# The influence of particle size

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# The influence of particle size on the absorption rate of catfish (*Clarias gariepinus*) bone calcium

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**Abstract**

The adequacy of calcium from food consumption is difficult to meet because of its low absorption rate. One of the ways to increase calcium absorption is to increase its solubility by decreasing its particle size to the nanometer scale. Nanoparticles have better performance due to their increased surface area-to-volume ratio. This study aimed to determine the effect of the particle size of fish bones on calcium absorption rate. The catfish bone flour was made by two methods with the same stages, but they differed in the use of tools (the machine method and the traditional method). The particle size analysis of the flour showed that the machine method was in the nanometer range. The nutritional content of the machine-milled flour was rich in protein and lipids, while the flour produced by the traditional method was rich in calcium, phosphorus, and carbohydrates. Based on the results of the absorption test, both catfish bone flours were proven to be able to gradually increase calcium levels to their peak at 2 h post-administration. In sum, bone flour with nanoparticle sizes produced by the machine was able to maximize the calcium absorption rate in rats.

**Keywords:** calcium absorption; fishbone; nanoparticle size; nutritional value.

**Practical Application:** This study will help to develop food waste (catfish bone) in nano-size to provide and enhance calcium absorption.

## 1. Introduction

An alternative to preventing osteoporosis is taking calcium supplements (Lee et al., 2017). Calcium is an essential mineral that has an important function in bone formation. Sources of calcium are widely associated with dairy and processed products such as yogurt and cheese. Milk and yogurt contain approximately 100–180 mg/100 g, while cheese provides 1 g of calcium/100 g (Ross et al., 2011). Nuts and seeds are also rich sources of calcium, especially almonds, sesame, and chia, which contain between 250 and 600 mg/100 g. Calcium-rich vegetables include kangkong (water spinach), broccoli, and watercress, which provide between 100 and 150 mg/100 g (U.S. Department of Agriculture, 2019). However, the adequacy of total calcium intake depends on the food consumption patterns of individuals.

Supplements are also a source of calcium, which is widely distributed in the community. The amount of calcium in a supplement is 1,000 mg per tablet, which is formulated to meet the calcium needs of adults. However, the use of calcium supplements is quite varied. A *systematic review* study reported that in the United States and Canada, about 40% of the adult population and 70% of older women consume calcium supplements (Cormick & Belizán, 2019). Whereas in Argentina and the Netherlands, only a small proportion of pregnant women take calcium supplements (Cormick et al., 2014). Calcium is generally consumed in the form of microcalcium. Because the absorption of calcium from food is only 50%, it often causes deficiency (Ranjan et al., 2019). Calcium absorption depends on the food consumed; several types of food can affect the degree of absorption or excretion of calcium (Guéguen & Pointillart, 2000). Some food sources can reduce the absorption of calcium such as asoxalic acid in spinach, sweet potatoes, and beans or phytic acid in high-fiber foods rich in whole grains and seeds. There are also food sources that increase calcium absorption, such as lactose and certain peptides that are formed during the digestion of milk (Cámara-Martos & Amaro-López, 2002).

Much smaller calcium reduction technology is currently being developed to increase the absorption of calcium in the body (Mosaddegh & Hassankhani, 2014). The synthesis of nanoparticles has attracted more attention because they have better performance due to their increased surface area and calcium absorption rate. Nanocalcium, which has a very small size ($10^{-9}$ m), can increase its solubility in the gastrointestinal

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tract, which further accelerates the absorption process into the circulatory system (Ranjan et al., 2019).

