PREPARATION AND CHARACTERIZATION OF FISH PROTEIN ISOLATEDTHROUGH PH SHIFT METHOD FROM TIGER TOOTH CROAKER (*Otholithus ruber*)

Abstract

Development of Fish Protein Isolate (FPI) from tiger tooth croaker (*Otholithus ruber*) fish meat using thepH shift method was carried outduring this study. Tiger tooth croaker was used as raw material because of their abundance and comparatively low price. During the study, thephysical characteristics and proximate composition of the fresh fish were analysed. The average length of thefish was 19.95 cm and weighed 94.6 g. respectively. FPI treated at different pH treatments (2.5, 4, 7, 11.5, and 12.5) were analyzed for proximate composition, peroxide value, and functional characteristics. In thepresent work, Tiger tooth croaker (*Otolithes ruber*) fish was found to be suitable for fish protein isolate production using acid or alkali processing and isoelectric precipitation.During storage at ambient temperature for 120 days in a200-gauge LDPE pouch, Fish Protein Isolates treated at different pH treatments (2.5, 4, 7, 11.5,and 12.5) indicated anincrease in moisture content, reduction in protein and lipid content, and nosignificant reduction in ash content.The total protein content was specifically high for pH 7 followed by pH 12.5, 11.5, 4, and 2.5. The effect of different pH on PV of fish protein isolates showed increasing content with increasing storage.The functional properties such as water-holding capacity (WHC), oil-holding capacity (OHC), emulsifying capacity (EC) and foam measurements (Foaming capacity (FC) and foam stability (FS) exhibit high values for all the samples of fish protein isolates. The quality attribute of functional properties of all fish protein isolates samples showed a trend of decreasing during the storage of 120 days.

Keywords: Fish Protein Isolate, Tiger tooth croaker, pH-shift method

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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 ofhigh importance and it plays crucial roles in normal development and maintenance. The sensory and physicochemical 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 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 that 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 theproduction 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

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processing by-products (Chen and Jaczynski, 2007; Shaviklo, 2012). This process involves selective 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 thefamily *Sciaenidae* of order Perciformes. In India, It constitutes 10–12% of the demersal catch and isfound in the both east and west coasts 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 the2018-19 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 nutraceutical industries for improving the economic value of these processing discards as they are one of the important bioresources.

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In this study, the alkali solubilization and precipitation technique to isolate proteins from tiger tooth croaker (*Otolithes ruber*) were used. The proximate composition, peroxide value, and functional 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 (temperature range of 0 to 2°C) to thefish processing laboratory of theCollege 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 from Central Drug House (CDH) Limited - New Delhi, Ranbaxy laboratories limited - SAS Nagar, Astron Chemical (INDIA), Rankem - New Delhi, Chemdyes Corporation, and 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 ground to mince in amixer 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

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8000 × g. for 15 mins at 4°C. After centrifugation, the obtained supernatant was removed and the precipitate was neutralized, andcompletely dried in aHot air oven at 60°C for 24 hours, the product was then ground in powder form, packed, and stored at ambient temperature until analysis. The samples were named as protein isolates 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 1990).

Functional characteristics

Water-holding capacity (WHC)

The water-holding capacity (WHC) of FPIs was analyzed following the procedure of Ozyurt *et al*. (2015). 2 g of the sample was dispersed in 20 ml of deionized water, andstirred 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 thesample.

Oil-holding capacity (OHC)

The oil-holding capacity (OHC) of FPIs was analyzed following the procedure described by Ozyurt *et al*. (2015). 1 g. of the sample was dispersed in 10 ml of vegetable oil, andstirred 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 was 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,

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andcentrifuged at 5000 × g. for 10 mins. and then poured into a 50 ml graduated measuring cylinder and allowed to stand for a 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

The 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) odometrically. 10 g. of thesample was taken and ground well with 15 g anhydrous sodium sulphate. Then transferred to a 100 ml stoppered flask and 30-40 ml chloroform was added and placed in adark place for about 15-20 mins. with occasionally shaking. 10 ml of chloroform extract, and 25 ml of solvent (2 volumes of glacial acetic acid and 35 ml of water) were added. The liberated iodine was titrated against standard sodium thiosulphate solution and explained as milliequivalent of peroxide/ kg of lipid.

