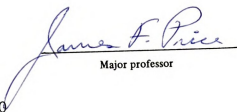




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PROPERTIES OF FISH MUSCLE PROTEINS
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FACTORS AFFECTING THE FUNCTIONAL PROPERTIES
OF FISH MUSCLE PROTEINS

By

Abdelbary Ahmed Dawood

A DISSERTATION

Submitted to
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ABSTRACT

FACTORS AFFECTING THE FUNCTIONAL PROPERTIES OF FISH MUSCLE PROTEINS

By

Abdelbary Ahmed Dawood

Freshwater suckers (Catostomidae family), an underutilized kind of fish, were obtained from Lake Huron (Saginaw Bay, Michigan) to study the possibility of developing new products utilizing its flesh. Suckers were mechanically deboned, then blast frozen and stored at -29°C . Minced sucker was analyzed for fat, protein, and moisture content. The effects of storage at 3°C for 7 days and -29°C for 90 days on the solubility of myofibrillar and sarcoplasmic proteins as well as nonprotein nitrogen of sucker flesh were also studied.

Experiments were designed to add sodium chloride, sodium tripolyphosphate and soy protein isolate in varying concentrations and combinations to study the influence on protein solubility, pH, percent swelling and gel formation of frozen mechanically deboned sucker flesh. The functional properties of fish sausages and canned minced fish were evaluated in terms of water holding



capacity using a centrifuge technique, texture using an Instron Universal Testing Machine and cooking loss.

Results showed that suckers have a lower caloric content than do red meats or poultry and, therefore, are an ideal source of animal protein for use in low calorie diets. Solubility of myofibrillar proteins decreased due to either refrigeration (3°C) or freezer (-29°C) storage, while sarcoplasmic proteins and nonprotein nitrogen were essentially unchanged by both storage methods as compared to that of prerigor muscle.

Results also indicated that both sodium chloride and sodium tripolyphosphate influence the solubility of fish muscle proteins. Total extracted protein and salt soluble protein increased with the addition of either sodium chloride or sodium tripolyphosphate, while the percentage of water soluble protein and nonprotein nitrogen decreased. Myosin heavy chain was solubilized in the presence of 3.0% and 3.6% sodium chloride and also with the addition of 0.45% sodium tripolyphosphate. The highest solubility of myosin heavy chain, that obtained from samples containing 0.45% sodium tripolyphosphate alone, was directly related to the high pH (7.8) of extraction solution. Solubility did not seem to be largely related to the ionic strength, since the ionic strength of 0.45% sodium tripolyphosphate is insufficient



to solubilize myosin. Apparently the 2% and 4% soy protein isolate added did not completely solubilize, since none of the protein fractions increased by 2% or 4% over a control containing no soy protein isolate.

Results revealed that changes in pH, swelling, and gel formation of sucker flesh were largely due to the addition of sodium tripolyphosphate and sodium chloride. Adding sodium tripolyphosphate increased pH and swelling and also improved gel formation, while those values decreased by the addition of sodium chloride. Adding soy protein isolate did not change either pH or gel formation, but did cause increased swelling. The functionality tests indicated samples that have the highest pH also have the highest values of solubility of myosin, greatest swelling, and the best gel forming ability.

In comparing the influences of sodium chloride and sodium tripolyphosphate, sodium chloride increased protein solubility, while sodium tripolyphosphate appeared to be more beneficial. For example, it increases myosin solubility, pH, swelling, and gel forming ability.

Comparing the water holding capacity, texture, and cooking loss of either sausage or canned product treatments, both product types showed low binding characteristics. However, adding corn meal and soy protein isolate in combination with sodium chloride and sodium tripolyphosphate improved their water holding capacity, texture

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and cook yield. It was concluded that sodium tripolyphosphate, corn meal, and fat should be used in manufacturing minced sucker products.

DEDICATION

To Ahmed Aly Dawood, my father.
His memory helped me complete this
study and will be with me forever.

ACKNOWLEDGMENTS

The author expresses sincere appreciation and gratitude to Dr. James Price for his advice, ideas, and suggestions throughout this study. His assistance in preparing this dissertation was particularly valuable.

In addition, because of his guidance and advice in the initial stages of this study, I would like to acknowledge Dr. Estes Reynolds.

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INTRODUCTION

In today's world there is major emphasis on efficiency and maximum utilization of energy resources. From a nutritional standpoint, new sources of protein are needed, and one source that is often overlooked is fish. Underutilized fish species and those species having low acceptance due to flavor defects, poor appearance, or problems of separating lean from bony tissue can be used successfully as raw materials for new fish products. In Japan during the last 25 years machines have been developed which separate edible flesh from skin and bones of eviscerated and deheaded fish and skeletal frames from filleting operations to produce minced fish muscle (Tanikawa, 1963). An increased amount of meat available for human food, increased market value of bony fish and savings in labor are some of the major benefits attributed to mechanical deboning. Mechanically separated minced muscle has been used in food products such as Kamaboko fish cake and fish sausages. In 1970, over one million metric tons of Kamaboko and fish sausages were produced in Japan (Okada et al., 1973). Also, other countries have recently begun to show interest in developing new food products from mechanically separated fish flesh.

Baker (1978) states that about 80-90% of the fish resources from the Great Lakes are wasted. So-called "trash" fish considered pollutants, are routinely thrown back by fishermen. The silver redhorse sucker (Moxostoma anisurum) and white sucker (Catostomus commersoni) taken from the Great Lakes were selected for this study on underutilized freshwater fish. These species are not used now commercially because of their bony nature, which complicates the separation of lean from skeletal tissue, lower consumer acceptability and the inferior binding properties of their flesh.

Binding properties of fish flesh are important not only from the standpoint of production practices but also because they influence quality characteristics such as texture, tenderness, juiciness, appearance, and palatability in finished products.

Freezing is generally used to preserve fish flesh, but freezing can cause physio-chemical changes in the colloidal structure of fish proteins. These changes lead to protein denaturation resulting in flesh that is inferior in functional performance as indicated by solubility of the myofibrillar proteins (mainly myosin) and water binding and holding characteristics. Technological problems induced by freezing include drip loss, and with prolonged storage, toughening of the muscle tissue causing

reduced acceptability and economic loss (Warrier et al., 1975).

Basic studies performed on frozen sucker muscle proteins have helped to define processing techniques that maintain quality and improve the economics of production. The use of phosphate salts and sodium chloride to improve water holding capacity and firmness of canned meat, and to obtain better structure and consistency in manufactured sausages, is common practice in the meat industry. Addition of soy protein isolate reduces cooking losses by absorbing some of the free water not bound by the functional proteins.

The aim of this study was to investigate the effect of added sodium chloride, sodium tripolyphosphate and soy protein isolate (Cenpro-P), in varying combinations and levels, on the functional properties of mechanically deboned frozen sucker flesh. These properties were evaluated in terms of the amount of different protein fractions extracted and the degree of swelling and gel formation of fish muscle proteins. Finally, canned and sausage-type products were developed to promote the utilization of suckers. Both products were analyzed for water holding capacity, cooking loss and texture. It was not within the scope of this study to adjust appearance and flavor of the products. However, these

characteristics could be altered to meet consumer demand by adding various flavor ingredients to the minced flesh.

LITERATURE REVIEW

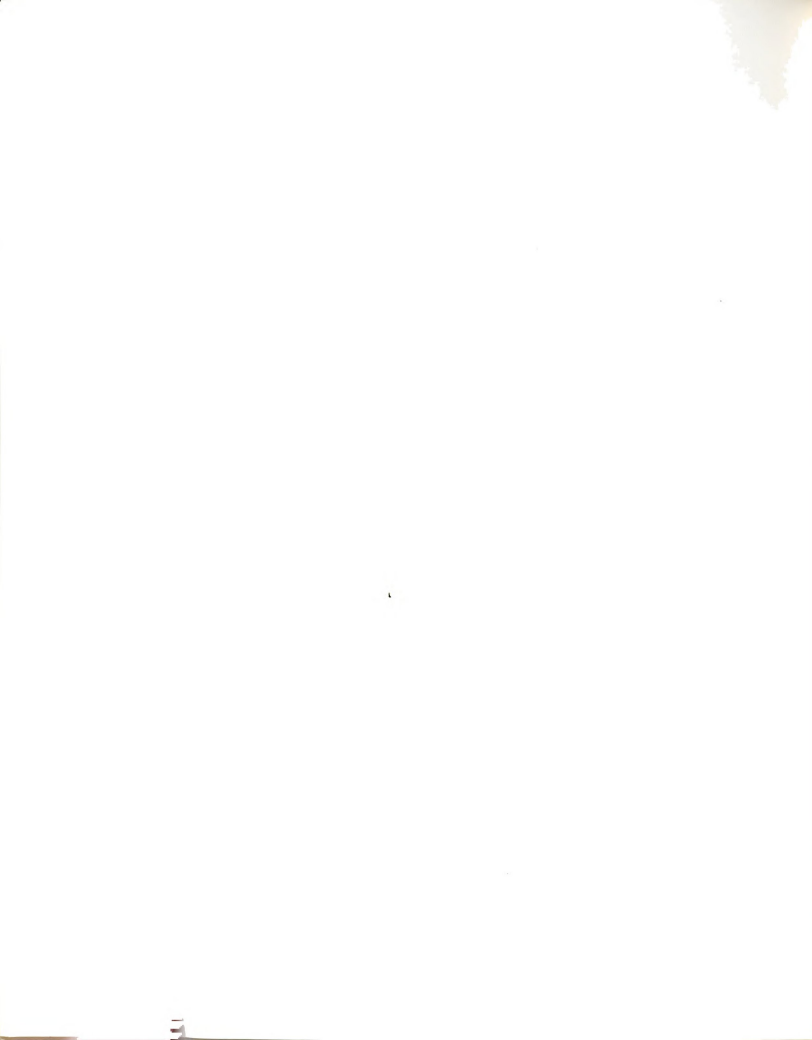
The Structure and Composition of Fish Muscle

Muscle is composed of fibers which are several centimeters in length and 0.01 to 0.1 millimeters in diameter. Individual muscle fibers are surrounded by membranes, called sarcolemma, and are arranged in bundles which enclose connective and fatty tissue. Structurally, these fibers include myofibrils, multiple nuclei, mitochondria, the fluid sarcoplasm, the sarcoplasmic reticulum and other bodies such as ribosomes, lysosomes and glycogen granules (Cassens, 1972). The proximate composition of fish muscle varies widely from species to species. Even within the same species the fat content of one individual may be more than ten times greater than that of another. The main constituents of the edible portion (skin and bone-free fillet) are as follows: moisture 28-90%, protein 6-28%; fat 0.2-64%, ash 0.4-1.5%. These percentages representing all species of fish illustrate individual variations due to season and geographical area of harvest, as well as age, sex and size of fish (Stansby and Olcott, 1963). The total protein of freshwater fish as classified by Moorjani et al., (1962),



consists of myofibrillar (59-73%), sarcoplasmic (about 35%) and connective tissue (4-6%) proteins. According to Goll et al., (1974) proteins identified thus far in the myofibrillar fraction are myosin (50-55%), actin (15-20%), tropomyosin (5-8%), troponin (5-8%), actinin (2-3%), β -actinin (0.5-1%), component C (2-3%), and M-Line proteins (3-5%). According to Connell and Howgate (1959) the ratio of myosin to actin in cod actomyosin is in the range of 2 to 4. Cod muscle myosin contributes 40% of the total protein (Connell, 1964), while actin contributes 15-20% of cod actomyosin (Connell and Howgate, 1959).

The myosin molecule is an elongated structure about $1600\text{\AA}^\circ$ in length and $15-40\text{\AA}^\circ$ in diameter (Rice, 1961; Zobel and Carlson, 1963). Its two major parts are the rod-like tail and globular head. The tail contains 2 or 3 stranded helical coiled coil, $12-1300\text{\AA}^\circ$ in length and $15\text{\AA}^\circ$ in diameter, while the globular head is $250-350\text{\AA}^\circ$ in length and $40\text{\AA}^\circ$ in diameter (Rice, 1961 and 1964). The head when split from the tail of the molecule is referred to as heavy meromyosin (HMM), and the rod-like tail is called light meromyosin (LMM). The molecular weight of myosin is about 500,000 dalton, and the weight of the head is about twice that of the tail (Gergely, 1966).



Functional Classification of Muscle Proteins

The role of muscle proteins in functional performance can be specified more precisely by dividing these into three major classes on the basis of their solubility (Goll, et al., 1974). The first class consists of the myofibrillar proteins which form the myofibril or contractible part of muscle. Ionic strengths greater than 0.30 are required to solubilize myofibrils. Because some myofibrillar proteins are soluble in water, they cannot be accurately defined on the basis of their solubility alone. The second class contains sarcoplasmic proteins, which are soluble at ionic strengths of 0.05 or less. They generally comprise the proteins of the muscle cell cytoplasm. Third are the stroma proteins which are those proteins insoluble in neutral solutions. Collagen and elastin constitute a large part of the stroma protein fraction.

Myofibrillar proteins are responsible for the quality attribute of texture and also have been identified with the properties essential for water binding, gel formation and emulsification characteristics in comminuted sausages (Briskey and Fukazawa, 1971; Goll et al., 1974). Bai and Radola (1977) reported that sarcoplasmic proteins of fish muscle have received great attention because they produce compounds responsible for flavor and color and

also because they contain enzymes causing post-mortem biochemical changes. [The water soluble proteins have a greater effect on functional properties of muscle proteins than has been previously thought (Schut and Brouwer, 1974).] The effect of these proteins on water binding, gel formation and emulsifying capacity is relatively minor compared to myofibrillar proteins (Hamm, 1960; Swift and Sulzbacher, 1963; and Schut and Brouwer, 1974).

Stroma proteins have poor water and fat binding ability. They shrink in the temperature range of 60-65°C, and form gelatin at higher temperatures causing problems such as instability and formation of jelly pockets in finished products. However, they are useful in reduction of the cost of finished products (Kramlich, 1972; Saffle et al., 1964).

The role of fish proteins has been described as being similar to that of red meat proteins (Learson et al., 1971). Also, the regulatory proteins such as tropomyosin, troponin and α -actinin affect the physical and chemical properties of actin and the combined actin-myosin complex on their capacity for heat-gelling or emulsification (Briskey and Fukazawa, 1971). Information reported on myofibrillar proteins, specifically for myosin and actin has aided in our understanding of how these proteins function in food systems.

The Extractability of Muscle Proteins

Protein extractability and solubility are useful parameters for determining the quantity of proteins available for emulsification (Saffle, 1968). A relationship between protein solubility, muscle color and hydration capacity was demonstrated by Borchert and Briskey (1965). Determination of soluble protein was used in another study as an index of frozen fish quality (Love, 1958). There is some confusion in the literature as to whether functionality depends upon the existence of these proteins in the soluble state. It has been shown that soluble proteins are required for good water binding (Sayre and Briskey, 1963; Cook, 1967). Sherman (1961) found a relationship between protein solubility and water retention as measured by water release during centrifugation. Trautman (1964) found that salt soluble proteins were the major emulsifying components in ham muscle. Saffle (1968) and Kramlich (1972) concluded that the presence of extensive amounts of protein in solution is required for good emulsification. Trautman (1966a) also concluded that protein solubility may be extremely important in finely comminuted sausages.

The extractability of protein from different muscles as a function of time post-mortem and physiological condition has been studied by a number of

investigators (Ironsides and Love, 1958; Sayre and Briskey, 1963; Scopes, 1964; Saffle and Galbreath, 1964; Borchert and Briskey, 1965; Cook, 1967; Dixon and Webb, 1966; Meinke et al., 1972). Factors which influence the extractability of meat proteins are cited as post-mortem age, pH, ionic strength, degree and type of masceration, extractant to muscle ratio, extraction time and temperature and freezing (Helander, 1957; Bard, 1965; Saffle, 1968). The extractability of muscle proteins is generally reduced during the early post-mortem period when pH is low and muscle temperature is still high (Borchert and Briskey, 1965). In 1964, Trautman developed a system of studying the effect of pH on muscle protein extracts. He found that a decreasing pH decreased the solubility of both water and salt soluble proteins in a linear manner. In this study the amount of salt soluble proteins extracted from pre-rigor muscle was 42% compared to 39% extracted from post-rigor muscle. Conversely, Cook (1967) found no difference in protein solubility of bovine muscle between pre- and post-rigor stretched tissue and noted that the contractile state of myofibrillar proteins greatly influences their solubility. Holding muscle at high temperature and low pH during the onset of rigor mortis reduces sarcoplasmic and myofibrillar protein solubility by about 55% and 75%, respectively compared to



amounts found immediately post-mortem (Sayre and Briskey, 1963). No change was reported for myofibrillar protein solubility however, and only a 17% reduction in sarcoplasmic protein solubility was found when pH remained high at the onset of rigor mortis. According to Meinke et al., (1972) solubility of fish muscle protein was minimal in the pH range 5.5-6.0 but increased on both the acidic and basic sides of this range. They found that only 20% of fish protein was soluble at pH 5.5, while 70% was soluble at pH 3 or pH 10. That the amount of extracted protein increases as muscle pH increases has been demonstrated by Saffle and Galbreath (1964).

Trautman (1964) reported that the amount of extractable salt soluble meat proteins is influenced by time post-mortem, while the amount of extractable water soluble and salt insoluble proteins is not influenced by this factor. Biochemical changes in fish muscle during rigor mortis have been reviewed by Nazir and Magar (1963). These authors found decreasing levels of glycogen, adenosine triphosphate and creatine phosphate, but a rise in inorganic phosphate in fish stored in crushed ice at about 2°C. The effects of fish size, season of harvest, reproductive cycle and starvation, on the amounts of extracted soluble proteins, were studied by Ironside and Love (1958). Saffle and Galbreath (1964) recorded the



influence of muscle collagen and elastin content on the amount of salt soluble meat protein extracted. The amount of extractable protein is known to increase with more muscle destruction because the structural protein of muscle becomes more available for solubilization as cell disruption and breakage of muscle becomes more extensive (Acton, 1972a).

Effect of Low Temperature
Storage on Protein
Extractability

Although frozen meat is used to a large extent in today's processed meat formulations (Kramlich, 1972), it has been shown to be less stable than fresh meat in sausage-type emulsions (Morrison et al., 1971). Jarenback and Liljemark (1975a) noted the disorder of myofibrils and a reduction in interfilament spacing after frozen storage of meat. This reduction and the observed disorders within and between myofibrils may reflect an impaired ability to reabsorb water following thawing. Freezing temperature has been shown to be an important factor in this problem. Jarenback and Liljemark (1975b) studied the effect of three temperatures on actomyosin filaments extracted from frozen cod. Decreases in number of actomyosin filaments as well as in length and amount of attached myosin to actomyosin were most extensive at -10°C , less at -20°C and hardly noticeable at -30°C .



These authors suggested that such reductions in extractable protein might be due to the effect of freezing and thawing rather than to storage above (-30°C), since freezing and thawing without storage and storage only at -30°C were not different in terms of reducing the amount of extractable proteins.

Changes in ice-stored fish are subtle and unlike those observed during frozen storage. Baliga et al. (1962) studied changes in soluble protein nitrogen of freshwater fish during iced storage. While total protein solubility decreased 20% after 15 days, sarcoplasmic protein decreased only slightly and nonprotein nitrogen increased. Moorjani et al., (1962) also recorded decreases in protein solubility for fish held in ice. They noted that non-protein nitrogen remained almost unchanged for 16 days of storage. Later, Baliga et al., (1969) reported a 10% decrease in protein solubility by the 5th day in ice followed by increases with further storage up to 13 days. The fall in solubility on the 5th day occurred at the same time as the highest level of precipitated actomyosin.

With increasing storage time, there is a progressive fall in the amount of protein extractable by neutral salt from frozen flesh. Several studies have been published confirming the decrease of protein extractability



during frozen storage. Solubility of protein decreased at a steady rate when fish fillets were stored at -14°C for 14 weeks, then decreased no further even in fillets stored for over 4 years (Love and Ironside, 1958). Poulter and Lawrie (1977) found that protein solubility decreased more rapidly during storage at -8°C than during storage at -30°C . The rate of decline in solubility varied from species to species, being much more rapid in some, while pH increased in all the species studied. Iwata and Okada (1971) noted that cold storage at -10°C decreased the amount of extractable fish protein. Protein solubility decreased from 72% to 45% in cod fillets stored for up to 82 months at -29°C (Cowie and Little, 1966). In 1968, Cowie and Mackie studied the effect of three temperatures (-7° , -14° , and -29°C) for various periods of times on the amount of extractable protein using two different extraction methods. After 20 weeks of storage at -7°C they reported that extractable protein content had fallen to 25-40% while after 34 weeks of storage at -14°C it dropped only to 40-60%. Protein extractability remained at 80% up to 49 months of storage at -29°C , then dropped gradually to about 70% after 82 months. On the other hand, contrasting results have been reported for both red meat and fish flesh. Acton and Saffle (1969) found no difference in the protein extractability of



frozen and corresponding unfrozen meat. As reported by Kato et al., (1974) there was also no change in extractability of myofibrillar protein in partially frozen fish muscle at -3°C . Sarcoplasmic proteins of fish remain mostly unchanged during prolonged frozen storage (Connell, 1960a; Babbitt et al., 1972; and Kato et al., 1974).

Several researchers have cited the decreased extractability of myofibrillar proteins during frozen storage as indicative of the denaturation of these proteins (Connell, 1960a; Cowie and Little, 1966; and Kato et al., 1974). This was questioned by Connell (1962), however, who found that the amount of actomyosin extracted from fish muscle falls during the development of rigor mortis and then increases again. Since this would imply a reversal of denaturation, Connell proposed that a change in the type of association between essentially native protein molecules was responsible. Hence, the use of tissue protein extraction for monitoring denaturation is complicated by the interaction of different proteins. The denaturation mechanism of myosin B (Actomyosin) was explained by Okitani et al., (1967) in two ways: Spontaneous aggregation and irreversible dissociation to myosin A and actin and denaturation caused by storage conditions such as temperature, pH and ionic strength.

Connell (1960a) found that the decrease in the amount of extractable protein from frozen fish is due mainly to a decrease in the amount of actomyosin dissolved. Dyer and Morton (1956) reported that extractable actomyosin appears to be a useful quality index for frozen fish. Myosin B extracted from pre-rigor muscle gave relatively high values for ATPase activity, ATP-sensitivity and reduced viscosity when compared to that extracted from post-rigor muscle (Fujimaki et al., 1965b). Kato et al., (1974) recorded a decrease in ATPase activity during storage of fish at -3°C and in ice. On the other hand, Connell (1960b) found no marked change in adenosine triphosphate (Myosin-ATPase) activity during frozen storage of cod flesh for up to 170 weeks at -29°C although a definite loss of activity was detected at -22° and -14°C . Seagran (1956) studied the changing properties of actomyosin from fish muscle that had been frozen and reported that solubility reached a maximum after one month of storage and declined continuously thereafter. Dyer and Morton (1956) and Oguni et al., (1975) confirmed the decreasing solubility of actomyosin with lengthening frozen storage time.

Myosin is the least stable component of frozen stored fish protein with denaturation intensified by repeated freezing and thawing of the muscle (Nikkila and

Linko, 1953). Connell (1960a) reported that cod myosin is a very unstable protein because it undergoes rapid denaturation and aggregation both in unfrozen solution at low temperature and, especially in frozen solution. Later Connell (1962) found that 70-80% of myosin from cod flesh became non-extractable at a rate similar to the extractability of myofibrillar protein during storage of this species at -14°C . Early in 1950, Snow reported that freezing and storing of myosin in the frozen state produced complex aggregation and reactions. According to Connell (1960a) the aggregation reaction involved two steps: a first-order step which does not affect the sedimentation coefficient, followed by a second or higher order step. Buttkus (1970) studied the aggregation of rabbit and trout myosin in frozen solutions and suggested a mechanism which involves disulphide-sulfhydryl exchange reactions between the activated myosin molecule and aggregates. This was concluded from observed behavior of sulfhydryl groups in the monomeric myosin molecule and from the influence of reducing conditions on high molecule weight aggregations.

Chu and Sterling (1970) characterized degree of myosin denaturation in fish on the basis of protein solubility, intrinsic viscosity, isoelectric point, ATPase activity, ultra-violet absorption spectrum and



optical rotatory dispersion of the purified myosin extract. These measurements showed that denaturation increased in the order: Fresh < Frozen < Frozen stored.

Actin, on the other hand, has a more stable nature. The amount extracted from cod flesh remained unchanged for up to 30 weeks of storage at -14°C (Connell, 1960C). It might be the globular structure of actin that make it seem to have characteristics as of sarco-plasmic proteins, which remain largely undenatured and soluble throughout storage.

Fatty acids reduce myofibrillar protein extractability. Jarenback and Liljemark (1975c) demonstrated that small quantities of linolic acid hydroperoxides caused precipitation of isolated myofibrillar proteins. Babbitt et al., (1972) explained the large decrease in total extractable protein of minced muscle after 60 days of frozen storage on the basis of higher concentrations of fatty acids in these samples. Anderson and Ravesi (1970) also found that extractability of protein decreased as free fatty acid content increased. These authors suggested that large amounts of free fatty acids could be formed during iced storage before freezing. In this study, protein extractability decreased most rapidly in the first eight to ten weeks of storage (-12°C and -18°C).



Noguchi and Matsumoto (1975a, b) and Noguchi et al., (1975) studied the preventive effect of amino acids, peptides, sulfur compounds, carboxylic acids, sorbitol and glucose on the freezing denaturation of proteins by determining solubility and superprecipitation of carp actomyosin. Acetylation, peptide formation and position and number of active groups were involved in the preventive effect of these compounds. Frozen samples containing some of these additives produced good quality kamaboko jelly after frozen storage.

Myofibrillar proteins of frozen cod were extracted at an ionic strength as low as 0.1 in the presence of sodium pyruvate (Tran, 1975). In frozen samples kept at -20°C for up to 14 days, added sodium pyruvate allowed greater protein extraction than potassium chloride. During subsequent storage, however, the reverse was true.

Protein-Protein interaction of tobacco mosaic virus has been studied by Lauffer (1964). Protein molecules interact with each other, therefore individual molecules influence and are influenced by other neighboring molecules. Associations between various proteins may be anything from specific covalent chemical bonds to weak intermolecular forces. Protein-Protein interactions have been found to be responsible for the formation of liquid crystalline phases (Lauffer, 1938). Other types



of protein-protein interaction result in the precipitation of proteins from solution (Oncley et al., 1952). According to Lauffer (1964) protein-protein interaction occurs between the molecules of a single protein in both amorphous and crystalline precipitations. Certain proteins in solution can exist in different states of polymerization depending upon composition, temperature, pressure and other characteristics of the solution (Oncley et al., 1952).

Effect of Sodium Chloride and Phosphates on Protein Extractability

Rigor mortis involves a type of aggregation of muscle protein in situ, characterized by the disorganization of tissue and loss of ATP from myofibrils. These changes may prevent the distribution of proteins into solution when salt solutions are used (Dixon and Webb, 1961). According to Asghar and Yeates (1974), the effect of rigor mortis on protein solubility apparently disappeared with the addition of phosphate and appropriate conditions of ionic strength, pH, and temperature during muscle protein extraction. Hermansson and Akesson (1975b) explained the influences of added salt on the properties of proteins in three ways: (1) by specific ion binding, (2) by influencing the ionic strength, and (3) by changing the properties of the solvent. In 1973, however,



Brotsky and Everson found that while a 2.5% sodium chloride concentration has considerable ionic strength, its ability to spread apart filaments is limited because of cross-linkages. Helander (1957) reported that with a 4.5% salt concentration a higher extractability of myofibrillar protein might be expected. Trautman (1964) extracted only 42 and 39% of salt soluble proteins from pre- and post-rigor pork muscle, respectively, using a 0.67 M sodium chloride solution, while Dyer et al., (1950) extracted up to 95% of fish muscle protein with 0.85 M sodium chloride. A pH of 7-9 and salt in the range of 3 to 5% represented optimum conditions for protein extraction in the latter study, with myosin the largest fraction extracted. It is recognized that the addition of sodium chloride to meat decreases its pH. Hamm (1957) found that adding sodium chloride to post-rigor ham muscle consistently lowers pH. Meinke et al., (1972) studied the relationship between protein solubility, salt concentration and pH. Their data indicated that protein solubility increased with increasing salt concentration at approximately pH 6, while the amount of extracted protein gradually decreased at 3.5, 4.2, and 11.1 pH values with increasing salt concentration up to 1.0 N. When salt was added to muscle tissue it dissolved in the water associated with the protein. Duerr and Dyer (1952) found



that myosin swelling was greatest in 2 to 5% salt solutions and that water becomes more firmly bound to myosin micelles in the tissues at these concentrations. These authors recorded the denaturation of myosin when salt concentrations reached 8 to 10% in muscle tissue.

