Physicochemical, morphological and functional properties of protein isolates obtained from four fish species

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Abstract In this study, we investigated the physicochemical, morphological and functional characteristics of fish protein isolates (FPIs) prepared from four fish species through alkali solubilization and isoelectric precipitation. Significant differences were observed in the protein content, physicochemical, morphological and functional characteristics among FPIs. Morphology and particle size analysis revealed a significant difference in the shape and size of FPIs. Lipid oxidation of FPIs varied between 0.206 and 0.305 mg MDA/kg. Furthermore, all FPIs exhibited two broad diffraction peaks at $\sim 8.6^\circ$ and 19.3° , revealing their amorphous nature. A significant difference was observed in the thermal properties of selected samples, including T_0 , T_P , T_C , and ΔH values. T_0 , T_P , T_C , and ΔH of the endothermic transition varied in the range of 40.9 – 43.0°C , 64.1 – 66.2°C , 99.1 – 100.6°C , and 237.1 – 267.2 J/g , respectively. All FPIs exhibited high water/oil absorption capacity, emulsifying capacity, foam stability and foaming capacity, signifying proteins potential to act as functional ingredients in the food industry.

Keywords Fish protein isolates · Functional properties · Morphology · Particle size · Thermal properties

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Introduction

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). In general, protein providing energy in terms of calories is redundant but contribution to protein synthesis is vital; thus, it plays crucial roles in normal development and maintenance. The physicochemical and sensory characteristics of any protein-rich food contribute to the overall structural behavior of the food (Foh et al. 2012). Based on their biological characteristics, sources of dietary protein can be classified into functional health-promoting foods (Kadam and Prabhasankar 2010).

The extraction of proteins from fish muscles is performed through several techniques, including repeated water washing and refining methods by the acid/enzyme hydrolysis method, the solvent extraction process and pH-shift method (Kobayashi and Park 2017; Shaviklo et al. 2010). A huge market exists for the utilization of processed fish by-products, including skin, viscera, bones, and product enhancement, but it is still in the nascent stage because during isolation, increased levels of lipids, microorganisms, and pro-oxidants are present as residues, which have negative effects on the quality and safety of the hydrolysate product (Campos et al. 2011). This problem can be avoided using the pH-shift method, which is based on the isoelectric solubilization and precipitation process. Alkali or acid solubilization is performed at the initial stage of processing after the homogenization of the raw meat, and all soluble proteins are extracted separately using high-speed centrifugation (Yongsawatdigul and Park 2004). In general, adjusting the pH to its isoelectric point leads to the

precipitation of protein. Furthermore, protein has been extracted from marine fish species, including silver carp and common carp by using the pH-shift method (Tian et al. 2017).

Food manufacturers fortify their processed foods with functional ingredients so that health-conscious consumer's needs are met. In the food industry, various technologies are used to incorporate the functional fish ingredient during processing. Functional fish ingredients as antioxidants or protein boosters or are used as nutraceutical ingredients. The common functional ingredients incorporated into food during processing include soy protein, fish protein and fish oils (Foh et al. 2012). The white flesh and low-fat fish species were contemplated the most suitable fish species for developing functional ingredients. The source and processing technique involved in isolating protein from fish determine the quality and characteristics of the fish protein used as a functional ingredient (Shaviklo et al. 2011).

Marine fish production accounts for 36 percent of the total fish production in India. Although inland fish contribute to a higher production rate in India, preference and consumption are always the highest for marine fish. Among various commercially important marine fish, Indian mackerel, squid, ponyfish, catfish, sardine, lobster, shrimp, and certain types of pelagic fish species with the highest consumption worldwide. The processing and preservation of fishes has been piloted since ancient times, but the isolation of proteins is a newer concept. Protein isolation is an essential process, as protein isolates are functional ingredients that contribute to the well-being of humans.

In this study, we used the alkali solubilization and precipitation technique to isolate proteins from catfish, Indian mackerel, striped ponyfish and sardine. We also investigated the physicochemical, morphological and functional properties of the protein isolates.

Materials and methods

Materials

Four fish species, namely catfish (*Batrachiocephalus mino*), Indian mackerel (*Rastrelliger kanagurta*), ponyfish (*Aurigequula fasciata*), and sardine (*Sardinella brachysoma*) were purchased from a local fish market in Puducherry, India. Fish samples were washed with a large quantity of water and were eviscerated and filleted, and the fillets were utilized in the preparation of fish protein isolates (FPIs). All chemicals and reagents were of analytical grade and were obtained from Hi-Media Chem. Co., Mumbai, India.

