

## The shelf-life of conventional surimi and recovery of functional proteins from silver carp (*Hypophthalmichthys molitrix*) muscle by an acid or alkaline solubilization process during frozen storage

Shabanpour B.<sup>1\*</sup>; Etemadian Y.<sup>1</sup>

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### Abstract

The shelf-life of conventional surimi and isolated proteins that modified by acidic pH (2.5) and by using alkali pH (11) from silver carp (*Hypophthalmichthys molitrix*) was studied during months of storage at  $-18\pm 2$  °C. For conventional surimi, three washing steps were used. In the third stage of washing, 0.2% NaCl was used to withdraw more water. The result showed that isolated protein by alkaline pH has a higher efficiency. In the obtained result of percent yield and the recovery of protein product, isolated proteins showed higher values than conventional surimi. Isolated protein by using acid-aided processes had lower lightness and whiteness score, compared with alkaline-aided process and surimi prepared by a conventional washing method during frozen storage. The concentration of myosin heavy chain and actin were varied with solubilizing pH. Also, the lowest downfall of protein and the best surimi quality were found in produced samples with alkaline-acid aided process.

**Keywords:** Silver carp, Conventional surimi, Acid-alkaline solubilization, Shelf-life, Frozen storage

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1- Faculty of Fisheries Science, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Golestan, Iran

\* Corresponding author's Email: bshabanpour@yahoo.com

## Introduction

Due to rapid growth, resistance to stress and diseases, having 15-18% protein with high nutritive value, white flesh and low price, Silver carp (*Hypophthalmichthys molitrix*) is as one of main species that are used widely in freshwater fish culture systems (Barrera *et al.*, 2002; Fu *et al.*, 2009). One of the main problems in these fish is presence many bone in them muscle. But today's, consumers are demanding products without bones and the smell. One of the main fish products is mince. It is an intermediate and inexpensive substance for producing other fish products (Shahidi, 2007). Maximum amount of mince consumption is in the surimi industry. Surimi is the wet concentrate of the myofibrillar proteins of fish muscle, that is mechanically deboned, water washed and frozen (Okada, 1992). It possesses some important functional properties such as gel-forming ability and water-holding capacity. Therefore, it has become the intermediate material for surimi-based products. Surimi can be produced from both marine and freshwater fish. In different stages of washing, part of fats, sarcoplasmic proteins and some myofibril proteins are extracted. Recently, new methods for recovery of muscle functional proteins by using of acid and alkaline solubilization are used. That is achieved the higher yield than conventional surimi. Surimi and isolated protein, both are used in manufacturing other products, bright and white in their texture are important, and in secondary products color

produced by them is efficient (Park, 2005). Bright and white in texture of these products, allow us to changed those to desired products color for customer. Silver carp has been used for surimi production due to its easy availability. During frozen storage, surimi may lose its functional properties as a result of denaturation or aggregation of myofibrillar proteins (Shenouda, 1980; Zhou *et al.*, 2006). However, the type of fish species in surimi production is very effective. But so far no study on the effects of freezing on the stability of concentrated protein has been performed. Therefore in this research, the effects of protein extraction process by using of pH changes on the stability of extracted protein from silver carp during frozen storage were evaluated and compared with conventional surimi.

## 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), thio-barbituric acid, trichloroacetic acid, 5-5'-dithiobis (2-nitrobenzoic acid), urea, ether, tris-hydrochloride buffer (Tris-HCl), sodium dodecyl sulfate (SDS) and  $\beta$ -mercaptoethanol ( $\beta$ ME) were purchased from Sigma Chemical Co. (St. Louis,

MO, U.S.A). All chemicals for electrophoresis were obtained from Bio-Rad (Richmond, CA, U.S.A).

#### *Production of surimi*

To produce surimi (washed silver carp muscle tissue), 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). Then, all produced surimi blended with the cryoprotectant mixture (4% sucrose, 4% sorbitol, and 0.3% sodium tripolyphosphate) (Undeland *et al.*, 2002). The final moisture content was 75%. The surimi was frozen in plastic bags at  $-18\pm 2^{\circ}\text{C}$ .

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

Ground muscles of silver carp (usually 120-300 g) were homogenized for 1 min (speed 24) with 9 volumes of ice-cold distilled water using an Ultra-Turrax T2; (IKA Working Inc., Willington, NC, U.S.A.). The proteins in the homogenate were solubilized by dropwise 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 2N NaOH or 2N 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:

$$\left[ \frac{\text{total muscle proteins} - \text{proteins of non-liquid fractions from the first centrifugation} - \text{proteins of supernatant from the second centrifugation}}{\text{total muscle proteins}} \right] \times 100$$

#### *Percent yield*

Percent yield of the washed mince from 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 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:

$$\% \text{ yield} = \left[ \frac{\text{weight of recovered washed mince}}{\text{weight of initial minced sample}} \right] \times 100$$

### Protein solubility

The solubility of protein obtained from different processes was measured according to the method of Rawdkuen, Sai-Ut *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 8000g for 5 min at 4°C, and the protein concentration of the supernatant was measured by the biuret method (Torten and Whitaker, 1964). Protein solubility (%) was defined as the fraction of the protein remaining soluble after centrifugation and calculated as follows:

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

### Color analysis

Color of the surimi and the isolated protein 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 PI. 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

filtered with a Whatman filter paper (No. 1), and the absorbance was read at 640 nm against an acid-acetone blank. Total pigment was calculated as hematin using the following formula: 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°C. The supernatant was filtered with Whatman filter paper (No. 1). 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). The myoglobin content was expressed as mg/g sample.

