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Fish ball is an extruded surimi-based product that is popular among Asian communities. In the manufacturing of fish ball, optimum floatation is extremely important for maximal production. Factors affecting floatation of fish ball in water were investigated. The density of threadfin bream surimi paste significantly decreased ($P<0.05$) as moisture content increased from 80% to 84% and temperature increased from 5°C to 40°C, to 90°C. Increased salt concentration from 2% to 3% also decreased surimi paste density. The ability of surimi paste to float or sink in water was observed according to changes in density. In gel texture measurement, when surimi was thawed for 1 h before chopping at 5°C with 2% salt the highest breaking force and deformation values were obtained. However, when surimi was thawed for 4 h before chopping at 20°C with 3% salt, the lowest breaking force and deformation values were found. Apparent viscosity of surimi paste decreased as moisture content

increased from 80% to 84%, salt concentration increased from 2% to 3%, and chopping temperature decreased from 20°C to 5°C. Setting gels in salt solution (5 or 10%) significantly reduced ($P<0.05$) their stickiness, which is the tendency to stick to one another.

Set fish ball is an extruded surimi-based product that is packaged after setting. Oversetting of set fish ball occurs at chilled temperature during storage. Shelf life extension of set fish ball by reducing oversetting conditions and microbial counts were examined. Encapsulated citric acid (CT) and Glucono-Delta-Lactone (GDL) were used to reduce oversetting. Parameters such as color, texture properties, pH, non-disulfide covalent bond, TGase activity, microbiological assay, viscosity, and water retention ability were examined to measure changes of fresh fish ball during refrigerated storage. Acetic acid, GDL, and chitosan were used to inhibit growth of microorganisms. Shelf life was measured for a period of 21 days. At Day 21, a reduction of 46%, 56%, and 26% in breaking force compared to the control was observed for 0.5GDL, 1.0GDL, and CT, respectively. GDL at 1.0% was shown to be the most effective in controlling oversetting of surimi. Chitosan (1%) dissolved in acetic acid maintained both aerobic plate and yeast counts at < 1 log CFU/g throughout 21 days of storage.

In summary, optimum processing conditions for set fish ball with uniform shape and maximum production were that frozen surimi was thawed for 1 h before chopping at 2% salt and 84% moisture until its final temperature reached 5°C and then surimi paste was held in 10% salt solution (40°C) for 20 min. The shelf life of set fish ball could be extended from 2 to 3 weeks with addition of 1% GDL in the surimi that controlled oversetting and 1% chitosan solubilized in 1% acetic acid that inhibited microorganisms for longer shelf life.

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Biochemical and Physical Factors Affecting Fish Ball

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Tiong Ngei Kok

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BIOCHEMICAL AND PHYSICAL FACTORS AFFECTING FISH BALL

CHAPTER 1

INTRODUCTION

Surimi is stabilized myofibrillar protein that can be comminuted with salt to form a three-dimensional protein network with heating. This mechanism is due to the denaturation of protein molecules followed by interaction of the denatured molecules to form cross-linkages (Ferry 1948; Powrie and Tung 1976).

Threadfin bream belongs to the family Nemipteridae. Ten species are commonly included as trawl catch (Guenneugues and Morrissey 2005). Thailand is the largest tropical fish surimi-producing country. Annual production of surimi from threadfin bream, which is also known as Itoyori in Japanese, and other tropical fish in Southeast Asia accounted for more than 150,000 t in 2003 (Guenneugues and Morrissey 2005).

Singapore has no surimi producing plants and annual surimi imports are estimated at about 14,000 t. Fish ball processing plants using surimi as an ingredient yielded an annual production of 18,000 t of fish ball in 2004. With four million people in Singapore consuming 70 t of fish ball/fish cake per day, the per capita consumption is about 6 kg (Tan SM, personal communication, 2004). The typical ingredients used in fish ball production include surimi, salt, sugar, monosodium glutamate (MSG), starch, and water.

Unlike other surimi seafood production, in fish ball production salt is added at the last minute of comminution. Otherwise, fish balls will sink to the bottom of the setting tank or float on the surface of the water, resulting in deformed fish ball that are flat or oval-shaped. Traditionally, the last-minute addition of salt during comminution prevents deformation by keeping extruded fish balls floating freely in the middle of the setting water. Due to limited studies on fish ball production, there is no established relationship between fish balls floating and salt addition during comminution.

In the production of fish ball, paste extruded in a ball shaped is set before placing in hot (95°C to 98°C) water for 10-30 min, depending on fish ball size (until the core temperature reaches 80°C). Then fish balls are chilled under running tap water. Fish balls are packed in a polyethylene (PE) bag before going through metal detection. Increasingly, Singaporean consumers prefer "fresh fish ball" to cooked fish ball because fresh fish ball is perceived as being more healthy and tasty. "Fresh fish ball" is not cooked but packaged with water after setting. Therefore, there is a great need to extend the shelf life of fish ball without sacrificing sensory and microbial quality.

Oversetting of fresh fish balls often occurs at chilled temperature during storage. Therefore fresh fish ball could only be kept for a few days. In order to overcome this oversetting problem, citric acid and Glucono-Delta-Lactone (GDL) were proposed to be tested in this study to determine whether the lower pH would delay setting of fresh fish ball in water. Inclusion of chitosan was also pursued as a method to extend the shelf life of chilled fish ball. The use of chitosan combined with acetic acid in shrimp salad showed the effectiveness at 5°C and ineffectiveness at 25 °C (Roller and Corvill 2000). However, information is lacking on the antibacterial activity of chitosan on surimi product such as fish ball.

In this current study, the overall objective was to improve fish ball processing methods in Singapore. Specific objectives involve evaluating: 1) factors that affect the floatation of fish ball during setting; 2) effect of citric acid and GDL on the setting phenomenon of fresh fish ball; and 3) effect of chitosan and low pH on the inhibition of microorganisms.

CHAPTER 2

LITERATURE REVIEW

Surimi-based products in Southeast Asia

Fish ball is a popular traditional food product in Southeast Asia. Each country has their own name for fish ball: Yu Huan in Singapore, Bebola Ikan in Malaysia and Indonesia, Nga Soke in Myanmar, Bola Bola in the Philippines, Look Chin Pla in Thailand (Kok and others 2002).

The industry is mainly centered in Chinese descendant communities. The scale of production varies from a one-man stall in the market preparing minced meat manually and selling the fish ball/fish cake directly to consumers, to large scale factories with production capacities of 1-2 t of products per day (Tan and others 1981).

In Singapore, the core of the industry lies in the production of fish balls and fish cakes for the numerous noodle stalls in the food centers. Fish balls are sold to noodle stalls and served with noodles. Fish cakes are fried and served in thin slices with noodles. Deep-fried fish balls at snack corners are becoming increasingly popular. The use of fish balls and fish cakes in other traditional dishes sold in food centers are also gaining popularity. Fish balls are also retailed directly to household consumers at wet markets and supermarkets. For retailing at supermarkets, fish balls are usually packed in styrofoam trays wrapped with a polyethylene (PE) sheet and vacuum packed. In the case of fresh fish balls they are packaged in PE bags with

water and displayed in refrigerated cabinets, with temperatures ranging from 3°C to 15°C.

Total surimi-based product includes fish ball, fish cake, chikuwa, and crabstick with an annual production of 22,000 t. The surimi-based product industry is valued at US$37.7 million and approximately 80% of the production is for the domestic market (USDA 2002). There are a total of 51 fish ball processing plants in Singapore, which comprises 80% of the total surimi-based production. The total daily production of fish balls and fish cakes in Singapore is estimated at about 70 t, retailing at a wholesale price of between US$6-8/kg. At wet markets, fish ball stalls produce fish balls from coral fish for direct retail to households at about US$7-8/kg. In recent years, leading processors from Thailand and Singapore have exported individually quick frozen fish balls to USA, Europe, and Australia, primarily for Asian communities (Kok and others 2002).

Previously, fish ball industries purchased fish daily from the fish market, landed by trawlers, as well as imported by truck from Malaysia. Low-cost and easily available fish species are used as raw materials. Higher value species like wolf herring (*Chirocentrus dorab*), coral fish (*Caesio spp.*), Spanish mackerel (*Scomberomorus spp.*), and conger eel (*Congresox spp.*) are often used for high priced and high quality fish balls (Tan and others 1981). In response to increasing labor costs and difficulty in obtaining labor to prepare fish for mincing, there is an increasing trend to use leached fish mince imported from Malaysia and Southern Thailand. Problems have also arisen with the insufficient supply of raw materials, as fish like coral fish and dorab (traditional raw materials) became increasingly expensive and limited in supply. This has led to the increasing use of frozen surimi imported from India, Indonesia, Malaysia, and Thailand. Due to the rising cost of

surimi, most of the processors are mixing surimi with fresh leached fish mince, thereby reducing cost and also enhancing the taste of final products.

The processing method involves grinding minced meat with salt and crushed ice is usually added to keep the paste cool. When a sticky paste is obtained, other ingredients like monosodium glutamate, sugar, and flour are added. The amount of water added is critical as it produces the soft and springy texture of the final products, and also lowers the cost of production. The paste is formed manually or with an extrusion machine into the desired shape (fish ball or fish cake). The product is then allowed to set in water (30°C for 2-3 h or 40-45°C for 20-30 min). The set products are boiled, cooled before being packed in plastic bags (3-5 kg/ bag), and stored in a chiller for distribution the following day.

The products are made for immediate delivery due to their relatively short shelf life of 4-5 days at around 5°C storage. Fish balls distributed in uncovered bamboo baskets without refrigeration have a shelf life of only two days. In a study on the bacteria flora of commercial cooked fish balls stored at 5°C, Lim (1989) reported that the bacterial level reached 10^6 CFU/g within 3 days, and exceeded 10^8 CFU/g in 4 days. Most of the isolated microorganisms were terrestrial bacteria, such as *Pseudomonas*, *Micrococcus*, *Moraxella*, and *Corynebacterium*.

Gelation characteristics of surimi

Proteins within the muscle are generally classified into three groups: myofibrillar, the contractile-element proteins; sarcoplasmic, the metabolic proteins; and stromal, the connective-tissue proteins. The predominant protein found in the

thick filament is myosin which constitutes 55-60% of the total myofibrillar protein. The myosin molecule is composed of two large subunits, myosin heavy chain (MHC) and four small subunits, myosin light chain (MLC) (Park and others 1997; Lanier 2000). Myosin has a molecular weight of 4.8×10^5 daltons and contains over 2000 amino acids (Rodger and Wilding 1990; Park and others 1997). Each MHC consists of a long α-helical and a globular region. The long α-helical portion of the MHC winds around each other to form the rod portion. The globular region was reported to be responsible for the ATPase activity and actin binding site (Park and others 1997). Globular actin, a globular protein of 40×10^3 daltons, is a double-stranded helical polymer of globular actin known as F-actin. Prerigor meat consists mainly of myosin, whereas postrigor meat consists of actomyosin (Ziegler and Acton 1984). Actomyosin is a complex of myosin, fibrous actin (F-actin), tropomyosin, troponins, and actinins (Briskey 1967).

Surimi is stabilized myofibrillar protein prepared by the mechanical washing of deboned fish to remove blood, lipids, enzymes, and other sarcoplasmic proteins and blended with cryoprotectants before freezing. A viscous sol is formed when myofibrillar proteins are mixed with salt. Under thermal conditions, the sol forms an elastic gel. The thermal gelation of the sol is responsible for the elasticity of comminuted myofibrillar gel products (Sano and others 1988). Thermal gelation of myofibrillar protein was studied in species such as beef, pork, rabbit, and chicken (Ziegler and Acton 1984). Myofibrillar proteins from cold-blooded animals such as cold water fish are less stable (Connell 1961), particularly in the presence of sodium chloride (Lanier 1986).

Both myosin and actomyosin had dominant roles in surimi gelation and showed species specification with regard to gelation properties (Shimizu and others

1983; Numakura and others 1985; Esturk and others 2004). MHC is responsible for the gelling properties of myofibrillar proteins (Ishioroshi and others 1979; Numakura and others 1985). Both the globular head (fragment S1) and α-helix region (rod) of the heavy chain were necessary for gelation of rabbit myosin (Samejima and others 1981; Ishioroshi and others 1979). The three dimensional network established during the setting process was due to the linkages in the tail portion of the molecule via hydrophobic interactions (Stone and Stanley 1992).

A more rigid but less elastic gel was obtained when sardine surimi, containing 3% NaCl, was quickly heated to 75°C or 90°C for 30 min without prior incubation at 35-40°C (Roussel and Cheftel 1988). This could be due to protein aggregation which predominated over unfolding. A gel with high rigidity and elasticity was obtained when incubation at 35-40°C was followed by cooking at 80-90°C (Ishikawa and others 1979; Roussel and Cheftel 1988). Cooking further increased gel rigidity, as shown with surimi from Atlantic croaker (Montejano and others 1983).

Gel setting, also known as "suwari" in Japanese, occurred when different types of surimi such as Alaska pollock, Atlantic croaker, sardine, threadfin bream, Pacific whiting, or round herring were comminuted with 2-3% sodium chloride and incubated at 5-40°C for 2-16 h (Ishikawa 1978; Shimizu and others 1981; Rossel and Cheftel 1988; Esturk and others 2004). Gel strength of surimi can be increased by subjecting surimi sol to setting below 40°C prior to cooking (An and others 1996; Kimura and others 1991). Setting or suwari has been widely applied in surimi product manufacture. Formation of stronger gel during setting has been attributed to non-covalent cross-linking between MHC induced by endogenous transglutamase (TGase) (Kumazawa and others 1995; Seki and others 1990), disulfide bonds (Hossain and others 1998), and hydrophobic interaction.

The setting response varies depending on fish species (Shimizu and others 1981). Setting temperature is related to the habitat temperature of the fish species (Morales and others 2001; Esturk and others 2004). The optimum temperature for setting among species may be determined by the heat stability of myosin. Fish myosin from cold water species is more vulnerable to thermal denaturation than warm water species (Johnston and others 1973; Hashimoto and others 1982; Tsuchimoto and others 1988; Howell and others 1991). Additionally, TGase has been reported to contribute to the polymerization of myosin (Araki and Seki 1993; Seki and others 1990). However, the rate of TGase mediated cross-linking of MHC may be primarily dependent on the conformation of substrate myosin at a given temperature rather than on the optimum temperature of TGase (Araki and Seki 1993; Kamath and others 1992). The TGase mediated cross-linking reaction of MHC varies, depending upon species (Araki and Seki 1993). Generally, setting can be performed at low (0-4°C), medium (25°C) and high (40°C) temperatures (Lanier 1992). Modori (gel-softening)-inducing proteinase is generally active at 50-60°C (Jiang 2000).

Benjakul and others (2003) studied the effect a medium-temperature setting of 25°C on gel properties and cross-linking of myofibrillar proteins in surimi from threadfin bream (*Nemipterus bleekeri*), bigeye snapper (*Priacanthus tayenus*), barracuda (*Sphyraena jello*), and bigeye croaker (*Pennahai macrophthalmus*). Increased setting time (0-8 h) resulted in a higher breaking force and deformation for all the surimi as non-disulfide covalent bond formation increased and MHC content decreased. Lee (1984) suggested that warm water species could tolerate higher washing water temperatures than cold water species without reducing protein functionality. Lee and Park (1998) reported optimum setting temperatures were 5°C for pollock and 25°C for Pacific whiting. Atlantic croaker (Kamath and others 1992)

and tilapia surimi (Yongsawatdigul and others 2000) achieved maximum gel strength when pre-incubation temperatures were 40°C. Esturk and others (2004) reported that chopping surimi from warm water species such as big eye, lizard fish, and threadfin bream at 20-25°C achieved maximum gel strength and cohesiveness. They found that the optimum setting temperature was 40°C for big eye and lizard fish and 25°C for threadfin bream surimi. Threadfin bream actomyosin exhibited major conformational changes at greater than 35°C. The addition of sodium chloride, which results in partial unfolding of actomyosin, is sufficient for TGase to cross-link amino acids at 25°C (Yongsawatigul and Park 2003).

Addition of reducing agents (sodium sulphite, thiosulphate or nitrite, 0-20 mmol/ kg) to Alaska pollock surimi prior to cooking (90°C, 60 min) was found to increase gel strength (Itoh and others 1979), suggesting that S-S bonds were involved in gel formation. Other studies indicated that SH group content of surimi remained constant during incubation at 38°C for 60 min (Alaska pollock) (Niwa and Miyake 1971), but decreased at higher temperatures (Atlantic croaker) (Liu and others 1982). However, the complete solubilization of the protein constituents of gels from Alaska pollock obtained either by incubation at 40°C or by cooking at 90°C, required both urea and mercaptoethanol (Niwa 1985). Since native myosin and actin contain several SH groups, but no (or very few) S-S bonds, it was likely that oxidation of SH into S-S occurs, mainly during cooking of surimi.

The role of hydrophobic interactions during the setting phenomenon was suggested by experiments showing that the covalent binding of apolar reagents on myofibrillar proteins from pork, chicken, dolphin, or carp promoted fast gel setting at 35-40°C in the presence of NaCl (Niwa and others 1981a, 1981b). Niwa (1975) also demonstrated that myofibrillar protein from flatfish (species known for fast setting

behavior) bound more naphtalene sulphonic acid (NSA; a hydrophobic fluorescent probe) after setting at 40°C and after cooking at 90°C for 15 min. Suwari is a unique property of fish muscle paste and is attributed to the TG catalyzed cross-linking of MHC (Seki 1990; Kimura 1991; Kamath 1992). Therefore, the optimal suwari pH must be compatible with TGase activity.

Typically, surimi gel quality is greatly affected by various intrinsic factors such as thermal denaturation and aggregation of muscle protein and the presence of proteinases and transglutaminase. TGase catalyzes the acyl-transfer reaction producing covalent bonds between proteins by the exchange of primary amines for ammonia at the γ-carboxylamide group of peptide-bound glutamine residues (Folk 1980). The peptide-bound lysine residues react with glutamine residues creating an ε-(γ-glutamyl)lysine (EGL) bond between proteins. Formation of intra- and inter-molecular covalent bonds resulted and these were reported to increase elasticity and firmness of various food proteins (Motoki and others 1980). An increase in gel strength of surimi after setting also resulted from endogenous TGase activity (Kamath and others 1992; Joseph and others 1994). Tsukamasa and others (1993) examined the quantitative change of EGL crosslink and the relationship between crosslink content and gel strength of salt-ground myofibril sol from sardine (*Sardinops melanostictus*) during incubation at 25°C. However, in the presence of ethyleneglycotetraacetic acid, gelation did not occur and no EGL cross-links were detected in myofibril sol. The EGL crosslink content and breaking strength of gels increased proportionally to incubation time. High correlation was observed between the logarithm of breaking strength and logarithm cross-links formed by transglutaminase, indicating the importance of EGL cross-links in the setting of sardine meat sol at <30°C.

The following processes are assumed to take place when surimi is kept at 35-40°C for 30 min in the presence of 0.5-0.6 M NaCl: (i) dissociation and solubilization of myosin filaments into individual molecules; (ii) partial unfolding of the α-helix region of myosin heavy chains; (iii) formation of an ordered gel network by aggregation of these regions through hydrophobic and electrostatic interactions (Liu and others 1982) (iv) formation of EGL bonds due to the function of TGase. Hydrogen bonds were important in gel stabilization during cooling, while hydrophobic interactions were important during heating when the myosin structure unfolds and becomes exposed to surrounding water (Park and others 1997). These processes were known to be influenced by temperature, pH, ionic strength, and the presence of $CaCl_2$ (Nishimoto and others 1987; Saeki and others 1988). A fifth process occurs during cooking at 70-90°C, namely aggregation of the globular heads of myosin heavy chain both through hydrophobic interactions and disulfide bonds (Taguchi and others 1987).

Effect of pH on the gelation of surimi

Surimi-based gel products are made from fish mince paste and or surimi paste by various thermal processes. Each stage involves a characteristic process with a combined physicochemical and enzymatic reaction. The pH of the salted paste is one of the most important factors in producing a strong elastic gel. There is a difference in the gelation mechanisms between acid- and salt-induced gelation. The mechanism of salt-induced surimi gelation was reviewed (Niwa 1992; Stone and Stanley 1992). Most studies were conducted at pH higher than the isoelectric point of protein, where protein molecules had a net negative charge. Alternations in the electrostatic and

hydrophobic interactions between protein molecules caused changes in protein structure. NaCl caused the myofibrillar protein initially to swell and subsequently break into actomyosin, myosin, and a variety of protein aggregates or complexes, thereby increasing the viscosity of the liquid phase and water-holding ability (Hamm 1975; Xiong and Blanchard 1994). The chloride ion was thought to be responsible for the effect of NaCl on myofibrillar proteins because it interacted strongly with the positive charge on the muscle protein, while the sodium ions were weakly bound (Puolanne and Terrell 1983; Asghar and others 1985). Belton and others (1987) found that chloride ion caused repulsion of myofibrillar proteins regardless of the presence of cation, based on the nuclear magnetic resonance study.

The gel strength of surimi-based products decreased with a decrease in pH to 6 regardless of the presence of salt (Trevino and Morrissey 1990). However, Fretheim and others (1985) found that myosin solution formed gels at 5°C when the pH decreased slowly. Venugopal and others (1994) and Chawla and others (1996) reported that the strength of threadfin bream or shark myofibrillar protein gel induced by organic acids (acetic, lactic, tartaric, or citric acids) increased with a decrease in pH and reached a maximum at pH 4.5. Acid-induced gelation occurred, which was less than the isoelectric point of myosin protein (pH 5.4), where protein molecules had a net positive charge. The addition of acid at the level used in the study might not cause the whole myofibril protein to swell and dissolve, but might cause partial solubilization of the outer layer of the myofiber or myofibrils. During gelation, solubilized proteins crosslink with other myofibrils, thus making the gel firmer.

The optimal pH of salted paste for strong gelation was pH 6.5-7.0 for flying fish muscle paste (Shimizu and others 1954). Miyake and Tanaka (1969) reported the

optimal pH for pelagic fish such as mackerel, tuna, and yellowtail was 6.2-6.7 and 7.0-7.5 for white meat fish such a walleye pollock, flatfish, Japanese sea bass, and grunt. Furthermore, horse mackerel muscle paste formed a strong gel at pH 7.0, but Lan and others (1995) reported that catfish muscle paste yielded its highest gel strength at pH 6.0. The optimal pH for all mammalian, chicken, and turkey muscle pastes was 6.0 (Lan and others 1995). The heat-induced gelation of purified myosin from rabbit muscle was optimally developed at pH 6.0 (Ishioroshi and others 1979), and this pH dependence by myosin gelation was derived from myosin rod, not S1. S1 gelation was independent over a wide range of pH from 5.0-8.0 (Samejima and others 1981).

Kim and others (1993) compared the gelling properties of walleye pollock surimi and beef myofibrils as a function of pH and found that the maximum gel strength was obtained at pH 7.0 for surimi and pH 6.0 for beef myofibrils (Kim and others 1993). The optimum pH for white meat fish of easy suwari species was approximately 7.0. The species difference (pH dependence) was linked to the various ways heat-induced gelation affected setting (Kim and others 1993; Nishimoto and others 1987).

