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Utilization of Tiger Tooth Croaker (*Otholithus ruber*) Fish Meat for Development of Fish Protein Isolate using pH Shifting Method

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ABSTRACT

Development of Fish Protein Isolate (FPI) from tiger tooth croaker (*Otholithus ruber*) fish meat using pH shift method was carried during this study. Tiger tooth croaker was used as raw material because of their abundance and comparatively low price. During the study, physical characteristics and proximate composition of the fresh fish were analyzed. The average length of fish was 19.95 cm and weighed 94.6 g. respectively. The proximate composition of raw material was 17.75% protein, 78.02% moisture, 2.39% total lipid and 1.37% ash content respectively. FPI treated at different pH treatments (2.5, 4, 7, 11.5 and 12.5) were analyzed for proximate composition, physico-chemical, functional and sensory characteristics. The total protein content was specifically high for pH 7 followed by pH 12.5, 11.5, 4 and 2.5. The functional properties exhibit high value for all the samples of fish protein isolates. Low lipid oxidation of FPI prepared through the pH-shift process imitates their functional characteristics. The alkali-aided method was found to be more effective for best physico-chemical and functional properties than acid-aided method.

Keywords Fish Protein Isolate, Tiger tooth croaker, pH-shift method, *Otholithus ruber*.

INTRODUCTION

As a source of animal protein, humans are highly dependent on seafood. Fishery by-products have received much consideration as an important protein source because of utilizing animal protein as a functional food ingredient (Chalamaiah *et al*. 2012). Generally, protein providing energy in terms of calories is not used but its contribution to protein synthesis is in highly importance and it plays crucial roles in normal development and maintenance. The sensory and physico-chemical characteristics of any protein-rich food contribute to the overall structural behavior of the food (Foh *et al*. 2012). Sources of dietary protein can be categorized into functional health promoting foods based on their biological characteristics (Kadam and Prabhasankar 2010).

Isolates are the most refined form of protein products containing the greatest concentration of

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protein and concentration contains no dietary fiber. They are very digestible and easily incorporated into different food products. Fish protein isolate is a protein concentrate which is prepared from fish muscle without retaining the original shape of the muscle. It is not generally consumed directly but used as raw material for production of other value added products. Humans are highly dependent on seafood as a source of animal protein. Fishery by-products, which are in huge supply, have received much consideration as a vital protein source as growing interest has been paid to utilizing animal protein as a functional food ingredient (Chalamaiah *et al*. 2012).

The pH-shift processing also called as the acid and/or alkaline solubilization followed by isoelectric precipitation (Hultin and Kelleher 2001), has been successfully recognized as a promising technique to recover direct protein from unconventional complex aquatic raw materials, including gutted fish (Taskaya *et al*. 2009, Marmon and Undeland 2010) and seafood processing by-products (Chen and Jaczynski 2007, Shaviklo 2012). This process involves selectively isolation of proteins from homogenized raw material using a high (> 10.5) or a low (< 3.5) pH to solubilize the muscle proteins followed by centrifugation to separate the solubilized proteins from high and low density undissolved material. Then, the recovery of solubilized proteins is done using isoelectric precipitation (usually pH 5.5) and dewatered by centrifugation or filtration. The recovered protein isolate can be mixed with cryoprotectants and then frozen like surimi or minced fish or might be directly dried into a fish protein powder (FPP) for further utilization.

Otolithes ruber commonly known as the tigertooth croaker, is a fish native to the Indian and Western Pacific Oceans and the Bay of Bengal. It belongs to family *Sciaenidae* of order Perciformes. In India, It constitutes 10–12% of the demersal catch and found in the both east and west coast throughout the year. It is a well-known edible marine fish. Croaker, being a *Carnivorous* species, its diet comprises a wide range of animals, such as crustaceans, polychaetes, mollusks, and small fish. In India, for surimi production, croaker is also one of the major raw materials. Croakers alone contributed 1.36 lakh tons during 2018-19 marine fish landing. For realizing the conversion of low-value processing discards into high-value byproducts, chemical characterization of croaker discards is important. At present, the croaker processing discards are mainly used for the production of fish manure, fish meal, and fish silage. The croaker discards can be used for the recovery of bioactive molecules that are utilized in food, healthcare, pharmaceutical, and nutraceuticals industries for improving the economic value of these processing discards as they are one of the important bio resources.

