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The effect of salt (0, 0.9, 1.7, 2.5%) and pH (range 4 to 10) on surimi gels made from Pacific Whiting (*Merluccius productus*) was investigated. Gel-forming ability was measured by the torsion test. In general, surimi gels increased in gel strength with increased pH. Breaking shear stress increased to a greater degree than breaking shear strain above pH 7.0. The increase in gel strength was greater at higher pHs for gels made without salt than those made with salt. At neutral pH, the salted surimi showed greater gel forming abilities than the unsalted whiting surimi. Poor gels were formed at low pH (pH 4 to 6) for both the salted and no-salt surimi. These results demonstrated that pH and salt concentration had an interactive effect on the gel-forming ability of the Pacific whiting surimi and that improved gel strength at low salt levels might be obtained by increasing the pH.

Effect of Salt and pH on Surimi Gels Made from Pacific Whiting (*Merluccius productus*)

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EFFECT OF SALT AND pH ON SURIMI GELS MADE FROM PACIFIC WHITING (*Merluccius productus*)

INTRODUCTION

Surimi is a Japanese term for mechanically deboned fish flesh which has been washed with water and mixed with cryoprotectants to extend frozen shelf life (Lee, 1984). Surimi-based products, such as kamaboko, tempura, chikuwa etc. are very popular in Japanese markets because of their unique texture properties (Suzuki, 1981). These surimi-based products are recognized as good nutritional products because of their high protein and low fat content. The technology of surimi processing was first commercialized in 1960 (Suzuki, 1981). The demand and consumer acceptance of surimi-based products in the United States have increased in recent years. Surimi production in the U.S. has grown from 2 million lbs in 1979 to 352 million lbs in 1991 (Kano, 1992). As a result of the shortage of raw materials for surimi production, primarily Alaska pollack, and increased market price, several groups have focused on the possibility of surimi production from Pacific whiting (Lee, 1984; Natural Resource Council, 1990; Morrissey et al., 1992).

Pacific Whiting (*Merluccius productus*) is an abundant fishery resource harvested off the Pacific Northwest coast of the United States and Canada from March through November (Nelson and Larkins, 1970; Fiscus, 1979; Patashnik et al.,

1982). Pacific whiting was an underutilized species for surimi production due to its soft-texture (Crawford and Law, 1972; Ryan, 1979; Anderson, 1985; Kabata and Whitaker, 1985; Nelson et al.,1985). The soft texture of Pacific whiting is caused by protease enzymes that may be related to myxosporidean parasites (Kudo et al., 1973). Research has shown that protease inhibitors are effective in preventing protease activity and maintaining good texture characteristics for Pacific whiting surimi (Chang-Lee et al., 1989). Advances in technology have increased utilization of Pacific whiting for surimi for both at-sea processing ships and shore-based plants (Morrissey et al., 1992).

During the conventional processing of surimi-based products, 2-3% salt is added to extract and solubilize the myofibrillar proteins, which are mainly responsible for heat-induced development of a functional protein matrix during surimi gel formation (Suzuki, 1981; Fukuzawa et al., 1961). Recent studies have shown that good gels can be prepared from fish muscle tissue without high concentration of NaCl (Hennigar et al., 1988a; Hennigar et al., 1989; Vareltzis et al., 1989). In general, production of high quality surimi based foods with low salt content would be beneficial to consumers health.

Surimi gel-forming ability is also significantly affected by pH value during processing (Asghar et al., 1985;

Wicker et al., 1986; Trevino et al., 1990; Morioka et al., 1992; Funatsu and Arai, 1991; Funatsu and Arai, 1992). Several reports showed that better surimi gels were formed under alkaline pH conditions (Wicker et al., 1986; Trevino et al., 1990; Morioka et al., 1992; Funatsu and Arai, 1992). Several investigators demonstrated that surimi gel strength is interactively influenced by both salt content and pH conditions (Trevino and Morrissey, 1991; Akahane and Schimizu, 1989). However, these effects still need further study.

Surimi gel-forming ability is also affected by heating temperature and heating rate (Lanier et al., 1982; Lee, 1984; Kim et al., 1986; Schmidt, 1988; Numakura et al., 1990). Several reports showed that low-temperature settings will increase surimi gel strength (Lanier et al., 1982; Wu et al., 1985a,b; Roussel and Cheftel, 1988; Numakaura et al., 1990; Niwa, 1991). In addition, better gels were formed by increasing the heating rate during the manufacture of surimi-based products (Lee, 1984).

The objectives of this study were to test the gel-forming properties of salted and no-salt surimi made from Pacific whiting, and to explore the interactions between salt concentrations and pH on surimi gels. The effects of different temperature settings on Pacific whiting surimi were also investigated and reported.

LITERATURE REVIEW

EFFECTS OF SALT AND pH ON MEAT GELS

Proteins within the muscle cell are generally classified into three groups: myofibrillar, the contractile proteins; sarcoplasmic, the metabolic proteins; and stromal, the connective-tissue proteins. Pan (1990) reported that the sarcoplasmic fraction possessed no gelation ability, as the fraction only coagulated upon heating to 80°C. Similar results were found for the stromal fraction. The myofibrillar fraction exhibited excellent gel-forming ability. Fukazawa et al. (1961) and Samejima et al. (1969) reported that among the myofibrillar proteins, myosin was essential in developing the desired binding properties in sausage prepared from whole myofibrils. This unique gelation process of meat proteins has been used to produce various sausage and surimi-based products with very distinct texture and flavor properties.

The mechanisms and interactions underlying the formation of the three dimensional protein network characteristics of gels are not fully understood. Practically all studies point to the necessity of protein denaturation and unfolding prior to the step of ordered protein-protein interaction and aggregation (Ferry, 1948; Hermansson, 1978). Hermansson (1978) stated that contrary to coagulation where aggregation of the protein molecules is

random, gelation involves the formation of a continuous network exhibiting a certain degree of order. The denaturation of proteins, prior to aggregation, resulted in a finer gel structure exhibiting greater elasticity than if random aggregation occurred simultaneously or prior to denaturation. The slower the aggregation step relative to the denaturation, the better the denatured chains orient themselves to a finer gel network (Hermansson, 1978). Lanier et al. (1982) stated that a setting below 40°C prior to heating to 60-80°C allows slow "ordering" of the protein molecules resulting in the formation of gels with greater firmness and cohesiveness.

It is important to understand and control the denaturation process in order to obtain the desired gel properties (Ziegler and Acton, 1984). The denaturation of native proteins involving the secondary, tertiary, or quaternary structure, in which alterations of hydrogen bonding, hydrophobic interactions, ionic linkages and disulfide bonds, occur during the transition to the denatured state (Anglemier and Montgomery, 1976). The major methods used in denaturation studies involve heat application and effects of pH, salts and detergents. Lee (1984) and Lanier (1986) reported that protein gel-forming ability was affected by the type of protein, protein quality, protein concentration, ingredient (e.g., starch, egg white, salt), pH, cooking temperature, cooking time and

setting condition. During the gelation of surimi paste, an ordered network structure is developed by new binding forces involving salt linkages, hydrogen bonds, disulfide bonds, and hydrophobic interactions (Niwa, 1991). Samejima et al. (1981) proposed that the heat induced gelation of myosin consists of two reactions: (1) aggregation of the globular head segments of the myosin molecule; and (2) network formation resulting from the unfolding of the helical tail segment. However, more research is needed to determine the binding forces involved in gelation, and the contribution of the subunit of myosin.

Effects of pH on Meat Gels

Several researchers have found that pH has a significant effect on the texture properties of various protein gels (Wicker et al., 1986; Trevino et al., 1990; Trevino and Morrissey, 1991; Morioka et al., 1992). Alkaline pH was found to improve the texture properties of fish protein gels in terms of increasing gel strength and/or elasticity. Wicker et al. (1986) showed that myosin gels from tilapia (*Serotherodon aureus*) at pH 7 and 8 had greater elasticity than those at pH 6. Trevino et al. (1990) also reported that sardine (*sardinops sagax*) surimi gels with high pH values (pH 9-10) appeared to have higher cohesiveness, solubility, and water holding capacity (WHC) than gels made at lower pH values. Funatsu and Arai (1991)

reported that walleye pollack surimi exhibited a maximum breaking strength and strain at pH 7.35 and pH 6.5-7.6 respectively; and the breaking strength and breaking strain both decreased when the pH was either decreased or increased from these optimum values. Trevino and Morrissey (1991) also found that salted red hake gels (1.5% salt) had a maximum hardness value at pH 7 and the hardness value was decreased over the alkaline range. It appears that the effect of alkaline pH on the texture properties of fish protein gels may be species dependent.

