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Variations in Milling Time and Their Impact on the Bio-Calcium Properties of Red Snapper Fish Bones

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Abstract

Calcium is a macromineral essential by human body, with deficiencies often leading to bone-related issues. Red snapper bone waste is a source of calcium. The bioavailability of calcium is maximized when present in microscopic particles, such as bio-calcium. This study aims to identify the optimal milling time to produce bio-calcium from red snapper bones, evaluating parameters such as water content, particle size, and yield. Bio-calcium was extracted from red snapper bone meal using a 1 N NaOH solution (1:3 ratio) with milling times of 1, 2, and 3 h. Analysis of the red snapper bone meal including yield, particle size, chemical composition, calcium, and phosphorus content. The bio-calcium was characterized based on water content, particle size, yield, Fourier-transform infrared spectroscopy (FTIR), and morphological structure using a scanning electron microscope (SEM). The results demonstrated that a 3-hour milling time yielded bio-calcium with a particle size of 410.8 nm, a water content of 4.51 ± 0.15%, and a yield of 4.12 ± 0.03%. SEM revealed a uniform morphology. FTIR spectra indicated the presence of carbonate, amine, hydrocarbon, and hydroxyl groups, suggesting residual organic substances such as protein, fat, and water. The uniform spectra across all samples highlight the consistency of the extraction process, ensuring a similar chemical composition.

Keywords: red snapper, micromineral, bio-calcium, fish bones, milling

Introduction

Calcium is an essential mineral critical for a variety of physiological functions, especially bone formation and maintenance (Upadhyay, 2017). In body fluids, calcium facilitates muscle contraction and relaxation, nerve impulse transmission, blood clotting, hormone regulation, and enzymatic activity. Additionally, calcium strengthens bones, serving as a reserve to maintain blood calcium levels (Noprisanti *et al.*, 2018). A deficiency in calcium intake can lead to bone health issues such as osteoporosis. According to data from the Indonesian Ministry of Health in 2019, the average calcium intake of Indonesians is only 300–350 mg per day for adults and 400–470 mg per day for pregnant women. These values fall short of international standards, which recommend 1,000–1,200 mg per day for adults and 1,200–1,500 mg per day for pregnant women. This imbalance leaves Indonesians vulnerable to calcium deficiency-related conditions (Husna et al., 2020).

In recent years, research has highlighted the advantages of calcium nanoparticles over conventional calcium forms, particularly their enhanced solubility and bioavailability. This has sparked interest in developing efficient and sustainable sources of bio-calcium (Media & Elfemi, 2021). Fish bones, a byproduct of the fisheries industry, are a promising source of bio-calcium. Among them, red snapper bones are particularly rich in calcium and other nutrients but are often underutilized (Anggraeni *et al.*, 2016).

Red snapper holds significant potential for contributing to food security and meeting dietary needs (Jampilek *et al.*, 2019). Red snapper production has shown consistent growth, increasing by 4.32% to 312,945 tons in 2021, with further growth predicted. However, the production processes, including filleting and surimi preparation, generate significant waste, such as heads, tails, skin, and bones. Utilizing red snapper bones not only reduces waste but also offers a sustainable alternative to traditional calcium sources like limestone and shells (Anggraeni & Handayani, 2022).

The milling process is critical in determining the particle size and other characteristics of bio-calcium derived from fish bones. Smaller, more uniform particle sizes enhance calcium's solubility and bioavailability, making it more effective for use in health and nutritional supplements (Kusumaningrum *et al.*, 2016). Despite the importance of milling, limited research exists on how variations in milling time affect the properties of bio-calcium.

This study addresses this gap by exploring different milling durations and their influence on the physicochemical properties of bio-calcium produced from red snapper bones. Specifically, the research aims to identify the optimal milling time based on parameters such as water content, particle size, and yield.

Materials and Methods

Preparation and Production of Red Snapper Fish Bone Powder

The preparation of red snapper fish bones was done refers to Anggraeni *et al.* (2016). Fresh bones were obtained from PT Kelola Laut Nusantara in Pati, Indonesia. The bones were thoroughly cleaned by rinsing with tap water to remove contaminants. They were then boiled for 7 h to eliminate any remaining flesh and subsequently dried at 50°C for 6 h. The dried bones were reduced in size using an HMR-50 hammer mill (AGROWINDO) with a 50 kg/hour throughput and equipped with 30 hammers. Analyses conducted on the resulting bone powder included yield, chemical composition, calcium and phosphorus content, and particle size.

Nanoscale Calcium Extraction from Red Snapper Fish Bones

The extraction of nanoscale calcium was done per Anggraeni *et al.* (2016). Red snapper fish bone powder was treated with a 1 N NaOH solution (Merck) in a 1:3 sample-to-solvent ratio. Extraction was performed at 100°C for 30 min. The resulting solution was filtered using Whatman No. 1 filter paper and adjusted to neutral pH. The filtrate was dried in an oven at 50°C for 12 h, ensuring the moisture content was reduced to below 8%. The dried material was subsequently milled using a ball mill with agate balls operating at 700 rpm for durations of 1, 2, and 3 h. Following milling, the material was subjected to a second drying process at 50°C for 3 h and sieved using a 200-mesh screen.

