Improvement of Gel Quality of Surimi from Bigeye snapper (*Priacanthus tayenus*)

Yuwathida Kwalumtharn

Master of Science Thesis in Fishery Products Technology

Prince of Songkla University

2002

Thesis Title	Improvement of Gel Quality of Surimi from Bigeye snapper (*Priacanthus tayenus*)
Author	Miss. Yuwathida Kwalumtharn
Major Program	Fishery Products Technology

Advisory Committee

.....Chairman

(Assoc. Prof. Dr. Soottawat Benjakul)

.....Committee

(Dr. Wonnop Visessanguan)

Examining Committee

.....Chairman

(Assoc. Prof. Dr. Soottawat Benjakul)

.....Committee

(Dr. Wonnop Visessanguan)

.....Committee

(Dr. Manee Vittayanont)

.....Committee

(Assoc. Prof. Dr. Nunta Churngchow)

The Graduate School, Prince of Songkla University, has approved this thesis as partial fulfillment of the requirement for the Master of Science degree in Fishery Products Technology.

.....

(Piti Trisdikoon, Ph.D.)

Associate Professor and Dean

Graduate School

ชื่อวิทยานิพนธ์	การปรับปรุงคุณภาพเจลซูริมิจากปลาตาหวาน
ผู้เขียน	นางสาวยุวธิดา ขวาลำธาร
สาขาวิชา	เทคโนโลยีผลิตภัณฑ์ประมง
ปีการศึกษา	2544

บทคัดย่อ

องค์ประกอบทางเคมี และคุณสมบัติของซูริมิแตกต่างกัน ขึ้นกับเกรดซูริมิและคุณภาพของวัตถุดิบ โปรตีนไมโอไฟบริลลาร์เป็นองค์ประกอบสำคัญที่พบในซูริมิ โดยแปรผกผันกับปริมาณสารประกอบไนโตรเจนที่ระเหยได้ทั้งหมดและปริมาณไตรเมทธิลเอมีน ซูริมิที่ผลิตจากปลาสดมีปริมาณสารประกอบไนโตรเจนที่ระเหยได้ทั้งหมดและไตรเมทธิลเอมีนต่ำสุด Tmax และเอนทัลปีของซูริมิเกรดต่ำและซูริมิที่ผลิตจากปลาคุณภาพต่ำมีค่าต่ำกว่าซูริมิเกรดสูงหรือซูริมิที่ผลิตจากปลาสด

จากการศึกษาคุณสมบัติของเจลของปลาตาหวานเกรด A และ B ที่เตรียมด้วยวิธีการต่างๆ พบว่าแอซซิเจลมีค่าแรงเจาะทะลุและระยะทางก่อนเจาะทะลุสูงสุด ตามด้วยเจลที่เตรียมโดยการให้ความร้อนโดยตรง ซูวาริเจล และโมโดริเจล โดยซูริมิเกรดสูงมีคุณภาพสูงกว่าซูริมิเกรดต่ำ ผลของการเติมโปรตีนเติมแต่ง (ไข่ขาว พลาสมาเลือดหมูและเลือดวัว) สารประกอบแคลเซียม (แคลเซียมคลอไรด์ แคลเซียมกลูโคเนตและแคลเซียมแลคเตท) และเอนไซม์ทรานส์กลูตามิเนสจากจุลินทรีย์ต่อคุณภาพเจลซูริมิจากปลาตาหวานพบว่าความเข้มข้นที่เหมาะสมของไข่ขาว พลาสมาเลือดหมูและเลือดวัว เป็น ร้อยละ 4 1 และ 1 ตามลำดับ เมื่อเติมโปรตีนพลาสมาความเข้มข้นสูงขึ้นพบว่าค่าแรงเจาะทะลุและค่าความขาวลดลง ส่วนการเติมไข่ขาวความเข้มข้นสูงถึงร้อยละ 4 ยังคงให้ค่าแรงเจาะทะลุเพิ่มขึ้นและค่าความขาวเพิ่มขึ้นเล็กน้อย โดยไม่มีผลต่อปริมาณของไมโอซินเส้นหนัก เมื่อศึกษาการใช้สารประกอบแคลเซียมพบว่าเมื่อความเข้มข้นสูงขึ้นค่าแรงเจาะทะลุสูงขึ้น ซูริมิที่เติมแคลเซียมกลูโคเนตมีค่าแรงเจาะทะลุสูงที่สุดเมื่อเปรียบเทียบกับแคลเซียมคลอไรด์และแคลเซียมแลคเตท ผลการเติมเอนไซม์ทรานส์กลูตามิเนสจากจุลินทรีย์ในซูริมิจากปลาตาหวาน พบว่าค่าแรงเจาะทะลุและระยะทางก่อนเจาะทะลุมีค่าเพิ่มขึ้นอย่างเด่นชัด การใช้สารเติมแต่งร่วมกันโดยการเติมไข่ขาวร้อยละ 4 แคลเซียมกลูโคเนต 50 มิลลิโมลต่อกิโลกรัม และเอนไซม์ทรานส์กลูตามิเนสจากจุลินทรีย์ ร้อยละ 0.1 และสภาวะในการเซ็ตตัวที่อุณหภูมิ 40

องศาเซลเซียส นาน 30 นาทีตามด้วยการให้ความร้อนที่อุณหภูมิ 90 องศาเซลเซียส นาน 15 นาที สามารถเพิ่มความแข็งแรงของเจลได้อย่างมีประสิทธิภาพ

จากการศึกษาการปรับปรุงความขาวของเจลซูริมิผสมระหว่างซูริมิปลาตาหวานเกรด SSA หรือ เกรด SA ร่วมกับซูริมิจากปลาทูแขก (อัตราส่วน 70:30 และ 50:50 ตามลำดับ) โดยใช้สารให้ความขาวต่างๆ พบว่าไทแทเนียมไดออกไซด์มีผลต่อการเพิ่มความขาวแก่ซูริมิได้อย่างมีประสิทธิภาพโดยไม่มีผลต่อคุณสมบัติการเกิดเจล น้ำมันพืชมีผลลดค่าความแข็งแรงของเจลแต่มีผลต่อความขาวของซูริมิ ขณะที่แคลเซียมคาร์บอเนตไม่มีผลต่อการเพิ่มความขาวแต่มีผลต่อการเพิ่มค่าความแข็งแรงของเจล

Thesis Title	Improvement of Gel Quality of Surimi from Bigeye snapper (*Priacanthus tayenus*)
Author	Miss. Yuwathida Kwalumtharn
Major Program	Fishery Products Technology
Academic Year	2001

Abstract

The compositions and property of bigeye snapper surimi varied, depending upon grades as well as raw material quality. Myofibrillar protein was found to be a major constituent in surimi. Trimethylamine and total volatile base conversely related with myofibrillar protein content in surimi. Surimi produced from very fresh fish had the lowest TVB and TMA. Tmax and enthalpy of surimi with lower grade and surimi produced from low quality fish were lower than higher grade surimi or surimi produced from very fresh fish.

The gelling characteristics of different grade surimi (A and B grade) of bigeye snapper were studied. The highest force and deformation were observed with ashi gel, followed by directly heated gel, suwari and modori gel. Higher grade surimi (A grade) exhibited higher quality gel. The effect of protein additives [egg white (EW), pig plasma protein (PPP), beef plasma protein (BPP)], calcium compounds (calcium choride, calcium gluconate, calcium lactate) and microbial transglutaminase (MTGase) on gel quality of surimi from bigeye snapper was investigated. The addition of protein additives at an appropriate concentration resulted in increased breaking force and deformation. Optimum concentrations of EW, BPP and PPP for both surimis (A and B grade) were found to be 4, 1 and 1 %w/w, respectively. When a higher amount of plasma protein added increased, the breaking force and whiteness decreased. However, addition of EW up to 4% the increased breaking force and deformation with a slight increase in whiteness. When a higher concentration of BPP and PPP were added more myosin heavy chain (MHC) was retained. However, no marked changes in MHC were obtained

with the increasing amount of EW. Among calcium compounds tested, calcium gluconate rendered the gel with higher breaking force, compared to calcium lactate and calcium chloride ($p<0.05$). Increased breaking force and whiteness were found as the concentration of calcium compounds increased ($p<0.05$). The addition of MTGase in bigeye snapper surimis resulted in a considerable increase in breaking force and deformation ($p<0.05$) without any changes in whiteness ($p>0.05$). The combination of 4% EW, 50 mmole/kg calcium gluconate and 0.1% MTGase and setting at 40°C for 30 min, prior to heating at 90°C for 15 min were found to effectively increase gel quality.

Whiteness improvement of mixed surimi between SSA or SA grade bigeye snapper surimi and mackerel surimi (70:30 and 50:50, respectively) using different whitening agents was investigated. Titanium dioxide was a promising whitening agent since it had no detrimental effect on gel forming ability, while oil reduced the gel strength even though whiteness could be improved. Calcium carbonate did not show the marked influence on whiteness improvement. However, it could increase the gel strength to some extent.

Acknowledgement

I wish to express my deepest and sincere gratitude to my advisor, Assoc. Prof. Dr. Sootawat Benjakul of the Department of Food Technology, Faculty of Agro-Industry, Prince of Songkla University, for his kindness, guidance and assistance in reading, correcting and criticizing the manuscript.

I also would like to express my profound gratitude to my co-advisor, Dr. Wonnop Visessanguan of BIOTEC Yothi-Research Unit, National Center for Genetic Engineering and Biotechnology, for his kindness, comments and helpful suggestion.

I am also very grateful to my examination committees, Dr. Manee Vittayanont of the Department of Food Technology, Faculty of Agro-Industry, Prince of Songkla University and Assoc. Prof. Dr. Nunta Churngchow of the Department of Biochemistry, Prince of Songkla University, for their kindness and helpful suggestion.

I am grateful to my dear friends and technicians who encouraged and helped in various ways.

This study could not be succeeded without the financial support from the Graduate School, Prince of Songkla University.

Finally, none of this would have been possible without love and encouragement of my parents and my brother. I thank them for their understanding during all of the times when I could not be with them and their steady love that supports me.

Yuwathida Kwalumtharn

Contents

Contents (Continued)

List of Tables

List of Tables (continued)

List of Figures

List of Figures (Continued)

Chapter 1

Introduction

Quality of surimi depends on the intrinsic and extrinsic factors such as species, season, harvesting etc. Gelation of fish proteins is the most important step in forming desired textures in many seafood products, particularly those from surimi. Various physical conditions and chemical additives can affect surimi gelling property. Food-grade additives have been widely used in surimi industry to improve the gel quality. Beef plasma protein, egg white and whey protein are common protein additives, mainly to retard the gel softening of surimi gel (Morrissey *et al.*, 1993; Weerasinge *et al.*, 1996) and acted as functional binders and fillers (Park, 2000). To maximize the endogenous transglutaminase (TGase) activity, setting at appropriate temperature and time is another means to enhance the gel strength via inducing non- disulfide covalent bonds. Furthermore, addition of calcium salts to activate the endogenous TGase, a calcium-dependent enzyme, has been found to improve the gel strength of surimi (Kishi *et al.*, 1991; Lee and Park, *1998*). TGase has also been found in a culture broth of variant of *Streptoverticillium mobaraense* (Ando *et al.*, 1989; Washizu *et al.*, 1994) and referred to as MTGase.

Due to a limited amount of endogenous TGase, TGase from other sources has been applied. Microbial TGase, which is capable of introduction covalent cross-linking between protein molecules, has became more popular for surimi industry (Seguro *et al.*, 1995; Jiang *et al.*, 2000). Quality of surimi gel can be varied among fish species or even the same species. Therefore, the addition of some potential additives can be another alternative to improve the gel quality.

Whiteness is another factor determining surimi gel quality. Some whitening agents such as titanium dioxide (TiO_2), vegetable oil and calcium carbonate ($CaCO_3$) are used to improve the whiteness of surimi. Different whitening agents render the different efficacy in whiteness improvement. Some whitener can lower affect the gel-forming ability.

Thus, the appropriate whitener should be chosen and applied properly to gain the high quality gel. The information gained will be beneficial for surimi industries in Thailand as well as ASEAN countries in order to improve the surimi gel quality with the use of proper additives.

Literature Review

Surimi

The surimi industry has changed dramatically over the past decade. A decrease in Alaska pollock harvests caused by stricter fisheries management has opened the door for the use of new species in the surimi industry. Southeast Asia initiated the expansion of surimi productions by using threadfin bream and other species (Itoyori, 1992). So far, the fish species used in Southeast Asia for production of surimi are mainly threadfin bream (*Nemipterus* spp.), bigeye snapper (*Priacanthus* spp.), croakers (*Pennahia* and *Johnius* spp.), and lizardfish (*Saurida* spp.) (Morrissey and Tan, 2000).

Threadfin Bream

The threadfin bream (*Nemipterus* spp.) belong to the family Nemipteridae, and about 10 species are commonly found in the Indo-West Pacific and subtropical waters. This threadfin bream surimi is now well accepted in Japan because of the white color, smooth texture, and strong gel-forming ability of the fish meat.

Bigeye Snapper

The bigeye snapper (*Priacanthus* spp.) belong to the family Priacanthidae and are represented by two species in the South China Sea area, *Priacanthus tayenus*, which is more abundant, and *P. macracanthus*. Both species have bright crimson color with a thick and tough skin. Unlike threadfin bream, which undergoes burst belly when kept in ice for a long time, the bigeye has a longer shelf life in ice. Minced meat is usually darker than that of threadfin bream because of the presence of a strip of dark meat on the caudal area. However, bigeye snapper has a high gel-forming ability. Furthermore, the bigeye snapper is abundant in the trawl catch, often landed in substantial quantities, and attains a size of about 30 cm, with the usual size ranging from 15-25 cm. Due to its appearance and thick skin, it is not consumed directly and therefore is a suitable raw material for surimi manufacture.

Croaker

Croaker (*Sciaenidae*) has been a preferred species for the traditional kamaboko industry in Japan. The species commonly used are blackmouth croaker (*Atrobucca nibe*), white croaker (*Argyrosomus argenteus*), and yellow croaker (*Pseudoscianena polytis*). Surimi from these species is generally a darker color than threadfin bream surimi but higher price.

Lizardfish

In South Japan, fresh lizardfish (*Saurida* spp.) have long been considered as a high-grade raw material for surimi and kamaboko, with high meat yield, white color, good flavor, and high gel forming ability. Nozaki *et al.* (1978) and Yasui *et al.* (1987) indicated that the gel forming ability of lizardfish falls rapidly during ice storage because of the formation of formaldehyde (FA) and dimethylamine (DMA) from trimethylamine oxide (TMAO). The main species of lizardfish landed are *Saurida tumbil* and *S. undosquamis* (usual size 20-30 cm).

Dark muscle fish

Several other fish species are sometimes used for surimi manufacture as well, although they are not commonly used as the proceeding species. These include the pike conger eel, the hairtail, barracuda, mackerel and sardine (Kano, 1992). Dark muscle fish species currently make up 40-50% of the total fish catch in the world. There is great interest in using the large quantities of these low-value, fatty, pelagic fish, which are currently available for human food (Hultin and Kelleher, 2000). Suzuki and Watabe (1987) reported that surimi prepared from fresh sardine has equal quality to the high grade Alaska pollock surimi. In addition, mackerel light muscle surimi had a higher and more constant sensory score, lower lipid oxidation, and less change in true strain values than surimi made from whole mackerel muscle (Kelleher *et al.*, 1994). Surimi with high fat content generally has lower quality than surimi prepared from the same species at a time when the fat content is lower (Ohshima *et al.*, 1993).

The dark muscle of pelagic species has been reported to contain higher concentrations of sarcoplasmic proteins than the light muscle. The presence of sarcoplasmic proteins has often been cited as one of the reasons for the poorer gelation characteristics of fish dark muscle compared with light muscle. Sarcoplasmic proteins are able to bind to the myofibrillar proteins and thus interfere with the formation of gel (Hultin and Kelleher, 2000). Dark muscle also has a higher proteolytic activity than white muscle (Shimizu *et al.*, 1992). Therefore, dark muscle is more susceptible to gel weakening that occurs if the gel is held too long at a temperature around 50-60°C. Alverez *et al.* (1999) found that thermal gel degradation in sardine surimi gels occurs at 50°C and 60°C.

Surimi processing technology

Surimi is minced fish, which has been extracted with water to remove strong flavor, pigments, and nonfunctional proteins and subsequently dewatered to reduce the moisture content to approximately that of intact fish muscle (Suzuki, 1981). When fish flesh is separated from bones and skin (usually mechanically), it is called "minced fish" . According to Pigott (1986), there are several methods to prepare fish for deboning. One is to remove the head, gut and thoroughly clean the belly walls before deboning the carcass. The other is to fillet the fish and then debone the fillet, which also contains skin and bones. The most important step of surimi processing to ensure maximum gelling, as well as colorless and odorless surimi, is efficient washing (Park and Morrissey, 2000). Many of the problems with color, taste, and odor that develop in minced meat are minimized or eliminated with washing. The washing process involves mixing minced meat with cold water (5°C) and removing water by screening and dehydrators or centrifuging to about 5-10% solids (Park and Morrissey, 2000). When the raw surimi are frozen, they will lose their functional properties rapidly. Therefore the raw surimi is generally mixed with cryoprotectants such as sucrose and sorbitol, alone or mixed at ~ 8% w/w. A mixture (1:1) of sodium tripolyphosphate and tetrasodium pyrophosphate at 0.2-0.3% is commonly added in combination with cryoprotectants (Lee, 1984). Surimi

blocks are placed in a contact plate freezer until the core temperature reaches –25°C (Park and Morrissey, 2000). Surimi can be used to produce a variety of products. Consumption is usually focused on four major product forms, namely: chikuwa, kamaboko, satsuma-age and hampen (Kano, 1992).

Gelation

The process of protein gelation involves two steps (Ziegler and Aton, 1984).

1. **Protein denaturation** Addition of salt in combination of heating are two major factors involved in denaturation and gelation of muscle proteins. Table 1 gives a summary of changes, which may occur during the heat denaturation of actomyosin.

Table 1 Conformational changes which may occur during the thermal denaturation of natural actomyosin

Temperature °C	Protein(s) or segment involved	Description of events
30-35	Native tropomyosin	Thermally dissociated from the F-actin backbone
38	F-actin	Super helix dissociates in to single chains
40-45	Myosin	Dissociates into light and heavy chains
	Head	Possibly some conformational change
	Hinge	Helix to random coil transformation
45-50	Actin, myosin	Actin-myosin complex dissociates
50-55	Light meromyosin	Helix to coil transformation and rapid aggregation
>70	Actin	Major conformational changes in the G-actin monomer

Source: Ziegler and Aton (1984)

2. Aggregation Denatured proteins begin to interact noncovalently to form a fine elastic network when surimi sol is subjected to heating process. Samejima *et al.* (1981) proposed the heat-induced gelation of myosin. It consists of two reactions as follows:

1. aggregation of the globular head segments of the myosin molecule, which is closely associated with the oxidation of sulfhydryl groups

2. network formation resulting from the unfolding of the helical tail segment. The head portions also associate to form "super-junctions' which provide extra cross-linking to the gel network. Since agents that block formation of disulfide bonds did not retard its onset, Samejima *et al.* (1981) concluded that another type of aggregation, perhaps due to intermolecular association of side chains, superimposes on the sulfhydryl-dependent reaction. Niwa (1992) recently reviewed the importance of the various bonds in gel network formation. The factors determining the number and kind of interactions or bonds include not only the species from which the surimi is derived (Suzuki, 1981; Shimizu, 1985) but also the heat conditions in which the gel is made (Ishikawa, 1978; Akahane and Shimizu, 1990; Lee *et al.*, 1990; Yamazawa, 1990). Chan *et al.* (1992) reported that thermal aggregation of fish myosin was coincidental with an increase in the surface hydrophobicity of the unfolded domains of myosin molecules and was affected by the temperature at which these domains unraveled. Temperature and ionic strength have a profound effect on the hydrophobic interaction of proteins. Chan *et al.* (1994) compared the denaturation and aggregation behaviors of cod and herring myosins, and reported that the inferior gel forming ability of herring muscle proteins was related to the unfolding profile of interior hydrophobic domains when heated. Extent of aggregation for fish myosin seems to depend on the amount of hydrophobic surface exposed on the heated molecules (Chan *et al.*, 1992; wicker *et al.*, 1986). Therefore, it is reasonable to assume that the extent of aggregation for poor gel forming species, i.e. herring, would be improved by finding a strategy to increase the surface hydrophobicity of heated myosins. Gill *et al.* (1992) reported that myosins from different fish species aggregated to different extents as temperature increased. Thus, the decrease in viscosity was probably due to thermal induced aggregation of myosin sub-fragments. Heating

actomyosin solution at 40 and 85°C resulted in reduction of ATPase activity. Similar trend was observed by Sano *et al.* (1994) who found that reactive SH increase from 20 to 50°C, suggesting that SH groups inside the actomyosin molecule emerged to the surface as a result of unfolding, thereby causing a gradual decrease in ATPase activity with the increase in temperature. A rapid loss in enzyme activity was found from 40 to 50°C, indicating conformational changes in active sites in actomyosin. Calcium ions can form salt linkages between negatively charged sites on two adjacent proteins (Wan *et al.*, 1994). Though the addition of calcium ions may contribute to the strengthening of surimi gels, but this type of bond will not cause gelation of surimi in itself. However, the addition of calcium salts to improve gelling properties of surimi may actually be more due to their effects on a crosslinking enzyme transglutaminase (TGase) in the muscle than from ionic linkages between proteins. TGase is a transferase, having the systematic name protein-glutamine γ-glutamyltransferase (EC 2.3.2.13). It catalyzes the acyl transfer reaction between γ-carboxyamide groups of glutamine residues in proteins, peptides, and various primary amines. When the ε-amino groups of lysine acts as acyl acceptor, it results in polymerization and inter- or intra-molecular crosslinking of proteins via formation of ε (γ-glutamyl) lysine linkages (Figure 1) (Ashie and Lanier, 2000). Transglutaminase (TGase) is an enzyme that catalyzes the polymerization and cross-linking of proteins through the formation of covalent bonds between protein molecules. This link enhances the physical strength (hardness and cohesiveness) of surimi gels.

Seki *et al.* (1990) isolated TGase from Alaska pollock and found that it could induce the gelation of minced fish. Tsukamasa and Shimizu (1990) further reported that the strong gel forming ability of sardine was due to the formation of the non-disulfide bond which later was shown to be due to the action of TGase (Tsukamasa *et al.*, 1993).

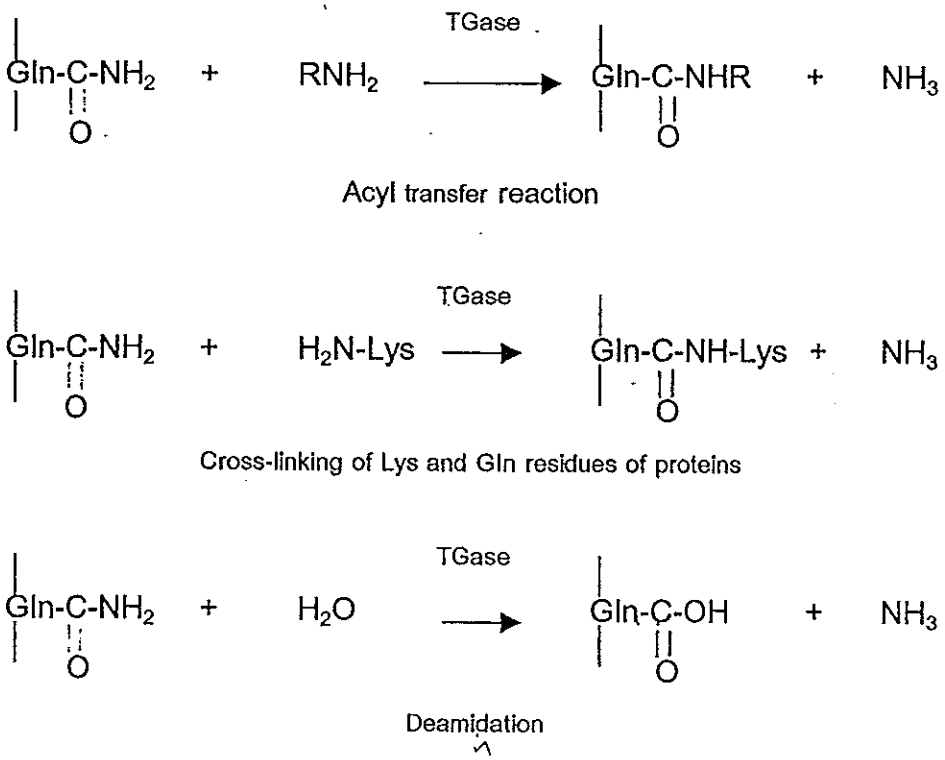


Figure 1 Reactions catalyzed by TGases

Source: Ashie and Lanier (2000)

As the temperature is increased over 45-50°C, gel network (suwari) is partially disrupted to form a broken network (modori). This process is species dependent (Shimizu *et al.*, 1981). The action of proteases has been found to promote this gel weakening (Lanier *et al.*, 1981; Lee, 1984). However, in systems without enzymes or containing enzyme inhibitors, softening was still apparent (Iwata *et al.*, 1974; Lee, 1984). Once the temperature is increased above 65-70°C, the gel becomes ordered and non-transparent. This stage is referred to as kamaboko (Suzuki, 1981). In this final stage, the cohesiveness and elasticity of the gel is enhanced.

Biological (Intrinsic) factors affecting surimi quality

Effects of Species

Quality of commercial surimi depends on the species used, however, the functional and compositional properties of the surimi vary. Therefore, it is important for processors to understand the relationships between the physicochemical functions of fish and the functional and compositional properties of surimi (Park and Morrissey, 2000).

The different gelling characteristics among various species are also dependent on the different endogenous enzymes present in the fish muscle. An *et al.* (1994) identified the enzymes in Pacific whiting as cathepsins B, H, and L. They behave differently with different environmental conditions, such as pH, temperature, and ionic strength. Cathepsin B and H are easily washed off during surimi processing, whereas cathepsin L remains in the muscle tissue (An *et al.*, 1994). Cathepsin L has an optimum temperature of 55°C and causes textural deterioration when the surimi paste is slowly heated. Therefore, enzyme inhibitors are required unless the surimi is cooked rapidly using an ohmic heater. (Yongsawatdigul *et al.*, 1995) Arrowtooth flounder (*Atheresthes stomias*) is another fish that requires enzyme inhibitors to minimize textural deterioration caused by a heat-stable enzyme (Greene *et al.*, 1990). Gel weakening at around 55-66°C has also been reported in threadfin bream (*Nemipterus bathybius*) (Toyohara *et al.*, 1988), Atlantic menhaden (*Brevoorti tyrannus*) (Boye and Lanier, 1988), white croaker (*Micropogon opercularis*), and oval filefish (*Navodon modestus*) (Toyohara *et al.*, 1990).

Araki and Seki (1993) showed that TGase activity varies between different fish species. White croaker (*Argyrosomus argentatus*) has the highest TGase activity followed in descending order by carp (*Cyprinus carpio*), walleye pollock (*Theragra chalcogramma*), chum salmon (*Oncorhynchus keta*), atka mackerel (*Pleurogrammus azonus*), and rainbow trout (*Oncorhynchus mykiss*).

Effects of Season

Generally, fish harvested during the feeding period produce the highest quality surimi. During this period, fish muscle has the lowest moisture content and low pH, as well as the highest total protein (Bandarra *et al.*, 1997). Fish harvested during and after

the spawning season produce the lowest quality surimi. It is established that spawning fish have a relatively higher pH and tend to retain more water. Consequently, it is difficult to remove the extra water from the washed meat. To easily remove the extra water, muscle tissue characteristics must be altered by either lowering the pH or increasing the salinity of the final wash water (Lee, 1984).

Effects of Freshness or Rigor Mortis

Freshness of fish is primarily time/temperature-dependent. The biochemical and biophysical changes during the development of rigor mortis induce significant changes in the functional properties of muscle proteins (Pigott, 1986). Fish should be processed as soon as possible after going through rigor mortis. Before passing through this stage, about 5 hr in the case of pollock surimi, it is difficult to remove the "fishy" odor, various membranes, and other contaminants that affect product quality (Pigott, 1986). However, Park *et al.* (1990) reported that significantly higher protein content and yield, reduced cooking loss, and enhanced gel-forming ability were associated with surimi processed from manually filleted prerigor tilapia fish. High quality surimi cannot be manufactured from insufficiently fresh fish with even the best available technology. The dependence of the gel-forming ability of surimi on the freshness of Alaska Pollock is shown in Table 2 (Toyoda *et al*, 1992).

Processing (extrinsic) factors affecting surimi quality

Harvesting

Surimi quality is affected by the harvesting conditions and methods used for capture, as well as the on-board handling methods and vessel storage conditions. Intrinsic quality factors (e.g. protein content, postspawning condition) are important considerations even before the nets are put in the water. The geographic location of the fishing grounds may also affect quality and determine factors such as the size of the fish or the amount of time required to deliver the fish to the processing plant (Park and Morrisey, 2000).

Several factors in the actual capture of fish can also affect final product quality, including weather conditions at-sea, capture methods, size of tow, length of tow, salt uptake, and temperature of the fish after capture. Most of these factors are interactive, and, at times, it is difficult to weigh the importance of each factor separately (Lee, 1984).

Table 2 Dependency of surimi gel strength on the freshness of raw fish (Alaska pollock)

	Fish condition (days of storage at 5°C)			
	Extremely fresh (0 day)	Very fresh (2 days)	Fairly fresh (4 days)	Not fresh (6 days)
	Gel strength (g cm)			
Unleached surimi	1100	600	350	150
Leached surimi	1200	850	650	400

Source: Adapted from Toyoda *et al.* (1992)

Water

The important quality factors associated with water are temperature, hardness or mineral content, pH, and salinity. The level of chlorination in the water should also be considered because of its bleaching and deodorizing effect (Lee, 1986). The surimi could be adjusted to obtain a standard moisture content such as 78% as suggested by Lanier *et al.* (1991). The water must be refrigerated to a low temperature, thus muscle proteins can retain their maximum functional properties. Temperature of water can vary based on the thermostability of fish proteins (Arai *et al.*, 1973).

Reppond and Babbitt (1997) reported that torsion stress and strain of surimi from pollock, herring, arrowtooth flounder and Pacific whiting torsion decreased linearly with increased moisture content but strain values were less sensitive to moisture than were stress values. According to Alverez *et al.* (1995), maximum gel strength was obtained at

lowest moisture when compared between 76 and 78% moisture content. Water addition affected lightness (L*) and yellowness (b*). Gels with higher moisture contents looked whiter (Park, 1995).

Time/Temperature of Processing

Fish are often kept in holding tanks with iced water (0°C) for up to another 6-14 hr. Thus, fish are usually 6-24 hr postharvest before they are subjected to surimi processing. During this holding period, the temperature can rise if fish are not handled properly. Prolonged holding time and elevated temperatures can cause severe proteolysis of myofibrillar proteins. Consequently, proteolysis before and during processing causes more myofibrillar proteins to be dissolved as water-soluble waste (Xiong *et al.*, 1989). The dependence of the gel forming ability of surimi on storage time of Tilapia (*Areochromis aureus*) is shown in Table 3. Pre-rigor stage produced the highest values for shear stress and true shear strain (Park *et al.*, 1990).

Table 3 Dependency of surimi gel-forming ability on storage time of raw fish (Tilapia)

Torsion	Pre-rigor (1 hr)	In-rigor (24 hr)	Post-rigor (72 hr)
Stress (KPa)	98.16	81.51	70.89
True strain	2.47	2.38	2.21

Source: Adapted from Park *et al.* (1990)

Lin and Park (1996) evaluated the effects of various postharvest storage temperatures and times on the proteolysis of Pacific whiting and its relationship to changes in protein solubility. Degradation of myosin heavy chain (MHC) increased rapidly during the postharvest storage period. This degradation occurred even when the temperature was maintained at 0°C. Greater degradation of MHC was observed with

prolonged storage. More than 70% MHC was degraded when fish were stored at 0°C for 72 hr (Lin and Park, 1996). Storage temperatures also affected degradation of MHC. When temperatures increased further, degradation occurred more rapidly. Both time and temperature were critical to MHC degradation. Consequently, to minimize proteolysis, fish should be processed promptly on landing or kept at 0°C if holding is necessary.

Washing cycle and wash water ratio

The proper degree of washing is required to produce good quality surimi. The color of surimi can be improved by increasing the washing cycles (Kim *et al.*, 1996), washing time and water quantity (Tejada *et al.*, 1981). This washing process is often repeated three to four times to ensure sufficient removal of sarcoplasmic proteins. The number of washing cycles and the volume of water will vary with the fish species (Lee, 1984). A recent report indicated that most sarcoplasmic proteins are fairly soluble and removed during the initial washing steps. Subsequent washing removes the residual sarcoplasmic proteins along with a small amount of myofibrillar proteins (Lin and Park, 1996). The loss of myofibrillar proteins during surimi processing could be due in part to the nature of their water solubility. Wu *et al.* (1991) reported that myofibrillar proteins were solubilized in water and low ionic strength solutions. In addition, extensive washing of muscle might cause decreased salt concentration in the tissue and allow myofibrillar proteins to become highly associated with water. Matsumoto (1959) reported that when protein extracted by salt solution was washed with water, most of the protein was solubilized. Also, increased washing time (Lee, 1986) or washing cycles using the same amount of water (Pacheco-Aguilar *et al*, 1989) resulted in more proteins, possibly myofibrillar proteins, being solubilized.

Lin and Park (1996) evaluated the effects of salt concentrations and washing cycles on the extraction of proteins. Sarcoplasmic proteins were readily soluble in water and easily removed in the initial washing steps. Washing with 0.25, 0.5, 1.0 % NaCl solutions also reduced the loss of myofibrillar proteins.

Lin and Park (1997) investigated minimizing water use for leaching by reducing the water/meat ratio and increasing the wash cycles and wash time. Increased wash time did not enhance the removal of sarcoplasmic proteins once equilibrium was reached. However, increased wash cycles continuously removed residual sarcoplasmic proteins from the mince. At the low water/meat ratio (2:1 or 1:1), regardless of wash cycles and wash time, no significant loss of myofibrillar proteins occurred. Myosin heavy chain content, water retention, and whiteness of the washed mince decreased when the water/meat ratio was reduced. Increasing wash cycles and/or washed time, however, enhanced these properties but also resulted in a higher moisture content (Park and Morrissey, 2000). For dark muscle fish, alkaline washing has been approached to improve gel-forming ability of surimi. Chen (1997) reported that the gel forming properties of horse mackerel (*Trachurus japonicus*) mince washed with alkaline solution increased mainly due to an increase in pH, rather than a decrease in oil content.

Improvement of gel quality of surimi

Gelation of fish proteins is the most important step in forming desired textures in many seafood products, particularly those from surimi. Various physical conditions and chemical additives can affect surimi gelling property. Enzyme inhibitors, such as beef plasma protein, egg whites, whey protein concentrates, or potato extracts, have been used in conjunction with cryoprotectants, gel enhancers, and color enhancers. It is common that the enzyme inhibitor is formulated with sucrose, sorbitol, sodium tripolyphosphate, tetrasodium pyrohosphate, calcium compounds (calcium lactate, calcium sulfate, calcium citrate, calcium caseinate), sodium bicarbonate, monoglyceride or diglyceride, and partially hydrogenated canola oil (Park and Morrissey, 2000).