Data Analysis

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

Results and discussion

Characteristics of raw materials

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The physical characteristics and proximate composition of fresh fish are 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, themean weight of fish was 94.6 ± 7.22 g. A 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 compare well with the results obtained by Zynudheen *et al.* (2010). The fish meat had aProtein content of17.36 %, lipid of4.74 %, moisture of77.28 %, and ash content was found to be 1.14 % respectively.

Table 1 Characteristics of raw material

A.	**PHYSICAL CHARACTERISTICS**		**Mean ± S.D.**
	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 change in fish protein isolatesduring theperiod of storage

Changes in proximate composition during theperiod of storage

Moisture

The moisture content in fish protein isolates at different pH (2.5, 4, 7, 11.5 and 12.5) showed increasing trends with increasing storage periods (Table 2).The interaction effect of

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treatments and storage period (days) was reported to be significant with aCV (%) of 7.537. The initial moisture content of pH 2.5, 4, 7, 11.5, and 12.5 were 3.17 ± 0.18, 3.18 ± 0.21, 3.18 ± 0.17, 3.02 ± 0.36 and 3.15 ± 0.19. At the end of thestorage period, moisture was found to be 3.60 ± 0.11, 3.60 ± 0.14, 3.59 ± 0.29, 3.44 ± 0.39, and 3.55 ± 0.15 for pH 2.5, 4, 7, 11.5 and 12.5 respectively (mean ± SD).It is due to anincrease in relative humidity of more than 70%. In a similar trend, Lone *et al*. (2015) reported the moisture content of RTFPI was 3.5%. Foh *et al*. (2010) reported amoisture content of 3.7% for FMMC (Freshly minced meat concentrate) of tilapia fish. The moisture content of FPIC (5.86 g/100 g), FPIIM (5.71 g/100 g), FPIP (5.65 g/ 100 g), and FPIS (6.05 g/100 g) (Kumarakuru *et al*., 2018).

Table 2Changes in moisture (%) in Fish Protein Isolate during storage at ambient temperature

Storage Period (Days)	Treatments					DX
	T1	T2	T3	T4	T5	
0	3.17 ± 0.18	3.18 ± 0.21	3.18 ± 0.17	3.02 ± 0.36	3.15 ± 0.19	**3.14**
30	3.26 ± 0.14	3.27 ± 0.17	3.26 ± 0.30	3.11 ± 0.42	3.23 ± 0.16	**3.22**
60	3.34 ± 0.13	3.36 ± 0.16	3.35 ± 0.25	3.20 ± 0.44	3.31 ± 0.13	**3.31**
90	3.48 ± 0.15	3.48 ± 0.15	3.46 ± 0.27	3.32 ± 0.38	3.42 ± 0.12	**3.43**
120	3.60 ± 0.11	3.60 ± 0.14	3.59 ± 0.29	3.44 ± 0.39	3.55 ± 0.15	**3.55**
TX	**3.37**	**3.37**	**3.36**	**3.21**	**3.33**	

Each value is represented bydry weight based on the mean ± SD of n=4.