In this study, the alkali solubilization and precipitation technique to isolate proteins from tigertooth croaker (*Otolithes ruber*) were used. The proximate composition, physico-chemical, functional and sensorial properties of the protein isolates were also evaluated.

MATERIALS AND METHODS

Materials

Tiger tooth croaker (*Otolithes ruber*) fish was purchased from the Veraval fish landing center and transported in iced condition with the temperature range of 0 to 2°C to fish processing laboratory of College of Fisheries Science, Veraval. It was washed thoroughly in potable chilled water to remove all adhering matters. Proximate analysis was carried out for the raw material. All chemicals and reagents were of analytical grade and were obtained of Central Drug House (CDH) limited - New Delhi, Ranbaxy laboratories limited - SAS Nagar, Astron chemical (INDIA), Rankem - New Delhi, Chemdyes Corporation or Baroda chemical industries (Baroda) limited.

Preparation of fish protein isolates

The extraction of FPIs was done by the method adopted by Hultin and Herbert (2005). Briefly, the fish fillets were grind to mince in mixer grinder and homogenized with ice-cold deionized water (1:9 ratio) for 3 mins. The pH of the suspension was adjusted to pH 2.5 using 1M HCL, pH 4 using 0.5 N 4C HCL, pH 7 using 0.5 N 4C HCL/NaOH, pH 11.5 using 1N NaOH and pH 12.5 using 1M NaOH. The homogenate was centrifuged at 8000 × g for 20

mins at 4°C. After centrifugation, three layers were produced; the upper layer and lower layer consist of lipid content and insoluble protein. The middle layer of the supernatant (soluble proteins) was filtered to remove neutral lipids and solid materials, particularly skin, bone and connective tissue. Subsequently, the filtrate pH was adjusted to 5.5 and the filtrate was again centrifuged at 8000 × g for 15 mins at 4°C. After centrifugation, the obtained supernatant was removed and the precipitate was neutralized, completely dried in Hot air oven at 60°C for 24 hrs, the product was then grinded in powder form, packed and stored at ambient temperature until analysis. The samples were named as protein isolate at pH 2.5 (T1), pH 4 (T2), pH 7 (T3), pH 11.5 (T4) and pH 12.5 (T5).

Proximate composition

The Proximate composition such as moisture, protein, lipid and ash contents of FPIs, was analyzed using standard AOAC methods (AOAC 2006).

Physico-chemical characteristics

Bulk density

The bulk density of FPIs was analyzed following the method of Joshi *et al*. (2011). Bulk density was analyzed by recording the volume occupied by FPIs in a pre-weighed 10 ml graduated cylinder up to the 10 ml mark. During FPIs filling, the cylinder was tapped 20 times and was weighed again and the bulk density of FPIs is expressed as kg/m^3.

pH

The pH of FPIs were analyzed 10 g. of the samples was weighed and mixed with 50 ml of deionized water; the mixture was stirred well for 5 mins and the suspension pH was measured using a digital pH meter.

Color analysis

Color analysis was done by using a colorimeter (CR-10, Konica Minolta Sensing, Inc., made in Japan), the color of FPIs were analyzed from three dimension: *L**, *a** and *b**. The chroma (C*) and hue angle (H°) values of FPIs were determined using the following formulas: C*= (*a**2 + *b**2)1/2 and H° = tan-1 (*b**/*a**), respectively.

Functional characteristics

Water-holding capacity (WHC)

The water-holding capacity (WHC) of FPIs were analyzed following the procedure of Ozyurt *et al*. (2015). 2 g of the sample was dispersed in 20 ml of deionized water, stirred for 20 mins at 30°C and centrifuged at 3000 × g for 15 mins The WHC is expressed as ml of water absorbed/g of sample.

