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Biochemical Properties of Fish Protein Isolate (FPI) from Silver Carp (*Hypophthalmichthys molitrix*) by Application of Acid-Alkali Processes Compared to Traditional Prepared Surimi

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ABSTRACT Some Biochemical properties of silver carp surimi prepared by application of acid-alkali aided methods were investigated and compared to that of derived by conventional method. In terms of total protein solubility and recovery, lipid reduction, and total pigment extractability and myoglobin removal there was a significant ($P<0.05$) difference among the treatments. Acid-aided method showed the most efficiency to recover more proteins (86.2%) in comparison to the alkaline-aided (79.8%) and conventional (76.7%) methods. The lipid reduction percentage was recorded as 43.6%, 58.4% and 72.3% for the conventional method, and acid-alkali aided methods, respectively. In terms of total pigment removal, the conventional method showed higher efficiency ($P<0.05$) compared to the pH-shifting methods. Conversely, fish protein solubilisation by acid-alkali aided techniques was more efficient ($P<0.05$) compared to the conventional method of making surimi. In conclusion, pH-shifting techniques were superior in comparison with the conventional method in order to recover more functional proteins and to efficiently reduce the lipid and myoglobin content of resultant fish protein isolate.

Key words: *Acid-alkali solubilisation, Biochemical Properties, Fish protein isolate, Silver Carp (Hypophthalmichthys molitrix), Surimi.*

1 INTRODUCTION

Universally, fish is considered as a valuable muscle protein resource, mainly due to its higher nutrition value, digestibility and functional properties compared to other protein sources (Friedman, 1996; Simopoulos, 1997). However, utilization of fish proteins as a food or food ingredient has been limited due to several reasons. These including rapid bacterial spoilage, lipid and protein oxidation, low stability and loss of functionality during processing and storage compared to mammalian and vegetable protein sources (Niki *et al.*, 1985; Baron CP, Kjaersgård IV, Jessen F, & Jacobsen C, 2007). Over the years, several methods have been developed in order to isolate proteins from the fish muscle and a good example is surimi processing which involves washing of fish mince and adding cryoprotectants to it before freezing to stabilize the proteins. Employing of

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this method, known as conventional surimi processing, results in a protein sol known as surimi paste is an intermediate value-added ingredient consist of about 18-20% protein, less than 1% lipid and about 75-78% moisture (venugopal, 2006).

In general terms, salt addition to surimi, with sufficient degree of grinding, breaks ionic bridges within the proteins, dissolves them and destabilizes their molecular structure toward subsequent thermal denaturation and promotes hydrophobic interaction. These hydrophobic interactions, rearrangement of hydrogen bonds as well as covalent bonds of proteins, play important roles in the formation of the protein network when the sol is heated (Ignacio Sa´nchez-Gonza´lez *et al.,* 2008). In conventional method and specifically during washing steps, many water soluble components (some prooxidant such as "haem" proteins and metal ions, some anti-oxidants such as ascorbate and glutathione peroxdiase), lipid and sarcoplasmic proteins along with some part of myofibrillar proteins are removed and consequently the yield of surimi decreases. Moreover, due to loss of some functional parts such as anti-oxidants and subsequent oxidation of pigments, flavor compounds, vitamins and more importantly reduction of surimi elasticity the surimi past will be unstable during frozen storage (Undeland *et al.*, 1998). In 1985, Niki and his colleagues collected the washed water from the first washing cycle of conventional surimi process and increased the pH to 10.0 followed by centrifugation to remove insoluble materials. Then, the pH of supernatant was adjusted to 5.5 and was heated up to 80°C in order to coagulate and collect the water soluble proteins. The authors reported that the mass of recovered proteins was about 20% of total surimi yield. Furthermore, considering the expansion of world surimi market and 12% annual growth of surimi import to Europe during 1996-2006 and increasing the price of surimi from 1.7 €/kg in 2006 to 2.5€/kg in 2012 (GLOBFISH, 2012) , there was a gap between surimi supply and its demand. To address this issue, it is necessary to introduce alternative low-value fish species as raw material for surimi preparation and more importantly to invent and develop more efficient processing techniques to isolate more proteins from the fish and other by-catch species.

