THE UNIVERSITY OF MANITOBA

FACTORS AFFECTING THE FORMATION OF PROTEIN FIBERS AND GELS FROM MECHANICALLY DEBONED WHITEFISH (*Coregonus clupeaformis*)

BY

WING - CHENG JOHN LAM

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ABSTRACT

Protein fibers and gels were made from white fish (Coregonus clupeaformis) flesh. Three kinds of mechanically deboned fish flesh (minced flesh, washed flesh and surimi) were prepared as raw materials in this study. Four heating systems (boiling water, steam kettle, oil bath and conventional gas oven) were evaluated for their ability to heat set fish fibers. The results showed that the washed flesh and high temperature (150°C) oven treatment may be the most suitable material and method for fiber making. Surimi flesh produced the softest texture of gel and fiber among these three materials. Effect of cations (Na^{+}, Ca^{2+}, Mg^{2+}, and Fe^{3+}) on fiber and gel preparations were compared. Ions with higher charge (Fe^{3+}) showed less positive effect to fiber formation and ions with higher R/C (radius/charge) ratio (Na^{+}) were more effective for fiber and gel preparation. In this study, 2% NaCl was suggested for fiber formation from washed fish flesh, because relatively higher tensile strength, and high cohesiveness (0.843 units, General Food Texturometer) and reduced hardness (2.84 units, General Food Texturometer) were shown.

Some processing factors such as mixing time, moisture content, pH, cooking time and addition of cryoprotectants,

sodium tripolyphosphate (STPP) and glucose, have been investigated. The cryoprotectants showed no effect on fiber formation but the water holding capacity of fish protein paste was improved by the addition of STPP. The optimization of these factors to the texture of a given product need further studies.

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter 1

INTRODUCTION

Fish has long been recognized as a very important protein source for human consumption. This protein has a large resource base throughout the world which includes marine and freshwater stocks. More than 60% of fish flesh is protein, dry basis, (Watt and Merrill, 1975) and the amino acid composition of this protein is similar to mammalian and avian meat (Bligh, 1981). However, because fish flesh is more prone to bacterial and biochemical spoilage, fish meat is less acceptable than other meat products in North America. On the other hand, a large section of the world's population has consumed a variety of fish products, such as fish sausage, for a long period of time (Erichi, 1970). More recently a new form of fish product, minced fish produced by mechanical deboning is gaining world wide acceptance. A variety of techniques have been developed which are designed to restructure minced fish to other forms; for example fish stick (Bligh, 1981). These techniques have the potential to increase the utilization of fish protein for human food.

A wet fish protein, surimi, has been developed for preparing a Japanese gel like sausage called kamaboko (Miyauchi et al., 1973). The surimi mince (fish flesh) has some uni-

que properties which enchance the potential of this product for wide usage as a food protein ingredient. These properties include a white color, reduced fish odor (almost bland odor), improved gel forming capability and improved long term frozen storage life (Suzuki, 1981).

The Food Science Department,University of Manitoba in cooperation with the Federal Department of Fisheries and Oceans, have been successful in producing high quality surimi and Kamaboko from white fish Coregonus clupeaformis and tullibee, Coregonus artedii (Hydamaka et al., 1984). Many of the lake stocks for these species have been highly infested with a parasite, Triaenophorous crassus, which renders the flesh aesthetically unacceptable but cause no harm to warm blooded animals (Lawler, 1959).

The surimi process was considered to have potential in up grading the quality of these fish stocks. Through processing the aesthetic effect of the parasite could either be eliminated or reduced to a level comparable with the accepted commercial standard for Canada.

This study was designed to

1. Evaluate three sources of comminuted fish flesh for their suitability in producing food fibers(spaghetti like or a coarse type of fiber) or food gels.
   a) Minced flesh (all components of fish meat excluding viscera and bone)

b) Washed flesh (all soluble components washed or floated from minced flesh)

c) Surimi flesh (washed flesh prepared by the traditional Japanese procedure)

2. Evaluate the effect of process modifications on the textural quality of fish protein fibers or gels.

a) Type of heat

b) Mixing time (incorporation of ingredients into fish flesh)

c) Added moisture

d) Cooking time

e) Fish flesh pH level

f) Use of specific cations

g) Use of cryoprotectants

Both ingredient forms (fibers and gels) are useful in the restructuring and production of various food products like kamaboko, simulated crablegs or simulated scallops . Therefore these forms were the most suitable for carrying out this particular study.

Chapter 2

LITERATURE REVIEW

2.1 PROTEIN TEXTURIZATION

2.1.1 Plant Protein

The present state of world food protein supplies is such that more food protein will be required in the future from both conventional and nonconventional sources (Burrows et al., 1972; Porter & Rolls, 1973; Kinsella, 1976). This is due to the need for solving protein-calorie malnutrition in many of the densely populated regions of the world. Thus the development of fabricated proteinaceous food, i.e. formulated texturized food, from plant protein sources was bound to occur.

Kellogg (around 1880) has been credited with pioneering the techniques of texturizing plant and animal protein for the purpose of improving their mouth-feel properties (Kinsella, 1978). Several methods have now been developed for texturizing food proteins, e.g. steam texturization, fiber spinning, thermal extrusion, chewy gel formation and fiber formation. Among these methods, fiber spinning pioneered the way for producing a simulated meat structure from plant protein by forming filaments or fibers which could be fabricated into meat-like products (Boyer, 1940).

Kinsella (1978) has described the conventional acid-base spinning process for producing fiber from soy bean protein or other isolates. Numerous patents have been granted for the production of spun fibers which utilized the spinning process based on Boyer's work (Boyer, 1954). Also, various proteins have been converted to spun fibers; the most common one being soy protein; others include casein, wheat protein and single cell protein (SCP). Plant proteins from more than one source have been combined during the texturizing or spun fiber process (Elmquist, 1965; Doly & Ruiz, 1974; Jaynes & Asan, 1976). These combinations helped to modify the texture of the spun fiber. However, fiber spinning requires modern equipment and highly skilled technicians. The process is comparatively expensive, and several technical and engineering problems can be encountered during spinning. Therefore, the more simple technique of extrusion has rapidly developed. Until now, the major technique for fabricating the textured vegetable protein was by extrusion of protein under heat and pressure. This process requires less equipment and less sophisticated technology when compared with the spinning process (Matz, 1971).

Currently, the largest market for textured plant protein is in their use as meat extenders, especially for school lunch programs in the United States which permits a 30% addition of plant products to meat items (Anon, 1971; USDA, 1972). Approximately 46 million pounds of rehydrated vege-

table protein were used in this program in 1972-1973 (Butz, 1974). Several other methods have been developed for the purpose of producing protein fibers. Anson and Pader (1958) published a simple process for manufacturing a chewy protein gel by thermal gelation of an aqueous dispersion of protein. This product was claimed to have the physical properties of resilience, elasticity and resistance to shear typical of meat. Steam texturization was another method which has been applied to soy protein. The mixture of soy protein isolate or concentrate and water was subjected to high pressure steam. This procedure also resulted in the evaporation of most undesirable flavors (Dunning et al., 1972; Strommer & Beck, 1973).

A patent for the production of rubbery and elastic protein fibers from vegetable protein micellar mass (PMM) has been described (Murray et al., 1978). The PMM was injected into hot water (>90°C) at atmospheric pressure for fiber formation by coagulation. The traditional method of high acid and alkali treatment to protein dope was proved to negatively affect native protein functionality and the whole process was somewhat more expensive and complicated than PMM procedure (Murray, et al., 1981). Therefore, the latter method is thought to have better potentiality in the future. Actually, some meat analog prototypes have already been produced by using this PMM fiber.

2.1.2 Fish protein

The development of the mechanical deboner for the production of minced fish flesh has become widely applied in the fish industry. This development has markedly increased the utilization of fish protein for human consumption (Bligh, 1981). A modification of the deboning method is the Japanese "Surimi" procedure. This procedure has been described as the biggest achievement in upgrading low quality fish. The deboned minced flesh is washed several times and mixed with salt and cryoprotective agents. This material is then the major ingredient in the traditional kamaboko products (Makiuchi, Y., 1981).

Kamaboko is a homogeneous fish protein gel which forms as a result of the functional properties of the fish muscle protein (Okada et al., 1973). The fundamental steps in processing kamaboko are washing, grinding with salt and heating. The gel forming ability is caused by the addition of salt into fish flesh which extracts myofibrillar protein from the muscle. The primary component of myofibrillar protein with respect to kamaboko gel formation is actomyosin which is formed by the interaction of actin and myosin (Suzuki, 1981). Actomyosin is the major component in the formation of the protein network in the kamaboko gel.

Fish sausage and ham are two more traditional Japanese textured fish protein products. The ingredients added to these products during processing include color compounds or

sodium nitrite, preservatives, salt and starch (Tanikawa, 1971). The starch was added to adjust the elasticity of the finished product. Following the casing step, the sausage was processed in a boiling water tank with steam. Recently, a co-extrudate produced from mixtures of soy and fish protein by thermal extrusion has been described (Murray & Stanley, 1980). This method has the advantages of raising the level of essential amino acids in the product and improving the extrudate texture by addition of fish protein. Sensory data, however, showed that the product would probably not be accepted by consumers in North America because of the fishy odor that was present in the starting fish minces which persisted through extrusion.

Lately the Japanese have developed a textured fish protein concentrate called "marinebeef" (Suzuki, 1981). Texture of this product was claimed to resemble that of livestock meat. The basic steps for producing marinebeef are; addition of salt as described previously for kamaboko, kneading and extrusion to modify fish texture, addition of ethanol to remove fat and moisture and to cause protein denaturation and coagulation. The "marinebeef" is recovered by dehydration. This product was made into chip or powder form for replacing animal meat at various levels in several products. The potential of this product is hard to predict although its market price in Japan was not expensive (U.S.$ 0.007 per gram of protein in Japan in 1979) (Suzuki, 1981). The ac-

ceptance by consumers of this product depends on their eating habits. However, because it enables the utilization of pelagic fish and shell fish, e.g. antartic krill, marinebeef has the potential for world wide utilization as a food ingredient.

2.2 FACTORS AFFECTING THE TEXTURE OF FISH PROTEIN GEL

2.2.1 Heat Energy

Experimental studies with sausages prepared from myofibrils (Hashimoto et al., 1959; Fukazawa et al., 1961a, 1961b) clearly showed that physico-chemical changes of myosin and actomyosin upon heating may play a major role in determining the binding properties of sausages. The protein-protein interactions in actomyosin solution obtained from mackerel have been studied by Deng et al. (1976). These interactions were affected by temperature. Also the rate and extent at which protein-protein interactions occurred could affect the performance of muscle protein in stabilizing water and the ability of the protein to impart the desired textural characteristics to the finished product (Tanfold, 1968; 1970). The results of these studies showed that the interaction of fish actomyosin would occur at room temperature (25°C). Therefore, it has been recommended that maintaining the temperature of a system below 10 degree C prior to cooking may improve water and fat binding of solubilized meat protein (Deng et al., 1976). Liu, et al. (1982) re-

ported that the croaker actomyosin started to coagulate at about 30°C. The differential temperature of protein coagulation for these two fish species strongly suggests that fish species will greatly affect the gel forming ability of mechanically deboned fish muscle (Cheng, et al., 1979b).

