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Biochemical and gelling properties of tilapia surimi and protein recovered using an acid-alkaline process

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ABSTRACT

The biochemical and gel properties of tilapia surimi prepared by a conventional washing method and protein isolated using alkaline-acid-aided processes were studied. Solubility and recovery of protein was found to be highest by using a conventional method, followed by an alkaline- and acid-aided process, respectively. Decreases in myoglobin and lipid contents were found in alkaline- or acid-aided process when compared to the conventional process ($p < 0.05$). The highest breaking force and deformation of kamaboko and modori gels was found in the gels prepared by the conventional washing method. Higher expressible water and whiteness were found in modori gels when compared to kamaboko gels. TCA-soluble peptide contents of conventional surimi gels were lower than those of acid- and alkaline-recovered protein gels. Degradation of myofibrillar protein was observed in acid-isolated protein. Microstructure of kamaboko gels showed more compact network than in modori gels in both conventional surimi and protein recovered using the pH-shift process.

1. Introduction

Surimi technology has been widely developed. Surimi is produced by repeatedly washing mechanically separated fish flesh with chilled water (5–10 °C) until most of the water-soluble protein is removed. The washing procedure is of great importance for surimi quality – not only for removing fat and undesirable materials, such as blood, pigments and odorous substances – but, more importantly, for increasing the concentration of myofibrillar protein, thereby improving gel-forming ability (Lanier & Lee, 1992). The gel-forming ability of surimi varies with the function of the myofibrillar proteins. A process that has been met with some success in recovering fish proteins is the production of surimi; however, yields are low because the process involves several washing steps (Kristinsson, Theodoure, Demir, & Ingadottir, 2005).

Acid- and alkaline-aided solubilisation has shown significant potential as a new method for maximal protein recovery from muscle food. The extraction mechanism of the two processes is to solubilise the muscle protein at low and high pH to separate soluble proteins, bone, skin, connective tissue, cellular membranes, and neutral storage lipids through the centrifugation. The solubilised proteins are collected and recovered by isoelectric precipitation to give a highly functional and stable protein isolate (Kristinsson & Ingadottir, 2006). The proteins recovered by this process have good functionality and in some cases better gelation properties than have proteins recovered by conventional surimi processing (Kristinsson et al., 2005).

Tilapia is one of the important economic freshwater fish of Thailand, constituting about 76% of the total aquaculture production of tilapia worldwide. Chiang Rai is the second largest tilapia production area in the northern part of Thailand and supports other provinces in that area. Tilapia muscle has a high content of pigments and non-structural lipids which can cause a high intensity of muddy and fishy odour. The presence of these components can strongly affect the flavour and colour of processed tilapia meat during storage and also affect consumer acceptability. Removal of the soluble sarcoplasmic proteins, lipids, fish blood, and other water-soluble materials from the flesh, as well as concentration the myofibrillar proteins by washing, have been investigated (Park, Lin, & Yongsawatdigul, 1997). This process can improve the functionalities and sensory characteristics of fish meat. However, a low yield is obtained with this process. The new approach of recovering protein by the pH-shift process can be used to encounter this problem. However, no information regarding the biochemical and gelling properties of muscle from tilapia cultured in Thailand has been reported. Therefore, the objective of this study was to investigate the effects of the pH-shift process and conventional washing on biochemical and gelling properties of tilapia muscle.

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2. Materials and methods

2.1. Chemicals

Bovine serum albumin (BSA), acrylamide, Tris, sodium dithionite were obtained from Fluka Chemika-BioChemika (Buchs, Switzerland). *L*-tyrosine, β-mercaptoethanol (βME), glutaraldehyde were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Sodium dodecyl sulphate (SDS), *N,N,N′, N′*-tetramethyl ethylene diamine (TEMED) and Coomassie Blue R-250 were procured from Bio-Rad Laboratories (Hercules, CA, USA). Sodium chloride, tricholoroacetic acid, calcium chloride, ethanol were obtained from Merck (Darmstadt, Germany).

2.2. Effect of processing conditions on biochemical properties of tilapia muscle

2.2.1. Fish sample

Fresh tilapia (weight 500–600 g/fish) was obtained from Pla Thong fish farm, Phan District, Chiang Rai, Thailand, and transported on ice to the Food Technology Department, Mae Fah Luang University. Fish were headed, eviscerated, and thoroughly washed before being subjected to mincing using a meat mincer (32 EI/80 Tre Spade, Torino, Italy) and kept in a freezer (−18 °C) until used.

2.2.2. Washed mince preparation

2.2.2.1. Conventional washing method. Washed mince (by conventional method) was prepared according to the method of Chaijan, Benjakul, Visessanguan, and Faustman (2006) with slight modifications. The washing was performed with a 1:3 (w/w) ratio of mince to cold distilled water (4 °C), and continuously stirred for 10 min in a cold room. The washed mince was filtered through four layers of cheese-cloth and then subsequently dewatered by using a hydraulic pressing machine (Owner Foods Machinery, Bangkok, Thailand). Washing was performed twice. The third washing step was carried out using 0.5% NaCl solution with a mince to NaCl solution ratio of 1:3 (w/w). Sample was referred to as the "*conventional washed mince: Con*" after dewatering.

