

Article

Recovery and Characterization of Calcium-Rich Mineral Powers Obtained from Fish and Shrimp Waste: A Smart Valorization of Waste to Treasure

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Abstract: With the increase in global aquaculture production, managing waste from aquatic biomass has become a significant concern. This research aimed to develop a sustainable valorization approach for recovering calcium-rich fish, including mackerel tuna and pangas bone and shrimp shell powders. The powders were characterized by various physicochemical and nutritional parameters, including proximate composition, amino acids, protein solubility, water holding capacity (WHC), oil holding capacity (OHC), and heavy metal contents. Color analysis and structural examination were carried out using field emission scanning electron microscopy (FE-SEM), energy dispersive X-ray spectroscopy (EDX), and Fourier-transform infrared spectroscopy (FT-IR), and in vitro radical scavenging activity was assessed. Significant protein content was observed in the powders, which was highest in shrimp shell powder (SSP) at 37.78%, followed by 32.29% in pangas bone powder (PBP) and 30.28% in tuna bone powder (TBP). The ash content was consistent in PBP and TBP at around 62.80%, while SSP had a lower ash content of 36.58%. Amino acid analysis detected 14 different amino acids in the recovered powders. Notably, SSP demonstrated the highest WHC and OHC values (2.90 and 2.81, respectively), whereas TBP exhibited the lowest values (1.11 for WHC and 1.21 for OHC). FE-SEM revealed the compact structure of TBP and PBP, contrasting with the porous surface of SSP. EDX analysis indicated higher calcium (24.52%) and phosphorus (13.85%) contents in TBP, while SSP was enriched in carbon (54.54%). All detected heavy metal concentrations were within acceptable limits. The recovered powders demonstrated significant ABTS free radical scavenging activity. The findings of this study suggest the suitability of the recovered powders for various food and pharmaceutical applications.

Keywords: fish bone powder; shrimp shell powder; fish waste; minerals; valorization; food applications

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1. Introduction

The global production of aquatic animals was estimated at about 178 MT (million tons) in 2020; over 157 MT were used for human consumption and the remainder were prescribed for non-food uses [1]. The continuation of processing of fisheries products has been responsible for a rising amount of by-products, which may represent up to 70% of processed fish, depending on the size, species, and type of processing [2]. The by-products are usually composed of heads (9–12%), viscera (12–18%), skin (1–3%), bones (9–15%), and scales (5%). Generally, these by-products are discarded in open areas, polluting the entire environment, or used for low-cost applications such as animal feed or on agricultural farms. To mitigate these issues, scientists are looking for alternative uses, such as the production of edible-grade fish bone powder. Due to their high mineral content, fish bones

are very interesting by-products that can be transformed into value-added products to achieve environmentally sustainable fishing and waste management [3].

Fish bones are the non-edible parts that are separated from the skeletal frame after removing the muscle. Fish bone powder, a by-product of the fish processing industry, is obtained through drying and grinding fish bones into a fine powder. Many studies have demonstrated the bioavailability of the inorganic constituents of fish bones, suggesting potential health advantages for both animals and humans [4]. Fish bone powder is rich in essential minerals like calcium (Ca), phosphorus (P), iron (Fe), and bio-functional collagen protein [5]. Earlier research indicated that fish bones boast high levels of minerals (21% to 57%), bioactive organic matter (between 20% and 30%), and trace amounts of carbohydrates [6]. Calcium, along with vitamin D, is essential for maintaining optimal bone health and achieving proper bone mass. Certain food ingredients and dietary adjustments can enhance bone density and prevent conditions like osteoporosis by ensuring sufficient calcium levels through natural pathways. Additionally, fish bone could serve as a valuable food ingredient or supplement of high quality, potentially aiding in preventing calcium deficiency and lowering the risk of osteoporosis [7]. Some researchers have utilized fish bone as a food ingredient in noodles, cookies, and crispy rice production; others have evaluated fish bone in food supplement production [3]. As a result of these studies, fish bones are suggested to be high Ca^{2+} food ingredients that can be integrated successfully into food and food supplement production. Therefore, fish bones and shrimp shell powders are applicable in the formulation of various food products as sources of biofunctional compounds, in food fortification, and in skin care or cosmetics. On the other hand, studies on the bioavailability of fish bones in the human body have shown that the calcium in fish bone is a well-absorbed source of Ca^{2+} [8].

The panga fish (*Pangasianodon hypophthalmus*) is one of the most popular riverine catfish species in Bangladesh, and is subject to huge demand worldwide due to its superior taste. It is commonly used as raw material in the surimi or fillet industries. Pangas weighing 1–2 kg consist of 47–55% flesh, with the remainder being waste such as head and bone (23–27%), abdominal fat tissue (5–6.5%), gills, and viscera (7–14%) [9], of which the head and bone parts can be used for fish bone powder. Regarding mackerel tuna (*Euthynnus affinis*), commercial processing of tuna either as canned tuna or tuna loins generates significant quantities of waste in the form of belly flap, off-cut meat, off-cut mince from the bone, meat mince, blood meat, head, gut, tail, skin, and bone. By-products of mackerel tuna provide a good source of fish bone powder [10,11]. According to a previous study, tuna bone powder is rich in protein, beneficial fat, calcium, and phosphorus [12]. Tiger shrimp (*Penaeus monodon*) is a marine crustacean and one of the most exportable seafood species in Bangladesh. The shrimp processing industries also produce a large quantity of waste, varying from 40–80% depending upon species and process. This waste comprises 48.5–56% shrimp raw material by weight, depending on the species [13]. The main components of shrimp head are protein (54.4%), minerals (21.1%), lipids (11.9%), vitamins (9.3%), and small quantities of valuable carotenoids [14]. There is a glorious opportunity to utilize this shrimp waste in the manufacture of value-added human food such as shrimp shell powder.

Despite various advantages, there have been very limited studies on the preparation and characterization of fish bones and shrimp shell powders, with some notable exceptions such as silver carp bone, grass carp bone powder, blue whiting fish bone powder, and seabream bone powder for food-grade applications [15–19]. Therefore, the aim of this study was to prepare bone powders from two different fish species, i.e., pangas fish (*P. hypophthalmus*) and mackerel tuna fish (*E. affinis*), and shrimp shell powder from tiger shrimp (*P. monodon*), and compare the various physicochemical characteristics, including proximate composition, amino acid contents, protein solubility, water and oil holding capacity, minerals and heavy metal contents, using FESEM-EDX analysis, FT-IR spectroscopy, color measurement, and free radical scavenging analysis.

2. Materials and Methods

2.1. Collection and Preparation of Sample

Fresh pangas fish (*P. hypophthalmus*) with a mean body weight of 2.1 ± 0.23 kg, mackerel tuna (*E. affinis*) with a mean body weight 2.24 ± 0.14 kg, and shrimp (*P. monodon*) with a mean body weight of 34.21 ± 6.23 g were purchased from a fish market named Boro Bazar in Jashore, Bangladesh. Tuna was harvested 200 nautical miles beyond the coastline and up to 200 m depth. Pangas and shrimp originated from a freshwater fish farm and coastal shrimp farm, respectively. The fishes and shrimp were brought to the laboratory in an insulated box with ice. The fishes and shrimp were thoroughly washed up with tap water to remove dirt and contaminants on the body surface. Then, the fishes were gutted and the bones and muscles were separated by filleting. Shrimp shells were also removed from the shrimp bodies. All these bones and shells were then subjected to alkaline treatment by boiling them with 0.2% NaOH at 90 °C for 30 min. The bones and shells were then rinsed with cold water several times and the water was drained properly. After that, they were autoclaved for 1 h (121 °C and 15 psi) and dried in a hot air oven for 8 h at 80 °C. The samples were ground using a pulverizer (Model: LG-30, Baixin Yaoji, Zhejiang, China) for 2 min to turn the bones and shell into a fine powder, which was then screened through a 297 µm sieve (H-4325, MATEST, Treviolo, Italy).