Additives to control texture and extend shelf life

Glucono-Delta-Lactone

GDL is commonly used in human food as a coagulant and a pH control agent. Compared with other food acids, GDL provides a gradual and continous decrease of

pH to equilibrium due to its slow hydrolysis to gluconic acid. Therefore, it is often used as a slow release acidulant. The taste characteristics change from sweet to slightly acidic when hydrolyzed GDL is converted into gluconic acid making it less tart than other acidulants (Schwertfeger and Buchheim 1999). The US Food and Drug Adminstration (USFDA 2005) assigned GDL the "generally recognized as safe" (GRAS) status and permitted its use in food without limitation other than good manufacturing practice. GDL is commercially produced by an aerobic oxidizing fermentation process to convert a carbohydrate source into gluconic acid. After fermentation a blend of gluconic acid and GDL is separated by crystallisation (USFDA 2005).

McGlynn and others (1993) reported that GDL was not hydrolyzed into gluconic acid without heat treatment and lowered the pH value of food only after heating. GDL is used as a preservative such as ascorbic acid to lower the pH value of products and prolong shelf life. It is also used for producing tofu by coagulating soymilk. Tofu, a soy protein gel, traps water, lipids, and other constituents in the matrix (Kim and Hans 2002). Gluconic acid was shown to be effective as acidulant that contributed little flavor to canned products (Kaercher 1989). Acidification and thermal processing were used to positively influence the texture of canned vegetables (Heil and McCarthy 1989). GDL caused milk proteins to aggregate due to pH reduction (Chen and others 2004).

Besides, tofu, whey protein, yoghurt, and vegetables, GDL has been used in fish. Milkfish has a characteristic light pink flesh due to the presence of myoglobin in the flesh. Addition of malic or citric acid improved whiteness of gels from milkfish surimi. However it had lower gel strength compared to using GDL at 0.4%, which improved whiteness without affecting gel strength. The results obtained also

suggested that acidification by GDL was relatively weak compared to that by malic and citric acids (Chen and others 1998).

The physical properties of fish sausage and other surimi-based products, such as elasticity and gel strength, are greatly dependent on meat pH. The best product is obtained in the pH range of 6.5 to 7.0. However providing mild acidity would be favorable from the stand point of prolonging shelf life, because spoilage bacteria have limited growth in acidic media. In addition, chemical preservatives are more effective in acidic compared to neutral media. When the pH of fish paste was adjusted below 5.8, in general, the paste transformed to a brittle gel with low gel strength and high expressible water. GDL, when added to water, is neutral, but on standing or by heating is converted to acids. The rate of hydrolysis increased with increased temperature. The increased acidity of meat during cooking did not affect the physical properties of the product. There was no syneresis of water, protein coagulation or loss of elasticity in the kamaboko (Okada and Komori 1965).

Chitosan

Chitin could be naturally obtained from shells of crustacean and shellfish, squid pen, and fungal cell walls (Knorr 1984). However, chitosan is only manufactured from crustaceans (crab, krill, and crayfish) primarily because a large amount of crustacean exoskeleton is available as by-product of food processing (Shepherd and others 1997). Worldwide production is estimated to be 3.9×10^4 t annually (Knorr 1991). Processing chitin from crustacean shellfish waste involves 2 main steps, protein separation and calcium carbonate separation (Muzzarelli 1977).

Waste from crustacean shell is usually ground and mixed with a dilute aqueous sodium hydroxide solution to dissolve the protein. The residual materials are then treated with dilute aqueous hydrochloric acid solution (up to 10%) to dissolve the calcium carbonate as calcium chloride. Deacetylization of chitin was carried out by treatment with hot 40-50% sodium hydroxide (Knorr 1984).

The deacetylated form of chitin (poly-β-1, 4-N-acetyl-D-glucosamine) is known as chitosan (2-deoxy-2-aminoglucose polymer). After deacetylation in alkali, chitosan with a specific degree of deacetylation was obtained. The higher the degree of deacetylation, the higher the ratio of free amino groups (NH^{+3}) on the chitosan molecule (Shahidi 1999). Chitosan was insoluble at neutral pH but soluble in weak acid (pH 6) solutions because of the positive charge on the C2 of the glucosamine monomer. Chitosan is one of the most abundant natural polysaccharides. Various types of chitosan are available and the difference mainly related to the molecular weight, viscosity, and degree of deacetylation (López-Caballero 2005). Most commercial chitosans have more than 70% degree of deacetylation and a molecular weight between 100,000 and 1.2 million daltons (Li and others 1997; Onsoyen and Skaugrud 1990).

Due to chitosan's unique poly-cationic nature, it has important industrial applications, such as waste water purification (Castellanos-Perez and others 1988; Knorr 1991), chelation of transition metals (Muzzarelli 1977), coacervate formation for cell entrapment (Knorr and Teutonico 1986), and coating of seeds for improved yield (Hadwiger and others 1984). The coagulating, lipid-binding, and antimicrobial properties made chitosan useful in food and nutritional applications (Shahidi and others 1999).

Chitosan inhibits the growth of a wide variety of fungi (Allan and Hadwiger 1979; Stossel and Leuba 1984; Hirano and Nagao 1989). The polycationic nature of chitosan interfered with negatively charged residues of macromolecules at the cell surface (Young and Kauss 1983). Chitosan and other polyamines interacted with the cell membrane to alter cell permeability (Young and others 1982). Chitosan with a higher degree of deacetylation generally showed higher solubility and more positive charges in an acidic environment (Chang and others 2003). The amount of absorbed chitosan onto the different bacteria determines the antibacterial activity of chitosan (Chen and others 2002; Loosdrecht and others 1987). The more chitosan absorbed would result in the greater changes in the structure of the cell wall and in the permeability of the cell membrane. Both result in the death of bacteria (López-Caballero and others 2005).

Chitosan had been used as a spray or coating to extend the shelf life and quality of fresh produce such as tomatoes and strawberries (El Ghaouth and others 1991, 1992). Growth rate of many fungal species in apple juice was reduced and the lag phase lengthened by chitosan (Roller and Corvill 1999). The growth of several bacteria and yeast found in mayonnaise and mayonnaise-based products was inhibited by chitosan glutamate dissolved in acetic acid (Roller and Corvill 2000).

Chen and others (2002) reported the effectiveness of chitosan as a natural disinfectant of bacteria associated with waterborne diseases, particularly gram-negative bacteria. The higher degree of deacetylation and higher concentration of chitosan generally relates to higher antibacterial activity. Contact time of at least twelve hours was required for inhibiting bacteria growth.

A reduction of 1 to 2 log cycles of total bacteria, *pseudomonads*, *staphylococci*, coliforms, gram-negative bacteria, and *micrococci* was reported in the presence of

1% chitosan when used in mince beef patties. Lower concentration of 0.2% and 0.5% chitosan had no effect on spoilage flora (Darmadji and Izumimoto 1994).

Katoaka and others (1998) reported the use of 1.5% chitosan on walleye pollock surimi with a combination of setting at 20°C, resulted in a twofold increase in gel strength. Breaking force and deformation of gel from barred garfish surimi significantly increased when added with 15 mg/g of chitosan with 65.6% degree of deacetylation. This resulted in the formation of protein-protein cross-links of and protein-chitosan conjugates by endogenous transglutaminase (Benjakul and others 2000).

The effects of 1 and 2% chitosan solutions on the shelf life of whole and headless shrimp stored at 4 to 7°C were studied by Simpson and others (1997). They found an increased lag phase of microbial growth with both concentrations of chitosan. The 2% chitosan solution had reduced microbial count of 1 log cycle when compared with the control.

Salmon fillet was dipped in various chitosan solutions prepared in 1% acetic acid. The results indicated that high molecular weight chitosan had lower aerobic plate counts than low molecular weight chitosan and control. However, the antimicrobial activity was only effective for the first 10 days of storage at refrigerated storage (Nicholas 2003).

A cold blend of gelatin and chitosan solution was used as a coating on cod patties. The coating delayed spoilage of cod patties. The coating did not impart any taste to the product since it melted away during the cooking process (López-Caballero 2005). The antimicrobial and functional properties of chitosan's solution and films depend on characteristics of the chitosan molecule itself (degree of deacetylation, molecular weight), other compounds in the system (type and

concentration of the acid, presence of proteins, lipids, ions, and other food ingredients), and environmental conditions (temperature and relative humidity) (Begin and Van Calsteren 1999; Kurita 2001; Synowiecki and Al-Khateeb 2003; Zheng and Zhu 2003).

CHAPTER 3

ELUCIDATING FACTORS AFFECTING FLOATATION OF FISH BALL

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ABSTRACT

Fish ball is an extruded surimi-based product that is popular among Asian communities. In the manufacturing of fish ball, optimum floatation is extremely important for maximal production. Factors affecting floatation of fish ball in water were investigated. The density of threadfin bream surimi paste significantly decreased ($P<0.05$) as moisture content increased from 80% to 84% and temperature increased from 5°C to 40°C, to 90°C. Increased salt concentration from 2% to 3% also decreased surimi paste density. The ability of surimi paste to float or sink in water was observed according to changes in density. In gel texture measurement, when surimi was thawed for 1 h before chopping at 5°C with 2% salt the highest breaking force and deformation values were obtained. However, when surimi was thawed for 4 h before chopping at 20°C with 3% salt, the lowest breaking force and deformation values were found. Apparent viscosity of surimi paste decreased as moisture content increased from 80% to 84%, salt concentration increased from 2% to 3%, and chopping temperature decreased from 20°C to 5°C. Setting gels in salt solution (5 or 10%) significantly reduced ($P<0.05$) their stickiness, which is the tendency to stick to one another.

Key words: Fish ball, surimi, density, gel texture measurement, viscosity, stickiness

INTRODUCTION

Fish ball is a popular, traditional food product in Southeast Asia. Fish ball consumption in Thailand is about 12,000 t annually (Park 2005a). In Singapore, approximately four million people consume 70 t fish ball/fish cake a day, resulting in about 6 kg per capita consumption (Park 2005a). The industry is mainly centered on Chinese descendant's communities. The scale of production may vary from a one-man stall in the market preparing minced meat manually and selling fish balls directly to consumers, to large scale factories with production capacities of 1-2 t of surimi-based products daily (Tan and others 1981).

The type of fish used for making high price and high quality fish ball included species such as wolf herring (*Chirocentrus dorab*), coral fish (*Caesio spp.*), Spanish mackerel (*Scomberomorus spp.*), and conger eel (*Congresox spp.*) (Tan and others 1981). Inconsistent and insufficient supplies of fresh fish in Singapore has lead to increased use of frozen surimi.

The most commonly used surimi for making fish ball is threadfin bream. Threadfin bream belongs to the family Nemipteridae and there are ten species commonly found in the tropical and subtropical waters of the Indo-West Pacific Region. In Thailand, India, Indonesia, the Philippines, and Malaysia, threadfin bream forms an integral part of trawl catch (Guenneugues and Morrissey 2005). Thailand is the largest tropical fish surimi-producing country. Annual production of surimi from threadfin bream and other tropical fish in Southeast Asia accounted for more than 150,000 t in 2003 (Guenneugues and Morrissey 2005).

In Singapore, annual surimi imports amounted to about 14,000 t. There are a total of 51 fish ball processing plants in Singapore with an annual production of

22,000 t surimi-based products such as fish ball, fish cake, chikuwa, and crabstick. Fish ball comprised 80% of total production. The surimi-based product industry is valued at US$37.7 million. The wholesale price of fish ball retails between US$6-8/kg. About 80% of the production is for the domestic market (USDA 2002). Typical ingredients used for fish ball include surimi, salt, sugar, monosodium glutamate (MSG), starch, and water.

Unlike other surimi seafood, in the manufacture of fish ball, salt is added at the last minute of comminution. Otherwise, fish balls sink to the bottom of the setting tank or float on the surface of the water. Either case can lead to deformation of fish balls, resulting in flat or oval-shaped products, or sometimes entanglement. Therefore, keeping a uniform shape during mass production is critical. Traditionally, last-minute addition of salt during comminution kept extruded fish balls floating freely in the middle of the setting water to prevent deformation.

Due to limited studies in fish ball manufacturing, there is no established relationship between fish balls floating in the middle of the water and addition of salt during comminution. Therefore, the objective of this study was to explore factors that affect floatation of fish ball during setting.

MATERIALS AND METHODS

Materials

Frozen surimi (grade AA) produced from threadfin bream (*Nemipterus spp.*) (TB) was obtained from Andaman Surimi Industries Co. Ltd (Bangkok, Thailand). Surimi was cut into approximately 1000 g blocks, vacuum packed, and stored in a freezer (-30°C) throughout the experiments. Moisture content of the surimi was 75.4 ± 0.2% (AOAC 1995).

Gel preparation

The first batch of frozen surimi was tempered at room temperature for 1 h before chopping to a final temperature of 5°C. The second batch of frozen surimi was tempered at room temperature for 4 h before chopping to a final temperature of 20°C. Tempered surimi was cut into approximately 4 cm cubes. Surimi cubes were placed in a Stephan vacuum cutter UM-5 (Stephan Machinery Corp., Columbus, OH, USA). In the first 1 min, surimi cubes were chopped at low speed. Salt, at various concentrations (2, 2.5, 2.75, and 3%) was sprinkled and chopping continued at low speed for 1 min. Ice/water was added to adjust moisture content to various levels (80, 82, and 84%), the samples were chopped at low speed for 1 min. During the final 3 min, chopping continued at high speed, with vacuum applied at 0.4 bar. After chopping, the paste was stuffed into stainless steel tubes (inner diameter, 1.9 cm;

length, 17.5 cm) with stainless steel screw caps, using a sausage stuffer (Sausage Maker, Buffalo, NY, USA). The interior wall of the tubes was coated with a film of PAM cooking spray (Boyle-Midway, Inc., NY). Paste was set at 40°C for 30 min followed by heating at 90°C for 15 min. Surimi gels were chilled quickly in ice water (0°C) for 15 min and refrigerated (5°C) overnight before analysis.

Density measurement

Surimi paste was extruded into stainless steel cylinder (inner diameter, 1.9 cm; length, 17.5 cm), with known weight and volume, using a sausage stuffer. Density of surimi paste was measured at four temperatures (5, 20, 40, and 90°C). Samples were placed in a controlled temperature water bath and allowed to expand freely as they gelled. At each test temperature, the expanded surimi gel outside the cylinder was trimmed off and the sample weight was accurately measured. Density was determined by weight of the surimi in the cylinder divided by the volume of the cylinder (AbuDagga and Kolbe 1997). Four replicates were used for each moisture content level.

Gel texture measurement

Gels were equilibrated to room temperature at 25°C and cut into 2.9 cm long. Gel samples were measured for breaking force and deformation to determine the strength and cohesiveness of gels, respectively, using a Texture Analyzer (TA-XT

plus, Texture Technologies Corp., NY, USA), equipped with a 5-mm spherical probe at a test speed of 1 mm/s (Park 2005b).

Stickiness of surimi gels

Gels were equilibrated to room temperature at 25°C and cut into 1.9 cm long. Stickiness was measured using a Texture Analyzer (TA-XT plus, Texture Technologies Corp., NY, USA) fitted with a compression plate (P/75) with 75 mm diameter. An applied force of 100 g was used with a contact time of 10 s and test speed of 0.5 mm/s. Stickiness was reported as the area under the curve as the plate moved upwards.

Viscosity measurement with capillary extrusion viscometer

Viscosity measurement was performed according to the method developed by Kim and Park (2005) using a Texture Analyzer (TA-XT plus, Texture Technologies Corp., NY, USA) fitted with a capillary fixture kit (TA-525) having an extrusion tube of 30 mm long and nozzle diameter of 6 mm. Surimi paste remaining after gel anaylsis was packed into the cylinder using a sausage stuffer (Sausage Maker, Buffalo, NY, USA). Surimi paste was extruded at different piston speeds (1, 5, 10, 15, and 20 mm/s). Maximum sustained force at each piston speed was recorded to calculate shear stress and shear rate.

$$\sigma = (\Delta PR/2L)$$

Where, σ = shear stress (N/m^2), ΔP = pressure (N), R = radius (mm), L = tube length (mm). VanWazer and others (1963) provided the Robinowitch-Mooney equation for shear rate of a non-Newtonian fluid as follows:

$$(dv/dr) = ((3+b)/4)Q$$

Where, (dv/dr) = shear rate (s^{-1}), $b = \Delta \log Q / \Delta \log \sigma$, $Q = (4q/\pi R^3)$, q = volumetric flow rate (s^{-1}), R= radius (mm), L = tube length (mm). Apparent viscosity of surimi paste was then determined by the slope of shear stress vs shear rate.

Determination of specific gravity

Specific gravity of the salt solution was measured using a pycnometer (Cole-Parmer Instrument Co., IL, USA). The net weight of the salt solution (g) was divided by the net weight of water (g) at a specific temperature.

Statistical analysis

Analysis of variance (ANOVA) was performed and Duncan multiple range test (Steel and Torrie 1980) was used to resolve a statistical difference between mean values. Data was analyzed using SPSS version 13.0 (SPSS Statistical Software, Inc., Chicago, IL, USA). A level of significance was set at $P<0.05$.

RESULTS AND DISCUSSIONS

Effect of moisture, chopping temperature, salt, and thermal treatment on density

The effect of moisture on density of surimi paste at various thermal treatments is given in Figure 3.1. The density of threadfin bream surimi paste significantly decreased ($P<0.05$) with increasing moisture and temperature. Density of surimi paste was affected by moisture content because the density of water is lower than the density of all the other components, except fat (AbuDagga and Kolbe 1997). A similar trend of increasing moisture and temperature caused decreased density of Pacific whiting surimi paste when measured at 30, 60, and 90°C (AbuDagga and Kolbe 1997). When surimi was heated, protein undergoes denaturation and caused a spatial arrangement of the native protein chains within the molecule to change to a more disordered arrangement thereby causing a change in density with increasing temperature (Ziegler and Acton 1984).

The effects of salt concentration on density of surimi paste at various thermal treatments are given in Figures 3.2 and 3.3. There were significant differences ($P<0.05$) in the densities of surimi paste at various thermal treatments having the same salt concentrations. Generally, density of surimi at 5°C was higher than surimi at 40°C and 90°C with the same salt concentration. The change in density between 5°C and 40°C were 17.27 kg/m^3, 12.04 kg/m^3, 19.59 kg/m^3, and 33.73 kg/m^3 for 2, 2.5, 2.75, and 3% salt concentration, respectively (Figure 3.2). The change in density between 40 °C and 90 °C were 27.76 kg/m^3, 48.13 kg/m^3, 55.76 kg/m^3 and 67.02 kg/m^3 for 2, 2.5, 2.75, and 3% salt concentration, respectively (Fig 3.2).

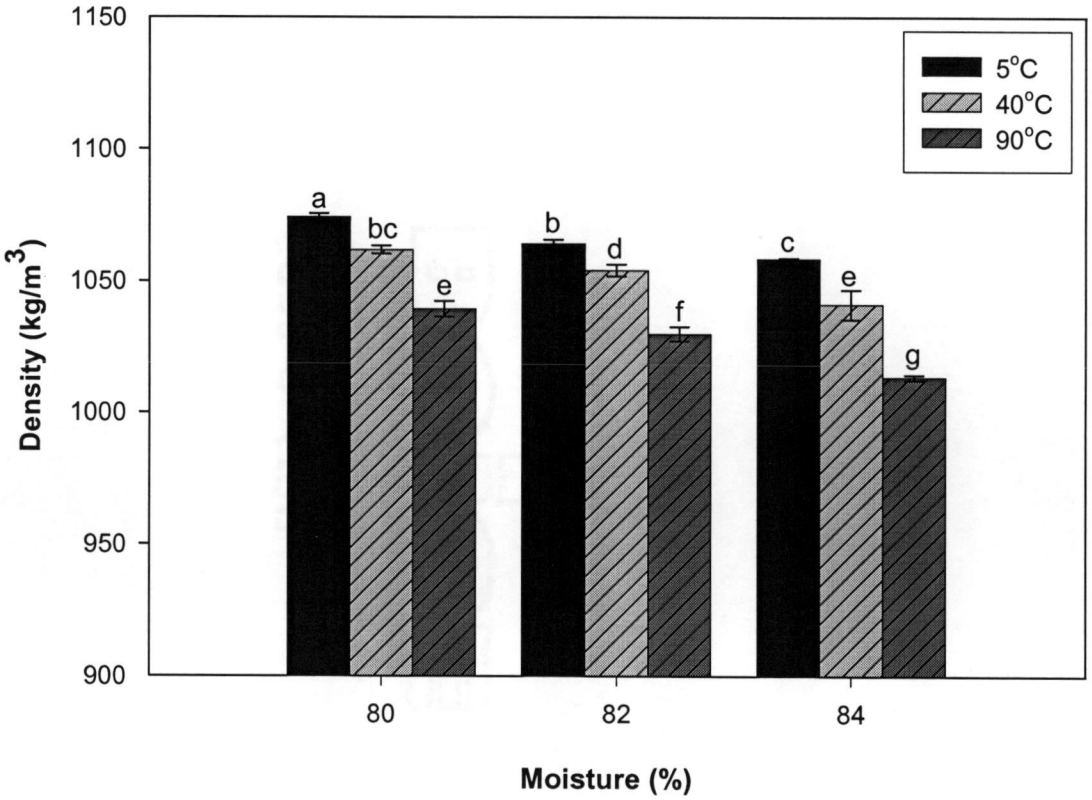

Figure 3.1 - Effect of moisture on density of surimi paste at various thermal treatments. Surimi paste was prepared after surimi was thawed for 1 h and chopped to a final temperature of 5°C with 2% salt. Different alphabetical letters indicate a significant difference ($P<0.05$)

Greater density change was observed between 40°C and 90°C than between 5°C and 40°C. With increasing salt concentrations, the change in density also increased.

The change in density between 20°C and 40°C was 5.73 kg/m^3, 6.79 kg/m^3, 13.36 kg/m^3, and 17.28 kg/m^3 for 2, 2.5, 2.75, and 3% salt concentration, respectively (Figure 3.3). The change in density between 40°C and 90°C was 32.33 kg/m^3, 40.02 kg/m^3, 37.57 kg/m^3, and 33.50 kg/m^3 for 2, 2.5, 2.75, and 3% salt concentration, respectively (Figure 3.3). The change in density of surimi paste was greater between 40°C and 90°C than between 20°C and 40°C. When surimi was thawed for 4 h and chopped to a final temperature of 20°C, densities reached above 1000 kg/m^3. Density of surimi paste decreased as temperature and salt concentration increased. Salt destabilized native proteins and caused the protein to unfold. This was suggested from the differential scanning calorimetry (DSC) in which the transition temperature of surimi paste was lowered by the addition of salt (Wu and others 1985).

