AN ABSTRACT OF THE THESIS OF

Maria Virginia Chang-Lee for the degree of Master of Science in Food Science and Technology presented on April 26. 1988

Title: The Production of Surimi from Pacific Whiting (Merluccius productus) and Evaluation of Kamaboko Gels

Abstract approved _____
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Yield, proximate composition, and proteolytic activity were determined in each unit operation during production of surimi from Pacific whiting. Preliminary studies involved the addition of protein adjuncts such as egg white (EW), whey protein concentrate (WPC), and soy protein isolate (SPI), at different levels, to kamaboko gels in order to select the main protein for further study. SDS-gel electrophoresis was performed to evaluate the effectiveness of egg white as a protease inhibitor in kamaboko gels.

Yields obtained from planked fish (42.4%) and minced flesh (35.7%) agree with those values reported from other studies in whiting. A reduced washed flesh yield was expected since washing is done to remove those constituents not desirable in surimi. Lower yields resulted in the refined flesh (17.4%), and surimi (19.9%). Proximate composition values through the unit operations were similar with other studies done with whiting, and were compared with other fish species indicating acceptable nutritional quality. Deboned and washed flesh (first wash) were significantly different in protease activity from the flesh (second

wash) and refined product ($P<0.05$). This demonstrates the importance of the washing process in surimi unit operations.

Protein adjuncts (EW, WPC, and SPI) in kamaboko gels at levels of 1 to 5% were evaluated based on hardness, cohesiveness and elasticity. Addition of egg white at levels ranging from 3 to 5% resulted in greater hardness ($P<0.05$), and elasticity ($P<0.005$) in comparison with WPC and SPI. Therefore EW at 3% level was selected for additional study.

Two stage heat treatments at 40^{o} C at varying times followed by a 90^{o} C for 20 min were tested with kamaboko gels containing 3% EW. No significant differences were found in hardness and elasticity compared to a one stage heat treatment at 90^{o} C for 40 min ($P<0.01$). Based on this study, a one stage heat treatment was selected for the cooking of kamaboko gels.

Potato starch at a level of 5% was also included in the following formulations due to its property to increase binding and elasticity. An increased hardness of 154% and elasticity 464% in kamaboko gels containing 3% EW was found compared to the control. An increase of 9% in hardness, and 23% in elasticity was found in gels containing 3% EW and 5% potato starch compared to gels containing only 3% EW. A significant difference was found in expressible moisture within the three formulations ($P<0.03$) due to the addition of the ingredients and their binding properties.

Egg white proved to have an inhibiting effect on proteolytic activity in kamaboko gels. This was demonstrated by SDS gel-electrophoresis in which the myosin band was present in formulations containing 3% egg white and indicated that no enzymatic breakdown was taking place.

The Production of Surimi from Pacific Whiting (*Merluccius productus*) and Evaluation of Kamaboko Gels

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DEDICATION

To my friend

the eternal Columbia River.

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THE PRODUCTION OF SURIMI FROM PACIFIC WHITING (*Merluccius productus*) AND EVALUATION OF KAMABOKO GELS.

INTRODUCTION

Surimi is the Japanese term for a product resulting from a traditional method of preparing fish by mincing and washing the flesh. For frozen storage, cryoprotectants are added. Surimi is mixed with salt for solubilization of myofibrillar proteins, followed by the formation of a continous gel by heat setting. A variety of products with desirable texture characteristics can be developed and has been called kamaboko.

Incorporation of ingredients, such as egg white, whey protein concentrate, soy protein isolate and potato starch improves the texture of the product. Such ingredients are presumed to participate in the gel network by filling the interstitial spaces of the solubilized myofibrillar proteins, resulting in an increase in hardness and elasticity.

The quality of the gels is evaluated from the raw surimi according to Japanese standards, and is based on the chemical, visual and physical properties. In the final product, instrumental methods have been developed to evaluate textural parameters such as hardness, cohesiveness and elasticity; and are frequently performed with the Instron Universal Testing Machine.

The development of surimi-based products has captured the interest of the U.S. market, and has encountered a high degree of acceptance. This has induced the seafood industry to evaluate available fisheries resources and to consider development of kamaboko products, with

emphasis on the underutilized species such as, Pacific whiting (*Merluccius productus*). Whiting, considered a trash fish due to its poor quality texture, is adversely affected by three main factors: parasitization by a Myxosporidian species; the activity of alkaline proteases that soften the gel when subjected to a specific temperature, and a high trimethylamine oxide content that toughens the product during frozen storage.

The main objectives of this research were to evaluate surimi production from Pacific whiting, and to develop kamaboko gels with acceptable texture characteristics.

LITERATURE REVIEW

Pacific whiting (Merluccius productus)

Resource

Pacific whiting (Merluccius productus) is one of the twenty-five species of fish in the cod family Gadidae, as listed by the American Fisheries Society (Bailey et al., 1970). Whiting range in size from 1 to 1.5 feet (30 to 46 cm) long, with a record length of about 3 feet. Their color ranges from gray to dusty brown with brassy overtone (Hart, 1973). Pacific whiting constitutes the most abundant groundfish resource off the West coast of the continental United States, and has been the target of a large foreign fishery (Nelson and Larkins, 1970). A Soviet fishery for whiting began in 1966 with a catch of 137,000 metric tons (mt). From 1973 to 1976, Poland and Bulgaria joined the fishery. Reported total catches peaked in 1976 at 238 kt. (Table 1). Due to the reduction in resource, information escalated rapidly from 1977 as the United States and Canada extended their fishery jurisdiction and developed comprehensive management plans for this species and other groundfish (Francis and Hallowed, 1985; Dark, 1985).

Table 1. Annual total catches (foreign, joint venture and domestic fisheries in kilotons) of Pacific whiting in U.S. and Canadian waters, 1966-83.

	U.S.	Canada	Combined
Year	Total	Total	Total
1966	137	1	138
1967	178	37	214
1968	61	61	122
1969	86	94	180
1970	160	75	235
1971	128	27	155
1972	74	43	118
1973	145	15	160
1974	194	17	211
1975	206	16	221
1976	232	6	238
1977	128	3	131
1978	98	6	105
1979	125	12	137
1980	72	18	90
1981	115	24	139
1982	76	32	108
1983	73	41	114

Table 1. (Francis and Hollowed. 1985).

Distribution

Pacific whiting is generally a cold water fish. It is found from the Gulf of California to the Gulf of Alaska from the surface to 491 fathoms [(900 m), (Hart, 1973)]. Four major spawning stocks have been identified: those off the California, Oregon, Washington and British Columbia coasts (Stauffer, 1985).

According to distribution and abundance studies, this species is normally found over the continental shelf slope of the California current system, roughly from latitude 25^{o} to 50^{o} N (Bailey and Francis, 1985). Most adults spend the summer months in the northern part of the range. In autumn, adults migrate southward from the Pacific

Northwest waters to spawn in winter off the coast of central, southern and Baja California. After spawning, adult fish migrate as far north as Vancouver Island, while eggs, larvae and juveniles are found off the coast of California.

Utilization

Pacific whiting is generally considered a trash fish (Ryan, 1979; Crawford and Law, 1972) due to a softly textured flesh (Anderson, 1985; Kabata and Whitaker, 1985; Nelson, et al., 1985). Consequently, it has been subjected to considerable interest and research for nearly 20 years (Nelson et al., 1985). This species has been evaluated for potential use, such as, fillets, animal feed, and for production of fish protein concentrate (Nelson et al., 1985).

From the nutritional standpoint Pacific whiting has a flesh protein content of approximately 16%, and a fat content less than 2%. This is similar to other fish species (Table 2), and makes an excellent food source. Once the abnormal (soft) textural conditions are completely resolved, it could have great demand as a high quality food fish.

Table 2. Average proximate composition of edible portions of various fish species (%).

Sample	Moisture (%)	Protein (%)	Fat (%)	Ash (%)
Whiting	82.18	15.78	1.67	0.86
Cod	80.90	18.42	0.42	0.92
Scup	77.65	17.51	3.97	1.46
Squid	81.34	16.33	1.67	1.09
Monkfish	83.29	15.85	0.53	1.21

(Jhaveri et al., 1984)

Despite these evaluations, Pacific whiting has not gained wide acceptance in the U.S. and remains an undertilized species in the domestic fisheries (Nelson et al., 1985). In many countries such as the USSR. Chile, Spain, Argentina and South Africa it is considered a valuable human food resource (Crawford and Law, 1972; Crawford et al., 1979).

Texture Defects

Possibilities for marketing Pacific whiting are dependent on overcoming the texture problem. One potential would be to offer the product as fillet blocks, for subsequent battering and deep fat frying. Another potential would be in the fabricated or analog food area, where minced flesh is evaluated for use as the raw material to prepare a wide variety of products such as imitation shrimp, crab legs and scallops (Nelson et al., 1985) .

During the 1960's and early 1970's, research on the whiting texture problem was limited due to the absence of industry interest in marketing this species. In recent years the enactment of the 200-mile fishery conservation zone legislation and the need to complement declining fishery resources have awakened industry interest in the potential domestic utilization of Pacific whiting (Patashnik et al., 1982; Nelson, 1985).

Parasitism

The abnormal (soft) muscle texture in Pacific whiting is caused by a Myxosporidian induced proteolysis. Almost 300 protozoan parasites have been reported to infect fishes (Lom, 1970). According to Kabata and Whitaker (1981), two species of Kudoa infecting Pacific whiting off North America shores are Kudoa thyrsitis and Kudoa paniformis. Both species penetrate the muscle fibers, disrupting them and gradually breaking up the tissue.

As demonstrated by Patashnik et al. (1982), once the parasite is detected by the host; the host reaction becomes histologically evident. The infected fiber gradually assumes a yellowish hue, passing into brown and eventually black. Observations with the naked eye show elongated structures or pseudocysts as white or black streaks. The cyst may be either uniformly or unevenly distributed through the interior of the flesh.

Proteolysis is capable of occurring at nearly any temperature above freezing. In the raw product, this mushiness could be seen after several hours of incubation, but with low temperature cooking methods, the liquefied product could be detected immediately. Frozen products thawed at room temperature would also exhibit tissue breakdown due to this proteoly sis (Erickson et al., 1983).

Tsuyuki et al. (1982) as cited by McFarlane and Beamish (1985), found that two optimum temperatures for the proteolytic activity of the enzyme produced by Kudoa are at 35 to 40^{o}C (pH 3.8), and at 55 to 60^{o}C (pH 6.7). Studies done by Patashnik et al. (1982), show that rapid

heating of the flesh to 70°C (158°F) for 10 minutes completely inactivates the protease and preserves its textural properties.

The effect of protease inhibitors has been investigated by Miller and Spinelli (1982) in parasitized Pacific whiting muscle. They found that naturally occurring protease inhibitors such as those found in soybean, lima bean, chicken egg white, turkey egg white and potato extract, in concentrations ranging from 1 to 5 mg/ml (incubated at 45° C for 90 min), did not show a significant inhibiting effect that would be suitable for use in food systems.

Health problems from the parasite have not been reported from areas such as Japan, where raw fish is consumed. Cooking readily destroys the organism if it is present in the fish muscle (Nelson et al., 1985).

The marketing potential of Pacific whiting is thus limited by the presence of Myxosporidian-induced proteolysis resulting in a poor quality product. A better product can be obtained if the fish has been immediately chilled, processed and rapidly cooked by methods such as deep fat frying. Cooking at temperatures between 375° F and 400° F and shortening the time intervals between the temperatures of maximum enzyme activity would produce a fillet with considerably better texture than by using slower methods of cooking (Nelson et al., 1985).

Alkaline Protease

Alkaline protease is present in the sarcoplasmic fraction of skeletal muscle, and also in the skin and internal organs in Pacific whiting (Erickson et al., 1983). It has been quantitated in other

fishes such as Atlantic croaker (Su et al., 1981a,b), carp (Makinodan et al., 1985), and barracuda (Iwata et al., 1974).

Alkaline protease hydrolyzes muscle protein optimally around 60^{o} C at neutral pH range, and influences the functionality of fish meat gels (Cheng et al., 1979; Lanier et al., 1981; Makinodan et al., 1985). Several factors influence the level of proteolytic activity in fish tissues: the species, harvest location, postharvest handling and processing and time and temperature of frozen storage.

Postmortem proteolytic reactions cause deterioration in fish muscle proteins, especially in the liver, alimentary canal and kidney tissues. These organs contain several hundred times more protease activity than muscle. Skin tissue was found to contain about the same total activity as muscle. Contamination from visceral residues during inadequate processing could lead to a high alkaline protease activity in the product, thereby affecting the quality of the product (Su et al., 1981a).

Studies reported by Cheng et al. (1979), show that rapid heating of fish gels above the optimum protease activity temperature produce a firmer, more springy texture in comparision with those heated at lower temperature for a longer time. Other researchers have also reported that prolonged heating of flesh in the 50 to 70^{o} C range caused a weakening of gel strength due to the activity of the alkaline protease (Lee and Toledo, 1976).

Improvement of the gel texture in white croaker meat gels by the addition of 0.5 mg/g of potato protease inhibitor has been reported by Lanier et al. (1981). However, Makinodan (1985), did not find any increase in hardness of gels made from the same species from a different

location by using the same inhibitor at the same concentration. Cheng et al. (1979) obtained satisfactory results using inhibitors such as the metal chelators ethylenediamine-tetraacetic acid (EDTA), and ethyleneglycol-bis-N,N'-tetraacetic acid (EGTA), in concentrations of 10mM. EGTA showed a greater inhibitory effect than EDTA since EGTA is more selective for calcium which often activates enzymes. EDTA chelates most heavy metals that are inhibitory to the enzymatic activity under assay conditions of pH 7.5 at 60^{o} C for 1 hour.

Trimethylamine Oxide

Trimethylamine oxide (TMAO) is found in most species of marine fishes, and in particularly high concentrations in the gadoid family. It is not well established whether this compound arises mainly from an exogenous source or from biosynthesis in the fish system (Bilinsky, 1964). Shenouda (1980) believed that TMAO has a similar function to uric acid in animals, that it is excreted to maintain nitrogen balance. The amount of TMAO present in fish is related to all the biological variability common in fish chemistry: species, storage time and temperature.

TMAO is broken down into the following compounds: trimethylamine (TMA), dimethylamine (DMA) and formaldehyde (FA) by different types of enzymatic and nonenzymatic action. Several possible mechanisms are discussed.

Bacterial enzymes (e.g. TMAOase) are released from some microorganisms, such as, Pseudomonas putrefaciens (Castell and Snow,

1951) and Vibrio parahaemolyticus (Unemoto et al., 1965). The reaction is more rapid at room temperature than at chill and frozen temperatures.

Endogenous enzyme is present in the muscle tissue of the gadoid family, it has been found to contain greater amounts of TMAO than any other non-gadoid North Atlantic species tested. TMAO-splitting enzyme is more active during frozen storage in the gadoid fishes, especially in Pacific whiting (Babbitt et al., 1972). Temperatures (from -5^{o} C to -40^{o} C) have been found to influence the activity of the enzyme TMAOase (Tokunaga, 1974).

The breakdown of TMAO into TMA, DMA, and FA compounds has been suggested to cause changes in the physicochemical properties of frozen stored gadoid muscle via interaction of protein with FA, producing crosslinks. This causes a significant decrease in the extractability of total protein, therefore resulting in a textural toughness (Gill et al., 1979).

Nonenzymatic reduction of TMAO has been reported to occur at temperatures between 22^{o} and 24^{o} C by cysteine only in the presence of sufficient amounts of iron or hemoglobin as a catalyst. TMA was the major product of this reduction, but appreciable amounts of DMA and FA were also formed (Vaisey, 1956).