Yield Analysis

Yield was calculated using the method described by Anggraeni *et al.* (2016). The percentage yield was determined with Formula 1.

$$Yield\ (\%) = \left(\frac{Final\ weight\ of\ sample}{Initial\ weight\ of\ sample}\right) \times 100 \quad (1)$$

This calculation helped assess how efficient the extraction process was and determine the nanoscale calcium yield from the red snapper fish bone powder.

Particle Size Analysis

Particle size was determined using a Particle Size Analyzer (PSA) as per the method described by Li *et al.* (2023). Particles were dispersed in a liquid medium, and the measured particle size represented the dimensions of individual particles. Particle size measurements were conducted using a Cordouan Vasco-PSA Arago DL 135 reflectometer, applying the Low-Angle Laser Light Scattering (LALLS) technique. This method utilizes light scattering, which is particularly relevant in size-exclusion chromatography.

The eluate from the size-exclusion chromatography (SEC) column was directed through a refractive index detector to measure the concentration of the solution over time and a laser scattering cell. The temporal variation of scattered light intensity was analyzed at low angles relative to the laser beam. Data obtained from low-angle light scattering were analyzed under the assumption that low-angle scattering approximates zero-angle scattering. This technique proved effective for measuring particle sizes ranging from 0.1 to 3,000 ìm when using a He-Ne laser beam with a wavelength (λ) of 0.63 μm.

Proximate, Calcium, and Phosphorus Analysis

Proximate analysis includes moisture, ash, crude protein, and fat content measurements (AOAC, 2005). Calcium levels were determined using Atomic Absorption Spectrometry (AAS) with a Perkin Elmer Analyst 100 burner, employing the wet ashing method at a wavelength of 422 nm (Idrus *et al.*, 2022). Phosphorus content was measured per the AOAC (2005) standards, using a UV-visible spectrophotometer at 660 nm.

Color Evaluation

Color evaluation described by Kusumawati *et al.* (2022). A Konica Minolta CR-400 colorimeter (Tokyo, Japan) was used to evaluate the sample's color.

Calibration was conducted using standard white and black measurements, yielding values of *L** = 92.87, *a** = “1.27, *b** = 3.33 for white and *L** = 21.30, *a** = “0.76, *b** = 1.27 for black.

Structural Analysis of Bio-Calcium

The structural characteristics of bio-calcium were examined using Scanning Electron Microscopy (SEM) as described by Arooj *et al.* (2014). Particle size was determined using the scale provided in the micrographs, and data were collected with ImageJ software, yielding a dataset of NN particle size measurements. A bar chart illustrating the particle size distribution was created using Origin-8 software, and the particle size values were derived through Gaussian analysis of the chart. Theaverage particle size was calculated by summing all x_i values in the dataset and dividing by N using the formula $\Sigma x_i /N$.

Data Analysis

A completely randomized design (CRD) was employed, with milling duration as the single factor. Data were analyzed using one-way Analysis of Variance (ANOVA), and significant differences ($p < 0.05$) were identified using Tukey’s Honest Significant Difference (HSD) test. For non-parametric data, Kruskal-Wallis analysis was applied. Statistical analyses were conducted using SPSS version 16.

Results and Discussion

Chemical and Physical Characteristics of Snapper Fish Bone Powder

The characterization of red snapper bone powder encompassed chemical composition, yield, calcium levels, phosphorus, and particle size. The chemical properties were analyzed to ensure the raw materials used in this study were of high quality. The results are presented in Table 1.

The study found that the yield of red snapper bone powder was 89.76%, achieved using a dry method with a hammer mill. This yield was higher than the 85.91% reported by Wijayanti *et al.* (2021a), likely due to differences in crushing methods. The dry method tends to produce higher yields compared to the wet method, as the latter dissolves more material in water (Lekahena *et al.*, 2014). The drying method and duration before milling play a critical role, as efficient drying reduces water content and enhances yield. Additionally, the fineness of the milling process significantly influences the yield. Finer grinding increases the surface area, facilitating nutrient extraction and further improving yield. In this study, the particle size of the red snapper bone powder was measured at 1,100.69 nm.

The particle size of the powder produced in this study does not fall within the nanoscale range. Abo El-Maaty *et al.* (2021) defined nanoparticles as having sizes between 10 and 100 nm. To achieve nanoparticle-sized red snapper bone powder, chemical processes such as demineralization (removal of minerals) and deproteinization (removal of proteins) are typically required. These processes can significantly affect particle size depending on the reagents and conditions used. Furthermore, size reduction techniques like ball milling could help achieve nanoscale dimensions, as more refined milling enhances particle size reduction.