2.2. Proximate Composition Analysis

The proximate composition including moisture, protein, lipid, and ash content of the samples was analyzed according to the guidelines provided by the Analytical Methods Committee [20], with slight modifications. The protein content of the samples was determined via the micro-Kjeldahl method using acid digestion (Kjeldhal digestion unit, Ra-158, Delhi, India), a Buchi distillation unit (K-350), and titration with N/10 NaOH. The moisture content was measured gravimetrically by drying at 105 °C for 12 h using a hot air oven (Mod: PSO-451, Company: MART, India). Ash content was determined via the gravimetric method, incinerating the residue by heating at 550 °C for 12 h in a muffle furnace (Model: L5/11, Nabertherm GmbH, Lilienthal, Germany). Lipid content was assessed using a Soxhlet apparatus (64825 Supelco, Sigma Aldrich, St. Louis, MO, USA), with *n*-hexane employed as the extracting agent.

2.3. Determination of Amino Acid Composition

The amino acid content of the fish bone and shrimp shell powders was analyzed following a previous method with some adjustments [21]. A high-speed amino acid analyzer (Model: LA 8080, Hitachi, Tokyo, Japan) equipped with a Hitachi high-performance cation-exchange column operating at a column temperature of 57 °C was employed. Exactly 1 g of prepared sample was combined with 25 mL of 6 M HCl in a glass tube. Subsequently, the tube underwent heating at 110 °C for 24 h using a heated sand bath. Following this, the solid residue was obtained by evaporating the excess HCl and then diluted with 6 mL of distilled water, before filtration using a 0.45 µm syringe filter.

2.4. Determination of Protein Solubility

The soluble protein content of fish bone and shrimp shell powders in water was determined using the method described by Wu et al. [22] with some modifications. The fish bone and shrimp shell powders were dispersed in water (1:10, *w/v*) and stirred at 300 rpm for different times (20, 40, 60, 80, and 100 min) at 50 °C. The mixture was then centrifuged at 3000 rpm for 10 min. The soluble protein content in the supernatant was determined using the Lowry method. The protein solubility of fish bone and shrimp shell powders in water was determined following Equation (1):

$$\text{Protein solubility (\%)} = \frac{\text{Protein content of supernatant}}{\text{Total protein content of powder}} \times 100$$

2.5. Water Holding Capacity (WHC)

The water holding capacity of fish bones and shrimp shell powders was determined according to the previously described method with some modifications [23]. Approximately, 1 g of sample was dissolved in 10 mL distilled water and stirred at 300 rpm for 24 h. The homogenized solution was centrifuged at 3000 rpm for 10 min. The supernatant was discarded, and the hydrated sample was weighed. The water holding capacity was calculated following the Equation (2).

$$\text{Water holding capacity} = \frac{W_2 - W_1}{W_1} \quad (2)$$

where, W_1 = Weight of dry sample. W_2 = Weight of hydrated sample.

The water solubility index (WSI) was calculated by measuring the aqueous supernatant after drying following the Equation (3).

$$\text{WSI (g/g)} = \frac{\text{Weight of sample recovered in supernatant (g)}}{\text{Weight of total sample dissolved in water (g)}} \quad (3)$$

2.6. Oil Holding Capacity (OHC)

Oil holding capacity of fish bones and shrimp shell powders were determined according to the previously described method with some modifications [23]. Approximately 1 g of powder was dissolved in 10 mL soybean oil and stirred for 30 min at room temperature. The homogenized solution was centrifuged at 3000 rpm for 10 min. The supernatant was discarded, and the residue was weighed. The oil holding capacity was calculated as Equation (4).

$$\text{Oil holding capacity} = \frac{W_2 - W_1}{W_1} \quad (4)$$

where, W_1 = Weight of dry sample, W_2 = Weight of oiled sample.

2.7. Determination of Heavy Metals Content

The heavy metal contents of fish bones and shrimp shell powders were determined using an ICP-OES optimum 2000 DV (PerkinElmer) equipped with winLab32 software, following the previously described method with some modifications [24]. Briefly, 1 g of dried powder sample was placed in a muffle furnace at 600 °C and heated for 6 h. After ashing, 4 mL of HNO_3 and 1 mL H_2O_2 were added to the ashed powder, and then distilled water was added to make the total volume 60 mL. The solution was heated on a hot plate and reduced to half (30 mL), after which 4 mL of distilled water and 1 mL of H_2O_2 were added, reheated, and reduced to half again (17.5 mL). After reducing the solution to half, distilled water was added to make a 20 mL solution, and filtration was performed using 125 mm filter paper. The filtering process was done twice. The samples were analyzed for lead (Pb), cadmium (Cd), arsenic (As), chromium (Cr), copper (Cu), and nickel (Ni).

2.8. Color Analysis

Color measurements were done using a portable colorimeter (Model: CS-826, Konica Minolta, Hangzhou, China) as previously described method [25]. Reported values are mean of five replicates and recorded as L^* (lightness), a^* (green to redness), and b^* (blue to yellowness).

2.9. Field Emission Scanning Electron Microscopy and Energy-Dispersive X-ray Analysis

The surface morphology and micro-structure of the samples was determined on the metallic stub using a field emission scanning electron microscopy (FE-SEM) (Zeiss Sigma300, Oberkochen, Germany) equipped with energy-dispersive X-ray (EDX) microanalysis using (X Flash Detector 630M, Berlin, Germany). At first, the carbon tape attached with stub and then very small amount of sample powders were dispersed on carbon tape. Then every stub was placed into gold sputtering machine for 2 min and attached on sample holder. Then sample holder was placed into FE-SEM machine, start pumping and images were captured with a magnification of 100 and electron accelerating voltage of 5.00

kV. For EDX the measurement was performed at 20.0 kV electron accelerating voltage with a magnification of 3000.

2.10. FTIR Spectroscopy

The chemical structure of sample powders was determined using a FTIR spectrometer (Nicolet IS-20, Thermos Scientific Inc., Waltham, MA, USA) at room temperature (20–22 °C) following the method described by Yin et al. [26]. Samples along with KBr (99% KBr + 1% sample) were prepared as a translucent pellet or into disk shape and scanned against a blank KBr palate background at the wavenumbers ranging from 400 to 4000 cm^{-1} . The OMNIC professional software version 8.3 for Windows was used to remove the background interference and the spectra obtained were analyzed using (Thermo Nicolette Corp., Madison, WI, USA).

2.11. ABTS Radical Scavenging Activity

ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activity of the sample powders was determined using the previously described method with some modifications [27]. The samples were extracted using distilled water 1:10 (*w/v*) for 24 h maintaining the 300 rpm stirring at room temperature. Then, the sample extracts were centrifuged at 3000 rpm for 10 min. The supernatant was evaluated for ABTS free radical scavenging capacity by mixing different ratio of sample extract (20, 40, 60, 80, 100 μL) and ABTS working solution (3.90, 3.92, 3.94, 3.96, 3.98 mL) to make 4.0 mL. The mixture was incubated for 30 min in dark condition at room temperature and the OD value was measured at 734 nm using a Synergy® HTX Multi-mode microplate reader (BioTeK, Winooski, VT, USA).

2.12. Statistical Analyses

Values are presented as means $\pm$ standard deviations of triple determinations. The data were analyzed by one-way analysis of variance (ANOVA) using SPSS 16.0 (SPSS Inc, Chicago, IL, USA). The difference between the means was determined by Duncan's Multiple Range Tests (DMRT) and $p \leq 0.05$ was regarded as significant level.