Surimi

History

The technique for making surimi dated from 1100 A.D. as a method to preserve fish. Traditionally Japanese surimi was prepared from fresh

fish, by deboning, mincing and washing of the flesh, and combining it with spices and salt. It was ground to a jellied consistency and cooked by steaming, broiling or frying. This variety of gelled products prepared from surimi is called kamaboko (Lee, 1984; Suzuki, 1981).

For several centuries, surimi was made on a day-to-day basis depending on the volume of fish available from day's catch, until 1959 when Japanese scientists discovered the role of sugars in the stabilization of frozen surimi (Matsumoto, 1978). Subsequently, frozen surimi production in Japan increased from 32 metric tons in 1965 to 2580 metric tons in 1984 (Sonu, 1986).

Surimi is prepared from over 60 fish species such as Alaska pollack, mackerel, croaker and shark (Suzuki, 1981). Each species requires slightly different processing techniques (Lee, 1984).

In recent years, about half of the Japanese frozen surimi has been processed on board and the other half by land-based operations (Okada, 1985). Manufacturing surimi on board results in superior quality to that processed in shore plants, due to the immediate processing on the same day of harvest that ensures a top quality product. Shore plants process fish that have been harvested 1 to 5 days earlier, depending on the distance of the fishing ground to the plant (Suzuki, 1981; Okada, 1985).

Recently, many countries have been interested in Japanese kamaboko products. Numerous researches have tried to adopt the surimi technology to underutilized species such as menhaden (Lloyd et al., 1985), red whiting and Pacific whiting (Groninger et al., 1985).

Surimi Operations

Today's surimi processing (Fig. 1) is achieved by mincing the skinless fillets in a belt-drum type meat deboner which separates the flesh from the bone and skin. The diameter of the drum perforations should not be larger than 3-4 mm to prevent the skin from passing through the holes (Takeda, 1971). The resulting minced flesh is washed mechanically with chilled water (5-10^{o} C), which causes removal of most of the water soluble proteins along with undesirable substances such as blood, pigments and trimethylamine oxide, to yield a uniform, white and odorless minced flesh. The number of washing cycles and the volume of water will vary with the fish species, the initial condition of the fish, type of washing equipment, and the desired quality of the surimi (Lee, 1984).

The washed product is dewatered under the pressure exerted in a screw press, that is controlled by the length of the screw barrel and residence time (Lee, 1984). The residence time is controlled by the rotation speed of the screw barrel. The slower the speed, the longer the meat receives the pressure resulting in the removal of a larger amount of moisture. Other methods to dewater flesh include centrifugation and an experimental vacuum system is being tested. The resulting product is refined in a strainer which separates residue of scales, bones, connective tissue and skin, and will give a higher quality surimi. Obviously, the smaller the perforation, the lower the yield. The perforation size within 1-2 mm is considered desirable.

In order to maintain the quality of surimi during frozen storage, cryoprotectants such as sucrose, sorbitol and polyphosphates (usually in concentrations of 4%, 4%, 0.3%, respectively) are added and mixed.

Sucrose and sorbitol prevent muscle protein, particularly actomyosin, from denaturation during frozen storage (Matsumoto, 1978). The levels of sugar and sorbitol can be adjusted depending upon the type of product to be made and the sweetness desired (Lee, 1984). The resulting product is packaged and blast frozen to -35^{o} C (Suzuki, 1981).

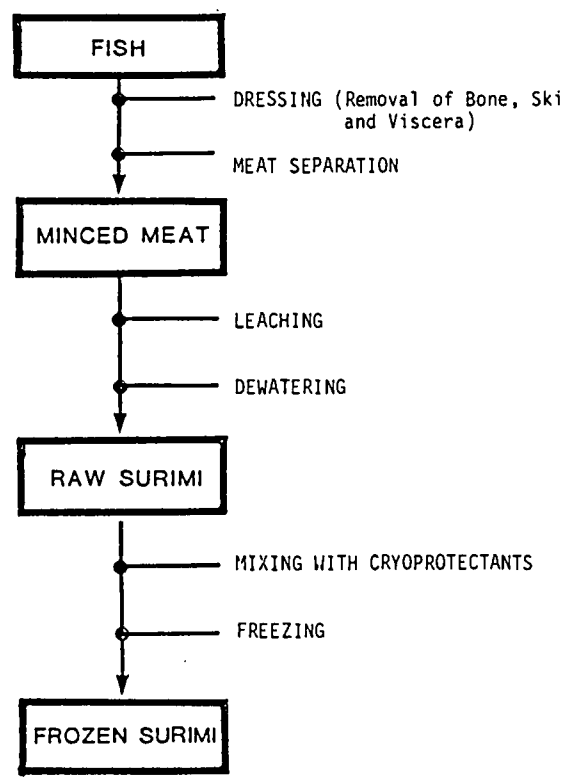


Figure 1. Surimi unit operations
(Sonu, 1986)

Kamaboko

Kamaboko is a generic term given to a variety of products prepared from surimi. The distinguishing property, is the capacity to form a continuous gel, which allows the formation of many desirable textures.

Gel formation

Kamaboko gel formation is a complex interaction of physicochemical changes of meat protein. Fukazawa et al. (1961) and Samejima et al. (1969) reported that of myofibrillar proteins of meat muscle, only myosin has the ability to influence the heat-induced gelation and suggested that the entire myosin molecule is required to develop desirable gel strength of the product. Therefore it is important to understand myosin's role in gel formation.

Myosin's native structure has been described as a double stranded alpha-helical rod with two globular heads at one end. These heads are responsible for its enzymatic (ATPase) activity and its ability to interact with actin during muscular contraction. Myosin can be cleaved near the head region by proteolytic enzymes such trypsin, producing two fractions of the protein: one of these is called light meromyosin and the other which contains the globular head structures of the myosin, is called heavy meromyosin (Lehninger, 1982; Hultin, 1985; Figure 2).

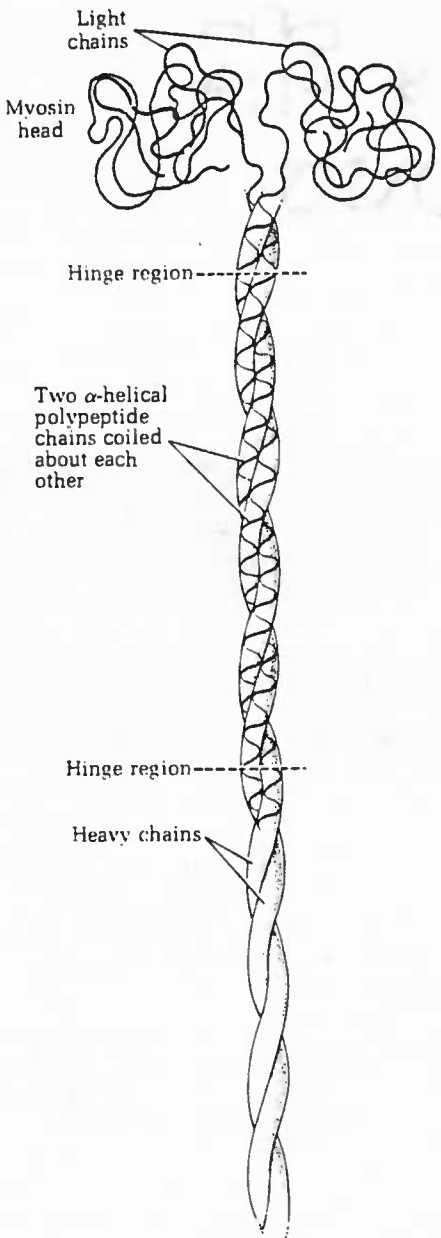


Figure 2. Myosin molecule.
(Lehninger, 1982)

The mechanism of gel formation is not well understood. The process is influenced by tissue pH, salt concentration, thermal energy input and protein concentration (Acton and Dick, 1984).

According to Niwa (1985) the gel formation in kamaboko is achieved by mixing partially frozen surimi with 2 to 3% NaCl; during this step the myofibrillar proteins are dissolved and extracted by the salt. The sodium chloride ions are bound to the acidic and basic amino acid residues, therefore intermolecular ionic bonds among molecules are ruptured, and as a result the proteins are dispersed in the water. During heating ionic bonds and hydrogen bonds are ruptured; and the formation of disulfide and hydrophobic bonds are promoted which results in a three dimensional gel network. However, studies done by Samejima, et al. (1981), concluded that thermal denaturation of the myosin tail portions results from the disruption of stabilizing forces of a noncovalent nature such as hydrogen and hydrophobic bonds and that disulfide cross-links or other covalent bonds are not involved.

Studies reported by Acton and Dick (1984) on the setting of fish proteins show the importance of the effect of heat on the activity of myosin ATPase. It was assumed that disulfide bonds are formed from free sulfhydryl groups present in the myosin ATPase at different heat settings.

Gel setting behavior and gel strength can vary from species to species (Makinodan and Ikeda, 1971). Gel setting takes place at temperatures up to 50^{o} C without cooking and occurs even at below room temperature. When comminuted flesh passes through a heating zone 60 to 70^{o} C, part of the gel structure is altered so that the gel becomes soft. This phenomenon is called softening (modori) and more often

occurs in unwashed fish tissue, where much of the water-soluble protein and proteases still remain. This did not occur in surimi gels prepared from Red whiting (Urophycis chuss) and Atlantic pollack (Pollachius virens) (Lee, 1984). However, surimi prepared from certain species is not affected by the "softening" phenomenon. Such softening is believed to be caused by an alkaline protease, since this enzyme has an optimum activity at 60 to 70^{o} C (Makinodan and Ikeda, 1971; Cheng et al., 1979; Lanier et al., 1981).

Surimi-Based Products

Surimi-based products are prepared by extruding the surimi paste into various shapes which resemble shellfish meat such as crab, lobster, scallop or shrimp. The closer the simulation desired, the greater sophistication of extrusion technique is required. The products may be divided into categories according to their fabrication and structural features (Lee, 1984).

According to Lee (1984), there are a number of important steps and considerations in making surimi-based products:

1. use of top-quality surimi is essential in making fiberized products which require a highly elastic and resilient texture.
2. water may be added so that the moisture level remains at 78 to 80%, as a maximum in the paste.
3. chopping must be sufficient for the maximum solubilization of the myofibrillar proteins.
4. setting (or tempering) of the paste extrudate should occur slowly and to a moderate degree without surface drying.

5. flavoring is usually achieved by using shellfish-extract-based flavor in combination with flavor enhancers such as monosodium glutamate or ribonucleotide.
6. colorings should be water-insoluble, such as lakes and carotenoid pigments.

Since the introduction of Japanese surimi-based products into the U.S. market from 4 to 5 years ago, the entire structure of the fabricated seafood market has changed. As a result, there is a high level of consumer acceptance and the market of surimi based products in the U.S. has grown from 2 million pounds in 1979 to 88 million pounds in 1985 (Parker, 1986). More research is needed in this area, however, with emphasis on the flavor and quality for Americanized surimi-based products.

The Effect of Ingredients on Gel Texture

The most important characteristic of kamaboko products is the quality of its gel texture, called "ashi" in Japanese. The ashi is related to the species, freshness of fish and the techniques used in processing. The formation of good ashi requires the extraction of the myofibrillar proteins from fish muscle and the gel formation of the extracted proteins (Okada, 1985). The sequence of incorporation of ingredients is an important factor that affects the textural quality of the final product, and varies with the type of formulation. The temperature of the process has to be kept below 10^{o} C to avoid protein denaturation.

Salt

Sodium chloride is an indispensable ingredient in kamaboko production. It participates in the solubilization of myofibrillar proteins in the fish muscle. The salt concentration used in commercially processed kamaboko ranges from 2 to 3% NaCl (Suzuki, 1981). This concentration has been considered optimum for the solubilization of myofibrillar proteins, and is called "salting in". The sodium chloride ions react with the charges of protein by decreasing the electrostatic attractions between opposite charges of neighboring molecules, therefore solubilizing the proteins. If the concentration of NaCl is greater than 2 to 3%, the protein displays a decrease in solubility, which may result in precipitation. This "salting out" effect results from the competition between protein and salt ions for water molecules necessary for their respective solvation (Cheftel et al., 1985).

Phosphates and pH

The quality of seafood is improved by the incorporation of phosphates which increase tenderness and binding, improving flavor and preventing microbial spoilage (Ellinger, 1972). According to Shimp (1985) and Lee and Toledo (1976), polyphosphates, such as pyrophosphates and tripolyphosphates, are effective in surimi-based products because they act by increasing pH and enhancing solubilization of the myofibrillar proteins. This improves water binding, therefore resulting in a better quality gel.

Generally about 0.2 to 0.5% phosphate is added directly to surimi during the grinding step. The extracted myosin in combination with other ingredients provides cohesive and elastic characteristics required for the molded structure of the final product. Another important factor is the regulation of the pH in the paste. Studies have shown that treating the meat paste with a strong base such as sodium hydroxide or a weak base such as sodium carbonate increases the gel strength. Tanaka et al. (1968) adjusted the pH of gels by adding sodium carbonate and determined that an optimum range of pH 7.1 to 7.3 for white meat species, and pH 6.1 to 6.5 for dark meat species, resulted in optimum gel strength.

Egg White

Egg white is an important ingredient in commercial food processing because of its many functional properties such as gel formation, and water binding capacity. Egg white is added to kamaboko to improve the gel strength, and give a glossiness to the finished product. Different concentrations are used depending on the fish species and the desired texture of the final product. When dry egg white is used, an appropriate amount of water should be added to adjust the moisture content of the finished product (Lee, 1984). Concentrations up to 20% of fresh egg white resulted in both a decrease in gel strength, and the development of an unpleasant hydrogen sulfide odor to the product (Okada, 1985).

Heat induced interactions among the heterogenous proteins in egg white have been little studied. The interactions may involve hydrogen,

hydrophobic, and disulfide bonds (Johnson and Zabik, 1981). Specific conditions of temperature, pH, protein concentration and ionic strength have profound influence in the mechanism of gel formation. Studies done by Burgarella et al. (1985 a,b), in the gel formation of egg white proteins in conjunction with fish muscle proteins, suggested that egg white proteins act as a "filler" by filling the interstitial spaces of the myofibrillar proteins.

Whey Protein Concentrate

Whey protein concentrate (WPC) has many functional properties which enable it to be used in a variety of food applications. One of these functional properties is the ability to form heat-induced gels under the appropriate conditions. Whey protein concentrate also contributes excellent nutritional (complete protein) and organoleptic properties, such as bland flavor and whiteness (Wingerd, 1979).

The main proteins participating in gel formation of WPC are beta-lactoglobulin and alpha-lactalbumin. These proteins have a compact, globular conformation with an alpha helical structure, and show a uniform distribution of acidic and basic, hydrophilic and hydrophobic amino acids along the polypetide chain (Morr, 1979). According to Kohnhorst and Magino (1985), the gel formation in the WPC is achieved as any other thermal gelation system; by disulfide bonds, ionic and hydrophobic interactions.

Soy Protein Isolate

Soy protein isolate (SPI) has been extensively used in comminuted meat and in deep fat fried kamaboko products because of its ability to form gels which provide a structural matrix for holding water, flavors and other added ingredients. The textural properties of kamaboko containing SPI are affected by the concentration used. As the levels of SPI increase, the product whiteness decreases, and an unacceptable flavor becomes distinct.

