

Effect of salt and alkaline on the physicochemical properties of the protein isolates extracted from lanternfish (*Benthoosema pterotum*)

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Introduction

Food proteins have long been recognized for their nutritional and functional properties. The nutritional properties of proteins are associated with their amino acid content. On the other hand, the functional properties of proteins relate to their contribution to the physicochemical and sensory properties of foods (Sila and Bougatef, 2016). Marine organisms contain proteins with high quantities of essential amino acids which can provide the high quality proteins for the human consumption (Cordova Murueta *et al.*, 2007). Fish muscle proteins are categorized in to three groups based on their solubility in aqueous solutions. Myofibrillar protein (soluble in high salt concentration), sarcoplasmic protein (soluble in water and weak salt solutions) and stromal protein

(insoluble proteins) (Karlsdottir, 2009). However, large amounts of protein-rich by-products are discarded during seafood processing without any attempt to recover the essential nutrients, becoming serious environmental pollution problems especially in developing countries (Chalamaiah *et al.*, 2010). Thus, the proteins can be isolated by providing optimum conditions. The development of protein recovered is an alternative to the reuse of large amounts of the generated by-products and allows the seafood industry to provide another source of protein of high nutritional value to meet human nutritional needs and reduce environmental problems associated with the processing of seafood (Rodrigues Freitas *et al.*, 2016).

Ionic strength and pH of the protein environment are the two most important

factors affecting protein solubility, conformation and functional properties (Thawornchinsombut and Park, 2005). The alternative method developed by Hultin and Kelleher (2000) involving acid or alkaline protein solubilization and isoelectric precipitation allows recovering functional proteins from underutilised species and by-products (Pires *et al.*, 2012). Solubilization of protein by alkali aided process is one of the simplest methods of protein extraction (Kumoro *et al.*, 2010). Alkaline treatment can improve the functional properties of protein. More researches have been done around alkaline isolation of protein from various fish species such as tilapia (Ingadottir, 2004), herring (Liang and Hultin, 2005), channel catfish (Kristinsson *et al.*, 2005), mackerel (Chaijan *et al.*, 2006), rainbow trout (Chen and Jaczynski, 2007), cod (Liang and Hultin, 2005; Brenner *et al.*, 2009), Cape hake (Pires *et al.*, 2012), bighead carp (Chang *et al.*, 2016). Although pH control is important for this process, ionic strength, which also affects the solubility and functionality of muscle proteins should not be ignored. Moreover, changing in physicochemical and functional properties of several proteins as a result of varying pH and ionic strength of the medium is reported (Thawornchinsombut and Park, 2007). For instance, myofibrillar proteins are soluble at high salt concentration (300–600 mM) at neutral or slightly alkaline or slightly acidic conditions. Solubilization of muscle proteins under high ionic strength involves two events: the depolymerization of the thick

filament backbone, and the subsequent dissociation of the myosin heads from the actin filaments (Thawornchinsombut and Park, 2005). However, few studies have employed both pH and NaCl changes with the fish protein isolate prepared using the pH-shift method.

Benthoosema pterotum, a small deep-water fish commonly called lantern fish (family: *Myctophidae*), is a low-value by-catch. This fish spoils easily and thus have not been utilized as food fish (Chai *et al.*, 2016). Lantern fish is the most common and abundant marines among the mesopelagic region that distribute all oceans. They usually are small and have large elliptical to round eyes. The characteristics that make them inhabit on mesopelagic area is the luminescent photophores on their body. Also, they can survive in low oxygen ambient. Lantern fish perform daily diel vertical migrations between the mesopelagic (200-2000 m) and epipelagic (10-100 m) regions (Catul *et al.*, 2011). The Oman sea is rich in fisheries resources with considerable amounts of mesopelagic fish and *B. pterotum* is the most common and important species in the area (FAO, 2001; Valinassab *et al.*, 2007). Recently Chai *et al.* (2016) claimed its protein hydrolysate showed antioxidative activity which could prevent the age-related neurodegenerative diseases. In other word, they study demonstrated that the protein hydrolysate isolated from *B. pterotum* containing active peptides, Phe-Tyr-Tyr and Asp-Trp, possessed neuron protection efficacy. Thus, it is necessary to consider the

isolation of lantern fish protein and their functional properties for using in food as a novel ingredient in future.

Overall, the aim of this study was production of fish protein isolate from lantern fish by combination of an alkaline and NaCl extraction and also evaluate the combined effect of pH and NaCl on the physicochemical properties of fish protein isolates.

Materials and methods

Raw material

10 kg of lantern fish (*B. pterotum*) was harvested from Oman Sea, (Bandar Abbas, south of Iran) and transferred to the Department of Food Science and Technology of Shiraz University. The whole un-gutted fish were frozen and then transported by insulated container to the laboratory and stored at -18 °C until use.

