Analyzing the Characteristics of Fishbone Powder Derived from *Pangasius* sp., *Thunnus tonggol,* and *Thunnus albacares* as Food Fortificant

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ABSTRACT

Pangasius sp., *Thunnus tonggol* (*T. tonggol*), and *Thunnus albacares* (*T. albacares*) are high-value commercial species widely used in fillet production, generating by-products, such as fishbone. The fishbone, rich in calcium (CA) and phosphorus (P), offers a valuable alternative for daily Ca intake. Using such by-products as a potential source of Ca presents a viable solution, offering food-fortificant ingredients applicable in various food products. Therefore, this study examined the physical and chemical properties of fishbone powder derived from *Pangasius* sp., *T. tonggol*, and *T. albacares*. The products preparations were subjected to cooking, autoclaving, pulverization, homogenization, drying, and grinding. The physical and chemical analysis of fishbone powder showed that *T. tonggol* and *T. albacares* had a darker color compared to *Pangasius* sp., with higher protein content and likely stimulation of a Maillard reaction during the drying process. All species maintained a neutral pH range, and their particle sizes significantly differed ($p< 0.05$), ranging from nm to µm. SEM images showed irregularly shaped and agglomerated particles in all fish species. The ash content was 54.35 g/100 g (*Pangasius* sp.), 53.62 g/100 g (*T. tonggol*), and 52.28 g/100 g (*T. albacares*), showing high mineral content, particularly in Ca exceeding 40%. The analysis of a Fourier transform infrared spectroscopy (FT-IR) showed carbonate and phosphate peaks, representing the presence of calcium salts. Based on the evaluation, fishbone powder for each species had the potential to serve as food fortificant.

Keywords: Characteristics; fishbone; fortificant; powder

INTRODUCTION

The global production of capture fisheries was 96.4 million tons (live weight eq.), while aquaculture fisheries reached 80.1 million tons (live weight eq.) in 2018. In this same year, Indonesia secured the second position after China in capture fisheries production (7.2 million tons/live weight eq.) and third after China and India in aquaculture fisheries production (5.4 million tons/live weight eq.) (FAO, 2021a). An essential economic commodity in Indonesia, with mass production, is derived from the *Thunnus* genus (capture product) and *Pangasius* (aquaculture product) (Hizbullah et al., 2019; Ramadhan et al., 2016). Data from KKP (Ministry of Marine Affairs and Fisheries) (2020) shows the production of these species reached 426.475 tons for *Pangasius* and 236.373 tons for *Thunnus* in 2020. The significant growth of the fishing industry has increased the processed fisheries products, including canned fish, fillet, and surimi-based products. For instance, total canned tuna processing production from January to March 2021 was approximately 291 (in

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1000 tons) from Thailand, Ecuador, Philippines, Spain, China, Netherlands, and Indonesia (FAO, 2021b). Furthermore, *Pangasius* frozen fillet products for export (based on harmonized system data no. 03043200, 03046200) were around 9.224.565 kg in October 2022 (BPS, 2022). These products use flesh as the primary raw material, generating waste, consisting of bones, heads, skin, viscera, and scales. Flesh constitutes about 50-60%, with the remainder being waste products (25-30% for head & bones, 10-15% for viscera), thereby contributing to environmental issues. Optimizing waste products by enhancing their properties can mitigate waste generation. Fishbone, by-products from the fish processing industry, contain abundant calcium (Ca) and minerals needed for human body growth (Cui et al., 2021; Shanshan, 2016).

Previous reviews have shown the richness of fishbone in Ca and minerals. Savlak et al. (2020) found that seabream fishbone contained 232-291 g/kg of Ca, 111-166 g/kg of phosphorous (P), 2.5-5.64 g/kg of magnesium (Mg), 72-102 mg/kg of zinc (Zn), and 72-748 mg/kg of potassium (K). Similarly, Nawaz et al. (2020) reported grass crap fishbone containing 231-238 mg/g of Ca, while Nemati et al. (2017) identified tuna fishbone with 38.16 g/100 g of Ca, 0.47 g/100 g of Mg, 0.08 g/100 g of Zn, 23.31 g/100 g of P, and 0.68 g/100 g of K. The study by Sumarto et al. (2021) observed that *Pangasius* fishbone contained 51.5% of Ca and 34.7% of P. However, nutritional deficiencies, including both macro and micro minerals, can significantly affect human health. Some essential nutrients, such as Ca, cannot be synthesized by the human body, requiring appropriate intake from food sources for growth, skeletal system rigidity, and various essential functions (Shkembi & Huppertz, 2022). Inadequate Ca intake can lead to preeclampsia, eclampsia during maternity, high blood pressure, increased LDL cholesterol, and a decreased prevention of fractures (Cormick & Belizán, 2019; Shlisky et al., 2022). This issue is evident in Indonesia, where teenagers in Agam Regency, West Sumatra, have an average Ca intake below the recommended dietary allowance (RDA) (only 882.7 mg/day from 1200 mg/day) (Desrida et al., 2017). Additionally, 64% of Indonesian women (60-64 years) have osteoporosis due to decreased bone density resulting from insufficient Ca intake (Sugianto et al., 2014). Using Ca from fishbone as food fortification for fortifying food products can address this issue. Food fortified with fishbone powder tends to sustain a healthy diet and become an alternative to daily Ca and mineral intake (Amitha et al., 2019; Handayani et al., 2020). Previous reviews on fishbone powder as food fortifier were conducted by Asikin et al. (2019), exploring knife (*Chitala* sp.) fishbone powder in the processing of *Amplang*. Benjakul & Karnjanapratum (2018) examined the development of whole wheat crackers fortified with tuna bone bio ca-powder, while Handayani et al. (2020) focused on creating dumplings fortified with gourami (*Osphronemus gouramy*).