The role that sodium pyrophosphate and sodium tripolyphosphate have in increasing protein solubility has been recognized by many investigators. Lee and Toledo (1976) found the solubility of myosin to increase in the presence of polyphosphates. Bendall (1954) explained the effect of these phosphates by the splitting of actomyosin into actin and myosin, and predicted that effective polyphosphates should have chemical structures similar to that of ATP. Salt-induced denaturation of Baltic Herring myosin was inhibited by the addition of various alkaline phosphates in a study by Linko and Nikkila (1961). When the concentration of salt in meat systems increases, higher concentrations of phosphates are required to prevent the denaturation of myosin. Mechanisms by which phosphates are able to inhibit the reduction of myosin solubility by sodium chloride and at the same time increase the hydration of this protein can be explained as follows: phosphates may be taken up directly by protein molecules, thereby increasing the number of polar groups on the protein and phosphates may be bound to



actomyosin, thereby increasing the myosin negative charge which causes its dissociation. Yasui et al., (1964b) reported that tripolyphosphate can dissociate actomyosin into its components after it has been converted to pyrophosphate by enzymatic hydrolysis. Yasui et al., (1964a) studied the effect of three inorganic polyphosphates on the solubility of myosin B (natural actomyosin) and on the extractability of structural protein from myofibrils in various conditions. Pyrophosphate or tripolyphosphate, which are low molecular weight inorganic polyphosphates, react with salt-free myosin B as a salt. The affinity of these inorganic salts for myosin B is greatly improved in the presence of high salt concentrations and divalent cations. Hexametaphosphate, on the other hand, is a highly polymerized polyphosphate compound whose binding with salt-free myosin is direct but somewhat inhibited by the presence of high salt concentration and divalent cations. According to Brotsky and Everson (1973) a polyphosphate concentration of 0.5% can break bonds, but cannot raise ionic strength sufficiently to spread apart filaments.

Effect of Heat on the Extractability of Muscle Proteins

Heating of meat causes the denaturation of sarcomeric and myofibrillar proteins with the degree of



denaturation dependent upon heating temperature (Hamm and Deatherage, 1960; Hamm, 1966). Solubility of actomyosin is reduced by heating and the greatest decrease occurs at temperatures between 40°C (104°F) and 60°C (140°F). The myofibrillar proteins became almost insoluble above 60°C (140°F) (Hamm, 1966). According to Hamm and Deatherage (1960) the effect of heat on extractability of muscle proteins expressed as percentage of fresh tissue was as follows:

Heating Temperature (°C)	Sarcoplasmic Proteins (%)	Myofibrillar Proteins (%)
20	100	100
40	86	69
60	23	2
80	1	12*

*Increase may be due to some breakdown of connective tissue.

In the study by Hamm and Deatherage (1960), most sarcoplasmic proteins coagulated between 40°C (104°F) and 60°C (140°F). At 50°C (122°F) a large part of these proteins are still soluble while at 66°C (150.8°F) only a few are not yet coagulated (Bendall, 1964). Cheng and Parrish (1979) studied the effect of heat on the solubility of



myofibrillar proteins in pre-rigor and post-mortem bovine muscle by using SDS-polyacrylamide gel electrophoresis. They indicated that α -actinin and myosin are heat labile, becoming insoluble at 50°C and 55°C, respectively. Actin, tropomyosin and troponin were more heat resistant, however. Actin became insoluble between 70°C and 80°C while tropomyosin and troponin became insoluble above 80°C. These data are in agreement with Locker (1956) who reported that myosin was completely coagulated at 55°C. Acton (1972b) recorded an 84 to 89% reduction in protein solubility when internal temperatures of meat reached 75-94°C. During cooking the quantity of protein extracted decreased slightly between 4°C-35°C, then decreased sharply between 35°C and 75°C. Further heating of the meat caused only slight changes in protein extractability.

In 1966 Hamm outlined several changes that occur when muscle protein is heated. He recorded no changes in the colloidal-chemical properties of tissue or in the solubility of ion-binding of muscle proteins at 20°C to 30°C, although the ATPase activity of myosin decreases at 30°C. Some changes in the myofibrillar proteins occur between 30°C-50°C which affect solubility, pH, ionic strength, water holding capacity, Ca^{++} and Mg^{++} binding capacity and the number of available sulfhydryl groups. The ATPase is completely inactivated between 30°C and 50°C.



A small portion of sarcoplasmic proteins are denatured between 30 and 40°C. At 50°C to 55°C there is a rearrangement of the myofibrillar proteins which delays changes in water-holding capacity and pH. At those temperatures new cross linkages begin to form which are quite stable and the denaturation of sarcoplasmic protein continues. At 55°C to 80°C most of the changes occurring at 50°C to 55°C continue to a lesser extent. At 65°C most myofibrillar and sarcoplasmic proteins are coagulated. Around 63°C collagen shrinks and at higher temperatures it is partially transformed to gelatin. Above 80°C the formation of disulfide bonds by oxidation of actomyosin sulfhydryl groups takes place and this continues with increasing temperature.

Smoke is another factor changing protein solubility and the electrophoretic behavior of meat proteins (Randall and Bratzler, 1970).

Functional Properties of Muscle Proteins

Swelling

Swelling is defined as the spontaneous uptake of water from any surrounding fluid (Hamm, 1960). Swelling and gelation measurements were negatively correlated with water loss (Hermansson and Akesson, 1975a). According to Hermansson (1972) swelling is strongly dependent on pH and ionic strength.



Several investigators found that phosphates have an effect on meat swelling. Shults et al., (1972) showed that swelling of meat was increased by adding phosphates, which in turn raised pH. Shults and Wierbicki (1973) also found that meat swelling was increased by the addition of different phosphate components. Shults et al., (1972) mentioned that meat swelling increased when meat was blended with low levels of sodium chloride (1-2%), but increasing the level of salt up to 3-5% caused decreases in swelling followed in turn by a rapid increase in swelling when salt levels increased from 5% to 10%. Even greater increases in meat swelling were caused by adding 0.5% tripolyphosphate to the various sodium chloride levels.

Bendall (1954) noted that the swelling effect of pyrophosphate and tripolyphosphate salts on comminuted whale meat was due to their ability to split actomyosin into its component proteins, actin and myosin, resulting in the uptake of water. Bendall noted that the effective polyphosphates have chemical structures similar to that of ATP. Shults et al., (1972) also reported that muscle ATP-ase converted some tripolyphosphate to pyrophosphate which had a specific swelling effect on meat in addition to its effect on pH elevation and its ability to split myosin B. Shults et al., (1972) reported that according

to Wierbicki et al., (1963) the effect of sodium chloride and polyphosphates at different levels and in varying combinations could be explained by the replacement of Ca^{++} by Na^+ on meat proteins causing an increase in meat swelling, while the exchange of Mg^+ and K^+ by Na^+ caused a decrease in meat swelling. The addition of high levels of sodium chloride (5-10%) caused a rapid increase in meat swelling due strictly to the ionic effect of sodium chloride on meat proteins.

Binding Properties and Gel-Forming Ability

The relationships between different protein fractions and binding properties were studied by Hegarty (1963) who attempted to discover which protein fractions had the greatest binding ability. Hegarty's results indicated that about 90% of actomyosin and myosin fractions were involved in binding, while about 50% and 60% of actin and sarcoplasmic protein respectively were used to bind oil added to the meat system. Fujimaki and Nakajima (1958) noted that the binding quality of sausage decreased as the quantity of sarcoplasmic protein decreased. Hegarty (1963) and Swift et al., (1961) found that oil binding increased as protein concentration decreased. McCready and Gunningham (1971) indicated that the emulsifying capacity of broiler dark meat which contains lower amounts



of total and salt-soluble proteins was higher than that of broiler light meat, which was higher in total and salt-soluble protein content. This higher emulsifying capacity of dark meat was due to its greater pH (6.5) which intensified certain salt-extractable protein fractions most instrumental in emulsifying capacity. Fukazawa et al., (1961a, b, c) compared the binding capacity of experimental beef sausage and its protein composition before and after cooking. They used tensile strength measurements as an index of binding capacity and found that myosin was the key to desirable binding quality.

The binding quality of meats was reported in a series of publications by Sato and Nakayama (1970) and by Nakayama and Sato (1971a, b, c). Sato and Nakayama (1970) stated that myofibrillar proteins and hydration were related to binding quality, and that myosin was the essential protein in the binding quality of sausage. Nakayama and Sato (1971a) found that native tropomyosin and actin influenced the binding capacity of meats when pyrophosphates were added to meat in processing sausages. The interaction between myosin A and F-actin was promoted by the presence of native tropomyosin. This interaction played an important role in protein fraction viscosity. In a second study Nakayama and Sato (1971b) found that heat set myosin and actomyosin gels had similar binding



properties but different physical properties. The binding quality of heated actomyosin gel increased with increasing myosin concentration indicating that the binding quality of sausage was influenced by the actomyosin content of meat. Nakayama and Sato (1971c) reported that myosin-A, F-actin, and tropomyosin also were important and should be controlled in sausage manufacture. The binding quality of heat-set gel increased when F-actin was present in a particular ratio to myosin, and a greater increase was found when tropomyosin was added. Pepper and Schmidt (1975) found that the binding strengths of beef rolls was increased as mixing time increased.

The heat gelling test is an excellent method to determine the changes which occur in muscle proteins during the transformation of muscle into meat. Trautman (1966b) found a definite relationship between the minimum concentration of protein able to form a gel by heating the protein sol at 80°C and post-rigor muscle pH. Fujimaki et al., (1965b) studied the effect of post-mortem aging on gel formation and emulsifying capacity. Myosin became more easily dissociated with post-mortem aging. Thus, the post-mortem age of muscle had a major influence on the role of extracted protein in gel formation and emulsification. Suzuki and Kanna (1963) found that myosin fraction extracted with salt solution from either full rigor



or post rigor fish muscle turned into gel. According to Nakayama and Sato (1971b); Samejima et al., (1969); Fujimaki et al., (1965b) and Fukazawa et al., (1961b) myosin fractions have a major effect on gel formation. Tong et al., (1975) found that alkaline extractions (pH 11) of squid protein concentrations were more effective than protein extracted by 4% salt in forming gels. These authors also indicated that the inability to form gels was probably related to the high salt content of their samples. In addition the poor gelation properties of salt extracts probably were related to residual salt content. The inability to form a gel at pH 6 was due primarily to the low solubility of protein at this pH. Ueda et al., (1968) reported that gel strength was determined during formation of the setting (a Japanese technical term, "suwari"). It is a well known phenomenon for ground muscles that homogeneous sols change into more viscous elastic structures when placed at about 30°C for a short period of time. They explain this phenomenon as follows: during heating of ground muscle sols to about 30°C, the closely folded peptide chains of myosin unfolded so that free radicals are exposed. Unfolded coils then combine with each other and form three-dimensional net structure. The strength and weakness of a gel is determined by formation and deformation of the setting, and the setting temperature required



for protein gel strength was found to be different from species to species. Myosin denaturation mechanisms, explained earlier by Buttkus (1974), were responsible for firming or gelling of protein structures in Kamaboko and meats from fish and mammals during cooking. According to Ferry (1948) formation of gels in denatured proteins involved first an unfolding of protein into more asymmetric shapes and exposure of reactive groups as well as nonpolar groups making them less hydrophilic. Association of the resulting chains by cross-links spaced some distance apart and by localized and nonlocalized attractive forces (including hydrogen bonding) produces a three-dimensional network throughout the sample in which the "gel fraction" approaches unity and in which rigidity is enhanced by further cross-linking taking place during heating.

Salt is necessary to extract salt-soluble protein from muscle tissues. These proteins play a major role in the binding properties of batter which in turn effects the texture, flavor, and other quality characteristics of finished sausage. Acton (1972a) found that binding strength increased when grinding was utilized for tissue breakdown. The increase of meat surface area and closer contact between surfaces in addition to the presence of salt and surface-soluble protein reduced



cooking loss and increased binding strength. Nakayama and Sato (1971c) found that the presence of 0.6 M sodium chloride in meat sol was necessary to produce high binding quality in heated actomyosin gel. Further study by Nakayama and Sato (1972c) showed that binding quality was greatly increased when gels prepared from "set" sols of minced hen meat were stored with 3.5% sodium chloride. The addition of sodium chloride and phosphates aided in extracting protein and setting up the protein for good binding properties (Brotsky and Everson, 1973; Swift and Ellis, 1957). Moore et al., (1976) found that binding strength of beef roll increased as salt level increased from 1% to 3%. An additional increase in binding strength was reported when 0.25% tripolyphosphate was added in combination with various salt levels. Suzuki and Kanna (1963) reported that fish myosin extracted with salt solution from flesh in full rigor and post-rigor had the ability to form gel. However, the presence of phosphate seemed to be essential for gel formation. Since polyphosphates have been shown to improve binding properties of red meats (Swift and Ellis, 1957), it is conceivable that fish treated with polyphosphates may produce fish products with improved binding properties. Fukazawa et al., (1961b) reported that the addition of polyphosphate resulted in the greatest amount of extracted protein both



before and after storage increasing the binding quality of sausage. Polyphosphates have been widely used in seafoods for many years. The phosphates commonly used today are trisodium orthophosphate, tetrasodium pyrophosphate, sodium tripolyphosphate and sodium metaphosphate. In the processing of seafood products, sodium tripolyphosphate is the most commonly used component of polyphosphate blends since it gives a desirable combination of properties such as improving texture and increasing water holding capacity (Brotsky and Everson, 1973). Improving the binding properties of meat by adding phosphates was studied by a number of investigators (Froning, 1965 a, b; Fukazawa et al., 1961a, b; Sato and Nakayama, 1971b). Fukazawa et al., (1961b) found that polyphosphates increased the binding capacity of structural proteins and thus provided a more stable emulsion. Polyphosphates may do this as a result of the dissociation of actomyosin into its components to increase the amount of available myosin (Fukazawa et al., 1961b, Yasui et al., 1964). Fukazawa et al., (1961b) mentioned that in denatured myofibrillar protein especially that of myosin A the effect of phosphate on binding quality was due to increased myosin A content rather than the quantity of extracted protein. According to Nakayama and Sato (1971b) improvements in the binding quality of meat via addition of phosphate



resulted in increases in the amount of force to produce the gel breaking point. This is due to decreases in gel elasticity and actomyosin viscosity thereby preventing formation of air pockets in stuffed sausage and producing a stronger gel matrix.

Water Holding Capacity

The ability of meat, particularly processed meat, to hold intrinsic or added moisture is an important factor in determining quality and product acceptability. According to Hamm (1960) the tightly bound water of hydration in meat is not readily released except under conditions resulting in extreme hydration such as protein denaturation, rigor mortis, changes in muscle pH, masking of charges of hydrophylic side chains, formation of salt bonds between hydrophilic side groups and the presence of salt. These factors also affect the water-holding capacity of meat. Further study by Hamm (1975) showed that the rheological behavior of beef muscle homogenates was influenced by time post-mortem, pH, addition of sodium chloride and water. Hamm (1975) further indicated that water holding capacity and swelling, myosin and actin interaction changes, and solubilization of myofibrillar proteins are the main factors affecting rheological properties of minced meat. Sherman (1961) reported that pH, time, temperature, additive concentration and the ability of alkaline



phosphates to split the bond between myosin and actin are factors influencing water binding capacity. Swift and Ellis (1956) also reported that factors influencing moisture retention of meat treated with phosphate salts are those which also influence solubilization of muscle proteins, such as temperature, time, ionic strength, and pH of treatments.

Several investigators have found that pH affects water retention and swelling of meat products. Minimum hydration is near the isoelectric point of muscle (pH 5.5) (Morse, 1955; Hamm and Deatherage, 1960; Miller et al., 1968), and hydration increases with increasing or decreasing pH. Similarly, Mahon (1961) found that the volume of cured meat was at a minimum at its isoelectric point (pH 5-5.5). Heating raw meat at its isoelectric point resulted in the lowest water holding capacity, while heating meat with pH values higher or lower than its isoelectric point resulted in higher water holding capacity (Hamm and Deatherage, 1960). Hamm (1960) also found that increasing the alkalinity of meat caused an increase in water retention properties. Sato and Nakayama (1970) reported that water holding capacity of batters was due mainly to hydration of myofibrillar protein, and that it decreased in proportion to decreases in breaking energy and myofibrillar protein content after heating. These

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factors thus directly affect binding quality in finished products.

Frozen storage of meat decreased its water holding capacity (Miller et al., 1968; Kato et al., 1974; Bremner, 1977). Differences in the ability of pre-rigor, post-rigor and frozen meat to hold intrinsic or added moisture during cooking were studied by Miller et al., (1968). These workers found that pre-rigor meat retained more moisture than either post-rigor or frozen meat during cooking. The water holding capacity of meat proteins is also directly related to drip in freezing and thawing and shrinkage on cooking. There is possibly a direct relation between drip on freezing and thawing and shrinkage on cooking (Wierbicki et al., 1957).

Coagulation of myofibrillar proteins during cooking causes the release of juice because heat affects the ability of meat to hold intrinsic or added moisture (Hamm, 1966). Most of the free water and that immobilized within the network of protein filaments found to become able to move freely and it is released from tissue during coagulation of myofibrillar proteins. The mono- and multi-molecular layers of water of hydration bound to polar groups in muscle proteins are not influenced by heat coagulation.

The pH of muscle tissue increases during heating (Wierbicki et al., 1957; Bendall, 1964). This change may be caused by charge changes or hydrogen bonding, or both, within myofibrillar proteins, since pH changes occur in both the tissue and myofibrils (Bendall and Pederson, 1962). Hamm (1960) studied the relationship between water binding capacity and pH. Heating at 40°C to 45°C decreases the water holding capacity of muscle tissue in its iso-electric range only. The fact that muscle tissue does not lose its water binding capacity at pH values 4.5 and 7.5 indicates that in heating up to 40°C the dehydration and shrinkage cannot be caused by the formation of stable cross linkages. It is probably that the release of juice and shrinkage of tissue in this range of temperatures are produced by unfolding of peptide chains. These changes may cause the formation of new hydrogen bonds around iso-electric point of actomyosin according to Hamm (1966). According to Acton (1972b) binding strength increased as internal temperature increased in the range of 35-82°C. Acton found that there was either no cooking loss, or less than 1% fluid loss at temperature up to 55°C, and then greater loss occurred when internal temperature ranged from 55-94°C. Cooking temperature has an important effect on binding capacity in poultry meat (Vadehra et al., 1970). Stronger bonds were developed when meat was heated for long periods at lower temperature.

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The addition of phosphate and sodium chloride was found to have an effect on the water holding capacity of cooked meat (Mahon, 1961; Shults et al., 1972; Brotsky and Everson, 1973; Shults and Wierbicki, 1973; Schwartz and Mandigo, 1976). Mahon (1961) concluded that salt concentration and net pH adjustment was the key to obtaining maximum water retention in meats. Wierbicki et al., (1957) studied the influence of adding chlorides of sodium, potassium, calcium, and magnesium to meat before heating on its water holding capacity and found that these salts increased water holding capacity, but shrinkage was less with divalent cations than with monovalent cations. However, the combination of sodium and magnesium chloride promoted the water holding capacity of cooked meat the most. Swift and Ellis (1956) also showed that an increase in water retention could be obtained by adding magnesium chloride to meat. Nakayama and Sato (1971c) reported that the presence of 0.6 M sodium chloride in solution was necessary to produce high binding quality and high water holding capacity in heated actomyosin gels. Sherman (1961) studied the effect of sodium chloride, pyrophosphate and polyphosphate on the water retention of ground pork at 0°C and 100°C. All three additives improved its water holding capacity. There were positive correlations between water holding capacity and anion absorption and



between pH with added phosphate and water holding capacity at 0°C. Phosphates affected the amount of actomyosin that entered solution at 0°C during aging. The greater the concentration of actomyosin, the stronger the gel formed at 100°C. This gel extends throughout the meat mass and retains moisture. Swift and Ellis (1956) reported that moisture retention of meat treated with sodium chloride was linearly related to the ionic strength of the solution applied. Water holding capacity of meat increased more when sodium chloride was added to meat in combination with pyrophosphate. Shults and Wierbicki (1973) found shrinkage could be reduced by 9% when 0.5% pyrophosphate was added, while the addition of 1.0% sodium chloride combined with 0.5% pyrophosphate resulted in a total shrinkage reduction of 18% in cooked meat. The addition of 1.0% sodium chloride also decreased shrinkage of meat. Shrinkage was reduced more, however, when salt concentration was increased to the range of 3 to 5%. Sodium chloride concentrations of more than 5% had an adverse effect on meat shrinkage. A slight reduction in meat shrinkage occurred when the concentration of sodium tripolyphosphate was increased from 0.5 to 1.0%, while Kena FP-28 (a mixture of sodium tripolyphosphate, tetrasodium pyrophosphate and sodium acid pyrophosphate) and tetrasodium pyrophosphate showed reduction of meat

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shrinkage in concentrations up to 1.0%. Further study was done by Shults et al., (1976) on pork rolls and chops, and these workers found cooking loss also was reduced by the addition of sodium chloride and tripolyphosphate. Also, Schwartz and Mandigo (1976) found decreases in moisture loss during cooking when salt was added to meat. Cooking loss decreased and juiciness increased as sodium tripolyphosphate levels increased.

Seafood treated with polyphosphate is generally more juicy and flavorsome on thawing and cooking, and natural moisture is still firmly bound to the muscle protein with less aggregation of myofilaments (Buttkus, 1970). Sutton (1973) mentioned that using polyphosphates in meat and fish products reduced cooking loss. It may be concluded that (Bendall, 1954; Hellendoorn, 1962; and Shults and Wierbicki, 1973) pyrophosphate and tripolyphosphate in combination with sodium chloride showed the highest increase in the water holding capacity of meat. The greatest effect of pyrophosphate and sodium chloride on moisture retention was due to the alkalinity of pyrophosphate solutions (Swift and Ellis, 1956).

Sutton (1973) studied the hydrolysis of triphosphate and pyrophosphate in muscle using P^{32} labelled triphosphate and found that rapid hydrolysis occurred in cod and beef muscle with the rate of hydrolysis approximately proportional to that protein concentration.



Several theories have been reported to explain how hydration is affected by the addition of sodium chloride or phosphates separately or in combination. According to Mahon (1961), the addition of 3% sodium chloride caused a detectable drop in pH. This may be explained as follows. On the acidic side of the isoelectric point proteins were positively charged and Cl^- ions were absorbed. While on the basic side of the isoelectric point there was a negative charge and Na^+ ions were absorbed with the liberation of some H^+ ions causing a drop in pH. Fish meat is normally on the alkaline side of its isoelectric point (pH 5-5.5) and consequently carries a net negative charge. Adding sodium chloride to negatively charged protein reduces the net negative charge to zero (Isoelectric point of meat), at which stage there is minimum hydration of muscle. Adding more sodium chloride causes the adsorption of more Na^+ ions and produce a net positive charge on protein. This positive charge reaches a maximum when 4 to 5% sodium chloride has been added, and at this stage the hydration of meat is also at a maximum. If yet more sodium chloride is added, Cl^- ions will be absorbed and the net positive protein charge is again reduced to zero. In the region of 10% salt the hydration of meat will be at a minimum. Experimentally, the maximum water holding capacity of meat has been



reached in the presence of 4% sodium chloride and 0.5% tripolyphosphate (Mahon, 1961).

Hamm (1971) reported the different ways that polyphosphate components affect meat hydration: (1) Raising pH of meat increases the negative charge on myofibrillar proteins. Negative charges on adjacent myofilaments repel each other allowing more water to enter the gel structure, (2) Increasing ionic strength up to certain limits causes a repulsion of adjacent molecules with resultant increases in hydration, (3) polyphosphates can break certain of the cross-links between myofibrillar proteins. Such as the calcium bonds between adjacent myofilaments also, pyrophosphate can directly effect the resolution of actomyosin into actin and myosin.

Several methods for measuring water holding capacity of muscle foods have been developed. Some methods measure the area occupied by moisture on filter paper when meat samples are subjected to standardized pressure for a specified time (Wiebicki and Deatherage, 1958; Karmas and Turk, 1975). Other workers have measured the amount of juice extracted from centrifuged meat (Sherman, 1961; Bremner, 1977). None of these methods give any physical-chemical quantity which identifies immobilized part of the water.



Labuza and Lewicki (1978) developed a new method of measuring water holding capacity of moist foods and gels which can be easily deformed under pressure. These authors placed the food to be measured in a special cup layered with filter paper of known water content and stored it at 6°C for 72 hr. A standard soil test cell was used to measure the water lost from the filter paper as a function of applied pressure.

Texture

According to Jowitt (1974), texture is an attribute of a substance caused by a combination of physical properties (including size and shape, number, nature, and conformation of constituent structural elements) and perceived by the senses of touch (including kinaesthesia and mouth feel), sight and hearing. The texture profile of a product can be described by studying its initial hardness, cohesiveness, elasticity, hardness of cross-section, ease of swallowing, moisture release, coarseness, and skin texture (Webb et al., 1975). These researchers also mentioned that no instrumental method is able clearly to assess all texture characteristics of a food product accurately indicating the individual characteristics contributing to the total textural properties, as perceived by human senses. DeMan (1975) reported that

texture is not only a property of a food, but that it depends on the person examining or consuming the food. In this respect, texture is as difficult to evaluate objectively as are the characteristics color and flavor.

Several types of instruments have been used to evaluate the texture of foods, however. The Instron Universal Testing Machine was applied to meat texture by several workers, e.g., Lee and Toledo (1976), Webb et al., (1976). This type of equipment is becoming increasingly popular with investigators since it has served a number of different purposes. By simply interchanging different test cells one can measure tensile strength, compressability, shearing strength, elasticity, etc. The Kramer Shear Press also has been used for meat texture measurements.

Several investigators have studied factors associated with meat texture, starting from the muscle composition of the intact animal and continuing through slaughter, rigor mortis and aging to cooking. Lee and Toledo (1976) indicated that amount of time spent in comminution, addition of sodium chloride or sodium chloride and tripolyphosphates, effect of mechanical deboning, temperature of cooking, and type of heating medium used are the factors affecting textural characteristics of fish sausage. Simon et al., (1965) found that the firmness



of frankfurter products increased as meat protein content increased. In terms of developing the desired texture of comminuted products utilizing muscle proteins, fractionation of the various types of protein has been classified as an important functional property (Webb, 1974). The texture of cooked fish muscle depends to a large extent on its pH (Cowie and Little, 1966; Love et al., 1974). It has been reported that storage temperature and the process of freezing affect meat texture (Heen and Karsti, 1965). Frozen fish is utilized to a large extent in canned fish today. Heen and Karsti (1965) indicate that undesirable changes, such as lipid oxidation and texture changes which may occur in canned salmon prepared from frozen fish, may be minimized or eliminated by careful handling of the frozen fish and limiting the storage period. Dyer and Morton (1956) showed an increase in toughness of fish flesh after frozen storage. This toughness may be due to a decrease in extractable actomyosin content. Ravesi and Anderson (1969), for example, found a correlation between the content of soluble protein and the texture of frozen fish muscle. Tokiwa and Matsumiya (1969) also found a relationship between fragmentation of myofibrils and changes in the texture of fish meat. Fragmentation was related directly to the quality of fish flesh and affected greatly by storage conditions.

Freezing and storage at -15°C or -28°C caused myofibrillar breakdown into small fragments more rapidly than storage at 0°C . According to Tran (1975) toughening of fish flesh during frozen storage could be prevented by treating the fish with sodium pyruvate.