Fish protein extraction

The protein extraction from lantern fish (*B. pterotum*) was applied according to the method described by Davenport and Kristinsson (2011) with slight modification. At first 200 g of frozen fish was thawed overnight in the refrigerator (4 °C) then minced by a domestic meat mincer through a 4 mm coarse grinding plate. The minced fish was dispersed with 6 volumes of distilled water and thoroughly homogenized (homogenizer IKA T25D). The homogenate was adjusted to pH 10 and 12 with 0.5 N NaOH and stirred gently for an hour under different salt concentrations (0.5%,

1.5%, 3% and 4.5% NaCl) at room temperature. The slurry was then centrifuged at 10000 rpm for 10 min at 4 °C to separate dissolved proteins from precipitated materials. Then the supernatants were collected and adjusted to the isoelectric point (PI) of fish proteins (pH 5) by using 0.5 N HCl, allowing time for precipitation of fish protein. The process was repeated once more and after readjustment, the solutions were centrifuged at 10000 rpm for 10 min at 4 °C. The precipitated proteins were collected and freeze dried. The freeze dried samples were ground to powder by a domestic mill and stored at 4 °C until use.

Proximate composition

Proximate analyses including protein and ash content of protein isolates were determined according to AOAC (AOAC, 2005).

Recovery yield

The yield as a percentage was calculated by dividing the weight of fish protein isolate (FPI) by the total wet weight of raw material used (Sathivel *et al.*, 2004).

$$\text{yield \%} = \frac{\text{weight of FPI (g)}}{\text{raw material (g)}} \times 100$$

Water-holding capacity

The water holding capacity of samples was calculated according the procedure of Foh *et al.* (2010), with slight modification. One g of each protein sample was stirred in 10 mL of distilled

water and vortexed for 30 s and then centrifuged at 2500 rpm for 25 min. Finally, the volume of the supernatant was measured. The water holding capacity was expressed as the milliliters of water absorbed per gram protein sample.

Oil-holding capacity

As in WHC measurement, 1 g of each sample was stirred in 10 mL of pure soybean oil and vortexed for 30 s and then centrifuged at 2500 rpm for 25 min. The free oil was decanted and oil-holding capacity was expressed by weight difference (Foh *et al.*, 2010).

Emulsifying capacity

Emulsifying capacity was measured according to the method of Foh *et al.* (2010) with slight modification. Five mL of soybean oil were mixed with 5 mL of a 1% fish protein isolate solution and homogenized (IKA model T25D homogenizer) at 20000 rpm for 2 min at room temperature. Then, the emulsion was centrifuged at 2800 rpm for 15 min. The volume of each fraction (water, oil and emulsion) was measured. The emulsification capacity was expressed as milliliters of emulsified oil per gram protein isolated (FPI).

$$\text{Emulsification capacity \%} = \frac{\text{emulsified oil (mL)}}{\text{fish protein isolated (g)}} \times 100$$

Foaming capacity

Foaming capacity was assessed following to the method of Diniz and Martin (1997). 0.25 g of fish protein isolated was dispersed in 25 mL of distilled water and then homogenized with laboratory homogenizer at 14000 rpm for 2 min at room temperature. Then the total volume of the liquid was measured after 30 s. the difference in volume was expressed as the volume of the foam.

Solubility

The method of Souissi *et al.* (2007) was applied with slight modification to evaluation the protein solubility. At first a solution of protein isolate samples (10 g L⁻¹) was prepared and then the pH of suspension was adjusted to 2 to 12 by drop wise addition of 0.1 N HCl and 0.1 N NaOH. The solutions were vortexed for 3 min at room temperature and centrifuged at 2800 rpm for 20 min. The protein content in the supernatant was assayed by the Biuret method using bovine serum albumin as the standard curve. Protein solubility was calculated according to the following equation:

$$\text{Protein solubility} = \frac{\text{Protein content in supernatant (mg)}}{\text{Protein content in initial sample (g)}} \times 100$$

Color evaluation

To evaluate the color fish protein isolates, samples were placed in a box (dimensions: 50×50×50 cm) with white-coloured walls. A low power (20 watt) white fluorescent lamp was used

to illuminate uniformly inside the box. The photography was carried out by a digital camera (canon, 16 Mega Pixels) with perpendicular distance of 30 cm and then colour components (L^* , a^* and b^*) were calculated using Photoshop software (CS3).

Statistical analyses

The data obtained from experiments were analyzed using analysis of variance (ANOVA) by using the SAS software and the differences between means were evaluated using Duncan's multiple test. All experiments were done in triplicates.

Results and Discussion

Proximate composition

Lantern fish (*B. pterotum*) with a mean body mass and length of 0.71 ± 0.11 g and 4.78 ± 0.04 cm, respectively, contained $75.34 \pm 0.24\%$ moisture, $69.66 \pm 1.95\%$ (dw) protein, $3.21 \pm 0.16\%$ (dw) lipid and $5.18 \pm 0.27\%$ (dw) ash. The proximate composition of fish protein isolates (FPI) which extracted at pHs 10 and 12 by adding different percentages of NaCl (0.5%, 1.5%, 3%, 4.5%) is shown in Table 1.