The protein-protein association by non-covalent forces would likely preceed the aggregation or coagulation step and also it was believed that hydrophobic interactions were the predominant force that brought about protein coagulation (Cheng, et al., 1979a). Lanier et al. (1982) also mentioned that setting of fish protein at temperatures below the coagulation temperature may be viewed as a process in which the solubilized proteins interact noncovalently to form gel network. This network formation has demonstrated that the interaction is mainly hydrophobic in nature (Samejima et al., 1982). However, the setting effect below coagulation temperature resulted in a loss of texture strength to the product as compared to an unset gel which is subsequently set by an extrusion process (Lanier et al., 1982). Therefore, the molding or shaping of fish gel products should be accomplished soon after comminution and protein extraction are completed.

It has been demonstrated that 60-70°C was the optimum temperature for protease activity in some fish flesh (Cheng, et al., 1979; Deng, 1981). Within this temperature range fish gel texture was weakened because of proteolytic hydro-

lysis. If the heating temperature was raised to 100°C, protein interaction was the predominant factor influencing the texture, and the softening effect caused by protease had disappeared. In general, the extent of protein-protein interaction or protein coagulation in situ, increased with increasing temperature (Deng, 1981).

2.2.2 Moisture Content

Practical experience (Tanikawa, 1971) and research results (Miyauchi et al., 1973; Lee and Toledo, 1977) have indicated that washing some mechanically deboned fish flesh can improve the quality and functional characteristics of minced muscle. Increased washing times and ratios of water volume to mince weight resulted in high springiness of washed mince (Tseo et al., 1983). Lee and Toledo (1976) reported that the textural strength of cooked fish mince decreased as the moisture content of the mince increased. The higher moisture content could increase the susceptibility of the material to lose binding characteristics with prolonged chopping. Their results also suggested that a possible critical moisture content may exist below which products were less cohesive. Lack of cohesiveness could result from insufficient water in the formulation to make adequate binding possible.

Pan et al. (1980) reported similar results with minced squid flesh; as the moisture content increased from 75% to

82%, the breaking force of fish gel decreased. This was explained since moisture affects the concentration and hydration of myofibrillar protein, thus affecting product texture.

Various commercial proteins such as soy protein isolates whey protein concentrates and caseinate have been used as binder blended with ground fillets for improving water binding capacity of cooked fish gel (Karmas & Turk, 1976). This seems to be a good method for overcoming excessive moisture problems mentioned previously and may also improve the product texture due to higher water binding capacity.

2.2.3 pH

It is well-known that charges on protein molecules are greatly affected by environmental pH (Conn & Stumpf, 1972). Thus fish protein functionality, e.g., solubility, protein-protein interactions, will certainly be affected by the pH level in processing systems. Meinke et al. (1972) reported that the pH values greatly influence fish protein solubility in the production of fish protein isolates. Fish protein had higher solubility near the two ends of the pH scale, i.e. under acidic or alkaline conditions. However, they also showed that protein solubility increased with increasing of salt concentration (0.1 N to 1.0N) at the pH of minimum solubility--approximately pH 6.0. From the report of Deng et al. (1976), more interactions of mackerel actomyosin

occurred at lower pH (5.8) than at higher pH (6.0). Also, the initial detectable interaction started at a lower temperature in lower pH range than higher pH. Squid mince has been used for detecting the effect of pH on product texture (Pan et al., 1980). There was an optimal pH (6.7) for the maximum breaking force of minced squid product when the force was measured by the Yamamoto Food Checker.

Protease action in muscle has been shown to influence muscle protein solubility (Chen et al., 1981). Optimum activity for alkaline protease in carp flesh was found to be at pH 8.0 and 65°C (Makinodan and Ikeda, 1977). Mullet alkaline protease had high activity at pH from 6-9 and at 60 -70°C (Deng, 1981). Deng (1981) concluded that the decrease in shear force at temperature between 55 and 85°C and at pH 8.0 was caused probably by protease hydrolysis.

It has been found that in Indian mackerel (Rastrelliger kanagurla) an extracellular protease released from Pseudomonas marinoglutinosa showed high activity against mackerel myofibrillar protein (Venugopal et al., 1983). The optimum pH range for this enzyme activity was 7.0-8.0 and the enzyme was released from the microorganism over a wide temperature range (2 -25°C). This enzyme could also act on actomyosin at low temperature (0 -2°C). The enzyme activity is sensitive to heat and gamma radiation treatment. Gamma radiation inactivation of this enzyme was suggested prior to refrigeration for enhancing the shelf-life of fish protein.

2.2.4 Salts

Various salts, e.g., NaCl, $CaCl_2$ should cause some influence on muscle protein functionality because they may impart different ionic strength to the system thereby affecting the solubility of salt soluble protein (Ohnishi & Rodger, 1980). Some of these cations (Na^+, Ca^{2+}, Mg^{2+}) have the ability to disrupt the tetrahedral structure of a pure water system and superimpose a new structure. This is termed a net structure forming effect (Fennema, et al., 1973) since the new structure formed by these ions more than compensates for any loss in normal water structure. Other ions (K^+, Cs^+, Cl^-) have rather weak electric field, disrupt the normal structure of water but fail to establish a compensating amount of new structure. Also water near readily hydratable ions assumes a structure similar to that found in crystalline salt hydrates. However, the expected effect of ions on water structure can be altered when the water is involved in collagen hydration. The concentration of NaCl in the system greatly affected the textural properties of Japanese fish cake kamaboko (Suzuki, 1981). The optimum range of salt concentration for kamaboko gel strength was 2.5%-3.0%. If the salt concentration in the system was too high, salting out of protein would occur in the system, thereby preventing kamaboko from forming.

Evans et al., (1975) reported that the addition of sodium phosphate in spaghetti had a deleterious effect on physical

properties. The author concluded that the major effect of phosphate seemed to be on the gluten network. The reduction of protein matrix cohesion appeared in the scanning electron micrographs as a retracted protein network. The combination effect of NaCl and polyphosphate salt to the texture of minced cooked fish muscle and other meat products have been demonstrated by some researchers (Lee & Toledo, 1976; Neer and Mandigo, 1977; Huffman et al., 1981). Significant increases in textural properties, e.g., compression strength and resilence were observed in samples containing both NaCl and polyphosphate as compared to samples containing only NaCl. Polyphosphates have been shown to dissociate actomyosin by formation of univalent metalmyosinate and soluble divalent metal-phosphate complexes (Yasui et al., 1964). Lee and Toledo (1976) stated that the synergistic effect of polyphosphate to NaCl probably occurred by cleavage of cross-linkages between protein molecules and the salt bridges formed by protein-bound divalent cations. Cleavage of the protein polymer results in increase of electrostatic repulsion between protein charges which may thus increase the hydration of soluble muscle protein (Hamm, 1971).

Deng and Tomaszewski (1980) studied the effect of salt, sodium alginate and tripolyphosphate on the quality of fish patties prepared from minced croaker and showed the positive effect of NaCl on hardness and flavor. However, sodium alginate had a negative effect on the breaking force and panel evaluation of firmness of the patties.

Some cryoprotective agents such as lactose, monosodium glutamate and sodium citrate have been used to prevent quality loss of minced cod (Rodger et al., 1980). These additives helped retain protein solubility. However, cryoprotective effect of the additives did not match the performance obtained by the Japanese in their studies on the preservation of surimi. This was probably due to reduced muscle tissue disintegration prior to additive addition where the activities were not well distributed throughout the protein matrix.

2.2.5 Others

Many other factors can affect the textural properties of fish protein gel. Some of them cause changes of protein in the raw material, and some affect the finished product texture during processing. Many research workers have demonstrated the close correlation between myofibrillar protein solubility and gel texture (Samejima et al., 1969; Tsai et al., 1972). Some reports, however, showed considerable variability in the relationship between myofibrillar protein solubility of raw fish muscle and texture of finished product (Webb et al., 1976; Cheng et al., 1979a).

It has been shown that the fish protein gel forming ability varied greatly with species. Gels made from various fish species showed different cohesiveness, firmness, shear force and springiness (Cheng et al., 1979b). During frozen

storage, fish minces undergo deteriorative changes in both texture and flavor (Award et al., 1969). The rate of deterioration is generally faster than for mammalian flesh but varies markedly from one species to another (Laird et al., 1980). Some of the published papers indicated that the formation of covalent cross-linked myosin occurred during frozen storage (Laird et al., 1980; Matthews et al., 1980), which could greatly influence the native protein functionality. Therefore, storage time is also an important factor which can harmfully affect the mince quality (Cheng et al., 1979b). Whether the covalent linkage formed during storage is of the disulphide type or some others has not been established.

It has been shown that trimethylamine oxide in fish muscle is converted into dimethylamine and formaldehyde (FA) during frozen storage even after heating (Hughes, 1959). Childs (1973) found that increasing FA concentration was associated with decreasing protein solubility. Some researchers also showed both myofibrillar and sarcoplasmic protein might be rendered insoluble by FA during frozen storage, and this effect was more evident under low ionic strength conditions (Ohnishi and Rodger, 1980).

Many factors other than those described previously have been demonstrated to affect textural characteristics of fish muscle gel during processing (Lee and Toledo, 1976,1979). Chopping time of fish muscle before cooking was found to

have a significant effect on shear strength (SS) of a gel. Shear strength increased with mixing time to a maximum value then decreased as chopping time was extended. The authors interpreted the extensive chopping could cause alterations in micro-structure of myofibrillar protein due to the elevated temperatures (caused by fiction) of the system or protein polymerization. Mechanical stress generated from the deboning machine has been mentioned to cause significant changes in physicochemical properties of muscle. Different types of cooking showed influence on product texture. Smoked, boiled and steamed have been tested (Lee & Toledo, 1976). Steamed product appeared to be more resilient and firmer than those cooked by other two methods. The weakening of texture in smokehouse cooked product was probably attributed to the long residence time at a temperature range below 80°C prior to final gelling at 95°C. The enzyme proteolysis effect, mentioned earlier, where the optimum temperature for the protease is 55 -85°C might explain this texture weakening effect (Deng, 1981).

Some ingredients such as shortening, soy protein fiber (SPF) and starch can modify the texture of fish protein gel (Lee & Toledo, 1979). The addition of shortening at the level of 11% (W/W) to mackerel minces could overcome the original mushy texture of protein gel. Fifteen percent of SPF addition significantly improved the gel texture and increased juiciness. Plastic fat has been used for improving

texture of tilefish protein gel (Lee & Abdollahi, 1981). The effect of this fat to the protein gel was influenced by fat distribution pattern which was affected by fat hardness.