Grabowska and Sikorski (1973) studied the difference between the texture of sausage made from fresh mechanically deboned Baltic cod and that made from frozen mechanically deboned Baltic cod. Sausages made from fresh cod yielded an acceptable finished product texture, while that made from frozen flesh had unappealing grainy texture. Moreover, separation of water and fat occurred in the finished product. Such poor texture characteristics were not improved even with the addition of carbohydrates, sodium chloride, and phosphates, separately or in combination. This lack of improvement may have been due to a decrease in protein solubility. Texture and binding of comminuted fish sausages were found to follow a pattern similar to solubility changes in myofibrillar proteins in minced frozen fish. Hashimoto et al., (1959) found that the elasticity of products decreased mainly in direct proportion to and in correspondence with the denaturation of myosin B.

Webb et al., (1975) reported that temperature and length of time spent chopping affected frankfurter texture



and solubility during cooking. Lee and Toledo (1976) also studied the effect of chopping on the texture characteristics of fish sausage. The texture of cooked comminuted products was improved by up to 12 minutes of chopping; however, longer periods resulted in a slight decrease in textural quality. The addition of sodium chloride and polyphosphates separately or in combination also improved texture characteristics. Two percent salt in the product increased shearing strength, compressibility and puncture strengths. Addition of polyphosphates increased solubilization of muscle protein and improved water binding and texture. In general, the mechanical strength of products was greatest with no additions, and declined progressively with the addition of sodium chloride and a sodium chloride-pyrophosphate mixture (Lee and Toledo, 1976).

Soy protein isolate improved the texture of meatballs as determined by instrumental and sensory evaluation (Hermansson, 1975). Lee and Toledo (1976) reported the same results indicating that soy protein isolate improved the texture of fish products. Textured soy flour also has been used in the formulation of acceptable mullet sausages (Daley and Deng, 1978; Daley et al., 1978).

It is known that certain changes occur in muscle when it is subjected to thermal energy, as mentioned

earlier. These changes affect meat texture during cooking. Texture is also influenced by the type of cooking. Lee and Toledo (1976) noted that cooking for long periods at temperatures below 80°C caused weakening of texture in smokehouse cooked products. Firmness of cooked comminuted fish muscle was generally greater in steamed products, less in smokehouse cooked products. Simon et al., (1965) found that firmness was increased by reducing the relative humidity in the smokehouse during processing.

Functional Properties of Soy Protein Isolate in Meat Systems

In the American meat industry, isolated soy protein is a widely used additive in sausage batters. Soy protein isolate has properties similar to those of meat proteins (Brown, 1972), and the use of soybean products in various foods is fashionable from the standpoint of the world food economy and dietetics. For example, large amounts of food grade soybean products are used in fish product industries in Japan (Yasumatsu et al., 1972b).

Soy protein isolates are the most refined forms of soybean proteins. They contain 90% or more protein on a dry basis and have a 2-4% ash content (Meyer, 1966). Many workers have investigated the functional properties of soy protein isolates utilized in different foods. Functional properties such as water and fat absorption,

oil emulsification, viscosity and gel formation are the most important characteristics of soy protein isolates as they are used in ground and canned meat formulations.

Wolf (1970) found that soybean proteins are a mixture of components with molecular weights ranging from 8000 to 600,000 and that the major soybean proteins are globulins which are insoluble at their isoelectric point (near pH 4.5). However, they are soluble in water or dilute salt solution at pH values above or below the isoelectric point (Wolf, 1969). The addition of soy protein isolate to a variety of foods supplies desirable functional properties, such as emulsifiability, ability to absorb fat, moisture holding capacity, and thickening and foaming ability (Wolf, 1970).

Some workers have claimed that extenders such as soy protein isolate increase emulsifying characteristics in batters and improve binding properties (Inkelaar and Fortuin, 1969). This is questionable, but they may absorb water not bound by the protein matrix, and reduce shrinkage or water loss (Pearson, et al., 1965; Van Eerd, 1971).

Soy protein isolates are prepared by extraction from undenatured flakes or flour with water near neutral pH, and precipitation of the proteins by adjusting the extract to the protein isoelectric pH. After isoelectric precipitation the isolates are no longer completely

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soluble in solutions with an ionic strength of 0.5 at pH 7.6. Wolf (1970) found that insolubilization may be due to a portion of the proteins being held together by disulfide bonds as aggregates, and further aggregate formation during isoelectric precipitation. He reported that this disulfide crosslinks must be broken to resolubilize these proteins.

Solubility is an important physical property of soy proteins, and is required if their desired functional properties are to be achieved. Oil seed proteins in general are a less soluble form of protein than animal proteins (Hagenmaier, 1972). Solubility of native soybean globulins strongly depends on pH (Wolf, 1970; Hagenmaier, 1972), temperature (Wolf, 1969) and salt content (Anderson et al., 1973, Van Megen, 1974). Even at the isoelectric point, soy protein can be dissolved easily up to very high concentrations with ionic strengths exceeding 0.7 with sodium chloride (Van Megen, 1974). However, Hermansson and Akesson (1975a) found a marked decrease in solubility in meat systems containing the soy protein isolate Promide-D, when sodium chloride was added. Solubility in this case was positively correlated to moisture loss. Pearson et al., (1965) found that a soy sodium proteinate was less soluble and was a poor emulsifier at the pH of meat.



Gel-forming ability is one of the important functional properties of isolated soy protein (Yasumatsu et al., 1972a), and the most important property of added proteins (Hermansson, 1975). Soy proteins form a gel which act as a matrix to hold moisture and fat (Wolf, 1970). The gel forming ability of soy proteins is dependent on temperature, length of time of heating, ionic strength, pH and protein concentration. Hermansson (1972) reported that when Promide-D solution (10%) was heated gelation occurred and strong gels were formed. Also water could not be pressed out of these gels even under very high pressure. The gel strength of Promide-D increased to a maximum with increasing temperature up to 80°C then decreased as the temperature was raised above this point. Gel strength also decreased rapidly with increasing ionic strength. At ionic strength of 0.5 gels were no longer formed.

The important properties of edible soybean proteins with respect to their use in food systems were summarized by Circle et al., (1964). Rate of gelling and gel firmness of a commercial edible isolated soy protein are dependent primarily on temperature, length of time of heating and protein concentration. In concentrations of 8-14%, gels are formed within 10 to 30 minutes at 70-100°C, but are disrupted if overheated to 125°C.



At concentrations at 16 to 17% the gels are firm, resilient and self-supporting and are less susceptible to disruption by overheating.

Several sodium salt additives may affect the heat-gelation of soy proteinate (Circle et al., 1964). At low concentrations (0.05 to 0.1%) these salts have a relatively minor effect on the viscosity of unheated and heated dispersions, but at higher concentrations (0.5 to 1%) the effect of the salts is to lower the viscosity of unheated dispersions and to raise that of the heated ones. The situation is different for the two reducing agents, sodium sulfite and cysteine, which markedly reduce the apparent viscosities of both unheated dispersions and heated gels. According to Yasumatsu, et al., (1972b), the additional effects of soybean proteins in fish products may be influenced by intrinsic amounts of protein and not by the amounts of fiber and fat in the soybean protein isolates. Saio et al., (1974) found that the expansion ratio of gels was increased as temperature increased and that their maximum expansion characteristics were obtained around 2.5 to 8.5 pH.

Hermansson and Akesson (1975b) showed that NaCl had a marked influence on both meat systems and the functional properties of added protein. In the absence of sodium chloride solubility was well correlated with



higher swelling, viscosity and gel strength. A reduction in swelling and gel strength was correlated with a decrease in protein solubility caused by protein association in the presence of salt. Hermanson and Akesson (1975b) found that Promine-D gels were smooth, and that it was impossible to press out water even under very high centrifugation forces.

The problems involved in the gelation of soybean proteins were discussed by Catsimpoolas and Meyer (1970) who found that the highest viscosity values were obtained at a neutral or mildly alkaline pH. An increase or a decrease in pH produced weaker gels. During heating at 65°, 70°, 75° and 80°C, the progel viscosity first increased rapidly with time and then continued to increase at a lower rate. Catsimpoolas and Meyer (1970) also studied the effect of ionic strength on gelation. At temperatures above 70°C the viscosities of progel and gels decreased with increasing concentrations of sodium chloride.

Swelling of soy proteins is strongly dependent on pH and ionic strength. Hermansson (1972) found a good positive correlation between gel strength and swelling ability, especially for soybean protein isolate. The addition of salt caused about a 60% decrease in solubility of Promide-D. This is understandable, as the amount of



water taken up decreased with ionic strength. Swelling increased as pH increased in the range of 4-10. Further study by Hermansson (1975) indicated that Promine-D had a high swelling capacity. Hermansson and Akesson (1975a) obtained positive correlations between solubility, swelling, viscosity, and moisture loss of model meat systems.

Meat systems in which part of the protein is replaced by soy protein isolates may show complex behavior with respect to water-binding properties. Fleming et al., (1974) reported that the water absorption and viscosity characteristics of untreated slurries of soy flour and concentrate were lower than those of the soy protein isolate because water absorption and viscosities tended to increase with the concentration of protein in a product. Judge et al., (1974) indicated that the addition of soy proteins to beef patties reduced shrinkage on cooking. Brown (1972) stated that Cook et al., (1969) pointed out that a meat-soy system will hold approximately 100% more free water than a pure meat system to which excess water was added. Yasumatsa et al., (1972b) found that the desirable effect of soy protein products in Kamaboko is their ability to remain as particles which can hold water. Cooked patties containing textured soy protein retained more moisture and less fat than all beef patties (Seideman et al., 1977). Karmas and Turk (1976) studied the water



binding properties of cooked fish containing various protein additives. The addition of soy protein to cooked fish increased its water binding capacity, and this capacity was not affected by sodium, calcium, and potassium associated with the soy protein. Karmas and Turk (1976) also indicated that fresh samples of soy protein had significantly better water binding capacity than old samples. Beef rolls containing 3% soy protein isolate retained more moisture which resulted in a higher cooked yield. Cooked yield of meat is closely associated with water binding capacity (Moore et al., 1976).



MATERIALS AND METHODS

Materials

Fish

Approximately 500 lbs. of fresh water sucker from Lake Huron (Saginaw Bay) were obtained for study in July of 1978. Roughly 50 percent of the fish were of the Silver Redhorse variety (Moxostoma anisurum), while the other half were White Suckers (Catostomus commersoni).

Fish were removed from nets and either kept alive in oxygenated tank for protein fractionation experiment or stored in wooden boxes in an ice house for one day before collection. They were then transported in ice to the meat laboratory at Michigan State University where they were held over night at 3°C and processed the following morning.

Additives and Spices

All additives used in this investigation were food grade materials. These consisted of "Cenpro-P-4540" soy protein isolate (Central Soya Co., Ft. Wayne, Ind. 46802), degerminated and enriched white cornmeal (Quaker Oats Co., Chicago, Ill. 60654), iodized sodium chloride (Hardy Salt Co., St. Louis, Mo. 63166), granular sodium tripolyphosphate (FMC Corp., Carteret, N.J. USA), 99% pure



monosodium glutamate (Accent International, Inc., Westwood, Mass. 02090) and sodium ascorbate of 91.7% purity (Permacurate-Roche Chemical Division, Hoffman-La Roche Inc., Nutley, N.J. 07110).

The spices employed were powdered onion, garlic, ground white pepper, allspice and ginger (Asmus Spice Co., Detroit, Mi. 48213) and ground paprika (McCormick & Co., Inc., Baltimore, Md. 21202). A smoke flavor was imparted to products by adding condensed liquid smoke (E. H. Wright Co., Kansas City, MO. 64108).

Preparation of Mechanically Deboned Fish Flesh

Fish were headed-gutted, rinsed under running cold water, split length wise, and passed through a Bibun mechanical deboning machine (Type SDX 13, Bibun Co., Fukuyama Hiroshima, Japan) equipped with a 5mm hole size drum. The minced flesh was then passed through the meat separator a second time to remove as many bone and scale fragments as possible. Yields of both the dressing and mechanical deboning operations were calculated.

Samples of minced flesh were collected after the second pass and analyzed for moisture, fat, protein, and nonprotein nitrogen. The sucker flesh was then vacuum packaged in 5 and 20 lb. lots in Cryovac (polyvinylidene chloride) bags which were blast frozen and stored at -29°C . The frozen fish was thawed in a 3°C cooler as needed.



Methods of Analysis

Protein Fractionation

Protein fractionation was performed according to a modification of the method of Helander (1957). All samples were extracted twice in duplicate. Extractions were carried out at 2 to 3°C with cold extracting solutions.

Sarcoplasmic protein.--Five grams of ground pre-rigor, chilled or frozen sucker muscle were weighed into a 125 ml beaker. Fifty ml of 0.015 M potassium phosphate buffer (pH 7.4) were added to the sample which was then extracted on a magnetic stirrer for 3 hrs. This was followed by centrifugation at 1400 xg for 20 min. The supernatant was filtered through eight layers of cheese cloth into 100 ml graduated cylinder and the residue resuspended in 50 ml of potassium phosphate buffer for a repeat of the procedure described above. The volume of the combined supernatants was recorded. Sarcoplasmic protein nitrogen was determined in duplicate 15 ml samples by the microkjeldahl method (A.O.A.C., 1975).

Nonprotein Nitrogen.--Fifteen ml duplicate samples were pipetted from the sarcoplasmic protein supernatant into 50 ml polyethylene centrifuged tubes, into which 5 ml of 10% (W/V) trichloroacetic acid were added. The

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mixture was allowed to stand for 2 to 4 hrs. at 3°C and was then centrifuged at 12,000 Xg for 20 mins. The supernatant was carefully decanted into kjeldahl flasks for nonprotein nitrogen determination by the microkjeldahl method.

Myofibrillar protein.--The residue from the sarco-plasmic protein extraction was suspended in 50 ml of 1.1 M potassium iodide (KI) phosphate buffer (pH 7.4) and extracted on a magnetic stirrer for 3 hrs, followed by centrifugation at 1400 X g for 20 mins. The supernatant was filtered through 8 layers of cheese cloth into a 100 ml graduated cylinder. The residue was resuspended in 50 ml of 1.1 M KI phosphate buffer and the procedure as described above repeated. The volume of the combined supernatants was recorded. Duplicate fifteen ml samples of the supernatant were used to determine the amount of myofibrillar protein nitrogen by the microkjeldahl method.

Extraction of Soluble Compon-
ents from Mechanically
Deboned Frozen Fish
(MDFF)

Total soluble, salt soluble, and water soluble proteins as well as nonprotein nitrogen, were extracted by a modification of the method described by Trautman (1964). Extractions were carried out two times at 3°C using cold extracting solutions.



One hundred grams of fish flesh, with or without soy protein isolate were blended with either six hundred grams of plain water or 600 grams of water mixed with sodium chloride, sodium tripolyphosphate or a combination of these in varying levels (Table 1). This was done with a Waring blender set at low speed (approximately 2300 rpm) for 90 seconds. The homogenate was centrifuged at 9500 X g for 10 minutes in an automatic refrigerator centrifuge (Sorvall Type RC2B, Rotor GSA--5008, Ivan Sorvall Inc., Norwalk, Conn.). The supernatant (soluble components) was then decanted and filtered through 8 layers of cheese cloth.

Fractionation of Soluble Components from Mechanically Deboned Frozen Fish

Duplicate 15 ml samples of the filtered supernatant were used for the determination of nonprotein nitrogen as previously described. The remaining filtered extract was divided into two parts to obtain the following components.

Soluble protein extract.--One part of the filtered extract was dialyzed against the same extracted buffer so that water and salt soluble proteins were retained in solution while dialyzable nonprotein nitrogen was removed. The volume of the dialyzed extracts was

TABLE 1.--Treatment Design and Formulations for the
Measurement of Various Protein Fractions,
pH, Swelling, and Gel-Forming Ability

Code No.	
A-1	No sodium chloride (NaCl), No sodium Tripolyphosphate (STPP), and No soy protein Isolate (SPI)
A-2	No NaCl, No STPP, 2% SPI
A-3	No NaCl, No STPP, 4% SPI
A-4	No NaCl, 0.225% STPP, No SPI
A-5	No NaCl, 0.225% STPP, 2% SPI
A-6	No NaCl, 0.225% STPP, 4% SPI
A-7	No NaCl, 0.45% STPP, No SPI
A-8	No NaCl, 0.45% STPP, 2% SPI
A-9	No NaCl, 0.45% STPP, 4% SPI
B-1	3.0% NaCl, No STPP, No SPI
B-2	3.0% NaCl, No STPP, 2% SPI
B-3	3.0% NaCl, No STPP, 4% SPI
B-4	3.0% NaCl, 0.255% STPP, No SPI
B-5	3.0% NaCl, 0.225% STPP, 2% SPI
B-6	3.0% NaCl, 0.255% STPP, 4% SPI
B-7	3.0% NaCl, 0.45% STPP, No SPI
B-8	3.0% NaCl, 0.45% STPP, 2% SPI
B-9	3.0% NaCl, 0.45% STPP, 4% SPI

TABLE 1.--Continued

Code No.	Treatment and Formulation
C-1	3.6% NaCl, No STPP, No SPI
C-2	3.6% NaCl, No STPP, 2% SPI
C-3	3.6% NaCl, No STPP, 4% SPI
C-4	3.6% NaCl, 0.225% STPP, No SPI
C-5	3.6% NaCl, 0.225% STPP, 2% SPI
C-6	3.6% NaCl, 0.225% STPP, 4% SPI
C-7	3.6% NaCl, 0.45% STPP, No SPI
C-8	3.6% NaCl, 0.45% STPP, 2% SPI
C-9	3.6% NaCl, 0.45% STPP, 4% SPI

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recorded. The total soluble protein present in duplicate 5 ml samples was determined by the microkjeldahl method.

Results for soluble protein nitrogen were expressed as a percentage of total protein nitrogen by using the formula:

$$\% \text{ Extractable Protein Nitrogen} = \frac{\text{Extractable N} - \text{Nonprotein N}}{\text{Total N} - \text{Nonprotein N}} \times 100$$

Water-soluble protein.--The second part of the filtered extract was dialyzed over night against 0.05 M NaCl to remove nonprotein nitrogen and to insolubilize the salt soluble protein. This was followed by centrifugation for 10 mins at 9500 Xg. The supernatant, containing the water soluble proteins, was then dialyzed against the same extraction buffer for 24 hours at 3°C. The volume of the dialyzed extracts was recorded. The total soluble protein present in duplicate 10 ml samples was determined by the microkjeldahl method.

Salt-soluble protein.--The residue from the centrifuged 0.05 M NaCl dialyzate containing the salt soluble protein was resuspended in 0.05 M sodium chloride and centrifuged again at 9500 Xg for 10 mins. to remove any contaminating water-soluble protein. The resuspension in 0.05 M sodium chloride was repeated yet another time,



followed by centrifugation, after which the residue was solubilized in 1.2 M sodium chloride and dialyzed against the same extracted buffer for 24 hrs. at 3°C.

Sodium Dodecylsulphate (SDS)
Polyacrylamide Gel Electro-
phoresis

Protein fractions of fresh fish and all total soluble protein fractions extracted from mechanically deboned frozen fish were compared using the technique described by Weber and Osborne (1969). In principle, the use of sodium dodecylsulfate (SDS) in combination with disulfide reducing agents (e.g., β -mercaptoethanol) causes the disruption of forces maintaining the secondary and tertiary structure of proteins resolving them into single polypeptide chains. Because of this and the fact that SDS combines with all proteins in a constant ratio, imparting a uniform charge, electrophoretic mobility is solely a function of protein size or molecular weight (Reynolds and Tanford, 1970).

The total extracted protein was combined with glycerol in a 50:50 ratio and stored at -80°C until used. Glycerol is able to attach a considerable amount of water to itself, rendering the latter unavailable for ice formation (Lovelock, 1954). Bound water can still act as a solvent for cell salts, however, so that the concentration of cell salts in the frozen material is

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kept low. Protein samples taken from the glycerol mixture were prepared for electrophoresis by heating solutions containing 20 μ g of total protein ranging from 40 μ l for higher protein content samples to 80 μ l for lower protein content sample with 80 μ l of tracking dye, composed of 1.0% SDS, 0.05 M Tris HCl at pH 7.1, 0.5% mercaptoetanol, 2% glycerol, and 0.01% Pyronin Y, in an 80°C water bath for 30 mins.

Ten percent polyacrylamide gels were prepared for a stock solution composed of 62.5g of electrophoresis grade acrylamide and 0.625g of N, N methylenebisacrylamide (0.25%) in 250 ml of distilled water. Ten ml of this stock solution were combined with 5 ml of tris-glycine stock solution composed of 0.5 M Trisma and 1.5 M glycine. Added to this were 1.25 ml of glycerol, 1 ml of 2.5% sodium dodecylsulfate, 10 μ l of N, N, N¹, N¹, tetramethylenediamine (TEMED) and 6.75 ml of H₂O. Finally, 1 ml of freshly made 1% ammonium persulfate was added to the gel solution to initiate polymerization, and the solution was deaerated using a water suction pump. The gel solution was immediately poured into glass running tubes (5mm I.D x 10 cm) which were held precisely vertical and then left for polymerization.

The tubes were placed in an electrophoresis apparatus (BIO-RAD, Model 150A, Gel Electrophoresis cell).

Samples containing 20 μ g of total soluble protein were layered on the exposed upper surface of a gel. Chamber buffer, composed of 100 ml of 20 M Tris-Glycine, 40 ml of 2.5% SDS and 860 ml water per liter, was carefully layered over the sample. Lower and upper chambers were filled with the same chamber buffer and a constant current applied at 3 mA per gel. The SDS treated proteins migrated toward the positive electrode in the lower chamber.

Approximately 18 hrs. were required for the completion of a run which was terminated when the tracking dye (red color) reached 1/2 cm away from the end of a gel. Gels were removed from running tubes by squirting water from a syringe between the gel and the tube wall. They were then immersed in staining solution (0.05 g comassie brilliant blue, 75 ml methanol, 65 ml water and 10.5 ml glacial acetic acid) for 16 hrs., followed by destaining (5% methanol, 7.5% glacial acetic acid) for 4 days.

The relative amounts of myosin heavy chains were estimated by measuring areas under corresponding curves produced by scanning the gels. Gels were scanned at a wavelength of 550 nm using scan speed of 1/2 cm per min. on a Beckman Spectrophotometer (Model 2400) equipped with a Gilford gel scanner (Model 2520). A horizontal cuvette holder was attached to the scanner. A Hewlett Packard Integrator (Model 3380) was programmed to correct

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for baseline drift by re-establishing baseline before and after each peak. Chart speed was adjusted at 2cm/min. Start delay and slope sensitivity were adjusted at 0.25 min. and 3.00 mv/min. respectively.

A standard curve (Appendix 1) was prepared in which areas of myosin heavy chain bands were plotted against the total protein extracted from mechanically deboned frozen fish samples. While this standard curve loses linearity at areas above about 500,000 total area, linearity of area could be achieved using mechanically deboned frozen fish protein samples containing up to 30 μ g total soluble protein.

Total Nitrogen

Total nitrogen was determined on approximately 0.5 grams of muscle by the microkjeldahl method (A.O.A.C., 1975).

Kjeldahl Method

The American Instrument Co. microkjeldahl method (1961) was used to determine both the concentration of protein in the different protein fractions and the amount of nonprotein nitrogen in the different treatments.

Moisture Determination

Approximately 5 grams of muscle sample were weighed into previously dried and tared aluminum dishes

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and dried in a 100°C air convection oven for 24 hrs. Weight loss was recorded after cooling the samples in a desicator and moisture was calculated as percentage of fresh tissue (A.O.A.C., 1975). Dried samples were saved for either extract determination.

Ether Extraction

The fat content of the fish muscle was determined by extracting dried samples with anhydrous ether for 4 hrs. on Goldfisch apparatus, as described by A.O.A.C (1975). Percentage fat was calculated on a fresh tissue basis.

Gel-Forming Measurement

Gel formation was determined using the least concentration endpoint (LCE) method reported by Trautman (1966b). Ten ml of total extracted protein containing different protein concentrations were heated in an 80°C water bath for 10 mins. and then transferred to an ice water bath for 1 hr. The strength of the coagulation was evaluated by inverting each tube, the least concentration endpoint being the lowest protein concentration forming a stable gel that remained in the inverted tube. Duplicate samples for each concentration of total protein were used to determine LCE, and all treatment measurements were repeated an additional time in duplicate.

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Swelling Measurement

Muscle swelling was determined by blending 25 grams of muscle, with or without soy protein isolate, with 100 ml of plain deionized water or with 100 ml of water containing sodium chloride, sodium tripolyphosphate or a combination of these in varying levels (Table 1). This was done with a waring blender set at low speed for 90 secs. Two homogenized 35 grams samples were weighed into two 50 ml centrifuge tubes and centrifuged at 480 x g for 15 mins. Supernatant volume was recorded.

Percent swelling was determined according to the following formula reported by Wierbicki et al. (1963).

$$\% \text{ Swelling} = 400 - (14.3 \times S)$$

where:

S = Supernatant in ml. (1 ml = 1 gram)

7 grams = Weight of meat in the slurry

(35-7) grams = amount of added weight in the slurry

$$\begin{aligned} \% \text{ Swelling} &= [(35-7) - S] / 7 \times 100 \\ &= \left[\frac{35-7}{7} - \frac{S}{7} \right] 100 \\ &= \left[4 - \frac{S}{7} \right] 100 \\ &= 400 - 14.29S \end{aligned}$$

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pH Measurement

The pH of total extracted proteins was measured with a pH meter (Beckman, Model 35600 Digital pH meter), using a glass electrode.

Chemicals

All chemicals used in this investigation were of reagent grade unless otherwise specified.

Smokehouse Shrinkage

The difference in weight of the stuffed product before and after smokehouse cooking was calculated as smokehouse shrinkage. Results are presented as percent cooking loss.

Baking Loss

Baking loss was calculated by the weight difference of filled cans before and after baking. Results are presented as percent baking loss.

Measurement of Water Holding Capacity

Water holding capacity of the cooked fish flesh was determined using the centrifuge technique of Bremner (1974). Both the smoked sausages and canned fish product were allowed to warm to approximately 25°C before testing. Cylindrical shapes were prepared by coring the center of products, providing a uniform cross section 13mm in

diameter. Ten grams of the core were weighed into 15 ml glass Corex^R (No. 8441) centrifuge tubes and centrifuged at 39100 Xg for 60 minutes. The supernatant was drained and the tubes inverted on paper toweling for 15 mins. to allow complete drainage of the remaining fluid (Zapata, 1978). The weight of the meat residue was recorded and water-holding capacity calculated according to the following formula.

$$\text{Water-holding Capacity} = 100 \times \frac{\text{weight of meat residue}}{10 \text{ g}}$$

Eight replicated measurements were made on each treatment for both products (four replicate measurements per trial).

Internal Texture Measurement

The texture of both products, measured as shear force, was determined on the Instron Universal Testing Machine (Model TTBB, Instron Corp., Canton, Mass.). Sample preparation, Fish Sausages: The edible casing and firm surface layer under the casing were removed in order that only resistance (i.e., internal texture) to the central mass of sausage was measured as the cell travelled through. The diameter of the skinless sausage was recorded. The sausage was then cut into a 10cm long cylinder and sheared using a compression load cell of the 1 to 50 kg range. A shear cell was attached to the upper moving fixture.



Drive speed and chart speed were adjusted at 20cm/minute and 5 cm/minute, respectively. The resistance force met by the cell was expressed as Kg-f per cm of sausage. Twelve replicated measurements were done on products from each treatment (six replicate measurements per trial).

Sample preparation, Canned Minced Fish: Canned products were cored in the center to achieve a uniform, cylindrical shaped sample, 18mm in diameter. Cylinders 10cm long were sheared across the width using the Instron as described earlier.

