendix 5). This corresponded to a microstructural value of 3 (Table 4.2) and a high tan delta value of 0.213 (Appendix 2). However, in general, there was no significant trends with the rigidity values over time and cryoprotectant type.

With whitefish, the most rigid sample occurred at week 4 with 0.1% TPP and 1% sucrose (Appendix 6). This corresponded to an intermediate microstructural value of 3 (Table 4.3) and relatively high tan delta value, 0.207 (Appendix 3). In general the samples appeared to become less rigid with time for each cryoprotectant regime. This pattern did not exist with the tan delta values i.e. they did not increase or decrease with time or with the microstructural ratings.

5.2 Effect of Storage Time

The amount of variation over the 16 week storage period was found to be minimal for all three methods of testing. Referring to Appendix 1 and Figure 5.1, we find
Figure 5.1 Effects of storage on Tan delta of mullet gels
FRESH  △ 0.1 TPP  □ 0.1 TPP 1% SUCROSE
* 0.1% TPP 5% SUCROSE + TRADITIONAL

TANδ (LOSS MODULUS/STORAGE MODULUS)

MULLET 5×7

WEEKS

0.40

0.30

0.20

0.10

0.00

for mullet only a slight increase in tan delta values over the storage period, with the exception of the fresh, 0.1% TPP and 1% sucrose plus 0.1% TPP and 5% sucrose. Some variation does exist at weeks 4 and 6 for particular cryoprotectant mixtures, specifically an increase in tan delta at week 4 for 0.1% TPP, 1% sucrose, and a decrease in tan delta at week 6 for 0.1% TPP, 4% sucrose, 4% sorbitol. Concerning the rigidity for mullet (Figure 5.2 and Appendix 4), the results have no set pattern. Initially there was an increase for all cryoprotectant combinations from week 0 to week 2, however at week 4, (with the exception of the sample containing only 0.1% TPP) a slight decrease in rigidity occurred. This trend continued at week 6 in all cases except for the sample with 0.1% TPP, 4% sucrose and 4% sorbitol where a significant improvement in rigidity appeared. In weeks 8 through 16, the gel structure seemed to stabilize somewhat. With the exception of 0.1% TPP 1% sucrose, all other samples were not significantly different when weeks 0 and 16 were compared.

With respect to the microstructure data, it also showed little change over the 16 week period except in the case of the fresh sample, where significant deterioration occurs from a rating of 3 at time 0 to a rating of 1 at week 16.

For tullibee (Figure 5.3 and Appendix 2), the tan delta data were found to significantly decrease at week 2, except for 0.1% TPP, 1% sucrose. This was followed by a significant increase at week 4 in all samples except the
Figure 5.2  Effect of storage on rigidity of mullet gels
Figure 5.3  Effect of storage on Tan delta of tullibee gels
0.1% TPP. The values decreased at week 6, and then stabilized themselves over the remaining weeks, giving values similar to those observed at week 0.

The rigidity data, (Figure 5.4 and Appendix 5) was somewhat more erratic having a significant increase at week 4 (except for 0.1% TPP, 5% sucrose), followed by a decrease at week 6 (again except for 0.1% TPP 5% sucrose). Some stabilization occurred at week 8, but this was followed by a slight increase at week 12, in all samples except for the extremes i.e. the fresh and the samples with 0.1% TPP, 4% sucrose and 4% sorbitol. Week 16 showed decreases in rigidity for all samples except for the fresh.

The microstructure data had similar trends with weeks 2, 4, 6 and 8 showing slight decreases in the ratings, but weeks 12 and 16 being only slightly different from the initial values.

For whitefish (Figure 5.5 and Appendix 3) very little change in tan delta values were seen over time, with weeks 0 and 16 being similar, except in the case of the fresh sample and the 0.1% TPP, 5% sucrose where significantly higher tan delta values were found.

The data for rigidity (Figure 5.6 and Appendix 6) were once again quite erratic having no definite trend. There did, however, appear to be some increase in rigidity in weeks 8 to 12, but this was followed by a decline at week 16. The microstructure data seem to indicate an increase in network strength with time. Overall, very little change was seen for the three fish
Figure 5.4  Effect of storage on rigidity of tullibee gels
Figure 5.5 Effect of storage on Tan delta of whitefish gels
TAN of (LOSS MODULUS/STORAGE MODULUS)

○ FRESH  △ 0.1TPP  □ 0.1TPP 1% SUCROSE

☆ 0.1% TPP 5% SUCROSE + TRADITIONAL

WHITEFISH

WEEKS
Figure 5.6  Effect of storage on rigidity of whitefish gels
over the 16 weeks. There were some erratic shifts in values with the O.T.M.S. methodology, but this was not as evident with either the Bohlin rheometer or microstructural analysis. Tonogai (1988) found that for whitefish stored at \(-40^\circ\) for 48 weeks, very little loss in protein recovery or solubility was apparent. It was concluded that \(-40^\circ\) was suitable to retard freeze denaturation in washed, minced whitefish. Iwata and Okado (1971), found that the gel forming ability of surimi made from fresh fish in good condition does not change significantly when held at a constant temperature below \(-20^\circC\). Based on this evidence, it would appear that the time period for this study was too short to see detrimental effects.

