Iranian Journal of Fisheries Sciences 14(4) 826- 845 2015

Physicochemical and rheological parameters changes for determining the quality of surimi and kamaboko produced by conventional, acid and alkaline solubilization process methods from common kilka (*Clupeonella cultriventris caspia*)

Shabanpour B.; Etemadian Y.*; Taghipour B.

Received: January 2014 Accepted: July 2014

Abstract

Physicochemical properties of surimi and kamaboko obtained of solubility in acid, alkaline and conventional methods were compared. The results indicated that the highest protein recovery was related to solubility in acid, alkaline and conventional methods, respectively. The highest removal of lipid and myoglobin was observed by solubility in alkali. Excretion of total pigment and sulfhydryl groups was not significantly different between solubility in alkali and acid methods. Whiteness of surimi prepared by acid method was more than the other two methods. Electrophoresis pattern in surimi produced by conventional method indicated loss of myofibril and sarcoplasmic proteins through the washing process. Solubility in acid and alkali methods showed myofibril proteins recovery along a part of the sarcoplasmic proteins and disintegration of myosin heavy chain. Physically, study of kamaboko showed that solubility in alkali generated features such as gel strength, expressible moisture, hardness, gumminess and elasticity was superior to the other two methods. About folding test and cohesiveness factor, there was no significant difference between solubility in alkali and conventional methods. In general, solubility in alkali method was better.

Keywords: Common kilka fish, Recovery, Solubility, Protein, Kamaboko

Faculty of Fisheries Science, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Beheshti Street. Iran

*Corresponding author's email: y.etemadian@gmail.com

Introduction

In recent decades the use of fish meat due to fatty acids and amino acids is highly advertised for the human body, as the health problems caused by the consumption of red meat have become ever more common. As a result, the demand for fish protein and diversity of aquatic products has increased around the world (Kristinsson and Rasco, 2000). Today, consumers are demanding products without bones and the smell of fish. In aquatics, further processing causes increase in the price of the final product; hence, the use of simple and inexpensive processes that will lead to product quality is considered a good practice. One of the main products of fish is minced meat that is the intermediate material and inexpensive compared to other aquatic products (Shahidi, 2007). The highest fish mice consumption is in the industry of surimi. Surimi is prepared preferably from the white-meat fish due to flavor, color and good gel properties. However, conventional methods of producing a good surimi from dark-meat fish have had little success. Unfortunately, in recent years, overfishing of white-meat fish has declined fish stocks massively As regards conventional surimi produced from high fat dark-meat fish there are problems such as low-grade of gel form, undesirable color and lipid oxidation. Development of new technologies to use fish meat as a source of protein is proposed (Hultin and Kelleher, 2000). Recently, new methods of extracting sustainable proteins from low fish muscle have been developed using solubility by acid and alkaline processes (Undel *et al*., 2002).

Protein extraction in both acid and alkaline processes is a form of the solubility of muscle protein at high or low pH for separating solution proteins, bone, skin, connective tissue, cell membranes and neutral storage lipids during the centrifuge. Then, the soluble proteins were collected and using isoelectric precipitation (pH approximately 5.5) a protein with high functional properties was produced (Rawdkuen *et al*., 2009). These processes are used for cheap species and dark-meat fish such as common kilka (*C.cultriventris caspia*). *Clupeonella* has been used in fish meal industry because it is not desirable and marketable. However, surimi production from *Clupeonella* for human nutrition; includes efforts for optimization of consumption and increasing of economics of the fish in Iran, is new. In this study, modern methods of protein recovery has been applied and compared with the conventional method of common kilka, surimi and kamaboko preparation.

Materials and methods

Chemicals

Ethylenediaminetetraacetic acid (EDTA), urea, tris-hydrochloride buffer (Tris-HCl), sodium dodecyl sulfate (SDS) and β-mercaptoethanol (βME), 5-5′-dithiobis (2-nitrobenzoic acid), petroleum ether, were purchased from Sigma Chemical Co. (St, Louis, MO/U.S.A).

Fish production, preparation, storage of mince and protein recovery

An amount of 20 kg common kilka (*C. cultriventris caspia*) was purchase from fishery cooperatives at the beach of Babolsar, Mazandaran Province and with a

mixture of ice and water was transferred immediately to the laboratory of fisheries. The fish was washed with water and kept on ice during the preparation process. Intestines and offal discharge, bone removal and be-heading was implemented manually and fillets were wheeled with a meat grinder with a pore diameter of 5 mm. Fish mince of was maintained in the zipper bag packs in the freezer at -80ºC until recovery of proteins. Thawing for protein recovery using conventional washing, acid and alkali solubility was performed by placing mince overnight at refrigerator temperature.

