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Natural sources of calcium and phosphorus in fish bones of *Plotosus canius* (Hamilton, 1822) and *Scomberomorus guttatus* (Bloch and Schneider, 1801) obtained from Banyuasin waters, South Sumatra, Indonesia

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Abstract

The fish meat production of *Plotosus canius* and *Scomberomorus guttatus* as a food source is worrying, as it increases the volume of bone waste, which damages the environment. This work aimed to determine the calcium and phosphorus content in fish bones of different species and size categories. Samples were collected from the coastal Banyuasin, South Sumatra. The samples were classified into three size categories: large (> 300 g), medium (150-250 g), and small (100 g). All trials were based on absorbance measurements using atomic absorption spectroscopy (calcium) and spectrophotometry UV -Vis (phosphorus). The statistical analysis used ANOVA, least significant difference (LSD), and independent sample T-test. Based on the results, the calcium content in fish bones of *P. canius* was 11.2%, 10.4%, and 9.3%, and phosphorus was 0.0238%, 0.0207%, and 0.0106%. The calcium content in fish bones of *S. guttatus* was 13.3%, 10%, and 7.4%, and phosphorus was 0.0271%, 0.0224%, and 0.0116%. The ANOVA results stated that the sample category had a real effect on calcium and phosphorus content (P = 0.05), followed by the results of the LSD test for each category were different, and the independent sample T-test Sig. (2-tailed) value exceeded 0.05, showing that there was no average difference in each fish bone. Fish bones of *P. canius* had a greater calcium content than *S. guttatus*, while *S. guttatus* had a greater phosphorus content than *P. canius*. According to the World Health Organization's calcium and phosphorus standards, fish bones from these two species can be developed into natural hydroxyapatite that is useful for human needs.

1. Introduction

Banyuasin coastal waters have a high potential for fishery products from the pisces, cephalopods, gastropods, and bivalves' classes (Rozirwan, Fauziyah, Nugroho *et al*., 2022; Rozirwan, Ramadani, Putri *et al*., 2023). Coastal people utilize fishery products as a source of livelihood to increase economic growth (Saputra *et al*., 2021). The rapid development of the industry in the field of processing fishery products has the potential to cause an increase in waste (Afreen and Ucak, 2020). The existence of this waste causes the formation of a decomposition process by sulfuric acid (H_2S), ammonia (NH_3), methane (CH_4), and CO_2, causing an unpleasant odor (Dewita *et al*., 2021). In addition, waste can lead to long-term degradation of the aquatic environment, potentially threatening the food security of coastal communities (Almaniar *et al*., 2021). The problem of fish waste has grown and has become a global concern in recent years. As much as 75% of fish biomass, including bones, heads, offal, skin, and fins, is not consumed because it requires further processing (Metwally *et al*., 2021). Fish bones can be used as raw materials to produce value-added compounds in various sectors, including agrochemical, biomedical, food, and pharmaceutical (Hlordzi *et al*., 2022). Fish bones as a source of calcium phosphate (CaP) ceramics have become the focus of many research studies because of their potential to produce quality biotechnological

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materials (Boutinguiza *et al*., 2012). Fish bones are a complex substance made of carbonated HAP, type-1 collagen, non-collagenous protein, and water (Ma *et al*., 2021). Calcium-phosphorus (Ca-P) based compounds are among the most widely used biomaterials for bone substitution (Corrêa and Holanda, 2019).

Calcium and phosphorus are essential minerals involved in key physiological functions, including metabolism, muscle contraction, and the formation of bones, scales, ATP, cell membranes, and nucleic acids (Manz *et al*., 2023). Chemical analysis revealed that fish bones are a valuable calcium phosphate source as an economical source for synthesizing hydroxyapatite (Pon-On *et al*., 2016). Bone is a biological composite consisting of an inorganic phase (calcium phosphate with a structure like carbonated hydroxyapatite) (Harvey *et al*., 2021). In general, hydroxyapatite ($Ca_5HO_{13}P_3$) is a mineral of calcium phosphate, is a significant component of bone, and can be used as a material for bone regeneration (Lee *et al*., 2021). Natural hydroxyapatite can be easily obtained from natural sources such as cow bones, pork bones, and fish bones (Prado *et al*., 2021). Research has shown that calcium and phosphorus in pelagic fish bones have potential as natural hydroxyapatite for bone repair and replacement (Prado *et al*., 2021). However, comparative data on calcium and phosphorus content in fish species with differing morphology, physiology, and habitat remain limited. Therefore, this study aims to compare the bone mineral content of *P. canius* and *S. guttatus* to evaluate their potential as sources of natural hydroxyapatite.