Table 1: Proximate composition of fish protein isolates at pHs 10 and 12 at different concentrations of NaCl.

FPI	NaCl (%)	Protein (dw%)	Ash (dw%)
pH 10	0	79.50 ± 1.17^b	2.43 ± 0.23^g
	0.5	73.01 ± 1.33^c	3.70 ± 0.39^f
	1.5	66.03 ± 1.72^d	4.29 ± 0.31^f
	3	59.28 ± 1.93^{ef}	4.36 ± 0.27^c
	4.5	58.40 ± 1.54^f	5.41 ± 0.32^a
pH 12	0	84.89 ± 1.28^a	1.94 ± 0.26^g
	0.5	65.56 ± 1.12^d	3.85 ± 0.34^f
	1.5	61.84 ± 1.63^e	4.35 ± 0.39^e
	3	58.79 ± 1.72^f	4.57 ± 0.39^d
	4.5	43.17 ± 2.05^g	5.69 ± 0.43^b

Data are expressed as mean \pm S.D. (n=3). Different letters in each column are significantly different ($p < 0.05$).

FPI= Fish Protein Isolate

Recovery yield

The recovery yield of fish protein isolated by alkaline condition at different salt concentrations is shown in Table 2. The results illustrated that the alkaline condition affected significantly on the protein extraction and the higher yields were obtained at pH 12. The solubility of muscle proteins has been increased at high alkaline pHs due to an increase in the negative charges of proteins and repulsions. According to

the results (Table 2) addition of different NaCl concentrations had influenced the yield of extraction ($p < 0.05$). The results also showed that recovery yield was decreased with increasing in NaCl concentration. However, the salt concentrations 1.5% and 3% NaCl had no significant effects on the protein recovery yield at pH 10 and then in 4.5% the yield achieved at least ($p < 0.05$). The same results were observed at pH 12. The lowest and the

highest yield extraction was obtained respectively at pH 10 with 4.5% NaCl and pH 12 without salt. Reduce in the recovery yield extraction maybe cause by salting out. Usually protein solubility decreased with increasing the NaCl concentration which due to electrostatic interactions. On other hand the binding proteins by water are influenced by adding salt. At high concentrations, the binding of proteins to water reduced, because salt ions compete with proteins for interaction by water. It means the reduction of interaction between protein and water occurs (Zayas, 1997; Leksrisompong, 2008; Shahiri Tabarestani *et al.*, 2012).

Water holding capacity

In Table 3 the results of water holding capacity (WHC) of the samples are illustrated. The results showed that WHC of isolated proteins were improved at extreme alkaline pH, so the WHC of protein isolated at pH 12 was higher than that isolated at pH 10 ($p < 0.05$). Moreover, salting had significant effect on the WHC of protein. According to the Table 3, WHC of proteins at two pHs (10 and 12) reduced with addition of 0.5% NaCl compared with the samples without

NaCl, so increasing the WHC was observed in high salt levels. However, the salt concentrations 1.5%, 3% and 4.5% had no significant effects on WHC at two pHs. The results revealed the addition of salt had high efficient on WHC of fish protein isolate. In low concentrates, sodium and chloride ions neutralized surface electrostatic charges of proteins that led to reduce the space of filaments proteins and thereby reduce the WHC. In high salt concentrates, the ion strong became increase which due to interaction of ions to proteins. Consequently the protein space increased that resulted to water flow to between the filaments and increased the WHC (Karlsdottir, 2009; Keever, 2011). With more increasing in salt concentration, proteins denatured gradually and exposed the hydrophobic groups at surface. The hydrophobic interactions led to proteins aggregation and precipitation. Thereby the WHC of proteins reduced (Pórarinsdóttir, 2010). Shaviklo *et al.* (2012) evaluated the effect of different salt percentages on WHC of proteins from cod. The results showed in 3% salt, WHC was the highest and the lowest WHC was observed in 1.2%.

Table 2: Effect of different concentrations of NaCl on protein recovery yield from lantern fish at pHs 10 and 12.

Sample	pH	NaCl (%)	Recovery yield (%)
FPI	10	0	29.09±0.84 ^d
		0.5	26.88±0.48 ^d
		1.5	22.16±0.16 ^e
		3	22.43±1.31 ^e
		4.5	19.15±0.17 ^f
FPI	12	0	48.62±0.54 ^a
		0.5	35.72±0.35 ^b
		1.5	32.85±0.61 ^c
		3	31.82±0.57 ^c
		4.5	21.51±0.28 ^{ef}

Data are expressed as mean ± S.D. (n=3). Different letters in each column indicate that the means are significantly different ($p < 0.05$).

Table 3: Water holding capacity of lantern fish protein isolates extracted at pHs 10 and 12 at different concentrations of NaCl.