Soy protein isolate is composed of globulins with minimum solubilities near pH 4.5, and has two major proteins, the 7S (conglycinin) and 11S (glycinin). Both proteins are important in participating in gel formation by covalent linkages i.e., disulfide linkages, and noncovalent interactions i.e., hydrophobic interactions, hydrogen bonding, and electrostatic attractions (Utsumi and Kinsella, 1985). Initial heating above 60^{o} C is necessary to induce dissociation of these proteins (Kinsella, 1979).

Starch

Starch is another basic ingredient in kamaboko products. Potato starch is most commonly used because of its higher water binding capacity and bland taste compared with either corn or wheat starch. When potato starch is cooked at temperatures greater than 57^{o} C, the high viscosity solutions obtained are very smooth and remain stable at low temperatures. These properties make it particularly well adapted for use in surimi gels where it reinforces the gel formation resulting

in a more firm and cohesive product and ensures that the original texture remains unchanged during frozen storage (Duxbury, 1986; Kim and Lee, 1987).

From a sensory aspect, potato starches tend to maintain the rubberiness of surimi gels, whereas egg white reduces the rubberiness. Thus it appears that they counteract each other, imparting a meaty texture (Lee, 1986).

Others

Other ingredients such as wheat proteins (gluten) are used to reinforce the gel strength by increasing the number of disulfide bonds (Lanier, 1986). Cysteine has been used as a reducing agent to promote the reduction of disulfide bonds formed during frozen storage (Yoshiaki et al., 1979). Potassium bromate has been used in the last stage of the mixing operation. During the heating process, oxidation takes place in the free sulfhydryl groups resulting in an increase of disulfide bonds. This, therefore, increases the gel strength of the final product.

Gel Texture Evaluation

Surimi quality can be evaluated by assessing the integrity of the myofibrillar proteins. Methods for this determination include, among others, percentage of extractable myofibrillar protein and measurement of ATPase activity (Holmquist et al., 1984).

The quality of surimi also can be evaluated according to Tokai Regional Fisheries Laboratory Methods (Lee, 1984) on the basis of chemical and visual conditions such as pH, impurities and whiteness; and physical properties such as viscosity in raw surimi. The gel forming ability can be evaluated based on the folding test in the finished product (Table 3).

Table 3. Japanese grading scale for the folding test.

Numerical score	Grade	Result of folding test	Degree of elasticity
5	AA	No cracks on folding into quarters	Extremely elastic
4	A	No crack on folding in half; crack on folding in quarters	Moderately elastic
3	B	Some cracking on folding in half	Slightly elastic
2	C	Breaks into pieces on folding in half	Not elastic
1	D	Breaks into fragments with finger pressure	Poor

Source: Nippon Suisan Kaisha Ltd. (1980).

Instrumental methods have been developed to compare physical attributes of gel strength in relation with the results from human sensory evaluation panels. Bourne, (1968) adapted methodology to the the Instron Universal Testing machine to perform a modified texture profile test by compressing standard size pieces of food two times in a manner analogous to that the General Foods Texturometer. The resulting profile is a force-distance curve called the Instron Texture Profile

Analysis (ITPA). ITPA is considered a simulation type test because it imitates the biting of a food sample in the human mouth. It has been shown to correlate well with sensory panel data for many foods and surimi-based gels (Daget and Collyer, 1984; Montejano et al., 1985).

Objective

The main objectives of this investigation were to evaluate surimi production from Pacific whiting (Merluccius productus), and to develop kamaboko gels with acceptable texture characteristics.

MATERIALS AND METHODS

Fish Processing

Fish Source

Pacific whiting (Merluccius productus) were caught off the coast of Astoria, Oregon during the summer, 1986 and summer and fall, 1987. The fish were iced until processing which was usually within 24 hours post catch.

Flesh Separation

Planking of the flesh was carried out by hand with a careful and complete removal of the fillets from the viscera, head, spine and black peritoneum. This was done to avoid visceral contamination of the planks which could cause an increase of proteolytic activity and adversely affect the gel formation in the kamaboko. Additionally materials from the spinal cord could influence color, and flavor and catalyze randicity. The planked flesh was deboned by using an Ikeuchi Deboner Model 805 (Ikeuchi Tekkosho, Ltd., Japan). The flesh was passed between a press and a perforated drum (0.4 cm in diameter), where it was forced through the holes of the drum as a minced flesh, the remaining material (bones, skin and adhering material) passed on to a conveyor belt and was deposited in an aluminum tray and discarded. The yield of the minced flesh from each lot was calculated on the basis of round fish

weight. Samples were taken from each lot for proximate analysis and protease activity determination.

Washing and Dewatering

Minced flesh was washed in polyethylene tanks (95 liter capacity) with water and ice at a ratio of one part flesh to three parts water by weight; and gently stirred for 5 minutes to leach out blood, pigments, water soluble proteins and low molecular weight nitrogenous compounds.

The washed minced flesh was dewatered in a Sano-Seisakusho screw press Model SD-8, (Ikeuchi Tekkosho, LTD., Japan), that allowed the product to flow through it continuously. Water was purged from the flesh slurry by squeezing the product into a progressive reducing chamber with the aid of a rotating screw (60 cm diameter) which allowed the pressurized water to escape through tiny drain holes (30 mesh market grade screen) in the chamber wall.

After the first wash the highest speed of the dewatering unit was used; after the second wash a lower speed was applied to obtain a moisture content of the product of approximately 78%. Samples were taken from the first and second dewatering procedures (flesh and water) for proximate analysis and protease activity determinations.

Refining

Refining of the dewatered minced flesh was carried out by an Akashi strainer Model S-1 (Akashi Tekkosho Co., Japan). The purpose of the strainer was to separate the foreign particles such as bones, connective tissue, and scales and give a high quality surimi (Suzuki, 1981). This machine has a partially jacketed screw device in which ice is used for cooling in order to prevent heating of the product due to friction.

Surimi Preparation

Surimi was prepared by mixing the refined flesh with cryoprotectants, 4% sucrose, 4% sorbitol and 0.5% Brifisol 414 (a mixture of sodium acid pyrophosphate, sodium pyrophosphate and sodium polyphosphate; BK Ladenburg Corp., North Hollywood, CA) in a Model VCM Hobart Silent Cutter (The Hobart Manufacturing Co., Troy., OH). The whole procedure was kept below 10^{o} C to prevent protein denaturation, particularly to actomyosin, during mixing (Matsumoto, 1978). Aliquots of surimi were packed into individual plastic trays (10.5 x 8.5 x 5.5 cm) of approximately 600 g, and vacuum sealed in a Cryovac bag (20 x 25 cm) with a Cryovac Model 6250-B vacuum chamber machine and frozen at -30^{o} C.

Gel Preparation

Formulation

Partially thawed surimi was used for the preparation of the fish gels (kamaboko). The formulations were calculated based on the percentage of surimi; and the moisture content of each was adjusted from 76 to 78%. Preliminary work involved the use of dry egg white (Melton G. Waldbaum Co. Wakefield., NE), whey protein concentrate (WPC Lacprodan-80, Danmark protein A.S., Danpro., Denmark), and soy protein isolate (SPI Ardex SP6 Fortified, Archer Daniels Midland Co. Decatur, IL). Concentrations ranged from 1 to 5% of each protein and 2% NaCl (total weight) was included in each formulation.

From the preliminary work, egg white was selected for the subsequent work because of its performance in improving the texture of the gels. Potato starch was another important ingredient selected. Levels of 5% or less of potato starch have been used traditionally by the Japanese to improve the gel strength and elasticity. According to Lee (1986), potato starch acts as a reinforcing factor in the composite gel matrix, due to its structural continuity upon gelatinization as well as its ability to fill the matrix. This results in an increased elasticity of the gels. On the other hand, it is presumed that egg white has a disrupting effect in the gel matrix, which causes structural discontinuity, thus increasing the hardness of the product.
From the sensory aspect, starch tends to maintain the rubberiness whereas egg white reduces the rubberiness, and it appears that they counteract each other. However, these resulting properties impart a

desirable meaty texture on the product, therefore it is necessary to have both in the formula (Lee, 1984).

In the final work, three formulations were prepared: the first formulation was comminuted with the addition of 3% NaCl (control); the second with 3% NaCl and 3% dry egg white, and the third one with 3% NaCl, 3% dried egg white and 5% potato starch (Western Polymer Corp., Tulelake, CA).

Each formulation was blended in a Hobart Kitchen Aid mixer (Model K5SS, Troy, OH), for 15 minutes after the addition of each ingredient. Caution was taken to keep the temperature below 10^{o} C to avoid protein denaturation. The batters for the preliminary work were packed in glass tubes (3 cm diameter and 15 cm long), sealed with rubber stoppers, and were subjected to two stage heat treatments. This consisted of 20, 40 or 60 min at 40^{o} C followed by heat processing for 20 min at 90^{o} C. In the final work the batters were poured into a plastic tray (10.5 x 8.5 x 2.5 cm) bagged and sealed in a vacuum chamber machine Cryovac Model 6250-B, and heat processed at 90^{o} C for 40 minutes in a Thelco Precision Scientific bath Model 83. Gels were cooled on ice post heat processing and prior to textural evaluation. This procedure was performed in triplicate for each formulation in the three separate lots of surimi.

Physical Analyses

Texture Profile Analysis

Fish gels were tempered to ambient temperature (21 to 23^{o} C) before the analysis was carried out. A compression analysis (double bite) was employed using a Model TM-M Instron Universal Testing Machine (Instron Corp., Canton, MA) in order to obtain values for hardness, cohesiveness and elasticity (Bourne, 1968). Cylinders 1.5 cm in diameter and 1.0 cm long were cut from the gels. Three replicate measurements were done on each different treatment.

In the preliminary work the samples were subjected to 75% compression by a 50 lb capacity load cell, with a crosshead speed of 50 cm/min, chart speed of 100 cm/min and a full scale load of 20. In the final work the samples were subjected to 80% compression with a 50 lb capacity load cell, a crosshead speed of 10 cm/min, and a chart speed of 100 cm/min with a full scale load of 20.

The results were calculated according to Bourne (1968) as follows. The force to compress each sample to 80% of its original length is reported in Newtons/gram (N/g) as hardness. Cohesiveness is calculated as the ratio of the area of the second compression curve (A2) to that of the first compression curve (A1) and is expressed in arbitrary units. Elasticity is the amount the sample recovers after compression. It is calculated as the ratio of the base length of the second curve (D2) to the base length of the first curve (D1) and is reported as a percentage. A dimensional analysis of the texture profile parameters is shown in Figure 3.

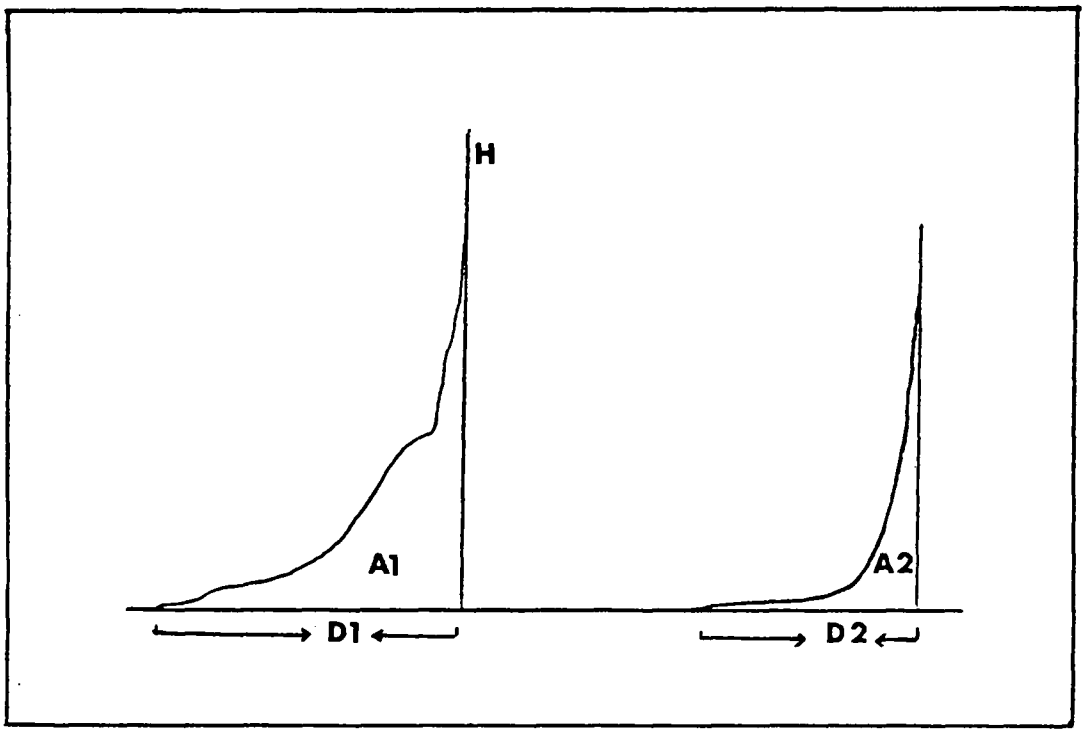


Figure 3. Instrumental Texture Profile Analysis (ITPA) from a Pacific whiting cooked gel. Double bite test. Hardness (H) in pounds; cohesiveness (A2/A1); elasticity (D2/D1) x (100).

Expressible Water Determination

Expressible water was measured using the method of Kudo et.al., (1973) with the following modifications: a sample approximately 2 mm in thickness weighing 100 mg was cut from the cooked gels and placed between two pieces of nylon filter (100 micron mesh, Filpaco Industries Inc., Chicago., IL). The nylon filters and sample were weighed and placed between two pieces of filter paper (Whatman No.1). It was pressed in a Carver Laboratory Press (Model 1300-193, F.S. Carver Inc.,NY, NY) at 1000 psi for one minute. The nylon filter and sample were removed from the paper and reweighed. Determinations were performed in triplicate for each treatment. The percentage of expressible water was calculated as weight after pressing divided by the weight before pressing, times 100.

Chemical Analyses

Proximate Composition

Proximate analyses were performed in duplicate according to A.O.A.C. methodology (1984). Total protein, lipid, ash and moisture were determined at each stage of processing for the three lots of surimi. Determinations were performed on samples taken after deboning; the first and second washings and dewatering (flesh and water); after refining and of the surimi.

Determination of pH

A ten gram sample was taken from each cooked and uncooked gel preparation and homogenized with 90 ml of distilled water for 30 seconds using a Kinematica CH-6010 Kriens-LU Homogenizer at 18,850±150 rpm. The pH was measured using a Corning pH meter Model 240, (Corning Ciba Diagnostics Co., Corning, NY).

Proteolytic Activity

Protease activity was determined according to Karvinen et al., 1982. The crude enzyme was prepared by weighing eight grams of muscle into 30 ml of 1 mM EDTA in 0.25 M sucrose buffer, pH 7.2, and homogenized with a Kinematica CH-6010 Kriens-LU homogenizer at 18,850±150 rpm. The homogenate was centrifuged in a Sorvall RC-5B Refrigerated Superspeed Centrifuge at 3,000 rpm (SS-34 rotor) for 10 minutes at 4^{o} C. The supernatant was rehomogenized for an additional two seconds and adjusted to pH 7.2 with 0.5 N KOH. Triton X-100 (0.4 ml, 10% solution) was added to rupture cell membranes, and the volume adjusted to 80 ml with the sucrose/EDTA buffer.

