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Comparative study on some biochemical characteristics of surimi from common carp and silver carp and proteins recovered using an acid-alkaline process

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Abstract

Some biochemical characteristics of common carp (*Cyprinus carpio*) and silver carp (*Hypophtalmichthys moltrix*) surimi prepared by a conventional washing method and protein isolated using alkaline-acid-aided processes were investigated. Solubility of protein in silver carp and common carp were found to be highest by using the conventional washing method. Decreases in myoglobin and lipid contents in both fish were found in the alkaline- or acid-aided process when compared to the conventional process ($p<0.05$). The percentage of protein recovery with the alkaline- aided process of silver carp was highest. The whiteness score in the alkaline solubilization process was higher than acid solubilization process and conventional method in silver carp. This result was different than the result obtained in common carp. There was no significant difference between treatments regarding total sulfhydryl (SH) groups ($p>0.05$). In protein patterns of Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), the lowest intensity of the myosin heavy chains (MHC) band was found in silver carp by the conventional washing process.

Keywords: Common carp, Silver carp, Acid- alkaline solubilization, Recovery of protein

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Introduction

Given the high nutritional value of aquatic foods and their importance in the world, most fisheries resources are destined for human consumption (Nolsøe and Undeland, 2009). A process that is almost successful in recovering fish proteins is the production of surimi. But gained yields from them are little because this process involves several washing steps (Kristinsson *et al*., 2005). Surimi production is aimed at concentrating myofibrillar proteins by removing lipids, blood, enzymes and sarcoplasmic proteins through lengthy washing of the fish mince (Julavittayanukul *et al*., 2006). In the conventional method, denaturation during surimi processing must be avoided. But a novel method of obtaining fish protein isolates induces denaturation by altering the pH during the process that is appropriate (Thawornchinsombut and Park, 2006). This process for isolating the fish proteins involves the solubilization of a disperse form of the fish tissue either in an acidic (pH≤3.5) or in an alkaline (pH≥11) aqueous solution. At these low or high pH conditions the protein net charge leads to the repulsion of protein chains and their solubilization. The protein rich aqueous solution is separated from solids (insoluble proteins, skin, bones, and scales) and from neutral lipids by centrifugation. The soluble proteins are then recovered through isoelectric precipitation by adjusting the pH at 5.5 and the precipitated proteins are removed by centrifugation (Batista *et al*., 2007). However, the information about the physicochemical changes of fish muscle proteins prepared under alkaline and acid solubilization is limited. Thus, our objective was to investigate the physicochemical characteristics of fish muscle proteins prepared by acidic and alkaline solubilization processes, using common carp and silver carp.

Materials and Methods

Chemicals

Sodium tripolyphosphate (STPP), sucrose, sorbitol, sodium chloride, hydrochloric acid, sodium hydroxide, boric acid, bovine serum albumin, potassium sodium tartrate, potassium iodide, copper sulfate, Whatman No. 1 filter paper, acetone, phosphate buffer, sodium dithionite, ethylenediaminetetraacetic acid (EDTA), 5-5′-dithiobis (2-nitrobenzoic acid), urea, ether, tris-hydrochloride buffer (Tris-HCl), sodium dodecyl sulfate (SDS) and β-mercaptoethanol (βME) were purchased from Sigma-Aldrich Chemical Company, United State. All chemicals for electrophoresis were obtained from Bio-Rad laboratories company, California.

Production of surimi

To produce surimi, a conventional laboratory scale process was used. Separately, minced muscles of common carp and silver carp, were gently mixed into 3 volumes of cold (4°C) water and slowly stirred with a rubber spatula for 15min, followed by a 15 min period of settling. The slurry was then dewatered by pouring it into a strainer lined with 2 layers of cheesecloth 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). Then, all the produced surimi was blended

with a cryoprotectant mixture (4% sucrose, 4% sorbitol, and 0.3% STPP) (Undeland *et al*., 2002). The final moisture content was 75.5 %. The surimi was frozen in plastic bags at -18±2 ˚C.