Salt is known to act with water molecules to strengthen hydrophobic interactions with sodium ions, water structure-making ions, and enhancing formation of clathrates (Niwa and others 1986b). With the progress of gel formation, free water in fish flesh converts to bound water. Change in the amount of bound water is measured by nuclear magnetic resonance (Suzuki 1973). Upon grinding surimi with salt, bound water increases. Presumably, water is bound to the protein surface as proteins dissolves. During setting, the bound water further increases (Niwa and others 1975). With the formation of a network structure, water becomes confined. On heating the set gel at high temperatures, rigidity increases as the portion of bound water is released. Consequently, the homogenous dispersion of the network structure is lost slightly. However, elastic gels are resistant to loss of water (Tagagi 1973; Akahane

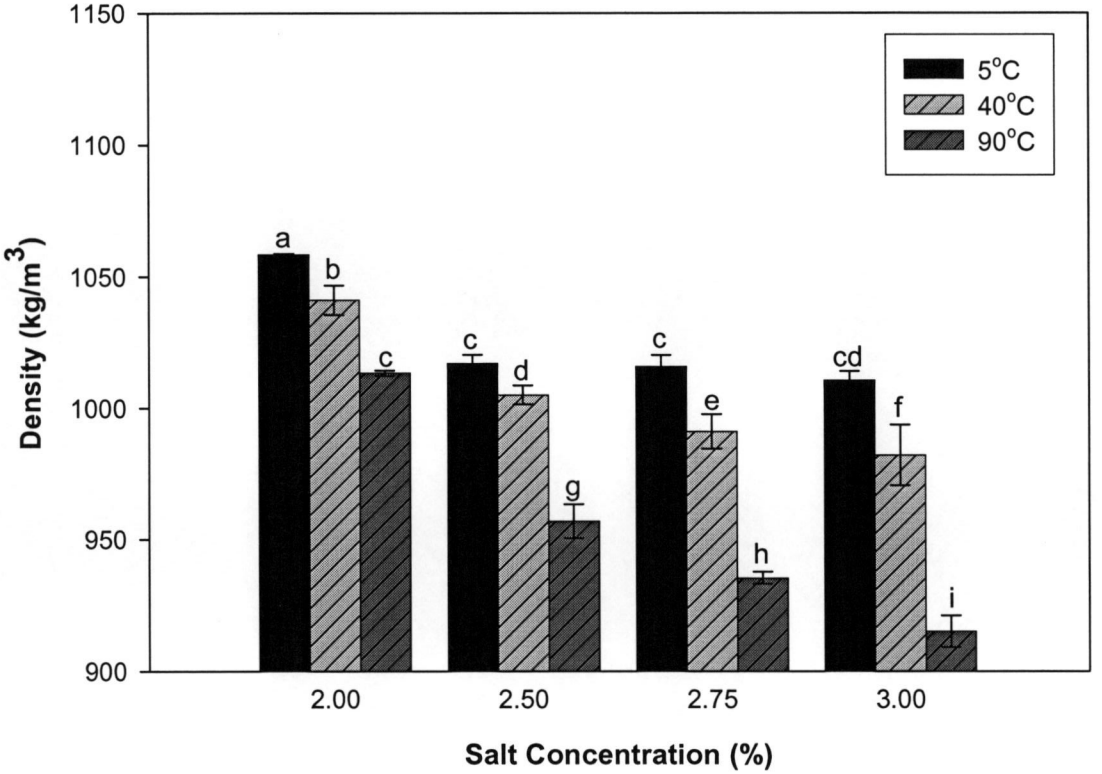

Figure 3.2 - Effect of salt concentration on density of surimi paste at various thermal treatments. Surimi paste was prepared after surimi was thawed for 1 h and chopped to a final temperature of 5°C with 84% moisture content. Different alphabetical letters indicate a significant difference ($P<0.05$)

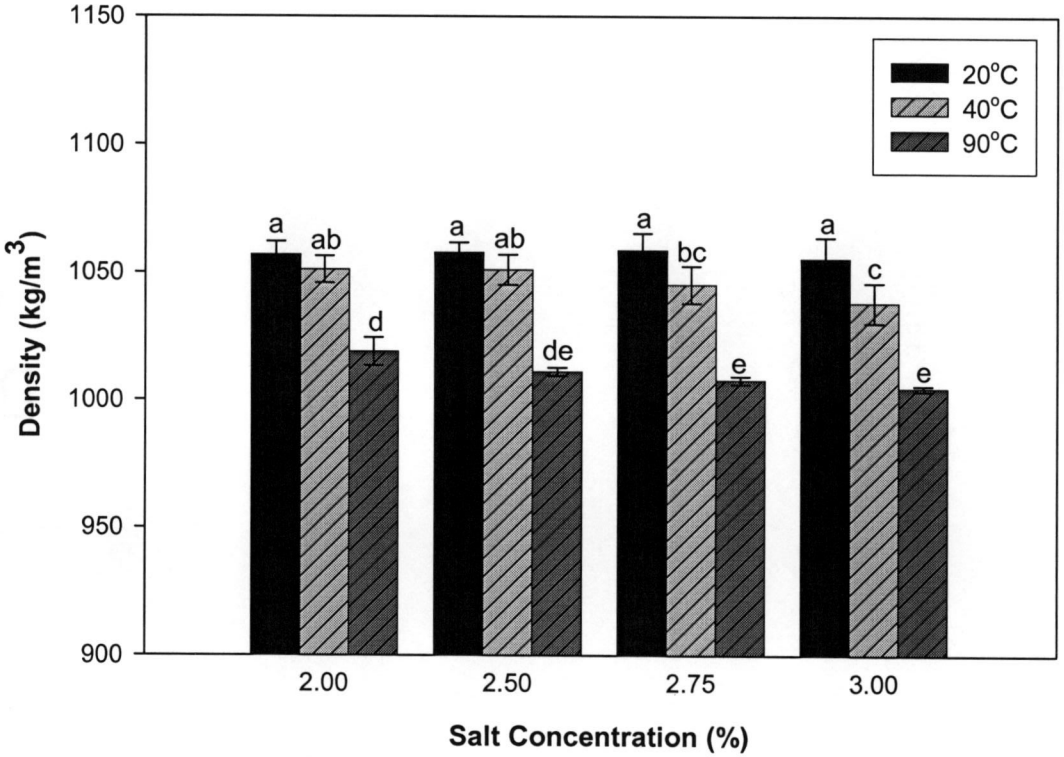

Figure 3.3 - Effect of salt concentration on density of surimi paste at various thermal treatments. Surimi paste was prepared after surimi was thawed for 4 h and chopped to a final temperature of 20°C with 84% moisture content. Different alphabetical letters indicate a significant difference ($P<0.05$)

and others 1981). Electrostatic measurements verified that total bound water increases as the polar amino acid residues are dispersed more homogenously within the surimi gel (Niwa and others 1984). Densities of surimi paste and gel were affected by salt concentration, moisture, thermal treatments, thawing time, and chopping temperature.

Floatation of set gel

Floatation of set gel is important especially when extruded surimi paste floats on the water surface or sinks to the bottom of the setting tank. The former cause extruded fish balls to stick to one another and the latter induces fish balls to flatten. Both cases result in deformed product.

Time required for surimi paste dropped into the setting tank to float is reported in Table 3.1. When surimi paste was chopped to a final temperature of 5°C after thawing for only 1 h, the effect of salt concentration on floatation time of set gel was observed only after adding salt at 2.5% or more. Set gel with 2% salt remained submerged at the bottom of the setting tank (40°C) even after 4 h. Time required for set gel to float decreased with increased salt and temperature. Only surimi thawed for 4 h and chopped to a final temperature of 20°C with 3% salt floats at 90°C. Surimi chopped with 2, 2.5 and 2.75% salt remained submerged after 4 h at both 40°C and 90°C. Attempts had been made to suspend set gel in salt water. This was achieved through measuring the density of surimi paste and matching equivalent specific gravity of salt solution through the use of a pycnometer. Surimi thawed for 4 h and

Table 3.1 – Effect of thawing conditions, salt concentration, and chopping temperature on the floatation of set gel. RS: remained submerged at the bottom of the setting tank after 4 h. Different alphabetical letters indicate a significant difference ($P<0.05$)

Thawing (h)/ Final Chopping Temperature (°C)	Salt Concentration (%)	Floatation Time (min) In Water	
		Water Temperature (°C)	
		40	90
1/5	2.00	RS	RS
	2.50	55.29[a]	2.45[d]
	2.75	10.39[b]	1.47[e]
	3.00	3.41[c]	1.48[e]
4/20	2.00	RS	RS
	2.50	RS	RS
	2.75	RS	RS
	3.00	RS	2.21[d]

chopped to a final temperature of 20°C with 84% moisture content and 2% salt was suspended in a 9.47% salt solution at room temperature.

Effect of thawing conditions, chopping temperature, and salt concentration on texture properties

The effects of salt concentration on breaking force and deformation values of cooked gels affected by thawing condition and final chopping temperature are given in Figures 3.4 and 3.5, respectively. Surimi thawed for 1 h and chopped at 5°C with 2% salt had the highest breaking force and deformation values. Surimi thawed for 4 h and chopped with 3% salt to a final chopping temperature of 20°C had the lowest breaking force and deformation value. This study indicated that there is a strong relationship between thawing conditions, chopping temperature, and salt concentration on protein stability of surimi.

Our observations were different from Esturk and others (2004) who studied the effects of thermal sensitivity of fish proteins from various species on rheological properties of cooked gels (78% moisture). Thermal sensitivity was tested at various final chopping temperatures (0, 5, 10, 20, 25, and 30°C) with 2% salt after surimi was tempered at room temperature for 1 h. Surimi paste was cooked at 90°C for 15 min. They found that cold-water species had maximum gel strength and cohesiveness at lower chopping temperatures (0°C), temperate water species such as Pacific whiting at 5°C to 10°C and warm water species such as big eye, lizard fish, and threadfin bream at 20-25°C final chopping temperature. Esturk and others (2004) indicated a strong relationship between habitat temperature and protein stability.

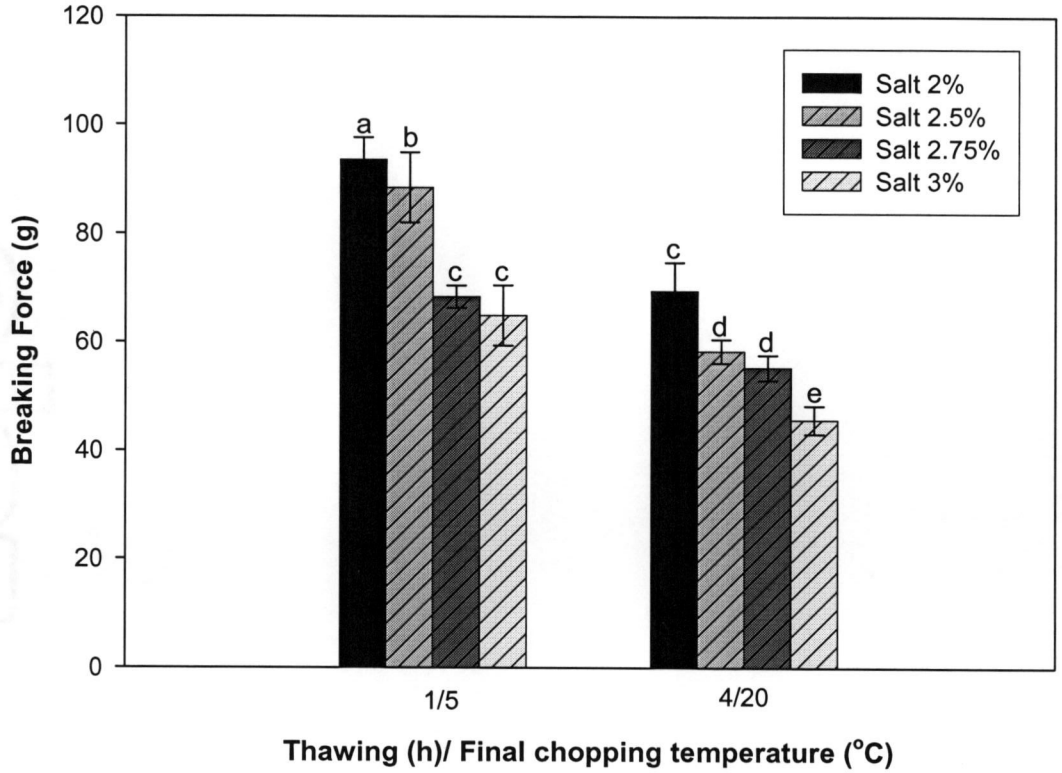

Figure 3.4 - Effect of salt concentration on breaking force of cooked gel affected by thawing condition and final chopping temperature. Surimi pastes were prepared after surimi was thawed for 1 h (final chopping temperature of 5°C) and 4 h (final chopping temperature of 20°C) with 84% moisture content. Different alphabetical letters indicate a significant difference ($P<0.05$)

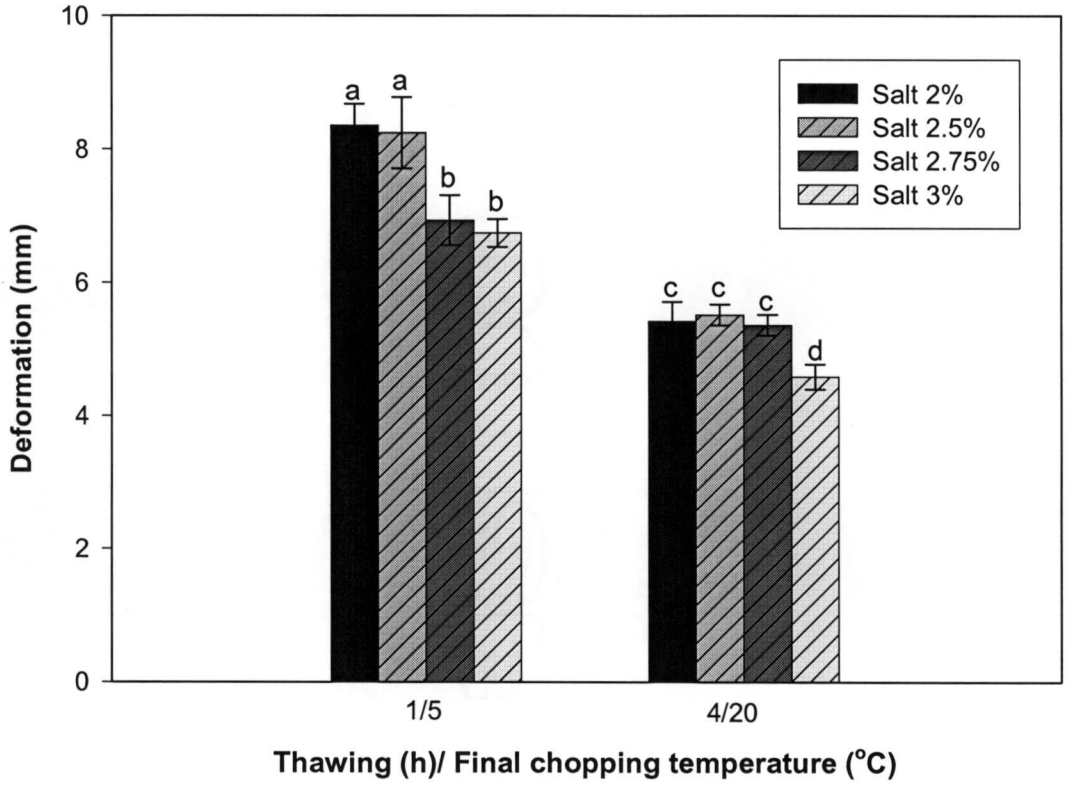

Figure 3.5 - Effect of salt concentration on deformation of cooked gel affected by thawing condition and final chopping temperature. Surimi pastes were prepared after surimi was thawed for 1 h (final chopping temperature of 5°C) and 4 h (final chopping temperature of 20°C) with 84% moisture content. Different alphabetical letters indicate a significant difference ($P<0.05$)

However, our additional study agreed with the findings by Esturk and others (2004). Threadfin bream surimi was tempered at room temperature for 1 h before chopping at 78% moisture and 2% salt. The paste was then heated at 90°C for 15 min. Cooked gels with a final chopping temperature of 20°C had higher breaking force and deformation values of 358.03 g and 13.74 mm, respectively than surimi with a final chopping temperature of 5°C, which had a breaking force of 232.58 g and 11.19 mm for deformation. Surimi tempered for 4 h at room temperature with final chopping temperature of 20°C also had higher breaking force and deformation of 283.84 g and 11.21 mm, respectively than surimi tempered for 1 h with final chopping temperature of 5°C. When surimi paste moisture content was adjusted to 84%, after 1 h thawing, a similar trend observed above occurred with a final chopping temperature of 20°C having higher breaking force (89.98 g) and deformation (10.77 mm) than a final chopping temperature of 5°C (breaking force 53.50 g and deformation 7.48 mm). Surimi that was thawed for 4 h (final chopping temperature of 20°C) and adjusted to 84% with 2% salt added had lower breaking force (44.93 g) and deformation (6.70) than surimi thawed for 1 h with a final chopping temperature of 5°C. Our additional study definitely indicated that texture properties of threadfin bream surimi gel were affected by thawing conditions and final chopping temperature and behaved differently when the moisture content was adjusted to 84%.

The function of salt is to help solubilize myofibrillar proteins (Okada 1986; Niwa 1992). When the protein unfolds, sodium cations interact with the anionic groups on several amino acids. The cation-anion interaction between carboxyl groups on the amino acids of fish proteins and the sodium ions may inhibit unfolding of the protein and the exposure of bonding sites important for the gelation process (Chung and others 1993). In order for surimi to gel well, salt, which is added to break the ionic

linkages and assist dispersion of the proteins, is necessary for the development of an elastic structure in heat-set gel (Niwa 1992). Myofibrillar proteins carry an overall negative charge at pH 7.0, which causes repulsion and better dispersion. Heat is needed to impart energy to vibrating molecules, which overcomes repulsion and aggregation so gelation can occur (Lanier and others 2005).

The rate of protein denaturation varies by species, according to its protein stability. Protein denaturation and aggregation, induced by heating under the proper conditions, drives gelation of surimi. However, denaturation of the proteins before the surimi is made into gel products will initiate interactions and the formation of bonds between proteins, allowing premature aggregation and preventing good gelation (Lanier and others 2005). Leaving surimi to thaw for 4 h at room temperature might have caused partial unfolding and premature aggregation to occur, rendering a lower breaking force and deformation than surimi thawed for 1 h.

Effect of moisture, salt concentration, thawing condition, and chopping temperature on viscosity

The effect of moisture on viscosity, slope of shear stress against shear rate, of surimi paste is described in Figure 3.6. With increased moisture content from 80% to 84%, apparent viscosity decreased. Similar trends were observed in the apparent viscosity of Alaska pollock, which proportionally decreased as moisture content increased from 76% to 86% (Kim and Park 2005). The effect of salt concentration, thawing conditions, and chopping temperature on the density of surimi paste is described in Figure 3.7. Overall, batches chopped at 20°C showed a higher apparent

viscosity compared to surimi chopped at 5°C using the same salt concentration. With increased salt concentration apparent viscosity also decreased.

Yongsawatigul and others (2002) found setting of threadfin bream could be induced at 25°C and 40°C. Threadfin bream exhibited major conformational changes at greater than 35°C (Yongsawatigul and others 2003). Addition of sodium chloride, which caused partial unfolding of actomyosin, induced TGase cross-linking of reactive amino acids at 25°C (Yongsawatigul and others 2003). At 4 h thawing and 20°C chopping temperature, protein was unfolded by heat denaturation and cross-linking occurred. Partial protein denaturation, which disrupts the protein matrix, occurs when the temperature rises during chopping (Rizvi 1981). Premature aggregation might have occurred and less salt ions would have to bind to fewer oppositely charged groups exposed on the protein surface. Less intermolecular ionic linkages among the myofibrillar proteins ruptured, and fewer proteins would be dissolved in the paste. Thereby the viscosity of the surimi paste with 84% moisture was higher after 4 h thawing and 20°C chopping temperature than 1 h thawing and 5°C chopping temperature.

Alternations in the electrostatic and hydrophobic interactions between protein molecules cause changes in protein structure. Sodium chloride initially causes myofibrillar protein to swell and subsequently to break into actomyosin, myosin, and a variety of protein aggregates or complexes, thereby increasing the viscosity of the liquid phase as well as the water-holding capacity (Hamm 1975; Xiong and Blanchard 1994). The chloride ion is thought to be responsible for the effect of sodium chloride on myofibrillar proteins because it interacts strongly with the positive charges on the muscle protein, while the sodium ions are weakly bound (Puolanne and Terrell 1983; Asghar and others 1985). Belton and others (1987) found that chloride ions cause

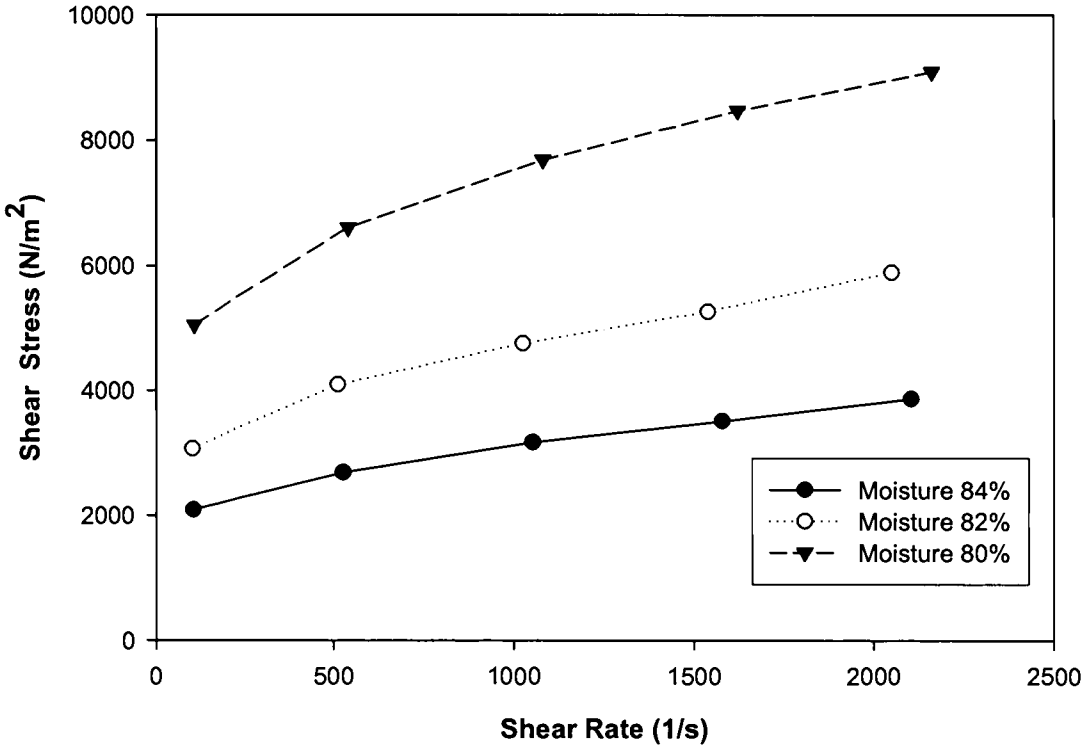

Figure 3.6 – Effect of moisture on surimi paste viscosity. Surimi paste was prepared after surimi was thawed for 1 h and chopped to a final temperature of 5°C with 2% salt.