Production of surimi by conventional method

To produce surimi; a conventional laboratory scale process was used. Separately, mince muscles of silver carp were gently mixed into 3 volumes of cold (4°C) water and slowly stirred with a rubber spatula for 15 min, following a 15 min period of settling. The slurry was then dewatered by pouring it into a strainer lined with two layers of cheesecloth followed by squeezing loosely bound water out of the washed material. This process was repeated 2 times, with the last wash including 0.2% NaCl to aid in dewatering. All steps were performed on ice (Kristinsson *et al*., 2005).

Protein isolation (PI) via the acid and alkaline solubility processes

Ground muscles of *Clupeonella* were homogenized for 1 min (speed 24) with 9 volumes of ice-cold distilled water using an Ultra-Turrax T2; (IKA Working Inc., Willington, N.C., U.S.A.). The proteins in the homogenate were solubilized by drop-wise addition of 2 N HCl or 2 N NaOH until a pH of 2.5 or 11 was reached. The protein suspension was centrifuged within 20 min at 10000g. The supernatant was separated from the emulsion layer by filtering these two phases through double cheesecloth. The soluble proteins were precipitated by adjusting the pH to 5.5 using 2 N NaOH or 2 N HCl. Precipitated proteins were collected via a second centrifugation at 10000g (20 min) (Undeland *et al*., 2002). To calculate the protein recovery (percent) in the acid and alkaline processes, the following formula was used:

[(total muscle proteins – proteins of non-liquid fractions from the first centrifugation – proteins of supernatant from the second centrifugation)/total muscle proteins] × 100

Moisture content

The plate was placed in the oven (105^0C) for half an hour. Cooled in desiccators and then weighed. 5-10g sample was weighed (M_0). The samples were placed into the plate and again weighed (M_1). Then the samples were placed in oven, and after 6 hours were removed and cooled in desiccators and weighed (M_2) (Parvaneh, 2007).

Moisture percent = $(M_1 – M_2) \times 100 / M_0$

Lipid content

Soxhlet apparatus was used to measure lipid. A 1g sample was used for this work (Parvaneh, 2007). The lipid percent was calculated using the following formula: lipid content in sample × 100 / 1g sample

Ash content

First, a porcelain crucible with lid was placed in the oven and then cooled in desiccators and weighed (M_0). Afterwards, 1g of dried sample was placed in it. It was placed in electric oven in 500-550^{0}C temperature and then cooled in desiccators and weighed again (M_1) (Parvaneh, 2007). Ash percent was calculated as ($M_1 – M_0$) × 100 / 1g sample.

Measurement of protein solubility

The solubility of protein obtained from different processes was measured according to the method of Rawdkuen, *et al*. (2009). Samples (2g) were homogenized 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 8000g for 5 min at4°C, and the protein concentration of the supernatant was measured by the Biuret method. Protein solubility (%) was defined as the fraction of the protein remaining soluble after centrifugation and calculated as follows: Protein solubility (%)=(protein concentration in supernatant/protein concentration in homogenate) × 100

Measurement of protein

An amount of 1ml of the unknown protein sample with 4 ml Biuret reagent was placed at room temperature after shaking for 20 min. Absorbance value of the unknown sample was put in the formula and the protein concentration of the unknown sample was obtained. In the case of dilution, the dilution rate was multiplied with the protein concentration and the actual amount of the protein in the sample is calculated. The accuracy of this method is between 0.5 to 10 mg/ ml protein. Also, for removing turbidity of the fat centrifuging with petroleum ether was applied.

Measurement of protein recovery

To determine the percentage of the protein recovery the following formula was used (Chen and Jaczynski, 2007):

$$\text{Protein recovery (\%)} = \frac{\text{The amount of protein in surimi}}{\text{The amount of protein in mince}} \times 100$$

Measurement of total pigment content

The total pigment content was determined according to the method of Lee *et al*. (1999). The washed mince (1g) was mixed with 9 ml of acid-acetone (90% acetone, 8% deionized 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 passed through 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 (Nolsøe and Undeland, 2009) using the following formula: Total pigment content (ppm)= $A_{640} \times 680$

Measurement of myoglobin

The myoglobin content was determined by direct spectrophotometric measurement, as described by Chaijan *et al*. (2005). A chopped sample of flesh (2g) was weighed inside a 50 ml polypropylene centrifuge tube and 20 ml of cold 40 mM phosphate buffer, pH 6.8, were added to it. The mixture was homogenized at 13,500 rpm for 10 s, followed by centrifuging at 3000g

for 30 min at 4^0C. The supernatant was filtered with Whatman No. 1 filter paper. The supernatant (2.5 ml) was treated with 0.2 ml of 1% (w/v) sodium dithionite to reduce the myoglobin. The absorbance was read at 555 nm against a cold 40 mM phosphate buffer blank. The myoglobin content was calculated from the milli molar extinction coefficient of 7.6 and a molecular weight of 16,110 (Gomez-Basauri and Regenstein, 1992). The myoglobin content was expressed as mg/g sample.