In a word, all of these factors including the great variation in fish species, physiological condition of fish, season and methods of harvesting, feeding ground, postharvest handling and processing can result in a considerably complicated system of fish protein processing. This will render the control of fish protein gel texture a very difficult task.

Chapter 3

MATERIALS AND METHODS

3.1 MATERIALS

White fish (Coregonus clupeaformis) from Boden lake (latitude x longitude = 5456 x 09383) and Utick lake (latitude x longitude = 5516 x 09600) were used for preparing raw materials for this research program. Fish classes, infestation rates, initial freshnesses and processing dates relevant to this experiment are presented in Table 1. The fish were gutted, boxed with crushed ice, transported to the processing laboratory, and kept under refrigeration (4°C) for 24 hours before further treatment.

3.1.1 Preparation of minced flesh

Whole, dressed fish were deheaded, scraped out and washed to remove remaining guts; then split into skin-on fillets with the backbone left in one side. The fillets were deboned by using a Baader Flesh Separator (BFS) equiped with a 5mm perforated drum using setting #2. The deboned flesh was simultaneously ground into minced flesh. The minced flesh was packed in block form (length x width x height = 50x30.5x2.5cm) in polyester bags with about 4kg for each block. Fish mince blocks were quickly frozen in a double

TABLE 1

Description of fish used in research program

Fish class	Infestation rate[2]	Initial freshness	Processing date	Product[3]
parasitized	281	4 days	5/Feb/1981	a,b,c
parasitized	128	1 day	13/Oct/1982	c
Commercial[1]	40-80	1 day	18/Feb/1983	a,b
Commercial[1]	40-80	1 day	25/Apr/1983	b

1: The fish were caught by Continental Cooperative and transported to the processing laboratory.

2: The unit is cysts/45kg.

3: a=minced flesh
b=washed flesh
c=surimi

plate contact freezer (Freeze-Cel by Dole) for 3 hours, then kept in frozen storage at -30°C until used for further experimental studies.

3.1.2 Preparation of washed flesh

After the deboning step (section 3.1.1.), the minced flesh was then washed in a large tank with chilled water at a ratio of four parts of water to one part by weight of minced flesh. During the washing procedure, blood, flesh pigments and water-soluble constituents were leached out. In addition, most of the fat floated to the surface and was drained away. The mince, water mixture was gently stirred with a mixing paddle for 2 minutes and allowed to settle for 2 minutes,then the water was decanted. This washing procedure was carried out six times. Food grade sodium chloride was added (0.1% by weight of fish and water) during the sixth washing step to facilitate the removal of water from the flesh. The washed flesh was dewatered by draining in fine mesh nylon bags. The bags with wet flesh were kneaded by hand and hung for one hour, then kneaded again at 1/2 hour, to remove water. The dewatered flesh was strained on the BFS through a 3mm perforated drum to remove any undesirable material present in the mince. Washed flesh was packed, frozen and stored as described for the minced flesh (section 3.1.1.).

3.1.3 Preparation of surimi

Surimi is a semi-processed wet fish protein. The initial stages for producing surimi have been discussed in section 3.1.1. and 3.1.2. Washed, strained fish flesh was mixed with 5% glucose and 0.2% sodium tripolyphosphate. The mixing was carried out in a Hobart LP-800 mixer for 15 minutes using a prechilled mixing bowl (4°C). Crushed ice was packed around the outer wall of the bowl to maintain a reduced temperature (4-5°C) during mixing. The blended flesh, surimi, was packed, frozen and stored as previously described (section 3.1.1.). A schematic description of the production of surimi is presented in Figure.1.

3.2 METHOD

3.2.1 Protein fiber formation

Minced, washed flesh and surimi were used, respectively, as raw materials for making protein fibers. The frozen flesh were defrosted in a cold room (4°C) for 48 hours before further treament.

To 120 g of each type of flesh (minced, washed and surimi), 2% NaCl by weight (reagent grade) was uniformly added by sprinkling. Each sample was mixed by a Hobart household 4C mixer for 15 minutes in the cold room (4°C) to obtain a viscous fish protein paste. The paste was then pressed into a 20 ml plastic syringe (without a needle)and extruded into boiling water for one minute to form spaghetti-like coarse fibers. Diameter of the orifice of syringe was 2 mm.

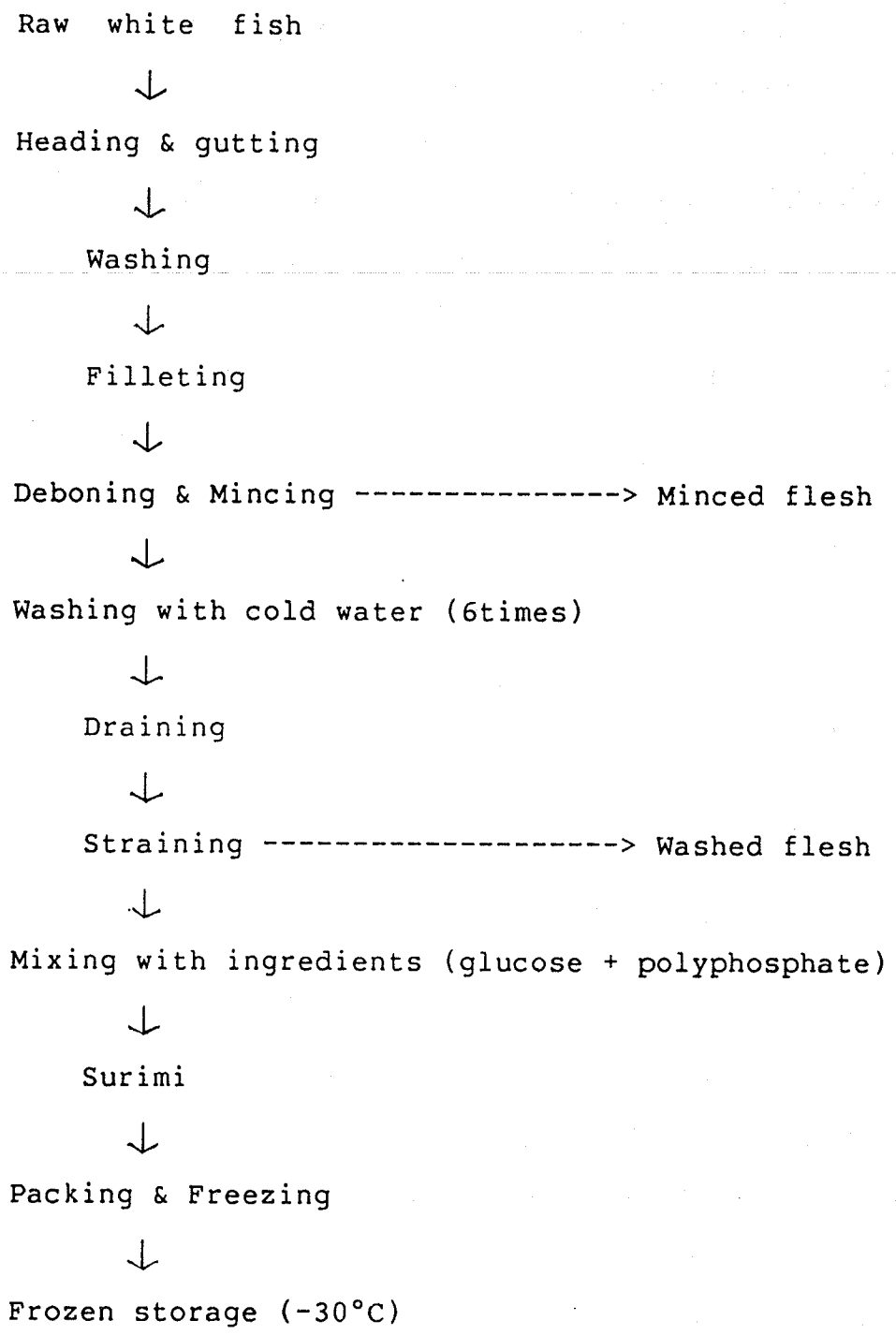

Figure 1: Processing procedure of surimi production

Several other methods were employed to heat denature the protein during fiber making process. These methods are described as follows :

3.2.1.1 Steam kettle

Fish protein paste, after 15 minutes mixing with NaCl, was extruded on a round aluminum plate in fibrous or noodle shape and then placed into preheated steam kettle 5 minutes at 104°C for fiber formation.

3.2.1.2 Oil bath

Fish paste was extruded into a hot vegetable oil bath for 30 seconds at 150±5°C for fiber formation.

3.2.1.3 Oven

Fish paste was extruded on a round aluminum plate in a fibrous or noodle shape, then put into a preheated standard oven (temperature controled) at 150±5°C for 3 minutes for fiber formation. The surimi used in the heating studies was produced in Oct, 1982 while the minced and washed flesh were produced in Feb, 1983.

3.2.2 Protein gel formation

Washed fish flesh was selected as the most ideal form for these studies based on the result of fiber strength test (section 3.2.3.1. and 4.2.2.). Several factors were

investigated for the purpose of developing an understanding of how they affect fish gel texture and fiber strength. Washed flesh used here was produced on Feb, 1983. The procedure used for each treatment is described below:

3.2.2.1 NaCl

Salt was uniformly and directly sprinkled on 200g washed flesh at seven rates (0%, 1.0%, 1.5%, 2.0%, 2.5 & 3.0% ,w/w basis). A Hobart household 4C mixer, speed 4 was used to blend the flesh and NaCl for 15 minutes in cold room (4°C). The time for blending was sufficient to cause the flesh to turn into a viscous paste. The paste (ca. 45g) was then placed into a plastic round bottom centrifuge tube (length x diameter = 10 x 2.7cm) by spoon and centrifuged at 14580g for 5 minutes in a Sorvall RC2-B automatic refrigerated centrifuge (5°C). This compressed all the paste in the tube, squeezed out some air bubbles in the paste and would obtain a more homogeneous gel texture. The tubes were then put into a preheated steam kettle (104°C) and cooked 15 minutes for gel formation. Fibers were made by boiling water treatment (section 3.2.1.).

3.2.2.2 Moisture

Distilled water at the levels of 5%, 10%, 20% and 30% was added to different aliquots of washed flesh before blending. Sodium chloride (2% by weight) was added to the

flesh in each moisture level to aid in gel formation, then the same procedure was followed as described in 3.2.2.1. for the production of fish gel. Fibers were made by boiling water treatment (section 3.2.1.).

3.2.2.3 pH

The original pH of washed fish flesh was 6.9. Flesh pH was adjusted by addition of 0.5N hydrochloric acid (reagent grade) or 0.5N sodium hydroxide (reagent grade) solution. The pH levels under investigation included 6.3, 6.9(control), 7.6 and 8.2. Acidic or alkali solutions were added to the flesh before blending with 2% NaCl. The procedure used to produce fish gel was the same as described in 3.2.2.1. and fiber formation same as outlined in 3.2.1.