The protease reaction mixture included 1 ml of the crude enzyme extract, 3 ml of 2.5% urea denatured hemoglobin as a substrate, and 1 ml of citrate buffer pH 6.5. After samples were incubated at 55^{o} C for 30 minutes, 3 ml of 10% TCA was added to stop the reaction. and the tubes were held on ice for 30 minutes. A blank was run using the same reaction mixture with the immediate addition of the TCA. The samples were filtered with Whatman No.1 paper and the absorbances were read at

280 nm in a Sargent-Welch SP6-550 UV/VIS Spectrophotometer. Concentrations were determined against a tyrosine standard curve in 1 mM EDTA in 0.25 M sucrose buffer pH 7.2.

To determine the effect of temperature on the proteolytic activity, 1% of Hammerstein casein (Nutritional Biochemical Co., Cleveland, OH) was used as a substrate and the procedure was the same as described. The incubation temperatures were performed at 5, 20, 40, 55, 70 and 90^{o} C for 30 minutes.

Protein Determination

Quantitation of protein was determined by the Biuret assay (Gornall et.al. 1949). A 2 ml sample was taken from each crude enzyme extract and was mixed with 8 ml of Biuret reagent. After standing for 30 minutes, the absorbances were read at 550 nm against a blank with a Sargent-Welch SP6-550 UV/VIS Spectrophotometer. Protein concentrations were determined against a bovine serum albumin (Sigma Chemical Co., St Louis, MO) standard curve.

Myofibrillar Protein Extraction

Extraction of myofibrillar proteins was carried out according to Hashimoto et al., (1979). The procedure was performed at 3 to 4^{o} C. Ten grams of surimi was homogenized with Kinematica CH - 6010 Kriens-Lu for two minutes in 100 ml of I=0.05 phosphate buffer pH 7.5 (15.6 mM Na_2HPO_4, 3.5 mM KH_2PO_4). The homogenate was centrifuged two times at

5,500 rpm (SS-34 rotor) for 20 minutes in a Sorvall RC-5B Refrigerated Superspeed centrifuge. The two supernatants were combined and identified as the sarcoplasmic protein fraction. The residue was homogenized with ten volumes of I=0.5 KCl phosphate pH 7.5 (0.45M KCl, 15.6 mM Na_2HPO_4. 3.5 mM KH_2PO_4) and centrifuged. This step was also performed two times. Both supernatants were collected and identified as a myofibrillar protein fraction. The quantitation of the protein was determined by the Biuret method as previously described.

Electrophoresis

Sample preparation for electrophoresis was carried out according to Cheng et al., (1979) with some modifications. Fish sols were prepared according to the specific procedures, as previously mentioned, and were subjected to two different treatments: an incubation for one hour at 55^{o} C and a heat treatment at 40^{o} C for 1 hour followed by 90^{o} C for 20 minutes. After each treatment the samples were cooled on ice. Two grams of sample were taken from each treatment in duplicate and were homogenized for 2 minutes with 24 ml of the following solution containing: 5.0 ml of 1M sodium phosphate sodium dodecyl sulfate buffer pH 7.2 (SDS buffer), 2.0 ml of mercaptoethanol and 2.0 g of SDS in 93 ml of distilled water.

Each preparation was heated in a boiling water bath for 5 minutes and centrifuged in a Sorvall RC-5B Refrigerated Superspeed centrifuge at 18,000 rpm (SS-34 rotor) for 30 minutes.

Protein determination of the samples was done by the method of Kalckar (1947); the samples were filtered in Whatman No. 542 and the

absorbances of 260, 280 and 340 nm were read and calculated by the following equation :

(OD280 - OD340)(1.45)-(OD260 - OD340)(0.74) = mg protein/ml

A 0.5 ml aliquot was taken from the supernatant fraction, 3 ml of SDS buffer pH 7.2, 1 ml of bromophenol blue, and 1 ml of 50% glycerol were added to the final preparation used for the electrophoretic evaluation. Average sample size ranged from 1.7 mg/ml to 2.9 mg/ml.

SDS-acrylamide gel electrophoresis was performed according to Weber and Osborn (1969). All chemicals were purchased from Bio-Rad Laboratories (Richmond, CA). A five percent acrylamide gel was prepared by mixing 22.2 g of acrylamide and 0.6 g of N'N'methylene-bis (acrylamide) in a final volume of 100 ml with SDS Buffer pH 7.2. After the elimination of the bubbles by vacuum, 1 ml of ammonium persulfate (35 mg in distilled water), 0.03 TEMED (N,N,N"-tetramethylethylene diamine) and 4.5 ml of distilled water was added. The gel solution was poured into glass tubes 12 cm in length and 5 mm ID, the gel length was 11.5 cm. A few drops of water were placed on the top to produce a flat surface, prevent dehydration and gel cracking.

After complete polymerization of the gels, 30 microliters of standard solution was applied to the top of a gel. The standard solution contained the following proteins: myosin, beta-galactosidase, phosphorylase b, bovine serum albumin and ovalbumin, with their corresponding molecular weights of 200,000, 116,000, 97,400, 66,200 and 42,699 daltons, respectively. From the prepared sample, 75 microliters was applied to the top of each gel. The electrophoresis was performed at 3 milliamps per tube for 20 minutes, and the current was increased to

8 ma/tube for 5 hours at room temperature, in an Electrophoretic Disk Unit (Holfer Model DE101, Portland, OR).

After this process, the gels were removed from the tubes and placed into a fixing solution (25% isopropanol, 10% glacial acetic acid) for three hours. Gels were then stained for 12 hours at room temperature with Comassie Blue R-250. The gels were rinsed and destained in a solution of 10% acetic acid, 5% methanol. After 48 hours complete destaining was achieved and the gels were stored in 7.5% acetic acid solution.

The resulting gels were analyzed by comparing the densitometric tracings using a Helena Laboratories Quick Scan Densitometer (Model 1020A). Quantitation of the protein bands in the gels was determined by automatic integration of the peak with the Quick Scan densitometer. The gels were photographed by Andrew Cier (Astoria, OR). Gels shrank over time and do not fit exactly into the scans illustrated.

Statistical Analyses

The statistical analyses were conducted by using ANOVA and simple linear regression with STATS Plus, a general statistics package for the Apple II/IIe computers (Human Systems Dynamics, North Ridge, CA). Duncan's Multiple range test was used for multiple comparision of means (O'Mahony, 1986).

RESULTS AND DISCUSSION

Product Yield in Surimi Unit Operations.

The main concerns during surimi operations are to maintain quality and to obtain maximum yield. Therefore, it is necessary to discuss each process in the unit operations as key factors in surimi production.

Pacific whiting was planked by hand (separation of the flesh from head, viscera and backbone), and mechanically deboned by using an Ikeuchi flesh separator. This process involved the separation of bone and skin from the flesh.

By using a mechanical flesh separator, Steinberg (1972) and Miyauchi (1972) reported minced yields (frames were included) of 60% on English sole versus 30% from whole fillets; 47% on minced flounder versus 31% for flounder fillets. It is important to note that the higher yield may contain materials detrimental to surimi quality. According to Martin (1976), a 30% yield of mechanically deboned minced flesh from whiting-related species such as Alaska pollack is necessary from an economic standpoint.

Studies done with Pacific whiting by Crawford et al. (1972), have shown a planked yield of 44.7%, and a minced yield of 35.5% based upon round weight using a Yanagiya flesh separator. Pacheco-Aguilar (1986) reported a planked yield of 42.9% and a minced yield of 32.4% based upon round weight in the same species using an Ikeuchi flesh separator.

In the current investigation, means of 42.4% from planks and 34.7% from minced flesh based upon round weight were obtained (Table 4). The planked flesh yield was lower compared to the planked yields reported

for other fish species by Steinberg (1972) and Miyauchi (1972). The yield of minced flesh was higher compared with Martin's (1976) results. The yield percentages of planked and minced flesh that resulted in the current investigation were within the ranges reported by Crawford et al. (1972) and Pacheco-Aguilar (1986) for Pacific whiting.

One of the most critical processes in surimi unit operations is the washing and dewatering of the minced flesh. During this process, blood, fat, water soluble proteins, pigments, trimethylamine oxide and other undesirable matter are removed. Lee (1985) reported that a volume of water 5 to 10 times that of minced flesh and at least three exchanges were necessary to obtain a satisfactory product. According to Babbitt et al. (1985), yields of washed minced flesh vary depending on the fish species, the initial condition of the fish, the ratio of water to flesh, and the number of washing cycles used.

Pacheco-Aguilar (1986), reported on the effectiveness of using a (3 :1) ratio of chilled water to minced flesh in two exchanges with Pacific whiting. These conditions were favorable from the standpoint of economy and from the reduction found in trimethylamine oxide (87%); lipid (15.8%) and ash (79.3%). Therefore in the present investigation, similar washings were used. The resulting product was a white, odorless and tasteless minced flesh.

In the current investigation a mean yield of 21.2% based upon round weight, was obtained as a dewatered, washed, minced flesh (Table 4). This value was slightly higher compared to the 19% yield obtained from Alaska pollack by Babbitt et al. (1985).

However, the mean yield resulting in the current investigation was mainly affected by the yield from lot 3. This was due to the

inefficiency of the dewatering system which requires a minimum operational load of 18 kg. Generally trials involved 40 kg; only 16 kg was available for lot 3.

The next process in the current investigation was to refine the dewatered washed product by using a strainer flesh separator, in which residues of bone, connective tissue and skin were removed.
The resulting product was then mixed with cryoprotectants (sucrose, 4%; sorbitol, 4%; phosphates, 0.3%) in order to avoid protein denaturation during frozen storage (Matsumoto, 1978).

Hilderbrand (1986) reported a yield of 25% surimi from Alaska pollack, and Lloyd et al. (1985) reported a 20% yield from menhaden surimi. Both were processed by conventional methods.
In the current investigation a mean yield of 17.4% from the refined product, and a mean yield of 19.9% surimi was obtained (Table 4). The mean yield for both products was affected from the values of lot 3 as previously discussed. When this value was not used to calculate the mean, the yield became 21.4%.

Table 4. Yield of flesh from surimi unit operations

Sample	Based on round weight (%) Lot 1	Lot 2	Lot 3	Lot 4	Mean
Round Fish	100.00	100.00	100.00	100.00	100.00
Planked	41.5	43.6	39.8	44.8	42.4 ± 2.2
Minced Flesh	39.8	33.5	30.2	35.2	34.7 ± 4.0
Washed Flesh	26.4	22.0	13.2	23.1	21.2 ± 5.7
Refined	21.6	15.8	11.2	21.0	17.4 ± 4.8
Surimi	23.5	17.7	15.3	22.8	19.9 ± 4.0
					21.4 ± 3.2[1]

[1] Mean calculated without the value from lot 3

Proximate Composition of Flesh in Surimi Unit Operations

In this section the proximate composition of Pacific whiting flesh in surimi unit operations is compared with other fish species. Table 5 illustrates the proximate composition of filleted flesh of different species, including Pacific whiting.

Table 5. Proximate composition of edible fillets from different species based on wet weight.

Species	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)
Whiting[a]	82.2	15.8	1.7	0.9
Cod[a]	80.9	18.4	0.4	0.9
Croaker[b]	79.4	18.1	1.9	1.1
Flounder[b]	77.0	21.2	1.2	1.2
Sardine[c]	77.1	19.0	3.7	2.6
Pacific Herring[c]	72.8	18.3	8.5	1.3

[a] Jhaveri et al. (1984)
[b] Anthony et al. (1983)
[c] Sidwell (1981)

Differences in proximate composition among fish species are clearly shown in Table 5. According to Love (1957) and Holdway and Beamish (1984), factors such as geographical location, seasonal variations, feeding habits and heterogeneity of tissue affect the proximate composition in different species and within the same species.

Cod and Pacific whiting are considered lean fishes due to their low lipid content that ranges from 0.42 to 1.67% (Jhaveri et al., 1984). In comparison, sardine and herring are considered fatty fishes with levels ranging from 3% to 8.5% (Sidwell, 1981).

In the current investigation proximate composition (Table 6) values in deboned flesh agree with those values for whiting reported by Jhaveri et al. (1984) and Pacheco-Aguilar (1986). Ranges of moisture (82.18 to 82.58%); protein (15.55 to 15.78%); lipid (0.82 to 1.67) and ash (0.86 to 1.05%) were found.

According to Lloyd et al. (1985), a final moisture content of 78% is necessary for the satisfactory performance of surimi in future products. Moisture content in the surimi product was decreased from 83.93% to 77.26% due to the addition of cryoprotectants (8.3%) by weight. This value is considered acceptable. A slight variation of protein content is shown during the whole process, although during washing most of the water soluble proteins were removed.

Lipid and ash content were mainly affected during the washing procedures, in which most of the lipids and inorganic matter were removed. There was a net reduction of 40.73% in lipid, and 77.36% in ash content, both from the deboned flesh to the refined product.

Table 6. Proximate composition of flesh during surimi unit operations.

Sample	Moisture	Protein	Lipid	Ash
	(%) wet weight			
Deboned	82.06 (0.61)[a]	15.29 (0.54)	1.53 (0.71)	1.06 (0.06)
Flesh, first wash	84.70 (0.84)	14.91 (0.65)	0.96 (0.34)	0.39 (0.01)
Flesh, second wash	81.89 (1.72)	15.40 (1.51)	1.08 (0.60)	0.40 (0.07)
Refined	83.93 (0.95)	14.37 (0.76)	0.91 (0.17)	0.24 (0.02)
Surimi [b]	77.26 (1.28)	13.34 (1.20)	0.64 (0.19)	0.50 (0.02)

[a] Figures in parentheses are the standard deviation for each value.
[b] Surimi is composed of refined product mixed with 4% sorbitol, 4% sucrose and 0.3% polyphosphates.
n = 9

Surimi production requires large quantities of fresh water during the washing process, and the volume used depends upon the the fish species and the required quality of the surimi (Lloyd et al., 1985). During this process the water is discharged as a waste water. It contains not only undesirable matter, but also water soluble proteins and other compounds that potentially have favorable utilization.

In this study, the water used for washing the minced flesh was collected and proximate composition was determined. Range values for moisture content for the first and second washes were from 99.05 to 99.45%; protein from 1.22 to 1.84%; lipid from 0.09 to 0.13% and ash from 0.06 to 0.15%. No significant differences from the first to the second washes were seen. These samples contained 0.8% total solids (estimated value dry weight basis), in which 87.87% are considered water soluble proteins. According to Lee (1984) and Suzuki (1981), studies for utilization of waste water are being done with emphasis on recovering the solids as a source of either animal feed or for human consumption.

Myofibrillar Proteins

As previously mentioned, the quantity of extractable myofibrillar proteins are a determinant in gel formation. Therefore, their functionality affects the textural integrity of the product. The myofibrillar proteins generally constitute 66 to 77% of the total protein in fish meat (Suzuki, 1981). Reported values of 76% of extractable myofibrillar proteins from cod, and a range of 70 to 79% in carp and flatfish in fresh fillets by Suzuki (1981), indicate variations

in species and processing conditions. In this study, an average of 54.04 $\pm$ 4.96 % extractable myofibrillar proteins was obtained from surimi, indicating low yield compared to the per cent extracted from fresh fillets. This reduction may be due to the handling of the fish and particle losses during the dewatering process.

Screening Protein Adjuncts

Preliminary work involved studies of egg white (EW), whey protein concentrate (WPC) and soy protein isolate (SPI), as ingredients at different concentrations in kamaboko gels and their effect on texture. The gels were prepared with 2% NaCl and individual treatments were mixed with different concentrations (1 to 5%) of each protein adjunct. The batters were adjusted to 78% moisture.