2. Materials and methods

2.1 Samples

Fish samples of *P. canius* and *S. guttatus* were obtained from fish collectors in Sungsang Village, Banyuasin, South Sumatra (Figure 1). Banyuasin waters are known as a major fishing area (Rozirwan, Fauziyah, Wulandari *et al*., 2022). Some criteria for fresh fish are red gills and scales, not slimy, transparent, convex eyes, clear eye corneas, and fishy smell (Issac *et al*., 2017). Identification of fish samples referred to White *et al*., (2013). There were 3 sizes of fish samples, namely large (>300 g), medium (150-250 g), and small (<150 g). Each size has three individuals.

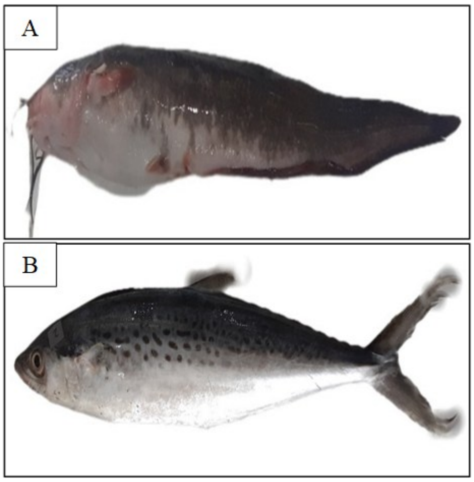

Figure 1. Fish morphology (A) *P. canius* (2) *S. guttatus*.

2.2 Sample preparation and destruction

The separation of fish bones from other organs of the body for further processing involves boiling fish bones at a temperature of 100°C for 1 hr to remove organic substances, blood, and meat attached (Boutinguiza *et al*., 2012). Then proceed with the alkaline extraction process with NaOH 1.5 N for 2 hrs by soaking at 60°C to remove protein, fat, and blood (Pon-On *et al*., 2016). After this, the fishbone samples were rinsed with distilled water and running water to neutralize the pH of the fish bones (Atma *et al*., 2018). The fish bones were dried in an oven at 65°C for 48 hrs to reduce the water content and were ground with a porcelain mortar and pestle (Sumarto *et al*., 2021). The preparation aimed to minimize the presence of impurities that would interfere with the analysis process by eliminating components other than the analyte (Rozirwan, Hananda, Nugroho *et al*., 2023). For calcium digestion, 1 g of the sample was mixed with 5 mL HNO_3, left at room temperature for 1 hr, then heated for 4 hrs and left overnight. Next, 0.4 mL H_2SO_4 was added and reheated for 1 hr. A few drops of $HClO_4$:HNO_3 (2:1) were added until the solution turned light yellow. The sample was cooled, mixed with 2 mL distilled water and 0.6 mL HCl, reheated for 15 minutes, then filtered into a 100 mL volumetric flask. For phosphorus digestion, 2 g of the sample was treated with Bray I extractant (30 mL 1 N ammonium fluoride and 5 mL 5 N HCl). The phosphate reagent was prepared by mixing ammonium molybdate, potassium antimonyl tartrate, ascorbic acid, and 5 N H_2SO_4, then diluted to 2 L with distilled water.

2.3 Determination of yield value

The fish bones sample that had been powdered was calculated for the yield value to determine the percentage ratio of the dry weight of fish bones (powder) to the wet weight of bones raw material. Yield was calculated based on the formula referring to Association of Official Analytical Collaboration International (1995).

2.4 Absorbance measurement of calcium and phosphorus content

Calcium content was measured using atomic absorption spectrophotometry (AAS) at a wavelength of 422.7 nm (Supriadi *et al*., 2021). Phosphorus was analyzed using a UV-Vis spectrophotometer by measuring light absorption in the UV (180–380 nm) or

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visible (380–780 nm) range (Pratiwi *et al*., 2022).