In this regards, a relatively novel processing technique were developed which are involved of acid or alkaline solubilisation and isoelectric precipitation of muscle proteins to give a highly functional and stable protein isolate from the low-value underutilized species and by-products). This technique has been widely used by many researchers (Choi *et al.,* 2002; Choi and Park, 2002; Undeland *et al.*, 2002; Kristinsson and Hultin, 2003a, b and c; Kristinsson and Hultin, 2004a, b; Yongsawatdugul and Park, 2004; Perez-Mateos *et al.*, 2004; Kristinsson *et al.*, 2005) and they are unanimously agreed on the advantage of this method over the conventional method of making surimi. Three major benefits of pH-shifting method are as (a) there is no need to manually separate the bones form the flesh, as the bones and scales debris removed easily as the sediment part after first centrifugation, (b) retention of more sarcoplasmic proteins and increasing of the surimi yield, and (c) efficient removal of lipid and hence minimizing the risk of lipid oxidation during frozen storage.

The new process has been shown to work well for various cold water species such as Pacific whiting (Choi & Park, 2002), Cod (Kristinsson & Hultin, 2003b), and Atlantic Croaker (Perez-Mateos *et al.*, 2004) but currently there is little data available for the potential of using these processes to produce functional proteins from warm water species such as silver carp. Silver carp (*Hypophthalmichthys molitirix*) is an abundant warm water fish and its increasing cultured supply in Iran means generating large amounts of by-products (and primary products) which could be utilized for its protein content, provided the proper

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process is used, hence, this fish is in sufficient quantities for further processing. Conventional surimi processing from silver carp has been somewhat successful although the yields are fairly low. To reach the goal of optimum utilization of silver carp muscle, it is essential to investigate the use of the newly developed acid and alkali-aided processes on whole muscle. The results from this research are expected to give important information based on protein recovery efficiency, fat and pigment removal percentage on the production of a high quality protein isolate from aquacultured silver carp.

2 MATERIALS AND METHODS

Silver carp with average weight of 475±23 g (Mean±SD) was freshly purchased from fish market, transported on the ice with the ratio of 1:1 (ice:fish) after about 30 min to the laboratory at Agricultural Sciences and Natural Resources University of Gorgan-Iran and kept at -20°C for 48 h. In order to thaw the fish, the carcass was kept inside the refrigerator (4°C) overnight. Then, fish was filleted and minced by application of a meat mincer (Braun, Triumph G3000, Czech Republic), equipped with a disk with 3 mm mesh size.

2.1 Surimi preparation procedure

2.1.1 Conventional method

Surimi was prepared following the method was described by (Lanier, 1992). Fish mince was mixed with cold (4°C) distilled water with the ratio of 3:1(water:fish mince). This mixture was stirred for 5 min following a 5 min period of settling; finally the slurry was drained with double folded cheese cloth. The whole procedure was repeated 3 times, and 0.3% (w/v) NaCl was added during the last washing cycle to dewater the surimi paste more efficiently.

2.1.2 pH-shifting methods

Following Nolsoe and Undeland (2008), in order to prepare a surimi gel by acid-alkali aided method, minced muscle was homogenized at ratio of 1:9 with cold distilled water while the pH was adjusted to 2.5 and 11.0 by application of 2M HCl and 2M NaOH, respectively, to solubilise the majority of fish muscle proteins via electrostatic repulsion. The soluble proteins were collected after centrifugation at 10000 ×g for 20 min as the supernatant. The middle layer, after removal of top layer (fat) and bottom layer (connective tissue only?) was recovered by isoelectric precipitation (subjecting the slurry to pH=5.5 and then collecting by centrifugation). The schematic diagram of this procedure is shown in Figure 1. The sediment (isolated proteins) was collected and its pH was neutralized to about pH 7.0 by addition of 2M NaOH.