The water content of the red snapper bone powder was 4.76%, aligning closely with the International Seafood of Alaska (ISA) standard for fish bone meal, which specifies a water content of 3.6%. The slightly higher water content observed in this study is attributed to the drying process, which used a temperature of 50°C, leaving some residual lumps of fishbone powder. Nonetheless, the 12-hour drying process resulted in a lower water content compared to the 4.34% reported by Bass *et al.* (2020), where sunlight was used for drying over 14 days. Oven drying at 50°C effectively reduces the moisture content, as it accelerates water

Table 1. Chemical and physical characteristics of red snapper fish bone powder

Parameters	Red snapper fish bone powder
Yield (%)	89.76 ± 1.65
Particle size (nm)	1,100.69
Moisture content (%)	4.76 ± 0.21
Ash content (%)	63.52 ± 1.45
Protein content (%)	19.03 ± 0.04
Fat content (%)	1.88 ± 0.34

Note: This experiment was conducted with 3 repetitions.

evaporation. Key factors affecting moisture reduction include drying temperature and duration. However, excessive heat or prolonged drying may degrade the material, while insufficient heat or time may leave residual moisture.

The ash content of the fish bone powder in this study was 63.52%, attributable to the high mineral content in the bones. This result is lower than the 85.44% reported by Abdullah *et al.* (2020), possibly due to variations in mineral composition. Aenglong *et al.* (2023) noted that ash content reflects the mineral concentration in a sample, while incomplete demineralization or deproteinization processes can leave organic residues, reducing ash levels.

The calcium content of the red snapper bone powder met the Indonesian National Standard (SNI 01-3158-1992), with quality I set at ≥30% and quality II at ≥20% by weight. This study's calcium content placed the bone powder in the quality I category. Nemati *et al.* (2017) highlighted that fish species and processing methods, such as boiling, significantly influence calcium levels in fish bone meal.

The fat content observed in this study was categorized as low. According to Husna *et al.* (2020), low-fat content enhances the stability and durability of the product. While fat in snapper bone powder contributes to its nutritional profile, particularly through beneficial essential fatty acids, the primary focus of bone powder is typically on its mineral and protein content rather than fat. A lower fat content is generally preferred, as it indicates a reduction in non-bone material and ensures higher purity. Excessive fat can impart a fishy flavor to the powder, promote oxidative rancidity, and result in a brownish appearance, diminishing its quality and acceptability.

Chemical and Physical Characteristics of Red Snapper Fish Bone Bio-Calcium

The milling time did not significantly affect the water content of bio-calcium derived from red snapper bones. However, slight variations were observed, with the highest water content recorded at 4.71 ± 0.01% for the 1-hour milling treatment and the lowest at 4.51 ± 0.12% for the 3-hour milling treatment (Table 2).

Table 2. Bio-calcium water content at different extraction times

Parameter	1 hour	2 h	3 h
Moisture content (%)	4.71±0.01^{a}	4.65±0.23^{a}	4.51±0.15^{a}
Yield (%)	6.75±0.11^{c}	5.23±0.76^{b}	4.12±0,03^{a}

Note: This experiment was conducted with 3 repetitions.

According to Anggraeni *et al.* (2016), the water content of bio-calcium extracted from tilapia bones using a base was 4.67%. Similarly, Benjakul and Karnjanapratum (2018) reported a water content of 7.35% for calcium extracted from fish bones. These findings suggest that milling duration influences the water content of bio-calcium derived from red snapper bones. However, the relationship between the two may be more pronounced and easier to discern compared to other parameters, such as homogeneity or particle size. Prolonged milling times may enhance the homogeneity and particle size uniformity of the material, potentially affecting its moisture absorption and retention properties (Wijayanti *et al.*, 2021b).

Drying the bio-calcium extracted from red snapper bones at 50°C was another step in the ball-milling refinement process. This step reduced residual moisture that otherwise caused aggregation on the milling balls and the mill walls, thereby hindering the refinement process. The milling procedure not only refined the material but also facilitated moisture reduction by increasing the surface area of the particles, which expedited moisture evaporation during sifting. The reduced particle size (200 mesh, equivalent to 75 ìm) increased the surface area, a key factor influencing reaction rates.