2.5 Statistical analysis

A one-way ANOVA was used to evaluate differences among group means for more than two samples, followed by the LSD test to identify specific differences (Rozirwan, Ramadani, Putri *et al*., 2023). For comparisons between two groups, an independent samples t-test was applied. A p-value below 0.05 showed a significant difference. Analyses were conducted using IBM SPSS Statistics v26.

3. Results and discussion

3.1 Yield value

The variation in yield values reflects the quantity of product obtained, indicating the efficiency of the extraction procedures applied (Figure 2). The results of yield value in each process through extraction with NaOH to remove fat, blood, and protein from the bone. Based on previous research Zainol *et al*., (2019), the extraction of fish scales with 5 N NaOH produced a yield value of 68%. However, after sintering at 1200°C, the yield was only 36%. The reduction in weight after sintering was probably due to the loss of organic residues in the fish scales after the alkaline treatment. Fishbone extraction can be used with several variations of 3% HCl at 10.1%, 3% H_3PO_4 at 9.6%, and 3% CH_3COOH at 9.3%. The higher the concentration of the acid solvent used, the resulting extraction will have an increased degree of acidity (Aisman *et al*., 2022). The difference in yield values produced can be caused by the method, solution concentration to remove non-collagen protein,

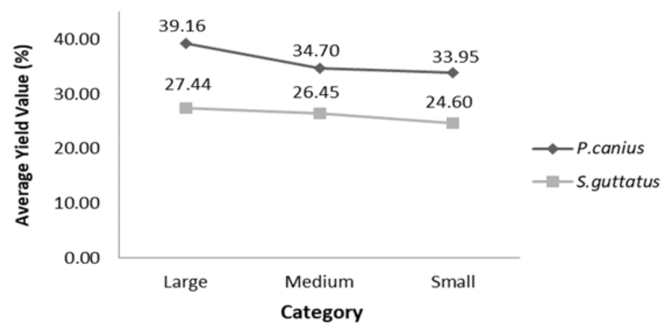

Figure 2. Average yield value in bones of *P. canius* and *S. guttatus.*

type of material, temperature, and production time (Wijaya and Junianto, 2021).

The yield values obtained in this study may reflect the quality of the bone meal produced from both fish species. A higher yield is generally associated with better flour quality. Fish bone meal is a solid product derived by removing most of the water content and a portion or all of the fat from fish bones (Ma *et al*., 2021). Fish meal could be used as the main component of aquaculture feed, which contains many nutrients such as protein, essential amino acids, omega-3 fatty acids, attractants, vitamins, and minerals (Hlordzi *et al*., 2022). The calculation of yield values was carried out to determine the success rate of food production. The higher the success of the production process, the better the quality of production and the more valuable the products become in various fields of fisheries (Atma *et al*., 2018).

Plotosus canius was classified as a demersal fish that prefers marine and brackish water habitats and is primarily found in estuaries, rivers, lagoons, and shallow waters (Prithiviraj and Annadurai, 2012). Mangrove forest waters have many *P. canius* fish for appropriate foraging, spawning, and enlargement (Rozirwan, Nugorho, Hendri *et al*., 2022; Rozirwan, Muhtadi, Ulqodry *et al*., 2023). *Scomberomorus guttatus* is a pelagic fish species typically found in muddy coastal waters, with a distribution range extending to depths of up to 50 meters (Al-Husaini *et al*., 2021). The distribution of pelagic fish is influenced by the environment, and pelagic fish tend to migrate to fertile seas (Welliken *et al*., 2021). Morphological observations were carried out on *P. canius*, in which the antennae functioned as a tactile tool to find food (Chakraborty and Yardi, 2020). The second dorsal fin is located on a vertical line between the anal and pelvic fins, and the tail type is pointed, has a dark brown color, no scales, and is slimy (Asriyana *et al*., 2020). *Scomberomorus guttatus* had a torpedo body shape, smooth skin, no scales, a select mouth type, and a semicircular tail type (Hakim *et al*., 2020).