### Total sulfhydryl (SH) groups determination

Total SH groups of samples treated at various treatments were determined according to Monahan and others (1995). Samples (1g) were homogenized in 9 mL of solubilizing

buffer (0.2 M Tris-HCl, 2% SDS, 10 mM ethylenediaminetetraacetic acid, 8 M urea, pH 8.0) (Ultra-Turrax T25; IKA Working Inc., Willington, NC, U.S.A.). The homogenates were heated at 100 °C for 5 min and centrifuged at  $10000 \times g$  for 15 min (Eppendorf Model 5810R; Westbury, NY, 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 and Park, 2004). The absorbance at 412 nm was measured to calculate the total SH groups using the extinction coefficient of  $13600 \text{ M}^{-1}\text{cm}^{-1}$ .

#### *Thiobarbituric acid-reactive substance (TBARS) analysis*

Malondialdehyde, formed from the breakdown of polyunsaturated fatty acids, serves as a convenient index for determining the extent of the peroxidation reaction. Malondialdehyde has been identified as the product of lipid peroxidation that reacts with thiobarbituric acid to give a red species absorbing at 535nm (Buege and Aust, 1978). The chopped fillet sample (0.5 g) was dispersed in 2.5 mL of 0.0375% thio-barbituric acid 15% trichloroacetic acid 0.25 N HCl solutions. The mixture was heated in boiling water for 15 min, followed by cooling in running tap water. The mixture was centrifuged at 3600g for 20 min and the absorbance was measured at 532 nm using a spectrophotometer (Biochrom, model Libra S12, UK) against a blank that contains all the reagents minus the lipid.

The malondialdehyde concentration of the sample can be calculated using an extinction coefficient of  $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ . TBARS was expressed as mg malondialdehyde/kg sample.

#### *Moisture content*

The plate was placed in the oven (105°C) for half an hour. Cooled in desiccators and then weighed. Five to ten grams of sample was weighed ( $M_0$ ). The samples were placed into the plate and again weighed ( $M_1$ ). Then were placed in oven, after 6 hours removed and cooled in desiccators and weighed ( $M_2$ ) (Parvaneh, 2007). Moisture percent =  $(M_1 - M_2) \times 100 / M_0$

#### *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  $\mu\text{L}$ ) were loaded to each well. Mobility of the protein bands were calibrated with standards of molecular weight markers. After staining and destaining, 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

Color and moisture changes in conventional surimi and protein isolates prepared of silver carp fish during frozen storage are shown in Table 1. The results showed that in first month, the highest  $L^*$  and whiteness values were observed in surimi samples prepared using alkaline solubilization process and conventional method, respectively. The  $a^*$  and  $b^*$  values (redness and yellowness) were less for conventional surimi with increase in storage time and there was a significant difference between samples ( $p < 0.05$ ). Moisture content of silver carp in samples prepared with alkaline and acid solubilization method increased during storage time, but in conventional method, moisture content was almost constant. Variations in values of total sulfhydryl groups during storage are depicted in Table 2. Total sulfhydryl groups were decreased during frozen storage, but there was no significant difference between three methods ( $p > 0.05$ ). Thiobarbituric acid-reactive substance changes in conventional surimi and protein isolates prepared of silver carp fish during frozen storage are shown in Table 2. The results showed that the conventional surimi had lower levels of thiobarbituric acid-reactive substance than acid-alkaline-processed isolates, but there was no difference significant between samples during frozen storage ( $p > 0.05$ ). With passing the time at 18°C for 5 months, protein yield was increased. The highest percentage of protein yield was showed

in samples prepared with the alkaline-aided method, but it was low in conventional method during frozen storage.

The highest protein solubility in silver carp was found in conventional surimi (0.89 mg/g) in first month of storage in freezer, followed by the alkaline-aided process (0.70 mg/g) and acid-aided process (0.59 mg/g), respectively (Table 2). Results showed that in total, except in first month, in this species there was no significant difference between acid- and alkaline-aided processes during frozen storage at 18°C for 5 months ( $p > 0.05$ ). In this study, with passing the time at 18°C for 5 months, myoglobin content decreased. There was a significant differences between the conventional method and acid-alkaline-aided processes ( $p < 0.05$ ). The total pigment contents of conventionally washed mince and protein isolated using the acid- or alkaline-aided process in silver carp were 132.62, 277.72 and 164.40 mg pigment/100g sample, respectively (Table 2).

But total pigment contents decreased during frozen storage at 18°C for 5 months. Also, results showed that the highest intensity of the myosin heavy chain and actin band were found in silver carp by the acid-aided process. With passing the time at 18°C for 5 months disappearance of myosin light chain band was observed in silver carp treated with the conventional method. There was no change in troponin-T band intensity. In all the months of