Total protein

The total protein content in fish protein isolates at different pH (2.5, 4, 7, 11.5, and 12.5) showed decreasing trends with increasing storage periods (Table 3). The interaction effect of treatments and storage period (days) was reported to be significant with aCV (%) of 1.766. The initial protein content of fish protein isolates at pH 2.5, 4, 7, 11.5, and 12.5 was found to be 80.90 ± 1.95, 82.30 ± 2.20, 87.42 ± 0.74, 83.15 ± 0.82 and 84.00 ± 0.95. At the end of thestorage

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period total protein was found to be 80.53 ± 1.91, 81.93 ± 2.22, 87.06 ± 0.71, 82.78 ± 0.91, and 83.63 ± 0.99 respectively (mean ± SD). The highest protein value was observed at pH 7 followed by pH 12.5, 11.5, 4, and 2.5. Kumarakuru *et al*. (2018) reported the protein content in FPIP (89.70 g/100g), FPIIM (87.27 g/100 g), FPIC (86.47 g/100 g) and FPIS (84.74 g/100 g). A protein content of 82.39-94.7% has been reported in fish protein isolates (Foh *et al*., 2010 ~~and~~ Liu *et al*., 2009). The protein content of RTFPI was 75.61 % (Lone *et al*., 2015). The decline in protein value in all samples is due to thedenaturation of protein during storage at ambient temperature.

Table 3 Changes in Total Protein (%) in Fish Protein Isolate during storage at ambient temperature

Storage Period (Days)	Treatments					DX
	T1	T2	T3	T4	T5	
0	80.90±1.95	82.30±2.20	87.42±0.74	83.15±0.82	84.00±0.95	**83.55**
30	80.84±1.87	82.24±2.24	87.37±0.76	83.09±0.84	83.94±0.93	**83.49**
60	80.75±1.92	82.15±2.19	87.28±0.78	83.00±0.86	83.85±0.91	**83.40**
90	80.65±1.84	82.05±2.16	87.18±0.75	82.90±0.88	83.75±0.97	**83.30**
120	80.53±1.91	81.93±2.22	87.06±0.71	82.78±0.91	83.63±0.99	**83.18**
TX	**80.73**	**82.13**	**87.26**	**82.98**	**83.83**	

Each value is represented bydry weight based on the mean ± SD of n=4.

Total lipid

The total lipid content in fish protein isolates at different pH (2.5, 4, 7, 11.5, and 12.5) showed decreasing trends with increasing storage periods (Table 4). The interaction effect of treatments and storage period (days) was reported to be significant with CV (%) 6.922. The initial lipid content of fish protein isolates at pH 2.5, 4, 7, 11.5, and 12.5 was found to be 2.45 ± 0.12, 2.37 ± 0.14, 2.07 ± 0.11, 2.16 ± 0.18 and 2.14 ± 0.19. At the end of thestorage period, total lipids were found to be 2.09 ± 0.18, 2.01 ± 0.14, 1.71 ± 0.19, 1.80 ± 0.17, and 1.81 ± 0.15

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respectively. The lipid content of FMMC of tilapia fish as reported by Foh *et al*. (2010) was 1.81 %. Tongnuanchan *et al*. (2011) reported 0.12 % lipid content in fish protein isolate from red tilapia. The lipid content of RTFPI was 2.35% respectively.

Table 4 Changes in Total Lipid (%) in Fish Protein Isolate during storage at ambient temperature

Storage Period (Days)	Treatments					DX
	T1	T2	T3	T4	T5	
0	2.45± 0.12	2.37± 0.14	2.07± 0.11	2.16± 0.18	2.14± 0.19	**2.243**
30	2.37± 0.15	2.29± 0.13	1.99± 0.15	2.08± 0.15	2.07± 0.21	**2.165**
60	2.28± 0.13	2.20± 0.15	1.90± 0.13	1.99± 0.21	1.98± 0.17	**2.075**
90	2.19± 0.17	2.11± 0.19	1.81± 0.17	1.90± 0.19	1.91± 0.18	**1.988**
120	2.09± 0.18	2.01± 0.14	1.71± 0.19	1.80± 0.17	1.81± 0.15	**1.888**
TX	**2.276**	**2.201**	**1.901**	**1.994**	**1.986**	

Each value is represented bydry weight based on the mean ± SD of n=4.