The mechanism of pH effect on protein gelation is not well understood. Most researchers associate the increase of gel strength and elasticity of protein gels to high water-holding capacity (WHC). WHC is the ability of a matrix of molecules, usually macromolecules, to entrap large amounts of water in a manner such that exudation is prevented (Fennema, 1985). It also can be expressed as a ratio of the amount of water removed by pressure to the total content of moisture in the muscle (Hamm, 1986). The increase in pH raise the net negative charges of protein; and the charged groups produce electrostatic repulsion expanding the protein network to entrap water. The water molecules are trapped inside the protein gel matrix decreasing its mobility, and allowing the protein gels to become stronger and more elastic (Hamm and Deatherage, 1960; Kinsella and Srinivasan, 1985; Pomeranz, 1985; Hamm, 1986). Certain fish species,

such as sardine (*Sardinopme lanosticta*), mackerel (*Pneumatophorus japonicus*) (Hashimoto et al., 1979), tilapia (*Tilapia mossambica*) and Atlantic croaker (*Nibea mitsukusii*) (Suzuki, 1981; Liu et al., 1982) contained high concentration of anionic amino acids (e.g., glutamic and aspartic acid) exhibiting higher WHC at alkaline pH. At alkaline pH conditions, these amino acids have functional groups that interact strongly with the surrounding water resulting in increased WHC (Kuntz, 1971; Trevino et al., 1990).

Acidic pH was found to decrease the gel strength and elasticity of fish protein gels. Funatsu and Arai (1992) found that acid treatment (pH 6.0) caused a decrease in gel-forming ability of pollack surimi, and an irreversible deterioration in the quality of surimi. Torley and Lanier (1992) reported that pollack surimi decreased in gel strength when pH decreased from 7.0 to 6.0. Nishino et al. (1991) showed a dramatic decrease in gel strength in pollack surimi with a decrease of pH from 6.75 to 3.77. At a pH close to 5, which is the isoelectric point of myofibrillar systems, a severe loss of WHC, solubility, and gel-forming ability was observed (Akahane and Shimizu, 1989; Hamm, 1986).

It is well known that proteins, at a pH close to the isoelectric point (IP), will lose their WHC due to loss of net electric charge on the molecules. At this pH, protein

molecules show minimal interactions with water and their net charges are sufficiently small to allow polypeptide chains to approach each other (Cheftel et al., 1985). The IP of muscle and fish protein was found to be approximately 5.0, an acidic pH (Cheftel et al., 1985; Hamm, 1986, Akahane and Shimizu, 1989). It is reasonable to expect the gel strength and elasticity of fish protein to decrease at acidic pH values.

Effects of Salt on Meat Gels

Salt has been found to enhance the solubility of the myofibrillar proteins, and also expand the shelf life of various meat products by retarding bacterial growth (Schmidt, 1988). Salt has become one of the most important ingredients in meat gels. The content of salt in meat gels varies from 1 to 5% depending on the preference of individual meat gel producers (Schmidt, 1986). In processing various surimi gels, adding salt was found to enhance the texture and flavor properties of the final products (Lee, 1984; Lanier, 1986; Amato et al., 1989; Ishioroshi et al., 1979). Salt levels of 2-3% have been reported as optimum for gel properties of mackerel surimi, sardine surimi and pollack surimi (Suzuki, 1981; Lee, 1984; Lanier, 1986; Morioka, 1992). Funatsu and Arai (1991) stated that at neutral pH 2-3% sodium chloride was essential to obtain the highest gel strength characteristic for

pollack surimi production. Amato et al. (1989) showed that 2% NaCl doubled shear stress and increased shear strain by 30-50% compared to 1% NaCl with muscle gels (breast, thigh, and drum) of chicken and turkey. However, Ishioroshi et al. (1979) reported that the gel forming ability of rabbit myosin, as measured by the shear modulus, did not increase with increased sodium chloride concentrations of 0.4-1.0 M. Shimizu and Simidu (1955) reported that a maximum gel strength was obtained at 1.0 M NaCl and a further increase in salt concentrations resulted in decreased gel strength. Investigating the effect of NaCl concentration on WHC of various meat products, Trout and Schmidt (1986) stated that salt concentrations above 2.9% had no beneficial effect on WHC.

The effect of salt addition on protein gelation is still not totally understood. Generally speaking, the purpose of adding salt to make protein gels is to solubilize the myofibrillar protein, primarily myosin, which is the main component in forming gels (Lee, 1984; Lanier, 1986). At the pH of postrigor meat, fish flesh or surimi, an intermolecular ionic linkage will be formed between the carboxyl groups of glutamic acid and the aspartic acid and amino groups of the lysine and the arginine causing an aggregation which is insoluble in water. Adding salt would rupture the intermolecular ionic linkages among the myofibrillar proteins dissolved in water because of their

increased affinity for water (Niwa 1991). The slight increase in ionic strength by adding salt tends to increase the solubility of proteins, a phenomenon also referred to as salting in. This salting in effect was found to be independent of the nature of the salt (Fox and Foster, 1957; Cheftel et al., 1982; Creighton, 1992). However, the addition of high concentrations of salt causes proteins to precipitate, a phenomenon referred to as salting out, thereby preventing protein gelation (Suzuki, 1981).

Salt not only affects the solubility of the myofibrillar proteins, but also destabilizes their molecular structure to thermal denaturation. Using differential scanning calorimetry (DSC) in the study of the transition temperatures of meat proteins, investigators showed that adding NaCl destabilized poultry breast meat (Kijowski and Mast, 1988), croaker surimi (Wu et al., 1985b), and beef muscle (Barbut and Findlay, 1991; Quinn et al., 1980; Stabursvik and Martens, 1980). The reduction of the heat stability of fish proteins by adding salt allows them to initiate gelation at low temperatures (Lanier, 1986; Pigott and Tucker, 1990).

Effects of Salt and pH on Meat Gels

Several studies reported that salt and pH showed a interactive effect on the meat gel-forming process. Akahane and Shimizu (1989) worked on the interaction of NaCl (0 and

3%) and pH (3-11) on surimi gels made from Alaska pollack. Their results showed a peak hardness value at pH 7 when 3% salt was added; and the hardness value decrease with increasing pH at alkaline range. The unsalted gels exhibited a peak hardness value at pH 9; and their hardness values were less than salted gels at pH 6-9, but higher than salted gels at pH 11. At acidic pH (3-5) range, both 3% salted and unsalted gels had very low hardness values. In a study on the influence of pH on the WHC of salted (2% NaCl) and unsalted comminuted beef at pH range from 3 to 7, Hamm (1986) showed that the WHC of unsalted comminuted beef was higher than that of salted comminuted beef at pH between 3 and 5. Lower WHC values were found for unsalted comminuted beef at pH 6-7. Trevino and Morrissey (1991) compared the effects of pH from 6-11 with addition of 0 and 1.5% NaCl on red hake (*Urophycis chuss*) surimi. Their results showed that 1.5% salted gels had higher hardness values than unsalted gels at pH 6-7, but lower hardness values than unsalted gels at alkaline pH (8-10). The peak hardness value for 1.5% salted and unsalted gels made from red hake occurs at pH 7 and pH 9.5 respectively. Trevino et al. (1990) reported that increasing ionic strength by adding salt would decrease WHC of sardine (*Sardinops sagax*) surimi gels at pH 9.

As mentioned in previous sections, both pH and salt have a significant effect on the heat induced gelation of

meat proteins. The interaction between pH and salt also played a very important role on protein functionality. The interaction between salt and pH can be generally classified into four types depending on the pH. At a pH lower than the isoelectric point (IP), adding salt decreased protein functionality (Akahane and Shimizu, 1989; Hamm, 1986). At pH equal to IP of myofibrillar protein (pH 5), adding salt shows no significant influence on protein functionality (Akahane and Shimizu, 1989; Hamm, 1986). At pH higher than IP (pH 6-7), adding salt was found to increase WHC and gel properties (Akahane and Shimizu, 1989; Hamm, 1986). The effect of NaCl at pH below 7 is predominantly due to the association of chloride ions with positively charged groups of myosin or actomyosin. This adsorption of chloride ions, which results in a shift of IP to lower pH, causes a weakening of the interaction between opposite charged groups at pH higher than IP and, therefore, an increase of WHC (Hamm, 1986; Acton et al., 1983). The shift of IP to lower pH also causes a weakening of intermolecular repulsive forces between protein molecules at pH lower than IP and results in a loss of WHC (Fig. 1; Hamm, 1986). In the alkaline pH range, adding salt appeared to decrease the protein functionality of surimi gels (Trevino and Morrissey, 1991; Trevino et al., 1990). According to Hamm (1986) and Trevino et al. (1990) the net negative charge of proteins at alkaline pH can be neutralized by sodium ions and the number

of available functional groups for water binding are decreased. There may be a reduction of gel strenth as well in this environment. The effects of salt and pH are important for a number of food protein systems and additional research is necessary to understand these interactions.