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

Ground muscles of common carp and silver carp (usually 120-300g) were homogenized for 1 min (speed 24) with 9 volumes of ice-cold distilled water using an Ultra-Turrax T2 homogenizer; (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, respectively, was reached. The protein suspension was centrifuged within 20 min at 10000×g. The supernatant was separated from the emulsion layer by filtering these 2 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 was collected via a second centrifugation at 10000×g (20 min). To calculate the protein recovery (percent) obtained by the acid and alkaline processes, the following formula was used (Undeland *et al*., 2002):

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

Percent yield

Percent yield of the washed mince from the different washing methods was determined according to the method of Kim *et al*, (2003). The yield was expressed as the weight of recovered protein divided by the weight of the minced fish (at the same moisture content). After an 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 assumed moisture content of fish muscle); the weight of recovered protein at the same moisture content was recorded.

The percent yield of protein was calculated as follows:

% yield = [weight of recovered washed mince/weight of initial minced sample] ×100

Protein solubility

The solubility of protein obtained from different processes was measured according to the method of Rawdkuen *et al*. (2009). Samples (2 g) 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 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 the protein remaining soluble after centrifugation and calculated as follows:

Protein solubility (%)= (protein concentration in supernatant/protein concentration in homogenate)× 100

Color analysis

Color of the surimi and the protein isolation was determined by using a Hunter lab (Lovibond, CAM-System 500). A minimum of 3 readings of Hunter L^*, a^*, and b^* values were taken from each batch of the surimi and protein isolation process.

Whiteness was calculated according to the following formula:

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

Total pigment determination

The total pigment content was determined according to the method of Lee *et al*. (1999). 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 (Nolsøe and Undeland, 2009).

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

Myoglobin analysis

The myoglobin content was determined by direct spectrophotometric measurement, as described by Chaijan *et al*. (2005). A chopped sample of flesh (2 g) was weighed into a 50 ml polypropylene centrifuge tube and 20 ml of cold 40 mM phosphate buffer, pH 6.8, were added. The mixture was homogenized at 13,500 rpm for 10 s, followed by centrifuging at 3000g for 30 min at 4 ^{0}C. 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. Myoglobin content was calculated from the millimolar extinction coefficient of 7.6 and a molecular weight of 16,110 (Gomez-Basauri and Regenstein, 1992).

Myoglobin (mg/100g)= (Absorbance× 16110)/(2g sample × 7.6)

Total sulfhydryl (SH) groups determination

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) with Ultra-Turrax T25 homogenizer; (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 15 min (Eppendorf Model 5810R; Westbury, N.Y., U.S.A.). To 1 mL aliquot of the supernatant was 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 *et al*., 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).

Moisture content

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

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

Ash content

First, a porcelain crucible with lid was placed in the oven. Cooled in a desiccator and weighed (M_0). 1g dried sample was placed in it. It was placed in an electric oven at 500-550˚C temperature, and heated for almost 12 hours to get a light gray color.

The sample was then cooled in a desiccator and weighed again (M_1) (Parvaneh, 2007).
Ash percent = ($M_1 - M_0$) × 100 / 1g sample

Lipid content

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

SDS-PAGE

The 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 on to each well. Mobility of the protein bands was calibrated with standards of molecular weight markers. After staining and distaining, the gel was scanned using a gel documentation system (Bio-Rad, USA).

Statistical analysis

Each experiment and each assay was done in triplicate. 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 16.0 for Windows, SPSS Inc, Chicago, IL, USA).

Results

In this study solubility of protein in common carp and silver carp recovered with different conditions are shown in Table 1. The highest protein solubility in common and silver carp was found in conventional surimi (0.89 mg/g, 0.94 mg/g), followed by the alkaline-aided process (0.38 mg/g, 0.86 mg/g) and acid-aided process (0.40 mg/g, 0.24 mg/g), respectively.