3. Results and Discussion

3.1. Proximate Composition of Fish Bone and Shrimp Shell Powders

There were significant differences found in the proximate composition among the fish bones and shrimp shell powders (Figure 1). The highest protein content was found in SSP 37.78%, followed by PBP 32.29%, and TBP 30.28. Fish bone is considered a good source of protein as it contains various proteins, including collagen. Nawaz et al. [28] investigated the protein content of grass carp fish bone powder depending on different treatments, finding contents ranging from 18.40% to 22.50%. Jasmadi et al. [9] reported slightly lower amounts of protein in pangas bone powder, ranging from 14.02% to 16.10%, depending on sonication and alkaline water bath treatments. The protein content in milkfish bone powder was reported to be 27.88% [29]. The total protein content in Atlantic salmon backbone and Baltic cod backbone powders were found to be 18.02% and 15.27%, respectively [30]. The protein content in seabream (*Sparus aurata*) fish bone powder was reported to be 26.07% [19]. In silver carp fish bone powder, the protein contents without milling and with milling were 20.44% and 20.52%, respectively [26]. However, another study reported a lower protein content in silver carp fish bone powder, at 13.27% [15]. Similarly, tilapia fish bone powder contained a low amount of protein (14.81%) [31]. The protein content in the fish bone and shrimp shell powders in this study was found to be higher compared with previous research. The protein content in fish bone powder can vary depending on factors such as the type of fish, processing methods, and any additives included in the powder. Alkaline solutions are more effective at solubilizing and leaching out more meat tissue and proteins from the bone. However, alkaline solutions are not completely effective in removing all protein, as some protein still remains in the bone powder. Proteins in bone

are typically categorized as stroma proteins. Collagen-associated amino acids, such as glycine, proline, and hydroxyproline, were found in bone powder from blue whiting, herring, and mackerel [5]. It is known that alkaline and acid treatments lead to a reduction in protein content due to protein degradation. A previous study found that NaOH treatment caused a considerable reduction in the protein content of Atlantic salmon backbone [30].

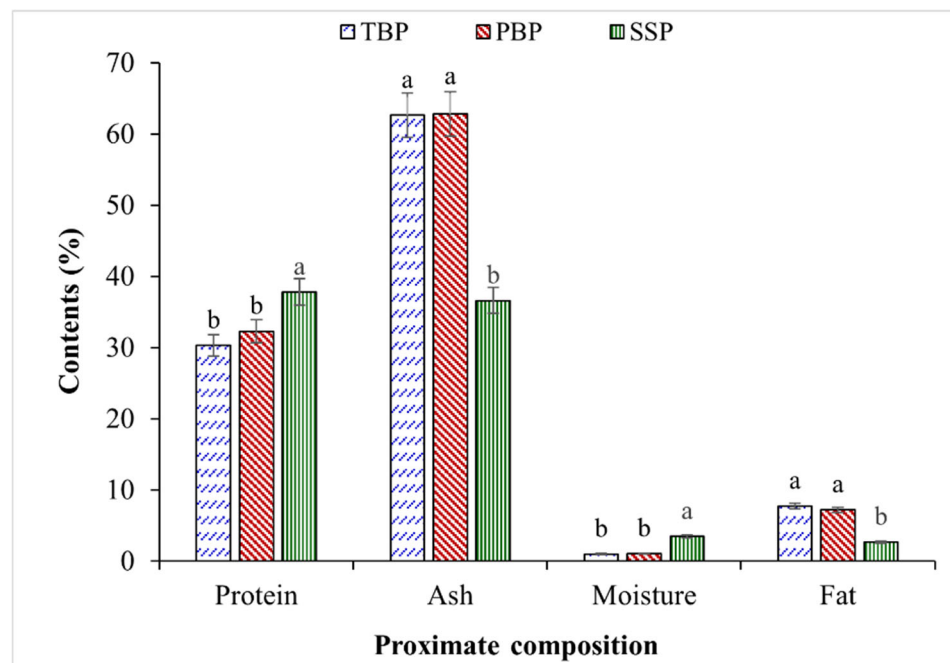


Figure 1. Proximate composition of fish bones and shrimp shell powder. Different small letters on groups of proximate composition indicate significant ($p \leq 0.05$) differences.

The ash contents of TBP, PBP, and SSP were found to be 62.63%, 62.80%, and 36.58%, respectively. Fish bones have a relatively high ash content compared with other parts of the fish, primarily because ash represents the inorganic mineral content remaining after organic matter has been burned off during combustion. Many researchers have reported high ash content in fish bone powders: 66.84% to 68.63% in pangas bone powder [9], 64.30% to 66.30% in grass carp bone powder [17], 51.42% to 80.84% in milkfish bone powder [29], 70.15% to 73.28% in salmon bone powder [30], 51.35% to 84.87% in seabream bone powder [19], 73.47% in silver carp [15], and 65.09% to 64.43% in silver carp bone powder [26]. Fish bones contain a significant amount of minerals like calcium and phosphorus, contributing to the ash content. The ash contents in the fish bone powder in the present study showed similar values compared to previous reports. The ash content in fish bone can vary depending on several factors such as the species of fish, processing methods, and the part of the bone being used. However, the ash content in SSP was found to be very low because shrimp shells are primarily composed of chitin, a polymer of N-acetyl-D-glucosamine, which has a lower mineral content compared with the mineralized structure of fish bones.

The moisture contents of TBP, PBP, and SSP were estimated to be 0.98%, 1.02%, and 3.45%, respectively. The moisture contents of fish bone powder from seabream, grouper, emperor, white snapper, Nile tilapia, and silver carp were reported to be 3.56% to 6.11% [19], 5.96%, 4.12%, 3.21% [32], 8.4% [33], and 3.49% to 3.59% [26], respectively. The bone powder in the present study revealed minimal moisture content, indicative of a well-executed drying process. Water molecules do not penetrate the bone tissue but rather loosely adhere to its surface, facilitating their near-complete removal during oven drying. As evidenced in Figure 1, the moisture content in the bone powder remained consistently low. This characteristic not only ensures stability at room temperature but also prevents the formation of clumps during storage. Beyond its physicochemical implications, the low

moisture content confers resistance to microbial degradation, as a moisture level of 2–3% is insufficient for microbial growth. This inherent stability renders fish bone powder a versatile ingredient suitable for various applications with ease and safety. Moisture content was high in shrimp shell powder, and there was no significant variation found between tuna fish bone powder (FBP) and pangas FBP ($p \leq 0.05$). Shrimp shell is rich in chitin, the structural component of shrimp shell, which consists of long-chain polysaccharides that can retain water. The chitin in the shell has hydrophilic (water-attracting) properties, contributing to the overall moisture content in shrimp shell. Fish bones generally have less moisture compared with shrimp shells because they are composed of minerals, collagen, and other proteins, which have a lower water content than the chitin found in crustacean exoskeletons. The mineralized nature of fish bones contributes to their reduced moisture content.

The highest fat content was found to be 7.70% in TBP, followed by 7.20% in PBP and 2.67% in SSP. Fish bone powder typically has a low fat content, as most of the fat in fish is concentrated in the flesh rather than the bones. Several previous studies have shown similar values in fat content in fish bone powders: 3.22% to 3.95% in pangas [9], 3.24% to 4.30% in grass carp [17], 1.83% to 7.85% in milkfish [29], 3.47% to 17.80% in seabream [19], and 3.12% in silver carp [26]. However, the exact fat content in fish bone powders can vary depending on factors such as the type of fish, processing methods, treatments used to create the powder, and any additional ingredients or additives. Freshwater and saltwater fish, as well as fatty fish like tuna and pangas, are rich in omega-3 fatty acids, which might cause higher fat content in their bones compared with shrimp shells. Alkaline treatment can also reduce the fat content in fish bone and shrimp shell powders. Benefiting from its low-fat content, MBP is less prone to oxidation, thereby extending its shelf life [29].