Shenouda (1980), has grouped the factors which cause protein denaturation during frozen storage into three categories, these include: a) factors related to changes in fish moisture, b) factors related to changes in fish lipids, and c) factors related to the activity of a specific enzyme - trimethylamine oxidase. To some extent these factors have been minimized in this study as a result of choice of fish species, processing or addition of cryoprotectants. By using freshwater fish we have virtually eliminated the threat of TMAO. Shenouda (1980) states that generally TMAO is extremely scant or entirely absent in freshwater fish. Other enzymes like alkaline proteases are removed during the washing process, so they too have little effect on denaturation.
The role of intact lipids on the stability or instability of fish myofibrillar proteins is unclear. Dyer and Dingle (1961) found that the presence of moderate levels of lipids may protect the fish proteins or increase their denaturation during frozen storage. Detrimental effects such as fish toughness and decreased protein extractability are related to free fatty acid accumulation. As well lipid oxidation may result in problems in some species. The surimi process, will to some extent decrease the effects of lipids, as much of the lipid material was removed during the successive washing steps.

With respect to changes related to fish moisture, these are as a result of ice crystal formation, damage due to dehydration or damage caused by salt concentration. In this case the addition of cryoprotectants and the use of a plate freezer, followed by constant storage at -40°C minimized the problems caused by these factors.

5.3 Relative Effectiveness of Cryoprotectants

Throughout the study, the "fresh" fish, for all three species was found to have the highest tan delta values, indicating the least elasticity. These samples also had poor networks with microstructural ratings of 2's and 3's, and with the exception of tullibee (Appendix 5), consistently the lowest rigidity values. The incorporation of cryoprotectants was found to slightly improve the network properties in most cases, however the level, type
of cryoprotectant and fish species seemed to have some impact.

For dynamic rheology, the addition of only TPP significantly improved the results obtained for whitefish (Appendix 3) when compared to the "fresh" sample (except at time 0). For tullibee (Appendix 2) significant improvements were found initially, at week 4 and then from weeks 8 through 16. Significant improvements in elasticity were found only initially and at weeks 8 and 12 for mullet (Appendix 1).

With respect to rigidity, it would seem that species had some impact on how well the TPP protected the fish. In whitefish a significant improvement for weeks two through sixteen (Appendix 6) was found when the TPP was compared to fresh. Mullet texture was found only to be significantly improved during weeks 4, and 8 to 16 (Appendix 4) and tullibee (Appendix 5) was only found to have notable differences at week 12. The addition of TPP, was also found to improve the microstructure, especially in mullet (Table 4.1) and whitefish (Table 4.3) but only slightly in tullibee (Table 4.2).

Polyphosphates have been found to affect protein gel structure in three ways (Brotsky and Swartz, 1980):
1/ pH, 2/ ionic strength 3/ specific protein interactions

The polyphosphates, by increasing the pH of the fish muscle, will increase the negative charge on the
myofibrillar protein. This will cause repulsion between myofilaments and more water will enter the gel structure. Polyphosphates are capable of increasing the ionic strength and thus increasing the electrostatic charge of the protein, again causing increased repulsion and hydration. Polyphosphates can also sequester chelators such as Ca$^{2+}$ or Zn$^{2+}$ which may catalyze protein aggregation during frozen storage.

It is quite evident that the TPP and sucrose or the sucrose/sorbitol combination improved both the microstructural ratings and the tan delta values over the 16 weeks. This was especially true at week 6 for mullet (Appendix 1), week 16 for tullibee (Appendix 2) and weeks 8, 12 and 16 for whitefish (Appendix 3). At these times, the inclusion of sucrose and sucrose/sorbitol had a positive impact on the tan delta values.

Based on microstructure (Tables 4.1 to 4.3) it is particularly evident that the 5% sucrose and the traditional blend improved or stabilized networks over time. This is indicated by a majority of the microscopic ratings being 4’s and 5’s.

Inclusion of the sugars had a negative impact on rigidity for mullet, week 4 and a positive impact at week 16 (Appendix 4). For the other weeks there was little significant difference among the values with the addition of sucrose or sucrose/sorbitol. There was no significant difference in rigidity for the various weeks with the inclusion of sugar for tullibee gels (Appendix 5). Once
again there was a negative influence on rigidity for whitefish at weeks 8 and 12 with the addition of the sugars, there was however an increase in rigidity for week 4 with the addition of 1% sucrose.