The protein recovery for each process of minced common carp and silver carp muscles are shown in Table 1. In common carp, the highest protein recovery was obtained in the acid-aided process (70.20%), followed by the alkaline-aided process (64.50%) and conventional method (60.50%). But in silver carp, the highest protein recovery was obtained in the alkaline-aided process (80.89%), followed by the acid-aided process (75.18%) and conventional method (74%). Also, in this examination, there were statistical differences between samples ($p<0.05$). On the whole, it was indicated that the alkaline method gave better results on percent yield (Table 1).

The color characteristics differed among the different protein preparations (Table 2). In common carp, protein isolated using alkaline-acid-aided processes had a lower L^* value (lightness), and thus lower whiteness score, compared with conventional surimi. But in silver carp, protein isolated using acid-aided processes had lowest L^* value and highest a^* value. b^* values showed a significant difference between the acid- and alkali-made isolates and conventional surimi ($p<0.05$).

The total pigment contents of conventionally washed mince and protein isolated using the acid- or alkaline-aided process in common carp were 205.59, 107.44 and 184.05 ppm pigment/g sample, respectively. In silver carp, the total pigment contents of conventionally washed mince and protein isolated using the acid- or alkaline-aided process in common carp

were 71.63, 83.64 and 275.85 ppm pigment/g sample, respectively. Also, about total SH groups the alkaline-aided process showed a lower level of total SH groups compared with the acid-aided process and conventional surimi in both fish (Table 3).

SDS-PAGE analysis for protein patterns of all processes is presented in Fig. 1. The most abundant protein recovered was MHC, followed by actin (AC), troponin-T (TN-T) and tropomyosin (TM). The lowest intensity of the MHC band was found in silver carp by the conventional washing process (Lane 4). Disappearance of TM bands was observed in common carp treated with the alkaline-aided process (Lane 1). High intensity of AC bands was observed in silver carp treated with the alkaline-aided process (Lane 5).

Discussion

Protein solubility in the samples

Good protein solubility is believed to be a prerequisite for many functional properties, including gelation and emulsification (Rawdkuen *et al*., 2009). Protein solubility in fish muscle has been used as a criterion for the alteration of proteins (Zayas, 1997). High solubility is a prerequisite for good extraction of muscle protein and their separation from undesirable components in the acid-aided or alkali-aided processes. Low solubility, on the other hand, is important in the protein-recovery step of the process in their isoelectric point range (Kristinsson *et al*., 2005). Solubility of protein in common carp and silver carp recovered with different conditions are shown in Table 1. The highest protein solubility in common and silver carp was found in conventional surimi (0.89 mg/g, 0.94 mg/g), followed by the alkaline-aided process (0.38 mg/g, 0.86 mg/g) and acid-aided process (0.40 mg/g, 0.24 mg/g), respectively. The high solubility in surimi conventional method was due to lower protein hydrolysis during the process than that in other methods. Overall, results showed that in common carp, there was no significant difference between acid- and alkaline-aided processes ($p>0.05$). However in silver carp there was found significant difference between acid- and alkaline-aided processes ($p<0.05$) which can be due to the role of lipid and its effect on hydrolysis in fish. In common carp this matter was almost obvious. Low protein solubility in alkaline-acid-aided processes is probably caused by the denaturation of muscle proteins induced by pH-shift (Rawdkuen *et al*., 2009). Kristinsson and Hultin (2004) reported that between protein isolates, the alkaline-aided process has higher protein solubility than the acid-aided process. Also, Zayas (1997) reported that protein solubility was greater at alkaline process than at acid process. These results are consistent with the results obtained from silver carp in this study. Also Zayas (1997) reported that protein solubility was higher at alkaline process than at acid process. 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.