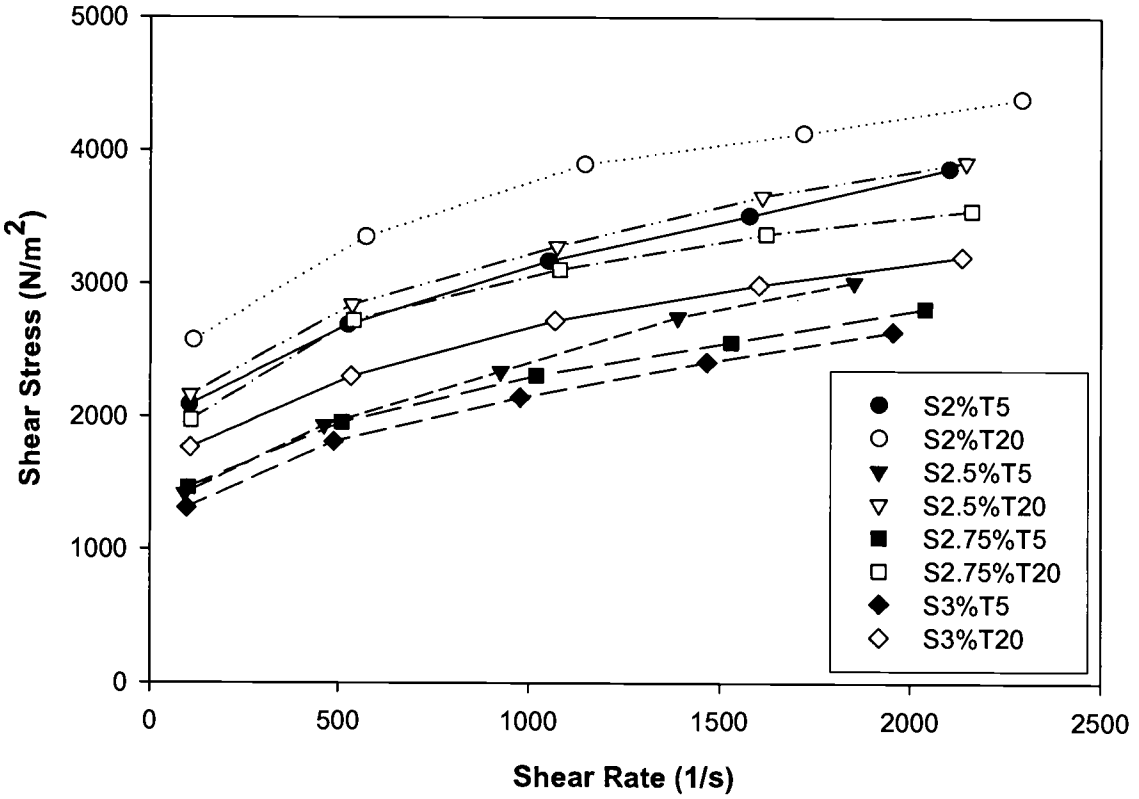

Figure 3.7 – Effect of thawing conditions, salt concentration and chopping temperature on surimi paste viscosity. Surimi pastes were prepared after surimi was thawed for 1 h (final chopping temperature of 5°C) and 4 h (final chopping temperature of 20°C) with 84% moisture content. S: salt concentration; T: final chopping temperature

repulsion of myofibrillar proteins regardless of the presence of cation, based on the nuclear magnetic resonance study.

Effect of setting in salt solution on stickiness of set gel

Stickiness is important because it determines the tendency of extruded fish ball to stick to one another thereby causing shape deformation. Holding set gels in a 40°C water bath with either 5% or 10% salt for 10, 20, 30 or 40 min significantly ($P<0.05$) affected stickiness of the gel as shown in Figure 3.8. Stickiness of the control sample, which was set in water for 30 min was significantly higher compared to other samples set in salt water. Stickiness was reduced by 44% when surimi paste was placed in a 40°C water bath with 10% salt for 10 min, 5% salt for 20 min, and 5% salt for 30 min. Stickiness decreased when setting time increased to 20 min but showed no further difference with further increases in setting time in a 10% salt water bath. In a 5% salt water bath, stickiness decreased with increased setting time.

No significant difference ($P>0.05$) in stickiness was observed between set gel in 5% salt solution for 40 min and in 10% salt solution for 20 min. Stickiness of the set gel was reduced with increased setting time and when 5% or 10% salt was added into the water bath.

Influence of salt addition and elevated temperature caused the protein to unfold slightly (Lanier and others 1982) to expose hydrophobic amino acid residues which interact with neighboring molecules to form intermolecular hydrophobic bonds. The high salt concentration of the salt water allows the surface of myofibrillar proteins

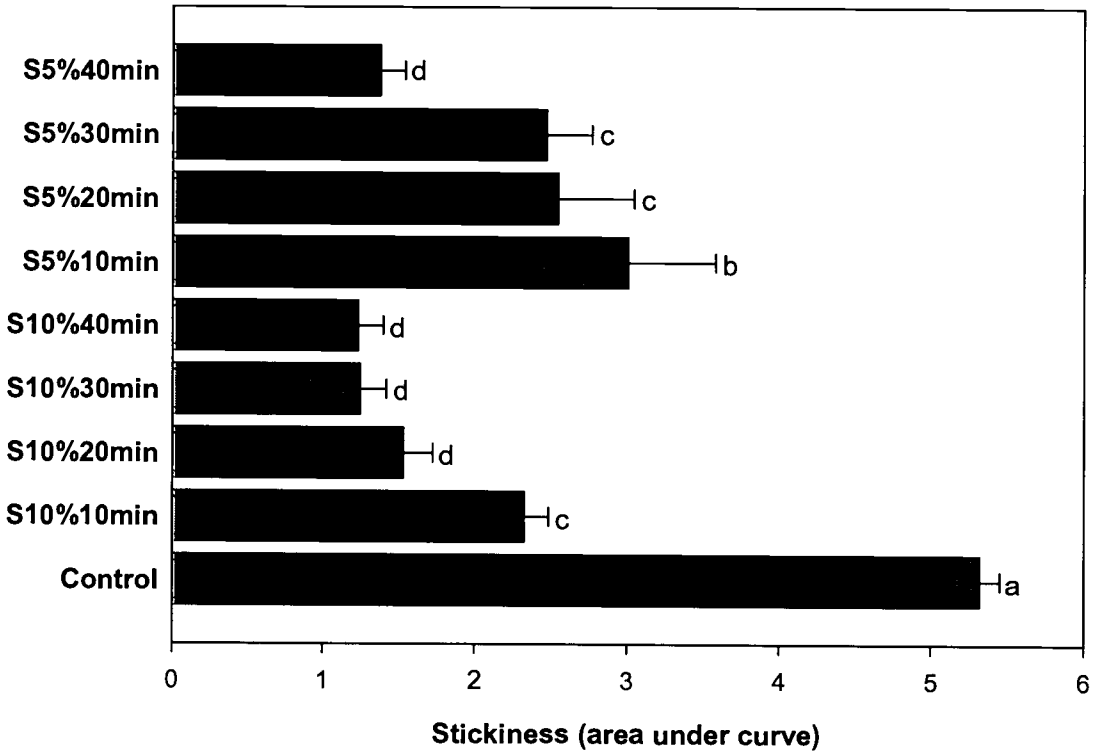

Figure 3.8 - Effect of setting time and salt concentration on stickiness of set gel. All samples were adjusted to 84% moisture and 2% salt added. Control was set in 40°C water for 30 min. S: Salt concentration setting solution. Different alphabetical letters indicate a significant difference ($P<0.05$)

to have high affinity for water molecules (Niwa and others 1986a). This accounted for the decreased stickiness of set gel.

CONCLUSION

The density of threadfin bream surimi paste significantly decreased ($P<0.05$) with increased moisture and temperature. Salt concentration, thawing conditions, and final chopping temperature also affected surimi paste density. Various factors affect surimi density and influence whether set gel floats or sinks in water. Texture of TB surimi gels was affected by salt concentration, thawing conditions, and chopping temperature. Increased moisture and salt concentration caused a decrease in the apparent viscosity of surimi paste. Setting surimi paste in a salt solution significantly reduced set gel stickiness. Maximal production of round shaped fish ball could be made when paste was prepared at 2% salt and 84% moisture chopped at 5°C, extruded into setting tank filled with 10% salt solutions, and held for 20 min.

CHAPTER 4

EXTENDING THE SHELF LIFE OF SET FISH BALL

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ABSTRACT

Set fish ball is an extruded surimi-based product that is popular in Singapore and other Southeast Asian countries. The product is packaged after setting. Oversetting of set fish ball occurs at chilled temperature during storage. Shelf life extension of set fish ball by reducing oversetting conditions and microbial counts were examined. Encapsulated citric acid (CT) and Glucono-Delta-Lactone (GDL) were used to reduce oversetting. Parameters such as color, texture properties, pH, non-disulfide covalent bond, TGase activity, microbiological assay, viscosity, and water retention ability were examined to measure changes of set fish ball during refrigerated storage. Acetic acid, GDL, and chitosan were used to inhibit growth of microorganisms. Shelf life was measured for a period of 21 days. At Day 21, a reduction of 46%, 56%, and 26% in breaking force compared to the control was observed for 0.5GDL, 1.0GDL, and CT, respectively. GDL at 1.0% was shown to be the most effective in controlling oversetting of set gel. Chitosan (1%) dissolved in acetic acid maintained both aerobic plate and yeast counts at < 1 log CFU/g throughout 21 days of storage.

Keywords: Fish ball, oversetting, glucono-delta-lactone, citric acid, chitosan

INTRODUCTION

Fish ball is an extruded surimi-based product that is popular in Singapore and other Southeast Asian countries. In Singapore the industry is valued at US$37.7 million annually (USDA 2002). Cooked fish ball is made by setting the fish ball followed by cooking at 90°C. The finished product is chilled under running tap water. The fish ball is packed in a polyethylene (PE) bag before going through metal detection. Increasingly, Singaporean consumers prefer "set fish ball" to cooked fish ball because of a more healthy and tasty perception. "Set fish ball" is not cooked but packaged with water after setting.

Oversetting of the set fish ball often occurs at chilled temperature during storage. Therefore set fish ball could only be kept for a few days. In order to overcome this oversetting problem, citric acid and Glucono-Delta-Lactone (GDL) were proposed to be tested in this study to determine whether the lower pH would delay setting of set fish ball in water. The US Food and Drug Adminstration (USFDA) assigned GDL "generally recognized as safe" (GRAS) status and permitted its use in food without limitation other than good manufacturing practice. Compared with other food acids, GDL provides a gradual, progressive, and continuous decrease of pH to equilibrium due to its slow hydrolysis to gluconic acid. Accordingly, it has been used as a slow release acidulant. During hydrolysis, its initial sweet taste becomes only slightly acidic, making the final flavor of an aqueous solution much less tart than other acidulants (Schwertfeger and Buchheim 1999). The increased acidity of the meat during cooking did not affect the physical properties of the product. GDL showed no effects on water syneresis, protein coagulation, or loss of elasticity occurred in the

kamaboko (Okada and Komori 1965). Chen and others (1998) reported that the use of GDL in milkfish improved whiteness without affecting gel strength.

Fish ball with high water activity is prone to the growth of microorganisms. Fish ball has a relatively short shelf life of 4-5 days at around 5°C storage. Fish ball distributed in uncovered bamboo baskets without refrigeration has a shelf life of only two days. In a study on the bacteria flora of commercial cooked fish balls stored at 5 °C, Lim (1989) reported that the bacterial level reached 10^6 CFU/g within 3 days, and exceeded 10^8 CFU/g in 4 days. Most of the isolated microorganisms were terrestrial bacteria, such as *Pseudomonas*, *Micrococcus*, *Moraxella*, and *Corynebacterium*. Therefore, there is a great need to extend the shelf life of fish ball without sacrificing sensory and microbial quality.

With an effort to find a way to extend the shelf life of set fish ball, the inclusion of chitosan was pursued. Chitin is the second most abundant polysaccharide in the world with a structure similar to cellulose. Chitosan is a β-1,4 linked N-acetyl-D-glucosamine biopolymer and a naturally occuring component in shells of crustaceans and cell walls of fungi (Knorr 1984). After deacetylation in alkali, chitosan, with a specific degree of deacetylation, is obtained. The higher the degree of deacetylation, the higher the ratio of free amino group (NH^{+3}) on the chitosan molecule. Chitosan is soluble in certain acid solutions, such as formic acid, acetic acid, and lactic acid. When dissolved in acid solution, chitosan becomes a polycationic polymer, which possesses many functional properties in food applications (Shahidi and others 1999). These applications include antibacterial and antifungal (Sudarshan and others 1992; Wang 1992; Chen and others 1998).

The effect of chitosan on spoilage in minced beef patties stored at 30°C for 2 days and at 4°C for 10 days was studied (Darmadji and Izumimoto 1994). A reduction

of 1 to 2 log cycles of total bacteria, *pseudomonads*, *staphylococci*, coliforms, gram-negative bacteria, and *micrococci* was reported in the presence of 1% chitosan. Lower concentrations of chitosan (0.2 and 0.5%) had no effect on spoilage flora. Roller and Covill (2000) reported the inhibiton of spoilage flora from log 8 CFU/g in the control to log 4 CFU/g throughout the 4 weeks of study at 5°C by the use of chitosan combined with acetic acid in shrimp salad. Chitosan was shown ineffective as a preservative at 25°C. However, information is lacking on the antibacterial activity of chitosan on surimi product such as fish ball.

Therefore the overall objective of this study was to extend the shelf life of set fish ball. Specific objectives were: 1) to investigate the effect of citric acid and GDL on the setting phenomenon of set fish ball; and 2) to measure the effect of chitosan and low pH on the inhibition of microorganisms.

MATERIALS AND METHODS

Materials

Frozen surimi (grade SA) produced from threadfin bream (*Nemipterus spp.*) was obtained from Andaman Surimi Industries Co. Ltd (Bangkok, Thailand). Surimi was cut into approximately 1000 g blocks, vacuum packed, and stored in a freezer (-25°C) throughout the experiments. The moisture content of the surimi was 74.0 ± 0.2% (AOAC 1995).

Encapsulated citric acid (Citrocoat A 4000 TP, 40% coated with palm oil) was

obtained from AppliChem GmbH (Darmstadt, Germany). Glucono-Delta-Lactone (GDL) was obtained from PURAC America, Inc. (Lincolnshire, IL, USA). Acid soluble chitosan with 85.6% deactetylation and molecular weight of 150,000 (Vanson Halosource, Inc., Redmond, WA, USA) was used in 1% acetic acid solution. Water soluble chitosan lactate (CL-60M) with 89% deacetylation and molecular weight of 50,000-100,000 was obtained from Kyowa Technos Co. Ltd (Chiba, Japan).

Gel preparation

Frozen surimi was tempered at room temperature for 1 h before cutting into approximately 4 cm cubes. Surimi cubes were placed in a Stephan vacuum cutter UM-5 (Stephan Machinery Corp., Columbus, OH, USA). In the first 1 min, frozen cubes were chopped at low speed. Salt (2%) was sprinkled and chopping continued at low speed for 1 min. Ice/water to adjust the moisture to 84% and various additives were added as shown in Table 4.1. The samples were chopped at low speed for 1 min. In the final 3 min, chopping continued at high speed, with vacuum applied at 0.4 bar. After chopping, the paste was stuffed into stainless steel tubes (inner diameter, 1.9 cm; length, 17.5 cm) with stainless steel screw caps, using a sausage stuffer (Sausage Maker, Buffalo, NY, USA). The interior wall of the tubes was coated with a film of PAM cooking spray (Boyle-Midway, Inc., NY, USA). Set gels were prepared by placing the surimi paste in 40°C water for 30 min. After setting, the gels were immediately cooled using ice water. Gels were cut into 2.9 cm lengths. Twenty-five pieces of cut set gels were then placed in a 3 mil polyamide-(nylon)-polyethylene vacuum pouch (Alpak Food Equipment, Portland, OR, USA) with various solutions as

shown in Table 4.1 and refrigerated (4°C). Cooked gels were obtained by subjecting set gel to heating at 90°C for 15 min. Cooked gels were chilled quickly in ice water (0°C) for 15 min and refrigerated (4°C) overnight before analysis. Water, 0.1% GDL, 1% acetic acid, and 1% chitosan in 1% acetic acid were individually sterilized at 121°C for 15 min.

Gels were subjected to various analyses every 3 days during 21 days of storage.

Table 4.1 - Experimental treatments for shelf life study of fish ball

Sample Name	Additives in surimi	Storage solutions
Control	-	Water
CT	0.2% Citrocoat A 4000TP	Water
0.5GDL	0.5% GDL	0.1% GDL solution
1.0GDL	1.0% GDL	0.1% GDL solution
A	-	1% acetic acid
CW	0.5% water soluble chitosan	Water
CA	-	1% acid soluble chitosan dissolved in 1% acetic acid

Gel texture measurement

Gels were equilibrated to room temperature at 25°C and cut into 2.9 cm long. Gel samples were measured for breaking force and deformation to determine gel

strength and cohesiveness, respectively, using a Texture Analyzer (TA-XT plus, Texture Technologies Corp., NY, USA), equipped with a 5-mm spherical probe at a test speed of 1 mm/s (Park 2005b).

Color measurement

Cooked gel was equilibrated to room temperature and color was measured according to the method described by Park (1995). Color L* (lightness) and b* (yellowness "+" or blueness "-") values were measured with a Chroma Meter (Model CR-310, Minolta Camera Co. Ltd., Osaka, Japan). Color a* was not reported because it was consistent regardless of processing parameters and moisture content of cooked gel (Park 1995). Whiteness of cooked gel was calculated using L*-3b* (Park 1994). Seven gel measurements per sample were used for color analysis.

Water retention ability

Water retention ability was measured according to the method developed by Kocher and others (1993). A microcentrifuge filtration unit consisted of a 2.0 mL microcentrifuge tube, which collected released fluid, and a filter insert (inner tube) which held the sample. The insert had a nylon screen with 0.45 μm pore size. Cooked gel (0.4 ± 0.05 g) was placed in the inner tube and the microcentrifuge filtration unit was spun in a microcentrifuge (Model: 5415C, Eppendorf, Hamburg, Germany).

Triplicate measurements were centrifuged for 10 min at 6000 rpm for each gel sample. Water retention ability was determined as:

WRA =(total g water in surimi gel – g water released)/ total g surimi gel

where, total g water = % moisture of surimi gel x surimi gel weight, g water released = (microcentrifuge tube weight + g of water) – microcentrifuge tube weight.

Determination of pH

Ten grams of set gel sample, in duplicate, were weighed and added to 90 mL of de-ionized water before homogenizing (Power Gen 700, GLH 115, Fisher Scientific Inc., Pittsburg, PA, USA) for 30 s. The pH was measured with a Hanna instruments pH meter (HI 9025, Whatman LabSales, OR, USA).

Aerobic and yeast/mold count

Stock Butterfield's buffered phosphate diluent was prepared by dissolving 34.0 g KH_2PO_4 in 500 mL H_20, adjusted to pH 7.2 with 175 mL 1N NaOH, and diluted to 1 L. The diluent 1.25 mL stock solution was diluted to 1 L with H_20 and dilution blanks prepared. The buffer was autoclaved for 15 min at 121°C (AOAC 1995).

For each sampling, 11 g of sample were added to 99 mL of sterile Butterfield's phosphate buffer and placed in a stomacher machine for 1 min. Serial dilutions were prepared using the dilution buffer. Diluted sample buffer was seeded into yeast and mold (YM) and aerobic plate count (APC) petrifilms (3M Microbiology, St Paul, MN,

USA). Appropriate dilutions inoculum (1 mL) was dispensed onto the center of the bottom film and a spreader used to evenly distribute the sample. The petrifilms were allowed to stand for 1 min to permit gel to form. Petrifilm YM plates were incubated at 25°C for 5 days while petrifilm APC were incubated at 35°C for 48 h. Triplicate plates in suitable range (30-300 colonies) were counted. Average counts were obtained and reported as colony forming unit (CFU)/g.

Viscosity measurement with capillary extrusion viscometer

Viscosity measurement was performed according to the method developed by Kim and Park (2005) using a Texture Analyzer (TA-XT plus, Texture Technologies Corp., NY, USA) fitted with a capillary fixture kit (TA-525) having an extrusion tube of 30 mm long and nozzle diameter of 6 mm. Surimi paste remaining after gel analysis was packed into the cylinder using a sausage stuffer (Sausage Maker, Buffalo, NY, USA). Surimi paste was extruded at different piston speeds (1, 5, 10, 15, and 20 mm/s). Maximum sustained force at each piston speed was recorded to calculate shear stress and shear rate.

$$\sigma = (\Delta PR/2L)$$

Where, σ = shear stress (N/m^2), ΔP = pressure (N), R = radius (mm), L = tube length (mm). VanWazer and others (1963) provided the Robinowitch-Mooney equation for shear rate of a non-Newtonian fluid as follows:

$$(dv/dr) = ((3+b)/4)Q$$

Where, (dv/dr) = shear rate (s^{-1}), b = Δlog Q/ Δlog σ, Q = ($4q/\pi R^3$), q = volumetric flow rate (s^{-1}), R= radius (mm), L = tube length (mm). Apparent viscosity of surimi paste was then determined by the slope of shear stress vs shear rate.

Preparation of crude TGase

Set gel samples were homogenized at setting 3 for 1 min (Power Gen 700, GLH 115, Fisher Scientific Inc., Pittsburg, PA, USA) with four volumes of extraction buffer (10 mM NaCl, 5 mM ethylenediaminetetraacetic acid (EDTA), 2 mM 1,4-Dithiothreitol (DTT), 10 mM Tris-HCl, pH 7.5). The homogenate was centrifuged at 10,000 x g (Rotor SS-34, Sorvall RC-5B DuPont Instruments, Newton, CT, USA) for 30 min at 4°C. The supernatant was used as crude TGase (Worratao and Yongsawatdigul 2003).

Determination of TGase activity

TGase activity was measured in terms of the incorporation of dansylcadaverine (MDC) into N, N'-dimethylated casein (DMC) according to the procedure of Takagi and others (1986) with a slight modification (Worrato and Yongsawatdigul 2003). The reaction mixture contained 1 mg/mL DMC, 15 µM MDC, 3 mM DTT, 5 mM $CaCl_2$, 50 mM Tris-HCl (pH 7.5), and 100 µL of crude TGase. The reaction was incubated at 37°C for 10 min and stopped by adding EDTA solution at a final concentration of 20 mM. The fluorescence intensity of MDC incorporated into

DMC was measured using a spectrophotofluorometer (LS50B, Perkin Elmer, Norwalk, CT, USA) at excitation and emission wavelengths of 350 and 480 nm, respectively. The control was performed as described above, except with EDTA added in the reaction before crude TGase. One unit of TGase activity was defined as the amount of enzyme that catalyzed the incorporation 1 nmol of MDC into DMC during 1 min incubation at 37°C. The enhancement factor used for the activity calculation, indicating fluorescence intensity, increased upon incorporation of MDC into DMC, was 1.26.

