www.ThePharmaJournal.com

The Pharma Innovation

**ISSN (E): 2277-7695**
**ISSN (P): 2349-8242**
**NAAS Rating: 5.23**
TPI 2023; SP-12(8): 1050-1055
© 2023 TPI
www.thepharmajournal.com
Received: 01-05-2023
Accepted: 12-06-2023

**Varsha Likhar**
Department of Fish Processing Technology, College of Fisheries Science, Kamdhenu University, Veraval, Gujarat, India

**BG Chudasama**
Department of Fish Processing Technology, College of Fisheries Science, Kamdhenu University, Veraval, Gujarat, India

**Siddharth Kumar Jatav**
Department of Fisheries Resource Management, College of Fisheries Science, Kamdhenu University, Veraval, Gujarat, India

**Tandel Mayur**
Department of Fish Engineering and Technology, College of Fisheries, Shirgaon, Ratnagiri, DBSKKV, Maharashtra, India

**Ajay E Sonvane**
Assistant Fisheries Development Officer, Department of Fisheries Govt. of Maharashtra, India

**Saiprasad Bhusare**
Fish Nutrition, Biochemistry and Physiology Division, ICAR-Central Institute of Fisheries Education, Mumbai, Maharashtra, India

**Corresponding Author:**
**Varsha Likhar**
Department of Fish Processing Technology, College of Fisheries Science, Kamdhenu University, Veraval, Gujarat, India

# Preparation and evaluation (Physico-chemical & sensory) of fish protein isolate from tiger tooth croaker (*Otolithes ruber*) obtained through pH shift method

**Varsha Likhar, BG Chudasama, Siddharth Kumar Jatav, Tandel Mayur, Ajay E Sonvane and Saiprasad Bhusare**

**Abstract**
Development of Fish Protein Isolate (FPI) from tiger tooth croaker (*Otolithes 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 analysed. The average length of fish was 19.95 cm and weighed 94.6 GRM. Respectively. FPI treated at different pH treatments (2.5, 4, 7, 11.5 and 12.5) were analyzed for physicochemical and sensory characteristics. In present 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 200 gauge LDPE pouch, Fish Protein Isolates treated at different pH treatments (2.5, 4, 7, 11.5 and 12.5) indicated increasing trends of physicochemical characteristics such as bulk density with not significant increase in pH. Color analysis showed decreasing trends with increasing storage periods in L*- value and increasing trends with increasing storage periods in a*- value and b*- value. Quality attributes of the stored samples found significant reduction in physicochemical properties of FPI. Gradual decrease in sensory score like appearance, odour and overall quality of all the samples were noted during four months of storage.

**Keywords:** Fish protein isolate, tiger tooth croaker, pH-shift method

## Introduction
Seafood is vital to human survival because it provides an abundant source of digestible protein. Due to the increasing popularity of using protein from animals as a functional food ingredient, fisheries by-products have gained a lot of attention as a potential source of protein (Chalamaiah *et al.*, 2012) [3]. While the oxidation of 1 gram of protein yields 4 kilocalories of energy, proteins are not typically thought of as primary energy sources. However, proteins' contributions to protein synthesis are very important, and they play major roles in proper growth and maintenance. Overall structural behavior is affected by both the sensory and physicochemical features of any kind of food that is being looked into as a protein source (Foh *et al.*, 2012) [5]. Biological properties may be used to classify protein sources as functional health enhancing foods (Kadam & Prabhasankar, 2010) [8].
Protein isolates, being the purest form of protein products, have the highest amount of protein and the lowest fiber content. They're simple to add into a variety of foods and have a high digestibility value. The term "fish protein isolate" refers to a protein concentrate made from fish muscle that has been processed such that its structure has been altered. In most cases, it is not ingested directly but rather serves as an ingredient in the production of value added products. Seafood is a major dietary staple since it provides animal protein. Since there is a rising interest in using animal-based protein as an essential component of food (Chalamaiah *et al.*, 2012) [3], fisheries by-products, which are abundant, have gained a lot of attention as a potential protein source.
Recovery of direct protein from unconventional complex aquatic raw materials, such as gutted fish (Taskaya *et al.*, 2009; Marmon and Undeland, 2010) [18, 13] and seafood processing by-products (Chen and Jaczynski, 2007; Shaviklo, 2012) [4, 16], has been recognized as a promising technique due to pH-shift processing, also known as the acid or alkaline solubilization followed by isoelectric precipitation (Hultin and Kelleher, 2001) [6]. The homogenized raw material is first treated with either a high (> 10.5) or low (3.5) pH to solubilize the muscle proteins, and then the solubilized proteins are separated from the high density and low density