The source of calcium that is generally consumed by the community is milk, even though there are other sources that have not been explored further, such as fish, one of which is catfish. Catfish is a freshwater fish commodity that is widely known in society, and the use of fish bones and catfish heads becomes the main source of waste that has not been given much attention. The fish production process generally produces 15%–30% of fish bone waste with a mineral content of 60%–70%. Fish bones are proven to be a source of halal gelatin and potentially becoming the source of other minerals such as calcium (Herpandi & Adzitey, 2011). The content of calcium and phosphorus in fish bones is classified as very high (20%–25%) of the fish's body weight, so fish bones can be an alternative food ingredient as a natural source of calcium to meet the daily calcium needs (Bechtel et al., 2019). In several studies, fish bone flour has the potential to be an ingredient for the fortification of food products to increase nutritional value. The calcium in the bones must be converted into simpler forms before the fortification process is carried out. Calcium is generally available in micro-size (μ) and is approximately 25% of the total calcium absorbed in the metabolic process (Hunt & Johnson, 2007). One of the alternatives to increase the maximum absorption of calcium is by converting it into nanocalcium. The form of nanocalcium allows a more efficient digestive process to enter the body's cells, which will increase the bioavailability of the mineral calcium (Park et al., 2007). Therefore, the aim of this study is to observe the effect of the particle size of calcium in catfish bone flour on the ability to absorb calcium in experimental animals. In addition, the nutritional content is also analyzed. The reduction of the particle size of fish bone flour is conducted by two different methods, namely, traditional and modern methods (by machine).

## 2. Materials and Methods

### 2.1. The making of catfish bone flour

The main ingredient used in this study was fresh catfish, *Clarias gariepinus*. In general, the process of producing catfish bone flour began with cleaning and washing the fresh catfish, separating the meat from the bone, softening the bone by boiling it at high pressure, draining the bone, drying the bone with an oven, flouring the bone, and finally filtering the bone. In this study, the production of fish bone flour was divided into two methods, namely, the traditional method by using home equipment and the modern method by using a high-power grinding machine.

### 2.2. Fish bone flour characteristic test

#### 2.2.1. Particle size of the flour

Particle size distribution was determined by dynamic light scattering (DLS) using a Nano ZS90 Analyzer (Malvern, Worcestershire, UK). Before analyzing, NFB was diluted to 0.1 g/100 g by using DI water containing sodium hexametaphosphate at 0.2 g/100 g, which acted as a dispersing agent, and then it was subjected to ultrasonic dispersing (FRQ-1008T, Front Ultrasonic Technology Co., Ltd., Hangzhou, China) for 15 min (Yin et al., 2015).

#### 2.2.2. Morphological analysis by scanning electron microscopy

The fishbone flour samples were placed in the wells of 6-well cell culture plates and sterilized for 4 h. Saos-2 cells (about $3 \times 10^5$ cells per well) were seeded in these plastic plates and incubated for 24 h in a humidified incubator at 37°C with 95% air and 5% $CO^2$. After 24 h of incubating, the media were removed, and specimens were fixed with a 3% volume fraction of glutaraldehyde, subjected to graded (30%–100%) alcohol dehydration, and kept at 20°C. Then they were examined with SEM (Deniz et al., 2015). According to Greiner (2009), the size of nanoparticles is in the range of 100–1,000 nm. The morphology of nanocalcium particles in catfish bone has a relatively similar crystal form to that of calcite (solid like a rock). The results of particle measurement by using the scanning electron microscope were 10,000× and 20,000× magnifications. The crystal itself has three different forms, namely, calcite, aragonite, and vaterite. Calcite is as solid as a rock; vaterite has a flower shape (flower-like); and aragonite has the shape of a group of needles (Sumarto et al., 2021).

### 2.3. Nutritional content analysis

#### 2.3.1. Proximate analysis

The proximate analysis of water, ash, calcium, lipid, phosphorus, protein, and carbohydrate contents was conducted according to the method of AOAC (AOAC, 2000).

#### 2.3.2. Calcium content

The calcium ($Ca^{2+}$) content of fish bone flour was determined using the wet ashing method described by Sembok (2013). A crucible containing 1 g of fish bone flour was placed in a muffle furnace and dried at 500°C for 12 h. It was allowed to cool down to 25°C, then 2 mL of concentrated HCL was added to the crucible, and evaporation to dryness was performed on a hot plate. Approximately 10 mL of $HNO_3$ (4.8M) was added to the crucible before it was placed in a water bath for 1 h. The mixture was transferred into a 100 mL-volumetric flask, and distilled water was added until a total volume of 100 mL was reached. It was then filtered by filter paper (no. 2, Whatman).