Measurement of non-disulfide covalent bond

Solubility of cooked gel was determined according to the method of Chawla and others (1996) with slight modification. The sample (1 g) was homogenized in 20 mL of 20 mM Tris-HCl, pH 8.0 containing 1% SDS (w/v), 8 M urea and 2% β-ME (v/v) for 1 min using a homogenizer (Power Gen 700, GLH 115, Fisher Scientific Inc., Pittsburg, PA, USA). The homogenate was heated in boiling water (100°C) for 3 min and stirred at room temperature for 4 h. The resulting homogenate was centrifuged (Rotor SS-34, Sorvall RC-5B DuPont Instruments, Newton, CT, USA) at 8,000 xg for 30 min at 4°C. Protein in the supernatant (10 mL) was precipitated by the addition of 50% (w/v) cold TCA to a final concentration of 10%. The mixture was kept at 4°C for 18 h and then centrifuged at 8,000 xg for 30 min. The precipitate was washed with 10% (w/v) TCA and solubilized in 0.5 M NaOH. The protein content was measured using the Biuret test (Uemoto 1966). Protein sample (5 mL) was added to 5 mL

Reagent A (0.4% $CuSO_4$. $5H_2O$, 8% NaOH, 0.2% glycerine). Another test tube was added with 5 mL protein sample and 5 mL Reagent B (8% NaOH, 0.2% glycerine). The mixtures were allowed to incubate at room temperature for 2 h. Absorbance was read at 545 nm with a UV-VIS spectrometer (UV 2401PC, Shimadzu Corp., Kyoto, Japan). Biuret color intensity was measured by subtracting the optical density of A from the optical density of B. Solubility was expressed as percent of the total protein.

Statistical analysis

Analysis of variance (ANOVA) was performed and the Duncan multiple range test (Steel and Torrie 1980) was used to resolve statistical difference between mean values. Data was analyzed using SPSS version 13.0 (SPSS Statistical Software, Inc., Chicago, IL, USA). A level of significance was set at $P<0.05$.

RESULTS AND DISCUSSIONS

Change of pH during storage

The control set gel at Day 0 was pH 7.0. Change of pH in set gel during 4°C storage is shown in Figure 4.1. In general, pH decreased during storage. Hashimoto and Arai (1985) reported that postmortem lowering of muscle pH was related to denaturation of myofibrillar protein due to conversion of muscle glycogen to lactic

acid. APC of control sample increased with storage shown in Table 4.2. The built up of pyschrotrophs during storage of set gel might have caused the decreased in pH through production of acid (Frazier and Westhoff 1978). Both the denaturation of myofibrillar protein and the growth of microorganisms accounted for the decreased in pH of the control sample. Control set gel reached pH 5.01 at Day 21. The lowest pH of set gel was 4.19 for A. The highest pH of set gel was obtained from sample CW. Use of acetic acid caused a dramatic drop in pH of set gel at Day 3 with CA (pH 4.65) and A (pH 4.56) from pH 7.0 at Day 0. The pH of CW was higher than the control throughout the 21 days. The pH of set gel with GDL at Day 0 was slightly lower compared to the control, pH 6.75 and 6.50 for 0.5GDL and 1.0GDL, respectively. With increased storage, the pH of 0.5GDL and 1.0GDL decreased further to pH 4.68 and 4.47, respectively at Day 21, indicating the formation of gluconic acid.

Change of pH in storage solutions is shown in Figure 4.2. The pH of storage solutions for the control, CW, and CT decreased during storage. Similar behaviors were observed for both 0.5GDL and 1.0GDL, whereby the pH of 0.1% GDL solution increased from Day 0 to Day 6 and decreased gradually, perhaps with the formation of gluconic acid. The pH of solutions containing acetic acid remained relatively constant throughout storage.

Microbiological assay

Aerobic plate count (APC) of set gel at Day 0 was 1.03×10^4 CFU/g. Factors such as season, source, grade, and processing procedures can result in differences in the microbial quality of surimi (Ingham and Potter 1987). APC of set gel during

storage at 4°C are shown in Table 4.2. APC of CW at Day 3 was < 1 log CFU/g, but increased logarithmatically with continued storage. Water soluble chitosan (CW) added into surimi paste temporarily inhibited the growth of microorganisms. The recommended microbiological safety criteria assigned to ready-to-eat fishery products is 10^6 CFU/g (ICMSF 1986). Various surimi samples exceeded 10^6 CFU/g on different days of storage; CT after 9 days; 0.5GDL and 1.0GDL after 12 days; control and CW after 15 days. Control, CW, CT, 0.5GDL and 1.0GDL were beyond 7 log CFU/g after 21 days of storage. Using 1% acetic acid as the storage solution caused a 3.72 log reduction in APC after 21 days of storage. APC of CA was < 1 log CFU/g throughout storage, > 6.44 log reduction in APC at Day 21. Dissolving 1% chitosan in 1% acetic acid was most effective in inhibiting the growth of microorganisms found in surimi.

Yeast was detected in the control on Day 18 with 3.55 log CFU/g. On Day 21, yeast counts were 4.57 log CFU/g for the control, CT (3.70 log CFU/g), 0.5GDL (3.49 log CFU/g), and 1.0GDL (2.42 log CFU/g). Yeast was <1 log CFU/g in CW, CA, and A. Chitosan and acetic acid inhibited the growth of yeast.

Gelation by acetic acid results in less susceptibility to microbial spoilage and hence better storage stability (Venugopal and others 1994). With the combination of acetic acid and refrigerated storage, chitosan was effective in inhibiting growth of bacteria and yeast in shrimp salad (Roller and Corvill 2000).

Chitosan with a higher degree of deacetylation generally had higher solubility and more positive charges in an acidic environment (Chang and others 2003). The amount of absorbed chitosan onto the bacteria cell wall determines the antibacterial activity of chitosan (Chen and others 2002; Loosdrecht and others 1987). The more chitosan absorbed would result in greater changes in the structure of the cell wall and

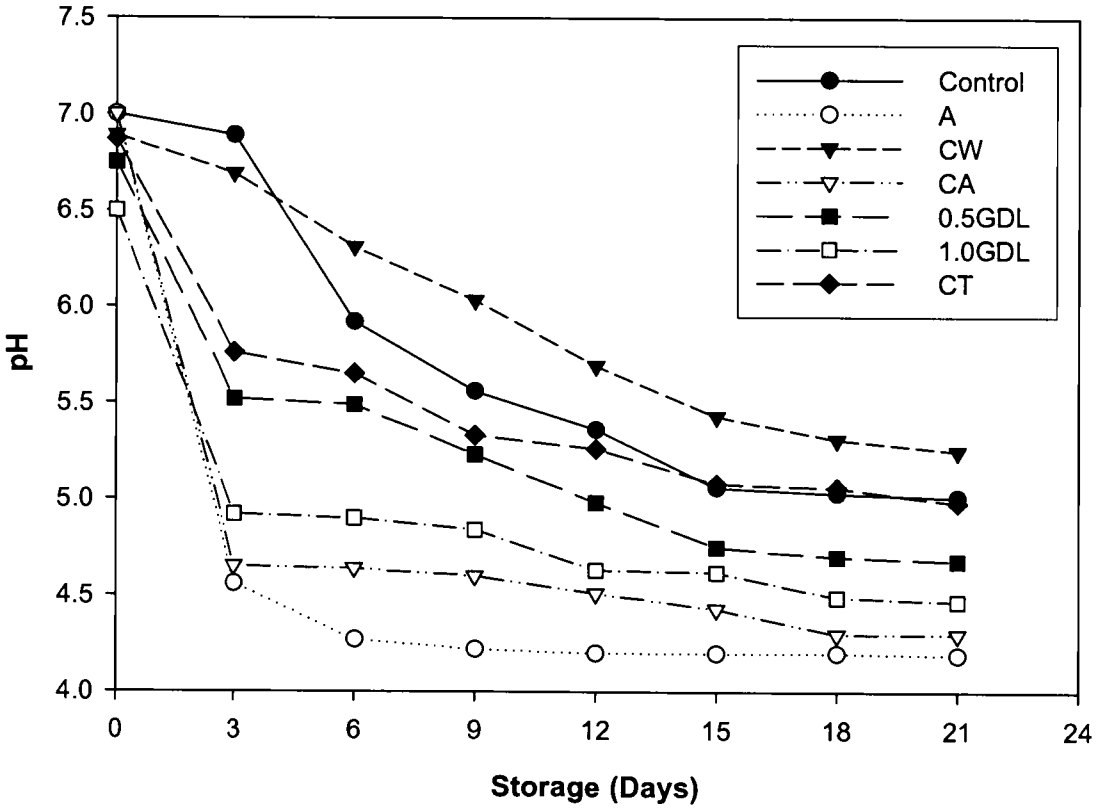

Figure 4.1 – Change of pH in set gel during 4°C storage

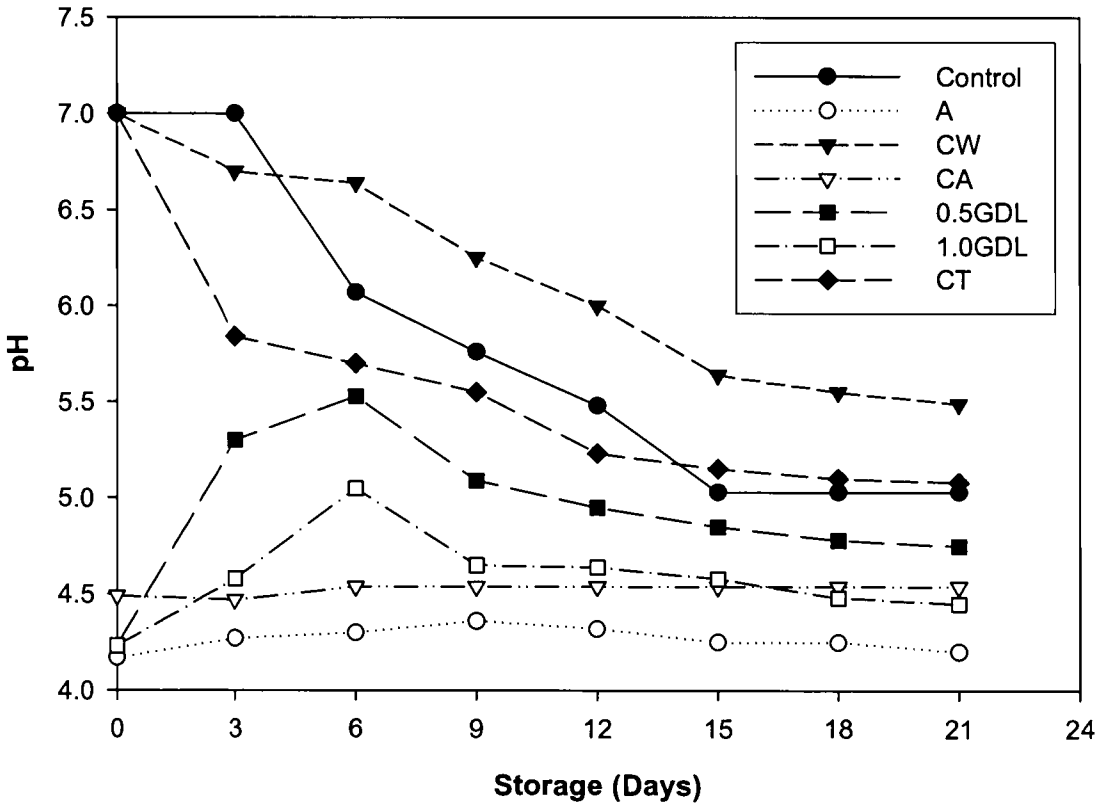

Figure 4.2 – Change of pH in storage solutions (water, 0.1% GDL, and 1% acetic acid) during 4°C storage

in the permeability of the cell membrane of bacteria. Both result in the death of bacteria. However, the addition of powdered chitosan to fish patties had no effect on bacterial growth (López-Caballero and others 2005).

Sudharshan and others (1992) studied the antimicrobial effect of water-soluble chitosans, such as chitosan lactate. Chitosan lactate had bactericidal properties against both gram-negative and gram-positive in the range of one to five log cycle reductions within one hour. However, their studies also showed that at pH 7, chitosan no longer had any bactericidal activity due to poor solubility at neutral pH and the presence of significant uncharged amino groups (Sudharshan and others 1992).

The permeabilizing effects of chitosan were demonstrated at slightly acidic conditions, in which it is protonated, and the carboxyl and phosphate group of the bacterial surface are anionic and offer potential sites for electrostatic binding of chitosan (Helander and others 2001). Water soluble chitosan (CW) was not effective in inhibiting bacterial growth due to less positive charges available. A recommended level of 0.5% by the manufacturer was only proved to have temporary inhibition on microorganisms in surimi. A reduction of 1 to 2 log cycles of total bacteria, *pseudomonads*, *staphylococci*, coliforms, gram-negative bacteria, and *micrococci* was reported in the presence of 1% chitosan when used in mince beef patties. Lower concentration of 0.2% and 0.5% chitosan had no effect on the spoilage flora (Darmadji and Izumimoto 1994).

Table 4.2 - Aerobic plate count of set gel during storage at 4°C. Different alphabetical letters within the same column indicate a significant difference (P<0.05). ND: not detectable with a detection limit of < 1 log CFU/g.

	Aerobic Plate Count Log CFU/g							
	Storage (Days)							
Treatment	**0**	**3**	**6**	**9**	**12**	**15**	**18**	**21**
Control	4.01 ± 0.07a	4.92 ± 0.08a	5.47 ± 0.07a	5.58 ±0.10c	5.63 ± 0.02c	6.72 ± 0.05c	7.36 ± 0.04a	7.44 ± 0.04b
A	4.01 ± 0.07a	ND	ND	2.78 ± 0.16e	3.04 ± 0.04e	3.26 ± 0.05e	3.36 ± 0.05d	3.72 ± 0.10d
CW	4.01 ± 0.07a	ND	4.79 ± 0.05c	5.04 ± 0.04d	5.17 ± 0.02d	6.57 ± 0.04d	7.32 ± 0.03a	7.32 ± 0.02c
CA	4.01 ± 0.07a	ND	ND	ND	ND	ND	ND	ND
0.5GDL	4.01 ± 0.07a	4.62 ± 0.03b	5.36 ± 0.05ab	5.96 ± 0.05a	6.16 ± 0.02b	6.84 ± 0.06b	6.92 ± 0.06b	7.62 ± 0.03a
1.0GDL	4.01 ± 0.07a	3.82 ± 0.10c	3.86 ± 0.09d	5.83 ± 0.02b	6.20 ± 0.17b	6.69 ± 0.09c	6.67 ± 0.06c	7.22 ± 0.10c
CT	4.01 ± 0.07a	4.93 ± 0.02a	5.27 ± 0.09a	6.03 ± 0.02a	7.30 ± 0.05a	7.34 ± 0.02a	7.37 ± 0.06a	7.45 ± 0.02b

Texture properties

Breaking force of set gel for the control, CW, and CT increased with increased storage (Figure 4.3). The breaking force of CA, A, and 1.0GDL increased to a maximum value on Day 6 of storage and decreased from Day 9 to 21. As for 0.5GDL, breaking force increased to a maximum value at Day 18. Highest breaking force was with CA and the lowest was with A at Day 21. GDL showed significantly lower ($P<0.05$) breaking force compared to the control at Day 21.

In general, deformation value of set surimi decreased with increased storage. Deformation value indicate the surimi quality. Maximum deformation of set gel was obtained at Day 3 for all samples except 0.5GDL, which was at Day 6 (Figure 4.4). Decreasing deformation value with storage is probably due to weakening of cross-links. CT and A had the lowest deformation values at Day 21. However, 0.5GDL, 1.0GDL, CW, and CA had significantly ($P<0.05$) higher deformation value than the control at Day 21.

Setting continues during storage, breaking force and deformation of cooked gel increased. Benjakul and others (2003) observed setting of threadfin bream surimi at 25°C up to 8 h and showed increased breaking force and deformation. Cooked gel, which was prepared by setting under the same conditions and subsequently heated at 90°C for 20 min, also showed increased breaking force and deformation value (Benjakul and others 2003). Improved gel properties via setting are due to polymerization of heavy chain myosin induced by TGase (Kimura and others 1991). The content of ε-(γ-glutamyl)lysine (EGL) in Alaska pollock surimi gel increased as setting time at 30°C increased, indicating the participation of endogenous TGase in the setting process (Kumazawa and others 1995).

Increased storage time also increased the breaking force of the cooked control sample. The highest breaking force was found for the control sample at Day 21 (Figure 4.5). Decreasing the pH of surimi affected the breaking force of cooked gels. All treatments had significantly lower ($P<0.05$) breaking force than the control at Day 21. At Day 21, breaking force decreased by 46%, 56%, and 26% compared to the control for 0.5GDL, 1.0GDL, and CT, respectively. GDL at 1.0% was shown to be the most effective in preventing oversetting of surimi. In general, deformation initially increased with storage time but decreased with further storage time (Figure 4.6). CW and CT had higher deformation value than the control at Day 21. While CA, A, 0.5GDL, and 1.0GDL had lower deformation value than the control at Day 21.

Gelation of muscle proteins occurred through two process: an initial unfolding (denaturation) followed by aggregation of the proteins into a three-dimensional network. The tail portion of the myosin molecules are first involved in aggregation through hydrophobic interactions (Niwa 1992). Venugopal and others (1994) suggested that weak acid could favor protein unfolding and facilitate the gelation process. Frethiem and others (1985) reported that gel formation of myosin was enhanced by slowly lowering pH and maximum gel strength was obtained at pH 4.5. They suggested the involvement of conformational changes of the protein during gelation by slowly lowering pH. The rate of TGase mediated cross-linking of myosin heavy chain may be primarily dependent on the conformation of substrate myosin at a given temperature rather than the optimum temperature of TGase (Araki and Seki 1993; Kamath and others 1992).

Yano (1990) explained the relationship between heating conditions and gel strength. The native state of the protein could be changed into a gelation-possible state, which further changed into a gelation-impossible or gelled state

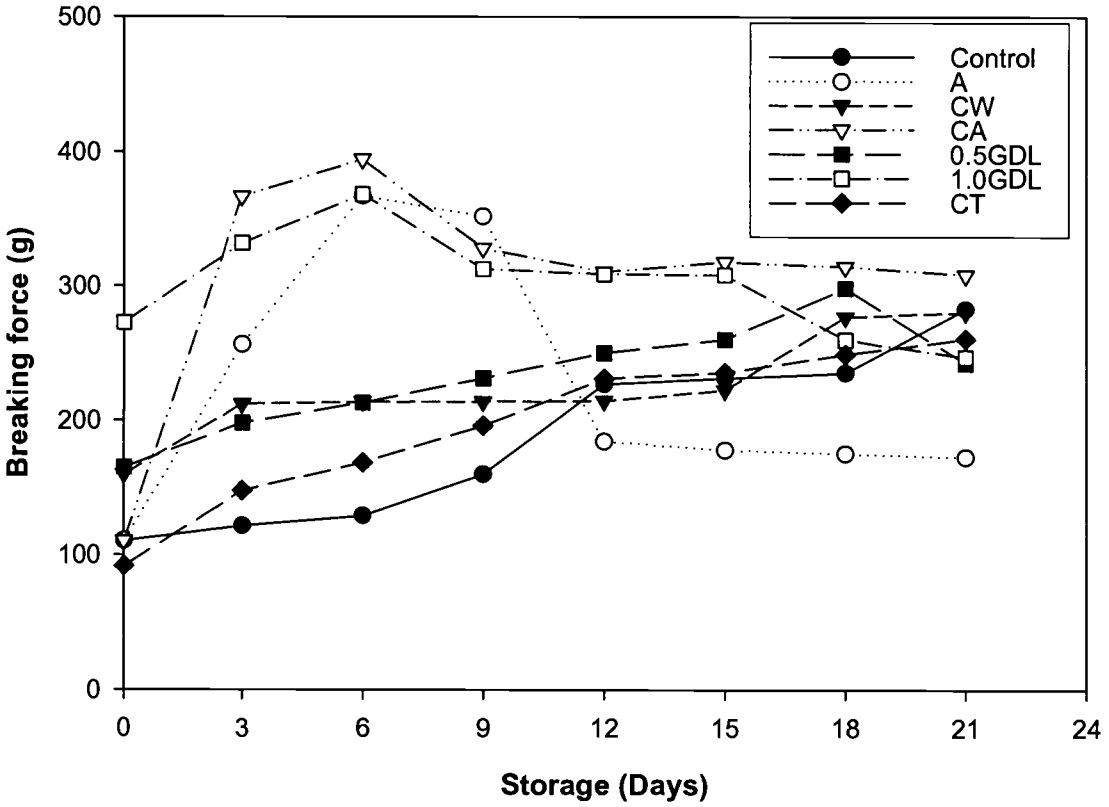

Figure 4.3 – Breaking force of set gel during 4°C storage

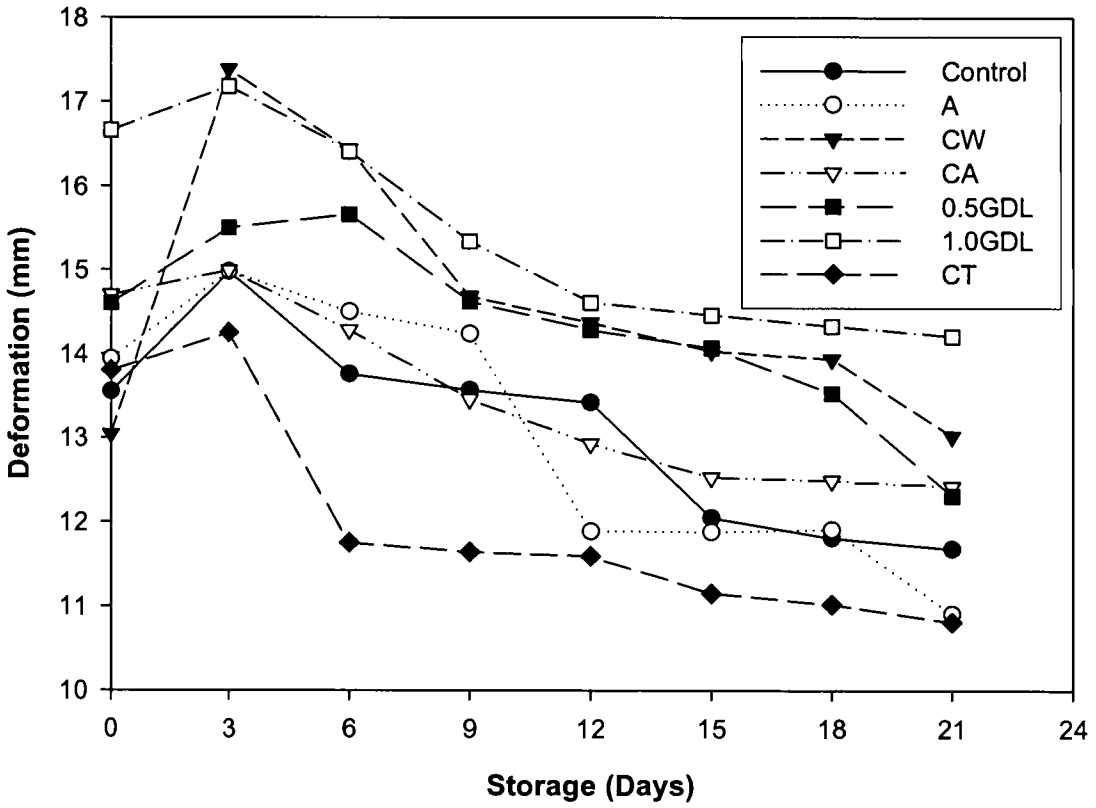

Figure 4.4 – Deformation value of set gel during 4°C storage

depending upon the environment (Yano 1990). Acid favored a gelation-possible state, which is converted into a gelled state by mild heat (Chawla and others 1996).