Protein recovery in the samples

The protein recovery for each process of minced common carp and silver carp muscles are shown in Table 1. In common carp, the highest protein recovery was obtained in the acid-aided process (70.20%), followed by the alkaline-aided process (64.50%) and conventional method (60.50%). But in silver carp, the highest protein recovery was obtained in the alkaline-aided process (80.89%), followed by the acid-aided process (75.18%) and conventional method (74%). Hultin and Kelleher (2000) demonstrated that 94.4% of mackerel light meat could be recovered using the acid-aided process. 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. 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. The lower recovery of surimi processing is reportedly due to the removal of water-soluble sarcoplasmic proteins during the washing steps (Xiong, 1997) and acceptable part of the myofibrillar proteins (Lin and Park, 1996). 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 *et al*., 2005; Kristinsson and Ingadottir, 2006). Obtained result of this study, showed that higher protein recovery was found when the mince was subjected to the acid- and alkaline-aided process in common carp and silver carp.

Percent yield

The protein yield obtained during acid and alkaline processing is primarily determined by three major factors, the solubility of the proteins at extreme acid or alkaline conditions, the size of the sediments formed during the centrifugations, and the solubility of the proteins at the pH selected for precipitation (Nolsøe *et al*., 2009). During conventional preparation of surimi, the exact yields depend mainly on the number of washes, the pH of the washing solution, and the ionic strength of the washing solution. Using the acid and alkaline processes, Undeland *et al*. (2002) found protein yields of 74±4.8% and 68±4.4%, respectively, from white muscle of herring (*Clupea harengus*). The lower yield on the alkaline side was linked to a larger sediment formation in the first centrifugation. In a similar comparison between acid- and alkali-aided processing, Kristinsson and Ingadottir (2006) investigated protein yields from tilapia (*Orechromis niloticus*). From repeated trials, they found yields from 56% to 61% with the acid process, and from 61% to 68% with the alkaline process. Also, in this study, there were statistical differences between samples ($p<0.05$). Overall, in contrast to these studies, results obtained indicate that the alkaline method gave better results (Table 1).

Table 1: Solubility and recovery of protein in common carp and silver carp with different condition.

	Treatment	Protein solubility (mg/g)	Recovery (%)	%Yield
Common carp	Conventional	0.89±0.015[a]	60.50±0.500[c]	47.94±0.145[d]
	Acid (pH 2.5)	0.40±0.042[b]	70.20±1.640[b]	55.16±0.166[b]
	Alkaline (pH 11)	0.38±0.038[b]	64.50±1.290[c]	77.88±0.262[a]
Silver carp	Conventional	0.94±0.007[a]	74.00±2.000[b]	48.24±0.389[d]
	Acid (pH 2.5)	0.24±0.007[c]	75.18±1.120[b]	51.76±0.183[c]
	Alkaline (pH 11)	0.86±0.012[a]	80.89±1.530[a]	54.42±0.298[b]

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 the treatments.

Color changes in the samples

One important parameter when comparing different processing methods is the color of the protein isolate (Nolsøe *et al*., 2009). In this study, the color characteristics differed among the different protein preparations (Table 2). In common carp, protein isolated using alkaline-acid-aided processes had a lower L^* value (lightness), and thus lower whiteness score, compared with conventional surimi. This lower whiteness likely stems from more retention of native heme proteins in the final material because redness (a^* value) was higher for protein isolated using alkaline-acid-aided processes compared with conventional surimi. b^* values (yellowness) were the same for the acid- and alkali-made isolates and conventional surimi. But in silver carp, protein isolated using acid-aided processes had lowest L^* value and highest a^* value. b^* values and showed a significant difference between the acid- and alkali-made isolates and conventional surimi (p<0.05). Choi and Park (2002) found the highest L^* and whiteness values for surimi washed three times followed by surimi washed once and then the acid produced protein isolate. Undeland *et al*. (2002) investigated the colors of acid and alkaline protein isolates from the light muscle of herring. They reported highest L^* and b* values were in the alkali-produced protein isolate. Also, in their examinations a^* value in the acid- and alkali-made isolates was equal. Kim *et al*. (1996) reported higher whiteness values and higher yellowness (b^* value) and redness values for catfish frame minced surimi. The higher L^* value could be attributed to the retention of connective tissue. More yellowness could be in part due to more retention of lipids. In both fish, more redness in acid-aided process is likely attributed to more co-precipitation of heme proteins.