Measurement of total sulfhydryl (SH) groups

Total SH groups of samples treated at various treatments were determined according to Monahan *et al*. (1995). Samples (1g) were homogenized in 9 mL of solubilizing buffer (0.2 M Tris-HCl,2% SDS, 10 mM EDTA, 8 M urea, pH 8.0) (Ultra-Turrax T25; IKA Working Inc., Willington, N.C., U.S.A.). The homogenates were heated at
100 °C for 5 min and centrifuged at 10000×g for 15min (Eppendorf Model 5810R; Westbury, N.Y., U.S.A.). To 1 mL aliquot of the supernatant we added 0.01 mL Ellman's reagent (10 mM 5, 5′-dinitrobis [2-nitrobenzoic acid]). The mixture was incubated at 40 °C for 25 min (Yongsawatdigul and Park, 2004). The absorbance at 412 nm was measured to calculate the total SH groups using the extinction coefficient of 13600 M–1cm–1 (Ellman, 1959).

Measurement of color

Color of the surimi and the protein isolation were determined by using a Hunter Lab (Lovibond, CAM-System 500). Whiteness was calculated according to the following formula: Whiteness=100–$[(100 – L^*)^2 + a^{*2} + b^{*2}]^{1/2}$

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out according to the method of Laemmli (1970) using 5% stacking gel and 15% separating gel. Proteins (30 µl) were loaded in this examination.

Gel preparation

Frozen surimi and protein isolates were partially thawed at 4C for 4–5h. The moisture content of samples was then adjusted to 80% (w/w) and then salt (3%, w/w) was added (Shabanpour *et al*., 2006). The mixture was chopped for 4 min at 4 C. The paste was stuffed into five layers of polyamide sausage casing with a diameter of 2.5 cm and both ends were sealed tightly. The paste was incubated at 40 C, followed by heating at 90C for 30 min in a water bath (Memmert, Schwabach, Germany). After heating, all gels were immediately cooled in cold water for 10 min and stored at 4C overnight prior the analysis (Jafarpour and Gorczyca, 2008a). Once cooled, the kamaboko gels were removed from the tubes with a plunger and sliced into the required dimensions for analysis of large-scale texture characteristics (gel strength and texture profile analysis).

Physicochemical properties of the gel (kamaboko)

Folding test

Six pieces of gel with thickness of 3 mm were prepared and according to the instructions using a tissue, tested and given a rating (Park, 2005). According to the guidelines, if samples folded twice without any cracks or fractures, the quality is AA with a score of 5, if samples folded once without any cracks or fractures, the quality is A with a score of 4, with the gradual breakdown of samples after folding, the quality is B with a score of 3, when the samples break into two parts, the quality is C with a score of 2 and breakage of samples with finger pressure without folding, lowers the quality to D with a score of 1 (Shabanpour *et al*., 2006).

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 (5kg) 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 percentage of sample weight using the following formula (Rawdkuen *et al*., 2009):

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

Measurement of texture profile

Texture profile analysis (TPA) was performed using a texture analyzer (Stable Micro Systems Ltd., Vienna Court, Lammas Road, Godalming, Surrey GU7 1YL, UK) equipped with a 10-kg load cell and Texture Pro Lite V1.0 software. Samples with thickness of 3 cm were compressed perpendicularly using a 50.8-mm diameter cylindrical probe and a speed of 1 mm/s. Texture variables (hardness, gumminess, cohesiveness and elasticity) were calculated as described by Jafarpour and Gorczyca (2008b).

Puncture test

Puncture test was carried out according to the method described by Kim *et al*., (2005). A texture analyzer (Stable Micro Systems Ltd., Vienna Court, Lammas Road, Godalming, Surrey GU7 1YL, UK), used for the puncture test, was equipped with a spherical-ended stainless steel plunger (P/5 S, Ø=5 mm) and had a cross-head speed (deformation rate) set at 60 mm/min with a 25-kg load cell. Breaking force (g) and breaking distance [deformation (mm)] were determined and gel strength was calculated. All results reported are an average of three replicates (Jafarpour *et al*., 2008a).

Breaking distance (mm) × Breaking force(g) = Gel strength

Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) and significant differences between mean values were determined by a least significant difference (LSD) test using the SPSS statistical package (SPSS 16.0 for Windows, SPSS Inc, Chicago, IL, USA). Levene's test was used to assess normality of the data. The folding test was performed through the nonparametric Kruskal-Wallis to compare the means.

Results

In this study, surimi and kamaboko was prepared from common kilka (*C.*

cultriventris caspia) using modern methods of acid and alkali solubility and compared with the conventional method.

According to Table 1, solubility of surimi obtained at pH=2.5 and 11 was 46% and 35%, respectively, whereas the solubility of protein in the conventional method was nearly double. These results are consistent with studies of Cortes-Ruiz *et al*. (2001) on sardine and Rawdkuen *et al*. (2009) on tilapia. Also, the electrophoresis pattern showed that the lowest hydrolysis in the conventional method and the highest hydrolysis were observed in the method of acid solubility.

