

Chemical changes and shelf-life of conventional surimi and proteins recovered using pH change method from common carp (*Cyprinus carpio*) muscle during 5 months storage at -18°C

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Abstract

Chemical changes and shelf-life of common carp (*Cyprinus carpio*) surimi prepared by conventional washing method and solubilization by acid and alkali processes were investigated during 5 months of storage at -18 °C. Results showed that surimi produced with acid and alkaline solubilization is significantly higher yield than the conventional surimi. Protein solubilization by using acid and alkaline process was high compared with conventional method during storage in freezer except in the first month. Gel electrophoresis patterns showed a significant difference in hydrolyzing of myosin and actin proteins that was observed with increasing storage time. Surimi samples prepared by conventional method indicated more hydrolyzation, especially in the light chain of myosin during frozen storage. Evaluating the color characteristics (L*, a*, b*) of surimi samples showed that protein produced by using of pH 11 has the highest lightness after conventional surimi. Also, more whiteness in surimi prepared by conventional method was due to effective removal of myoglobin during washing. At zero month, total fat in the surimi samples prepared with acid and alkaline process was low than the surimi samples prepared by conventional method that it can intent to shelf-life increase and health of the product during storage.

Keywords: Common carp, Shelf-life, Conventional surimi, Acid and alkaline process, Frozen storage

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Introduction

The high nutritional value of aquatic foods and its importance in the world, has proved for human during years (Nolsøe and Undeland, 2009). The production of surimi is a process that has had some success in recovering fish proteins (Kristinsson *et al.*, 2005). Surimi is the wet concentrate of the myofibril proteins of fish muscle, that is mechanically deboned, water washed and frozen (Okada, 1992). In order to increase the production of surimi in world, along with conventional method, demand for new methods like pH change has increased (Thawornchinsombut and Park, 2006). At these low or high pH conditions, the protein net charge leads to the repulsion of protein chains and their solubility. Also, this method needs less water for washing flesh fish. In this method of pH change, the rate of product and protein stability isolated from muscle is high (Hultin and Kelleher, 1999). pH change method has some advantages compared to the conventional method. It does not require mechanical removal of skin or bone, partial recovery of sarcoplasmic proteins, increasing yield and removing neutral lipids and membrane is to minimize oxidation. Also, another advantage of this method is that the effluents are containing low solids content and O_2 demand compared to conventional surimi method (Park, 2005; Nolsøe *et al.*, 2009). With the conversion of inexpensive fish and low consumption into value-added products such as surimi and or isolated protein, not only it can minimize loss of marine products, but also to prevent loss of protein-rich sources. Cold storage avoid the risk of microbial spoilage and

minimize the rate of biochemical reactions (Matsumoto, 1980) in surimi. Also, during storage, surimi may lose its functional properties as a result of denaturation or aggregation of myofibril proteins (Shenouda, 1980; Zhou *et al.*, 2006). The knowledge about the physicochemical changes of fish muscle proteins prepared by using of alkali and acid solubility is still limited. Therefore the purpose of this study was to investigate the protein extraction process using pH change or conventional method on stabilizing the surimi production during 5 months of storage at -18°C .

Materials and methods

Production of surimi

To produce surimi (washed common carp muscle tissue), a conventional laboratory scale process was used. Separately, minced muscle of common carp was gently mixed into 3 volumes of cold (4°C) water and slowly stirred with a rubber spatula for 15 min, 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 with squeezing to separate 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% sodium tripolyphosphate) (Undeland *et al.*, 2002). The final moisture was 75.5%. The surimi was frozen in plastic bags at -18°C .

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

A ground muscle of common carp (usually 120-300 g) was 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 solved by drop wise addition of 2N HCl or 2N NaOH until a pH of 2.5 or 11, respectively. 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 2N NaOH or 2N HCl. Precipitated proteins were collected via a second centrifugation at 10000×g (20 min) (Undeland *et al.*, 2002). To calculate the protein recovery (percent) obtained by the acid and alkaline processes, the following formula was used:

$$[(\text{total muscle proteins} - \text{proteins of non liquid fractions from the first centrifugation} - \text{proteins of supernatant from the second centrifugation}) / \text{total muscle proteins}] \times 100$$

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

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:

$$\text{Yield (\%)} = [\text{weight of recovered washed mince} / \text{weight of initial minced sample}] \times 100$$

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 supernatant was measured by Biuret method. Protein solubility (%) was defined as the fraction of the protein remaining soluble after centrifugation and calculated as follows:

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

Color analysis

Color of the surimi and isolated protein were 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:

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

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 1h 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 *et al.*, 2009) by the following formula:

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

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 3000 g for 30 min at 4°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). 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 *et al.* (1995). Sample (1g) was homogenized in 9 mL of solubilizing buffer (0.2 M Tris-HCl, 2% SDS, 10 mM ethylenediaminetetraacetic acid, 8M urea, pH 8.0) (Ultra-Turrax T25; IKA Working Inc., Willington, NC, USA.). The homogenates were heated at 100 °C for 5 min and centrifuged at 10000 × g for 15 min (Eppendorf Model 5810R; Westbury, NY, USA). To 1 mL aliquot of the supernatant was added 0.01 mL Ellman's reagent (10 mM 5, 5'-dinitro bis [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).

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 535 nm (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 0.25N 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 oven (105°C) 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 were placed in oven, after 6 hours removed and cooled in desiccators and weighed (M_2) (Parvaneh, 2007).

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

Ash content

First, a porcelain crucible with lid was placed in the oven, cooled in desiccators and weighed (M_0). One gram dried sample was placed in it. It was placed in electric oven at 500-550 °C. And heated enough to get a light gray color. Then cooled in desiccators and weighed again (M_1) (Parvaneh, 2007).

$$\text{Ash percent} = (M_1 - M_0) \times 100 / 1\text{g sample}$$

Lipid content

Soxhlet apparatus was used to measure lipid. One gram sample was used for this work (Parvaneh, 2007). The lipid percent was calculated using the following formula:

$$\text{Lipid percent} = \text{lipid content in sample} \times 100 / 1\text{g sample}$$