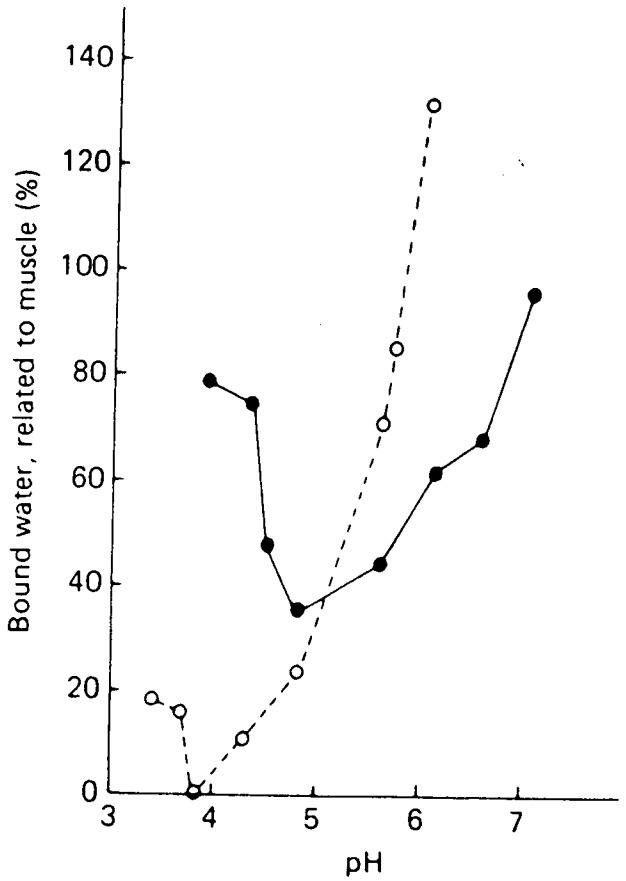

Fig.1 Influence of pH on the water-holding capacity of salted (dash line) (2% NaCl) and unsalted (solid line) comminuted beef. (50% added water; filter paper press method; (Hamm, 1986)

MATERIALS AND METHODS

Fish Source

Pacific whiting (*Merluccius productus*) were captured off the Oregon coast and processed into fillets at Astoria Seafood Sales, Inc., Astoria, OR. within 24 hr of capture. The fish fillets were immediately stored on ice and transferred to the OSU Seafood Lab upon purchase. The fish fillets were stored at 4°C until processing. Surimi production was initiated within 8 hr of fish fillets off-loading.

Surimi Production

The fish fillets was washed in polyethylene tanks with water and ice (4:1) at a ratio of 1 part flesh to 3 parts water (w/w) with gentle stirring for 5 min. The mixture was allowed to set another 5 min, then the suspension was decanted. The flesh was dewatered at high speed in a Sano-Seisakkusho screw press (Model SD-8, Ikeuchi Tekkosho, Ltd., Japan). The washing and dewatering process was repeated twice. During the third wash, 0.25% salt was added to facilitate dewatering, and the press was operated at low speed to reduce the moisture content. The dewatered mince was refined with a surimi strainer (Model S-1, Akashi Tekkosho Co., Japan). The refined mince was placed in the freezer for one hr to remove heat gained during the refining process. The mince was removed from the freezer and split

into two equal parts, mixed with a specific cryprotectant formulation in a Hobart Silent Cutter (Model VCM, Hobart Manufacturing Co., Troy, OH) for 5 min to produce surimi separately. The two surimi production formulations were as the follows: (a) mince mixed with 4% sorbitol (ICI Specialties, New Castle, DE), 4% sucrose (C&H Pure Cane Sugar, CA and Hawaiian Sugar Company, Concord, CA) and 0.3% Brifisol s-1 (tetrasodium pyrophosphate and sodium tripolyphosphate; B.K. Ladenburg Corp., Cresskill, NJ), (b) mince was mixed with 4% sorbitol, 4% sucrose, 0.3% Brifisol s-1, and 1% Beef Plasma Protein (BPP) (American Meat Protein Corp., Ames, Iowa). The sorbitol and sucrose acted as cryoprotectants; whereas, Brifisol s-1 acted as a water binding agent. BPP acted as a protease inhibitor. The temperature was maintained below 10°C during surimi production. The surimi samples were packed into 600 g aliquots in plastic containers, vacuum packed and frozen in a blast freezer overnight at -30°C. Samples were transferred to frozen storage at -20°C.

Fish Gel Preparation

The frozen surimi packages were removed from frozen storage and kept at room temperature for 2 hr to reach -5°C, then cut into slices for testing. The moisture of the surimi was determined by heating 5 g thawed surimi at 40 °C using an Ohaus Moisture Determination Balance (Model 6010,

Ohaus Scale Company, Union, NJ) until the percentage of moisture kept constant. The moisture content of fish gel formulations was kept constant at approximately 78% by adding ice. Final NaCl concentrations of fish gels were approximately 0, 0.9, 1.7 and 2.5% by weight. The pH of prepared gels were from 4-10 with an increment of one pH unit and adjusted by addition of 1.0 M NaOH, or 1.0 M HCl. The surimi was blended at low speed for 30 sec without vacuum in a vacuum mixer (Model 5289, Stephan Machinery Corportion, Columbus, Ohio). NaCl, ice, 1.0 M NaOH and 1.0 HCl were blended into the surimi mixture at low speed with no vacuum for an additional 1 min. The pH of the blended surimi paste was measured and adjusted as necessary by adding 1.0 M NaOH and 1.0 M HCl and blended again for 30 sec. An additional adjustment of pH was often required for proper fish gel preparations. The pH was measured by pH meter (Model 240, Corning pH Meter, Corning, NY) with a spear tip combination electrode (Corning BNC U.S. Std., Corning, NY). Blending of surimi paste was continued and vacuum blending was initiated when the surimi paste became uniform. The paste was blended to a final temperature of 5-7°C, and the total blending time was approximately 4 min. The surimi paste was transferred to a sausage stuffer (Model 14208, The Sausage Maker, Buffalo, NY) carefully to avoid incorporation of air pockets in the paste. The paste was then extruded into stainless steel cooking tubes (i.d. = 19

mm) which had previously been sprayed with a lecithin-based releasing agent to facilitate the removal of gels after cooking. Both ends of the tubes were sealed and the tubes with surimi paste were cooked in a 90°C water bath for 15 min. The tubes were transferred to an ice water bath for 15 min. Fish gels were removed from the cooking tubes and refrigerated at 4°C in sealed plastic bags overnight. Gel quality tests were carried out the next day.

Torsion Test

The gel properties were measured by torsion test. The results of torsion tests are expressed as shear stress and shear strain. Shear stress is a measure of gel strength, while shear strain is related to cohesiveness of gels. The fish gels were taken from the refrigerator and allowed to reach room temperature prior to testing. Gel properties were then determined by cutting samples to the length of 28 mm. The sample ends were glued to styrene discs with cyanoacrylate glue. Samples were shaped into a hourglass geometry with the Torsion Cutter (Model 91, Accu-Tool Corp., Cary, NC) and the sample narrow diameter was measured by a caliper. The hourglass-shaped samples were subjected to torsional shear in the modified Brookfield viscometer (Model DV-1, Brookfield Engineering Laboratories, INC., Stoughton, MA) at 2.5 rpm as described by Lanier (1991). All samples were tested in replicates of eight. Shear stress and shear

strain were calculated using the equation developed by Hamann (1983) as:

Stress = τ = 1581 * (viscometer max digital torque units)

Strain = γ = 0.150 * (chart travel distance, mm/chart vel, s) - 0.00847 * (viscometer max digital torque units)

Shear Strain = $\ln [1 + (\gamma^2/2) + \gamma(1 + \gamma^2/4)^{0.5}]$

Water-Holding Capacity Determination

The water-holding capacity was determined according to the method described by Jiang et al. (1985). Five g of fish gel were sliced into 3 mm thick and wrapped with 3 pieces of filter paper (Whatman No.44). The wrapped samples were centrifuged at 3000 X g for 20 min. The percentage ratio of the moisture content in centrifuged fish gels to the original moisture content provided a water-holding capacity index.

Sodium Analysis

Sections of the surimi gels prepared for torsion tests were set aside for sodium analysis. Sodium analysis was determined in the surimi gels by the flame photometric method as described in AOAC (1990) using an Atomic Absorption spectrophotometer (Model 403, Perkin Elmer, Norwalk, CT).

Statistical Analysis

Statistical analysis of data was carried out by using one way analysis of variance. Difference among mean values were determined by using the Least Significant Difference (LSD) multiple range test (Steel and Torrie, 1980). Values were considered significant when $p<0.05$.

RESULTS

Effects of Salt and pH on Gel Strength

The effects of pH on Pacific whiting surimi gels made at three different salt levels and measured by torsion methods are shown in Fig. 2 and 3. At the lowest pH tested (pH 4), only the surimi paste made without salt formed a gel strong enough to undergo shaping and testing. At pH 5, no measurable gels were formed at all salt levels. This pH is near the isoelectric point of fish muscle tissue and gelling forces are very weak. Above pH 5 surimi gels were strong enough to be shaped and measured. Breaking stress values increased steadily with increasing pH. The lower salt concentrations (0% and 0.9%) showed the greatest increase from pH 5 to pH 10. The breaking shear stress values for 0 and 0.9% salt surimi were significantly greater ($p<0.05$) than the high salt surimi at the upper end of the alkaline range. Breaking shear strain values also increased markedly from pH 5 through pH 8. However, the increases in breaking shear strain values above pH 8 were more gradual for most surimi gels. The high salt surimi gel (2.5%) decreased slightly in breaking shear strain over the final pH unit. The greatest difference in breaking shear strain values among surimi was at pH 6 where the high salt surimi had a breaking shear strain value twice as great as the 0% salt surimi.