Table 2: Color properties in common carp and silver carp recovered with different conditions

	Treatment	L*	a*	b*	Whiteness
Common carp	Conventional	68.20±0.231[c]	4.57±0.267[bc]	2.97±0.267[a]	67.73±0.250[c]
	Acid (pH 2.5)	59.07±0.267[e]	6.70±0.000[a]	2.47±0.233[a]	58.45±0.257[e]
	Alkaline (pH 11)	62.83±0.133[d]	6.17±0.267[a]	2.23±0.233[a]	62.25±0.073[d]
Silver carp	Conventional	71.67±0.133[b]	4.03±0.267[c]	1.47±0.267[b]	71.34±0.110[b]
	Acid (pH 2.5)	68.07±0.133[c]	5.10±0.000[b]	2.70±0.000[a]	67.55±0.130[c]
	Alkaline (pH 11)	72.93±0.590[a]	4.83±0.267[b]	2.47±0.233[a]	72.39±0.554[a]

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

Myoglobin contents of the samples

Myoglobin extractability of common carp and silver carp muscle, processed by the conventional washing method, acid-aided and alkaline-aided processes are shown in Table 3. The retained myoglobin contents in common carp were 78.44, 39.57 and 46.29 mg/100 g by using the conventional method, acid-aided and alkaline-aided processes, respectively. In silver carp, retained myoglobin contents were 40.99, 28.97 and 14.49 mg/ 100g by using the conventional method, acid-aided and alkaline-aided processes, respectively. Probably due to protein hydrolyze in the method of solubility by acid and alkali, muscle myoglobin effectively removed. Also, Chaijan *et al.* (2006) reported the alkaline solubilising process could remove myoglobin most effectively from sardine and mackerel muscles. Decreases in myoglobin contents were found in alkaline- or acid-aided process when compared to the conventional process (Rawdkuen *et al.*, 2009). In general, myoglobin extracting efficiency depended on fish species, muscle type, storage time and washing process (Chaijan and Benjakul, 2006).

Total pigment contents of the samples

Chromoprotiens are mainly composed of a porphyrinic group conjugated with a transition metal and are responsible for color of muscle foods. However, carotenes and carotenoproteins exist alongside chromoproteins and also play an important part in meat color (Perez-Alvarez and Fernandez-Lopez, 2006). The two major pigments in muscle foods responsible for the red color are myoglobin and hemoglobin (Razavi-Shirazi, 2001). In this study, the total pigment contents of conventionally washed mince and protein isolated using the acid- or alkaline-aided process in common carp were 205.59, 107.44 and 184.05 ppm pigment/g sample, respectively. In silver carp, the total pigment contents of conventionally washed mince and protein isolated using the acid- or alkaline-aided process in common carp were 71.63, 83.64 and 275.85 ppm pigment/g sample, respectively (Table 3). The highest total pigment removal was found in common carp mince processed by conventional washing process. But in silver carp, the highest total pigment removal was found in the acid-aided process ($p<0.05$). The result indicated that washing process in silver carp could remove myoglobin and other pigments in minced fish, leading to lower pigment content in the fish muscle. But this result was not compatible with results obtained in common carp. Chaijan *et al.* (2006) noted that total extractable

pigment content in sardine and mackerel muscles gradually decreased as the storage time increased.

Total SH groups contents of washed mince and protein isolates

Another way to elucidate protein aggregation is to monitor changes in SH groups (LeBlanc and LeBlanc, 1992). A change in total SH is attributed to oxidation of SH, reduction of disulfide bonds (S-S), and S-S/SH interchange reactions (Thawornchinsombut *et al*., 2006). In this examination, in both fish the alkaline-aided process showed a lower level of total SH groups compared with the acid-aided process and conventional surimi (Table 3). But there were no significant differences between the three methods used ($p>0.05$). Yongsawatdigul and Park (2004) suggested that oxidation and SH/S-S interchange reactions could occur during acid solubilization. Furthermore, a number of studies have reported a decrease in SH content of alkali-treated proteins (Monahan *et al*., 1995; Kim *et al*., 1996; Yongsawatdigul *et al*., 2004).