Total ash

The total ash content in fish protein isolates at different pH (2.5, 4, 7, 11.5, and 12.5) showed increasing trends with increasing storage periods (Table 5). The interaction effect of treatments and storage period (days) was reported to be not significant with CV (%) 3.434. The initial ash content of fish protein isolates at pH 2.5, 4, 7, 11.5, and 12.5 was found to be 3.57 ± 0.13, 3.59 ± 0.14, 3.45 ± 0.11, 3.52 ± 0.06 and 3.50 ± 0.07. At the end of thestorage period, total lipids were found to be 3.72 ± 0.21, 3.74 ± 0.15, 3.60 ± 0.14, 3.67 ± 0.09, and 3.65 ± 0.09 respectively.

Lone *et al*. (2015) reported the ash content of RTFPI was 4 %. The ash content of FPIIM (0.91 g/100 g), FPIP (0.90 g/100 g), FPIC (0.83 g/100 g), and FPIS (0.88 g/100 g) (Kumarakuru *et al*., 2018). The non-significant increase in ash content could be due to the bones present during the mincing operation.

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Table 5 Changes in Ash (%) in Fish Protein Isolate during storage at ambient temperature.

Storage Period (Days)	Treatments					DX
	T1	T2	T3	T4	T5	
0	3.57±0.13	3.59±0.14	3.45±0.11	3.52±0.06	3.50±0.07	**3.52**
30	3.61±0.15	3.63±0.16	3.49±0.13	3.56±0.05	3.54±0.06	**3.56**
60	3.64±0.17	3.66±0.17	3.52±0.17	3.59±0.08	3.57±0.10	**3.59**
90	3.69±0.19	3.71±0.19	3.57±0.19	3.64±0.04	3.62±0.12	**3.64**
120	3.72±0.21	3.74±0.15	3.60±0.14	3.67±0.09	3.65±0.09	**3.67**
TX	**3.64**	**3.66**	**3.52**	**3.59**	**3.57**	

Each value is represented bydry weight based onthe mean ± SD of n=4.

The result indicated that all the parameters were within the prescribed limit signifying the freshness of thefish used in the study.

Changes in Peroxide value during theperiod of storage

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 (Balachandran, 2001). The effect of different pH on thePV of fish protein isolates is depicted in Table 6 showing increasing trends with increasing storage. At the end of thestorage period, PV values were found to be 5.36 ± 0.23(meq/kg), 5.33 ± 0.25 (meq/kg), 5.11 ± 0.13 (meq./kg), 5.24 ± 0.08(meq./kg), and 5.29 ± 0.13 (meq./kg) at pH 2.5, 4, 7, 11.5 and 12.5 respectively (mean ± SD). The lowest value was recorded for thepH 7 sample followed by pH 11.5, 12.5, 4, and 2.5 samples. The interaction effect of treatments and storage period (days) was found to be not significant with CV (%) 4.503. The results are in agreement with the work done by Panpipat and Chaijan (2016).

Table 6 Changes in Peroxide Value (mEq/kg) in Fish Protein Isolate during storage at ambient temperature.

Storage	Treatments	

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Period (Days)	T1	T2	T3	T4	T5	DX
0	3.63±0.18	3.54±0.16	3.41±0.12	3.48±0.14	3.51±0.19	**3.51**
30	4.09±0.21	4.01±0.13	3.78±0.16	3.87±0.06	3.89±0.06	**4.36**
60	4.46±0.15	4.42±0.17	4.25±0.19	4.34±0.11	4.36±0.10	**3.93**
90	4.79±0.13	4.78±0.21	4.68±0.09	4.72±0.03	4.73±0.05	**4.74**
120	5.36±0.23	5.33±0.25	5.11±0.13	5.24±0.08	5.29±0.13	**5.27**
TX	**4.46**	**4.41**	**4.24**	**4.33**	**4.35**	

Each value is represented bydry weight based on the mean ± SD of n=4.