3.2 Calcium and phosphorus content

The average calcium content in *P. canius* was 11.2%, 10.4%, and 9.3% for large, medium, and small sizes, respectively, while in *S. guttatus* it was 13.3%, 10.0%, and 7.4% for the corresponding size categories (Figure 3). Phosphorus content in *P. canius* was

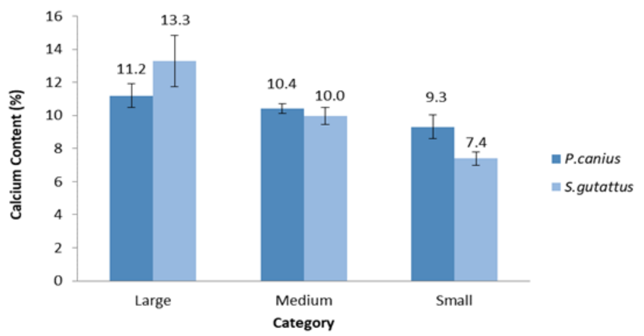

Figure 3. Calcium content in bones of *P. canius* and *S. guttatus.*

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Table 1. The mineral content of some fish species from previous studies.

Categories	Species	Part of body fish	Mineral content	References
Freshwater fish	*Oncorhynchus mykiss*	Meat	Ca (0.21±0.17 g·kg^{-1}), P (2.26±0.14 g·kg^{-1})	Kiczorowska *et al.* (2019)
	O. mossambicus	Meat	Ca (1.62±0.02%) P (1.21±0.06%)	Ullah *et al.* (2022)
	P. paradiseus	Whole fish	Ca (1.67±0.03%) P (1.21±0.03%)	
	Cyprinus carpio	Meat and skin	Ca (1232.98±31.62 mg/100 g), P (4767.49±47.16 mg/100 g)	Manz *et al.* (2023)
Sea and ocean fish	*Trachurus capensis*	Meat	Ca (62.47 mg), Mg (46.98 mg)	Maulu *et al.* (2021)
	Capros aper	Muscle	Ca (5073.6±163.2 mg/kg), P (3952.5±110.5 mg/kg)	Pinto *et al.* (2022)
	Neoepinnula orientalis	Whole fish ex.scales	Ca (4247±16 mg.kg^{-1}), Mg (2253±21 mg.kg^{-1})	Vijayan *et al.* (2016)
	Sparus aurata	Bones	Ca (9.23±0.34 mg/g), Mg (0.33±0.06 mg/g)	Kandyliari *et al.* (2020)
	Argyrosomus regius	Bones	Ca (6.93±0.93 mg/g), Mg (0.67±0.013 mg/g)	
	Sardinella maderensis	Meat and skin	Ca (1364.47±36.24 mg/100 g), P (2170.09±15.26 mg/100 g)	Manz *et al.* (2023)
	Scomber scombrus	Bones	Ca (143 g/kg), P (86 g/kg)	Toppe *et al.* (2007)
	Clupea harengus	Bones	Ca (197 g/kg), P (95 g/kg)	
	Gadus morhua	Bones	Ca (190 g/kg), P (113 g/kg)	
Brackish water fish	*Scatophagus argus*	Whole body ex. scales and intestines	Ca (4247±16 mg.kg^{-1}), Mg (1415±25 mg.kg^{-1})	Vijayan *et al.* (2016)
	Ilisha africana	Meat and skin	Ca (462.78±34.85 mg/100 g), P (2548.32±57.96 mg/100 g)	Manz *et al.* (2023)
	Ethmalosa fimbriata	Meat and skin	Ca (468.05±21.15 mg/100 g), P (1569.43±86.57 mg/100 g)	

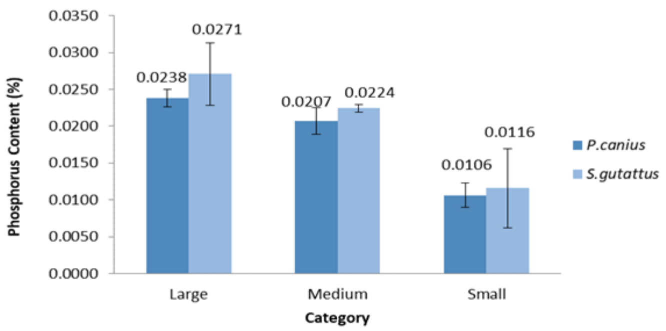

Figure 4. Phosphorus content in bones of *P. canius* and *S. guttatus.*

0.0238%, 0.0207%, and 0.0106% for large, medium, and small sizes, respectively, while in *S. guttatus* it was 0.0271%, 0.0224%, and 0.0116% for the same size categories (Figure 4). The mineral content of each species shows considerable variation, as reported in previous studies summarized in Table 1.