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 on 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 triplicates. Data were subjected to analysis of variance. 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 changes in surimi samples produced by three methods of conventional, alkaline and acid solubilization from common carp muscle during frozen storage are presented in Table 1. The results showed that most whiteness and lightness (L^*) were observed in surimi samples prepared by conventional method and then in surimi samples prepared by alkaline solubilization process. The lowest shelf redness (a^*) and yellowness (b^*) were found in conventional surimi with increase in storage time. A significant difference were observed between samples ($p < 0.05$). The obtained results of moisture changes showed that in common carp, moisture content in surimi samples increases from

zero month to first month and after that this changes had a proven process in conventional and acid solubilization methods (Fig. 1). Ash changes of common carp increased in all treatments especially in surimi samples prepared by acid solubilization process, but there was no significant difference between storage months (Fig. 2). Lipid changes in surimi samples produced by three methods of conventional, alkaline and acid solubilization from common carp muscle during frozen storage are presented in Fig. 3. The result showed that in the first month lipid content in conventional surimi samples was higher than the method of pH change. This process in the surimi samples prepared using pH change was proven from the first month to the end. Changes of total sulfhydryl content of surimi samples prepared by three methods of conventional, solubilization by using of acid and alkali process from common carp during storage in -18°C are shown in Table 2. Total sulfhydryl content of all samples decreased. The greatest decrease was related to the method of solubility by alkaline. Thiobarbituric acid-reactive substance values of common carp surimi samples at zero month were less than 0.5

mg malonaldehyde/ 100g muscle (Fig. 4). This amount increased with increasing storage time in freezer ($p<0.05$). In Fig. 5, protein solubility in surimi samples produced by conventional, alkaline and acid solubilization from common carp muscle during frozen storage showed that there was significant difference between samples from the first month to the end of storage. The lowest protein solubility was observed in conventional surimi. Myoglobin content decreased with increasing storage time (Fig. 6) and this decrease was more in pH change process than the conventional method. A significant difference was observed between samples ($p<0.05$). Total pigment changes in surimi samples produced by three methods of conventional, alkaline and acid solubilization from common carp muscle during frozen storage are presented in Fig. 7. The results showed that total pigment content decreased with increasing storage time. The least amount of pigment was observed in surimi samples prepared by conventional method. So that the maximum amount of pigment removal was observed during the washing process.

Table 1: Color changes in common carp recovered with different conditions during 5 months storage at -18°C.

Treatment-carp		0	1	2	3	4
L*	Conventional	78.20 ^a ±0.231	76.73 ^{ab} ±0.939	71.20 ^{dc} ±2.656	60.89 ^{fg} ±0.220	77.93 ^a ±1.143
	Alkaline	69.07 ^{bc} ±0.133	68.10 ^{de} ±0.907	54.05 ^{hi} ±1.662	56.65 ^{hig} ±1.520	64.27 ^{ef} ±0.797
	Acid	72.83 ^c ±3.474	65.63 ^c ±1.162	52.92 ⁱ ±0.198	58.39 ^{hg} ±0.905	66.01 ^e ±1.021
a*	Conventional	3.57 ^h ±0.371	3.77 ^{hg} ±0.267	4.66 ^f ±0.235	4.39 ^{gf} ±0.090	3.94 ^{hg} ±0.087
	Alkaline	5.70 ^e ±0.115	6.70 ^d ±0.462	8.06 ^b ±0.289	7.23 ^{dc} ±0.153	8.82 ^a ±0.177
	Acid	5.90 ^e ±0.000	6.97 ^{dc} ±0.267	7.65 ^{bc} ±0.162	6.97 ^{dc} ±0.153	8.06 ^b ±0.087
b*	Conventional	2.70 ^{def} ±0.000	3.23 ^{dc} ±0.267	3.23 ^{dc} ±0.000	1.38 ^g ±0.321	4.39 ^{ab} ±0.387
	Alkaline	2.47 ^f ±0.233	3.77 ^{bc} ±0.267	3.15 ^{dec} ±0.177	2.22 ^f ±0.136	3.59 ^c ±0.319
	Acid	2.23 ^{ef} ±0.233	4.30 ^{ab} ±0.000	3.14 ^{dec} ±0.235	2.39 ^f ±0.080	4.92 ^a ±0.236
Whiteness	Conventional	77.74 ^a ±0.183	76.21 ^a ±0.985	70.64 ^{bc} ±2.645	60.62 ^{hg} ±0.212	77.16 ^a ±1.186
	Alkaline	72.11 ^b ±0.131	67.17 ^{dc} ±0.883	53.24 ^{ki} ±1.663	56.00 ^{ji} ±1.495	63.01 ^{gf} ±0.742
	Acid	68.45 ^{dc} ±0.252	64.67 ^{ef} ±1.179	52.20 ^k ±0.193	57.74 ^{hi} ±0.875	64.72 ^{ef} ±0.957

Values are given as means ± SD from triplicate groups.

Small letters in the same column indicate significant differences ($p < 0.05$) between treatments.

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

Table 2: Total sulfhydryl groups (SH) changes in common carp recovered with different conditions during 5 months storage at -18°C.

Treatment-carp		0	1	2	3	4
SH (Mol/ 10 ⁵ g protein)	Conventional	6.66 ^a ±0.546	3.77 ^{bc} ±0.228	3.71 ^{bcd} ±0.137	3.76 ^{bc} ±0.300	3.20 ^{dec} ±0.895
	Alkaline	6.01 ^a ±0.079	2.74 ^{de} ±0.113	3.70 ^{bcd} ±0.202	4.10 ^{bc} ±0.153	2.60 ^e ±0.106
	Acid	6.93 ^a ±0.194	4.49 ^b ±0.059	3.66 ^{bcd} ±0.142	3.78 ^{bc} ±0.128	2.59 ^e ±0.059

Values are given as means ± SD from triplicate groups.

Small letters in the same column indicate significant differences ($p < 0.05$) between treatments.

Table 3: Protein yield (%) changes in common carp recovered with different conditions during 5 months storage at -18°C.

Treatment-Carp		0	1	2	3	4
Protein yield (%)	Conventional	47.94 ^k ±0.145	50.41 ⁱ ±0.015	49.40 ^j ±0.046	49.96 ⁱ ±0.081	49.87 ⁱ ±0.139
	Alkaline	77.88 ^d ±0.262	78.87 ^c ±0.110	82.16 ^a ±0.110	80.09 ^b ±0.370	79.98 ^b ±0.064
	Acid	55.21 ^h ±0.166	55.41 ^h ±0.066	58.24 ^f ±0.133	57.67 ^g ±0.390	61.18 ^e ±0.150

Values are given as means ± SD from triplicate groups.