The main process for using fishbone as Ca fortification consisted of softening and restructuring. Various methods, comprising acidic, alkaline, enzymatic, physical, or combined treatments, were used. The physical treatment was considered safer due to the absence of chemical reagents in the process. For instance, Nawaz et al. (2020) adopted a colloidal mill for grass crap bone powder, Cui et al. (2021) used a steam explosion apparatus for tuna products, Putri et al. (2020) utilized a multi-mill machine for *Pterygoplichthys pardalis*, Yin et al. (2017) used coarsely milled for silver crap bone powder, and Handayani et al. (2020) adopted a blender for gourami. Therefore, this study distinguishes itself from previous ones by using three different species, including *Pangasius* sp., *T. tonggol,* and *T. albacares*. The extraction method consisted of cooking (boiling), autoclaving, pulverizing (meat-grinder), and high-speed homogenization to produce pasta, subsequently dried in the oven and ground to create fishbone powder. The objective of this study was to determine the characteristics of fishbone powder as food fortificant from *Pangasius* sp., *T. tonggol*, and *T. albacares*. Furthermore, the characteristics of the products, including physical aspects (color, images, size, and particle distribution) and chemical (pH, proximate, minerals, and structure components), were thoroughly evaluated.

METHODS

Material

Three fish species, comprising *Pangasius* sp, *T. tonggol,* and *T. albacares* were used in creating fishbone powder*. Pangasius* sp. was sourced from a local culture pond in Sleman-Yogyakarta, while *T. tonggol* and *T. albacares* were obtained from the Sadeng fish market in Gunungkidul-Yogyakarta, Indonesia. Each species was obtained in quantities of approximately 1 to 2 kg/head. The fish were transported under iced conditions from Sleman and Sadeng to the laboratory. Fishbone was extracted through heading, evisceration, filleting, washing with tap water, and subsequently stored at -20 °C for further analysis. Additional materials included Kjeldahl tablets (Merck, Germany), H_2SO_4 (95-97% Merck, Germany), HCl (25% Merck, Darmstadt-Germany), NaOH (EMSURE, Germany), petroleum ether (Merck, Germany), and boric acid (EMSURE, Germany).

Equipment used in the study included an autoclave (Model TOMY SS-325, TOMY Kogyo co., LTD, Tokyo, Japan), a meat grinder (Tummy capsule cutter quatre OLL-688, China and Hand Blender Neo Tokebi, Korea), a high-speed homogenizer (IKA T25 Digital Ultra Turrax, Germany), an oven (Memmert, Germany, flow 100%), and a Chroma Spectrophotometer CM-5 (Konica Minolta, Japan linked with Color Management Software SpectraMagic™ NX CM-S100w). There was also a digital pH meter (Eutech Instruments PC 700, Singapore) equipped with a probe (Eutech Thermo Scientific, Singapore), particle analyzer (Horiba Scientific SZ 100, Japan), a scanning electron microscope (SEM Hitachi SU3500, Japan, acceleration 10 kV, vacuum: 6 Pa, x30.000 magnification) attached with an energy-dispersive X-ray spectrometer (EDX), and Images measurement observed by SEM SU3500 software package). Additionally, the equipment included a moisture analyzer (OHAUS MB 120, USA), water bath (Memmert, Germany), an X-ray Fluorescence (XRF) (Epsilon 4, linked with Epsilon software, Malvern Panalytical Ltd, UK), a Fourier transform infrared spectroscopy (FT-IR) spectrometer (Vertex 80, Bruker, Germany, linked with the OPUS software program), and statistical software (R studio 4.1.2).