2.2 Surimi gel preparation

The surimi paste after dewatering was then put in a food processor and chopped for about 60s to create a homogen paste. After adding salt (2% w/w) while it was still being homogenized, ice-water was sprinkled over the mince to adjust the moisture content of the paste to 80%. During blending, temperature of resultant surimi gel was maintained between 4-10ºC as mentioned above. Afterward, the surimi paste was put in icing bag and squeezed into stainless steel tubes (Length 20 cm, internal diameter 2.50 cm) which had previously been sprayed with canola oil to lubricate the inner surface of the tube. Both ends of the tubes were then sealed with screw thread caps. To obtain low temperature setting (Lanier, 1992), the surimi in the tubes was refrigerated (2-4ºC) overnight. To convert the surimi paste to surimi gel known as kamaboko, the tubes were placed in a hot water bath at 90ºC ± 2ºC for 30 min. After heating, the tubes were cooled in an ice water bath to *ca*. 10ºC to stop any further effect of heat on texture and once cooled the kamaboko gels were removed from the tubes with a plunger and sliced to required dimensions for measuring large scale texture characteristics.

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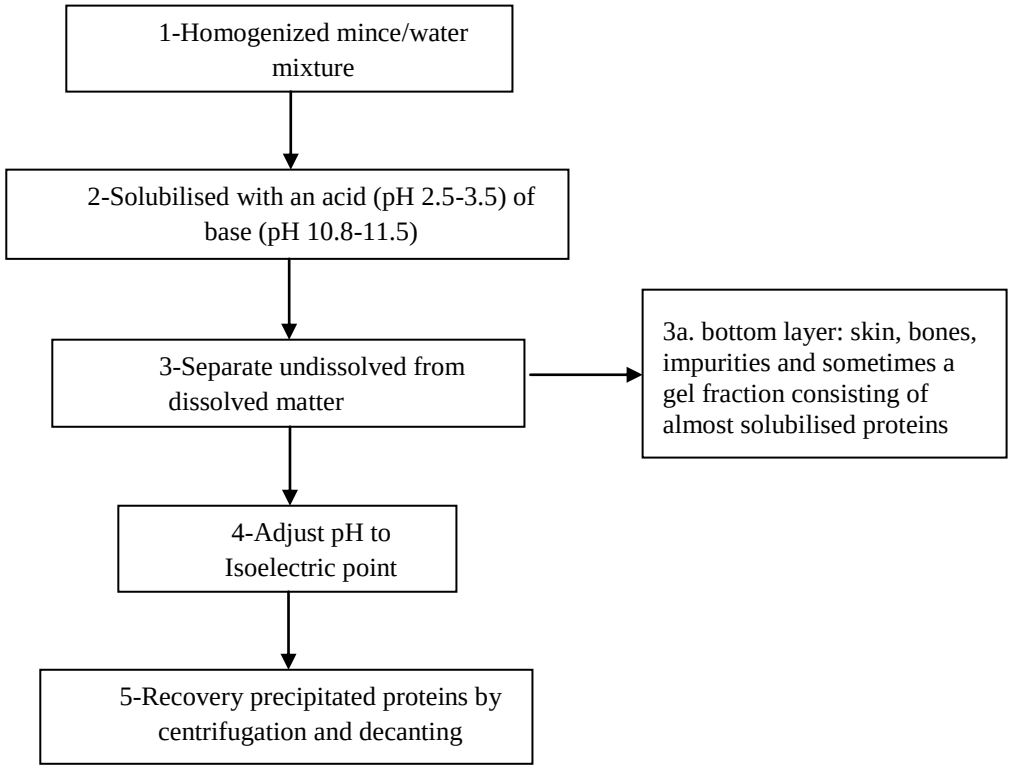

Figure 1 Schematic diagram of surimi preparation by application of acid-alkali aided method (Adapted from Nolsoe and Undeland, 2008)

2.3 Chemical characteristics of surimi gel

2.3.1 Moisture content

10 g of surimi gel was weighetd and placed in a hot oven set at 105°C during overnight and the moisture content was measured by application of following method (Association of Official Analytical Chemists, AOAC, 2000):

$$\text{Moisture content} = (1 - Wf/Wi) \times 100 \quad (1)$$

where Wf is final weight of surimi gel sample and Wi is initial weight of surimi gel sample