2.2.2.2. Alkaline-aided process. The alkaline-aided process was carried out as described by Hultin and Kelleher (2000) with slight modifications. Minced fish was homogenised (IKA Labortechnik homogenizer, Selangor, Malaysia) at a 1:9 (w/v) ratio with cold distilled water for 60 s. The pH of the homogenates was adjusted to 11.2 by using 2N sodium carbonate. Homogenates was centrifuged at 10,000*g* for 20 min at 4 °C. The alkaline-soluble fraction was collected and adjusted to the isoelectric point of muscle proteins (pH 5.5) by using 2N HCl. The precipitate was then filtered through 4-folded cheese-cloth and was dewatered by centrifugation at 12,000*g* for 20 min at 4 °C. The final pH of the sample was adjusted to pH 7.0 using 2N NaOH. The sample was referred to as the "*alkaline washed mince: Alk*"

2.2.2.3. Acid-aided process. The acid-aided process was done using the method of Hultin and Kelleher (2000) with slight modifications, as described previously, except that the pH of sample was adjusted to 3.0 with 2N acetic acid. Soluble proteins were recovered by isoelectric precipitation at pH 5.5 and collected as described above. The precipitate was adjusted to pH 7.0 by using 2N NaOH. The sample was referred to as the "*acid washed mince: Acid*".

2.2.3. Physicochemical properties of minced fish

2.2.3.1. Protein solubility. The solubility of protein obtained from different processes was measured according to the method of Choi and Park (2002) with slight modifications. Samples (2 g) were homogenised with 18 ml of 0.5 M borate buffer solution, pH 11.0, for 60 s and stirred for 30 min at 4 °C. The homogenates were centrifuged at 8000*g* for 5 min at 4 °C, and the protein concentration of the supernatant was measured by the Biuret method. Protein solubility (%) was defined as the fraction of protein remaining soluble after centrifugation and calculated as follows:

$$\text{Protein solubility}\ (\%) = \frac{\text{protein concentration in supernatant}}{\text{protein concentration in homogenate}} \times 100$$

2.2.3.2. Protein recovery. Protein recovery (% yield) of the washed mince from different washing methods was determined according to the method of Kim, Park, and Choi (2003). The recovery was expressed as the weight of recovered protein divided by the weight of the minced fish (at the same moisture content). After a acid-aided, alkaline-aided or conventional washing process, the moisture content of washed mince and protein isolates was equally adjusted to 79% moisture (the initial moisture content of fish muscle); the weight of recovered protein at the same moisture content was recorded. The recovery of protein was calculated as follows:

$$\text{Protein recovery}\ (\%\,\text{yield}) = \frac{\text{weight of recovered washed mince}}{\text{weight of initial minced sample}} \times 100$$

2.2.3.3. SDS-Polyacrylamide gel electrophoresis (SDS–PAGE). The pattern of protein obtained from different methods was determined by using electrophoresis according to the method of Laemmli (1970). To 3 g of sample were added with 27 ml of 5% SDS (85°C). The mixtures were homogenised at a speed of 11,000 rpm for 1 min using an IKA Labortechnik homogenizer (Selangor, Malaysia). The homogenate was incubated at 85°C for 60 min, followed by centrifugation at 8000*g* for 5 min at room temperature, using a Mikro 20 centrifuge (Hettich Zentrifugen, Tuttlingen, Germany). The supernatants were mixed at 1:1 (v/v) ratio with the sample buffer (0.5 M Tris–HCl, pH 6.8, containing 4% SDS, 20% glycerol and 10% BME), and boiled for 3 min. The samples (20 μg protein) were loaded into the polyacrylamide gel (10% running and 4% stacking gel) and subjected to electrophoresis at a constant current of 15 mA per gel, using a Mini Protean II unit (Bio-Rad Laboratories, Inc., Richmond, CA, USA). After separation, the proteins were stained with 0.02% (w/v) Coomassie Brilliant Blue R-250 and destained with 50% methanol (v/v) and 7.5% (v/v) acetic acid, followed by 5% methanol (v/v) and 7.5% (v/v) acetic acid.

2.2.4. Total pigment and myoglobin analysis

2.2.4.1. Total pigment determination. The total pigment content was determined according to the method of Lee, Hendricks, and Cornforth (1999). Washed mince (1 g) was mixed with 9 ml of acid-acetone (90% acetone, 8% deionised water and 2% HCl). The mixture was stirred with a glass rod and allowed to stand for 1 h at room temperature. The extract was filtered with a Whatman No. 1 filter paper, and the absorbance was read at 640 nm against an acid-acetone blank. The total pigment was calculated as hematin using the following formula:

$$\text{Total pigment content}\ (\text{ppm}) = A_{640} \times 680$$

2.2.4.2. Myoglobin content determination. The myoglobin content was determined by direct spectrophotometric measurement, as described by Chaijan et al. (2006). Two grams of washed mince were weighed into a 50 ml centrifuge tube and 20 ml of 40 mM phosphate buffer, pH 6.8, were added. The mixture was homogen-

ised at 20,000 rpm for 10 s, followed by centrifuging at 3000*g* for 30 min at 4 °C. The supernatant was filtered with a Whatman No. 1 filter paper. The supernatant was treated with 0.2 ml of 1% (w/v) sodium dithionite to reduce the myoglobin and then absorbance measured at 555 nm. Myoglobin content was calculated from the millimolar extinction coefficient of 7.6 and a molecular weight of 16,111.

$$\text{Myoglobin content (mg/100 g mince)} = A \times 16,111 \times F \times Ws \times 7.6 \times 100$$

where A = absorbance, F = dilution factor, Ws = weight of mince in grammes.