Fishbone Powder Preparations (Modification from Nawaz et al. (2018))

The bones were initially cut into smaller pieces (15-20 cm), washed with tap water, and boiled for 30 minutes at 90-100 °C. After boiling, they were rinsed with warmed-aquadest three times to remove any remaining meat, fat, and blood. Subsequently, the bones were cut into minor pieces (about 5-10 cm) and autoclaved at 121 °C and 15 psi for approximately 45 minutes. Autoclaved bones were subjected to grinding in a meat grinder until they were transformed into mashed/pureed fishbone for size reduction. Following this, ice-deionized water was added to the mashed fishbone in a ratio of 0.9:1 (Nawaz et al., 2018), and the mixture was blended in a meat grinder for one minute to achieve homogeneity. The mashed products were further subjected to high-speed homogenization at 10,000 rpm for 20 minutes (Nawaz et al., 2018) to create bone-pasta products. The pasta was dried in an oven for two days at 50 °C until it reached a maximum moisture content of 8%, adhering to the National Standardization Agency *01-3158-1992* for fishbone powder (Febriani et al., 2021). The dried pasta was ground using a meat grinder to pass through a 100-mesh sieve. The resulting fishbone powder was packed in a ziplock-plastic standing pouch and stored at room temperature for further analysis.

Analysis

The color of fishbone powder was assessed using a Chroma Spectrophotometer, and the data were expressed as *L**, *a**, *b**. In the analysis, the *L** value showed lightness/brightness, the *a** represented redness to greenness, and the *b** signified yellowness to blueness. The whiteness index (WI) was determined using the Equation 1 (Baycar et al., 2022).

$$WI = 100 - [(100-L)^2 + a^2 + b^2]^{1/2} \quad (1)$$

The pH of fishbone powder was measured with a digital pH meter, and the data were recorded by immersing the pH-meter probe into 20 ml of the powder solution (Simbolon et al., 2019). A particle size analysis was conducted using a dynamic light scattering particle analyzer (Yin et al., 2015), while the morphology was observed through scanning electron microscopy linked with EDX (Benjakul & Karnjanapratum, 2018). Moisture content was determined by adopting a Moisture analyzer at 105 °C (Cebeci et al., 2020), and ash content was determined gravimetrically by incinerating 1-2 g of the sample at 550 °C for 6 hours (Cebeci et al., 2020). The ash content was calculated using the Equation 2.

$$Ash\ content = \frac{Ash\ weight\ (g)}{Sample\ weight\ (g)} \times 100\% \quad (2)$$

The crude protein content was analyzed through the Micro-Kjeldahl method (AOAC, 2005). The Kjeldahl method consisted of destruction/digestion, distillation, and titration processes (Putri et al., 2020). The percentage of crude protein content was calculated by multiplying total nitrogen by the protein factor (Pf), which was specifically 5.6 for fishery products (Mariotti et al., 2008). Total carbohydrates were determined using an alternative method (Ooi et al., 2012; Putri et al., 2020). The calculation for total carbohydrates applied the Equation 3.

$$\text{Carbohydrates by diff} = 100 - (\text{crude protein in DW} + \text{crude fat in DW} + \text{ash in DW}) \quad (3)$$

Mineral contents (Ca, P, Mg, K, Na) were analyzed using XRF, a non-destructive method measuring the fluorescent X-ray emitted when the element was excited by a primary X-ray source (Feng et al., 2020). The chemical structure was assessed using FT-IR, with the sample examined in the frequency range of 400 to 4000 cm^{-1}. The Middle IR spectrum (400-4000cm^{-1}) was widely used in the sample analysis (Nandiyanto et al., 2019).

Statistical Analysis

All experiments were conducted in triplicates, and the results were presented as means ± standard

deviation. The analysis of variance (ANOVA) and Duncan multiple range tests (DMRT) were performed (p<0.05) using R studio 4.1.2 software.

RESULTS AND DISCUSSION

The Color Evaluation of Fishbone Powder

Table 1 provided the color evaluation of fishbone powder, which was presented in *L**, *a**, and *b** values. The *L** signified lightness/brightness, *a** showed redness to greenness, and *b** represented yellowness to blueness. Furthermore, the *L** value for *Pangasius* sp., *T. tonggol*, and *T. albacares* bone powder was 91.51 ± 0.12, 81.24 ± 0.15, and 84.28 ± 0.41, respectively. Each color parameter showed significant differences ($p<0.05$) among the species. A higher *L** value suggested a lighter, brighter, or whiter product, influencing the highest WI. Moreover, higher *a** and *b** values showed a darker product, with *T. tonggol* having the highest values among the species. A lower *L** value corresponded to higher *a** and *b** values. This was in line with the observation of Nawaz et al. (2018) and Desai et al. (2018) stating that the lower lightness (*L** value) led to higher redness (*a** value) and yellowness (*b** value).