2.3.2 Protein solubility and recovery percentage

Minced muscle of silver carp was homogenized in a Waring blender (Waring commercial, Model 32BL80 (8011), USA) with cold distilled water in ratio of 1:9 (fish mince:water) for two times period of 60s with 30s interval time. The homogenate was then separated in two fractions; pH of each fraction was adjusted to pH 2.5 and pH 11 by application of 2M HCl and 2M NaOH, respectively. Afterward, protein homogenate was subjected to centrifugation at 10000 g for 20 min. After serial dilution of the supernatant, the protein content was measured by Biuret method, following Torten and Whitaker (1964) and the solubility percentage was calculated based on the following formula (Kristinsson and Liang, 2006):

$$\text{Protein solubility (\%)} = (P_f/P_i) \times 100 \quad (2)$$

Where P_f is protein content of supernatant after centrifugation and P_i is protein content of supernatant before centrifugation

To determine the recovery percentage of protein, the content of surimi protein was divided

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by protein content of fish mince, multiplied by 100 (Kristinsson and Liang, 2006).

2.3.3 Lipid reduction

In order to determine the efficiency of pH-shifting method to reduce the lipid content of fish mince, the crude lipid of one g of dried fish mince and/or surimi sample was analyzed by ether extraction in a Soxhlet apparatus by application of petroleum ether (AOAC, 2000). After separation of extraction balloon from the apparatus, the solvent residuals were evaporated in a hot water bath and finally after drying of balloon in a hot oven and cooling it down in a desiccators, the total crud lipid content was calculated and subsequently the lipid removal percentage was determined based on the below formula:

$$\text{Lipid reduction (\%)} = (1 - L_S/L_M) \times 100 \quad (3)$$

where L_S is lipid content of surimi sample and L_M is lipid content of fish mince sample.

2.3.4 Total pigment content and its removal percentage

Following the method described by Rawdkuen *et al*. (2009), for determination of total pigment content, one g of fish mince, fish protein isolate or surimi sample was mixed and stirred with 9 ml acetone acid (90% acetone, 8% distilled water and 2% hydrochloric acid). After one hour resting time at ambient temperature, the mixture was filtered through a No.1 Whatman filter paper. Absorbance was read at 640 nm wavelength and acetone was used as blank. Haematin percentage as total pigment content was calculated as below:

$$\text{Total pigment content (\%)} = A_{640} \times 680 \quad (4)$$

To calculate the percentage of pigment removal, the following formula was used:

$$\text{Pigment removal percentage} = (1-P_S/P_M) \times 100 \quad (5)$$

Where P_S is total pigment content of surimi sample and P_M is total pigment content of fish mince.

2.3.5 Myoglobin content and its removal percentage

Following the method of Chaijan and Benjakul (2006) as was described by Rawdkuen *et al.,* (2009) two grams of fish mince, fish protein isolate or surimi sample were weighed into a 50 ml centrifuge tube and 20 ml of phosphate buffer (40 mM, pH 6.8) were added on it. The mixture was homogenized at 3000 ×g for 30 min at 4°C. The supernatant was filtered with a Whatman No.1 filter paper. In order to reduce the extracted myoglobin, 0.2 ml of 1% (w/v) sodium dithionite was added to the supernatant and then absorbance was measured at 555 nm. By considering the millimolar extinction coefficient of 7.6 and a molecular weight of 16.111, myoglobin content was calculated as below:

$$\text{Myoglobin content (\%)} = (A \times 16.111 \times F \times W_S \times 7.6) \times 100 \quad (6)$$

where A is absorbance, F is dilution factor and W_S is weight of sample (g).

The percentage of myoglobin removal was calculated based on the following formula:

$$\text{Myoglobin removal percentage} = (1-M_S/M_M) \times 100 \quad (7)$$

where M_S is myoglobin content in sample and M_M is myoglobin content in sample.