Small letters in the same column indicate significant differences ($p < 0.05$) between treatments.

The results indicated that in the pattern of electrophoresis, the band intensity of myosin, actin and tropomyosin proteins with increasing storage time had significant differences among different treatments. Each month, the most band intensity in the heavy chain of myosin and actin of surimi samples prepared by acid solubility was observed. But over time, at -18°C , the intensity of the band of heavy chain of myosin and actin decreased in samples prepared by alkaline solubility. Troponin-T had no change in band intensity. In all the months of storage, molecular weight of actin and myosin did not change much (Fig 8-A/8-D).

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 *et al.*, 2009). In this study, the color characteristics were differed (Table 1). There was a significant difference between L^* , a^* and b^* values ($p < 0.05$) in different conditions of preparing surimi during frozen storage. In common carp, surimi prepared by conventional washing method had a higher L^* value (lightness), and also higher whiteness score compared with alkaline-acid-aided processes during frozen storage. a^* value was higher for protein isolated using alkaline-aided processes compared with conventional surimi and acid-aided process from second months to the end of storage. Choi and Park (2002) in their studies also 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^* value showed a significant difference between the acid- and alkali-made isolates and conventional surimi ($p < 0.05$). More yellowness could be due to more retention of lipids. Undeland *et al.* (2002) investigated the colors of acid and alkaline protein isolates from the light muscle of herring. They reported highest L^* values, b^* values for the alkali-produced protein isolate. Also in their examination a^* value was the same for the acid- and alkali-made isolates. More redness in alkaline-aided process is likely attributed to more co-precipitation of heme proteins.

Moisture, ash and lipid changes in conventional surimi and protein isolates during frozen storage

Chemical indicators of surimi prepared by three methods of conventional, solubility using an acid-alkaline process from common carp during 5 months storage in -18°C changed significantly, indicating a decline in product quality. The results show that in common carp, the content of moisture in surimi samples prepared increased from zero month to first month and after that in conventional surimi samples and solubilization using an acid process this process was invariant to the end of the period. However this increase in the method of solubility by alkaline was maintained until the end ($p < 0.05$). Based on the results of the approximate constituent compounds of common carp surimi, the moisture content of the samples isolated with acid process during storage was higher than conventional surimi that it could be due to active groups in the protein structure that will attract retain more

water. Also reducing the moisture content of fish protein isolate at high pH (11) in some fish can be due to protein structural changes. Changes in protein structure affect the function of the protein structure in the maintenance of water. Similar results about the protein samples isolated from rainbow trout has been reported by Chen and Jaczynski (2007).

Changes of ash content in surimi samples prepared by three methods of conventional, solubility by acid and alkaline process from common carp during storage at -18°C showed that in all treatments until one month, especially in samples of surimi prepared by the acid process, the ash content increased ($p < 0.05$), but after that, a significant difference between the samples was not observed during storage until the end. Ash content in samples of surimi from common carp in zero day was about 2-2.5% per one gram dried surimi sample that these values increased with increasing storage time. Ash content prepared by the alkaline process was lower. Changes of total lipid content in surimi samples prepared by three methods of conventional, solubility by acid and alkaline process from common carp during storage in -18°C showed that in the first month, total lipid content of the surimi samples prepared by the conventional method was higher than the surimi samples prepared by pH. But in the surimi samples prepared by acid-alkaline process, total lipid content from one month to the end of storage was invariable. Reason of that is because of higher fat reduction in the protein isolated samples at zero month, the production method of protein isolated and fat removal procedure

at the end of the first phase of centrifuge process. During the preparation of protein isolated from herring, Undeland *et al.* (2002) found that the protein isolated samples that were prepared by the alkaline method were lower in fat than the protein isolated samples that were prepared by the acid method. This means that in preparation of fish protein isolated by the alkaline method, more fat is removed that these results are different during storage. In this study, the protein isolated samples prepared by alkaline method in comparison with the protein isolated samples prepared by acid method had a higher fat content. However, it can be concluded that using a centrifuge to remove the fat of fish, especially in the first months of processing is considered one advantage in preparation of fish protein isolated by pH shift.

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

Histological and functional properties of proteins depend on mainly myofibril proteins. Actomyosin, is the major protein found in myofibril (Montecchia *et al.*, 1997). Actomyosin shifts with changes in the functional groups such as sulfhydryl groups, hydrophobic groups and the physicochemical properties such as ATBase is accompanied. Accordingly, it is possible that the changes in the active sulfhydryl groups and total sulfhydryl groups occur and the formation of disulfide bonds that indicating the aggregation between proteins and their effects on functional properties realized. Changes in total sulfhydryl content of

surimi samples prepared by three methods of conventional, solubility by acid and alkali process from common carp during storage in -18°C indicated total sulfhydryl content of all samples decreased. The greatest decrease was related to the method of solubility by alkaline. Reduction in the total sulfhydryl content is due to the formation of disulfide bonds or oxidation of sulfhydryl groups or exchange of disulfide bond (Benjakul *et al.*, 1997) or due to the formation of hydrogen and hydrophobic bonds that is the reaction barrier of sulfhydryl structure in actomyosin molecular (Benjakul *et al.*, 2009). Also when a side of protein chain is included the amino acids with sulfhydryl groups react with other chain and disulfide covalent bond create between them. This binding is very important at the coagulation of fish protein and improves the texture of value-added foods (Jaczynski, 2008). From the first month to the third month a gradually increasing process was observed which could be due to the opening of the protein structure (Lichen *et al.*, 1985) and this process again decreased from the third month to the fourth month. Change of pH leads to the appearance of sulfhydryl groups in the three-dimensional structure of the protein. But removing sarcoplasmic proteins and the part of myofibril proteins during the washing process in the surimi samples prepared by conventional method is decreased sulfhydryl groups (Kim *et al.*, 2003). These results are not consistent with the results of common carp in this study.

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

TBARS changes in surimi samples prepared by three methods of conventional, solubility by acid and alkali process from common carp during 5 months storage in freezer showed that TBARS values of common carp surimi samples at zero month were less than 0.5 mg malonadialdehyde/ 100g muscle. This amount increased with increasing storage time in freezer that it is indicative of oxidation ($p < 0.05$). Lipid oxidation can lead to unsuitable appearance, inappropriate color and problems in the taste. Furthermore, it is possible cause tissue problems by reacting with products resulting from the oxidation of proteins (Kristinsson *et al.*, 2005). In studies that reported by Kristinsson and Liang (2006), Kristinsson and Demir (2003), processing using acid-treated cause more lipid oxidation compared to alkaline-treated in variable species. These results correspond with obtained results of this study. Also, in this study we observed that the TBARS values after using acid-alkaline process have more effect on subsequent oxidation of protein isolated during storage in freezer compared to conventional surimi.