3.2.2.4 Mixing time

Six different periods of time, 10, 15, 20, 25, 30 and 40 minutes, were used for detecting the effect of mixing time on gel texture and fiber strength. Sodium chloride (2%) was added into the flesh and minced at 4°C. The methods used for producing fibers and gels were the same as 3.2.1. and 3.2.2.1.

3.2.2.5 Cooking time

Different cooking times (10, 15, 20, 25, 30, 40 minutes) were used for gel preparations. The procedures for producing the gel were the same as described in section 3.2.2.1.

3.2.2.6 Other salts and cryoprotectants

Calcium chloride ($CaCl_2$), magnesium chloride ($MgCl_2$), ferric chloride ($FeCl_3$), sodium tripolyphosphate (STPP) and glucose (reagent grade) were added separately at various levels (Table 2) to washed flesh. The individual Salts and sugar were pre-dissolved in 3ml distilled water in a beaker and poured uniformly on the flesh. An additional 2ml water were used to wash residual material from the beaker onto the sample. The procedure used to produce fish gels and fibers was the same as described in 3.2.2.1. and 3.2.1. Percentages of the salts added in each treatment are listed in Table 2.

3.2.3 Measurement of fiber strength and gel texture properties

3.2.3.1 Fingers test for fiber strength

There is no well accepted method for measuring the strength of protein fibers. In addition texture measuring devices at the University of Manitoba are not sensitive enough to detect differences in the strength of soft fibers. Therefore a subjective test using an individual's hands was established. One end of the formed fiber was held by thumb and index finger of one hand and another end by the other

hand approximately 3cm apart. Then the fiber was pulled to the breaking point and the tensile strength was measured by subjective evalution. This procedure was repeated 3 times for each sample in order to compare the fiber strength of every treatment more accurately.

The strength of the fiber was arbitrarily established by a 0+, 1+, 2+, 3+, 4+ system for the convenience of comparison. If two samples were of the same strength then an equal score would be given; 0+ represented the case where fibers could not be formed. Score were assigned from 1+ representing the weakest fibers and : 4+ representing the strongest fibers. This measurement was made after the formed fibers were cooled to room temperature.

3.2.3.2 Measurement of hardness and cohesiveness of protein gel

The steam cooked protein gel was carefully removed from the plastic centrifuge tube with the help of a small spatula. The gel was cut into a cylindrical shape (diameter x height = 2.7 x 1.5cm) for texture evaluation.

A General Foods Texturometer (GFT) was used to measure hardness and cohesiveness. The cut gel was placed in a given position on the platform resting on the lower arm of the masticator on the GFT. The chewing forces were exerted through the up and down movements of the lucite plunger (diameter x height = 1.8 x 2.5cm) mounted on the masticator.

TABLE 2

Percentages of various salts added in each treatment

Salts	T1 (%)	T2 (%)	T3 (%)	T4 (%)	T5 (%)
		Without NaCl			
$CaCl_2$	0.5	1.0	1.5	2.0	2.5
$FeCl_3$	0.5	1.0	1.5	2.0	2.5
STPP	0.5	1.0	1.5	2.0	2.5
Glucose	1.0	2.0			
		With NaCl			
$CaCl_2$	0.0	0.001	0.01	0.1	0.5
$MgCl_2$	0.0	0.001	0.01	0.1	0.5
$FeCl_3$	0.0	0.001	0.01	0.1	0.5
STPP	0.0	0.001	0.01	0.1	0.5
Glucose	2.5	5.0			

T = treatment ; percentage was w/w (salt/flesh) basis.

A typical texture-profile curve for two bites obtained on the GFT is presented in Figure 2. The curve reads from right to left. Area A1 represents the first chew and area A2 the second chew. The area A3 represents the amount of adhesiveness in a sample. The texture-profile curve for a fish protein gel developed in this research program is presented in Figure 3. The lack of an A3 area in Figure 3 indicates that fish gels have no adhesiveness detectable by the GFT.

Definition of hardness and cohesiveness and the calculation of these two parameters for the GFT system are shown as follows (Bourne, 1978):

Hardness

Def: Force necessary to attain a given deformation of product.

$$\text{Hardness} = \frac{\text{Height of first peak (A1)}}{\text{Volts input*}}$$

(*: Volts input=1 for all measurements in this study)

Cohesiveness

Def: Strength of internal bonds making up the body of the product.

$$\text{Cohesiveness} = \frac{\text{Area (cm}^2\text{) of second peak}}{\text{Area (cm}^2\text{) of first peak}}$$

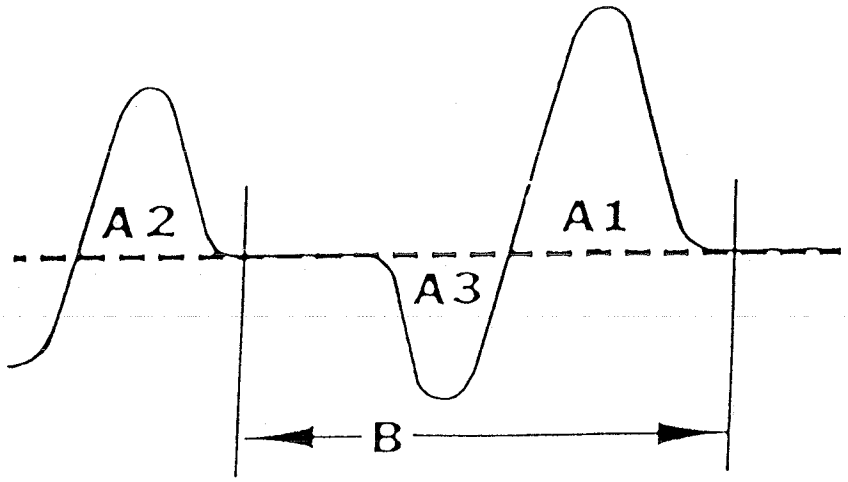

Fig 2. A typical texture-profile curve from GF Texturometer

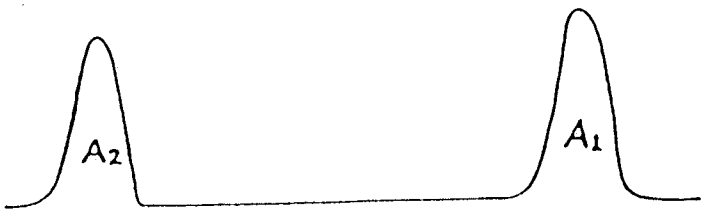

Fig 3. Texture-profile curve of a fish gel sample

A polar planimeter (Keuffel & Esser Co. 4236M) was used to calculate the area of A1 and A2. Peak area was measured two times and the average was taken. All treatments were replicated four times. When the difference of hardness between two gels is approximately greater than 0.5 units and cohesiveness is greater than 0.02 units, the strength of the corresponding fibers showed different fingers test scores.

3.2.3.3 Measurement of water holding capacity of fish paste

Water holding capacity of fish paste after mixing with salt is denoted by the quantity of water loss after 5 minutes of centrifugation (14580g) of fish paste (45g). The water forced out from the paste by centrifugal force was decanted into a breaker and weighed. The higher water loss indicates lower water holding capacity of protein paste. The unit of this parameter is g water loss per 45g of fish paste.

Chapter 4

RESULTS AND DISCUSSION

4.1 INTRODUCTION

This research program was designed to study the factors affecting the production of fibers and gels produced from mechanically deboned white fish flesh. The average proximate compositional analysis of the raw fish flesh, minced flesh,washed flesh and surimi used in the overall program are shown in Table 3 (Watt and Merrill, 1975 ; Hydamaka, et al.,1984). Production of minced and washed flesh and surimi are described in sections 3.1.1., 3.1.2., and 3.1.3. The main features of the washed flesh and surimi are high protein, low fat and no carbohydrate content. Protein, being the major component of these meats has a significant effect on the formation of gels and fibers produced from the fish flesh material.

Many factors that affect the functional properties of protein were investigated in this study. These include source of heat, kind of raw material, mixing time, added moisture, pH, cooking time, type of cation, concentration of cation, sodium tripolyphosphate and glucose.

Table 3

Composition of raw white fish, minced flesh, washed flesh and surimi (100g edible portion)

Material	Water(g)	Protein(g)	Fat(g)	Carbohydrate (g)	Ash(g)	Ca(mg)	P(mg)	Fe(mg)	Na(mg)	K(mg)
Raw	71.7	18.9	8.2	0	1.2	*	270	0.4	52	299
Minced	76.6	18.7	4.3	0	1.08	-	-	-	-	-
Washed	85.0	16.5	0.9	0	0.26	-	-	-	-	-
Surimi	82.0	12.5	0.8	0	0.62	-	-	-	-	-

*: Asterisk denotes lack of reliable data for the constituent believed to be present in measurable amount.

-: Dash denotes the constituent has not been measured.

4.2 PRODUCTION OF FIBERS AND GELS

4.2.1 Incorporation of sodium chloride into fish flesh

Sodium chloride is known to extract myofibrillar protein from meat and affect protein-protein interactions (Suzuki, 1981). Therefore NaCl (at six levels from 0.5 to 3.0%) was incorporated into washed fish flesh (Tables 4,5 & 6) to determine the effect on fiber formation, fiber strength and gel texture. It was observed that differences in fiber strength could be detected by the finger test where the corresponding gel hardness was greater than 0.5 units and/or cohesiveness greater than 0.02 units.

The addition of NaCl to washed fish flesh reduced the hardness of the cooked protein gel (Table 4). At the same time the cohesiveness of this gel increases to a maximum and then starts to diminish. Incremental increases of NaCl from 0 to 1.0% significantly decreased (Table 4, Appendix A1) hardness by 5.35 units and from 1.0 to 3.0% by 1.28 units . On the other hand, incremental increases of NaCl from 0 to 1.0% showed little change in cohesiveness (0.017 units), but this value significantly increased 0.168 units (Table 5, Appendix A2) when NaCl content increased to 2.5%. Cohesiveness declined by 0.06 units when 3% NaCl was added. Suzuki (1981) reported that high NaCl concentrations which caused the salting out of protein prevented formation of kamaboko. This effect may have contributed to the results observed in these experiments.

TABLE 4

Effect of added NaCl to washed flesh on gel hardness

NaCl (%)	Hardness	Unit change	Grouping[1]
0	8.93[2]		A
0.5	6.14	5.35	B
1.0	3.58		C
1.5	2.98		D
2.0	2.84	1.28	D
2.5	2.54		E
3.0	2.35		E

1: The result of Duncan's Multiple Range Test at $P<0.05$. Same letter indicates the hardness are not significantly different.

2: Average of four observations.

TABLE 5

Effect of added NaCl to washed flesh on gel cohesiveness

NaCl (%)	Cohesiveness	Unit change	Grouping[1]
0	0.681[2]	--	A
0.5	0.696	0.017	A
1.0	0.698	--	A
1.5	0.714		A
2.0	0.843	0.168	B
2.5	0.866	--	B
3.0	0.806	0.06	B

1: The result of Duncan's Multiple Range Test at $P<0.05$. Same letter indicates the cohesiveness are not significantly different.