Changes in functional properties during storage

Water-holding capacity

Proteins have both hydrophilic and hydrophobic properties therefore it can interact with water and oil in foods (Butt and Batool, 2010). The water-holding capacity of all fish protein isolates samples showed a trend of decreasing during the storage. The interaction effect of different pH treatments and storage period (days) were found to be significant with aCV (%) of 1.204. Initially water-holding capacity at pH 2.5, 4, 7, 11.5, and 12.5 was 2.18 ± 0.02, 2.33 ± 0.04, 2.42 ± 0.03, 2.45 ± 0.12 and 2.46 ± 0.11 (mean ± SD). Which changed to 1.82 ± 0.17, 1.98 ± 0.21, 2.08 ± 0.15, 2.10 ± 0.23, and 2.10 ± 0.21 (mean ± SD) for pH 2.5, 4, 7, 11.5, and 12.5 respectively on the last day of thestorage period of 120 days (Table 7). The WHC is affected by pH and ionic strength. Similar observations were made by Foh *et al*. (2010) while studying FMMC of tilapia fish and Kumarakuru *et al*. (2018) while studying functional properties of protein isolates obtained from four fish species.

Table 7 Changes in Water Holding Capacity (mL g^{-1}) in Fish Protein Isolates during storage at ambient temperature.

Storage Period (Days)	Treatments					DX
	T1	T2	T3	T4	T5	
0	2.18±0.02	2.33±0.04	2.42±0.03	2.45±0.12	2.46±0.11	**2.373**
30	2.10±0.06	2.25±0.07	2.35±0.05	2.37±0.09	2.38±0.17	**2.295**
60	2.01±0.09	2.17±0.13	2.26±0.11	2.28±0.04	2.29±0.09	**2.206**

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90	1.92±0.13	2.08±0.17	2.18±0.09	2.20±0.15	2.20±0.05	**2.119**
120	1.82±0.17	1.98±0.21	2.08±0.15	2.10±0.23	2.10±0.21	**2.019**
TX	**2.009**	**2.166**	**2.260**	**2.287**	**2.291**	

Each value is represented bydry weight based on the mean ± SD of n=4.

Oil-holding capacity (WHC)

The OHC determines the capacity of food materials to absorb oil. The OHC of proteins is important functional property as it improves the mouthfeel and retains flavor in food. High oil absorption is essential in the formulation of food systems like sausage, cake batters, and mayonnaise and salad dressing (Butt and Batool, 2010). The oil-holding capacity of all fish protein isolates samples showed a trend of decreasing during the storage. The interaction effect of different pH treatments and storage period (days) were found to be significant with aCV (%) of 0.677. Initially oil-holding capacity at pH 2.5, 4, 7, 11.5, and 12.5 was 1.43 ± 0.08, 1.54 ± 0.01, 2.11 ± 0.02, 2.42 ± 0.12, and 2.48 ± 0.09 (mean ± SD). Which changed to 1.08 ± 0.21, 1.19 ± 0.12, 1.75 ± 0.01, 2.07 ± 0.26,and 2.11 ± 0.23 (mean ± SD) for pH 2.5, 4, 7, 11.5, and 12.5 respectively on the last day of thestorage period of 120 days (Table 8). It is because ~~of~~OHC reflects the extent of denaturation of the protein. As the protein denaturation increased during storage the OHC of fish protein isolates decreased. Similar results were also reported by Kumarakuru *et al*. (2018); Elsohaimy *et al*. (2015); Foh *et al*. (2010, 2012) with anOHC range of 5.32 – 5.83 mL/g, 1.88 mL/g, 2.43 mL/g, and 3.38 mL/g respectively.

Table 8 Changes in Oil Holding Capacity (mL g^{-1}) in Fish Protein Isolates during storage at ambient temperature.