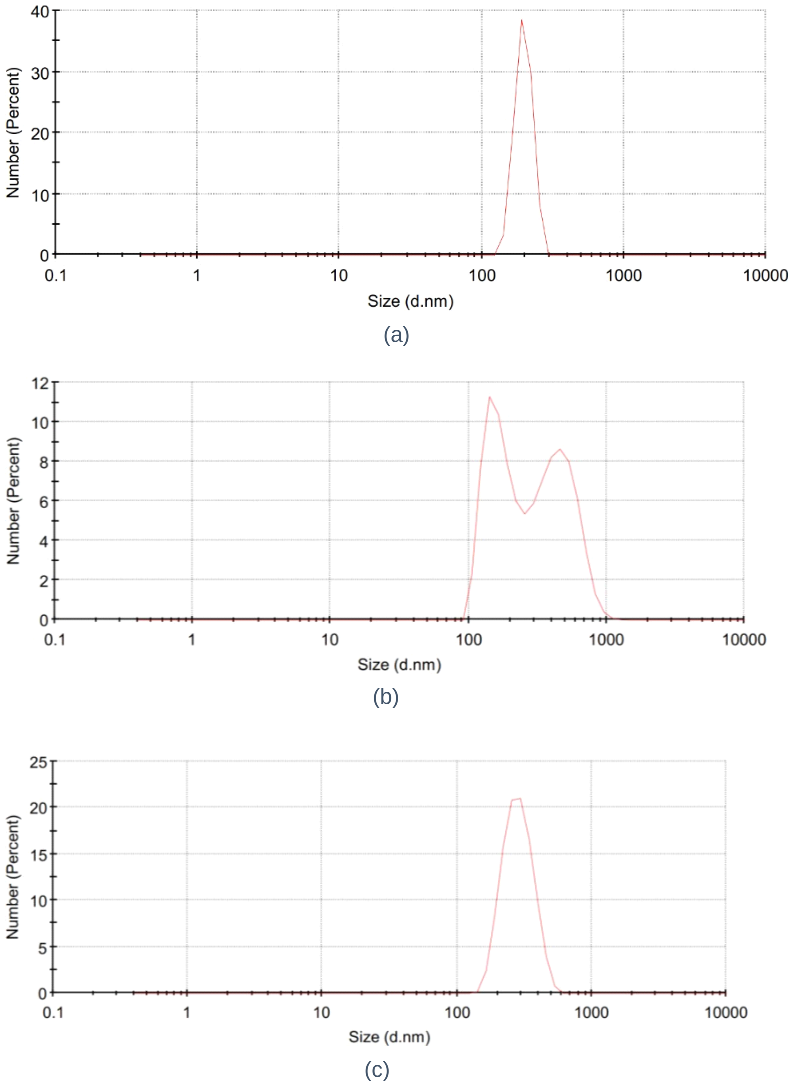
Variations in milling duration significantly affected the yield of bio-calcium. The highest yield, 6.75 ± 0.11%, was obtained after 1 hour of milling, while the lowest yield, 4.12 ± 0.03%, was recorded for the 3-hour treatment (Table 2).

The findings highlight considerable differences in bio-calcium yield between treatments. Compared to yields of 5.91% (base extraction) and 4.41% (acid extraction) for red snapper bones reported by Han *et al.* (2023), the current study's yields were lower. The yield of bio-calcium depends on milling duration, as prolonged milling can enhance particle size reduction and increase the amount of material that can be powdered (Yin & Park, 2015). However, there is an efficiency limit to milling; beyond this threshold, further milling may not improve yield and can result in material loss due to dust formation and excessive energy consumption (Chandran *et al.*, 2019).

Red Snapper Fish Bone Bio-calcium Particle Size

The study found that the particle size of bio-calcium derived from red snapper bones ranged from a minimum of 410.8 nm after 3 h of treatment to a maximum of 776.9 nm after 1 hour (Figure 1). Since the particle size was below 1,000 nm, the bio-calcium produced in this study is classified as nano. This finding is consistent with Mehmood *et al.* (2022), who noted that nanoparticles typically range from 1 to 1,000 nm. Additionally, Arooj *et al.* (2014) reported that nanoparticles generally fall within a size distribution of 200 to 400 nm. The decrease in particle size with increased extraction duration is a result of continued size reduction during the extraction process. According to Anggraeni *et al.* (2016), smaller particle sizes are associated with higher bioavailability in the body, suggesting that bio-calcium extracted from tilapia bones for 90 min could offer greater bioavailability.

The size analysis results confirm the effectiveness of the ball mill for reducing particle size and producing bio-calcium particles in the nano-scale range. The size reduction occurs through the combined impact and friction of the milling balls, which break down the particles. As milling time increases, the intensity of impact and friction also increases, resulting in finer particles. However, milling efficiency may decrease beyond a certain point due to excessive workload or heat buildup. The bio-calcium particles in this study were larger than those reported by Benjakul *et al.* (2017), who found a particle size of 20.29 nm for calcium derived from tuna bones. This discrepancy can be attributed to the distinct characteristics of the fish bones and differences in milling conditions post-extraction. The particle size distribution of bio-calcium derived from red snapper bones is shown in Figure 1.



(a)

(b)

(c)

Figure 1. The particle size of red snapper fish bone bio-calcium: (a) 1 h, (b) 2 h, (c) 3 h.

Bio-Calcium Morphology Derived from Red Snapper Fish Bones

Figure 2 shows the morphology of bio-calcium particles derived from red snapper fish bones. The analysis reveals that increasing the milling duration can lead to more uniformity of particle size distribution, resulting in a more consistent sample, as observed in SEM images. Intensive milling induces physical and chemical changes in the particles, including alterations to the crystal structure, which are discernible in SEM analyses. The SEM images indicate that the bio-calcium exhibits distinct characteristics, featuring uniform particle shapes and sizes. The most prevalent morphologies observed include vaterite crystals, with some aragonite also present. Calcium crystals typically exist in several polymorphic phases, such as calcite, aragonite, and vaterite.