Processing Procedures

I. Smoked Fish Sausages.--The following ingredients were mixed at medium speed for 8 mins. in a Kitchen Aid Food Preparer (Model A-200, Hobart Mfg. Co., Troy, Ohio) with a paddle attachment (Table 2): Mechanically Deboned Frozen Fish, sodium chloride, sodium tripolyphosphate, monosodium glutamate, sodium ascorbate, condensed smoke and spices, either with or without soy protein isolate and white corn meal or the combination of these two included. Spices consisted of 0.10% garlic powder, 0.30% white pepper powder, 0.30% paprika pepper powder and 0.05% allspice. Hydrogenated vegetable oil was added and the mixture blended at low speed for 7 mins. more. The fish paste was stuffed into collagen casings (Brechtien,

TABLE 2.--Design and Formulation of Smoked Fish Sausage Treatments

Code No.	Treatment and Formulations ¹
A	97% Mechanically Deboned Frozen Fish (MDFF), 2% sodium chloride (Nacl), No hydrogenated vegetable oil (HVO).
B	88% MDFF, 3.0% Nacl and 8% HVO
C	84% MDFF, 3.0% Nacl, 4% soy protein isolate (SPI) and 8% HVO
D	84% MDFF, 3.0% Nacl, 4% white corn meal and 8% HVO
E	80% MDFF, 3.0% Nacl, 4% SPI, 4% white corn meal and 8% HVO

¹In all formulations (A, B, C, D, E) the following additives and spices are present: 0.45% sodium tripolyphosphate, 0.1% monosodium glutamate, 0.05% sodium ascorbate, 0.5% condensed smoke, and the spices.



Box 411, Mt. Clemens, Mich.) 35mm in diameter which were then linked.

Sausages were cooked in an Elek-Trol laboratory Smokehouse (Drying Systems Inc., Chicago, Ill.) according to the schedule shown in Table 3. The cooking process was followed by a 10 min. cold water shower.

TABLE 3.--Smokehouse Cooking Schedule

Time (min.)	Temperature (°F)		Relative Humidity %
	Dry Bulb	Wet Bulb	
60	120	84	22
120	140	100	25
90	160	120	30
60	175	133	30
60	190	154	40

II. Canned Frozen Minced Fish.--The following ingredients were mixed in the same manner described earlier for sausages (Table 4): Mechanically Deboned Frozen Fish, ground beef tripe, sodium chloride, sodium tripolyphosphate and spices, either with or without soy protein isolate and white corn meal or the combination of these two included. Hydrogenated vegetable oil was added and the mixture blended at low speed for 7 mins. more. The fish paste was then placed into size 211 x 304,

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TABLE 4.--Design and Formulation of Canned Minced Fish Treatments

Code Number	Treatment and Formulations ¹
A	86.5% Mechanically Deboned Frozen Fish (MDFF)
B	82.5% MDFF, 4% soy protein isolate (SPI)
C	82.5% MDFF, 4% white corn meal
D	78.5% MDFF, 4% SPI, 4% white corn meal

¹In all formulations (A, B, C, D) the following additives and spices are present: 4% beef tripe, 8% hydrogenated vegetable oil, 1% sodium chloride, 0.45% sodium tripolyphosphate, 0.30% white pepper powder, 0.15% onion powder, 0.15% garlic powder, 0.30% paprika pepper powder and 0.05% ground ginger.

c-enamel coated cans containing about 15% zinc oxide (American Can Company, Greenwich, Conn.). The filled cans were baked in a dry air oven (Thelco, Model 18, GCA/Precision Scientific, Chicago, Ill.) for 30 mins at 167°F (75°C). The product was then pressed and drained to remove any free liquid. Immediately after baking cans were closed using an automatic closing machine (Canning Devices Inc., Manitowoc, Wis.).

The sealed cans were processed in a still retort (Food Machinery Corp., Sprague-Sells Division, Hoopeston, Ill.) at 250°F (121°C) for 75 mins. The processed cans were immediately cooled in water until the temperature at the center of the cans was reduced to approximately 100°F.

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Statistical Analysis

A factorial experiment was designed with three levels of sodium chloride, three levels of sodium tri-polyphosphate and three levels of soy protein isolate. Analysis of variance (Steel and Torrie, 1960) was performed to assess the effect of main variables and their interactions. When significant differences were observed between more than two means, either Duncan's Multiple Range Test (Duncan, 1955), the student "t" test or comparing all means with a control (Dunnett's procedure) were performed to determine which means were significantly different. Analysis of variance was conducted at the Michigan State University Computer Center using SPSS (Statistical Package for the Social Science) version 7.0 (MSU, March 18, 1978).

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RESULTS AND DISCUSSION

Yield of Mechanically Deboned Fish and Its Chemical Composition

Table 5 shows some of the physical and chemical characteristics of mechanically deboned sucker flesh after it was processed by the deboning machine twice, for a yield of 47.60% of the round weight of the fish. This amount is similar to that reported by Morris (1977) and Zapata (1978), who obtained a 50% yield of mechanically deboned flesh for the same fish species using the same technique. However, in this study eviscerated and deboned suckers yielded approximately 80% of their weight as mechanically deboned flesh. Yield is an important consideration in determining the final costs of a given product. In general, yields vary considerably depending on the species, amount of waste from heading and gutting and the type of machinery used.

Moisture, fat and protein content of minced sucker flesh (Table 5) is similar to that reported by Zapata (1978). According to Stansby and Olcott (1963), fresh-water suckers are among those fish with high protein low oil content (15-20% and less than 5%, respectively). Suckers have a lower caloric content than do red meats or



TABLE 5.--Average Percentage Yields Per Total Weight of Fish for Dressing and Deboning Operations, and Average Percentage of Chemical Composition and Standard Deviation of Mechanically Deboned Freshwater Sucker Flesh (Catostomus and Moxostoma Spp.)

Characteristics	Percentage
Eviscerated and deheaded fish (% of round weight)	67.60
Mechanically deboned meat (% of round weight)	47.60
Moisture	80.70 $\pm$ 0.52
Nitrogen x 6.25	16.65 $\pm$ 0.02
Fat	2.03 $\pm$ 0.15
Nonprotein Nitrogen	1.70 $\pm$ 0.05

poultry, and thus are an ideal source of animal protein for use in low caloric diets. Generally, up to one-third of the oil in fish is comprised of polyunsaturated fatty acids with four to six double bonds per fatty acid (Osterhaug et al. 1963). Due to the relatively high moisture content of suckers (80.70%), the ratio of moisture to protein content (16.65%) is approximately 5 to 1 (Table 5).

Effects of Refrigeration and Freezing Storage
on the Extractability of Fish Muscle Proteins

Seasonal fluctuations in supplies of fish make it necessary to store quantities of minced fish for future use. Unless the raw fish is utilized immediately, freezing

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is the first choice of storage methods to preserve proper gelling of the fish proteins for production of texturized products. According to the generally accepted concepts of freezing technology, slow freezing usually causes a serious deterioration of fish flesh due to the rupture of cells by the formation of large ice crystals in the muscle tissue. However, fast freezing is thought not to have this effect on fish flesh (Slavin, 1963). Since refrigeration is so important in the fish industry, Table 6 and Figure 1

TABLE 6.--Mean* and Standard Deviation of Protein Fractions Extracted from Prerigor, Refrigerated and Frozen Sucker flesh (Catstomus and Moxostoma spp.) by the Helander Method (N = 4).

	Salt Soluble Protein	Water Soluble Protein	Nonprotein Nitrogen
Prerigor	50.15 ^a ± 1.38	29.04 ^a ± 0.43	12.28 ^a ± 0.53
Refrigerated	43.11 ^b ± 0.47	27.57 ^a ± 1.34	11.66 ^a ± 0.45
Frozen	37.20 ^c ± 0.32	29.53 ^a ± 3.09	11.99 ^a ± 0.52

*Means in the same column not followed by the same superscript are significantly different (p < 0.01).

compare refrigeration and fast freezing treatments to prerigor muscle with respect to the degree of muscle protein denaturation. The extractability of myofibrillar protein (salt soluble protein) from frozen and refrigerated muscle was less than that from prerigor muscle

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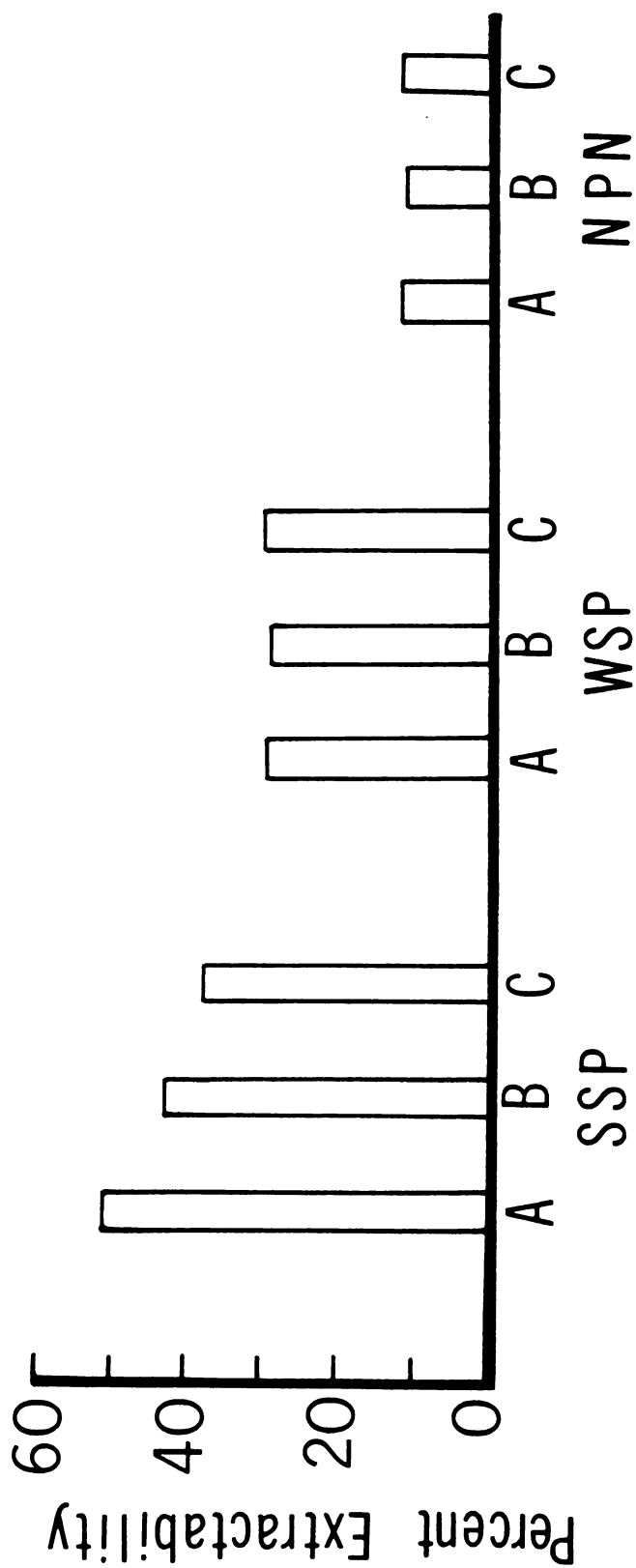
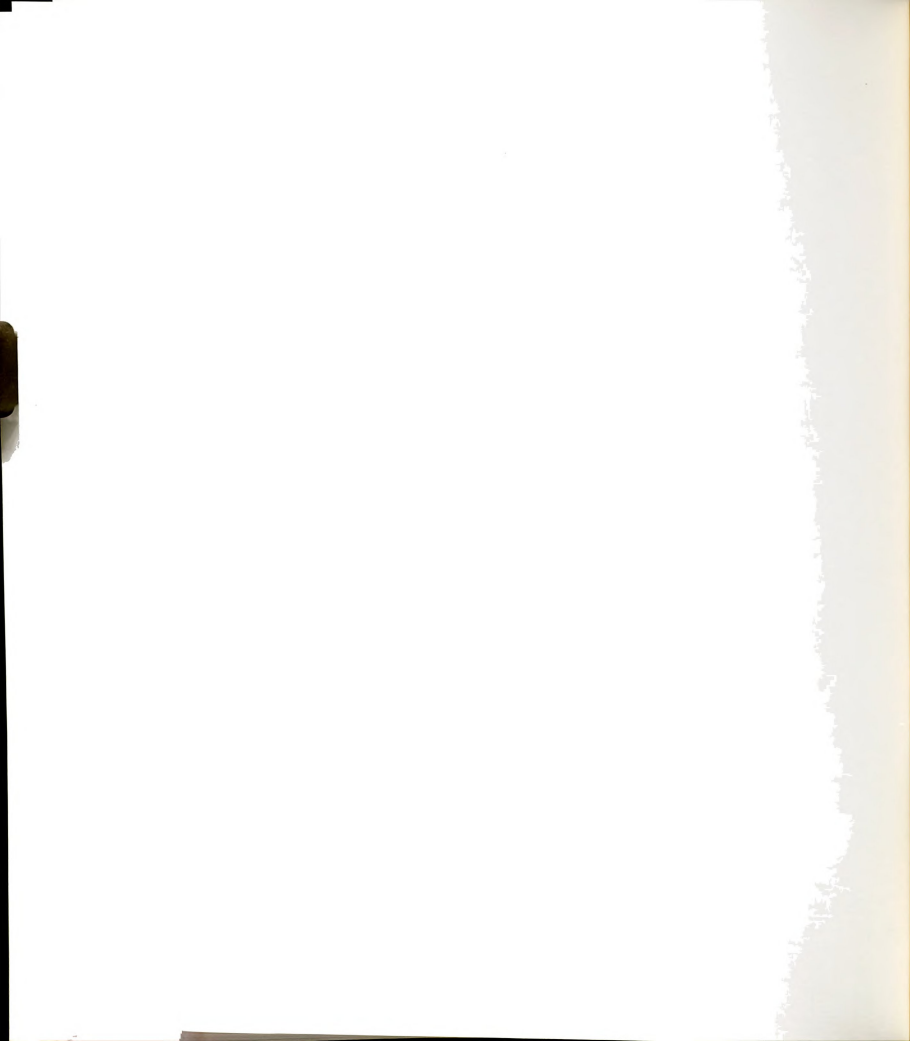


Figure 1.--Effect of Low Temperature Storage on Salt Soluble Protein (SSP), Water Soluble Protein (WSP) and nonprotein Nitrogen (NPN) Extracted from Sucker Flesh (Catostomus and Moxostoma spp.) by the Helander Method: A = prerigor, B = Refrigerated at 3°C and C = frozen at -29°C.



indicating that some denaturation occurred in the myofibrillar proteins. In refrigerated muscle stored at 3°C for seven days the rate and extent of the protein denaturation were much smaller. There was approximately a 7% reduction in extracted myofibrillar protein compared to 13% in the frozen muscle. Moreover, neither refrigeration nor freezing significantly affected the amount of sarcoplasmic protein (water soluble protein) or nonprotein nitrogen extracted from muscle (Table 6). An analysis of variance (Table 7) indicated that freezing and refrigeration significantly decreased the amount of myofibrillar protein extracted from fish muscle ($p < 0.01$). Results of the studentized range test showed that the amount of myofibrillar protein extracted from either prerigor or refrigerated muscle was significantly higher than that extracted from frozen muscle ($p < 0.01$). Freezing had greater an effect in decreasing the extractability of myofibrillar protein (Table 6).

These results are in agreement with the findings of Dyer (1951), Connell (1964), and Babbitt et al. (1972). According to Goll et al. (1969), prerigor conditions are optimal for myofibrillar protein extraction due to the presence of ATP which maintains disassociation between actin and myosin. Rigor and post rigor extractability, according to these workers, would be reduced by

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TABLE 7.--Analysis of Variance of the Effect of Low Temperature Storage on
Extracted Salt Soluble Protein

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F-Value	Significance of F
Between Groups	2	335.8641	167.9321	221.2487	0.00001
Within Groups	9	6.8312	0.7590		
Total	11	342.6953			



interactions between myofibrillar proteins, particularly those between myosin and the relatively inextractable actin, which result in the rate of myosin extraction becoming dependent upon the rate of actin extraction. In a review of fish muscle proteins Connell (1964) indicated that the myofibrillar protein (actomyosin) fraction of fish flesh is subject to the greatest protein insolubilization by freezing, whereas the solubility of sarco-plasmic proteins is not influenced by freezing. Protein denaturation is defined as a change in protein such that it is no longer soluble or extractable by salt solutions under conditions in which native protein would be soluble or extractable (Dyer and Dingle, 1961). The amount of denaturation depends primarily on temperature and pH. Fish protein is sensitive to denaturation, being more labile than most mammalian muscle, and will become denatured at lower temperature and higher pH than required for red meat protein denaturation (Forrest et al., 1975). Denaturation of fish muscle proteins at low temperatures is caused by the formation of a strong salt solution in the muscle at the eutectic point (Dyer, 1951; Love, 1958). This occurs because inorganic salts of cells, such as magnesium chloride, can remain in solution even at very low temperature. It, therefore, seems likely that the dehydration of muscle cells during frozen

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storage is the main phenomenon favoring conditions for denaturation (Dyer, 1951).

Effects of Additives on the Protein
Extractability of Frozen
Mechanically Deboned Fish

The value of measurements of protein solubility in various salt solutions lies in the functional role of proteins in processing high quality products. Since rigor mortis is thought to decrease extractability of some proteins (Dixon and Webb, 1961) and because phosphates or increasing ionic strength enhance extractability, the effects of adding sodium chloride, sodium tripolyphosphate and soy protein isolate were investigated.

Total Extracted Protein

Sodium chloride is the most common nonmeat ingredient added to fish products. This salt serves many functions in meat systems, one of which is to solubilize myofibrillar proteins. The effects of adding several concentrations of sodium chloride (0.0, 3.0, and 3.6%) on total protein extracted from minced frozen fish are presented in Table 8 and Appendix 2. Total protein fraction was isolated by dialyzing the filtered extract to retain both salt soluble and water soluble proteins, while dialyzable nonprotein nitrogen was removed. Total extracted protein increased significantly ($p < 0.05$) when sodium

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TABLE 8.--Effects of Adding Sodium Chloride and Sodium Tripolyphosphate on the Amount of Total Extracted Protein of Frozen Mechanically Deboned Fish (Total Extracted Protein = mg Extracted Protein/100 mg Total Protein)

		% Sodium Chloride			
		0.0	3.0	3.6	Mean ^b
	0.000	13.67 ^a	67.40	62.17	47.63
% Sodium	0.225	15.20	54.70	54.81	41.57
Tripolyphosphate	0.450	47.84	58.80	61.11	55.91
	Mean ^b	25.48	60.30	59.36	

^aStandard error for each cell mean (N = 12) is 0.07.

^bStandard error for each row or column mean (N = 36) is 0.04.

chloride was added. The greatest increase occurred when 3.0% sodium chloride was used in the extraction.

Comparison between the highest extractability values obtained from frozen fish samples with addition of sodium chloride (Table 8), without taking into account optimum pH and ionic strengths, and those values obtained earlier by Helander's (1957) method (Table 6) indicated that both methods yielded almost the same amount of protein.

Total extracted protein curves (Figure 2) indicate a minimum solubility of minced frozen fish at zero

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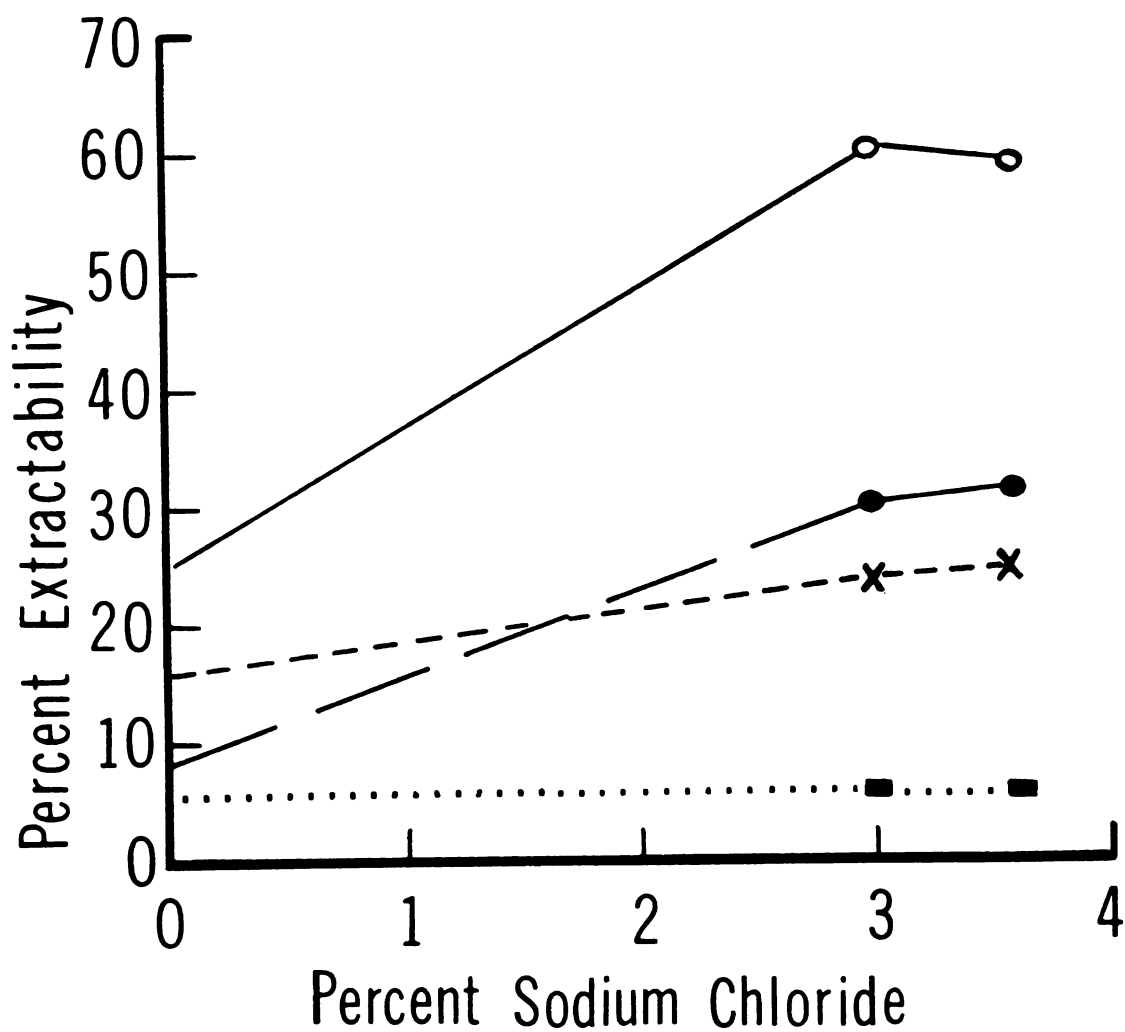


Figure 2.--Effect of Sodium Chloride in Varying Concentrations on the Protein Fractions of Frozen Minced Fish. 0-0 (Total Extracted Protein), ●-● (Salt Soluble Protein), X-X (Water Soluble Protein) and ■-■ (Nonprotein Nitrogen).

percent sodium chloride (data from this study). Using an extraction solution without sodium chloride, only 14-48% of the fish protein was solubilized, the amount depending on the phosphate level. Much of this solubility value was due to the presence of sodium tripolyphosphate, since only 13.67% total protein was extracted with plain water. Table 8 shows a rapid increase in protein extractability from 0.0% to 3.0% sodium chloride, a phenomenon noted also by Dyer et al. (1950). The effect of increasing sodium chloride from 3.0 to 3.6% on protein solubility was small, and may be associated with slight decreases in solubility in the absence of tripolyphosphate. Adding sodium chloride to the extraction solution increased ionic strength and high ionic strengths are required to solubilize myofibrillar proteins (Dyer et al., 1950). According to Bendall (1954), solutions of 3.0% and 3.6% sodium chloride have ionic strengths of 0.51 and 0.61 respectively. Most myofibrillar proteins require ionic strengths greater than 0.3 to be solubilized (Goll et al., 1974); or as noted by Helander (1957) and Goll et al., (1969), myofibrillar proteins are soluble only in solutions with ionic strengths ranging from 0.4 to 1.5. In addition to providing the necessary ionic strength, sodium chloride has the ability to spread protein filaments apart in the extraction solution (Brotsky and Everson, 1973). Thus,

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the increase in total extracted protein is a result of adding sodium chloride.

Sodium tripolyphosphate is the most widely used component of phosphate blends in the manufacture of processed meats since it has a desirable combination of properties. In samples containing no sodium chloride, approximately 50% of the fish proteins were extracted when 0.45% sodium tripolyphosphate was added, but only 13% and 15% respectively were obtained when 0.0% and 0.225% sodium tripolyphosphate were added (Table 8). However, with sodium chloride concentrations of 3.0% and 3.6%, total extracted protein was lower when sodium tripolyphosphate was used. The low extractability (Table 8) obtained with sodium tripolyphosphate alone might be due to low ionic strength (Dyer et al., 1950), and low pH. Therefore, the very low extractability values of 15% (Table 8) obtained might be due to both low ionic strength and lower pH. Meinke et al. (1972) noted that the highest solubilization of fish myofibrillar proteins required extreme pH conditions (< 4.0 but > 8.0). The lower extractability of protein at lower ionic strengths was attributed to the presence of strong associations between myofibrillar proteins (Goll et al., 1969). Sodium tripolyphosphate concentrations of 0.225% and 0.45% were not strong enough to spread protein filaments apart at

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low ionic strength. However, 0.45% sodium tripolyphosphate raised the pH to 7.8, which was more favorable for extraction of fish muscle proteins (Meinke et al., 1972), as indicated by the sharp increase in the percent extractability of fish protein (Figure 3). There were no increases in the amounts of extracted protein by adding sodium tripolyphosphate in the presence of sodium chloride (Figure 3).

There was no effect on the total extracted protein when 2% and 4% soy protein isolate was added to samples containing no sodium chloride (Table 9). However, an analysis of variance revealed highly significant differences between soy protein isolate treatments (Table 10). With further analysis using Dunnet's Test, it was established that significant differences existed between individual treatments. The effects of 2% and 4% soy protein isolate treatments were significantly greater ($p < 0.05$) than that of the control (0.0% soy protein isolate). Adding 2% and 4% soy protein isolate did not increase the extracted protein greatly since soy protein isolate contains approximately 90% protein. According to Wolf (1970), insolubilization of soy protein isolates may be caused by that portion of those proteins held together by disulfide bonds as aggregates, and more aggregates are formed during isoelectric precipitation.



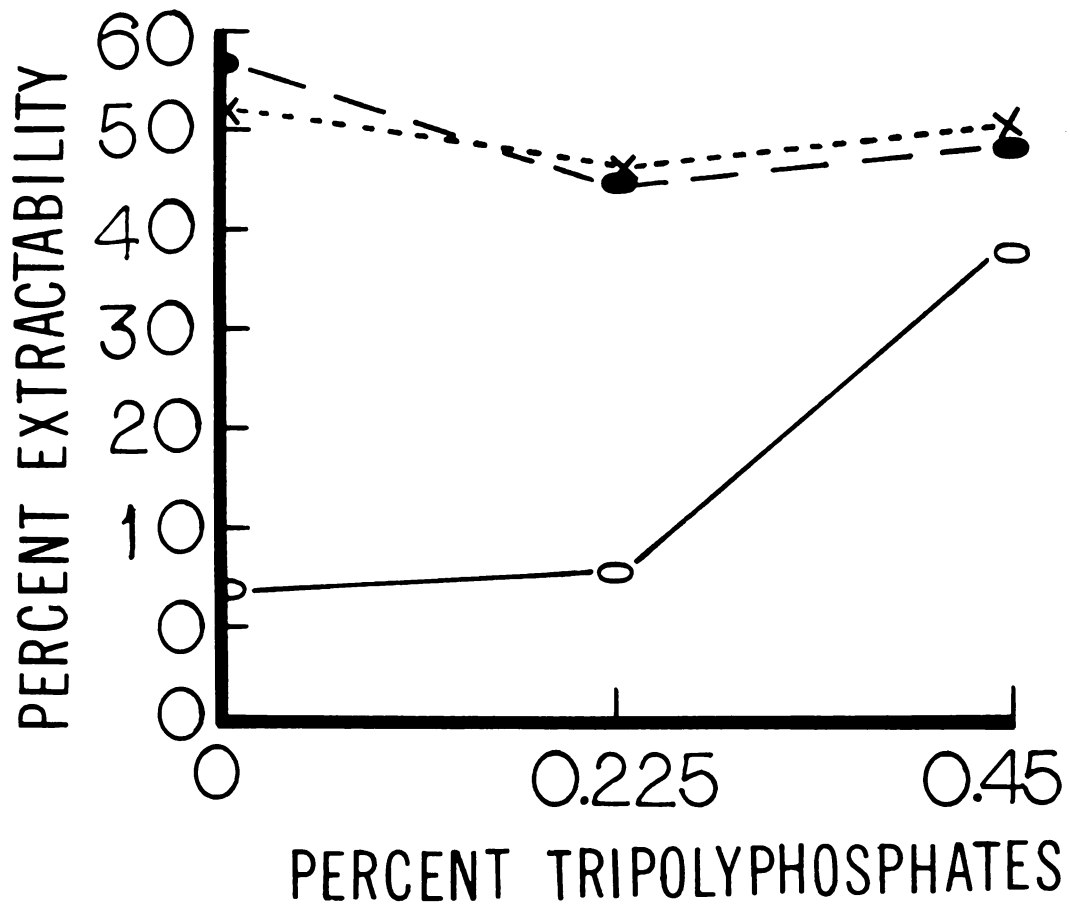


Figure 3.--Effects of Sodium Chloride and Sodium Tripolyphosphate, in Varying Concentrations and Combinations, on the Total Extractable Protein of Frozen Mechanically Deboned Fish. 0-○ 0.0% NaCl, ●-● 3.0% NaCl, X---X 3.6% NaCl.