Recovery of protein in the method of the acid and alkali solubility and the conventional method was 84.19%, 73.41% and 64.54%, respectively (Table 1). The percentage of protein loss during preparing surimi from minced meat of common kilka was different in all treatments (p<0.05). According to the amount of protein of minced fish meat (22%) and Table 1, the amount of protein loss during washing process by conventional method was about 35%. These amounts were about 16% for solubility by acid method and were about 27% for solubility by alkali method. The results showed that the loss of protein in the conventional method compared to solubility by acid method was more than 2 times.

Table 1: Solubility of protein recovery, protein percent and protein recovery from minced meat of common kilka.

Treatment / Factor	Acid	Alkaline	Conventional
Protein solubility (mg/g)	$0.35^{c}\pm0.02$	$0.46^{b}\pm0.02$	$0.77^{a}\pm0.06$
Protein (%)	$18.52^{a}\pm0.06$	$16.47^{b}\pm0.49$	$14.48^{c}\pm0.21$
Protein recovery (%)	$84.19^{a}\pm2.31$	$73.41^{a}\pm1.11$	$64.54^{c}\pm1.53$

Letters (a-c) indicate significant differences according to LSD test at the 5% level.

The result of lipid content, total pigment and myoglobin is shown in Tables 2 and 3. Based on the results, solubility by acid and alkali methods were more efficient than conventional methods in lipid and pigment removal. But in both factors, there were not significantly different between acid and alkaline solubility methods (p<0.05), and the percentage of pigment removal and lipid from minced meat of common kika using acid and alkaline solubility methods was similar. The difference between the percentages of myoglobin removal in the method of solubility by acid was not significantly different with conventional methods and solubility by alkali process, but myoglobin removal in both methods of conventional methods and solubility by alkali process was significant (p<0.05).

Table 2: Lipid and pigment contents of surimi prepared from common kilka by conventional method and pH changes.

Treatment / Factor	Acid	Alkaline	Conventional
Lipid percent (g dry weight)	2.38^{b}±0.28	1.88^{c}±0.15	6^{a}±1.15
Pigment (mg/100g)	106.08^{b}±26.93	113.9^{b}±23.56	444.38^{a}±38
Myoglobin (mg/100g)	74.9^{ab}±21.78	54.06^{b}±19.8	93.63^{a}±8.23

Letters (a-c) indicate significant differences according to LSD test at the 5% level.

Table 3: Percentage of lipid removal and pigment of surimi prepared from common kilka by conventional method and pH changes.

Treatment / Factor	Acid	Alkaline	Conventional
Percentage of lipid removal	80.32^{b}±0.22	84.31^{a}±1.41	50.11^{c}±7.12
Percentage of pigment removal	78.49^{a}±3.35	79.99^{a}±4.07	19.39^{b}±0.9
Percentage of myoglobin removal	83.3^{ab}±4.1	88.07^{a}±3.98	79.13^{b}±1.17

Letters (a-c) indicate significant differences according to LSD test at the 5% level.

Minced fish lipid from common kilka was based on the dry weight of 13g per 100g sample. The washing process and pH changes reduced the amount of lipid in the muscle. The reduction of lipid in the conventional method, solubility by alkali and acid methods were 50.11, 80.32 and 84.31 respectively. These results were consistent with other studies.

According to Table 3, the removal efficiency of total pigment in the conventional method, solubility by acid and alkali was 19.39%, 78.49% and 79.99%, respectively and the difference was not significant between acid and alkaline solubility (p>0.05). Also, the difference was not significant between removal of myoglobin in solubility by acid method with conventional method and solubility by alkali method (p>0.05). This was probably due to protein hydrolyze in the method of solubility by acid and alkali, and the muscle myoglobin effectively removed (Rawdkuen *et al*., 2009). According to Table 4, a^* index shows red color. The results obtained in the present study, showed that a^* index decreased in all treatments. But this decrease was observed in solubility by acid method more. This decrease is a result of removal of heme proteins. b^* index shows yellow color (Choi and Park, 2002b). Increase in b^* index of surimi samples prepared by acid and alkaline methods is a result of denaturation and oxidation of heme proteins (Yongsawatdigul *et al*., 2004). However, this parameter did not change in the conventional method. Also in this study, colorimetric parameters of L^* and whiteness were significantly increased (p<0.05). Between treatments, the lowest whiteness was observed in the conventional method and then in the method of solubility by alkali. According

to the results, the total pigment removal and myoglobin in solubility by acid and alkali method was similar. However, the whiteness of surimi samples prepared by acid solubility process was more. In the conventional method, despite the removal of myoglobin and sarcoplasmic proteins, the remaining dorsal skin of kilka muscle that contains high levels of melanin; the whiteness content is less than the other two processes (Table 4).