undissolved material by centrifugation. Dewatering by centrifugation or filtration follows isoelectric precipitation (often pH 5.5) for the recovery of solubilized proteins. For future use, the recovered protein isolate may be mixed with cryoprotectants and frozen as surimi or minced fish, or it may be immediately dry into a fish protein powder (FPP) (Likhar *et al.*, 2022) [11].

Originally from the Bay of Bengal, the Indian Ocean, and the Western Pacific, *Otolithes ruber* is more commonly referred to as the tigertooth croaker. It is classified in the family *Sciaenidae* of the order Perciformes. It may be caught year-round off of India's east and west coasts and accounts for 10–12% of the country's demersal catch. It is a popular marine food source. A vast variety of species, including crustaceans, polychaetes, mollusks, and tiny fish, constitute the bulk of the food of the carnivorous croaker. One of the most significant components in making surimi in India is croaker. In terms of marine fish landing in 2018-2019, croakers alone accounted for 1.36 lakh tons. Chemical characterisation of croaker waste is useful for turning otherwise worthless industrial wastes into profitable ones. Discards from processing croaker are now being utilized for fish meal, fish manure and fish silage production. Croaker processing waste is one of the key bio resources since it may be used to recover bioactive compounds used in the food, health care, pharmaceuticals, and nutraceuticals sectors.

In the present study, the alkali solubilization and precipitation approach was utilized in order to separate the proteins from the tigertooth croaker (*Otolithes ruber*). Protein isolates were also tested for their physicochemical and sensory qualities.

## Materials and Methods

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) [6]. 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. Centrifugation at 8000 g for 20 minutes at 4°C was used to separate the homogenate. There were three distinct layers separated by centrifugation, the upper layer containing the lipid content and the lower layer containing the insoluble protein. Solid components, such as skin, bone, and connective tissue, were separated from the soluble proteins by filtering the intermediate layer of the supernatant. After lowering the pH of the filtrate to 5.5, it was centrifuged once more at 8000 g for 15 minutes at 4 °C. Following the removal of the acquired supernatant using centrifugation, the precipitate was next neutralized before being thoroughly dried in a hot air oven at a temperature of 60 °C. For 24 hours. After this, the product was ground into a powder, packaged, and kept at room temperature until it was analyzed. 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 of raw material

We used the standard AOAC procedures (AOAC 1990) to conduct an analysis on the proximate composition of FPIs. This included analyzing the amounts of moisture, protein, lipids, and ash.

### Physicochemical characteristics Bulk density

FPI bulk density has been examined using the same technique as Joshi *et al.* (2011) [7]. In order to conduct an analysis of bulk density, we recorded the amount of space that was taken up by FPIs in a graduated cylinder that was pre-weighed and set to 10 milliliters. The cylinder was tapped 20 times and weighed again during the FPIs filling process, and the bulk density of FPIs is given in kg/m3.

### pH

FPIs' pH levels were measured. After weighing and measuring 10 grams of the samples, they were combined with 50 ml of deionized water and given a thorough stirring for a period of 5 minutes before the pH of the suspension was determined 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.

### 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. According to the conventional statistical procedures provided by Snedecor & Cochran (1967) [17], we conducted analysis of variance to identify statistically significant differences in the samples between the treatments.

## 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) [19]. 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 *et al.* (2010) [21]. 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 ± 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 changes in fish protein isolates during period of storage**

Result indicated that all the parameters were within the prescribed limit signifying the freshness of fish used in the study.