This study showed that both *Thunnus* species had a darker powder compared to *Pangasius* sp. Pelagic fish, such as *Thunnus*, possess darker muscle containing more myoglobin than freshwater fish, particularly *Pangasius* (Kannaiyan et al., 2019). Myoglobin, a key protein influencing meat color (Mancini & Hunt, 2005), contributed to the reddish-brown color of fish muscle (Kannaiyan et al., 2019), showing in the higher *a** value. Despite cooking (boiling) and various size reduction processes, the inner bone tissue of pelagic fish retained more myoglobin than freshwater fish. Additionally, the Maillard reaction during the drying process contributed to *Thunnus* lower WI value, causing browning. The Maillard reaction, a non-enzymatic process, occurred between amino groups (amino acids) and carbonyl groups (reducing sugars) (S. Liu et al., 2022), resulting in brown-black colors. Amino groups in the Maillard reaction were amine groups from amino acids, while reducing sugars comprised monosaccharides, disaccharides, five-carbon sugars, six-carbon sugars, aldehyde compounds, and keto-based compounds (X. Liu et al., 2020). *Thunnus*, with a higher crude protein content (Table 3), including amino acids, had a darker colored powder due to the browning phase of the Maillard reaction during the drying process.

The pH, Particle, and Distribution Size Evaluation of Fishbone Powder

Table 2 presented the pH and particle size evaluations. Fishbone powder from three different species had a neutral pH range, of approximately 6 to 7. In consideration of safety, fishbone extraction was conducted without applying any solvent, resulting in neutral pH conditions. Both pH and particle size showed significant differences (p<0.05) among all the species. Despite variations in values among species, the pH remained within the neutral range, without showing acidity or alkalinity. These differences might be attributed to the initial pH conditions of the raw materials used in the preparation of the products. A study conducted by Talib et al. (2014) reported a lower pH value because acidic treatments were adopted to enhance bioavailability.

Particle sizes had a broad distribution (Table 2), ranging from nano to micron sizes due to size-reducing processes such as cooking, autoclaving, pulverizing,

Table 1. Color evaluation of fishbone powder

Species	Parameter				
	L	*a**	*b**	WI	Color images*)
Pangasius sp.	91.51 ± 0.12^{a}	0.17 ± 0.01^{c}	7.28 ± 0.03^{c}	88.81 ± 1.10^{a}	
T. tonggol	81.24 ± 0.15^{c}	3.13 ± 0.02^{a}	20.02 ± 0.08^{a}	72.39 ± 0.12^{c}	
T. albacares	84.28 ± 0.41^{b}	1.95 ± 0.03^{b}	19.29± 0.32^{b}	75.03 ± 0.09^{b}	

Notes: Analysis was performed in triplicates. Data were expressed as means ± standard deviations. The same letters were not significantly different based on DMRT (p<0.05). *) sign showed the color appearance of fishbone powder taken by color measurement instruments.

Table 2. pH and particle size evaluation of fishbone powder

Parameter	pH	Particle size (n-µm)
Pangasius sp.	7.08 ± 0.01[a]	639.65 ± 2.75[b]
T. tonggol	6.03 ± 0.01[c]	555.8 ± 3.30[c]
T. albacares	6.65 ± 0.02[b]	1020.8 ± 3.30[a]

Notes: Analysis was done in three replicates. Data were expressed as means ± standard deviations. The same letters were not significantly different based on DMRT (p<0.05).

homogenizing, and drying. According to Harmain et al. (2018), nanoparticles were designed for easy absorption or solubilization by the body, typically ranging between 10-1000 nm (Anonim, 2015; Carvalho et al., 2018). Meanwhile, particle size distribution represented the cumulative concentration of particles in a range of sizes (Anonim, 2015). The type of calcium salts present in food fortificant also influenced absorption. Palacios et al. (2021) mentioned that several calcium salts approved for use as food fortificant (including calcium carbonate, calcium chloride, calcium phosphate, calcium sulfate, and calcium citrate), had a bioavailability ranging from 20% to 40%.

The results showed that the particle size of *Pangasius* sp. bone powder was distributed widely, ranging from 402.44 to 1363.97 nm, with an average maximum accumulation rate of 639.65 ± 2.75 nm (Figure 1.a). *T. tonggol* had a particle size distribution spanning 356.20 to 1741.10 nm, with an average maximum accumulation rate of 555.8 ± 3.30 nm (Figure 1.b). Meanwhile, *T. albacares* bone powder had a particle size range of 454.69 to 5901.02 nm, with an average maximum accumulation rate being 1020.8 ± 3.30 nm (Figure 1.c). The observed differences in sizes among the species might be attributed to the varying rigidity of the bone matrix structure. Cui et al. (2021) reported a broad particle size range from 710 to 1782.502 µm for tuna fishbone powder produced through autoclaving, drying (oven), and grinding (using a high-speed blade mill).