TABLE 9.--Effect of Adding Soy Protein Isolate on the Amount of Total Extracted Protein of Frozen Mechanically Deboned Fish (Total Extracted Protein = mg Extracted Protein/100 mg Total Protein)

		% Sodium Chloride			
		0.0	3.0	3.6	Mean ^b
% Soy	0.0	25.35 ^a	58.87	56.74	46.99
Protein Isolate	2.0	25.20	60.53	60.28	48.67
	4.0	25.83	61.36	61.08	49.42

^aStandard error for each cell mean (N = 12) is 0.07.

^bStandard error for each column mean (N = 36) is 0.04.

Disulfide crosslinks must be broken to resolubilize these proteins. As described earlier, protein molecules interact with each other, and one type of interaction results in the precipitation of protein from solution (Oncley, et al., 1952). Precipitation of proteins may have occurred when soy protein isolate was added to frozen minced fish since large quantities of pellets were found in centrifuge bottles in samples containing soy protein isolate. Pearson et al. (1965) found that a soy sodium proteinate was less soluble at the pH of meat. There were significant interactions ($p < 0.01$) between either sodium chloride and sodium tripolyphosphate or sodium chloride



TABLE 10.--Analysis of Variance of the Effects of Sodium Chloride, Sodium Tripolyphosphate and Soy Protein Isolate in Varying Concentrations and Combinations on Total Extracted Protein of Frozen Mechanically Deboned Fish

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F-Value	Significance of F
Sodium Chloride (V_1)	2	786.333	393.167	2472.705	0.001
Sodium Tripolyphosphate (V_2)	2	103.159	51.580	6534.861	0.001
Soy Protein Isolate (V_3)	2	3.122	1.561	25.945	0.001
$V_1 \times V_2$	4	185.383	46.346	770.318	0.001
$V_1 \times V_3$	4	1.573	0.393	6.538	0.001
$V_2 \times V_3$	4	2.360	0.590	9.804	0.001
$V_1 \times V_2 \times V_3$	8	4.370	0.546	9.079	0.001
Explained	26	1086.300	41.781	694.442	0.001
Residual	81	4.873	0.060		
Total	107	1091.174	10.198		

and soy protein isolate or sodium tripolyphosphate and soy protein isolate on the extractability of protein from frozen minced fish (Table 10).

Salt Soluble Protein

In samples without sodium chloride, no salt soluble protein was extracted unless sodium tripolyphosphate was present (Table 11 and Appendix 3). Salt soluble protein comprised approximately 51% and 54% of the total extracted protein in samples containing 3.0 and 3.6% sodium chloride, respectively, while about 8-50% of the total extracted proteins were salt soluble in the presence of sodium tripolyphosphate alone (Table 11).

TABLE 11.--Effects of Adding Sodium Chloride and Sodium Tripolyphosphate on the Amount of Salt Soluble Protein Extracted from Frozen Mechanically Deboned Fish (Salt Soluble Protein = mg salt Soluble Protein/100 mg Total Extracted Protein)

		% Sodium Chloride			
		0.0	3.0	3.6	Mean ^b
	0.000	0.00	47.65	51.47	33.04
% Sodium	0.225	8.17 ^a	57.02	54.84	40.01
Tripolyphosphate	0.450	52.17	47.75	55.59	51.83
	Mean ^b	20.11	50.80	53.96	

^aStandard error for each cell mean (N = 6) is 0.08.

^bStandard error for each row or column mean (N = 13) is 0.05.

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As noted earlier, salt soluble protein extracted from frozen minced fish increased as sodium chloride concentration increased, with the largest increase obtained between 0.0% and 3.0% sodium chloride (Figure 2). The data indicated that salt soluble protein increased as sodium tripolyphosphate concentration increased. The data also showed that salt soluble protein comprised 8.2% to 57.0% of the total extracted protein in samples containing sodium tripolyphosphate, while lower percentages of the total extracted protein were salt soluble protein in the samples without sodium tripolyphosphate (Table 11). In addition, the data suggest that adding sodium tripolyphosphate sharply increased the extractability of salt soluble protein in the absence of sodium chloride (Figure 4). However, the addition of sodium tripolyphosphate in the presence of 3.0% sodium chloride had slight adverse effect on the extractability of salt soluble protein. These results may be explained by the fact that pyrophosphate can directly effect the resolution of actomyosin into its components actin and myosin and increase the solubility of those dissociated proteins (Fukazawa et al., 1961b). Sodium tripolyphosphate can cause this after it has reverted to pyrophosphate by enzymatic hydrolysis (Yasui et al., 1964b). An analysis of variance revealed significant effects on salt soluble protein

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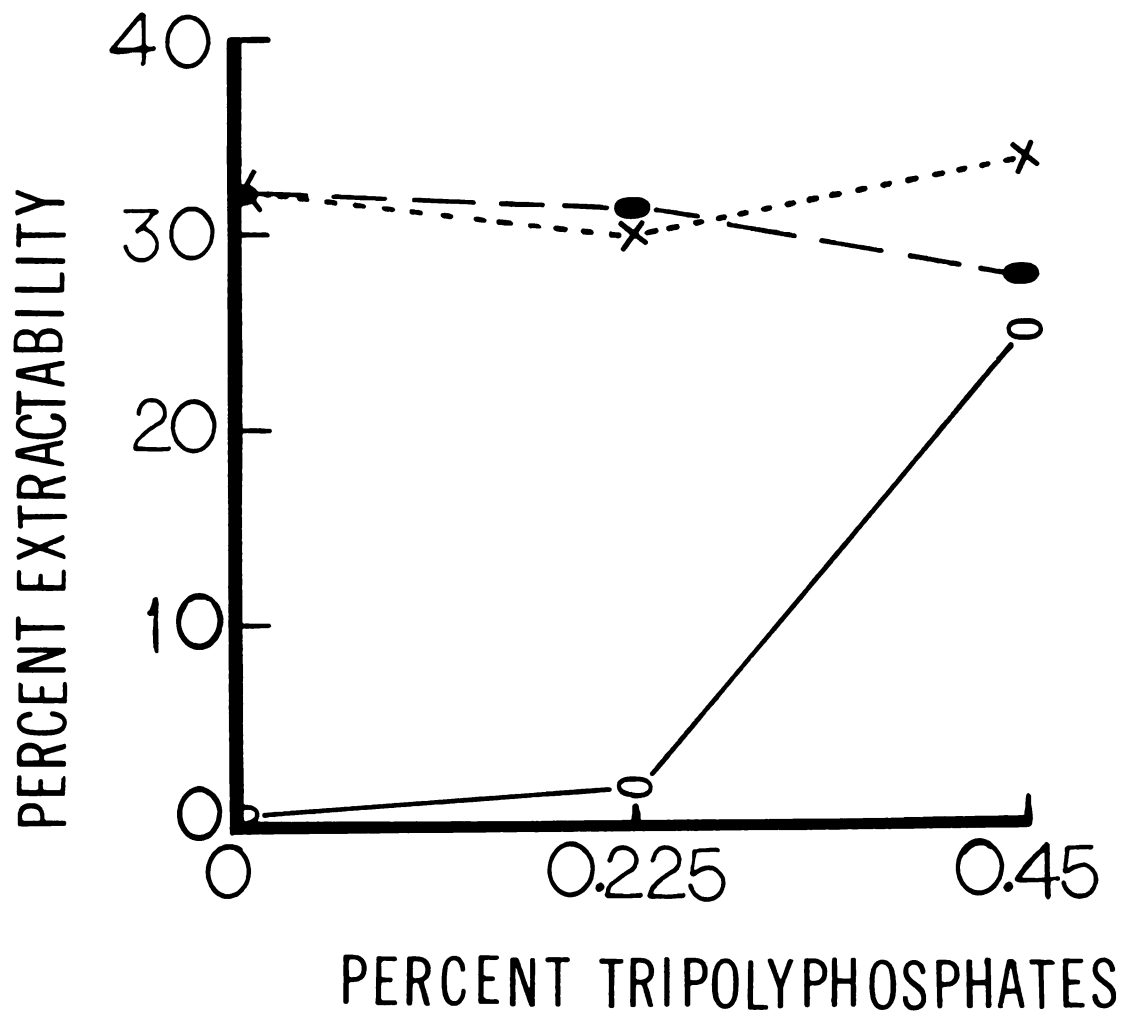


Figure 4.--Effects of Sodium Chloride and Sodium Tripolyphosphate, in Varying Concentrations and Combinations, on the Salt-Soluble Protein Fraction Extracted from Frozen Mechanically Deboned Fish. 0-○ 0.0% NaCl, ●- -● 3.0% NaCl and X---X 3.6% NaCl.



caused by adding either sodium chloride or sodium tri-polyphosphate ($p < 0.01$) (Table 12).

The data do not indicate a very large change in the amounts of salt soluble protein from frozen minced fish attributable to addition of soy protein isolate (Table 13). However, analysis of variance (Table 12) shows a significant effect caused by adding soy protein isolate ($p < 0.01$). Here again, further analysis comparing the effects of 2% and 4% soy protein isolate to the control using Dunnet's test indicated that both levels significantly increased the extractability of salt soluble protein ($p < 0.05$).

The interactions between sodium chloride and sodium tripolyphosphate or between sodium tripolyphosphate and soy protein isolate were significant ($p < 0.01$) (Table 12). However, salt soluble protein was not significantly affected by interaction between sodium chloride and soy protein isolate.

Water Soluble Protein

Water soluble protein fractions were obtained from the supernatant of the filtered protein extract, which was dialyzed overnight against 0.05M sodium chloride to remove nonprotein nitrogen and to insolubilize the salt soluble proteins. Adding sodium chloride decreased the percentage of extracted water soluble protein in the total

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TABLE 12.--Analysis of Variance of the Effects of Sodium Chloride, Sodium Tripolyphosphate and Soy Protein Isolate in Varying Concentrations and Combinations on Salt Soluble Protein Extracted from Frozen Mechanically Deboned Fish

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F-Value	Significance of F
Sodium Chloride (V_1)	2	63.636	34.318	857.950	0.001
Sodium Tripolyphosphate (V_2)	2	17.617	8.809	220.225	0.001
Soy Protein Isolate (V_3)	2	0.824	0.412	10.300	0.001
$V_1 \times V_2$	4	32.656	8.165	204.125	0.001
$V_1 \times V_3$	4	0.445	0.111	2.775	0.071
$V_2 \times V_3$	4	1.462	0.365	9.125	0.001
$V_1 \times V_2 \times V_3$	8	3.116	0.519	12.975	0.001
Explained	26	137.479	5.287	132.175	0.001
Residual	27	1.079	0.040		
Total	53	138.558	2.614		

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APR 1 2001
Level of Detail: 100%
Consent

TABLE 13.--Effect of Adding Soy Protein Isolate on the Amount of Salt Soluble Protein Extracted from Frozen Mechanically Deboned Fish (Salt Soluble Protein = mg salt soluble protein/100 mg total extracted protein)

		% Sodium Chloride			
		0.00	3.00	3.60	Mean ^b
% Soy	0.0	18.82 ^a	51.55	52.63	41.00
Protein Isolate	2.0	20.66	49.88	55.62	42.05
	4.0	20.87	50.98	53.63	41.82

^aStandard error for each cell mean (N = 6) is 0.08.

^bStandard error for each column mean (N = 18) is 0.05.

extracted protein. Water soluble protein decreased from approximately 77% to 40% and 43% of the total extracted protein by adding 3 and 3.6% sodium chloride, respectively (Table 14). This effect was expected, since salt soluble protein content increased as sodium chloride concentration increased (Figure 5). The quantity of water soluble protein also increased as sodium chloride concentration increased (Figure 2 and Appendix 4). The increase in water soluble protein caused by adding sodium chloride may be due to the lack of precipitation of myofibrillar protein during dialysis. This interpretation is in agreement with

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TABLE 14.--Effects of Adding Sodium Chloride and Sodium Tripolyphosphate on the Amount of Water Soluble Protein Extracted from Frozen Mechanically Deboned Fish (Water Soluble Protein = mg Water Soluble Protein/100 mg Total Extracted Protein)

		% Sodium Chloride			
		0.00	3.00	3.60	Mean ^b
	0.000	99.21 ^d	36.68	35.13	57.00
% Sodium	0.225	88.91	46.11	50.51	61.84
Tripolyphosphate	0.450	44.26	37.41	43.78	41.82
	Mean ^b	77.46	40.07	43.14	

^aStandard error for each cell mean (N = 6) is 0.08.

^bStandard error for each row or column mean (N = 18) is 0.04.

the finding of Goll et al., (1974), who reported that some myofibrillar proteins, such as troponin, are soluble in water. There is an increase in water soluble protein, and a decline in the rate of increase in salt soluble protein between 3.0 and 3.6% sodium chloride (Figure 5). This may be caused by a mechanical effect during the extraction process, or by development of cross-linked structures inhibiting extraction of salt soluble protein.

As sodium tripolyphosphate increased, water soluble protein decreased in samples without sodium chloride (Table 14). The protein fraction extracted (99.21%) at 0.0% sodium chloride and 0.0% sodium tripolyphosphate was

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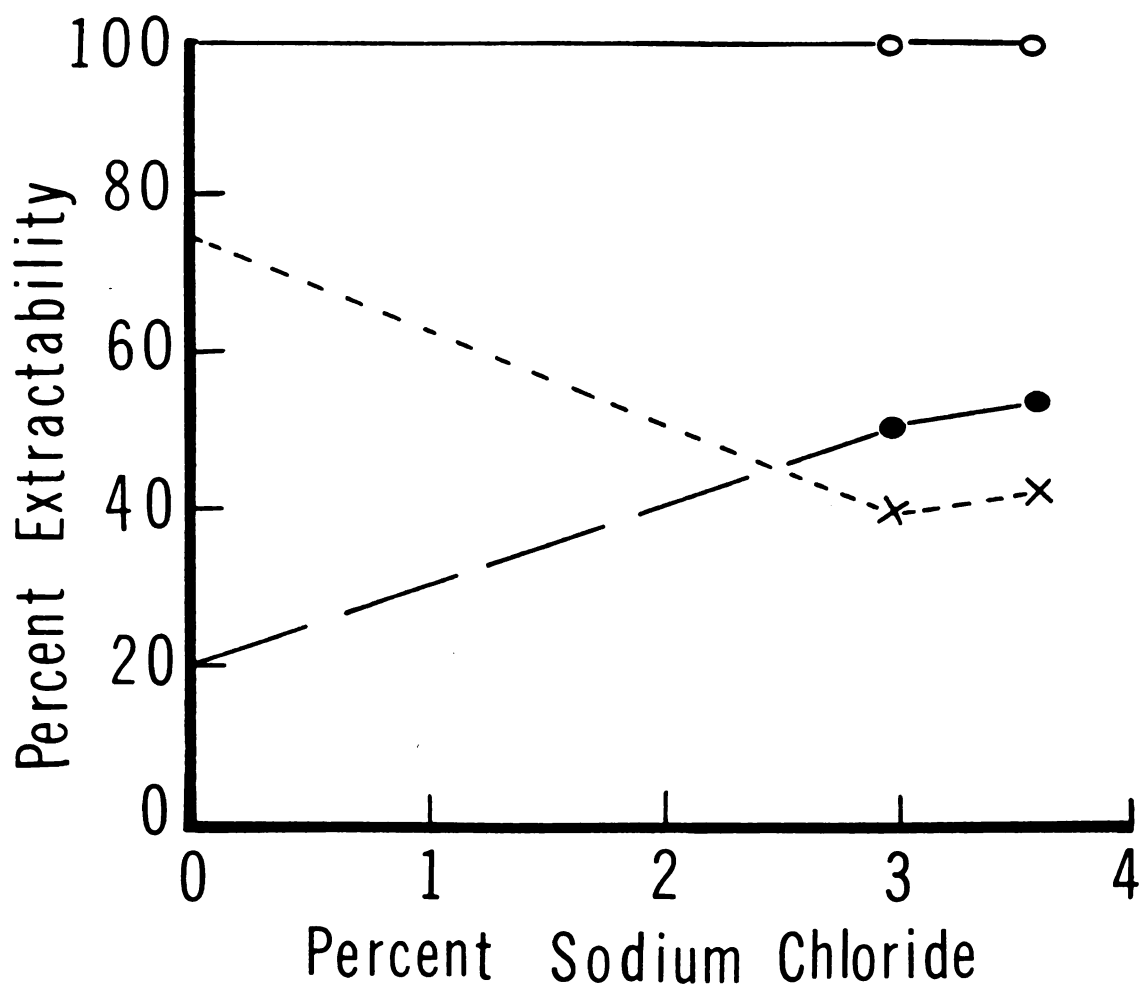


Figure 5.--Effect of Sodium Chloride in Varying Concentrations on the Ratio between the Amounts of Salt Soluble Protein and Water Soluble Protein Extracted from Frozen Minced Fish. 0-0 Total Extracted protein, ●- -● salt soluble protein and X---X water soluble protein.

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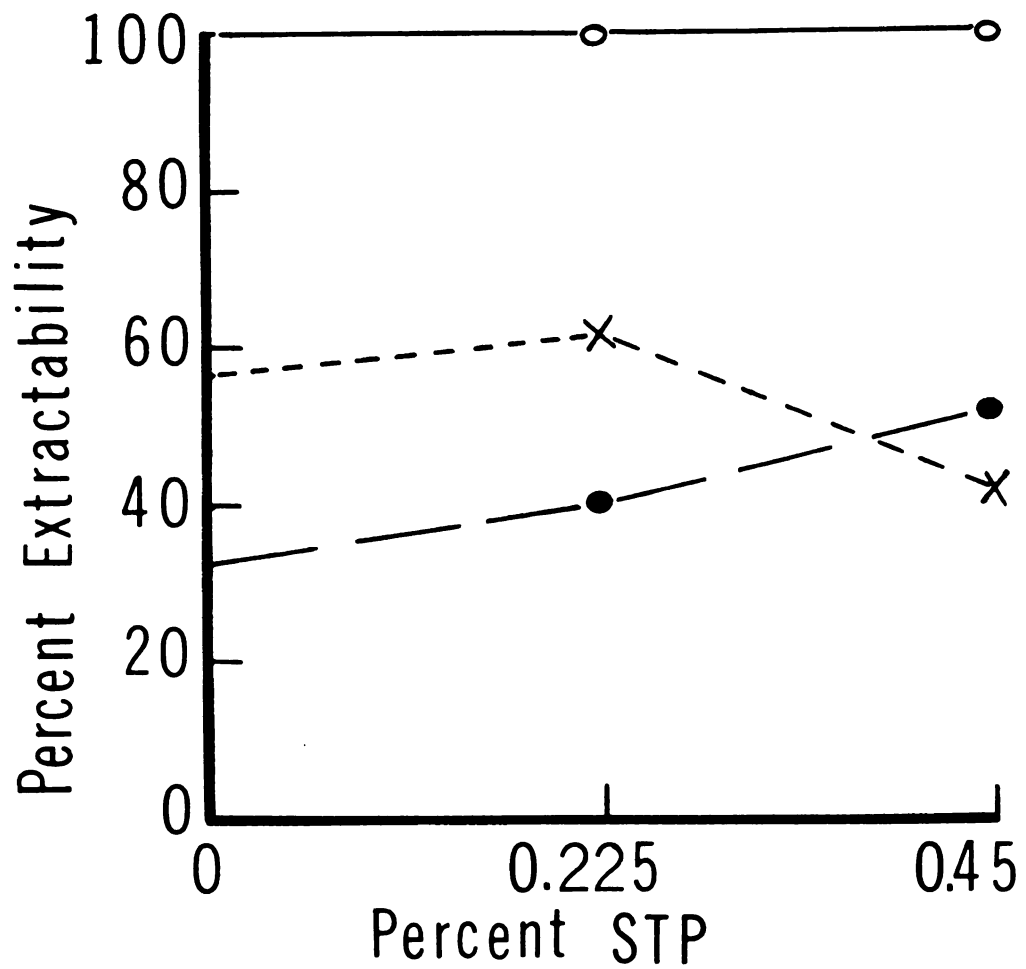


Figure 6.--Effect of Sodium Tripolyphosphate (STP) in Varying Concentrations on the Ratio Between the Amounts of Salt Soluble Protein and Water Soluble Protein Extracted from Frozen Minced Fish. 0-0 Total Extracted Protein, ●- -● Salt Soluble Protein and X---X water soluble Protein.



water soluble protein. The mean values of water soluble protein over all salt levels were 57.6%, 61.84% and 41.81% of the total extracted protein in samples containing 0.0%, 0.225% and 0.45% sodium tripolyphosphate, respectively (Table 14). Generally, water soluble protein was lowest at the 0.45% level of sodium tripolyphosphate (Figure 6). Once again, this effect was expected, since the amount of salt soluble protein increased as sodium tripolyphosphate increased (Figure 6).

Addition of soy protein isolate resulted in slight increases in the water soluble protein extracted from frozen minced fish (Table 15). This effect was apparent

TABLE 15.--Effect of Adding Soy Protein Isolate on the Amount of Water Soluble Protein Extracted from Frozen Mechanically Deboned Fish (Water Soluble Protein = mg Water Soluble Protein/100 mg Total Extracted Protein)

		% Sodium Chloride			
		0.0	3.0	3.6	Mean ^b
% Soy	0.0	74.78 ^a	41.08	42.67	52.88
Protein Isolate	2.0	78.57	38.07	43.49	53.37
	4.0	78.91	41.05	43.26	54.41

^aStandard error for each cell mean (N = 6) is 0.08.

^bStandard error for each column mean (N = 18) is 0.04.

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only in samples without sodium chloride, since water soluble protein increased from 74.89% to 78.57% and 78.91% by adding 2% and 4% soy protein isolate.

An analysis of variance established that adding soy protein isolate significantly affected the extractability of water soluble protein ($p < 0.01$). A Dunnet test showed that 2% and 4% soy protein isolate treatments resulted in significantly ($p < 0.05$) higher amounts of water soluble protein being extracted. There were also significant interactions ($p < 0.01$) between sodium chloride and either sodium tripolyphosphate or soy protein isolate (Table 16). The interaction between sodium tripolyphosphate and soy protein isolate was not significant.

Nonprotein Nitrogen

Microkjeldahl nitrogen analysis was used to determine the amount of nonprotein nitrogen in 15 ml samples of filtered total extracted protein of the supernatant after precipitation of proteins with trichloroacetic acid (TCA). Average percentages of soluble nonprotein nitrogen decreased significantly ($p < 0.05$) as either sodium chloride or sodium tripolyphosphate concentrations increased (Table 17). The largest amount of nonprotein nitrogen was obtained from samples with plain water (Table 17 and Appendix 5).

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TABLE 16.--Analysis of Variance of the Effects of Sodium Chloride, Sodium Tripolyphosphate and Soy Protein Isolate in Varying Concentrations and Combinations on Water Soluble Protein Extracted from Frozen Mechanically Deboned Fish

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F-Value	Significance of F
Sodium Chloride (V_1)	2	24.647	12.323	334.220	0.001
Sodium Tripolyphosphate (V_2)	2	2.104	1.052	28.527	0.001
Soy Protein Isolate (V_3)	2	1.299	0.650	17.620	0.001
$V_1 \times V_2$	4	9.484	2.371	64.304	0.001
$V_1 \times V_3$	4	1.037	0.259	7.031	0.001
$V_2 \times V_3$	4	0.421	0.105	2.858	0.043
$V_1 \times V_2 \times V_3$	8	2.724	0.341	9.236	0.001
Explained	26	41.717	1.605	43.515	0.001
Residual	27	0.996	0.037		
Total	53	42.713	0.806		

TABLE 17.--Effects of Adding Sodium Chloride and Sodium Tripolyphosphate on the Soluble Nonprotein Nitrogen of Frozen Mechanically Deboned Fish (Nonprotein Nitrogen = mg Nonprotein Nitrogen/100 mg Total Protein "N x 6.25")

		% Sodium Chloride			
		0.0	3.0	3.6	Mean ^b
% Sodium	0.000	7.77 ^a	6.11	5.15	6.34
Tripolyphosphate	0.225	6.07	6.07	6.06	6.07
	0.450	5.75	5.60	5.61	5.65
	Mean ^b	6.53	5.93	5.61	

^aStandard error for each cell mean (N = 12) is 0.01.

^bStandard error for each row or column mean (N = 36) is 0.005.

Adding soy protein isolate significantly ($p < 0.05$) decreased the amount of soluble nonprotein nitrogen (Table 18).

Analysis of variance (Table 19) indicated that there were significant interaction between sodium chloride and sodium tripolyphosphate, and also between sodium chloride and soy protein isolate ($p < 0.01$).

Myosin Heavy Chain

Myosin heavy chain bands did not appear on SDS gel electrophoresis patterns of samples of total extracted protein without either sodium chloride or sodium



TABLE 18.--Effect of Adding Soy Protein Isolate on the Nonprotein Nitrogen of Frozen Mechanically Deboned Fish (Nonprotein nitrogen = mg non-protein nitrogen/100 mg Total Protein "N x 6.25")

		% Sodium Chloride			
		0.0	3.0	3.6	Mean ^b
	0.0	6.75 ^a	6.21	5.89	6.28
% Soy	2.0	6.62	5.88	5.64	6.05
Protein Isolate	4.0	6.22	5.69	5.29	5.75

^aStandard error for each cell mean (N = 12) is 0.01.