The measurement of calcium and phosphorus content for the fish bones of *P. canius* and *S. guttatus* with different size categories indicates that the sample size affects the mineral content in the fish bones. The smaller the sample category, the lower the calcium and phosphorus mineral content. Maulu *et al.* (2021) found that smaller fish contain higher amounts of minerals than most large and medium fish, regardless of processing. This is because small fish still contain components rich in minerals, such as bones, heads, and viscera. Based on the categories of size of *P. pardalis*, large fish have the highest concentration of calcium, and the lowest calcium content is found in medium-sized fish (Wijayanti *et al.*, 2023). Previous research has also found that the mineral content of deep-sea fish was similar to brackish water fish (Vijayan *et al.*, 2016). Fish need trace elements for physiological and biochemical functions to maintain their normal life processes (Lall and Kaushik, 2021). Macrominerals play an important role in cellular, tissue, and organ function, and their levels in the fish's body are influenced by various factors, including the fish's size (Weyh *et al.*, 2022). Mineral absorption by fish is carried out by drinking seawater and stored by endocrine homeostatic regulatory mechanisms that optimally improve cell, tissue, and organ systems (Pinto *et al.*, 2022). Macro-minerals in fish bodies are directly related to the development and maintenance of the skeletal system (Hlordzi *et al.*, 2022). Large fish are more likely to take large quantities of food because they adapt the type of food to their mouth opening (Harvey *et al.*, 2021). Nutrients in the developmental system of fish are known to interact with minerals because of their ability and tendency to form chemical bonds (Baeverfjord *et al.*, 2019). Such interactions are broadly classified as positive, synergistic, harmful, or antagonistic. Direct positive interactions between elements in structural processes, such as the requirement for copper (Cu) and iron (Fe) for hemoglobin formation, calcium (Ca), phosphorus (P), and magnesium (Mg) for bone hydroxyapatite formation, and Mn-Zn interaction for conformation RNA molecule exact (Lall and Kaushik, 2021). The absorption of minerals in fish through their food and habitat is essential in promoting growth, resulting in good body composition, meat quality, and maintaining fish health (Pinto *et al*., 2022).

3.3 Analysis of variance and least significant difference

The ANOVA results indicated significant differences in calcium and phosphorus concentrations in fish bones across size categories for both species (Table 2). In *P. canius*, calcium levels were notably different between large and small fish (p = 0.009), but not between medium and small fish (p = 0.075). For phosphorus levels in *P. canius*, there were significant differences between both large-small and medium-small groups (p = 0.000). Further analysis using the LSD test (Table 3), revealing that calcium levels in *P. canius* were significantly different between large and small sizes (p = 0.009), but not between medium and small sizes (p = 0.075). Phosphorus levels in *P. canius* differed significantly between both large-small and medium-small groups (p = 0.000). In *S. guttatus*, both calcium and phosphorus showed significant differences across all size comparisons ($p < 0.05$). Based on the results of the ANOVA test ($P<0.05$), fish size had a significant effect on the calcium and phosphorus content in the bones of *P. canius* and *S. guttatus*. Furthermore, the LSD test showed significantly different calcium and phosphorus contents in each fish size. Differences in calcium and phosphorus content between categories based on the size of each type of fish bone depend on its ability to absorb inorganic elements from food and the living environment (Manz *et al*., 2023). Mineral absorption in fish may vary depending on gastric physiology, particularly between gastric and agastric species, as well as from direct absorption of minerals from water (Weyh *et al*., 2022). Habitat also plays a significant role; for example, freshwater species such as whitefish and trout have been reported to possess mineral levels comparable to those of marine species like halibut, mackerel, and herring (Kiczorowska *et al*., 2019). Additionally, variations in mineral content may result from differences in catch locations, physiological traits, taxonomic classification, analytical procedures, and even the timing of sample analysis (Pinto *et al*., 2022).

Table 2. ANOVA results for calcium and phosphorus in fish bones

Species	Element	df	F	Sig.
P. canius	Calcium	2	7.154	0.026
S. guttatus	Phosphorus	2	57.116	0.000
P. canius	Calcium	2	27.216	0.001
S. guttatus	Phosphorus	2	12.088	0.008

Table 3. LSD test results based on fish size category.