2: Average of four observations

TABLE 6

Effect of added NaCl to washed flesh on fiber strength and water holding capacity of fish paste

NaCl (%)	B W[1]	W L[2]
0	0+	9.1
0.5	1+	7.4
1.0	2+	0.3
1.5	3+	0.0
2.0	4+	0.0
2.5	4+	0.0
3.0	3+	0.0

1: BW = Boiling water treatment for fiber formation.

2: WL = Water loss, which represents the water holding capacity of fish paste. The unit is g water loss / 45g fish paste. Each value is the average of duplicates.

Washed fish flesh without added NaCl was able to produce a very hard gel (Table 4), but unable to form fibers in boiling water (Table 6). This material lost much of its water content (9.1g per 45g of paste , Table 6). The paste was not viscous after the mixing step. The addition of NaCl to the paste reduced the loss of water significantly (above 1.5% NaCl no loss was recorded). Maximum fiber strength was observed in paste with 2.0 to 2.5 % added NaCl. Fish paste with good water holding capacity was essential for fiber formation in this test. Briskey and Fukazawa (1981) stated that water retention was due to the solubilization of myofibrillar protein, especially actomyosin, during the mixing step. It has been established that components of myofibrillar protein such as myosin, actin and actomyosin will be extracted from muscle at higher ionic strength salt solutions (usually 0.3 to 0.4 M) (Suzuki, 1981). For different sources of protein, different optimum salt concentration for protein extration may be needed. According to the results of this experiment, 2% NaCl was selected for further gels and fibers texture studies.

4.2.2 Comparison of different heating methods and raw materials for fiber formation

Four methods of heating were used to heat set fibers formed from minced, washed and surimi flesh. The relative strength of these fibers was measured as described in section 3.2.3.1. Higher tensile strengths were produced in fi-

bers heated in oil bath or in a standard oven (Table 7) when compared with boiling water or steam treatments. This indicates that the higher temperature treatment has a positive effect on fiber formation and strength. A similar temperature effect has been demonstrated with a vegetable protein texturization process (Kinsella, 1978). Heating in the oil bath had the highest temperature (155°C) and the shortest heating time (30 sec) among the four methods. Heating in a conventional oven has a different environment from an oil bath although the temperatures of both treatments were similar. With the oven method the fibroid shaped fish paste was exposed to a hot dry air environment. Some moisture and entrapped air would escape to this environment. This might have improved the protein-protein interaction and resulted in reduced diameter of the formed fiber and increased fiber strength (Table 7), but the results are inconclusive. Utilization of boiling water is the most convenient of these methods for fiber fromation. Boiling water and steam kettle treatments showed similar effects in the development of fiber strength , although the latter was a few degrees higher in temperature.

One of the characteristics of oil heated fibers was a shrinking or collapsing effect. This was probably due to a replacement of moisture in flesh with oil and a loss of air from the flesh during cooking. This affected the diameter of the fiber but not the overall length. The absorbed oil

may affect the storage of this type of product. The loss of volume may affect its usefullness if one were making a layered texturized product. Washed and minced flesh produced fibers with more tensile strength than fibers produced from surimi (Table 7). This suggests that the ingredients (0.2% STPP and 5% glucose) incorporated during the surimi process in some way interfered with binding among the protein molecules (fiber formation).

Three different raw materials were compared for fiber formation and fiber strength. The results (Table 8) show that minced flesh produces somewhat stronger fibers than the other two materials. Minced flesh has lower moisture and higher fat content than washed and surimi flesh (Table 3). Lee and Toledo (1976) found that high levels of moisture (>75%) in fish flesh reduced compression force and shear force values. Others reported that fat or shortening would improved the textural properties of minced fish gel (Lee and Toledo, 1979 ; Lee and Abdollahi, 1981). However, the complicated composition of minced flesh gives large variability in the textural properties of formed fibers and creates many difficulties for quality control during frozen storage.

Fibers formed from surimi flesh had the weakest strength of all the materials by all the heating methods except for the oil bath treatment. Actually, surimi paste had a lower viscosity after mixing with NaCl than minced and washed

TABLE 7

Comparison of four heating methods for fiber formation in each raw material

Material methods	Minced[1] flesh	Washed[1] flesh	Surimi[1]
Boiling water	3+[2]	2+[2]	1+[2]
Steam kettle	1+	2+	1+
Oil bath	4+	3+	2+
Oven	2+	1+	3+

1: Contains 2% NaCl.

2: Average of three observations.

TABLE 8

Comparison of three raw materials for fiber formation in each heating method

Materials	Methods: Boiling water	Steam kettle	Oil bath	Oven
Minced flesh[1]	2+[2]	3+	2+	3+
Washed flesh[1]	1+	2+	1+	2+
Surimi[1]	1+	1+	3+	1+

1: Contains 2% NaCl.

2: Average of three observations.

flesh. It may be that the extraction of myofibrillar protein, which has been described as the major component contributed to fish protein gel formation (Suzuki, 1981), from surimi was obstructed by the addition of 5% glucose and 0.2% polyphosphate. However, glucose and STPP may also interfere with protein gel or fiber formation and produce softer textured products. This property may be an advantage in the use of surimi as an ingredient in the simulation of some marine products. All additional studies in this program were carried out with washed fish flesh since surimi produced soft textured products and minced flesh was too variable in composition.

4.2.3 Mixing time

The mixing procedure has been demonstrated to affect the microstructure of myofibrillar protein and ultimately the shear strength of protein gels (Lee and Toledo, 1976 ; 1979). The optimum mixing time for washed fish flesh (2% NaCl incorporated) was between 15 and 25 minutes (Table 9 & 10). When the mixing time is shortened or extended beyond this range fiber strength and gel cohesiveness declined. A mixing time of 15 minutes was used in all subsequent studies. However, the optimum mixing time for a certain gel texture on fiber strength is not necessarily the range described here. This will depend on the texture that is specified for that product.

TABLE 9

Effect of mixing time of washed flesh on gel texture

Time(min)	Hardness		Cohesiveness	
10	3.25[2]	A[1]	0.788[2]	A B[1]
15	2.98	C	0.810	A B
20	2.61	B	0.832	A B
25	2.61	B	0.837	A
30	2.75	B C	0.807	A B
40	2.53	B	0.782	B

1: The capital letters represent the result of Duncan's Multiple Range Test at $P<0.05$.

2: Average of four observations.

TABLE 10

Effect of mixing time of washed flesh on fiber strength

Time(min)	B W[1]	O V[2]
10	2+[3]	2+[3]
15	3+	3+
20	3+	3+
25	3+	3+
30	2+	2+
40	1+	1+

1: BW = Boiling water treatment for fiber formation.

2: OV = Oven treatment for fiber formation.

3: Average of three observations.

4.2.4 Moisture

Moisture content has a significant effect on the texture of fish protein gel (Lee and Toledo, 1976 ; Pan et al., 1980). Insufficient or higher moisture contents in the formulation will be deleterious to gel texture and fiber strength. In this study adding water to the formulation reduced gel hardness and fiber strength (Table 11 & 12). Increasing the moisture content from 86.2 % to 87.5 % decreased the hardness from 3.13 to 2.27 units. Additional increases in moisture to 89.4 % significantly reduced hardness, cohesiveness and fiber strength (1.25 units, 0.65 units and 0+) (Table 11 & 12 ; Appendix A5 & A6).

The addition of water to washed fish flesh (2% NaCl added) has not improved the functional properties of this product. This is not surprising considering the initial starting level of 86.2 % for this component. Lee and Toledo (1976) have reported that reduced moisture levels may increase fiber strength. On the other hand, too low a moisture content may also have a negative effect on fiber strength. Further studies are required to identify an optimum moisture level for washed fish flesh when it is processed to a specific texturized product. Both boiling water and oven treatments were used for fiber formation. Similar results were obtained within each treatment (Table 12). However, the fiber formed by oven treatment appeared to have stronger tensile strength than the fiber from the boiling water treatment (subjective measurement).

TABLE 11

Effect of added moisture(with 2% NaCl) to washed flesh on gel texture

Added water	Calculated moisture content	Hardness		Cohesiveness	
0%	86.2[3]%	3.13[2]	A[1]	0.789[2]	A[1]
5	86.9	2.53	A	0.783	A
10	87.5	2.27	B	0.803	A
20	88.5	1.59	B	0.779	A
30	89.4	1.25	C	0.650	B

1: The capital letters represent the result of Duncan's Multiple Range Test at $P<0.05$. Same letter indicates the hardness are not significantly different.

2: Average of four observations.

3: Original moisture content of washed flesh.

TABLE 12

Effect of added moisture (with 2% NaCl) to washed flesh on fiber strength

Added water	Calculated moisture content	B W[1]	O V[2]
0%	86.2%	2+[3]	2+[3]
5	86.9	2+	2+
10	87.5	1+	1+
20	88.5	1+	1+
30	89.4	0+	0+

1: BW= Boiling water treatment for fiber formation.

2: OV= Oven treatment for fiber formation.

3: Average of three observations.

4.2.5 pH

The functional properties of protein systems can be significantly affected by pH. The pH of washed fish flesh was adjusted to three levels and tested with the control at pH 6.9 (Table 13). At pH 6.3 the gels were harder and less cohesive than at pH 6.9 or 7.6. Gels produced from washed flesh adjusted to pH 6.9, 7.6 & 8.2 were statistically of similar hardness (2.53-2.63). On the other hand, the gel from the pH 8.2 formulation was less cohesive than the other two. The optimum pH range was 6.9-7.6 for the production of stronger fibers and more cohesive gels (Table 13 & 14). Meinke et al. (1972) found that the pH range greatly influenced fish protein solubility. Each species of fish may have a separate optimum pH range for best protein solubility and textural properties (Deng, et al., 1976 ; Pan, et al.,1980 ; Chen, et al., 1981 ; Deng, 1981). Protease activity in fish muscle, which is influenced by pH may also affect the protein solubility (Chen, et al., 1981). The extent of protease activity in white fish muscle and its effect on gel texture has not been studied yet.

The oven heat treatment was observed to improve fiber strength when compared with the boiling water treatment. This indicates that high temperature dry air heat dried the fiber slightly and achieves a stronger heat set within the protein-water matrix. This was also observed in section 4.2.4.

TABLE 13

Effect of adjusted pH (with 2 % NaCl) of washed flesh on gel texture

pH	Hardness		Cohesiveness	
6.3	2.96[3]	A[2]	0.762[3]	A[2]
6.9[1]	2.63	B	0.828	A B
7.6	2.58	B	0.847	B
8.2	2.53	B	0.793	A B

1: Original pH of washed flesh is 6.9.

2: Capital letters represent the results of Duncan's Multiple Range Test at $P<0.05$. Same letter indicate the hardness are not significantly different.

3: Average of four observations.