Preparation of fish protein isolates

The extraction of FPIs was done by the method described by Kobayashi and Park (2017) with minor modifications. Briefly, fish fillets were homogenized with ice-cold deionized water (1:9 ratio) in a homogenizer (Ika Ultra Turrax T25, Germany) for 3 min. The pH of the suspension was adjusted up to 11.5 using 1 N NaOH. The homogenate was centrifuged (C-24BL, Remi, India) at $8000 \times g$ for 20 min 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 using cheesecloth and Whatman no. 1 paper to remove neutral lipids and solid materials, particularly skin, bone, and connective tissue. Subsequently, the filtrate pH was adjusted to 5.5 using 1 N HCl and the filtrate was centrifuged at $8000 \times g$ for 15 min at 4 °C. After centrifugation, the obtained supernatant was removed and the precipitate was neutralized, freeze-dried and stored at -20 °C until analysis. The samples were named as catfish protein isolate (FPIC), Indian mackerel fish protein isolate (FPIIM), ponyfish protein isolate (FPIP), and sardine fish protein isolate (FPIS).

Chemical composition

The chemical composition, including moisture, ash, protein and lipid contents of FPIs, was analyzed using standard AOAC methods (AOAC 1990).

Physicochemical 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 preweighed 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

To analyze the pH of FPIs, accurately 10.0 g of the samples was weighed and mixed with 50 mL of deionized water; the mixture was stirred well for 5 min, and the suspension pH was measured using a digital pH meter (MetrohmAG, Switzerland).

Water activity

Using a water activity meter (Aqualab Series 4TE, Decagon Devices, Inc., USA), the water activity of FPIs were determined at room temperature until the values were concurrent.

Color analysis

Using a colorimeter (D-25, Hunter Associates Laboratory, USA), the color of FPIs were analyzed from three dimensions: 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 $H^\circ = \tan^{-1}(b^*/a^*)$, respectively.

Functional characteristics

Water-holding capacity

The water-holding capacity (WHC) of FPIs were analyzed following the procedure of Özyurt et al. (2015). In this assay, 2.0 g of the sample was dispersed in 20 mL of deionized water, stirred for 20 min at 30 °C, and centrifuged at $3000 \times g$ for 15 min. The WHC is expressed as mL of water absorbed/g of sample.

Oil-holding capacity

The oil-holding capacity (OHC) of FPIs were analyzed following the procedure described by Özyurt et al. (2015). The accurately weighed (1.0 g) sample was dispersed in 10 mL of vegetable oil, stirred well for 5 min, and centrifuged at $3000 \times g$ for 15 min. The OHC was displayed as the weight difference.

Emulsifying capacity

The emulsifying capacity (EC) of FPIs were determined according to the procedure of Özyurt et al. (2015). In this assay, 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 $5000 \times g$ for 10 min, 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 using the following equation. $EC \text{ (mL/100 g)} = (\text{Height of emulsifier layer/Height of total volume}) \times 100$.

Foam measurements

The foaming capacity (FC) and foam stability (FS) of FPIs were analyzed according to the method of Foh et al. (2012). In this assay, 1.0 g of FPIs was added to 50 mL of distilled water in a 100-mL graduated cylinder. The mixture was stirred for 3 min, and the generated foam volume was noted and was considered as FC. Furthermore, the foam volume noted after 15, 20 and 30 min was considered the percentage of FS.

Particle size analysis

The particle size of FPIs were analyzed using a Zetasizer (Malvern Instruments Co. Ltd., Worcestershire, UK), and ethylene glycol were used as the dispersant. The protein suspension was sonicated for 3 min and was used in the analysis. The resulting data were analyzed using MasterSizer software (Version 5.54, Malvern Instruments Co. Ltd., Worcestershire, UK), and the particle size of FPIs is displayed in nm.

Morphological characteristics

The morphologies of FPIs were observed under a scanning electron microscope (SEM; Hitachi, S-3400H, Tokyo, Japan) at an accelerating voltage of 15 kV. For analysis, the samples were sprinkled on double-sided sticky tape placed on aluminum stubs and were coated with a carbon layer.