2.4 Statistical analysis

By application of SPSS software package, data were subjected to the analysis of variance (One-Way ANOVA), and the Levene's test was used to assess the homogeneity of variance in different samples. The significance of

[DOR: 20.1001.1.23222700.2013.1.3.4.8]

difference between means was measured at confidence level of 95 percent (probability 0.05) by LSD test.

3 RESULTS AND DISCUSSION

3.1 Chemical analysis

The proximate composition of fish mince, total pigment and myoglobin content before any further processing step is shown in Table 1. Accordingly, silver carp fish mince is composed of about 78% moisture, 18% protein, 0.8% fat and 1.0% ash. Considering the protein content and crude lipid of silver carp muscle, this fish can be potentially turned to a surimi with the proper quality characteristics.

3.2 Protein solubility and recovery

According to Table 2 there was a significant ($P<0.05$) difference among the treatments in terms of protein loss during preparation of surimi from silver carp mince. Considering the protein content of fish mince (18.0%), after three washing cycles, nearly 23.3% of protein content was removed in the waste water and the majority (75%) of protein loss was happened after the first washing cycle.

Table 1 Proximate composition of silver carp mince

Treatment type	Measured parameters						
	Moisture content (%)	Protein (%)	Crude lipid (%)	Ash (%)	Total pigment content (mg/100 g)	Myoglobin content (mg/100 g)	
Silver carp mince	78.55 ± 0.36	18.05 ±	0.83 ± 0.12	1.02 ± 0.23	1139 ± 187	271	± 32

Table 2 Solubility and recovery percentage of proteins from Sliver carp mince prepared by conventional method and pH-shifting processes

Treatments (different Methods)	Sample source	Amount of protein in g/100g ± Standard deviation remaining after the following steps			Protein solubility (%)	Protein recovery (%)
		I	II	III		
Traditional method	Mince	17.96 ± 0.73	-	13.79 ± 0.02	NA*	76.76 ± 1.09
	Water (lost protein)	3.07 ± 0.15	0.78 ± 0.03	0.32 ± 0.04		
Acid-aided method	Supernatant (step1) or Sediment (step II)	15.47 ± 0.15	13.34 ± 0.08	NA	92.17 ± 0.97	86.23 ± 0.81
	Sediment (step1) or Supernatant (step II)	1.41 ± 0.17	1.07 ± 0.12	NA	NA	NA
Alkaline-aided method	Supernatant (step1) or Sediment (step II)	14.54 ± 0.21	13.07 ± 0.15	NA	89.92 ± 2.28	79.82 ± 1.15
	Sediment (step1) or Supernatant (step II)	1.81 ± 0.41	1.81 ± 0.27	NA	NA	NA

*NA: Not Applicable

[DOR: 20.1001.1.23222700.2013.1.3.4.8]

It is believed that this part of protein is mostly comprised of sarcoplasmic protein (water soluble protein fraction) in which are the target of washing process during surimi preparation, since according to some researchers these proteins have negative impact on surimi gelling properties (Okada, 1964, Nakagawa *et al.*, 1989). On the other hand, in case of pH-shifting methods, the amount of protein loss was equal to 13.7% and 20.1% after the second centrifugation step, corresponding to the acid-aided and alkaline-aide methods, respectively.