**Changes in physicochemical properties during storage**

**Bulk density**

The effect of different pH on bulk density of fish protein isolates is depicted in Table 2 showing increasing trends with increasing storage. Because of this, it is possible that differences in the bulk density of protein isolates are attributable to differences in the structure of proteins. At the end of storage period bulk density value were found to be 0.86 ± 0.10 (mL-1), 0.82 ± 0.07 (mL- 1), 0.68 ± 0.07 (mL-1), 0.85 ± 0.10 (mL-1), and 0.82 ± 0.05(mL-1) at pH 2.5, 4, 7, 11.5, and 12.5 respectively (mean ± SD). Lowest value was recorded for pH 7 sample followed by pH 12.5, 11.5, 4 and 2.5 samples. There was a statistically significant (CV = 9.133) interaction effect between treatments and storage duration (in days). The bulk density of a food sample may be used to determine how much handling is required, what kind of packaging is best, and where the food will be stored and transported (Kumarakuru *et al.*, 2018) [10]. According to Lone *et al.* (2015) [12], the formulation of weaning foods should avoid using ingredients with a high bulk density since these foods should have a low bulk density. So the bulk density increased with increased in protein denaturation. Foh *et al.* (2012) [5] who studied bulk density of FMMC of tilapia fish and Lone *et al.* (2015) [12] who studied bulk density of RTFPI.

**Table 2:** Changes in Bulk Density (mL-1) in Fish Protein Isolate during storage at ambient temperature

| Storage Period (Days) | Treatments | | | | | DX |
|---|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | T5 | |
| 0 | 0.59±0.05 | 0.55±0.04 | 0.40±0.06 | 0.57±0.03 | 0.55±0.05 | 0.532 |
| 30 | 0.64±0.08 | 0.60±0.06 | 0.45±0.08 | 0.62±0.05 | 0.60±0.03 | 0.582 |
| 60 | 0.70±0.06 | 0.66±0.08 | 0.51±0.09 | 0.68±0.07 | 0.66±0.06 | 0.642 |
| 90 | 0.77±0.09 | 0.74±0.05 | 0.59±0.10 | 0.76±0.09 | 0.73±0.09 | 0.718 |
| 120 | 0.86±0.10 | 0.82±0.07 | 0.68±0.07 | 0.85±0.10 | 0.82±0.05 | 0.806 |
| TX | 0.712 | 0.674 | 0.526 | 0.696 | 0.672 | |

Each value is represented dry weight based as the mean ± SD of n=4.

**pH**

The pH changes can be used as a spoilage indicator in fishery products. The pH values of fish protein isolates play an important role in determining their shelf life and foaming and emulsification properties. The fish protein isolates at pH 2.5, 4, 7, 11.5, and 12.5 showed increasing trends of pH with increasing storage periods (Table 3). With a CV (%) of 2.035, we observed that the interaction effect of treatments and storage period (days) was not significant. On day 0, there were no noticeable differences between treatments. At the end of storage period pH value were found to be 5.82 ± 0.21, 4.58 ± 2.57, 6.33 ± 0.23, 5.87 ± 0.15 and 5.91 ± 0.16 at pH 2.5, 4, 7, 11.5 and 12.5 respectively (mean ± SD). Kumarakuru *et al.* (2018) [10] reported the similar trends of pH value in FPIC, FPIIM, FPIP and FPIS was 5.70, 5.52, 5.51 and 5.65 respectively.

**Table 3:** Changes in pH in Fish Protein Isolate during storage at ambient temperature

| Storage Period (Days) | Treatments | | | | | DX |
|---|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | T5 | |
| 0 | 5.67±0.18 | 5.75±0.08 | 6.06±0.13 | 5.72±0.09 | 5.77±0.04 | 5.794 |
| 30 | 5.70±0.16 | 4.46±2.50 | 6.22±0.21 | 5.75±0.12 | 5.80±0.08 | 5.586 |
| 60 | 5.73±0.14 | 4.49±2.47 | 6.25±0.28 | 5.78±0.16 | 5.83±0.10 | 5.616 |
| 90 | 5.77±0.15 | 4.53±2.38 | 6.29±0.25 | 5.82±0.19 | 5.87±0.05 | 5.656 |
| 120 | 5.82±0.21 | 4.58±2.57 | 6.33±0.23 | 5.87±0.15 | 5.91±0.16 | 5.702 |
| TX | 5.738 | 4.762 | 6.23 | 5.788 | 5.836 | |

Each value is represented dry weight based as the mean ± SD of n=4.