Calcite is characterized by a cubic morphology, aragonite by needle-like clusters, and vaterite by a spherical structure (Li *et al.*, 2023). According to Stepankova *et al.* (2023), calcite may also display rhombohedral, scalenohedral, cubic, and prismatic morphologies. Aragonite tends to form acicular clusters or agglomerations, while vaterite often appears as globular shapes. Notably, the morphology of the bio-calcium produced in this study differs from prior findings. For instance, Yusuf *et al.* (2019) reported that bio-calcium derived from local clamshells exhibited a uniform, needle-shaped aragonite morphology. Similarly, Shi *et al.* (2018) documented flower-like or vaterite crystal shapes in bio-calcium derived from fish bone. These discrepancies can be attributed to variations in the bio-calcium production processes. Furthermore, EL-Sokkary *et al.* (2012) noted that calcite crystals exhibit a strong affinity for surfaces, which contributes to their stability. In contrast, aragonite is prone to detachment from surfaces, whereas vaterite is unstable and can convert into calcite when exposed to a solvent.

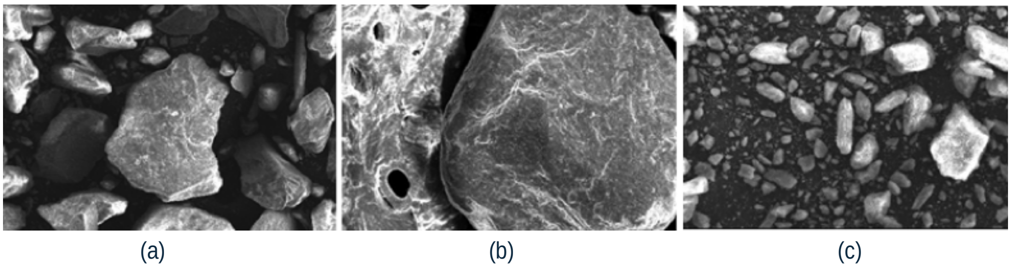

(a) (b) (c)

Figure 2. Morphology of red snapper fish bone bio-calcium: (a) 1 h, (b) 2 h, (c) 3 h.

Functional Structure of Bio-Calcium by FTIR

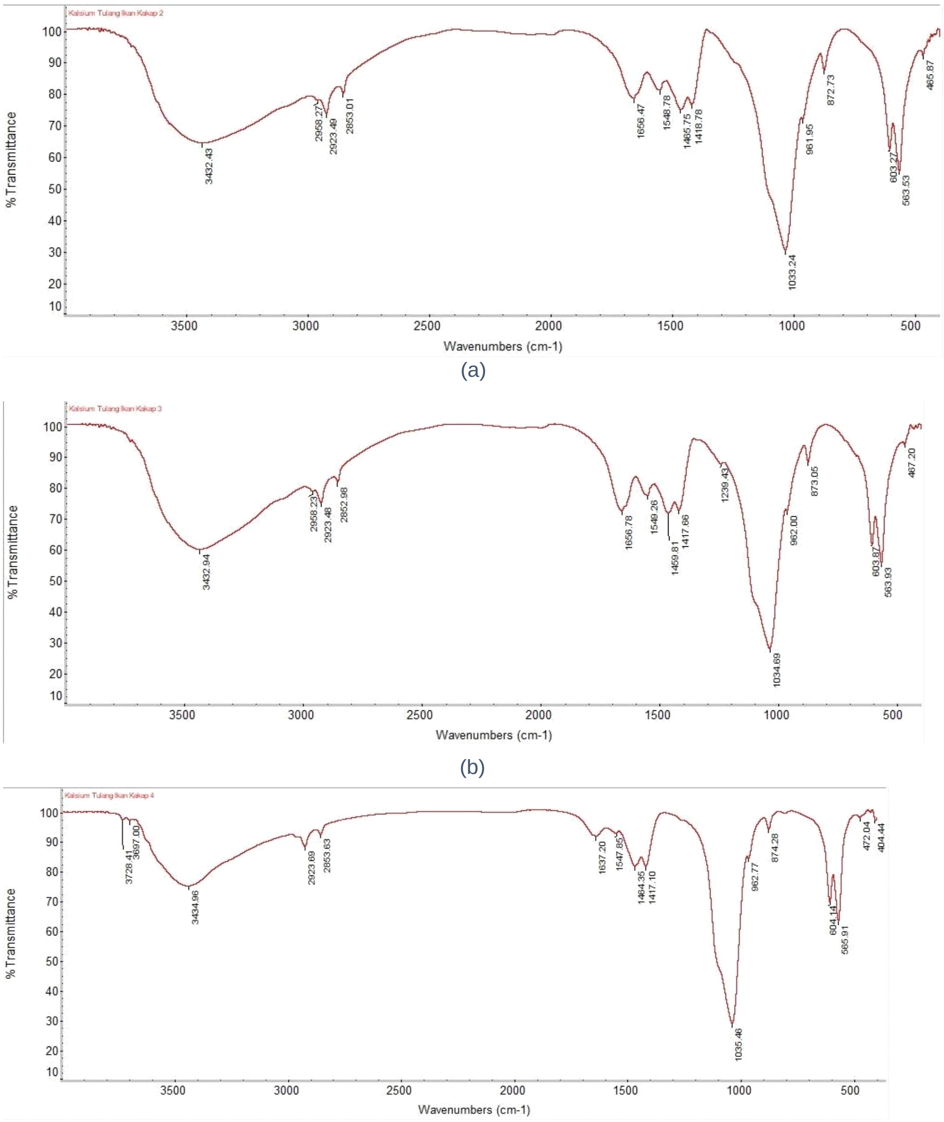
Figure 3 presents the FTIR spectra of bio-calcium derived from red snapper fish bones at various milling durations. The analysis reveals a prominent phosphate absorption band at approximately 1,035 cm^{-1}. Additionally, the v4 phosphate absorption band, representing asymmetric bending vibrations, had a split-shaped absorption band at 563 and 603 cm^{-1} across all spectra, confirming the presence of hydroxyapatite (HAP) crystals (Lekahena *et al.*, 2014).