Protein recovery changes

Protein recovery content using the solubility by acid-alkaline process and conventional method in common carp was 70.20%, 64.50% and 60.50%, respectively. In all studies, the recovery of protein in conventional washing method for producing surimi than pH changes method has been less observed. However, the

recovery of protein by acid and alkaline process was different. Recovery of protein using solubility by acid and alkali process is related to three main factors including: 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, solubility by alkali process has helped higher efficiency in protein recovery compared to the solubility by acid process (Park and Morrissey, 2000; Kim *et al.*, 2003, Kristinsson and Ingadottir, 2006; Palafax *et al.*, 2009). However, in most studies, efficiency of solubility by acid process was more than solubility by alkali process. In this case, has been reported studies by Pacheco-Aguilar *et al.*, (2001) on sardine, Undeland *et al.*, (2002) on herring (*Clupea harengus*) light muscle, Batista *et al.*, (2003) on blue whiting, Ingadottir (2004) on tilapia white muscle, Kristinsson *et al.*, (2005) on channel catfish muscle, Kristinsson and Liang (2006) on Atlantic croaker (*Micropogonias undulates*) muscle, Rawdkuen *et al.*, (2009) on tilapia and Shirvani *et al.*, (2010) on silver carp muscle. Their results are consistent with obtained results from common carp in this study. In general, these results are the fact that effect of changes in pH is higher than conventional method due to the higher recovery of sarcoplasmic proteins and effective prevention of the loss of myofibril proteins during the subsequent washing and dewatering process. This subject leads to more efficient of muscle protein in pH changes. In the conventional method of producing surimi, during the washing process in successive cycles, sarcoplasmic

proteins are easily dissolved in water and then extracted and with increasing the wash, the myofibril proteins are dissolved and lost. Thus, protein yield is reduced in the conventional method (Choi *et al.*, 2002). In common carp, the most protein yield was found in the method of solubility by alkaline.

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

Myofibril protein solubility has important role in the coagulation and water holding capacity of muscle protein. In Fig. 5, the solubility of carp surimi obtained using the method of acid and alkaline process at the zero and the first month was lower than the conventional method. In common carp a significant difference was observed between treatments until the end of the storage period ($p < 0.05$). High solubility in conventional surimi in the early months was due to less hydrolysis of proteins during processing and storage in freezer. These results corresponded with previous reports of Cortes-Ruiz *et al.* (2001) on sardine and Rawdkuen *et al.* (2009) on tilapia.

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

Myoglobin extracted from surimi samples prepared by three methods of conventional, solubility by acid and alkali process during 5 months storage in -18°C showed that myoglobin levels decreased with increasing storage time and the reduction of myoglobin was more in the surimi samples prepared by acid-alkaline

process in comparison with conventional surimi. Statistically, a significant difference was observed between samples until the end of storage. Increase in the extraction of myoglobin is probably due to further degradation of muscle proteins. Chaijan *et al.* (2006) reported that the solubility by alkaline process can lead to the effective removal of myoglobin from sardine and mackerel muscles. But extraction of myoglobin depends on the species, muscle type, storage time and washing process. Yongsawatigol and Park (2004), Jafarpour and Gorczyca (2008) stated that more white in surimi prepared by conventional method is due to the effective removal of myoglobin during washing. The results of this study were consistent with their results.

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

Total pigments contents changes of surimi samples prepared by three methods of conventional, solubility by alkali and acid process from common carp during storage in freezer showed that total pigment content decreased with increasing storage time. The minimum amount of pigment was observed in the surimi samples that were prepared by conventional. It means that there was the highest pigment removal in the conventional surimi during the washing process. Pigment removal in all samples had a decrease trend and this reduction was observed in the samples prepared by conventional method more.

Chromoproteins are often composed of a group with a symmetrical crystal structure and a variable metal, and they can cause color of muscle foods. Although, there are carotenes and carotenoproteins along with chromoproteins that play an important role in meat color (Perez-Alvarez and Fernandez-Lopez, 2006). Hemoglobin and myoglobin are two main pigments that constitute red color in muscle foods. In this study the highest pigment removal was observed in the samples prepared by conventional method and alkaline process ($p < 0.05$). The results showed that washing process can be extracted the myoglobin and other pigments from minced fish and leading to less pigment in the fish muscle. Chaijan *et al.* (2006) reported that the removal of total pigments in sardine and mackerel muscles gradually decreased as the storage time increased. Also, Chen (2003) found that the removal efficiency of myoglobin in lionfish decreased with increasing storage time on ice. Insolubility and binding of myoglobin oxidized with muscle caused lower removal of myoglobin during the washing process (Chen, 2003). It was presumed that the alkaline process could extract more pigment from the muscle. These extracted pigments could be denatured during alkaline treatment and could not be co-precipitated at pH 5.5, therefore they were removed from the muscles (Rawdkuen *et al.*, 2009).