**Color analysis**

***L**-value**

The *L** value 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). It was shown that the interaction effect of treatments and storage term (days) did not provide a meaningful result, with a CV (%) of 3.061. The initial *L** value of fish protein isolates at pH 2.5, 4, 7, 11.5 and 12.5 were found to be 72.6 ± 2.13, 74.8 ± 2.89, 71.3 ± 2.08, 69.9 ± 1.80, and 70.9 ± 1.92. At the end of storage period *L** value

were found to be 53.3 ± 2.16, 55.7 ± 3.25, 52.0 ± 2.22, 50.6 ± 1.79, and 51.6 ± 1.76 respectively (mean ± SD). Yongsawatdigul and Park (2004) [20] reported *L** value for FPI (alkali process) of Rockfish (fish fillet) was 76.2 and reported 70.1 (*L** value) for FPI (acid process) of Pacific whiting (fish fillet). Correlated trend of the results were also observed by Kristinsson *et al.* (2005) [9]; Panpipat and Chaijan (2016) [14] and Shaviklo *et al.* (2008) [15].

**Table 4:** Changes in Color Analysis (*L** -value) in Fish Protein Isolates during storage at ambient temperature

| Storage Period (Days) | Treatments | | | | | DX |
|---|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | T5 | |
| 0 | 72.6±2.13 | 74.8±2.89 | 71.3±2.08 | 69.9±1.80 | 70.9±1.92 | 71.93 |
| 30 | 68.3±2.18 | 70.7±3.21 | 67.0±2.12 | 65.6±1.75 | 66.5±1.95 | 67.68 |
| 60 | 63.7±2.19 | 66.1±3.20 | 62.5±2.16 | 61.1±1.77 | 62.0±1.90 | 63.13 |
| 90 | 58.6±2.21 | 61.0±3.28 | 57.3±2.19 | 56.0±1.76 | 56.9±1.86 | 58.01 |
| 120 | 53.3±2.16 | 55.7±3.25 | 52.0±2.22 | 50.6±1.79 | 51.6±1.76 | 52.69 |
| TX | 63.358 | 65.693 | 62.070 | 60.708 | 61.632 | |

Each value is represented dry weight based as the mean ± SD of n=4.

### *a**-value

The *a** value 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). There was no statistically significant relationship between treatment and storage duration (in days), as measured by CV (%), of 3.585. The initial *a** value of fish protein isolates at pH 2.5, 4, 7, 11.5 and 12.5 were found to be 10.7 ± 0.51, 11.1 ± 0.33, 10.7 ± 0.45, 10.5 ± 0.25, and 10.4 ± 0.31. At the end of storage period *a** value were found to be 25.8 ± 0.54, 26.1 ± 0.25, 25.7 ± 0.56, 25.6 ± 0.18 and 25.4 ± 0.36 respectively (mean ± SD). Similar results were reported by Kumarakuru *et al.* (2018) [10] and Panpipat and Chaijan (2016) [14].

**Table 5:** Changes in Color Analysis (*a** -value) in Fish Protein Isolates during storage at ambient temperature

| Storage Period (Days) | Treatments | | | | | DX |
|---|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | T5 | |
| 0 | 10.7±0.51 | 11.1±0.33 | 10.7±0.45 | 10.5±0.25 | 10.4±0.31 | 10.73 |
| 30 | 14.2±0.49 | 14.6±0.26 | 14.2±0.57 | 14.0±0.28 | 13.9±0.30 | 14.23 |
| 60 | 17.9±0.45 | 18.3±0.27 | 18.0±0.54 | 17.8±0.31 | 17.6±0.29 | 17.97 |
| 90 | 21.7±0.58 | 22.0±0.29 | 21.7±0.59 | 21.5±0.22 | 21.4±0.35 | 21.70 |
| 120 | 25.8±0.54 | 26.1±0.25 | 25.7±0.56 | 25.6±0.18 | 25.4±0.36 | 25.76 |
| TX | 18.117 | 18.465 | 18.103 | 17.937 | 17.788 | |

Each value is represented dry weight based as the mean ± SD of n=4.