Similar trends were demonstrated, as shown in Fig. 4 and 5, for Pacific whiting surimi gels made with 1% beef plasma protein (BPP), a commercially used protease inhibitor. Only the no-salt surimi gel formed a measurable gel at pH 4. No measurable gels were formed for all salt concentrations at pH 5. All samples showed increased gel strength as pH was increased to neutrality. Again, there was noticeable improvement in breaking shear stress for the no salt and the 0.9% salt surimi in the alkaline range. The breaking shear stress values for the lower salt surimi samples were significantly greater than the higher salt surimi samples above pH 7 ($p<0.05$). The breaking shear strain values of Pacific whiting surimi made with 1% BPP were significantly improved for all samples when pH was adjusted from 5 to 7 ($p<0.05$). Surimi made with 0%, 0.9% and 1.7% were significantly higher in breaking shear strain at pH 8 than corresponding samples measured at neutral pH ($p<0.05$). There were no significant differences in breaking shear strain among the surimi samples made with different salt concentrations at pH 8 ($p<0.05$). As before, the largest differences in breaking shear strain among surimi samples was seen at pH 6 with the high salt surimi close to twice the value of the no-salt surimi.

Effects of Protease Inhibitor

Addition of BPP, as a protease inhibitor, to Pacific

whiting surimi greatly improves its gel characteristics at all pH's and salt concentrations. At pH 7, the breaking shear stress values of surimi gels with BPP were approximately twice the value as surimi gels made without BPP (Fig. 2 and 4). Breaking shear strain values at neutral pH also increased between 25-40% in whiting surimi gels depending on the salt concentrations (Fig 3 and 5). The breaking stress values, for the most part, continued to increase in the alkaline range for surimi made with and without BPP. Surimi made with inhibitor reached a peak in breaking shear strain at neutral pH and showed little further improvement in the alkaline range while samples made without inhibitor showed their highest breaking shear strain values above pH 7.

Water-holding Capacity

The results on water-holding capacity (WHC) are shown in Fig. 6 and 7. At pH 4 the no-salt surimi had the highest WHC at this pH. At pH 5, close to the isoelectric point, all samples had low values. A one unit pH increase from pH 5 to 6 showed a dramatic increase in WHC for the two higher salt surimi. At neutral pH, all surimi had WHC between 75 and 81%. There were only minor increases in WHC for the various surimi samples tested in the alkaline range. Similar results are shown in WHC for surimi made with BPP.

Sodium Analysis

Sodium analysis was undertaken to determine if the addition of NaOH for pH adjustment would contribute significant amounts of sodium ions and affect gel strength, compared to the addition of NaCl. Table 1 describes the sodium content for Pacific whiting surimi made with and without BPP at pH 6 and pH 10. At pH 6, there was no addition of NaOH in the surimi paste while at pH 10 the surimi paste was made with the maximum amounts of NaOH. Although there were differences in Na content between the surimi made at these different pH extremes, the increases were minor. Therefore, the changes in torsion results were due to pH effects and not to changes in sodium concentration.

DISCUSSION

The results of this study showed that an increase in pH of Pacific whiting surimi gels improved its gel-forming ability and resulted in a increase in shear stress, shear strain, and WHC values. Similar results have been reported by other researchers. Trevino et al. (1990) showed that sardine (*sardinops sagax*) surimi appeared to have higher functionality at pH values of 9-10. Torley and Lanier (1992) reported that pollack surimi increased gel strength when the pH increased from 6 to 7. Wicker et al. (1986) stated that myosin gels from tilapia (*Serotherodon aureus*) at pH 7 and 8 had greater elasticity than those at pH 6. Morioka et al. (1992) showed that the optimum pH region of gelation for sarcoplasmic protein of Pacific mackerel (*Scomber japonicus*) was between 7.5 and 9.0. Funatsu and Aria (1991) found that maximum gel strength occurred closer to neutrality for pollack surimi. They measured gel strength for Alaska pollack surimi made at 2.5% NaCl and at various pHs between 5.76-8.4. The pH values for pollack surimi were at 7.35 for the maximum breaking strength and between a range of 6.5-7.6 for the maximum breaking strain.

Possible reasons for improvement in gel-forming ability and WHC at a higher pHs may be due to an increase in negative charges on the amino acids of the myofibrillar proteins. This would allow protein chain to be repelled

from each other and open up space within gel structure for binding water; thereby, increasing WHC by raising the pH of surimi gels (Schmidt, 1988). At the alkaline pHs, the numerous anionic groups in fish protein were thought to facilitate the expansion of the gel network due to repulsion of negatively charged groups. This would allow anionic groups to be free to bind with water molecules and increase the WHC (Hamm and Deatherage, 1960).

There are other factors in Pacific whiting surimi gels that favor gelation at alkaline pH. It has been demonstrated by a number of researchers that Pacific whiting undergo textural damage due to an active protease system in the muscle tissue (Nelson et al., 1985; Patashnik et al., 1982). This proteolysis will adversely affect the gel strength of surimi made from this species (Chang-Lee et al., 1989; Morrissey, 1992). Work by Porter (1992) demonstrated that the main protease enzyme in Pacific whiting surimi has a pH peak at 5.5. A shift to alkaline pH would diminish the proteolysis effect of the protease on surimi gels. During cooking of the gels, protease activity is briefly increased as the temperature passes through the 50°-60°C range. At neutral or slightly acidic pH, proteolytic activity would be higher than activity found at alkaline pH. At higher pHs, less proteolysis of myofibrillar proteins should occur in Pacific whiting surimi.

A decrease in pH of whiting surimi gels by adding HCl

resulted in poor gel-forming ability and decreased shear stress, shear strain and WHC values. Nishino et al. (1991) showed a dramatic decrease in gel strength in pollack surimi as the pH was lowered stepwise from 6.75 to 3.77 with the addition of glucono delta lactone. Funatsu and Arai (1992) also found that acid treatment (pH 6.0) caused a decrease in gel-forming ability in pollack surimi, and an irreversible deterioration in the quality of surimi. Surimi lost gel-forming ability over the acid pH range because low pH will decrease the extractability of myofibrillar proteins, which are mainly responsible for protein gelation. Myofibrillar proteins tend to increase filament-forming ability and become larger in size in the acid pH range. These larges of myofibrillar proteins are difficult to extract (Fukuzawa et al., 1961; Kaminer and Bell, 1966; Suzuki, 1981; Matsurra and Aria, 1986). At pH 5, the isoelectric point (IP) of myofibrillar proteins, whiting surimi gels exhibited no gel-forming ability and very low WHC due to no net charge. Similar IP value of muscle proteins was also reported by Hamm (1986), Cheftel et al. (1985), and Akahane and Shimizu (1989). At the IP of myofibrillar protein, the net charge is at a minimum and less polar groups are available for binding water because of stearic effects (Hamm, 1986). A decrease in the electrostatic repulsion between the peptide chains causes a tighter protein network and low WHC (Hamm and Deatherage, 1960).

The gel-forming ability of Pacific whiting surimi is also affected by the addition of NaCl. The results of this study showed that the increase in ionic strength by the addition of NaCl increased gel forming ability at pH 6-7. This agrees with the common knowledge that adding 2-3% salt is necessary to optimize the gel properties of surimi product (Lee, 1984; Lanier, 1986; Nishimoto et al., 1987; Akahane and Shimizu, 1989). The purpose of adding salt is to solubilize the myofibrillar protein, primarily actomyosin, which are the main components in forming gels (Lee, 1984; Lanier, 1986). However, at alkaline pH range 8-10, the increase of NaCl concentration appears to decrease gel-forming ability of myofibrillar proteins. Trevino et al. (1990) reported that an increase of ionic strength at pH 9 decreased WHC of sardine (*Sardinops sagax*) surimi. Trevino and Morrissey (1991) stated that adding 1.5% NaCl decreased the hardness of gels made from red hake (*Urophycis chuss*) at pH 8-10. According to Trevino et al. (1990) the net negative charge of proteins at alkaline pH can be neutralized by sodium ions with sodium chloride and the number of available functional groups to water are decreased.

This study showed the effects of salt and pH on the gel-forming ability of myofibrillar proteins in Pacific whiting surimi. Lanier (1986) stated that a shear strain higher than 1.8 was needed in order to pass the traditional

Japanese folding test as a good quality surimi product. This study showed the important of pH control in the production of surimi, it also demonstrated the feasibility of producing good quality surimi-based seafood products at lower salt concentrations by pH adjustments.