TABLE 14

Effect of adjusted pH (with 2 % NaCl) of washed flesh on fiber strength

pH	B W[1]	O V[2]
6.3	1+[4]	1+
6.9[3]	2+	2+
7.6	2+	2+
8.2	1+	1+

1: BW = Boiling water treatment for fiber formation.

2: OV = Oven treatment for fiber formation.

3: Original pH of washed flesh is 6.9.

4: Average of three observations.

4.2.6 Cooking time

The effect of the cooking time on the hardness and cohesiveness of protein gels was investigated. In general, gel cohesiveness did not vary greatly (0.023 units) between 10 and 40 minutes of cooking (Table 15). Also, gel hardness declined 0.06 units only as cooking time increased. The gels developed a honeycomb appearence when the cooking time was extended beyond 20 minutes. One possible reason for this effect could be overheating of the protein-water gel matrix causing a partial collapse as the protein molecules become extensively denatured. A cooking time of 15 minutes was used in subsequent studies.

TABLE 15

Effect of cooking time on gel texture

Time(min)	Hardness				Cohesiveness	
10	2.45[2]	A[1]			0.803[2]	A[1]
15	2.10	A	B		0.823	A
20	2.19	A	B		0.807	A
25	1.96		B		0.826	A
30	1.39			C	0.813	A
40	1.76		B		0.810	A

1: The result of Tukey's Studentized Range Test at $P<0.05$. Same letter indicates the values are not significantly different.

2: Average of four observations.

4.2.7 Summary

Several factors were found to modify the hardness and cohesiveness properties of gels produced from washed fish flesh. These factors also affected the strength of fibers produced from this material. Subseqently, all further studies were conducted using the following system. Washed fish flesh (pH 6.9)(produced on Feb. 1983) was mixed for 15 minutes with 2 % NaCl (and the other ingredients were included). This material was cooked for 15 minutes in a steam bath for gels or with the standard oven or boiling water system for fibers. This procedure ensured that the processing system had minimal effect on variability observed in gels and fibers during these studies.

4.3 EFFECT OF SPECIFIC CATIONS ON FIBERS AND GELS PRODUCED FROM WASHED FISH FLESH

The effects of mono, di and trivalent cations on fibers and gels were evaluated in this study. The ionic strengths of the various systems were kept as constant as possible (Table 16). Sodium chloride was studied separately (section 4.2.1) and with $CaCl_2$, $MgCl_2$ and $FeCl_3$; $CaCl_2$ and $FeCl_3$ were also studied separately.

TABLE 16

Calculated ionic strength of salt system at various concentrations

Salt	Conc. (%) 0.5	1.0	1.5	2.0	2.5
NaCl	0.1	0.2	0.3	0.4	0.5
$CaCl_2$ [1]	0.12	0.24	0.36	0.48	0.60
$FeCl_3$ [2]	0,12	0.25	0.37	0.49	0.62

1: Calcium chloride was calculated by $CaCl_2 \cdot 2H_2O$.

2: Ferric chloride was calculated by $FeCl_3 \cdot 6H_2O$.

4.3.1 Calcium chloride ($CaCl_2$)

4.3.1.1 Incorporation of $CaCl_2$

The addition of $CaCl_2$ to washed fish flesh produced effects that are distinctly different from the addition of NaCl (Table 17). Fibers were not formed from washed fish flesh paste with 0.5 and 1.0 % added $CaCl_2$. Also, water losses at these two concentration levels were higher than those with NaCl added at the same levels (Table 6 & 18).

Fiber formed from paste with 1.5 % $CaCl_2$ was not elastic i.e. had weak tensile strength although water loss from the paste was zero. When 2.0 and 2.5 % $CaCl_2$ were added, the tensile strength of the fibers increased. However, these fibers had a gritty texture when they were chewed. Measurement of hardness and cohesiveness in the $CaCl_2$ gels was conducted at levels of 1.5 % and above. The gels with 0.5 and 1.0 % $CaCl_2$ shrank in the centrifuge tubes during cooking. The deformed gels would not have given representative data with the GF texturometer.

The cohesivensess of gels containing 1.5 and 2.0 % $CaCl_2$ was similar to NaCl gels at the same concentration. However, the $CaCl_2$ gels were softer (Table 4 & 17). The tensile strengths of the $CaCl_2$ fibers were softer than the NaCl fibers. The cohesiveness of the $CaCl_2$ gels reached a maximum (0.82 units) at 2.0 % and then declined. The hardness of these gels decreased greatly from the control at all levels.

TABLE 17

Effect of added $CaCl_2$ to washed flesh on gel texture

$CaCl_2$ (%)	Hardness		Cohesiveness		
0	8.93[2]	A[1]	0.681[2]	A[1]	
0.5	--[3]		--		
1.0	--		--		
1.5	2.26	B	0.711	A	
2.0	1.88	C	0.823		B
2.5	1.31	D	0.775	A	B

1: The result of Duncan's Multiple Range Test at $P<0.05$. Same letter indicates the values are not significantly different.

2: Average of four observations.

3: Dash denotes the fiber could not be formed on that $CaCl_2$ addition level. No gel was prepared for texture measurement.

TABLE 18

Effect of added $CaCl_2$ to washed flesh on fiber strength and water holding capacity of fish paste

$CaCl_2$ (%)	B W[1]	W L[2]
0	0+[3]	--[4]
0.5	0+	14.0
1.0	0+	13.5
1.5	1+	0
2.0	2+	0
2.5	2+	0

1: BW = Boiling water treatment for fiber formation.

2: WL = Water loss (g water loss /45g fish paste).

3: Average of three observations.

4: Dash denotes the result was not measured.

4.3.1.2 Incorporation of $CaCl_2$ plus NaCl

Calcium has been used during the processing of fruits and vegetables to increase the firmness of tissues or for the formation of protein gels (Ellinger, 1977). Therefore, the synergistic effect of Ca^{2+} combined with NaCl and incorporated in washed fish flesh on gel texture and fiber strength was tested. At low concentrations (0.001% and 0.01%),the effect of $CaCl_2$ to fiber strength or hardness and cohesiveness of gel are not significant (Table 19 & 20 ; Appendix A11 & A12). When 0.1% of $CaCl_2$ was added the cohesiveness of gel increased (compared to 0.01%) and the fiber strength increased when boiling water was used as the heat source. However, when 0.5% of $CaCl_2$ was added the gel became firmer and more cohesiveness but the fiber was weakened with both moist and dry heat.

Higher concentrations of $CaCl_2$ (about 0.5%)added to the system negatively affected the fiber strength. This may due to the change in ionic strength in the system which could affect protein extractability. The increase in hardness and cohesiveness of the 0.5% $CaCl_2$ gel may be due to the binding effect of $Ca^{2+}$to protein molecules. Addition of $CaCl_2$ to washed fish flesh (with 2% NaCl) did not have any synergistic effect on the functional properties of this system.

Addition of $CaCl_2$ changed the sensory properties of both gels and fibers, both had a gritty texture, when chewed (at

TABLE 19

Effect of added $CaCl_2$ (with 2% NaCl) to washed flesh on gel texture

$CaCl_2$ (%)	Hardness		Cohesiveness	
0	2.31[2]	A[1]	0.790[2]	A[1]
0.001	2.29	A	0.785	A
0.01	2.30	A	0.789	A
0.1	2.40	A	0.825	A
0.5	2.68	B	0.836	A

1: The capital letters represent the result of Duncan's Multiple Range Test at $P<0.05$. Same letter indicates the hardness are not significantly different.

2: Average of four observations.

TABLE 20

Effect of added $CaCl_2$ (with 2% NaCl) to washed flesh on fiber strength

$CaCl_2$ (%)	B W[1]	O V[2]
0	2+[3]	2+[3]
0.001	2+	2+
0.01	2+	2+
0.1	3+	2+
0.5	1+	1+

1: BW = Boiling water treatment for fiber formation.

2: OV = Oven treatment for fiber formation.

3: Average of three observations.

0.1 and 0.5% $CaCl_2$). A slightly gritty texture may be accepted by consumers especially the orientals.

4.3.2 Magnesium chloride ($MgCl_2$)

4.3.2.1 Incorporation of $MgCl_2$ plus NaCl

Magnesium chloride was used to compare the effect of this bivalent salt with the effect of $CaCl_2$ on fiber strength and gel texture. The data (Table 21 & 22) illustrated that $MgCl_2$ reduced the hardness of the protein gel, had little effect on cohesiveness and slightly improved fiber strength (0.1% $MgCl_2$)(Table 22). On the other hand, $CaCl_2$ increased the hardness and cohesiveness of these gels (Table 19). Therefore, the effect of $MgCl_2$ to protein extractability and protein-protein interactions may be different from $CaCl_2$ although both are bivalent salts. The strength of fiber made by the oven treatment was higher than those by the boiling water treatment at the same addition level.

TABLE 21

Effect of added $MgCl_2$ (with 2% NaCl) to washed flesh on gel texture

$MgCl_2$ (%)	Hardness		Cohesiveness	
0	2.31[2]	A[1] C	0.790[2]	A[1]
0.001	2.00	B	0.801	A
0.01	2.13	A B C	0.808	A
0.1	2.35	A	0.818	A
0.5	2.08	B C	0.779	A

1: The capital letters represent the result of Duncan's Multiple Range Test at $P<0.05$. Same letter indicates the values are not significantly different.

2: Average of four observations.

TABLE 22

Effect of added $MgCl_2$ (with 2% NaCl) to washed flesh on fiber strength

$MgCl_2$ (%)	B W[1]	O V[2]
0	2+[3]	1+[3]
0.001	2+	1+
0.01	2+	2+
0.1	3+	2+
0.5	1+	1+

1: BW = Boiling water treatment for fiber formation.

2: OV = Oven treatment for fiber formation.

3: Average of three observations.

4.3.3 Ferric chloride ($FeCl_3$)

4.3.3.1 Incorporation of $FeCl_3$

Fibers could not be produced from washed fish flesh with $FeCl_3$ incorporated. Upon addition of $FeCl_3$, the paste lost much of its water holding capacity (Table 23). Water holding capacity was maximum between 1.5 and 2.0% $FeCl_3$ but declined quickly at 2.5%. These data suggest that water holding capacity is not the only essential factor in fiber and gel formation. The trivalent cation (Fe^{3+}) seems to disrupt the functional properties essential for fiber and gel formation while the monovalent cation (Na^{+}) seems to assist in this system. The effects of the divalent cation (Ca^{2+}) are intermediate to the above two. At the higher addition levels of $FeCl_3$ (1.5%, 2.0% and 2.5%), the paste turned to a brown color which was imparted by the $FeCl_3$ solution. This would detrimentally affect the acceptability of the product even though fiber could have been made through the addition of this salt.