3.2. The Composition and Contents of Total Amino Acids

The amino acid composition and contents in TBP, PBP, and SSP are presented in Table 1. A total of 17 amino acids were detected in the TBP, PBP, and SSP samples, including 9 essential amino acids and 8 non-essential amino acids. The highest total essential amino acid content was found to be 15.47 g/100 g in SSP, followed by 11.77 g/100 g in PBP and 10.92 g/100 g in TBP. The total non-essential amino acid contents in TBP, PBP, and SSP were 15.07 g/100 g, 15.47 g/100 g, and 15.54 g/100 g, respectively. Among the essential amino acids, lysine had the highest individual content, with 3.62 g/100 g in SSP, 2.02 g/100 g in PBP, and 1.93 g/100 g in TBP. Glycine had the highest content among the non-essential amino acids in the studied samples, with 4.78 g/100 g in TBP, 3.72 g/100 g in PBP, and 3.26 g/100 g in SSP.

Table 1. The total amino acids composition and contents (g/100 g) of TBP, PBP, and SSP.

Name of Amino Acid	TBP	PBP	SSP
Essential amino acids (EAAs)			
Histidine	0.46 ± 0.01 ^a	0.46 ± 0.01 ^a	n.d.
Threonine	1.38 ± 0.11 ^b	1.76 ± 0.02 ^a	1.98 ± 0.14 ^a
Arginine	1.85 ± 0.12 ^c	2.23 ± 0.14 ^b	2.83 ± 0.11 ^a
Valine	1.13 ± 0.05 ^b	1.34 ± 0.12 ^a	1.59 ± 0.12 ^a
Phenylalanine	1.13 ± 0.08	1.31 ± 0.13 ^b	1.70 ± 0.06 ^a
Isoleucine	1.17 ± 0.10 ^b	1.17 ± 0.11 ^b	1.41 ± 0.04 ^a
Leucine	1.87 ± 0.13 ^a	1.48 ± 0.08 ^b	1.98 ± 0.14 ^a
Lysine	1.93 ± 0.12 ^b	2.02 ± 0.06 ^b	3.62 ± 0.17 ^a
Histidine	n.d.	n.d.	0.36 ± 0.02 ^a
ΣEAAs	10.92	11.77	15.47
Non-essential amino acids (NEAAs)			
Aspartic acid	1.78 ± 0.04 ^b	1.54 ± 0.14 ^a	2.21 ± 0.05 ^b
Glutamic acid	1.22 ± 0.12 ^b	2.88 ± 0.11 ^c	4.94 ± 0.14 ^a
Serine	1.54 ± 0.11 ^c	1.02 ± 0.17 ^b	2.43 ± 0.11 ^a

Glycine	4.78 ± 0.08 ^a	3.72 ± 0.15 ^b	3.26 ± 0.10 ^c
Proline	3.17 ± 0.07 ^a	3.12 ± 0.10 ^a	n.d.
Alanine	1.36 ± 0.14 ^b	1.43 ± 0.11 ^b	1.77 ± 0.14 ^a
Taurine	1.22 ± 0.12 ^a	0.29 ± 0.05 ^c	0.54 ± 0.10 ^b
Tyrosine	n.d.	1.47 ± 0.10 ^a	0.39 ± 0.03 ^b
ΣNEAAs	15.07	15.47	15.54

Values are presented as means ± standard deviation of triple determinations. Different superscript small letters in each row indicate significant differences ($p \leq 0.05$). n.d. = not detected.

The fish bone powders were rich in collagen-type protein, which contributed to the substantial content of glycine and proline in TBP and PBP; however, proline was absent in SSP. The primary protein in fish bone is type I collagen, characterized by repetitive Gly-Pro-Hyp sequences [34]. Yin et al. [16] reported a very high content of glycine and proline in silver carp fish bone powder following milling treatment, with the contents being $17.42 \pm 0.05\%$ and $9.19 \pm 0.12\%$ of total amino acids, respectively. The findings of the present study align with this previous report. Amino acids are an important criterion for determining protein quality because they perform vital roles in the body, including maintaining nutritional balance, functioning as enzymes and hormones, supporting immune function, and providing disease protection.

3.3. Protein Solubility

The protein solubility in water of the fish bone powder and shrimp shell powder is presented in Figure 2. The protein solubility varied depending on the sources of the powders and increased with prolonged soaking periods. The maximum protein solubility was observed in TBP, reaching 14.53%, followed by 12.25% in PBP and 8.25% in SSP, with a soaking period of 100 min. The lowest protein solubility was 3.25% in SSP, 4.35% in PBP, and 5.25% in TBP, after a soaking period of 20 min. Similar protein solubility results were reported by Qin et al. [35], who found that protein solubility in yak bone powder increased with prolonged soaking time, with a maximum solubility of approximately 12% after 100 min. The *in situ* steam explosion of chicken bone powder significantly increased the water-soluble protein content from 3.28 to 3.73 mg/g [36]. There are no previous reports available on the protein solubility of fish bone powders. The water solubility of fish bone powders is an important indicator of the nature and quality of its proteins. The soluble protein content of fish bone powder in water can be used as an important indicator to evaluate the protein solubility of powders, which is important for consumers. The extent of protein solubility of fish and shrimp shell powders depends on the type of powder, particle size, temperature, and duration of exposure to liquids.

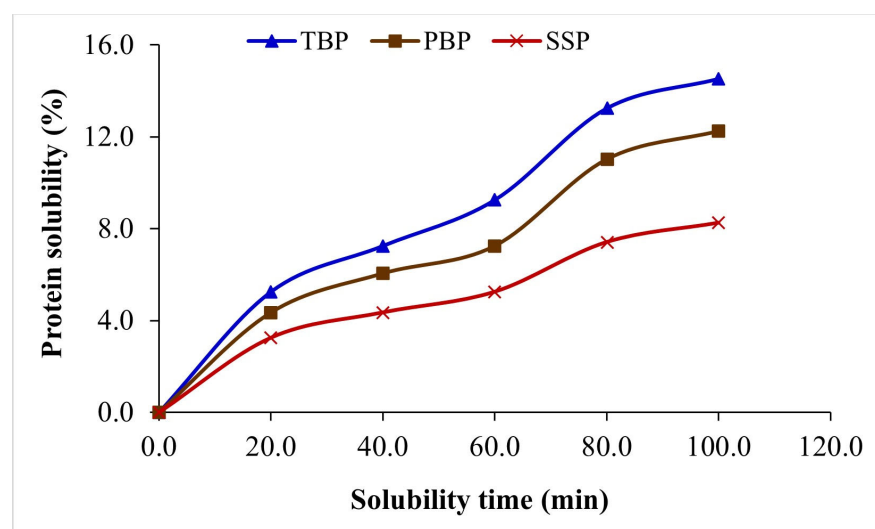


Figure 2. The changes in the water solubility of FBP and SSP over the soaking time.

3.4. Water and Oil Holding Capacity

The water holding capacity (WHC) of fish bone and shrimp shell powder varied depending on the species (Figure 3). SSP showed the highest WHC of 2.85 g/g, followed by PBP and TBP, with values of 1.56 g/g and 1.21 g/g, respectively. Kong et al. [37] reported that the WHC of steam-exploded chicken bone powder (1.99 g/g) was significantly ($p < 0.05$) higher than that of native chicken bone powder (1.91 g/g). The WHC of yak bone powder increased from 0.90 to 1.02 g/g with an increase in the soaking period from 10 to 50 min [35]. The WHC of fish bone powder is affected by the type of fish bone, particle size, and soaking period. Moreover, the ratio of polar to non-polar amino acids in protein molecules also influences the WHC [38]. This might result from changes in the surface properties of the powder, such as increased surface energy, surface area, and protein exposure area [22]. The WHC of fish bone powder is a critical property that influences its functionality, usability, and potential applications in the food, pharmaceutical, and cosmetic industries.