Sugars added to surimi have a two-fold purpose; i.e. to add sweetness to the product and as a cryoprotectant. Sugars are added to prevent protein denaturation which impacts the water holding capacity and gel forming ability of the fish. Protein denaturation is mainly caused by water migration from between proteins to form ice crystals. The cryoprotective effects of sugars have been studied by several researchers and recently reviewed by MacDonald and Lanier (1991). Lee and Timasheff (1981), plus Arakawa and Timasheff (1982) determined that the denaturation of protein is less thermodynamically favorable in a sugar solution than it is in water. These researchers further showed that the stabilizing solute molecules (sugars, low molecular weight polyols) were excluded from the surface of the protein, thus preferentially hydrating the protein. While this preferential hydration of the protein has sometimes been identified as the primary protective effect of the solute exclusion (implying that the protein is thus protected against surface dehydration during freezing), the true protective effect is explained thermodynamically. The addition of protective solutes results in a positive free energy change because the sugar is excluded from the protein surface. The magnitude of this unfavorable free
energy shift is assumed to be in proportion to the surface area of the protein i.e. the volume of the cavity occupied by the protein and its hydration shell. Since the protein cavity is greater when the protein is unfolded, this means that the native state of the protein is thermodynamically favored in a sugar/low molecular weight polyol solution.

In addition, many of the cryoprotectant sugars and polyols increase the surface tension of water, thus leading to protein stabilization. Back et al. (1979) noted that hydrophobic interactions between pairs of hydrophobic groups are stronger in sucrose or glycerol than in pure water. They concluded that this is the mechanism by which sugars and polyols in general may stabilize proteins to heat denaturation.

Thus, it may be concluded that those polyols and sugars which do increase the surface tension of water may act to stabilize proteins dually - by favoring solute exclusion from the protein surface and by enhancing the strength of intramolecular hydrophobic interactions. The latter effect arises from the unfavorable decrease in entropy that occurs when water molecules experience a decreased mobility (enhanced hydrophobic bonding) in the vicinity of exposed hydrophobic sidechains.

5.4 Effect of Species

Overall the differences between the three fish species was minimal, however several trends were apparent regardless of the cryoprotectants added. The whitefish
gels (Appendix 3) were initially more elastic (lower tan delta) than either mullet (Appendix 1) or tullibee (Appendix 2). The tan delta values for mullet were almost consistently the highest over the 16 week study, indicating that this species exhibited the least elasticity. This species also had the highest rigidity values when cryoprotectants were included except for the initial week (Figure 4.10) and week 4 (Figure 4.12).

Another trend which became apparent, was that changes which occurred during storage did not affect the three species equally. For example initially the "fresh" tullibee had a tan delta of 0.252 and a firmness of 1.028. After six weeks of storage this had changed to 0.196 for elasticity and 1.736 for firmness, indicating an improvement. However for whitefish the reverse is true, the elasticity values changes from 0.194 to 0.224 after 6 weeks and the firmness from 2.438 to 1.254.

It is also evident from the study that cryoprotectants had different effects on the three species. In whitefish, for example, at week 6 (Figures 4.5 and 4.13), the inclusion of cryoprotectants caused a decrease in tan delta and an increase in rigidity. However in mullet, the tan delta values remained fairly constant regardless of cryoprotectants, whereas the rigidity values increased. Similarly at week 12 (Figure 4.7) and week 16 (Figure 4.8), the addition of cryoprotectants improved the elasticity of whitefish and tullibee but had little effect
on mullet.

Overall, while whitefish gels may have been the best, they were also the most susceptible to changes during storage and benefitted most from the inclusion of cryoprotectants. Mullet, seemed to maintain its original network throughout the study period, and in most instances was unaffected by the inclusion of cryoprotectants. Tullibee was consistently the least rigid and its elasticity was generally similar to whitefish.
CONCLUSIONS AND RECOMMENDATIONS

Stanley (1987) has stated that any study of food texture will profit from being accompanied by a consideration of the underlying framework producing the responses being investigated. In response to this, the use of light microscopy proved to be valuable in monitoring the texture of gels produced from three species of freshwater fish. This technique showed very little change between the three species, however the impact of cryoprotectants was evident by the structural integrity maintained. Samples which contained the higher levels of sucrose or the sucrose/sorbitol combination seemed to consistently have the more well-formed networks.

The use of tan delta values from dynamic rheology also provided a good assessment of network quality. This type of testing is of a nondestructive nature, and produced relatively similar values for the three species. However the results obtained using large deformation compression testing appeared to be more subjective to variations in sample preparation. Paulson and Tung (1989) found a high correlation between rupture slope and the loss and storage moduli of canola protein isolate gels, this study seems to concur with their results.