In terms of protein solubility, there was no significant ($P>0.05$) difference between pH-shifting methods, however, by application of acid-aided method, significantly ($P<0.05$) more protein was recovered. In the acid-alkali aided processes protein recovery is significantly higher compared to the conventional method due to recovery of both myofibrillar and part of sarcoplasmic proteins. Quoting from Undeland *et al.* (2002) "the theory behind acid and alkaline protein solubilisation is the formation of net positive and net negative charges, respectively, on the proteins. This creates electrostatic repulsive forces, which drives the molecules of the protein aggregates apart". Protein recovery using conventional technique is usually lower due to loss of sarcoplasmic proteins (~30% of total protein) during washing steps. Undeland *et al.* (2002) reported that in the case of herring fish about 72% of the total protein was solubilised by the alkali method (pH 10.8) and 68% of that was recovered after precipitation (pH 5.5 treatments). These results are similar to those found in study conducted by Kristinsson *et al.* (2005) on the channel cat fish mince in which protein recovery was increased from 70% to 82% in an alkaline method by skipping of the 1st centrifugation step. These authors also found that alkaline-aided method resulted in a greater protein recovery than a laboratory–based conventional method which only recovered at about 62% protein. Similarly, Jafarpour and Gorczyca (2008) reported that solubilisation percentage of common carp myofibrillar proteins by alkaline-aided method was at about 82% (w/w of initial mass) of which about 74% was recovered after precipitation at pH 5.5 and this was significantly ($P<0.05$) higher than the protein recovery percentage of traditional method.

By reviewing the results of previous research studies it is noticeable that some researchers such as Kim *et al.* (2005), Kristinsson and Liang (2006), Nolsoe and Undeland (2008), and Palafox *et al.* (2009) it can be concluded that alkaline-aided method is superior to acid-aided method in terms of protein recovery percentage. However, majority of researchers such as Kristinsson and Demir (2002), Undeland *et al.* (2002), Kristinsson and Liang (2006), Batista *et al.* (2007), and Rawdkuen *et al.* (2009) studying wide range of fish and other aquatic species such as squid, stated that the acid-aided method is the proper method for isolation and recovery of protein. The possible explanation is that the higher pk_a value (9.1-10.8) in alkaline-aided method cause lower level of ionization of protein residuals compared to acid-aided method, corresponding to lower protein denaturation and subsequently, lower protein aggregation and precipitation at isoelectric pH (5.5). Another possibility is that in alkaline solubilisation of protein, there is higher tendency in formation of protein-lipid emulsions in comparison with acid-aided method, which it means that more proteins have been trapped in form of emulsions and as a result it is more difficult to recover them from the supernatant fraction (Kristinsson *et al.* 2005).

3.3 Lipid reduction

In terms of lipid reduction, the acid-alkali aided methods were more efficient compared to the conventional method of making surimi (Table 3) and also the difference between acid and alkaline methods was significant ($P<0.05$).

[DOR: 20.1001.1.23222700.2013.1.3.4.8]

Table 3 Percentage of lipid, pigment and myoglobin of silver carp mince that was treated by conventional method and pH-shifting methods for making surimi

Surimi characteristics	Surimi making methods		
	Conventional method	Acid-aided method	Alkaline-aided method
Lipid * (%)	2.37±13.0^{C}	1.75±25.0^{b}	1.17±14.0^{a}
Total pigment (mg/100g)	306±34^{a}	748±5.41^{c}	508±2.153^{b}
Myoblobin content (mg/100g)	100.7±7.28 ^{b}	19±4.3^{a}	17.7±5.4^{a}

*Calculation was done based on the dry matter.

Different superscripts in the same row are correspondent to the significant difference at 95% confidence level.

The lipid content of mince from silver carp was calculated as 4.21g/100g in which it was significantly reduced after washing steps during conventional method and pH-shifting techniques of making surimi. Such a reduction was calculated as 43.6%, 58.4% and 72.3% for conventional method and acid-alkali aided methods, respectively.

In 2005, Kristinsson *et al.* stated that pH shifted techniques are more efficient in terms of lipid removal compared to the conventional method. In another study, Kristinsson and Liang (2006) reported that alkaline aided method of making surimi removed the lipid content of surimi 4 times more than the conventional method. According to Batista and his co-workers (2007), application of acid and alkaline techniques for making surimi from sardine caused 51% and 61.3% reduction in the lipid content, respectively. Similarly, Rawdkuen *et al.* (2009) reported 67.8%, 82.5% and 88.6% reduction in fat content of tilapia surimi prepared by the conventional method, acid and alkaline aided methods, respectively.