Protein crystallinity

The X-ray diffraction (XRD) patterns of FPIs were obtained using a diffractometer (Shimadzu, XRD 7000) with a copper anode with a $K\alpha$ value of 1.54. The diffractograms were recorded between 10° and 80° (2 θ), with a speed of 1.5°/min at 40 kV and 30 mA.

Thermal characteristics

The thermal characteristics of FPIs were examined using a differential scanning calorimeter (DSC, TA-Q20, Newcastle, USA). Approximately 3.0 mg of the sample was mixed with 7.0 μ L of distilled water, placed in an aluminum pan, and equilibrated for 2 h at 4 °C. The pan was heated at a rate of 10 °C/min from 30 to 200 °C. An empty pan was used as the reference. From the DSC curve, the transition temperatures and enthalpy values were determined using Universal Analysis 2000 3.9A software.

Lipid oxidation

Thiobarbituric acid reactive substances (TBARS) of FPIs were analyzed using the procedure described by Qiu et al. (2015). After the addition of HCl, the sample (10 g) was distilled; subsequently, deionized water and thiobarbituric acid (0.02 M, 10 mL) were added to the distillate. The mixture was placed in a water bath at 100 °C for 30 min, and absorbance was measured at 538 nm. Malondialdehyde-bis-(dimethyl acetate) was used as the standard.

Statistical analysis

In this study, all assays were performed in triplicate. The resulting data are expressed as mean with standard deviation. The variance between the mean values was considered significant at $p < 0.05$. The data were analyzed using SPSS 20.0 (SPSS Inc. Chicago, USA).

Results and discussion

Chemical composition

The chemical composition of FPIC, FPIIM, FPIP and FPIS is shown in Table 1. The chemical composition, including moisture, protein, ash and lipid contents were found to significantly differ among FPIs isolated in the present study ($p < 0.05$). Alkali extraction method resulted in 89.70 g/100 g protein content in FPIP followed by 87.27 g/100 g in FPIIM, 86.47 g/100 g in FPIC and 84.74 g/100 g in FPIS ($p < 0.05$). Table 1 also presents 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). The ash content of FPIIM (0.91 g/100 g) was the highest, followed by FPIP (0.90 g/100 g), and the lowest values ($p < 0.05$) were noted for FPIC (0.83 g/100 g) and FPIS (0.88 g/100 g). Furthermore, the lipid content of FPIS (2.65 g/100 g) was higher than that of FPIC (2.12 g/100 g), followed by FPIIM (1.86 g/100 g), and the lowest value was noted for FPIP

(1.61 g/100 g). The variation in chemical composition in FPIs could be attributed to the variety of fish species, which could also be presumed to contribute to the efficiency of alkali extraction method as observed from chemical composition varying to the species of fishes.

Physicochemical 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. Bulk density indicates the behavior of the product and the influence of numerous factors including the preparation method, drying procedure, fineness of particles, and moisture content (Foh et al. 2012). As shown in Table 2, bulk density of FPIC, FPIIM, FPIP and FPIS were 338.3 kg/m³, 310.2 kg/m³, 269.3 kg/m³ and 359.6 kg/m³, respectively, and found to differ significantly ($p < 0.05$). The variance in the bulk density of protein isolates is possibly due to the structure of proteins. Associated factors such as particle size, number of contact point of protein isolate, intensity of attractive inter particle forces and their combined effects play a vital role in determining the bulk density. Paste thickness is one important parameter that is desirable in product development for convalescent and infant formulas (Ogunwolu et al. 2009). Protein isolates with higher bulk density are desirable, as it aids in reducing paste thickness. Furthermore, the bulk density of protein isolates is influenced by the hydrophobicity, solubility, and hydrodynamic properties of proteins (Mohamed et al. 2009).

pH and water activity

The pH and water activity of FPIs is shown in Table 2. FPIs has the acidic pH values as pH value were 5.70, 5.52, 5.51 and 5.65 for FPIC, FPIIM, FPIP and FPIS respectively. Furthermore, FPIC had the highest pH, and the