The addition of enzyme inhibitors or calcium compounds before freezing surimi is not necessary, especially because added calcium compounds can actually enhance protein denaturation during frozen storage. Instead, these compounds can be added when the surimi paste is prepared to make gels (Lee and Park, 1998).

Use of NaCl

Gels could be prepared using fish muscle without NaCl, which also suggests that myofibrillar proteins from fish muscle could be soluble in water or very low ionic strength solutions (Hennigar *et al.*, 1988). The addition of salt shifted the denaturation transitions to lower temperatures and decreased the enthalpies of heat denaturation. These results suggest that the addition of salt might cause a partial unfolding of proteins and increase sensitivity to denaturation (Park and Lanier, 1989). Gill *et al.* (1992) concluded that thermal aggregation of herring myosin showed little dependence on salt concentration (0.6-1.4 M NaCl, pH 6.5) but salt enhanced aggregation of cod myosin at heating temperatures higher than 50°C.

Alvarez *et al.* (1995) reported the effects of salt concentration of sardine surimi gels. Maximum gel strength was obtained at salt concentrations of 2.24% NaCl. The formation of a firm and elastic kamaboko gel from sardine surimi requires the addition of 2-4% NaCl (Roussel and Cheftel, 1990). Chen (1995) found that the breaking force of surimi increased with increasing NaCl concentration, but deformation increased at lower NaCl concentration and then decreased when NaCl concentration reached 1.6 M.

Use of calcium ions

Sufficient calcium ions must be present for the endogenous TGase to be active and induce setting (Lee *et al*, 1998). Calcium compounds are commonly added as a gel enhance. Yamamoto *et al.* (1991) patented the use of a mixture of sodium bicarbonate, calcium citrate, and calcium lactate as gel quality improving agents. Nowadays, calcium compounds are often used in surimi gels as a gel enhancer. Yasuyuki *et al.* (1993) reported that ε (γ-glutamyl) lysine crosslink content and breaking strength of gels from sardine surimi increased when $CaCl_2$ was added in combination with setting at 25°C.

Ashie and Lanier (2000) showed differences in TGase sensitivity to Ca^{2+} ions. The concentration of Ca^{2+} required to express maximum TGase activity in the walleye pollock was 3 mM, while TGase from red sea bream liver and carp muscle required 0.5 mM and 5 mM Ca^{2+} for full activity, respectively. The optimal Ca^{2+} concentrations for *Limulus*

hemocyte, guinea pig liver, and Japanese oyster were found to be 8, 10 and greater than 25 mM, respectively. Walleye pollack surimi pastes having different Ca^{2+} concentrations, incubated at 25°C prior to heating at 90°C had different cross-linked myosin heavy chains and varied incorporation of MDC into surimi paste. The maximal cross-linking and MDC incorporation were obtained in the presence of Ca^{2+} in the range of 2-5 mM (Wan *et al.*, 1994).

Lee and Park (1998) concluded that the textural properties of surimi could be improved maximally with a 25°C preincubation and the addition of 0.1% calcium lactate or 0.05% calcium acetate for Alaska pollock and 0.2% calcium lactate for Pacific whiting.

Use of microbial transglutaminase (MTGase)

The commercial development of a low cost source of TGase from microbial culture offers a means of upgrading the gelling quality of surimi (Ando *et al*, 1989). This microbial TGase is not calcium sensitive; therefore, neither chelating agents nor calcium salts have any marked effect on its activity (Lanier, 2000). MTGase from the genus *Streptoverticillium* has molecular weight of 40 kDa (Ando *et al.*, 1989). The Ca^{2+}-independent MTGase from *Streptoverticillium mobarense* (Nonaka *et al.*, 1989; Huang *et al.*, 1992; Gerber *et al.*, 1994) or from *Streptoverticillium ladakanum* (Tsai *et al*, 1996) has shown potential to increase the gel strength of fish surimi.

The bacterial transglutaminase caused cross-linking of a wider range of proteins and thus exhibited lower substrate specificity than the blood transglutaminases (Govardus *et al.*, 2001). Fungal TGase induced stronger gels (higher stress) in pollock surimi when 5 mM calcium was added, whereas calcium had little effect on the performance of microbial TGase. Effective concentrations of microbial TGase on the formation of cross-links was observed at 1.5-3.0 unit/g protein for high grade pollock surimi and 3.0-5.0 unit/g protein for low grade pollock surimi (Soeda *et al.*, 1996). Crosslinking of myosin heavy chains via ε (γ-glutamyl) lysine formation increased when MTGase was added (Seguro *et al.*, 1995). Effect of MTGase was investigated by

evaluating breaking strength and deformation of gels from MTGase treated surimi with and without setting at 30°C. Quantitative analysis of ε (γ-glutamyl) lysine crosslink was also carried out to monitor the MTGase reaction. In set gels, breaking strength and ε (γ-glutamyl) lysine crosslink increased, and myosin heavy chain decreased correspondingly with MTGase concentration. Surimi gel could be generally improved through the formation of crosslinks by the addition of MTGase in surimi (Sakamoto *et al.*, 1995).

Jiang *et al.* (2000a) investigated the properties of surimi gels from threadfin bream and pollack surimi set at 30°C or 45°C with MTGase from *Streptoverticillium ladakanum*. The optimal amounts of MTGase and setting conditions were 0.3 unit/g surimi either at 30°C for 90 min or at 45°C for 20 min for threadfin bream. For pollack surimi, MTGase at a level of 0.2 unit/g surimi with setting at 30°C for 60 min were found to be optimum condition. MTGase catalyzed MHC cross-linking of both pollack and threadfin bream surimi and increased the gel forming ability of surimi.

Ramirez *et al.* (2000) reported that optimal condition for the addition of MTGase to improve gel quality from striped mullet (*Mugil cephalus*) involved: a concentration of MTGase of 9.3 g/kg of surimi, and setting at 37°C for 3.9 hrs. Under these conditions, maximum shear strain was observed. However, the addition of MTGase at 5 g/kg of surimi in combination with setting at 34.5°C for 1 hr rendered the maximum shear stress. Concentration of microbial TGase, temperature and time were optimized to improve the mechanical properties of surimi from silver carp. Optimal predicted properties were obtained by employing the following setting conditions: a concentration of microbial TGase of 8.8 g/kg of surimi, at 39.6°C for 1 hr. Under these conditions, a surimi from silver carp with shear stress of 146 kPa and shear strain of 1.59 was obtained. Shear stress was strongly affected by temperature and time, while shear strain was moderately affected (Ramirez *et al.*, 2000).

Jiang *et al.* (1998) investigated the effect of combining MTGase from *Streptoverticillium ladakanum* with ultraviolet (UV) irradiation on the gelation of minced mackerel. The gel strength of minced mackerel with MTGase alone at a concentration of

0.47 unit/g reached 1789 g.cm, which was greater than that control. When MTGase-supplemented minced mackerel was exposed to UV light for the optimal irradiation time of 20 min, the gel strength could be further increased by 25%. MTGase causes the cross-linking of MHC of mackerel actomyosin and UV irradiation enhances this polymerization of MHC by MTGase. Accordingly, the gel strength of minced mackerel increased significantly when MTGase and UV irradiation were used in combination.

Jiang *et al.* (2000b) reported that a combination of MTGase, reducing agent and protease inhibitor was employed to improve the quality of underutilized fish surimi. MTGase could catalyze the MHC cross-linking and increase the gel forming ability of hairtail surimi. The texture degradation caused by the endogenous proteases could be inhibited by the addition of inhibitor. The best solution to improve gel-forming ability of frozen hairtail surimi was the combination of 0.35 units MTGase/g, 0.1% sodium bisulfite and 0.01mM E-64.

Use of proteinase inhibitors

Endogenous cysteine proteinases in fish muscle, such as lysosomal cathepsins and cytoplasmic calpains are still active during postmortem storage or thermal processing in surimi-based products (An *et al.*, 1994). The most active proteinases in fish muscle that can soften the surimi gels vary with species, but are generally categorized into two major groups: cathepsin and heat stable alkaline proteinases (Jiang, 2000). Cathepsins B and L could cause the thermal degradation of mackerel surimi gels (Lee *et al.*, 2000).

In surimi production, proteolysis of myosin will lead to loss of gel strength unless controlled by additives such as dried bovine plasma protein (BPP), dried egg white, whey protein concentrate, or potato flour (Akazawa *et al.*, 1993; Morrissey *et al.*, 1993). The main mechanism of BPP in controlling modori is believed to be protease inhibition by components of the protein additives. (Seymour *et al.*, 1997). α_2-macloglobulin is a major inhibitor contributing to inhibition towards most of proteinase. Inhibitory effect of BPP is possibly due to

α_2-macloglobulin (Hamann *et al.*, 1990)

Specific protease inhibitors in egg white are shown in Table 4 (Garcia-Carreno and Hernandez-Cortes, 2000).

Table 4 Protease inhibitors in Egg white

Inhibitor	Specificity
Cystatin	Cysteine protease: several cathepsins, ficin
Ovoinhibitor	Serine proteases: elastase and chymotrypsin
Ovomacroglobulin	Aspartic proteases: pepsin and renin
Ovomucoid	Trypsin

Source: Garcia-Carreno and Hernandez-Cortes (2000).

Morrissey *et al.* (1993) reported that beef plasma protein showed the highest inhibitory activity in whiting surimi as compared to egg white and potato extract. Similar results have been found in arrowtooth flounder (Reppond and Babbit, 1993). Specific protease inhibitors in plasma protein are shown in
Table 5 (White *et al.*, 1973). Weerasinghe *et al.* (1995) found that BPP showed higher percentage of papain (a cysteine proteinase) inhibitors followed by whey protein concentrate, potato powder, and egg white. Egg white was found to have higher concentration of trypsin (i.e., serine proteinase) inhibitors followed by BPP, potato powder, and whey protein concentrate. BPP is very effective in decreasing autolytic activity in Pacific whiting and arrowtooth flounder surimi.

Table 5 Protease inhibitors in beef plasma proteins

Inhibitors	Specificity
α_2-macloglobulin	Cysteine, Serine, Aspartic and metallo proteases
Kininogens	Cysteine proteases
Bovine serum albumin	Nonspecific competitive inhibitor

Source: Adapted from White *et al.* (1973)

Kininogen, a cysteine proteinase inhibitor from pig plasma (kininogen) with a molecular weight (MW) of 55 kDa, purified to electrophoretical homogeneity, inhibited calpains, cathepsins B, L and L-like and papain but did not inhibit trypsin, chymotrypsin and cathepsin D (Lee *et al.*, 2000) .

Purified L-kininogen was found to prevent actomyosin degradation by cathepsins (Lee *et al.*, 2000). Pig plasma containing L-kininogen, could significantly prevent the MHC degradation and improve the gel properties of mackerel surimi (Lee *et al.*, 2000). The texture of mackerel gel increased to 2 fold and 1.7 fold in breaking force and deformation, respectively with the addition of 1% pig plasma protein. Benjakul *et al.* (2000) found that porcine plasma protein shows the highest inhibitory activity in Pacific whiting surimi as compared to egg white and beef plasma protein. Proteinase inhibitor from pig plasma was found to have a molecular weight (MW) of 60-63 kDa by inhibitory activity staining with both papain and trypsin.

Seymour *et al.* (1997) reported that gel strength enhancement of Pacific whiting surimi could be achieved by using α_2-macroglobulin enriched plasma fraction (FIV-1) and transglutaminase-enriched plasma fraction (FI-S). Enhancement of Pacific whiting surimi by BPP is the result of a combination of factors, including inhibition of proteolytic degradation by plasma proteins, and protein cross-linking by TG. Maximum hardness and elasticity of the heat induced gels of bluefish meat pastes were higher for the α_2-

macroglobulin containing samples than the control samples (Sareevoravikul *et al.*, 1996).

When using a recombinant-derived cystatin (cysteine proteinase inhibitor) in Pacific whiting surimi, its inhibitory activity (using azocasein as substrate) was 10 times greater than that of the 1% BPP typically used in the industry (Kang and Lanier, 1999)

Whitening agents

Some whiteners such as titanium dioxide (TiO_2), vegetable oil and calcium carbonate ($CaCO_3$) are used to improve the whiteness of surimi seafood. The levels used, however, vary depending on product specification, but common levels are 0.5-1.5% for calcium carbonate, 0.02-0.06% for TiO_2, and 2-6% for vegetable oil (Park, 2000). TiO_2 is an approved colorant with the restriction of 1 % (w/w) in the finished product. When TiO_2 is used, careful measurement of pH is needed. At higher levels, TiO_2>0.2%, the pH of the meat approaches 6.7 or below. At these conditions, fish proteins do not form an elastic gel; therefore, during crab stick production, the sheet of surimi paste keeps breaking. The optimal level found was 1 g kg^{-1} of TiO_2 in fish mince, which renders the most acceptable level of whiteness (Meacock *et al.*, 1997).

Vegetable oil is often used as a colorant. Vegetable oil improves the perceived whiteness of surimi gels, possibly through the light scattering effect of the paste or oil emulsion. As the percentage of oil increased, the lightness and yellow hue linearly increased (Lauro, 2000).

Calcium carbonate is also a synthetically prepared powder, composed primarily of precipitated $CaCO_3$. This pigment has considerably less whitening strength compared with TiO_2. Carbon dioxide gas could be released in products with $CaCO_3$. This makes the product appear to be suffering from microbial deterioration (Lauro, 2000). Thus the use of calcium carbonate as a whitening agent is not recommended. Calcium carbonate produces carbon dioxide in the presence of acid and phosphate. As a result, air bubbles often appear on the product.

Objectives

1. To study the effect of some additives and heating conditions on gel strengthening of surimi from bigeye snapper.
2. To study the effect of some whitening agents on whiteness and gel forming ability of mixture between bigeye snapper surimi and mackerel surimi

Chapter 2

Materials and Methods

1. Samples and preparation

Frozen surimi (SSA, SA, A, B grade) produced from bigeye snapper (*Priacanthus tayenus*) and frozen surimi produced from mackerel (*Trachurus japonicus*) were purchased from P.F.P. Trading Co, Ltd., Maung, Songkhla. The surimi samples were cut into blocks (0.5 kg), placed in polyethylene bags and kept at –20°C until used.

Bigeye snapper (*Priacanthus tayenus*) were purchased from the dock in Songkhla. The fish, off-loaded approximately 36-48 h after capture, were transported to Department of Food Technology, Prince of Songkla University in ice with a fish/ice ratio of 1:2 (w/w) within 1 h. Fish were immediately washed and stored in ice with a fish/ice ratio of 1:2 (w/w) for 10 days. Fish/ice ratio was kept constant throughout the storage by changing the ice every 2 days.

2. Chemicals

Various calcium compounds including Ca-chloride, Ca-lactate, Ca-gluconate were purchased from Fluka (Buchs, Switzerland). Microbial TGase was obtained from Ajinomoto Co., Ltd. (Kawasaki , Japan). Sodium dodecyl sulfate (SDS), β-mercaptoethanol (βME), brilliant blue R, glycerol and high molecular weight marker were purchased from Sigma (St. Louis. Mo., U.S.A.). Coomassie blue R-250, penta-sodium triphosphate, sodium hexametaphosphate, tris (hydroxymethyl) aminomethane, Folin-Ciocalteu's phenol reagent, trichloroacetic acid (TCA) were obtained from Merck (Darmstadt, Germany).

3. Instruments

Instruments	Model	Company
Electrophoresis apparatus	Mini-Protein II	Bio-Rad, USA
Double-beam spectrophotometer	UV-16001	SHIMADZU, Australia
pH meter	Denver 15	Scientific, USA
Refrigerated centrifuge	RC-5B plus	Sorvall, USA
Water bath	W350	Memmert, Germany
Magnetic stirrer	RO 10 power	KIKAL labortechnik,Germany
Homogenizer	T25	Ultra turrax, Malaysia
Texture analyzer	TA-XT2	Stable Micro Systems, England
Basket centrifuge	CE 21 K	Grandimpianti, Italy
Scanning Electron Microscope	JSM5800LV	JEOL, Japan

4 Determination of chemical compositions and property of surimi

4.1 Chemical compositions of surimi

Chemical compositions of surimi were determined as followed:

- Protein (AOAC, 1999)
- Moisture (AOAC, 1999)
- Ash (AOAC, 1999)
- Fat (AOAC, 1999)
- pH (Lee *et al.*, 1975)
- Total Volatile Base (TVB) and trimethylamine (TMA) (Conway and Byrne, 1936)

4.2 Nitrogenous constituents in surimi

Fractionation of surimi was carried out according to the method of Hashimoto *et al.* (1979), as shown in Figure 1. Each fraction containing different composition, e.g. non-protein nitrogen, sarcoplasmic protein, myofibrillar protein, alkali-soluble protein and stroma was subjected to nitrogen determination using Kjeldahl method (AOAC, 1999).

4.3 Thermal denaturation

Thermal denaturation of muscle proteins was studied using Differential Scanning Colorimetry (DSC). The scanning range was 25-90°C and a heating rate of 10°C/min was used. T_{max} was measured and the enthalpy of denaturation (ΔH) was calculated from thermogram.

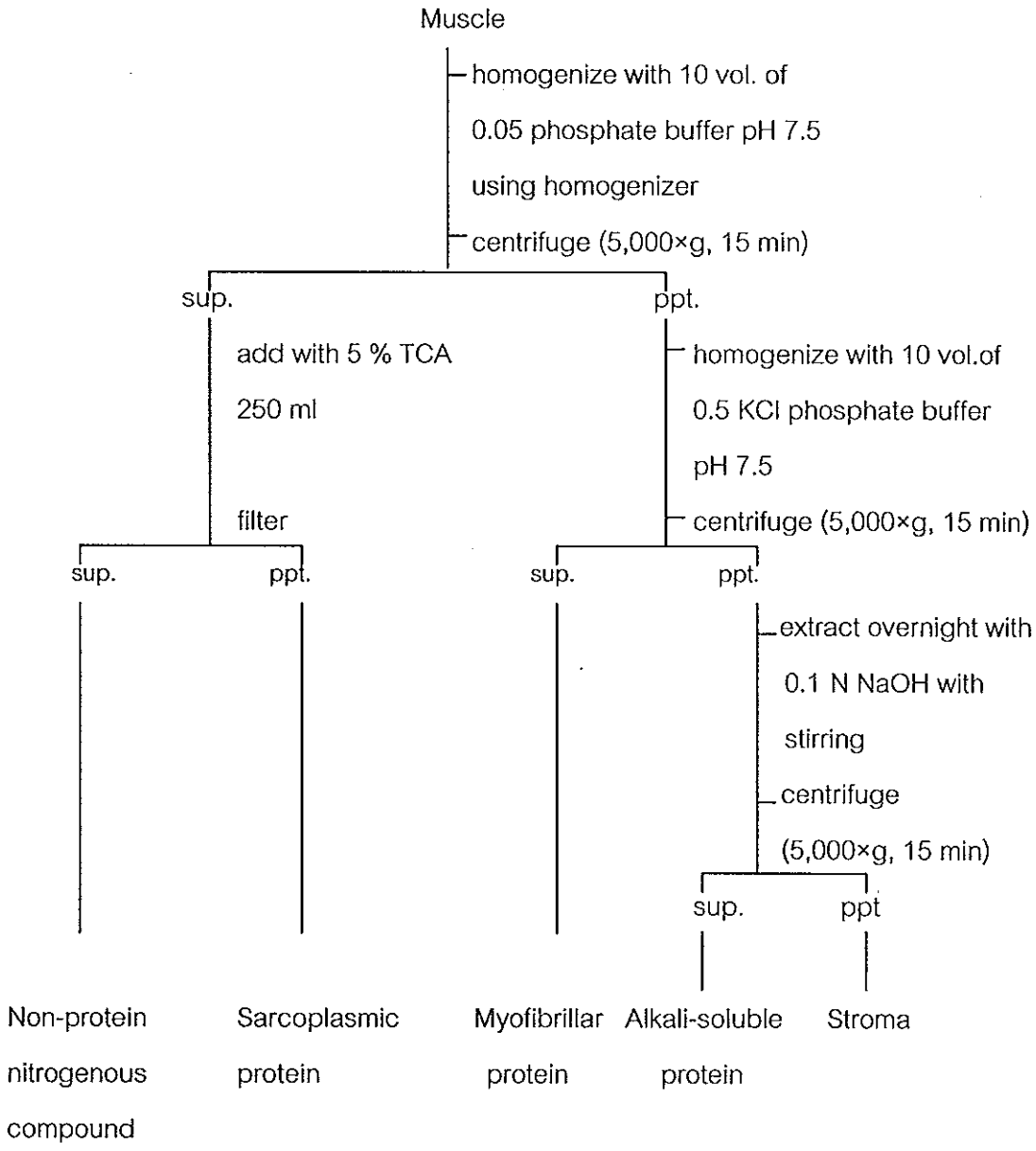


Figure 2 Fractionation procedure of muscle proteins

Source: Hashimoto *et al.* (1979)

5. Production of surimi from bigeye snapper

Fish stored in ice for 0 and 10 days were used for surimi preparation as described in the following scheme (Figure 3)

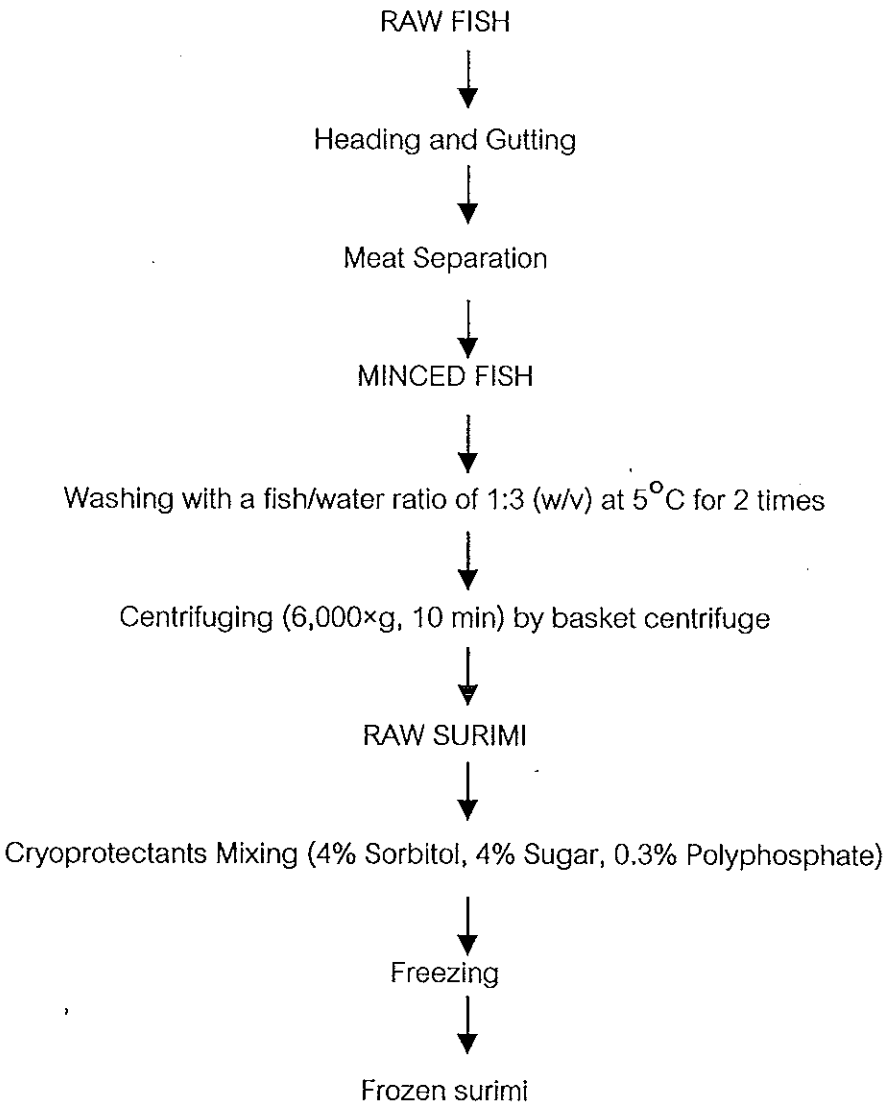


Figure 3 Scheme for surimi production

6. Determination of surimi gel quality

Frozen surimi produced from flesh fish and fish stored in ice for 10 day was partially thawed at 4°C overnight (8-10 h). Surimi was then cut into small pieces and mixed with 2.5 % salt in a mixer. The moisture content was adjusted to 80% with iced water. During chopping, the temperature was maintained below 10°C. The sol was stuffed into casing with a diameter of 3 cm. Surimi sol was subjected to setting/heating under different conditions as follows:

- Direct heating 90°C for 20 min.
- Setting at 40°C for 30 min (Suwari gel).
- Setting at 60°C for 30 min, followed by heating at 90°C for 20 min (Modori gel).
- Setting at 40°C for 30 min, followed by heating at 90°C for 20 min (Ashi gel).

After setting/heating, the gel was cooled down immediately using iced water. Surimi gel properties were then determined as follows:

6.1 Force and deformation were measured using Texture analyzer (Benjakul *et al.*, 2001).

6.2 Colors were measured by Hunter lab. Values were expressed as L*, a*, b* color system. Whiteness was calculated by the following formula:

$$\text{Whiteness} = 100-\{(100-L^*)^2+a^{*2}+b^{*2}\}^{1/2}$$

6.3 Expressible moisture was analyzed according to the method of Wierbicki and Deatherage (1958).

6.4 TCA-soluble peptides was determined according to the method of Morrissey *et al.* (1993).

6.5 Solubility of surimi gel was determined in solvent mixtures (20 mM Tris-HCl containing 1 % sodium dodecyl sulfate, 8 M urea and 2% β-mercaptoethanol, pH 8) according to the procedure of Roussel and Cheftel (1990).

7. Improvement of gel quality of different grade surimi from bigeye snapper

7.1 The effect of some additives on gel quality improvement

A and B grade surimi from bigeye snapper was partially thawed at 4°C overnight (8-10 h). Surimi was then cut into small pieces and mixed with 2.5 % salt in a mixer. The moisture content was adjusted to 80% with iced water. During chopping, various chemicals or enzymes at different concentrations were added and mixed thoroughly. Those chemicals used are shown as follows:

7.1.1 Calcium ion

7.1.1.1 Type of calcium:

calcium gluconate, calcium choride and calcium lactate

7.1.1.2 Concentrations (0, 10, 20, 50, 100 mmole/kg)

7.1.2 Protease inhibitors

7.1.2.1 Type of protease inhibitors:

Pig plasma protein, Beef plasma protein and Egg white

7.1.2.2 Concentrations (0, 0.5, 1, 2, 3 and 4 %w/w)

7.1.3 Microbial transglutaminase (0, 0.01, 0.02, 0.05, 0.1 and 0.2 %w/w)

The sol was stuffed into casing with a diameter of 3 cm. After setting at 40°C for 30 min, followed by heating at 90°C for 20 min, the gels were cooled down immediately using iced water. Then gels were subjected to determination as mentioned before:

7.2 Combination effect of additives on gel quality

Selected chemicals (calcium ions, protease inhibitors, MTGase) at optimum concentrations which showed the highest breaking force were added into surimi sol. The sol was then subjected to setting at 40°C for different times (0, 15, 30, 60 and 90 min), followed by heating at 90°C for 20 min. After cooling, surimi gel samples were tested for force and deformation, color and protein pattern using SDS-PAGE as described above.

8. Improvement of whiteness of mixture between white and dark flesh surimi

8.1 Preparation of mixed surimi

Surimi from white-flesh fish (bigeye snapper Grade SA, SSA) and dark-flesh fish (mackerel) were mixed at different ratio of white flesh: dark flesh surimi (100:0, 90:10, 80:20, 70:30, 60:40, 50:50, 0:100). Surimi gels were prepared by setting at 40°C for 30 min, followed by heating at 90°C for 20 min. Cooled gel samples were then analyzed for force, deformation and whiteness. Ratio of white flesh surimi to dark flesh surimi, which rendered the whiteness value more than 70, but still had the breaking force comparable to that of white flesh surimi for each grade, was chosen for whiteness improvement.

8.2 Effect of whitening agents on whiteness and gel forming ability

8.2.1 Effect of whitening agents on whiteness

Various whitening agents at different concentrations were added into the sol of mixed surimi with a proper ratio as follows:

- Calcium carbonate (0.5, 1.0, 1.5, 2.0 % w/w)
- Titanium dioxide (0.01, 0.02, 0.05, 0.1 % w/w)
- Vegetable oil (1, 2, 3, 5 % w/w)

The sol was subjected to setting at 40°C for 30 min, followed by heating at 90°C for 20 min. The gel was cooled suddenly in iced water. Gels were tested for force and deformation and whiteness.

8.2.2 Effect of whitening agents on protein composition, properties and micro structure

Individual whitening agent at the optimum concentration, which rendered the highest whiteness, was added into surimi sol and mixed thoroughly. The sol obtained was then determined for thermal denaturation using DSC. The scanning range was 25-90°C with a heating rate of 10°C/min. T_{max} and enthalpy of denaturation (ΔH) was determined. In addition, sols were then set at 40°C for 30 min, prior to heating at 90°C for 20 min. Gels obtained were subjected to following analysis:

- Solubility was determined in solvent mixtures (20 mM tris-HCl containing 1% sodium dodecylsulfate, 8 M urea and 2% β-mercaptoethanol, pH 8) according to the procedure of Roussel and Cheftel (1990).
- Microstructure was determined using scanning electron microscopy. Samples were fixed with 2% glutaraldehyde for surimi added with calcium carbonate and titanium dioxide, and with 2% osmium tetroxide for surimi added with vegetable oil in a phosphate buffer (pH 7.2) (Hayat, 1981). Fixed samples were then dehydrated in solutions of acetone of increasing strength and critical point dried (Balzers mod. CPD 030, Balzers Process Systems, Liechtenstein) using CO_2 as transition fluid. The prepared samples were mounted on copper specimen holders, sputter-coated with gold (Balzers mod. SCD 004) and examined on a JSM 5200 (JEOL, Ltd., Akishima, Japan).

9. Statistical analysis

Data were subjected to analysis of variance (ANOVA) and the mean comparison were performed using Duncan's multiple range test (Steel and Torrie, 1980).

Chapter 3

Results and discussion

1. Chemical compositions and property of surimi

Chemical compositions of surimi produced from fresh and 10 days stored bigeye snapper were compared with those commercially surimi produced from bigeye snapper and mackerel (Table 6). As a major constituent in surimi, water accounted for approximately 74-77%, followed by protein (16.02-18.61%) and trace amounts of ash and lipids. Regardless of grade, fish species and freshness of raw fish material, pH and moisture content of surimi were found to be the same ranges of 6.8–7.0 and 74-77%, respectively. Depending on the species and the nutritional status of the animal, the muscle of marine fish and invertebrates contain 50-80% water (Sikorski *et al.* 1990). In addition, the composition of muscle can vary depending on the season, size, sex, spawning and feeding condition (Pigott and Tucker 1990). Water is the important quality factors. Surimi is generally adjusted to obtain standard moisture content ranging from 78-80% as suggested by Lanier et al. (1991). Reppond and Babbitt (1997) reported that the increasing moisture content decreased torsion stress and strain of surimi from pollock, herring, arrowtooth flounder and Pacific whiting. Based on dry basis, protein constituted as the major solid component in surimi in which the protein content varied depending on grade of surimi and freshness of raw fish material. Among surimi commercially produced, SSA grade (the best grade available in the market) had the highest protein content, followed by SA, A and B grades, respectively. Storage of fish material for 10 days prior to processing to surimi resulted in a significant decrease in protein content. Similar to SSA grade surimi, surimi produced from fresh fish contained higher protein content than that produced from fish iced-stored for 10 days ($P<0.05$). The results suggested that freshness of fish material is primarily important to obtain a premium grade surimi bigeye snapper. Similar to those produced from white flesh fish, mackerel surimi had similar protein content, comparable to high grade bigeye snapper surimi. Except whiteness, surimi from dark meat fish had a comparable proximate

composition and gelling ability to those produced from white flesh fish (Hultin and Kelleher, 2000). Further fractionation of muscle proteins based on solubility classified muscle proteins into four fractions (Table 7). Myofibrillar proteins were found to be a major constituent in surimi, comprising 60.0 to 71.3%. It was noted that the protein content varied depending on the grade or freshness of raw material used for surimi production. Myofibrillar proteins were found to be major constituent ranging from 66 to 67% of the total protein in fish meat (Careche *et al.*, 1995). Since most of sarcoplasmic proteins and connective tissues are generally removed during surimi processing, myofibrillar proteins in muscle from both species can be increased and thus solely play an essential role in muscle functional properties, especially gelling property (Foegeding *et al.* 1996). Surimi with high grade or produced from fresh fish contained a higher content of myofibrillar protein, while surimi with lower grade or prepared from unfresh fish had a lower content. Since myosin has been reported to play an important role in gelation (Careche *et al.*, 1995), the results clearly indicated the differences in gel forming ability between high and low grade surimi as well as that between surimi produced from fresh and unfresh fish. Stroma and alkali-soluble proteins were also found in all surimi in the range of 6.75-18.47% and 6.20-10.91%, respectively. Stroma proteins consist of not only collagen and elastin, but also connectin and other proteins (Sikorski and Borderias 1990). Teleost and elasmobranch generally contain 3 and 10% stroma, respectively. Large connective tissues are normally removed during surimi processing. The protein pattern of alkali-soluble fraction was somewhat similar to that of myofibrillar fraction. The result was in agreement with Hashimoto *et al.* (1979) who reported that alkali-soluble fraction of sardine and mackerel muscle exhibited the similar protein pattern to myofibrillar fraction. However, a protein band with higher molecular weight than MHC was found as a major band. The content of non-protein nitrogen in surimi is ranging from 0.17 to 1.12 mg N/g (1.18 – 2.94% total nitrogen). Although non-protein components were found at very low content, these components may include trimethylamine oxide, ammonia and other low molecular weight compounds which can contribute to the flavor of surimi (Hashimoto *et al.*, 1979). Generally, non-protein

nitrogenous compounds in white fish muscle make up 9-15% of total nitrogen (Sikorski 1994). Generally, low grade surimi are generally produced from low quality fish which proteins underwent degradation and denaturation. As a consequence, small peptides were generated and could be extracted with low ionic strength solvent. From the result, sarcoplasmic proteins increased with the lower grade surimi. It was noted that surimi produced from 10 days iced-stored fish constituted lower myofibrillar protein, but higher sarcoplasmic protein. Sarcoplasmic proteins are soluble in water or solutions of diluted salt. The dark muscle of pelagic species has been reported to contain higher concentrations of sarcoplasmic proteins than the light muscle (Hultin and Kellener, 2000) but washing process can remove most of sarcoplasmic proteins. Such differences in protein compositions possibly contributed to the different gel-forming ability of different surimi.

Total volatile base (TVB) and trimethylamine (TMA) content in surimi are shown in Table 8. High grade surimi generally had the lower TMA and TVB than low grade surimi. It is evident that surimi produced from very fresh fish had the lowest TVB and TMA content, whereas B grade surimi contained similar TMA and TVB content to surimi produced from fish stored in ice for 10 days. TVB and TMA content conversely related with myofibrillar protein content in surimi. It was postulated that the decomposition of nitrogenous compounds, especially myofibrillar protein was accompanied with the increased TVB or TMA obtained. TMA is associated with the fishy odor of spoilage and is clearly part of the spoilage pattern of many fish. Fresh fish generally have very low TMA values (e.g. less than 1.5 mg/100g cod) (Hebard *et al.*, 1982). TVB involves all volatile amines produced during spoilage. TVB content would estimate the amount of ammonia (NH_3) present, which is formed mainly as a product of protein breakdown, the amount of dimethylamine, which is produced during storage, as well as the amount of TMA (Hebard *et al.*, 1982).