Reduction in lipid content is function of the initial lipid content of mince, viscosity of homogen after pH modification, and more importantly the centrifugation speed in order to separate and precipitate the cell lipids (Nolsoe and Undeland., 2008). According to Kristinsson *et al.* (2005) in conventional method, as membrane phospholipids are in a close affinity with the cell membrane, and part of storage lipids form a stable complex with the protein during washing cycles, it is not a surprise to expect more lipid in the resultant surimi, whereas, by application of low and high pH in the pH-shifting methods, solubilised proteins detach more easily from the storage lipids and membrane phospholipids. By application of centrifugation, based on "density and solubility differences", membrane phospholipids precipitate in the bottom of the centrifuge tubes and some neutral lipids float on the top of the supernatant in which both fractions can be removed easily from the protein isolate (Kristinsson *et al.*, 2005). The higher efficiency of alkaline pH to reduce more lipid can be attributed to its higher emulsification ability, on the other hand, more solubilised protein are lost along with the lipid phase (superficial layer) (Kristinsson and Hultin, 2003b). Hence, as an advantage, the alkaline-aided method reduces more lipid and make the resultant surimi more stable during freezing storage, but the disadvantage is that due to higher protein loss, the yield percentage is lower compared to the acid-aided method.

3.4 Total Pigment content and myoglobin analysis

The conventional method showed higher ability to extract more pigment in comparison with the

[DOR: 20.1001.1.23222700.2013.1.3.4.8]

pH-shifting methods (Table 3). However, in case of myoglobin, the acid and alkaline aided methods removed the myoglobin more efficiently compared to the conventional method, but the difference between pH-shifting treatments was not significant (P>0.05). Interestingly, the visual whiteness was in coincidence with total pigment removal but oppose to the protein recovery results, i.e. more protein recovery, less pigment removal and subsequently less whiteness in colour.

The pigment content is directly related to the colour of surimi and as the colour becomes whiter, the surimi would be more popular; nevertheless the colour of surimi is dependent to its application types (Tabilo-Munizaga and Barbosa-Canovas, 2004, Nolsoe and Undeland, 2008). Even though, carotenes and cartenoproteins play an important role in colour of muscle, however, chromoprotiens in which are a "porphyrinic group conjugated with a transition metal" are responsible for the colour of fillet (Perez-Alvarez and Fernandez-Lopez, 2006, Rawdkuen *et al.,* 2009). According to Table 3, the pigments removal efficiency of all three methods of making surimi, conventional method, acid- and alkaline aided methods was 73%, 34% and 55%, respectively. Many researchers believe that the whiteness of surimi prepared by conventional method is higher than the one was prepared by pH-shifting methods (Choi & Park, 2002; Undeland *et al.,* 2002; Yongsawatdigul & Park, 2004; Perez-Mateos & Lanier, 2006; Rawdkuen *et al.,* 2009). This is confirmed in this study, even though, Kristinsson and his colleagues (2005) and Kristinsson and Liang (2006) stated that colour of channel cat fish and Atlantic croaker surimi, respectively, was prepared by alkaline-aided methods was whiter than that derived by conventional method.

In terms of the myoglobin contents, the result was not in accordance to the pigment removal and whiteness of surimi. In this study, acid and alkaline aided methods removed the myoglobin more efficiently compared to the conventional method (93% vs. 63%), but there was no significant (P>0.05) difference between pH-shifting treatments. This is in agreement with the results of Rawdkuen *et al.* (2009). These researchers stated that in acid-alkali aided method, more myoglobin comes out of intracellular structure of muscle tissue due to higher degradation of muscle and solubilisation of myofibrillar proteins, whereas, insolubility and oxidation of myoglobin cause its lower extraction and removal in conventional method (Chen, 2003, Rawdkuen *et al.,* 2009). However, as it was mentioned earlier in the text, the whiteness of surimi is not associated with the myoglobin content, but total pigment removal, hence, the colour of surimi derived from pH-shifting methods would be less white than the one prepared by conventional method. Furthermore, more whiteness of conventional surimi can be contributed to the removal of more haemoglobin from the intercellular structures (Yongsawatdigul & Park, 2004; Perez-Mateos & Lanier, 2006; Jafarpour & Gorczyca, 2008), Chaijan and *et al.* (2006) reported the higher efficiency of alkaline pH to remove more myoglobin compared to the conventional method and also mentioned that this efficiency is dependent to the fish species, type of fillet, preserving time and condition of fish mince washing, etc.