Storage Period (Days)	Treatments					DX
	T1	T2	T3	T4	T5	
0	1.43±0.08	1.54±0.01	2.11±0.02	2.42±0.12	2.48±0.09	**2.001**
30	1.35±0.02	1.45±0.05	2.02±0.07	2.33±0.05	2.39±0.13	**1.913**
60	1.27±0.11	1.38±0.09	1.94±0.13	2.25±0.09	2.30±0.19	**1.832**

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90	1.18±0.17	1.29±0.03	1.85±0.21	2.17±0.21	2.21±0.31	**1.745**
120	1.08±0.21	1.19±0.12	1.75±0.25	2.07±0.26	2.11±0.23	**1.645**
TX	**1.268**	**1.375**	**1.940**	**2.253**	**2.298**	

Each value is represented bydry weight based on the mean ± SD of n=4.

Emulsifying capacity (EC)

The emulsifying capacity (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 emulsifying capacityof all fish protein isolates samples showed a trend of decreasing during the storage. The interaction effect of different pH treatments and storage period (days) were found to be significant with aCV (%) of 0.128. Initially emulsifying capacity at pH 2.5, 4, 7, 11.5, and 12.5 was 76.2 ± 0.12, 77.5 ± 0.09, 78.1 ± 0.12, 81.0 ± 0.11 and 81.6 ± 0.08 (mean ± SD). Which changed to 75.7 ± 0.27, 77.1 ± 0.29, 77.6 ± 0.31, 80.61 ± 0.26,and 81.14 ± 0.28 (mean ± SD) for pH 2.5, 4, 7, 11.5, and 12.5 respectively on the last day of thestorage period of 120 days (Table 9). EAI (Emulsifying activity index) of RTFPI was 281.0, 207.3, and 535.0 m^2/g, while ~~as~~ the ESI (emulsionstability index) was 11.30, 4.17 and 7.0 min at pH 3, 5, and 7 respectively (Lone *et al*., 2015). Gulzar *et al*. (2017) reported the EC of soy protein (52.5 mL/100 g) and marama protein (53.4 mL/100 g) respectively.

Table 9 Changes in Emulsifying Capacity (mL /100 g) in Fish Protein Isolates during storage at ambient temperature.

Storage Period (Days)	Treatments					DX
	T1	T2	T3	T4	T5	
0	76.2±0.12	77.5±0.09	78.1±0.12	81.0±0.11	81.6±0.08	**78.92**
30	76.1±0.17	77.4±0.03	78.0±0.09	80.9±0.21	81.4±0.15	**78.81**
60	76.0±0.19	77.3±0.14	77.9±0.21	80.8±0.16	81.3±0.19	**78.70**
90	75.9±0.21	77.2±0.18	77.7±0.28	80.7±0.19	81.2±0.24	**78.58**
120	75.7±0.27	77.1±0.29	77.6±0.31	80.6±0.26	81.1±0.28	**78.46**
TX	**76.02**	**77.34**	**77.89**	**80.84**	**81.37**	

Each value is represented bydry weight based on the mean ± SD of n=4.

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Foam measurements

Foaming capacity (FC) and Foam stability (FS)

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).The foaming capacityof all fish protein isolates samples showed a trend of decreasing during the storage. The interaction effect of different pH treatments and storage period (days) were found to be significant with aCV (%) of 0.291. The highest foaming capacity was at pH 12.5 followed by pH 11.5, 7, 4, and 2.5.Initially foaming capacity at pH 2.5, 4, 7, 11.5, and 12.5 was 41.5 ± 0.12, 42.9 ± 0.09, 45.5 ± 0.13, 47.8 ± 0.10 and 48.5 ± 0.17(mean ± SD). Which changed to 40.9 ± 0.39, 42.5 ± 0.19, 45.1 ± 0.19, 47.5 ± 0.27, and 48.1 ± 0.34 (mean ± SD) for pH 2.5, 4, 7, 11.5, and 12.5 respectively on the last day of thestorage period of 120 days (Table 10).

Table 10 Changes in Foaming capacity (mL /100 g) in Fish Protein Isolates during storage at ambient temperature.