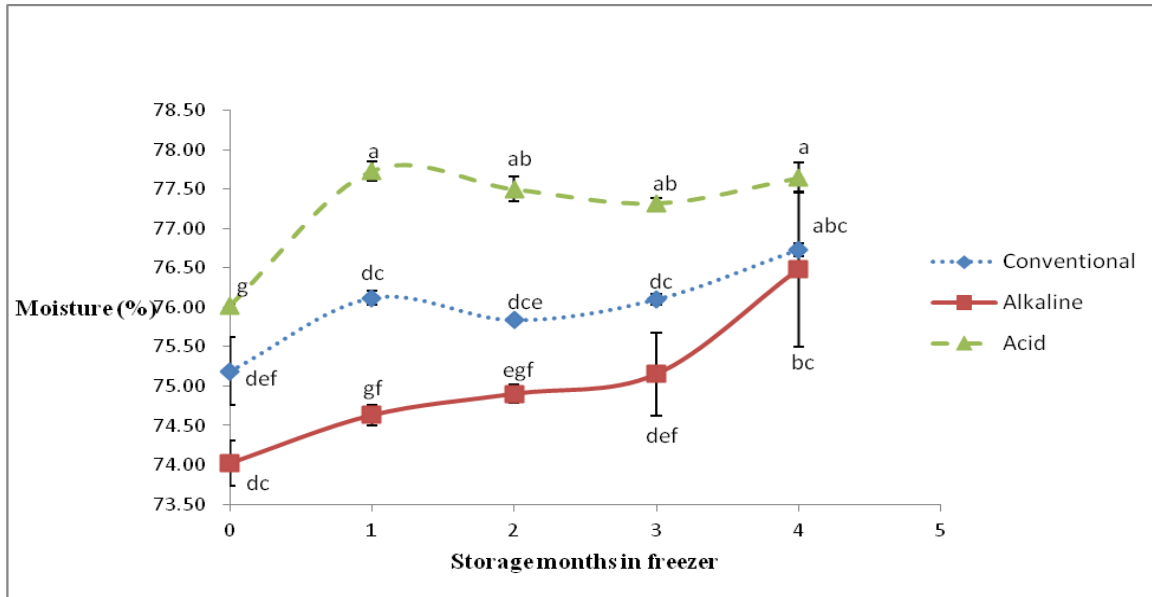


Figure 1: Formed proximate composition (moisture) of common carp surimi prepared with different conditions during 5 months of storage at -18°C.

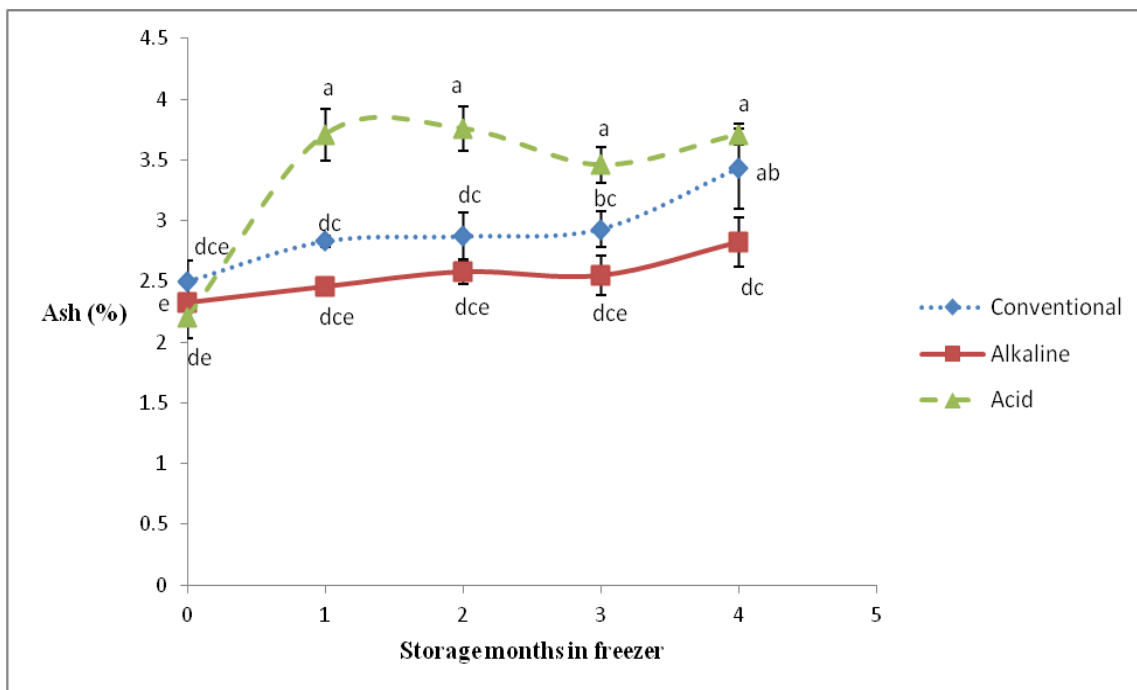


Figure 2. Formed proximate composition (ash) of common carp surimi prepared with different conditions during 5 months of storage at -18°C.

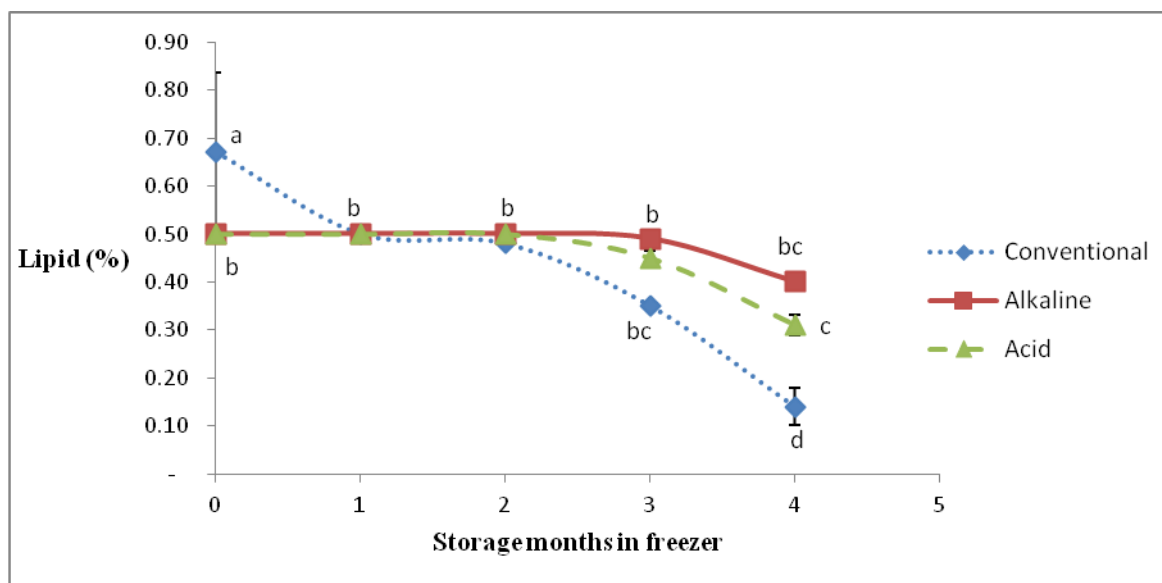


Figure 3: Formed proximate composition (lipid) of common carp surimi prepared with different conditions during 5 months of storage at -18°C.