^bStandard error for each column mean (N = 36) is 0.005.

tripolyphosphate and in samples containing 0.225% sodium tripolyphosphate (gels 1-6, Figure 7). These data indicated that myosin heavy chains insolubilize at low ionic strength. However, 0.45% sodium tripolyphosphate solubilized myosin (gels Nos. 7, 8 and 9, Figure 7). The effect of the latter concentration of sodium tripolyphosphate may be due to the high pH (7.8) of the extraction solution, since the ionic strength of 0.45% sodium tripolyphosphate is insufficient to solubilize myosin (Goll, et al., 1969). In combination or alone, 3 percent sodium chloride with sodium tripolyphosphate extracted myosin, but myosin heavy chain bands were light (gels Nos. 1, 2

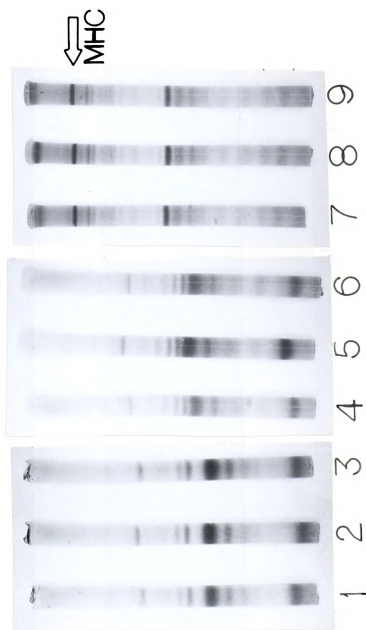
TABLE 19.--Analysis of Variance of the Effects of Sodium Chloride, Sodium Tripolyphosphate and Soy Protein Isolate in Varying Concentrations and Combinations on Nonprotein Nitrogen Extracted from Frozen Mechanically Deboned Fish

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F-Value	Significance of F
Sodium Chloride (V_1)	2	0.543	0.272	323.177	0.001
Sodium Tripolyphosphate (V_2)	2	0.308	0.154	183.495	0.001
Soy protein Isolate (V_3)	2	0.104	0.052	61.668	0.001
$V_1 \times V_2$	4	0.906	0.227	269.618	0.001
$V_1 \times V_3$	4	0.013	0.003	3.730	0.008
$V_2 \times V_3$	4	0.003	0.001	0.774	0.545
$V_1 \times V_2 \times V_3$	8	0.026	0.003	3.881	0.001
Explained	26	1.902	0.073	87.085	0.001
Residual	81	0.068	0.001		
Total	107	1.970	0.018		



Figure 7. --SDS Polyacrylamide Gel Electrophoresis of
Total Protein Extracted from Frozen Mechanically Deboned Fish with the Following Additives.

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|----|---------------------------------|------------------------|
| 1. | No sodium tripolyphosphate, | no soy protein isolate |
| 2. | No sodium tripolyphosphate, | 2% soy protein isolate |
| 3. | No sodium tripolyphosphate, | 4% soy protein isolate |
| 4. | 0.225% sodium tripolyphosphate, | no soy protein isolate |
| 5. | 0.225% sodium tripolyphosphate, | 2% soy protein isolate |
| 6. | 0.225% sodium tripolyphosphate, | 4% soy protein isolate |
| 7. | 0.45% sodium tripolyphosphate, | no soy protein isolate |
| 8. | 0.45% sodium tripolyphosphate, | 2% soy protein isolate |
| 9. | 0.45% sodium tripolyphosphate, | 4% soy protein isolate |





and 3) when 3.0% sodium chloride alone was used (Figure 8). Adding 3.6% sodium chloride with or without sodium tri-polyphosphate also extracted myosin from frozen minced fish (Figure 9).

Changes in the total peak area of soluble myosin heavy chains from frozen mechanically deboned fish are shown in Table 20. The gel scanner was equipped with an integrator to give total peak area of myosin heavy chain bonds. Base line positioning (A and B, Figure 10) is critical in obtaining accurate peak areas (Figure 10). As sodium chloride increased from 3.0% to 3.6%, myosin heavy chain total area increased from 0.74×10^5 to 1.00×10^5 in samples containing no sodium tripolyphosphate. On the other hand, myosin heavy chain total peak area decreased as sodium chloride increased in samples containing either 0.225% or 0.45% sodium tripolyphosphate. These results are in agreement with those of Linko and Nikkila (1961) who found that salt induced denaturation of Baltic Herring myosin was inhibited by the addition of various alkaline phosphates. When salt concentration in meat systems increased, higher concentrations of phosphates are required to prevent the denaturation of myosin. This effect might be due to the lowering of pH, since pH decreased as sodium chloride increased (Table 23). It was considered that the findings of Meinke et al. (1972)



Figure 8.--SDS Polyacrylamide Gel Electrophoreses of Total Protein Extracted from Frozen Mechanically Deboned Fish with 3.0% Sodium Chloride and the Following Additives

- | | |
|------------------------------------|------------------------|
| 1. No sodium tripolyphosphate, | No soy protein isolate |
| 2. No sodium tripolyphosphate, | 2% soy protein isolate |
| 3. No sodium tripolyphosphate, | 4% soy protein isolate |
| 4. 0.225% sodium tripolyphosphate, | No soy protein isolate |
| 5. 0.225% sodium tripolyphosphate, | 2% soy protein isolate |
| 6. 0.225% sodium tripolyphosphate, | 4% soy protein isolate |
| 7. 0.45% sodium tripolyphosphate, | No soy protein isolate |
| 8. 0.45% sodium tripolyphosphate, | 2% soy protein isolate |
| 9. 0.45% sodium tripolyphosphate, | 4% soy protein isolate |

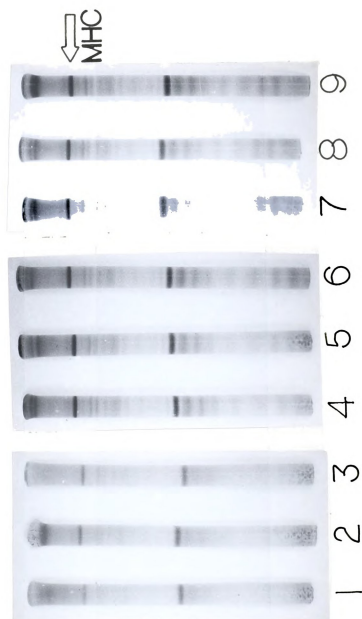




Figure 9.--SDS Polyacrylamide Gel Electrophoresis of Total Protein Extracted from Frozen Mechanically Deboned Fish with 3.6% sodium chloride and the following Additives.

1. No sodium tripolyphosphate, No soy protein isolate
2. No sodium tripolyphosphate, 2% soy protein isolate
3. No sodium tripolyphosphate, 4% soy protein isolate
4. 0.225% sodium tripolyphosphate, No soy protein isolate
5. 0.225% sodium tripolyphosphate, 2% soy protein isolate
6. 0.225% sodium tripolyphosphate, 4% soy protein isolate
7. 0.45% sodium tripolyphosphate, No soy protein isolate
8. 0.45% sodium tripolyphosphate, 2% soy protein isolate
9. 0.45% sodium tripolyphosphate, 4% soy protein isolate

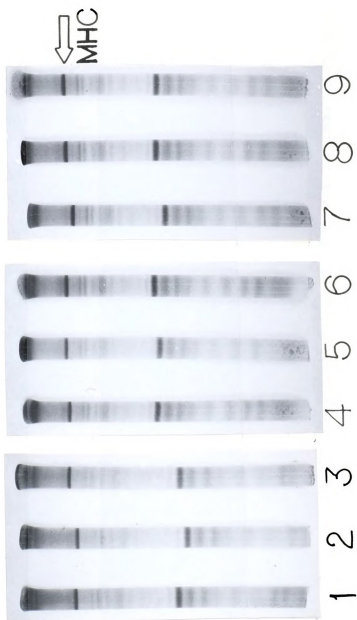


TABLE 20.--Effects of Adding Sodium Chloride and Sodium Tripolyphosphate on Solubility of Myosin Heavy Chain of Frozen Mechanically Deboned Fish (Myosin Heavy Chain = Total Peak Area Measured by Scanning Myosin Heavy Chain Bonds Obtained from 20 Micrograms Total Extracted Protein Sample)

	% Sodium Chloride			Mean ^b
	0.0	3.0	3.6	
0.000	---	0.74×10^5	1.00×10^5	0.58×10^5
0.225	---	2.14×10^5	1.14×10^5	1.09×10^5
0.450	2.59×10^{5a}	1.85×10^5	1.45×10^5	1.96×10^5
Mean ^b	0.86×10^5	1.57×10^5	1.19×10^5	

^aStandard error for each cell mean (N = 9) is 269.

^bStandard error for each row or column mean (N = 27) is 155.



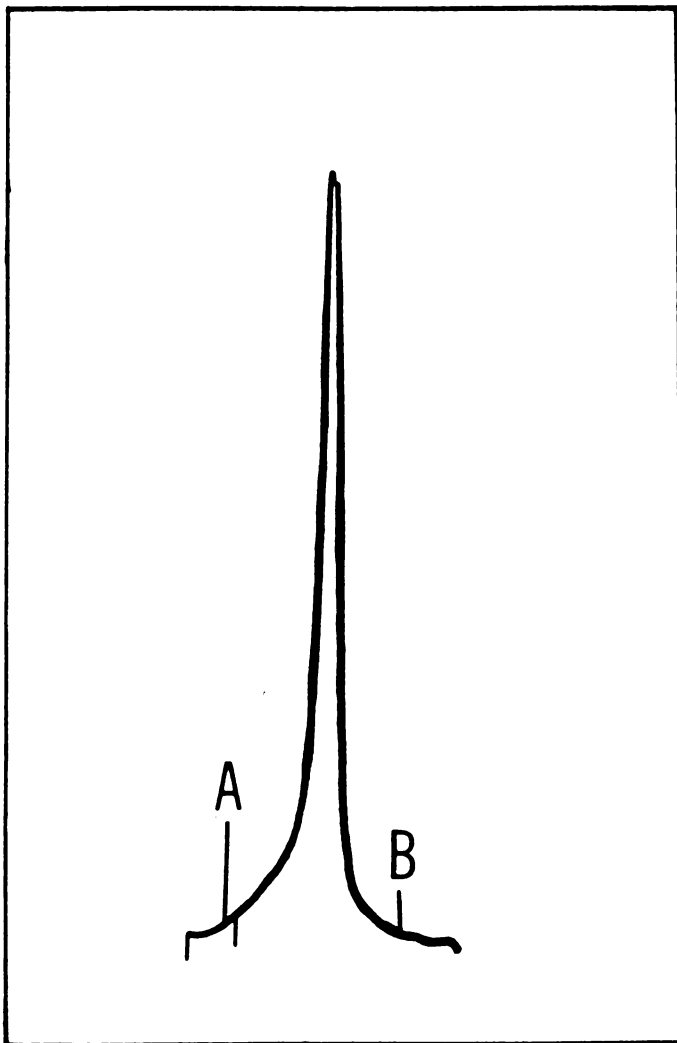


Figure 10.--Typical Absorbance Peak for Heavy Chain Total Area of Myosin Extracted from Frozen Mechanically Deboned Fish.

A = Baseline before establishing myosin heavy chain peak

B = Baseline after establishing myosin heavy chain peak.

could offer an interpretation of the results. They noted that the highest solubilization of fish myofibrillar proteins required extreme pH conditions (< 4.0 or > 8.0), and as mentioned earlier, that myosin comprised 40% of Fish (Cod) muscle protein (Connell, 1964). In general, adding either sodium chloride or sodium tripolyphosphate increased the solubility of myosin heavy chains (Table 20).

Soy protein isolate had no effect on myosin heavy chain solubility (Table 21).

Figure 11 and Appendix 6 show the effects of sodium chloride, sodium tripolyphosphate and soy protein isolate in varying concentrations and combinations on the solubility of myosin heavy chain. The largest myosin heavy chain peak area was obtained from samples containing 0.45% sodium tripolyphosphate alone (Nos. 7, 8, and 9, Figure 11-A).

An analysis of variance (Table 22) indicated significant differences in peak area caused by sodium chloride, sodium tripolyphosphate and soy protein isolate. There was also significant interaction between sodium chloride and sodium tripolyphosphate ($P < 0.01$). However, peak area was not significantly affected by interactions between either sodium chloride and soy protein isolate or sodium tripolyphosphate and soy protein isolate (Table 22).

TABLE 21.--Effect of Adding Soy Protein Isolate on the Solubility of Myosin Heavy Chains of Frozen Mechanically Deboned Fish
(Myosin Heavy Chain = Total Peak Area Measured by Scanning Myosin Heavy Chain Bonds Obtained from 20 Micrograms of Total Extracted Protein Sample)

	% Sodium Chloride				Mean ^b
	0.0	3.0	3.6		
% Soy	0.0	0.82 x 10 ^{5a}	1.59 x 10 ⁵	1.10 x 10 ⁵	1.17 x 10 ⁵
	2.0	0.88 x 10 ⁵	1.68 x 10 ⁵	1.25 x 10 ⁵	1.27 x 10 ⁵
Protein Isolate	4.0	0.88 x 10 ⁵	1.46 x 10 ⁵	1.24 x 10 ⁵	1.19 x 10 ⁵

^aStandard error for each cell mean (N = 9) is 269.

^bStandard error for each column mean (N = 27) is 155.

Figure 11.--Effects of Sodium Chloride, Sodium Tripolyphosphate and Soy Protein Isolate, in Varying Concentrations, and Combinations on Myosin Heavy Chain (MHC) Total Area per 20 Mg Protein Sample Extracted from Frozen Mechanically Deboned Fish.

A = No sodium chloride, B = 3.0% sodium chloride and C = 3.6% sodium chloride

- 1, 2 and 3 of A, B and C were 0.0% Sodium Tripolyphosphates.
- 4, 5 and 6 of A, B and C were 0.225% Sodium Tripolyphosphates.
- 7, 8 and 9 of A, B and C were 0.45% Sodium Tripolyphosphates.
- 1, 4 and 7 of A, B and C were 0.0% Soy Protein Isolate .
- 2, 5 and 8 of A, B and C were 2.0% Soy Protein Isolate .
- 3, 6 and 9 of A, B and C were 4.0% Soy Protein Isolate .

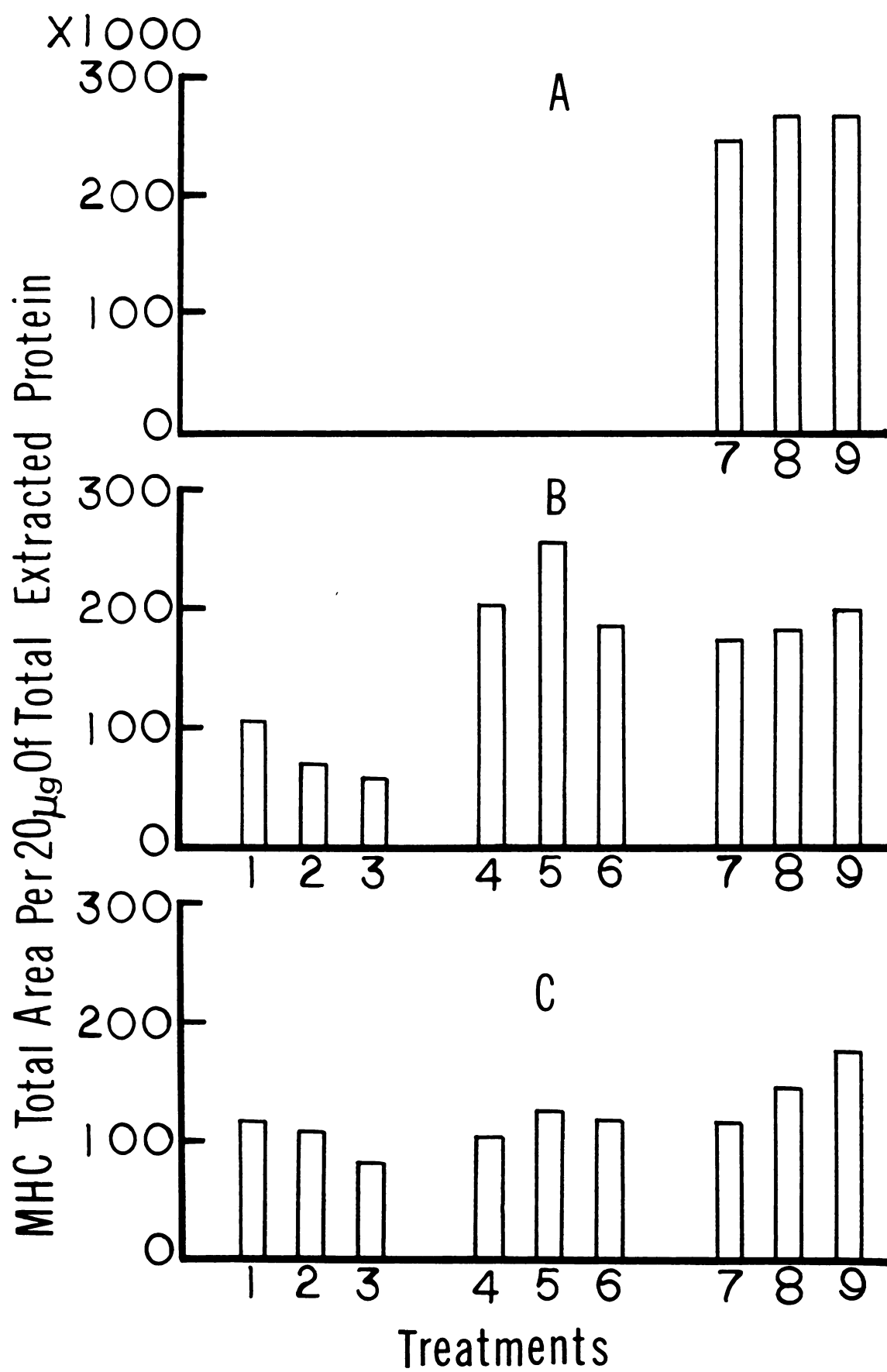


TABLE 22.--Analysis of Variance of the Effects of Sodium Chloride, Sodium Tripolyphosphate and Soy Protein Isolate in Varying Concentrations and Combinations on Myosin Heavy Chain Total Area Extracted from Frozen Mechanically Deboned Fish

Source of Variance	Degree of Freedom	Sum of Squares	Mean Square	F-value	Significance of F
Sodium Chloride (V_1)	2	2123371.969	1061685.984	16.945	0.001
Sodium Tripolyphosphate (V_2)	2	553753.288	276876.644	4.419	0.017
Soy Protein Isolate (V_3)	2	459924.501	229962.251	3.670	0.032
$V_1 \times V_2$	4	1786740.353	446685.088	7.129	0.001
$V_1 \times V_3$	4	171357.397	42839.349	0.684	0.606
$V_2 \times V_3$	4	309392.963	77348.241	1.235	0.307
$V_1 \times V_2 \times V_3$	8	912488.202	114061.025	1.820	0.093
Explained	26	6317028.673	242962.641	3.878	0.001
Residual	54	3383387.407	62655.322		
Total	80	9700416.081	121255.201		



Effects of Additives on pH of Frozen
Mechanically Deboned Fish

It is interesting to note changes in the pH of frozen minced fish flesh caused by adding varying concentrations and combinations of sodium tripolyphosphate, sodium chloride and soy protein isolate, since protein solubility, swelling, gel formation and water holding capacity in fish muscle are affected by pH (Hermanson, 1972; Trautman, 1964; Hamm, 1960). The minimum fish protein solubility falls within a pH range of 5.5-6.0; however, protein solubility increases on both the acidic and basic sides of this range (Meike, et al., 1972).

In general, as sodium chloride was increased, the pH of frozen deboned fish decreased (Table 23). These

TABLE 23.--Effects of Adding Sodium Chloride and Sodium Tripolyphosphate on pH of Frozen Mechanically Deboned Fish

		% Sodium Chloride			
		0.0	3.0	3.6	Mean ^b
	0.000	6.86 ^a	6.20	6.14	6.40
% Sodium	0.225	7.27	6.41	6.38	6.69
Tripolyphosphate	0.450	7.80	6.61	6.67	7.05
	Mean ^b	7.31	6.43	6.40	

^aStandard error for each cell mean (N = 6) is 0.002.

^bStandard error for each row or column mean (N = 18) is 0.001.



results agree with those of Hamm (1957), who found that adding sodium chloride to post-rigor ham muscle consistently lowered its pH. This reduction in pH was explained as follows by Mahon (1961). On the acidic side of the isoelectric point protein is positively charged and Cl^- ions are absorbed, while on the basic side, there are negative charges and Na^+ ions are absorbed with the liberation of some H^+ ions causing a drop in pH. Frozen mechanically deboned fish has a pH of 6.68 which is on the alkaline side of the isoelectric point of some proteins, and consequently carries a net negative charge. Adding sodium chloride to negatively charged protein causes Na^+ ions to be absorbed with the liberation of H^+ ions, causing a drop in pH.

As sodium tripolyphosphate increased, pH also increased (Table 12 and Appendix 7). pH increased to 7.8 with the addition of 0.45% sodium tripolyphosphate in samples without sodium chloride (Table 23). Brotsky and Everson (1973) reported similar results. Adding sodium tripolyphosphate to meat increased its pH, which in turn increased the negative charge on myofibrillar proteins. The data also showed that addition of sodium tripolyphosphate raised the pH of frozen minced fish even in the presence of sodium chloride with the largest increase when no sodium chloride was added (Figure 12). There



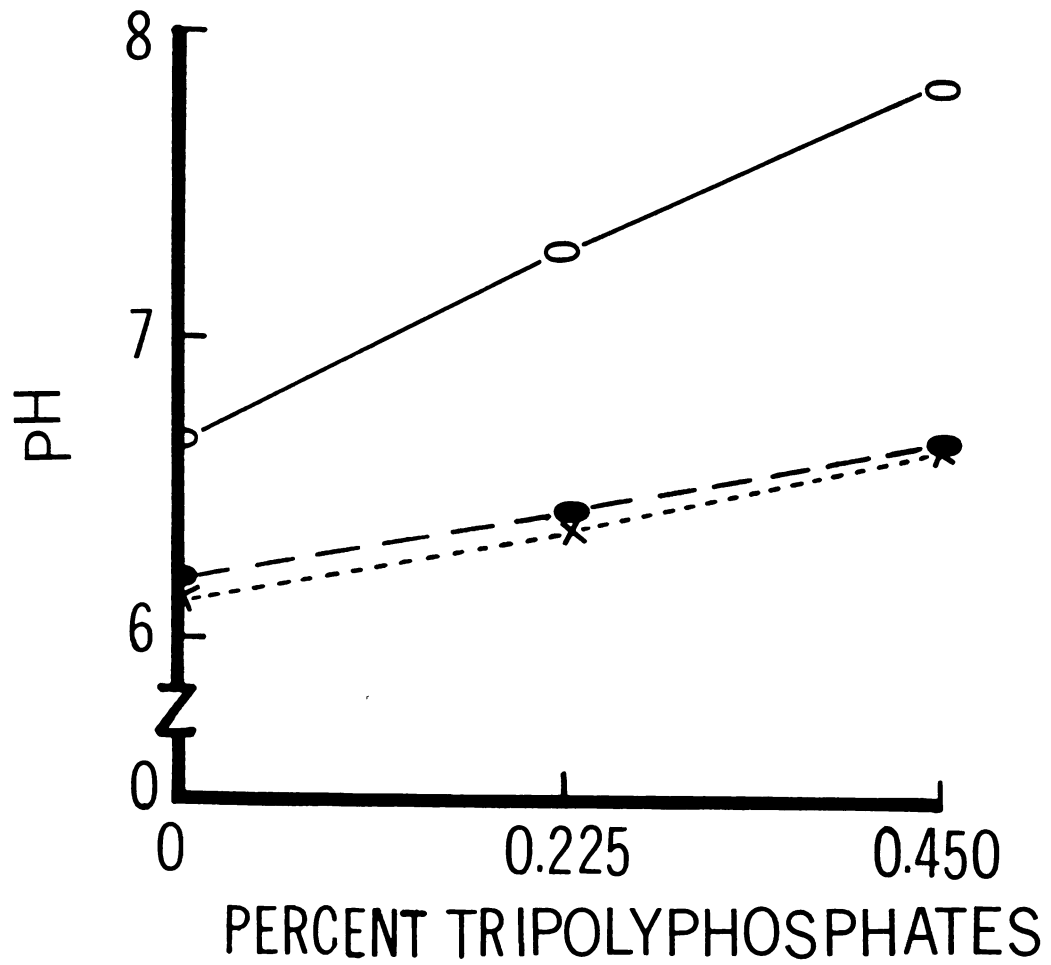


Figure 12.--Effects of Sodium Chloride and Sodium Tri-polyphosphate, in Varying Concentrations and Combinations on pH of Frozen Mechanically Deboned Fish. 0-0 0.0% NaCl, ●- -● 3.0% NaCl and X---X 3.6% NaCl.

were no remarkable changes in pH caused by adding soy protein isolate to frozen minced fish in samples with or without sodium chloride (Table 24).

TABLE 24.--Effect of Adding Soy Protein Isolate on pH of Frozen Mechanically Deboned Fish

		% Sodium Chloride			
		0.0	3.0	3.6	Mean ^b
% Soy Protein Isolate	0.0	7.27 ^a	6.46	6.37	6.70
	2.0	7.33	6.42	6.40	6.72
	4.0	7.33	6.39	6.42	6.71

^aStandard error for each cell mean (N = 6) is 0.002.

^bStandard error for each column mean (N = 18) is 0.001.

From the results of an analysis of variance (Table 25), it was evident that blending either sodium chloride, sodium tripolyphosphate, or soy protein isolate significantly affected the pH of frozen minced fish ($P < 0.01$). The analysis also indicated that there were significant effects on the pH of fish muscle caused by the interaction of those three additives.



TABLE 25.--Analysis of Variance of the Effects of Sodium Chloride, Sodium Tripolyphosphate and Soy Protein Isolate, in Varying Concentrations and Combinations, on pH of Frozen Mechanically Deboned Fish

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F-Value	Significance of F
Sodium Chloride (V_1)	2	9.672	4.836	0.2E+0.5*	0.001
Sodium Tripolyphosphate (V_2)	2	3.770	1.885	0.1E+0.5**	0.001
Soy Protein Isolate (V_2)	2	0.005	0.002	14.865	0.001
$V_1 \times V_2$	4	0.383	0.096	580.888	0.001
$V_1 \times V_3$	4	0.032	0.008	48.287	0.001
$V_2 \times V_3$	4	0.009	0.002	13.483	0.001
$V_1 \times V_2 \times V_3$	8	0.037	0.005	27.817	0.001
Explained	26	13.907	0.535	3245.328	0.001
Residual	27	0.004	0.000165		
Total	53	13.911	0.262		

*0.2E + 0.5 is 20,000.

**0.1E + 0.5 is 10,000.



Effects of Additives on Percent Swelling of
Frozen Mechanically Deboned Fish

The effects of sodium chloride, sodium tripolyphosphate and soy protein isolate on frozen minced fish swelling are shown in Appendix 8. As sodium chloride increased, swelling decreased, with the lowest mean values observed in the presence of 3.6% sodium chloride (Table 26). Shults et al., (1972) reported similar

TABLE 26.--Effects of Adding Sodium Chloride and Sodium Tripolyphosphate on Percent Swelling of Frozen Mechanically Deboned Fish

		% Sodium Chloride			
		0.0	3.0	3.6	Mean ^b
	0.000	55.68 ^a	53.34	47.27	52.10
% Sodium	0.225	72.60	59.78	48.45	60.28
Tripolyphosphate	0.450	108.40	61.57	63.95	77.85
	Mean ^b	78.77	58.23	53.22	

^aStandard error for each cell mean (N = 12) is 1.10.

^bStandard error for each row or column mean (N - 36) is 0.63.

results, stating that meat swelling decreased with the addition of 3-5% sodium chloride. The decrease in swelling was attributed to the exchange of Mg^{++} and K^{+} by Na^{+} .

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As amounts of sodium tripolyphosphate blended with frozen minced fish increased, swelling also increased (Table 26). Bendall (1954), Hermansson (1972), and Shults et al., (1972) recorded similar findings. It is thought that sodium tripolyphosphate causes frozen mechanically deboned fish to swell because of its relative effect on pH. Swelling increased as pH increased in the range of 4-10 (Hermansson, 1972). Moreover, Bendall (1954) stated that swelling is due to the ability of inorganic pyrophosphate to split actomyosin into its component proteins, actin and myosin, resulting in the uptake of water. This phenomenon resembles the action of adenosine triphosphate on disassociation of actomyosin. The data indicated that swelling increased sharply in the presence of both levels of sodium tripolyphosphate in samples without sodium chloride (Figure 13). Swelling was also caused by the combined effect of sodium tripolyphosphate and sodium chloride (Table 26).

Swelling of frozen minced fish increased as soy protein isolate levels increased, with the highest values occurring at the 4.0% level of soy protein isolate (Table 27, Figure 14). Hermansson (1975) found that soy protein isolate has a high capacity to cause swelling. Pearson et al., (1965) also noted the tendency of this substance to absorb water.