Gels were heated at 90^{o} C for 1 hour. There was a significant difference ($P<0.05$) in hardness among EW, SPI and WPC for concentrations 3 to 5%. Addition of egg white resulted in the highest degree of hardness. Cohesiveness among the three protein adjuncts was not significantly different ($P>0.05$) despite the concentrations used. Elasticity was significantly different among EW, SPI and WPC for concentrations from 2 to 5% ($P<0.001$). Elasticity with EW resulted in a significant difference from 3 to 5% compared to the 1 to 2% levels ($P<0.005$). However, gels containing levels of 4% and 5% EW showed a decrease in elasticity and in addition, developed a strong odor of EW. For SPI concentrations, no significant differences in elasticity ($P>0.05$) resulted. At levels of 1 to 2% WPC, the elasticity was significantly lower compared to the other levels studied ($P<0.05$).

Based on these studies, 3% egg white was selected as the preferred protein adjunct for the final preparation of kamaboko gels.

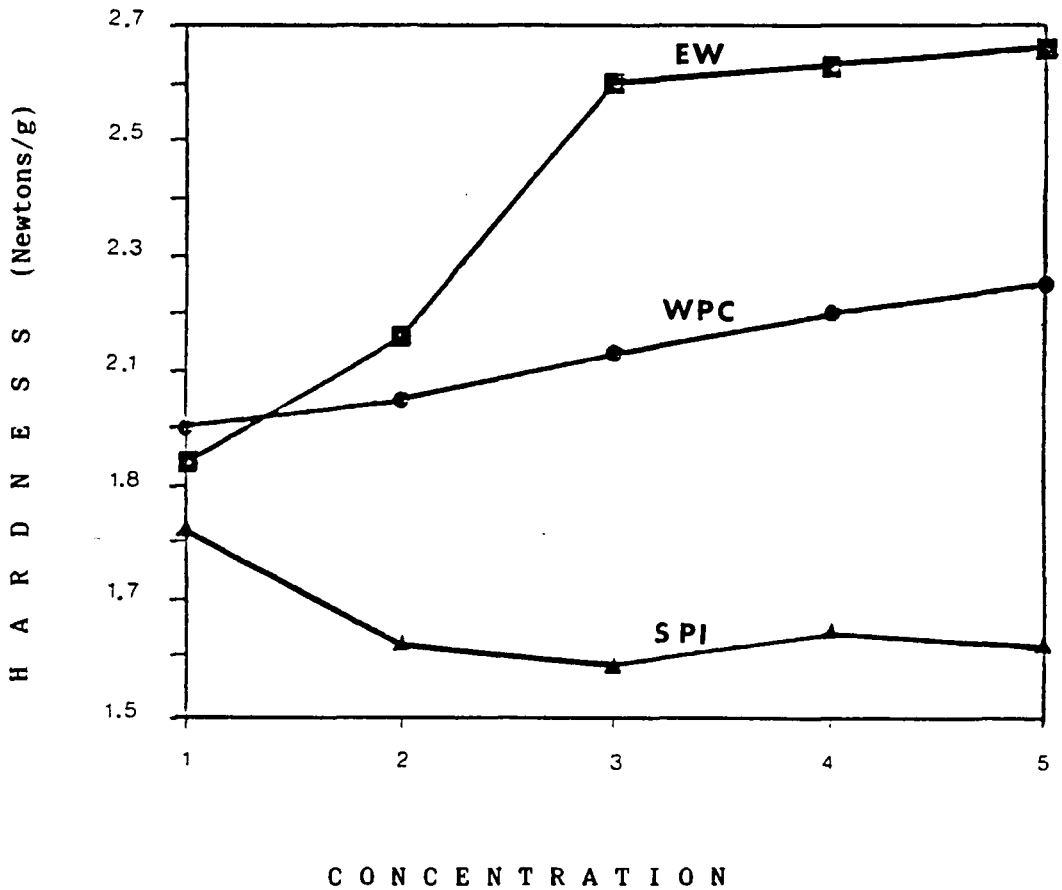


Figure 4. Effect of concentration of protein adjuncts egg white (EW), whey protein concentrate (WPC) and soy protein isolate (SPI) on the hardness of cooked kamaboko.

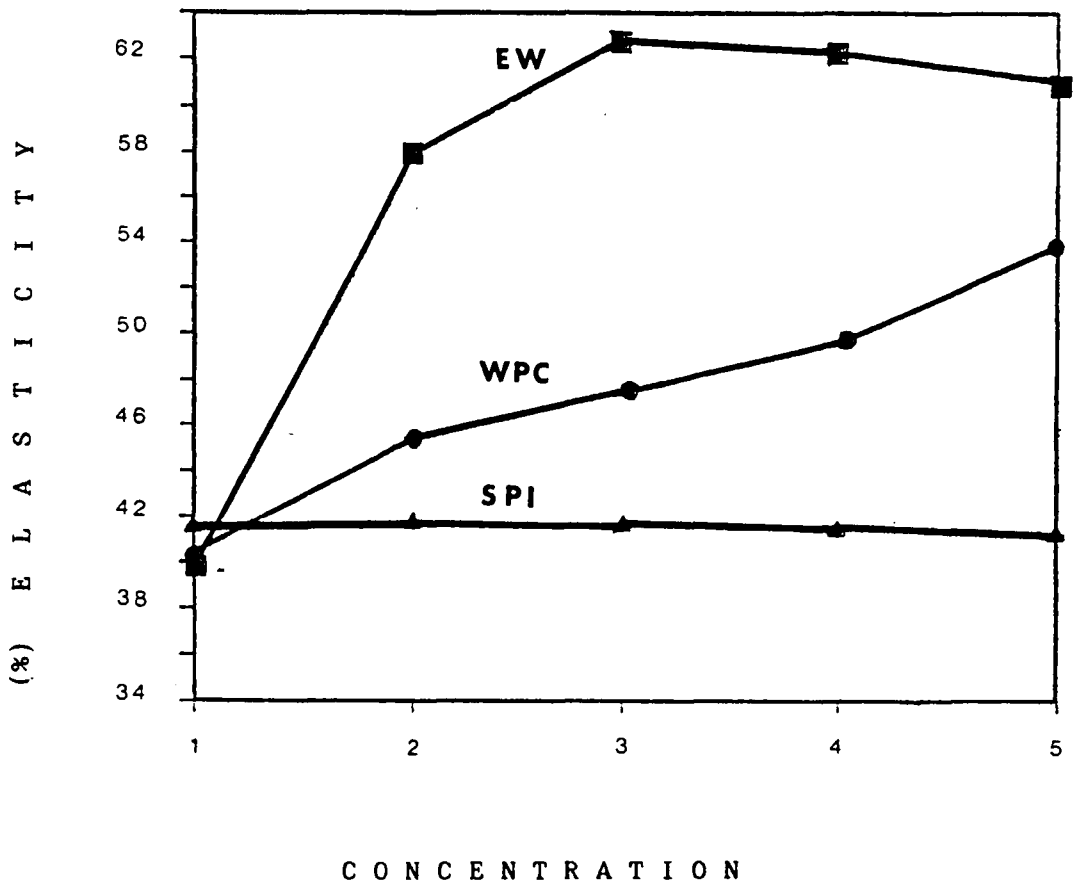


Figure 5. Effect of concentration of protein adjuncts egg white (EW), whey protein concentrate (WPC) and soy protein isolate (SPI) on the elasticity of cooked kamaboko.

Effect of Temperature

Gels containing 3% NaCl, 3% EW and 78% moisture were subjected to two stage heat treatments at 40^{o} C for varying times followed by 90^{o} C for 20 minutes. No significant differences were found in these treatments ($P<0.01$). However, when the gels were subjected to a one stage heat treatment of 90^{o} C for either 40 min or 1 hour, no significant differences in hardness and elasticity were found ($P<0.05$) compared to the two stage treatments. Based on these studies, the single stage heat treatment was selected for the subsequent investigation of kamaboko gels.

Preparation and Evaluation of Kamaboko Gels

As previously indicated, egg white was selected for use in the subsequent work to improve the texture profile of kamaboko gels. Three formulations were calculated based on surimi weight and adjusted to 76% moisture; the control or A (surimi, 3% NaCl), B (surimi, 3% NaCl, 3% egg white), and C (surimi, 3% NaCl, 3% egg white, 5% potato starch). Determinations of pH and moisture were conducted on uncooked and heat set gels. Table 7, shows these results indicating that changes in pH and moisture after processing the gels were not significantly different ($P>0.05$). Moisture content differed slightly in the three formulations (A, B, C) due to the addition of dry ingredients.

Table 7. Moisture and pH determinations in raw and cooked kamaboko.

	Raw		Cooked	
Formulation	% Moisture	pH	% Moisture	pH
A	75.82 (0.63)[1]	6.96 (0.24)	75.59 (0.20)	6.97 (0.21)
B	76.35 (0.30)	6.93 (0.25)	76.05 (0.36)	7.01 (0.21)
C	77.16 (0.40)	6.94 (0.19)	77.09 (0.65)	6.97 (0.18)

A = Surimi. 3% NaCl, B = Surimi, 3% NaCl, 3% egg white, C = Surimi, 3% NaCl, 3% egg white, 5% potato starch.

[1] Figures in parentheses are the standard deviation for each value. Moisture content was adjusted to 76 $\pm$ 1%.

The interaction of protein and water is extremely important in comminuted fish flesh products, and it has been expressed as water holding capacity (WHC). According to Hamm (1986), WHC is defined as the ability of meat to hold its own or added fluid during application of any force. After comminution, the cell wall of the muscle fibers is destroyed. Therefore differences between extracellular and intracellular water are eliminated and the absorbancy of the thick filaments or myosin determines the WHC of meat (Fukazawa, 1961).

Regenstein (1984), discussed different methods to measure water retention properties by means of water binding potential (a synonym for WHC) and expressible moisture (EM). Expressible moisture, is the amount of water that is entrapped but not bound to the protein system, and can be determined by pressing the sample with a fixed compression.

Lee and Toledo (1976) found a correlation between EM with texture measurements made from Spanish mackerel. This indicates that EM also

can be used as a reliable measurement with relation to binding properties of water and protein in comminuted product.

The addition of ingredients also affects the textural parameters by improving the quality of kamaboko gels, according to their individual composition. As previously mentioned, the addition of egg white and potato starch are important ingredients in Japanese products since, in combination, they impart a meaty texture to the final product.

In this investigation, texture parameters as well as expressible moisture were determined on the cooked gels. They were prepared from the formulations previously described, and subjected to 90^{o} C for 40 min. Table 8 shows the following results: formulation (B) increased in hardness (154%) and elasticity (464%) compared to the control (Formulation A). Formulation (C) increased in hardness (9%) and elasticity (23%) compared to formulation (B). The EM was significantly reduced ($P<0.003$) from formulation (A) to formulation (C), due to the addition of the ingredients and their competition for available water.

Table 8. Texture and expressible water measurements in cooked kamaboko.

Formulation	Hardness[1] N/g	Cohesiveness[1]	Elasticity[1] (%)	Expressible[2] Moisture (%)
A	15.06a (4.34)[3]	0.10a (0.03)	10.95a (0.37)	67.95 (2.07)
B	38.24b (6.73)	0.31b (0.02)	61.71b (8.50)	64.60 (1.80)
C	42.12c (3.57)	0.31b (0.01)	80.50c (4.76)	62.74 (1.62)

A = surimi, 3% NaCl, B = surimi, 3% NaCl, 3% egg white, C = surimi, 3% NaCl, 3% egg white, 5% potato starch.
Moisture content was adjusted to 76±1%.
[1] Different letter within columns indicate significant differences (P<0.001).
[2] (P<0.003)
[3] Figures in parentheses are the standard deviation for each value.

The effect of egg white and potato starch on the gel texture can clearly be seen. Some researchers have speculated whether or not these ingredients could also have an inhibiting effect on alkaline proteases. Makinodan et al. (1985), reported an inhibiting effect of potato extract, but not an increased hardness in gels made from white croaker. However, Lanier et al. (1981), by using the same extract with the same species found an improvement of the gel texture as well as an inhibiting effect on alkaline proteases. Miller and Spinelli (1982), used egg white and potato extract in low concentrations (1 to 5 mg/ml), and did not find a significant inhibitory effect on alkaline proteases of Pacific whiting.

Based on these reports, it is possible that the concentrations of egg white and potato starch are determinants in the inhibitory effect of proteases. This point will discussed in a later section.

Protease Activity

The presence of the Myxosporidian parasite is considered to be the major cause of the poor texture quality in Pacific whiting. There are two possible mechanisms of action: first, that the parasite produces a proteolytic enzyme that breaks down the muscle fibers resulting in an undesirable soft texture. The second possibility is the presence of proteases that are released from the lysozymes as an immune response, and may also contribute to the post mortem breakdown of muscle proteins.

The quality of kamaboko gels greatly depends on the efficiency of the processing and the degree of contamination by viscera, skin and blood residues during processing. According to Su et al. (1981a), higher proteolytic activity is developed in mechanically separated tissue compared with hand-separated tissue, due to retention of bits of skin and contamination with residues from internal organs during the mechanical deboning process. Su et al. (1981b), also indicated that pproteolytic activity still remains even in washed and skinned, minced flesh from Atlantic croaker, therefore affecting the texture, especially the hardness of the cooked gels.

Proteolytic activity in Pacific whiting from the sarcoplasmic fraction is affected by temperature and incubation time (Erickson et al., 1983). Here, Pacific whiting surimi enzyme extract was incubated at different temperatures for 30 min. Preliminary studies related with incubation time did not show any significant effect after 60 and 90 minutes ($P>0.05$). The optimum temperature was found to be 55^{o} C (Figure 6). Therefore these conditions were used to determine the proteolytic activity in samples taken during each surimi unit operation.

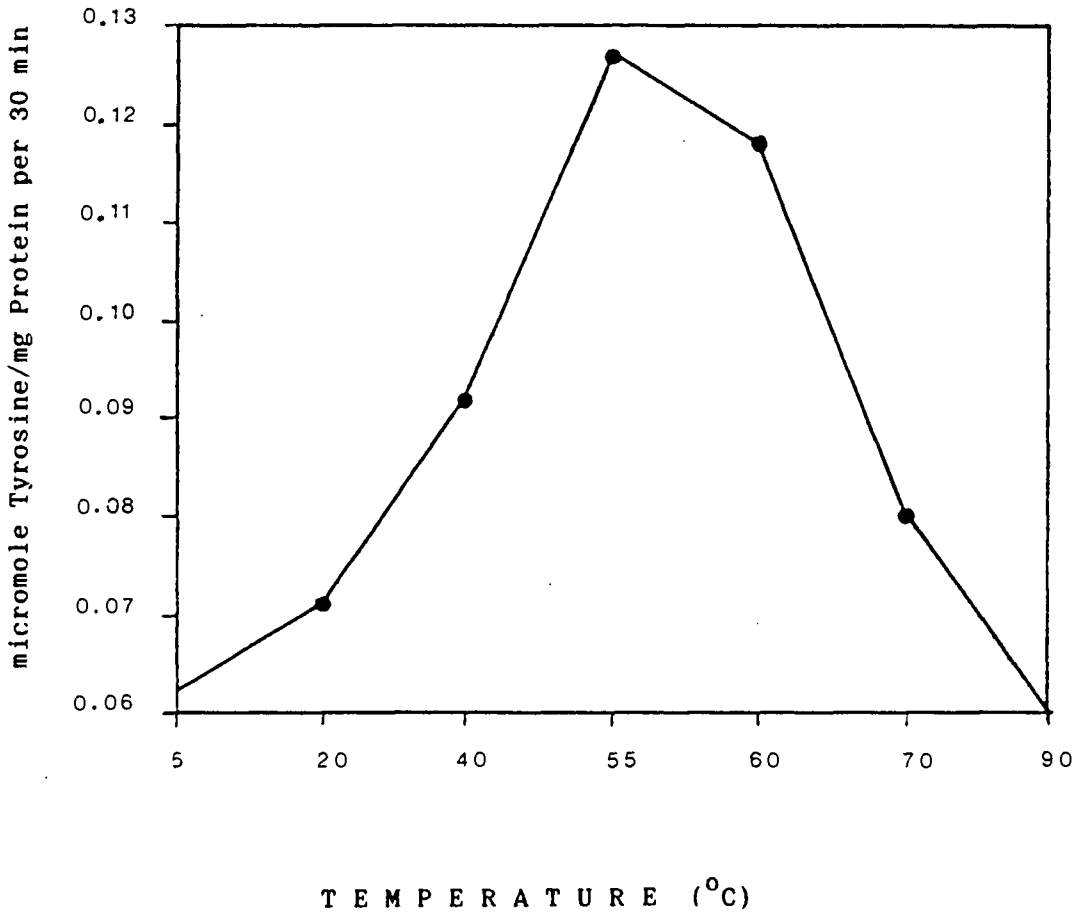


Figure 6. The effect of temperature on proteolytic activity of a crude extract from Pacific whiting surimi.