2.2.5. Lipid content determination

Lipid content in all sample treatments was determined by using Soxhlet extraction according to the method of AOAC 920.39B (2000). Lipid content, on a dry weight basis, was calculated by using the following equation:

$$\%\text{Fat (dry weight basis)} = (\text{g of fat in sample/g of dried sample}) \times 100$$

2.3. Effect of processing conditions on gelling properties of tilapia surimi

2.3.1. General

After processing, the moisture content of surimi and protein isolates was adjusted to around 79%. Cryoprotectants (4% sucrose, 4% sorbitol) were added to washed mince and protein isolates accordingly, during 60 s of mixing in a mixer (T.D Chemical Trader, Birmingham, UK). Sample preparation was conducted at <5 °C. Surimi paste was then packed into polyethylene bags and frozen in a freezer. Frozen samples were stored at −18 °C until used.

2.3.2. Gel preparation

Frozen surimi and protein isolates were partially thawed at 4 °C for 4–5 h, cut into small pieces with an approximate thickness of 1 cm and then placed in a mixer (Moulinex Masterchef 350, Paris, France). The moisture content of samples was then adjusted to 80% (w/w) and salt (2.5%, w/w) was added. The mixture was chopped for 4 min at 4 °C. The paste was stuffed into polyvinylidine chloride casing with a diameter of 2.5 cm and both ends were sealed tightly. The paste was incubated at 40 °C (kamaboko gel) and 65 °C (modori gel) for 30 min, followed by heating at 90 °C for 20 min in a water bath (Memmert, Schwabach, Germany). After heating, all gels were immediately cooled in iced water for 30 min and stored at 4 °C overnight prior to analysis.

2.3.3. Measurement of gel properties

2.3.3.1. Texture analysis. Texture analysis of gels was carried out using a Model TA-XT2 texture analyser (Stable Micro System, Surrey, UK). Gels were equilibrated at room temperature (25–27°C) before analysis. Five cylindrical samples (2.5 cm in length) were prepared and tested. Breaking force (strength) and deformation (cohesiveness/elasticity) were measured by the texture analyser equipped with a spherical plunger (5-mm diameter) with a depression speed of 60 mm/min.

2.3.3.2. Whiteness determination. Five gel samples from each treatment were subjected to whiteness measurement using a Hunter-Lab instrument (Colour QuestXE, Virginia, USA). Illuminant C was used as the light source of measurement. CIE L^*, a^* and b^* values were measured. Whiteness was calculated using the following equation:

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

2.3.3.3. Determination of expressible moisture. Cylindrical gel samples were cut to a thickness of 5 mm, weighed (X) and then the sample placed between 1 piece of filter paper on top and 2 pieces of filter paper below. A standard weight (5 kg) was placed on the top of the sample for 2 min, and then the sample was removed from the papers and weighed again (Y). Expressible moisture was calculated and expressed as a percentage of sample weight as follows:

$$\text{Expressible moisture } (\%) = [(X - Y)/X] \times 100$$

2.3.3.4. Determination of TCA-soluble peptides. To 2 g of finely chopped gel samples, 18 ml of 5% TCA were added and the mixture homogenised for 2 min using an IKA Labortechnik homogeniser (Selangor, Malaysia) at a speed of 11,000 rpm for 2 min. The homogenate was incubated at 4°C for 1 h and centrifuged at 8000*g* for 5 min (25°C) using a Mikro 20 centrifuge (Hettich Zentrifugen, Tuttlingen, Germany). TCA-soluble peptides in the supernatant were measured according to the Lowry method (Lowry, Rosebrough, Farr, & Randall, 1951) and expressed as mol tyrosine/g sample.

2.3.3.5. Scanning electron microscopy (SEM). Microstructure of gels prepared by the different methods was determined using a scanning electron microscope (LE01450VP, Cambridge, UK). Samples with a thickness of 2–3 mm were fixed with 2.5% (v/v) glutaraldehyde in 0.2 M phosphate buffer (pH 7.2) for 2 h. The samples were then rinsed for 1 h in distilled water before being dehydrated in ethanol with serial concentrations of 50, 70, 80, 90 and 100% (v/v). Dried samples were mounted on a bronze stub and sputter-coated with gold (Sputter coater, SC7620, Polaron, UK). The specimens were observed with a SEM at an acceleration voltage of 10 kV.

2.4. Statistical analysis

All chemical analyses were preformed in triplicate. In physical analyses, e.g. expressible moisture, whiteness and textural properties, at least 5 determinations at each treatment were conducted. Data were subjected to analysis of variance (ANOVA). Comparison of means was carried out by Duncan's multiple-range test. Analysis was performed using a SPSS package (SPSS 10.0 for Windows, SPSS Inc, Chicago, IL, USA).

3. Results and discussion

3.1. Effect of processing conditions on biochemical properties of tilapia muscle

3.1.1. Protein solubility

The solubility of protein obtained from conventionally washed mince and the pH-shift process is shown in Table 1. Good protein solubility is believed to be a prerequisite for many functional properties, including gelation and emulsification. Protein solubility in fish muscle has been used as a criterion for the alteration of proteins (Zayas, 1997). The highest protein solubility was found in conventional surimi (3.91 mg/g), followed by the alkaline-aided process (0.57 mg/g) and acid-aided process (0.23 mg/g), respectively. Low protein solubility in alkaline-acid-aided processes is probably caused by the denaturation of muscle proteins induced by pH-shift. Decrease of protein solubility, as a result of protein denaturation, subsequently increased hydrophobic interactions, which caused precipitation of the proteins (Zayas, 1997). Kristins-

Table 1
Biochemical properties of minced tilapia recovered with different conditions