Table 6 Proximate compositions of surimi from bigeye snapper and mackerel

Samples	pH	Constituent (% wet weight basis)			
		Moisture	Protein	Fat	Ash
Bigeye snapper SSA grade surimi	7.00±0.00 [a]	75.02±0.80 [a]	18.60±0.24 [a]	0.17±0.01 [a]	1.41±0.15 [a]
Bigeye snapper SA grade surimi	7.00±0.01 [a]	75.11±0.10 [b]	16.53±0.32 [b]	0.15±0.02 [b]	1.56±0.02 [b]
Bigeye snapper A grade surimi	7.00±0.14 [a]	74.84±0.02 [c]	16.30±1.25 [c]	0.16±0.01 [c]	1.69±0.06 [c]
Bigeye snapper B grade surimi	7.14±0.01 [e]	76.21±0.32 [d]	16.17±0.62 [d]	0.15±0.01 [b]	1.87±0.01 [d]
Surimi from fresh bigeye snapper	7.04±0.27 [a]	75.02±0.37 [a]	18.61±0.94 [a]	0.18±0.03 [d]	1.16±0.05 [e]
Surimi from bigeye snapper kept for ten days	6.86±0.03 [b]	77.06±0.10 [e]	16.02±0.56 [e]	0.23±0.04 [e]	1.52±0.32 [f]
Mackerel surimi	7.00±0.00 [a]	75.02±0.05 [a]	17.02±0.56 [f]	0.25±0.04 [f]	1.03±0.12 [g]

Mean ± SD from triplicate determinations.

The different superscripts in the same column denote the significant differences ($p<0.05$)

Table 7 Nitrogenous compositions of surimi from bigeye snapper and mackerel

Samples	Compositions				
	Non-protein nitrogen	Sarcoplasmic protein	Myofibrillar protein	Alkali-soluble protein	Stroma
Bigeye snapper SSA grade surimi	0.26±0.01 [a]	1.74±0.37 [a]	20.43±0.42 [a]	4.09±0.09 [a]	2.20±0.07 [a]
	(0.90)*	(6.06)	(71.13)	(14.24)	(7.66)
Bigeye snapper SA grade surimi	0.23±0.00 [b]	1.20±0.14 [b]	16.82±0.63 [b]	4.50±0.34 [b]	2.70±0.12 [b]
	(0.90)	(4.71)	(66.09)	(17.68)	(10.61)
Bigeye snapper A grade surimi	0.21±0.06 [c]	1.81±0.22 [a]	10.23±0.05 [c]	3.02±0.41 [c]	1.08±0.49 [c]
	(1.28)	(11.07)	(62.57)	(18.47)	(6.60)
Bigeye snapper B grade surimi	0.17±0.00 [d]	1.53±0.06 [d]	9.15±0.46 [d]	2.61±0.18 [d]	0.89±0.09 [c]
	(1.18)	(10.66)	(63.76)	(18.19)	(6.20)
Surimi from fresh bigeye snapper	1.12±0.06 [e]	11.58±0.72 [c]	21.43±0.64 [e]	2.05±0.09 [e]	3.20±0.05 [e]
	(2.94)	(8.49)	(70.54)	(6.75)	(10.53)
Surimi from bigeye snapper kept for ten days	0.66±0.12 [f]	8.14±0.12 [f]	13.47±0.03 [f]	2.72±0.44 [f]	2.45±0.16 [f]
	(0.87)	(13.99)	(60.03)	(12.12)	(10.91)
Mackerel surimi	0.22±0.03 [g]	1.66±0.13 [g]	17.12±0.33 [g]	4.12±0.22 [g]	2.11±0.03 [g]
	(3.68)	(6.58)	(67.86)	(16.33)	(8.36)

Mean ± SD from triplicate determinations

*Numbers in parenthesis represent percentage distribution.

The different superscripts in the same column denote the significant differences ($p<0.05$)

Table 8 TMA and TVB of surimi from bigeye snapper and mackerel

Samples	TMA(mgN/100g)	TVB(mgN/100g)
Bigeye snapper SSA grade surimi	1.83±0.12 [a]	19.78±0.32 [a]
Bigeye snapper SA grade surimi	2.32±0.02 [b]	22.13±0.11 [b]
Bigeye snapper A grade surimi	2.94±0.20 [c]	29.40±2.97 [c]
Bigeye snapper B grade surimi	4.90±0.99 [d]	32.90±2.97 [d]
Surimi from fresh bigeye snapper	1.37±0.00 [e]	12.32±0.00 [e]
Surimi from bigeye snapper kept for ten days	4.91±0.15 [f]	30.67±1.13 [f]
Mackerel surimi	2.00±0.12 [g]	20.12±0.51 [g]

Mean ± SD from triplicate determinations.

The different superscripts in the same column denote the significant differences ($p < 0.05$)

Thermal stability of muscle protein

Thermal stability is one of the most important characteristics of muscle proteins related to thermal denaturation and aggregation during heating. Analysis of DSC thermograms of muscle proteins from both species of bigeye snapper revealed two major endothermic peaks with the peak maximum temperatures (T_{max}) of 37.17-50.33 and 68.50-71.87 °C (Table 9). The observed T_{max} was within the temperature range observed among various fish species in which the first and second peaks were postulated to be the transitions of myosin and actin, respectively. Fish myosin is very nonstable in comparison with that of mammal (Connell, 1961; Ogawa *et al.*, 1994). The different T_{max} of the transitions among fish species seem to be correlated with the habitat temperature of the fish (Akahane *et al.* 1985; Davies *et al.* 1988).

From the results, T_{max} and enthalpy of low grade surimi were generally lower than those found in high grade surimi or surimi produced from very fresh fish. The results suggested that both myosin and actin of high grade surimi were relatively more stable to

thermal denaturation, compared to that of low grade surimi. The thermal stability of fish proteins from different species resulted in varied thermal gelation of meat paste. A small quantity of heat was used for the easy-setting meat and the easy-disintegrating meat (Iso *et al.* 1991). Shift in T_{max} and a decrease in enthalpy required for denaturation suggested that both myosin and actin in A and B grade surimi underwent higher denaturation, compared to other surimi. Discrepancies in thermal stability may affect how the proteins behave or interact with each other during heating, which directly relates to their ability to form a gel under a particular condition. Beas *et al.* (1991) found that T_{max} of all pre-spawning fish muscle extracts were less than those from post spawning. The result was in agreement with Davies *et al.* (1988) who found that the thermal stability of fish muscle proteins varies between species with considerable implications for storage and processing properties.

Table 9 T_{max} and enthalpy of surimi from bigeye snapper and mackerel

Samples	Peak 1		Peak 2	
	T_{max} (°C)	ΔH(J/g)	T_{max} (°C)	ΔH(J/g)
Bigeye snapper SSA grade surimi	50.22±0.21^{a}	0.201±0.04^{a}	71.80±0.12^{a}	0.401±0.10^{a}
Bigeye snapper SA grade surimi	49.52±0.14^{b}	0.164±0.02^{a}	71.64±0.32^{a}	0.311±0.05^{a}
Bigeye snapper A grade surimi	41.42±0.59^{c}	0.149±0.10^{a}	69.33±0.24^{a}	0.251±0.02^{a}
Bigeye snapper B grade surimi	37.17±0.24^{d}	0.101±0.12^{a}	68.75±0.35^{a}	0.201±0.12^{a}
Surimi from fresh bigeye snapper	50.33±0.70^{e}	0.210±0.11^{a}	71.87±0.06^{a}	0.411±0.02^{a}
Surimi from bigeye snapper kept for ten days	48.33±0.56^{f}	0.148±0.19^{a}	68.50±0.14^{a}	0.300±0.21^{a}
Mackerel surimi	48.25±0.59^{f}	0.150±0.13^{a}	71.75±0.12^{a}	0.290±0.06^{a}

Mean ± SD from triplicate determinations.

The different superscripts in the same column denote the significant differences ($p < 0.05$)

2. Gelling characteristics of different grade surimi from bigeye snapper

Force and deformation of surimi gels in relation with grade, freshness of raw fish materials, and heating regimes are shown in Figure 4. Two-step heated gels with setting at 40 °C or ashi gels had the highest force and deformation, followed by directly heated gel, suwari (gel set at 40°C) and modori gel, respectively. Properties of ashi gels were similar to suwari gels, however, much higher force and deformation were observed with ashi gel (Alverez and Tejada, 1997). From the results, higher grade surimi (A grade) provided the higher quality gel. Breaking force and deformation of A grade surimi were higher than B grade surimi. Surimi prepared from fresh bigeye snapper also rendered the higher breaking and deformation than that produced from bigeye snapper stored in ice for 10 days. The results reflected the importance of raw material quality on gelling characteristic of surimi.

Further heating of suwari gels to produce ashi gels caused the aggregation of a structure formed in the setting stage, probably via the formation of disulfide bridges and hydrophobic interactions at high temperatures (Alverez and Tejada, 1997). Setting was necessary to prepare surimi gels of acceptable quality (Alverez *et al.*, 1995). Setting has been reported to be associated with endogenous transglutaminase, which induced non-disulfide covalent bond, especially ε-(γ-glutamyl lysine) linkage (Seki *et al.*, 1990; Tsukamasa and Shimizu, 1990, 1991; Kimura *et al.*, 1991; Sato *et al.*, 1992; Kumazawa *et al.*, 1993; Sakamoto *et al.*, 1995). When suwari properties were compared, it was found that suwari from lower grade and that produced from unfresh fish had the lower breaking force and deformation. This was probably caused by the loss in transglutaminase activity as well as the increased disintegration of myosin, which is the major contributor to gel formation.

Modori occurred when the formation of stable and temporary crosslinks among proteins exceeded that optimal for ashi (thermally irreversible surimi gels) forming ability (Alverez *et al.*, 1995). It was also caused by the hydrolysis of protein molecules due to fish muscle proteases (An *et al.*, 1994). Niwa (1992) described three mechanisms responsible for modori; proteolytic degradation due to enzymes; thermal coagulation of

myofibrillar proteins and involvement of specific protein. Enzymatic degradation of the gel with the breakdown of MHC has been shown to depend on species and season (Park, 2000). Modori is mainly attributed to heat denaturation of myofibrillar proteins (Toyohara and Shimizu, 1988). Modori has been reported to be induced by endogenous thermal stable proteinases that can degrade myosin rapidly (An *et al.*, 1995). Proteolysis disintegration of surimi gel is characterized by high activity at temperature above 50°C and by the rapid and severe degradation of myofibrillar proteins, particularly myosin (Tsukamasa and Shimizu, 1991; Alverez, 1999b). Their disintegration has ditrimental effects on surimi quality, which substantially lowers the gel strength and elasticity (Niwa, 1992). For modori, surimi with lower grade or that from unfresh fish had the lower breaking force and deformation.

The quality of directly cooked gel is generally poorer than those with prior setting. The lower quality of such directly cooked gels is thought to be due to rapid formation of disulfide and hydrophobic protein-protein bonds in the absence of the conditions required for the protein to orient to form a network (Niwa, 1985). Protein coagulation becomes more general in directly heated gel than in set gels (Niwa, 1975). Furthermore, endogenous transglutaminase was found to induce the ε–(γ-glutamyl lysine) linkage during setting. Subsequently, stronger gel was found, particularly after heating to 90°C.

From the result, suwari gels had the lowest whiteness when compared with other gels (ashi, modori and directly heated gel). This was probably due to the fact that higher temperature caused the denaturation of protein, especially pigments remained in the muscle, leading to more turbidity as shown by higher whiteness. When the temperature used for modori gel preparation was increased (60°C for 30 min, followed by 90°C for 20 min), more whiteness was observed. Directly heated gel had similar whiteness to modori gel. However, Ashi gel had slightly lower whiteness than directly heated gel. Park (1995) found that colors and whiteness of surimi gels were related to species, compositional and physical conditions during preparation and measurements. When comparing the whiteness of ashi gels between A and B grade surimi, it was noted that

the former had higher whiteness. The same result was found with surimi produced from fish with different freshness. Surimi produced from fresh fish rendered the higher whiteness than that prepared from fish stored in ice for 10 days. This was probably due to the extensive oxidation of myoglobin or hemoglobin during the extended storage, leading to the more adduction of those pigments to the muscle proteins. As a result, washing could not remove those adducts. Generally, whiteness was in accordance with L* value. Surimi processing requires extensive washing to remove fat and other undesirable compounds, inducing pigments. Thus, raw surimi is naturally mild in odor and translucent in appearance (Park and Morrissey, 1994). Myoglobin and hemoglobin responsible for the red hue of fish meat, are thoroughly removed during the washing and dewatering step of surimi processing. However, commercial surimi has four to five different grades based on the L* and b* values of cooked gels (Park and Morrissey, 1994).

Expressible moistures

Among all surimi gels tested, modori gel showed the highest expressible moistures than other gels (Table 11). The result suggested that water could not be retained in gel matrix potentially. Modori gel matrix did not have the well-organized structure, since the degraded polypeptide could not form the strong three-dimensional matrix, which can imbibe water effectively. The result was in accordance with Alverez *et al.* (1999b) who found that modori occurring in sardine surimi has maximum expressible moisture when compared with directly cooked gel without prior setting. Alverez and Tejada (1997) found that suwari gel had lower water holding capacity than kamaboko gels. From the result, suwari and ashi gels tended to have lower expressible moisture than modori and directly cooked gels indicating the higher water holding capacity of the gels. During setting at 40°C, proteins underwent some denaturation and aligned themselves gradually to form the network, which can imbibe water. The heating process after setting could ruin some hydrogen bonding, which involved in water holding in gel matrix, though heating could induce the aggregation of protein to form the ordered

network. As a result, similar water holding capacity in both gels, suwari and ashi gels, was observed.

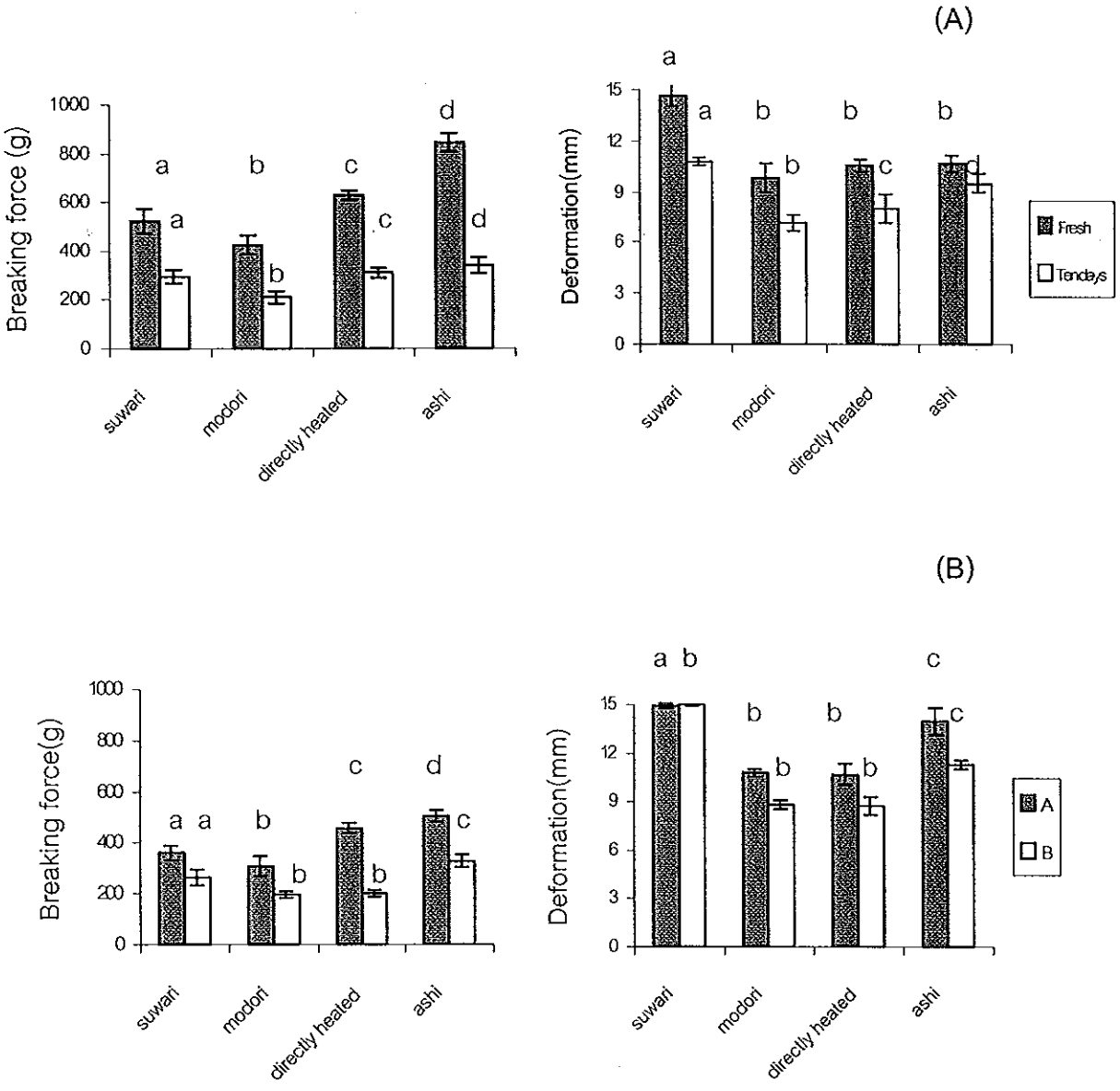


Figure 4 Breaking force and deformation of surimi gel from fresh and ten days stored bigeye snapper (A) and commercial A and B grade surimi from bigeye snapper (B) The different superscripts on the bar within the same grade denote the significant differences ($p<0.05$)

Table 10 L*, a*, b* value and whiteness of different bigeye snapper surimi gels prepared under various condition

Gel types	Samples	L*	a*	b*	whiteness
Suwari	Surimi from fresh fish	67.94±0.47$^{ab, A}$	-1.76±0.04$^{f, A}$	6.78±0.30$^{ab, AB}$	67.92±0.57$^{ab, A}$
	Surimi from ten days stored fish	67.33±0.33$^{a, A}$	-1.20±0.12$^{i, B}$	8.52±0.16$^{de, C}$	67.19±0.49$^{a, A}$
	A grade surimi	69.54±1.16$^{ab, B}$	-2.61±0.06$^{a, C}$	6.63±1.42$^{a, A}$	68.70±1.40$^{b, B}$
	B grade surimi	69.96±0.23$^{b, B}$	-1.46±0.04$^{g, D}$	8.08±0.17$^{cd, BC}$	68.83±0.23$^{b, B}$
Modori	Surimi from fresh fish	82.47±0.81$^{efg, A}$	-2.18±0.13$^{d, A}$	8.38±0.63$^{de, A}$	80.43±0.48$^{e, A}$
	Surimi from ten days stored fish	80.24±0.63$^{cde, B}$	-1.71±0.06$^{fg, B}$	9.70±0.13$^{g, B}$	79.22±0.29$^{cd, B}$
	A grade surimi	85.02±0.52$^{g, C}$	-2.33±0.12$^{a, A}$	7.45±0.10$^{bc, A}$	83.11±0.49$^{h, C}$
	B grade surimi	84.30±0.25$^{g, C}$	-1.47±0.05$^{g, C}$	9.70±0.08$^{g, C}$	81.48±0.20$^{fgh, D}$

Continued

Gel types	Samples	L*	a*	b*	whiteness
Directly heated	Surimi from fresh fish	82.62±0.30 fg, A	-2.23±0.04 cd, A	8.34±0.16 d, B	80.58±0.14 e, A
	Surimi from ten days stored fish	83.19±0.04 fg, A	-1.85±0.06 ef, B	9.27±0.16 fg, C	80.60±0.04 e, A
	A grade surimi	83.80±0.56 g, A	-2.31±0.05 bcd, A	7.32±0.28 abc, A	82.07±0.62 gh, A
	B grade surimi	83.03±0.28 fg, A	-1.60±0.02 gh, C	9.28±0.08 fg, C	80.59±0.27 e, A
Ashi	Surimi from fresh fish	80.69±0.92 de, BC	-2.30±0.13 bcd, A	8.68±0.17 de, A	78.71±0.86 de, C
	Surimi from ten days stored fish	78.12±0.67 c, A	-1.98±0.50 e, B	9.15±0.30 efg, A	76.20±0.68 c, A
	A grade surimi	80.99±0.33 de, C	-2.38±0.07 b, A	7.47±0.04 bc, A	79.44±0.31 de, C
	B grade surimi	79.62±0.50 cd, B	-1.56±0.09 g, C	9.59±0.13 g, A	77.42±0.39 cd, B

Mean±SD from triplicate determinations

Different letters in the same column denote the significant differences ($p<0.05$). Different capital letters in the same column within the same gel denote the significant differences ($p<0.05$)

Table 11 Expressible moisture of different bigeye snapper surimi gels prepared under various conditions.

Gel types	Samples	Expressible moisture (%)
Suwari	Surimi from fresh fish	$7.50\pm1.65^{ab, AB}$
	Surimi from ten days stored fish	$6.21\pm0.14^{a, A}$
	A grade surimi	$7.49\pm0.38^{ab, AB}$
	B grade surimi	$8.27\pm0.45^{c, B}$
Modori	Surimi from fresh fish	$7.56\pm0.19^{ab, A}$
	Surimi from ten days stored fish	$8.47\pm1.07^{c, A}$
	A grade surimi	$8.63\pm0.40^{c, A}$
	B grade surimi	$10.47\pm0.85^{e, B}$
Directly heated	Surimi from fresh fish	$7.19\pm0.31^{ab, A}$
	Surimi from ten days stored fish	$8.18\pm1.75^{c, A}$
	A grade surimi	$8.32\pm0.42^{c, AB}$
	B grade surimi	$9.99\pm0.93^{d, B}$
Ashi	Surimi from fresh fish	$7.47\pm0.82^{ab, A}$
	Surimi from ten days stored fish	$7.19\pm0.31^{c, AB}$
	A grade surimi	$7.66\pm0.23^{c, A}$
	B grade surimi	$8.75\pm0.11^{c, B}$

Mean±SD from triplicate determinations

Different letters in the same column denote the significant differences ($p<0.05$). Different capital letters in the same column within the same gel denote the significant differences ($p<0.05$)

Protein degradation in surimi from bigeye snapper

An increase in TCA soluble peptides indicated the degradation of muscle protein during thermal process (Table 12). The results revealed that proteins were degraded to a higher extent in modori gels. The modori was found to be associated with proteolysis induced by heat-activated proteases, which had optimum temperature around 50-60°C. Furthermore, modori is mainly attributed to heat denaturation of myofibrillar proteins (Tsukamasa and Shimizu, 1991). Modori is a common term for thermal gel degradation occurring when the gel structure is irreversibly destroyed during heating when surimi paste is incubated at temperatures close to 60 °C. The resulting gel is a very brittle, non elastic gel (Alverez *et al.*, 1999b). The myofibril associated proteinase is regarded as much more important since it still retains after washing process. Consequently, those enzymes directly contributed to the degradation of proteins, resulting in poor gel network. Heat-activated proteinases were present in both sarcoplasmic fluid as well as were associated with myofibrils, causing the degradation of myofibrils *P. macracanthus* generally contained a higher proteolytic activity of sarcoplasmic and myofibril-associated proteinase, compared to *P. tayenus*. Major alkaline heat-activated sarcoplasmic proteinase in *P. macracanthus* was serine proteinase, while different proteinases were found in sarcoplasmic fluid of *P. tayenus* muscle. Major myofibril-associated proteinase in both species was classified as a serine proteinase. When comparing the autolytic activity between mince and washed mince, it was found that washed mince showed a lower activity. This was presumed to be due to a removal of sarcoplasmic proteinase, but the proteinase associated with myofibrils still remained (Benjakul *et al.*, 2002). Shimizu *et al.* (1981) reported the diversity of modori inducing proteinase in fish muscle. This may lead to the remarkable species-specificity of modori phenomenon.

Table 12 TCA soluble peptides of different bigeye snapper surimi gels prepared under various conditions.

Gel types	Samples	TCA soluble peptides (μmol tyrosine / 10 g samples)
Control	Surimi from fresh fish	$1.37\pm0.28^{a,A}$
	Surimi from ten days stored fish	$1.89\pm0.04^{b,B}$
	A grade surimi	$1.77\pm0.10^{b,C}$
	B grade surimi	$2.01\pm0.19^{b,D}$
Suwari	Surimi from fresh fish	$2.66\pm0.02^{cde,A}$
	Surimi from ten days stored fish	$3.06\pm0.26^{de,A}$
	A grade surimi	$2.75\pm0.14^{cde,A}$
	B grade surimi	$3.06\pm0.16^{e,A}$
Modori	Surimi from fresh fish	$2.82\pm0.07^{cde,A}$
	Surimi from ten days stored fish	$3.17\pm0.06^{fgh,A}$
	A grade surimi	$2.88\pm0.55^{cde,A}$
	B grade surimi	$3.22\pm0.04^{h,A}$
Directly heated	Surimi from fresh fish	$2.66\pm0.07^{cde,A}$
	Surimi from ten days stored fish	$3.14\pm0.06^{fgh,B}$
	A grade surimi	$2.63\pm0.11^{cd,A}$
	B grade surimi	$3.04\pm0.13^{de,B}$
Ashi	Surimi from fresh fish	$2.63\pm0.32^{cd,A}$
	Surimi from ten days stored fish	$3.00\pm0.06^{de,A}$
	A grade surimi	$2.47\pm0.14^{c,A}$
	B grade surimi	$2.78\pm0.13^{cde,A}$

Control: surimi paste without setting and heating

Mean±SD for triplicate determinations

Different letters in the same column denote the significant differences ($p<0.05$)

Different capital letters in the same column within the same gel denote the significant differences ($p<0.05$)

Solubility

Among all surimis tested, modori showed the highest solubility in a mixture solvent containing SDS, urea and β-mercaptoethanol (Table 13). The highest solubility was in accordance with the lowest breaking force observed in modori gel from all surimis. On the other hand, gel with lowest solubility had the highest breaking force and deformation. Mixtures containing sodium dodecyl sulfate, urea and β-mercaptoethanol were used to solubilize proteins via destroying all bonds, except non-disulfide covalent bond, particularly ε-(γ-glutamyl) lysine linkage. Therefore, the low solubility indicated the presence of such a linkage, which was formed to a higher extent when setting was performed under optimum condition. It has been known that endogenous transglutaminase play a crucial role in formation of ε-(γ-glutamyl) lysine linkage, which is presumed to be a major contributor for strengthening the gel matrix. The high proportion of remaining insoluble protein may indicate that non-disulfide covalent bonds played an important in the formation of suwari and ashi gels (Careche *et al.*, 1995). When comparing the solubility of modori gel prepared from different grade surimis or surimi prepared from different raw materials, it was found that higher solubility was observed in modori prepared from unfresh fish, compared to that from fresh fish. Also, modori B grade surimi had the higher solubility than modori from A grade surimi. During handling or storage of raw materials, protein could be denatured or degraded to some extent. Those proteins were much more susceptible to degradation caused by protease. As a result, modori from low grade surimi or surimi prepared from unfresh fish occurred to a higher extent, compared to those from high grade surimi or surimi produced from very fresh fish

Ashi gels generally had the lowest solubility, followed by suwari gel. This suggested the ashi gels had a highest amount of non- disulfide covalent bond formed during both setting and heating. However, setting played a paramount role in the formation of non- disulfide covalent bond as evidenced by the decreased solubility (Table 13).

Table 13 Solubility of different bigeye snapper surimi gels prepared under various conditions.

Gel types	Samples	Solubility (%)*
Suwari	Surimi from fresh fish	$88.26\pm2.77^{a, A}$
	Surimi from ten days stored fish	$93.14\pm1.43^{b, B}$
	A grade surimi	$94.51\pm0.58^{c, C}$
	B grade surimi	$96.10\pm1.48^{d, D}$
Modori	Surimi from fresh fish	$94.31\pm0.13^{a, E}$
	Surimi from ten days stored fish	$98.67\pm1.23^{b, F}$
	A grade surimi	$97.37\pm0.18^{c, G}$
	B grade surimi	$99.87\pm0.04^{d, H}$
Directly heated	Surimi from fresh fish	$90.20\pm0.28^{a, I}$
	Surimi from ten days stored fish	$95.43\pm2.73^{b, J}$
	A grade surimi	$96.40\pm0.02^{c, K}$
	B grade surimi	$97.60\pm0.14^{d, L}$
Ashi	Surimi from fresh fish	$78.87\pm0.11^{a, M}$
	Surimi from ten days stored fish	$89.50\pm1.64^{b, N}$
	A grade surimi	$90.13\pm1.43^{c, O}$
	B grade surimi	$93.74\pm0.07^{d, P}$

Mean±SD for triplicate determinations

*Solubility in solvent mixtures (20 mM tris-HCl containing 1% SDS, 8M urea and 2% β-ME, pH 8.0)

Different letters in the same column denote the significant differences ($p<0.05$)

Different capital letters in the same column within the same gel denote the significant differences ($p<0.05$)

3. Effect of some additives on gel quality improvement

3.1 Beef plasma proteins (BPP)

Breaking force and deformation of bigeye snapper ashi gel added with various BPP concentrations are depicted in figure 5. Increased breaking force and deformation were observed as BPP was added up to 1%. BPP at a level higher than 1% resulted in decreased breaking force and deformation, especially when a higher concentration was used. Therefore, optimum concentration of BPP was 1% for both grade surimi. With addition of 1% BPP, breaking force and deformation were found to increase by 58 and 21% for A grade surimi and 49 and 20% for B grade surimi, respectively.

The increase in gel strength by addition of BPP was presumed to be due to the binder effect of BPP in the gel matrix. The decrease in breaking force and deformation as BPP concentration increased was postulated to be due to the dilution effect of non-myofibrillar proteins. Slightly increased MHC band was observed as the BPP added increased (Figure 6). The result suggested that the proteolysis was inhibited to some extent by BPP. Proteolysis in surimi was shown to be caused by the lysosomal cysteine proteinase cathepsin L (An *et al.*, 1996; Masaki *et al.*, 1993; Seymour *et al.*, 1994). In surimi production, proteolysis of myosin will lead to loss of gel strength unless controlled by additives such as BPP, dried egg white whey protein concentrate or potato flour (Akazawa *et al.*, 1993; Morrissey *et al.*, 1993). Mammalian blood plasma contains cysteine protease inhibitors including α_2-macroglobulin (Harpel and Brower, 1983) and kininogens (Kato *et al.*, 1981; Turpeinen *et al.*, 1981). The main mechanism of BPP in controlling modori is believed to be protease inhibition by components of the protein additives. Weerasinghe *et al.* (1995) found BPP to have a higher percentage of papain (cysteine proteinase) inhibitors, followed by whey protein concentrate, potato powder, and egg white. Egg white was found to have a higher concentration of trypsin (i.e. serine proteinase) inhibitors, followed by BPP, potato powder and whey protein concentrate. BPP is very effective in decreasing autolytic activity in Pacific whiting and arrowtooth flounder surimi (Weerasinghe, 1995; Morrissey *et al.*, 1993; Reppond and Babbitt, 1993) and it has been proposed that inhibition of protease by inhibitors found in plasma,

mainly α_2-macroglobulin, is the primary mechanism of gel strength enhancement (Hamann *et al.*, 1990). Seymour *et al.* (1997) noted that 1% BPP inhibited only 78% of Pacific whiting surimi autolysis. From the result, it can be inferred that high gel strength can be attained from greater inhibition of proteolytic activity and/or from gel enhancing effects of cogelling proteins and/or crosslinking activities. Though it was quite effective as a gel enhancing/inhibitor additive for surimi, BPP has limited consumer acceptance as a food additive because of religious taboos, esthetic objections and a tendency to add color and flavor components to bland foods at higher incorporation levels (Kang and Lanier, 1999).

L*, a*, b* value and whiteness of both grade bigeye snapper surimis added with various BPP concentrations are shown in Table 14. This result suggested that L* and whiteness decreased but a* and b* value increased when higher concentrations of BPP were added. Addition of 1-1.5% BPP decreases whiteness and increases yellowness of surimi, which is undesirable color (Park, 1995). Furthermore, samples containing BPP at concentration greater than 1% are seldom accepted by Japanese consumers (Akazawa *et al.*, 1993).

When a higher concentration of BPP was added, more MHC band was retained. The lowest MHC band was found with the samples without BPP addition. This result revealed that proteolysis occurred during gel preparation, leading to the disintegration of gel matrix. The result was coincidental with the gel strength, which increased as MHC was more retained. Therefore, BPP was shown to inhibit the degradation of MHC. The same result was observed for both grade surimis (Figure 6).

3.2. Porcine plasma proteins (PPP)

Breaking force and deformation of bigeye snapper surimi gel added with various PPP concentrations are depicted in figure 7. Breaking force and deformation of bigeye snapper surimi increased as PPP concentration increased and the addition of PPP at a level higher than 1% decreased the gel strength significantly, especially when the higher concentration of PPP was applied (Figure 7). From the result, the optimum concentration

of PPP was found to be 1% for both grade surimis. This result was in agreement with Lee *et al.* (2000) who found that the texture of mackerel gel increased to 2 fold and 1.7 fold in breaking force and deformation, respectively with 1% PPP addition. The addition of PPP significantly improved the gel property of bigeye surimi. The breaking force and deformation of bigeye snapper surimi gels with 1% PPP were increased by 42% and 22% for A grade surimi and 34 and 10% for B grade surimi, respectively (Figure 7). Benjakul *et al.* (2001) found that PPP showed inhibitory activity towards trypsin, papain, digestive enzymes and modori proteinases from bigeye snapper. At a level of 0.5%, PPP effectively prevented the degradation of MHC in fish muscle. Additionally, the addition of PPP at a level of 0.5% resulted in an increase in breaking force and deformation of kamaboko gel from bigeye snapper (Benjakul and Visessanguan, 2000)

L* and whiteness of bigeye snapper surimi added with PPP decreased as the concentration of PPP increased ($p<0.05$). However, a* and b* values of surimi gel increased when PPP at a higher amount was added (Table 15) From the SDS-PAGE pattern, it was found that slightly more MHC was retained as PPP added was increased (Figure 8). This was probably due to the inhibitory activity of PPP toward proteinase, especially modori inducing proteinase. Benjakul and Visessanguan (2000) reported that PPP had the inhibitory activity against proteinase and autolysis of Pacific whiting. MHC in Pacific whiting was more retained when higher concentrations of PPP were used. The inhibitory component was found to be 60,000-63,000 dalton as tested by inhibitory activity staining. From SDS-PAGE pattern (Figure 8), similar effect of PPP on inhibition of proteolysis was observed between two grade surimis.

3.3 Egg white (EW)

Breaking force and deformation of bigeye snapper surimi gel added with various concentrations of EW are shown in Figure 9. When the increasing amount of EW was added, breaking force and deformation increased proportionally. From the result, 4% EW rendered the highest breaking force and deformation. It suggested that EW was a good protein additive, which could enhance the gel strength. Frozen and dried egg

white acted as functional binders which might increase both shear stress and shear strain values of surimi gels (Park, 1998). EW may functioned as gel binder between myofibrillar protein network. Since EW has been reported to contain various proteinase inhibitors, including ovoinhibitors, ovomucoid, etc. (Garcia-Carreno and Hernandez-Cortes), gel enhancing effect was also possibly caused by proteinase inhibition.