Among the produced powders, SSP showed the highest oil holding capacity (OHC) of 2.91 g/g, followed by PBP and TBP with values of 1.15 g/g and 1.11 g/g, respectively. The OHC of cuttlefish bone powder ranged from 1.05 ± 0.18 g oil/g sample to 1.47 g oil/g sample [38]. The oil holding capacity of steam-exploded chicken bone powder (1.78 g/g) was significantly ($p < 0.05$) higher than that of native chicken bone powder (1.73 g/g) [37]. The reported values for OHC closely fit the values obtained in the present study. The OHC of fish bone powders depends on the particle size, processing method, composition, surface area, etc. The oil holding capacity of fish bone powder is a critical functional property that influences the sensory, nutritional, and processing attributes of food products, making it an important ingredient in various food applications. The high WHC and OHC of an ingredient determine its ability to enhance the texture and flavor of a product. The OHC of fish bone and shrimp shell powders obtained in the present study reveals their potential applications as emulsifiers in food formulations such as bakery products.

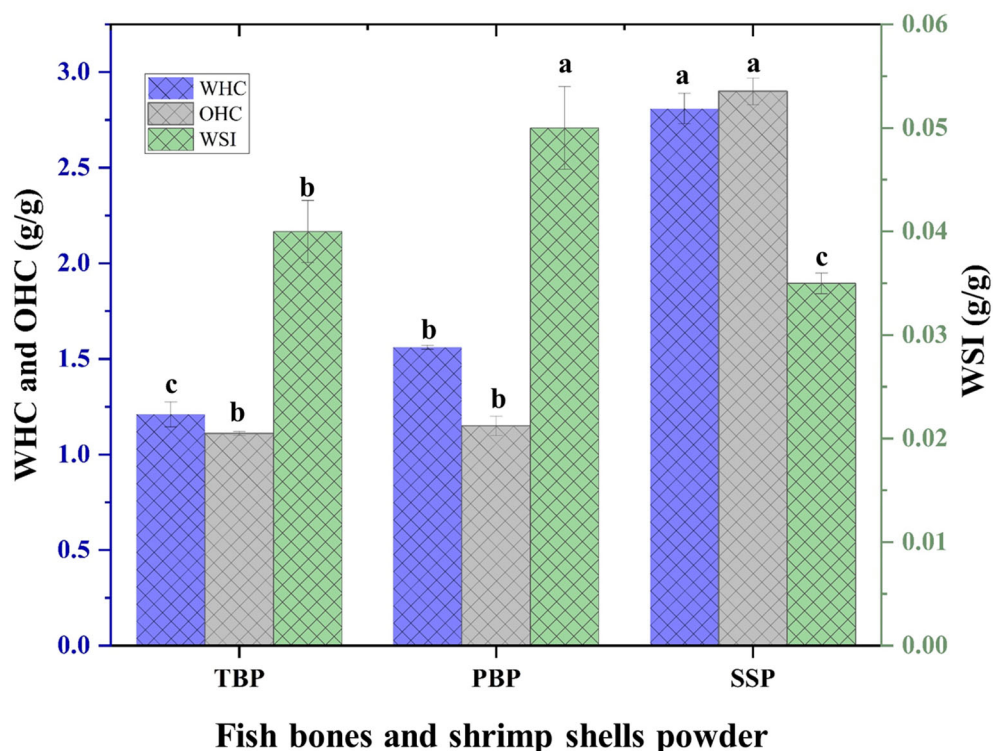


Figure 3. Hydration and oil holding properties of fish bone and shrimp shell powders. Values are presented as means $\pm$ standard deviation ($n = 3$). Different small letters on each column bar of same parameters indicate significant differences ($p \leq 0.05$).

PBP showed the highest water solubility index (WSI) value of 0.05 g/g, followed by TBP and SSP with values of 0.04 g/g and 0.035 g/g, respectively. Kong et al. [37] reported that the WSI value of steam-exploded chicken bone powder (0.044 g/g) was significantly ($p \leq 0.05$) higher than that of native chicken bone powder (0.02 g/g). The WSI values obtained in our study closely align with previously reported values. The water solubility index of fish bone powder is a key characteristic that influences its functionality, applicability, and potential benefits in various industrial, nutritional, and biomedical applications.

3.5. Color Analysis of Fish Bones and Shrimp Shell Powder

The color characteristics of fish bone and shrimp shell powders play a significant role in consumer acceptance. Figure 4 illustrates the color values (L^* , a^* , and b^*) of various fish bone and shrimp shell powder samples. These values varied significantly among the three samples, probably due to differences in pigment presence and the abundance of minerals, proteins, and lipids. PBP exhibited the highest lightness (L^*) value, followed by TBP and SSP. The higher transparency of PBP was probably due to the composition of the bone matrix, which consisted mainly of minerals such as calcium and phosphorus. These minerals contributed to the overall color of the bone, giving it a whitish appearance. Conversely, SSP showed greater redness (a^*), attributed to carotenoids, especially astaxanthin [39]. TBP tends to have a yellowish hue due to lipid content, notably in the bone marrow. TBP and PBP exhibited less yellowness and redness, possibly due to lower pigment and substance levels. The present findings showed superior color properties compared with sea-bream fish bone powder [19]. Consumer preference generally favors white or transparent bone powder, as observed in a previous report [40]. Sefrienda et al. [9] also noted similar color attributes in pangas bone powder, emphasizing its white powder-like appearance. The L^* value of cuttlefish bone powder was found to be similar to the present study, ranging from 88.1 to 89.23 [38]. Additionally, a study on chicken bone powder found specific color values ($L^* = 27.93$, $a^* = 21.97$, $b^* = 10.40$) [36]. Whiteness indicates the quality of powders, with white being preferred for incorporation into various food products [38].

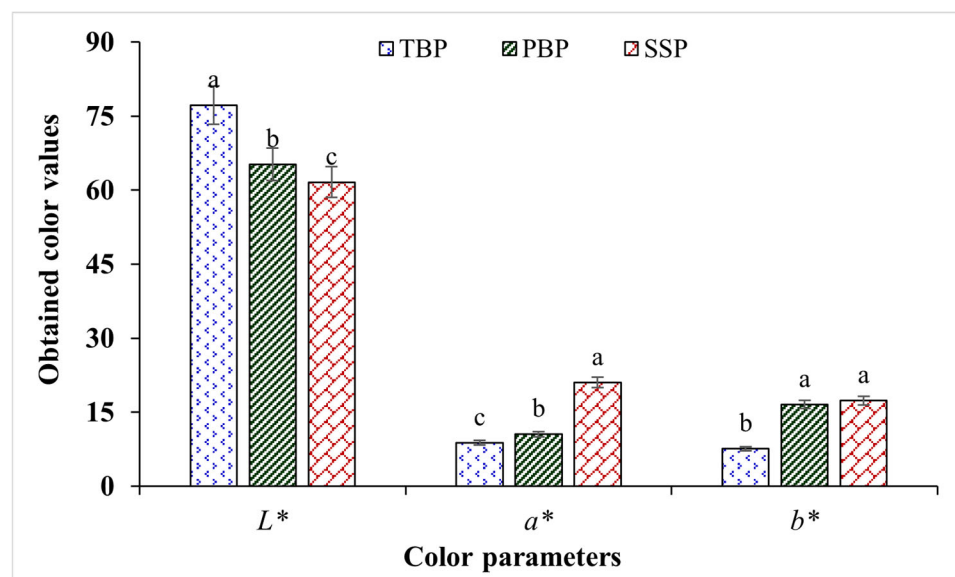


Figure 4. L^* (lightness), a^* (redness), and b^* (yellowness) of fish bone and shrimp shell powders. Different small letters on each color parameter column indicate significant ($p \leq 0.05$) differences.