Table 1 Chemical composition of protein isolates from different fish species

Sample	Chemical composition			
	Moisture (g/100 g)	Proteins (g/100 g)	Ash (g/100 g)	Lipids (g/100 g)
FPIC	5.86 ± 0.09 ^b	86.47 ± 0.12 ^c	0.83 ± 0.05 ^c	2.12 ± 0.04 ^b
FPIIM	5.71 ± 0.07 ^c	87.27 ± 0.05 ^b	0.91 ± 0.10 ^a	1.86 ± 0.03 ^c
FPIP	5.65 ± 0.04 ^d	89.70 ± 0.10 ^a	0.90 ± 0.03 ^a	1.61 ± 0.19 ^d
FPIS	6.05 ± 0.05 ^a	84.74 ± 0.02 ^d	0.88 ± 0.02 ^b	2.65 ± 0.15 ^a

Values expressed are mean ± SD. Values with the same superscripts in a column did not differ significantly ($p < 0.05$) by DMRT

FPIC protein isolate from catfish, FPIIM protein isolate from Indian mackerel, FPIP protein isolate from pony fish, FPIS protein isolate from sardine

Table 2 Physico-chemical characteristics of protein isolates from different fish species

Parameters	Types of fish protein isolates			
	FPIC	FPIIM	FPIP	FPIS
Bulk density (kg/m ³)	338.3 ± 3.21 ^c	310.2 ± 5.12 ^b	269.3 ± 8.12 ^a	359.6 ± 5.32 ^d
pH	5.70 ± 0.15 ^c	5.52 ± 0.10 ^a	5.51 ± 0.17 ^a	5.65 ± 0.25 ^b
Water activity	0.343 ± 0.05 ^a	0.323 ± 0.02 ^b	0.315 ± 0.06 ^c	0.339 ± 0.07 ^a
<i>Color parameters</i>				
<i>L*</i>	52.3 ± 0.38 ^b	58.8 ± 0.54 ^a	57.4 ± 1.66 ^a	50.6 ± 0.20 ^c
<i>a*</i>	1.48 ± 0.03 ^c	1.59 ± 0.15 ^b	1.68 ± 0.05 ^a	1.34 ± 0.10 ^d
<i>b*</i>	25.2 ± 0.59 ^a	19.4 ± 0.10 ^b	19.6 ± 0.81 ^b	25.8 ± 0.20 ^a
Chromaticity	25.26 ± 0.25 ^b	19.92 ± 0.10 ^c	19.73 ± 0.81 ^c	25.83 ± 0.27 ^a
Hue angle (°)	86.62 ± 0.14 ^b	85.45 ± 0.11 ^c	85.11 ± 0.29 ^d	87.01 ± 0.18 ^a

Values expressed are mean ± SD. Values with the same superscripts in a row did not differ significantly ($p < 0.05$) by DMRT

FPIC protein isolate from catfish, FPIIM protein isolate from Indian mackerel, FPIP protein isolate from pony fish, FPIS protein isolate from sardine

lowest pH was observed for FPIIM and FPIP. The pH values of FPIs play a significant role in determining their shelf life and foaming and emulsification properties.

In general, the shelf life of food system is calculated based on its water activity, which is an intrinsic property that denotes the availability of free water in the food system. In the food system, lower water activity provides protection from microbial growth and contamination and delays deterioration through biochemical reactions (Primo-Martín et al. 2010). As shown in Table 2, FPIC, FPIIM, FPIP and FPIS had varying water activities of 0.343, 0.323, 0.315 and 0.339, respectively, with a significant difference ($p < 0.05$). In a food system, moisture migration between domains can be avoided by adding an edible layer between the domains, resulting in a change in the water activity of the food ingredients (Suriya et al. 2017).

Color analysis

In addition to taste and texture, color parameters are important characteristics that influence the acceptability of food products. Color parameters (L^* , a^* and b^*) of FPIs from four fish species is presented in Table 2. Additionally, these values were used to determine color properties such as chromaticity and the hue angle. As shown in Table 2, FPIIM had the highest L^* value (58.8), followed by FPIP (57.4) and FPIC (52.3). The lowest L^* value was noted for FPIS (50.6). These lightness values are lower than those of Alaska pollock protein (76.0) (Sathivel and Bechtel 2006), whereas these values are higher than those of saithe protein (41.2) (Shaviklo et al. 2012). The a^* values of FPIP, FPIIM, FPIC and FPIS were up to 1.68, 1.59, 1.42 and 1.34, respectively, with a significant difference ($p < 0.05$). These values are comparable to those of kidney bean and pea proteins (Shevkani et al. 2015), whereas these values

are higher than those of saithe fish protein (− 1.12) (Shaviklo et al. 2012). Furthermore, significant differences were noted in b^* values among FPIs, and the values ranged between 19.4 and 25.8. Chromaticity was calculated as chroma (C^*), which denotes the fullness of color. The protein isolates from sardine fish exhibited maximum chromaticity (25.83). Hue angle represents the perception of color. From the results, isolated samples were found to lie in the first quadrant of the hue angle (0°–90°) corresponding to the range of reddish-purple to yellow.

