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Recovery of protein from Threadfin Bream (*Nemipterus japonicus*) Surimi leached water using pH shifting technique

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Abstract

The pH-shift method is a recent process used to isolate proteins from animal muscle with good functional properties. It can be used to recover proteins from cheap, underutilized sources and/or process byproducts. Large amount of leached water containing dissolved protein and fat is drained out as effluent of Surimi industry. An attempt was made to recover dissolved proteins by pH shifting method and assess its nutritional composition and storage stability for its better utilization. The proteins recovered from Surimi leached water were found to contain 63.93 ± 0.07% % protein, 1.62 ± 0.19 % fat, 24.43 ± 0.35 % ash, and 8.87 ± 0.14 % moisture. Solubility, water holding capacity **(**WHC**)** and fat binding capacity **(**FBC) were also assessed to find its suitability as food ingredient. Recovered samples were quite stable for a period of three months without any significant sign of deterioration. pH shifting methods were found to be effective in recovering dissolved materials in leached water. A very high protein (63.93 to 60.40%) and mineral rich (24.43 to 26.35 %) fine powder of good functional properties was recovered from large amounts of leached water drained out by Surimi industry. The results of this work clearly show that the process of alkaline solubilization can successfully be employed to recover proteins with good functional properties from Surimi leached water.

Keywords: Threadfin Bream, Surimi, pH shifting and protein recovery

Introduction

Washing is one of the most critical steps in surimi manufacturing. Once the raw fish flesh has been obtained, cyclic washings are applied to remove sarcoplasmic proteins (enzymes and heme proteins) fat, and other nitrogenous compounds from the minced fish flesh. Increased water usage for washing usually results in more protein loss and increased wastewater disposal (Hazen *et al*., 1988). The protein lost into wastewater by leaching accounts for 15 to 30% of the total protein of minced meat (Okazaki, 1994), which is discharged as effluent. It was estimated that approximately 40-50% of total proteins are lost during washing (Yang & Froning, 1992 and Macdonald et al., 1996). It has been reported that surimi wash-water typically contains 0.5–2.3% protein (Morrissey *et al.,* 2000). Some of the soluble

solids, lost in wash water, could be highly functional myofibrillar proteins (Hazen *et al.*, 1988; Lin & Park, 1996 and Huidobro *et al.*, 1998) and also water soluble vitamins B and minerals which are important nutrients lost during washing (Sen, 2005). The protein removed in the process is an edible protein (Niki *et al.*, 1985). Attempt has been made in recent past to recover protein from surimi waste water by several researchers (Mohammad *et al.*, 2002 and Stine *et al.*, 2011) using different technology. A new pH shifting method has been developed at the University of Massachusetts (Hultin & Kelleher, 1999). Effluents from seafood processing plants have become a crucial issue due to the presence of a large amount of organic matters and major sources of coastal environmental pollution (Sagar & Naikwade, 2012).

Methods for recovery of washed water proteins include heat, complexing agents, electro-coagulation (Hasegawa *et al.*, 1982), membrane filtration (Green *et al.*, 1984) and air flotation (Beck *et al.*, 1974). Attempts were made by many to recover protein from surimi waste water (Niki *et al.*, 1985 and Mohammad *et al.*, 2002) using different technologies. pH shifting is one of methods used in food processing industry for recovery of ingredients from aqueous extract. The aim of this study was to recover protein from leached water generated in surimi industry, pH shifting technique to assess the quality of recovered protein.

Materials and methods

Fresh threadfin bream (*Nemipterus japonicus*) was collected from Veraval, fishing harbor and transported in chilled condition to the laboratory. Fish was dressed and meat was collected. The meat was washed repeatedly for three times with chilled (6 to 8 ºC) water each cycle of 10 min. Leaching ratio of water and fish was 3:1. Leached water was taken in glass beaker and then sufficient quantity of 1N NaOH was added to adjusted pH of leached water to 11-12 where precipitation occurs. The precipitated sample was washed to reduce its pH and centrifuge for 4000 rpm to separate precipitated protein. After discarding supernatant solid settled at bottom of centrifuge tube was collected which was dried in hot air oven temperature maintained at 40ºC. The pH shifting method was followed by (Hultin & Kelleher, 2000) in acid/alkaline solubilization process.

Powder collected was stored at room temperature in a pet jar for storage study for analysis of proximate composition, color, Trimethylamine (TMA), Total Volatile Base Nitrogen (TVB-N), Total Plate Count (TPC) and functional properties. Storage study of 90 days was carried out by analyzing the physical, chemical and microbiological parameters at 15 days interval of time. Proximate composition was analyzed by following AOAC (2006). TMA, TVB-N of protein powder were analyzed by adopting the method described by Beatty & Gibbons (1937).