In other studies that was conducted by Kristinsson and Hultin (2004b) and Kristinsson and Liang (2006) these authors postulated that there is high possibility of haemoglobin denaturation at low pH and its subsequent precipitation along with isolated proteins at isoelectric point (pH = 5.5), whereas at alkaline pH, haemoglobin mostly remains intact and it is difficult to separate it by centrifugation following adjusting the pH to 5.5. In 2005, Kristinsson and his colleagues by studying the protein content of supernatant after second

[DOR: 20.1001.1.23222700.2013.1.3.4.8]

centrifugation at pH 5.5 in absorbance spectra of 350-700 nm, reported that in contrast to the acid-aided method, in case of alkaline solubilisation, many "Haem" proteins remained in the supernatant. Hence, the whiteness of surimi derived from acid solubilisation of fish protein would be lower mainly due to denaturation and precipitation of haemoglobin plus retention of some other sarcoplasmic proteins.

4 CONCLUSION

It can be concluded that by application of acid-alkali aided methods to prepare surimi from silver carp, more protein recovery (more yield), more lipid reduction and more myoglobin removal is achievable compare to the conventional method. Furthermore, reduction of lipid contents in acid and alkaline solubilisation of fish protein can hinder the lipid oxidation and quality deterioration of resultant surimi. However, in terms of colour of surimi, the conventional method is superior to the pH-shifting methods, because of better removal of "Haem" proteins, in which are responsible in reduction of the surimi whiteness.

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ویژگی‌های بیوشمیایی ایزوله پروتئینی تهیه شده از ماهی فیتوفاگ (*Hypophthalmichthys molitrix*) به روش حلالیت به کمک اسید و قلیا در مقایسه با سوریمی آماده شده به روش سنتی

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چکیده برخی از ویژگی های بیوشیمیایی سوریمی تهیه شده از ماهی فیتوفاگ با استفاده از روش حلالیت به کمک اسید و قلیا بررسی شده و با سوریمی تهیه شده از روش سنتی مقایسه گردید. اختلاف در شاخص‌های پروتئین بازیابی شده، کاهش سطح چربی و خروج رنگ‌دانه کل و میوگلوبین گوشت چرخ شده بین سه تیمار آزمایشی معنی‌دار بود. روش حلالیت به کمک اسید دارای بالاترین کارآیی در بازیابی پروتئین (۸۶/۲ درصد) در مقایسه با روش حلالیت به کمک قلیا (۷۹/۸درصد) و روش سنتی (۶۷/۷ درصد) بود ($P<0/05$). درصد کاهش چربی در مقادیر ۴۳/۶، ۵۸/۴ و ۷۲/۳ درصد به‌ترتیب برای روش‌های سنتی تهیه سوریمی، روش اسیدی و قلیایی ثبت گردید. از لحاظ حذف رنگ‌دانه کل، روش سنتی به‌طور معنی‌داری ($P<0/05$) از قابلیت بالاتری در کاهش رنگ‌دانه گوشت در مقایسه با روش حلالیت به کمک اسید و قلیا برخوردار بود. اما در مورد درصد کاهش میوگلوبین، این روند بر عکس بود، به‌طوری‌که روش حلالیت به کمک تغییرات pH از کارآیی بالاتری ($P<0/05$) در کاهش میوگلوبین در مقایسه با روش سنتی تهیه سوریمی برخوردار بود. در مجموع روش حلالیت به کمک اسید و قلیا در میزان بازیابی پروتئین و خارج ساختن چربی و درصد میوگلوبین گوشت چرخ شده نسبت به روش سنتی برتری داشت.

کلمات کلیدی: بازیابی پروتئین، حلالیت به کمک اسید و قلیا، سوریمی، ماهی فیتوفاگ *(Hypophthalmichthys molitrix)*

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