CONCLUSION

Our study demonstrated that it was possible to produce good quality low salt Pacific whiting surimi by adjusting pH to alkaline condition. Both salt and pH had significant effects on the gel-forming ability of whiting surimi gels ($p<0.05$). For whiting surimi, increased pH resulted in increased shear stress and shear strain, and maintained water-holding capacity. At neutral pH, the 2.5% salted whiting surimi had higher gel-forming ability than surimi made with lower salt concentrations. Low-salt surimi gels made at alkaline pHs showed equal or greater gel strength characteristics than high-salt suirmi made under similar conditions. Additional research is needed to explore the mechanism of protein gelation and the roles of salt and pH in the gelation process.

Table 1. Sodium content (mg/g) on Pacific whiting surimi gels after adding salt and adjusting pH.

%NaCl Added	Gel pH adjusted to 6.0		Gel pH adjusted to 10.0	
	Na (mg/g) surimi 0% BPP	Na (mg/g) surimi 1% BPP	Na (mg/g) surimi 0% BPP	Na (mg/g) surimi 1% BPP
0	1.631 (0.149)	2.263 (0.158)	2.597 (0.130)	2.988 (0.150)
0.9	4.626 (0.146)	4.961 (0.180)	5.763 (0.258)	5.767 (0.363)
1.7	7.810 (0.225)	7.022 (0.219)	7.796 (0.075)	7.994 (0.654)
2.5	9.548 (0.198)	9.601 (0.541)	10.586 (0.629)	10.101 (0.805)

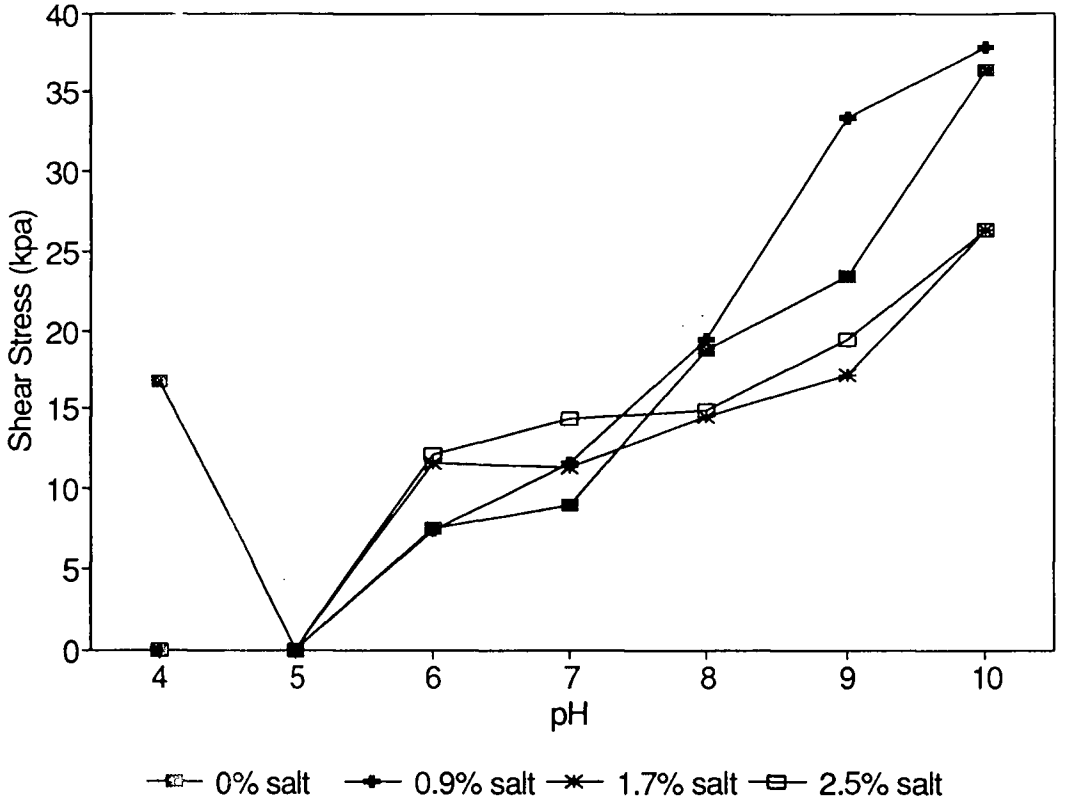

Fig.2 Shear stress values (KPa) on surimi gels made from Pacific whiting without beef plasma protein.

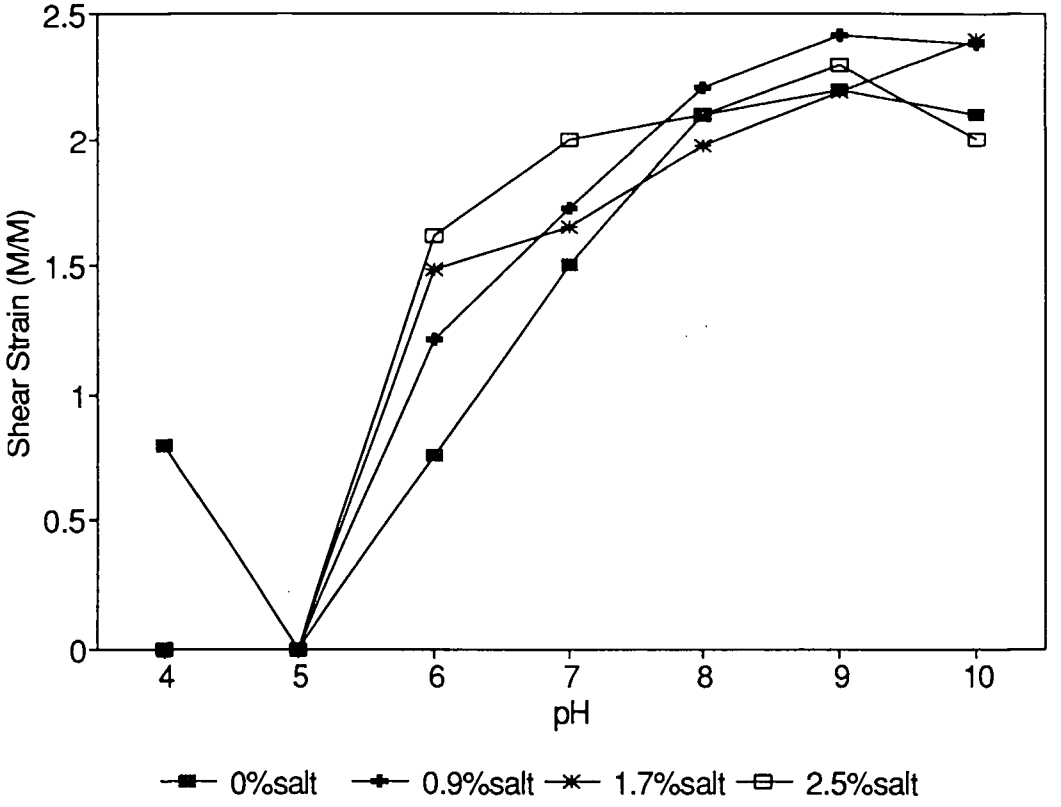

Fig.3 Shear strain values (M/M) on surimi gels made from Pacific whiting without beef plasma protein.

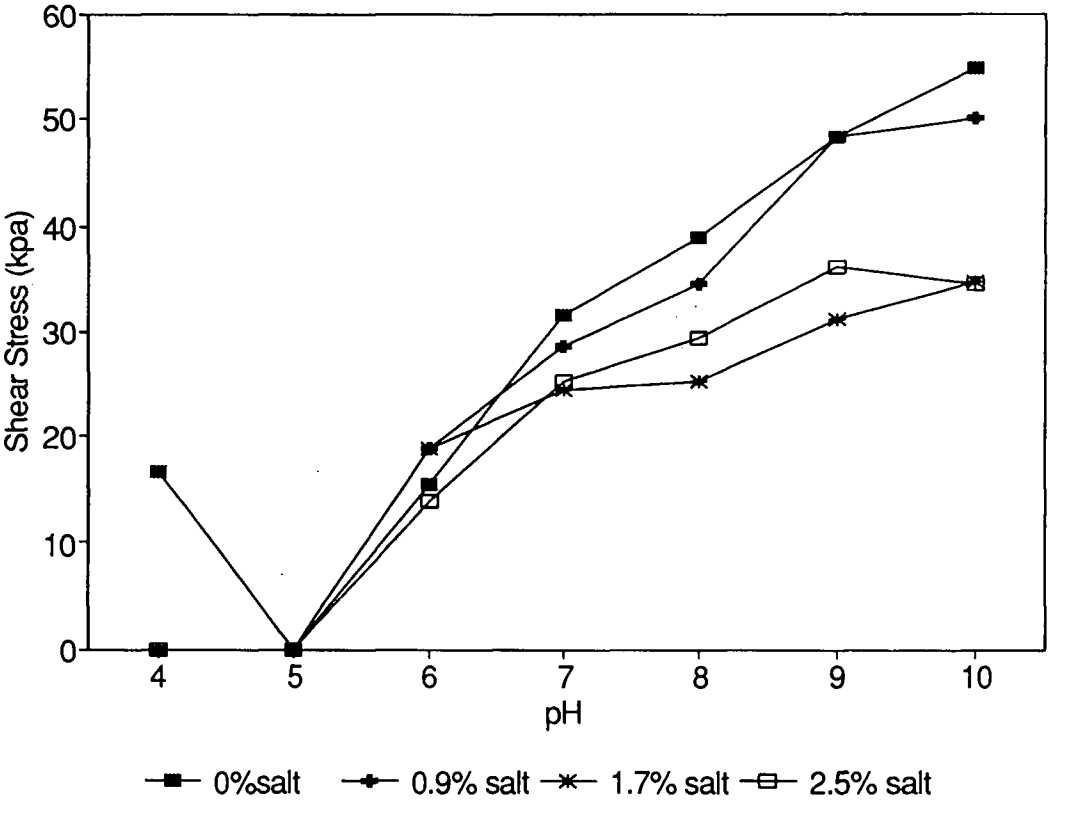

Fig.4 Shear stress values (KPa) on surimi gels made from Pacific whiting adding 1% beef plasma protein.