Table 4: Colorimetric parameters of surimi prepared from common kilka by conventional method and pH changes

Treatment / Factor	Acid	Alkaline	Conventional
L*	71.27 [a] ±0.46	66.17 [b] ±0.46	46.8 [c] ±1.57
a*	3.73 [b] ±0.25	5 [a] ±0.17	5.43 [a] ±0.42
b*	0.4[c]	1.2[a]	-1.2[b]
Whiteness	71.02 [a] ±0.49	65.8 [b] ±0.43	46.58 [c] ±1.42

Letters (a-c) indicate significant differences according to LSD test at the 5% level.

In Table 5, the obtained results of measuring total sulfhydril groups are shown. There was no significant difference between the method of solubility by acid and alkali (p>0.05). But there was statistically a significant difference between these methods with the conventional washing method.

Table 5: Total sulfhydryl groups (SH) of surimi prepared from common kilka by conventional method and pH changes.

Treatment / Factor	Acid	Alkaline	Conventional
SH (mol/10^5 g protein)	4.93 [a] ±0.078	4.6 [a] ±0.02	3.7 [b] ±0.021

Letters (a-c) indicate significant differences according to LSD test at the 5% level.

In Fig. 1, polypeptide pattern of kilka proteins recovered by the conventional washing method, and solubility by acid and alkali processes is shown. The band intensity of myosin heavy chain and actin using the conventional washing method did not change, but protein hydrolysis was obvious in the method of acid and alkali solubility.

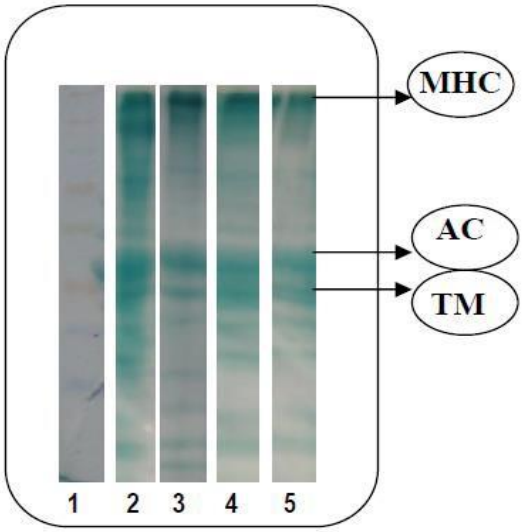

Figure 1: SDS-PAGE pattern of minced common kilka prep ared with different condition. Lane 1: Marker, lane 2: minced common kilka without processing, Lane 3: common kilka pretreated with conventional method, Lane 4: common kilka pretreated with acid-aided process, Lane 5: common kilka pretreated with alkaline-aided process. MHC: myosin heavy chains, AC: actin and TM: tropomyosin.

In Table 6, the difference between gel strength and breaking force of kamaboko produced was significant at each of the three treatments, whereas the difference between gel failure distance was not statistically significant in the method of acid solubility compared to the conventional method (p>0.05). About the folding test, gel produced by the conventional method and the solubility by alkali process were rated 4 and showed a significant difference with the method of solubility by acid. Expressible moisture content was significantly different among all treatments (p<0.05). Maximum gel strength was related to surimi produced using solubility by alkali process, the conventional method and solubility by acid process, respectively. Results of the studies are different in this field due to different responses of proteins to separation processes.

Table 6: Gel strength and expressible moisture of kamaboko prepared from common kilka after spending setting stage until the next morning in the refrigerator and bake for 30 min at 90°C.

Factor \ Treatment	Acid	Alkaline	Conventional
Breaking force (g)	182.00^{c}±4.24	222.5^{a}±3.54	197.67^{b}±2.08
Distance (mm)	4.7^{b}±0.13	5.75^{a}±0.05	5.04^{b}±0.08
Gel strength (g×mm)	846.4^{c}±31.96	1284.03^{a}±26.19	996.79^{b}±7.41
Folding test	3^{b}	4^{a}	4^{a}
Expressible moisture (%)	6.4^{a}±0.56	4.74^{c}±0.28	5.42^{b}±0.26

Letters (a-c) indicate significant differences according to LSD test at the 5% level.

The rating of folding test, being indicative of the cohesiveness parameter of the gel texture was implemented according to Shabanpour *et al*. (2006). In this study, gels obtained by the conventional method and the solubility by alkali process were rated 4 and the method of solubility by acid was rated 3 out of 5. This indicated that the acidic gel is weaker than other gels. In other research, gel obtained using the solubility by acid and alkali was received the full score (Undeland *et al*., 2002; Kristinsson *et al*., 2006c).

Expressible moisture of gels obtained by the conventional method, and the solubility by acid and alkali processes was 4.52%, 6.4% and 4.74% respectively (Table 6). The difference between hardness, gumminess and elasticity of kamaboko produced using conventional method and solubility by acid and alkali process was significant (p<0.05). But the difference between cohesiveness of kamaboko produced using conventional method and solubility by alkali process was not significant (p>0.05), and in all parameters, solubility by alkali method was higher (Table 7). Also, in this study, the most hardness, cohesiveness, elasticity and gumminess were observed in pH 11 and the least contents of factors were observed in pH 2.5.