Sample	pH	NaCl (%)	WHC (mL g ⁻¹)
FPI	10	0	2.83±0.28 ^{bc}
		0.5	2.33±0.76 ^c
		1.5	4.66±0.57 ^a
		3	5.50±0.50 ^a
		4.5	5.33±0.28 ^a
FPI	12	0	3.66±0.29 ^b
		0.5	2.83±0.57 ^{bc}
		1.5	5.16±0.28 ^a
		3	5.33±0.57 ^a
		4.5	4.83±0.28 ^a

Data are expressed as mean ± S.D. (n=3). Different letters in each column are significantly different ($p<0.05$).

Oil holding capacity

The results of the oil holding capacity (OHC) of the proteins isolated by alkali-aided process are given in Table 4. According to the results alkaline extraction process had considerable effect on OHC and the proteins isolated at pH 12 had higher OHC than that extracted at pH 10 ($p<0.05$). Furthermore the results showed salting (0.5% NaCl) led to reduce the OHC of proteins substantially compared with samples without salt ($p<0.05$). Following the addition of salt up to 3%, the OHC significantly increased ($p<0.05$). The results revealed the 4.5% and 3% levels have no significant effect

on OHC. The highest OHC at two pHs was observed at 3%. Pórarinsdóttir (2010) reported with increasing salt concentrates, hydrophobic surface groups are exposure. By salt increasing extremely, the protein interactions become strongly and aggregation of proteins occur which leading to reduce the space between filaments (Thorarinsdottir *et al.*, 2004). It seems these interactions can effect on OHC. It is apparent that treated by alkaline and NaCl led to denaturation of muscle proteins and unfolding the proteins structure. Thereby the hydrophobic interactions increase which increase the OHC subsequently.

Table 4: Oil holding capacity of lantern fish protein isolated at pHs 10 and 12 at different concentrations of NaCl.

Sample	pH	NaCl (%)	OHC (mL g ⁻¹)
FPI	10	0	2.81±0.28 ^b
		0.5	2.19±0.27 ^c
		1.5	2.25±0.20 ^c
		3	2.91±0.03 ^{ab}
		4.5	2.81±0.12 ^b
FPI	12	0	3.22±0.16 ^a
		0.5	2.25±0.23 ^c
		1.5	2.29±0.13 ^c
		3	3.22±0.20 ^a
		4.5	2.97±0.18 ^{ab}

Data are expressed as mean ± S.D. (n=3). Different letters in each column are significantly different ($p<0.05$).

Emulsifying capacity

Table 5 represents the emulsifying capacity of fish protein isolate samples. As can be seen from the Table, the alkaline condition had significantly effect on emulsifying capacity and could be improved when the pH shift to extreme alkalinity so the proteins isolated at pH 12 had higher emulsifying properties than that treated at pH 10 ($p < 0.05$). The results also showed the addition of 0.5% NaCl led

to reduce slightly the emulsifying capacity of proteins isolated compared with isolates without NaCl then at 1.5% salt increase in emulsifying capacity was observed. At high salt concentrations (3% and 4.5%), reduce in the protein emulsifying capacity at two pHs was noted. The maximum and minimum emulsifying capacity at two pHs was observed respectively at 1.5% and 4.5% NaCl.

Table 5: Emulsification and foaming capacity of lantern fish protein isolated at pHs 10 and 12 at different concentrations of NaCl.

Fish protein isolate	NaCl (%)	Emulsifying capacity (mL g ⁻¹)	Foaming capacity (mL g ⁻¹)
pH 10	0	26.66±5.77 ^e	41.67±7.22 ^b
	0.5	23.33±5.77 ^{bcde}	58.33±7.22 ^a
	1.5	29.33±6.11 ^{cde}	70.83±7.22 ^a
	3	20.00±4.00 ^{bcde}	70.83±7.22 ^a
	4.5	18.66±4.61 ^{de}	70.83±7.22 ^a
pH 12	0	36.66±5.77 ^{ab}	58.33±7.22 ^a
	0.5	32.00±7.21 ^{bc}	66.67±7.22 ^a
	1.5	42.66±4.61 ^a	62.50±12.50 ^a
	3	30.66±6.11 ^{bcd}	66.67±14.43 ^a
	4.5	29.33±6.11 ^{bcde}	70.83±7.22 ^a

Data are expressed as mean ± S.D. (n=3). Different letters in each column are significantly different ($p < 0.05$).

The researchers showed the addition of salt had effected on hydrophobic interaction of proteins. When the salt concentration and alkaline pHs increase, the protein structure unfolded and denaturated that led to exposure hydrophobic groups and helped to the protein – oil binding (Pórarinsdóttir, 2010; Davenport and Kristinsson, 2011; Pires *et al.*, 2012). Reducing the protein emulsifying capacity at high levels of salt maybe caused by the strong protein binds and protein aggregations. Because the space of protein filaments became reduce and thereby reduced of proteins functionality (Thorarinsdottir *et al.*, 2004).