Due to very different testing methodologies, none of the actual test results obtained in this study can be compared to marine fish results. Sribhibhadh (1985), in
reporting on the prospects of developing countries making surimi from local species, pointed out that work to date shows poor potential for freshwater species. Kudo et al (1973) found that salt water fish like white flounder, hake and dogfish did not have good gel-forming properties. However, Lanier (1984) has indicated that red hake and silver hake are good potential sources for surimi. This would indicate that there are mixed successes, with both marine and freshwater fish being tested for surimi production. This study’s results seem to indicate that networks are obtainable using these three species of freshwater fish and relatively little change in the network structure occurred over a 16 week storage at -40°.

The effectiveness of cryoprotectants is difficult to assess from this study. Generally it appears that the increased concentration of sucrose gives a slightly better gel than those without cryoprotectants or only TPP. However the differences between the cryoprotectants were minimal, and the storage study may have been too short for a full assessment of the cryoprotectant mixtures.

With respect to the three species, it would appear that the whitefish has the greatest potential for production of a commercial surimi base. This species gave the best gels initially and seemed to benefit the most from the addition of cryoprotectants. Both mullet and tullibee were affected to a lesser extent by cryoprotectants.
Further work which could be done includes:

1) Follow test procedures which have been used for salt-water surimi, in order to make an accurate comparison with fresh water results. One example could be to analyze the sol to gel formation using the Bohlin rheometer.

2) A longer storage period is required in order to find out the true benefits of the cryoprotectants.

3) For future work it is recommended that several batches of the different species be processed on separate days. One of the shortfalls in this project was that the experimental design did not allow for the comparison of the three species statistically.
LITERATURE CITED


APPENDIX 1  Effects of 16 weeks of storage on the tan delta of mullet gels with various levels of cryoprotectants

<table>
<thead>
<tr>
<th>WEEKS</th>
<th>Fresh#</th>
<th>0.1% TPP</th>
<th>0.1% TPP</th>
<th>0.1% TPP</th>
<th>0.1% TPP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.239$^{ab1}$</td>
<td>0.203$^{a3}$</td>
<td>0.224$^{ab12}$</td>
<td>0.227$^{ab12}$</td>
<td>0.210$^{a23}$</td>
</tr>
<tr>
<td></td>
<td>±.004</td>
<td>±.002</td>
<td>±.014</td>
<td>±.010</td>
<td>±.003</td>
</tr>
<tr>
<td>2</td>
<td>0.227$^{c1}$</td>
<td>0.225$^{bc12}$</td>
<td>0.228$^{ab1}$</td>
<td>0.224$^{bc12}$</td>
<td>0.215$^{ac2}$</td>
</tr>
<tr>
<td></td>
<td>±.008</td>
<td>±.002</td>
<td>±.011</td>
<td>±.010</td>
<td>±.006</td>
</tr>
<tr>
<td>4</td>
<td>0.230$^{bc1}$</td>
<td>0.224$^{bc1}$</td>
<td>0.256$^{c2}$</td>
<td>0.217$^{bc1}$</td>
<td>0.221$^{bc1}$</td>
</tr>
<tr>
<td></td>
<td>±.006</td>
<td>±.006</td>
<td>±.032</td>
<td>±.004</td>
<td>±.004</td>
</tr>
<tr>
<td>6</td>
<td>0.229$^{c1}$</td>
<td>0.229$^{bd1}$</td>
<td>0.214$^{a2}$</td>
<td>0.213$^{c2}$</td>
<td>0.202$^{e3}$</td>
</tr>
<tr>
<td></td>
<td>±.002</td>
<td>±.005</td>
<td>±.002</td>
<td>±.004</td>
<td>±.002</td>
</tr>
<tr>
<td>8</td>
<td>0.235$^{bc1}$</td>
<td>0.222$^{c2}$</td>
<td>0.225$^{ab2}$</td>
<td>0.223$^{bc2}$</td>
<td>0.223$^{bc2}$</td>
</tr>
<tr>
<td></td>
<td>±.006</td>
<td>±.001</td>
<td>±.007</td>
<td>±.009</td>
<td>±.003</td>
</tr>
<tr>
<td>12</td>
<td>0.245$^{a1}$</td>
<td>0.221$^{c3}$</td>
<td>0.235$^{abc2}$</td>
<td>0.235$^{a2}$</td>
<td>0.233$^{d2}$</td>
</tr>
<tr>
<td></td>
<td>±.005</td>
<td>±.004</td>
<td>±.012</td>
<td>±.004</td>
<td>±.002</td>
</tr>
<tr>
<td>16</td>
<td>0.235$^{bc12}$</td>
<td>0.235$^{d12}$</td>
<td>0.243$^{bc1}$</td>
<td>0.235$^{a12}$</td>
<td>0.227$^{bd2}$</td>
</tr>
<tr>
<td></td>
<td>±.007</td>
<td>±.007</td>
<td>±.010</td>
<td>±.005</td>
<td>±.002</td>
</tr>
</tbody>
</table>

# Indicates no cryoprotectants.

* Mean values in each column followed by the same letters are not significantly different (P<0.05).