L*, a*, b* value and whiteness of bigeye snapper surimi added with various EW concentrations are shown in Table 16. L* value and whiteness of A grade surimi gel increased, when EW concentrations increased. However, no marked changes in whiteness of B grade surimi gel were found with addition of EW.

Figure 10 shows SDS-PAGE pattern of bigeye snapper surimi gel added with EW. No differences in protein pattern of both grade surimis were found. EW possibly worked as inhibitor for modori inducing proteinase to some extent. Egg white was found to have a higher concentration of trypsin (i.e. serine proteinase) inhibitors followed by BPP, potato powder and whey protein concentrate. EW is very effective in decreasing autolytic activity in surimi made from tropical fish (Weerasinghe *et al*, 1995). EW is expensive and provides an undesirable sulfur like odor at the levels needed for effective inhibition (Porter *et al.*, 1993) but EW must be added at higher concentration as compared to BPP (Chang-Lee *et al.*, 1989; Chang-Lee *et al.*, 1990; Akazawa *et al.*, 1993; Morrissey *et al.*, 1993)

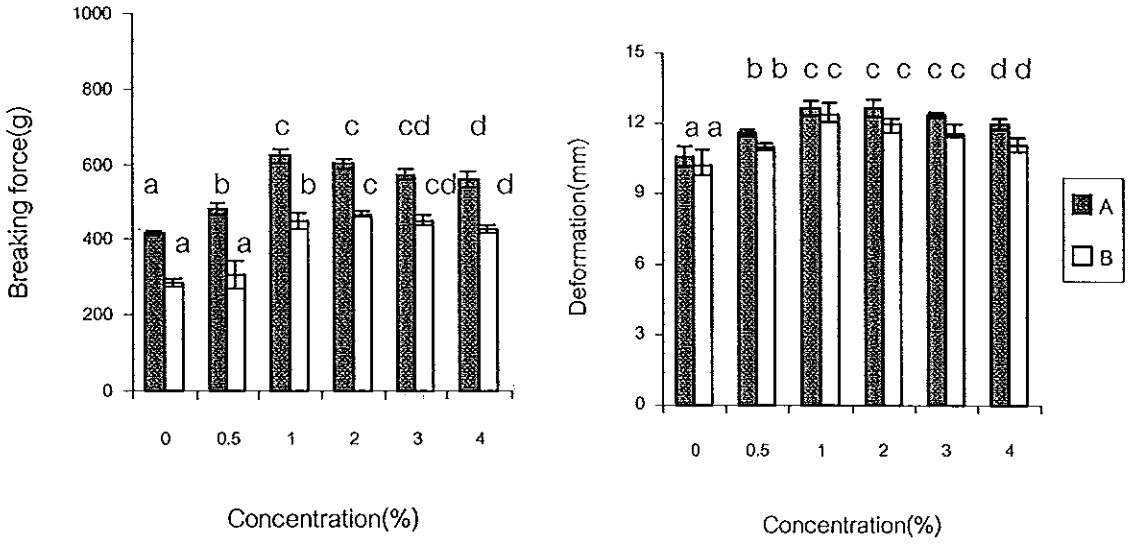


Figure 5 Breaking force and deformation of different grade bigeye snapper surimi gels added with various BPP concentrations and set at 40 °C for 30 min, followed by heating at 90 °C for 15 min

Different letters on the bars within the same grade surimi denote the significant differences ($p<0.05$)

Table 14 L*, a*, b* value and whiteness of different grade bigeye snapper surimi gels added with various BPP concentrations

Concentration (%)	Grade	L*	a*	b*	whiteness
0	A	81.59±1.47^{a}	-0.82±0.09^{a}	8.36±0.06^{a}	79.69±1.34^{a}
	B	80.87±0.09^{x}	-1.93±0.09^{u}	10.61±0.09^{u}	78.11±0.04^{u}
0.5	A	81.65±0.51^{a}	-0.47±0.07^{b}	10.28±0.09^{b}	78.91±0.44^{a}
	B	80.47±0.40^{x}	-1.46±0.03^{v}	11.98±0.11^{v}	77.09±0.38^{v}
1	A	80.59±0.27^{a}	-0.17±0.07^{c}	11.35±0.11^{c}	77.48±0.18^{b}
	B	80.08±0.56^{xy}	-1.22±0.10^{w}	12.79±0.11^{w}	76.32±0.41^{w}
2	A	80.46±0.14^{a}	0.41±0.03^{d}	13.45±0.24^{d}	76.27±0.18^{c}
	B	79.19±0.92^{y}	-0.31±0.06^{x}	14.57±0.04^{x}	74.59±0.75^{x}
3	A	78.94±0.23^{b}	0.83±0.02^{e}	15.10±0.05^{e}	74.08±0.22^{d}
	B	78.35±0.36^{z}	0.31±0.02^{y}	15.57±0.10^{y}	73.31±0.24^{x}
4	A	77.69±0.22^{c}	1.46±0.06^{f}	16.14±0.20^{f}	72.45±0.22^{e}
	B	77.67±0.19^{z}	0.79±0.01^{z}	16.68±0.65^{z}	72.09±0.51^{y}

Mean±SD from triplicate determinations.

The different superscripts in the same column within the same grade denote the significant differences (p<0.05)

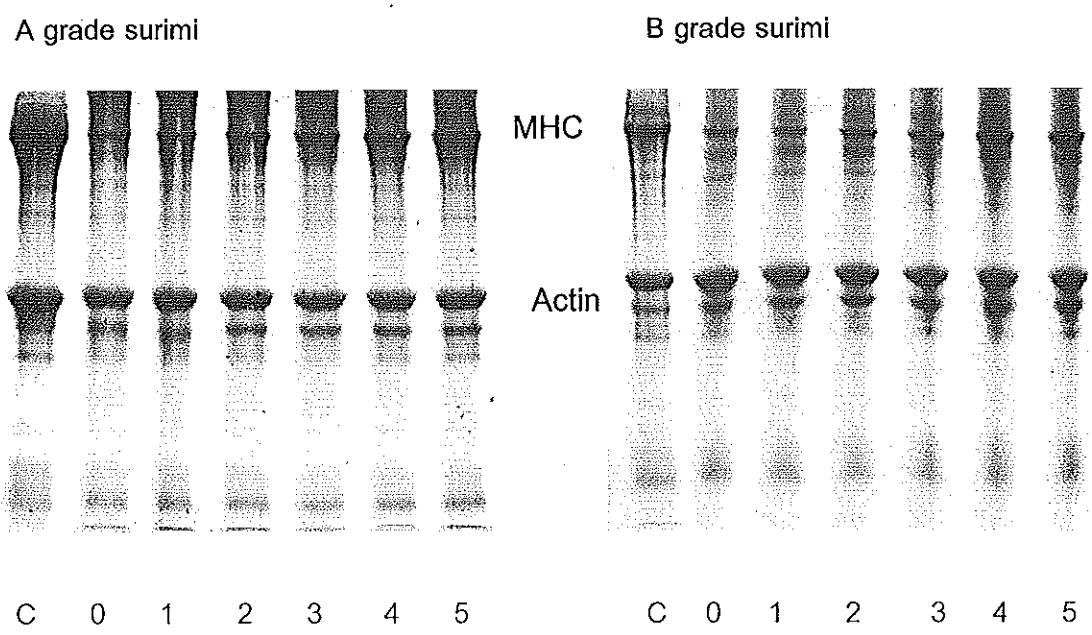


Figure 6 SDS-PAGE pattern of different grade bigeye snapper surimi gels added with BPP at different concentrations. C: surimi paste without setting and heating. Number 0, 1, 2, 3, 4, 5 represents 0, 0.5, 1, 2, 3, 4 % w/w, respectively

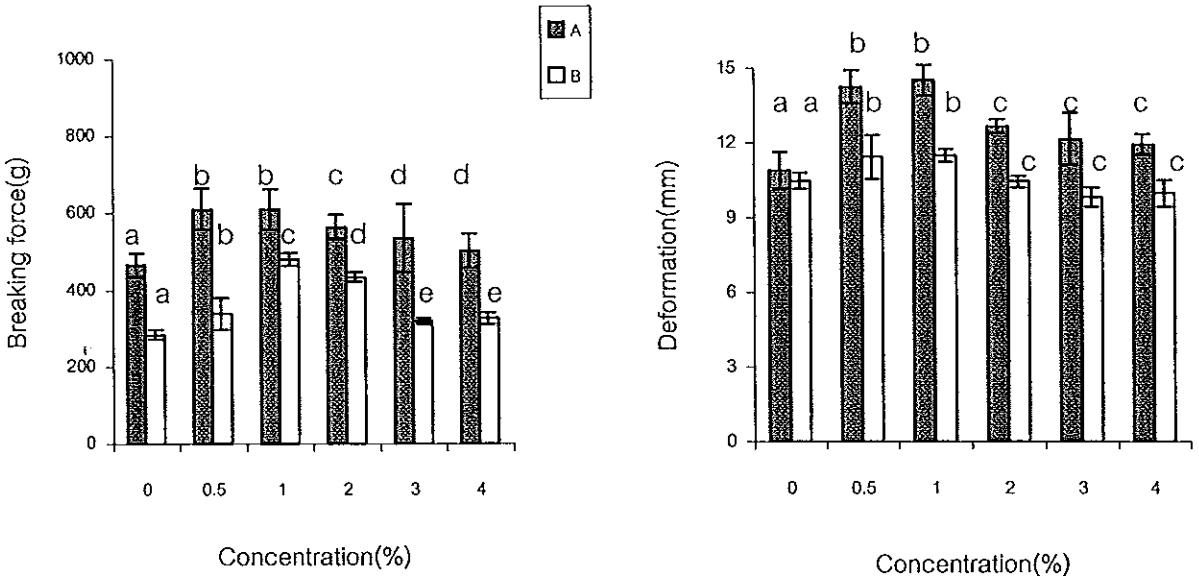


Figure 7 Breaking force and deformation of different grade bigeye snapper surimi gels added with various PPP concentrations and set at 40 °C for 30 min, followed by heating at 90 °C for 15 min

Different letters on the bars within the same grade surimi denote the significant differences ($p<0.05$)

Table 15 L*, a*, b* value and whiteness of different bigeye snapper surimi gels added with various PPP concentrations

Concentration (%)	Grade	L*	a*	b*	whiteness
0	A	82.64±0.45^{a}	-0.76±0.09^{a}	9.28±0.14^{a}	80.31±0.33^{a}
	B	80.62±1.15^{a}	-1.01±0.14^{a}	11.05±0.09^{a}	77.67±1.01^{a}
0.5	A	80.55±1.93^{a}	-0.67±0.05^{a}	10.47±0.10^{b}	77.88±1.72^{b}
	B	77.82±0.75^{b}	-1.22±0.07^{a}	11.70±0.10^{b}	74.91±0.67^{b}
1	A	78.39±0.79^{a}	-0.65±0.12^{a}	11.18±0.10^{c}	75.66±0.71^{c}
	B	76.83±1.75^{b}	-1.23±0.05^{a}	12.59±0.16^{c}	73.61±1.50^{b}
2	A	73.99±0.77^{b}	-0.64±0.11^{b}	12.44±0.17^{d}	71.16±0.73^{d}
	B	74.13±0.87^{c}	-1.48±0.11^{a}	13.16±0.11^{d}	70.97±0.74^{c}
3	A	74.14±0.29^{b}	-0.67±0.18^{b}	12.42±0.16^{d}	69.48±0.22^{d}
	B	71.86±1.12^{d}	-1.49±0.09^{a}	13.21±0.42^{e}	68.90±0.85^{d}
4	A	69.62±0.27^{c}	-0.48±0.21^{c}	12.75±0.05^{e}	67.03±0.27^{e}
	B	68.84±0.79^{e}	-1.67±0.04^{ab}	13.70±0.05^{f}	65.95±0.74^{e}

Mean±SD form triplicate determinations

The different superscripts in the same column within the same grade denote the significant differences (p<0.05)

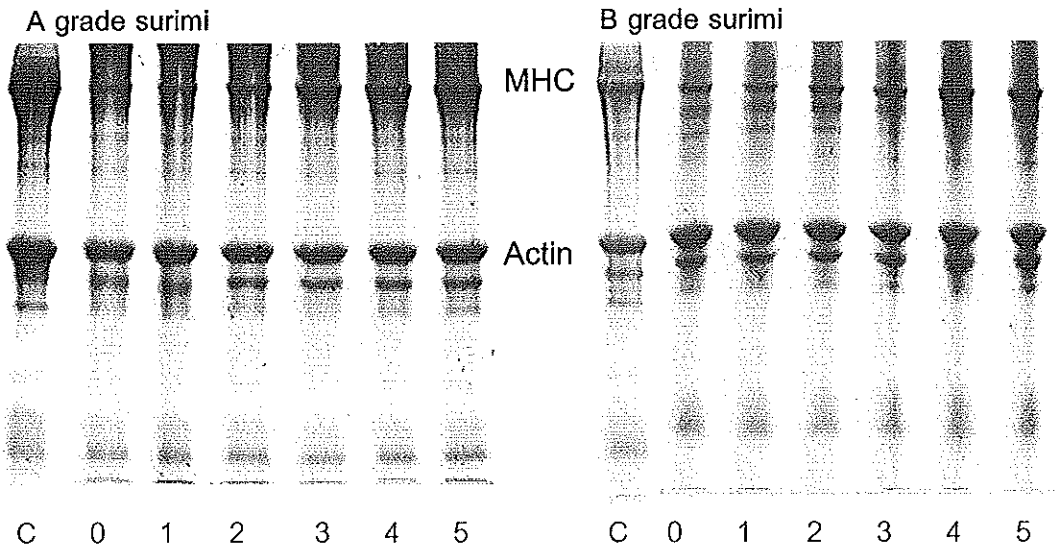


Figure 8 SDS-PAGE pattern of different grade bigeye snapper surimi gels added with PPP at different concentrations. C: surimi paste without setting and heating. Number 0, 1, 2, 3, 4, 5 represents 0, 0.5, 1, 2, 3, 4 % w/w, respectively

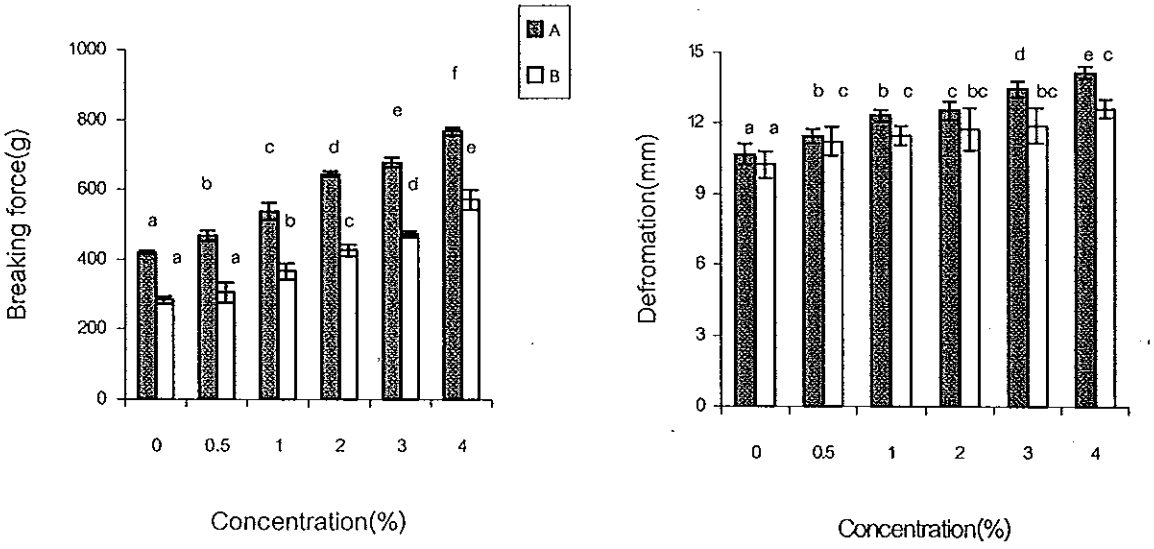


Figure 9 Breaking force and deformation of different grade bigeye snapper surimi gels added with various EW concentrations and set at 40 °C for 30 min, followed by heating at 90 °C for 15 min

Different letters on the bars within the same grade surimi denote the significant differences ($p<0.05$)

Table 16 L*, a*, b* value and whiteness of different grade bigeye snapper surimi gels added with various EW concentrations

Concentration (%)	Grade	L*	a*	b*	whiteness
0	A	80.70±0.24^{a}	-0.71±0.06^{a}	8.15±0.04^{a}	79.03±0.24^{a}
	B	80.54±0.33^{a}	-1.92±0.06^{a}	10.38±0.17^{a}	77.86±0.29^{a}
0.5	A	81.51±0.12^{ab}	-0.98±0.26^{a}	7.67±0.26^{b}	79.95±0.24^{b}
	B	80.70±0.28^{a}	-2.03±0.06^{b}	10.46±0.34^{ab}	77.95±0.08^{a}
1	A	81.91±0.95^{b}	-1.02±0.06^{a}	8.63±0.30^{c}	79.92±0.93^{b}
	B	80.54±0.25^{a}	-2.08±0.17^{b}	10.52±0.01^{ab}	77.78±0.23^{a}
2	A	82.42±0.29^{bc}	-1.06±0.03^{a}	9.42±0.05^{d}	80.20±0.26^{b}
	B	80.82±0.30^{ab}	-1.93±0.06^{b}	10.71±0.16^{b}	77.95±0.32^{a}
3	A	82.17±0.09^{c}	-0.86±0.05^{a}	9.42±0.05^{e}	79.81±0.06^{b}
	B	81.20±0.09^{b}	-2.01±0.03^{ab}	11.27±0.04^{c}	77.99±0.09^{a}
4	A	82.55±0.45^{c}	-0.91±0.02^{a}	9.74±0.10^{f}	80.00±0.40^{b}
	B	81.51±0.25^{bc}	-1.97±0.08^{ab}	11.46±0.16^{c}	78.15±0.13^{a}

Mean±SD from triplicate determinations.

The different superscripts in the same column within the same grade denote the significant differences (p<0.05)

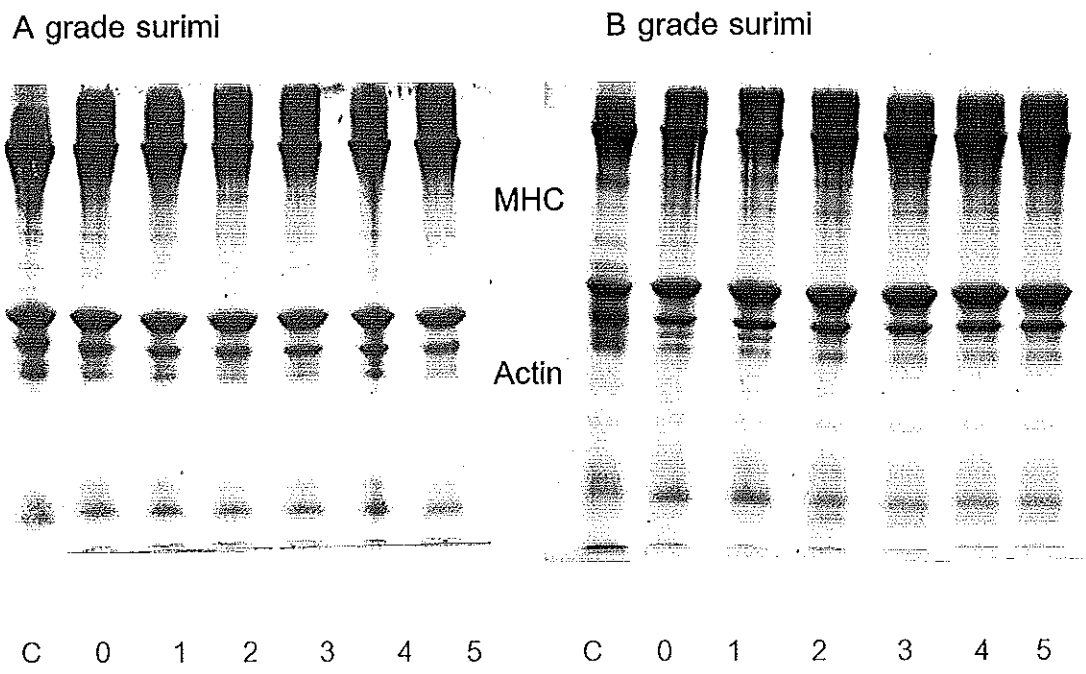


Figure 10 SDS-PAGE pattern of different grade bigeye snapper surimi gels added with EW at different concentrations. C: surimi paste without setting and heating. Number 0, 1, 2, 3, 4, 5 represents 0, 0.5, 1, 2, 3, 4 % w/w, respectively

3.4 Calcium compounds

Different calcium compounds at various concentrations were added into bigeye snapper surimi with different grades. Addition of all calcium compounds (calcium chloride, calcium lactate, and calcium gluconate) at the concentrations ranging from 10 to 100 mM resulted in the increase in breaking force and deformation ($P<0.05$) (Figure 11). More increase in both breaking force and deformation was observed as the concentration increased ($P<0.05$). At the same concentration tested, calcium gluconate generally rendered the superior breaking force and deformation to other calcium salts. Deformation of surimi gel added with calcium salts showed the same trend with breaking force. From the result, it revealed that calcium directly affected the gel property. It has been known that fish TGase is Ca^{2+}-dependent (Seki *et al.*, 1990). Therefore, calcium ion effectively activated TGase activity, leading to higher efficiency in inducing the cross-linking of muscle proteins. Furthermore, calcium ions can also form ionic linkages

between negatively charged sites on two adjacent proteins (Lanier, 2000). Participation of TGase reaction during the setting and its effect on gel strength was elucidated (Wan *et al.*, 1994). This result was in agreement with Kamath *et al.* (1992) who reported that an endogenous TGase was largely responsible for the setting phenomenon. Wan *et al.* (1995) confirmed the essential role of TGase in the setting of walleye pollack surimi paste compared to that of salmon, non-setting fish. Even though the breaking force increased with the addition of calcium salts used, magnitude of breaking force of A grade surimi was much higher than that of B grade surimi. Therefore, grade of surimi, which is associated with protein integrity, determined the efficiency of gel improvement by calcium salts. More degraded protein could not form the gel with prime quality, even with the addition of calcium salt at the high concentrations.

L*, a*, b* value and whiteness of bigeye snapper surimi added with various calcium compounds concentrations are present in Table 17. Generally, L* and whiteness increased with addition of all types and concentrations of calcium salts tested. This was presumed to be due to high scattering effect of calcium particles in surimi gels. An increase in whiteness was observed as higher concentration of calcium salts was added. At the same concentration of calcium salt, no differences in whiteness of surimi gel were observed among calcium salts used. Park (2000) stated that calcium compounds are often used in surimi as gel quality improvement. For B grade surimi, whiteness could be improved to be equivalent to that of A grade surimi by addition of proper calcium salts at an appropriate concentration.

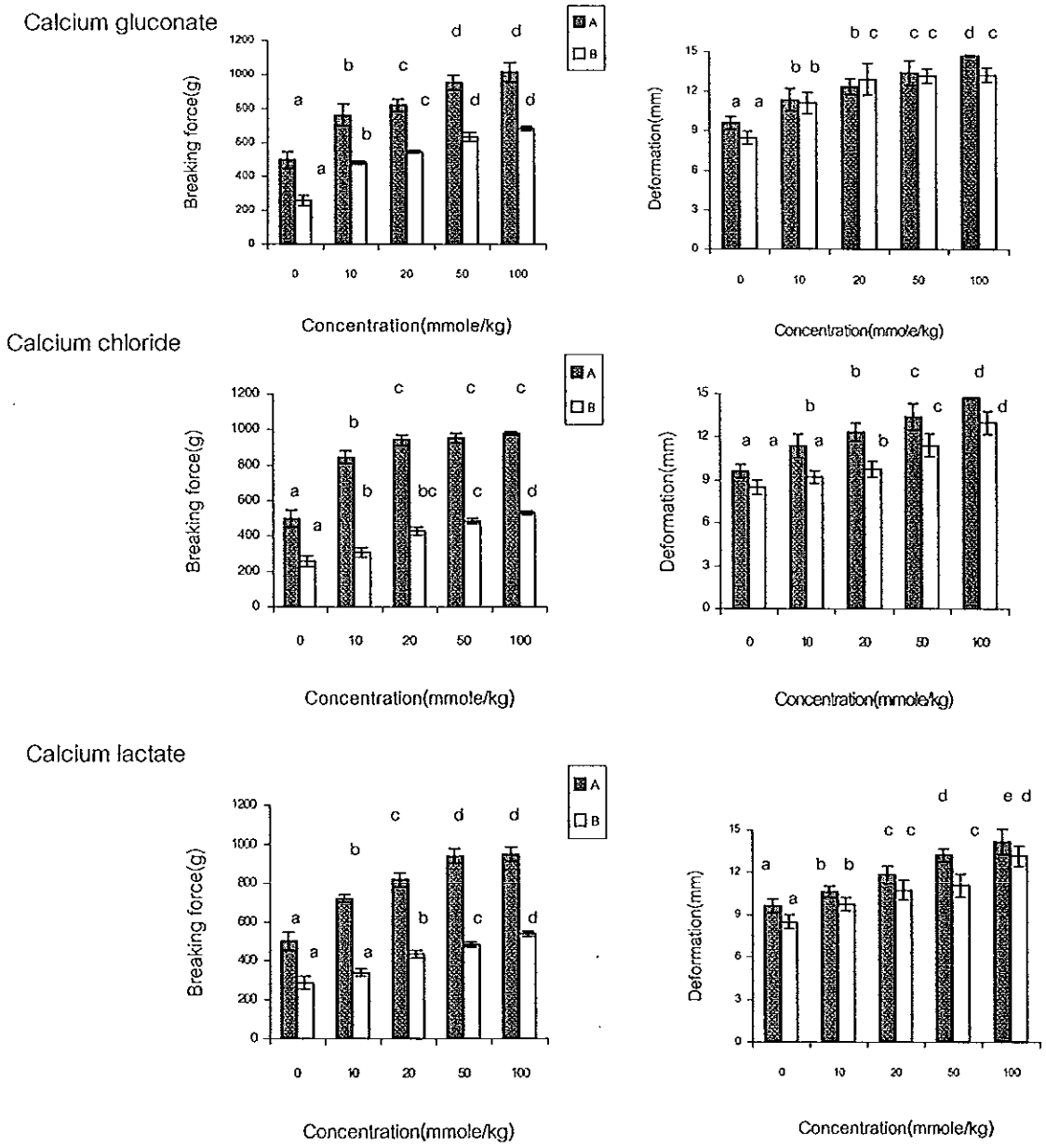


Figure 11 Force and deformation of different grade bigeye snapper surimi gels added with various calcium compounds at different concentrations and set at 40 °C for 30 min, followed by heating at 90 °C for 15 min

Different letters on the bars within the same grade surimi denote the significant differences ($p<0.05$)

Table 17 L*, a*, b* value and whiteness of different grade bigeye snapper surimi gels added with various calcium compounds at different concentrations

Calcium compounds	Concentration (mmole/kg)	Grade	L*	a*	b*	whiteness
Control	0	A	82.13±0.44^{a}	-1.81±0.09^{a}	10.59±0.20^{a}	79.14±0.29^{a}
		B	79.53±0.37^{a}	-1.96±0.06^{a}	10.57±0.15^{a}	76.88±0.28^{a}
Calcium gluconate	10	A	82.55±0.39^{a}	-1.15±0.01^{b}	8.86±0.04^{b}	80.40±0.32^{b}
		B	80.45±0.34^{b}	-1.93±0.10^{ab}	10.03±0.20^{bc}	77.94±0.39^{b}
	20	A	83.19±0.06^{b}	-0.95±0.03^{c}	8.58±0.07^{c}	81.12±0.02^{c}
		B	81.82±0.11^{c}	-1.84±0.03^{b}	10.13±0.13^{c}	79.12±0.17^{c}
	50	A	83.80±0.15^{c}	-1.03±0.05^{c}	8.67±0.09^{bc}	81.60±0.27^{d}
		B	82.03±0.07^{c}	-1.71±0.02^{c}	9.81±0.08^{cd}	79.46±0.04^{c}
	100	A	83.94±0.15^{c}	-0.94±0.06^{c}	8.57±0.16^{c}	81.77±0.17^{d}
		B	81.87±0.86^{c}	-1.66±0.05^{c}	9.64±0.14^{d}	79.40±0.82^{c}
Calcium chloride	10	A	82.06±0.25^{a}	-1.21±0.15^{b}	8.63±0.15^{a}	79.99±0.15^{b}
		B	81.34±0.26^{b}	-1.92±0.12^{a}	10.19±0.15^{b}	78.66±0.30^{b}
	20	A	83.25±0.13^{b}	-1.00±0.02^{c}	8.58±0.19^{a}	81.09±0.03^{c}
		B	81.34±0.15^{b}	-1.73±0.06^{b}	9.96±0.19^{bc}	78.78±0.22^{b}
	50	A	84.73±0.61^{c}	-0.93±0.02^{c}	8.70±0.02^{a}	82.35±0.52^{d}
		B	81.58±0.25^{c}	-1.65±0.05^{b}	9.81±0.25^{bc}	79.83±0.24^{c}
	100	A	84.39±0.51^{c}	-0.86±0.07^{c}	8.48±0.13^{b}	82.17±0.42^{d}
		B	83.02±0.11^{c}	-1.45±0.04^{c}	9.81±0.25^{c}	80.33±0.21^{d}
Calcium lactate	10	A	81.93±0.35^{a}	-1.01±0.02^{b}	9.08±0.07^{b}	79.69±0.33^{ab}
		B	81.27±0.24^{b}	-1.82±0.03^{a}	10.19±0.02^{b}	78.48±0.17^{b}
	20	A	82.96±0.37^{ab}	-1.07±0.03^{b}	8.80±0.06^{bc}	80.74±0.32^{bc}
		B	82.29±0.44^{c}	-1.71±0.17^{b}	9.96±0.17^{bc}	79.66±0.40^{c}
	50	A	83.86±0.16^{c}	-0.92±0.07^{c}	8.62±0.10^{c}	81.63±0.19^{c}
		B	82.59±0.37^{c}	-1.66±0.04^{b}	9.72±0.34^{c}	79.80±0.33^{cd}
	100	A	83.94±0.15^{c}	-0.94±0.06^{d}	8.57±0.16^{d}	81.77±0.17^{d}
		B	82.98±0.36^{c}	-1.38±0.03^{c}	9.05±0.01^{d}	80.38±0.43^{d}

Mean±SD from triplicate determination

The different superscripts in the same column within the same grade and same calcium salt denote the significant differences (p<0.05)

When the protein patterns of surimi gels were determined using SDS-PAGE, it was found that MHC decreased continuously as the higher concentrations of calcium salts were used (Figure 12). However, no changes in actin were observed, though calcium salts were added at a higher level. From the result, it indicated that MHC underwent cross-linking to a much higher extent, compared to actin. This was possibly because MHC contained the acyl donor and acceptor aligned in the appropriate position for reaction. No MHC remained with the addition of calcium salts even at the lowest concentration used (10 mmole/kg). Generally, no different protein pattern was observed among samples added with different calcium salts and concentrations. The decrease in MHC was coincidental with the increase in breaking force and deformation. Therefore, calcium salts were presumed to activate endogenous transglutaminase, which effectively induce cross-linking, especially ε–(γ-glutamyl lysine). Wan *et al.* (1994) reported that amount of cross-linked MHC in A grade surimi paste was observed with the addition of calcium chloride up to 5 mM. The crosslinking of MHC in the surimi appeared to be dependent on the Ca^{2+} concentration. The addition of 5 mmole/kg $CaCl_2$ sharply increased the formation of cross-linked MHC (Wan *et al.*, 1994).

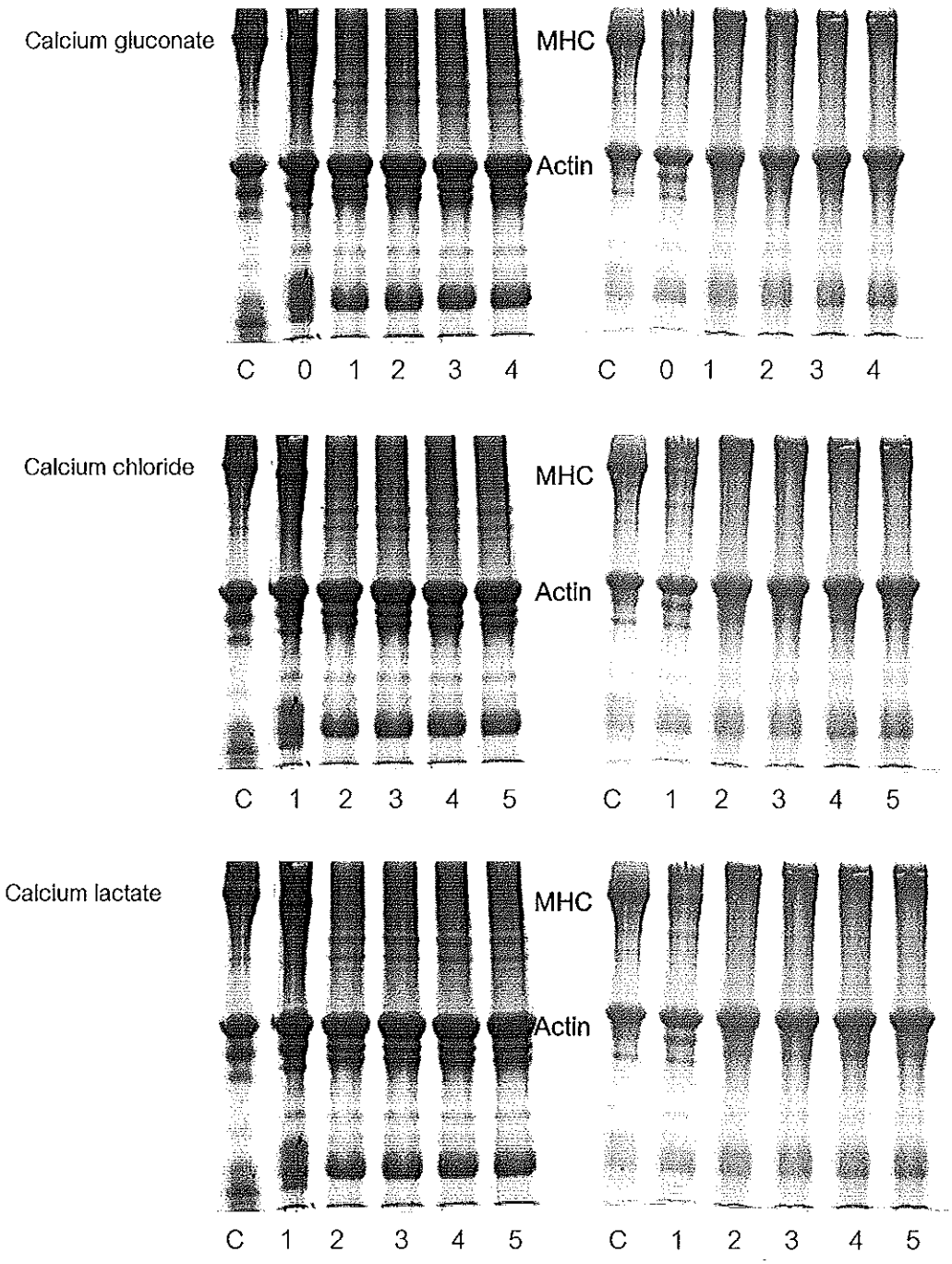


Figure 12 SDS-PAGE pattern of different grade bigeye snapper surimi gels added with calcium compounds at different concentrations. C: surimi paste without setting and heating. Number 0, 1, 2, 3, 4 represents 0, 10, 20, 50, 100 mmole/kg, respectively

3.5 Microbial transglutaminase (MTGase)

The addition of MTGase at a concentration ranging from 0 to 0.2% (w/w) resulted in a significant increase in breaking force and deformation ($P<0.05$) (Figure 13). At a level of 0.01%, breaking force and deformation were increased by 111 and 37% for A grade surimi and 64 and 34% for B grade surimi, respectively. No changes in breaking force were observed when MTGase was added at a concentration above 0.1%. For deformation, no significant increase was found with the addition of MTGase above 0.01%. From the result, MTGase was very effective in bigeye snapper surimi gel improvement. The effects of MTGase on surimi gel varied with fish species (Asagami *et al.*, 1995). The increase in breaking force and deformation of gels added with MTGase was presumably caused by the increased cross-linking induced by MTGase. Jiang *et al.* (2000a) found that MTGase catalyzed the MHC cross-linking of both pollack and golden threadfin bream surimi and increased the gel forming ability of surimi. MTGase at a level of 0.3 and 0.2 unit/g was adequate to improve gel strength of frozen golden threadfin bream and pollack surimi, respectively. However, the addition of MTGase at a high level (higher than 0.03%) resulted in the decrease in breaking force of surimi made from some fish species (Asagami *et al.*, 1995). Tsai *et al.* (1996) found that the gel strength of minced mackerel product increased with the addition of purified TGase, and reached the highest level, which was about 3.9 fold over that of control, when the amount of TGase was 0.34 units/g of meat. When the added TGase was higher than this amount, although the rigidity (breaking force) increased, the elasticity (deformation) decreased. This phenomenon was not observed in this study. This might be because the concentrations of MTGase were not high enough to affect the texture. Excess cross-linking of proteins stabilized by non-disulfide covalent bonds possibly resulted in the rigid gel, leading to the loss in elasticity as observed by the decrease in deformation of gel added with very high MTGase concentration. Therefore, the addition of MTGase at a level of 0.02-0.05% was found to be an appropriate level to improve bigeye snapper surimi gel.