3.6. FE-SEM Analysis of Fish Bones and Shrimp Shell Powders

FE-SEM was used to analyze the morphology of TBP, PBP, and SSP, as shown in Figure 5. The images revealed a distinct variance in morphology and size among the samples. Smaller particles were observed in PBP (8.64 μm) and TBP (41.59 μm), whereas larger

particles were found in SSP (395.9 μm). The size and shape of the particles in the powder can affect its bioavailability, which is the amount of a substance that is absorbed by the body. Smaller particles tend to be more bioavailable than larger particles. The surface morphologies of PBP and TBP showed compact structures with smaller particle sizes compared with SSP. This might be due to the breakdown of collagen and organic matter during boiling and milling [41]. In contrast, SSP was quite large, flat, and slightly porous. The possible reason may be that it mostly comprised chitin, a thin-layer structure that did not turn into small particles after applying heat and milling treatment. The particle size of grass carp (*Ctenopharyngodon idella*) fish bone powder was found to be larger and relatively compact in structure [17]. Another study by Zhang et al. [42] investigated the effect of thermal treatment on fish bone powder and reported that a compact structure was observed.

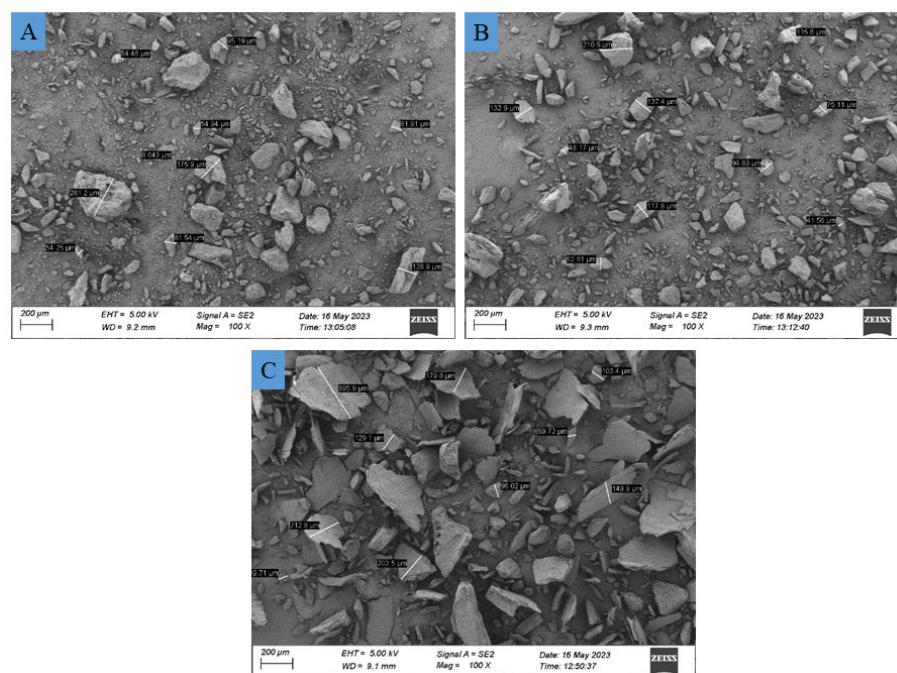


Figure 5. SEM images of the microparticles: (A) pangas fish bone powder, (B) tuna fish bone powder, (C) shrimp shell powder.

3.7. EDX Spectra of Fish Bones and Shrimp Shell Powders

Energy dispersive X-ray spectroscopy (EDX) was conducted to determine the elemental composition of the prepared bone powder. This method expresses the movement of components in the bone particles. As shown in Figure 6, carbon (C), oxygen (O), sodium (Na), potassium (K), magnesium (Mg), phosphate (P), and calcium (Ca) were found in all samples. The intensities of calcium and phosphorus peaks were higher in TBP and PBP, whereas carbon and oxygen peak intensities were higher in SSP compared with PBP and TBP. This could be attributed to the presence of chitin in shrimp shells, in addition to the richness of collagen in fish bone powder. The elemental compositions of TBP were as follows: C (22.19%), O (36.86%), Na (1.90%), Mg (0.64%), P (13.85%), K (0.02%), Ca (24.52%), and Fe (0.02%), and for PBP, C (25.94%), O (37.32%), Na (1.04%), Mg (0.43%), P (12.90%), K (0.07%), Ca (22.28%), and Fe (0.02%). Similarly, Yin et al. [26] prepared ultrafine fish bone powder from silver carp (*Hypophthalmichthys molitrix*) and found 23.39% calcium, 15.27% phosphorus, 0.24% magnesium, 0.02% potassium, and 0.11% sodium. The calcium and phosphorus contents in yellowfin tuna (*Thunnus albacares*) fish bone powder were reported to be 38.16% and 23.31%, respectively [43]. Toppe et al. [5] reported 23.3% calcium, 11.1% phosphorus, and 0.36% magnesium in horse mackerel bone powder. The Ca/P atomic ratio values for TBP and PBP were 1.77 and 1.73, respectively, slightly higher

than the stoichiometric hydroxyapatite (HAp) ratio of 1.67. This deviation may be attributed to the substitution of B-type carbonate ions, which replaced phosphate ions in the hydroxyapatite lattice structure. In contrast, the elemental composition of SSP was C (54.54%), O (34.54%), Na (1.53%), Mg (0.37%), P (1.26%), K (0.14%), Ca (7.58%), and Fe (0.04%). The atomic ratio of Ca/P obtained for SSP was 6.01.