* Mean values in each row followed by the same numbers are not significantly different (P<0.05)

+ Mean values based on a minimum of four determinations.
APPENDIX 2  Effects of 16 weeks of storage on the tan delta of tullibee gels with various levels of cryoprotectants

<table>
<thead>
<tr>
<th>WEEKS</th>
<th>Fresh#</th>
<th>0.1%TPP</th>
<th>0.1%TPP</th>
<th>0.1%TPP</th>
<th>0.1%TPP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1%Sucrose</td>
<td>5%Sucrose</td>
<td>4%Sucrose</td>
<td>4%Sorbitol</td>
</tr>
<tr>
<td>0</td>
<td>.252a1</td>
<td>.207a2</td>
<td>.197a2</td>
<td>.178a2</td>
<td>.207a2</td>
</tr>
<tr>
<td></td>
<td>± .035</td>
<td>± .007</td>
<td>± .016</td>
<td>± .015</td>
<td>± .006</td>
</tr>
<tr>
<td>2</td>
<td>.198dl</td>
<td>.196bc1</td>
<td>.188a1</td>
<td>.194b1</td>
<td>.178b1</td>
</tr>
<tr>
<td></td>
<td>± .003</td>
<td>± .022</td>
<td>± .003</td>
<td>± .010</td>
<td>± .003</td>
</tr>
<tr>
<td>4</td>
<td>.223bc1</td>
<td>.194c2</td>
<td>.213b1</td>
<td>.221c1</td>
<td>.231c1</td>
</tr>
<tr>
<td></td>
<td>± .003</td>
<td>± .006</td>
<td>± .012</td>
<td>± .013</td>
<td>± .019</td>
</tr>
<tr>
<td>6</td>
<td>.196dl</td>
<td>.192c1</td>
<td>.189a1</td>
<td>.173a2</td>
<td>.174b2</td>
</tr>
<tr>
<td></td>
<td>± .006</td>
<td>± .002</td>
<td>± .005</td>
<td>± .003</td>
<td>± .003</td>
</tr>
<tr>
<td>8</td>
<td>.214cd1</td>
<td>.192c2</td>
<td>.193a2</td>
<td>.182ab3</td>
<td>.180b3</td>
</tr>
<tr>
<td></td>
<td>± .003</td>
<td>± .004</td>
<td>± .003</td>
<td>± .001</td>
<td>± .003</td>
</tr>
<tr>
<td>12</td>
<td>.227bc1</td>
<td>.193c2</td>
<td>.192a23</td>
<td>.184ab4</td>
<td>.199a3</td>
</tr>
<tr>
<td></td>
<td>± .002</td>
<td>± .022</td>
<td>± .005</td>
<td>± .003</td>
<td>± .003</td>
</tr>
<tr>
<td>16</td>
<td>.242ab1</td>
<td>.206ab2</td>
<td>.195a3</td>
<td>.186ab34</td>
<td>.180b4</td>
</tr>
<tr>
<td></td>
<td>± .014</td>
<td>± .004</td>
<td>± .005</td>
<td>± .007</td>
<td>± .002</td>
</tr>
</tbody>
</table>

# Indicates no cryoprotectants.

* Mean values in each column followed by the same letters are not significantly different (P<0.05).

* Mean values in each row followed by the same numbers are not significantly different (P<0.05).

† Mean values based on a minimum of four determinations.
APPENDIX 3  Effects of 16 weeks of storage on the tan delta of whitefish with various levels of cryoprotectants