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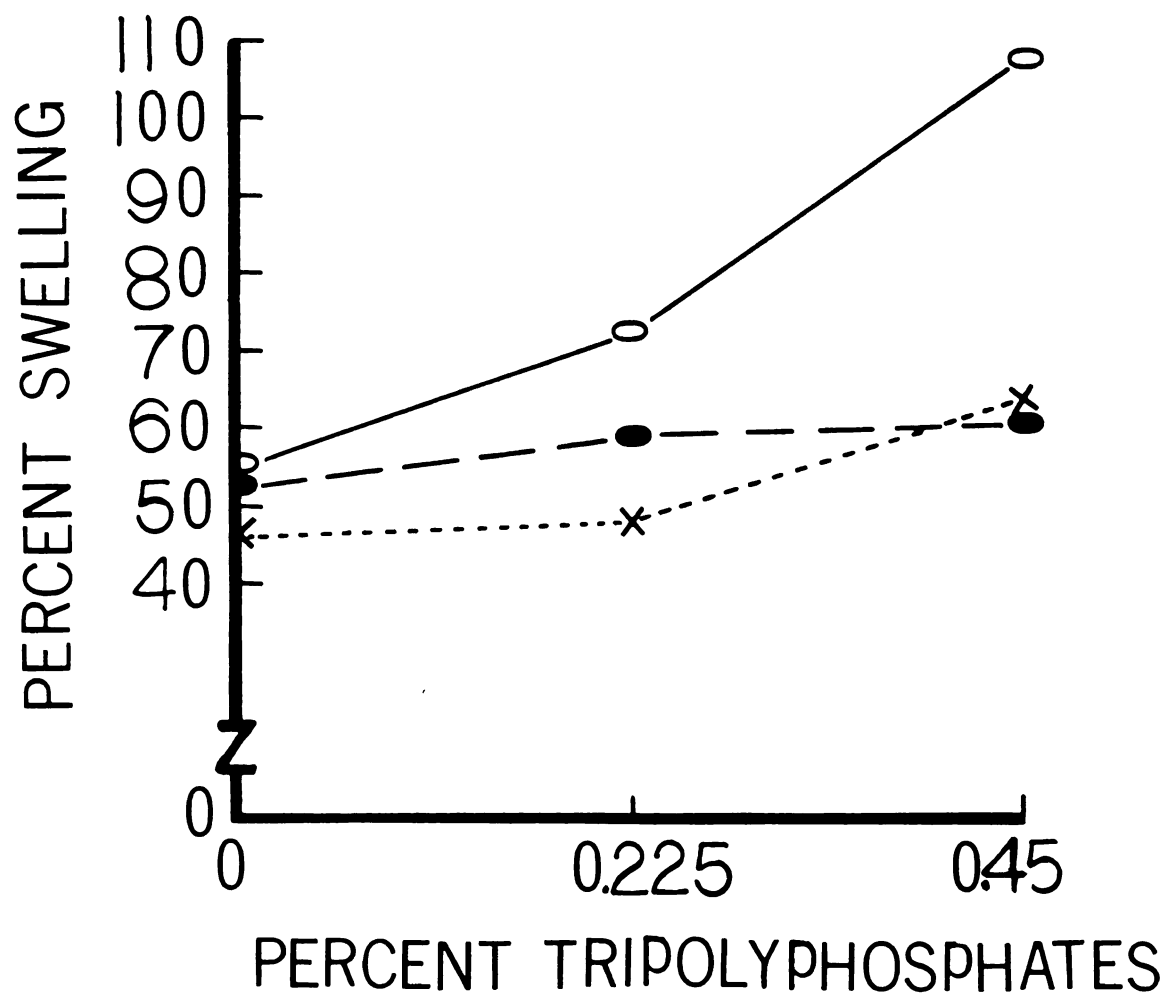


Figure 13.--Effects of Sodium Chloride and Sodium Tri-polyphosphate in Varying Concentrations and Combinations on the Swelling of Frozen Mechanically Deboned Fish. 0-0 0.0% NaCl, ●- -● 3.0% NaCl and X---X 3.6% NaCl.



TABLE 27.--Effect of Adding Soy Protein Isolate on Percent Swelling of Frozen Mechanically Deboned Fish

		% Sodium Chloride			
		0.0	3.0	3.6	Mean ^b
	0.0	72.19 ^a	49.65	42.50	54.78
% Soy	2.0	73.48	54.42	52.63	60.18
Protein Isolate	4.0	88.97	70.62	63.54	74.38

^aStandard error for each cell mean (N = 12) is 1.10.

^bStandard error for each column mean (N = 36) is 0.63.

Proteins have the ability to absorb large amounts of water, and the ratio of water to protein can be very important to the binding properties of protein. Brown (1972), citing Cook et al., (1969), pointed out that a meat-soy system will hold approximately 100% more free water than a pure meat system to which excess water was added.

Proteins vary widely in their ability to bind water, and therefore in their degree of hydration. As with electric charge, hydration is a source of stability to protein macromolecules in an aqueous system. Water

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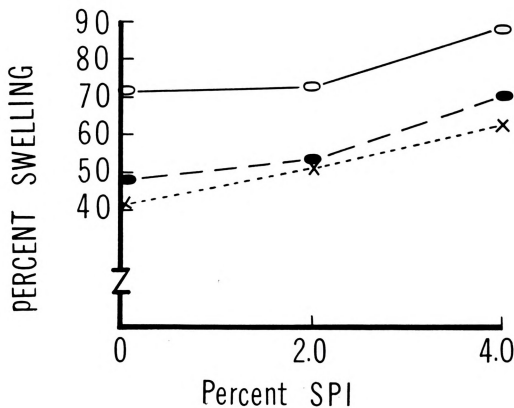


Figure 14.--Effects of Sodium Chloride and Soy Protein Isolate in Varying Concentrations and Combinations, on the Swelling of Frozen Mechanically Deboned Fish. 0-0 0.0% NaCl, ●-● 3.0% NaCl and X---X 3.6% NaCl.

is held on proteins by hydrogen bonding to backbone structure, and by hydration of polar side chains.

Lauffer (1964) suggested that ions on the protein surface may bind water by electrostriction or that water may be organized in "iceberg" form around hydrophobic groups or that both phenomena occur at the same time. Also, insoluble proteins possibly absorb water, changing the degree of hydration, and consequently altering their physical and chemical properties. This could have occurred when soy protein isolate was blended with frozen minced fish and water. Therefore, soy protein isolate, itself, was responsible for the uptake of a considerable quantity of added water.

An analysis of variance (Table 28) indicated significant differences in swelling caused by sodium chloride, sodium tripolyphosphate and soy protein isolate. There were also significant interactions between those three additives ($p < 0.01$).

Effects of Additives on Gel Forming Ability of Frozen Mechanically Deboned Fish

One of the most important functional properties of comminuted fish meat is its ability to form gels which are stable after heating in the presence of salts. It is

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TABLE 28.--Analysis of Variance of the Effects of Sodium Chloride, Sodium Tripolyphosphate, and Soy Protein Isolate, in Varying Concentrations and Combinations, on Swelling of Frozen Mechanically Deboned Fish

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F-Value	Significance of F
Sodium Chloride (V_1)	2	13141.323	6570.661	457.615	0.001
Sodium Tripolyphosphate (V_2)	2	14334.181	7167.090	499.154	0.001
Soy Protein Isolate (V_3)	2	6316.469	3158.234	219.956	0.001
$V_1 \times V_2$	4	8619.173	1704.793	118.731	0.001
$V_1 \times V_3$	4	284.014	71.003	4.945	0.001
$V_2 \times V_3$	4	1332.025	333.006	23.192	0.001
$V_1 \times V_2 \times V_3$	8	4302.810	537.851	37.459	0.001
Explained	26	46529.995	1789.615	124.638	0.001
Residual	81	1163.037	14.358		
Total	107	47693.032	445.729		



well known that the rheological characteristics of such gels depend primarily on the concentration of muscle protein and its physiochemical state, which is, in turn, related to the ionic strength and pH of the muscle.

The effects of varying concentrations and combinations of sodium chloride, sodium tripolyphosphate and soy protein isolate on the gel forming ability of minced frozen fish are given in Appendix 9. The least concentration endpoint is the minimum protein concentration required to form a gel. The least concentration endpoint of total extracted protein required for gelation increased as the concentration of sodium chloride increased (Table 29). Poor gelation properties of total extracted

TABLE 29.--Effects of Adding Sodium Chloride and Sodium Tripolyphosphate on the Least Concentration Endpoint of Frozen Mechanically Deboned Fish Extracted Protein

		% Sodium Chloride			
		0.00	3.00	3.60	Mean ^b
	0.000	1.41 ^a	4.36	5.62	3.40
% Sodium	0.225	1.40	5.53	6.87	4.60
Tripolyphosphate	0.450	1.22	7.75	7.31	5.43
	Mean ^b	1.34	5.88	6.60	

^aStandard error for each cell mean (N = 12) is 0.11.

^bStandard error for each row or column mean (N = 36) is 0.06.



protein in samples containing sodium chloride were probably related to high ionic strength and possibly lower pH in these samples. These results agree with those of Tong et al., (1975), who reported that poor gelation properties were related to residual salt content.

The least concentration endpoint was decreased by adding 0.45% sodium tripolyphosphate only in samples containing no sodium chloride (Table 29 and Figure 15). Tong, et al., (1975) also noted that alkaline protein extracts were more effective than salt protein extracts in gel forming ability. In the present study, those samples with only 0.45% sodium tripolyphosphate had pH values as high as 7.8. Improved gelling ability with 0.45% sodium tripolyphosphate may also be related to myosin content, since the largest myosin heavy chain total peak areas were obtained only from protein samples extracted from fish in the presence of 0.45% sodium tripolyphosphate (Figure 11). These findings are similar to those of Fukazawa et al., (1916c) and Nakayama and Sato (1971b), who noted that myosin had a major effect on gel formation. However, in samples containing sodium chloride the presence of sodium tripolyphosphate increased least concentration endpoint values. The least concentration endpoint significantly increased ($p < 0.01$) as sodium chloride increased (Table 29).

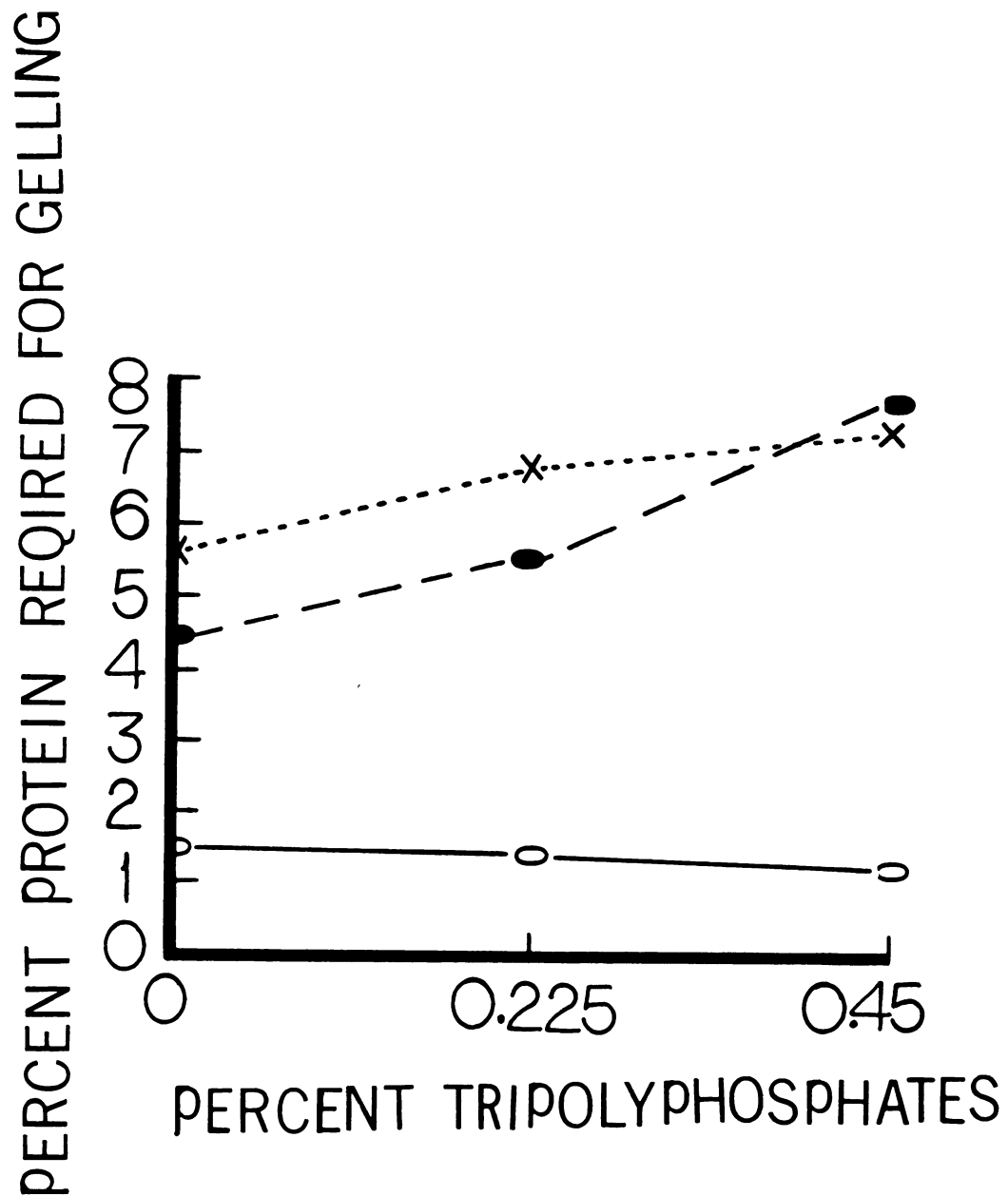


Figure 15.--Effects of Sodium Chloride and Sodium Tripolyphosphate, in Varying Concentrations and Combinations, on Least Concentration Endpoint of Total Extracted Protein Fraction Extracted from Frozen Mechanically Deboned Fish. 0-0 0.0% NaCl, ●- -● 3.0% NaCl and X---X 3.6% NaCl.

It has been shown that soy protein isolate has the ability to form a gel when heated (Hermansson, 1972). Soy protein isolate slightly improved gel forming ability in samples of frozen minced fish without sodium chloride. The least concentration endpoint decreased slightly from 1.49% to 1.19% and 1.34% with the addition of 2% and 4%, respectively, of soy protein isolate (Table 30).

TABLE 30.--Effect of Adding Soy Protein Isolate on Least Concentration Endpoint of Frozen Mechanically Deboned Fish Extracted Protein

		% Sodium Chloride			
		0.0	3.0	3.6	Mean ^b
% Soy Protein Isolate	0.0	1.49 ^a	5.24	6.32	4.35
	2.0	1.19	6.31	6.74	4.75
	4.0	1.34	6.09	6.74	4.72

^aStandard error for each cell mean (N = 12) is 0.11.

^bStandard error for each column mean (N = 36) is 0.06.

Gelation of soy protein isolate does not occur at concentrations of 6% or lower, although there is a small increase in viscosity (Circle et al., 1964). The lower levels of 2% and 4% soy protein isolate were selected for blending with frozen minced fish to avoid a

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strong soy flavor in finished products. Moreover, since fish flesh contained approximately 16% total protein content, there was no need to add higher concentrations of soy protein isolate for gelation. As noted above, in samples containing sodium chloride the presence of soy protein isolate did not improve gel formation (Table 30) and the least concentration endpoint was increased by adding soy protein isolate. Catsimpoolas and Meyer (1970) reported similar results, noting that at temperatures above 70°C the viscosities of soy protein gels decrease as sodium chloride concentration increase from 1.18% to 11.76%. This reduction in gel strength in the presence of sodium chloride was correlated with a decrease in protein solubility caused by protein aggregations such as the precipitation of soy proteins (Hermansson and Akesson, 1975b) and a decrease in pH (Catsimpoolas and Meyer, 1970). These workers found that the highest viscosity values were obtained at a neutral or mildly alkaline pH.

The water soluble protein fraction extracted from frozen minced fish with plain water formed gels at a low protein concentration (1.60%) with the application of heat at 80°C for 10 minutes (Appendix 9). This is an indication that freezing had no major effect on the physiochemical properties of sarcoplasmic protein in mechanically deboned fish.

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Analysis of variance indicated that there were significant effects on gel formation caused by sodium chloride, sodium tripolyphosphate and soy protein isolate blended with frozen minced fish ($p < 0.01$, Table 31). There were also significant interactions between those additives on the gel forming ability of fish muscle protein ($p < 0.01$).

Further Processing of Frozen Mechanically Deboned Fish

Fish Sausages

The development of new products such as sausage from frozen mechanically deboned fish requires a basic knowledge of the functionality of muscle proteins, since denaturation during frozen storage causes changes in the quality of finished products. In addition, studies done on protein solubility, swelling, gel formation and the pH of frozen mechanically deboned fish indicated that 3% sodium chloride, 0.45% sodium tripolyphosphate and 4% soy protein isolate were adequate for fish sausage formulation. The study further indicated that those concentrations are needed for quality maintenance and improvement in the economics of sausage production. However, 4% soy protein isolate did not improve the functional properties of frozen mechanically deboned fish as was expected, and the use of more than 4% soy protein isolate in product

TABLE 31.--Analysis of Variance of the Effects of Sodium Chloride, Sodium Tripolyphosphate and Soy Protein Isolate, in Varying Concentrations and Combinations on Least Concentration Endpoint of Total Extracted Protein of Frozen Mechanically Deboned Fish

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F-Value	Significance of F
Sodium Chloride (V_1)	2	582.362	291.181	1899.369	0.001
Sodium Tripolyphosphate (V_2)	2	47.596	23.798	155.234	0.001
Soy Protein Isolate (V_3)	2	3.224	1.613	10.524	0.001
$V_1 \times V_2$	4	42.647	10.662	69.547	0.001
$V_1 \times V_3$	4	5.579	1.395	9.098	0.001
$V_2 \times V_3$	4	9.066	2.278	14.785	0.001
$V_1 \times V_2 \times V_3$	8	16.088	2.011	13.118	0.001
Explained	26	706.565	27.176	177.266	0.001
Residual	81	12.418	0.153		
Total	107	718.983	6.719		



formulation imparted a soy flavor to the finished product. Because of this flavor, 4.0% white corn meal was used in product formulation.

In comparing treatments, fish sausages containing only 2.0% sodium chloride and 0.45% sodium tripolyphosphate (Treatment-A, Table 2 and Figure 16) shrank more when heated to 82°C internal temperature for 30 minutes than any of the suasages made with 3.0% sodium chloride, 0.45% sodium tripolyphosphate and 8% hydrogenated vegetable oil and any of the combinations of soy protein isolate and white corn meal (i.e., treatments B, C, D and E, Table 2). Among sausages made according to the five treatments and heated to an internal temperature of 82°C, the one with only salt and phosphate (treatment A) had poor physical structure and shape, whereas the others appeared normal (Figure 16).

The effect of overheating on the binding characteristics of sausages is well known in the meat industry. According to Swift and Ellis (1957), heating to internal temperatures much in excess of 65.5°C should be avoided. However, regulation No. 541.2 of the Michigan Department of Agriculture, Food Inspection Division, requires that all smoking should be accomplished so that the internal temperature of the coldest part of a fishpreparation remains at 82°C for not less than 30 minutes. Also,

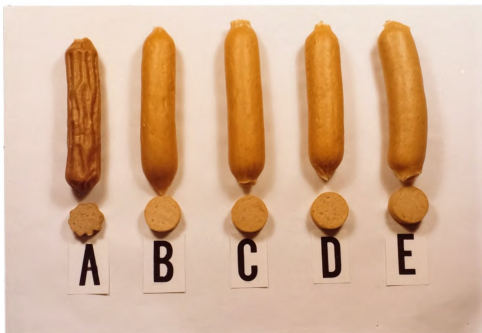


Figure 16.--Whole Fish Sausages and Cross-sections:¹

A - 2% sodium chloride (NaCl), B - 3% NaCl and 8% hydrogenated vegetable oil (HVO), C - 3% NaCl, 4% soy protein isolate (SPI) and 8% HVO, D - 3.0% NaCl, 4% corn meal and 8% HVO and E - 3% NaCl, 4% SPI, 4% corn meal and 8% HVO.

¹In all sausages (A, B, C, D, and E) the following additives and spices are present: 0.45% sodium tripolyphosphate, 0.1% mono-sodium glutamate, 0.05% sodium ascorbate, 0.5% condensed smoke, and mixed spices.

U. S. Government regulations require an F_{180} of 30 minutes to control Clostridium botulinum type E found naturally in fish (FDA, 1970).

Adding 8% hydrogenated vegetable oil, 4% soy protein isolate and 4% corn meal separately or in combination reduced the cooking loss of fish sausages (Table 32), but in comparing mean differences between treatments B, C, D and E only treatment E, which contained 4% soy protein isolate and 4% corn meal, significantly decreased cooking loss ($p < 0.05$).

The quality of the meat used is one of the most important concerns facing the processor in manufacturing sausage (Hasimoto et al., 1959). Sodium tripolyphosphate is thought to improve certain physical characteristics of sausage products and, permits wider latitude in utilizing raw materials that naturally lack the ability to bind.

Sodium chloride and sodium tripolyphosphate also can be used in fish products to reduce percentage of cooking loss during processing. The greatest cooking loss that occurred with frozen mechanically deboned fish containing only 2% sodium chloride and 0.45% sodium tripolyphosphate (Treatment-A) may be caused by the poor binding capacity and higher moisture content of fish



TABLE 32.--Average Percentages* and Standard Deviations of Cooking Loss, Water Holding Capacity and Shear Force (Kg-f/3.0 cm Sectional Ø) of Smoked Fish Sausages

Treatments	A	B	C	D	E
Cooking Loss % (N=2)	34.65±0.49	23.20 ^{bfg} ±0.14	21.15 ^{ceg} ±1.77	22.43 ^{def} ±1.34	18.83 ^{acd} ±1.64
Water Holding Capacity % (N=8)	83.10±1.62	90.20 ^a ±1.47	93.00 ^b ±1.20	98.04 ^c ±0.95	99.53 ^d ±0.17
Shear Force (N=12)	0.94±0.06	0.80 ^a ±0.09	1.03 ^{bc} ±0.05	1.03 ^{ac} ±0.05	1.52 ^d ±0.10

*Average percentage in the same row not followed by the same superscript are significantly different ($p < 0.05$).

A = 2% NaCl
 B = 3% NaCl, 8% hydrogenated vegetable oil (HVO)
 C = 3% NaCl, 8% HVO, 4% soy protein isolate (SPI).
 D = 3% NaCl, 8% HVO, 4% corn meal.
 E = 3% NaCl, 8% HVO, 4% SPI, 4% corn meal.

In all sausages (A, B, C, D and E) the following additives and spices are present: -0.45% sodium tripolyphosphate, 0.1% monosodium glutamate, 0.05% sodium ascorbate, 0.5% condensed smoke, and mixed spices.



muscle. Higher moisture content also causes shrinkage to be greater during cooking. Therefore, addition of 2% sodium chloride and 0.45% sodium tripolyphosphate did not improve the water binding capacity of frozen mechanically deboned fish, as was also reported by Grabowska and Sikorski (1973), who found that the addition of 0.6%-5.0% sodium chloride and 0.2%-0.5% polyphosphate, separately and in combination, did not improve the binding quality of mechanically deboned frozen fish. Nevertheless, high shrinkage observed in this study (34.65%) does not mean that sodium chloride and sodium tripolyphosphate did not reduce cooking loss, since there were no samples without either sodium chloride or sodium tripolyphosphate for comparison. One purpose of this study was to investigate improvement in frozen mechanically deboned fish sausages quality, but not to assess the effect of sodium chloride and sodium tripolyphosphate on quality. According to Hashimoto et al., (1959). Sausages made from poor quality meat are usually not acceptable, mainly because the water and fat content of the meat have been released from the products. Grabowska and Sikorski (1973) investigated the water and fat binding capacities of mechanically deboned fish and found that its suitability for sausage emulsions

deteriorates during frozen storage more rapidly than that of fillets or round fish. It may be that denaturation of myofibrillar protein, mainly actomyosin, is closely related to the quality of the sausage since actomyosin is the largest constituent of muscle protein (Hashimoto et al., 1959).

The quality of fish sausages made during this study was evaluated by estimating water holding capacity (WHC) and texture of the finished products (Table 32). It will be noted that all additives, including hydrogenated vegetable oil, soy protein isolate, and corn meal, improved WHC of sausages. Adding corn meal produced a greater increase in the WHC of the sausage more than the addition of soy protein isolate. The greatest water holding capacity (99.53%) was obtained when frozen mechanically deboned fish contained a combination of the higher salt level and 0.45% sodium tripolyphosphate, vegetable oil and both soy protein isolate and corn meal (Treatment-E). It was established that adding either 4% soy protein isolate or 4% corn meal separately or in combination significantly increased WHC ($p < 0.05$). In products based on comminuted meats, such as sausages, sodium chloride is important not only for its antimicrobial action, but also because it aids in the improvement of WHC. The ability of muscle proteins to hold fat as

well as water is also very important in the sausage industry, and one of the functions of sodium chloride is to enhance the power of myofibrillar proteins to bind water and emulsify fat.

Regulation No. 541 of the Michigan Department of Agriculture, Food Inspection Division, indicated that all smoked fish should be processed so that the water phase portion of the finished product has a sodium chloride content of not less than 5%. This means that the sodium chloride content of the finished product, when divided by the sum of the sodium chloride and moisture contents and multiplied by 100, will yield a number of at least 5. Adding 3% sodium chloride to minced fish brings sodium chloride in the water phase portion of the finished product to approximately 5%. Sodium chloride and sodium tripolyphosphate increase WHC of sausages through the combined effects of relatively high ionic strength (Hamm, 1960) and an increase in the solubility of muscle proteins, caused by the ability of phosphate to dissociate actomyosin into myosin and actin (Bendall, 1954).

When the sodium chloride concentration of minced fish was increased to 3% and 8% hydrogenated vegetable oil was added, WHC improved by 7% (Treatment-B). An unexpected result of this study was that the WHC of sausage increased with increasing fat content, for fat should not contribute to water binding capacity

(Hamm, 1960). Swift et al., (1954) noted this effect of fat on WHC up to a fat to protein ratio of 2.8:1. At higher ratios (>3.4:1) the WHC decreased. Hamm (1960) noted that the amount of sodium chloride required in sausage formulas is usually calculated in proportion to the total amount of lean meat and fat. Fat takes up much less sodium chloride than muscle tissue, and adding fat to meat increases the amount of sodium chloride per unit weight of lean meat. This, in turn, increases the WHC of muscle. Sodium chloride may also improve the emulsifying properties of muscle proteins because of high hydration, and perhaps begins a dissolution process on the surfaces of protein particles, thereby promoting interfacial reaction between proteins and fat.

Soy protein isolate is thought to bind water and fat when it forms a gel upon heating. Adding soy protein isolate improved swelling of minced fish as mentioned earlier (Table 27), and it significantly increased WHC of fish sausage ($p < 0.05$, Table 32). Hamm (1960) found a highly significant correlation between swelling and WHC. Increased swelling is a desirable effect because high absorption usually tends to increase the unit yield of the finished product (Yasumatsu et al., 1972b). The latter researchers also reported that an increase in water absorption is directly proportional to the amount of protein added. Corn meal, however, is not classified

as a binder, although it may bind water it is incapable of emulsifying fat. Adding 4% corn meal to fish sausage formulations significantly increased their WHC ($p < 0.05$). According to Kramlich (1972) the ability of starch to bind water may be affected adversely by meat amylases. These enzymes are capable of degrading starch, but degradation did not occur when starch was heated above 80°C, the inactivation temperature of meat amylases.

The texture of the central mass of sausages was determined as a shear value, obtained from the resultant force/distance curve (Figure 17). The force measured at the yield point (A, Figure 17) indicated firmness (Table 32), and the greater the shear force, the greater the firmness of the product. Texture and binding in comminuted fish sausage has been found to follow a pattern similar to that of solubility changes in myofibrillar proteins in minced frozen fish (Grabowska and Sikorski, 1973). The relationship between texture, binding and the solubility of myofibrillar proteins might explain the effect of sodium chloride and sodium tripolyphosphate on texture, since the solubility of myofibrillar proteins is enhanced by adding sodium chloride and sodium tripolyphosphate to frozen minced fish.