Functional properties

Water- and oil-holding capacity

The WHC of FPIC, FPIIM, FPIP and FPIS is shown in Table 3. From the findings, WHC values of all FPIs ranged between 4.23 and 4.80 mL/g, with a significant difference ($p < 0.05$). FPIP had the highest WHC value, and the lowest value was observed for FPIS. These values are slightly higher than those of tilapia fish protein (2.63 mL/g) (Foh et al. 2012), whey protein (0.5 mL/g) (Kaushik et al. 2016), soy protein (4.05 mL/g), and wheat protein (3.67 mL/g) (Ashraf et al. 2012). In general, the binding capacity between food materials and water molecules plays a major role in food systems because it improves mouth-feel, flavor retention, and texture. Additionally, the carbohydrate content greatly influences the WHC of foods.

Furthermore, the OHC determines the capacity of food materials to absorb oil. As shown in Table 3, FPIIM, FPIP, FPIC and FPIS had varying OHC values of 5.83 mL/g, 5.71 mL/g, 5.52 mL/g and 5.32 mL/g respectively with a significant difference ($p < 0.05$). These values are higher than those of quinoa protein (1.88 mL/g) (Elsahaimy et al. 2015), tilapia fish protein (3.38 mL/g) and soya protein

Table 3 Functional characteristics of fish protein isolates from different fish species

Parameter	Type of fish protein isolates			
	FPIC	FPIIM	FPIP	FPIS
WHC (mL/g)	4.47 ± 0.08 ^b	4.70 ± 0.10 ^a	4.80 ± 0.05 ^a	4.23 ± 0.15 ^c
OHC (mL/g)	5.52 ± 0.10 ^b	5.83 ± 0.05 ^a	5.71 ± 0.16 ^a	5.32 ± 0.10 ^c
EC (mL/100 g)	82.3 ± 0.09 ^b	82.5 ± 0.12 ^b	84.3 ± 0.15 ^a	77.9 ± 0.13 ^d
FC (mL/100 g)	46.2 ± 0.10 ^b	45.2 ± 0.17 ^c	47.7 ± 0.20 ^a	44.2 ± 0.12 ^d
FS at 15 min (mL/100 g)	32.6 ± 0.16 ^b	32.5 ± 0.20 ^b	34.0 ± 0.15 ^a	30.5 ± 0.13 ^c
FS at 20 min (mL/100 g)	22.0 ± 0.17 ^b	21.1 ± 0.15 ^c	22.5 ± 0.17 ^a	0.3 ± 0.17 ^d
FS at 30 min (mL/100 g)	14.8 ± 0.12 ^b	16.5 ± 0.20 ^a	17.1 ± 0.15 ^a	15.3 ± 0.08 ^b

Values expressed are mean ± SD. Values with the same superscripts in a row did not differ significantly ($p < 0.05$) by DMRT

FPIC protein isolate from catfish, FPIIM protein isolate from Indian mackerel, FPIP protein isolate from pony fish, FPIS protein isolate from sardine, WHC water holding capacity, OHC oil holding capacity, EC emulsifying capacity, FC foaming capacity, FS foaming stability

(2.81 mL/g) (Foh et al. 2012). The OHC of food materials is affected by numerous factors such as type and amount of proteins, degree of hydrolysis, temperature and type of oil (Cumby et al. 2008). Furthermore, the greater capability of FPIs to hold or absorb water and oil improves their application in the formulation of food products such as cakes, sausages and mayonnaise, there by improving their functional characteristics.

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 can be influenced by the isolation procedure and the physico-chemical properties of proteins (i.e., size and type, pH, hydrophobicity, solubility and surface charge of proteins). The EC of FPIC, FPIIM, FPIP and FPIS was up to 82.3 mL/100 g, 82.5 mL/100 g, 84.3 mL/100 g, and 77.9 mL/100 g, respectively (Table 3), with a significant difference ($p < 0.05$). These values are higher than those of soy protein (52.5 mL/100 g) and marama protein (53.4 mL/100 g) (Gulzar et al. 2017), whereas they are comparable to those of tilapia fish protein (83.7 mL/100 g) (Foh et al. 2012). The interaction between the water and oil phase is higher in protein isolates with higher solubility.