---

**CRYOPROTECTANTS**

<table>
<thead>
<tr>
<th>WEEKS</th>
<th>Fresh#</th>
<th>0.1%TPP</th>
<th>0.1%TPP</th>
<th>0.1%TPP</th>
<th>0.1%TPP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1%Sucrose</td>
<td>5%Sucrose</td>
<td>4%Sucrose</td>
<td>4%Sorbitol</td>
</tr>
<tr>
<td>0</td>
<td>0.194_{a1}^{a2}</td>
<td>0.198_{b1}^{a1}</td>
<td>0.184_{a1}^{a2}</td>
<td>0.176_{a2}^{a2}</td>
<td>0.176_{a2}^{ab2}</td>
</tr>
<tr>
<td></td>
<td>± 0.008</td>
<td>± 0.025</td>
<td>± 0.007</td>
<td>± 0.006</td>
<td>± 0.003</td>
</tr>
<tr>
<td>2</td>
<td>0.217_{bc1}^b</td>
<td>0.199_{b2}^{a2}</td>
<td>0.181_{b2}^{a3}</td>
<td>0.175_{b3}^{a3}</td>
<td>0.18{c3}^b</td>
</tr>
<tr>
<td></td>
<td>± 0.012</td>
<td>± 0.011</td>
<td>± 0.003</td>
<td>± 0.002</td>
<td>± 0.009</td>
</tr>
<tr>
<td>4</td>
<td>0.233_{d1}^d</td>
<td>0.167_{b4}^b</td>
<td>0.207_{b2}^b</td>
<td>0.18{b3}^b</td>
<td>0.176_{ab2}^{ab2}</td>
</tr>
<tr>
<td></td>
<td>± 0.009</td>
<td>± 0.026</td>
<td>± 0.013</td>
<td>± 0.003</td>
<td>± 0.003</td>
</tr>
<tr>
<td>6</td>
<td>0.224_{bcd1}^{bc}</td>
<td>0.203_{a2}^{a2}</td>
<td>0.188_{a3}^{a3}</td>
<td>0.171_{a4}^{a4}</td>
<td>0.16{d4}^b</td>
</tr>
<tr>
<td></td>
<td>± 0.012</td>
<td>± 0.007</td>
<td>± 0.003</td>
<td>± 0.004</td>
<td>± 0.002</td>
</tr>
<tr>
<td>8</td>
<td>0.217_{bcd1}^{bcd}</td>
<td>0.198_{a2}^{a2}</td>
<td>0.182_{a3}^{a3}</td>
<td>0.177_{a3}^{a3}</td>
<td>0.176_{ab3}^{ab3}</td>
</tr>
<tr>
<td></td>
<td>± 0.007</td>
<td>± 0.004</td>
<td>± 0.004</td>
<td>± 0.003</td>
<td>± 0.006</td>
</tr>
<tr>
<td>12</td>
<td>0.229_{bd1}^{bd}</td>
<td>0.194_{a2}^{a2}</td>
<td>0.186_{a2}^{a2}</td>
<td>0.18{b2}^{b2}</td>
<td>0.183_{bc2}^{bc2}</td>
</tr>
<tr>
<td></td>
<td>± 0.014</td>
<td>± 0.004</td>
<td>± 0.003</td>
<td>± 0.002</td>
<td>± 0.003</td>
</tr>
<tr>
<td>16</td>
<td>0.212_{c1}^{c}</td>
<td>0.198_{a2}^{a2}</td>
<td>0.193_{a2}^{a2}</td>
<td>0.19{b2}^{b2}</td>
<td>0.173_{a3}^{a3}</td>
</tr>
<tr>
<td></td>
<td>± 0.006</td>
<td>± 0.009</td>
<td>± 0.016</td>
<td>± 0.009</td>
<td>± 0.004</td>
</tr>
</tbody>
</table>

# Indicates no cryoprotectants.

* Mean values in each column followed by the same letters are not significantly different (P ≤ 0.05).

* Mean values in each row followed by the same numbers are not significantly different (P ≤ 0.05).

+ Mean values based on a minimum of four determinations.
APPENDIX 4  Effects of 16 weeks of storage on the rigidity (N/mn) of mullet gels with various levels of cryoprotectants