Foaming capacity

In Table 5 the foaming capacity of fish proteins isolated obtained from alkaline extraction at different NaCl concentrations are illustrated. The results revealed the alkaline had effective influence on foaming properties and the protein isolated at pH 12 (without NaCl) showed better foaming capacity than that isolated at pH 10. According to the results, foaming capacity of fish protein isolates at pH 10 substantially increased with adding 0.5% NaCl ($p < 0.05$). At high NaCl levels foaming capacity increased though no significant difference between proteins foaming capacity at different concentration of salt was observed. Furthermore, by increasing

salt percentages the foaming capacity improved and the maximum foaming capacity was observed at 4.5%. More researches have been showed at high pHs and high salt concentration protein denaturation occurred (Lauritzsen, 2004; Thawornchinsombut and park, 2006). Salting led to increase the surface activity due to increase in negative charges and repulsion of protein filaments. Thereby, adsorption of protein between water and air increased and led to improve the foaming capacity (Keever, 2001; Foh *et al.*, 2010).

Solubility

The solubility curves of the fish protein isolates by adding salt are shown in Figs. 1 and 2. The results illustrated the protein solubility increased significantly at extreme pHs either acidic or alkaline conditions and was

the least at the isoelectric point (pH 5). As can be seen, salting had substantially effect on protein solubility ($p < 0.05$). According to the curves the protein solubility increased significantly at extreme acidic and alkaline pHs then reduced around pH 4. An almost U-shape solubility profile was observed in fish protein isolates. There were no significant differences at acidic pHs (2 and 3) and alkaline pHs (10, 11 and 12) in solubility of proteins isolates at pH 10 and 0.5% NaCl. With increasing the salt concentration up to 1.5% solubility increased. Also 3% and 4.5% levels led to enhance the protein solubility in the pH range of 2 to 12 ($p < 0.05$). The similar solubility pattern was observed that isolate at pH 12. The highest solubility was considered at 4.5% NaCl for both kinds of fish protein isolates.

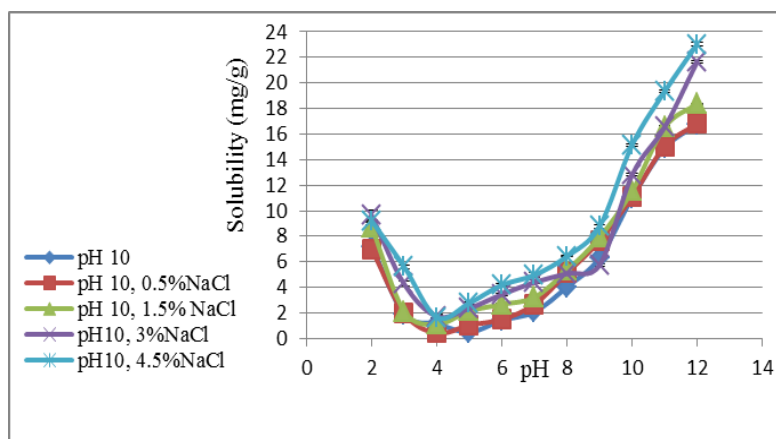


Figure 1: Solubility (mg g⁻¹) profile of lantern fish proteins isolated at pH 10 at different NaCl concentrations. Data are expressed as mean \pm S.D. (n=3).

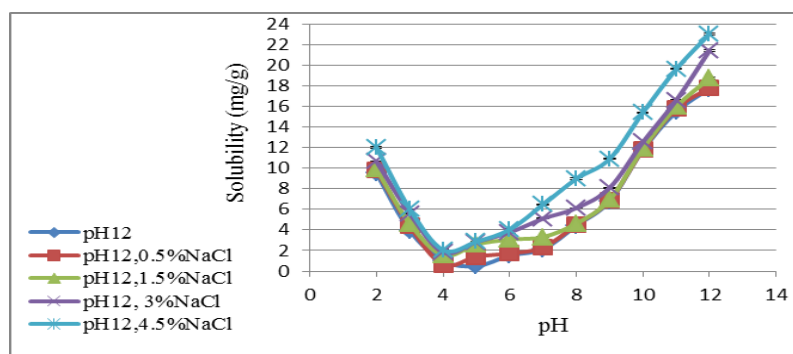


Figure 2: Solubility (mg g⁻¹) profile of lantern fish proteins isolated at pH 12 at different NaCl concentrations. Data are expressed as mean \pm S.D. (n=3).

At pHs higher or lower than isoelectric point, the solubility of the proteins increases because the proteins contain net negative or positive charges which provide more water binding sites and then around isoelectric point decrease as result in the negative and positive charges are (Tadpitchayangkoon, 2008; Palafox *et al.*, 2009). In the other research, Kim *et al.* (2005) described the lowest solubility at 0.1 M NaCl was observed around pH 4 and then slightly increased at pH 3. The researches of Leksrisonpong (2008) showed by adding NaCl, chloride ions selectively neutralize the positively charged regions of protein surface and effectively shift isoelectric pH to lower point. Because the negative charge of system was enhanced and should attract more H⁺ ion to reach the isoelectric point.