#### 2.3.3. Phosphorus content

Phosphorus was estimated spectrophotometrically by using the molybdovanadate method (AOAC, 1990), with slight modifications described by Nwanna et al. (2008). Approximately 1.5 g of the sample was ashed for 48 h at 480°C. After the ash had cooled to room temperature, 2 mL of 6 N HCl was added, and the mixture was boiled to reach complete evaporation of the acid. After being cooled down to room temperature, 2.5 mL of 6 N HCl was added to the mixture. The mixture was warmed to dissolve all the solutions present. The solution was then cooled and diluted to 50 mL using distilled deionized water.

The phosphorus concentration was analyzed using the Canada molybophosphoric acid colorimetric method with slight modifications (Nwanna & Schwarz, 2007). Approximately 3 mL of vanadate-molybdate reagent was added to 3 mL of the diluted solution of the sample. The phosphorus concentration was measured spectrophotometrically at 430 nm after the reaction mixture was thoroughly mixed with a machine (Heidolph REAX 2000, No. 54119, Germany) and allowed to stand at room temperature for 10 min.

### *2.4. Study of calcium absorption in experimental animals*

#### *2.4.1. Preparation*

The experimental animals in this study were white male Wistar strain rats (*Rattus norvegicus*) aged 8–12 weeks. The weight of the rats ranged from 200 to 250 g. The experimental animals were acclimatized by feeding them basal rations and distilled water *ad libitum*. The composition of the basal ration was prepared according to the AIN 93 standard.

#### *2.4.2. Intervention*

The intervention was performed in accordance with the objective to determine the effect of particle size of catfish bone flour on absorption rate, so the intervention was conducted by giving a sample of catfish bone flour as much as 2.46 g (containing 500 mg of calcium for a human dose). If it was converted into a rat experimental animal dose, then 44.28 mg/200 g BW of rats were given orally. The intervention was repeated three times per group. This study was approved by the Health Research Ethical Clearance Commission, Faculty of Dental Medicine, Universitas Airlangga (number: 295/HRECC.FODM/VI/2021).

#### *2.4.3. Blood sampling*

Blood samples were obtained at every time interval after the rats were given fish bone flour products, and then 0.3 mL of blood was taken from the veins of the tail each time using a 1-mL *disposable syringe*. Blood sampling time started at pre-30 min, post-30 min, 1 h, 2 h, 4 h, and 24 h. The rat blood sample taken from the heart was put into a test tube and waited for 3 h to separate the blood and the serum. Furthermore, it was centrifuged (*Hettich Zentrifugen*) at 3,000 rpm for 15 min.

#### *2.4.4. Analysis of blood calcium levels*

A volume of 1 mL of serum sample was pipetted into a test tube, and then 4 mL of 5% TCA was added. The solution was vortexed (homogenized) and then centrifuged at 3,000 rpm for 30 min. The supernatant result was pipetted 1 mL each into a test tube, then 1 mL of 5% strontium (Sr) solution and 8 mL of aquadest were added. The analysis was performed by an atomic absorption spectrophotometer (AAS) at a wavelength of 422.4 nm. The calcium standard solution, namely, calcium carnionate ($CaCO_3$), was made with concentrations of 0, 0.5, 1, 3, and 5 ppm. The reading results were then compared with the standard curve so that the calcium levels in mg/dl or ppm could be obtained.