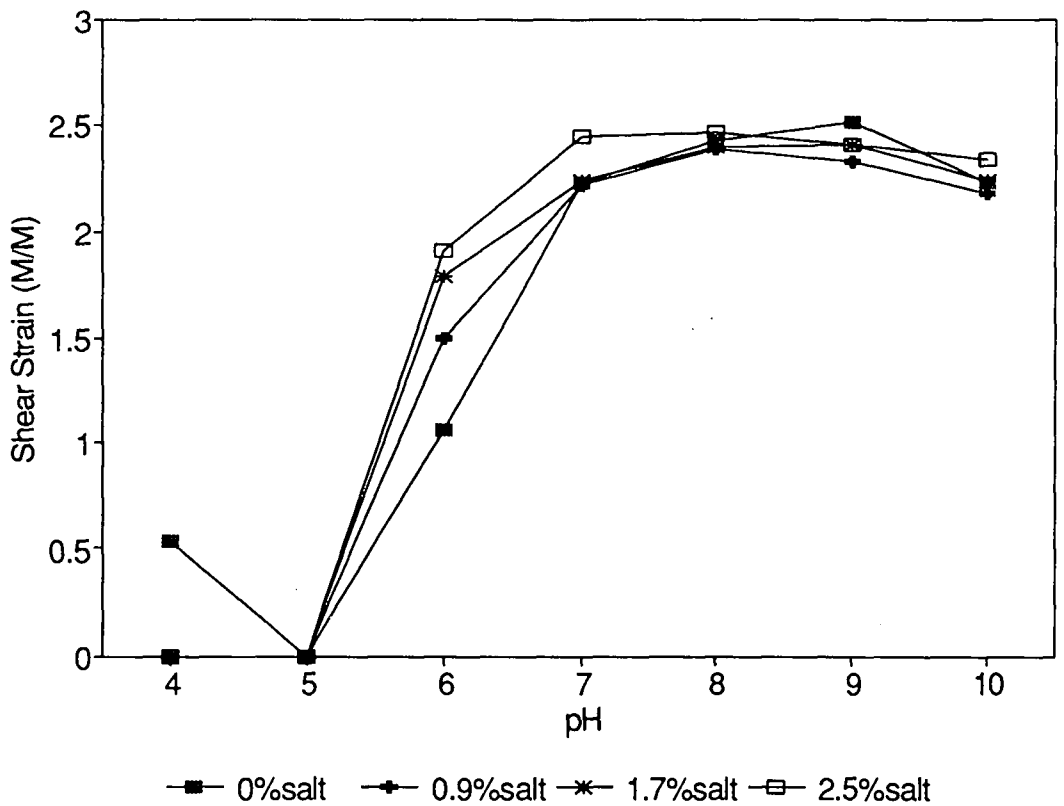

Fig.5 Shear strain values (M/M) on surimi gels made from Pacific whiting adding 1% beef plasma protein.

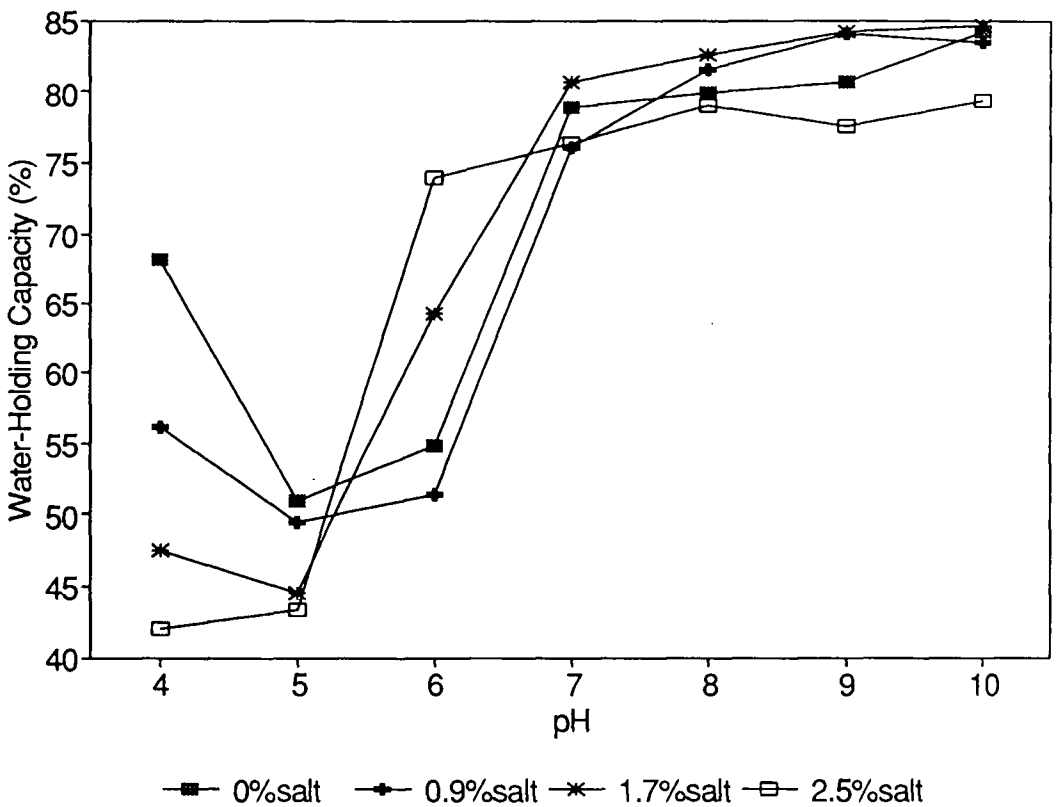

Fig.6 Water-holding capacity on surimi gels made from Pacific whiting without beef plasma protein.

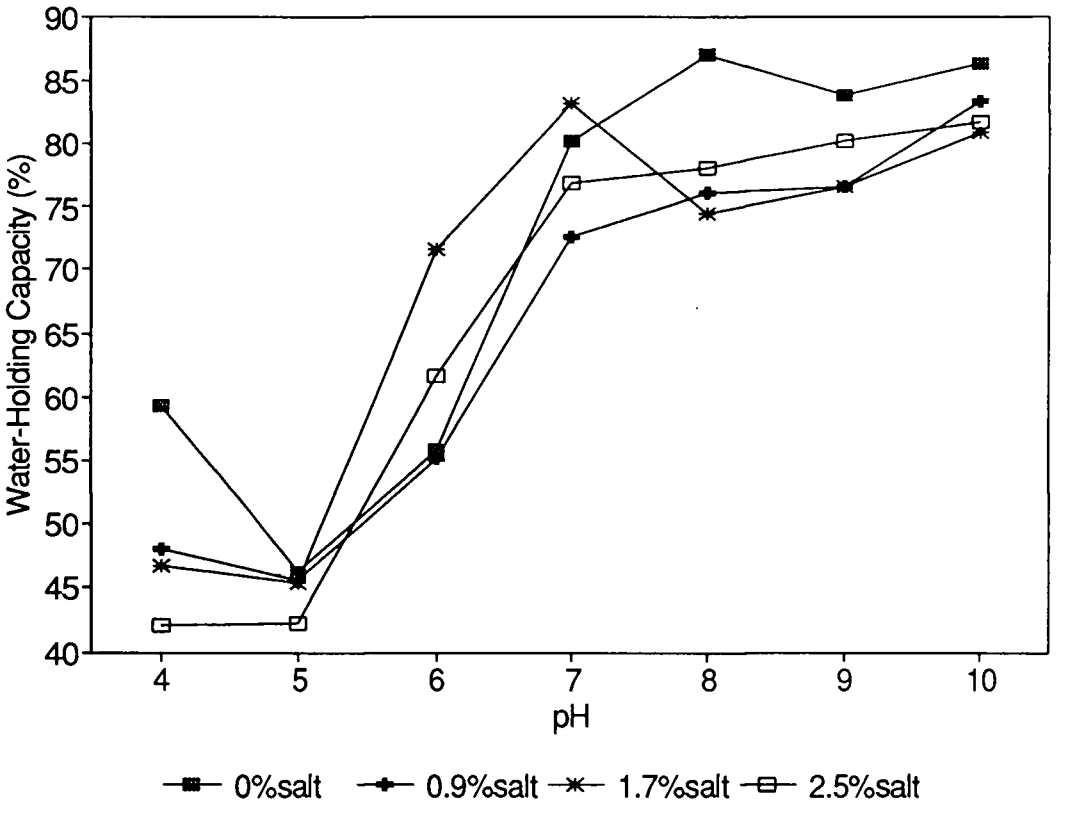

Fig.7 Water-holding capacity on surimi gels made from Pacific whiting with beef plasma protein.