**Table 1. Moisture and color contents in silver carp recovered with different conditions during frozen storage.**

|           |                  | Months of storage in freezer |                           |                           |                           |                           |
|-----------|------------------|------------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| Treatment |                  | 0                            | 1                         | 2                         | 3                         | 4                         |
| Moisture  | Conventional     | 75.95±0.086 <sup>de</sup>    | 76.05±0.096 <sup>de</sup> | 76.04±0.089 <sup>de</sup> | 76.00±0.050 <sup>de</sup> | 76.04±0.043 <sup>de</sup> |
|           | Acid (pH 2.5)    | 74.76±0.056 <sup>f</sup>     | 74.80±0.320 <sup>f</sup>  | 76.35±0.854 <sup>de</sup> | 77.74±0.333 <sup>ab</sup> | 77.22±0.160 <sup>bc</sup> |
|           | Alkaline (pH 11) | 75.00±0.000 <sup>f</sup>     | 75.25±0.442 <sup>fe</sup> | 78.19±0.431 <sup>a</sup>  | 78.58±0.288 <sup>a</sup>  | 78.66±0.075 <sup>a</sup>  |
| L *       | Conventional     | 71.67±0.133 <sup>b</sup>     | 72.03±0.328 <sup>d</sup>  | 80.69±1.129 <sup>a</sup>  | 78.70±0.717 <sup>a</sup>  | 68.78±0.811 <sup>e</sup>  |
|           | Acid (pH 2.5)    | 68.07±0.133 <sup>c</sup>     | 69.93±0.133 <sup>c</sup>  | 76.40±0.384 <sup>b</sup>  | 76.44±0.687 <sup>b</sup>  | 66.81±0.419 <sup>f</sup>  |
|           | Alkaline (pH 11) | 72.93±0.590 <sup>a</sup>     | 73.97±0.353 <sup>c</sup>  | 79.57±0.992 <sup>a</sup>  | 78.80±0.387 <sup>a</sup>  | 68.92±0.685 <sup>e</sup>  |
| a *       | Conventional     | 4.03±0.267 <sup>c</sup>      | 3.77±0.267 <sup>c</sup>   | 3.63±0.133 <sup>c</sup>   | 3.76±0.267 <sup>c</sup>   | 3.70±0.115 <sup>c</sup>   |
|           | Acid (pH 2.5)    | 5.10±0.000 <sup>b</sup>      | 5.37±0.267 <sup>a</sup>   | 5.10±0.000 <sup>ab</sup>  | 4.66±0.357 <sup>b</sup>   | 5.01±0.180 <sup>ab</sup>  |
|           | Alkaline (pH 11) | 4.83±0.267 <sup>b</sup>      | 4.83±0.267 <sup>ab</sup>  | 5.19±0.090 <sup>ab</sup>  | 5.37±0.153 <sup>a</sup>   | 5.19±0.090 <sup>ab</sup>  |
| b *       | Conventional     | 1.47±0.267 <sup>b</sup>      | 1.20±0.000 <sup>f</sup>   | 2.09±0.327 <sup>e</sup>   | 3.70±0.115 <sup>bc</sup>  | 1.07±0.371 <sup>f</sup>   |
|           | Acid (pH 2.5)    | 2.70±0.000 <sup>a</sup>      | 2.97±0.267 <sup>dce</sup> | 4.39±0.090 <sup>ab</sup>  | 5.01±0.180 <sup>a</sup>   | 3.32±0.495 <sup>dc</sup>  |
|           | Alkaline (pH 11) | 2.47±0.233 <sup>a</sup>      | 2.97±0.267 <sup>dce</sup> | 3.77±0.533 <sup>bc</sup>  | 5.19±0.090 <sup>a</sup>   | 2.62±0.077 <sup>de</sup>  |
| Whiteness | Conventional     | 71.34±0.110 <sup>b</sup>     | 71.75±0.318 <sup>d</sup>  | 80.23±1.126 <sup>a</sup>  | 78.26±0.707 <sup>ab</sup> | 68.54±0.827 <sup>e</sup>  |
|           | Acid (pH 2.5)    | 67.55±0.130 <sup>c</sup>     | 69.31±0.181 <sup>c</sup>  | 75.46±0.354 <sup>c</sup>  | 75.52±0.713 <sup>c</sup>  | 68.34±0.658 <sup>e</sup>  |
|           | Alkaline (pH 11) | 72.39±0.554 <sup>a</sup>     | 73.35±0.357 <sup>d</sup>  | 78.58±1.04 <sup>ab</sup>  | 77.93±0.403 <sup>b</sup>  | 66.31±0.396 <sup>f</sup>  |

Values are given as means ± SD from triplicate groups.

a,b,c,d,e Different letters in the same column indicate significant difference ( $p < 0.05$ ) between treatments.

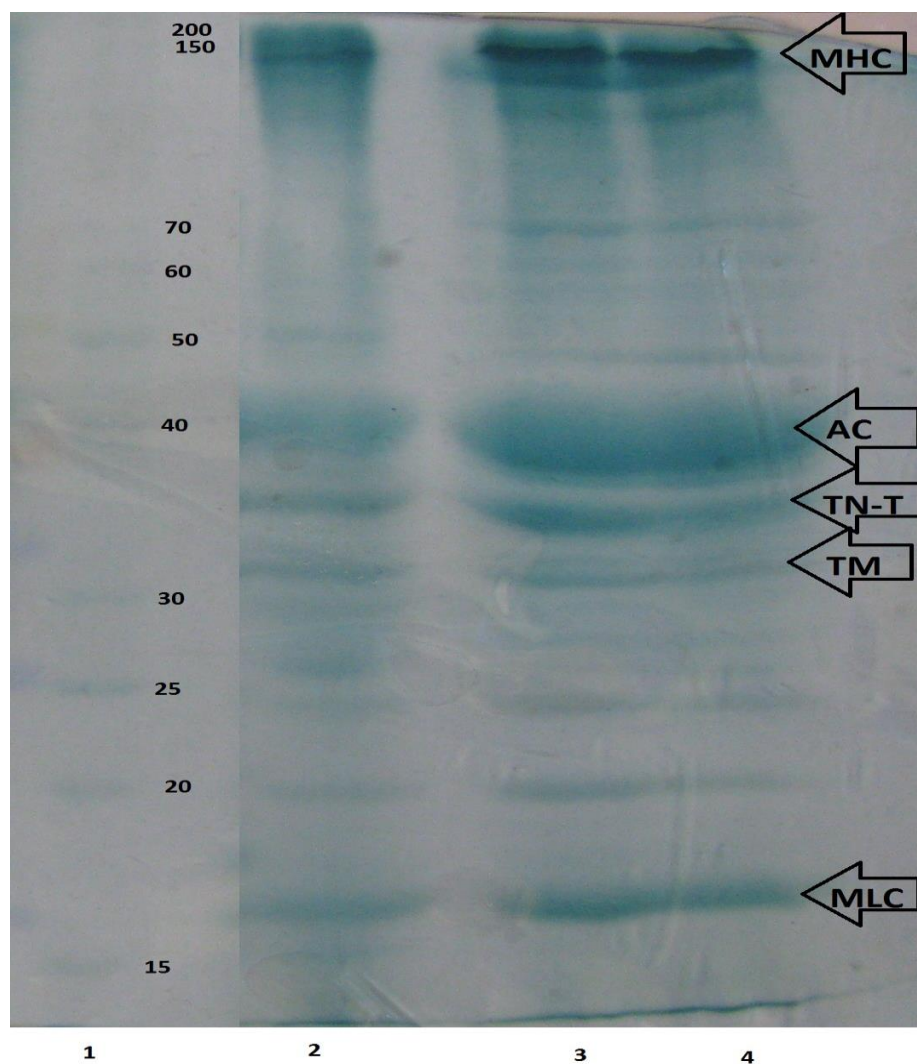
L : \*Lightness index, a : \*Redness index, b : \*Yellowness index.