Table 9 shows the difference in protease activity in each of the three lots at the different steps during surimi processing. The results indicate differences among the lots and the samples taken during the process. A significant decrease in proteolytic activity was found after the second washing ($p<0.05$). This demonstrates the importance of washing the minced flesh in the process, in which water soluble proteins i.e., enzymes are leached out. The resulting product is a white, minced flesh highly concentrated in myofibrillar proteins. The flesh from the second washed and refined product showed no significant differences ($p>0.05$) in proteolytic activity from one another.

Table 9. Reduction of protease activity during surimi unit operations.

	Tyrosine/mg prot. (μ mol)		Remaining Protease Activity (%)
Lot 2			
Deboned	0.28	(0.01)[1]	100.0
Flesh, first wash	0.20	(0.05)	71.4
Flesh, second wash	0.17	(0.04)	60.7
Refined	0.13	(0.02)	46.4
Lot 3			
Deboned	0.38	(0.02)	100.0
Flesh, first wash	0.32	(0.01)	84.2
Flesh, second wash	0.25	(0.03)	65.8
Refined	0.23	(0.01)	60.5
Lot 4			
Deboned	0.31	(0.02)	100.0
Flesh, first wash	0.28	(0.01)	90.3
Flesh, second wash	0.21	(0.02)	67.4
Refined	0.17	(0.01)	54.8
Mean[2]			
Deboned	0.32	(0.05)[a]	100.0
Flesh, first wash	0.26	(0.06)[a]	82.0
Flesh, second wash	0.21	(0.04)[b]	64.4
Refined	0.18	(0.05)[b]	53.9

[1] Figures in parentheses are the standard deviation for each value.
[2] Different letters within columns indicate significant differences. ($P<0.05$)

Table 10 shows the correlation coefficients (R), indicating the high correlation effect of proteolytic activity in the refined flesh on the hardness, elasticity and expressible moisture of the cooked gels in the three different lots.

Table 10. Correlation coefficients (R) relating texture parameters, expressible moisture in cooked kamaboko, with protease activity in the refined flesh, (lots 2, 3, 4).

Formulation	Hardness vs. Protease	Elasticity vs. Protease	% Exp moist vs. Protease
A	-0.98	-0.98	0.99
B	-0.95	-1.00	0.97
C	-0.96	-0.97	0.94

A = surimi. 3% NaCl: B = surimi. 3% NaCl. 3% egg white;
C = surimi, 3% NaCl, 3% egg white, 5% potato starch.

Effect of Temperature and Ingredients on Gelation in Kamaboko

The function of the myofibrillar proteins in a comminuted product is their ability to form a continuous gel, and among these proteins myosin is the primary agent in this process (Fukazawa, 1961). The quality of kamaboko gels is affected in particular by the fish species, processing conditions and heat treatments.

Studies on protein changes during heating and the effect on textural parameters have been reported by Makinodan and Ikeda, (1971); Cheng et al., (1979) and Lanier et al., (1981). All have demonstrated that gel setting of fish muscle proteins can take place at temperatures above 55^{o} C. and also can occurr at chill temperatures. However, when

the gels pass through a heating zone from 60 to 70^{o} C, part of the gel structure is altered, and the gel becomes soft (Makinodan and Ikeda, 1971; Cheng, et al., 1979). This phenomenon more often occurs in unwashed fish tissues, where much of the soluble proteins and proteases still remain (Lee, 1984). According to Cheng et al., (1979), kamaboko gels made from mullet, ribbon and sand trout were not as sensitive as those from croaker gels heated to 70^{o} C or lower.

From these studies, it can be clearly seen that gel setting behavior is a temperature dependent factor. Lee (1984), pointed out that a fast heating rate, a tight cohesive network with a large number of small aggregates is formed. Whereas at a slow heating rate, a loose network with a small number of large aggregates is formed.

As previously mentioned, studies have been done using food ingredients such as egg white, soybean trypsin inhibitor and potato starch in kamaboko gels to investigate their effect as protease inhibitors. A great controversy has resulted from these studies, because of the difference in results, that make it difficult to generalize whether or not these ingredients have an inhibitory effect This may be due to the inconsistent experimental conditions as well as the difference in fish species.

In this study, SDS-gel electrophoresis was performed in order to evaluate the effect of egg white and potato starch during thermal processing in the kamaboko gels. Table 11 shows surimi and the gel formulations under two different heat treatments used for this study.

Table 11. Surimi and gel formulations under two heat treatments.

Formulation	Heat treatment	
Thawed surimi (control 1)	-	-
Surimi (control 2)	I	II
Surimi, 3% NaCl, (A)	I	II
Surimi, 3% NaCl, 5% potato starch (B)	I	II
Surimi, 3% NaCl, 3% EW (C)	I	II
Surimi, 3% NaCl, 3% EW, 5% potato Starch (D)	I	II

I. heat treatment at 55^{o} C for 1 hour.
II. heat treatment at 40^{o} C for 1 hour followed by 90^{o} C for 20 min.

Figures 7a, 10a, 13a show the control 1 in lots 2, 3 and 4, respectively. The presence of the myosin band indicates that no enzymatic breakdown has taken place. However, when control 2, (Figures 7b, 10b, 13b) and gel formulations (Figures 7c, 10c, 13c) in their respective lots, were subjected to the first heat treatment (55^{o} C for 1 hour), disappearance of their myosin band indicated enzymatic breakdown.

Figures 8a, 11a, 14a (lots 2, 3 and 4, respectively) show gel formulation (B) subjected to the first heat treatment, in which the myosin band is not present. This indicates enzymatic breakdown of myosin due to the non inhibitory effect of potato starch. However, formulations C and D show the inhibition effect of egg white under the first heat treatment due to the appearance of myosin band in Figures 8b, 11b, 14b; 8c, 11c, 14c (in lots 2, 3, 4, respectively).

The second heat treatment (40^{o} C for 1 hour followed by 90^{o} C for 20 minutes) was applied to the gel formulations A, C, and D in which the following results were obtained: gel formulation (A) in Figures 9a, 12a, 15a (lots 2, 3, 4, respectively) showed enzymatic breakdown of the

myosin due to the disappearance of its band. Low molecular weight compounds were also visible due to the appearance of more bands. This indicates that hydrolysis has taken place in proteins of high molecular weight.

The effect of egg white present in formulations C and D (Figures 9b, 12b, 15b; 9c, 12c, 15c (lot 2, 3, and 4 respectively) clearly shows its inhibiting effect by the appearance of the myosin band.

Figure 7. Gel electrophoresis scans representing various heat treatments in lot 2. A) Surimi at 4^{o} C; B) Surimi at 55^{o} C for 1 hour; C) Surimi, 3% NaCl at 55^{o} C for 1 hour. The molecular weight of 200,000 corresponds to myosin.

Figure 7

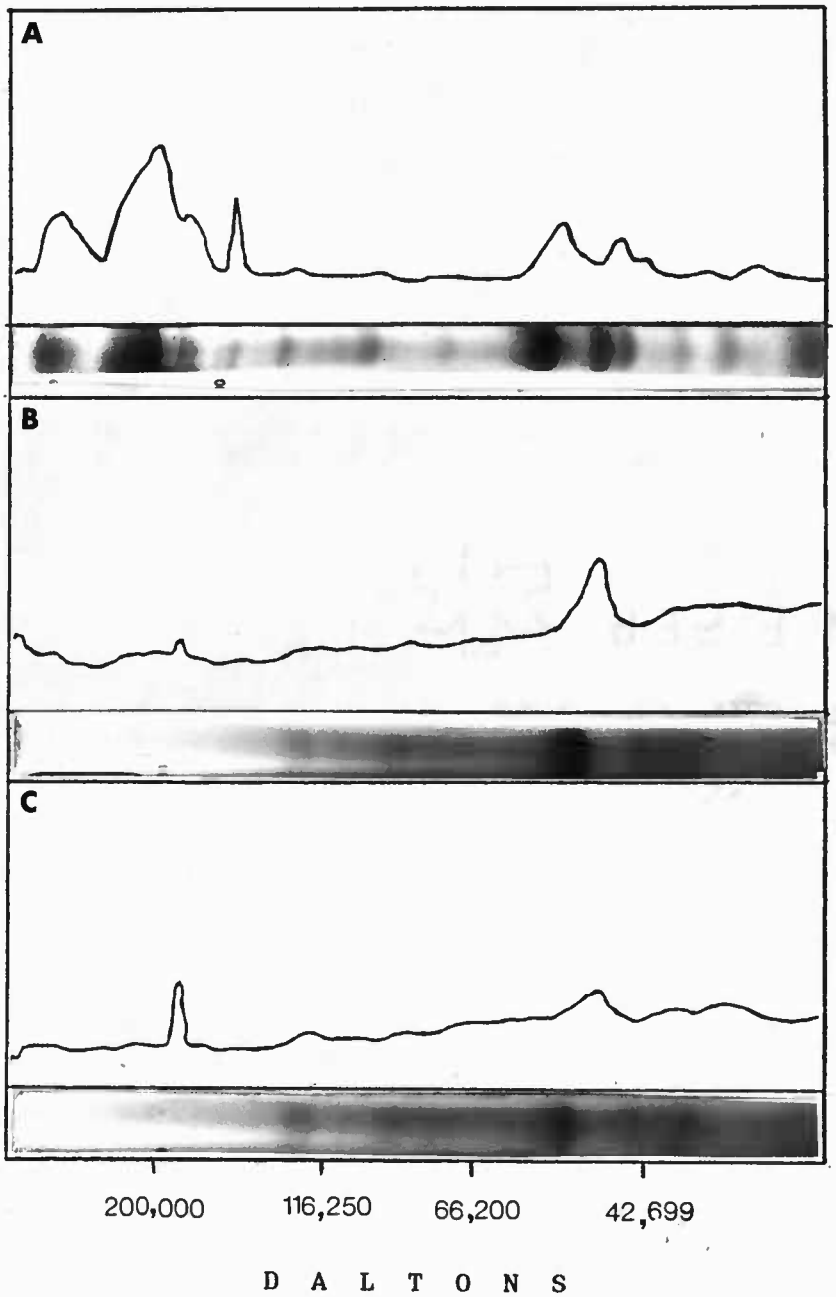


Figure 8. Gel electrophoresis scans representing first heat treatment (55^{o} C for 1 hour) in lot 2. A) Surimi, 3% NaCl, 5% potato starch; B) Surimi, 3% NaCl, 3% egg white; C) Surimi, 3% NaCl, 3% egg white, 5% potato starch. The molecular weight of 200,000 corresponds to myosin.

Figure 8

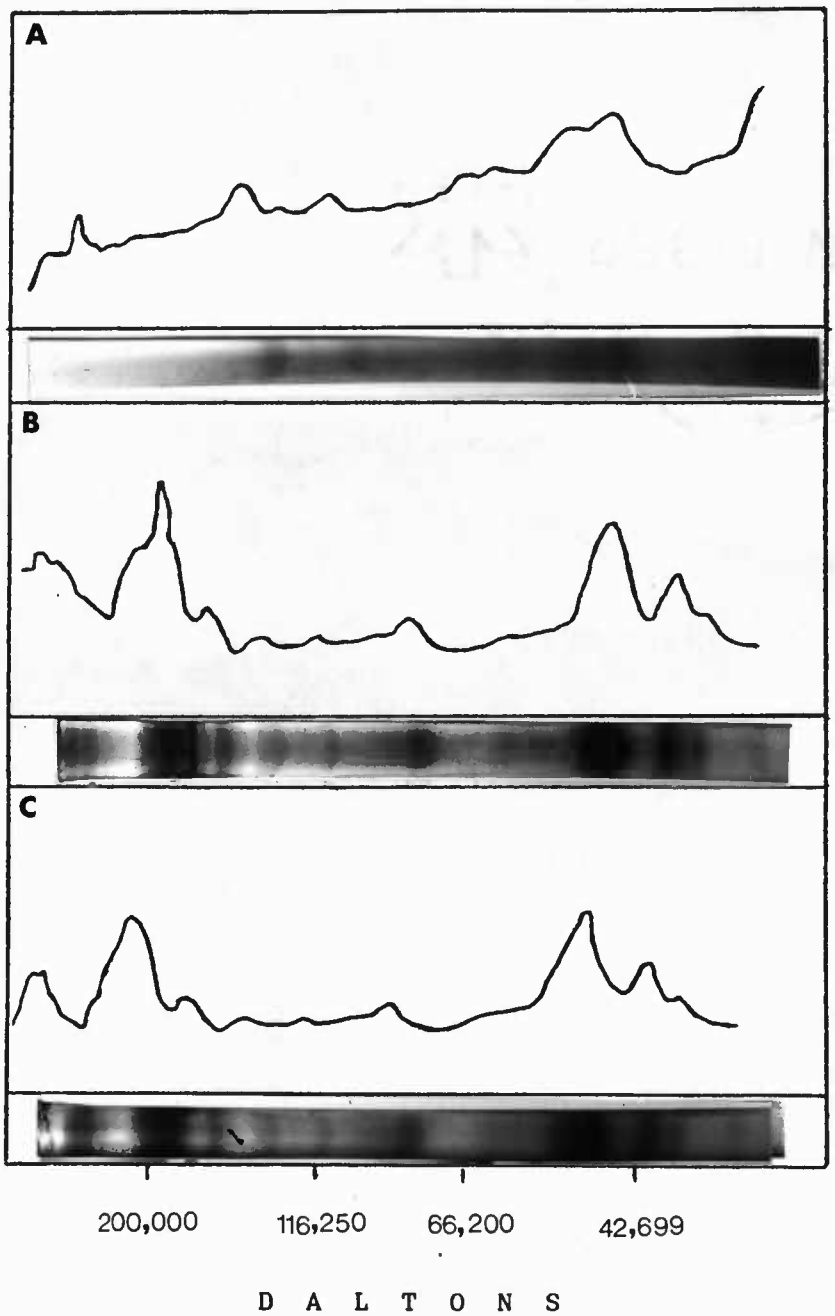


Figure 9. Gel electrophoresis scans representing second heat treatment (40^{o} C for 1 hour followed by 90^{o} C for 20 minutes) in lot 2. A) Surimi, 3% NaCl; B) Surimi, 3% NaCl, 3% egg white; C) Surimi, 3% NaCl, 3% egg white, 5% potato starch. The molecular weight of 200,000 corresponds to myosin.