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APPENDIX A

Table 1. Shear stress values (kPa) on surimi gels made from Pacific whiting without beef plasma protein.

NaCl (%)	pH4	pH5	pH6	pH7	pH8	pH9	pH10
0	17.9^{x} (1.5)	---	7.5^{aw} (0.6)	9.0^{aw} (0.8)	9.0^{bx} (2.3)	23.0^{by} (3.5)	36.5^{bz} (3.8)
0.9	---	---	7.3^{aw} (1.3)	11.7^{bcw} (0.4)	19.5^{bx} (1.4)	33.3^{cy} (4.2)	37.7^{bz} (4.3)
1.7	---	---	11.7^{bwx} (0.8)	11.4^{bw} (0.6)	14.5^{awx} (0.7)	17.1^{ax} (1.7)	26.3^{az} (2.3)
2.5	---	---	12.1^{bw} (1.1)	14.4^{cwx} (2.5)	14.9^{ax} (0.9)	19.4^{ay} (1.2)	26.4^{az} (3.2)

^{abc} Means in the same column followed by the different superscripts were significantly different within the the same pH treatment ($p<0.05$).

^{wxyz} Means in the same row followed by the different superscripts were significantly different within the the same NaCl concentration treatment ($p<0.05$).

Table 2. Shear strain values (M/M) on surimi gels made from Pacific whiting without beef plasma protein.

NaCl (%)	pH4	pH5	pH6	pH7	pH8	pH9	pH10
0	0.8^{v} (0.1)	---	0.8^{av} (0.1)	1.5^{aw} (0.1)	2.1^{abx} (0.2)	2.2^{ax} (0.2)	2.1^{ax} (0.2)
0.9	---	---	1.2^{bv} (0.1)	1.7^{bw} (0.1)	2.2^{bx} (0.2)	2.4^{bxy} (0.2)	2.4^{by} (0.1)
1.7	---	---	1.5^{cv} (0.1)	1.6^{bw} (0.1)	2.0^{ax} (0.1)	2.2^{ay} (0.1)	2.4^{bz} (0.1)
2.5	---	---	1.6^{dv} (0.1)	2.0^{cw} (0.2)	2.1^{bwx} (0.1)	2.3^{abxy} (0.1)	2.0^{ay} (0.0)

^{abcd} Means in the same column followed by the different superscripts were significantly different within the the same pH treatment ($p<0.05$).

^{vwxyz} Means in the same row followed by the different superscripts were significantly different within the the same NaCl concentration treatment ($p<0.05$).

Table 3. Shear stress values (kPa) on surimi gels made from Pacific whiting with 1% beef plasma protein.

NaCl (%)	pH4	pH5	pH6	pH7	pH8	pH9	pH10
0	16.6^{v} (3.0)	---	15.3^{av} (1.5)	31.6^{bw} (3.8)	38.9^{cx} (2.3)	48.3^{by} (5.4)	54.8^{cz} (3.6)
0.9	---	---	18.9^{bv} (1.4)	28.6^{bw} (1.9)	34.5^{cx} (2.7)	48.2^{by} (5.5)	50.0^{by} (3.2)
1.7	---	---	18.7^{bv} (1.9)	24.5^{aw} (3.5)	25.2^{aw} (1.7)	31.0^{ax} (1.4)	34.7^{ax} (3.4)
2.5	---	---	13.8^{av} (2.6)	25.2^{aw} (1.5)	29.4^{bx} (3.6)	36.0^{ay} (3.6)	34.5^{ay} (4.8)

^{abc} Means in the same column followed by the different superscripts were significantly different within the the same pH treatment ($p<0.05$).

^{vwxyz} Means in the same row followed by the different superscripts were significantly different within the the same NaCl concentration treatment ($p<0.05$).

Table 4. Shear strain values (M/M) on surimi gels made from Pacific whiting with 1% beef plasma protein.

NaCl (%)	pH4	pH5	pH6	pH7	pH8	pH9	pH10
0	0.5^{v} (0.1)	---	1.1^{aw} (0.0)	2.2^{ay} (0.1)	2.4^{az} (0.1)	2.5^{bz} (0.1)	2.2^{aby} (0.2)
0.9	---	---	1.5^{bv} (0.1)	2.2^{awx} (0.1)	2.4^{ay} (0.1)	2.3^{axy} (0.2)	2.2^{aw} (0.1)
1.7	---	---	1.8^{cv} (0.1)	2.2^{aw} (0.2)	2.4^{ay} (0.1)	2.4^{aby} (0.1)	2.2^{abw} (0.1)
2.5	---	---	1.9^{cv} (0.2	2.4^{bw} (0.1)	2.5^{aw} (0.1)	2.4^{abw} (0.1)	2.3^{by} (0.2)

[abc] Means in the same column followed by the different superscripts were significantly different within the the same pH treatment ($p<0.05$).

[vwxyz] Means in the same row followed by the different superscripts were significantly different within the the same NaCl concentration treatment ($p<0.05$).

APPENDICES

APPENDIX B

EFFECTS OF DIFFERENT TEMPERATURE SETTINGS ON SURIMI GELS

Introduction

Muscle proteins of many fish species possess a unique ability to set or gel at lower temperatures than other animal proteins (Wu, 1985). Lanier et al. (1982) and Kim et al. (1986) reported that salted homogenates using the muscles of certain fish species from cold-temperature habitats have the ability to form gels as low as 0°C. Addition of salt lowers the heat stability of proteins, allowing initiation of gelation at lower temperatures (Lanier, 1986).

This low temperature gelation was found to improve the gel properties of various fish protein gels. Niwa (1991) reported that 25°C was the optimum setting temperature for Alaska pollack surimi. Numakura et al. (1985) reported that the 30°C setting resulted in better gel strength than the 20°C setting for Alaska pollack surimi. Numakura et al. (1990) stated that increased gel strength of pollack surimi reached its maximum at 30°C; whereas, gel strength was found to initially increase and subsequently decrease after the setting at 40°C or above. Lanier et al. (1982) reported that a 40°C setting increased both hardness and cohesiveness of Atlantic Croaker surimi, however, a 60 or 80°C cook showed an increase in hardness and a decrease in

cohesiveness. Roussel and Cheftel (1988) showed that sardine surimi, quickly heated at 75°C or 90°C for 30 min without pre-setting at 35-40°C, formed a more rigid but less elastic gel. Douglas-Schwarz and Lee (1988) studied the thermostability of red hake and Alaska pollack surimi in the range of 40-90°C during processing, and found that a 40°C pre-setting temperature produced the most cohesive gels for both surimis.

The mechanism of low temperature setting effects on gel properties of fish proteins is not totally clear. Hermansson (1978) stated that the denaturation of proteins prior to aggregation resulted in a finer gel structure, exhibiting greater elasticity than if random aggregation occurs simultaneously prior to denaturation. The slower the aggregation step relative to the denaturation, the better the denatured chains orient themselves resulting in finer gel network. Setting of fish proteins at temperatures below the coagulation temperature, approximately 40°C, (Liu et al., 1982) may be viewed as a process in which the solubilized proteins partially unfold to an increased degree with increased temperature, and interact noncovalently to form a fine, elastic, and translucent gel network. Further processing at higher temperatures caused the gel to become firmer and more opaque as disulfide bonds formed and further unfolding and exposure of hydrophobic regions took place (Itoh et al., 1979; Lanier et al., 1982).

The loss of gel forming ability for some surimi pastes incubated at near 60°C has been reported (Makinodan and Ikeda, 1971; Lee and Toledo, 1976). Niwa (1991) proposed three possible factors that might cause a very brittle heat-set gel (modori) for some surimi pastes incubated at near 60°C, which were: (1) thermal coagulation of myofibrillar protein during heating, (2) a heat activated proteolytic degradation of myosin (3) the participation of nonenzymatic modori-inducing protein. Makinodan and Ikeda (1971) suggested that hydrolysis of meat protein by muscle proteinase at 50-70°C caused weakening of gel strength. Also several researchers reported that heat stable alkaline proteases were considered to cause textural degradation when fish paste was processed at 55-75°C (Cheng et al., 1979a,b; Lanier et al., 1981; Su et al., 1981). The phenomenon often occurs in unwashed fish tissues where most of the soluble proteins and proteases still remain (Lee, 1984).