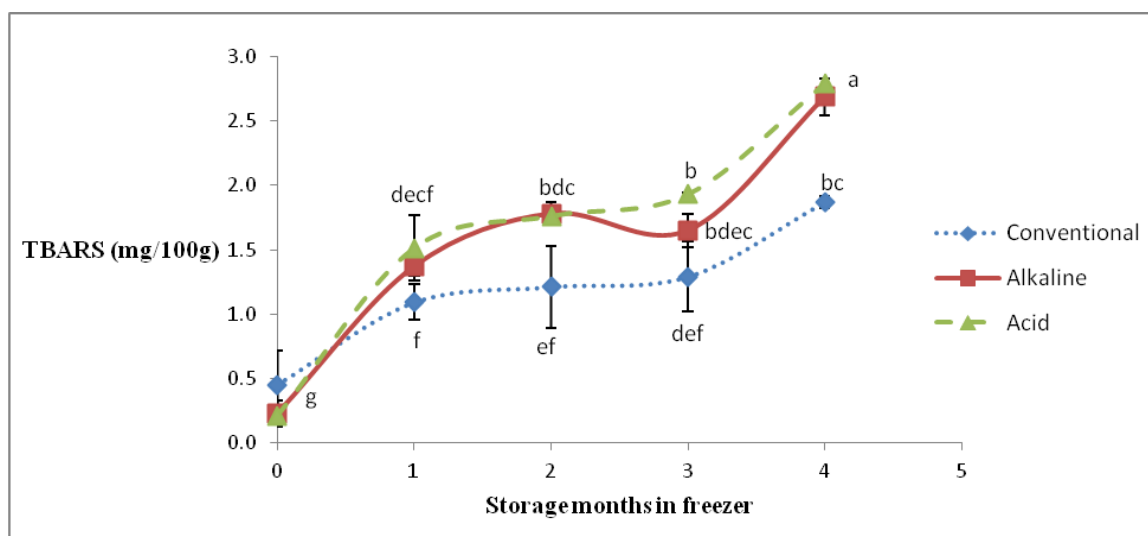


Figure 4: Thiobarbituric acid-reactive substance (TBARS) changes of common carp surimi prepared with different conditions during 5 months of storage at -18°C.

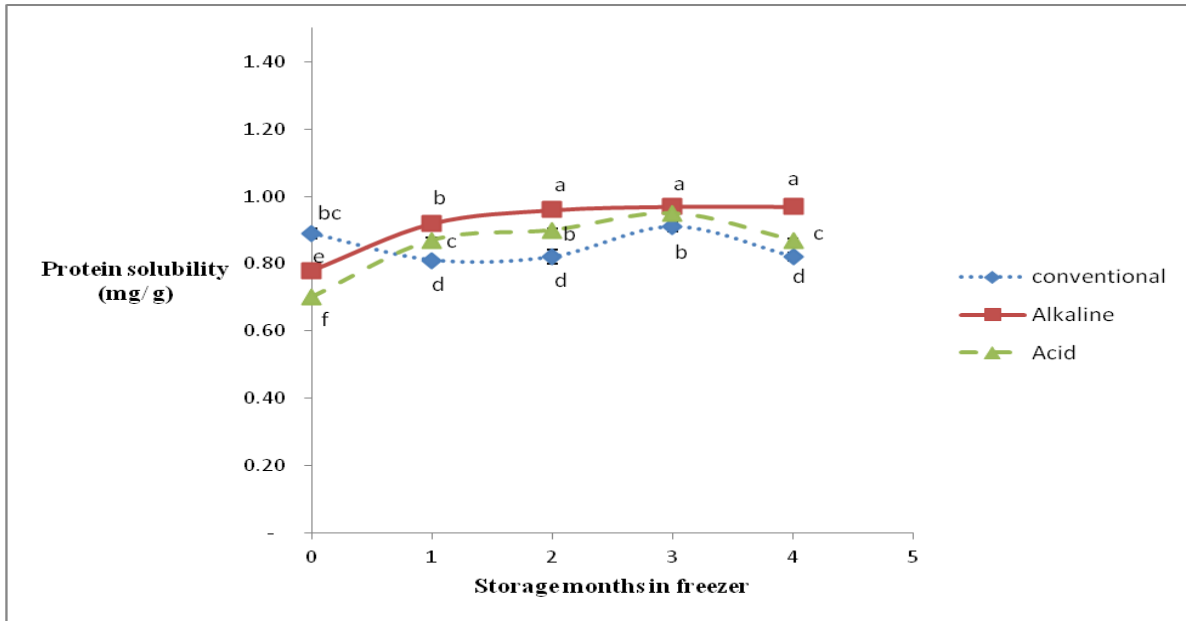


Figure 5: Protein solubility changes of common carp surimi prepared with different conditions during 5 months of storage at -18°C.

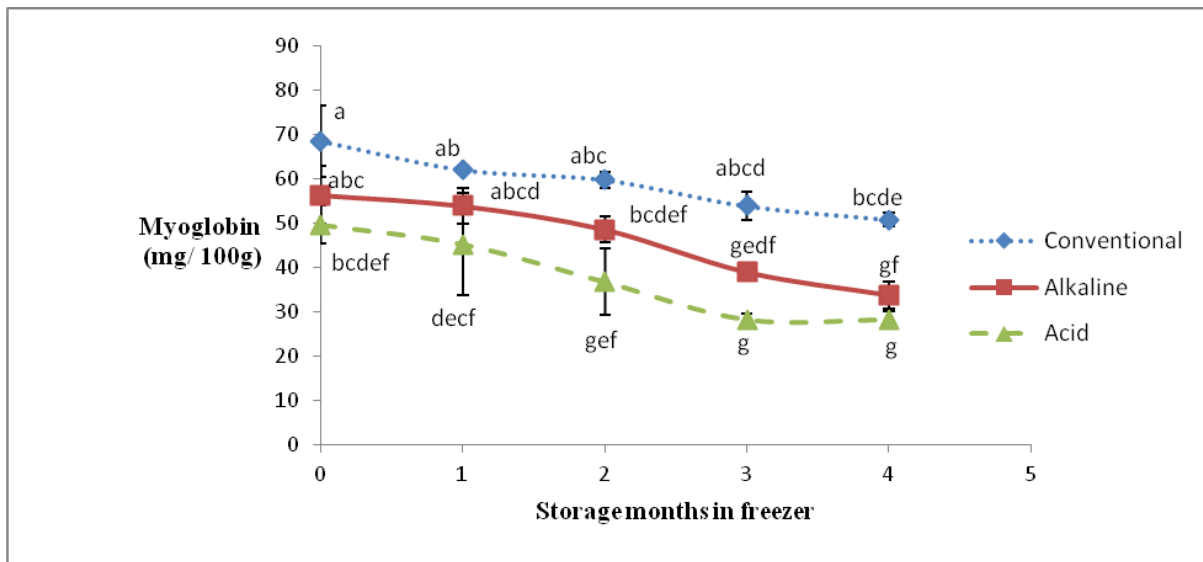


Figure 6: Myoglobin changes of common carp surimi prepared with different conditions during 5 months of storage at 18°C.