Storage Period (Days)	Treatments					DX
	T1	T2	T3	T4	T5	
0	41.5±0.12	42.9±0.09	45.5±0.13	47.8±0.10	48.5±0.17	**45.26**
30	41.4±0.17	42.8±0.13	45.4±0.20	47.7±0.16	48.4±0.13	**45.20**
60	41.3±0.23	42.7±0.08	45.3±0.27	47.6±0.09	48.3±0.21	**45.09**
90	41.2±0.14	42.6±0.11	45.2±0.15	47.5±0.21	48.2±0.27	**44.99**
120	40.9±0.39	42.5±0.19	45.1±0.19	47.5±0.27	48.1±0.34	**44.85**
TX	**41.29**	**42.74**	**45.36**	**47.66**	**48.34**	

Each value is represented bydry weight based on the mean ± SD of n=4.

The foam stabilityof all fish protein isolates samples showed a trend of rapidly decreasing during the storage. The interaction effect of different pH treatments and storage period (days) were found to be significant with a CV (%) of 0.549. Initially,foam stabilityat pH 2.5, 4, 7, 11.5, and 12.5 was 26.3 ± 0.15, 27.3 ± 0.21, 28.1 ± 0.27, 29.2 ± 0.31, and 29.5 ± 0.17 (mean ± SD).

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Which changed to 13.9 ± 0.23, 14.7 ± 0.29, 15.5 ± 0.21, 16.9 ± 0.19, and 17.3 ± 0.31 (mean ± SD) for pH 2.5, 4, 7, 11.5, and 12.5 respectively on the last day of thestorage period of 120 days (Table 11). The highest foam stability was at pH 12.5 followed by pH 11.5, 7, 4, and 2.5.

Table 11 Changes in Foam stability (mL /100 g) in Fish Protein Isolates during storage at ambient temperature.

Storage Period (Days)	Treatments					DX
	T1	T2	T3	T4	T5	
0	26.3±0.15	27.3±0.21	28.1±0.27	29.2±0.31	29.5±0.17	**28.12**
30	23.3±0.04	24.1±0.15	24.9±0.11	26.3±0.27	26.7±0.13	**25.11**
60	20.2±0.11	21.0±0.19	21.8±0.18	23.2±0.09	23.6±0.21	**22.01**
90	17.1±0.03	17.9±0.26	18.6±0.05	20.1±0.16	20.5±0.27	**18.88**
120	13.9±0.23	14.7±0.29	15.5±0.21	16.9±0.19	17.3±0.31	**22.06**
TX	**21.466**	**22.302**	**23.091**	**24.507**	**24.836**	

Each value is represented bydry weight based on the mean ± SD of n=4.

Kumarakuru *et al*. (2018) reported the FC of FPIC, FPIIM, FPIP, and FPIS was up to 46.2 mL/100 g, 45.2 mL/100 g, 47.7 mL/100 g, and 44.2 mL/100 g respectively, and FS of FPIC, FPIIM, FPIP, and FPIS was ranged between 30.5 and 34.0 mL/100 g, 20.3 and 22.5 mL/100 g and 14.8 and 17.1 mL/100 g for foam intervals at 15, 20 and 30 mins respectively, with a significant difference ($p < 0.05$). The FC value of quinoa protein was 58.37 mL/100 mL (Elsohaimy *et al*., 2015). The FS of FMMC of tilapia fish ~~was~~ ranged from 90.17 to 52.63 % as reported by Foh *et al*. (2010).

CONCLUSIONS

The fish protein isolates are the most refined form of protein products containing the greatest concentration of protein. 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 thealkali-aided method specifically

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at pH 7. The results revealed that thealkali-aided method exhibited more favorable functional properties than theacid-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 apowerful tool to recover functional proteins from tiger tooth croaker (*Otolithes ruber*).

Hence the study proved the alkali extraction method in the isolation of fish protein isolates with favourable functional characteristics to be applicable in the development of protein-rich food products satisfying the present need for isolation of functional nutrients in the area of functional food.

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