Treatment	Protein solubility (mg/g)	Recovery (%)	Lipid reduction (%)	Myoglobin (mg/100 g)	Total pigment (mg/100 g)
Conventional	3.91 ± 0.21[c]	67.9 ± 0.65[a]	67.8 ± 1.33[b]	39.5 ± 3.03[b]	451 ± 91.3[a]
Acid (pH 3.0)	0.23 ± 0.08[a]	81.4 ± 4.12[b]	85.2 ± 3.13[a]	21.4 ± 2.12[a]	682 ± 152[b]
Alkaline (pH 11.2)	0.57 ± 0.07[b]	71.5 ± 9.71[a]	88.6 ± 2.13[a]	18.5 ± 2.95[a]	448 ± 76.0[a]

Values are given as means ± SD from triplicate determinations.
[a,b,c] Different letters in the same column indicate significant differences ($p < 0.05$) between treatments.

son and Hultin (2004) concluded that the more improperly unfolded the protein (hemoglobin), the lower was its solubility. Among protein isolates, the alkaline-aided process provided higher protein solubility than did the acid-aided process. Zayas (1997) reported that protein solubility and yield of extract was greater at alkaline pH than at acid pH. However, Kristinsson and Hultin (2003) reported that the acid and alkaline unfolding of cod myosin had no impact on the solubility characteristics of myosin refolded at pH 7.5. This is likely due to the fact that the rod portion of the protein was in a native configuration after acid and alkaline treatments (Kristinsson & Hultin, 2003).

3.1.2. Protein recovery

The protein recovery for each process of minced tilapia muscle is shown in Table 1. The highest protein recovery was obtained in the acid-aided process (85.4%), followed by the alkaline-aided process (71.5%) and conventional method (67.9%). Kristinsson et al. (2005) reported that the acid- and alkaline-aided processes of channel catfish muscle gave higher protein recoveries than did the conventional surimi process. However, Kristinsson and Ingadottir (2006) found no significant difference between acid- and alkaline-aided processes for protein recoveries of tilapia light muscle. Batista, Pires, and Nelhas (2007) reported that the global yields of sardine muscle achieved were 77% and 73% for the alkaline- and acid-solubilisation. Choi and Park (2002) reported that the recoveries of Pacific whiting were about 60% and 40% by using acid-aided and conventional surimi processes, respectively. Hultin and Kelleher (2000) demonstrated that 94.4% of mackerel light meat could be recovered using the acid-aided process. Kristinsson and Liang (2006) also showed that the acid-aided process let to higher recoveries than did the alkaline-aided process for Atlantic croaker muscle proteins. The increasing yield of acid- and alkaline-aided processes was probably due to recovery of sarcoplasmic proteins in muscle. In the washing process of surimi production, sarcoplasmic proteins are readily soluble in water and removed at the washing steps. Upon extensive washing, myofibrillar proteins can also become readily soluble and then lost, resulting in a lower yield. In addition, since most sarcoplasmic proteins were retained during acid-aided processing, a maximum yield can be obtained as compared to the conventional process (Choi & Park, 2002). The alkaline-aided process shows more protein denaturation and thus less precipitate of proteins when adjusted to pH 5.5 than does the acid-aided process (Kristinsson & Hultin, 2004). Studies on catfish and tilapia demonstrated a significantly higher amount of soluble proteins left in the supernatant after the second centrifugation for the alkaline-aided process, while more sarcoplasmic proteins were recovered with the muscle proteins when using the acid-aided process (Kristinsson & Ingadottir, 2006; Kristinsson et al., 2005). This finding shows that higher protein recovery was obtained when the mince was subjected to the acid-aided process.

3.1.3. Protein pattern of tilapia washed mince and protein isolates

SDS-PAGE analysis for protein patterns of all processes is presented in Fig. 1. The most abundant protein recovered was myosin heavy chains (MHC), followed by actin (AC), troponin-T (TN-T) and tropomyosin (TM). The lowest intensity of the MHC band was found in the protein isolated by the acid-aided process. A new molecular band with a molecular weight below the MHC band was clearly seen in the acid- and alkaline-aided processes (lanes 3–4). However, disappearance of TM and high intensity of AC bands were observed in the alkaline-aided process. It could be hypothesised that a reduction of those bands was induced by either acid or alkaline hydrolysis during the solubilisation process. Kelleher and Hultin (2000) believed that the small protein bands obtained in muscle extract were a result of myosin hydrolysis induced by the activation of enzymes. Choi and Park (2002) reported that greatly reduced MHC and AC concentrates were obtained when the acid-aided process was used, with appearance of new molecular bands of 124, 78 or 70 kDa in Pacific whiting muscle. Yongsawatdigul and Park (2004) also reported that acid and alkaline solubilisation processes of rockfish muscle induced degradation of MHC, resulting in a protein band of 120 kDa. Lower intensity of AC was also found in the alkaline-aided process compared with the acid-aided process. This might reflect AC hydrolysis in the acid-aided process. Kristinsson and Ingadottir (2006) reported that more actin was found at high pH (25.8% at pH 11) compared with low pH (16.9% at pH 2.5). Hydrolysis at low pH during the acid-aided process has been observed for other species, such as herring and Pacific whiting (Choi & Park, 2002).