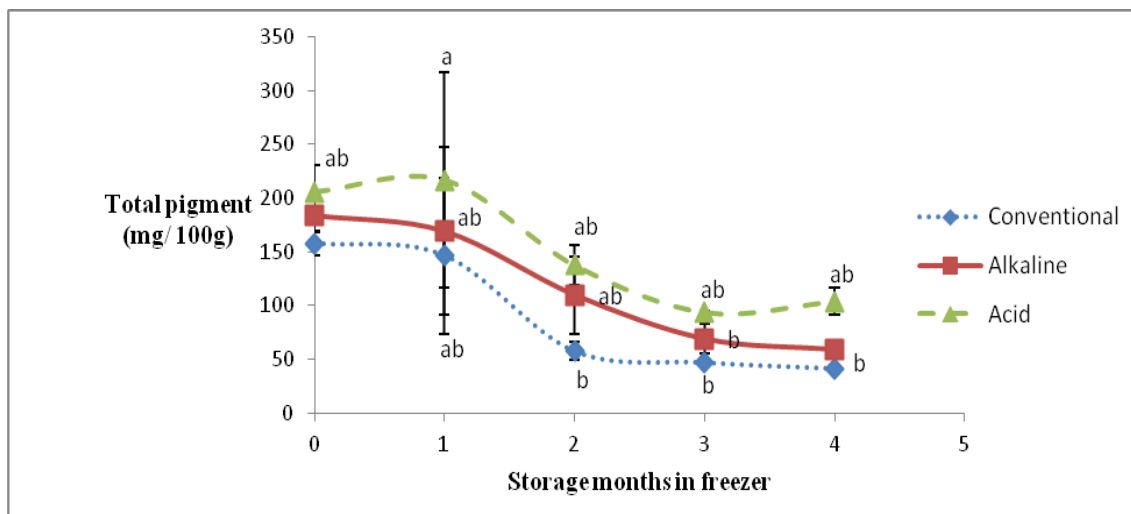


Figure 7: Total pigment changes of common carp surimi prepared with different conditions during 5 months of storage at -18°C.