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 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 (Table 3), with a significant difference ($p < 0.05$). From the findings, the FC of protein isolates was greatly improved with the increase in protein concentration. In addition, the FC values are lower than those of quinoa protein (58.37 mL/100 mL) (Elsohaimy et al. 2015).

Furthermore, the FS of all FPIs 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 min, respectively, with a significant difference ($p < 0.05$). Foam formation of proteins can be regulated by three major factors including transportation, penetration, and reorganization of molecules at the air/water interface (Wilde and Clark 1993). In addition, these processes are influenced by numerous factors, such as protein solubility, equilibrium between the rigidity and flexibility of proteins, pH, hydrophobicity, temperature and ionic strength (Shevkani et al. 2015).

Particle size distribution

Particle size distribution is one of the primary characteristic of proteins and can affect the functional properties of food systems. The mean particle size of all FPIs is presented in Table 4. From the findings, the particle size distribution of FPIC, FPIIM, FPIP and FPIS was up to 704.85 nm, 1453.23 nm, 1957.32 nm and 584.01 nm, respectively, with a significant difference ($p < 0.05$). These values are slightly higher than those of soy protein (~ 100 nm) (Morales et al. 2015) and pea protein (474.7 nm) (Zhang et al. 2014). The difference in the particle size distribution of FPIs may be because of the formation of aggregates through precipitation or treatment during the isolation of proteins.

Table 4 Particle size, lipid oxidation and thermal properties of fish protein isolates

Parameter	Type of fish protein isolates			
	FPIC	FPIIM	FPIP	FPIS
Particle size (nm)	704.85 ± 13.2 ^c	1453.23 ± 10.4 ^b	1957.32 ± 12.7 ^a	584.01 ± 13.5 ^d
TBARS (mg MDA/kg)	0.255 ± 0.010 ^b	0.231 ± 0.021 ^c	0.221 ± 0.015 ^d	0.287 ± 0.018 ^a
<i>Thermal properties</i>				
T ₀ (°C)	41.07 ± 0.10 ^b	42.85 ± 0.15 ^a	41.84 ± 0.02 ^b	41.92 ± 0.05 ^b
T _P (°C)	64.24 ± 0.14 ^b	64.79 ± 0.05 ^b	64.53 ± 0.03 ^b	65.60 ± 0.60 ^a
T _C (°C)	99.90 ± 0.05 ^b	100.3 ± 0.21 ^a	99.78 ± 0.07 ^b	100.5 ± 0.03 ^a
ΔH (J/g)	267.3 ± 0.27 ^a	255.2 ± 0.32 ^b	247.2 ± 0.35 ^c	237.6 ± 0.45 ^d

Values expressed are mean ± SD. Values with the same superscripts in a row did not differ significantly ($p < 0.05$) by DMRT

FPIC protein isolate from catfish, *FPIIM* protein isolate from Indian mackerel, *FPIP* protein isolate from pony fish, *FPIS* protein isolate from sardine, *T₀* onset temperature, *T_P* peak temperature, *T_C* conclusion temperature, ΔH enthalpy, TBARS thiobarbituric acid reactive substances

Morphological characteristics

Figure 1 depicts the surface morphology of FPIC, FPIIM, FPIP and FPIS isolated using the alkaline method. No significant difference was observed in shape; particles in all isolated proteins showed a flaked structure. Moreover, the surface of particles was smooth without wrinkles and folding. However, the particle size showed differences,

which may be because of differences in the aggregation of protein particles. Particle size may influence the dissolution rate of the isolate protein besides other factors. Extensive cellular disruption as reported in surface morphology may contribute to the movement of cell constituents into the aqueous phase resulting in enhancement of the extraction yield of protein. FPIC had a relatively larger particle size.

Fig. 1 Scanning electron micrographs of fish protein isolates: *FPIC* protein isolate from catfish, *FPIIM* protein isolate from Indian mackerel, *FPIP* protein isolate from ponyfish, *FPIS* protein isolate from sardine

Foh et al. (2012) reported similar morphological characteristics of protein isolates from *Tilapia* fish.