3.1.4. Lipid reduction

Lipid content of fresh tilapia mince was 8.57 g/100 g sample. The washing and pH-shift processes caused reduction of lipid content in fish muscle. It was found that 67.8%, 85.2% and 88.6% of lipid was reduced in the muscle after being processed with conventional washing, acid- and alkaline-aided treatments, respectively (Table 1). Batista et al. (2007) reported that the fat content

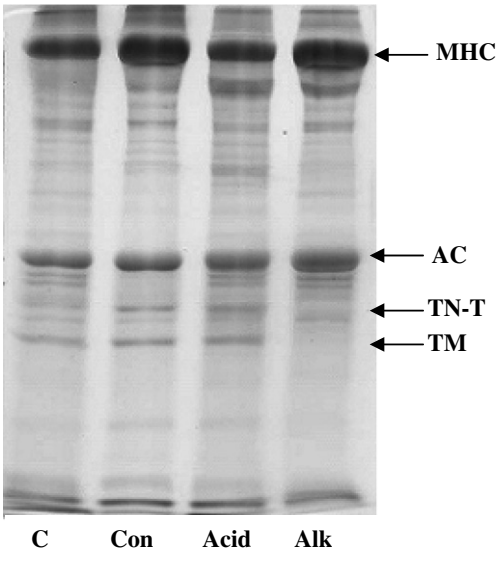

Fig. 1. SDS-PAGE of minced tilapia prepared with different conditions. C: control minced, Con: conventional method, Acid: acid-aided process, Alk: alkaline-aided process. MHC: myosin heavy chains, AC: actin, TN-T: troponin-T, and TM: tropomyosin.

reductions in muscle sardine were 65.3% and 51.0% for the proteins recovered after alkaline and acidic solubilisation, respectively. Acid-solubilisation (pH 3.0) of catfish protein removed 74% of the fat (Dewitt, Nabors, & Kleinholz, 2007). Kristinsson et al. (2005) reported that the acid- and alkaline-aided processes of channel catfish led to more reduction in lipids than did the surimi process. Lipid reduction in Atlantic croaker was more than 4 times greater when using the alkaline-aided process compared with the conventional process (Kristinsson & Liang, 2006). The lower lipid reduction for the conventional method was not surprising because membrane lipids are retained and a portion of the storage lipids co-aggregates with the proteins during the washing process. Larger reduction for the acid- or alkaline-aided processes was obtained because, at low and high pH, the solubilised proteins are separated from the storage lipids and the membrane phospholipids. On centrifugation, these components separate on the basis of density and solubility differences (Kristinsson et al., 2005). The alkaline-aided process gave the lowest lipid content when compared with other processes. Kristinsson and Hultin (2003) suggested the higher lipid removal by the alkaline process may be due to the greater emulsification ability of the proteins at alkaline pH. Hultin and Kelleher (2000) also supposed that the first centrifugation step will cause a portion of the membrane phospholipids to sediment in the bottom layer of the centrifuge tube, and also cause significant separation of neutral lipids to the top.

3.1.5. Myoglobin and total pigment contents of washed mince and protein isolates

Myoglobin extractability of tilapia muscle, processed by the conventional washing method, acid-aided and alkaline-aided processes, is shown in Table 1. A greater content of myoglobin was found in protein recovered using acid-alkaline-aided processes than in conventional surimi. The retained myoglobin contents were 39.5, 21.4 and, 18.5 mg/100 g by using the conventional method, acid-aided and alkaline-aided processes, respectively. The increase in myoglobin extractability was possibly due to the increased degradation of muscle proteins, leading to an enhanced efficiency of myoglobin removal from the disintegrated muscle. Chaijan et al. (2006) reported the alkaline solubilising process could remove myoglobin most effectively from sardine and mackerel muscles. However, myoglobin extracting efficiency depended on fish species, muscle type, storage time and washing process.

The total pigment contents of conventionally washed mince and protein isolated using the acid- or alkaline-aided process were 451, 682 and 448 mg pigment/100 g sample, respectively (Table 1). Chromoprotiens are mainly composed of a porphyrinic group conjugated with a transition metal and are responsible for colour of muscle foods. However, carotenes and carotenoproteins exist alongside chromoproteins and also play an important part in meat colour (Perez-Alvarez & Fernandez-Lopez, 2006). The two major pigments in muscle foods responsible for the red colour are myoglobin and hemoglobin. The highest total pigment removal was found in tilapia mince processed by the alkaline-aided process and conventional washing process ($p < 0.05$). The result indicated that washing could remove myoglobin and other pigments in minced fish, leading to lower pigment content in the fish muscle. Chaijan et al. (2006) noted that total extractable pigment content in sardine and mackerel muscles gradually decreased as the storage time increased. Chen (2003) also reported that myoglobin extracting efficiency in milkfish decreased with increasing iced storage time. Insolubility and binding of oxidised myoglobin to the muscle resulted in less removal of myoglobin during the washing process (Chen, 2003). It was presumed that the alkaline process could extract more pigment from the muscle. These extracted pigments could be denatured during alkaline treatment and could not be co-precipitated at pH 5.5. Therefore, they were removed from the muscle.

3.2. Effect of processing conditions on gelling properties of tilapia surimi

3.2.1. Textural properties

Gel prepared from conventional surimi showed a greater breaking force and deformation than did that from acid- and alkaline-aided processes (Fig. 2). The lowest breaking force and deformation were found in the gels prepared by the acid-aided process ($p < 0.05$). The results suggested that fish protein is extremely denatured due to the pH-shift process, especially in acidic condition. Choi and Park (2002) reported that acid processing resulted in low breaking force due to the activity of retained cathepsin L enzymes. Shikha, Hossain, Morioka, Kubota, and Itoh (2006) concluded that the neutralisation of acidified protein did not recover the gel strength to the level before acidification. However, no significant difference in breaking force of modori gels prepared from protein isolated with acid- and alkaline-aided processes was found ($p > 0.05$). When comparing kamaboko and modori gels, the breaking force of the former was higher than that of the latter ($p < 0.05$). Perez-Mateos and Lanier (2006) reported that the Atlantic menhaden gels made from acid- or alkaline-aided processes gave generally lower punch force and deformation values because those gels exhibited no apparent of transglutaminase/setting activity. In addition, apparently acid- or base-induced solubilisation leads