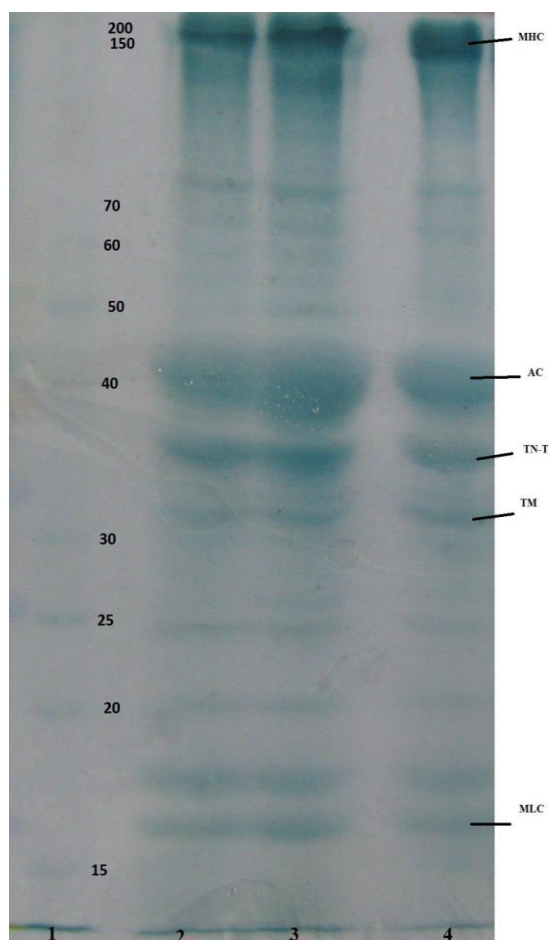


Figure. 8-A- First month

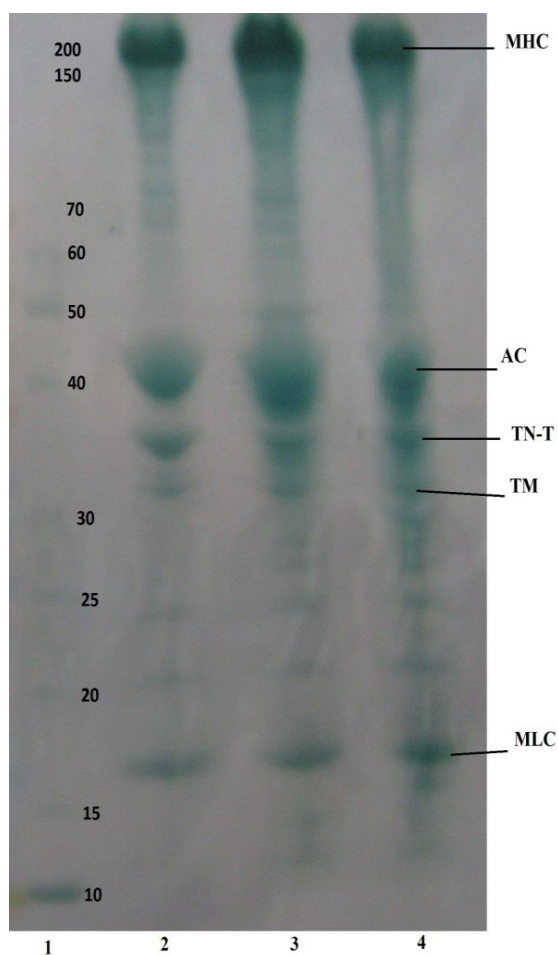


Figure. 8-B- Second months

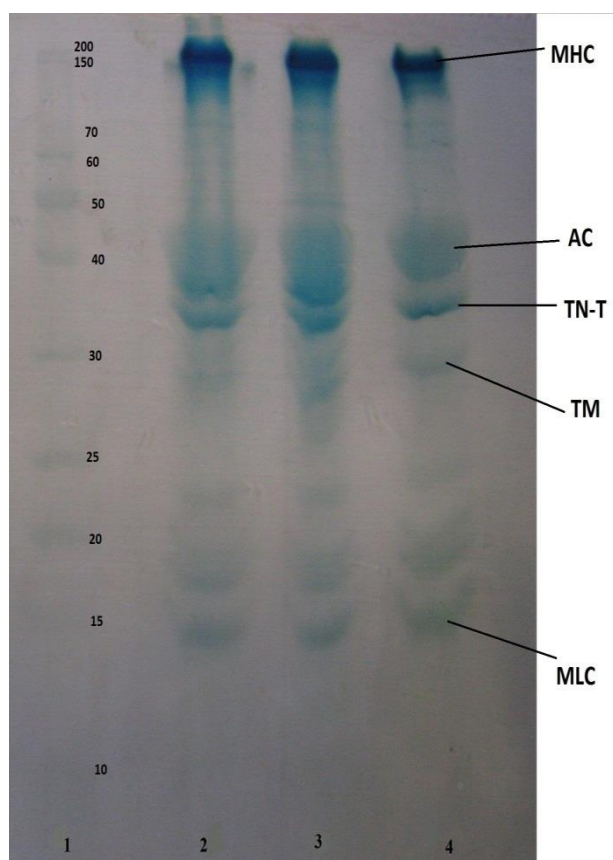


Figure. 8-C- Third months

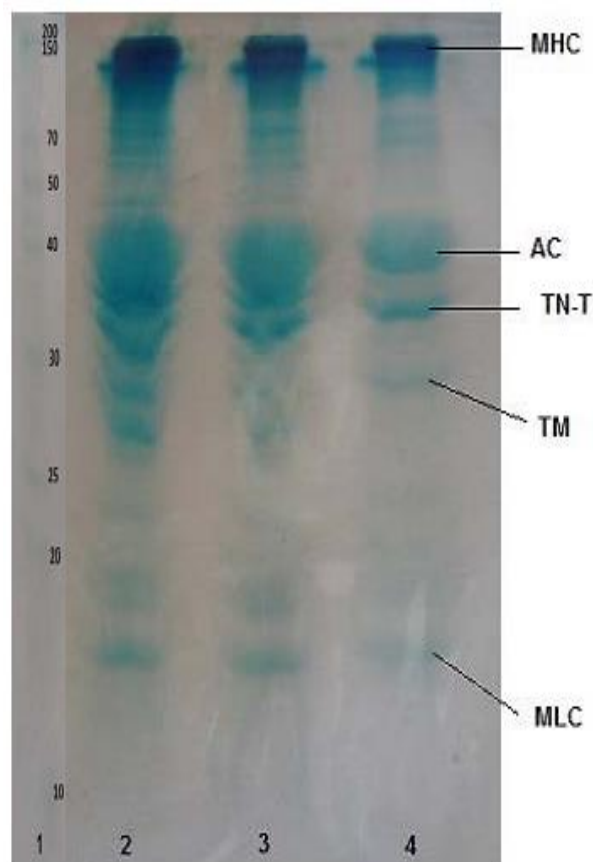


Figure. 8-D- Forth months

Figure 8:A/B/C/D. SDS-PAGE of minced common carp prepared with different conditions during frozen storage at -18°C from first month to four months. Lane 1: Marker, lane 2: common carp prepared with alkaline-aided process, Lane 3: common carp pretreated with acid-aided process, Lane 4: common carp pretreated with conventional method. MHC: myosin heavy chains, MLC myosin light chains: AC: actin, TN-T: troponin-T, and TM: tropomyosin.

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

Fish protein changes are effective on nutritional properties. On the other hand, textural and functional properties of meat are essentially depending on myofibril proteins (Thippeswamy *et al.*, 2002). So, changes in proteins during storage on ice is indication of hydrolyze activity in fish muscle (Benjakul *et al.*, 1997). Myosin

and actin are main proteins involved in most of the functional properties of myofibril proteins (Mohan *et al.*, 2008). According to Fig. 8-A to 8-D, SDS-PAGE electrophoresis patterns show that in common carp, the band intensity of the heavy chain of myosin and actin in surimi samples prepared by conventional method and change of pH is varied. The results show that in the pattern of electrophoresis,

band intensity of myosin, actin and tropomyosin proteins with increasing storage time showed a significant difference among different treatments. In this study, the most band intensity was observed in the heavy chain of myosin and actin of the surimi samples prepared using the acid solubility in every month. That can be indicative of less hydrolysis. But with time at -18°C , the band intensities of the heavy chain of myosin and actin decreased in the samples prepared by the alkaline solubility. Band intensity of troponin-T had no change. In all the months of storage, the molecular weight of actin and myosin did not change more. In the surimi samples prepared by conventional method during storage, we observed more hydrolysis especially in light chain of myosin, which is compatible with the obtained results of protein solubility in this study. Hultin and Kelleher (2000) believed that the weak bands of protein are the result of hydrolysis effect of enzyme activity. Park and Morrissey (2000) on Atlantic whiting fish, indicated that the largest reduction in band intensity of myosin (myosin heavy chain) and actin was observed when they used the acid solubility method. Also, Yongsawatdigul and Park (2004) on rock fish reported that the acid-alkaline solubility process lead to decrease in the band intensity of myosin high chain. Kristinsson and Ingadottir (2006) reported that the highest band intensity of actin proteins was observed at high pH (11) compared with low pH (5.2).

Results of chemical analysis that were performed at certain intervals during 5 months of storage in the freezer showed that TBARs and sulfhydryl groups as chemical indicators of fish quality control in surimi samples prepared by pH changes in common carp were better than conventional method. Also, pH changes compared to the conventional method indicated the highest protein recovery, protein yield and solubility of the protein. Therefore, the production of protein isolates using acid and alkaline process is a promising way of increasing the utilization of cultivated fish for food production. The color changes in surimi samples prepared by conventional method during storage at -18°C , indicated that a^* factor decreased and overall whiteness increased. Myoglobin and total pigments showed a gradual decrease in all treatments, but during the washing process, the maximum amount of pigment removal was related to in the conventional surimi. Therefore, one of important suggestions for future researches is using of fish protein isolate in the preparation of various products such as sausages.

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