X-ray diffraction

The XRD patterns of different FPIs is shown in Fig. S1. From the findings, all FPIs exhibited low diffraction peaks at approximately 8.6° and 19.5°. The diffraction profile revealed the partial crystallinity of FPIs. Diffraction curves of FPIC, FPIIM, FPIP and FPIS showed some resemblance in their shapes. Typically, food materials in the solid state may be either crystalline, semi-crystalline, or amorphous. The XRD patterns of FPIs are slightly different from that of soy protein isolate, which displays three peaks at 8.5°, 19.5° and 24.4° (Wang et al. 2006). As shown in Fig. S1, all FPIs (FPIC, FPIP, FPIIM and FPIC) exhibited only two diffraction peaks, and the peak at approximately 24.4° was absent. Furthermore, all FPIs showed XRD profiles with similar intensities and shapes. Previous studies have reported two similar peaks at the same diffraction angles (8.5° and 19.5°) observed in the present study for protein isolate from cotton seed meal (Ma et al. 2018).

Thermal properties

The thermal transition properties of FPIs (FPIC, FPIIM, FPIP and FPIS) is presented in Table 4. From the DSC thermogram, during thermal scanning, all FPIs exhibited a single endothermic transition with higher enthalpy. Transition temperatures indicate the thermal stability of proteins. Previous studies reported that the denaturation of meat proteins occurs in three regions of the DSC curve: peaks at ~ 54–58 °C (myosin), 65–67 °C (collagen and sarcoplasmic proteins) and 80–83 °C (actin) (Tornberg 2005). As shown in Table 4, different FPIs showed significant variations in their onset, peak, and conclusion temperatures (denoted as T_0 , T_p , and T_c , respectively). The gelatinization temperatures, such as T_0 , T_p , and T_c , and ΔH were 41.07–42.85 °C, 64.24–65.60 °C and 99.78–100.5 °C and 237.1–267.2 J/g, respectively. These values are comparable to those of the tilapia FPI (Foh et al. 2012). Due to the lower denaturation temperature, FPI utilization is limited in the food industry because FPIs are unstable against heating, organic solvents, and proteolysis. Furthermore, the difference in the transition temperatures of FPIs might be due the difference in isolation procedure, and protein concentration used for testing.

Lipid oxidation of FPIs

The primary factor limiting the utilization of processed fish by-products in food industry is their susceptibility to lipid oxidation, which directly influences the shelf life of

the product. The removal of the heme protein and iron in the alkali solubilization process may be the reason for the high oxidative stability of the recovered protein. Dark meat contains the other heme protein, namely myoglobin, which functions as a pro-oxidant in the muscle tissues of silver carp (Kristinsson and Liang 2006). Alkali-treated mullet and spanish mackerel showed lower TBARS than acid-processed mullet and spanish mackerel (Li et al. 2013). The lipid oxidation of protein isolates was evaluated by calculating the TBARS level (Table 4). This level is an indication of the concentration of MDA, which is one of the major end products of lipid autoxidation. The alkaline-aided method has been reported to reduce fat and active pro-oxidants in protein isolates (Tokur and Korkmaz 2007). The MDA level was significantly different among the four protein isolates, and it ranged from 0.206 to 0.305 mg MDA/kg FPI. FPIS showed the highest MDA level, and lowest level was found for FPIP. The variation in the MDA level may be due to the total lipid content has been present in on the protein isolates and the roll of polar lipids in the isolates enough, leading to a significant increase in oxidation products.

Conclusion

This study demonstrated that alkali-aided processing and isoelectric precipitation can be successfully used to extraction of protein isolates from four fish species, namely catfish, Indian mackerel, ponyfish, and sardine. The results revealed that FPIP and FPIIM exhibited more favorable physicochemical properties than FPIC and FPIS. Low lipid oxidation of protein isolates prepared through the pH-shift process replicates their functional characteristics. XRD showed the amorphous nature of FPIs. The DSC thermogram of the FPIs samples showed a single endothermic transition with higher enthalpy. Moreover, all FPIs displayed high EC, WHC, OHC and FS. Therefore, the pH-shift process can be used as a powerful tool to recover functional proteins from fish species. Hence the study evidenced the alkali extraction method in the isolation of fish protein isolate with favourable physiochemical and functional characteristics to be applicable in the development of protein rich food products satisfying the present need of isolation of functional nutrients in the arena of functional food.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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