Table 3: Total sulfhydryl groups, myoglobin and total pigment content in common carp and silver carp recovered with different conditions.

	Treatment	SH	Myoglobin (mg/100g)	Total pigment (mg/100g)
Common carp	Conventional	6.66±0.546[a]	78.44±8.023[a]	205.59±3.536[b]
	Acid (pH 2.5)	6.60±0.194[a]	39.57±4.298[bc]	107.44±2.069[d]
	Alkaline (pH 11)	5.85±0.079[a]	46.29±6.544[b]	184.05±2.613[c]
Silver carp	Conventional	6.80±0.044[a]	40.99±0.353[bc]	71.63±0.852[f]
	Acid (pH 2.5)	6.29±0. 930[a]	28.97±3.140[dc]	83.64±.2.943[e]
	Alkaline (pH 11)	5.77±0.092[a]	14.49±0.707[d]	275.85±8.581[a]

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

Lipid, moisture and ash contents of washed mince and protein isolates

There was a significant difference between surimi prepared by a conventional washing method and protein isolated using alkaline-acid-aided processes. In this study, moisture content in common carp by use of acid-aided process showed higher value, whereas in silver carp this value was showed lower (Table 4). About lipid, decrease in lipid content in both fish were found in alkaline- or acid-aided process when compared to the conventional process ($p<0.05$). About ash, there was no significant difference between surimi prepared by a conventional washing method and protein isolated using alkaline-acid-aided processes ($p>0.05$). These factors can be important in the measurement of biochemical properties in every species of fish.

Table 4: Moisture, lipid and ash content in common carp and silver carp recovered with different conditions.

	Treatment	Moisture	Lipid (%)	Ash
Common carp	Conventional	75.85±0.105^{c}	2.17±0.167^{a}	2.93±0.133^{a}
	Acid (pH 2.5)	77.14±0.109^{b}	1.17±0.167^{b}	2.93±0.470^{a}
	Alkaline (pH 11)	73.02±0.391^{e}	1.00±0.000^{bc}	2.20±0.173^{a}
Silver carp	Conventional	75.95±0.086^{c}	1.33±0.167^{b}	2.93±0.120^{a}
	Acid (pH 2.5)	74.76±0.056^{d}	1.00 ±0.000^{bc}	2.63±0.291^{a}
	Alkaline (pH 11)	78.70±0.131^{a}	0.67±0.167^{c}	2.43±0.186^{a}

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

Protein pattern of common carp and silver carp 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 MHC, followed by AC, TN-T and TM. The lowest intensity of the MHC band was found in silver carp by the conventional washing process (Lane 4). Disappearance of TM bands was observed in common carp treated with the alkaline-aided process (Lane 1). High intensity of AC bands was observed in silver carp treated with the alkaline-aided process (Lane 5). It could be hypothesized that a reduction of those bands was induced by 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 solubilization processes of rockfish muscle induced degradation of MHC, resulting in a protein band of 120 kDa. These results were not compatible with the results of the present study. Lower intensity of AC was found in common carp treated with alkaline-aided process compared with the acid-aided process. This might reflect AC hydrolysis in the alkaline-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 and Park, 2002).