## 3. Results and Discussion

### *3.1. Process of Producing Catfish Bone Flour*

The main material used in this study was fresh catfish, *Clarias gariepinus*. The process of producing catfish bone flour is shown in Figure 1. The stages of catfish bone flour production by traditional methods or by using machines were basically the same. The two methods were distinguished by their production capacity and the tools used. The first stage was the process of cleaning and washing the fresh catfish to remove dirt and unused stomach contents. The second stage was the process of separating the parts of the catfish, which were the meat, skin, head, and fish bones. Furthermore, the parts used for the flour production were the bones and spines of the catfish, which were then softened by boiling them at a high temperature and pressure (presto) for 45 min. In this high-pressure boiling stage, a different tool was used between the traditional and machine methods; the traditional method used a pressure pan, while the machine method used a pressure cooker with a capacity of 51 L. After that, in the traditional method, the fish bones that still contained water were drained manually by using a cloth filter; in the machine method, a spinner machine with a capacity of 10 kg was used. In the next stage, the fish bones were dried for 2 h in an oven to reduce the water content to its maximum. The oven used for the traditional method was a small oven commonly used by households, in contrast to the oven used for the machine method, which used a multilevel oven. The dried fish bones were then ground in a grinding process using a grinder and a disc mill. The last stage was filtering the fish bone flour to produce really fine flour. The sieve used for the traditional

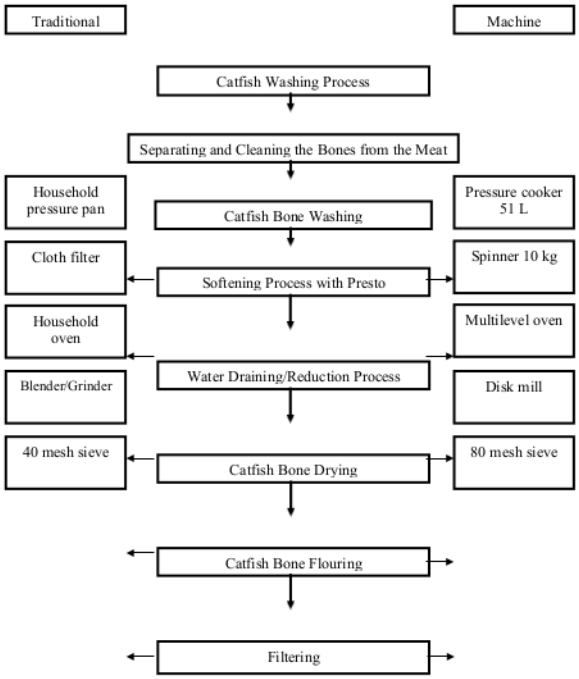



**Figure 1**. Stages of catfish bone flour production.

method was an ordinary one, while an 80-mesh sieve was used for the machine method.

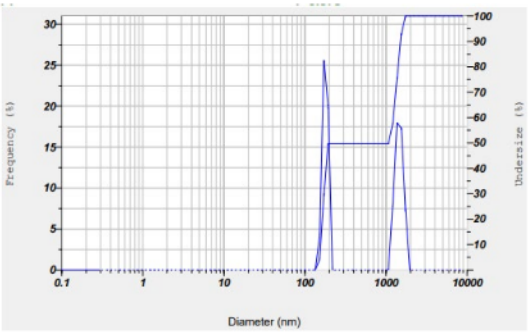
### *3.2. Characteristics of catfish bone flour*

According to Greiner (2009), the size of nanoparticles is in the range of 1–100 nm, and the size of microparticles is in the range of 1–1,000 µm (Greiner, 2009). Table 1 shows the results of the particle size characterization test of catfish bone flour with the machine and the traditional method. The graph shows two peaks: the first peak in the fishbone flour machine group shows 50% of the particles in the range of 160 nm and the second peak in the range of 1,300–1,400 nm. The European Commission has adopted a recommendation on the definition of a nanomaterial. The recommendation defines a nanomaterial as having a proportion of particles whose size ranges from 1 to 100 nm that is at least 50% (European Commission, 2011). In addition, this product is made from fish bones, which, based on nutritional content (Table 1), contain 9.3% phosphorus. Based on research conducted by Li et al. (2019), flour based on phosphorus and processed by large-scale production has a particle size of 100–300 nm, referred to as nanoparticles. So, based on these findings, machine-made fishbone flour meets the requirements for nanoparticles. On the contrary, the particle