The addition of MTGase did not cause the significant changes in color as well as whiteness for both grade bigeye snapper surimis (P>0.05) (Table 18). Then the use of MTGase is a potential approach for increasing gel strength without causing the changes in whiteness.

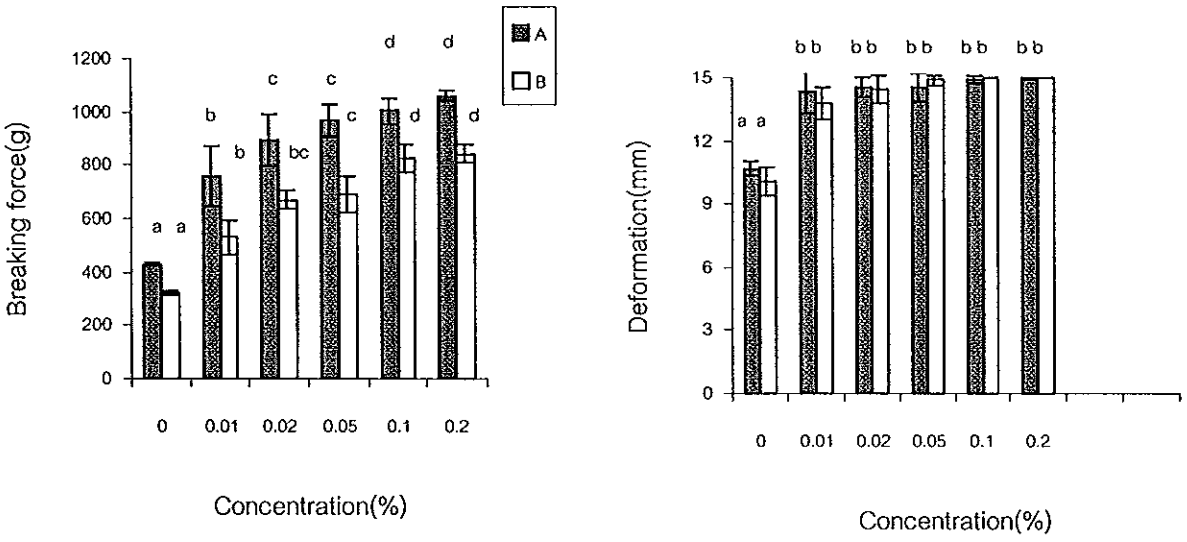


Figure 13 Breaking force and deformation of different grade bigeye snapper surimi gels added with various MTGase concentrations. Gels were set at 40 °C for 30 min, followed by heating at 90 °C for 15 min.
Different letters on the bars within the same grade surimi denote the significant differences (p<0.05)

Table 18 L*, a*, b* value and whiteness of different grade bigeye snapper surimi gels added with various MTGase concentrations

Concentration (%)	Grade	L*	a*	b*	whiteness
0	A	82.56±0.27^{a}	-1.84±0.07^{a}	10.64±0.16^{a}	79.48±0.31^{a}
	B	81.66±0.13^{x}	-2.87±0.07^{xy}	13.26±0.03^{x}	77.30±0.14^{x}
0.01	A	82.49±0.21^{a}	-1.78±0.01^{a}	10.81±0.75^{a}	79.34±0.22^{a}
	B	82.14±0.21^{xy}	-2.87±0.10^{x}	13.31±0.38^{x}	77.54±0.05^{x}
0.02	A	82.64±0.06^{a}	-1.77±0.02^{ab}	10.93±0.01^{a}	79.41±0.06^{a}
	B	81.74±0.21^{xy}	-2.91±0.04^{x}	13.56±0.25^{x}	77.08±0.32^{x}
0.05	A	82.45±0.01^{a}	-1.82±0.00^{ab}	10.88±0.33^{a}	79.24±0.17^{a}
	B	82.01±0.03^{xy}	-2.81±0.03^{x}	13.52±0.05^{xy}	77.32±0.02^{x}
0.1	A	82.29±0.15^{a}	-1.74±0.01^{ab}	10.92±0.08^{a}	79.12±0.17^{a}
	B	82.19±0.16^{y}	-2.92±0.03^{y}	13.24±0.41^{xy}	77.61±0.11^{x}
0.2	A	82.24±0.07^{a}	-1.37±0.41^{b}	11.06±0.33^{a}	79.03±0.21^{a}
	B	81.94±0.24^{xy}	-2.87±0.04^{x}	14.05±0.13^{y}	76.92±0.26^{x}

Mean±SD from triplicate determination

The different superscripts in the same column within the same grade surimi denote the significant differences (p<0.05)

Electrophoretic pattern revealed that MHC underwent cross-linking when MTGase was added (Figure 14). No MHC was remained when only 0.01% MTGase was added. This indicated that MTGase was very powerful for induction of MHC crosslinking (Figure 14). Nevertheless, no marked changes in actin were obtained. The decrease in MHC was concomitant with the increased breaking force and deformation, particularly when the higher concentration of MTGase was added. Nakahara *et al.* (1999) reported that MTGase and carp TGase could not cross-link actin molecules. However, MTGase

rapidly polymerized MHC. This result indicated that MTGase effectively induced the polymerization of MHC, which is a major protein contributing to the gel network formation. As a consequence, the stronger gel network was formed. The result was in accordance with Jiang *et al.* (1998) who reported that cross-linking of MHC occurred rapidly in mackerel surimi added with MTGase.

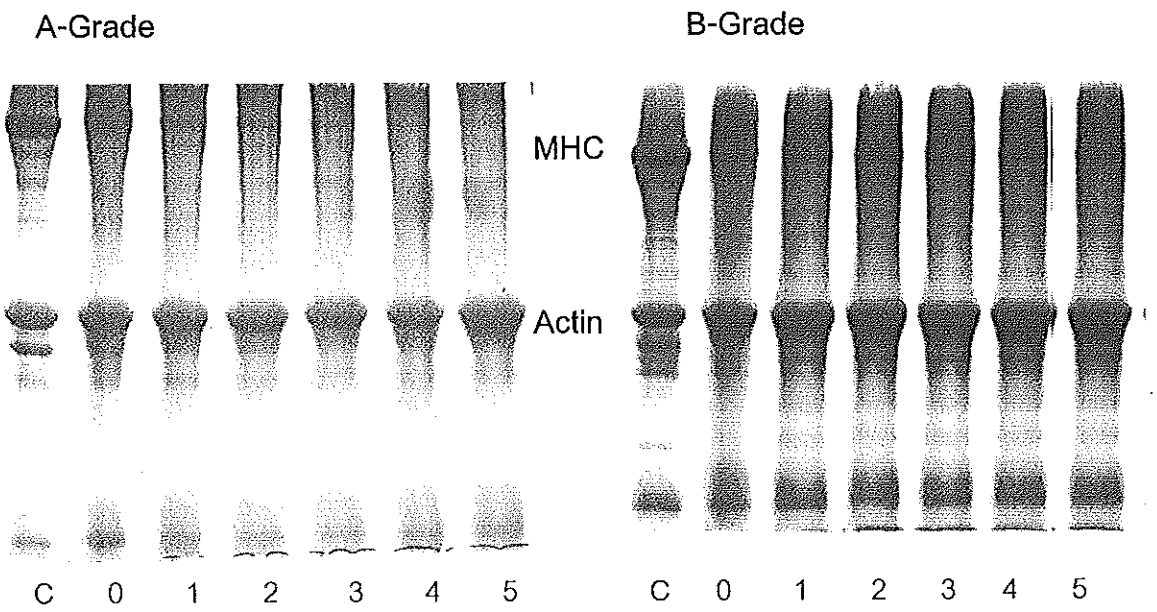


Figure 14 SDS-PAGE pattern of different grade bigeye snapper surimi gels added with various MTGase concentrations. C: surimi paste without setting and heating. Number 0, 1, 2, 3, 4, 5 represents 0, 0.01, 0.02, 0.05, 0.1, 0.2 % w/w, respectively

3.6 Mixed additives

To enhance the surimi gel improvement, calcium compounds, protein additives, and MTGase at an appropriate concentration were used in combination. From the previous results, EW (4%), calcium gluconate (50 mmole/kg) and MTGase (0.1%) was individually the optimum concentration for both grade surimis. A grade surimi was shown to render the higher gel quality than B grade surimi. Surimi was mixed with calcium compounds, protein additive and MTGase and subjected to setting for different

times. From the result, breaking force and deformation of bigeye snapper gels containing calcium gluconate, EW and MTGase increased as setting time at 40°C increased up to 30 min ($p<0.05$). No significant changes were observed when the setting time was longer than 30 min. Thus, setting time of 30 min was the optimum time for both grade surimis added with additives. This suggested that the inter-intra linkages of muscle proteins were gradually constructed and accordingly the gel strength was increased. The low temperature setting was responsible for the polymerization of MHC catalyzed by TGase (Nowsad *et al.*, 1994). However, the muscle proteins were also gradually hydrolyzed by the endogenous proteases, especially when the setting time was too long. Therefore, the optimal setting time needs to be determined for each individual meat paste. Kumawawa *et al.* (1995) reported that the gel strength of kamaboko made from FA grade surimi with 3% NaCl increased as setting time increased. From the result, it suggested that breaking force and deformation of bigeye snapper surimi gel for both grades of surimi increased when 4% EW, 50 mmole/kg calcium gluconate and 0.1% MTGase were added. EW was a good protein additive and was found to have a higher concentration of trypsin inhibitors (Weerasinghe *et al.*, 1995). Egg white also worked as the filler or binder in the gel matrix. Calcium gluconate generally activated endogenous, whereas MTGase was used as external source of cross-linking enzymes. As a result, gel matrix could be synergistically strengthened by the combination of all additives. Breaking force and deformation were found to increase by 117 and 17% for A grade surimi, and 67 and 16% for B grade surimi, respectively when combined additives were used and setting time of 30 min was implemented.

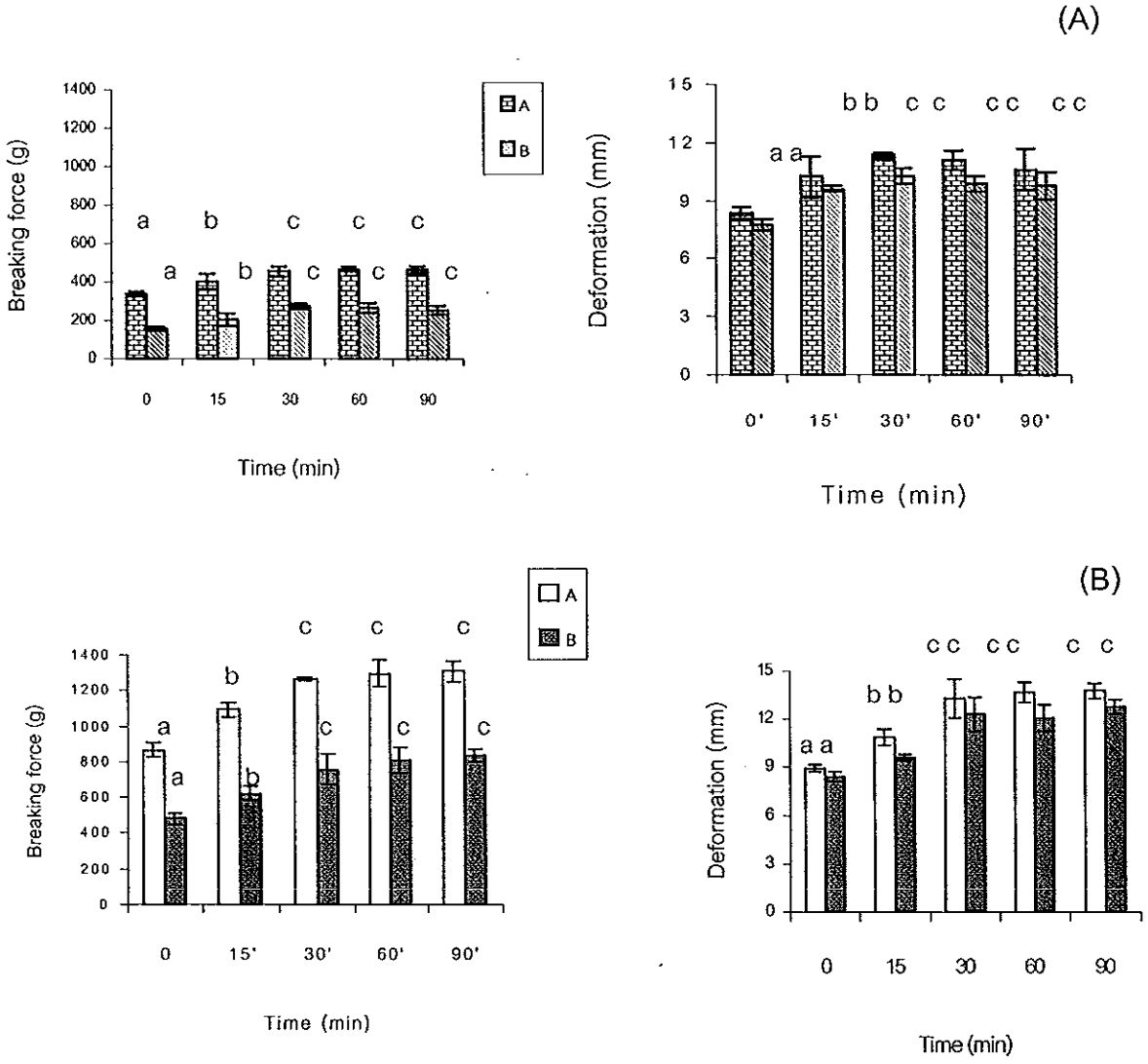


Figure 15 Force and deformation of different grade bigeye snapper surimi gels without (A) and with (B) the addition of egg white, calcium gluconate and MTGase and set at 40°C for different times, prior to heating at 90°C for 15 min Different letters on the bars within the same grade surimi denote the significant differences ($p<0.05$)

L*, a*, b* and whiteness of both grade bigeye snapper surimi added with combined additives and set for different times at 40°C, prior to heating are shown in Table 19 and 20. It was found that whiteness and L* value decreased significantly ($p < 0.05$) when the surimi gel with or without additives was set for 15 to 90 min prior to heating. This was possibly caused by the non-enzymatic browning reaction, which could occur during setting. As a consequence, whiteness was decreased to some extent. However, no marked differences in whiteness and L* value were observed among samples subjected to setting for different period of times. Also, no differences in whiteness between surimi with and without additives were noted. Though egg white was found to reduce the whiteness to some extent, calcium compounds added was shown to improve the whiteness. As a result, no significant changes in whiteness between surimi gels with and without additives were observed.

From the SDS-PAGE pattern, it was found that no different protein pattern was observed between samples without and with additives (Figure 16 and 17). In general, MHC decreased as the setting time increased, indicating that MHC underwent much more cross-linking induced by endogenous TGase for gel without additives, and by both endogenous TGase and MTGase for gel without additives respectively, when the setting time increased. The MHC was more retained in B grade surimi, compared with A grade surimi. This result suggested that protein with more integrity was a preferable substrate for both endogenous and microbial transglutaminase.

Table 19 L*, a*, b* and whiteness of different grade bigeye snapper surimi gel set at 40°C for different times, prior to heating at 90°C for 15 min

Time (min)	Grade	L*	a*	b*	whiteness
Control					
0	A	83.30±0.56^{a}	-2.31±0.05^{a}	7.32±0.28^{b}	82.07±0.62^{a}
	B	83.03±0.28^{x}	-1.60±0.02^{x}	9.28±0.08^{x}	80.59±0.27^{x}
15	A	81.51±0.12^{b}	-0.98±0.26^{b}	7.67±0.26^{b}	79.95±0.24^{b}
	B	80.70±0.28^{y}	-2.03±0.06^{y}	10.46±0.34^{xy}	77.95±0.08^{y}
30	A	81.91±0.95^{b}	-1.02±0.06^{a}	8.63±0.30^{c}	79.92±0.93^{b}
	B	80.54±0.25^{y}	-2.08±0.17^{y}	10.52±0.01^{xy}	77.78±0.23^{y}
60	A	82.42±0.29^{bc}	-1.06±0.03^{a}	9.42±0.05^{d}	80.20±0.26^{b}
	B	80.82±0.30^{y}	-1.93±0.06^{y}	10.71±0.16^{y}	77.50±0.32^{y}
90	A	82.17±0.09^{b}	-0.86±0.05^{a}	9.42±0.05^{c}	79.81±0.06^{b}
	B	81.20±0.09^{y}	-2.01±0.03^{xy}	11.27±0.04^{z}	77.49±0.09^{y}

Mean ± SD from triplicate determinations.

The different superscripts in the same column within the same grade surimi denote the significant differences (p<0.05)

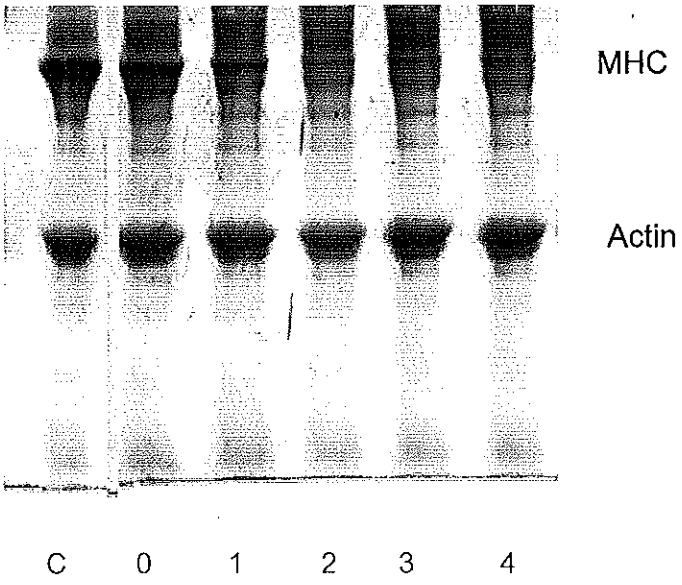
Table 20 L*, a*, b* and whiteness of different grade bigeye snapper surimi gels added with egg white, calcium gluconate and MTGase and set at 40°C for different times, prior to heating at 90°C for 15 min

Time	Grade	L*	a*	b*	whiteness
0	A	83.91±0.47^{a}	-0.58±0.09^{a}	10.50±0.15^{ab}	81.98±0.29^{a}
	B	82.71±0.18^{x}	-1.73±0.04^{x}	8.38±0.03^{xy}	79.89±0.06^{x}
15	A	81.81±0.37^{b}	-0.71±0.02^{a}	10.49±0.04^{ab}	79.07±0.37^{a}
	B	80.22±0.08^{y}	-1.83±0.09^{y}	8.16±0.13^{y}	78.52±0.07^{x}
30	A	81.79±0.89^{b}	-0.55±0.04^{a}	10.14±0.49^{ab}	79.14±0.68^{a}
	B	80.00±0.70^{y}	-1.90±0.22^{x}	8.14±0.19^{y}	78.32±0.61^{x}
60	A	81.72±0.48^{b}	-0.61±0.01^{a}	10.60±0.11^{ab}	78.86±0.47^{a}
	B	80.15±0.26^{y}	-1.75±0.02^{x}	8.45±0.11^{xy}	78.36±0.28^{x}
90	A	81.97±0.32^{b}	-0.58±0.03^{a}	10.63±0.08^{b}	79.06±0.26^{a}
	B	80.39±0.37^{y}	-1.75±0.10^{x}	8.28±0.19^{xy}	78.64±0.30^{x}

Mean ± SD from triplicate determinations.

The different superscripts in the same column within the same grade surimi denote the significant differences (p<0.05)

A grade surimi



B grade surimi

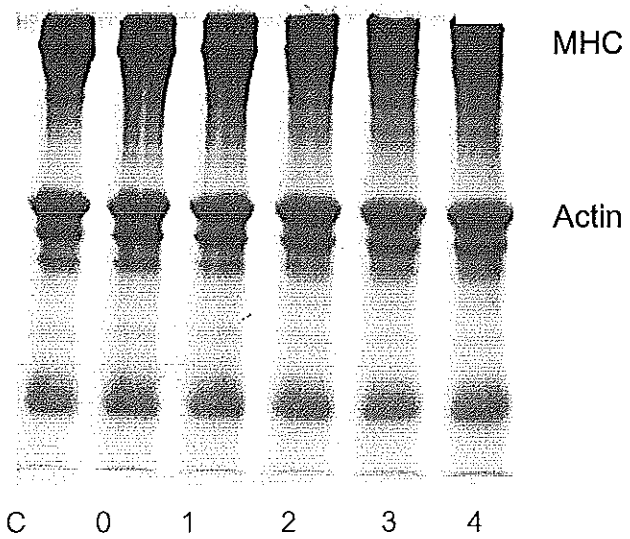
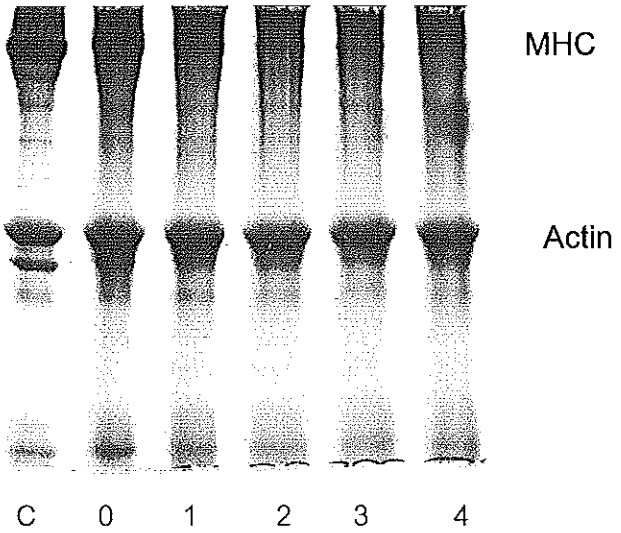


Figure 16 SDS-PAGE pattern of different grade bigeye snapper surimi gels set for different times, C: surimi paste without setting and heating, Number 0, 1, 2, 3, 4 represents 0, 15, 30, 60 and 90 min of setting time

A grade surimi



B grade surimi

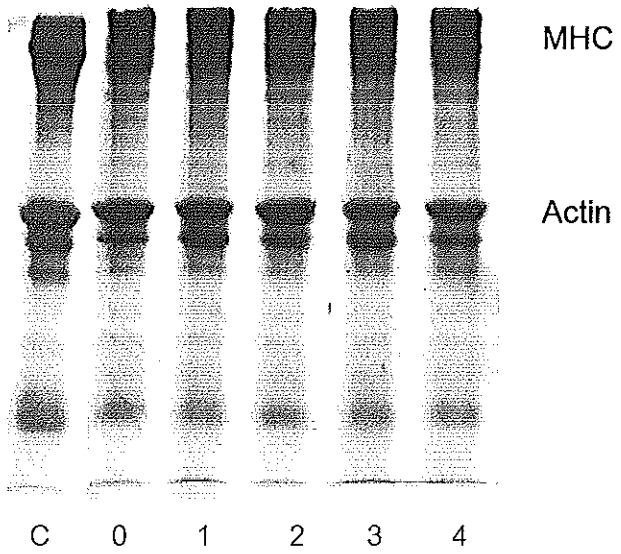


Figure 17 SDS-PAGE pattern of different grade bigeye snapper surimi gels added with egg white, calcium gluconate and MTGase and set for different times. C: surimi paste without setting and heating, Number 0, 1, 2, 3, 4 represents 0, 15, 30, 60 and 90 min of setting time

4. Improvement of whiteness of mixture between white and dark flesh surimi

4.1. Effect of white: dark flesh surimi ratio on gel properties

Ratio of white flesh surimi to dark flesh surimi, which did not reduce the whiteness value to less than 70, but still had the breaking force comparable to that of white flesh surimi for each grade was selected. Generally, the decrease in breaking force was observed in SSA grade bigeye snapper surimi as the increased ratio of mackerel surimi was added (Figure 18). Ratio of SSA grade surimi to mackerel surimi at 70:30 (w/v) showed the breaking force of 933 g, which is the minimum value of Grade SSA surimi. The increase in breaking force was observed in SA grade surimi as the increased ratio of mackerel surimi was added. Ratio of SA grade bigeye snapper surimi to mackerel surimi at 50:50 (w/v) showed the breaking force of 743 g, which still had the breaking force comparable to that of SA grade surimi. Conversely, the addition of mackerel surimi to a higher extent tended to increase the breaking force, but no significant increase was found (p>0.05). For whiteness, it was found that an increase in mackerel surimi ratio led to a decrease in whiteness and L-values of surimi gels (Table 21).

Therefore, the proper ratio of bigeye snapper surimi to mackerel surimi were 70:30 for SSA grade and 50:50 for SA grade. The mixed surimi with such a ratio was used throughout the study for whiteness improvement using different whitening agents including calcium carbonate, titanium dioxide and vegetable oil at various concentrations.

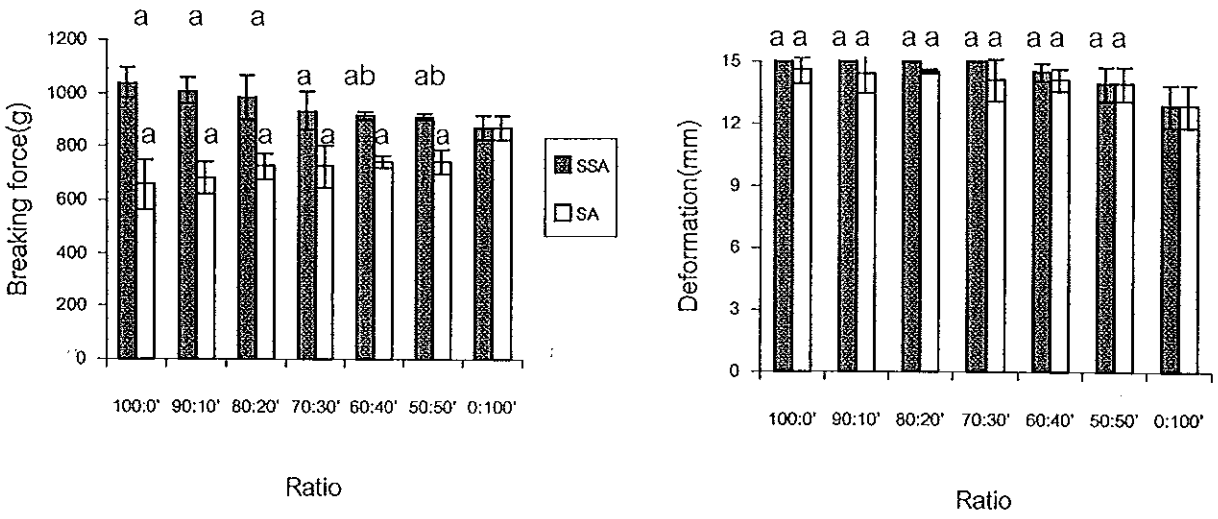


Figure 18 Breaking force and deformation of mixture between SSA or SA grade bigeye snapper surimi and mackerel surimi at different ratios. Gels were prepared by setting at 40°C for 30 min, followed by heating at 90°C for 15 min
Different letters on the bars within the same grade surimi denote the significant differences ($p<0.05$)

Table 21 L*, a*, b* and whiteness of mixture between SSA or SA grade bigeye snapper surimi and mackerel surimi at different ratios

Ratio (white:dark)	Grade	L*	a*	b*	Whiteness
100:0	SSA	86.27±0.41^{a}	-2.93±0.05^{a}	9.37±0.45^{a}	83.11+0.31^{a}
	SA	82.36±0.15^{f}	-3.10±0.09^{f}	10.18±0.12^{f}	79.40±0.08^{f}
90:10	SSA	80.50±0.38^{b}	-2.94±0.06^{a}	9.08±0.18^{a}	78.29±0.33^{a}
	SA	77.31±0.33^{g}	-2.67±0.09^{g}	9.01±0.25^{g}	75.43±0.30^{g}
80:20	SSA	76.50±0.38^{c}	-2.69±0.06^{b}	8.23±0.15^{b}	74.95±0.31^{b}
	SA	75.27±0.06^{h}	-2.53±0.13^{h}	8.24±0.31^{h}	73.81±0.14^{h}
70:30	SSA	71.77±0.89^{d}	-2.51±0.13^{c}	7.68±0.42^{c}	70.63±0.81^{c}
	SA	70.84±0.17^{i}	-2.25+0.02^{i}	7.47±0.15^{i}	69.82±0.15^{i}
60:40	SSA	70.85±0.13^{d}	-2.25±0.02^{d}	7.30±0.10^{cd}	69.86±0.13^{c}
	SA	69.95±1.11^{j}	-2.26±0.07^{i}	7.06+0.31^{j}	69.05±1.01^{i}
50:50	SSA	69.07±0.73^{e}	-2.18±0.03^{d}	6.93±0.17^{d}	68.22±0.75^{d}
	SA	67.37±0.38^{k}	-2.11±0.05^{i}	6.87±0.66^{j}	66.59±0.38^{j}
0:100	SSA	63.26±0.20	-1.5±+0.06	6.39±0.09	62.68±0.12
	SA	63.26±0.20	-1.52±0.06	6.39±0.09	62.68±0.12

Mean±SD from triplicate determinations.

The different superscripts in the same column within the same grade surimi denote the significant differences (p<0.05)

4.2. Effect of whitening agent on whiteness and gel forming ability

Force and deformation of mixed surimi gel added with various whitening agents at different concentrations are depicted in Figure 19. Higher breaking force and deformation were obtained with mixed SSA grade surimi gel added with calcium carbonate, particularly when the concentration used increased. However, calcium carbonate did not increase breaking force and deformation for mixed lower grade surimi (SA grade). Breaking force and deformation of mixed SSA grade surimi was found to be increased by 17 and 6%, respectively with the addition of 1.5% calcium carbonate. Calcium carbonate contains calcium ion, which functioned as gel enhancer by activation of endogenous transglutaminase. Nevertheless calcium carbonate has been reported to render the lowest breaking force and deformation when compared with other calcium salt (Park, 2000). Calcium carbonate produces carbon dioxide and air bubbles often appear on the samples (Park, 2000). In essence, air bubbles may interfere crosslinking of protein. However, addition of carbonate, similarly to addition of inorganic pyrophosphate, produces a meat pH increase which would promote swelling and loosening of protein structure (Shults *et al*, 1972), which in turn increase the water-binding capacity of the mince (Kolakowski *et al.*, 1994)

When comparing between mixed SSA and SA grade surimi, it was noted that calcium carbonate had no effect on gel improvement of mixed SA grade surimi. This was probably due to the fact that mixed SA grade surimis had the poorer protein integrity, which could not be polymerized effectively by the induction of endogenous TGase. Also, lower amount of TGase in mixed SA grade was postulated, leading to the lower setting phenomenon.

When vegetable oil at different concentrations was added into mixed surimi, breaking force gradually decreased as the amount of oil increased (Figure 19). However, no changes in deformation were observed with the addition of vegetable oil. The increased amount of oil added possibly impeded the cross-linking of proteins, resulting in less aggregation of protein with loosen bonding. Therefore, amount of oil

added is needed to taken into consideration in order to obtain the gel with desirable textural property.

The addition of titanium dioxide did not cause any changes in breaking force and deformation of both mixed surimis (Figure 19). The result indicated that titanium dioxide did not interfere the gel matrix formation. This may be due to the small particle size, which could disperse uniformly throughout the gel. As a consequence, the aggregation still occurred among the protein molecules.

L*, a*, b* and whiteness of mixed surimi with different whitening agent at various concentrations are shown in Table 22. Generally L* and whiteness increased with addition of all whitening agents, especially at the higher concentrations. Vegetable oil ranging from 0 to 5% increased L* value and whiteness of mixed surimi similarly with titanium dioxide ranging from 0-0.1%. The result suggested that titanium dioxide was very promising whitening agent since it had no detrimental effect on gel forming ability, while oil reduced the gel strength even though the whiteness could be improved. However, calcium carbonate did not show the marked influence on whiteness improvement though it could increase the gel strength to some extent. In general, those chemicals were not soluble and uniformly distributed well during mixing and thus make the products somewhat chalky or opaque white (Park, 1998). Titanium dioxide had smaller particles than calcium carbonate. As a result, titanium dioxide might disperse better than calcium carbonate. Vegetable oil sometime contributes to improved whiteness through the light scattering effect of highly comminuted surimi paste (Park, 1998). Park (2000) found that calcium carbonate and titanium dioxide showed the ability to make opaque surimi gels. Based on whiteness improvement, proper amount of individual whitening agent was chosen as follows: 1.5% calcium carbonate, 5% vegetable oil and 0.1% titanium dioxide. However, those whitening agents affected the whiteness of surimi gel differently.

Among all surimi tested, surimi added with vegetable oil showed the highest solubility in a mixture solvent containing, SDS, urea, β-mercaptoethanol (Table 23). Surimi added with calcium carbonate exhibited the lowest solubility, followed by surimi

added with titanium dioxide. From the result, gel with lowest solubility had the highest breaking force and deformation. This result confirmed the role of TGase in protein crosslinking during setting, especially when Ca ion (calcium carbonate) was added. Vegetable oil used at high concentrations possibly impeded the crosslinking of ε–(γ-glutamyl) lysine linkage due to the dilution effect. Solubility of surimi added with titanium dioxide was comparable with the control. The result indicated that titanium dioxide had no effect on gel-forming ability. The result was coincidental with gel strength, which was not altered when titanium dioxide was added. Mixed surimi gel (SA grade) had the higher solubility than SSA grade surimi gel, when added with the same whitening agent was added. From the result, it can be inferred that high grade surimi (SSA grade) with better protein integrity showed the superior gel forming ability to the lower grade surimi (SA grade).