### *b**-value

The *b** value in fish protein isolates at different pH (2.5, 4, 7, 11.5 and 12.5) showed increasing trends with increasing storage periods (Table 6). With a CV (%) of 4.312, it was found that there was no statistically significant interaction between treatments and storage duration (in days). The initial *b** value of fish protein isolates at pH 2.5, 4, 7, 11.5 and 12.5 were found to be 16.70 ± 0.64, 17.40 ± 0.76, 16.70 ± 0.45, 18.10 ± 1.08 and 19.10 ± 0.71. At the end of storage period *a** value were found to be 30.57 ± 0.67, 31.27 ± 0.75, 30.69 ± 0.51, 31.99 ± 1.14 and 32.95± 0.86 respectively (mean ± SD). Similar results were reported by Shaviklo *et al.* (2008) [15]; Abdollahi and Undeland (2019) [1]; Panpipat and Chaijan (2016) [14] and Kumarakuru *et al.* (2018) [10].

**Table 6:** Changes in Color Analysis (*b** -value) in Fish Protein Isolates during storage at ambient temperature

| Storage Period (Days) | Treatments | | | | | DX |
|---|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | T5 | |
| 0 | 16.70±0.64 | 17.40±0.76 | 16.70±0.45 | 18.10±1.08 | 19.10±0.71 | 17.60 |
| 30 | 19.81±0.68 | 20.51±0.77 | 19.82±0.41 | 21.22±1.05 | 22.20±0.75 | 20.71 |
| 60 | 23.16±0.71 | 23.86±0.79 | 23.02±0.58 | 24.57±1.10 | 25.54±0.78 | 24.03 |
| 90 | 26.71±0.62 | 27.41±0.74 | 26.83±0.45 | 28.13±1.03 | 29.11±0.81 | 27.64 |
| 120 | 30.57±0.67 | 31.27±0.75 | 30.69±0.51 | 31.99±1.14 | 32.95±0.86 | 31.49 |
| TX | 23.395 | 24.093 | 23.416 | 24.804 | 25.783 | |

Each value is represented dry weight based as the mean ± SD of n=4.

### Sensory characteristics

The variation in appearance of fish protein isolates with different pH exhibited a sample decreasing trend during the storage. After 120 days of storage period highest score 5.55 was recorded in pH 7. pH 2.5, 4, 11.5 and 12.5 had comparatively lower score record as shown in Table 7.

All of the samples of fish protein isolates showed a declining trend of score for odor quality as storage time increased. Initial samples of fish protein isolates had a pH of 7.35, which dropped to 4.20 ± 0.08 throughout the course of storage. At pH 2.5, 4, 11.5 and 12.5 decreased to 4.14 ±0.25, 4.15 ± 0.35, 4.15 ± 0.28 and 4.18 ± 0.31 respectively (mean ± SD) at the end of storage period.

The overall quality of all the fish protein isolates samples progressively decreased as storage time increased (Table 9). All samples of fish protein isolates scored same 7.67 on first day of storage period. At the end of 120 days the values decreased to 4.37 ± 0.28, 4.37 ± 0.35, 4.65 ± 0.27, 4.45 ± 0.29 and 4.45 ± 0.32 for pH 2.5, 4, 7, 11.5 and 12.5 respectively (mean ± SD). The performance of fish protein isolates was

highest at pH 7, followed by pH 11.5, 12.5, 4, and 2.5. This was despite the fact that the general acceptability of the product deteriorated as the storage time progressed.

All the fish protein isolates sample showed decreasing trend in their sensory quality, possibly because of lipid oxidation, physicochemical and functional changes of all group.