SEM Images and SEM-EDX Spectroscopy of Fishbone Powder Particles

Figure 2 presented scanning electron microscopy images of fishbone powder particles derived from

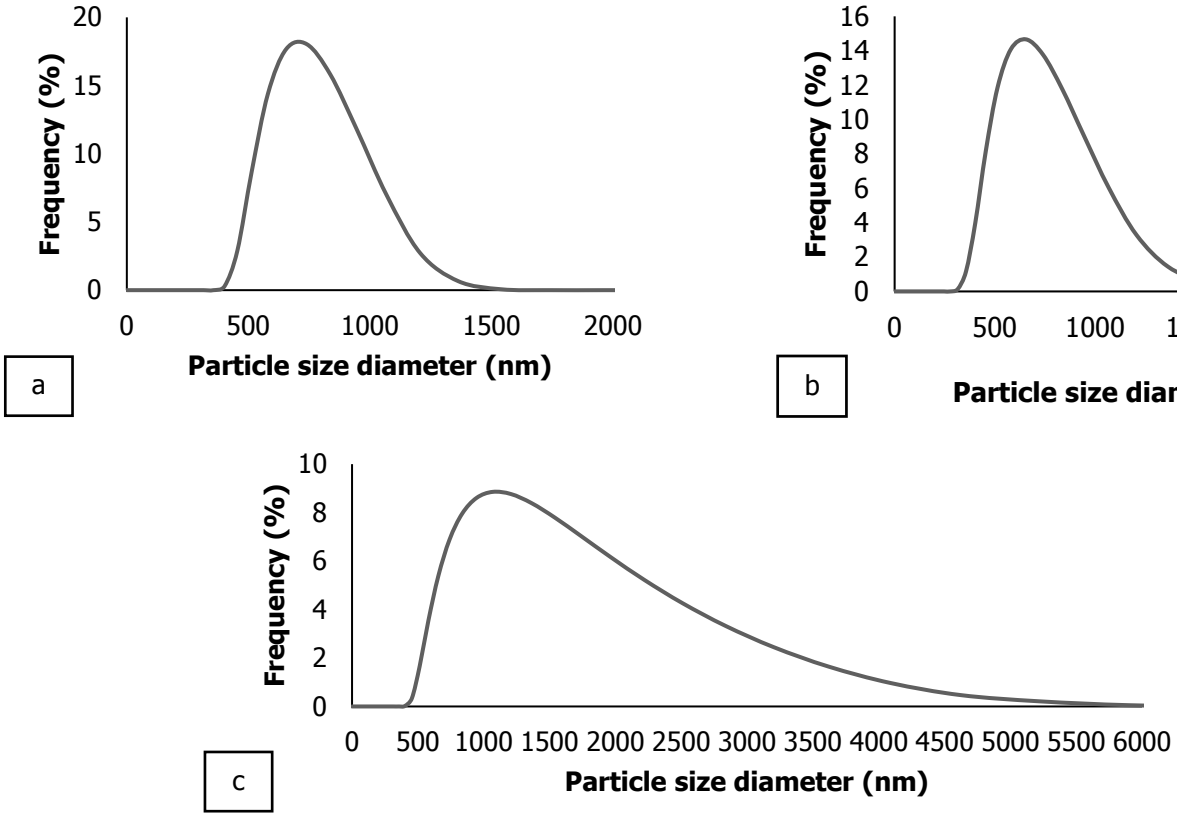
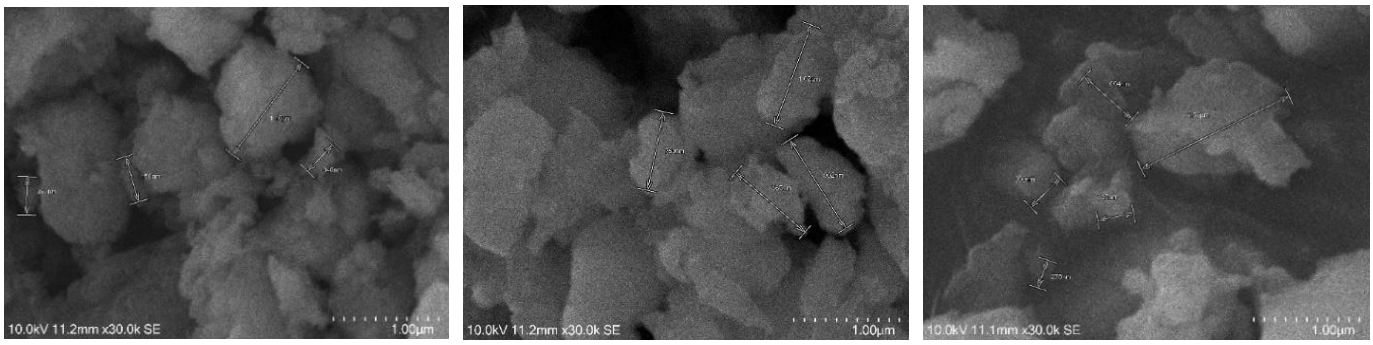


Figure. 1. Particle size distribution chart of *Pangasius* sp. bone powder (a), *T. tonggol* bone powder (b), *T. albacares* bone powder (c)



(a) (b) (c)