Oil-holding capacity (OHC)

The oil-holding capacity (OHC) of FPIs were analyzed following the procedure described by Ozyurt *et al*. (2015). 1 g of the sample was dispersed in 10 ml of vegetable oil, stirred well for 5 mins and centrifuged at 3000 × g for 15 mins. The OHC was displayed as the weight difference.

Emulsifying capacity (EC)

The emulsifying capacity (EC) of FPIs were determined according to the procedure of Ozyurt *et al*. (2015). 0.5 g of the sample was added to 50 ml of 0.1 M NaCl and was stirred well and 10 ml of vegetable oil was added. The suspension was homogenized for 5 min, centrifuged at 5000 × g for 10 mins and then poured into a 50 ml graduated measuring cylinder and allowed to stand for few mins until the emulsified layer was stable. The EC was calculated as EC (ml/100g) = (Height of emulsifier layer/Height of total volume)* 100.

Foam measurements

Foaming capacity (FC) and foam stability (FS) of FPIs were analyzed according to the method of Foh *et al*. (2012). 1 g of FPIs was added to 50 ml of distilled water in a 100 ml graduated cylinder. The mixture was stirred for 3 mins and the generated foam volume was noted and was considered as FC. Furthermore, the foam volume noted after 15, 20 and 30 mins was considered the percentage of FS.

Lipid oxidation

The peroxide value (PV) of lipid was determined from

the lipid extract according to Jacobs (1958) iodomatrically. 10 g of sample was taken and ground well with 15 g anhydrous sodium sulfate. Then transferred to a 100 ml stoppered flask and 30-40 ml chloroform was added and placed in dark place for about 15-20 mins with occasionally shaking. 10 ml of chloroform extract and 25 ml of solvent (2 volume of glacial acetic acid and 35 ml of water) were added. The liberated iodine was titrated against standard sodium thiosulfate solution and explained as milliequivalent of peroxide/ kg of lipid.

Sensory quality

The FPIs were evaluated for freshness using descriptive scoring for appearance, texture and odour. The overall acceptance of FPIs were also assessed. The mean score was calculated for each attribute.

Data analysis

Data was statistically analyzed as per factorial Completely Randomized Design. Analysis of variance was used to find out significant difference in sample between the treatments as per the standard statistical methods described by Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

Characteristics of raw materials

Physical characteristics and proximate composition of fresh fish is shown in Table 1. The fresh fish measured 19.95 ± 0.86 cm on an average. The standard length of fish was 17 ± 0.74 cm whereas, mean weight of fish was 94.6 ± 7.22 g. Similar range of length and weight of tiger tooth croaker (*Otolithes ruber*) was recorded by Vijayakumar *et al*. (2016). The yield of picked meat was 34% from whole fish.

The fish fillets were used for proximate composition analysis; moisture content was about 78.02 ± 1.21 %, protein content 17.75 ± 0.61 %, lipid content 2.39 ± 0.06 % and ash content was 1.37 ± 0.08 % respectively. The results of the proximate composition compares well with the results obtained by Zynudheen (2010). The fish meat had protein content 17.36 %, lipid 4.74 %, moisture 77.28 % and ash content were found to be 1.14 % respectively.

Table 1. Characteristics of raw material.

A.	Physical characteristics	Mean ± SD
1	Total length(cm)	19.95 ± 0.86
2	Standard length (cm)	17 ± 0.74
3	Weight of fish (g)	94.6 ± 7.22
4	Yield of picked meat(from whole fish)	34%
B.	Proximate composition	
1	Moisture (%)	78.02 ± 1.21
2	Total protein (%)	17.75 ± 0.61
3	Total lipid (%)	2.39 ± 0.06
4	Total ash (%)	1.37 ± 0.08