Figure 9

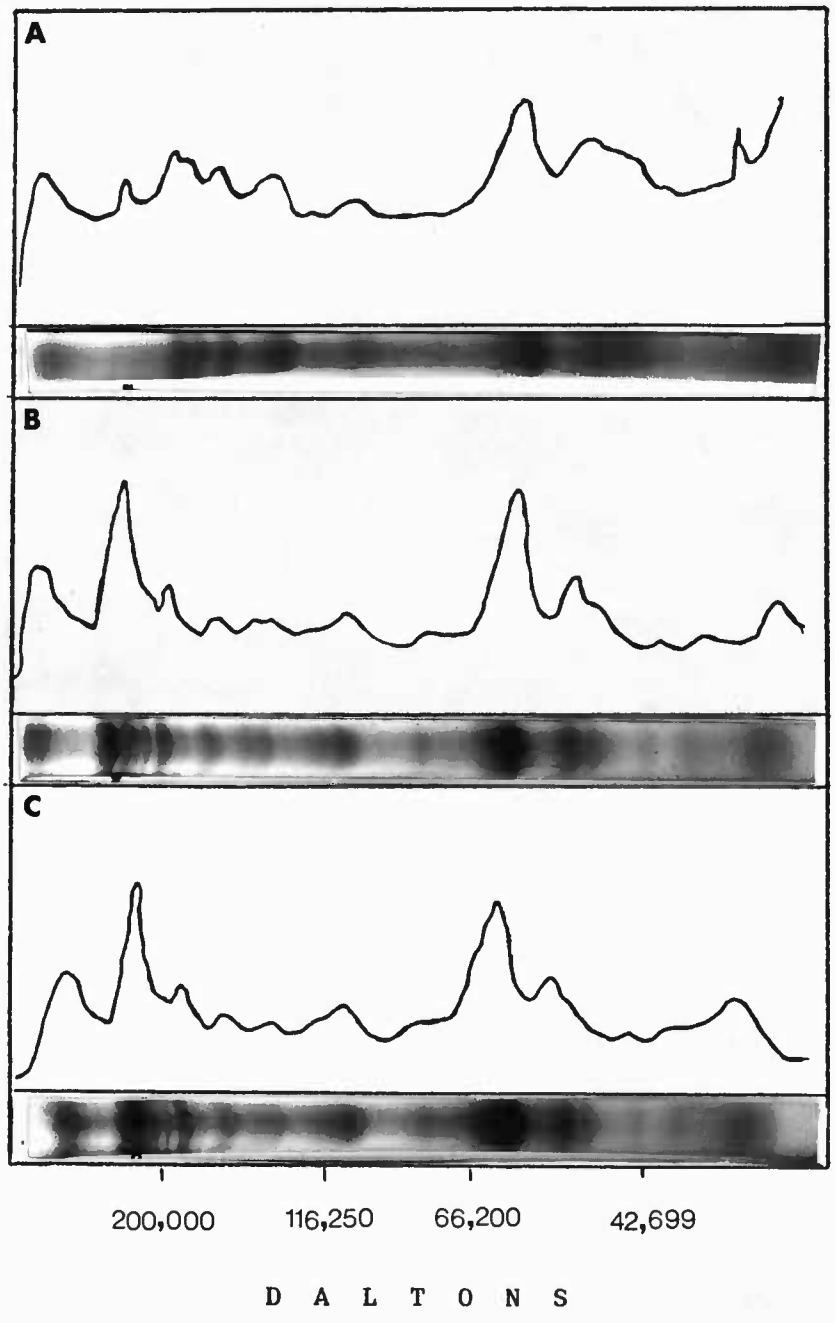


Figure 10. Gel electrophoresis scans representing various heat treatments in lot 3. A) Surimi at 4° C: B) Surimi at 55° C for 1 hour; C) Surimi, 3% NaCl at 55° C for 1 hour. The molecular weight of 200,000 corresponds to myosin.

Figure 10

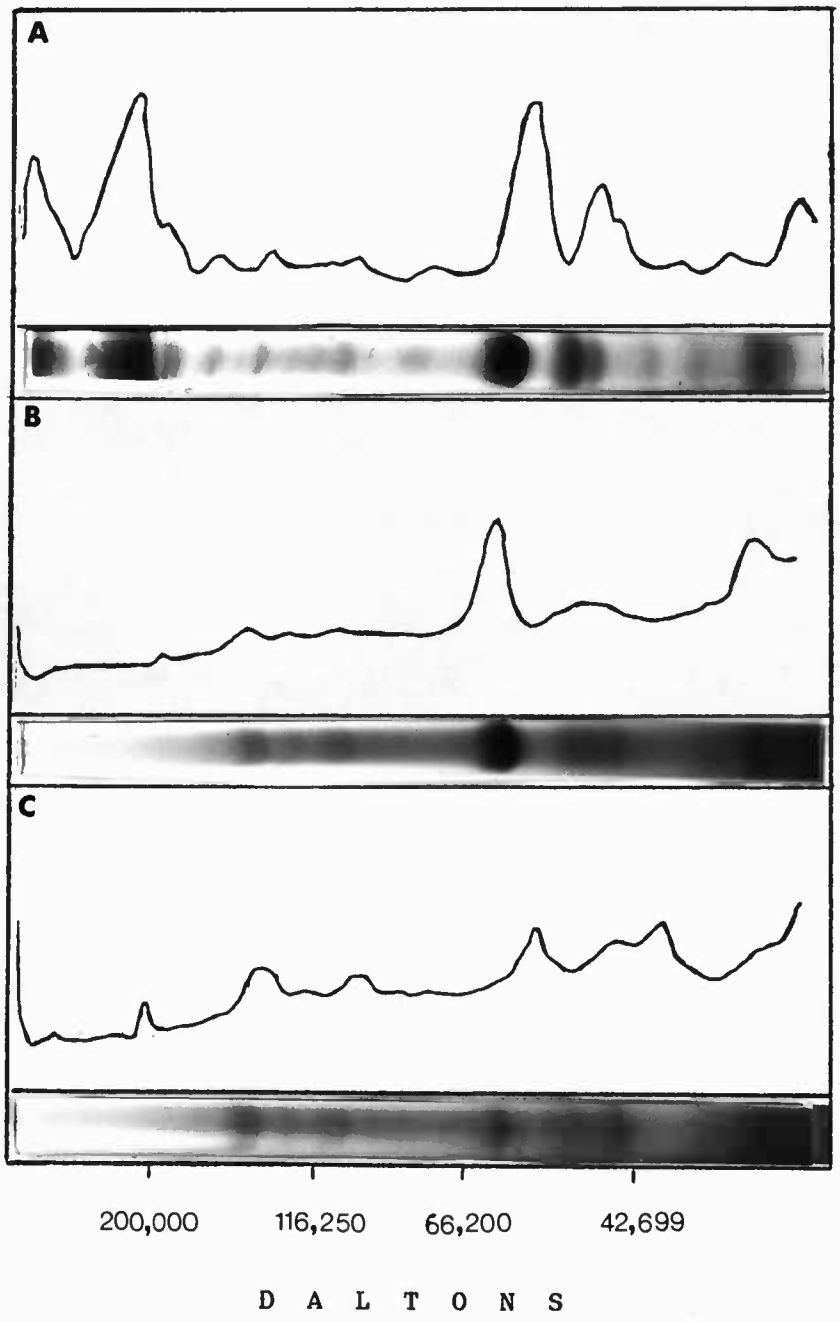


Figure 11. Gel electrophoresis scans representing first treatment (55^{o} C for 1 hour) in lot 3. A) Surimi, 3% NaCl, 5% potato starch; B) Surimi, 3% NaCl, 3% egg white; C) Surimi, 3% NaCl, 3% egg white, 5% potato starch. The molecular weight of 200,000 corresponds to myosin.

Figure 11

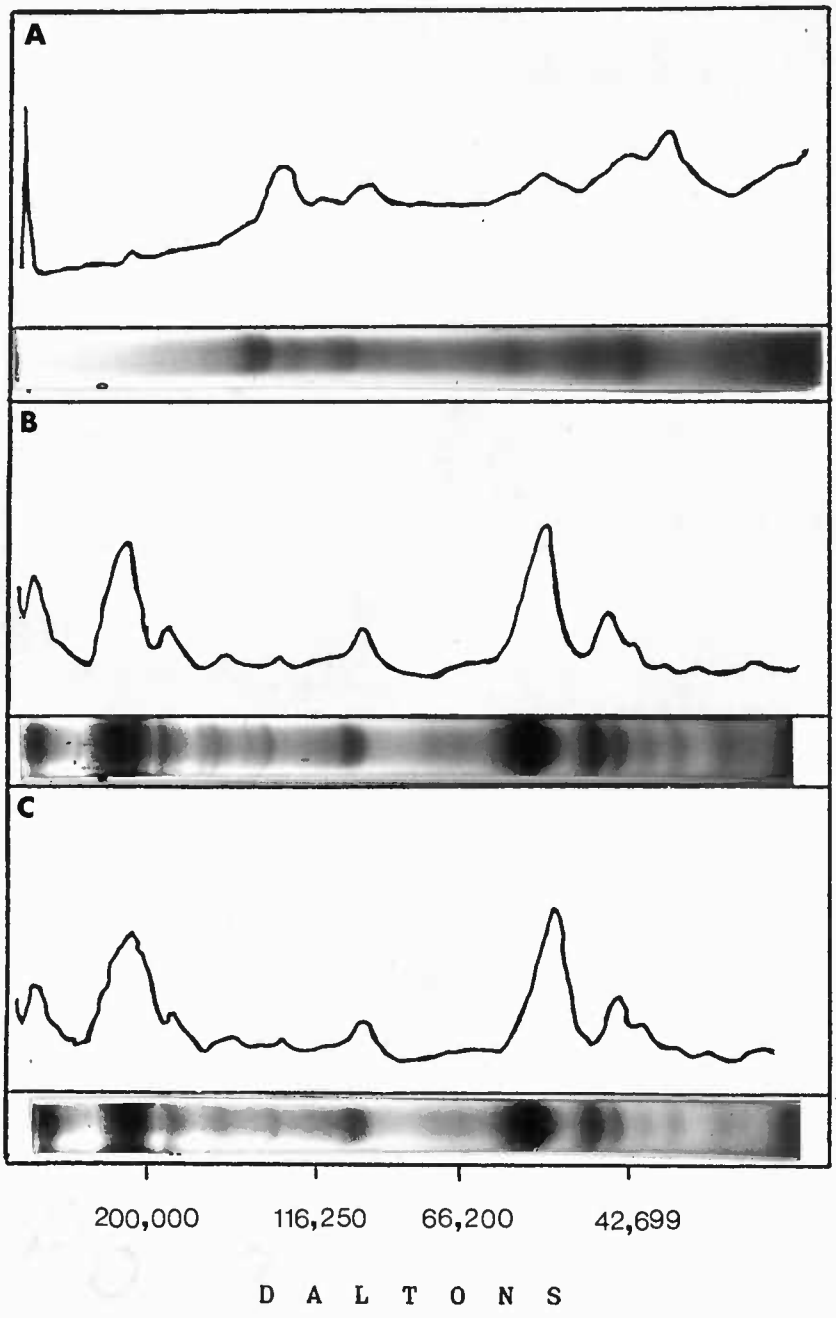


Figure 12. Gel electrophoresis scans representing second heat treatment (40^{o} C for 1 hour followed by 90^{o} C for 20 minutes) in lot 3. A) Surimi, 3% NaCl; B) Surimi, 3% NaCl, 3% egg white; C) Surimi, 3% NaCl, 3% egg white, 5% potato starch. The molecular weight of 200,000 corresponds to myosin.

Figure 12

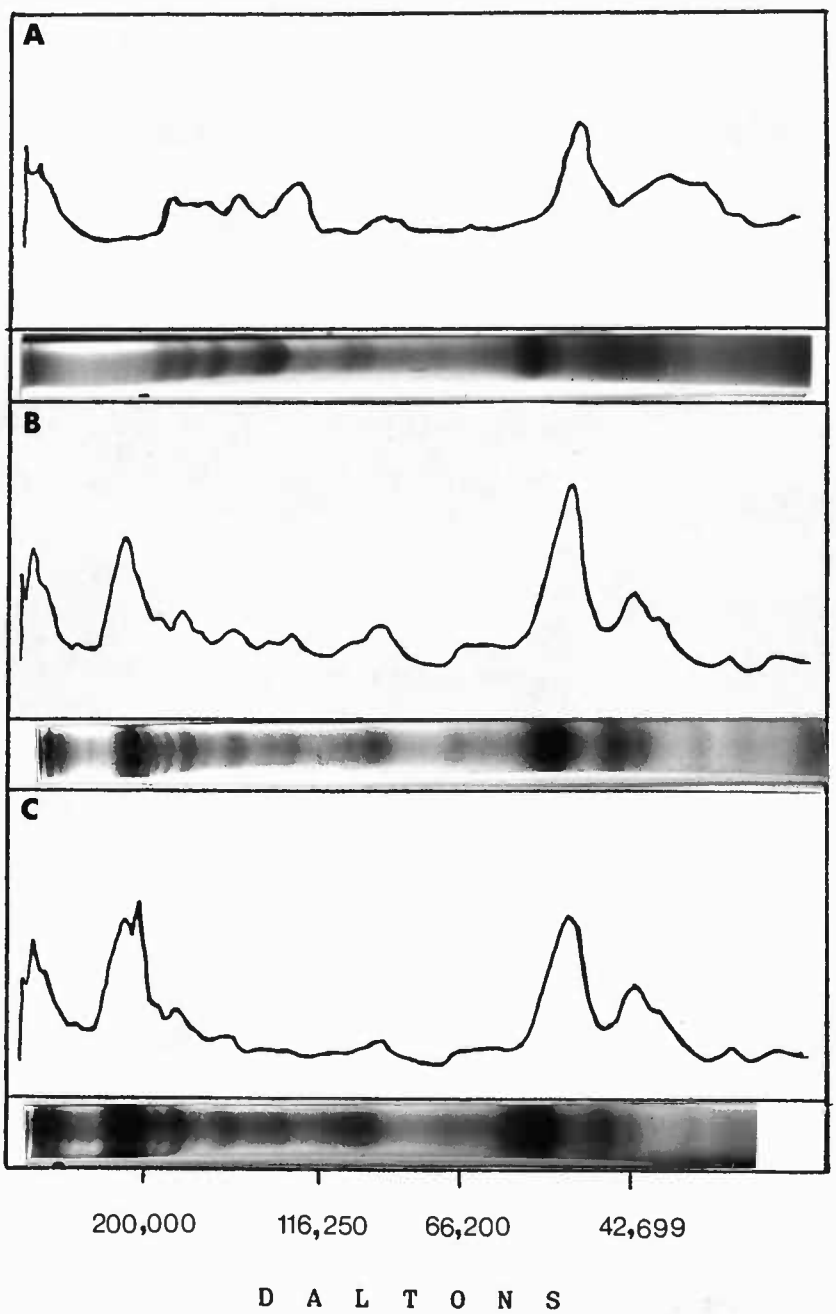


Figure 13. Gel electrophoresis scans representing various heat treatments in lot 4. A) Surimi at 4° C; B) Surimi at 55° C for 1 hour; C) Surimi, 3% NaCl at 55° C for 1 hour. The molecular weight of 200,000 corresponds to myosin.