<table>
<thead>
<tr>
<th>CRYPOTPROTECTANTS</th>
<th>Fresh#</th>
<th>0.1%TPP</th>
<th>0.1% TPP 1% Sucrose</th>
<th>0.1%TPP 5% Sucrose</th>
<th>0.1%TPP 4% Sucrose</th>
<th>0.1%TPP 4% Sorbitol</th>
</tr>
</thead>
<tbody>
<tr>
<td>WEEKS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>2.140&lt;sup&gt;ab1&lt;/sup&gt;&lt;sup&gt;l&lt;/sup&gt; ±1.148</td>
<td>2.657&lt;sup&gt;a1&lt;/sup&gt; ±1.425</td>
<td>2.019&lt;sup&gt;a1&lt;/sup&gt; ±1.143</td>
<td>2.497&lt;sup&gt;ab1&lt;/sup&gt; ±1.476</td>
<td>2.644&lt;sup&gt;ab1&lt;/sup&gt; ±1.526</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2.310&lt;sup&gt;ab1&lt;/sup&gt;&lt;sup&gt;l&lt;/sup&gt; ±1.444</td>
<td>3.017&lt;sup&gt;a1&lt;/sup&gt; ±1.533</td>
<td>3.430&lt;sup&gt;b1&lt;/sup&gt; ±1.299</td>
<td>3.439&lt;sup&gt;a1&lt;/sup&gt; ±1.213</td>
<td>2.647&lt;sup&gt;ab1&lt;/sup&gt; ±1.568</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1.947&lt;sup&gt;ab1&lt;/sup&gt;&lt;sup&gt;l&lt;/sup&gt; ±1.577</td>
<td>3.393&lt;sup&gt;b3&lt;/sup&gt; ±1.213</td>
<td>3.394&lt;sup&gt;bd2&lt;/sup&gt; ±1.270</td>
<td>3.263&lt;sup&gt;a12&lt;/sup&gt; ±1.821</td>
<td>2.481&lt;sup&gt;b1&lt;/sup&gt; ±1.658</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>1.759&lt;sup&gt;b1&lt;/sup&gt;&lt;sup&gt;l&lt;/sup&gt; ±1.497</td>
<td>2.009&lt;sup&gt;c1&lt;/sup&gt; ±1.109</td>
<td>2.516&lt;sup&gt;c12&lt;/sup&gt; ±1.264</td>
<td>2.455&lt;sup&gt;ab12&lt;/sup&gt; ±1.403</td>
<td>3.151&lt;sup&gt;a2&lt;/sup&gt; ±1.656</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>2.015&lt;sup&gt;ab1&lt;/sup&gt;&lt;sup&gt;l&lt;/sup&gt; ±1.056</td>
<td>2.932&lt;sup&gt;a2&lt;/sup&gt; ±1.139</td>
<td>3.528&lt;sup&gt;b4&lt;/sup&gt; ±1.247</td>
<td>2.522&lt;sup&gt;ab3&lt;/sup&gt; ±1.076</td>
<td>3.130&lt;sup&gt;a2&lt;/sup&gt; ±1.185</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>2.402&lt;sup&gt;a1&lt;/sup&gt;&lt;sup&gt;l&lt;/sup&gt; ±1.444</td>
<td>3.654&lt;sup&gt;b2&lt;/sup&gt; ±1.315</td>
<td>2.923&lt;sup&gt;cd1&lt;/sup&gt; ±1.310</td>
<td>3.066&lt;sup&gt;ab12&lt;/sup&gt; ±1.215</td>
<td>2.779&lt;sup&gt;ab1&lt;/sup&gt; ±1.520</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>1.750&lt;sup&gt;b1&lt;/sup&gt;&lt;sup&gt;l&lt;/sup&gt; ±0.098</td>
<td>2.530&lt;sup&gt;a23&lt;/sup&gt; ±0.257</td>
<td>2.938&lt;sup&gt;cd4&lt;/sup&gt; ±0.090</td>
<td>2.220&lt;sup&gt;b2&lt;/sup&gt; ±0.219</td>
<td>2.641&lt;sup&gt;ab34&lt;/sup&gt; ±0.361</td>
<td></td>
</tr>
</tbody>
</table>

# Indicates no cryoprotectants.

* Mean values in each column followed by the same letter are not significantly different (P ≤ 0.05).

* Mean values in each row followed by the same number are not significantly different (P ≤ 0.05).

+ Means based on triplicate determinations.

---

101
APPENDIX 5 Effects of 16 weeks of storage on the rigidity (N/mm) of tullibee gels with various levels of cryoprotectants

<table>
<thead>
<tr>
<th>CRYOPROTECTANT</th>
<th>Fresh#</th>
<th>0.1%TPP 1%Sucrose</th>
<th>0.1%TPP 5%Sucrose</th>
<th>0.1%TPP 4%Sucrose</th>
<th>0.1%TPP 4%Sorbitol</th>
</tr>
</thead>
<tbody>
<tr>
<td>WEEKS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>2.028abc1 ±1.118</td>
<td>1.885ab1 ±1.169</td>
<td>1.816ab1 ±1.144</td>
<td>2.083a1 ±0.69</td>
<td>2.150ab1 ±0.449</td>
</tr>
<tr>
<td>2</td>
<td>2.915b1 ±1.22</td>
<td>2.143ab1 ±0.812</td>
<td>2.284b1 ±0.564</td>
<td>2.225a1 ±0.056</td>
<td>2.042ab1 ±0.204</td>
</tr>
<tr>
<td>4</td>
<td>2.587bc12 ±0.716</td>
<td>3.447c1 ±0.325</td>
<td>3.904c3 ±0.366</td>
<td>2.100a2 ±0.285</td>
<td>2.358ab12 ±0.765</td>
</tr>
<tr>
<td>6</td>
<td>1.736aced12 ±0.223</td>
<td>1.586a2 ±0.170</td>
<td>1.738ab12 ±0.211</td>
<td>2.128a1 ±0.307</td>
<td>2.446ab1 ±0.049</td>
</tr>
<tr>
<td>8</td>
<td>2.013abc1 ±0.096</td>
<td>1.858ab1 ±0.183</td>
<td>2.041ab1 ±0.453</td>
<td>2.151a1 ±0.111</td>
<td>2.208ab1 ±0.547</td>
</tr>
<tr>
<td>12</td>
<td>1.650ad1 ±0.492</td>
<td>2.406b2 ±1.108</td>
<td>2.195b12 ±0.370</td>
<td>2.669b2 ±0.247</td>
<td>2.108ab12 ±1.133</td>
</tr>
<tr>
<td>16</td>
<td>2.032abc1 ±0.412</td>
<td>1.752a1 ±0.225</td>
<td>1.96ab1 ±0.503</td>
<td>1.647c1 ±0.072</td>
<td>1.456bccl ±0.175</td>
</tr>
</tbody>
</table>