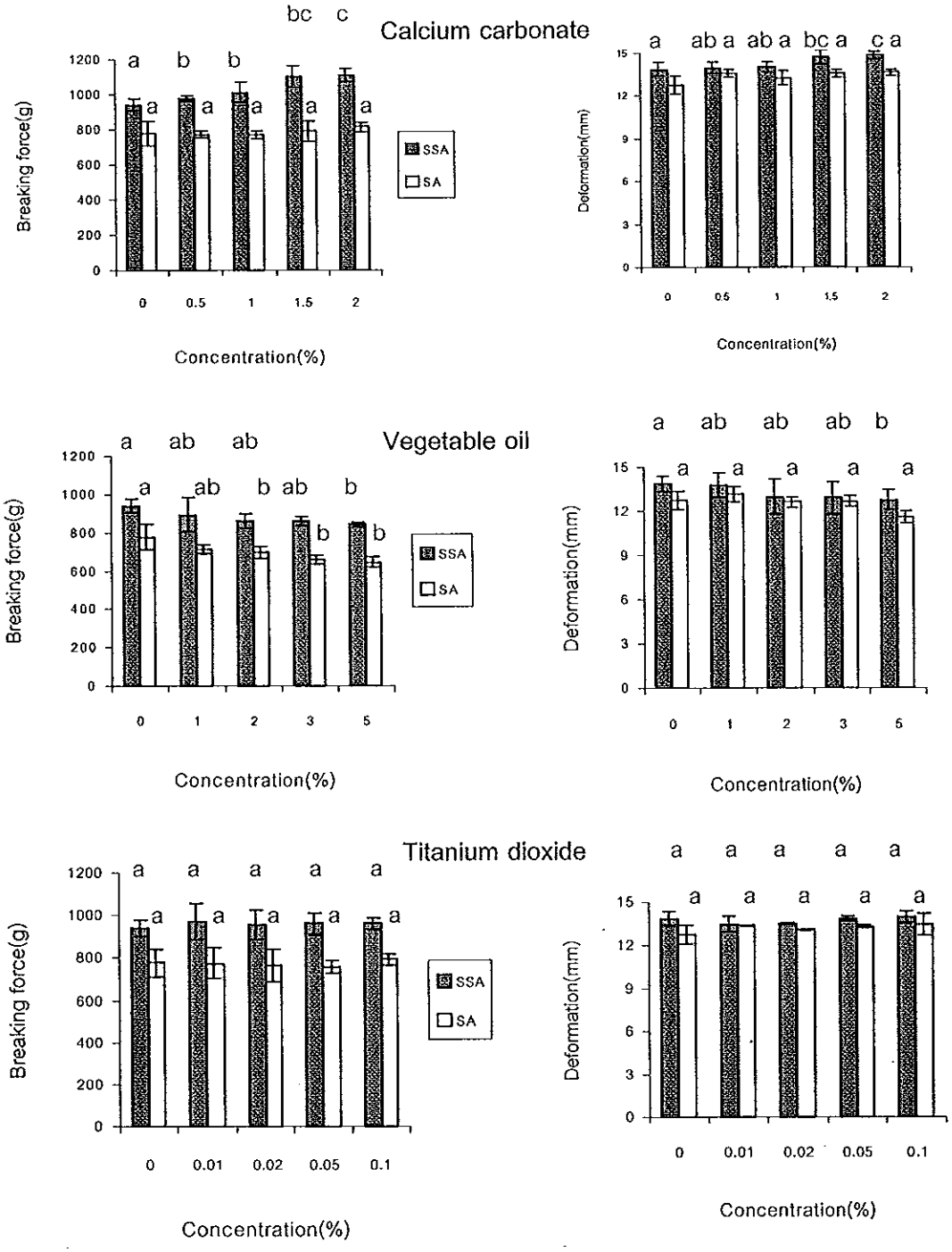


Figure 19 Force and deformation of mixture between SSA or SA grade bigeye snapper surimi and mackerel surimi added with whitening agents at different concentrations. Gels were set at 40 °C for 30 min, followed by heating at 90 °C for 15 min Different letters on the bars within the same grade surimi denote the significant differences ($p<0.05$)

Table 22 L*, a*, b* and whiteness of mixture between SSA or SA grade bigeye snapper surimi and mackerel surimi gels added with whitening agents at different concentrations

Whitening agents	Concentration (%)	Grade	L*	a*	b*	whiteness
	0	SSA	69.80±0.32^{a}	-2.28±0.08^{a}	7.95±0.04^{a}	68.68±0.26^{a}
		SA	67.37±0.38^{u}	-2.11±0.04^{u}	6.87±0.6^{u}	66.59±0.38^{u}
Calcium carbonate	0.5	SSA	71.89±0.68^{b}	-2.20±0.04^{ab}	8.17±0.08^{a}	70.64±0.63^{b}
		SA	70.68±0.13^{v}	-2.02±0.04^{v}	7.15±0.09^{v}	69.75±0.15^{v}
	1	SSA	72.72±0.34^{bc}	-2.16±0.01^{ab}	8.24±0.11^{a}	71.42±0.31^{bc}
		SA	70.01±0.70^{vw}	-2.02±0.06^{v}	7.06±0.02^{v}	69.12±0.68^{vw}
	1.5	SSA	73.07±0.06^{c}	-2.02±0.06^{c}	8.25±0.21^{a}	71.76±0.07^{c}
		SA	71.47±0.55^{wx}	-1.93±0.03^{w}	7.26±0.11^{w}	70.49±0.50^{wx}
	2	SSA	72.54±0.98^{bc}	-2.08±0.12^{bc}	8.36±0.32^{a}	71.22±0.10^{bc}
		SA	71.86±0.50^{y}	-1.95±0.01^{w}	7.48±±0.03^{x}	70.82±0.48^{x}
Vegetable oil	1	SSA	73.20±0.33^{b}	-2.21±0.12^{a}	8.23±0.15^{a}	71.88±0.34^{b}
		SA	73.02±0.39^{y}	-2.12±0.04^{uv}	7.61±0.08^{v}	71.88±0.36^{v}
	2	SSA	74.00±0.20^{b}	-2.23±0.13^{a}	8.05±0.27^{a}	72.69±0.25^{b}
		SA	72.91±0.68^{v}	-2.05±0.04^{v}	7.53±0.14^{v}	71.80±0.69^{v}
	3	SSA	75.40±0.30^{c}	-2.02±0.02^{ab}	8.23±0.24^{a}	73.98±0.32^{c}
		SA	73.81±0.22^{w}	-2.07±0.04^{v}	7.54±0.14^{v}	72.67±0.17^{w}
	5	SSA	77.23±0.06^{d}	-2.11±0.08^{b}	8.87±0.12^{b}	75.47±0.56^{d}
		SA	76.16±0.04^{x}	-1.96±0.02^{w}	7.45±0.02^{v}	74.95±0.05^{x}
Titanium dioxide	0.01	SSA	72.31±0.16^{b}	-2.29±0.15^{a}	8.04±0.24^{ab}	71.07±0.21^{b}
		SA	71.51±0.14^{v}	-2.02±0.08^{uv}	6.94±0.11^{uv}	70.61±0.14^{v}
	0.02	SSA	72.19±0.51^{b}	-2.27±0.11^{a}	7.81±0.26^{ab}	71.02±0.54^{b}
		SA	71.64±0.64^{v}	-2.05±0.02^{v}	7.10±0.09^{v}	70.69±0.65^{v}
	0.05	SSA	73.83±0.44^{c}	-2.08±0.02^{b}	8.34±0.14^{b}	72.45±0.43^{c}
		SA	73.39±0.25^{w}	-2.01±0.01^{v}	7.37±0.08^{w}	72.32±0.25^{w}
	0.1	SSA	76.54±0.56^{d}	-1.96±0.03^{b}	8.41±0.11^{b}	75.00±0.56^{d}
		SA	75.55±0.52^{x}	-1.81±0.04^{w}	7.33±0.10^{w}	74.41±0.52^{x}

Mean±SD from triplicate determinations

The different superscripts in the same column within the same grade surimi in the same whitening agent denote the significant differences (p<0.05)

Ratio of bigeye snapper grade SSA to mackerel was 70:30

Ratio of bigeye snapper grade SA to mackerel was 50:50

Table 23 Solubility of mixture between SSA or SA grade bigeye snapper surimi and mackerel surimi gel added with various whitening agents.

Samples	Solubility(%)
SSA:Black (70:30)+ 2.5% NaCl	83.26±0.25[a]
SSA:Black (70:30)+ 5% Oil	87.33±0.15[b]
SSA:Black (70:30)+ 1.5% Calcium carbonate	81.23±0.05[c]
SSA:Black (70:30)+ 0.1% Titanium dioxide	83.00±0.11[d]
SA:Black (50:50)+ 2.5% NaCl	85.32±0.14[a]
SA:Black (50:50)+ 5% Oil	90.01±0.34[b]
SA:Black (50:50)+ 1.5% Calcium carbonate	83.90±0.32[c]
SA:Black (50:50)+ 0.1% Titanium dioxide	84.84±0.12[d]

Mean±SD from triplicate determinations

The different superscripts in the same column within the same grade surimi denote the significant differences ($p<0.05$)

Thermal denaturation

In order to determine thermal denaturation of surimi proteins as affected by various whitening agents, surimi sols added with whitening agents were subjected to DSC analysis. T_{max} and enthalpy of surimi with lower grade were lower than those of surimi with higher grade. Salt addition reduced both myosin and actin enthalpies. Beas *et al.* (1991) found that myofibrillar protein from pre and post-spawning hake added with NaCl had an important drop of denaturation enthalpy and T_{max} also shifted to lower temperatures. T_{max} of peak 1 of mixed surimi for both grades was shifted to lower temperature when oil was added. With addition of calcium carbonate, reduction of T_{max} was found in mixed surimi (SSA grade). However, no changes in T_{max} were found with the addition of titanium dioxide. From the result, no marked change in T_{max} of peak 2 were found with addition of whitening agents. The similar trend was noted between the

changes in T_{max} and enthalpy. The peak with T_{max} of 49.00-50.33 was presumed to be myosin peak, whereas that with T_{max} of 70.75 was postulated to be actin peak. For intact muscle, peak 1 is believed to arise from a myosin transition and peak 2 is from actin transition (Stabursvik and Martens 1980; Akahane *et al.*, 1984; Davies *et al.*, 1988).

Microstructure of gels from mixed surimi added with 1.5% calcium carbonate, 5% vegetable oil and 0.1% titanium dioxide (Figure 20) are illustrated, in comparison with gels without any whitening agents. In general, fibrous structure was observed for both mixed surimis. However, mixed surimi from SA grade had a larger void, compared with that from SSA grade surimi. Larger particles of calcium carbonate were found to be dispersed uniformly in the gel matrix, compared with smaller particle of titanium dioxide. For oil droplet, spherical shape was observed continuously in the gel. However, varied sizes of droplet were found. This droplet was contributable to light scattering, leading to whitening of gel. Therefore, different whitening agents resulted in different whiteness of surimi gel, due to the different size, shape and surface properties of those compounds in the way, which reflect the light.

Table 24 T_{max} and enthalpy of mixture between SSA or SA grade from bigeye snapper surimi and mackerel surimi added with various whitening agents

Samples	Peak 1		Peak 2	
	T_{max} (°C)	ΔH (J/g)	T_{max} (°C)	ΔH (J/g)
SSA:Black (70:30)	50.33±0.47^{b}	0.152±0.03^{a}	70.75±0.12^{e}	0.029±0.01^{a}
SSA:Black (70:30)+ 2.5 % NaCl	50.74±0.11^{b}	0.036±0.04^{b}	64.83±0.47^{b}	0.192±0.01^{b}
SSA:Black (70:30)+ 5% Oil	45.72±0.00^{a}	0.030±0.01^{c}	65.00±0.94^{b}	0.147±0.03^{c}
SSA:Black (70:30)+ 1.5% $CaCO_3$	46.74±0.11^{a}	0.072±0.10^{d}	64.42±0.83^{b}	0.244±0.06^{d}
SSA:Black (70:30)+ 0.1% TiO_2	50.06±2.03^{b}	0.035±0.08^{e}	65.08±0.12^{b}	0.180±0.12^{e}
SA:Black (50:50)	49.00±0.71^{a}	0.147±0.05^{a}	70.75±0.59^{a}	0.245±0.02^{a}
SA:Black (50:50)+ 2.5% NaCl	46.67±0.24^{b}	0.027±0.10^{b}	65.25±0.59^{b}	0.137±0.03^{b}
SA:Black (50:50)+ 5% Oil	43.17±0.94^{b}	0.020±0.04^{c}	65.67±0.71^{b}	0.209±0.12^{c}
SA:Black (50:50)+ 1.5% $CaCO_3$	46.33±0.00^{b}	0.059±0.20^{d}	65.79±0.42^{b}	0.165±0.01^{d}
SA:Black (50:50)+ 0.1% TiO_2	47.00±0.47^{b}	0.020±0.01^{c}	65.42±0.12^{b}	0.142±0.05^{e}

Mean ±SD from triplicate determinations

The different superscripts in the same column within the same grade surimi denote the significant differences (p<0.05)

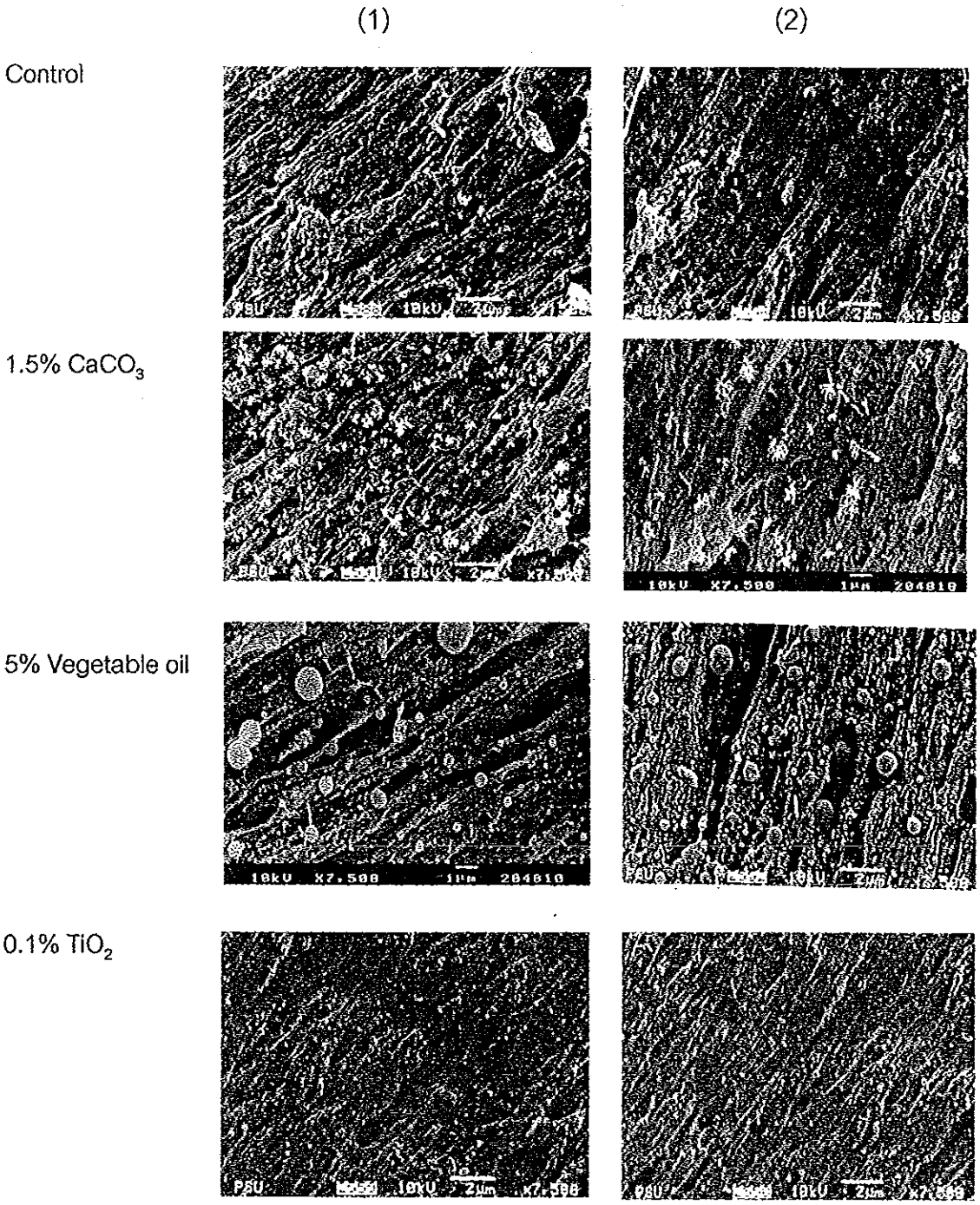


Figure 20 Microstructure of surimi gels containing various whitening agents

(1) mixed surimi between SSA grade bigeye snapper surimi and mackerel surimi (70: 30)

(2) mixed surimi between SA grade bigeye snapper surimi and mackerel surimi (50: 50)

Chapter 4

Conclusions

1. Chemical composition and gelling property of bigeye snapper surimi were different, depending on grade of surimi and the quality of raw material. Surimi with lower grade or produced from unfresh fish had the lower quality than high grade or surimi produced from fresh fish.
2. Much higher force and deformation were observed with ashi gel, compared to other gels. Modori had the lowest quality gel. The properties of directly cooked gel were generally poorer than those with prior setting.
3. Addition of protein additives, calcium salts and MTGase could effectively improve bigeye snapper surimi gel. The combination use of 4% EW, 50 mmole/kg calcium gluconate and 0.1% MTGase could be a potential means to improve the gel forming ability. The optimum setting time for both grade surimis containing combined additives was 30 min.
4. Titanium dioxide was very promising whitening agent for mixture between bigeye snapper surimi and mackerel surimi since it had no detrimental effect on gel forming ability, while oil reduced the gel strength ever though the whiteness could be improved. However, calcium carbonate did not show the marked influence on whiteness improvement though it could increase the gel strength to some extent.

References

A.O.A.C. 1999. Official Method of analysis 15th ed. Association of Agricultural Chemists. Washington, DC.

Akahane, Y. and Shimizu, Y. 1990. Effect of setting incubation on the water-holding capacity of salt-ground fish meat and its heated gel. Nippon Suisan Gakkaishi. 56: 139-143.

Akazawa, H., Miyauchi, Y., Sakurada, K., Wasson, K.H. and Reppond, K.D. 1993. Evaluation of proteinase inhibitors in Pacific whiting surimi. J. Aquat. Food Prod. Technol. 2 (3): 79-95.

Alvarez, C. and Tejada, M. 1997. Influence of texture of suwari gels on kamaboko gels made from sardine (*Sardina poilchardus*) surimi. J. Sci. Food Agric. 75: 472-480.

Alverez, C., Couso, I. and Tejada, M. 1995. Sardine surimi gels as affected by salt concentration, blending heat treatment and moisture. J. Food Sci. 60 : 622-626.

Alvarez, C., Couso, I. and Tejada, M. 1999a. Microstructure of suwari and kamaboko sardine surimi gels. J. Sci. Food Agric. 79: 839-844.

Alvarez, C., Couso, I. and Tejada, M. 1999b. Thermal gel degradation (modori) in sardine surimi gels. J. Food Sci. 64: 633-637.

An, H., Peters, M.Y. and Seymour, T.A. 1996. Role of endogenous enzymes in surimi gelation. Trends Food Sci. Technol. 7: 320-327.

An, H., Weerasinghe, V., Seymour, T.A. and Morrissey, M.T. 1994. Cathepsin degradation of Pacific whiting surimi proteins. J. Food Sci. 59: 1013-1017.

Ando, H., Adachi, M., Umead, K., Matsuura, A., Nonaka, M., Uchio, R., Tanaka, H. and Motoki, M. 1989. Purification and characteristics of novel transglutaminase derived from microorganisms. J. Agric. Biol. Chem. 53: 2613-2617.

Arai, K., Kawamura, K. and Hayashi, C. 1973. The relative thermostabilities of the actomyosin ATPase from the dorsal muscles of various fish species. Bull. Jap. Soc. Sci. Fish. 39: 1077-1082.

Araki, H. and Seki, N. 1993. Comparison of reactivity of transglutaminase to various fish actomyosins. Nippon Suisan Gakkaishi. 95: 711-716.

Asagami, T., Ogiwara, M., Wakameda, A. and Noguchi, S. 1995. Effect of microbial transglutaminase on the quality of frozen surimi made from various kinds of fish species. Fish. Sci. 61: 267-272.

Ashie, I.N.A. and Lanier, T.C. 2000. Transglutaminase in seafood processing. In Haard, N.F. and Simpson, B.K. (eds.), Seafood enzymes utilization and influence on postharvest seafood quality, pp. 147-166. New York: Marcel Dekker.

Bandarra, N.M., Batista, M.L., Nunes, M.L., Empis, J.M. and Chistie, W.W. 1997. Seasonal changes in lipid composition of sardine (*Sardina pilchardus*). J. Food Sci. 62: 40-42.

Beas, V.E., Wagner, J.R., Anon, M.C. and Crupkin, M. 1991. Thermal denaturation in fish muscle proteins during gelling : effect of spawning condition. J. Food Sci. 56: 281-284.

Benjakul, S. and Visessanguan, W. 2000. Pig plasma protein: potential use as proteinase inhibitor for surimi manufacture; inhibitory activity and the action components. J. Sci. Food Agric. 80: 1351-1356.

Benjakul, S., Visessanguan, W and Srivilai, C. 2001. Porcine plasma protein as proteinase inhibitor in bigeye snapper (*Priacanthus tayenus*) muscle and surimi. J. Sci. Food Agric. 81: 1039-1046.

Benjakul, S., Leelapongwattana, K. and Vissessanguan, W. 2002. Comparative study on proteolysis of two species of bigeye snapper, Priacanthus macracanthus Priacanthus tayenus. J. Sci. Food Agric. (Inreview)

Boye, S.M. and Lanier, T.C. 1988. Effect of heat stable alkaline protease activity of Atlantic menhaden (*Brevoorti tyrannus*) on surimi gels. J. Food Sci. 53: 1340-1342, 1398.

Carache, M., Alvarez, C. and Tejada, M. 1995. Suwari and kamaboko sardine gels: effect of heat treatment on solubility of networks. J. Agric. Food Chem. 43: 1002-1010.

Chan, J.K. and Gill, T.A. 1994. Thermal aggregation of mixed fish myosins. J. Agric. Food Chem. 42: 2649-2655.

Chan, J.K., Gill, T.A. and Paulson, A.T. 1992. Cross-link ability of myosin heavy chains from cod, herring and silver hake during thermal setting. J. Food Sci. 57: 906.

Chang-Lee, M.V., Lamila, L.E. and Crawford, D.L. 1990. Yield and composition of surimi from Pacific whiting (*Merluccias Productus*) and the effect of various protein additive on gel strength. J. Food Sci. 55: 83-86.

Chang-Lee, M.V., Pacheco-Aguilar, R., Lamila, L.E. and Crawford, D.L. 1989. Proteolytic activity of surimi from Pacific whiting (*Merluccias Productus*) and heat set texture. J. Food Sci. 54: 1116-1119.

Chen, H.H. 1995. Thermal stability and gel-foaming ability of shark muscle as related to ionic strength. J. Food Sci. 60: 1237-1240.

Chen, H.H., Chui, E.M. and Huang, J.R. 1997. Color and gel-forming properties of horse mackerel (*Trachurus japoricus*) as related to washing conditions. J. Food Sci. 62: 985-990.

Ching, H.L. and Linot, A. 1993. Surimi composition and technology: present status and nutritional considerations. Int. J. Food Sci. Nutri. 44: 555-563.

Connell, J.J. 1961. The relative stabilities of the skeletal muscle myosin of some animals. J. Biochem. 80: 503-509.

Conway, E. J. and Byrne, A. 1936. An absorption apparatus for the micro-determination of certain volatile substances. The micro-determination of ammonia. Biochem. J. 27: 413-423.

Davies, J.R., Bardsley, R.O. and Ledward, D.A. 1988. Myosin thermal stability in fish muscle. J. Sci. Food Agric. 45: 61-68.

Garcia-Carreno, F. and Hernandez-Cortez, P. 2000. Use of protease inhibitors in seafood products. In Haard, N.F. and Simpson, B.K. (eds.), Seafood Enzymes, pp. 531-548. New York: Marcel Dekker.

Gerber, U., Jucknischke, U., Putzein, S. and Fuchsbauer, H.L. 1994. A rapid and simple method for the purification of transglutaminase from *Streptoverticillium mobaraense*. J. Biochem. 299: 825-829.

Gill,T.A., Chan, J.K., Phonchareon, K.F. and Paulson, A.T. 1992. Effect of salt concentration and temperature heat-induced aggregation and gelation of fish myosin. Food Res. Int. 25: 333-341.

Greene, D.H. and Babbitt, J. 1990. Control of muscle softening and proteinase parasite interactions in arrowtooth flounder (*Atheresthes stomias*). J. Food Sci. 55: 579-580.

Govardus, A.H de Jong, Wijngaards, G., Boumans, H., Koppelman, S.J. and Hessing, M. 2001. Purification and substrate specificity of transglutaminases from blood and *Streptoverticillium mobaraense*. J. Agric. Food Chem. 49: 3389-3393.

Hamann, D.D., Amato, P.M., Wu, M.C. and Foegeding, E.A. 1990. Inhibition of modori (gel weakening) in surimi by plasma hydrolyzate and egg white. J. Food Sci. 55: 665-669.

Harpel, P.C. and Brower, M.S. 1983. α_2-Macroglobulin: an introduction. In (Boland, B., Hitchcock, J. and Scholnick, R. eds.), Chemistry and Biology of α_2-Macroglobulin, pp. 1-9. The New York Academy of Science. New York.

Hashimoto, K., Watabe, S., Kono, M. and Shiro, K. 1979. Muscle protein composition of sardine and mackerel. Bull. Jap. Soc. Sci. Fish. 45: 1435-1441.

Hayat, M.A. 1981. Fixation for electron microscopy. In Harris, J. R. (ed.), Electron Microscopy in Biology, pp. 1-14. New York: Oxford University Press.

Hebard, C.E., Flick, G.J. and Martin, R.E. 1982. Occurrence and significance of trimethylamine oxide and its derivatives in fish and shellfish. . In Martin, R.E., Flick, G.J., Hebard C.E. and Ward, D.R. (eds.), Chemistry and Biochemistry of Marine Food Products, pp. 149-304. Westport, CT: AVI Publ Comp.

Henningar, C.J., Buck, E.M., Hultin, H.O., Peleg, M. and Vaeltzis, K. 1988. Effect of washing and sodium chloride on mechanical properties of fish muscle gels. J Food Sci. 53: 963-964.

Huang, Y.P., Seguro, K., Motoki, M. and Tawada, K. 1992. Cross-linking of contractile proteins from skeletal muscle by treatment with microbial transglutaminase. J. Biochem. 112: 229-234.

Hultin, H.O. and Kelleher, H.D. 2000. Processing from dark muscle fish. In Park, J.W. (ed.), Surimi and Surimi Seafood, pp. 59-78. New York: Marcel Dekker.

Ishikawa, S. 1978. Fish jelly product (kamaboko) and frozen minced meat (frozen surimi) made of sardine-II. Influence of the temperature during the manufacturing process on the jelly strength of kamaboko. Bull. Tokai Reg. Fish Res. Lab. 94: 37-44.

Itoyori, K. 1992. The situation of global surimi, with special emphasis on the Japanese market. Pacific Whiting: Harvesting, Processing, Marketing and Quality Assurance. Oregon Sea Grant, Corvallis, OR.

Iwata, K., Kobashi, K. and Hase, J. 1974. Studies on muscle alkaline protease III. Distribution of alkaline protease in muscle of fresh water fish, marine fish and in I nternal organs of carp. Bull. Jap. Soc. Sci. Fish. 40: 201-209.

Jiang, S.H., Ho, M.L., Jiang, S.H., Lo, L. and Chen, H.C. 1998. Color and quality of mackerel surimi as affected by alkaline washing and ozonation. J. Food Sci. 63: 652-655.

Jiang, S.T., Hsieh, J.F., Ho, M.L. and Chung, Y.C. 2000a. Microbial transglutaminase affects gel properties of golden threadfin-bream and pollack surimi. J. Food Sci. 65: 694-699.

Jiang, S.T., Hsieh, J.F., Ho, M.L. and Chung, Y.C. 2000b. Combination effects of microbial transglutaminase, reducing agent, and protease inhibitor on the quality of hairtail surimi. J. Food. Sci. 65: 241-245.

Jiang, S.T., Leu, S.Z. and Tsai, G.J. 1998. Cross-linking of mackerel surimi actomyosin by microbial transglutaminase and ultraviolet irradiation. J. Agric. Food. Chem. 46: 5278-5282.

Kamath, G.G., Lanier, T.C., Foegeding, E.A. and Hamann, D.D. 1992. Nondisulfide covalent cross-linking of myosin heavy chain in setting of Alaska pollock and Atlantic croaker surimi. J. Food Biochem. 16: 151-172.

Kang, I.S. and Laneir, T.C. 1999. Bovine plasma protein functions in surimi gelation compared with cysteine protease inhibitors. J. Food Sci. 34: 842-846.

Kang, I.S. and Lanier, T.C. 1999. Bovine plasma protein functions in surimi gelation compared with cysteine protease inhibitors. J. Food Sci. 64: 842-846.

Kano, I. 1992. Tropical surimi. INFOFISH international. 1: 21-25.

Kato, H., Nagasawa, S., Iwanaga, S. 1981. HMW and LMW kininogens. Methods Enzymol. 80: 172-198.

Kelleher, S.D., Hultin, H.O. and Wilhelm, K.A. 1994 Stability of mackerel surimi prepared under lipid-stabilizing processing conditions. J. Food Sci. 59: 269-271.

Kim, J.M., Hamann, D.D., Lanier, T.C. and Wu, M.C. 1996. Effects of freeze-thaw abuse on the viscosity and gel-forming properties of surimi from two species. J. Food Sci. 51: 951-956.

Kim, J.M., Lui, C.H., Eun, J.B., Park, J.W., Oshimi, R., Hayashi, K., Ott, B., Aramaki, T. 1996. Surimi from fillet frames of channel catfish. J. Food Sci. 61: 428-431.

Kishi, H., Nozawa, H. and Seki, N. 1991. Reactivity of muscle transglutaminase on carp myofibrils and myosin B. Nippon Suisan Gakkaishi. 57: 1203-1210.

Kolakowski, E., Kolakowski, A., Lachowicz, K., Bortnowska, G. and Wianecki, M. 1994. The use of sodium carbonates to improve textural properties of cod minces. J. Sci. Food Agric. 66: 429-437.

Kumazawa, Y., Numazawa, T., Seguro, K. and Motoki, M. 1995. Suppression of surimi gel setting by transglutaminase inhibitors. J. Food Sci. 60: 715-717.

Laneir, T.C. 2000. Surimi gelation chemistry. In Park, J.W. (ed.), Surimi and Surimi Seafood, pp. 343-349. New York: Marcel Dekker.

Laneir, T.C., Lin, T.S., Hamann, D.D. and Thomas, F.B. 1981. Effects of alkaline protease in mince fish on texture of heat-processed gels. J. Food Sci. 47: 1643-4649.

Lanier, T.C., Hart, K. and Martin, R.E. 1991. A manual of standard methods for measuring and specifying the properties of surimi. U. North Carolina Sea Grant College Program. Raleigh, NC. 1495-1540.

Lauro, G.J. 2000. Natural colorants for surimi seafood. In In Park, J.W. (ed.), Surimi and Surimi Seafood, pp. 417-444. New York: Marcel Dekker.

Lee, C.M. 1984. Surimi process technology. Food Technol. 38 (11): 69-80.

Lee, C.M. 1986. Surimi manufacturing and fribrication of surimi-based products. Food Technol. 40 (2): 115-118.

Lee, J.J., Tzeng, S.S. and Jiang, S.T. 2000. Purification and characterization of low molecular weight kininogen from pig plasma. J. Food Sci. 65: 81-86.

Lee, J.J., Tzeng, S.S., Wu, J. and Jiang, S.T. 2000. Inhibitions of thermal degradation of mackerel surimi by pig plasma protein and L-kininogen. J. Food Sci. 65: 1124-1129.

Lee, N.G. and Park, J.W. 1998. Calcium compounds to improve gel functionality of Pacific whiting and Alaska pollock surimi. J. Food Sci. 63: 969-974.

Lee, N.H., Seki, N., Kato, N., Nakagawa, S., Terui, S. and Arai, K. 1990. Gel forming ability and cross-linking ability of myosin heavy chain in salted meat paste from threadfin bream. Nippon Suisan Gakkaishi. 56: 329-336.

Lin, D. and Morrissey, M.T. 1995. Northern squawfish (*Ptychocheilus oregonensis*) for surimi production. J. Food Sci. 60: 1245-1247.

Lin, T.M. and Park, J.W. 1996. Protein solubility in Pacific whiting affected by proteolysis during storage. J. Food Sci. 61: 536-539.

Lin, T.M. and Park, J.W. 1997. Effective washing conditions reduce water for surimi processing. J. Aquat Food Product Technol. 6: 65-79.

Lou, Y.K., Kuwahara, R., Kaneniwa, M., Murata, Y. and Yokoyama, M. 2001. Comparison of gel properties of surimi from Alaska Pollock and three freshwater fish species: effects of thermal processing and protein concentration. J. Food Sci. 66: 548-554.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265-275.

Masaki, T., Shimomukai, M., Miyaushi, Y., Ono, S., Tuchiya, T., Mastuda, T., Akazawa, H. and Soejima, M. 1993. Isolation and characterization of protease responsible for jellification of Pacific hake muscle. Nippon Suisan Gakkaishi. 59: 683-690.

Matsumoto, J.J. 1959. Identity of M-actomyosin from aqueous extract of the squid muscle with the actomyosin-like protein from salt extract. Bull. Jap. Soc. Sci. Fish. 25: 38-43.

Meacock, G., Taylor, K.D.A., Knowles, M.J. and Himonides, A. 1997. The improved whitening of minced cod flesh using dispersed titanium dioxide. J. Sci. Food Agric. 73: 221-225.

Morrisey, M.T. and Tan, S-M. 2000. World resources for surimi. In Park, J.W. (ed.), Surimi and Surimi Seafood, pp. 1-58. New York: Marcel Dekker.

Morrissey, M.T., Wu, J.W., Lin, D. and An, H. 1993. Protease inhibitor effects on torsion measurement and autolysis of Pacific whiting surimi. J. Food Sci. 58: 1050-1054.

Nakahara, C., Nozawa, H. and Seki, N. 1999. A comparison of cross-linking of fish myofibrillar proteins by endogenous and microbial transglutaminase. Fish. Sci. 65: 138-144.

Niwa, E. 1975. Role of hydrophobic bonding in gelation of fish flesh paste. Bull. Jap. Soc. Sci. Fish. 41: 907-908.

Niwa, E. 1992. Chemistry of surimi gelation. In Lanier, T.C. and Lee, C.M. (eds.), Surimi Technology, pp. 389-428. New York: Marcel Dekker.