**Table 2. Total sulfhydryl groups, thiobarbituric acid-reactive substance, solubility of protein, % yield, myoglobin and total pigment determination in silver carp recovered with different conditions during frozen storage.**

|                           |                  | Months of storage in freezer |                            |                           |                           |                            |
|---------------------------|------------------|------------------------------|----------------------------|---------------------------|---------------------------|----------------------------|
| Treatment                 |                  | 0                            | 1                          | 2                         | 3                         | 4                          |
| SH                        | Conventional     | 6.80±0.044 <sup>a</sup>      | 3.00±0.154 <sup>ab</sup>   | 2.97±0.069 <sup>ab</sup>  | 2.51±0.765 <sup>b</sup>   | 2.40±0.298 <sup>b</sup>    |
|                           | Acid (pH 2.5)    | 7.22±0.930 <sup>a</sup>      | 3.43±0.137 <sup>ab</sup>   | 3.11±0.216 <sup>ab</sup>  | 3.55±0.084 <sup>a</sup>   | 3.01±0.131 <sup>ab</sup>   |
|                           | Alkaline (pH 11) | 5.93±0.092 <sup>a</sup>      | 3.34±0.037 <sup>ab</sup>   | 2.97±0.124 <sup>ab</sup>  | 3.21±0.251 <sup>ab</sup>  | 2.82±0.485 <sup>ab</sup>   |
| TBARS                     | Conventional     | 0.27±0.055 <sup>c</sup>      | 1.16±0.226 <sup>b</sup>    | 1.35±0.199 <sup>b</sup>   | 1.41±0.199 <sup>ab</sup>  | 1.29±0.038 <sup>b</sup>    |
|                           | Acid (pH 2.5)    | 0.32±0.079 <sup>c</sup>      | 1.39±0.214 <sup>ab</sup>   | 1.56±0.245 <sup>ab</sup>  | 1.98±0.291 <sup>a</sup>   | 1.31±0.078 <sup>b</sup>    |
|                           | Alkaline (pH 11) | 0.13±0.015 <sup>c</sup>      | 1.28±0.194 <sup>ab</sup>   | 1.61±0.096 <sup>ab</sup>  | 1.72±0.183 <sup>ab</sup>  | 1.60±0.263 <sup>ab</sup>   |
| Protein solubility (mg/g) | Conventional     | 0.84±0.007 <sup>e</sup>      | 0.89±0.006 <sup>dc</sup>   | 0.92±0.003 <sup>bac</sup> | 0.91±0.003 <sup>bac</sup> | 0.90±0.006 <sup>bacd</sup> |
|                           | Acid (pH 2.5)    | 0.54±0.012 <sup>g</sup>      | 0.59±0.006 <sup>dc</sup>   | 0.93±0.010 <sup>bac</sup> | 0.92±0.027 <sup>bac</sup> | 0.93±0.003 <sup>bacd</sup> |
|                           | Alkaline (pH 11) | 0.67±0.003 <sup>f</sup>      | 0.70±0.009 <sup>de</sup>   | 0.89±0.012 <sup>bac</sup> | 0.93±0.015 <sup>bac</sup> | 0.92±0.017 <sup>bacd</sup> |
| % yield                   | Conventional     | 49.00±0.389 <sup>d</sup>     | 48.10±0.058 <sup>g</sup>   | 45.09±0.049 <sup>ef</sup> | 39.86±0.023 <sup>f</sup>  | 30.38±0.015 <sup>e</sup>   |
|                           | Acid (pH 2.5)    | 51.88±0.183 <sup>c</sup>     | 51.19±0.020 <sup>d</sup>   | 50.53±0.049 <sup>a</sup>  | 47.63±0.017 <sup>b</sup>  | 45.22±0.069 <sup>a</sup>   |
|                           | Alkaline (pH 11) | 54.01±0.298 <sup>b</sup>     | 53.27±0.020 <sup>d</sup>   | 52.82±0.081 <sup>c</sup>  | 52.76±0.045 <sup>c</sup>  | 51.99±0.009 <sup>c</sup>   |
| Myoglobin (mg/100g)       | Conventional     | 60.98±1.224 <sup>a</sup>     | 56.18±1.224 <sup>a</sup>   | 42.75±1.413 <sup>b</sup>  | 34.52±2.12 <sup>c</sup>   | 33.81±3.689 <sup>c</sup>   |
|                           | Acid (pH 2.5)    | 57.82±0.147 <sup>b</sup>     | 43.81±4.298 <sup>b</sup>   | 24.73±3.140 <sup>d</sup>  | 21.20±2.668 <sup>de</sup> | 19.08±1.060 <sup>def</sup> |
|                           | Alkaline (pH 11) | 44.49±1.120 <sup>a</sup>     | 39.12±1.010 <sup>bc</sup>  | 14.29±2.119 <sup>gf</sup> | 11.66±0.000 <sup>g</sup>  | 15.55±1.413 <sup>gef</sup> |
| Total pigment (mg/100g)   | Conventional     | 171.63±19.338 <sup>c</sup>   | 132.62±11.000 <sup>c</sup> | 49.01±6.683 <sup>d</sup>  | 36.66±1.188 <sup>d</sup>  | 35.73±2.090 <sup>d</sup>   |
|                           | Acid (pH 2.5)    | 283.64±10.227 <sup>b</sup>   | 277.72±40.833 <sup>a</sup> | 146.44±1.835 <sup>c</sup> | 75.14±18.897 <sup>d</sup> | 49.98±8.693 <sup>d</sup>   |
|                           | Alkaline (pH 11) | 275.85±7.505 <sup>b</sup>    | 164.45±16.222 <sup>c</sup> | 66.87±2.776 <sup>d</sup>  | 44.44±1.618 <sup>d</sup>  | 41.41±0.816 <sup>d</sup>   |