Table 7: Texture profile of kamaboko prepared from common kilka after spending setting stage until the next morning in the refrigerator and bake for 30 min at 90ºC.

Treatment / Factor	Acid	Alkaline	Conventional
Hardness (N)	$28.03^{c}\pm1.38$	$35.03^{a}\pm0.47$	$31.07^{b}\pm0.7$
Cohesiveness (-)	$0.28^{b}\pm0.01$	$0.35^{a}\pm0.02$	$0.33^{a}\pm0.01$
Elasticity (-)	$0.53^{c}\pm0.03$	$0.66^{a}\pm0.02$	$0.61^{b}\pm0.02$
Gumminess (N)	$12.18^{c}\pm0.05$	$15.15^{a}\pm0.05$	$14.15^{b}\pm0.04$

Letters (a-c) indicate significant differences according to LSD test at the 5% level.

Discussion

Global demand for surimi consumption was 600 thousand tons in 2008, while the production of surimi was only about 500 thousand tons. Lack of resources for surimi production and 70% increase in raw material, and the cost of fishing, has made the use of new materials for the production of surimi and or surimi products improvement essential (Leksrisompong, 2008).

Myofibrils protein solubility plays an important role in the coagulation and water holding capacity of muscle proteins (Hultin and Kelleher, 1999). The high solubility in surimi produced through the conventional method was due to lower protein hydrolysis during the process.

The high solubility of obtained surimi had direct correlation with gel formation. The high solubility of proteins leads to uniform distribution in the gel and the resulted product is high quality. This

subject is almost consistent with the results of kamaboko (Table 6) that showed the gel obtained from the solubility of alkaline and the conventional method is higher quality than the gel obtained from the solubility of acid. In all studies, the effectiveness of conventional washing method for producing surimi was lower than the method of pH changes, while the efficiency of the solubility by acid and alkaline processes were different. Recovery of protein using solubility by acid and alkaline processes is related to three main factors: protein solubility at high and low pH, the amount of material deposited on the first centrifugation and isoelectric point of the protein solubility. In some studies, such as those of Park and Morrissey (2000), Kim *et al*. (2003), Kristinsson and Ingadottir (2006a) the solubility by alkaline process had higher efficiency in protein recovery compared to the solubility by acid process. However, in most studies, efficiency of the solubility by acid process was more than the solubility by alkaline process. In this regard, we can point out to reported studies by Undeland *et al*. (2002) on herring light muscle, Ingadottir (2004) on tilapia white muscle, Kristinsson and Liang (2006c) on Atlantic croaker muscle, Rawdkuen *et al*. (2009) on tilapia and Shirvani *et al*. (2010) on silver carp muscle. These results suggest that the effect of pH change method is more than conventional method due to more recovery of sarcoplasmic proteins that effectively prevent the loss of myofibril proteins during washing and dewatering sequential processes. This will lead to more recovery of muscle proteins in the pH changes. In the conventional method of producing surimi, during the washing process in successive cycles, sarcoplasmic proteins are easily dissolved in water and removed. With increase in washing, myofibril proteins are dissolved and removed. So, protein recovery is reduced in the conventional method (Choi and Park, 2002a). In the method of alkaline solubility due to more PK_a and less ionization groups, compared to the solubility by acid less denaturation occurred. When the pH adjusted to 5.5, the protein was less precipitated. Also, the property of fat emulsions in the alkaline pH was more than acidic pH. This caused most of protein being trapped in the method of the solubility by alkali process that reduced protein recovery (Kristinsson *et al*., 2005).

The decrease in lipid content led to increase in surimi shelf-life. Due to high contents of mitochondria, kilka fish has great phospholipids membranes that are the main material of oxidation in muscle tissue. So, the processing is effective for protein recovery from kilka muscle that increase the removal of phospholipids membranes (Shahidi, 2007). Kristinsson and Demir (2003) reported that the removal of lipid from four different species of fish in the method of alkaline solubility was higher than other methods, and found that solubility by alkali process had more oxidative stability than the conventional washing method and solubility by acid process. Petty and Kristinsson (2004) studied on Spanish mackerel and reported that the oxidation was high in the method of acid solubility due to further compound of homogenous solution. More effective removal of lipid

in solubility by acid and alkali process is due to proteins dissolved in low and high pH and proteins separated from stored lipid and phospholipids membranes (Kristinsson, 2002; Kelleher *et al*., 2004). These compounds were separated during centrifugation based on the difference in density. Phospholipids were deposited on the substrate of centrifuge tube, and a lot of neutral lipid was floating in the surface layer (Kristinsson *et al*., 2005). Also, this process is in relation to factors such as fat content in the first materials, homogenized viscosity of adjusting pH and centrifuge speed. Centrifuge speed in the removal of lipid from membranes and their deposition was more effective than other factors (Nolsøe *et al*., 2009). Less removal of lipid from minced muscle in washing by the conventional method is due to remaining lipid of membranes and stored lipid that attached to portions. The cause of more effective lipid removal in solubility by alkali method was in relation with more emulsification of proteins in high pH. Kristinsson and Hultin (2003) and Kristinsson *et al*. (2005) reported that alkali treatment (pH=7.5-11) compared to acidic treatment (pH=2.5-7), dramatically improved properties of the emulsion formed by myosin in minced and washed muscle of cod. Also, in the method of solubility by alkali, lipid is saponified and dissolves (Shabanpour *et al*., 2006), and based on the differences in density, floats in the surface layer and causes more effective removal of lipid.