Characteristics of fish protein isolates

Proximate composition

The proximate compositions such as moisture, protein, ash and lipid contents were found to significantly differ among FPIs isolated at different pH treatment in the present study ($p< 0.05$) is given in Table 2. At different pH treatment moisture content was 3.17 ± 0.18 % (T1) 3.18 ± 0.21 % (T2) 3.18 ± 0.17 % (T3) 3.02 ± 0.36 % (T4) and 3.15 ± 0.19 % (T5). pH-shift method resulted in 87.42 ± 0.74 % protein content in T3 followed by 84.00 ± 0.95 % in T5, 83.15 ± 0.82 % in T4, 82.30 ± 2.20 % in T2 and 80.90 ± 1.95 % in T1. The lipid content of T1 (2.45 ± 0.12 %) was higher followed by T2 (2.37 ± 0.14 %), T4 (2.16 ± 0.18 %), T5 (2.14 ± 0.19 %) and T3 (2.07 ± 0.11 %). The ash content of T2 (3.59 ± 0.14 %) was the highest, followed by T1 (3.57 ± 0.13 %), T4 (3.52 ± 0.06 %), T5 (3.50 ± 0.07 %) and the lowest value was noted for T3 (3.45 ± 0.11 %).

Physico-chemical properties

Bulk density

Bulk density is commonly used to analyze the sample mass, handling requisite and types of packaging materials suitable for the storage and transportation of food materials (Kumarakuru *et al*. 2018). As shown in Table 3, bulk density of T1, T2, T3, T4 and T5

Table 2. Proximate composition of fish protein isolates. T1: pH 2.5, T2: pH 4, T3(C): pH 7, T4: pH 11.5, T5: pH 12.5.

Proximate Composition (%)	T1	T2	T3 (C)	T4	T5
Moisture (%)	3.17±0.18	3.18±0.21	3.18±0.17	3.02±0.36	3.15±0.19
Protein (%)	80.90±1.95	82.30±2.20	87.42±0.74	83.15±0.82	84.00±0.95
Lipid (%)	2.45±0.12	2.37±0.14	2.07±0.11	2.16±0.18	2.14±0.19
Ash (%)	3.57±0.13	3.59±0.14	3.45±0.11	3.52±0.06	3.50±0.07

were 0.59 ± 0.05 mL^{-1}, 0.55 ± 0.04 mL^{-1}, 0.40 ± 0.06 mL^{-1}, 0.57 ± 0.03 mL^{-1} and 0.55 ± 0.05 mL^{-1} respectively and found to differ significantly ($p< 0.05$). The difference in the bulk density of protein isolates is possibly due to the structure of proteins. High bulk density is unfavorable for the formulation of weaning foods, where low bulk density is required (Lone *et al*. 2015). The results are in agreement with the work done by Foh *et al*. (2010) who studied bulk density of FMMC of tilapia fish and Lone *et al*. (2015) who studied bulk density of RTFPI.

pH

The pH changes can be used as a spoilage indicator in fishery products. The pH values of FPIs play a significant role in determining their shelf life and foaming and emulsification properties. The pH of FPIs is shown in Table 3. FPIs has the acidic pH values as pH value were 5.67 ± 0.18, 5.75 ± 0.08, 6.06 ± 0.13, 5.72 ± 0.09 and 5.77 ± 0.04 for T1, T2, T3, T4 and T5 respectively. Furthermore, T3 had the highest pH, and the lowest pH was observed for T1. Kumarakuru *et al*. (2018) reported the similar trends of pH value in FPIC, FPIIM, FPIP and FPIS was 5.70, 5.52, 5.51 and 5.65 respectively.