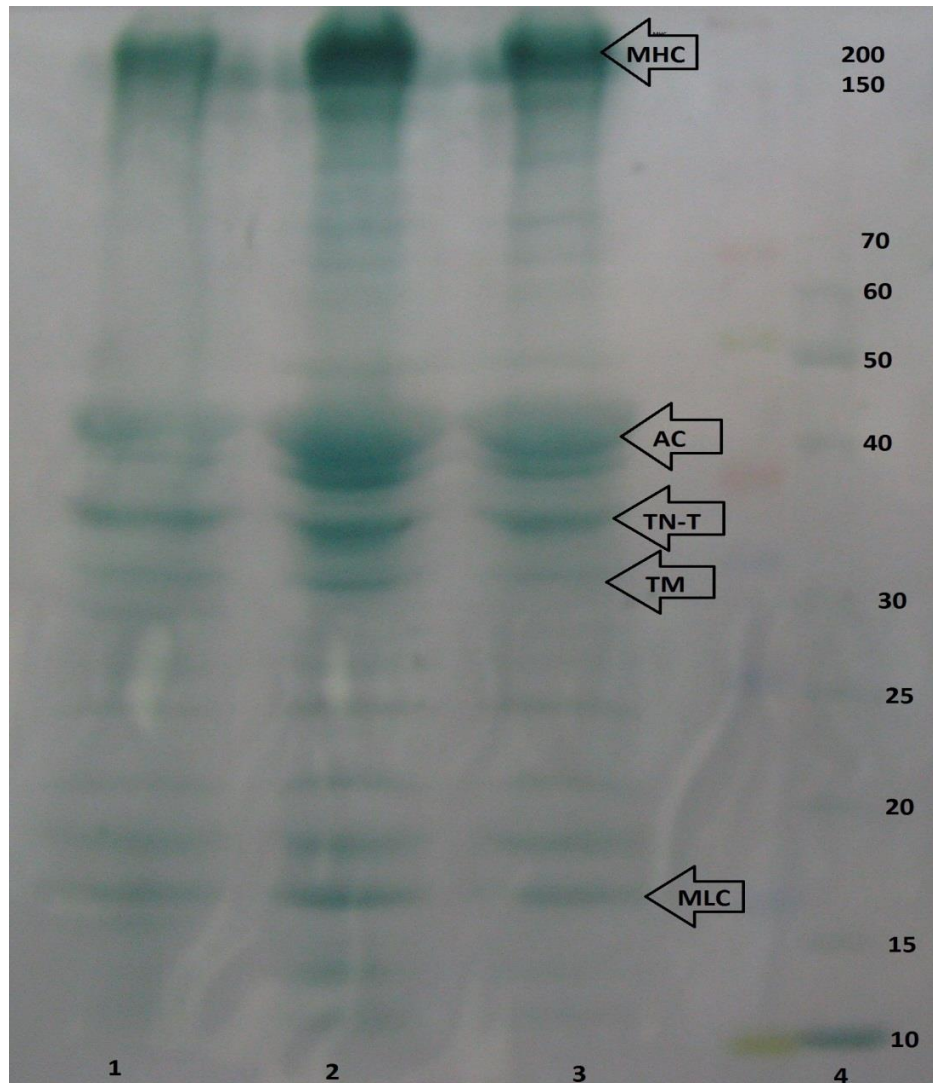
Values are given as means ± SD from triplicate groups.

a,b,c,d,e,fg Different letters in the same column indicate significant difference ( $p < 0.05$ ) between treatments.