Recent evidence by Morrissey et al. (1992) confirmed that loss of gel forming ability in Pacific whiting surimi was due to proteolytic activity. In several surimi samples heated at 60°C and run on gel electrophoresis, there was a disappearance of all high molecular weight proteins which were critical for gel formation (Cheng et al., 1979a; Chang-Lee,1988; Kinoshita et al., 1992). Erickson et al. (1983) and Chang-Lee et al. (1988) also reported that the optimal temperature for protease enzyme in whiting was approximately

55°C.

The purpose of this study was to evaluate the textural property changes of Pacific whiting (*Merluccius productus*) surimi gels caused by different temperature settings and the effect of adding beef plasma protein on these gels.

Material and Methods

Commercially frozen Pacific whiting surimi was purchased from an Oregon processing plant and was stored at -20°C until processing. Two groups of fish gels were prepared mixing 2% salt, and adding either 1% or 0% beef plasma protein (BPP), a protease inhibitor. The ingredients were blended in a Stephan vacuum mixer (Model UM5 universal, Stephan Machinery Corporation, Columbus, OH) about 4 min. Final moisture content for each formulation was kept at 78% by adding ice. The well mixed fish paste was extruded from a sausage stuffer (5-lb Capacity, The Sausage Maker, Buffalo, NY) into stainless steel cooking tubes (17.8 x 2.2 cm inside diameter), and cooked in a water bath at different temperature settings: (1) 90°C 15 min (2) 25°C 2 hr followed by 90°C 15 min (3) 40°C 1 hr followed by 90°C 15 min (4) 60°C 30 min followed by 90°C 15 min. After cooking, the gels were transferred to an ice water bath for 15 min. The gel-forming properties were measured by the torsion test described by Lanier (1991). Shear stress and shear strain were calculated using the equations given by Hamann (1983).

Results and Discussion

Effects of different temperature settings on whiting surimi gels are shown in Fig. 8 and 9. At a 60°C/30 min pre-setting, whiting surimi gels were too weak to run torsion tests. The loss of gel forming ability of whiting surimi gels cooked at 60°C was thought to be the response of the protease enzymes (Morrissey et al., 1992; Hamann et al., 1990; Erickson et al., 1983; Chang-Lee et al., 1988). Adding 1% beef plasma protein (BPP), as a protease inhibitor, greatly increased both breaking shear stress and breaking shear strain of whiting gels made at all temperature settings ($p<0.05$). Similar results were reported by Hamann et al. (1990) who showed BPP improved the gel properties of menhaden and low grade pollack surimi gels.

Both breaking shear stress and breaking shear strain values of whiting surimi gels showed no significant difference between gels made at 25°C/2 hr or 40°C/1 hr pre-setting and gels prepared without pre-setting. Cooking at 25°C/2 hr or 40°C/1 hr did not cause significant changes in breaking shear stress and breaking shear strain values for whiting gels with 1% BPP. For the gels made at 60°C/30 min pre-setting causing a slight decrease of breaking shear stress values. Lanier (1986) showed that a 40°C pre-setting increased the gel properties of surimi made from warm water fish species such as menhaden. However, our results did not

show a significant increase of breaking shear stress or breaking shear strain either at 40°C or 25°C pre-setting for the whiting surimi gels with or without BPP. This difference in the pre-setting effect in Pacific whiting from other fish may be due to differences in species and/or harvest locations (Cheng et al., 1979).

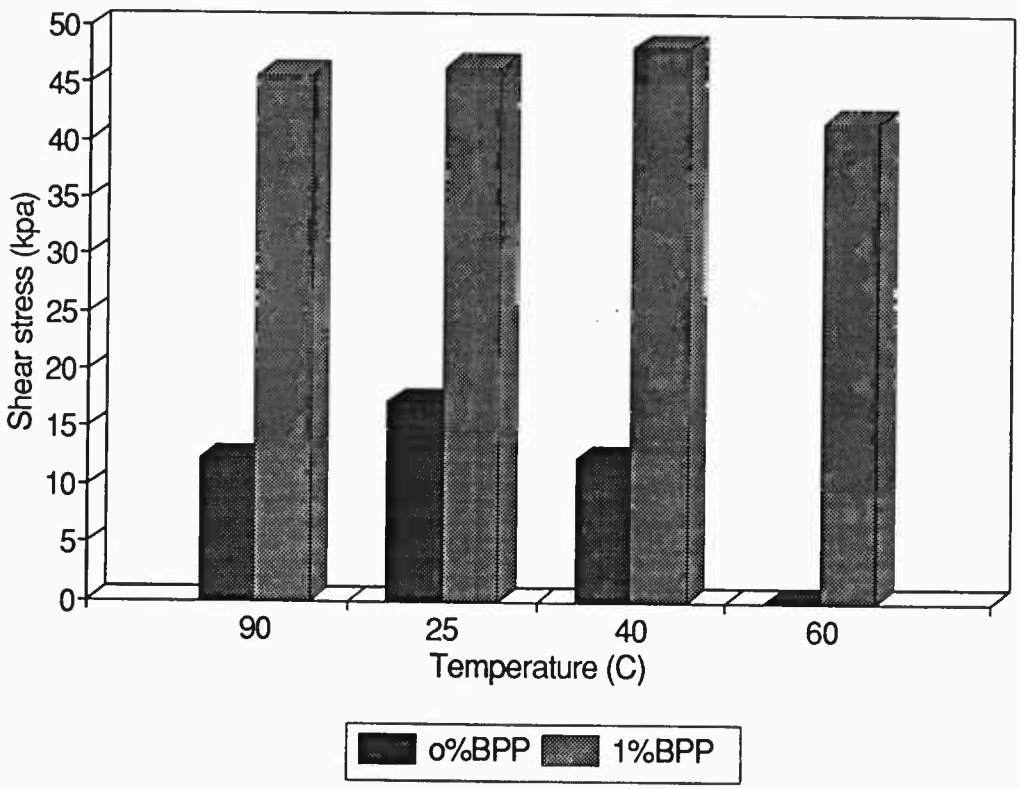

Fig.1 Effect of different temperature settings on surimi gels made from Pacific whiting.

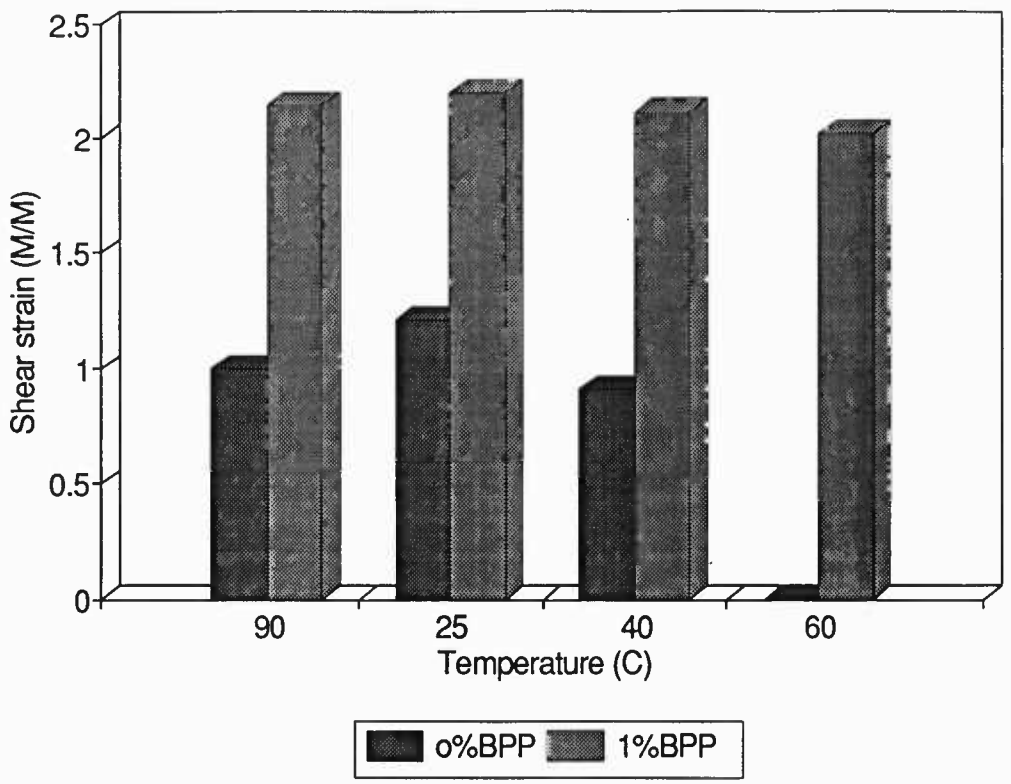

Fig.2 Effect of different temperature settings on surimi gels made from Pacific whiting.