Surimi and protein isolate, both are intermediate products that are used in the manufacture of other products, and lightness and whiteness in their texture are very important. Lightness and whiteness of these products, allow change in the color of products (Park, 2005). Color of the product is influenced by factors such as dark muscle tissue, blood and pigments such as melanin. Melanin can enter through the eyes, skin or black lines around the abdomen to produce texture (Hultin *et al*., 2005). So, one of the important parameters for processing is color. Frequently, surimi with higher whiteness is more desirable. Of course, it depends on the use of surimi (Tabilo-Munizaga and Barbosa-Canovas, 2004). Chaijan and Benjakul (2006a) reported that the solubility by alkali method has more efficiency in removal of myoglobin from sardine and mackerel muscle than the conventional method. They noted that species type, muscle type, storage time and washing conditions in removal of myoglobin are efficient.

Chromoproteins are often composed of a group with a symmetrical crystal structure and a variable metal that cause color of muscle foods. Also, carotenes and carotenoproteins play important role in meat color. However, emoglobin and myoglobin are two main pigments that constitute red color in muscle foods. They have the essential role in whiteness content and are one of the important factors in the quality of surimi gel (Perez-Alvarez and Fernandez-Lopez, 2006; Rawdkuen *et al*., 2009). Myoglobin is located on the inner structure of muscle, and is not easily removed by the conventional method. However, hemoglobin is easily lost during manipulation and maintenance (Karayannkidis *et al*., 2007). Also, being insoluble and oxidation of muscle

myoglobin will result in removal of less myoglobin during washing process. So, discoloration of muscle is higher because of the reaction of myoglobin with other muscle components, especially myofibril proteins (Kristinsson and Liang, 2006c). The whiteness of surimi samples prepared by acid solubility process was more. It is probably due to the degradation of pigments in low pH than high pH. In other studies, the relationship between the whiteness content with direct removal of myoglobin, hemoglobin and total pigment has not been investigated. Rawdkuen *et al*. (2009) studies on tilapia indicated that despite the effective removal of total pigment and myoglobin in the method of solubility by alkali, the kamaboko produced had less whiteness. They noted denaturation and oxidation of heme proteins are main reasons. Cortes-Ruiz *et al*. (2001) reported that the higher whiteness is related to surimi prepared by the conventional method and the gel produced compared to the method of solubility by acid process.

When a side of protein chain is containing an amino acid with sulfhydril group, if reacted with other chain, disulfide bond is created between them. This bond is very important in the coagulation of fish protein and improves value-added food texture (Jaczynski, 2008). pH change leads to the emergence of sulfhydryl groups in the three-dimensional structure of protein. But the removal of sarcoplasmic and myofibril proteins during the washing process in the conventional method reduces total sulfhydryl groups. This is consistent with the results obtained in this study and the studies of Choi and Park (2002a) and Ingadottir (2004).

Myofibril proteins of fish contain myosin, actin, tropomyosin, troponin, actin and a set of different proteins with lower percentage (Razavi-Shirazi, 2001). In the SDS-PAGE, the pattern of surimi prepared by the conventional method can be inferred that the heavy chain of myosin does not decompose but the band intensity of proteins such as actin and tropomyosin is reduced during washing process. However, it is clear that the myosin heavy chain in the method of acid and alkali solubility is decomposed. However, decomposing value in the alkaline pH was more than the acidic pH. Undeland *et al*. (2002) stated that there was an important difference between solubility by acid and alkali methods. This difference was due to further decomposition of myofibril proteins in the method of acid solubility compared to solubility by alkali method, but they were unable to determine the main reason for this decomposition. Also, Rawdkuen *et al*. (2009) studying on tilapia surimi reported that a band with lower molecular weight than myosin heavy chain was observed in the method of acid and alkali solubility.

In the recovery of proteins from fish muscle, some functional properties of the protein may have reduced and or often significantly improved. The main functional properties of fish protein are its ability to form elastic and strong gel with high water holding capacity (Shahidi, 2007). Gel is an intermediate state between solid and liquid in protein fibers that provides a protein network through cross-linking. Stability of the structure is

provided by hydrophobic and covalent interactions (Razavi-Shirazi, 2001). Studies have shown that changes in the formation of the gel depend on the species and production conditions.