Venugopal and others (1994) and Chawla and others (1996) reported strength of threadfin bream or shark myofibrillar protein gel induced by organic acids (acetic, lactic, tartaric, or citric acids) increased with decreased pH and reached a maximum at pH 4.5. Acid-induced gelation occurred lower than the isoelectric point of myosin protein (pH 5.4), where protein molecules have a net positive charge. The addition of acid at the level used in the study might not cause the whole myofibril protein to unfold and dissolve, but might cause only partial solubilization of the outer layer of the myofiber or myofibrils. During gelation, the solubilized proteins crosslinked with other myofibrils, thus making the gel firmer. Acid treatment could cause both muscle shrinkage (leading to toughening) and protein hydrolysis (leading to softening). This could account for increased breaking force at the beginning of storage and decreased in breaking force upon extended storage, respectively. The net result is dependent on acid concentration (Forrester and others 2002). Prolonged storage in an acidic environment caused the breaking force to decrease, and protein hydrolysis could have occurred due to protein denaturation (Figure 4.5).

GDL became hydrolyzed into gluconic acid in water and formed relatively weak hydrogen bonds (Chang and others 2003). Nishino and others (1991) showed dramatic decrease in gel strength in pollock surimi as the pH was lowered stepwise from 6.75 to 3.77 with the addition of GDL. Addition of up to 0.4% GDL increased gel strength of milkfish, however at 0.6% GDL, decreased gel strength and increased expressible drip were observed (Chen and others 1998).

In the presence of chitosan, protein-polysaccharide conjugates were formed between the reactive group of glucosamine (acyl acceptor) and the glutaminyl residue

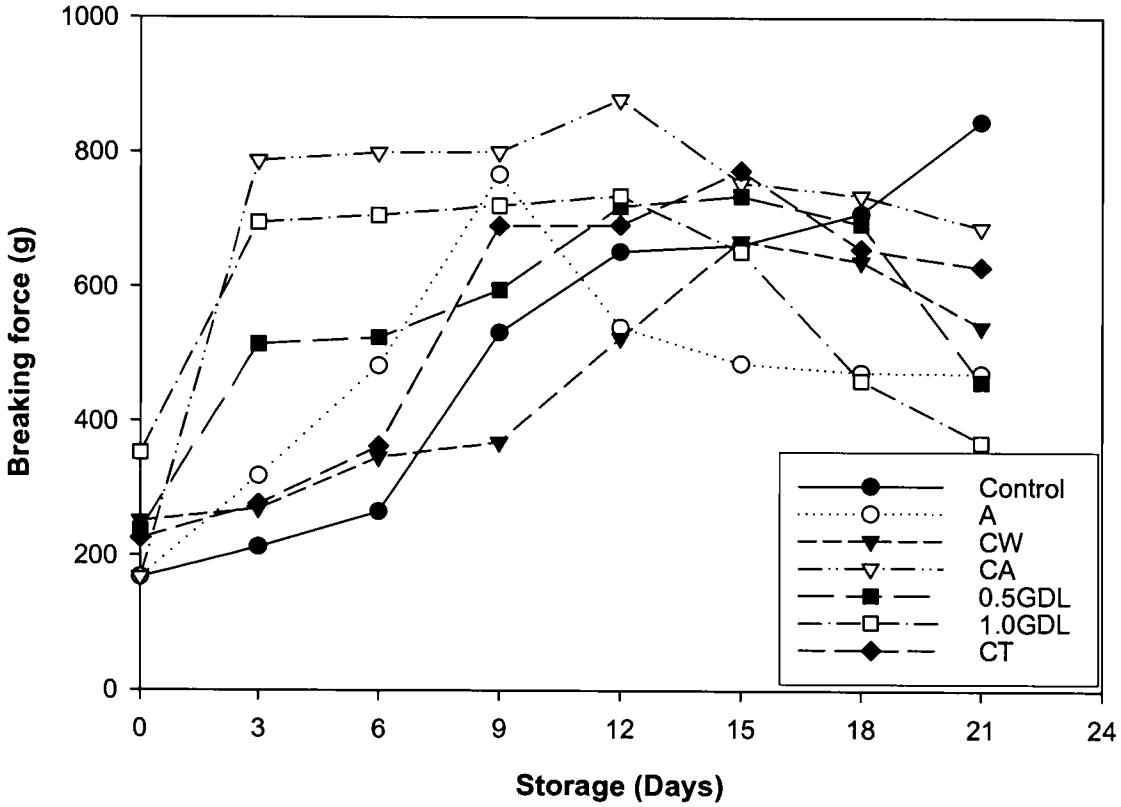

Figure 4.5 – Breaking force of cooked gel

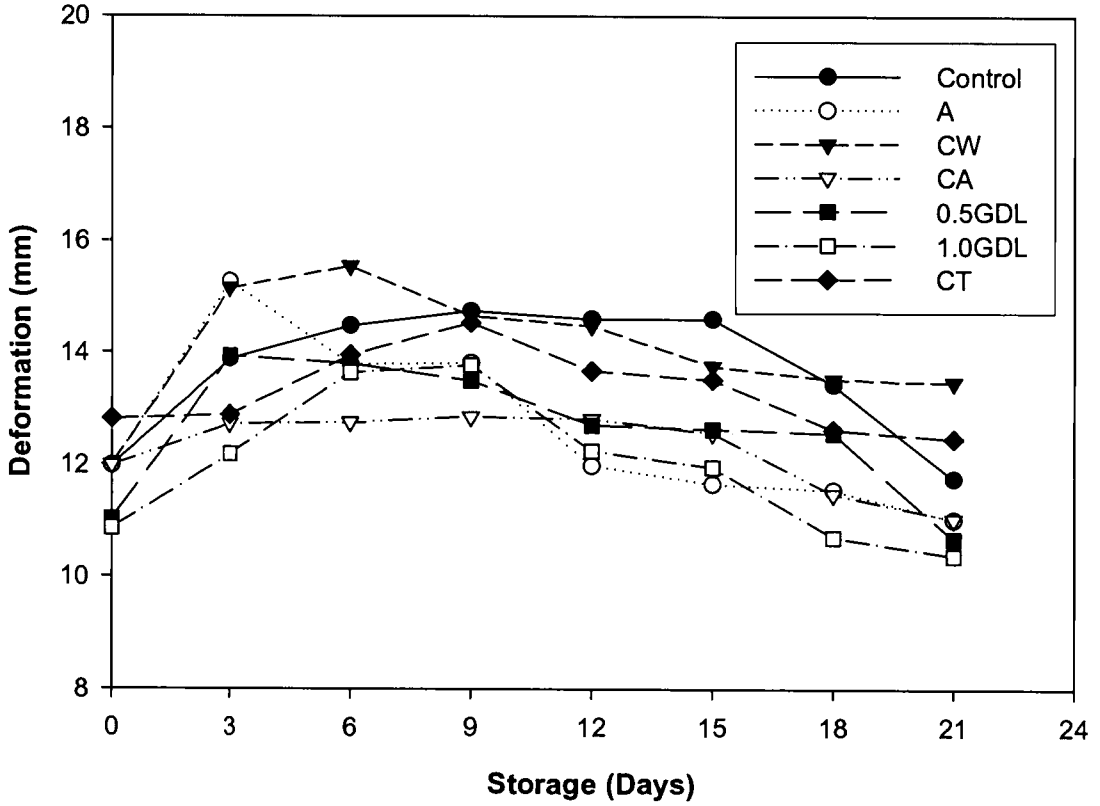

Figure 4.6 – Deformation value of cooked gel

of myofibrillar proteins, with the subsequent formation of monosubstituted γ-amides of peptide-bound glutamic acid (Greenberg and others 1991). When chitosan amino groups undergo more crosslinking with glutamyl residues of myofibrils, induced by endogenous TGase, fewer glutamyl residues remained in the myofibrils resulting in less polymerization of myofibrils inter- or intramolecularly (Benjakul and others 2000).

The balance of protein-chitosan and protein-protein conjugates determines surimi gel strength (Benjakul and others 2000). Xiong and Blanchard (1993) reported interaction of salt soluble protein (SSP) and polysaccharide via noncovalent bonding and thus interfered with SSP thermal gelation resulting in a decrease in gel strength. Protein gelation was interupted at a pH near the pI of myofibrillar proteins due to electrostatic attraction among the molecules. Huang and Li (1998) indicated that ionic interaction between the positively charged amino group of chitosan and SSP affected SSP gelation in the lower-temperature region (below 50°C) resulting in lower gel strength. This could explain the decrease in breaking force and deformation for CW.

Kataoka and others (1998) suggested the enhancing effect of chitosan on gel formation of walleye pollock surimi was due to the activity of the endogenous transglutaminase known to be present in surimi. Low pH from the addition of acetic acid, could favor unfolding of myofibrillar proteins. The pKa of the amino group of the glucosamine residue in chitosan is 6.3 (Poole 1989). CA, with pH of less than 5, caused both the amino group of myofibrillar protein and chitosan to be positively charged, thereby reducing the ionic interaction. Therefore, a significantly higher breaking force ($P<0.05$) was shown in CA than CW and A (Figure 4.5).

Changes in color

L* value (lightness) of cooked gel is shown in Figure 4.7. L* value of the control increased from 80.84 to 88.48. L* value of CW also increased significantly ($P<0.05$) during the first 15 days of storage and decreased until Day 21. Significantly higher L* value ($P<0.05$) was observed for 0.5GDL and 1.0GDL than the control until Day 18. Chen and others (1998) observed improved L* when GDL was added to milkfish surimi. However at Day 21, no significant difference in L* value ($P<0.05$) was observed between 0.5GDL, 1.0GDL and the control. GDL affected the L* value of cooked gel. L* value of CA remained relatively constant between Day 9 and 21. L* value of A also remained relatively constant during 12 to 21 days of storage. However, L* value of the control was significantly higher ($P<0.05$) than CA and A after 12 days of storage.

b* value of cooked gel is shown in Figure 4.8. In general, b* value of cooked gel decreased during the first 6 days of storage. Higher b* value was observed for CW compared to other samples with the addition of chitosan affecting the yellow hue of the cooked gels. CT, 0.5GDL, and 1.0GDL had the lowest b* value. CW and CA generally had higher b* value. With chitosan added directly to surimi in the case of CW, the b* value was affected starting from Day 0. For CA, chitosan in the storage solution caused an increase in b* value of cooked gel during storage.

Whiteness of cooked gel is shown in Figure 4.9. Whiteness value of the control, A, CA, and CW rapidly increased for the first 3 days, slowly increased for the next 6 days, and remained constant during 9 to 21 d of storage. CT, 0.5GDL and 1.0GDL had significantly higher ($P<0.05$) whiteness value than the control at Day 0. Adding citric acid and GDL improved whiteness of cooked gel. CT whiteness

increased to a maximum value at Day 12 but decreased continuously until Day 21. GDL at 0.5% and 1.0% also increased to a maximum value on Day 6 and remained relatively constant during the rest of storage. At Day 21, CW, and CA had the lowest whiteness value probably due to the effect of chitosan. GDL at 0.5% had the highest whiteness value among all the samples.

Water retention ability

Change in the water retention ability (WRA) of cooked gels is shown in Figure 4.10. GDL at 1.0% had the lowest water retention ability throughout 21 days of storage. Generally, the water retention ability of all samples decreased with increased storage time. Significant decreases ($P<0.05$) of WRA were observed for A, CW, CA, 0.5GDL, and CT at Day 3 and gradually decreased during 6 to 21 days of storage. The control showed only a gradual change in WRA from Day 0 to 6 and a significant change after 9 days, followed by a gradual change until Day 21. At Day 21, A had the highest WRA whereby 0.5GDL and 1.0GDL had the lowest WRA.

Akahane and Shimizu (1989) reported that water holding capacity (WHC) of Alaska pollock surimi was minimal at pH 5. When pH increased above 5, WHC of surimi paste markedly increased and the amount of free water decreased in salt-ground surimi paste. The amount of free water in surimi at pH 7.0 reached a minimum after addition of 2-3% salt. A decrease in the negative charge of myofibrillar protein, as the acidity increased (Chen and others 1998), could account for reduced WRA with increased storage time. As citric acid solution caused denaturation, resulting in

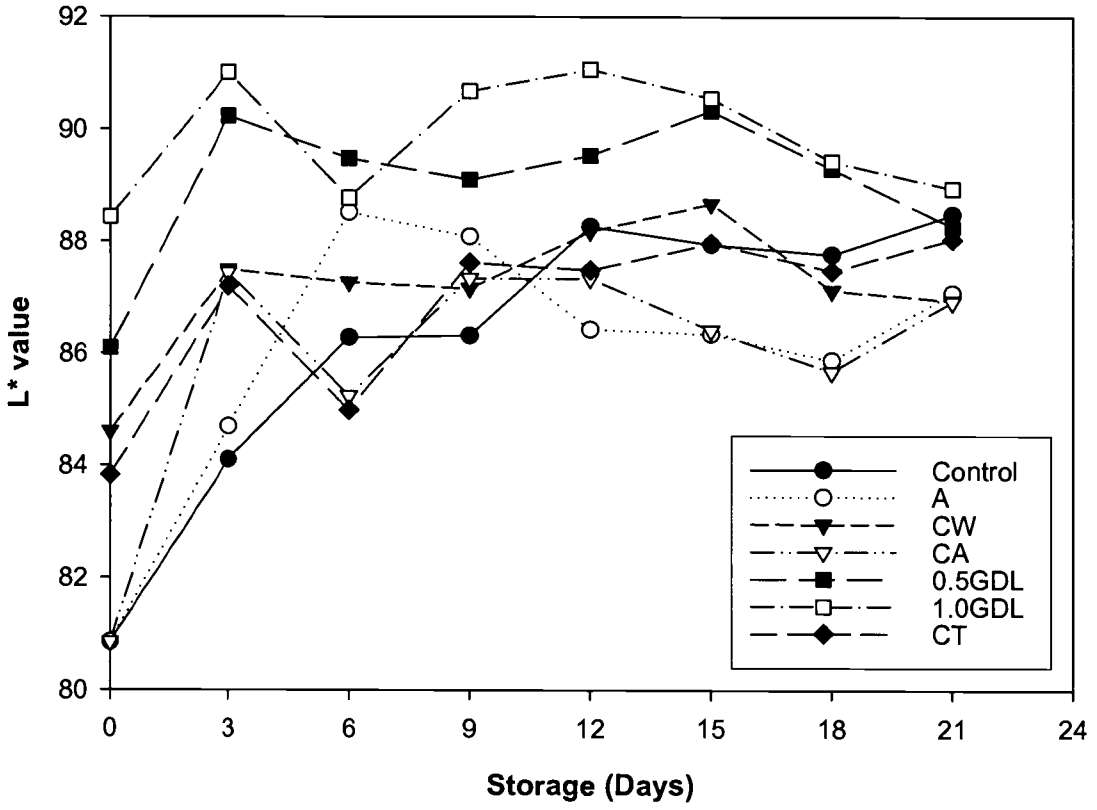

Figure 4.7 – L* value of cooked gel

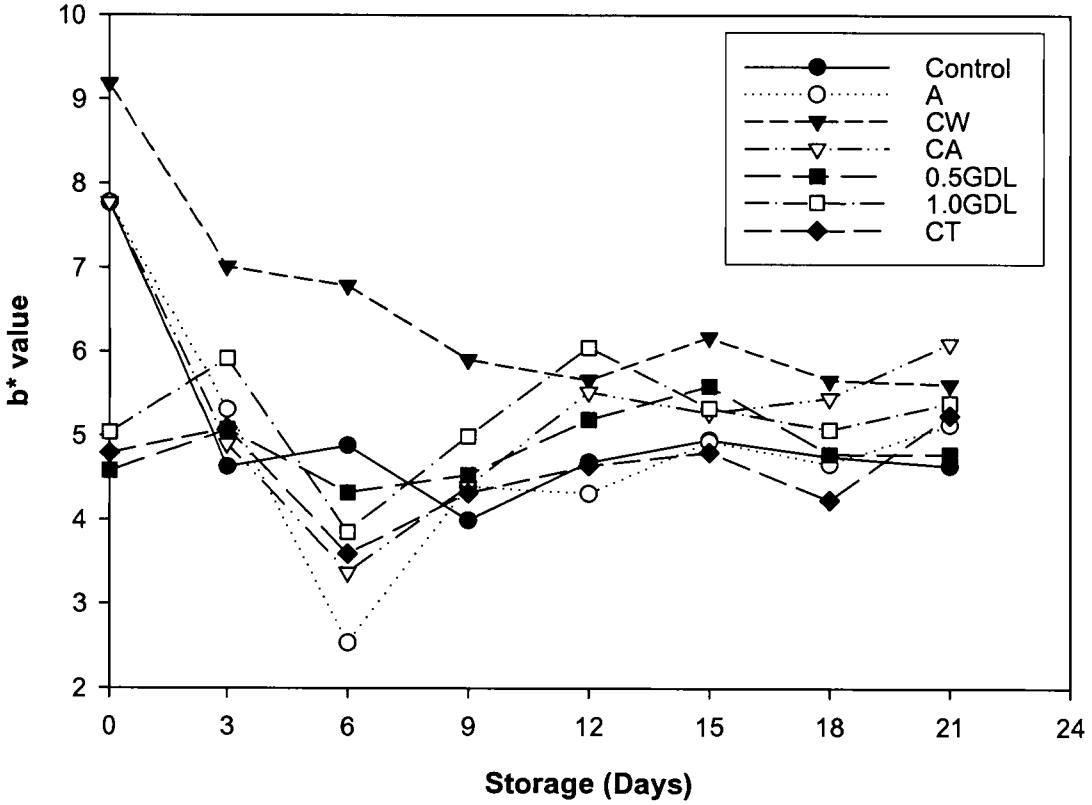

Figure 4.8 – b* value of cooked gel

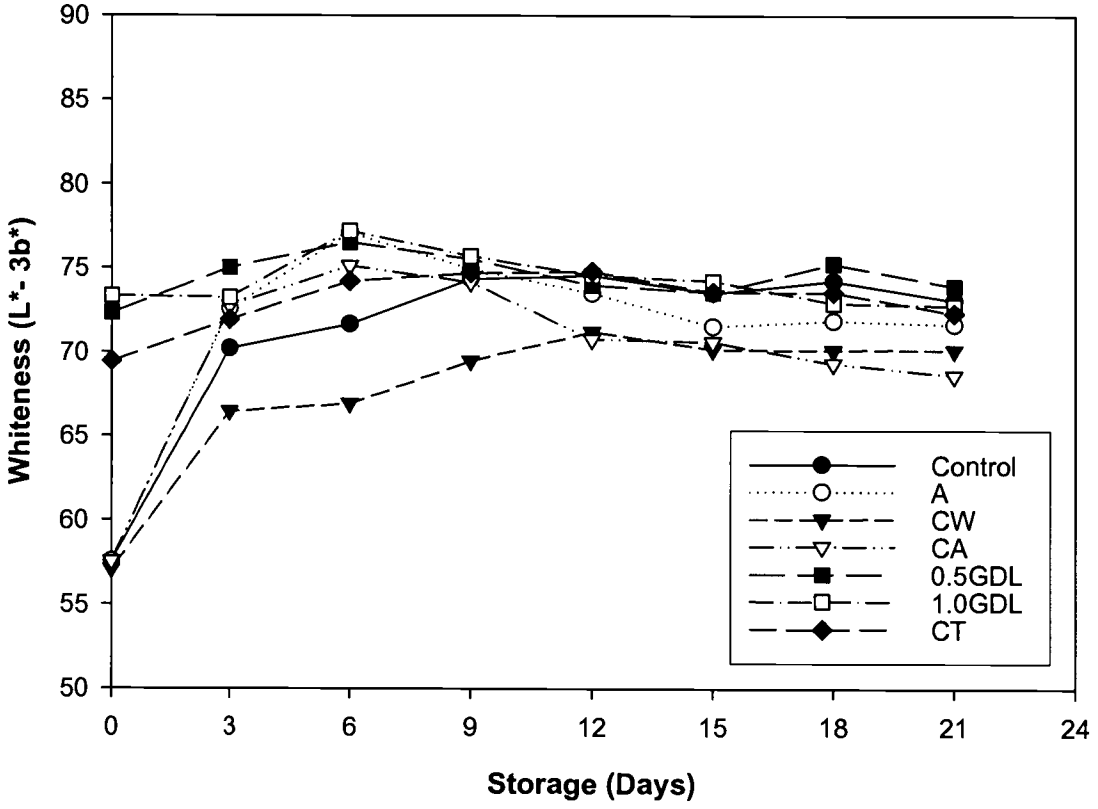

Figure 4.9 – Whiteness value of cooked gel

decreased WHC (Forrester and others 2002), CT showed a trend of continuously reduced WRA.

TGase activity

Change in TGase activity in set gel during storage is shown in Figure 4.11. At Day 0, the control, CA, and A had the highest TGase activity. Addition of citric acid (CT), water soluble chitosan (CW), and GDL significantly decreased ($P<0.05$) TGase activity. Set gel with 1% GDL had the lowest TGase activity at Day 0. With increased storage time, TGase activity significantly decreased ($P<0.05$). At the end of 3 weeks, CT had the lowest TGase activity, while the other samples had similar TGase activities. Addition of citric acid (CT) and GDL affected the pH of set gel, thereby TGase activity was reduced.

Yongsawatdigul and others (2002) demonstrated TGase activity of threadfin bream (TB) mince was 99.6 units/g of dry weight and after washing and dewatering, only 44% residual activity was retained in the surimi. The optimum conditions of crude TGase activity from TB were 55°C at pH 7.5 and 37°C at pH 7.0 (Yongsawatdigul and Park 2004). Activity of TGase at 55°C was two times greater than at 37°C. However incubation at 55°C induced proteolysis of muscle proteins and produced TCA-soluble oligopeptides of 7.66 nmol/mg/h and reached a maximum of 10.11 nmol/mg/h at 60 °C (Yongsawatdigul and others 2002). Therefore incubation for TGase activity of TB surimi for this study was chosen at 37°C.

Measurement of non-disulfide covalent bond

Solutions containing SDS, urea, and β-mercaptoethanol were used to solubilize protein gels by destroying all bonds, except non-disulfide covalent bonds, particularly the EGL linkage (Benjakul and others 2003). Low solubility indicated an increase in the formation of non-disulfide, covalent cross-links. The viscoelastic properties of surimi gels are influenced by several bonds, including EGL linkages (Niwa 1992). The formation of EGL linkages was found with the addition of microbial tranglutaminase during the setting process (Kimura and others 1991; Seguro and others 1995; Sakamoto and others 1995).