Figure 2. SEM images of fishbone powder particles: *Pangasius* sp. (a), *T. tonggol* (b), *T. albacares* (c)

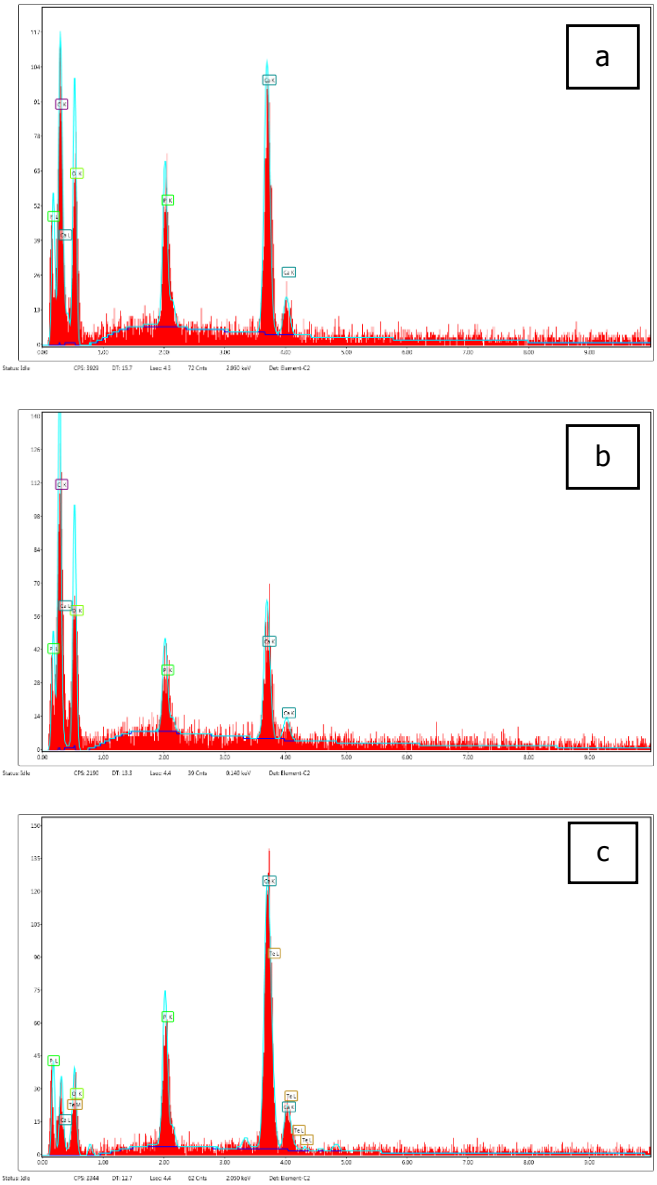


Figure 3. EDX spectra of bone powder particle: *Pangasius* sp. (a), *T. tonggol* (b), *T. albacares* (c)

three species at 30,000x magnification, representing irregular morphological structures and agglomerated particles. The term agglomerated referred to particles holding together (Anonim, 2015). The shapes might have arisen from the processes of cooking, autoclaving, pulverizing, homogenizing, and drying fishbone matrix to reduce its size. According to Nawaz et al. (2020), these physical processes might have caused the inner matrix to disintegrate, leading to calcium release. The uneven shape, agglomerated particles, and various particle sizes were likely attributed to variations in bone matrix density between species and the applied physical treatments.

In Figure 3, the EDX images showed prominent peaks associated with the presence of Ca and P, with higher proportions than other elements (Table 3). The intense peaks were line in with the results in Table 3, showing elevated levels of Ca and P. Detection challenges for trace minerals with SEM-EDX, having a limit of 1-10% wt, precluded the presence of other elements (Choěl et al., 2005; Idowu et al., 2020). This result, similar to a previous study by Yin et al. (2015), showed prominent peaks of nanosilver carp fishbone extracted using the physical milling process, emphasizing the high-intensity elements of Ca and P.

Chemical Characteristics Evaluation (Proximate And Minerals) of Fishbone Powder

The chemical composition of fishbone powder was presented in Table 3. Several reduction size processes, including cooking, autoclaving, pulverizing, homogenizing, and drying, were used to preserve the nutritional percentages. Fishbone powder from *Pangasius* sp., *T. tonggol*, and *T. albacares* had respective moisture contents of 4.21 g/100 g, 3.69 g/100 g, and 5.10 g/100 g. The drying procedure maintained a moisture level of 3-5 g/100 g, impacting the storage stability of the products. According to Sumarto et al.