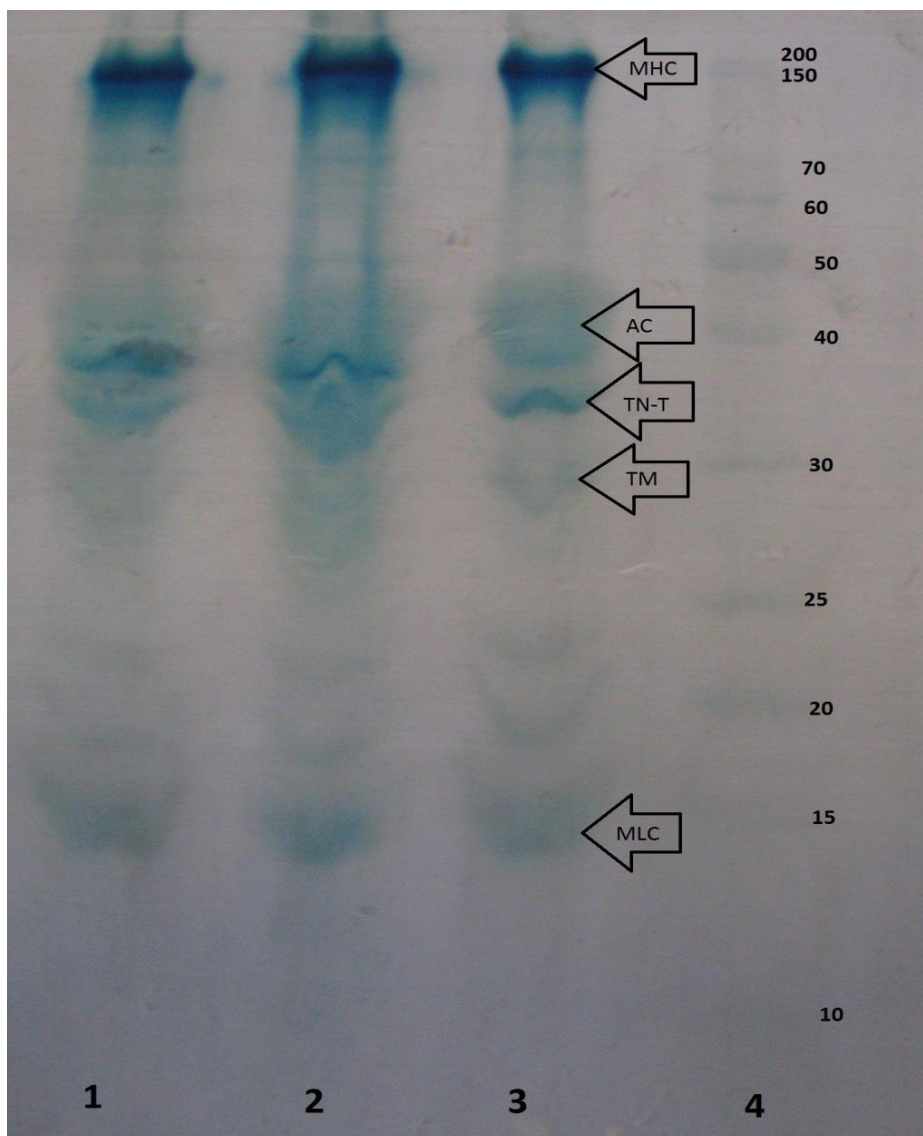


**Figure 1:** SDS-PAGE of minced silver carp prepared with different conditions during frozen storage at 18°C for first month. Lane 1: Marker, lane 2: silver carp prepared with conventional method, Lane 3: silver carp treated with alkaline-aided process, Lane 4: silver carp treated with acid-aided process. MHC: myosin heavy chains, MLC: myosin light chains, AC: actin, TN-T: troponin-T, and TM: tropomyosin.

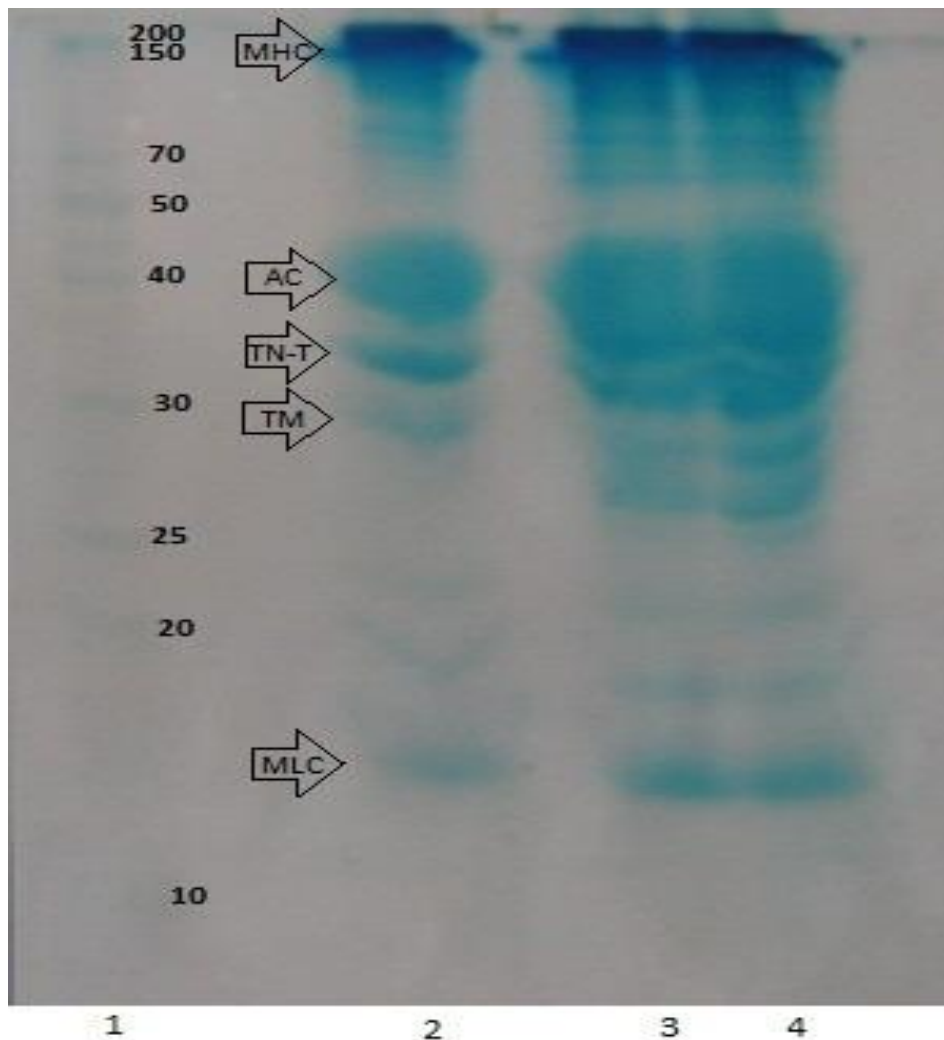




**Figure 2:** SDS-PAGE of minced silver carp prepared with different conditions during frozen storage at 18°C for second months. Lane 1: silver carp treated with alkaline-aided process, lane 2: silver carp treated with acid-aided process, Lane 3: silver carp prepared with conventional method, Lane 4: Marker. MHC: myosin heavy chains, MLC: myosin light chains, AC: actin, TN-T: troponin-T, and TM: tropomyosin.