Protein Solubility:

An amount of 0.5 g of powder (W1) was placed in a 100 ml glass beaker and added with 5 ml distilled water. The powder was gently mixed by a spatula for 1 min or until no more fine particles was seen. The solution was then filtered through a whatman filter paper No.4 that was known for its weight (W2). The filter paper was dried at 100°C for 4 h in a hot air oven, cooled in a desiccator and weighed again (W3). The solubility of powder was calculated by the procedure of Kahtani & Hassan (1990) as:

$$\% \text{ Solubility} = 100 - \frac{\{[W3 - W2] \times 100\}}{W1}$$

Fat Binding Capacity:

The fat binding capacity of the powder samples were determined by placing 200 mg

of each sample into a 15-ml centrifuge tube and adding 10 ml of soya bean oil. The sample was thoroughly mixed with a small steel spatula, kept for 30 min with intermittent mixing every 10 min, and then centrifuged at 3000 rpm for 20 min. Free oil was then decanted and the fat adsorption capacity was expressed as milliliters of fat adsorbed by 1 g of protein in sample (Sathivel et al., 2005).

Water Holding Capacity:

One gram of powder was added to 10 ml distilled water in a 15-ml centrifuge tube. Tubes were centrifuged at 3000 rpm for 15 min. The supernatant was poured through a funnel into a calibrated beaker. The volume of supernatant was subtracted from the original 15-ml. The results were reported in terms of ml of water held by 1g of protein powder (Miller & Groninger, 1976).

Colour:

Colour measurement was made using a colorimeter (CR-10 Konica Minolta sensing, ING japan). Color values were expressed using the International Commission on Illumination '*L*' (lightness), '*a*' (+a is red, -a is green) and '*b*' (+b is yellow, -b is blue) (CIE, 1978).

Statistical analysis:

Data were analyzed statistically as per factorial complete randomized design. Analysis of variance was worked out using standard statistical procedures as described by Snedecor & Cochran (1967).

Results and discussion

Proximate composition:

The proximate composition of protein powder was recorded as 8.87 ± 0.14% moisture, 63.92 ± 0.07% protein, 1.62 ± 0.19% fat, and 24.43 ± 0.35% ash (Table 1). Rahman *et al.*, (2012) has reported the moisture content of 7.89% in dried fish powder. Jeyasanta *et al.*, (2013) also reported that edible fish powder contained protein 55.6%. Ash content 4.8–17.7% was reported by Sathivel *et al.*, (2004) for protein powders from herring and arrow tooth flounder tissues. Proximate composition of recovered protein power from surimi leached water 6.72% moisture, 75.55% protein, 1.03 % fat and 13.04 % ash reported by Mulye *et al.*, (2015). Protein and lipid content was 68.2% and 2.6% in alkaline produced protein isolate with centrifugation in Sardine by pH shifting method. Reported protein recovery for whole carp was 49 to 66% (Taskaya *et al.*, 2009), 59 to 63% for Cape hake by-products (Ireneu *et al.*, 2006), 57-59% from gutted herring (Marmon & Undeland, 2010), 45-50% from Antarctic krill (Chen *et al.*, 2009) and 33% from shrimp processing waste (head and body shell) (Khumallambam *et al.*, 2011). Gradual increase in the moisture content and decrease in protein content noted during storage period was also reported by Jeyasanta *et al.*, (2013) in edible fish powder from trash fishes in which 55.6% protein initially which decreased to 48.72% in 5^{th} month of storage period. The ash content of species is an indication of the mineral concentration in the organisms (Agoes & Hamami, 2007). Ash content of 4.8–17.7% was reported by Sathivel et al. (2004) in protein powders from herring and arrow tooth flounder tissues. Proximate composition of recovered protein remained more or less stable during 90 days period of storage.

Table 1: Changes in proximate composition of protein powder during storage period.

Parameters (%)	Storage period (days)						
	0	**15**	**30**	**45**	**60**	**75**	**90**
Moisture	08.87± 0.14	09.65± 0.33	09.83± 0.15	10.06± 0.06	10.83± 0.12	11.23± 0.34	12.17± 0.13
Protein	63.92± 0.07	63.48± 0.22	63.15± 0.08	62.89± 0.09	62.39± 0.27	61.50± 0.29	60.40± 0.32
Fat	01.62± 0.19	01.56± 0.06	01.35± 0.05	01.12± 0.07	00.90± 0.05	00.81± 0.09	00.64± 0.06
Ash	24.43± 0.35	24.58± 0.30	24.92± 0.05	25.16± 0.12	25.22± 0.15	25.88± 0.16	26.35± 0.19

Results are mean ± SD of four samples.

Table 2: Change in functional properties of protein powder during storage period.