The difference between the structures of the gel is a result of differences in all of proteins and formed bands during the heating process. In some studies, the gel strength of surimi produced by the conventional method was more. In this regard, this can be related to the removal of Cathepsin B and L during repeated washing (Choi and Park, 2002a), removal of sarcoplasmic proteins, lipid and other insoluble materials and increasing concentration of myosin heavy chain with more activity of Ca^{+2}-ATPase (Chaijan *et al*., 2006b; Kristinsson and Ingadottir, 2006a), the formation of large bands of sulfhydryl and high concentration of myofibril proteins after washing (Yongsawatdigul and Park, 2004). Also, researchers have noted the cause of weak gel in surimi prepared by pH shift method, the absence of transglutaminase enzyme activity in the stage of clotting gel (Perez-Mateos *et al*., 2006), negative effects of sarcoplasmic proteins and their binding with myofibril proteins, and protein denaturation in pH changes (Rawdkuen *et al*., 2009). Also in some other studies, the gel strength of surimi produced using solubility by alkali method was more. Its reasons can be increase in the reaction between proteins (Kristinsson, 2002), the low activity of proteases in the method of alkaline solubility (Kristinsson and Demir, 2003; Nolsøe and Undeland, 2009), remains of sarcoplasmic proteins and their role in increasing the break force of gel (Hultin *et al*., 2005; Nolsøe and Undeland, 2009), suggesting the formation of disulfide bands between myofibril and sarcoplasmic proteins and non-interference of sarcoplasmic proteins in gel texture characteristic (Yongsawatdigul *et al*., 2004). These researchers reported that the cause of low gel strength in the method of acid solubility is higher recovery of sarcoplasmic proteins in this method (Hultin *et al*., 1999) incomplete removal of cathepsin B and L and activity of both enzymes (Choi and Park, 2002a), further oxidation of sulfhydryl groups and limiting disulfide reaction during gel formation (Yongsawatdigul and Park, 2004), and the effect of low pH on protein structure (Davenport and kristenson, 2003). Proteolysis is a main problem in the process of extracting protein from muscle that has negative effects on protein functionality, gel formation and water holding capacity. Kristinsson and Ingadottir (2006a) and Undeland *et al*. (2002) reported proteolysis of myosin at low pH. Mireles *et al*., (2007) stated that unfolding proteins using solubility by acid and alkali process and again twisting of proteins with adjusting the pH to the isoelectric point and then to neutral pH, leads to change in proteins structure. Also, the appearance of a large number of hydrophobic sites in a good gel and especially the gel produced using solubility by acid method leads to produce gel with low water holding capacity compared to gel produced by the conventional method. The gel strength between the three treatments (conventional method, solubility by acid and alkali process) largely depends on the species

and processing conditions. Transglutaminase (TGase) is one of the sarcoplasmic enzymes that cause the phenomenon of sitting in surimi paste at low temperature. The activity of this enzyme leads to the cross-linking between proteins and increase in the gel strength. In the solubility by acid and alkali processes, the main section of sarcoplasmic proteins remains and possibly structural changes of myofibril proteins during protein recovery processes using acid and alkaline expose more functional groups to cross-link and other reactions of protein-protein by TGase (Perez-Mateos *et al*., 2006). In this study, the gel strength in the method of solubility by alkali was higher than the solubility by acid method (Table 6) due to high protein solubility and ability in forming a homogeneous gel and also due to higher water holding capacity in this method (Batista *et al*., 2007). Complete destruction of muscle structure by acid and alkali solubility may play a key role in helping to improve proteins in forming a gel by heat which in a similar effect also reduced the need for salt (Chang *et al*., 2001; Wright and Lanier, 2005). In the present study it appears that sarcoplasmic proteins and myofibril maintained in the pH changes has increased gel strength.

In studies conducted by Chaijan *et al*. (2006b), Kristinsson and Liang (2006c) and Rawdkuen *et al*. (2009) the expressible moisture was consistent with the gel strength. These researchers stated that when expressible moisture is higher, the gel network is weaker and water holding capacity is lower. Karayannkidis *et al*. (2007) washed and prepared surimi from sardine muscle in acidic and alkaline pH. They reported that the most hardness was observed in pH 2.5 and the least hardness was observed in pH 10 and 11.5. Gel cohesiveness prepared by pH 2.5 was greater than 10 and 11.5; also these researchers achieved more elastic gel in acidic conditions than alkaline conditions.

The results of the physicochemical properties study about surimi and kamaboko produced through changes of pH and the conventional method, confirmed the hypothesis of more efficiency of product solubility by acid process from minced meat of common kilka. Also, the percentage of more lipid removal was observed in the method of solubility by alkali. There was no significant difference in removal of total pigment using solubility by acid and alkali method, but it was significantly higher than the conventional method. It should be noted that depending on the species used and processing condition the results are different. According to the present study, we recommend that surimi prepared by alkaline solubility and the conventional methods from kilka fish can be used to produce a good product in spite of the poor color but due to the high strength of the gel, in products such as burgers, cutlets and sausages.

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