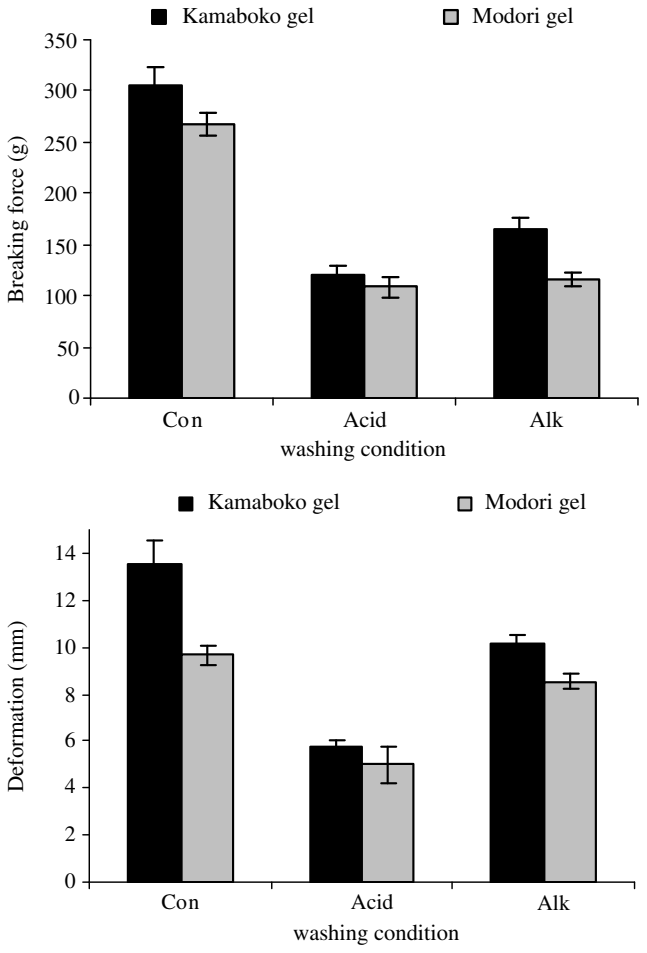

Fig. 2. Breaking force and deformation of gel from tilapia prepared with different conditions. *Bar indicated standard deviation from five determinations. **Different letters indicate significant differences between treatments ($p < 0.05$).

to substantial changes in the conformation and structure of fish proteins, leading to different properties (Kristinsson & Hultin, 2003). Chaijan et al. (2006) also reported that the surimi gel prepared by the conventional method showed greater breaking force and deformation than did that from the alkaline solubilising process. Kim et al. (2003) found higher breaking force in fish protein gel treated under alkaline conditions (pH 11) than that under acid conditions (pH 2). Yongsawatdigul and Park (2004) also reported higher breaking force and deformation were obtained in rockfish muscle protein gels prepared by alkaline solubilisation. Batista et al. (2007) reported the protein recovered after acid- or alkaline-aided processes showed poorer gelling properties than those of surimi and the acidic-recovered proteins had the lowest gel strength. With the appropriate washing, sarcoplasmic proteins could be removed, resulting in concentrated myofibrillar proteins, and consequently increased breaking force of surimi gel (Yongsawatdigul & Park, 2004). Small quantities of sarcoplasmic proteins can have adverse effects on the strength and deformability of myofibril protein gels (Hultin & Kelleher, 2000). Some sarcoplasmic proteins that remain in pH-shifting processes may be bound to the myofibrils during the heat treatment, thus decreasing the strength of the gel.

3.2.2. Whiteness of surimi and protein isolate gels

Whiteness of gels from tilapia processed with different conditions is shown in Table 2. The highest whiteness was found in surimi gels prepared by the conventional washing method ($p < 0.05$). The acid-treated gel showed a higher whiteness than that of the alkaline-treated ($p < 0.05$). Perez-Mateos and Lanier (2006) reported that conventionally washed surimi gels exhibited a higher whiteness than did the acid- or alkaline-aided processes. A similar result was reported by Chaijan et al. (2006) for the gel prepared from mackerel muscle. Kristinsson and Liang (2006) reported that both acid- and alkaline-aided isolates of croaker meat had significantly higher lightness. The lower whiteness of gels treated with acid and alkali was due to the remaining hemoproteins in the recovered protein (Choi & Park, 2002). The result also showed that higher whiteness occurred in modori gels than in kamaboko gels. Higher whiteness in the surimi gels could be due to heat treatment and changes of native heme protein during protein gelation. The lowest whiteness was probably due to higher levels of denatured and oxidised heme proteins in the alkaline-aided process than in other methods.

3.2.3. Expressible moisture of gels

The expressible moisture of kamaboko gels made from minced tilapia processed with conventional method, acid- and alkaline-aided processes were 5.39%, 7.85%, and 5.90%, respectively (Table 2). The lowest expressible moisture was found in the gel made from muscle protein washed by the conventional method. The result indicated that the protein network of that gel was higher in water-holding properties. Dewitt et al. (2007) reported that the water-holding ability values of catfish gels from treated protein (pH 3.0) were significant lower than those of gels from the control. In general, the lower expressible moisture was coincidental with the increased breaking force. High expressible moisture was found in both kamaboko and modori gels prepared with acid- or alkaline-aided processes. This was possibly due to the poor gel network of pH-shifted mince. Therefore, gel matrices that could not imbibe water led to high water releases. In addition, adjusting the pH of protein isolate to neutral can enhance the unfolding of proteins to some extent and alter the water-binding property of protein. Different expressible moisture suggested difference in the water-holding capacity of the gel network. Chaijan et al. (2006) reported that a higher expressible moisture occurred in the gels of sardine and mackerel muscle prepared by the alkaline process than in those from the conventional washing method.