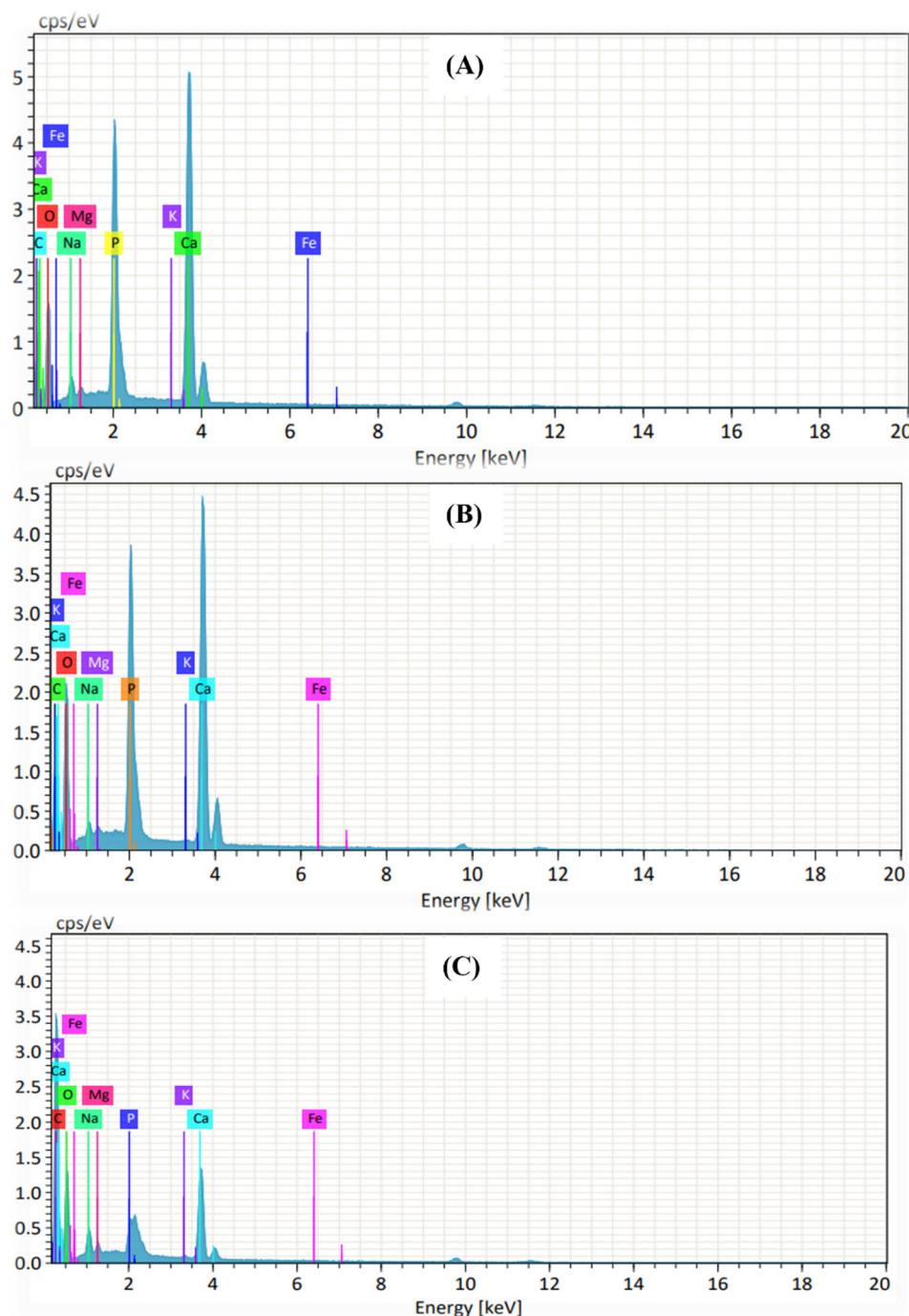


Figure 6. EDX spectra of fish bone and shrimp shell powders: (A) tuna bone powder; (B) pangas bone powder; and (C) shrimp shell powder.

Peak intensities of calcium and phosphorus were higher in pangas FBP and tuna FBP, while peak intensities of carbon and oxygen were higher in shrimp shell powder than in pangas and or tuna FBP. This might be due to the shrimp shell being composed of chitin, whereas FBP is rich in collagen. The elemental composition of TBP was C (22.19%), O

(36.88%), Na (1.89%), Mg (0.64%), P (13.85%), K (0.02%), Ca (24.52%), and Fe (0.02%). For PBP, the values were C (27.94%), O (37.32%), Na (1.04%), Mg (0.43%), P (12.90%), K (0.07%), Ca (22.26%), and Fe (0.02%). The Ca/P atomic ratio values of tuna FBP and pangas FBP were 1.77 and 1.73, respectively, which were slightly higher than that of stoichiometric HAp (Ca/P = 1.67). This might be due to the substitution of B-type carbonate ions that replaced phosphate ions in the HAp lattice structure. The elemental composition of SSP was C (54.54%), O (34.54%), Na (1.53%), Mg (0.37%), P (1.26%), K (0.14%), Ca (7.58%), and Fe (0.04%). Yin et al. [26] prepared ultrafine fish bone powder from silver carp (*H. molitrix*) and found 23.39% calcium, 15.27% phosphorus, 0.24% magnesium, 0.02% potassium, and 0.11% sodium, aligning closely with the present study. Nemati et al. [44] found 38.16% calcium and 23.31% phosphorus in yellowfin tuna (*Thunnus albacares*) bone powder, showing slight dissimilarities to the present research. Horse mackerel bone powder showed 23.3% calcium, 11.1% phosphorus, and 0.36% magnesium, which was similar to the current study [5]. Therefore, the current study of fish bone and shrimp shell powders mostly agrees with previous reports.

3.8. FT-IR Analysis of Fish Bones and Shrimp Shell Powder

Fourier-transform infrared spectroscopy (FT-IR) is an analytical method that can be used to identify specific functional groups in bone powder. Figure 7 shows the FT-IR spectra of different fish bone and shrimp shell powders. The FT-IR spectra of TBP, PBP, and SSP did not show any significant differences. All samples exhibited peaks characteristic of the phosphate group (PO_4^{3-}) band, ranging from 500 to 800 cm^{-1} . TBP displayed prominent phosphate absorption peaks at 564 cm^{-1} and 1040 cm^{-1} , while PBP showed peaks at 562 cm^{-1} and 1030 cm^{-1} . SSP exhibited very weak bands at 1030 cm^{-1} for phosphate absorption. Peaks around 1000 cm^{-1} indicated phosphate absorption and C-N stretching [40]. Major absorption peaks were found in two different regions: 872 cm^{-1} and 1400 cm^{-1} to 1650 cm^{-1} for TBP, and 874 cm^{-1} and 1410 cm^{-1} to 1660 cm^{-1} for PBP, indicating carbonate ion substitution. The bands ranging from 1500 to 1650 cm^{-1} were identified as amide I, amide II, and amide III bands, respectively, present in all samples due to the presence of collagen [17]. The bands at 2860 cm^{-1} and 2930 cm^{-1} for TBP and PBP were assigned to organic material (C-H) absorption.

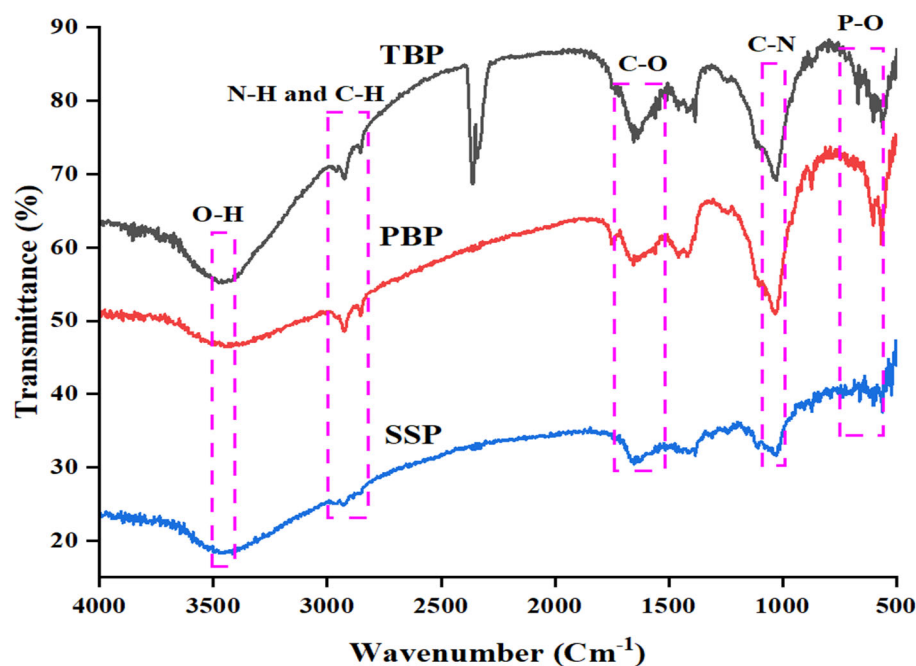


Figure 7. FT-IR spectra of fish bone and shrimp shell powders.

Furthermore, a distinctive peak at $3450\text{--}3460\text{ cm}^{-1}$, associated with the hydroxyl stretching (OH^-) mode of hydroxyapatite (HA), was clearly detected [44]. In SSP, the spectral bands were slightly different from those of TBP and PBP due to their structural features. In SSP, the (O-H) absorption band was found at 3460 cm^{-1} . The spectral bands of SSP showed a similar trend to previous studies [45]. All treatments of the bone powder exhibited distinct spectral bands, indicating that the chemical structure of the bone powder remained intact. Peaks at 875 cm^{-1} and 1639 cm^{-1} showed carbonate bands [44]. The peak band at 1453 cm^{-1} indicated the presence of carbonate ions [16], while the peak band at 1533 cm^{-1} represented the amide group [46]. TBP showed a characteristic peak at around 2300 cm^{-1} which was absent in PBP and SSP. Such a peak typically signifies the presence of carbon dioxide (CO_2) or other similar molecules in the sample. The peak bands at 2852 and 2922 cm^{-1} showed the presence of organic material at a relatively high intensity [44]. The bands around 3300 cm^{-1} indicated a sufficiently low water content [47].