**Table 7:** Changes in appearance in Fish Protein Isolates during storage at ambient temperature

| Storage Period (Days) | Treatments | | | | | DX |
|---|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | T5 | |
| 0 | 8.25±0.15 | 8.25±0.09 | 8.25±0.11 | 8.25±0.19 | 8.25±0.25 | 8.25 |
| 30 | 7.15±0.05 | 7.20±0.18 | 7.47±0.16 | 7.25±0.24 | 7.25±0.19 | 7.26 |
| 60 | 6.35±0.09 | 6.35±0.23 | 6.62±0.06 | 6.55±0.29 | 6.55±0.15 | 6.48 |
| 90 | 5.35±0.21 | 5.35±0.31 | 5.50±0.18 | 5.45±0.31 | 5.45±0.11 | 5.42 |
| 120 | 5.42±0.27 | 5.35±0.29 | 5.55±0.25 | 5.45±0.07 | 5.45±0.28 | 4.44 |
| TX | 6.305 | 6.300 | 6.480 | 6.390 | 6.390 | |

Each value is represented dry weight based as the mean ± SD of n=4.

**Table 8:** Changes in Odour in Fish Protein Isolates during storage at ambient temperature

| Storage Period (Days) | Treatments | | | | | DX |
|---|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | T5 | |
| 0 | 7.35±0.06 | 7.35±0.12 | 7.35±0.15 | 7.35±0.21 | 7.35±0.19 | 7.35 |
| 30 | 6.45±0.11 | 6.45±0.24 | 6.60±0.23 | 6.47±0.13 | 6.50 ±0.12 | 6.49 |
| 60 | 5.35±0.16 | 5.32±0.27 | 5.55±0.19 | 5.37±0.16 | 5.40±0.21 | 5.40 |
| 90 | 4.65±0.19 | 4.62±0.31 | 4.75±0.11 | 4.62±0.31 | 4.62 ±0.27 | 4.65 |
| 120 | 4.14±0.25 | 4.15±0.35 | 4.20±0.08 | 4.15±0.28 | 4.18±0.31 | 4.16 |
| TX | 5.58 | 5.58 | 5.69 | 5.59 | 5.61 | |

Each value is represented dry weight based as the mean ± SD of n=4.

**Table 9:** Changes in Overall Quality in Fish Protein Isolates during storage at ambient temperature

| Storage Period (Days) | Treatments | | | | | DX |
|---|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | T5 | |
| 0 | 7.67±0.11 | 7.67±0.16 | 7.67±0.21 | 7.67±0.26 | 7.67±0.19 | 7.67 |
| 30 | 6.95±0.08 | 6.95±0.31 | 7.05±0.15 | 7.00±0.08 | 7.00±0.23 | 6.99 |
| 60 | 6.15±0.15 | 6.15±0.29 | 6.35±0.35 | 6.20±0.13 | 6.20±0.28 | 6.21 |
| 90 | 5.25±0.21 | 5.25±0.21 | 5.45±0.23 | 5.27±0.05 | 5.27±0.15 | 5.30 |
| 120 | 4.37±0.28 | 4.37±0.35 | 4.65±0.27 | 4.45±0.29 | 4.45±0.32 | 4.46 |
| TX | 6.08 | 6.08 | 6.23 | 6.12 | 6.12 | |

Each value is represented dry weight based as the mean ± SD of n=4.

## Conclusions

This study demonstrates that tiger tooth croaker (*Otolithes ruber*) protein isolates may be effectively extracted using the pH-shift approach. According to the findings, the alkali-aided method has better physicochemical qualities than the acid-aided method. Fish protein isolates had the greatest sensory scores in pH 7 for both appearance and odor. As storage times lengthened, a general decline in quality was seen across all of the fish protein isolates samples. Therefore, tiger tooth croaker (*Otolithes ruber*) may be effectively processed with acid or alkali, and isoelectric precipitation can be employed to extract functional proteins.

Thus, the study confirmed the efficacy of the alkali extraction method in the isolation of fish protein isolate with important physicochemical and functional characteristics, which can be used to create protein-rich food products that meet the current demand for the isolation of functional nutrients in the field of functional food.