**Table 1**. Fishbone flour particle size test results.

**Fishbone Flour (Machine)**

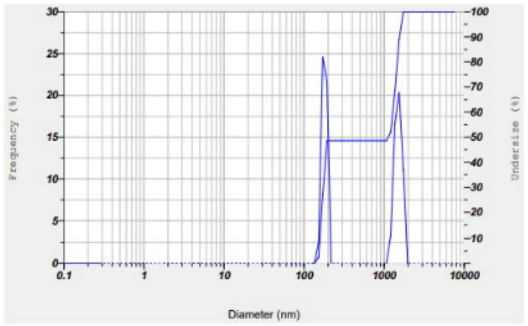
**First test**




| Peak No. | S.P.Area Ratio | Mean (nm) | SD (nm) |
|---|---|---|---|
| 1 | 0.50 | 168.0 | 12.5 |
| 2 | 0.50 | 1,368.0 | 154.1 |

Z-Average particle size: 462.9 nm
PI: 0.578

**Second test**

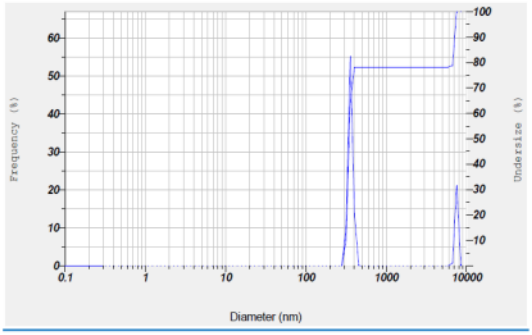



| Peak No. | S.P.Area Ratio | Mean (nm) | SD (nm) |
|---|---|---|---|
| 1 | 0.50 | 169.6 | 11.8 |
| 2 | 0.50 | 1,416.2 | 148.0 |

Particle size: 484.4 nm
PI: 0.594

**Fishbone Flour (Traditional)**

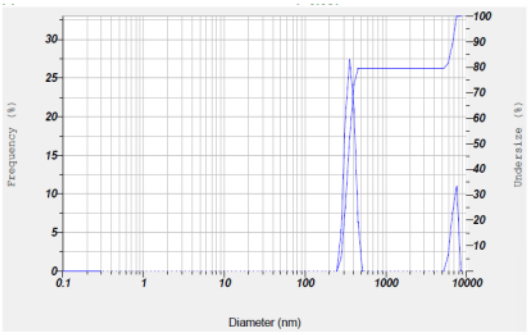
**First test**




| Peak No. | S.P.Area Ratio | Mean (nm) | SD (nm) |
|---|---|---|---|
| 1 | 0.78 | 337.6 | 22.3 |
| 2 | 0.22 | 7,063.1 | 136.6 |

Z-Average particle size: 1,493.5 nm
PI: 0.692

**Second test**

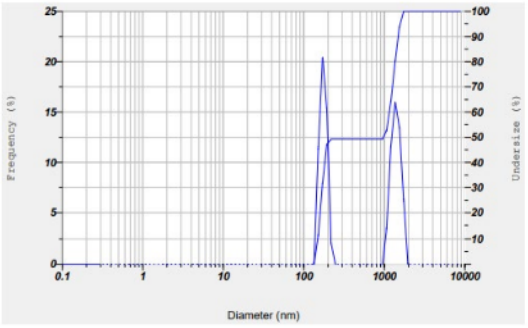
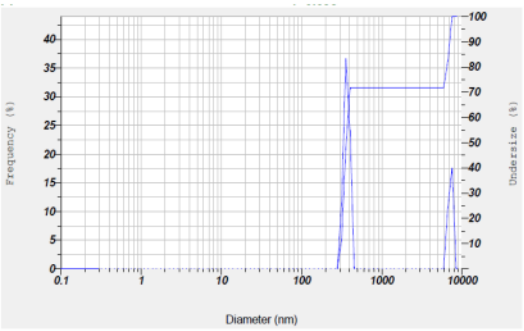



| Peak No. | S.P.Area Ratio | Mean (nm) | SD (nm) |
|---|---|---|---|
| 1 | 0.80 | 339.5 | 43.1 |
| 2 | 0.20 | 6,637.0 | 523.0 n |

Particle size: 1,510.3 nm
PI: 0.687

Continue...