Adding 8% hydrogenated vegetable oil to fish sausage decreased its firmness (Table 32). Swift et al.,

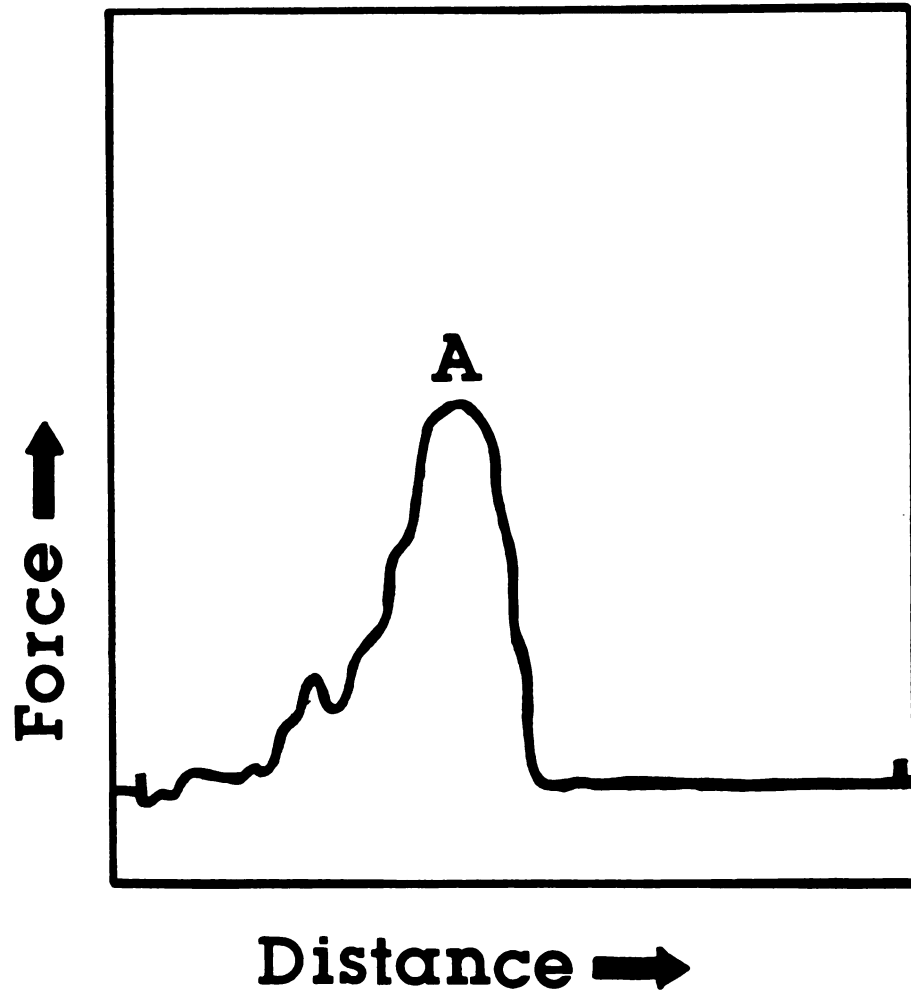


Figure 17.--Typical Force/Distance Curve for Central Mass of Fish Products. A = Yield point indicative of firmness.

(1954), reported similar results, noting that variations in fat content produced changes in the tenderness of bologna. They found that as fat content increased, product tenderness also increased. On the other hand, in this study the greatest firmness in fish sausage (1.52Kg-f) was obtained with a blend of 4% soy protein isolate and 4% corn meal in addition to 3% sodium chloride and 0.45% sodium tripolyphosphate. Texture measurements indicated that adding either 4% soy protein isolate or 4% corn meal, or a combination of both significantly increased the firmness of sausages ($p < 0.05$). Increased firmness may be caused by improved swelling due to the presence of soy protein isolate, as reported earlier (Table 27), and also by the absorption of water by corn meal.

Canned Minced Fish

Canned fish is commercially sterile since it is heated to temperatures at which most microorganisms and their spores, particularly Clostridium botulinum, are killed. Such high temperatures cause denaturation, amino acid destruction, H_2S liberation, metmyoglobin formation, collagen solubilization, Maillard-type browning, and accompanying undesirable changes in flavor. These effects are more marked in canned products than in normal cooked fish products (Lawrie, 1968). It is generally recognized that canned foods produced from frozen

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fish especially from fish held for long periods on ice or in refrigerated brine, are inferior in quality to those made from absolutely fresh fish. Denaturation of protein which occurs in round fish and frozen minced fish during such a holding period may affect the canned product in many ways. Generally, holding frozen fish results in decrease protein solubility, lower water and fat binding capacities, and poor texture. Water holding capacity and textural properties were investigated in this study of canned minced fish. The earlier study reported in this dissertation indicated that the addition of 0.45% phosphate without sodium chloride extracted approximately 50% of the total protein, solubilized the greatest quantity of myosin heavy chains, produced the highest pH and swelling, and gave the best gel forming ability to frozen minced fish (Tables 8, 20, 23, 26, and 29). Therefore, it was decided to use the same blend of frozen minced fish in a canned product, with the addition of 1.0% sodium chloride to improve product taste and to obtain the improved functional properties mentioned earlier under practical conditions. In addition to the amounts of sodium chloride and sodium tripolyphosphate mentioned above, the canned minced fish paste blend contained 4% beef tripe and 8% hydrogenated vegetable oil. Some of the batter also contained 4% soy protein isolate

or 4% corn meal, or a combination of these two ingredients. The product was processed at 121°C for 75 minutes. After the cans were filled, the fish paste was baked in a dry air oven at 75°C for 30 minutes to remove most of the oxygen and portions of its moisture content in order to obtain the proper texture.

The average percentages of baking loss for each treatment were 0.75, 0.69, 0.41, and 0.33 (Table 33). These values were calculated by averaging the cooking loss percentage of two trials. There was a slight reduction in moisture content (0.33% to 0.75%), which resulted from evaporation during heating. The fast surface coagulation occurring during the dry heating process prevented much evaporation from fish paste. Fish paste that contained 4% soy protein isolate and 4% corn meal in addition to the other ingredients (Treatment-D) had the lowest baking loss (0.33%). This reduction in baking loss may be due to an improvement in the water binding capacity of fish paste through the addition of soy protein isolate and corn meal. Coagulation of protein and gelatinization of starch that occurred at the product surface also prevented baking loss. Moreover, the moisture content of fish paste containing soy protein isolate and corn meal was less than that of the other treatments since some of the fish flesh was replaced in them by these two ingredients, both of which contain little moisture. Baking loss

TABLE 33.--Average Percentages* and Standard Deviations of Baking Loss, Water Holding Capacity and Shear Force (Kg-f/1.8 cm sectional Ø) of Canned Minced Fish

Treatments ¹	A	B	C	D
Baking Loss % (N=2)	0.73 ^C ±0.05	0.69 ^{b,C} ±0.01	0.41 ^a ±0.00	0.33 ^a ±0.09
Water Holding Capacity % (N=8)	79.24 ^a ±1.27	92.94 ^b ±1.00	95.33 ^C ±0.73	98.79 ^d ±0.21
Shear Force (N=12)	0.18 ^a ±0.02	0.29 ^b ±0.01	0.31 ^C ±0.02	0.41 ^d ±0.02

*Average percentage in the same row not followed by the same superscript are significantly different ($p < 0.05$).

A = 86.5% mechanically deboned frozen fish (MDFF)
 B = 82.5% MDFF, 4% soy protein isolate
 C = 82.5% MDFF, 4% corn meal
 D = 78.5% MDFF, 4% soy protein isolate, 4% corn meal

¹In all treatments (A, B, C, D) the following additives and spices are present: 4% beef tripe, 8% hydrogenated vegetable oil, 1% sodium chloride, 0.45% sodium tripolyphosphate, 0.30 white pepper powder, 0.15% onion powder, 0.15% garlic powder, 0.30% paprika pepper powder and 0.05% ground ginger.

of fish paste was significantly decreased ($p < 0.05$) by adding corn meal or a combination of corn meal and soy protein isolate, but not by soy protein isolate alone.

Canned minced fish containing 1.0% sodium chloride, 0.45% sodium tripolyphosphate, 4% beef tripe and 8% hydrogenated vegetable oil (Treatment-A) shrank more than that containing 4% soy protein isolate and/or 4% corn meal in addition to the other ingredients. When the finished products had been processed at 121°C for 75 minutes, all but those subject to treatment A showed relatively firm structure with few air pockets (Figure 18). Treatment-A containing neither soy protein isolate nor corn meal produced more meat exudate. When fish paste was heated, moisture was released due to the denaturation of muscle proteins and the syneresis of gelation gel. Meat that has a high water holding capacity should reabsorb some of the released moisture. Canned minced fish that contained either soy protein isolate or corn meal or a combination of both reabsorbed the released moisture, whereas minced fish alone failed to do so.

Treatment A showed approximately 80% water holding capacity. Further improvement in water holding capacity was obtained by adding soy protein isolate and/or corn meal (Treatment A vs. B, C and D). In this study corn meal appeared to be a better water binder than

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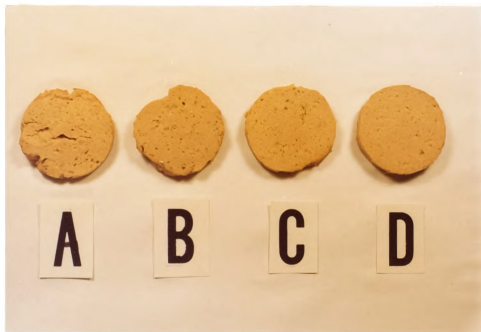


Figure 18.--Cross-sections of Canned Minced Fish.

A¹ - 86.5% minced fish, B¹ - 82.5% minced fish and 4% soy protein isolate (SPI),
C¹ - 82.5% minced fish and 4% corn meal,
D¹ - 78.5% minced fish, 4% SPI and 4% corn meal.

¹In all formulations (A, B, C and D) the following additives are present: 4% beef tripe, 8% hydrogenated vegetable oil, 1.0% sodium chloride, 0.45% sodium tripolyphosphate and mixed spices.

soy protein isolate in fish paste. This may be because corn meal has a greater capacity to swell and form gels than soy protein isolate. The greatest water holding capacity (98.79%) was obtained by using 4% soy protein isolate and 4% corn meal in combination with the other ingredients.

The force required to shear a 1.8 cm sectional diameter of canned fish product made according to treatment A was 180 grams. This product was soft and mushy and difficult to slice. Firmness increased with the addition of 4% soy protein isolate (Treatment-B), 4% corn meal (Treatment-C) and a combination of the two (Treatment-D). The force needed to shear 1.8 cm sectional diameters of products made according to treatments B, C and D was 290, 310, and 410 grams, respectively. Statistical analysis indicated that each of the four treatment means for firmness were significantly different from each other ($p < 0.05$).

SUMMARY AND CONCLUSION

Some of the physical and chemical characteristics of minced sucker flesh were examined. Prerigor sucker muscle protein was fractionated into myofibrillar and sarcoplasmic proteins as well as nonprotein nitrogen. The effects of refrigeration and freezer storage on the extractability of those protein fractions were examined. It was found that the solubility of myofibrillar proteins decreased during both refrigerator and freezer storage, but that the solubility of sarcoplasmic proteins and nonprotein nitrogen was not influenced by either method of storage.

Frozen minced sucker protein was fractionated into salt and water soluble proteins and nonprotein nitrogen fractions, as well as myosin heavy chain to study the interrelated effects of adding sodium chloride, sodium tripolyphosphate and soy protein isolate on protein solubility, pH, swelling and gel forming properties. Total extracted protein and salt soluble protein increased with the addition of either sodium chloride or sodium tripolyphosphate. On the other hand, levels of water soluble protein and nonprotein nitrogen decreased when either

sodium chloride or sodium tripolyphosphate were added to extraction solution. Myosin heavy chain was solubilized with the addition of 3% or 3.6% sodium chloride and also in the presence of 0.45% sodium tripolyphosphate, but not when a concentration of 0.225% of this chemical was used. The values of total extracted protein, water soluble and salt soluble proteins and nonprotein nitrogen extracted from frozen minced fish containing 2% and 4% soy protein isolate indicated that soy protein isolate did not completely solubilize, since none of the protein fractions increased by 2% or 4% over a control containing no soy protein isolate.

Swelling and pH of frozen minced fish increased as sodium tripolyphosphate concentration increased, but as sodium chloride concentration increased swelling and pH decreased. Adding soy protein isolate did not change the pH, but did cause increased swelling. Poor gelation properties resulted from adding sodium chloride, but the addition of sodium tripolyphosphate alone improved gel forming ability. Measurement of gel formation did not seem to be a suitable way to estimate the effect of adding soy protein isolate on the quality of frozen minced fish.

The functionality tests indicated that frozen minced fish has some desirable properties. Samples which had the highest ionic strength did not exhibit the highest

overall ratings of functional properties. It is not certain that added sodium chloride is in itself responsible for the resulting functional behavior of fish muscle protein. If this were the case, the presence of salt would have both beneficial and detrimental aspects. Taking protein solubility as an example, the salt-rich samples produced high values in solubility, but also showed the lowest values for swelling, gel forming and pH. In comparison, sodium tripolyphosphate appears to cause many beneficial effects, such as increases in protein solubility, pH, swelling and gel forming ability.

Frozen minced sucker was blended with different additives and sausage-type and canned products were processed and their functional properties evaluated in terms of water-holding capacity and texture. Fish sausages and canned minced fish showed poor water holding capacity, low binding characteristics and high cooking losses. However, 3.0% salt in fish sausages and 1.0% salt added to canned products as well as 0.45% sodium tripolyphosphate in combination with hydrogenated vegetable oil, soy protein isolate and corn meal in both products improved their functional properties. Since soy protein isolate has an objectionable flavor, and because the addition of corn meal improved water holding capacity, texture and cooking losses at least as much as soy protein isolate,

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it was concluded that corn meal should be used in manufacturing processed fish products.

If canned minced fish were accepted throughout the world, especially in the less-developed countries, it would significantly contribute to the efficient use of underutilized fish species for food, and extend world supplies of quality protein.

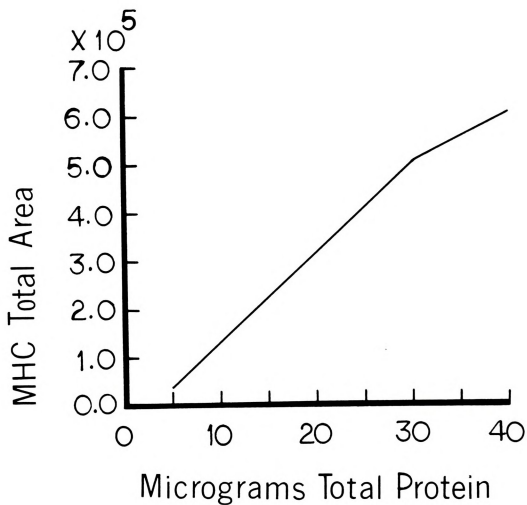
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APPENDICES

APPENDIX 1

STANDARD CURVE FOR MYOSIN HEAVY CHAIN

TOTAL PEAK AREA



Appendix 1.--Standard Curve for Myosin Heavy Chain Total Peak Area.

APPENDIX 2

AVERAGE PERCENTAGE OF TOTAL PROTEIN EXTRACTED
FROM MECHANICALLY DEBONED FROZEN FISH



APPENDIX 2.--Average Percentage and Standard Deviation
of Total Protein Extracted from Mechanically
Deboned Frozen Fish (N = 4)

Percent Additives				
Sodium Tripoly- phosphate	Soy Protein Isolate	Sodium Chloride		
		0.00	3.00	3.60
0.000	0.0	13.65 [*] ±0.25	67.81±1.92	58.54±0.68
0.000	2.0	13.45±0.20	66.14±2.22	64.26±1.28
0.000	4.0	13.91±0.14	68.24±1.53	63.72±0.74
0.225	0.0	14.17±0.08	49.13±1.04	52.79±1.18
0.225	2.0	14.83±0.36	55.99±1.64	55.79±0.51
0.225	4.0	16.61±0.06	58.95±2.40	55.85±2.24
0.450	0.0	49.24±2.12	59.67±1.24	58.90±2.41
0.450	2.0	47.31±1.66	59.74±1.08	60.78±1.66
0.450	4.0	46.96±2.27	56.88±0.97	63.66±1.64

*Total extracted protein = mg extracted protein/
100 mg total muscle protein.

APPENDIX 3

AVERAGE PERCENTAGE OF SALT-SOLUBLE PROTEIN
EXTRACTED FROM MECHANICALLY DEBONED
FROZEN FISH



APPENDIX 3.--Average Percentage and Standard Deviation
of Salt-Soluble Protein Extracted From
Mechanically Deboned Frozen Fish (N = 2)

Percent Additives				
Sodium Tripoly- phosphate	Soy Protein Isolate	Sodium Chloride		
		0.00	3.00	3.60
0.000	0.0	0.00	26.57*±0.98	29.97±0.51
0.000	2.0	0.00	32.70 ±2.42	34.53±0.55
0.000	4.0	0.00	37.07 ±0.40	31.53±0.25
0.225	0.0	0.78±0.00	32.07 ±2.46	29.37±0.20
0.225	2.0	1.17±0.04	32.19 ±0.85	30.33±1.87
0.225	4.0	1.85±0.08	28.47 ±0.51	30.45±1.02
0.450	0.0	25.10±4.16	29.97 ±1.95	30.09±0.68
0.450	2.0	25.59±1.19	25.52 ±0.51	35.73±0.81
0.450	4.0	24.17±0.55	28.62 ±1.23	36.04±1.08

*Salt soluble protein = mg salt soluble protein/
100 mg total muscle protein.



APPENDIX 4

AVERAGE PERCENTAGE OF WATER-SOLUBLE
PROTEIN EXTRACTED FROM MECHANICALLY
DEBONED FROZEN FISH



APPENDIX 4.--Average Percentage and Standard Deviation
of Water-Soluble Protein Extracted from
Mechanically Deboned Frozen Fish (N = 2)

Sodium Tripoly- phosphate	Soy Protein Isolate	Percent Additives		
		Sodium Chloride		
		0.00	3.00	3.60
0.000	0.0	13.39*±0.00	25.91±0.39	19.28±0.17
0.000	2.0	13.39 ±0.00	22.31±0.38	22.04±0.30
0.000	4.0	13.96 ±0.04	26.00±0.43	24.32±0.51
0.225	0.0	13.18 ±0.04	22.94±2.12	27.81±2.37
0.225	2.0	13.06 ±3.18	24.83±0.55	29.67±0.17
0.225	4.0	14.23 ±0.34	27.89±0.03	25.52±0.55
0.450	0.0	16.54 ±0.81	22.88±0.93	24.98±0.34
0.450	2.0	22.76 ±0.25	21.59±6.06	26.13±0.04
0.450	4.0	23.99 ±0.38	21.47±3.26	29.25±0.89

*Water soluble protein = mg water soluble protein/
100 mg total muscle protein.

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APPENDIX 5
AVERAGE PERCENTAGE OF NONPROTEIN NITROGEN
EXTRACTED FROM MECHANICALLY DEBONED
FROZEN FISH



APPENDIX 5.--Average Percentage and Standard Deviation
of Nonprotein Nitrogen Extracted from
Mechanically Deboned Frozen Fish (N = 4)

Percent Additives				
Sodium Triphosphosphate	Soy Protein Isolate	Sodium Chloride		
		0.00	3.00	3.60
0.000	0.0	8.00*±0.45	6.59±0.17	5.48±0.29
0.000	2.0	8.15 ±0.11	5.88±0.07	5.10±0.21
0.000	4.0	7.15 ±0.14	5.87±0.20	4.88±0.23
0.225	0.0	6.36 ±0.00	6.16±0.20	6.35±0.16
0.225	2.0	5.97 ±0.03	6.08±0.16	6.13±0.10
0.225	4.0	5.88 ±0.05	5.96±0.13	5.69±0.16
0.450	0.0	5.88 ±0.05	5.88±0.16	5.84±0.20
0.450	2.0	5.73 ±0.18	5.67±0.10	5.70±0.18
0.450	4.0	5.63 ±0.18	5.25±0.08	5.30±0.06

*Nonprotein nitrogen = mg nonprotein nitrogen/100
mg total muscle protein (N X 6.25).



APPENDIX 6

MEAN VALUES OF MYOSIN HEAVY CHAIN

TOTAL AREA EXTRACTED FROM

MECHANICALLY DEBONED

FROZEN FISH



Appendix 6.--Mean Values and Standard Deviation of Myosin
Heavy Chain Total Area Extracted from
Mechanically Deboned Frozen Fish (N = 3)

Percent Additives				
Sodium Tripoly- phosphate	Soy Protein Isolate	Sodium Chloride		
		0.00	3.00	3.60
0.000	0.0	0.00	1.02×10^5 ± 0.01	1.14×10^5 ± 0.23
0.000	2.0	0.00	0.66×10^5 ± 0.00	1.04×10^5 ± 0.15
0.000	4.0	0.00	0.55×10^5 ± 0.02	0.82×10^5 ± 0.14
0.225	0.0	0.00	2.02×10^5 ± 0.08	1.02×10^5 ± 0.07
0.225	2.0	0.00	2.55×10^5 ± 0.14	1.25×10^5 ± 0.16
0.225	4.0	0.00	1.85×10^5 ± 0.14	1.16×10^5 ± 0.06
0.450	0.0	2.46×10^5 ± 0.30	1.74×10^5 ± 0.16	1.14×10^5 ± 0.03
0.450	2.0	2.66×10^5 ± 0.06	1.82×10^5 ± 0.28	1.46×10^5 ± 0.03
0.450	4.0	2.66×10^5 ± 0.48	1.99×10^5 ± 0.09	1.75×10^5 ± 0.17

*Total peak area that measured by scanning myosin heavy chain bond obtained from 20 micrograms total extracted protein sample.



APPENDIX 7

AVERAGE OF PH OF MECHANICALLY
DEBONED FROZEN FISH



APPENDIX 7.--Average and Standard Deviation of pH of
Mechanically Deboned Frozen Fish (N = 2)

Percent Additives				
Sodium Tripoly- phosphate	Soy Protein Isolate	Sodium Chloride		
		0.00	3.00	3.60
0.000	0.0	6.80±0.00	6.30±0.00	6.09±0.03
0.000	2.0	6.90±0.00	6.20±0.00	6.15±0.00
0.000	4.0	6.90±0.00	6.10±0.00	6.19±0.01
0.225	0.0	7.20±0.00	6.40±0.02	6.35±0.01
0.225	2.0	7.30±0.00	6.39±0.01	6.39±0.02
0.225	4.0	7.30±0.00	6.43±0.01	6.40±0.01
0.450	0.0	7.80±0.00	6.68±0.03	6.67±0.02
0.450	2.0	7.80±0.00	6.68±0.01	6.67±0.02
0.450	4.0	7.80±0.00	6.65±0.00	6.68±0.00



APPENDIX 8

AVERAGE PERCENT OF SWELLING OF
MECHANICALLY DEBONED FROZEN
FISH

APPENDIX 8.--Average Percent and Standard Deviation of
Swelling of Mechanically Deboned Frozen
Fish (N = 4)

Percent Additives				
Sodium Tripoly- phosphate	Soy Protein Isolate	Sodium Chloride		
		0.00	3.00	3.60
0.000	0.0	65.74±3.57	49.65±5.84	28.20±0.00
0.000	2.0	42.50±0.00	40.71±3.57	56.80±0.00
0.000	4.0	56.80±0.00	69.67±4.13	56.80±0.00
0.225	0.0	57.72±4.13	56.80±0.00	42.50±0.00
0.225	2.0	71.10±0.00	51.44±6.84	42.50±0.00
0.225	4.0	88.97±4.13	71.10±0.00	60.37±4.13
0.450	0.0	96.12±7.15	42.50±0.00	56.80±0.00
0.450	2.0	106.85±8.25	71.10±0.00	56.58±3.57
0.450	4.0	121.15±5.84	71.10±0.00	76.46±6.84



APPENDIX 9

AVERAGE OF LEAST CONCENTRATION ENDPOINT OF MECHANICALLY DEBONED FROZEN FISH



APPENDIX 9.--Average and Standard Deviation of Least Concentration Endpoint of Mechanically Deboned Frozen Fish (N = 4)

Percent Additives				
Sodium Tripoly- phosphate	Soy Protein Isolate	Sodium Chloride		
		0.00	3.00	3.60
0.000	0.0	1.60±0.56	4.45±0.40	5.19±0.39
0.000	2.0	1.43±0.29	4.65±0.00	6.04±0.65
0.000	4.0	1.20±0.28	3.99±0.14	5.63±0.24
0.225	0.0	1.81±0.10	4.45±0.47	7.12±0.22
0.225	2.0	0.82±0.11	7.07±0.93	6.82±0.21
0.225	4.0	1.57±0.23	5.06±0.47	6.67±0.49
0.450	0.0	1.07±0.24	6.82±0.66	6.66±0.36
0.450	2.0	1.33±0.52	7.21±0.11	7.35±0.36
0.450	4.0	1.25±0.70	9.21±0.02	7.91±0.14

Note: Least concentration endpoint is the minimum protein concentration required to form a gel.

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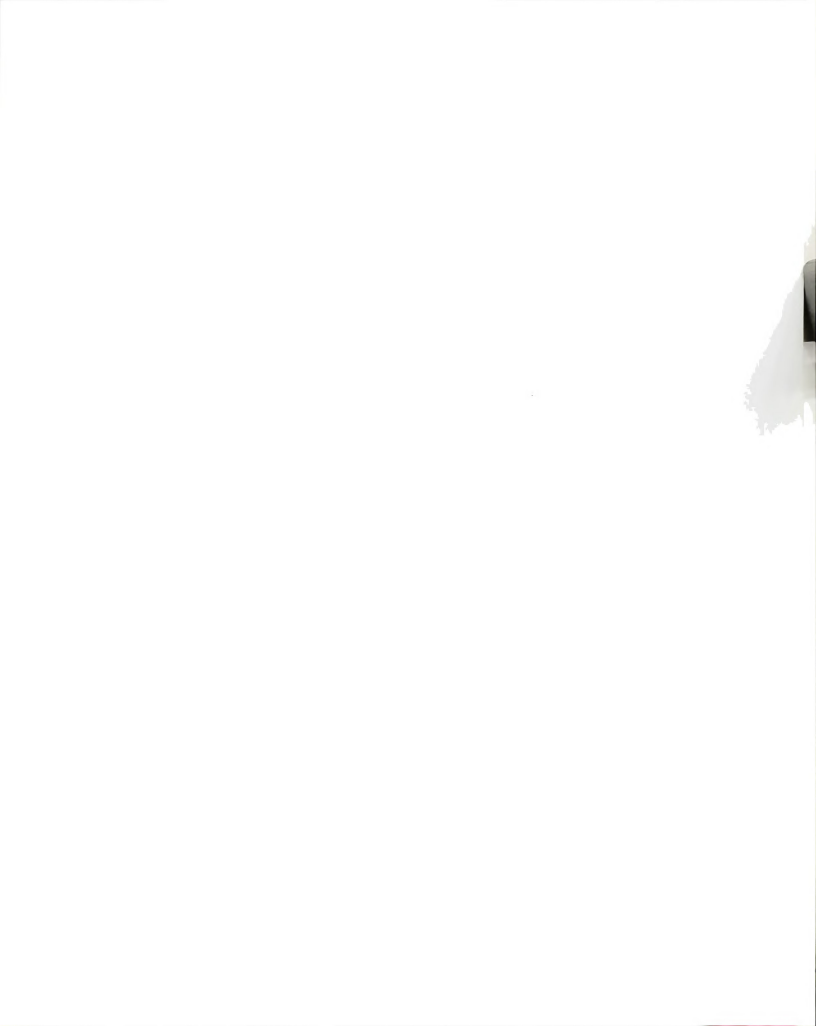
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