(2021), the moisture content of fishbone powder could be influenced by the physical and chemical properties of the raw material and the drying process. The ash content of the products was 54.35 g/100 g (*Pangasius* sp.), 53.62 g/100 g (*T. tonggol*), and 52.28 g/100 g (*T. albacares)*, showing richness in mineral content. The variation in the ash concentration was influenced by the mineral composition of each species (Sumartono et al., 2021). Furthermore, the presence of high crude fat content in fishbone powder might be attributed to its retention during the processing steps. The crude fat content of the products was 12.10 g/100 g, 10.49 g/100 g, and 12.99 g/100 g for *Pangasius* sp., *T. tonggol, and T. albacares*. The crude protein of fishbone powder ranged from 20.36 g/100 g for *Pangasius*, 21.53 g/100 g for *T. tonggol*, 21.77 g/100 for *T. albacares*, while carbohydrate ratios for *Pangasius* sp., *T. tonggol*, and *T. albacares* were 12.79 g/100 g, 14.36 g/100 g, and 12.95 g/100 g, respectively. The nutritional values had similarities, with significant differences among them. Essentially, the protein content in this study was lower than in previous reviews. Sumartono et al. (2021) reported a protein content of 24.39 g/100 g for *Pangasius*, and Cui et al. (2021) found a protein content of 26.6 g/100 g for *Thunnus* fishbone powder. The difference in protein content between the present study and previous ones could be attributed to distinct extraction methods used in fishbone powder production.

The Ca content exceeded 40% in all species (Table 3). The fish extraction method facilitated the breakdown of the inner bone matrix, leading to the release of Ca. As observed by Sumartono et al. (2021), bones contain living cells and intracellular matrices in the form of inorganic salts. P constituted the second-highest percentage, exceeding 12% across all species, while other minerals were present as trace elements. Similar to previous reviews (Cui et al., 2021; Nemati et al., 2017; Savlak et al., 2020; Sumarto et al., 2021), this study showed that Ca and P were predominant elements in fishbone powder products.

There was currently no national standard for fishbone powder intended for use as food fortificant, there was a standard only for feed (*National Standardization Agency 01-3158-1992*) (Table 4). It was essential to evaluate the study based on the standard requirements of specifications for comparable products. Some evaluations were in line with the specifications of the *National Standardization Agency 01-3158-1992*, such as moisture and Ca. The investigation unveiled additional factors not specified in the standards, but the factors played a significant role in determining the products quality (table 3). As of then, the quality of the products had been maintained in accordance with standards.

FT-IR of Fishbone Powder

Figures 4, 5, and 6 outlined subtle variations in absorption peaks and chemical structures among the species. The absorption peaks in the range of 1490-1410 suggested the presence of the carbonate ion (Nandiyanto et al., 2019), exemplified by the peaks at 1411.74 (Figure 4), 1412.52 (Figure 5), and 1450.55 (Figure 6). The peaks around 1100-1000 signified the phosphate ion (Nandiyanto et al., 2019), as evidenced

Table 3. Chemical characteristics evaluation (in dry basis) of *Pangasius* sp., *Thunnus tonggol*, *Thunnus albacares* bone powder

Parameter	*Pangasius* sp.	*T. tonggol*	*T. albacares*
Moisture (g/100 g)	4.21 ± 0.19^{b}	3.69 ± 0.02^{b}	5.10 ± 0.49^{a}
Ash (g/100 g)	54.35 ± 0.68^{a}	53.62 ± 0.08^{a}	52.28 ± 0.11^{b}
Crude fat (g/100 g)	12.10 ± 0.64^{a}	10.49 ± 0.55^{b}	12.99 ± 0.20^{a}
Crude protein (g/100 g)	20.36 ± 0.22^{b}	21.53 ± 0.24^{a}	21.77± 0.45^{a}
Carbohydrates by diff (g/100 g)	12.79 ± 0.27^{b}	14.36 ± 0.74^{a}	12.95 ± 0.41^{b}
Calcium (%)	46.915±0.09^{a}	44.830±0.034^{c}	45.277±0.279^{b}
Phosphor (%)	13.126±0.027^{a}	12.907±0.031^{ab}	12.862±0.183^{b}
Potassium (%)	0.189±0.0004^{c}	0.214±0.000^{b}	0.369±0.001^{a}
Sodium (%)	0.482±0.032^{b}	0.413±0.002^{c}	0.549±0.015^{a}
Magnesium (%)	0.565±0.007^{a}	0.531±0.009^{b}	0.516±0.003^{b}

Notes: Analysis was done in three replicates. Data are expressed as means ± standard deviations. The same letters are not significantly different based on DMRT (p<0.05). All parameters were calculated in the dried weight.