Species	Element	Category	Sig.
P. canius	Calcium	Large-Small	0.009*
		Medium-Small	0.075
P. canius	Phosphorus	Large-Small	0.000*
		Medium-Small	0.000*
S. guttatus	Calcium	Large-Small	0.000*
		Medium-Small	0.018*
S. guttatus	Phosphorus	Large-Small	0.003*
		Medium-Small	0.015*

*The mean difference is significant at 0.05 level.

3.4 Independent sample T-test

The calcium and phosphorus content of *P. canius* and *S. guttatus* were tested for normality and homogeneity. They obtained $p>0.05$ in both, in which the data were normally distributed and homogeneous. The independent sample T-test showing the difference in the average content of calcium and phosphorus in the two species of fish bones is summarized in Table 4. The results show that no significant difference in calcium and phosphorus content between fish bones in *P. canius* and *S. guttatus*. Based on the Sig. (2-tailed) values obtained, namely 0.908 and 0.551, meaning that the significant value exceeds the value of 0.05, indicating no difference in the average calcium and phosphorus content in *P. canius* and *S. guttatus*. The results for calcium and phosphorus content in these two species did not have a significant average comparison; they were only 0.11% and 0.002% different. The data on the calcium content of *P. canius* and *S. guttatus* bones showed that the calcium content of *P. canius* was higher than that of *S. guttatus*. Meanwhile, the phosphorus content in *S. guttatus* was slightly higher than in *P. canius*.

Several internal and external factors influence the mineral composition of fish bones (Table 1). Mineral concentrations in fish tissues are affected by size, age, sex, maturity, habitat, environmental parameters, and food availability. Fish bones are known to be rich in minerals (Boutinguiza *et al*., 2012), and dietary factors may contribute to variations in calcium content between *P. canius* and *S. guttatus*. Lall and Tibbetts (2009) also emphasized that mineral absorption is influenced by the surrounding environment, making the fish's origin a determining factor. Furthermore, mineral content in fish is associated with metal absorption from the environment (Weyh *et al*., 2022). Overall, larger fish exhibited higher calcium and phosphorus levels compared to smaller individuals. These two essential minerals are vital for

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Table 4. Independent sample T-test statistics for differences in average calcium phosphorus content in fish bones of *P. canius* and *S. guttatus*.

Variances		T-test for equality of means		
		Sig. (2-tailed)	Mean difference	Std. error difference
Calcium	Equal variances assumed	0.908	0.11111	0.94582
Phosphorus	Equal variances assumed	0.551	-0.00200	0.00328

human health particularly in bone development and maintenance (Loughrill *et al*., 2017). Phosphorus as a component of ATP supports energy metabolism, bone and tooth structure, and physiological processes such as acid-base balance, muscle contraction, and nerve transmission (Corrêa and Holanda, 2019; Manz *et al*., 2023).

According to WHO, the recommended daily calcium intake is 400–500 mg for adults, increasing to 700–800 mg with high protein intake, and up to 1200 mg for pregnant women, breastfeeding mothers, children, and adolescents. Intake should not exceed 2500 mg/day to avoid hypercalciuria. The recommended for phosphorus intake is 700 mg for adults and 1250 mg for adolescents. According to Metwally *et al*. (2021), fish by-products can be utilized to reduce reliance on external nutrient sources. Fish bones are also a promising source of hydroxyapatite, a bioceramic material widely applied in medical, health, and food industries (Harvey *et al*., 2021).

4. Conclusion

Fish bones of *P. canius* have more significant potential as a source of calcium than *S. guttatus* fish bones. The bones of *S. guttatus* have a more significant phosphorus content than those of *P. canius*. The large fish category has a higher mineral content than medium and small fish. Based on the different tests on the average range of calcium and phosphorus, the two types of fish bones did not have a significant average difference with the Sig. 2-tailed values of 0.908 and 0.551 were tested at the 0.05 level of confidence (α). Based on the nutritional adequacy ratio, this study's calcium and phosphorus content can be used as a natural source of minerals for the body's daily needs and has potential for further development in the health sector as bone substitutes or natural hydroxyapatite.

Conflict of interest

The authors declare no conflict of interest.

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