Color analysis

Color parameters (*L**, *a** and *b**) of FPIs from tiger tooth croaker is presented in Table 3. The *L** value indicates whiteness, *a** value indicates redness and *b** value indicates yellowness. As shown in Table 3, T2 had the highest *L** value (74.8 ± 2.89), followed by T1 (72.6 ± 2.13), T3 (71.3 ± 2.08) and T5 (70.9 ± 1.92). The lowest *L** value was noted for T4 (69.9 ± 1.80). The *a** values of T1, T2, T3, T4 and T5 were up to 10.7 ± 0.51, 11.1 ± 0.33, 10.7 ± 0.45, 10.5 ± 0.25 and 10.4 ± 0.31, respectively, with a not significant difference ($p< 0.05$). Furthermore, not significant differences were noted in *b** values among FPIs and the values ranged between 16.70 ± 0.64 and 19.10 ± 0.71. Similar results were reported by Shaviklo (2008), Abdollahi and Undeland (2019), Panpipat and Chaijan (2016) and Kumarakuru *et al*. (2018).

Peroxide value

Lipid oxidation in muscle foods is predominantly detrimental to overall quality and storage stability respectively. Peroxide value (PV) is used to express the oxidative state of lipid-containing foods. It measures the first stage of oxidative rancidity (Balachan-

Table 3. Physico-chemical properties and peroxide value of fish protein isolates.

Parameters	Treatments				
	T1	T2	T3	T4	T5
Bulk density (kg/m^3)	0.59±0.05	0.55±0.04	0.40±0.06	0.57±0.03	0.55±0.05
pH	5.67±0.18	5.75±0.08	6.06±0.13	5.72±0.09	5.77±0.04
Color parameters					
*L**	72.6±2.13	74.8±2.89	71.3±2.08	69.9±1.80	70.9±1.92
*a**	10.7±0.51	11.1±0.33	10.7±0.45	10.5±0.25	10.4±0.31
*b**	16.70±0.64	17.40±0.76	16.70±0.45	18.10±1.08	19.10±0.71
Peroxide value (meq/kg)	3.63±0.18	3.54±0.16	3.41±0.12	3.48±0.14	3.51±0.19

dran 2001). The effect of different pH on PV of fish protein isolates is depicted in Table 3. The PV value were found to be 5.63 ± 0.18 (meq/kg), 3.54 ± 0.16 (meq/kg), 3.41 ± 0.12 (meq/kg), 3.48 ± 0.14 (meq/kg) and 3.51 ± 0.19 (meq/kg) at T1, T2, T3, T4 and T5 respectively. Lowest value was recorded for T3 sample followed by T4, T5, T2 and T1. Alkali-aided method showed lower PV value as compared to acid-aided method and the lowest PV value was at pH 7. The results are in agreement with the work done by Panpipat and Chaijan (2016).

Functional properties

Water- and oil-holding capacity

The WHC of T1, T2, T3, T4 and T5 is shown in Table 4. From the findings, WHC values of all FPIs ranged between 2.18 ± 0.02 and 2.46 ± 0.11 mL/g, with a significant difference ($p<0.05$). T5 had the highest WHC value and the lowest value was observed for T1. Similar observations were made by Foh *et al*. (2012) while studying FMMC of tilapia fish. Generally, the binding capacity between food materials and water molecules plays a major role in food systems because it improves mouthfeel, flavor retention and texture.

Furthermore, the OHC determines the capacity of food materials to absorb oil. As shown in Table 4, T1, T2, T3, T4 and T5 had varying OHC values of 1.43 ± 0.08 mL/g, 1.54 ± 0.01 mL/g, 2.11 ± 0.02 mL/g, 2.42 ± 0.12 mL/g and 2.48 ± 0.09 mL/g respectively with a significant difference ($p<0.05$). Similar results were also reported by Kumarakuru *et al*. (2018), Elsohaimy *et al*. (2015), Foh *et al*. (2012) with OHC range of 5.32 – 5.83 mL/g, 1.88 mL/g, 2.43 mL/g and 3.38 mL/g respectively.

Emulsifying capacity

The EC reveals the capacity of a sample to swiftly adsorb at oil/water interfaces during the formation of an emulsion by avoiding flocculation and coalescence. The EC of T1, T2, T3, T4 and T5 was up to 76.2 ± 0.12 mL/100 g, 77.5 ± 0.09 mL/100 g, 78.1 ± 0.12 mL/100 g, 81.0 ± 0.11 mL/100g and 81.6 ± 0.08 mL/100 g, respectively (Table 4), with a significant difference ($p<0.05$). Gulzar *et al*. (2017) reported the EC of soy protein (52.5 mL/100 g) and marama protein (53.4 mL/100 g) respectively.