Table 4. National standard for fishbone powder for feed industry (*National Standardization Agency 01*-3158-1992)

No	Characteristics of bone powder	Requirements	
		Quality 1	Quality 2
1	Moisture content (%) (max)	8	8
2	Crude fat content (%)	3	6
3	Calcium content (%) (min)	30	20
4	Phosphate content (as P_2O_5), (weight/dry weight) (Min)	20	20
5	Phosphate content (P), % (weight/dry weight)	8	8
6	The fineness of sand/silica, % (weight/dry weight) (Max	1	1
7	Fineness (Mesh 25), (weight/dry weight) (Min)	90	90

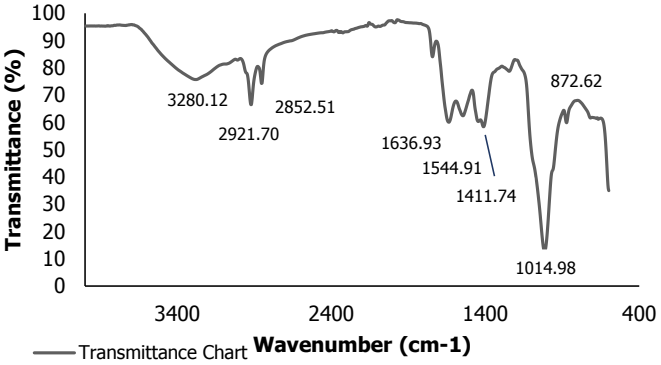


Figure 4. FTIR spectra of *Pangasius* sp. bone powder

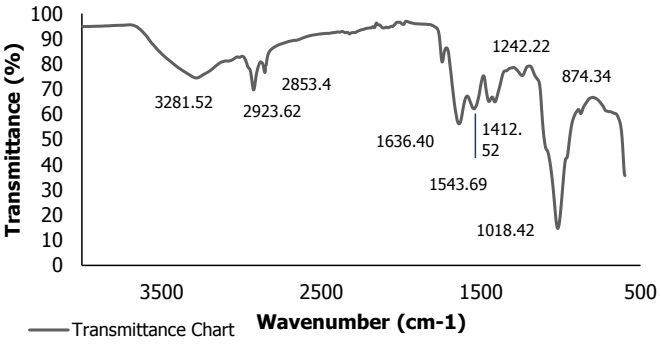


Figure. 5. FT-IR spectra of *T. tonggol* bone powder

by strong peaks at 1014.98 (Figure 4), 1018.42 (Figure 5), and 1017.34 (Figure 6). Additionally, the peaks in the 995-850 range showed phosphate for P-O-C stretch (Nandiyanto et al., 2019), exemplified by peaks at 872.62 (Figure 4), 874.34 (Figure 5), and 873. 85 (Figure 6). The peaks at 1590–1720 cm^{-1} represented amide I, showing characteristic collagen peaks (Nawaz et al., 2020; Qin et al., 2022), evident in peaks around

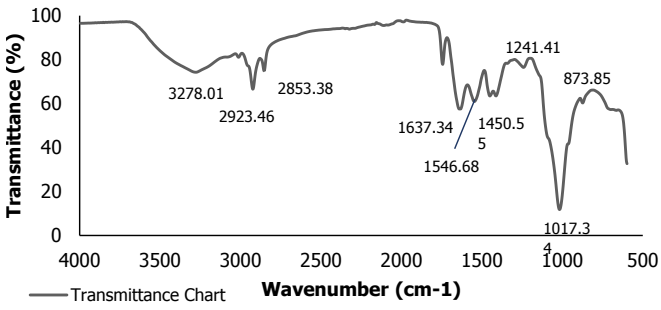


Figure. 6. FT-IR spectra of *T. albacares* bone powder

1636.93 (Figure 4), 1636.40 (Figure 5), and 1637.34 (Figure 6). Moreover, the peaks at 2852 showed the absorption of organic material (C-H) (Boutinguiza et al., 2012), evident in the peaks at 2852.51 (Figure 4), which had a low-intensity. The peaks representing 3273-3293 showed stretching O-H symmetric, possibly related to water (Nandiyanto et al., 2019), exemplified by peaks at 3280.12 (Figure 4), 3281.52 (Figure 5), and 3278.01 (Figure 6). This presence of adsorbed water was due to the use of iced deionized water in fishbone extraction. The analysis of FT-IR showed that the chemical structure of fishbone powder was influenced by the extraction method and the applied species but it had a relatively similar pattern. Based on the analysis, calcium salt types present in fishbone powder of each species included carbonate and phosphate, two types widely used in food fortification (Palacios et al., 2021).

CONCLUSION

In conclusion, based on an investigation of its properties, fishbone powder made from *Pangasius* sp., *T. tonggol*, and *T. albacares* showed potential as a food fortificant. The analyses of proximate elements

(protein, fat, ash, moisture, and carbohydrates) were similar for all species, but *Thunnus* had a higher protein content. All species had a varied mineral composition, with Ca containing more than 40%. Pangasius had the highest whiteness index (WI). The fishbone powder from all species had a neutral pH range, varied sizes ranging from nano to micron, irregular morphological structures, and agglomerated particles. The FT-IR analysis revealed carbonate and phosphate in each species' fishbone powder, representing types of calcium salts suitable for food fortification.

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CONFLICT OF INTEREST

The authors declared no known competing financial or personal relationships that could have influenced the work reported in this paper.

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