3.2.4. Protein degradation in surimi and protein isolate gels

The TCA-soluble peptide contents of surimi gels varied, depending on the type of gel and the processing applied for minced fish preparation. Differences in the TCA-soluble peptide content of the gels obtained from different processings were observed (Table 2). The highest TCA-soluble peptide content (2.41 mol) was found in the gels produced from acid-aided washed mince ($p < 0.05$). When comparing kamaboko and modori gels, the former had lower TCA-soluble peptide contents than had the latter ($p < 0.05$). The presence of soluble peptides, indicated that proteolytic degradation occurred during heating of the gel sample. Kristinsson and Liang (2006) reported that, in acid- and alkaline-aided processes, there was a protein band of 150 kDa, representing partial hydrolysis of myosin. Choi and Park (2002) also reported the degradation of MHC to smaller fragments of 124, 78, 70, and 43 kDa when Pacific whiting mince was solubilised under acidic conditions

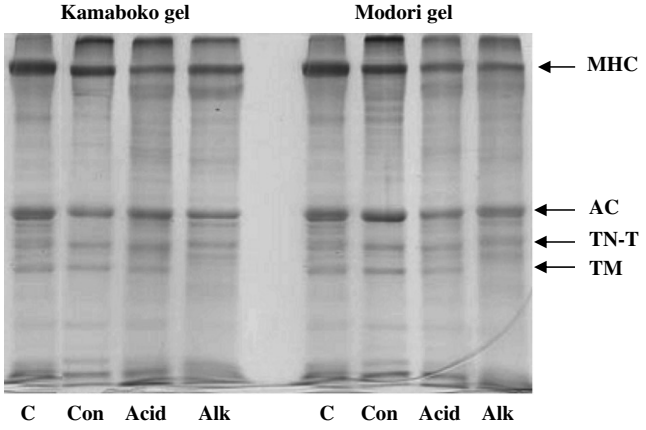

Fig. 3. Protein pattern of surimi gels prepared by using different conditions. C: control minced, Con: conventional method, Acid: acid-aided process, Alk: alkaline-aided process. MHC: myosin heavy chains, AC: actin, TN-T: troponin-T, and TM: tropomyosin.

Table 2
Whiteness, expressible moisture and TCA-soluble peptide content of surimi gels

Gel	Treatment	Whiteness	Expressible moisture (%)	TCA-soluble peptide (μmol Tyr/g)
Kamaboko	Conventional	78.33 ± 0.17^{d}	5.39 ± 0.39^{a}	1.68 ± 0.05^{a}
	Acid (pH 3.0)	76.62 ± 0.05^{c}	7.85 ± 1.14^{b}	2.06 ± 0.10^{b}
	Alkaline (pH 11.2)	60.58 ± 0.32^{a}	5.90 ± 0.16^{a}	2.05 ± 0.06^{b}
Modori	Conventional	78.59 ± 0.13^{e}	4.92 ± 0.23^{a}	1.74 ± 0.07^{a}
	Acid (pH 3.0)	77.52 ± 0.03^{d}	10.11 ± 1.61^{c}	2.41 ± 0.05^{c}
	Alkaline (pH 11.2)	64.37 ± 0.07^{b}	6.51 ± 0.86^{ab}	2.29 ± 0.17^{c}

Values are given as means ± SD from five determinations (triplicate determinations for TCA-soluble peptide content).
^{a,b,c,d} Different letters indicate significant differences ($p < 0.05$) between conditions.

(pH 2.5). High TCA-soluble peptide content indicated a greater hydrolysis of muscle proteins. The TCA-soluble peptide content in surimi produced from the conventional washing process was lower than that of other treatments ($p < 0.05$). Acidic treatments might produce small peptides, resulting in an increase in TCA-soluble peptide content. Proteolytic degradation of myofibrillar proteins, especially myosin, resulted in reduction in molecular weight and the loss of structural domains, which are essential for molecular interaction and binding (Visessanguan & An, 2000). From the result, though the incubating was conducted at 65 °C (for modori gel), which is generally a temperature suitable for heat-activated proteinases, degradation led to high TCA-soluble peptides.

3.2.5. Protein pattern of surimi and protein isolate gels

A decrease in MHC band intensity was observed in gels from all treatments compared with the minced fish (Fig. 3). The lowest MHC band intensity was found in kamaboko and modori gels prepared by the acid-aided process. However a cross-linking protein band with molecular weight higher than MHC was obtained with surimi gels from both kamaboko and modori gel prepared by the