**Figure 3:** SDS-PAGE of minced silver carp prepared with different conditions during frozen storage at 18°C for third months. Lane 1: silver carp treated with alkaline-aided process, lane 2: silver carp treated with acid-aided process, Lane 3: silver carp prepared with conventional method, Lane 4: Marker. MHC: myosin heavy chains, MLC: myosin light chains, AC: actin, TN-T: troponin-T, and TM: tropomyosin.



**Figure 4:** SDS-PAGE of minced silver carp prepared with different conditions during frozen storage at 18°C for fourth months. Lane 1: Marker, lane 2: silver carp prepared with conventional method, Lane 3: silver carp treated with alkaline-aided process, Lane 4: silver carp treated with acid-aided process. MHC: myosin heavy chains, MLC: myosin light chains, AC: actin, TN-T: troponin-T, and TM: tropomyosin.

## Discussion

### *Color changes in conventional surimi and protein isolates during frozen storage*

One important parameter when comparing different processing methods is the color of the protein isolate (Nolsøe and Undeland, 2009). In this study, the color characteristics differed

among different protein preparations. There was significant difference between  $L^*$ ,  $a^*$  and  $b^*$  values in different conditions of preparing surimi during frozen storage. In this fish, protein isolated using acid-aided processes had a lower  $L^*$  value (lightness), and also lower whiteness score, compared with alkaline-aided process and surimi

prepared by a conventional washing method during frozen storage. 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-aided processes compared with conventional surimi and acid-aided process. 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.

The  $b^*$  values (yellowness) were less for conventional surimi. The  $b^*$  values showed a significant difference between the acid- and alkali-made isolates and conventional surimi. Undeland *et al.* (2002) investigated the colors of acid and alkaline protein isolates from the light muscle of herring. They reported the highest  $L^*$  values,  $b^*$  values for the alkali-produced protein isolate. Also in their examination  $a^*$  values were the same for the acid- and alkali-made isolates. Kim *et al.* (1996) reported higher whiteness values and higher yellowness ( $b^*$  value) and redness values for catfish frame mince surimi. The higher  $L^*$  value could be attributed to retention of connective tissue. More yellowness could be in part due to more retention of lipids.

More redness in alkaline-aided process is likely attributed to more co-precipitation of heme proteins.

#### *Moisture changes in conventional surimi and protein isolates during frozen storage*

In this study, with pass of time, moisture content of silver carp in alkaline-aided process was showed higher of others. This factor is different in various species (Nolsøe *et al.*, 2009). Also, there was a significant difference between surimi prepared by a conventional washing method and protein isolated using alkaline-acid-aided processes during storage in freezer at  $-18\pm 2^\circ\text{C}$ . These factors can were important and different in measurement of biochemical properties in every species of fish.

#### *Total sulfhydryl groups (SH) changes in conventional surimi and protein isolates during frozen storage*

Variations in values of SH during storage are depicted in Table 2. In fact, another way to elucidate protein aggregation is to monitor changes in total sulfhydryl groups. SH groups are located in both head and tail portions of myosin (Somponges *et al.*, 1996; Yamaguchi and Sekine, 1996). A change in total SH is attributed to oxidation of SH, reduction of disulfide bonds (S-S), and S-S/SH interchange reactions (Thawornchinsombut and Park, 2006). In this examination, silver carp minces prepared with acid-aided process showed a higher level of total sulfhydryl groups compare with alkaline-aided process and conventional surimi. SH groups were decreased during frozen storage, but there was no significant difference between three done methods. Yongsawatdigul and Park (2004) reported a marked decrease in SH groups was observed in the acid-

treated samples. The decrease in total SH content can be due to the formation of disulfide bond via oxidation of SH group or disulfide interchanges (Hayakawa and Nakai, 1985). Also, they 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).

*Thiobarbituric acid-reactive substance (TBARS) changes in conventional surimi and protein isolates during frozen storage*

Kristinsson *et al.* (2005) reported that lipid oxidation in conventional surimi process is like acid- and alkali-aided process. Their results showed that none of the processes gave significantly higher TBARS values than the starting material. Alkaline processing without the first centrifugation was an exception in that it yielded an isolate with a very low TBARS value. Also, Kristinsson and Liang (2006) investigated lipid oxidation in unprocessed ground Atlantic croaker (*Micropogonias undulates*) and in conventional surimi as well as acid- and alkali-processed isolates made thereof. The highest increase in TBARS was also here seen for the acid-processed protein isolate followed by the conventional surimi and the alkaline protein isolate. Undeland *et al.* (2005) studied how lipid oxidation progressed under acid processing of herring fillet mince. They tested how changes in the process and

the use of different antioxidants influenced lipid oxidation both under the processing itself and subsequential ice storage of protein isolates. Inhibition of lipid oxidation during acid and alkaline isolation of cod muscle proteins was also studied by Varelziz and Hultin (2007). In their research, they showed that both acid and alkaline processing enhanced the oxidative stability of protein isolates. They suggested that acid processing caused in situ aggregation of the cod muscle membranes rendering them less susceptible to lipid oxidation. In total, in several studies, conventional surimi had the lowest level of oxidation.

*Protein recovery changes*

The protein recovery for each process of minced silver carp muscles investigated only in first month. 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%). Obtained result of this study shows that higher protein recovery was found when the mince was subjected to the alkaline-aided process in silver carp. The lower recovery of surimi processing is reportedly due to the removal of water-soluble sarcoplasmic proteins during the washing steps (Xiong, 1997) and possibly part of the myofibrillar proteins (Lin and Park, 1996). Kristinsson and Ingadottir (2006) found no significant difference between acid- and alkaline-aided processes for protein recoveries of tilapia light muscle.