Color evaluation

The results of color evaluation of fish protein isolates are given in Table 6. According to the results the protein isolated at pH 12 had higher “L” value and lower “a” value than that isolated at pH 10. Also the results showed salting

had significantly effect on protein color properties. The color evaluation of protein isolated at different NaCl percentages in our study showed that with increasing the salt concentration, “L” value increased. Also there were no significant different between various salt concentrations (1.5%, 3% and 4.5%) at protein isolated at pH 10. The highest “L” value was observed at pH 12 and 3% NaCl. It seems salting help to remove the pigments. Removing the dark pigments from different parts of fish at alkaline conditions maybe led to increase the lightness of protein isolates (Rawdkuen *et al.*, 2008; Pires *et al.*, 2012). Furthermore “a” value (redness) was reduced by increasing the NaCl concentration and no significantly different was observed between 3% and 4.5% NaCl in protein isolated at pH 10. The lowest “a” value was observed at 3% salt at pH 10 that probably caused by reduction of amounts of hemoglobin. The redness is associated to some pigments such as hemoglobins. More researches showed extraction of fish protein by alkali- aided process could improve the color properties of protein isolated (Kristinsson *et al.*, 2005;

Kristinsson and Liang, 2006; Tahergorabi *et al.*, 2012). The evaluation of “b” value showed by increasing the NaCl concentration “b” value slightly increased though no significantly different at various levels of NaCl was observed. It seems during the protein isolation at alkaline pHs with adding salt, hemoglobin slightly denaturated. The de-naturated proteins have brownish. Also some myoglobin

proteins might denaturated at high pHs. This protein denaturated maybe coprecipitated with muscle proteins at 5.5 and led to increase the “b” value (Kristinsson *et al.*, 2005; Chaijan *et al.*, 2006). Also addition of salt maybe led to lipid and protein oxidation. Thereby produce brownish pigments which influence on production color properties (Ceballos, 2012).

Table 6: Color evaluation of lantern fish proteins isolated at pHs 10 and 12 at different NaCl concentrations.

Sample	NaCl (%)	L	a	b
pH 10	0	46.22±1.07 ^{bcd}	2.67±0.51 ^{bc}	34.22±1.64 ^{abc}
	0.5	37.22±1.02 ^e	7.11±0.77 ^a	32.56±1.84 ^c
	1.5	45.56±3.91 ^{cd}	4.22±1.71 ^b	35.00±1.86 ^{abc}
	3	47.44±3.10 ^{bc}	0.78±0.38 ^c	36.22±1.95 ^a
	4.5	46.56±0.96 ^{bcd}	1.89±1.26 ^c	36.00±1.00 ^a
pH 12	0	53.33±1.33 ^a	1.67±0.33 ^c	33.33±1.20 ^{bc}
	0.5	35.22±2.59 ^e	1.67±1.15 ^c	35.22±0.77 ^{ab}
	1.5	43.22±1.50 ^d	1.44±1.64 ^c	34.78±0.84 ^{abc}
	3	49.89±0.69 ^{ab}	1.78±0.19 ^c	34.67±0.67 ^{abc}
	4.5	48.33±1.45 ^{bc}	1.11±0.19 ^c	35.66±0.58 ^{ab}

Data are expressed as mean ± S.D. (n=3). Different letters in each column are significantly different ($P<0.05$).

The fish proteins isolated by alkali-aided processing have suitable functional properties and could be used as ingredients in value added products. This research showed addition of NaCl during extraction led to improve the functional properties. According to the results the WHC, OHC, emulsifying capacity and foaming of proteins isolated with salting were better than that isolated without salting. In addition, the solubility curves showed the protein solubility was dependent on pH and the solubility curves of protein isolated at pH 12 was higher than pH 10. Moreover, the protein solubility was enhanced substantially by increasing the salt concentration and the isoelectric

point reduced and shifted to acidic pH (4). Furthermore, the fish protein isolates had desirable color properties that probably cause by heme protein denaturation during alkaline extraction.

References

- AOAC. 2005.** Official methods of analysis. Edited by Horwitz, W. and Latimer, G.W. (18th ed.). Association of Official Analytical Chemists, Incorporated. Maryland, U.S.A. 1-7.
- Brenner, T., Johannsson, R. and Nicolai, T., 2009.** Characterisation and thermo- reversible gelation of cod muscle protein isolates. *Food Chemistry*, 115, 26–31.