## References


1. Abdollahi M, Undeland I. Structural, functional, and sensorial properties of protein isolate produced from salmon, cod, and herring by-products. Food and bioprocess technology. 2018;11(9):1733-1749.
2. AOAC. Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC) International 18th edition.; c2006.
3. Chalamaiah M, Kumar BD, Hemalatha R, Jyothirmayi T. Fish protein hydrolysates: proximate composition, amino acid composition, antioxidant activities and applications: a review. National Library of Medicine. 2012;135(4):3020-38.
4. Chen YC, Jaczynski J. Protein recovery from rainbow trout (*Oncorhynchus mykiss*) processing byproducts via isoelectric solubilization/precipitation and its gelation properties as affected by functional additives. Journal of Agricultural and Food Chemistry. 2007;55(22):9079-9088.
5. Foh MBK, Wenshui X, Amadou I, Jiang Q. Influence of pH Shift on from silver carp (Hypophthalmichthys molitrix) by isoelectric solubilization/precipitation. LWT-Food Science and Technology. 2012;42(6):1082-1089.
6. Hultin HO, Kelleher SD. High Efficiency Protein Extraction from Adipose Hultin, T. L. and Herbert, O. Process for recovery of functional proteins by pH-shifts. Surimi and Surimi Seafood; c2001-2005. p. 107-139.
7. Joshi M, Adhikari B, Aldred P, Panozzo JF, Kasapis S. Physicochemical and functional properties of lentil protein isolates prepared by different drying methods. Food Chemistry. 2011;129(4):1513-1522.
8. Kadam SU, Prabhasankar P. Marine foods as functional ingredients in bakery and pasta products. Food Research International. 2010;43(8):1975-1980.
9. Kristinsson HG, Theodore AE, Demir N, Ingadottir B. 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. 2005;70(4):C298-C306.
10. Kumarakuru K, Reddy CK, Haripriya S. Physicochemical, morphological and functional properties of protein isolates obtained from four fish species. Journal of food science and technology. 2018;55(12):4928-4936.
11. Likhar V, Chudasama BG, Bhola DV. Utilization of Tiger Tooth Croaker (*Otolithes ruber*) Fish Meat for Development of Fish Protein Isolate using pH Shifting Method. Environment and Ecology. 2022;40(3):1197-1205. ISSN 0970-0420.
12. Lone DA, Wani NA, Wani IA, Masoodi FA. Physico-chemical and functional properties of rainbow trout fish protein isolate. International Food Research Journal. 2015;22(3):1112.
13. Marmon SK, Undeland I. Protein isolation from gutted herring (*Clupea harengus*) using pH-shift processes. Journal of Agricultural and Food Chemistry. 2010;58(19):10480–10486.
14. Panpipat W, Chaijan M. Biochemical and physicochemical characteristics of protein isolates from bigeye snapper (*Priacanthus tayenus*) head by-product using pH shift method. Turkish Journal of Fisheries and Aquatic Sciences. 2016;16(1):41-50.
15. Shaviklo GR. Evaluation and utilization of fish protein isolate products. 2008.
16. Shaviklo GR. Characteristics of freeze-dried fish protein isolated from saithe (*Pollachius virens*). Journal of Food Science and Technology. 2012;49(3):309-318.
17. Snedecor GW, Cochran WG. Statistical Methods, lowa State University Press, lowa, U.S.A.; 1967. p. 435.
18. Taskaya L, Chen Y, Jaczynski CJ. Functional properties of proteins recovered Tissues. Invent Biotechnologies Inc. 2009;63(5):235-235.
19. Vijayakumar R, Zynudheen AA, Panda SK, Ravishankar CNR. Nutritional evaluation of processing discards from tiger tooth croaker (*Otolithes ruber*). Food science and biotechnology. 2016;25(5):1251-1257.
20. Yongsawatdigul J, Park JW. Effects of Alkali and Acid Solubilization on Gelation Characteristics of Rockfish Muscle Proteins. Journal of Food Science. 2004;69(7):499–505.
21. Zynudheen AA. Utilization of fishery waste in India. Central Institute of Fisheries Technology. Conservation and Sustainability of Coastal Living Resources of India, 1-3 December 2009, Cochin. 2010.