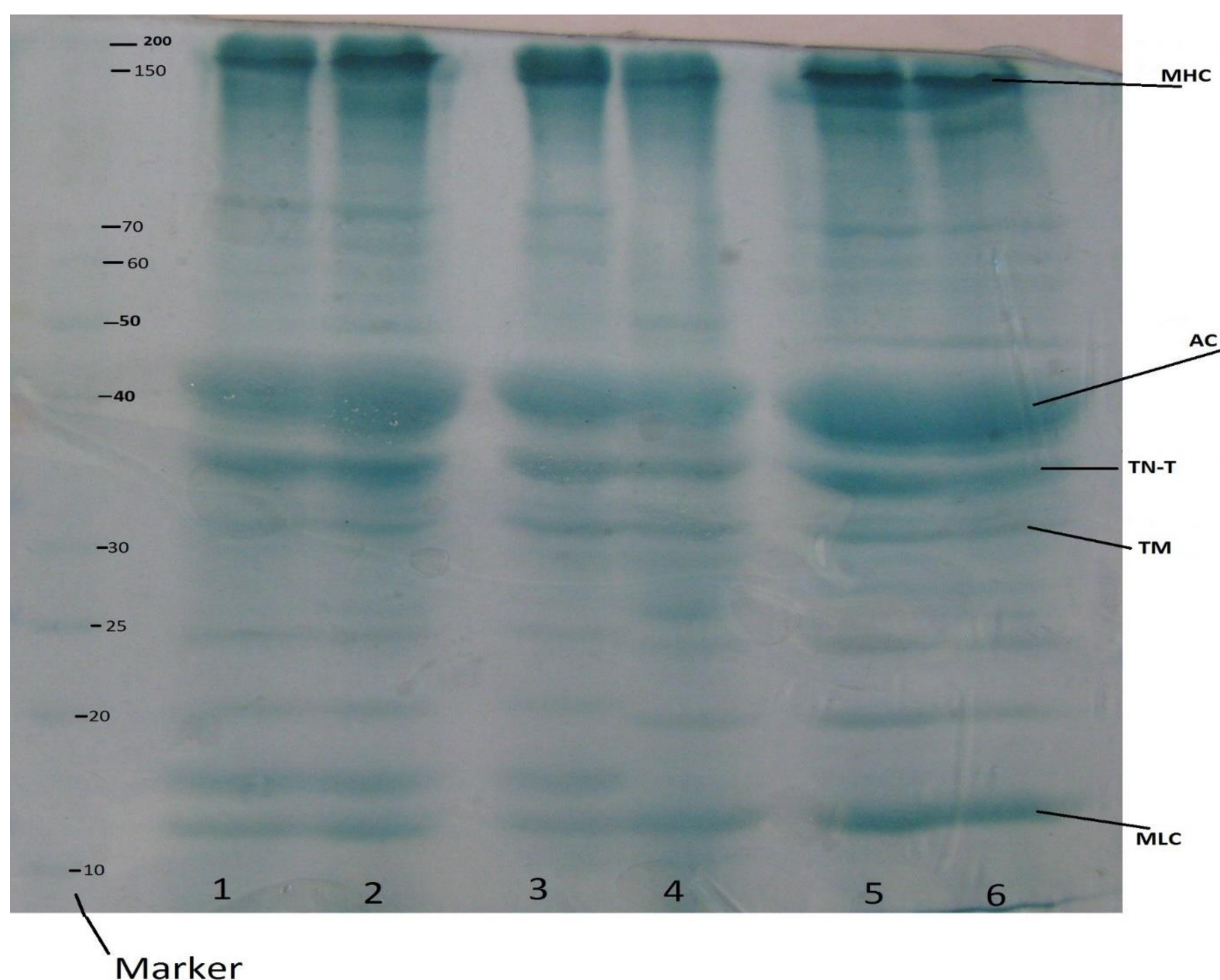

Figure 1: SDS-PAGE of minced common carp and silver carp prepared with different conditions. Lane 1: common carp treated with alkaline-aided process, Lane 2: common carp treated with acid-aided process, Lane 3: common carp prepared with conventional method, Lane 4: silver carp prepared with conventional method Lane 5: silver carp treated with alkaline-aided process Lane 6: silver carp treated with acid-aided process. MHC: myosin heavy chains, AC: actin, TN-T: troponin-T, and TM: tropomyosin.

The results of this paper illustrate that acid and alkali processing were more successful than surimi prepared by the conventional washing method for the recovery of proteins from common carp and silver carp muscles. Therefore, acid and alkaline production of protein isolates is a promising way for increasing the utilization of cultivated fish for food production. Also the use of a fresh raw material can help to do so and attain the lowest relative downfall in protein. The best surimi quality was produced with alkaline-acid aided process.

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