The control showed a trend of decreased solubility as storage increased (Figure 4.12). Decreased solubility indicated that non-disulfide bond formation occurred to a greater extent as storage increased. This accounted for increased breaking force during storage (Figure 4.5). After 1 wk of storage, breaking force of CA, A, 0.5GDL, and 1.0GDL showed higher breaking force (Figure 4.5) compared to the control. Reduced solubility ($P<0.05$) was also observed for A, CA, 0.5GDL, and 1.0GDL compared to the control after 1 wk (Figure 4.12). The acidic environment could enable proteins to unfold, allowing the reactive lysine and glutamine residues to be exposed for EGL linkage formation.

CA also had lower solubility than CW throughout the 21 days of storage. The acidic environment in CA allowed for the myofibrillar proteins to unfold and also caused the chitosan to have polycationic properties, which could cause ionic interactions between protein and chitosan. In CW, the non-charged chitosan might have hindered TGase formation of EGL linkages between myofibrillar proteins. In general at 3 wk, solubility of all samples was higher compared to the

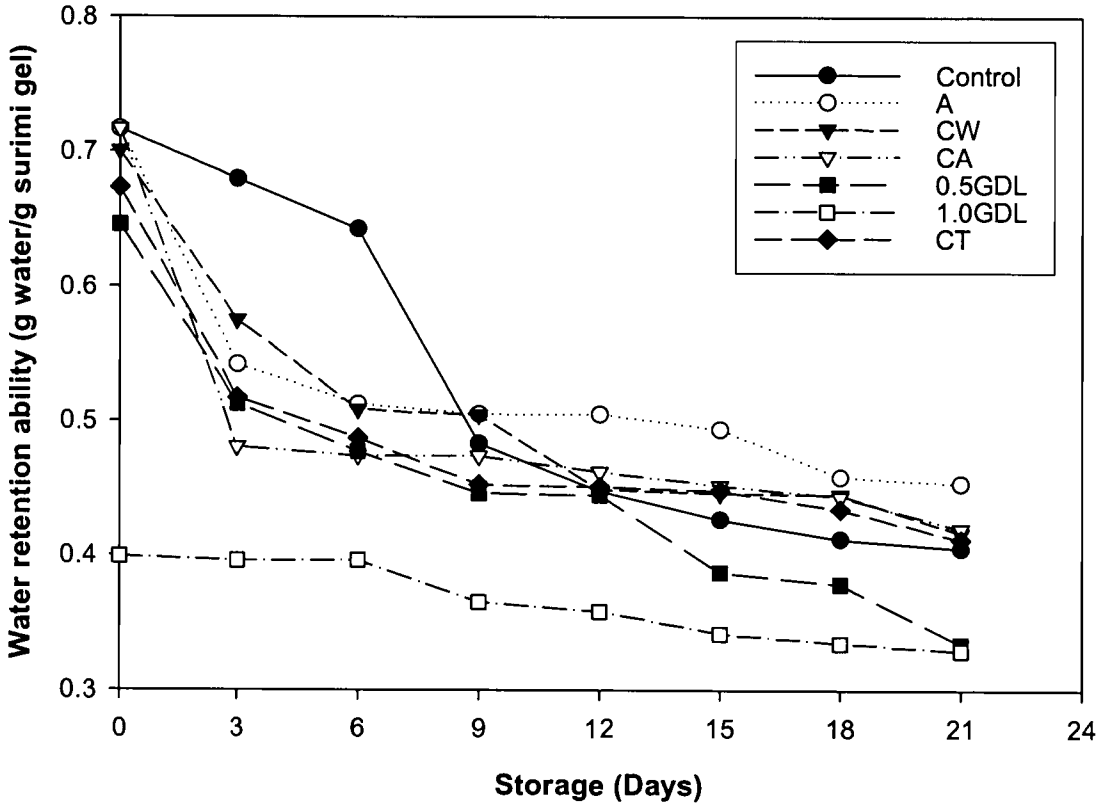

Figure 4.10 – Water retention ability of cooked gel

control. This accounted for the higher breaking force for the control compared to the other samples in Figure 4.5.

Viscosity

The addition of chitosan, citric acid, and GDL in surimi affected viscosity (Figure 4.13). At 5°C surimi paste temperature, addition of citric acid did not affect viscosity since encapsulated citric acid will only be released upon heating above 60°C (Millison J, personal communication, 2005).

With increased GDL, the apparent viscosity of surimi paste decreased. GDL caused partial unfolding of the native protein. Frethiem and others (1985) suggested the involvement of conformational changes of the protein during gelation in an acidic environment. Addition of water soluble chitosan affected surimi paste viscosity. Therefore, CW had the lowest viscosity.

Conclusion

Extension of shelf life of set fish ball by reducing the oversetting conditions and microorganisms was examined. GDL was found to be most effective at 1.0% in controlling oversetting of fish ball. GDL effectively improved the whiteness of fish ball at 0.5%. Growth of microorganisms was successfully inhibited by packaging the fish ball in 1% chitosan solubilized in 1% acetic acid.

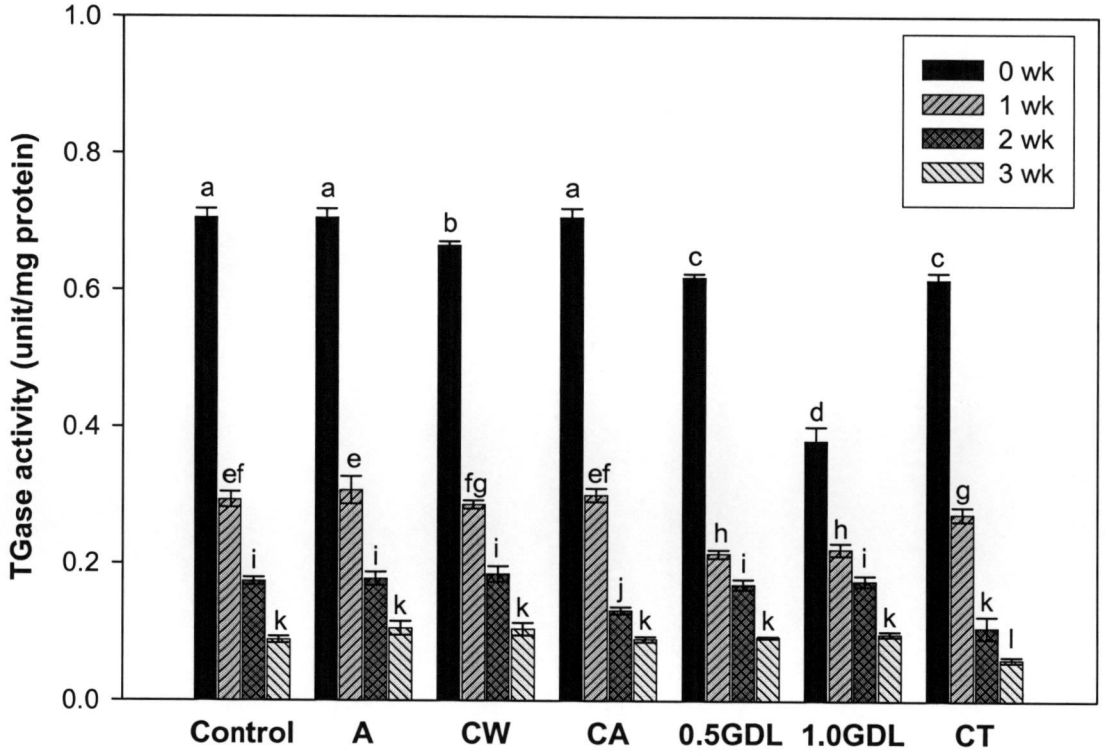

Figure 4.11 – Change in crude TGase activity of set gel during 4°C storage. Different alphabetical letters indicate a significant difference ($P<0.05$)

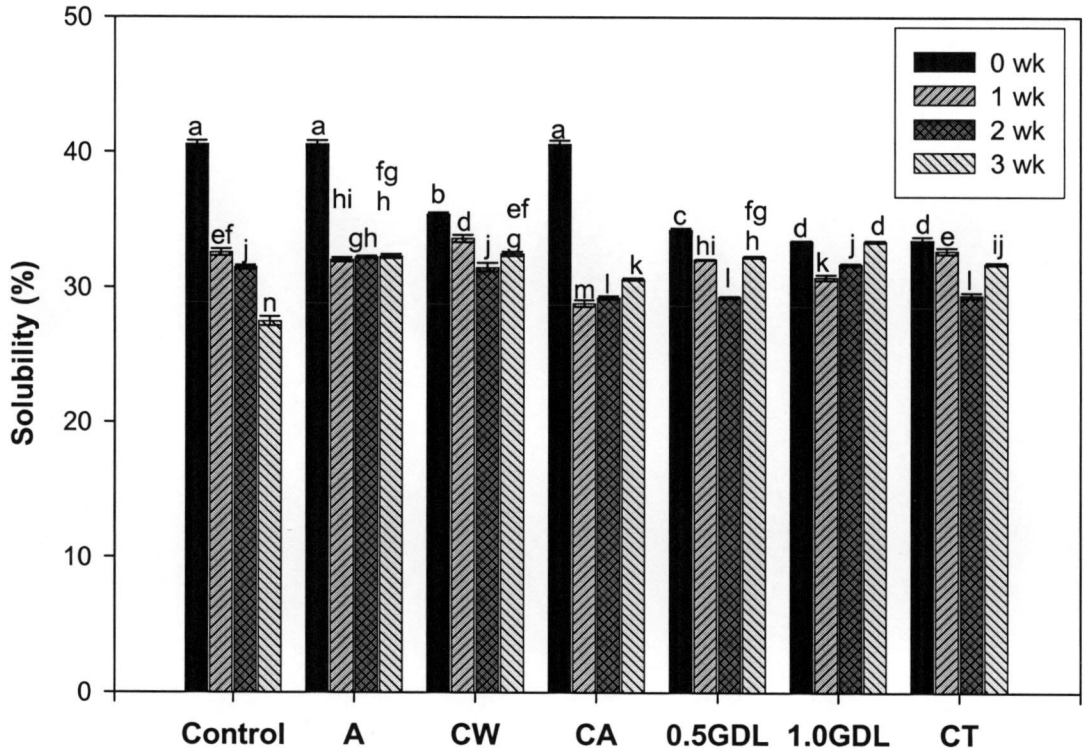

Figure 4.12 – Change in solubility of cooked gel. Different alphabetical letters indicate a significant difference ($P<0.05$)

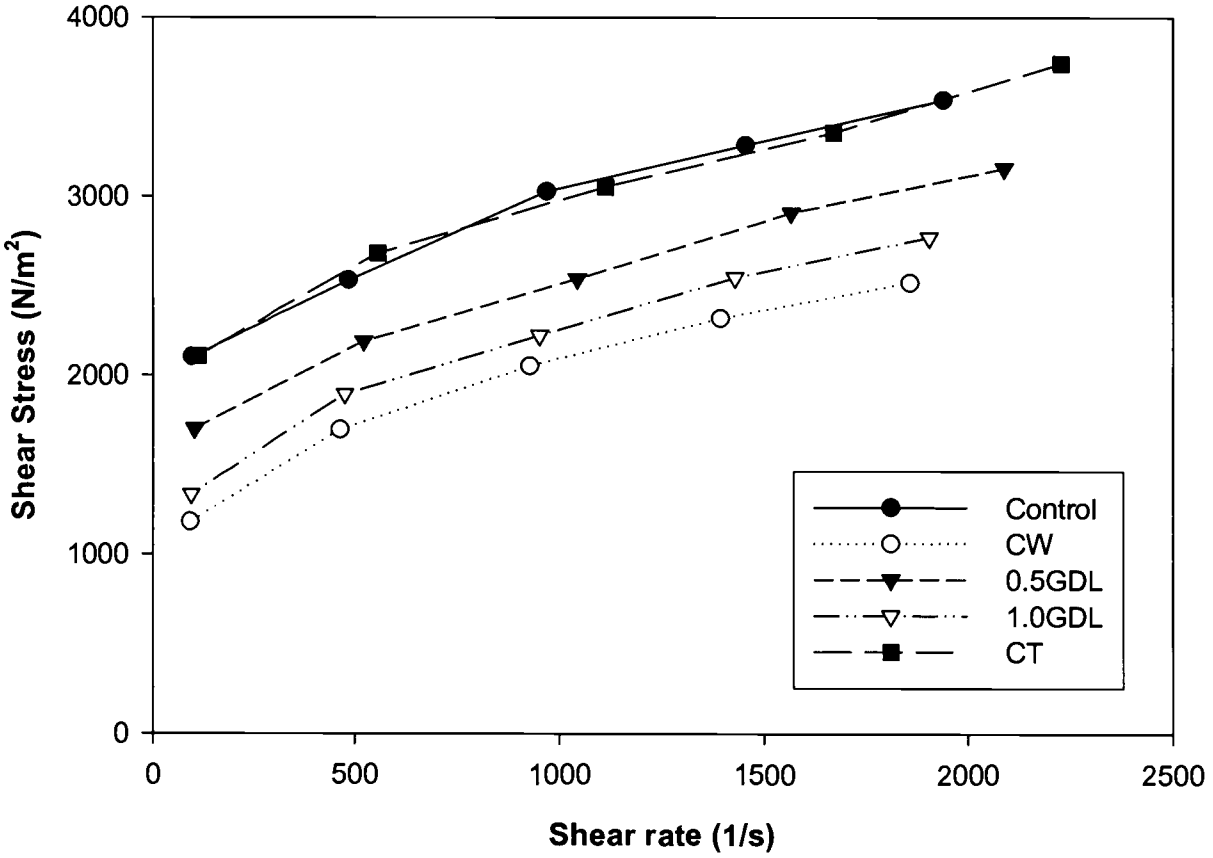

Figure 4.13 – Effect of chitosan, encapsulated citric acid, GDL on the viscosity of surimi paste

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APPENDIX

MULTIDISCIPLINARY APPROACHES FOR EARLY DETERMINATION OF GELATION PROPERTIES OF FISH PROTEINS

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ABSTRACT

Multidisciplinary approaches for early determination of fish protein quality were evaluated based on various biochemical and physical methods. A good correlation for deformation against ATPase activity was found with R^2=0.74. Another good correlation was obtained between storage moduli G' for gelling point and gel deformation at R^2=0.74. However, others showed positive trends, but with lower correlations coefficients ranging 0.55 and 0.63: between storage moduli G' at its initial increase and gel breaking force (0.55); between storage moduli G' at gelling point and gel deformation (0.63); between storage moduli G' at gelling point and gel breaking force (0.55).

Keywords: Multidisciplinary approaches, ATPase, oscillatory dynamic rheology, fish protein

INTRODUCTION

Texture of fish proteins is evaluated by the functional characteristics of surimi as influenced by intrinsic and extrinsic factors. Texture is evaluated using instruments to measure rheological properties. The most common rheological methods for texture analysis use torsion as a fundamental test and punch/penetration as an empirical test. Gel strength is represented by breaking force, obtained from punch test or shear stress obtained from torsion test. Cohesiveness and the degree of protein interaction in gel formation are represented by deformation, obtained from punch test or shear strain obtained from the torsion test. In the conventional method of gel analysis, gels are cooked in water bath at 90°C for 15-40 min, depending on the sample size. Gels are kept at 4°C overnight and gelation properties are evaluated in the following day. Texture data are available approximately 24 h later. By then, the production might have been completed or the products shipped to market. The amount of time required for conventional gel analysis does not allow prompt decision-making during production when there is an obvious problem in quality.

Based on the quantity of frozen surimi (200,000 t) produced in the US annually, a significant value could be saved if prompt evaluation is made and a decision is made in a short period of time. Multidisciplinary approaches, including biochemical measurement, oscillatory dynamic tests, and micro differential scanning calorimetry were thought to show a short cut for the quality assessment.

Surimi gels are characterized by dynamic rheological tests as both viscous and elastic. The continuous monitoring of the mechanical viscoelastic properties of proteins during heating provides valuable information on the molecular transformation

and the chemical forces involved in structure formation and breakdown (Hamann and others 1990). Small strain studies performed with the oscillatory dynamic test can be used to monitor changes of fish proteins during thermal processing, revealing the timing rates and significance of gelation events.

Although Akahane and others (1981) found some positive correlation between results from conventional DSC and gel strength, there is little practical application in the surimi industry due to low sensitivity and low reproducibility of conventional DSC. Recently, the micro DSC (Setaram Inc, Lyon, France), which is highly sensitive, has demonstrated that accurate measurements of transition enthalpies can be obtained for milligram quantities of protein. The probes of micro DSC are derived from the Calvet principle, a symmetrical heat-flux design. Based on this new design, the sample is surrounded by a heat flux detector that measures the entire heat flux resulting from the transformation of the sample, which conventional DSC cannot detect. The calibration, which is always a difficult problem with the conventional DSC technique, can also be easily achieved using ohmic heating technique (Welzel 1999; Parlouër and Chan 2001). This newly developed micro DSC should give accurate reproducibility, resulting in better correlation and easier application for early determination of texture quality.

The overall objective was to determine the quality of surimi (fish proteins) at an early stage of production using multidisciplinary approaches. Detailed objectives were: 1) To investigate the quality of fish proteins using differential scanning calorimetry; 2) To investigate the quality of fish proteins using oscillatory dynamic test; 3) To understand the effects of the biochemical properties of fish proteins on gelation

properties; 4) To determine a relationship between three different approaches and correlate with fracture gel analysis.

MATERIALS AND METHODS

Surimi samples

Different grades of Alaska pollock (*Theragra chalcogramma*) surimi were obtained from American Seafoods Company (Seattle, WA, USA), Trident Seafoods Corporation (Seattle, WA, USA) and UniSea, Inc. (Redmond, WA, USA).

Different grades of Pacific whiting (*Merluccius productus*) surimi were obtained from Pacific Surimi (Warrengton, OR, USA) and American Seafoods Company. Frozen surimi was cut into approximately 1000 g blocks, vacuum packed, and stored in a freezer (-25°C) throughout the experiments.

For the entire study, 60 batches of surimi chopping conducted and evaluated for their biochemical and rheological/thermal analysis to determine their relationship trends.

Actomyosin (AM) extraction

Actomyosin extraction was done based on the method described by MacDonald and Lanier (1994) with slight modifications. A small portion (2.5 g) of Alaska pollock surimi was homogenized in 50 mL of 0.6 M KCl (pH 7.0) using a PowerGen 700 homogenizer (Fisher Scientific, Pittsburg, PA, USA) at its speed 3 for 2 min. Homogenization process was done in a 5°C cold room to prevent excessive heat during the process. The homogenate was extracted for 30 min in a 5°C cold room and centrifuged at 8,000 xg for 30 min. However, for Pacific whiting, homogenization was done using 0.6 M KCl containing 20 mM tris-HCl (pH 7.0) for 30 s and extraction was done for 3 h according to Choi and Park (2002). The supernatant was collected and analyzed for protein content, surface hydrophobicity, and total sulfhydryl group.

Protein assay

Protein concentration of the supernatant was determined by the dye binding method (Bradford, 1976) using bovine serum albumin as a standard.

Assay of Ca^{2+}-ATPase activity

To a 0.25 mL AM solution (1.5-5mg/mL) was added 0.125 mL of 0.5M Tris-maleate buffer (pH 7.0), 0.125 mL of 0.1 M $CaCl_2$, and 1.875 mL of deionized water.

The mixture was incubated for 5 min at 25°C before adding 0.125 mL of 20 mM ATP solution. The ATPase assays of actomyosin were carried out for 8 min at 25°C. The reaction was stopped by the addition of 1.25 mL chilled 15% trichloroacetic acid. The mixture was centrifuged at 3,000 xg for 5 min and the supernatant was analyzed for liberated inorganic phosphate by the method described by MacDonald and Lanier (1994). The Ca^{2+}-ATPase activity was defined as micromoles inorganic phosphate liberated per milligram protein (uM Pi/mg protein/min) at 25°C. Phosphate determinations were performed in duplicate.

Total sulfhydryl (SH) group

Total sulfhydryl group was determined by the method established by Ellman (1959) using 5-5'-dithiobis-(2-nitrobenzoic acid) (DNTB). To 1 mL actomyosin solution (4 mg/mL) was added 9 mL of 0.2M tris-Cl (pH 7.0) containing 8 M urea, 2% SDS, and 10 mM EDTA. An actomyosin mixture (4 mL aliquot) was mixed with 0.1% DNTB (0.4 mL). The reaction mixture was incubated at 40°C for 25 min before measuring the absorbance at 412 nm. Total sulfhydryl groups were determined using a molar extinction coefficient of 13,600 $M^{-1}cm^{-1}$.

Surface hydrophobicity

Surface hydrophobicity (S_o) of actomyosin was determined by the method of Kato and Nakai (1980). Actomyosin solution was diluted to a series of different

concentration from 0.1 to 1 mg/mL in an aqueous solution (0.6 M KCl, 0.04 M $NaHCO_3$). After stabilizing at 25°C, 10 µL of 1-anilinonaphthalene-8-suflonic acid (ANS) (8 mM in 0.1 M phosphate buffer, pH 7.0) was added to 2 mL of the diluted protein. The relative fluorescent intensity of ANS-protein conjugates was measured using a luminescence spectrophotometer (Perkin Elmer LS-50B, Norwalk, CT, USA) at wavelengths (λ_{ex}, λ_{em}) of 374 nm and 485 nm, respectively. Protein hydrophobicity was calculated from the initial slopes of plots of relative fluorescence intensity and protein concentration (w/v) using linear regression analysis.

Gel preparation

Frozen surimi was tempered at room temperature for 1 h before being cut into 4 cm cubes. Surimi cubes were placed in a Stephan vacuum cutter UM-5 (Stephan Machinery Corp., Columbus, OH, USA). In the first 1 min, frozen cubes were chopped at low speed. 2% of salt was sprinkled and chopping continued at low speed for 1 min. Ice/water was added to adjust moisture to 78% to allow evaluation of gels at an equal moisture content. Beef plasma protein (BPP) was added at 1% for Pacific whiting surimi and the samples were chopped at low speed for 1 min. For the final 3 min, chopping continued at high speed while a vacuum was maintained at 0.5-0.6 bar. After chopping, the paste was stuffed into stainless steel tubes (inner diameter, 1.9 cm; length, 17.5 cm) with stainless steel screw caps, using a sausage stuffer (Sausage Maker, Buffalo, NY, USA). The interior wall of the tubes was coated with a film of PAM cooking spray (Boyle-Midway, Inc., NY). The tubes were heated in a

water bath at 90°C for 15 min. Cooked gels were chilled quickly in ice water (0°C) for 15 min and kept refrigerated (5°C) overnight.

Gel fracture assessment

Gels were equilibrated to room temperature at 25°C, for a minimum of 2 h and cut into 2.9 cm long. Gel samples were measured for breaking force and deformation to determine the strength and cohesiveness of gels, respectively. Texture analyzer (TA-XT plus, Texture Technologies Corp., NY, USA) equipped with a 5-mm spherical probe was used at a crosshead speed of 1 mm/s (Park 2005).