4.3.3.2 Incorporation of $FeCl_3$ plus NaCl

Ferric chloride has an negative effect on gel formation. This is similar to the result when $FeCl_3$ was used alone (section 4.3.3.). Gel hardness and cohesiveness of control (0% $FeCl_3$) were not significantly different with low levels of $FeCl_3$ (0.001 - 0.01%) (Table 24). Gel cohesiveness dropped significantly when 0.1% $FeCl_3$ was added and fi-

TABLE 23

Effect of added $FeCl_3$ to washed flesh on fiber strength and water holding capacity of fish paste

$FeCl_3$ (%)	B W[1]	W L[2]
0	0+[3]	--[4]
0.5	0+	19.8
1.0	0+	14.0
1.5	0+	0.0
2.0	0+	1.3
2.5	0+	8.7

1: BW = Boiling water treatment for fiber formation.

2: WL = Water loss (g water loss / 45g fish paste).

3: Average of three observations.

4: Dash denotes the fiber could not be formed at that $FeCl_3$ addition level.

ber strength was also reduced (Table 25). The fish paste had a very low viscosity as 0.5% of $FeCl_3$ was added and fiber could not be formed by both boiling water and oven treatment. The mechanism for this negative effect of $FeCl_3$ to fiber formation is not clear.

According to the results presented in section 4.3.3., the change in ionic strength within the system as $FeCl_3$ is increased does not seem to be the only reason for producing this phenomenon. Ferric chloride does not only impart a negative effect to fiber formation, but also imparts a rusty odor and orange color rendering the product aesthetically unacceptable.

TABLE 24

Effect of added $FeCl_3$ (with 2% NaCl) to washed flesh on gel texture

$FeCl_3$ (%)	Hardness		Cohesiveness	
0	2.31[2]	A[1]	0.790[2]	A[1]
0.001	2.32	A	0.785	A
0.01	2.53	A	0.808	A
0.1	2.47	A	0.717	A
0.5	--[3]		--	

1: The result of Duncan's Multiple Range Test at $P<0.05$. Same letter indicates the values are not significantly different.

2: Average of four observations.

3: Dash denotes the fiber could not be formed on that $FeCl_3$ addition level. No gel was prepared for texture measurement.

TABLE 25

Effect of added $FeCl_3$ (with 2% NaCl) to washed flesh on fiber strength

$FeCl_3$ (%)	B W[1]	O V[2]
0	2+[3]	2+
0.001	2+	2+
0.01	2+	2+
0.1	1+	1+
0.5	0+	0+

1: BW = Boiling water treatment for fiber formation.

2: OV = Oven treatment for fiber formation.

3: Average of three observations.

4.3.4 Summary

Incorporation of mono-(Na^+), bi-(Ca^{2+}, Mg^{2+}) and trivalent (Fe^{3+}) ions into washed fish flesh markedly affected the properties of fibers and gels. When the charge on the ion increased a negative effect was observed on fiber formation (Tables 6, 18, 23). A direct proportional relationship was observed between the ratio of cation radius to charge (R/C value) (Table 26) and fiber formation and gel texture. The reason for this relationship is unknown. However, it is known that cations and anions disrupt the tetrahedral structure of water in pure aqueous systems (Fennema, 1973) and depending on the R/C values can impose a new structure, called a net structure former or disrupt structure , called a net structure breaker. Sodium (Na^+), calcium (Ca^{2+}) and magnesium (Mg^{2+}) are considered to be net structure formers while chloride (Cl^-) is a net structure breaker. High concentrations of chloride (Cl^-) in $FeCl_3$ systems may be deleterious to fiber and gel formation while $CaCl_2$ and $MgCl_2$ were able to modify these systems. Control of these ions should enable one to produce specific textures in fish fibers and gels.

TABLE 26

Ionic radius, charge and the ratio of radius to charge of cations[1]

Cations	Radius (R)	Charge (C)	R/C
Na^{+}	0.97	+1	0.97
Ca^{2+}	0.99	+2	0.50
Mg^{2+}	0.66	+2	0.33
Fe^{3+}	0.64	+3	0.21

1: Data are obtained from " Handbook of Chemistry and Physics", CRC Press.

4.4 EFFECT OF CRYOPROTECTANTS ON FIBERS AND GELS PRODUCED FROM WASHED FISH FLESH

4.4.1 Sodium tripolyphosphate (STPP)

4.4.1.1 Incorporation of STPP

Sodium tripolyphosphate (STPP) was one of the ingredients in surimi which may have affected the gel and fiber forming properties of this material. When STPP was added to washed flesh, fiber formation was inhibited (Table 28). Polyphosphate was able to improve water holding capacity of the paste as no water was lost even at the lowest level of addition (0.5%). STPP has been reported to have very strong hydration properties in water and interacts with protein molecules (Ellinger, 1972). It was possible to prepare gels with STPP incorporated washed fish flesh but these gels were softer than those prepared with the mono-(Na^+) and divalent(Ca^{2+}) systems (Tables 4, 17 & 28). The cohesiveness of the gels was similar to those produced with the divalent (Ca^{2+}) system and the slightly less cohesive than the monovalent (Na^+) system. Cohesiveness reached a maximum at 1.5% STPP and declined.

The failure of the STPP systems to produce fiber in boiling water is interesting. The necessity for a system to have good water retention was identified as an essential component for producing good fibers and gels. Myofibrillar protein was suggested to interfere with gelling and the fiber forming properties. Perhaps STPP was unable to extract enough of this component during the mixing operation thereby

TABLE 27

Effect of added STPP to washed flesh on gel texture

STPP (%)	Hardness		Cohesiveness	
0.5	1.83[2]	A[1]	0.752[2]	A[1]
1.0	1.76	A B	0.784	A B
1.5	1.64	B C	0.820	B
2.0	1.57	C	0.799	A B
2.5	1.43	D	0.775	A B

1: The result of Duncan's Multiple Range Test at $p<0.05$. Same letter indicates the values are not significantly different.

2: Average of four observations.

TABLE 28

Effect of added STPP to washed flesh on fiber strength and water holding capacity of fish paste

STPP (%)	B W[1]	W L[2]
0.5	0+[3]	0
1.0	0+	0
1.5	0+	0
2.0	0+	0
2.5	0+	0

1: BW = Boiling water treatment for fiber formation.

2: WL= Water loss (g water loss/45g fish paste).

3: Average of three observations.

resulting in weak gels and a lack of fiber formation. Also polyphosphate has been reported (Evans et al., 1975) to have a deleterious effect on the physical properties of protein.

4.4.1.2 Incorporation of STPP plus NaCl

The incorporation of STPP at various levels with 2% NaCl into washed fish flesh did not significantly affect hardness or cohesiveness (Table 29). The control samples were prepared from a separate lot of fish (caught and processed in April, 1982) and this may be the reason for the lower scores for hardness (2.84 in Table 4) and cohesiveness (0.843 in Table 5) observed in Table 29. Cohesiveness increased with addition of STPP to 0.1% then decreased. Hardness increased with additions of STPP to 0.01% then declined. Similar affects for STPP and NaCl were observed by Lee and Toledo (1976) and Huffman et al. (1981). Higher concentration of STPP may cause some dissociation of actomyosin (Yasui, et al., 1964) and produce a softer texture in the protein gel.

Fibers were produced with STPP when 2% NaCl was incorporated(Table 30) even though STPP apparently prevented formation when no other cations were added (Table 15). Little effect on fiber strength was observed at low levels of STPP (0.001-0.01%) but higher concentrations (0.1-0.5%) noticeably weakened the fibers. STPP was found to improve water holding capacity in fish flesh but its ability to extract myofibrillar protein is uncertain. As noted in section

TABLE 29

Effect of added STPP (with 2% NaCl) to washed flesh on gel texture

STPP (%)	Hardness		Cohesiveness	
0	1.49[2]	A[1]	0.796[2]	A[1]
0.001	1.74	A	0.814	A
0.01	1.75	A	0.807	A
0.1	1.60	A	0.818	A
0.5	1.46	A	0.772	A

1: The result of Tukey's Studentized Range Test at $p<0.05$. Same letter indicates the values are not significantly different.

2: Average of four observations.

TABLE 30

Effect of added STPP (with 2% NaCl) to washed flesh on fiber strength

STPP (%)	B W[1]	O V[2]
0	3+[3]	2+
0.001	3+	2+
0.01	3+	3+
0.1	2+	1+
0.5	1+	1+

1: BW = Boiling water treatment for fiber formation.

2: OV = Oven treatment for fiber formation.

3: Average of three observations.

4.4.1.1. this property may contribute to the weakened fiber observed in this study.

4.4.2 Glucose

4.4.2.1 Incorporation of glucose

Glucose (1.0% and 2.0%) was incorporated into washed fish flesh. Fibers were not formed in boiling water and the gels shrank in the centrifuge tube during cooking in steam kettle. As a result experimental data such as fiber strength, hardness and cohesiveness were not collected. Glucose combined with STPP are incorporated into surimi as protein antidenaturant (Miyauchi et al., 1973). However, glucose does affect protein extraction from fish muscle. On the other hand, glucose (reducing sugar) could react with amino group to produce Maillard browning (Hodge and Osman, 1980) in cooked surimi products, e.g. Chinese fish balls.

4.4.2.2 Incorporation of glucose plus NaCl

Fibers were not able to be produced when only glucose was incorporated into fish flesh (section 4.4.2.1.). The addition of 2% NaCl (Table 31 & 32) permitted the development of fibers and gels. In the standard surimi process, 5% glucose is added to fish flesh. This level did not affect hardness and cohesiveness as compared to the control (2% NaCl added). The 2.5% level of addition slightly increased hardness and cohesiveness. Fiber strength was reduced by both levels.

TABLE 31

Effect of added glucose (with 2% NaCl) to washed flesh on gel texture

Glucose (%)	Hardness		Cohesiveness	
0	1.67[2]	A[1]	0.797[2]	A[1]
2.5	1.80	A	0.819	A
5.0	1.64	A	0.807	A

1: The result of Duncan's Multiple Range Test at $p<0.05$. Same letter indicates the values are not significantly different.

2: Average of four observations.

TABLE 32

Effect of added glucose (with 2% NaCl) to washed flesh on fiber strength

Glucose (%)	B W[1]	O V[2]
0	3+	1+
0.5	2+	1+
5.0	1+	1+

1: BW = Boiling water treatment for fiber formation.

2: OV = Oven treatment for fiber formation.

3: Average of three observations.

Glucose alone apparently interfers with gel and fiber formation. Addition of NaCl helps overcome the problems involved with producing both products. It is not as effective however in the fiber formation process.

Chapter 5

CONCLUSIONS AND RECOMMENDATIONS

The potential for utilizing commercial and non-commercial (parasitized) sources of white fish (Coregonus clupeaformis) as a bland protein ingredient in other food systems has been investigated. Fish, caught in Manitoba lakes, was transported to the Freshwater Institute, University of Manitoba and processed to comminuted fish flesh. Studies were conducted on the production of food fibers and gels from this high protein material. Three types of comminuted material (minced, washed and surimi flesh) were compared for production of food fibers. While all materials were able to be reformed to fibers the washed material was considered to be the more appropriate raw ingredient for this purpose.