- Catul, V., Gauns, M. and Karuppasamy, P.K., 2011.** A review on mesopelagic fishes belonging to family Myctophidae. *Reviews in Fish Biology and Fisheries*, 21, 339–354.
- Ceballos, M.J., 2012.** Effect of protein and lipid oxidation in the changes of color in salted and dried herring and klippfish. Master Thesis, Norwegian University of Science and Technology, Department of Biotechnology. 22-29.
- Chai, H.J., Wu, C.J., Yang, S.H., Li, T.L. and Pan, B.S., 2016.** Peptides from hydrolysate of lantern fish (*Benthoosema pterotum*) proved neuroprotective in vitro and in vivo. *Journal of Functional Foods*, 24, 438-449.
- Chaijan, M., Benjikul, S., Visessanguan, W. and Faustman, C., 2006.** Physicochemical properties, gel-forming ability and myoglobin content of sardine (*Sardinella gibbosa*) and mackerel (*Rastrelliger kanagurta*) surimi produced by conventional method and alkaline solubilisation process. *Journal European Food Research and Technology*, 222, 58–63.
- Chalamaiah, M., Rao, G.N., Rao, D.G. and Jyothirmayi, T., 2010.** Protein hydrolysates from meriga (*Cirrhinus mrigala*) egg and evaluation of their functional properties. *Food Chemistry*, 120(3), 652-657.
- Chang, T., Wang, C., Yang, H., Xiong, S., Liu, Y. and Liu, R., 2016.** Effects of the acid-and alkali-aided processes on bighead carp (*Aristichthys nobilis*) muscle proteins. *International Journal of Food Properties*, 19(8), 1863-1873.
- Chen, Y.C. and Jaczynski, J., 2007.** Protein recovery from rainbow trout (*Oncorhynchus mykiss*) processing by-products via isoelectric solubilization/precipitation and its gelation properties as affected by functional additives. *Journal of Agricultural and Food Chemistry*, 55(22), 9079–9088.
- Co'rdova Murueta, J.H., Navarrete del Toro, M.A. and Garc'ia Carren'õ, F., 2007.** Concentrates of fish protein from bycatch species produced by various drying processes. *Food Chemistry*, 100, 705–711.
- Davenport, M.P. and Kristinsson, H.G., 2011.** Channel catfish (*Ictalurus punctatus*) muscle protein isolate performance processed under different acid and alkali pH values. *Journal of Food Science*, 76, E240, 3.
- Diniz, F.M. and Martin, A.M., 1997.** Effects of the extent of enzymatic hydrolysis on the functional properties of shark protein hydrolysate. *LWT Food Science and Technology*, 30, 266–272.
- FAO. 2001.** Report of the: Trilateral Workshop on lanternfish in the Gulf of Oman. FAO Fisheries Report No. 665, FIIT/R665, ISSN 0429-9337.
- Foh, M.B.K., Wenshui, X., Amadou, I. and Jiang, Q., 2010.** Influence of pH shift on functional properties of protein isolated of Tilapia (*Oreochromis niloticus*) muscles and of soy protein isolate. *Food*

- Bioprocess Technology*, 5(6), 2192-2200.
- Hultin, H.O. and Kellehr, S.D., 2000.** High efficiency alkaline protein extraction. United States Patent, 6, 136,959. issued October 24
- Ingadottir, B., 2004.** The use of acid and alkali-aided protein solubilization and precipitation methods to produce functional protein ingredients from Tilapia. PhD diss., University of Florida.
- Karlsdottir, M.G., 2009.** Application of additives in chilled and frozen white fish fillets- Effects on chemical and physicochemical properties. Master Thesis in Food Science. Faculty of Food Science and Nutrition, School of Health Sciences, University of Iceland.
- Keever, B.D., 2011.** Salt concentration and species affects protein extractability and processed meat characteristics. MSc Thesis. Graduate College of the University of Illinois at Urbana-Champaign.
- Kim, Y.S., Yongsawatdigul, J., Park, J.W. and Thawornchinsombut, S., 2005.** Characteristics of sarcoplasmic proteins and their interaction with myofibrillar proteins. *Journal of Food Biochemistry*, 29, 517–532.
- Kristinsson, H.G., Theodore, A.E., Demir, N. and Ingadottir, B., 2005.** A comparative study between acid- and alkali-aided processing and surimi processing for the recovery of proteins from channel catfish muscle. *Journal of Food Science*, 70, C298– C306.
- Kristinsson, H.G. and Liang, T., 2006.** Effect of pH-shift processing and surimi processing on Atlantic croaker (*Micropogonias undulates*) muscle proteins. *Journal of Food Science*, 7, 298–306.
- Kumoro, A.C., Nurul Aini, S., Retnowati, D.S. and Sri Budiayati, C., 2010.** Effect of temperature and particle size on the Alkaline extraction of protein from chicken bone waste. *Reaktor*, 13(2), 124-130.
- Lauritzsen, K., 2004.** Quality of salted cod (*Gadus morhua* L.) as influenced by raw material and salt composition. PhD Thesis, Norwegian College of Fishery Science University of Tromsø.
- Leksrisonpong, Ph., 2008.** Effect of precipitating conditions on floc size, recovery yield and gelation properties of meat isolates prepared by pH-shifting. MSc Thesis. Graduate Faculty of North Carolina State University.
- Liang, Y. and Hultin, H.O., 2005.** Separation of muscle membrane from alkali-solubilized fish muscle proteins. *Journal of Agricultural and Food Chemistry*, 53(26), 10012-10017.
- Palafox, H., Co´rdova-Murueta, J.H., Navarrete del Toro, M.A. and Garcı´a-Carren˜o, F.L., 2009.** Protein isolates from jumbo squid (*Dosidicus gigas*) by pH-shift processing. *Process Biochemistry*, 44, 584-587.
- Pires, C., Costa, S., Batista, A.P., Nunes, M.C., Raymundo, A. and Batista, I., 2012.** Properties of protein powder prepared from Cape