Torsion test

Gels were equilibrated to room temperature at 25°C for a minimum of 2 h and cut into 2.9 cm long. Gels were cut into pieces (length 2.9 cm) and the dumbbell geometry (end diameter: 1.9 cm; diameter at the center 1.0cm) made from stainless steel mold. Then gels were subjected to the Hamann Torsion gelometer (Gel Consultants, Raleigh, NC. USA) set at 2.5 rpm (NFI, 1991). Torque values were converted to shear stress, indicating gel strength, while angular displacement was calculated as shear strain, denoting gel cohesiveness.

Oscillatory dynamic measurement

Development of an actomyosin gel network was measured as a function of temperature using a CS-50 rheometer (Bohlin Instruments, Inc., East Brunswick, NJ). A CP-4/40 (4° angle, 40 mm diameter) cone and plate was used. To prevent moisture loss from the sample during heating, a plastic cover with a moistened sponge inside was used. The sample was heated from 20 to 90°C at a heating rate of 1 °C/min. Based on predetermined the linear viscoelastic region, 1 Pa torque value and 0.1 Hz frequency were selected during temperature sweep under oscillatory test mode.

Thermodynamic properties

Surimi paste was subjected to micro differential scanning calorimetry (micro DSC III, Setaram, Inc., Lyon, France). Calibration for temperature accuracy was done using deionized water and naphthalene. Calibration with the sample was also performed to determine the amount of deionized water required as a reference. Surimi paste weighing 500 ± 5 mg was sealed in a hastelloy sample vessel. Samples were scanned with a reference vessel containing deionized water at a heating rate of 1 °C/min over a temperature range of 20-90°C.

RESULTS AND DISCUSSION

Ca^{2+}-ATPase activity and gel fracture analysis

Sulfhydryl groups, SH_1, and SH_2 at active sites of myosin are responsible for the actomyosin Ca^{2+}-ATPase activity (Yamaguichi and Sekine 1966). Either group is sufficient to measure the Ca^{2+}-ATPase activity (Reisler and others 1974). Oxidation of SH groups was reported to inactivate the Ca^{2+}-ATPase of actomyosin in iced and frozen storage (Jiang and others 1988; Sompongse and others 1996).

Ca^{2+}-ATPase activity was used to evaluate the gel forming capacity of actomyosin as an indicator of the biochemical quality of muscle proteins (MacDonald and Lanier 1994; Carvajal and others 1999). A decrease of Ca^{2+}-ATPase activity, which measures the conformational changes of myosin, relates to the oxidation of sulfhydryl groups at the active sites of myosin (Sompongse and others 1996).

With the analysis of the punch test results and ATPase activity, an interesting linear relationship was found. As ATPase activity increased, gel deformation increased with the correlation coefficient (R^2) values at 0.74 (Figure 1). Breaking force values also increased as ATPase activity increased, however, at a lower determination coefficient (R^2) of 0.57 (Figure 2). This result suggests that a quickly determined ATPase activity could be used to estimate the cohesiveness of surimi gels.

Katoh (1979) used ATPase activity to evaluate the quality of frozen surimi from Alaska pollock. High Ca^{2+} ATPase activity was noted for high quality surimi, corresponding well to gel strength. He also found that Mg^{2+} ATPase activity was an

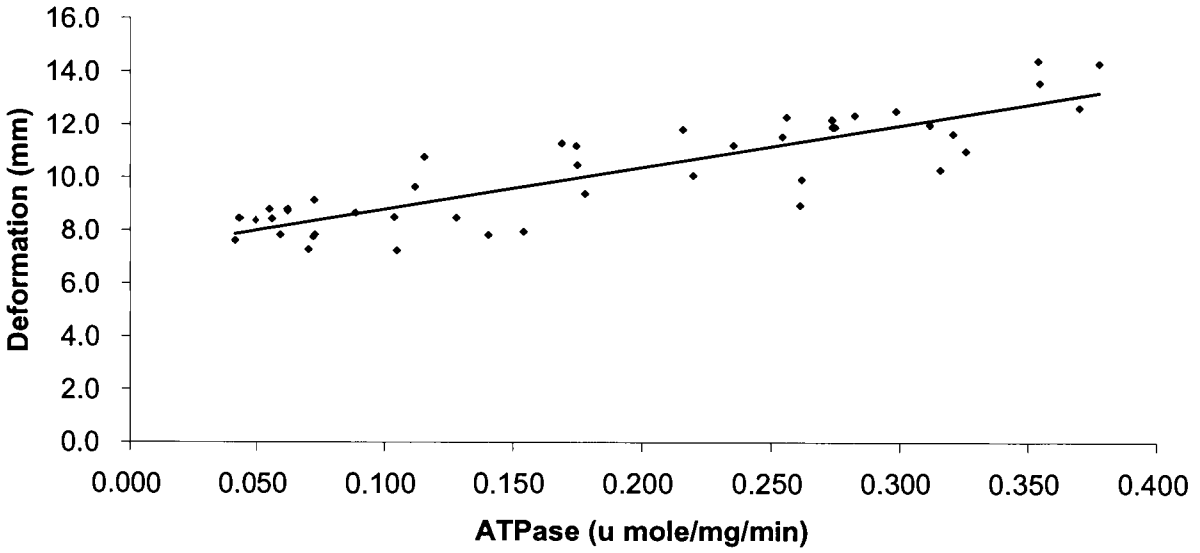

Figure 1 - Relationship of deformation (mm) vs concentrations of Ca^{2+}-ATPase (umole/mg/min) of different grade of surimi samples (R^2= 0.74)

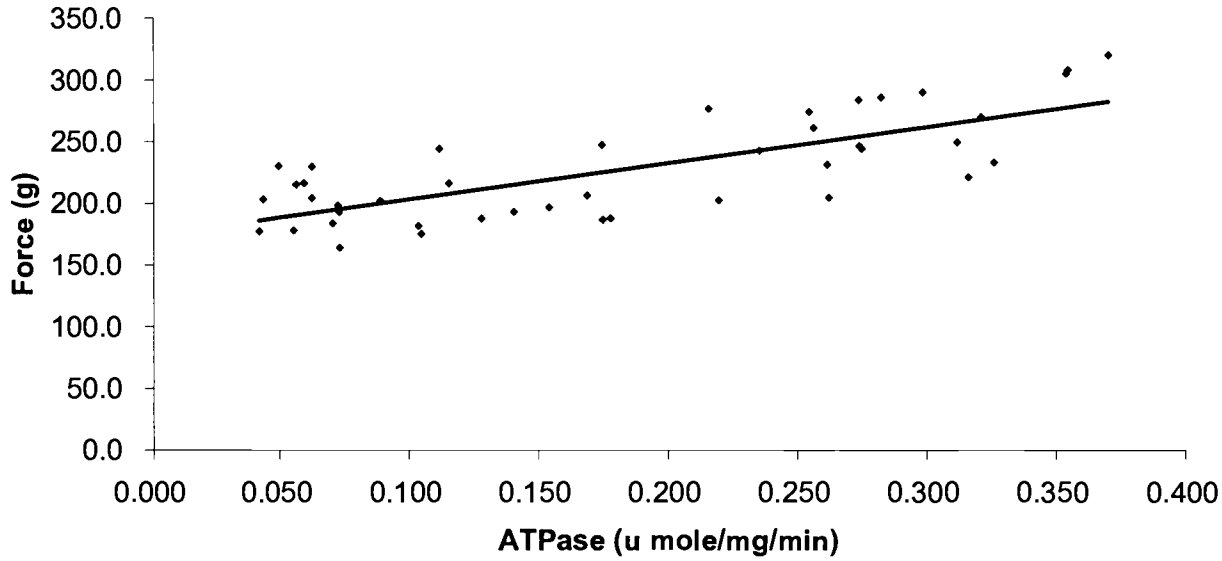

Figure 2 - Relationship of breaking force (g) vs concentrations of Ca^{2+} - ATPase (umole/mg/min) of different grade of surimi samples (R^2= 0.57)

excellent index for assessing quality of surimi as well as for estimating the freshness of raw material used for surimi preparation.

However, no acceptable correlation was found between fundamental torsion test and ATPase activity with its coefficient (R^2) values 0.10 and 0.16 for shear stress and shear strain, respectively against ATPase activity.

Surface hydrophobicity and gel fracture analysis

Surface hydrophobicity correlates with the degree of protein unfolding and denaturation, resulting in changed functional properties (Hayakawa and Nakai 1980). As the protein unfolds, non-polar amino acid groups are exposed at the molecular surface and the aqueous environment resulting in an increase in surface hydrophobicity (Kato and Nakai 1980). Surface hydrophobicity was applied to study the unfolding of various proteins such as milk, soy, and muscle proteins (Kato and Nakai 1980; Hayakawa and Nakai 1985; Wicker and others 1986; Yongsawatigul and Park 1999). Roura and others (1992) found that surface hydrophobicity of whiting actomyosin increased as viscosity decreased.

Surface hydrophobicity measured using an ANS probe (S_o-ANS) for surimi samples ranged from 785 to 1300. However, a linear regression analysis to correlate surface hydrophobicity and fracture gel analysis did not yield a significant relationship. The coefficient values were less than 0.1.

Total sulfhydryl (SH) groups and gel fracture analysis

Yongsawatdigul and Park (2003) found that total SH remain unchanged up to 30°C and gradually decreased from 40-80°C for threadfin bream actomyosin. Formation of disulfide linkages occurred simultaneously with protein unfolding and aggregation causing a stronger gel network indicated through increased in G' at >46.3 °C. However, our efforts to determine the correlation between total SH content and fracture gel analysis resulted in a poor result. Correlation coefficients with total SH content were 0.29 for gel breaking force, 0.17 for gel deformation, 0.20 for shear stress, and 0.18 for shear strain

Oscillatory dynamic measurement and gel fracture analysis

Our attempts were made to see if there is any correlation between non-fracture gel analysis which takes a relatively short time and time-consuming conventional fracture gel analysis. Storage moduli (G') of Alaska pollock started to increase at 28-29 °C while that of Pacific whiting at 30 °C. Storage moduli (G') of threadfin bream (warm water fish) actomyosin started to increase at 34.5 °C (Yongsawatigul and Park 2003). Initial increases of G' indicated the formation of myosin cross-linking resulting in the transformation from a viscous sol to an elastic network (Egelansdal and others 1986). Hamann (1992) showed that pollock proteins observed a near zero slope section near 28 °C. The zero slope section was likely produced by the combination of conformation loss and structure building so that little change in structural rigidity occurs. Rapid structure building started at 28 °C. The

protein structure developed via myosin head groups at temperatures below 38°C loosened up and caused a change in protein native conformation. These changes associated with the loss of G' values were found after the initial peak (Wu and others 1991).

Figure 3 showed strong correlations between the storage moduli at 28-29°C (Alaska pollock) and 30°C (Pacific whiting) referred to as G'a. A good correlation coefficient of (R^2) 0.74 was obtained with G'a against gel deformation. A trend whereby higher G'a values produced low deformation values was found. Figure 4 showed a similar trend for G'a against gel breaking force, but with a lower correlation coefficient of 0.55. The relationships between parameters of punch test were inversely proportional to the storage moduli G'a.

G'b refers to the lowest point of storage moduli or the point where the moduli started to increase observed normally at 43-45°C. G' initial peak detected at 35-37°C. A similar peak observed for croaker indicates some transition in protein conformation and/or protein-protein association occurred at that temperature. This transition is related to the high temperature "setting" phenomenon (Lanier and others 1982). A relationship of G'initial peak (G'b) against punch test results (Figures 5 and 6) provided a correlation coefficient of 0.63 (deformation) and 0.55 (force), respectively. The relationships between fracture analysis results were inversely proportional to the storage moduli, G'initial (G'b).

Figures 3, 4, 5, and 6 showed negative slopes. The lower gel strength provided higher storage moduli at the earlier stage of temperature sweep. Kim and others (1986) showed a strong peak at 38°C for freeze/thawed pollock surimi, while no peak for control sample. They suspected that the control (without freeze/thaw) would be nearer the low entropy native stage and required more energy to unfold

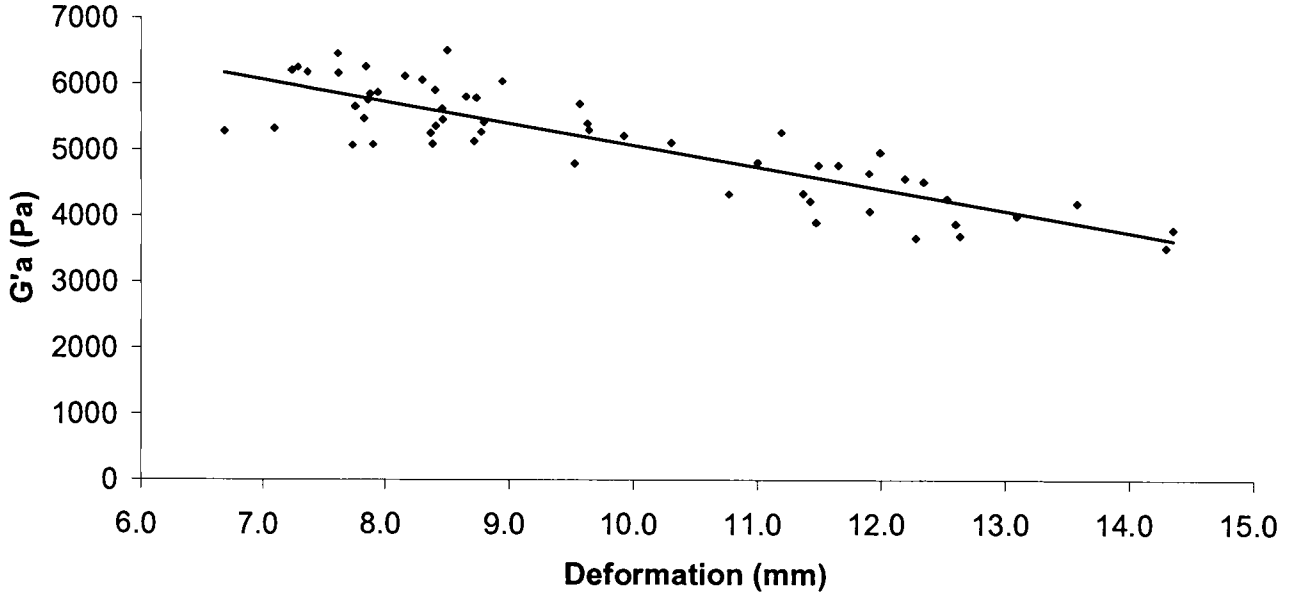

Figure 3 - Relationship of G'a (Pa) vs deformation (mm) of different grades of surimi samples (R^2=0.74)

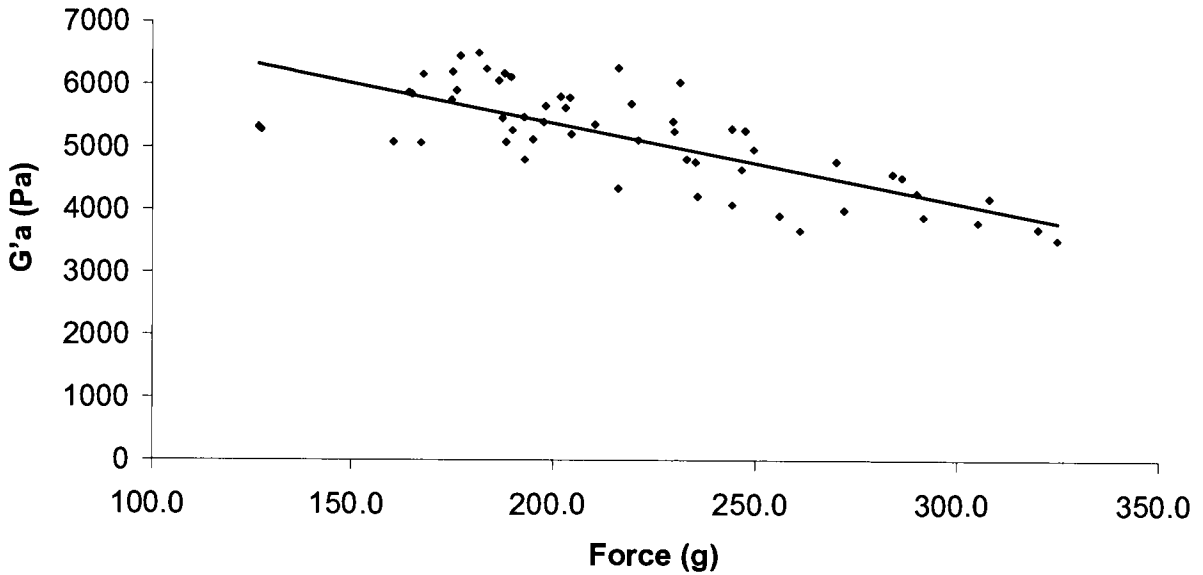

Figure 4 - Relationship of G'a (Pa) vs breaking force (g) of different grades of surimi samples (R^2=0.55)

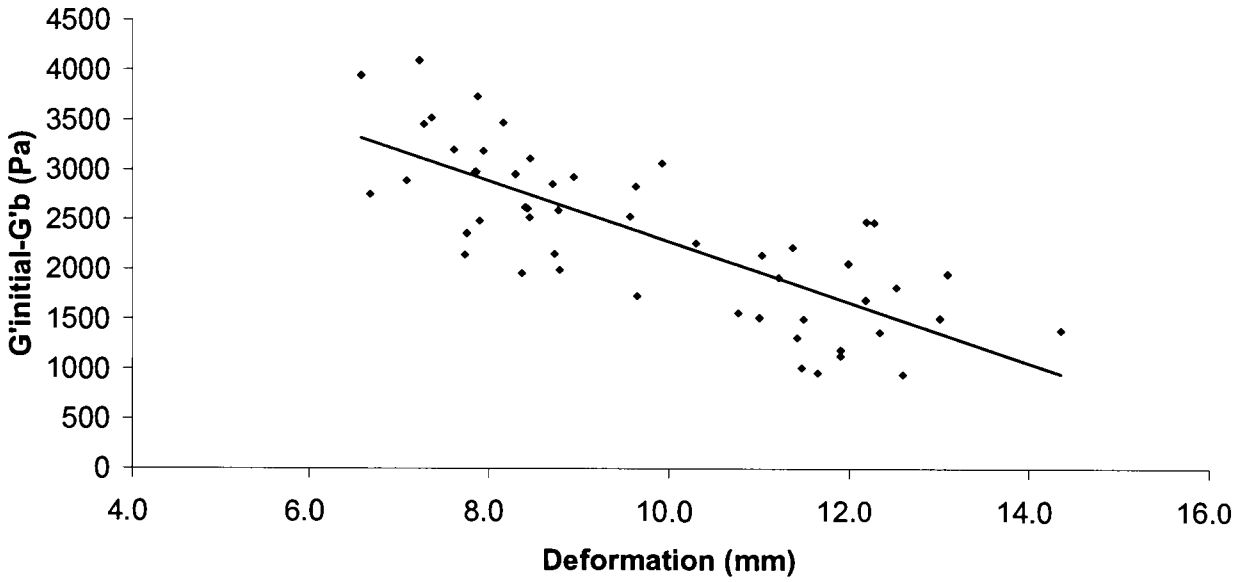

Figure 5 - Relationship of G'initial-G'b (Pa) vs deformation (mm) of different grades of surimi samples (R^2=0.60)

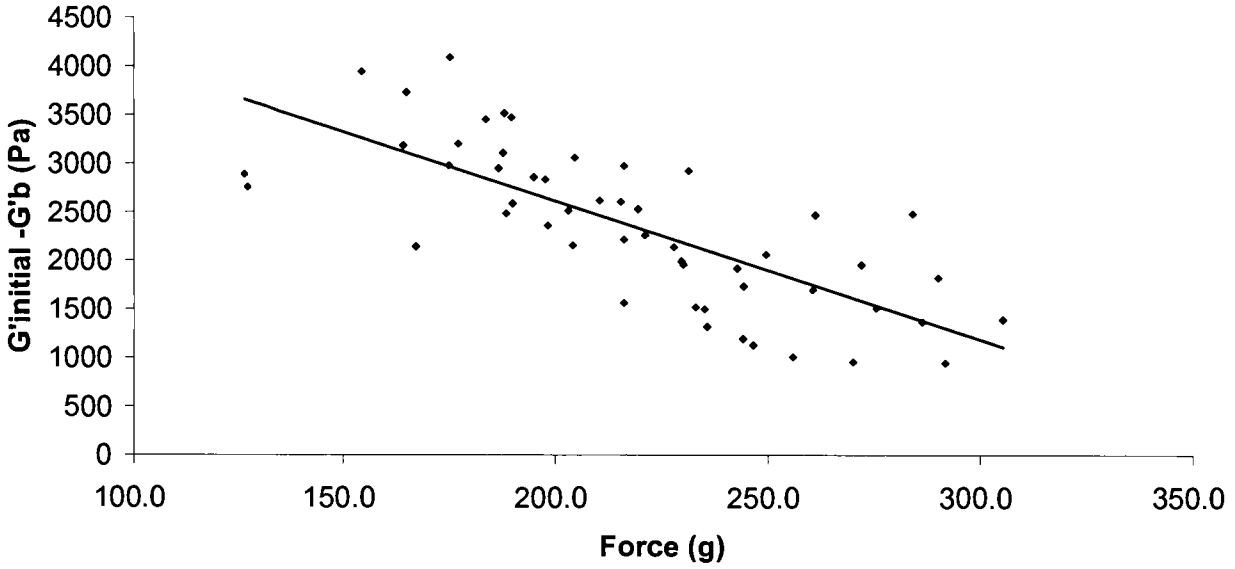

Figure 6 - Relationship of G'initial-G'b (Pa) vs breaking force (g) of different grades of surimi samples (R^2=0.55)

prior to aggregation producing a more gradual change. The decline of G' after reaching the first peak had also been reported in myosin (Egelandsdel and others 1986; Wu and others 1991; Yongsawadigul and others 1999) and myofibrillar proteins (Xiong and Blachard 1994). Helix to coil transformation of myosin leads to a large increase in the fluidity of semi-gels and may disrupt some proteins network that had already been formed, resulting in a declined storage modulus (Xiong and Blanchard 1994). Subsequently, formation of new bonds produced a more permanent protein network structure observed at 45°C or higher.

Micro differential scanning calorimetry measurement and fracture analysis

Efforts to correlate the total enthalpy change of myosin and actin with fracture gel analysis did not yield a reasonable relationship. The correlation coefficient was less than 0.1.

The setting and disintegrating reactions were endothermic. A small amount of ΔH means the completion of structural or conformational changes of proteins with a small quantity of heat (Iso and others 1991). Shimuzu and others (1981) also found a low correlation between gel strength obtained using a mechanical testing and ΔH measured using a conventional DSC.

CONCLUSION

Two methods, ATPase activity and dynamic oscillatory rheology, were found to be useful for early determination of fish protein quality. The results showed a good correlation corresponding to fracture gel values. The industry could implement the outcome successfully in its daily operation.

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