Storage day	Solubility (%) (mean ± S.D)	FBC (ml/g) (mean ± S.D)	WHC (ml/gm) (mean ± S.D)
0	36.24 ± 0.04	7.07 ± 0.19	10.51 ± 0.36
15	36.09 ± 0.06	6.46 ± 0.05	10.27 ± 0.30
30	35.73 ± 0.30	6.11 ± 0.10	9.71 ± 0.10
45	35.54 ± 0.57	5.89 ± 0.10	9.41 ± 0.14
60	33.76 ± 0.09	5.46 ± 0.15	9.24 ± 0.18
75	31.89 ± 0.40	5.01 ± 0.12	8.17 ± 0.07
90	30.86 ± 0.18	4.67 ± 0.12	7.76 ± 0.16

Results are mean ± SD of four samples.

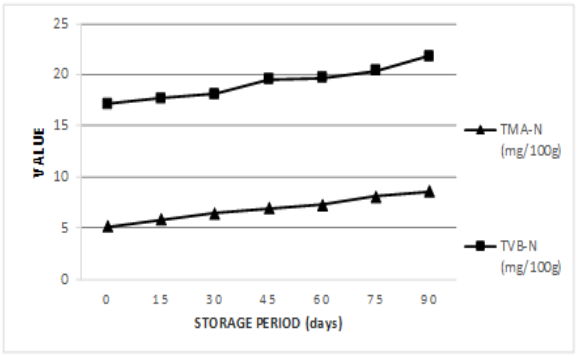

Fig. 1: Change in TMA-N and TVB-N value of protein powder during storage period.

TMA-N and TVB-N significantly changed ($p<0.05$) from 5.12 ± 0.05 to 8.56 ± 0.26 mg/100g and 17.12 ± 0.15 to 21.81 ± 0.19 mg/100g respectively during 90 days of storage period (Figure 1), which is in the limit of acceptability (Mukundan & Balasubramanian, 2011). TVB-N values increased with the storage period but never crossed the limit of acceptability of 35-40 mg/100g as suggested by Lakshmanan (2000). Similar trend of increase in TMA-N content during storage was reported by Chacko *et al.*, (2005) in soup powder of squid during storage in laminated packaging. Even after 90 days of storage TVB-N did not cross the critical limit of 35-40 (mg/100g) which indicates the stability and quality of stored samples.

The colour parameters were viz., *L* (lightness) 78.23 ± 0.04, *a* (redness/greenness) 0.04 ± 0.05 and *b* (yellowness/blueness) 16.86 ± 0.06 (Figure 2). However, both *L* and *a* were significantly changed ($p<0.05$) while b was not significantly changed ($p>0.05$) by the storage periods. *L* value in 68.20 in found in leached mince of *Clupeonella cultiventriscaspia* reported by Rahmanifarah et al. (2014) by pH shifting method. Huda et

al. (2001) reported '*L*' 85.59, *'a'* 0.30 and *'b'* 16.38 for lizardfish surimi powder. No notable changes in colour were noted during period of storage.

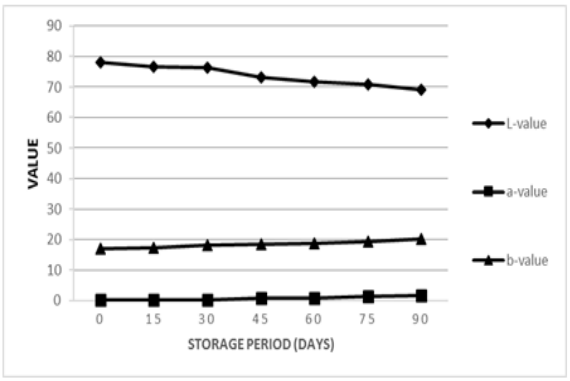

Fig. 2: Change in '*L*', *'a'* and '*b*' value of protein powder during storage period.

Functional properties:

The recovered protein from surimi leach water had mean solubility and mean WHC of 36.24 ± 0.04 and 10.51 ± 0.36 ml/g (Table.2). Solubility of 30.21 found in leached mincemeat reported by Rahmanifarah et al. (2014) in pH shifting method. FBC 7.07 ± 0.19 ml/g from this study was similar to finding of Kristinsson & Rasco (2000) in Atlantic salmon protein hydrolysates ranging from 2.86 to 7.07 ml/g also Mulye *et al.* (2015) show that solubility and WHC decrease with increase of storage period. The mechanism of fat binding capacity is thought to be mainly because of the physical entrapment of the oil (Sathivel & Bechtel, 2008).

Conclusion

The investigation indicated that three litters of water were used during leaching process of one kg of threadfin bream surimi production. Leached water was contained 4.25g solid. Recovered solid in the form of creamy white powder by pH shifting method contains 63.92% protein. The rest being minerals and fat content. Fine recovered protein powder being rich in minerals and also good in functional properties like solubility, WHC and FBC makes it an ideal functional food ingredient. Considering the enormous amount of leached water generated during surimi production, an appreciable amount of functional protein and mineral rich food ingredients can be recovered for better utilization as food rather than waste. Finally, recovered fine powder from leached water, by pH shifting method from leached water was a nutritionally rich in protein, fat and minerals as were as stable to be exploited as functional ingredients for foods and feed formulation.

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