Nonaka, M., Tanaka, H., Okiyama, A., Motoki, M., Anso, H., Umeda, K. and Matsukuwa, A. 1989. Polymerization of several proteins by Ca^{2+}-independent transglutaminase derived from microorganisms. Agric. Biol. Chem. 36: 1078-1081.

Nowsad, A.AKM., Katoh, E., Kanoh, S. and Niwa, E. 1994. Setting of transglutaminase-free actomyosin paste from Alaska pollack surimi. Fish. Sci. 60: 295-297.

Nozaki, Y., Kanazu, R. and Tabata, Y. 1978. Freezing storage of lizardfish for kamaboko preparation. Refrigeration. 53: 473-480.

Ogawa, M., Tamiya, T. and Tsuchiya, T. 1994. Structural changes of carp myosin during heating. Fish. Sci. 60: 723-727.

Ohshima T., Suzuki, T., Koizumi, C. 1993. New developments in surimi technology. Trends Food Sci. Technol. 4: 157-163.

Pacheco-Aguilar, R., Crawford, D.L. and Lampila, L.E. 1989. Procedures for the efficient washing of minced whiting (*Miraculous productus*) flesh for surimi production. J. Food Sci. 54: 248-252.

Park, J.W. 1995. Surimi gel colors as affected by moisture content and physical conditions. J. Food Sci. 60 : 15-18.

Park, J.W. 1998. Advanced technology in surimi seafood manufacturing. *The 2nd OSU Surimi Technology School*, Bangkok, 18-20 August 1998, pp. 1-237.

Park, J.W. 2000. Ingredient technology and formulation development. In Park, J.W. (ed.), Surimi and Surimi Seafood, pp. 343-329. New York: Marcel Dekker.

Park, J.W. and Lanier, T.C. 1989. Scanning calorimetric behavior of tilapia myosin and actin due to processing of muscle and protein denaturation. J. Food Sci. 54: 49-51.

Park, J.W. and Morrissey, M.T. 1994. The need for developing surimi standard. Quality Assurance and Quality Control for Seafood. (Sylvia, G. and Morrissey, M.T. eds.). Corvallis, OR. Oregon Sea Grant.

Samejima, K., Ishioroshi, M. and Yasui, T. 1981. Relative roles of the head and tail portions of the molecule in heat induced gelation of myosin. J. Food Sci. 46: 1412-1416.

Sano, T., Ohno, T., Fuchino, H.O., Matsumoto, J. and Tsuchiya, T. 1994. Carp natural actomyosin: thermal denaturation mechanism. J. Food Sci. 59: 1002-1008.

Sareevoravitkul, R., Simpson, B.K. and Ramaswamy, H. 1996. Effect of crude α_2-macroglobulin on properties of bluefish (*Pomatomus saltatrix*) gels prepared by high hydrostatic pressure and heat treatment. J. Food Biochem. 20: 49-63.

Seguro, K., Kumazawa, Y., Ohtsuka, T., Toiguchi, S. and Motoki, M. 1995. Microbial transglutaminase and ε-(γ-glutamyl) lysine crosslink effects on elastic properties of kamaboko gels. J. Food Sci. 60: 305-311.

Seki, N., Uno, H., Lee, N., Kimura, I., Toyoda, K., Fujita, T. and Arai, K. 1990. Transglutaminase activity in Alaska pollack muscle and surimi and its reaction with myosin B. Nippon Suisan Gakkaishi. 56: 126-128.

Seymour, T.A., Morissey, M.T., Peter, M.Y. and An, H. 1997. Surimi gel enhancement by bovine plasma proteins. J. Agric. Food Chem. 42: 2421-2427.

Seymour, T.A., Morrissey, M.T., Peters, M.Y. and An, H. 1994. Purification and characterization of Pacific whitening protease. J. Agric. Food Chem. 42: 24-27.

Shimizu, Y. 1985. Biochemical and functional properties of material fish. In W.A., Seattle, W.A., Martin, R.E. and Collete, R.L. (eds.), Proceedings of the International Symposium on Engineered Seafood Including Surimi, Nov 19-21, pp. 148-167. Washington DC: National Fish. Institute.

Shimizu, Y., Machida, R. and Takenami, S. 1981. Species variations in the gel-forming characteristics of fish meat paste. Nippon Suisan Gakkaishi. 47: 95-104.

Shimizu, Y., Toyohara, H. and Lanier, T.C. 1992. Surimi production from fatty and dark-flesh fish species. In Lanier, T.C. and Lee, C.M. (eds.), Surimi Technology, pp. 181-207. New York: Marcel Dekker.

Shults, G.W., Russell, D.R., Wierbicki, E. 1972. Effect of condensed phosphate on pH, swelling and water-holding capacity of beef. J. Food Sci. 37: 860-864.

Soeda, T., Toiguchi, S., Mumazawa, T., Sakaguchi,S. and Kuhara, C. 1996. Studies on the functionalities of microbial transglutaminase for food processing. II. Effects of microbial transglutaminase on the texture of gel prepared from suketoudara surimi. J. Jap. Soc. Food Sci. Tech. 43: 780-786.

Stabursvik, E. and Marten, H. 1980. Thermal denaturation of proteins in post rigor muscle tissue as studied by differential scanning calorimetry. J. Sci. Food Agric. 31: 1034-1042.

Steel, R.D.D. and Torrie, J.H. 1980. Principle and procedure of statistics: a biometrical, end ed. McGraw-Hill, New York.

Suzuki, T. 1981. Fish and krill proteins, processing technology. p. 1-61. Applied Science: London.

Suzuki, T. and Watabe, S. 1987. New processing technology of small pelagic fish protein. Food Res. Int. 2: 271-307.

Tejada, M., Borderias, A.J. and Moral, A. 1981. Effects of washing of horse mackerel (*Trachurustrachurus L.*) minces on the removal of substances detrimental to preservation in cold storage. Refrigeration Sci. Technol. 4: 371-375.

Toyoda, K., Kimura, I., Fujita, I., Noguchi, S.F. and Lee, C.M. 1992. The surimi manufacturing process. In Lanier, T.C. and Lee, C.M. (eds.), Surimi Technology, pp. 79-112. New York: Marcel Dekker.

Toyohara, H. and Shimizu, Y. 1988. Relation between the modori phenomenon and myosin heavy chain breakdown in threadfin-bream gel. Agric. Biol. Chem. 52: 255-257.

Toyohara, Y., Sakata, T., Yamashita, K., Kinishita, M. and Shimizu, Y. 1990. Degradation of ovalfilefish meat gel caused by myofibrillar proteinase. J. Food Sci. 55: 364-368.

Tsai, G.J., Lin, S.M. and Jiang, S.T. 1996a. Transglutaminase from *Streptoverticillium ladakanum* and application to minced fish product. J. Food Sci. 61: 1234-1238.

Tsai, G.J., Lin, S.M. and Jiang, S.T. 1996b. Screening the microorganism and some factors for the production of transglutaminase. J. Chinese Agric. Chem. Soc. 34: 228-240.

Tsukamasa, Y., Sato, K., Shimizu, Y., Imai, C., Sugiyama, M., Minegishi, Y. and Kaqabata, M. 1993. ε-(γ-glutamyl) lysine crosslink formation in sardine myofibril sol during setting at 25^0C. J. Food Sci. 59: 785-787.

Turpeinen, U., Syvanen, A.C. and Hamberg, U. 1981. Kininogen molecular heterogeneity by affinity chromatography on concanavalin A and *Ricinus communis* lectins. FEBS lett. 131: 137-142.

Wan, J., Kimura, I. and Seki, N. 1995. Inhibitory factors of transglutaminase in salted salmon meat paste. Fish. Sci. 61: 968-972.

Wan, J., Kimura, I., Satake, M. and Seki, N. 1994. Effect of calcium ion concentration on the gelling properties and transglutaminase activity of walleye pollack surimi paste. Fish. Sci. 60: 107-114.

Weerasinghe, V.C., Morrissey, M.T. and An, H. 1995. Characterization of active components in food-grade protease inhibitor for surimi manufacture. J. Agric. Food Chem. 44: 2584-2590.

Weerasinghe, V.C., Morrissery, M.T., Chung, C.Y. and An. H. 1996. Whey protein concentrate as a proteinase inhibitor in Pacific whiting surimi. J. Food Sci. 61: 367-371.

White, A., Handler, P. and Smith, E. L. 1973. Principles of Biochemistry. McGraw-Hill Book Company. New York.

Wicker, L., Lanier, T.C., Hamann, D.D. and Akahane, T. 1986. Thermal transitions in myosin-ANS fluorescence and gel rigidity. J. Food Sci. 51: 1540-1543.

Wierrbicki, E. and Deatherage, F.E. 1985. Denaturation of water holding capacity of fresh meats. J. Agric. Food Chem. 58: 387-392.

Wu, Y.J., Atallah, M.T. and Hultin, H.O. 1991. The properties of washed, minced fish muscle have significant solubility in water. J. Food Biochem. 15: 209-218.

Xiong, Y.L. and Brekke, C.J. 1989. Changes in protein solubility and gelation properties of chicken myofibrils during storage. J. Food Sci. 54: 1141-1146.

Yamamoto, Y., Okubo, T., Hatayama, S., Naito, M. and Rbizu, T. 1991. Frozen surimi product and process for preparing. US patent. 5,028,444.

Yamazawa, M. 1990. Effect of high temperature heating on physical properties of kamaboko gel. Nippon Suisan Gakkaishi. 56. 497-503.

Yasui, A. and Lim, P.Y. 1987. Changes in chemical and physical properties of lizardfish meat during ice & frozen storage. Nippon Shokuhin Kyogo Gakkaishi. 34: 54-60.

Yasuyuki, T., Sato, K., Shimizu, Y., Imai, C., Sugiyama, M., Minegishi, Y. and Kawabata, M. 1993. ε-(γ-glutamyl) lysine crosslink formed in sardine myofibril sol during setting at 25^{0}C. J. Food Sci. 58: 785-787.

Yongsawatdigul, J., Park, J.W., Kolbe, E., Dagga, Y.A. and Morrissey, M.T. 1995. Ohimic heating maximizes gel functionality of Pacific whiting surimi. J. Food Sci. 60: 10-14.

Zeigler, G.R. and Acton, J.C. 1984. Machanism of gel forming by proteins of muscle tissue. Food Technol. 38 (5): 77-82.

Appendix 1

Analytical Methods

Moisture content (AOAC, 1991)

Method

1. Dry the empty dish and lid in the oven at 105^{0}C for 30 min and transfer to desiccator to cool (30 min). Weigh the empty dish and lid.
2. Weigh about 5 g of sample to the dish. Spread the sample with spatula.
3. Place the dish with sample in the oven. Dry for 16 h. or overnight at 105^{0}C.
4. After drying, transfer the dish with partially covered lid to the desiccator to cool. Reweigh the dish and its dried content.

Calculation

$$\% \text{ Moisture} = \frac{(W_1 - W_2) \times 100}{W_1}$$

Where: W_1 = weight (g) of sample before drying

W_2 = weight (g) of sample after drying

Ash (AOAC, 1991)

Method

1. The crucible and lid is first placed in the furnace at 550^{0}C overnight to ensure that impurities on the surface or crucible is burn off. Cool the crucible in the desiccator (30 mins).
2. Weigh the crucible and lid to 3 decimal places.
3. Weigh about 5 g sample into the crucible. Heat over low bunsen flame with lid half covered. When fumes are no longer produced, place crucible and lid in furnace.

4. Heat at 550^{0}C overnight. During heating, do not cover with the lid. Place the lid on after complete heating to prevent loss of fluffy ash. Cool down in the desiccator.
5. Weigh the ash with crucible and lid until turning to gray. If not, return the crucible and lid to the furnace for further ashing.

Calculation

$$\% \text{ Ash content} = \frac{\text{Weight of ash} \times 100}{\text{Weigh of sample}}$$

Protein (AOAC, 1991)

Reagents

- Kjeldahl catalyst : Mix 9 parts of potassium sulphate (K_2SO_4) anhydrous, nitrogen free with 1 parts of copper sulphate ($CuSO_4$)
- Sulfuric acid (H_2SO_4)
- 40% NaOH solution (w/v)
- 0.02 N HCL solution
- 4% H_3BO_3 solution (w/v)
- Indicator solution : Mix 100 ml of 0.1% methyl red (in 95% ethanol) with 200 ml of 0.2% bromocresol green (in 95% ethanol)

Method

1. Place sample (0.5-1.0 g) in digestion flask.
2. Add 5 g of kjeldahl catalyst, and 20 ml of conc. H_2SO_4.
3. Prepare a tube containing the above chemical except sample as blank. Place flasks in inclined position and heat gently until frothing ceases. Boil briskly until solution clears.
4. Cool and add 60 ml distilled water cautiously.
5. Immediately connect flask to digestion bulb on condenser, and with tip of condenser immersed in standard acid and 5-7 indicator in receiver. Rotate flask to mix content thoroughly; then heat until all NH_3 has distilled

6. Remove receiver, wash tip of condenser, and titrate excess standard acid in distillate with standard NaOH solution.

Calculation

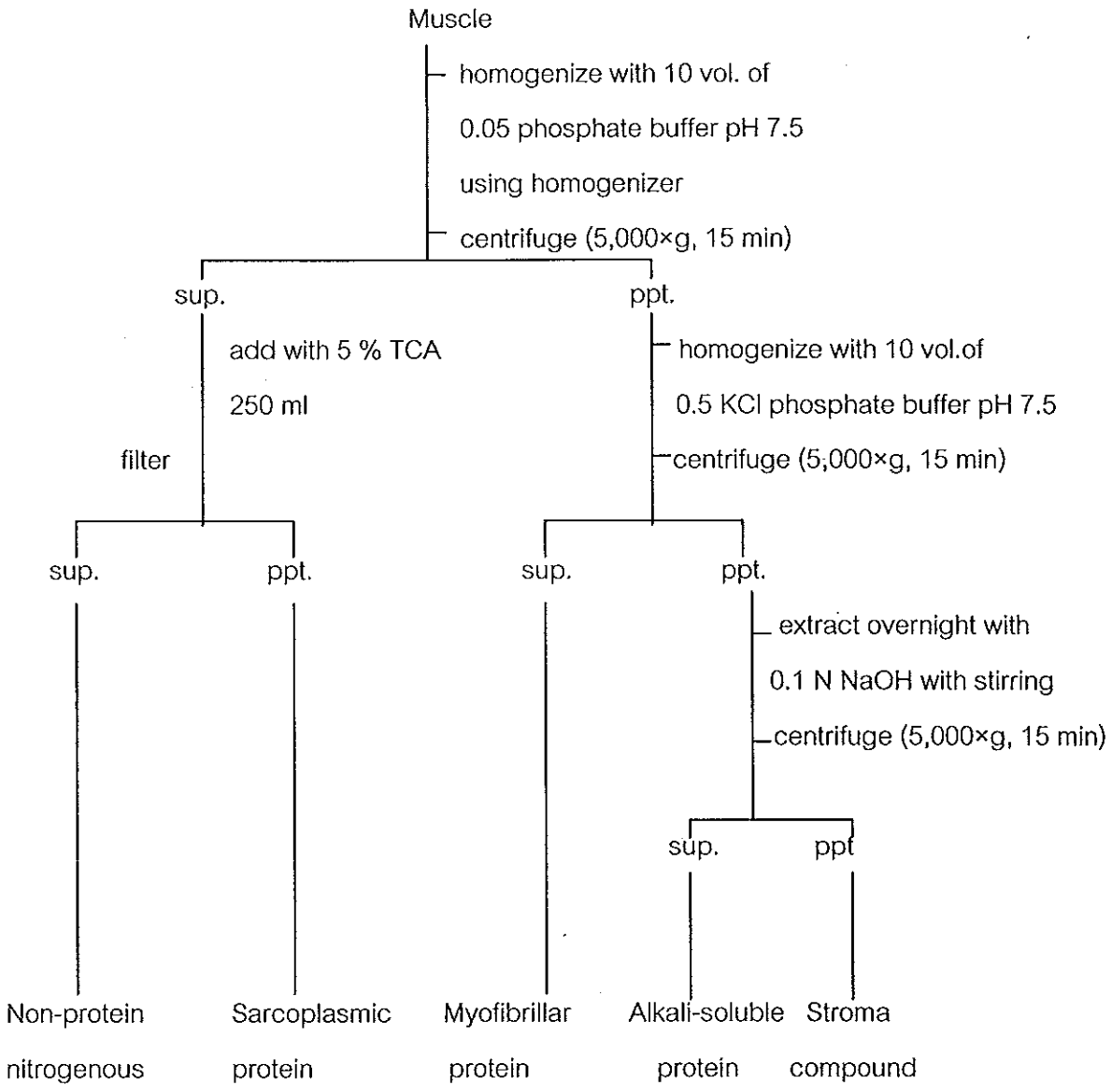
$$\% \text{ Protein} = \frac{(A-B) \times N \times 1.4007 \times 6.25}{W}$$

Where:
- A = volume (ml) of 0.02N HCL used sample titration
- B = volume (ml) of 0.02N HCL used in blank titration
- N = Normality of HCL
- W = weight (g) of sample
- 14.07 = atomic weight of nitrogen
- 6.25 = the protein-nitrogen conversion factor for fish and its by-products

Determination of protein composition (Hashimoto *et al.*, 1979)

Reagents

- 0.05 M phosphate buffer, pH 7.5
- 0.5 M KCl phosphate buffer, pH 7.5
- Trichloroacetic acid
- 0.1N NaOH



pH determination (Benjakul *et al.*, 1997)

Method

1. Weigh 5 g of sample. Add 10 volumes of distilled water (w/v).
2. Homogenize for 20 min.
3. Measure pH using pH meter.

Determination of trimethylamine (TMA-N), total volatile basic nitrogen (TVB-N) by Conway's method (Conway and Byrne, 1936)

Regents

- Inner ring solution (1% boric acid solution containing indicator): Take 10 g of boric acid in 1litre flask, add 200 ml of ethanol. After dissolving boric acid, add 10 ml of mixed indicator solution, then make up to 1 litre with distilled water.
- Mixed indicator solution: Disolve bromocresol green (BCG) 0.01 g and methyl red (MR) 0.02 g in 10 ml of ethanol
- 0.02N HCl : Dilute 20 ml of 1N HCl standard solution with distilled water and make up to 1000 ml.
- Saturated K_2CO_3 solution: Take 60 g of potassium carbonate (K_2CO_3), and add 50 ml of distilled water. Boil gently for 10 min. after cooling down, obtain filtrate through filter paper.
- 50% K_2CO_3 solution: Dilute saturated K_2CO_3 solution twice with distilled water.
- 4% trichloroacetic acid (CCl_3COOH) (TCA) solution: Dissolve 40 g of TCA in 960 ml of distilled water.
- Sealing agent: Take 3 g Trangacanth gum, add 30 ml of distilled water, 15 ml of glycerine and 15 ml of 50% saturated K_2CO_3 solution and mix well.
- Neutralized 10 % formaldehyde solution: Add 10 g of $MgCO_3$ to 100 ml of formaline (35% formaldehyde solution) and shake in order to neutralize the acidity of formaline. Filter and dilute filtrate 3 times with distilled water.

Method

Sample extraction:

1. Take 2 g of fish meat in a mortar and grind well.
2. Add 8 ml of 4% TCA solution and grind well.
3. Stand for 30 min at ambient temperature with occasional grinding.
4. Filter through filter paper (Whatman No. 41) (or centrifuge at 3,000 rpm, for 10 min).

5. Keep the filtrate in –20°C freezer if necessary.

Determination of TVB-N:

1. Apply sealing agent ot Conway's unit.
2. Pipette 1 ml of inner ring solution into inner ring
3. Pipette 1 ml of sample extract into outer ring.
4. Slant the Conway's unit with cover.
5. Pipette 1 ml of saturated K_2CO_3 solution into outer ring.
6. Close the unit.
7. Mix gently.
8. Stand for 60 min at 37°C in incubator.
9. Titrate inner ring solution with 0.02N HCl using a micro-burette until green color turns to pink.
10. Do blank test using 1 ml of 4% TCA instead of sample extract.

Determination of TMA-N:

1. Apply sealing agent to Conway's unit.
2. Pipette 1 ml of inner ring solution into inner ring.
3. Pipette 1 ml of sample extract into outer ring.
4. Pipette 1 ml of neutralized 10% formaldehyde into outer ring.
5. Slant the Conway's unit with cover.
6. Pipette 1 ml of saturated K_2CO_3 solution into outer ring.
7. Close the unit.
8. Mix gently.
9. Stand for 60 min at 37°C in incubator.
10. Titrate inner ring solution with 0.02N HCl using a micro-burette until green color turns to pink.
11. Do blank test using 1 ml of 4% TCA instead of sample extract.

Calculation

$$\text{TMA-N or TVB-N (mg N/100 g)} = \frac{(V_S - V_B) \times (N_{HCl} \times A_N) \times V_E \times 100}{W_S}$$

where: V_S =Titration volume of 0.02N HCl for sample extract (ml)

V_B =Titration volume

N_{HCl} = Normality of HCl (= 0.02 N×f, factor of HCl)

A_N =Atomic weight of Nitrogen (× 14.00)

W_S = Weight of muscle sample (g)

V_E =Volume of 4% TCA ued in extraction

Measurement of breaking force and deformation

Equipment

1. Texture analyzer
2. Computer

Method

Measure breaking force and deformation of the inspection sample of surimi gel with a squeeze stress tester (rheometer). Use a spherical plunger, of which diameter was 5 mm and speed was 60 mm/minute. Remove film off the inspection sample of surimi gel, cut into 25 mm long test specimen and place test specimen on the sample deck of the tester so the center of the test specimen will come just under the plunger. Apply load to the plunger and measure the penetration force in g and the deformation in mm at breakage.

Record the obtained value of the penetration and deformation in mm in g by integral number. Record the obtained value of the deformation in mm to the first decimal place.

Prepare six or more test specimens from the same inspection sample of surimi gel and test each of them. Record the average values obtained thereby.

Measure of color and whiteness

Equipment

1. Color analyzer (CIE Lab)
2. Computer

Method

Temper frozen surimi completely to room temperature (near 25°C). Fill into a 50 ml glass beaker (4 cm diameter, 5.5 cm height) and measure color values of L*, a*, and b* (CIE Lab system) to the first decimal point. Complete contact between the test specimen and the colorimeter measurement port. Measure three samples and record the average value.

$$\text{Whiteness} = 100-\left[(100-L^*)^2+a^{*2}b^{*2}\right]^{0.5}$$

Measurement of expressible moisture (Hasegawa, 1987)

Method

1. Place the muscle sample (Xg) between 2 filter paper on top and three filter paper on the bottom.
2. Increase the pressure to 10 kg/cm^2 within 30 sec.
3. Maintain at 10 kg/cm^2 constant pressure for 2 min, then remove the sample, and weigh the pressed sample (Z g).

Calculation

$$\text{Expressible moisture (\%)} = \frac{(X-Z) \times 100}{X}$$

Measurement of autolytic degradation products (Morrissey *et al.*, 1993; Benjakul *et al.*, 1997)

Reagents

- 5% Trichloroacetic acid (TCA) (w/v)
- Tyrosine

Method

1. Weigh 3 g of fish muscle and homogenize in 27 ml of 5% TCA.
2. Keep on ice for 1 hr, and centrifuge at 5000×g for 5 min.
3. Measure tyrosine in the supernatant as an index of autolytic degradation products and express as μmol tyrosine/g muscle.

Lowry's procedure for qautitation of proteins (with a slight modified Lowry *et al.*, 1951)

Reagents

- A: 2% sodium carbonate in 0.1 N NaOH
- B: 0.5% $CuSO_4.5H_2O$ in 1% sodium citrate
- C: 1 N Folin Phenol reagent
- D: 1 ml reagent B + 50 ml reagent A (or similar ratio)
- Standard reagent: Bovine serum albumin (BSA) at concentration of 1 mg/ml

Method

1. Add 2 ml reagent D to each of the standards and unknown tubes (sample = 200 μl). Vortex immediately.
2. Incubate precisely 10 min at room temperature.
3. Add 0.2 ml reagent C (previously dilute 1: 1 with distilled water) and vortex immediately.
4. Incubate 30 min at room temperature (sample incubated longer than 60 min should be discarded).
5. Read absorbance at 750 nm.
6. Plot standard curve and calculate the unknown.

Standards

Bovine serum albumin (BSA) at concentration of 1 mg/ml (used to determine protein concentration)

Tyrosine at concentration 1 mM (used to determine enzymatic activity)

Standard volumes (μl): 0, 20, 40, 60, 100, 140 and 200

Biuret method quantitation of proteins (Copeland, 1994)

Reagents

- Biuret reagent: Combine 1.50 g $CuSO_4.5H_2O$, 6.00 g sodium potassium tartrate, and 500 ml distilled water in a beaker and stir, add while stirring 300 ml of 10% NaOH (w/v), transfer to a 1 liter volumetric flask and bring to 1 liter with distilled water, transfer to a plastic bottle for storage.
- Distilled water
- Standard reagent: 10 mg/ml bovine serum albumin (BSA)

Method

1. To each of eight disposible cuvette, add the following reagents according to the table.
2. To tubes 6-8, 50 µl of protein sample were added, Mix the contents of each tube well by using the closed end of

the capillary tube as a stirring rod.

3. Add 2.0 ml of the biuret reagent to each tube, and mix well.
4. Incubate the mixture at room temperature for 30-45 min, then read the absorbance of each tube at 540 nm.
5. For tube 1-5, plot the absorbance at 540 nm as a function of effective BSA concentration, and calculate the best fit straight line from data. Then, using the average absorbance for the three sample of unknown, read the concentration of sample from the plot.

Table: Experimental set up for the Biuret's assay.

Tube number	Water (µl)	10 mg/ml BSA (µl)	Effective BSA Concentration (mg/ml)
1	500	0	0
2	400	100	2
3	300	200	4
4	200	300	6
5	100	400	8
6	450	0	unknown
7	450	0	unknown
8	450	0	unknown

Solubility (Roussel and Cheftel, 1990)

Reagents

- Tris-HCL buffer (pH 8.0) containing 1% (w/v) SDS, 8 M urea and 2% (v/v) β–mercaptoethanol
- 0.5 M NaOH
- 50% trichloroacetic acid (TCA)

Method

To a weighed amount (1 g) of sample (mince or gel), cut into small pieces, in a 100 ml conical flask, 20 ml of solvent was added, then homogenize for 1 min. Heat in boiling water bath for 2 min, then incubate at 40^0C for 4 h. All samples were then centrifuged at 12,100 xg for 30 min in a Sorvall-RC2 centrifuge. To 10 ml of the supernatant (soluble fraction), cold 50% (w/v) TCA was added to give a final TCA concentration of 10%. Samples were kept at 0-3^0C for 18 h, centrifuged at 1,000 xg for 20 min in a table-top clinical centrifuge and supernatant was removed. The precipitate was washed with 10% TCA and solubilized in 0.5 M NaOH. Protein content was estimated by the biuret method using BSA as standard.

Samples were also solubilized in 0.5 M NaOH. Protein content in 0.5 M NaOH extract was used as reference value, i.e., 100%.

Electrophoresis (SDS-PAGE) (LeammLi, 1970)

Reagents

- Protein molecular weight standards
- 30% Acrylamide-0.8% bis Acrylamide
- Sample buffer: Mix 30 ml of 10% of SDS, 10 ml of glycerol, 5 ml of β–mercaptoethanol, 12.5 ml of 50 mM Tris-HCL, pH 6.8, and 5-10 mg Bromophenol blue (enough to give dark blue color to the solution). Bring the volume to 100 ml with distilled water. Divide into 1 ml aliquots, and stored at -20^{0}C.
- 2% (w/v) Ammonium persulfate
- 1% (w/v) SDS
- TEMED (N,N,N'N'-tetramethylenediamine)
- 0.5 M Tris-HCL, pH 6.8
- 1.5 M Tris-HCL, pH 8.8
- 0.1 M EDTA
- Electrophoresis buffer: Dissolve 3 g of Tris, 14.4 g of glycine and 1 g of SDS in distilled water. Adjust to pH 8.3. Add water to 1 liter total volume
- Staining solution: Dissolve 0.04 g of Coomassie blue R-250 in 100 ml of methanol. Add 15 ml of glacial acetic acid and 85 ml of distilled water.
- Destaining solution I: 50% methanol-7.5% glacial acetic acid
- Destaining solution II: 5% methanol-7.5% glacial acetic acid

Method

Pouring the seperation gel:

1. Assemble the minigel apparatus according to the manufacture's detailed instructions. Make sure that the glass and other components are rigorously clean and dry before assembly.

2. Mix the separation gel solution by adding, as defined to following Table.
3. Transfer the separating gel solution using a Pasture pipette to the center of sandwich is ~ 1.5 to 2 cm from the top of the shorter (front) glass plate.
4. Cover the top of the gel with a layer of isobutyl alcohol by gently squirting the isobutyl alcohol against the edge of one of the spacers. Allow the resolving gel to polymerize fully (usually 30-60 min).

Pouring the stacking gel:

1. Pour off completely the layer of isobutyl alcohol.
2. Preparation a 4% stacking gel solution by adding as defined in table.
3. Transfer stacking gel solution to tickle into the center of the sandwich along an edge of spacers.
4. Insert a comb into the layer of stacking gel solution by placing one corner of the comb into the gel and slowly lowering the other corner in. Allow the stacking gel solution to polymerize 30 to 45 min at room temperature.

Reagents	10% running gel	4% stacking gel
30% Acrylamide-bis	1.167 ml	0.4 ml
1.5 M Tris-HCL buffer, pH 6.8	0.875 ml	-
0.5 M Tris-HCL buffer, pH 6.8	-	1.0 ml
1% SDS	0.35 ml	0.3 ml
Distilled water	0.7585 ml	0.9 ml
0.1 EDTA	-	0.8 ml
2% Ammonium persulfate	0.35 ml	0.4 ml
TEMED	6 µl	5 µl

Sample preparation:

1. Weigh 3 g of sample and homogenize with 5% (w/v) SDS in a final volume of 30 ml.
2. Incubate the mixture at 85^{0}C for 1 h.

3. Centrifuge at 3,500 *xg* for 5 min at ambient temperature and collect supernatant.

Loading the gel:

1. Dilute the protein to be 4:1 (v/v) with sample buffer in microcentrifuge tube and boil for 1 min at 100^{0}C.
2. Remove the comb without tearing the edge of the polyacrylamide wells.
3. Fill the wells with electrode buffer.
4. Place the upper chamber over the sandwich and lock the upper buffer chamber to the sandwich. Pour electrode buffer into the lower buffer chamber. Place the sandwich attached to the upper buffer chamber into the lower chamber.
5. Fill the upper buffer chamber with electrode buffer so that the sample wells of the stacking gel are filled with buffer.
6. Use a 10-25 μl syringe with a flate-tipped needle, load the protein sample into the wells by carefully applying the sample as a thin layer at the bottom of well.
7. Fill the remainder of the upper chamber with additional electrode buffer.

Running the gel:

1. Connect the power supply to the anode and cathode of the gel apparatus and run at 50 V and 150 V.
2. After the bromophenol blue tracking dye has reached the bottom of the separating gel, disconnect the power supply.

Disassembling the gel:

1. Remove the upper buffer chamber and attached sandwich.
2. Orient the gel so that the order of the sample well is known, remove the sandwich from the upper buffer chamber, and lay the sandwich on a sheet of

absorbent paper or paper towels. Carefully slide the spacers out from the edge of the sandwich along its entire length.

3. Insert a spatula between the glass plates at one corner where the spacer was, and gently pry the two plates apart.
4. Remove the gel from the lower plate. Place the plate with the gel attached into the shallow dish of fixing agent or dye and swishing the plate.

Staining the gel:

1. Place the gel in a small plastic box and cover with the staining solution. Agitate slowly for 1 h or more on a rotary rocker.
2. Pour off the staining solution and cover the gel with a solution of destaining solution I. Agitate slowly for ~ 15 min.
3. Pour off the destaining solution I and cover the destaining solution II. Discard destaining solution and replace with fresh solution. Repeat until the gel is clear except for the protein bands.