Figure 13

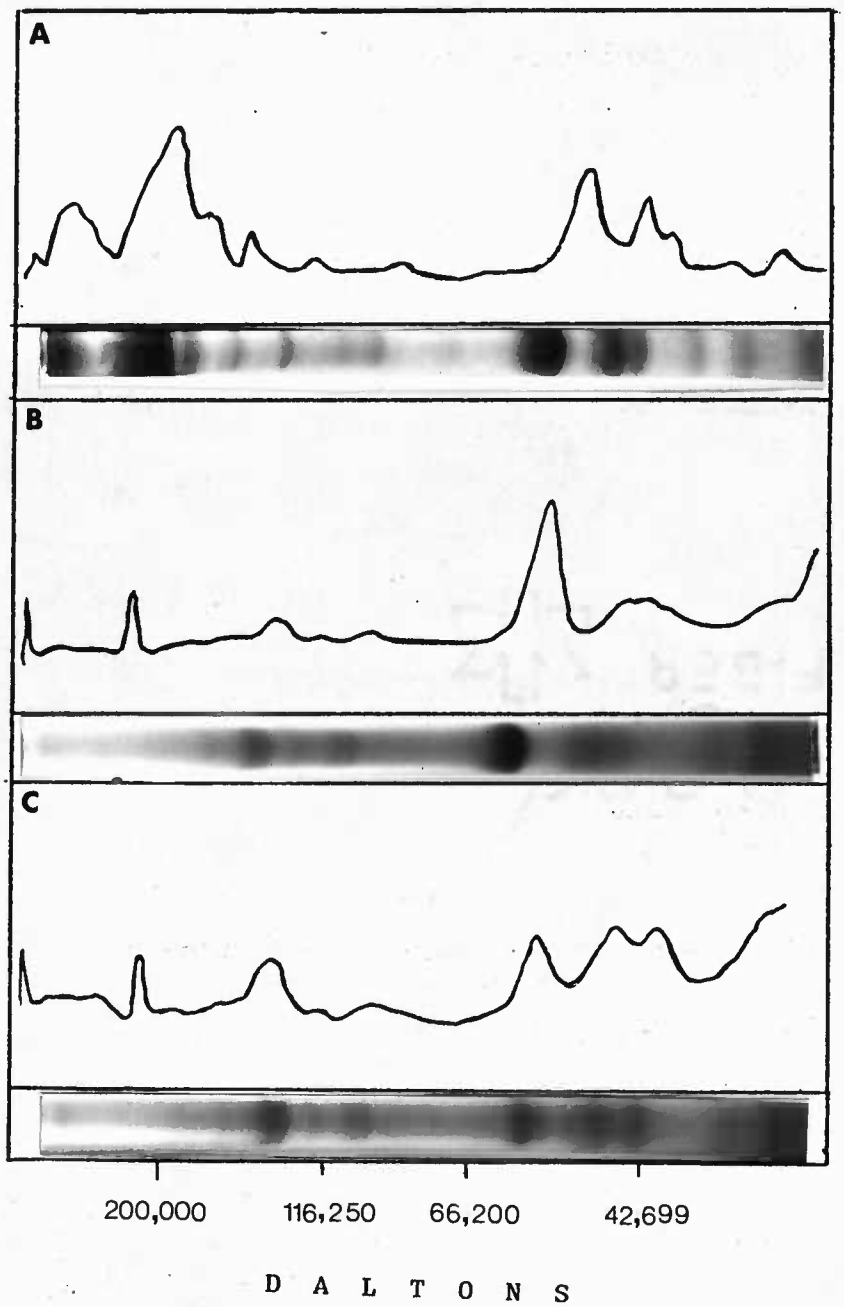


Figure 14. Gel electrophoresis scans representing first heat treatment (55° C for 1 hour) in lot 4. A) Surimi, 3% NaCl, 5% potato starch; B) Surimi, 3% NaCl, 3% egg white; C) Surimi, 3% NaCl, 3% egg white, 5% potato starch. The molecular weight of 200,000 corresponds to myosin.

Figure 14

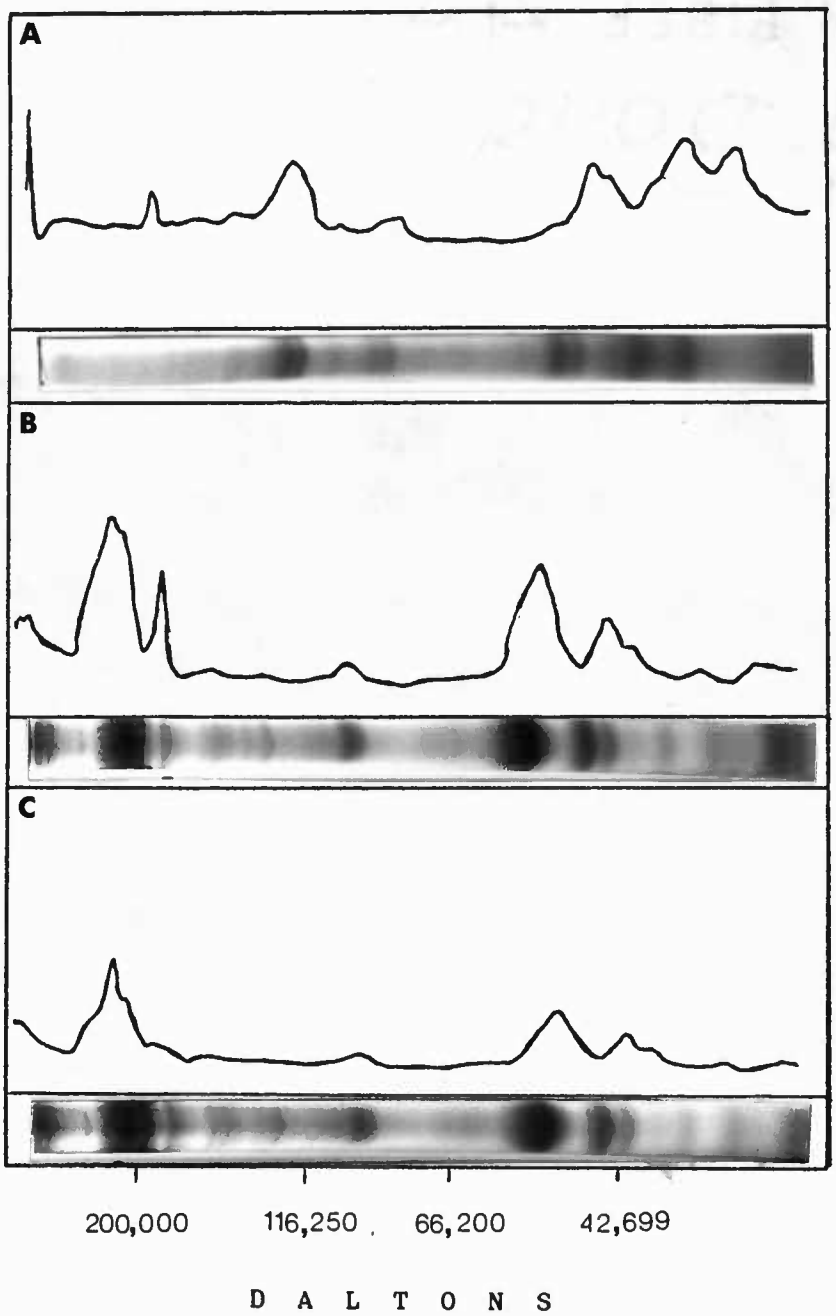
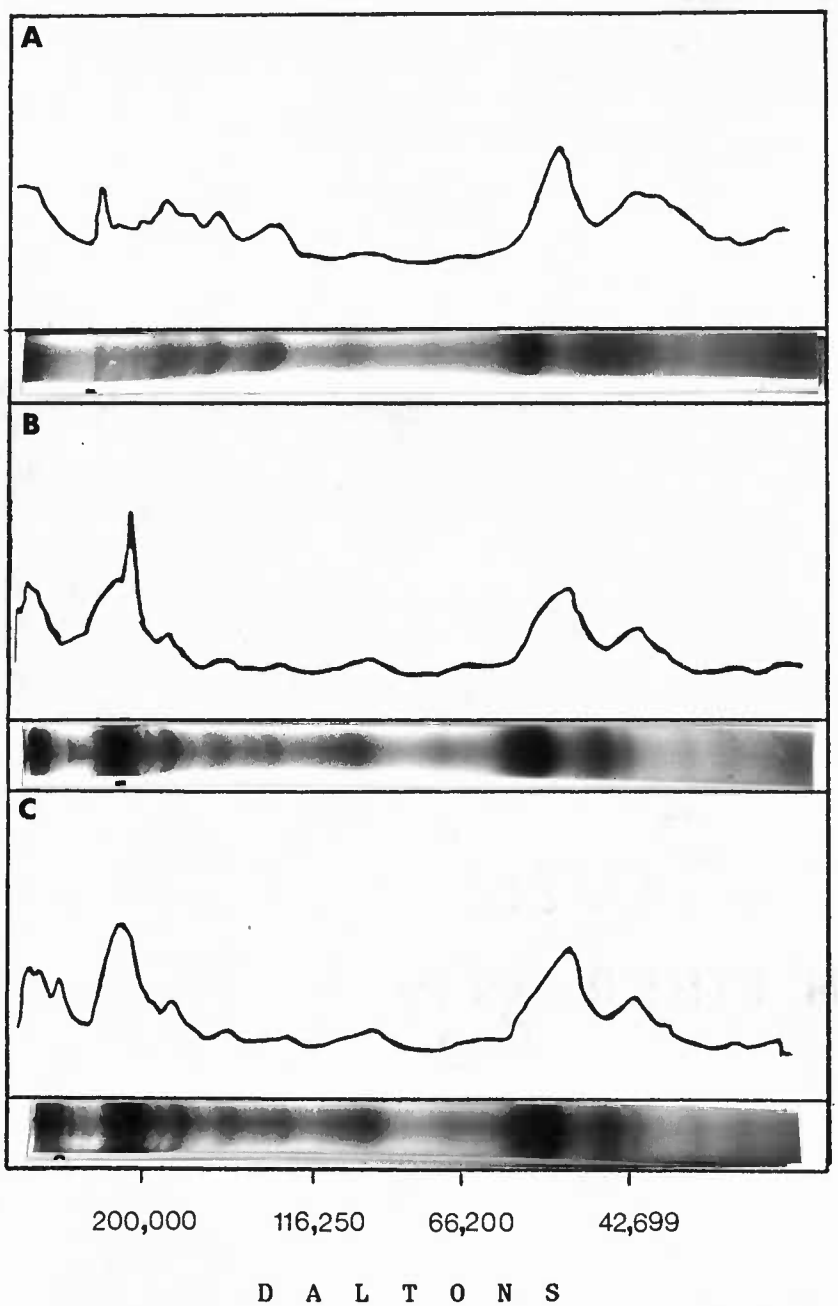


Figure 15. Gel electrophoresis scans representing second heat treatment (40^{o} C for 1 hour followed by 90^{o} C for 20 minutes) in lot 4. A) Surimi, 3% NaCl; B) Surimi, 3% NaCl, 3% egg white; C) Surimi, 3% NaCl, 3% egg white, 5% potato starch. The molecular weight of 200,000 corresponds to myosin.

Figure 15



This indicates that egg white has an inhibitory effect on proteolysis with either a single stage or a dual stage heat treatment. In addition, this explains ressults from the preliminary work, in which no significant differences between single and two stage heat treatments were found in gels containing egg white.

Miller and Spinelli (1982) reported no significant difference in the inhibitory effect from either egg white or potato extract (1 to 5 mg/ml) in a crude protease extract prepared from Pacific whiting muscle. However, Lanier et al. (1981) and Makinodan et al. (1985) worked with white croaker from different locations and at concentrations of 0.5 mg/g and both found an inhibitory effect of egg white and potato extract on enzymatic activity. From these reports it is clear, that the inhibitory effect of these ingredients depends basically on the concentrations used. In the current study, concentrations greater than those previously reported were used. It was found that only egg white had an inhibitory effect on the proteolytic activity.

Many researchers have done studies in the heat denaturation of egg white and its behavior in the coagulation process. This could be a possible explanation for the inhibitory effect seen in this study.

According to Matsudomi et al., (1986), ovalbumin and lysozyme are the major proteins in egg white and interact electrostatically in nature. The effect of heating temperature is another factor that influences the aggregation and coagulation of ovalbumin and lysozyme when both are mixed. These proteins did not show any changes in turbidity until heating to 60^{o} C. however when the temperature exceeded 70^{o} C aggregation of both proteins occurred. This was demonstrated by changes in the surface hydrophobicity, indicating that aggregation

occurred prior to the heat denaturation of these proteins. There was. however a linear inhibiton of lysozyme and ovalbumin aggregation in the presence of serial concentrations of NaCl (Cunningham and Lineweaver, 1967; Matsudomi et al. 1986). Native ovalbumin contains four cysteine residues and one residue of cystine per molecule (Matsudomi et al. 1986); lysozyme has no cysteine residues, but contains four cystine residues. When these proteins were subjected to heat treatment at 65^{o} C, ovalbumin alone did not show significant changes in sulfhydryl groups; but started to decrease at a heating temperature greater than 70^{o} C. This indicates that cysteine residues in ovalbumin existed in the interior of the protein molecule, and were exposed during heat denaturation (Matsudomi et al. 1986)..

In lysozyme, the amount of sulfhydryl groups did not show any changes during heating, consequently, intramolecular disulfide bonds were not cleaved by heating. The amount of surface sulfhydryl groups in the mixture of ovalbumin and lysozyme did not show significant changes up to 72^{o} C, but increased to the heating temperature of 74^{o} C; and began to decrease in the mixture. Thus it was suggested from these results that the reactivity of ovalbumin was enhanced by the addition of lysozyme. Therefore these proteins participate in the heat-induced aggregation by sulfhydryl disulfide interchange reaction (Matsudomi et al., 1986). It is important to note that other proteins containing SH groups such as bovine serum albumin, beta-lactoglobulin and s-ovalbumin inactivate lysozyme at 60^{o} C at rates similar to that given by native ovalbumin (Cunningham and Lineweaver, 1967). Assuming that the whiting protease reacts in a manner analogous to lysozyme in the presence of egg white, a sulfhydryl-disulfide interchange reaction in the kamaboko gels

may occur when they are subjected to a single stage heat treatment. More research is needed in this area.

CONCLUSIONS

From these results the following can be concluded:

1) Yield and the efficiency of the unit operations are important factors in the economy of surimi production. An overall yield of 19.9% was obtained.

2) Proximate composition of the flesh in surimi unit operations values agree with those reported by other studies done with Pacific whiting. From a nutritional standpoint, whiting surimi can be considered a nutritional source as good as marketed analogs manufactured from other fish species.

4) Egg white, WPC and SPI in serial concentrations were evaluated for potential enhancement of textural parameters. Egg white at 3% was selected as a main ingredient in the kamaboko gel in the subsequent trials for this work based upon its greater effect on hardness and elasticity.

5) Egg white and potato starch in combination proved to be important ingredients for kamaboko gels. The matrix reinforcing effect of potato starch tends to mantain the rubberiness, while egg white reduces the rubberiness. Both were necessary to impart a meaty texture.

6) The proteolytic activity was reduced throughout each surimi unit operation. In the refined flesh, 50.9% of the proteolytic activity was reduced. The balance is potentally deleterious to the gel formation at specific temperatures.

7) The inhibitory effect of egg white on the proteolytic activity was demonstrated by SDS-polyacrylamide gel electrophoresis. Potato starch showed no inhibitory effect.

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