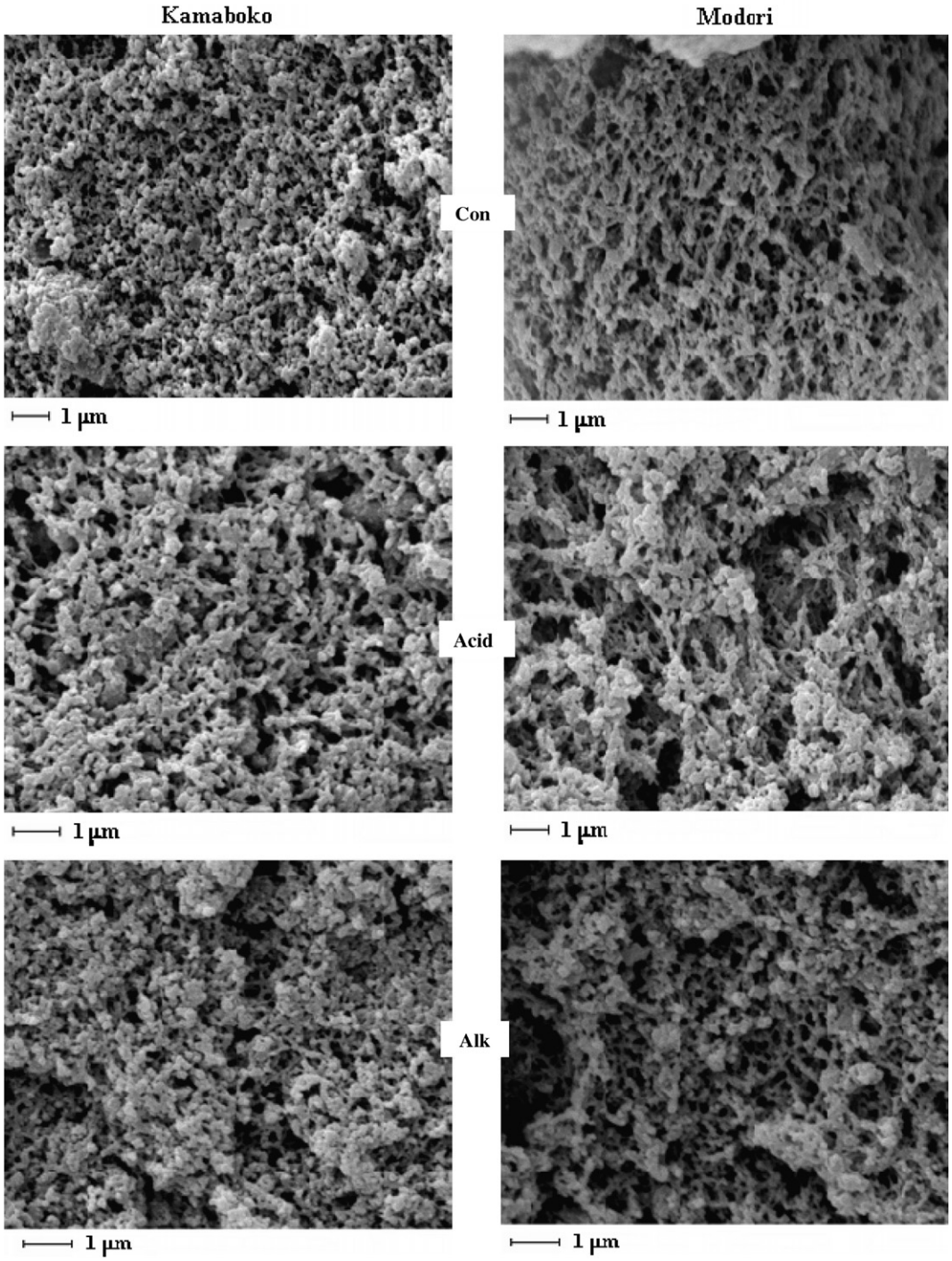

Fig. 4. Electron microscopic image of surimi gels prepared by using different conditions (magnification: 10,000×, EHT: 10 kV). Con: conventional method, Acid: acid-aided process, and Alk: alkaline-aided process.

conventional washing method. In addition, a higher intensity of cross-linking protein was found in kamaboko gel than in modori gel. The disappearance of TN-T and TM was observed with both kamaboko and modori gels prepared by using the alkaline-aided process. Yeung and Jinx-Soo (2005) suggested that degradation of MHC in croaker and jack mackerel, under acidic conditions, was higher than that under alkaline conditions. This result contributed to decrease of breaking force of the heat-induced gel from acidic processing. Decrease of MHC in gels with the acid-aided process was coincidental with decrease in the breaking force. The decrease in MHC of gels prepared using the acidic solubilisation process was coincidental with increase of TCA-soluble peptide content. However, it was not clear whether the reduction of MHC resulted from the degradation of myosin by acid protease or acid hydrolysis. Kim et al. (2003) reported that strong cathepsin L-like activity was found in Pacific whiting fish proteins treated with the alkaline process. Cathepsins B and L showed higher activities in acid-aided processes than did conventional surimi produced from Pacific whiting (Choi & Park, 2002). When comparing the MHC intensity of gels made by the conventional method and pH-shifting processes, it was found that more MHC was retained in samples prepared by the conventional method, indicating more efficiency in removal of some proteinases.

3.2.6. Microstructures of gels

The microstructures of modori and kamaboko gels, prepared by using conventional washing and acid-alkaline-aided processes, were observed by using a scanning electron microscope (SEM). Overall microstructures of gels were not significantly different from each other (Fig. 4). The gels displayed a smooth protein gel matrix with numerous small holes, which formed a porous network structure. Modori and kamaboko gels, prepared by conventional, acid and alkaline-aided processes, showed a structure with aggregates of sparse packed spherical proteins, arranged in clusters. When compared sizes of holes distributed in the matrix, the conventional gels showed the smallest hole in the structure. The regularly ordered and fine fibrillar structures in such surimi gels are likely responsible for the higher breaking force and deformation. The structure of the samples was regular, which is a characteristic of good gels that exhibit high elasticity and smooth texture (Lanier & Lee, 1992). This may be a causal factor in the change of textural properties (for example lower gel strength), considering the relative expressible moisture in samples and concentration of myofibrillar protein.

4. Conclusion

Higher protein yields, and greater lipid and pigment reductions of tilapia muscle were achieved with the acid-alkaline-aided processes than with the conventional washing process. However, conventional surimi showed a higher gel strength with lower TCA-soluble peptides. In addition, higher water-holding capacity with a more compact network, was found in kamaboko gels but a greater whiteness was obtained in modori gels.

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