Appendix 2

Analysis of variance

Table 1-A. Analysis of variance for proximate compositions of surimi from bigeye snapper and mackerel

pH

SV	DF	SS	MS	F
Treatment	6	0.088	1.35E-02	368.737**
Error	7	2.56E-04	3.65E-05	
Total	13	8.10E-02		

**= Significant at 1% level

Moisture

SV	DF	SS	MS	F
Treatment	6	8.42	1.40	893249.5**
Error	7	0.002	1.57	
Total	13	8.42		

**= Significant at 1% level

Protein

SV	DF	SS	MS	F
Treatment	6	14.99	2.49	165794.25**
Error	7	1.050	1.507	
Total	13	16.04		

**= Significant at 1% level

Fat

SV	DF	SS	MS	F
Treatment	6	2.019	2.0032	1663.53**
Error	7	1.35	1.92	
Total	13	2.14		

**= Significant at 1% level

Ash

SV	DF	SS	MS	F
Treatment	6	1.03	0.17	79919.52**
Error	7	1.5E-05	2.14286E-06	
Total	13	1.03		

**= Significant at 1% level

Table 2-A Analysis of variance for Nitrogenous compositions of surimi from bigeye snapper and mackerel

Non-protein nitrogen

SV	DF	SS	MS	F
Treatment	6	2.26693	0.37782	25594.35**
Error	14	0.00021	1.5E-05	
Total	20	2.26714		

**= Significant at 1% level

Sarcoplasmic protein

SV	DF	SS	MS	F
Treatment	6	311.45	51.9083	10292453**
Error	14	7.1E-05	5E-06	
Total	20	311.45		

**= Significant at 1% level

Myofibrillar protein

SV	DF	SS	MS	F
Treatment	6	408.18	68.03	89289343**
Error	14	1.1E-05	7.6E-07	
Total	20	408.18		

**= Significant at 1% level

Alkali-soluble protein

SV	DF	SS	MS	F
Treatment	6	15.7236	2.6206	131342.7**
Error	14	0.00028	2E-05	
Total	20	15.7239		

**= Significant at 1% level

Stroma

SV	DF	SS	MS	F
Treatment	6	12.8186	2.13644	45921.41**
Error	14	0.00065	4.7E-05	
Total	20	12.8193		

**= Significant at 1% level

Table 3-A Analysis of variance for TMA of surimi from bigeye snapper and mackerel

SV	DF	SS	MS	F
Treatment	6	38.0756	6.34594	19958.8**
Error	14	0.00445	0.00032	
Total	20	38.0801		

**= Significant at 1% level

Table 4-A Analysis of variance for TVB of surimi from bigeye snapper and mackerel

SV	DF	SS	MS	F
Treatment	6	976.309	162.718	7493597**
Error	14	0.0003	2.2E-05	
Total	20	976.309		

**= Significant at 1% level

Table 5-A Analysis of variance for Tmax and enthalpy of surimi from bigeye snapper and mackerel

Tmax (peak 1)

SV	DF	SS	MS	F
Treatment	4	159.226	39.8065	2.29257^{ns}
Error	9	156.269	17.3633	
Total	13	315.496		

^{ns}= Non significant

Tmax (peak 2)

SV	DF	SS	MS	F
Treatment	4	12.0745	3.01863	1.52109^{ns}
Error	9	17.8607	1.98452	
Total	13	29.9352		

^{ns}= Non significant

Enthalpy (peak 1)

SV	DF	SS	MS	F
Treatment	4	0.00532	0.00133	0.88038^{ns}
Error	9	0.0136	0.00151	
Total	13	0.01892		

^{ns}= Non significant

Enthalpy (peak 2)

SV	DF	SS	MS	F
Treatment	4	0.04222	0.01055	1.34957^{ns}
Error	9	0.07038	0.00782	
Total	13	0.1126		

^{ns}= Non significant

Table 6-A Analysis of variance for Breaking force and deformation of surimi gel from fresh and ten days stored bigeye snapper and commercial A and B grade surimi from bigeye snapper

SV	DF	SS	MS	F
Treatment	3	437531	109323	235.77**
Error	8	5741.75	342.783	
Total	11	443273		

**= Significant at 1% level

Table 7-A Analysis of variance for whiteness of different bigeye snapper surimi gels prepared under various condition in the same column

SV	DF	SS	MS	F
Treatment	15	1475.988	98.39919	61.8686**
Error	32	50.89454	1.590454	
Total	47	1526.882		

**= Significant at 1% level

Table 8-A Analysis of variance for L*, a*, b* value and whiteness of different bigeye snapper surimi gels prepared under various condition in the same column within the same gel

Suwari gel

SV	DF	SS	MS	F
Treatment	3	14.217	4.738999	8.014855**
Error	8	4.730216	0.591277	
Total	11	18.94721		

**= Significant at 1% level

Modori gel

SV	DF	SS	MS	F
Treatment	3	42.7175	14.23917	68.27384**
Error	8	1.668477	0.20856	
Total	11	44.38598		

**= Significant at 1% level

directly heated gel

SV	DF	SS	MS	F
Treatment	3	4.94441	1.64814	0.31692^{ns}
Error	8	41.6045	5.20056	
Total	11	46.5489		

^{ns}= Non significant

Ashi gel

SV	DF	SS	MS	F
Treatment	3	18.3559	6.11863	16.9295**
Error	8	2.89134	0.36142	
Total	11	21.2472		

**= Significant at 1% level

Table 9-A Analysis of variance for expressible moisture of different bigeye snapper surimi gels prepared under various conditions in the same column

SV	DF	SS	MS	F
Treatment	15	48.7492	3.24995	5.91038**
Error	32	17.5959	0.54987	
Total	47	66.3451		

**= Significant at 1% level

Table 10-A Analysis of variance for expressible moisture of different bigeye snapper surimi gels prepared under various conditions in the same column within the same gel

Suwari gel

SV	DF	SS	MS	F
Treatment	3	6.48362	2.16121	2.819^{ns}
Error	8	6.13327	0.76666	
Total	11	12.6169		

^{ns}= Non significant

Modori gel

SV	DF	SS	MS	F
Treatment	3	13.8402	4.6134	13.276**
Error	8	2.78	0.3475	
Total	11	16.6202		

**= Significant at 1% level

directly heated gel

SV	DF	SS	MS	F
Treatment	3	12.1589	4.05296	4.85369^{ns}
Error	8	6.6802	0.83503	
Total	11	18.8391		

**= Significant at 1% level

Ashi gel

SV	DF	SS	MS	F
Treatment	3	2.98729	0.99576	3.97828^{ns}
Error	8	2.0024	0.2503	
Total	11	4.98969		

^{ns}= Non significant

Table 11-A Analysis of variance for TCA soluble peptides of different bigeye snapper surimi gels prepared under various conditions in the same column

SV	DF	SS	MS	F
Treatment	19	9.887048	0.520371	16.94661**
Error	20	0.61413	0.030707	
Total	39	10.50118		

**= Significant at 1% level

Table 12-A Analysis of variance for TCA soluble peptides of different bigeye snapper surimi gels prepared under various conditions in the same column within the same gel

Control

SV	DF	SS	MS	F
Treatment	3	0.46322	0.15441	82350.3**
Error	4	7.5E-06	1.9E-06	
Total	7	0.46323		

**= Significant at 1% level

Suwari gel

SV	DF	SS	MS	F
Treatment	3	0.26349	0.08783	3.09647^{ns}
Error	4	0.11346	0.02836	
Total	7	0.37694		

^{ns}= Non signicant

Modori gel

SV	DF	SS	MS	F
Treatment	3	0.36722	0.12241	1.53747^{ns}
Error	4	0.31846	0.07962	
Total	7	0.68568		

^{ns}= Non signicant

directly heated gel

SV	DF	SS	MS	F
Treatment	3	0.41038	0.13679	15.0829**
Error	4	0.03628	0.00907	
Total	7	0.44665		

**= Significant at 1% level

Ashi gel

SV	DF	SS	MS	F
Treatment	3	0.31206	0.10402	2.85134^{ns}
Error	4	0.14593	0.03648	
Total	7	0.45799		

^{ns}= Non significant

Table 13-A Analysis of variance for solubility of different bigeye snapper surimi gels prepared under various conditions in the same column

SV	DF	SS	MS	F
Treatment	15	1198.1	79.8734	8E+07**
Error	32	3.2E-05	1E-06	
Total	47	1198.1		

**= Significant at 1% level

Table 14-A Analysis of variance for solubility of different bigeye snapper surimi gels prepared under various conditions in the same column within the same gel

Suwari gel

SV	DF	SS	MS	F
Treatment	3	103.132	34.3773	3.4E+07**
Error	8	8E-06	1E-06	
Total	11	103.132		

**= Significant at 1% level

Modori gel

SV	DF	SS	MS	F
Treatment	3	51.5001	17.1667	1.7E+07**
Error	8	8E-06	1E-06	
Total	11	51.5001		

**= Significant at 1% level

directly heated gel

SV	DF	SS	MS	F
Treatment	3	95.732	31.9107	3.2E+07**
Error	8	8E-06	1E-06	
Total	11	95.732		

**= Significant at 1% level

Ashi gel

SV	DF	SS	MS	F
Treatment	3	369.231	123.077	1.2E+08**
Error	8	8E-06	1E-06	
Total	11	369.231		

**= Significant at 1% level

Table 15-A Analysis of variance for breaking force of A grade bigeye snapper ashi gel added with various BPP concentrations and set at 40°C for 30 min, followed by heating at 90°C for 15 min.

SV	DF	SS	MS	F
Treatment	5	174064.8	34812.96	151.1861**
Error	18	4144.78	230.2656	
Total	23	178209.6		

**= Significant at 1% level

Table 16-A Analysis of variance for deformation of A grade bigeye snapper ashi gel added with various BPP concentrations and set at 40°C for 30 min, followed by heating at 90°C for 15 min.

SV	DF	SS	MS	F
Treatment	5	12.3927	2.47855	33.1061**
Error	18	1.3476	0.07487	
Total	23	13.7403		

**= Significant at 1% level

Table 17-A Analysis of variance for breaking force of B grade bigeye snapper ashi gel added with various BPP concentrations and set at 40°C for 30 min, followed by heating at 90°C for 15 min.

SV	DF	SS	MS	F
Treatment	5	355359	71071.8	192.913**
Error	18	6631.46	368.414	
Total	23	361990		

**= Significant at 1% level

Table 18-A Analysis of variance for deformation of B grade bigeye snapper ashi gel added with various BPP concentrations and set at 40°C for 30 min, followed by heating at 90°C for 15 min.

SV	DF	SS	MS	F
Treatment	5	12.3005	2.46011	14.0925**
Error	18	3:14223	0.17457	
Total	23	15.4428		

**= Significant at 1% level

Table 19-A Analysis of variance for whiteness of A grade bigeye snapper ashi gel added with various BPP concentrations and set at 40°C for 30 min, followed by heating at 90°C for 15 min.

SV	DF	SS	MS	F
Treatment	5	117.8123694	23.5625	65.6971**
Error	12	4.303839608	0.35865	
Total	17	122.116209		

**= Significant at 1% level

Table 20-A Analysis of variance for whiteness of B grade bigeye snapper ashi gel added with various BPP concentrations and set at 40°C for 30 min, followed by heating at 90°C for 15 min.

SV	DF	SS	MS	F
Treatment	5	80.65308507	16.1306	80.681**
Error	12	2.399169094	0.19993	
Total	17	83.05225417		

**= Significant at 1% level

Table 21-A Analysis of variance for breaking force of A grade bigeye snapper ashi gel added with various PPP concentrations and set at 40°C for 30 min, followed by heating at 90°C for 15 min.

SV	DF	SS	MS	F
Treatment	5	87016.3	17403.3	4.9789**
Error	18	62917.3	3495.4	
Total	23	149934		

**= Significant at 1% level

Table 22-A Analysis of variance for deformation of A grade bigeye snapper ashi gel added with various PPP concentrations and set at 40°C for 30 min, followed by heating at 90°C for 15 min.

SV	DF	SS	MS	F
Treatment	5	19.4752	3.89505	8.16451**
Error	18	8.58727	0.47707	
Total	23	28.0625		

**= Significant at 1% level

Table 23-A Analysis of variance for breaking force of B grade bigeye snapper ashi gel added with various PPP concentrations and set at 40°C for 30 min, followed by heating at 90°C for 15 min.

SV	DF	SS	MS	F
Treatment	5	115336	23067.1	52.5836**
Error	18	7896.16	438.676	
Total	23	123232		

**= Significant at 1% level

Table 24-A Analysis of variance for deformation of B grade bigeye snapper ashi gel added with various PPP concentrations and set at 40°C for 30 min, followed by heating at 90°C for 15 min.

SV	DF	SS	MS	F
Treatment	5	7.02804	1.40561	5.93332**
Error	18	4.26422	0.2369	
Total	23	11.2923		

**= Significant at 1% level

Table 25-A Analysis of variance for whiteness of A grade bigeye snapper ashi gel added with various PPP and set at 40°C for 30 min, followed by heating at 90°C for 15 min.

SV	DF	SS	MS	F
Treatment	5	400.542	80.1083	113.617**
Error	12	8.46088	0.70507	
Total	17	409.002		

**= Significant at 1% level

Table 26-A Analysis of variance for whiteness of B grade bigeye snapper ashi gel added with various PPP and set at 40°C for 30 min, followed by heating at 90°C for 15 min.

SV	DF	SS	MS	F
Treatment	5	272.689	54.5379	58.3697**
Error	12	11.2122	0.93435	
Total	17	283.902		

**= Significant at 1% level

Table 27-A Analysis of variance for breaking force of A grade bigeye snapper ashi gel added with various EW concentrations and set at 40°C for 30 min, followed by heating at 90°C for 15 min.

SV	DF	SS	MS	F
Treatment	5	269299	53859.8	255.556**
Error	18	2529.07	210.756	
Total	23	271828		

**= Significant at 1% level

Table 28-A Analysis of variance for deformation of A grade bigeye snapper ashi gel added with various EW concentrations and set at 40°C for 30 min, followed by heating at 90°C for 15 min.

SV	DF	SS	MS	F
Treatment	5	24.9917	4.99833	37.4162**
Error	18	1.60305	0.13359	
Total	23	26.5947		

**= Significant at 1% level

Table 29-A Analysis of variance for breaking force of B grade bigeye snapper ashi gel added with various EW concentrations and set at 40°C for 30 min, followed by heating at 90°C for 15 min.

SV	DF	SS	MS	F
Treatment	5	24.9917	4.99833	37.4162**
Error	18	1.60305	0.13359	
Total	23	26.5947		

**= Significant at 1% level

Table 30-A Analysis of variance for deformation of B grade bigeye snapper ashi gel added with various EW concentrations and set at 40°C for 30 min, followed by heating at 90°C for 15 min.

SV	DF	SS	MS	F
Treatment	5	15.792	3.1584	8.44309**
Error	18	8.97793	0.37408	
Total	23	24.7699		

**= Significant at 1% level

Table 31-A Analysis of variance for whiteness of A grade bigeye snapper ashi gel added with various EW concentrations and set at 40°C for 30 min, followed by heating at 90°C for 15 min.

SV	DF	SS	MS	F
Treatment	5	2.46178	0.49236	2.55967^{ns}
Error	12	2.30822	0.19235	
Total	17	4.77		

**= Significant at 1% level

Table 32-A Analysis of variance for whiteness of B grade bigeye snapper ashi gel added with various EW concentrations and set at 40°C for 30 min, followed by heating at 90°C for 15 min.

SV	DF	SS	MS	F
Treatment	5	0.23806	0.04761	1.04094^{ns}
Error	12	0.54887	0.04574	
Total	17	0.78693		

ns = Non significant

Table 33-A Analysis of variance for breaking force of A grade bigeye snapper ashi gel added with various calcium gluconate concentrations and set at 40°C for 30 min, followed by heating at 90°C for 15 min.

SV	DF	SS	MS	F
Treatment	4	645371	161343	63.0466**
Error	15	38386.5	2559.1	
Total	19	683757		

**= Significant at 1% level

Table 34-A Analysis of variance for deformation of A grade bigeye snapper ashi gel added with various calcium gluconate concentrations and set at 40°C for 30 min, followed by heating at 90°C for 15 min.

SV	DF	SS	MS	F
Treatment	4	61.2124	15.3031	34.5962**
Error	15	6.63502	0.44233	
Total	19	67.8474		

**= Significant at 1% level

Table 35-A Analysis of variance for breaking force of B grade bigeye snapper ashi gel added with various calcium gluconate concentrations and set at 40°C for 30 min, followed by heating at 90°C for 15 min.

SV	DF	SS	MS	F
Treatment	4	437531	109383	285.757**
Error	15	5741.75	382.783	
Total	19	443273		

**= Significant at 1% level

Table 36-A Analysis of variance for deformation of B grade bigeye snapper ashi gel added with various calcium gluconate concentrations and set at 40°C for 30 min, followed by heating at 90°C for 15 min.

SV	DF	SS	MS	F
Treatment	4	66.7901	16.6975	28.8426**
Error	15	8.68378	0.57892	
Total	19	75.4738		

**= Significant at 1% level

Table 37-A Analysis of variance for whiteness of A grade bigeye snapper ashi gel added with various calcium gluconate concentrations and set at 40°C for 30 min, followed by heating at 90°C for 15 min.

SV	DF	SS	MS	F
Treatment	4	13.7526	3.43814	60.0583**
Error	10	0.57247	0.05725	
Total	14	14.325		

**= Significant at 1% level

Table 38-A Analysis of variance for whiteness of B grade bigeye snapper ashi gel added with various calcium gluconate concentrations and set at 40°C for 30 min, followed by heating at 90°C for 15 min.

SV	DF	SS	MS	F
Treatment	4	15.0428	3.76069	20.1607**
Error	10	1.86536	0.18654	
Total	14	16.9081		

**= Significant at 1% level

Table 39-A Analysis of variance for breaking force of A grade bigeye snapper ashi gel added with various calcium choride concentrations and set at 40°C for 30 min, followed by heating at 90°C for 15 min.

SV	DF	SS	MS	F
Treatment	4	636185.9688	159046.4922	149.186**
Error	15	15991.47354	1066.098236	
Total	19	652177.4423		

**= Significant at 1% level

Table 40-A Analysis of variance for deformation of A grade bigeye snapper ashi gel added with various calcium choride concentrations and set at 40°C for 30 min, followed by heating at 90°C for 15 min.

SV	DF	SS	MS	F
Treatment	4	32.8329523	8.208238075	13.8059**
Error	15	8.9182145	0.594547633	
Total	19	41.7511668		

**= Significant at 1% level

Table 41-A Analysis of variance for breaking force of B grade bigeye snapper ashi gel added with various calcium choride concentrations and set at 40°C for 30 min, followed by heating at 90°C for 15 min.

SV	DF	SS	MS	F
Treatment	4	216749.8736	54187.4684	106.916**
Error	15	7602.311138	506.8207425	
Total	19	224352.1847		

**= Significant at 1% level

Table 42-A Analysis of variance for deformation of B grade bigeye snapper ashi gel added with various calcium choride concentrations and set at 40°C for 30 min, followed by heating at 90°C for 15 min.

SV	DF	SS	MS	F
Treatment	4	53.4981413	13.37453533	33.4631**
Error	15	5.9952025	0.399680167	
Total	19	59.4933438		

**= Significant at 1% level

Table 43-A Analysis of variance for whiteness of A grade bigeye snapper ashi gel added with various calcium choride concentrations and set at 40°C for 30 min, followed by heating at 90°C for 15 min.

SV	DF	SS	MS	F
Treatment	4	25.1888	6.2972	18.162**
Error	10	3.46724	0.34672	
Total	14	28.6561		

**= Significant at 1% level

Table 44-A Analysis of variance for whiteness of B grade bigeye snapper ashi gel added with various calcium choride concentrations and set at 40°C for 30 min, followed by heating at 90°C for 15 min.

SV	DF	SS	MS	F
Treatment	4	15.6382	3.90955	32.2442**
Error	10	1.21248	0.12125	
Total	14	16.8507		

**= Significant at 1% level

Table 45-A Analysis of variance for breaking force of A grade bigeye snapper ashi gel added with various calcium lactate concentrations and set at 40°C for 30 min, followed by heating at 90°C for 15 min.

SV	DF	SS	MS	F
Treatment	4	551494	137873	99.6667**
Error	15	20750.2	1383.35	
Total	19	572244		

**= Significant at 1% level

Table 46-A Analysis of variance for deformation of A grade bigeye snapper ashi gel added with various calcium lactate concentrations and set at 40°C for 30 min, followed by heating at 90°C for 15 min.

SV	DF	SS	MS	F
Treatment	4	54.8951	13.7238	40.7685
Error	15	5.04941	0.33663	
Total	19	59.9445		

**= Significant at 1% level

Table 47-A Analysis of variance for breaking force of B grade bigeye snapper ashi gel added with various calcium lactate concentrations and set at 40°C for 30 min, followed by heating at 90°C for 15 min.

SV	DF	SS	MS	F
Treatment	4	199817	49954.2	120.304**
Error	15	6228.51	415.234	
Total	19	206045		

**= Significant at 1% level

Table 48-A Analysis of variance for deformation of B grade bigeye snapper ashi gel added with various calcium lactate concentrations and set at 40°C for 30 min, followed by heating at 90°C for 15 min.

SV	DF	SS	MS	F
Treatment	4	48.0028	12.0007	28.1521**
Error	15	6.39422	0.42628	
Total	19	54.3971		

**= Significant at 1% level

Table 49-A Analysis of variance for whiteness of A grade bigeye snapper ashi gel added with various calcium lactate concentrations and set at 40°C for 30 min, followed by heating at 90°C for 15 min.

SV	DF	SS	MS	F
Treatment	4	22.9397	5.7349247	52.4302**
Error	10	1.09382	0.109382171	
Total	14	24.0335		

**= Significant at 1% level

Table 50-A Analysis of variance for whiteness of B grade bigeye snapper ashi gel added with various calcium lactate concentrations and set at 40°C for 30 min, followed by heating at 90°C for 15 min.

SV	DF	SS	MS	F
Treatment	4	21.1608	5.290212211	85.1795**
Error	10	0.62107	0.062106635	
Total	14	21.7819		

**= Significant at 1% level

Table 51-A Analysis of variance for breaking force of A grade bigeye snapper ashi gel added with various MTGase concentrations and set at 40°C for 30 min, followed by heating at 90°C for 15 min.

SV	DF	SS	MS	F
Treatment	4	499417	124854	23.2007**
Error	15	80722.2	5381.48	
Total	19	580139		

**= Significant at 1% level

Table 52-A Analysis of variance for deformation of A grade bigeye snapper ashi gel added with various MTGase concentrations and set at 40°C for 30 min, followed by heating at 90°C for 15 min.

SV	DF	SS	MS	F
Treatment	4	1.63259	0.40815	1.03049^{ns}
Error	15	5.94103	0.39607	
Total	19	7.57361		

ns = Non significant

Table 53-A Analysis of variance for breaking force of B grade bigeye snapper ashi gel added with various MTGase concentrations and set at 40°C for 30 min, followed by heating at 90°C for 15 min.

SV	DF	SS	MS	F
Treatment	4	209306	52326.4	20.4361**
Error	15	38407.3	2560.48	
Total	19	247713		

**= Significant at 1% level

Table 54-A Analysis of variance for deformation of B grade bigeye snapper ashi gel added with various MTGase concentrations and set at 40°C for 30 min, followed by heating at 90°C for 15 min.

SV	DF	SS	MS	F
Treatment	4	3.56835	0.89209	4.21448^{ns}
Error	15	3.17508	0.21167	
Total	19	6.74343		

ns = Non significant

Table 55-A Analysis of variance for whiteness of A grade bigeye snapper ashi gel added with various MTGase concentrations and set at 40°C for 30 min, followed by heating at 90°C for 15 min.

SV	DF	SS	MS	F
Treatment	5	0.29966	0.05993	1.46664^{ns}
Error	6	0.24518	0.04086	
Total	11	0.54485		

ns = Non significant

Table 56-A Analysis of variance for whiteness of B grade bigeye snapper ashi gel added with various MTGase concentrations and set at 40°C for 30 min, followed by heating at 90°C for 15 min.

SV	DF	SS	MS	F
Treatment	5	0.72391	0.14478	4.40668^{ns}
Error	6	0.19713	0.03286	
Total	11	0.92104		

ns = Non significant

Table 57-A Analysis of variance for breaking force of A grade bigeye snapper surimi gel set at 40°C for different times, prior to heating at 90°C for 15 min

SV	DF	SS	MS	F
Treatment	4	556807	139202	17.5122**
Error	9	103335	7948.83	
Total	13	660141		

**= Significant at 1% level

Table 58-A Analysis of variance for deformation of A grade bigeye snapper surimi gel set at 40°C for different times, prior to heating at 90°C for 15 min

SV	DF	SS	MS	F
Treatment	4	47.7836	11.9459	6.57752**
Error	9	23.6102	1.81617	
Total	13	71.3938		

**= Significant at 1% level

Table 59-A Analysis of variance for breaking force of B grade bigeye snapper surimi gel set at 40°C for different times, prior to heating at 90°C for 15 min

SV	DF	SS	MS	F
Treatment	4	1051780	262945	78.9719**
Error	9	46614.4	3329.6	
Total	13	1098395		

**= Significant at 1% level

Table 60-A Analysis of variance for deformation of B grade bigeye snapper surimi gel set at 40°C for different times, prior to heating at 90°C for 15 min

SV	DF	SS	MS	F
Treatment	4	86.8902	21.7226	26.3676**
Error	9	7.41451	0.82383	
Total	13	94.3048		

**= Significant at 1% level

Table 61-A Analysis of variance for breaking force of A grade bigeye snapper surimi gels added with EW, calcium gluconate and MTGase set at 40°C for different times, prior to heating at 90°C for 15 min.

SV	DF	SS	MS	F
Treatment	4	767399	191850	66.149**
Error	9	26102.4	2900.27	
Total	13	793502		

**= Significant at 1% level

Table 62-A Analysis of variance for deformation of A grade bigeye snapper surimi gels added with EW, calcium gluconate and MTGase set at 40°C for different times, prior to heating at 90°C for 15 min.

SV	DF	SS	MS	F
Treatment	4	30.7198	7.67994	3.56152^{ns}
Error	9	21.5637	2.15637	
Total	13	52.2834		

ns = Non significant

Table 63-A Analysis of variance for breaking force of B grade bigeye snapper surimi gels added with EW, calcium gluconate and MTGase set at 40°C for different times, prior to heating at 90°C for 15 min.

SV	DF	SS	MS	F
Treatment	4	1038752	259688	72.4241**
Error	13	46613.5	3585.66	
Total	17	1085365		

**= Significant at 1% level

Table 64-A Analysis of variance for deformation of B grade bigeye snapper surimi gels added with EW, calcium gluconate and MTGase set at 40°C for different times, prior to heating at 90°C for 15 min.

SV	DF	SS	MS	F
Treatment	4	103.266	25.8166	30.6616**
Error	13	8.41983	0.84198	
Total	17	111.686		

**= Significant at 1% level

Table 65-A Analysis of variance for whiteness of A grade bigeye snapper surimi gel set at 40°C for different times, prior to heating at 90°C for 15 min

SV	DF	SS	MS	F
Treatment	4	11.4777	2.86942	5.4157**
Error	10	5.29833	0.52983	
Total	14	16.776		

**= Significant at 1% level

Table 66-A Analysis of variance for whiteness of B grade bigeye snapper surimi gel set at 40°C for different times, prior to heating at 90°C for 15 min

SV	DF	SS	MS	F
Treatment	4	14.0838	3.52094	9.74746**
Error	10	3.61216	0.36122	
Total	14	17.6959		

**= Significant at 1% level

Table 67-A Analysis of variance for whiteness of A grade bigeye snapper surimi gels added with EW, calcium gluconate and MTGase set at 40°C for different times, prior to heating at 90°C for 15 min.

SV	DF	SS	MS	F
Treatment	4	11.1251	2.78128	24.3531**
Error	10	1.14206	0.11421	
Total	14	12.2672		

**= Significant at 1% level

Table 68-A Analysis of variance for whiteness of B grade bigeye snapper surimi gels added with EW, calcium gluconate and MTGase set at 40°C for different times, prior to heating at 90°C for 15 min.

SV	DF	SS	MS	F
Treatment	4	29.0423	7.26058	44.141**
Error	10	1.64486	0.16449	
Total	14	30.6872		

**= Significant at 1% level

Table 69-A Analysis of variance for breaking force of mixture between different SSA grade grade bigeye snapper surimi and mackerel surimi at different ratios.

SV	DF	SS	MS	F
Treatment	5	41231	8246.21	2.70747**
Error	12	36548.7	3045.73	
Total	17	77779.8		

**= Significant at 1% level

Table 70-A Analysis of variance for deformation of mixture between SSA grade bigeye snapper surimi and mackerel surimi at different ratios.

SV	DF	SS	MS	F
Treatment	5	3.07624	0.61525	4.06496^{ns}
Error	12	1.81624	0.15135	
Total	17	4.89248		

ns = Non significant

Table 71-A Analysis of variance for breaking force of mixture between SA grade bigeye snapper surimi and mackerel surimi at different ratios.

SV	DF	SS	MS	F
Treatment	5	29640.4	5928.07	1.15711^{ns}
Error	12	61478.1	5123.18	
Total	17	91118.5		

ns = Non significant

Table 72-A Analysis of variance for deformation of mixture between SA grade bigeye snapper surimi and mackerel surimi at different ratios.

SV	DF	SS	MS	F
Treatment	5	1.10458	0.22092	0.39566
Error	12	6.70017	0.55835	
Total	17	7.80475		

ns = Non significant

Table 73-A Analysis of variance for whiteness of mixture between SSA grade bigeye snapper surimi and mackerel surimi at different ratios.

SV	DF	SS	MS	F
Treatment	5	409.63	81.9261	320.932**
Error	12	3.0633	0.25528	
Total	17	412.694		

**= Significant at 1% level

Table 74-A Analysis of variance for whiteness of mixture between SA grade bigeye snapper surimi and mackerel surimi at different ratios.

SV	DF	SS	MS	F
Treatment	5	300.954	60.1908	275.064**
Error	12	2.62589	0.21882	
Total	17	303.58		

**= Significant at 1% level

Table 75-A Analysis of variance for breaking force of mixture between SSA grade bigeye snapper surimi and mackerel surimi gels added with various calcium carbonate.

SV	DF	SS	MS	F
Treatment	4	146133	36533.2	18.098**
Error	10	20186.3	2018.63	
Total	14	166319		

**= Significant at 1% level

Table 76-A Analysis of variance for deformation of mixture between SSA grade bigeye snapper surimi and mackerel surimi gels added with various calcium carbonate.

SV	DF	SS	MS	F
Treatment	4	2.39253	0.59813	3.49532^{ns}
Error	10	1.71124	0.17112	
Total	14	4.10377		

ns = Non significant

Table 77-A Analysis of variance for breaking force of mixture between SA grade bigeye snapper surimi and mackerel surimi gels added with various calcium carbonate.

SV	DF	SS	MS	F
Treatment	4	3862.69	965.673	0.50053^{ns}
Error	10	19293.1	1929.31	
Total	14	23155.8		

ns = Non significant

Table 78-A Analysis of variance for deformation of mixture between SA grade bigeye snapper surimi and mackerel surimi gels added with various calcium carbonate.

SV	DF	SS	MS	F
Treatment	4	1.55074	0.38768	0.83554^{ns}
Error	10	4.63991	0.46399	
Total	14	6.19065		

ns = Non significant

Table 79-A Analysis of variance for whiteness of mixture between SSA grade bigeye snapper surimi and mackerel surimi gels added with various calcium carbonate.

SV	DF	SS	MS	F
Treatment	4	6.05713	1.51428	18.2829**
Error	10	0.82825	0.08283	
Total	14	6.88539		

**= Significant at 1% level

Table 80-A Analysis of variance for whiteness of mixture between SA grade bigeye snapper surimi and mackerel surimi gels added with various calcium carbonate.

SV	DF	SS	MS	F
Treatment	4	9.60976	2.40244	59.2044**
Error	10	0.40579	0.04058	
Total	14	10.0155		

**= Significant at 1% level

Table 81-A Analysis of variance for breaking force of mixture between SSA grade bigeye snapper surimi and mackerel surimi gels added with various vegetable oil.

SV	DF	SS	MS	F
Treatment	4	536999	134250	31.4498**
Error	10	42686.9	4268.69	
Total	14	579686		

**= Significant at 1% level

Table 82-A Analysis of variance for deformation of mixture between SSA grade bigeye snapper surimi and mackerel surimi gels added with various vegetable oil.

SV	DF	SS	MS	F
Treatment	4	3.97525	0.99381	1.95409^{ns}
Error	10	5.0858	0.50858	
Total	14	9.06105		

ns = Non significant

Table 83-A Analysis of variance for breaking force of mixture between SA grade bigeye snapper surimi and mackerel surimi gels added with various vegetable oil.

SV	DF	SS	MS	F
Treatment	4	127988	31997.1	20.7688**
Error	10	15406.3	1540.63	
Total	14	143395		

**= Significant at 1% level

Table 84-A Analysis of variance for deformation of mixture between SA grade bigeye snapper surimi and mackerel surimi gels added with various vegetable oil.

SV	DF	SS	MS	F
Treatment	4	0.45602	0.114	0.5736^{na}
Error	10	1.98752	0.19875	
Total	14	2.44354		

ns = Non significant

Table 85-A Analysis of variance for whiteness of mixture between SSA grade bigeye snapper surimi and mackerel surimi gels added with various vegetable oil.

SV	DF	SS	MS	F
Treatment	4	5.71146	1.42787	32.8607**
Error	10	0.43452	0.04345	
Total	14	6.14598		

**= Significant at 1% level

Table 86-A Analysis of variance for whiteness of mixture between SA grade bigeye snapper surimi and mackerel surimi gels added with various vegetable oil.

SV	DF	SS	MS	F
Treatment	4	11.3177	2.82943	10.407**
Error	10	2.71877	0.27188	
Total	14	14.0365		

**= Significant at 1% level

Table 87-A Analysis of variance for breaking force of mixture between SSA grade bigeye snapper surimi and mackerel surimi gels added with various titanium dioxide.

SV	DF	SS	MS	F
Treatment	4	1581.54	395.385	0.12345^{ns}
Error	10	32026.7	3202.67	
Total	14	33608.2		

ns = Non significant

Table 88-A Analysis of variance for deformation of mixture between SSA grade bigeye snapper surimi and mackerel surimi gels added with various titanium dioxide.

SV	DF	SS	MS	F
Treatment	4	0.61208	0.15302	0.23811^{ns}
Error	10	6.42646	0.64265	
Total	14	7.03854		

ns = Non significant

Table 89-A Analysis of variance for breaking force of mixture between SA grade bigeye snapper surimi and mackerel surimi gels added with various titanium dioxide.

SV	DF	SS	MS	F
Treatment	4	2078.01	519.504	0.14849^{ns}
Error	10	34986.6	3498.66	
Total	14	37064.6		

ns = Non significant

Table 90-A Analysis of variance for deformation of mixture between SA grade bigeye snapper surimi and mackerel surimi gels added with various titanium dioxide.

SV	DF	SS	MS	F
Treatment	4	0.89641	0.2241	0.36627^{ns}
Error	10	6.11848	0.61185	
Total	14	7.01489		

ns = Non significant

Table 91-A Analysis of variance for whiteness of mixture between SSA grade bigeye snapper surimi and mackerel surimi gels added with various titanium dioxide.

SV	DF	SS	MS	F
Treatment	4	36.3005	9.07511	92.3776**
Error	10	0.98239	0.09824	
Total	14	37.2829		

**= Significant at 1% level

Table 92-A Analysis of variance for whiteness of mixture between SA grade bigeye snapper surimi and mackerel surimi gels added with various titanium dioxide.

SV	DF	SS	MS	F
Treatment	4	35.9551	8.98878	173.656**
Error	10	0.51762	0.05176	
Total	14	36.4727		

**= Significant at 1% level

Table 93-A Analysis of variance for solubility of mixture between SSA grade bigeye snapper surimi and mackerel surimi gel added with various whitening agents.

SV	DF	SS	MS	F
Treatment	3	39.9134	13.3045	1004111**
Error	4	5.3E-05	1.3E-05	
Total	7	39.9135		

**= Significant at 1% level

Table 94-A Analysis of variance for solubility of mixture between SA grade bigeye snapper surimi and mackerel surimi gel added with various whitening agents.

SV	DF	SS	MS	F
Treatment	3	44.5951	14.865	1.7E+07**
Error	4	3.5E-06	8.8E-07	
Total	7	44.5951		

**= Significant at 1% level

Table 95-A Analysis of variance for Tmax and enthalpy of mixture between SSA grade from bigeye snapper surimi and mackerel surimi added with various whitening agents

Tmax (peak 1)

SV	DF	SS	MS	F
Treatment	4	42.7502	10.6875	12.161**
Error	5	4.3942	0.87884	
Total	9	47.1444		

**= Significant at 1% level

Tmax (peak 2)

SV	DF	SS	MS	F
Treatment	4	56.5355	14.1339	38.5339**
Error	5	1.83395	0.36679	
Total	9	58.3694		

**= Significant at 1% level

Enthalpy (peak 1)

SV	DF	SS	MS	F
Treatment	4	0.02114	0.00529	264280**
Error	5	1E-07	2E-08	
Total	9	0.02114		

**= Significant at 1% level

Enthalpy (peak 2)

SV	DF	SS	MS	F
Treatment	4	0.05159	0.0129	921211**
Error	5	7E-08	1.4E-08	
Total	9	0.05159		

**= Significant at 1% level

Table 96-A Analysis of variance for Tmax and enthalpy of mixture between SA grade from bigeye snapper surimi and mackerel surimi added with various whitening agents

Tmax (peak 1)

SV	DF	SS	MS	F
Treatment	4	8.63846	2.15962	6.51782**
Error	5	1.6567	0.33134	
Total	9	10.2952		

**= Significant at 1% level

Tmax (peak 2)

SV	DF	SS	MS	F
Treatment	4	43.9185	10.9796	39.3789**
Error	5	1.3941	0.27882	
Total	9	45.3126		

**= Significant at 1% level

Tmax (peak 1)

SV	DF	SS	MS	F
Treatment	4	0.02341	0.00585	266074**
Error	5	1.1E-07	2.2E-08	
Total	9	0.02341		

**= Significant at 1% level

Tmax (peak 2[)

SV	DF	SS	MS	F
Treatment	4	0.01713	0.00428	225366**
Error	5	9.5E-08	1.9E-08	
Total	9	0.01713		

**= Significant at 1% level

Vitae

Name	Miss Yuwathida Kwalumtharn
Birth Date	22 March 1978
Place of Birth	Roi-Et, Thailand

Educational Attainment

Degree	Name of Institution	Year of Graduation
Bachelor of Science (Fisheries)	Kasetsart University	1999