Kelleher and Hultin (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 the conventional surimi process. 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).

*Protein solubility changes in conventional surimi and protein isolates during frozen storage*

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. 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 silver carp recovered with different conditions is shown in Table 2. The highest protein solubility in silver carp was found in conventional surimi (0.89 mg/g) in first month of storage in freezer, followed by the alkaline-aided process (0.70 mg/g) and acid-aided process (0.59 mg/g), respectively. Result showed that in total, except in first month, in this species there was no significant difference between acid- and alkaline-aided processes during frozen storage at 18°C for 5 months. 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). Zayas (1997) reported that protein solubility was greater at alkaline pH than acid pH. These results are consistent with the results obtained from silver carp in this study. Kristinsson and Hultin (2004) reported that among protein isolates, the alkaline-aided process provided higher protein solubility than the acid-aided process. Also, Zayas (1997) reported that protein solubility 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.

*Changes of % protein yield in conventional surimi and protein isolates during frozen storage*

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 surimi preparation, the exact yields depend mainly on the number of washes, the pH of the washing solution, and the ionic strength of the washing solution. Changes of % protein yield have indicated in Table 2. In this study, there was a significant difference between treatments during frozen storage at 18°C for 5 months. With passing the time at 18°C for 5 months, % protein yield was increased. The results showed that % protein yield in the acid-alkaline- aided processes were higher than conventional method during frozen storage. Also, in this study, there were significant differences between samples. 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*). In a similar comparison between acid- and alkali-aided processing, Kristinsson and Ingadottir (2006) investigated protein yields from tilapia (*Oreochromis niloticus*). From repeated trials, they found yields 56% to 61% with the acid process, and 61% to 68% with the alkaline process.

*Myoglobin contents changes in conventional surimi and protein isolates during frozen storage*

Myoglobin extractability of silver carp muscle, processed by the conventional washing method, acid-aided and alkaline-aided processes, is shown in Table 2. In first month, the retained myoglobin contents in silver carp were 56.18, 43.81 and 49.11 mg/100 g by using the conventional method, acid-aided and alkaline-aided processes, respectively. The increase in myoglobin extractability in conventional method was possibly due to the increased degradation of muscle proteins, leading to an enhanced efficiency of myoglobin removal from the disintegrated muscle. Decreases in myoglobin contents were found in alkaline- or acid-aided process when compared to the conventional process (Rawdkuen *et al.*, 2009). Chaijan *et al.* (2006) reported the alkaline solubilising process could remove myoglobin most effectively from sardine and mackerel muscles. But in total, myoglobin extracting efficiency depends on fish species, muscle type, storage time and washing process. In this study, with passing the time at 18°C for 5 months, myoglobin content was decreased. There was a significant difference between the conventional method and acid-alkaline-aided processes.

*Total pigment contents changes in conventional surimi and protein isolates during frozen storage*

Chromoproteins are mainly composed of a porphyrinic group conjugated with

a transition metal and are responsible for color of muscle food. However, carotenes and carotenoproteins exist alongside chromoproteins and also play an important role 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. In this study, total pigment contents of conventionally washed mince and protein isolated using the acid- or alkaline-aided process in silver carp were 132.62, 277.72 and 164.40 mg pigment/100g sample, respectively (Table 2). But total pigment contents decreased during frozen storage at 18°C for 5 months. The highest total pigment removal was found in silver carp mince processed by acid-aided process. The result indicated that washing process in silver carp could remove pigments in minced fish, leading to lower pigment content in the fish muscle, but regarding to myoglobin the result was inverse. Chaijan *et al.* (2006) noted that total extractable pigment content in sardine and mackerel muscles gradually decreased as the storage time increased, which those results were compatible with our results in the present study. Chen (2003) reported that 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.

*Protein pattern of silver carp in conventional surimi and protein isolates during frozen storage*

The most abundant protein recovered was myosin heavy chains (MHC), followed by actin (AC), troponin-T (TN-T), tropomyosin (TM) and myosin light chains (MLC). The concentration of myosin heavy chain and actin varied with solubilizing pH. SDS-PAGE analysis indicated that little apparent hydrolytic degradation took place between the start of the acidification/alkalization. In this study, the highest intensity of the MHC band was found in silver carp by the acid-aided process. With passing the time at 18°C for 5 months disappearance of MLC bands was observed in silver carp treated with the conventional method. High intensity of AC bands was observed in silver carp treated with the acid-aided process. It could be hypothesized that a reduction of those bands was induced by hydrolysis during the solubilization 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. 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). But in this study, actin in pH 11 decreased during frozen storage for 5 months. Therefore, solubility and integrity of bands should be taken into consideration in selecting the optimal solubilizing pH values; pH 2.5 and 11 were chosen as the optimal pH values for acidic and alkaline solubilization in this study.

In total, purpose of this study, was production of surimi and fish protein isolate of silver carp and also search on some properties in related to produce surimi with three methods (conventional, acid and alkaline process). Protein isolate obtained from fish waste has high nutritional value and can be used in food production. But, Protein isolate obtained from fish waste is consumed less by consumers. So, more research is needed in this case. The most important recommendation for future researches, is extensive use of fish protein isolate in preparation other various products such as sausages and salami, fish cakes, noodles, fish burgers and also use of fish protein isolate powder in the formulation of various snacks like puffs, potato chips and ice cream. On the other hand, the effect of fish protein isolate powder in increasing functional properties such as viscosity and consistency of the other products can be achieved. The results of this paper illustrate that acid and alkali processing were more successful than surimi prepared by a conventional washing method for the recovery of proteins from silver carp muscles.

Therefore, acid and alkaline production of protein isolates is a promising way of increasing the utilization of cultivated fish for food production. Also, the lowest protein degradation and the best surimi quality were observed in surimi samples prepared with alkaline-acid aided process.

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