# Indicates no cryoprotectants.

* Mean values in each column followed by the same letter are not significantly different (P≤ 0.05).

* Mean values in each row followed by the same number are not significantly different (P≤ 0.05).

+ Means based on triplicate determinations.
APPENDIX 6 Effects of 16 weeks of storage on the rigidity (N/mm) of whitefish gels with various levels of cryoprotectants

<table>
<thead>
<tr>
<th>CRYOPROTECTANTS</th>
<th>Fresh#</th>
<th>0.1%TPP</th>
<th>0.1%TPP</th>
<th>0.1%TPP</th>
<th>0.1%TPP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1%Sucrose</td>
<td>5%Sucrose</td>
<td>4%Sucrose</td>
<td>4%Sorbitol</td>
</tr>
<tr>
<td>WEEKS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>2.438$^{a1}$</td>
<td>2.693$^{a1}$</td>
<td>2.537$^{a1}$</td>
<td>2.584$^{a1}$</td>
<td>2.418$^{a1}$</td>
</tr>
<tr>
<td></td>
<td>±.260</td>
<td>±.001</td>
<td>±.182</td>
<td>±.103</td>
<td>±.155</td>
</tr>
<tr>
<td>2</td>
<td>2.115$^{b1}$</td>
<td>2.705$^{a2}$</td>
<td>2.780$^{b2}$</td>
<td>2.399$^{a2}$</td>
<td>2.322$^{ab2}$</td>
</tr>
<tr>
<td></td>
<td>±.195</td>
<td>±.458</td>
<td>±.119</td>
<td>±.197</td>
<td>±.347</td>
</tr>
<tr>
<td>4</td>
<td>2.173$^{b1}$</td>
<td>3.538$^{b2}$</td>
<td>3.471$^{c2}$</td>
<td>2.898$^{a3}$</td>
<td>3.762$^{c2}$</td>
</tr>
<tr>
<td></td>
<td>±.243</td>
<td>±.123</td>
<td>±.403</td>
<td>±.619</td>
<td>±.797</td>
</tr>
<tr>
<td>6</td>
<td>1.254$^{c1}$</td>
<td>2.109$^{c2}$</td>
<td>2.298$^{a2}$</td>
<td>2.411$^{a2}$</td>
<td>2.912$^{bc3}$</td>
</tr>
<tr>
<td></td>
<td>±.118</td>
<td>±.205</td>
<td>±.272</td>
<td>±.508</td>
<td>±.316</td>
</tr>
<tr>
<td>8</td>
<td>2.051$^{b1}$</td>
<td>2.934$^{a3}$</td>
<td>2.577$^{ab23}$</td>
<td>2.297$^{a12}$</td>
<td>2.227$^{ab12}$</td>
</tr>
<tr>
<td></td>
<td>±.020</td>
<td>±.376</td>
<td>±.054</td>
<td>±.349</td>
<td>±.211</td>
</tr>
<tr>
<td>12</td>
<td>2.562$^{a1}$</td>
<td>3.176$^{ab2}$</td>
<td>2.827$^{b12}$</td>
<td>2.634$^{a1}$</td>
<td>2.699$^{bc1}$</td>
</tr>
<tr>
<td></td>
<td>±.205</td>
<td>±.182</td>
<td>±.314</td>
<td>±.148</td>
<td>±.314</td>
</tr>
<tr>
<td>16</td>
<td>1.293$^{c1}$</td>
<td>1.896$^{c23}$</td>
<td>2.199$^{a3}$</td>
<td>2.005$^{a23}$</td>
<td>1.699$^{a12}$</td>
</tr>
<tr>
<td></td>
<td>±.180</td>
<td>±.333</td>
<td>±.336</td>
<td>±.109</td>
<td>±.030</td>
</tr>
</tbody>
</table>

# Indicates no cryoprotectants.

* Mean values in each column followed by the same letter are not significantly different (P≤ 0.05).

* Mean values in each row followed by the same number are not significantly different (P≤ 0.05).

+ Means based on triplicate determinations.