- hake by-products. *Journal of Food Engineering*, 108, 268–275.
- Pórarinsdóttir, K.A., 2010.** The influence of salting procedures on the characteristics of heavy salted cod. PhD Thesis, Department of Food Technology, Engineering and Nutrition, Lund University, Sweden.
- Rawdkuen, S., Sai-Ut, S., Khamsorn, S., Chaijan, M. and Benjakul, S., 2008.** Biochemical and gelling properties of tilapia surimi and protein recovered using an acid-alkaline process. *Food Chemistry*, 112(1), 112-119.
- Rodrigues Freitas, I., Cortez-Vega, W.R. and Prentice, C., 2016.** Physicochemical and functional properties of protein recovered from fish waste. *Journal of Aquatic Food Product Technology*, 25(7), 1034-1044.
- Sathivel, S., Bechtel, P.J., Babbitt, J., Prinyawiwatkul, W., Negulescu, I. I. and Reppond, K.D., 2004.** Properties of protein powders from arrowtooth flounder (*Atheresthes stomias*) and herring (*Clupea harengus*) byproducts. *Journal of Agricultural and Food Chemistry*, 52(16), 5040-5046.
- Shahiri Tabarestani, H., Maghsoudlou, Y., Motamedzadegan, A., Sadeghi Mahoonak, A.R. and Rostamzad, H., 2012.** Study on some properties of acid-soluble collagens isolated from fish skin and bones of rainbow trout (*Onchorhynchus mykiss*). *International Food Research Journal*, 19(1), 251-257.
- Shaviklo, A.R., Thorkelsson, G. and Arason, S., 2012.** Quality changes of fresh and frozen protein solutions extracted from Atlantic Cod (*Gadus morhua*) trim as affected by salt, cryoprotectants and storage time. *Turkish Journal of Fisheries and Aquatic Sciences*, 12, 41-51.
- Sila, A. and Bougatef, A., 2016.** Antioxidant peptides from marine by-products: Isolation, identification and application in food systems. A review. *Journal of Functional Foods*, 21, 10-26.
- Souissi, N., Bougatef, A., Triki-Ellouz, Y. and Nasri, M., 2007.** Biochemical and functional properties of Sardinella (*Sardinella aurita*) by-product hydrolysates. *Food Technology and Biotechnology*, 45(2), 187–194.
- Tadpitchayangkoon, P., 2008.** Effect of pH and pH shift process on conformational changes and gelation of sarcoplasmic proteins from striped catfish (*Pangasius hypophthalmus*). PhD Thesis, University of Technology. Thailand.
- Tahergorabi, R., Beamer, S.K., Matak, K.E. and Jaczynski, J., 2012.** Functional food products made from fish protein isolate recovered with isoelectric solubilization/precipitation. *LWT - Food Science and Technology*, 48, 89-95.
- Thawornchinsombut, S. and Park, J.W., 2005.** Role of ionic strength in biochemical properties of soluble fish proteins isolated from cryoprotected Pacific whiting

mince. *Journal of Food Biochemistry*, 29(2), 132-151.

Thawornchinsombut, S. and Park, J.W., 2006. Effect of NaCl on gelation characteristics of acid and alkali treated Pacific Whiting fish protein isolates. *Journal of Food Biochemistry*, 31, 427-455.

Thawornchinsombut, S. and Park, J.W., 2007. Effect of NaCl on gelation characteristics of acid-and alkali-treated pacific whiting fish protein isolates. *Journal of Food Biochemistry*, 31(4), 427-455.

Thorarinsdottir, K.A., Arason, S., Bogason, S.G. and Kristbergsson, K., 2004. The effects of various salt concentrations during brine curing of cod (*Gadus morhua*). *International Journal of Food Science and Technology*, 39, 79-89.

Valinassab, T., Pierce, G.J. and Johannesson, K., 2007. Lanternfish (*Benthoosema pterotum*) resources as a target for commercial exploitation in the Oman Sea. *Journal of Application Ichthyology*, 23, 573-577.

Zayas, J.F., 1997. Functionality of proteins in food. Berlin: Springer Science and Business Media. pp. 79-82.