3.9. Heavy Metals Contents Analysis of FBP and SSP

Heavy metal contamination is a critical issue, especially in marine organisms, including fish and shrimp. Therefore, the prepared fish bone and shrimp shell powders were investigated to determine the presence of six commonly occurring heavy metals in the aquatic environment: lead (Pb), cadmium (Cd), arsenic (As), chromium (Cr), copper (Cu), and nickel (Ni). Among them, Pb, Cd, and As were not detected in any samples (Figure 8). The Cr concentrations were found to be $0.20\text{ mg}/100\text{ g}$ and $0.22\text{ mg}/100\text{ g}$ in TBP and SSP, respectively, whereas no Cr was detected in PBP. The highest Cu content was found to be $0.84\text{ mg}/100\text{ g}$ in SSP, followed by $0.52\text{ mg}/100\text{ g}$ in TBP and $0.27\text{ mg}/100\text{ g}$ in PBP. The Cu content was lower than the maximum permissible value according to Codex regulation levels ($1\text{--}10\text{ mg}/100\text{ g}$) [48]. Cu is a cofactor for several enzymes (known as cupro-enzymes) involved in energy production, iron metabolism, neuropeptide activation, connective tissue synthesis, and neurotransmitter synthesis. The heavy metal concentrations found in fish bone and shrimp shell powders were within safety levels for human consumption. The variation in heavy metal concentrations in FBP and SSP observed in the current study might be due to the different habitats of the species (pangas in freshwater, tuna in saltwater, shrimp in brackish water).

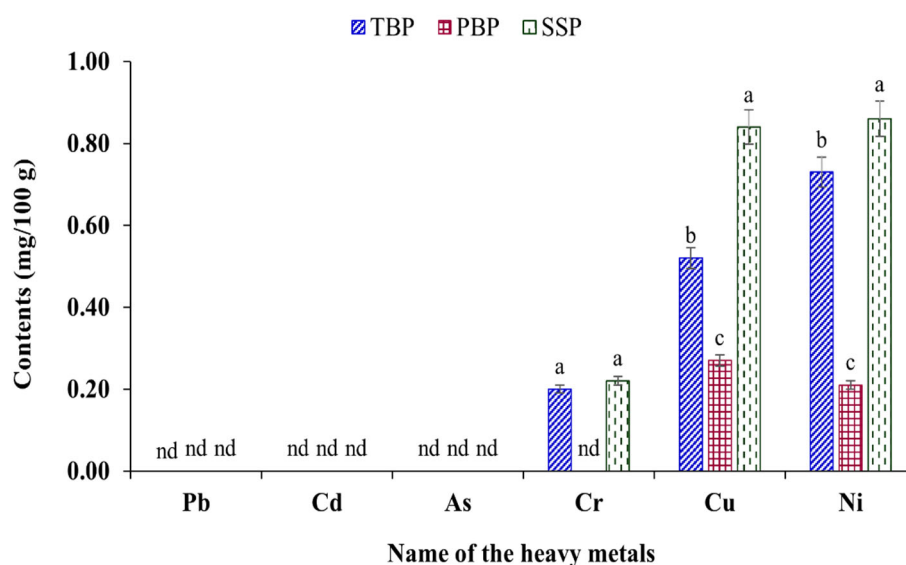


Figure 8. Heavy metal contents in fish bone and shrimp shell powders. Different small letters on the columns representing heavy metals indicate significant ($p \leq 0.05$) differences. nd = not detected.

Toppe et al. [5] found Cr levels ranging from 2.4 to 6.7 mg/kg in fatty fish (salmon, trout, herring, mackerel, and horse mackerel), similar to the findings of the present study. The acceptable level of chromium in fish bone powder or any food product depends on various factors such as regulations set by governmental agencies, the intended use of the product, and potential health risks associated with chromium exposure.

3.10. ABTS Radical Scavenging Activity of Fish Bones and Shrimp Shell Powder

The fish bone and shrimp shell powders exhibited significant ABTS free radical scavenging activity. ABTS is an *in vitro* biochemical assay that is based on chromogenic changes due to the scavenging of free radicals, which are determined spectrophotometrically. Figure 9 shows the reduction in OD values due to scavenging in the ABTS solution in a dose-dependent manner. Among the produced powders, SSP showed the highest ABTS radical scavenging activity, followed by PBP and TBP in increasing concentrations. The OD value of the ABTS working solution was 1.087, which was reduced to 0.16, 0.21, and 0.27 for SSP, TBP, and PBP, respectively, at a 100 μ L concentration. Fish bones are abundant in minerals like calcium, magnesium, and phosphorus, which exhibit antioxidant effects by neutralizing free radical ions. Peptides derived from collagen and other proteins found in fish bones also demonstrate antioxidant properties by scavenging free radicals. Additionally, shrimp shell powder contains chitin and chitosan, polysaccharides with antioxidant attributes capable of scavenging free radicals. The combined actions of these bioactive compounds contribute to the ABTS radical scavenging activity of fish bone powder. Kong et al. [37] assessed the ABTS free radical scavenging activity of chicken bone powder and observed significant free radical scavenging capacity. Culinary applications of fish bone powders rich in antioxidant capacity represent feasible approaches for various uses in the food industry [49]. Nevertheless, the precise composition and concentration of these compounds may vary depending on factors such as fish species, processing methods, and environmental conditions.

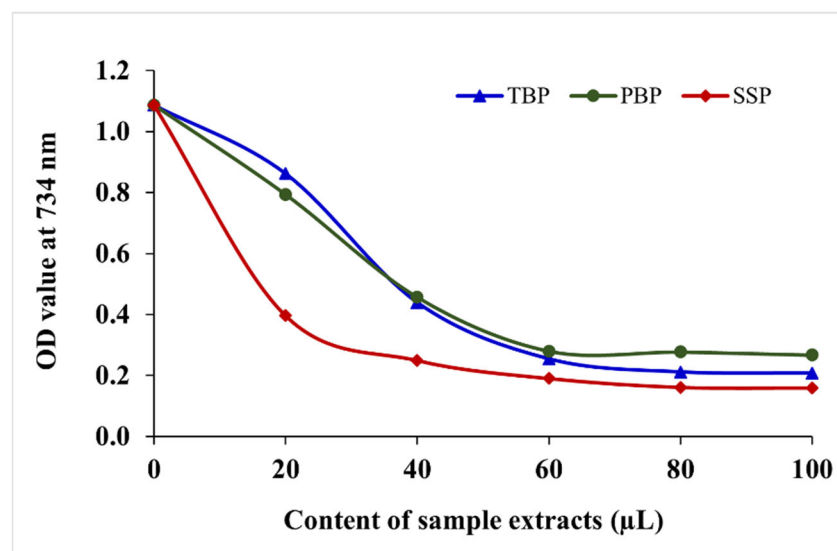


Figure 9. *In vitro* ABTS free radical scavenging ability of fish bone and shrimp shell powders.

4. Conclusions

The goal of the present study was to utilize fish and shrimp by-products as mineral supplements for human consumption. In the current study, it was observed that NaOH treatment during the preparation of fish bones and shrimp shell powders acted as an organic detergent to remove residues from bones and also reduce the fishy smell. It was found that TBP and PBP were rich in minerals (Ca and P), which are beneficial for teeth and bone growth in the human body and might increase their acceptability to consumers.

Conversely, SSP contained a high amount of protein, which could serve as a high-quality protein source for people suffering from malnutrition, and a carbon complex (chitin), which is beneficial to the gut and immune system in the human body. The physicochemical and nutritional properties of the recovered powders suggest their applications in various food and pharmaceutical industries. To popularize this product among consumers as a mineral powder, further studies are required to evaluate in vitro digestibility, organoleptic acceptability, and toxicity. Additionally, further research is needed to test the safety of the samples in animal model studies and establish the feasibility of value-added fishery products using the powders.

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