Sodium chloride was found to significantly affect production of fibers and gels. Incorporation at a level of 2% was found to produce relatively strong fibers and gels with good cohesiveness (0.843 units) and reduced hardness (2.84) after being set by heat. Four methods for heating the fibers and gels were compared. The gas fired oven (temperature at 150°C) was considered the appropriate procedure for producing a high tensile strength fiber. Further studies need to be conducted on temperature-time relationships and their effect on fiber quality.

It was found that water holding capacity (or water loss) of the fiber paste was an important factor affecting fiber formation when using washed flesh, although it was not the only influential factor. The higher the water holding capacity, the easier it was to form fibers and these had higher tensile strengths. Other factors which affected fiber production and gel texture included mixing time, pH, moisture content of the flesh, cooking time, specific cations and cryoprotectants and these were investigated. The results demonstrated that these factors could modify fiber strength or gel texture. Further studies are required to predict the optimization of these factors in order to develop a specific product texture. Specific cations incorporated into the washed flesh were able to modify the textural quality of both fibers and gels. The charge on the ion apparently was inversely proportional and R/C ratio of the ion was directly proportional to the overall quality of fibers in this study. Further work is necessary in this area.

Some recommendations are as follows:

1. Design equipment similar to that used for production of pasta long products. The type of equipment required includes specialized extruders and air dryers. This system would enable the industry to mass produce a heat set high protein fiber that should be stable in long term storage because of its reduced moisture content.

2. Evaluate other fresh water and marine fish species for utilization as a protein ingredient .
3. Development of a suitable instrumental method to measure fiber strength in order to obtain more accurate and reliable results.
4. The mechanism of fiber formation related to protein-protein and protein-water interactions requires more basic research. A fuller understanding of these relationships and also the R/C ratio of cation to the system may permit improved control of the textural quality of fibers and gels produced from fish protein material.

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Appendix A

A.1 ANALYSIS OF VARIANCE OF THE EFFECT OF ADDED NACL TO WASHED FLESH ON GEL HARDNESS

Source	DF	SS	MS	F
Conc.	6	121.7329	20.2888	605.62*
Replications	3	0.0478	0.0159	0.48 ns
Error	17	0.5695	0.0335	
Total	26	122.3502		

*: significantly different at P<0.05.

ns= not significantly different at P<0.05.

A.2 ANALYSIS OF VARIANCE OF THE EFFECT OF ADDED NACL TO WASHED FLESH ON GEL COHESIVENESS

Source	DF	SS	MS	F
Conc.	6	0.1400	0.0233	11.56*
Replications	3	0.0159	0.0053	2.63 ns
Error	17	0.0343	0.0020	
Total	26	0.1903		

*: significantly different at P<0.05.

ns= not significantly different at P<0.05.

A.3 ANALYSIS OF VARIANCE OF THE EFFECT OF MIXING TIME OF WASHED FLESH ON GEL HARDNESS

Source	DF	SS	MS	F
Time	5	1.2932	0.2586	11.41*
Replications	3	0.0141	0.0047	0.21 ns
Error	14	0.3175	0.0227	
Total	22	1.6247		

*: significantly different at P<0.05.

ns= not significantly different at P<0.05.

A.4 ANALYSIS OF VARIANCE OF THE EFFECT OF MIXING TIME OF WASHED FLESH ON GEL COHESIVENESS

Source	DF	SS	MS	F
Time	5	0.0094	0.0019	1.99 ns
Replications	3	0.0010	0.0003	0.37 ns
Error	14	0.0131	0.0009	
Total	22	0.0235		

ns= not significantly different at P<0.05

A.5 ANALYSIS OF VARIANCE OF THE EFFECT OF ADDED MOISTURE TO WASHED FLESH ON GEL HARDNESS

Source	DF	SS	MS	F
Percentages	4	0.0627	8.9078	44.67*
Replications	3	0.0453	2.2269	0.30 ns
Error	12	0.5983	0.0498	
Total	19	9.5514		

*: Significantly different at P<0.05

ns= not significantly different at P<0.05

A.6 ANALYSIS OF VARIANCE OF THE EFFECT OF ADDED MOISTURE TO WASHED FLESH ON GEL COHESIVENESS

Source	DF	SS	MS	F
Percentages	4	0.0627	0.0156	4.07*
Replications	3	0.0145	0.0005	1.26 ns
Error	12	0.0462	0.0038	
Total	19	0.1234		

*: significantly different at P<0.05

ns= not significantly different at P<0.05

A.7 ANALYSIS OF VARIANCE OF THE EFFECT OF PH OF WASHED FLESH ON GEL HARDNESS

Source	DF	SS	MS	F
pH	3	0.4477	0.1492	5.36*
Replications	3	0.0399	0.0133	0.48 ns
Error	9	0.2503	0.0278	
Total	15	0.7380		

*: significantly different at P<0.05

ns= not significantly different at P<0.05

A.8 ANALYSIS OF VARIANCE OF THE EFFECT OF PH OF WASHED FLESH ON GEL COHESIVENESS

Source	DF	SS	MS	F
pH	3	0.0171	0.0057	2.72 ns
Replications	3	0.0029	0.0009	0.46 ns
Error	9	0.0189	0.0021	
Total	15	0.0389		

*: significantly different at P<0.05

ns= not significantly different at P<0.05

A.9 ANALYSIS OF VARIANCE OF THE EFFECT OF COOKING TIME ON GEL HARDNESS

Source	DF	SS	MS	F
Time	5	2.5960	0.5192	21.22*
Replications	3	0.2548	0.0849	3.47*
Error	14	0.3425	0.0245	
Total	22	3.1933		

*: significantly different at P<0.05

A.10 ANALYSIS OF VARIANCE OF THE EFFECT OF COOKING TIME ON GEL COHESIVENESS

Source	DF	SS	MS	F
Time	5	0.0016	0.00003	0.30 ns
Replications	3	0.0071	0.0006	2.12 ns
Error	14	0.0155	0.0011	
Total	22	0.0242		

ns= not significantly different at P<0.05

A.11 ANALYSIS OF VARIANCE OF THE EFFECT OF ADDED CACL TO WASHED FLESH ON GEL HARDNESS

Source	DF	SS	MS	F
Conc.[1]	2	1.8250	0.9125	40.46*
Replications	3	0.0476	0.0158	0.70 ns
Error	5	0.1128	0.0225	
Total	10	1.9854		

1: Only 1.5, 2.0 & 2.5% of CaCl were compared.

*: significantly different at P<0.05

ns= not significantly different at P<0.05

A.12 ANALYSIS OF VARIANCE OF THE EFFECT OF ADDED CACL TO WASHED FLESH ON GEL COHESIVENESS

Source	DF	SS	MS	F
Conc.[1]	2	o.o227	o.o113	3.77 ns
Replications	3	0.0011	0.0004	0.12 ns
Error	5	0.0151	0.0030	
Total	10	0.0389		

1: Only 1.5, 2.0, & 2.5% of CaCl were compared

ns= not significantly different at P<0.05

A.13 ANALYSIS OF VARIANCE OF THER EFFECT OF ADDED CACL (WITH 2% NACL) TO WASHED FLESH ON GEL HARDNESS

Source	DF	SS	MS	F
Conc.	4	0.3556	0.0889	3.59*
Replications	3	0.0977	0.0325	1.31 ns
Error	11	0.2727	0.0248	
Total	18	0.7261		

*: significantly different at P<0.05

ns= not significantly different at P<0.0

A.14 ANALYSIS OF VARIANCE OF THE EFFECT OF ADDED CACL (WITH 2% NACL) TO WASHED FLESH ON GEL COHESIVENESS

Source	DF	SS	MS	F
Conc.	4	0.0080	0.0020	0.70 ns
Replications	3	0.0052	0.0017	0.62 ns
Error	11	0.0312	0.0028	
Total	18	0.0444		

ns= not significantly different at P<0.05

A.15 ANALYSIS OF VARIANCE OF THE EFFECT OF ADDED MGCL (WITH 2% NACL) TO WASHED FLESH ON GEL HARDNESS

Source	DF	SS	MS	F
Conc.	4	0.3543	0.0885	4.26*
Replications	3	0.1048	0.0349	1.68 ns
Error	12	0.2498	0.0208	
Total	19	0.7089		

*: significantly different at P<0.05

ns= not significantly different at P<0.05

A.16 ANALYSIS OF VARIANCE OF THE EFFECT OF ADDED MGCL (WITH 2% NACL) TO WASHED FLESH ON GEL COHESIVENESS

Source	DF	SS	MS	F
Conc.	4	0.3543	0.0885	4.26*
Replications	3	0.1048	0.0349	1.68 ns
Error	12	0.2498	0.0208	
Total	19	0.7089		

*: significantly different at P<0.05

ns= not significantly different at P<0.05

A.17 ANALYSIS OF VARIANCE OF THE EFFECT OF ADDED FECL (WITH 2% NACL) TO WASHED FLESH ON GEL HARDNESS

Source	DF	SS	MS	F
Conc.	3	0.1246	0.0415	2.07 ns
Replications	3	0.0407	0.0135	0.67 ns
Error	8	0.1610	0.0201	
Total	14	0.3263		

ns= not significantly different at P<0.05

A.18 ANALYSIS OF VARIANCE OF THE EFFECT OF ADDED FECL (WITH 2% NACL) TO WASHED FLESH ON GEL COHESIVENESS

Source	DF	SS	MS	F
Conc.	3	0.0178	0.0059	6.32*
Replications	3	0.0077	0.0025	2.75 ns
Error	8	0.0075	0.0009	
Total	14	0.0330		

*: significantly different at P<0.05

ns= not significantly different at P<0.05

A.19 ANALYSIS OF VARIANCE OF THE EFFECT OF ADDED STPP (WITH 2% NACL) TO WASHED FLESH ON GEL HARDNESS

Source	DF	SS	MS	F
Conc.	4	0.2721	0.0680	3.93*
Replications	3	0.0087	0.0029	0.17 ns
Error	12	0.2079	0.0173	
Total	19	0.4887		

*: significantly different at P<0.05

ns= not significantly different at P<0.05

A.20 ANALYSIS OF VARIANCE OF THE EFFECT OF ADDED GLUCOSE (WITH 2% NACL) TO WASHED FLESH ON GEL HARDNESS

Source	DF	SS	MS	F
Conc.	2	0.3950	0.1975	0.12 ns
Replications	3	0.0209	0.0070	0.004 ns
Error	6	9.6785	1.6131	
Total	11	10.0944		

ns= not significantly different at P<0.05

A.21 ANALYSIS OF VARIANCE OF THE EFFECT OF ADDED GLUCOSE (WITH 2% NACL) TO WASHED FLESH ON GEL COHESIVENESS

Source	DF	SS	MS	F
Conc.	2	0.0010	0.0005	0.56 ns
Replications	3	0.0013	0.0004	0.44 ns
Error	6	0.0052	0.0009	
Total	11	0.0075		

ns= not significantly different at $P<0.05$