The FTIR spectra of the six nano-calcium samples demonstrate the presence of carbonate, amine, hydrocarbon, and hydroxyl functional groups, indicative of small amounts of organic material, such as proteins, fats, and water. The uniformity of the spectra across samples suggests that the consistent extraction process resulted in a homogeneous chemical composition. Previous studies have identified distinct spectral peaks at specific wavenumbers, such as 564 cm^{-1} (Yin *et al.*, 2015), 603 cm^{-1}, 700–725 cm^{-1} (Cahyanto *et al.*, 2017), and 1,033 cm^{-1} (Bonadio *et al.*, 2013), corroborating the presence of phosphate groups. Prominent peaks at 875 and 1,639 cm^{-1} indicate the presence of carbonate bands (Boutinguiza *et al.*, 2012).

The carbonate ions are further confirmed by the peak at 1,453 cm^{-1} (Yin *et al.*, 2015), while the amide group is evidenced by the peak at 1,533 cm^{-1} (Zainol *et al.*, 2019). Peaks at 2,852 and 2,922 cm^{-1} reflect the organic material's reasonably high intensity (Boutinguiza *et al.*, 2012). The bands above 3,300 cm^{-1} indicate water content within the sample, albeit at a relatively low intensity (Nam *et al.*, 2019).

Conclusion

The duration of milling does not significantly affect the moisture content of bio-calcium derived from red snapper fish bones. However, it substantially influences the yield and particle size. A 3-hour milling treatment was the most effective, producing bio-calcium with a particle size of 410.8 nm, a moisture content of 4.51 ± 0.15%, a yield of 4.12 ± 0.03%, and consistent morphology.



(a)

(b)

(c)

Figure 3. FT-IR spectra of red snapper fish bone bio-calcium at different milling times: (a) 1 h, (b) 2 h, (c) 3 h.

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References

Abdullah, N. H., Mohamed Noor, A., Mat Rasat, M. S., Mamat, S., Mohamed, M., Mohd Shohaimi, N. A., Ab Halim, A. Z., Mohd Shukri, N., Abdul Razab, M. K. A., & Mohd Amin, M. F. (2020). Preparation and characterization of calcium hydroxyphosphate (Hydroxyapatite) from tilapia fish bones and scales via calcination method. Materials Science Forum, 1010 MSF, 596–601. https://doi.org/10.4028/www.scientific.net/MSF.1010.596

Abo El-Maaty, H. A., El-Khateeb, A. Y., Al-Khalaifah, H., El-Sayed, E. S. A., Hamed, S., El-Said, E. A., Mahrose, K. M., Metwally, K., & Mansour, A. M. (2021). Effects of eco-friendly synthesized calcium nanoparticles with biocompatible *Sargassum latifolium* algae extract supplementation on egg quality and scanning electron microscopy images of the eggshell of aged laying hens. *Poultry Science*, 100(2), 675–684. https://doi.org/10.1016/j.psj.2020.10.043

Aenglong, C., Ngasakul, N., Limpawattana, M., Sukketsiri, W., Chockchaisawasdee, S., Stathopoulos, C., Tanasawet, S., & Klaypradit, W. (2023). Characterization of novel calcium compounds from tilapia (*Oreochromis niloticus*) by-products and their effects on proliferation and differentiation of MC3T3-E1 cells. *Journal of Functional Foods*, 10(5), 70–85. https://doi.org/10.1016/j.jff.2022.105361

Anggraeni, N., & Handayani, H. T. (2022). Consumer acceptance and nutritional value of catfish cendol (Clarias batracus) with the addition of coffee powder. *Agromix*, 13(1), 1–8. https://doi.org/10.35891/agx.v13i1.2655

Anggraeni, N. (2020). Utilization of eels (Monopterus albus) in making protein-rich cendol. *Jurnal Agercolere*, 2(2), 47–52. https://doi.org/10.37195/jac.v2i2.118

Anggraeni, N., Sastro, D. Y., & Riyadi, P. H. (2016). Utilization of bio-calcium from tilapia (Oreochromis niloticus) bones in rice analogues from various types of sweet potatoes (*Ipomoea batatas L.*). *Jurnal Aplikasi Teknologi Pangan*, 5(4), 112–120. https://doi.org/10.17728/jatp.187