Foaming properties

During protein foaming, the interfacial area that can be produced by a protein is referred to as FC, whereas FS denotes the capability of a protein to stabilize air bubbles against gravitational stress (Benelhadj *et al*. 2016). Commonly, FC and FS are the functional characteristics of protein isolates and regulate their utilization in food systems. The FC of T1, T2, T3, T4 and T5 was up to 41.5 ± 0.12 mL/100 g, 42.9 ± 0.09 mL/100 g, 45.5 ± 0.13 mL/100 g, 47.8 ± 0.10 mL/100g and 48.5 ± 0.17 mL/100 g, respectively (Table 4), with a significant difference ($p<0.05$). The FC value of quinoa protein was 58.37 mL/100 mL (Elsohaimy *et al*. 2015).

Furthermore, the FS of all FPIs were 26.3 ± 0.15 mL/100 g, 27.3 ± 0.21 mL/100 g, 28.1 ± 0.27 mL/100 g, 29.2 ± 0.31 mL/100 g and 29.5 ± 0.17 mL/100 g at T1, T2, T3, T4 and T5 for foam intervals at 15 mins respectively, with a significant difference ($p<0.05$). The FS of FMMC of tilapia fish was ranged from 90.17 to 52.63 % as reported by Foh *et al*. (2010).

Table 4. Functional properties of fish protein isolates. WHC: Water-holding Capacity. OHC: Oil-holding Capacity. EC: Emulsifying Capacity. FC: Foaming Capacity, FS: Foam Stability.

Parameters	Treatments				
	T1	T2	T3	T4	T5
WHC	2.18±0.02	2.33±0.04	2.42±0.03	2.45±0.12	2.46±0.11
OHC	1.43±0.08	1.54±0.01	2.11±0.02	2.42±0.12	2.48±0.09
EC	76.2±0.12	77.5±0.09	78.1±0.12	81.0±0.11	81.6±0.08
FC	41.5±0.12	42.9±0.09	45.5±0.13	47.8±0.10	48.5±0.17
FS	26.3±0.15	27.3±0.21	28.1±0.27	29.2±0.31	29.5±0.17

Table 5. Sensory characteristics of fish protein isolates.

Parameters	Treatments				
	T1	T2	T3	T4	T5
Appearance	8.25±0.15	8.25±0.09	8.25±0.11	8.25±0.19	8.25±0.25
Odor	7.35±0.06	7.35±0.12	7.35±0.15	7.35±0.21	7.35±0.19
Overall quality	7.67±0.11	7.67±0.16	7.67±0.21	7.67±0.26	7.67±0.19

Sensory characteristics

Sensory evaluation is the most reliable test for raw material and processed fishery products (Ryder *et al*. 1993). The application of the FPI is strongly dependent on its sensory attributes and it depends on the quality of raw materials (Abdollahi and Undeland 2019). The samples were analyzed for appearance, odor and overall quality is given in Table 5. The scores at T1, T2, T3, T4 and T5 were same for appearance (8.25), odor (7.35) and overall quality (7.67) respectively.

CONCLUSION

This study demonstrated that acid or alkali- aided processing and isoelectric precipitation can be successfully used to extraction of protein isolates from tiger tooth croaker (*Otolithes ruber*) fish. Protein recovery was highest for alkali-aided method specifically at T1 (pH 7). The results revealed that alkali-aided method exhibited more favorable physico-chemical and functional properties than acid-aided method. Low lipid oxidation of protein isolates prepared through the pH-shift process replicates their functional characteristics. Therefore, the pH-shift process can be used as powerful tool to recover functional proteins from tiger tooth croaker (*Otolithes ruber*).

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