Arooj, N., Dar, N., & Samra, Z. Q. (2014). Stable silver nanoparticles synthesis by Citrus sinensis (orange) and assessing activity against food poisoning microbes. *Biomedical and Environmental Sciences*, 27(10), 815–818. https://doi.org/10.3967/bes2014.118

Bas, M., Daglilar, S., Kuskonmaz, N., Kalkandelen, C., Erdemir, G., Kuruca, S. E., Tulyaganov, D., Yoshioka, T., Gunduz, O., Ficai, D., & Ficai, A. (2020). Mechanical and biocompatibility properties of calcium phosphate bioceramics derived from salmon fish bone wastes. International Journal of Molecular Sciences, 21(21), 1–14. https://doi.org/10.3390/ijms21218082

Benjakul, S., Mad-Ali, S., Senphan, T., & Sookchoo, P. (2017). Bio-calcium powder from precooked skipjack tuna bone: Production and its characteristics. *Journal of Food Biochemistry*, 41(6), 20–30. https://doi.org/10.1111/jfbc.12412

Benjakul, S., & Karnjanapratum, S. (2018). Characteristics and nutritional value of whole wheat cracker fortified with tuna bone bio-calcium powder. *Food Chemistry*, 259, 181–187. https://doi.org/10.1016/j.foodchem.2018.03.124

Bonadio, T. G. M., Sato, F., Medina, A. N., Weinand, W. R., Baesso, M. L., & Lima, W. M. (2013). Bioactivity and structural properties of nanostructured bulk composites containing Nb2O5 and natural hydroxyapatite. *Journal of Applied Physics*, 113(22). https://doi.org/10.1063/1.4809653

Boutinguiza, M., Pou, J., Comesaña, R., Lusquiños, F., De Carlos, A., & León, B. (2012). Biological hydroxyapatite obtained from fish bones. *Materials Science and Engineering C*, 32(3), 478–486. https://doi.org/10.1016/j.msec.2011.11.021

Cahyanto, A., Kosasih, E., Aripin, D., & Hasratiningsih, Z. (2017). Fabrication of hydroxyapatite from fish bone waste using reflux method. *IOP Conference Series: Material Science and Engineering*, 172(012006). https://doi.org/10.1088/1757-899X/172/012006

Chandran, K., Song, S., & Yun, S. Il. (2019). Effect of size and shape controlled biogenic synthesis of gold nanoparticles and their mode of interactions against foodborne bacterial pathogens. *Arabian Journal of Chemistry*, 12(8), 1994–2006. https://doi.org/10.1016/j.arabjc.2014.11.041

Darmanto, Y. S., Kurniasih, R. A., Romadhon, R., Riyadi, P. H., & Anggraeni, N. (2022). Characteristic of analogue rice made from arrowroot (*Maranta arundinaceous*) and seaweed (*Gracilaria verrucosa*) powder fortified with fish collagen. *Food Research*, 6(5), 370–379. https://doi.org/10.26656/fr.2017.6(5).473

El-Sokkary, T. M., Khalil, K. A., & Ahmed, I. A. (2012). Preparation of â-dicalcium silicate (â-C2S) and calcium sulfoaluminate phases using non-traditional nanomaterials. *HBRC Journal*, 8(2), 91–98. https://doi.org/10.1016/j.hbrcj.2012.09.004

Han, F., Li, T., Li, M., Zhang, B., Wang, Y., Zhu, Y., & Wu, C. (2023). Nano-calcium silicate mineralized fish scale scaffolds for enhancing tendon-bone healing. *Bioactive Materials*, 20, 29–40. https://doi.org/10.1016/j.bioactmat.2022.04.030

Hasan, L. A. (2021). Evaluation of the properties of orthodontic adhesive incorporated with nano-hydroxyapatite particles. *The Saudi Dental Journal*, 33(8), 1190–1196. https://doi.org/10.1016/j.sdentj.2021.01.001

Husna, A., Handayani, L., & Syahputra, F. (2020). Utilization of goatfish bones (Abalistes stellaris) as a source of calcium in fish bone meal products. *Acta Aquatica: Aquatic Sciences Journal*, 7(1), 13. https://doi.org/10.29103/aa.v7i1.1912

Idrus, I., Wahab, S., Mustapa, F., & Wibowo, D. (2020). Comparative analysis of calcium content in milkfish by varying of wet and dry conditions using AAS instrumentation. *Journal of Applied Food Technology*, 7(2), 41–45. https://doi.org/10.17728/jaft.8455

Kusumaningrum, I., Sustono, D., & Pamungkas, B. F. (2016). Utilization of Belinda fish bones as a source of calcium powder using the alkaline method. *Journal of Fisheries and Marine Research*, 19(2), 148–155.

Kusumawati, Pipin., Priyanto, Triwitono., Sri, Anggrahini., & Yudi, Pranoto. (2022). Nano-calcium Powder Properties from Six Commercial Fish Bone Waste in Indonesia. *Squalen Bull. Mar. Fish. Postharvest Biotech*, 17(1), 1-12. https://doi.org/10.15578/squalen.601

Jampilek, J., Kos, J., & Kralova, K. (2019). Potential of nanomaterial applications in dietary supplements and foods for special medical purposes. *Nanomaterials*, 9(2), 11–20. https://doi.org/10.3390/nano9020296

Lekahena, V., Faridah, D. N., Syarief, R., & Peranginangin, R. (2014). Physicochemical characterization of bio-calcium result of tilapia fish bone extraction using alkaline and acid solutions. *Jurnal Teknologi Dan Industri Pangan*, 25(1), 57–64. https://doi.org/10.6066/jtip.2014.25.1.57

Li, X., Heng, B. C., Bai, Y., Wang, Q., Gao, M., He, Y., Zhang, X., Deng, X., & Zhang, X. (2023). Electrical charge on ferroelectric nanocomposite membranes enhances SHED neural differentiation. *Bioactive Materials*, 20, 81–92. https://doi.org/10.1016/j.bioactmat.2022.05.007

Media, Y., & Elfemi, N. (2021). Socio-cultural problems and policy alternatives in efforts to reduce stunting among toddlers in Solok Regency, West Sumatra Province. *Jurnal Ekologi Kesehatan*, 20(1), 56–68. https://doi.org/10.22435/jek.v20i1.4130

Mehmood, S., Kausar Janjua, N. K., Tabassum, S., Faizi, S., & Fenniri, H. (2022). Cost-effective synthesis approach for green food packaging coating by gallic acid conjugated gold nanoparticles from Caesalpinia pulcherrima extract. *Results in Chemistry*, 4(2), 20–27. https://doi.org/10.1016/j.rechem.2022.100437

Muijah, S., & Safitri, D. E. (2019). Nutritional status and micronutrient intake (thiamine, pyridoxine, calcium, magnesium) associated with premenstrual syndrome.

ARGIPA (Arsip Gizi Dan Pangan), 4(1), 45–53. https://doi.org/10.22236/argipa.v4i1.3850

Nam, P. V., Hoa, N. Van, & Trung, T. S. (2019). Properties of hydroxyapatites prepared from different fish bones: A comparative study. *Ceramics International*, 45(16), 20141–20147. https://doi.org/10.1016/j.ceramint.2019.06.280

Nemati, M., Huda, N., & Arifin, F. (2017). Development of calcium supplement from yellowfin tuna (*Thunnus albacares*) fishbone wastes and characterization of nutrition quality. *International Food Research Journal*, 24(6), 22–28.

Noprisanti, Masrul, & Defrin. (2018). Relationship between protein, calcium, phosphorus, and magnesium intake with bone density in adolescent girls at SMP Negeri 5 Padang. *Jurnal Kesehatan Andalas*, 7(Supplement 3), 29–36.

Shi, P., Liu, M., Fan, F., Yu, C., Lu, W., & Du, M. (2018). Characterization of natural hydroxyapatite originated from fish bone and its biocompatibility with osteoblasts. Materials Science and Engineering C, 90(September 2017), 706–712. https://doi.org/10.1016/j.msec.2018.04.026

Stepankova, H., Michalkova, H., Splichal, Z., Richtera, L., Svec, P., Vaculovic, T., Pribyl, J., Kormunda, M., Rex, S., Adam, V., & Heger, Z. (2023). Unveiling the nanotoxicological aspects of Se nanomaterials differing in size and morphology. *Bioactive Materials*, 20, 489–500. https://doi.org/10.1016/j.bioactmat.2022.06.014

Upadhyay, R. K. (2017). Chronic kidney diseases and nanoparticle therapeutics. *Journal of Tissue Science & Engineering*, 08(03), 120–130. https://doi.org/10.4172/2157-7552.1000209

Wijayanti, I., Benjakul, S., & Sookchoo, P. (2021a). Effect of high-pressure heating on physical and chemical characteristics of Asian sea bass (*Lates calcarifer*) backbone. *Journal of Food Science and Technology*, 58(8), 3120–3129. https://doi.org/10.1007/s13197-020-04815-6

Wijayanti, I., Sookchoo, P., Prodpran, T., Mohan, C. O., Aluko, R. E., & Benjakul, S. (2021b). Physical and chemical characteristics of Asian sea bass bio-calcium powders as affected by ultrasonication treatment and drying method. *Journal of Food Biochemistry*, 45(4), 78–85. https://doi.org/10.1111/jfbc.13652

Yin, T., & Park, J. W. (2015). Textural and rheological properties of Pacific whiting surimi as affected by nano-scaled fish bone and heating rates. *Food Chemistry*, 180, 42–47. https://doi.org/10.1016/j.foodchem.2015.02.021

Zainol, I., Adenan, N. H., Rahim, N. A., & Jaafar, C. N. A. (2019). Extraction of natural hydroxyapatite from tilapia fish scales using alkaline treatment. *Materials Today: Proceedings*, 16, 1942–1948. https://doi.org/10.1016/j.matpr.2019.06.072