**Table 1**. Continuation.

| Fishbone Flour (Machine) | Fishbone Flour (Traditional) |
|---|---|
| **Third test** | **Third test** |
|  |  |
| Peak No. / S.P.Area Ratio / Mean (nm) / SD (nm) | Peak No. / S.P.Area Ratio / Mean (nm) / SD (nm) |
| 1 / 0.50 / 165.2 / 16.8 | 1 / 0.72 / 343.6 / 28.1 |
| 2 / 0.50 / 1,318.7 / 178.4 | 2 / 0.28 / 6,777.5 / 395.2 |
| Particle size: 462.1 nm<br>PI: 0.559 | Particle size: 1,520.2 nm<br>PI: 0.693 |
| **Average**<br>Particle size: 469.8 nm<br>PI: 0.577 | **Average**<br>Particle size: 1,508 nm<br>PI: 0.6907 |

Note: Dispersants using ethanol.

size of the traditional method for the first peak was >70% of the particles in the range of 300 nm, and the second peak is in the range of 6,000–7,000 nm, so they were categorized as microparticles. The processing method of using a large-capacity machine produced a better product quality, including a smaller particle size and homogeneous surface area (Yin et al., 2016). This was different from the traditional method, which still involved simple processes such as using a sieve with a larger mesh and using a blender for the flouring process, which produced a coarser product compared to the power of a disc mill machine. The power of different machines caused the particle breakage energy also different.

Research by Muryati et al. (2019) stated that the characteristics of nanocalcium oxide determined by XRD had an average particle size of 38.94445 nm, which indicated that the sample had met the requirements as a nanomaterial. The fishbone flour particle size of *Micromesistius potassium* by using Mastersizer 3,000 (± SD) showed 26.6 nm with Dx (90 µm) (Busca et al., 2021). A study by Zhu et al. (2021), which implemented *thermoultrasonic treatment* on halibut fish bones, resulted in a particle size of 605.92 nm. Hydrophobic intermolecular interactions could destroy or reduce particle size. The distribution of nanoparticle size in fish bone flour by special treatment resulted in a more homogenous size. This was possible because the treatment responded to the collision rate and impact strength so that it produced the expected nanoparticle size (Zhu et al., 2021). The treatment of catfish bone flour produced by using a machine contributed to the homogenization of particle size and a more stable final result, so that the results shown were in accordance with the nanoparticle size criteria.

The nanocalcium particle morphology was determined using SEM JEOL JSM 6510-LA. The results of particle measurement were obtained using the scanning electron microscope at 10,000× and 20,000× magnifications. The crystal has three different crystal forms, namely, calcite, aragonite, and vaterite. Calcite is as solid as a rock; veterit has a flower-like shape; and aragonite has the shape of a group of needles.

In Figure 2, the microscopic appearance of fishbone flour is shown using a machine (Figure 2A) and traditional (Figure 2B) methods. Particles in fishbone flour by machine method (Figure 2A) appear to be scattered with a smaller size than the particles in fishbone flour by the traditional method (Figure 2B), which are larger in size. In addition, the particles in Figure 2B appear to be sticking to each other. Other research showed microstructure photos of fishbone flour *Micromesistius poutassou* particles look smaller and are similar to photos of control products, namely, the microstructure of milk flour (Busca et al., 2021).

### *3.3. The proximate analysis of catfish bone flour*

Based on the result of the experiment using a different production of nano-calcium from fishbone, both the machine and traditional methods significantly affect the characteristics of water, ash, calcium, phosphorus, energy, and carbohydrate content. The results of the proximate test of catfish bone flour

are shown in Table 2. The difference in the production method of catfish bone flour between traditional and machine methods shows different nutritional values.

### 3.3.1. Water content

The percentage of water content in catfish bone flour using the machine method is 5.63%, higher than the traditional method, which is 4.51%. Different results found that water content reaches 10.35% because the drying process only uses sunlight (Taufiq & Fadlila, 2021). The low water content produced in this research is due to the use of a household oven where the temperature has a good heat cycle. According to another study, the higher the drying temperature, the lower the water content of a food source. This is because the water content in the material is different from the surrounding air, so it will be easier to evaporate (Ikhsan & Patang, 2016).

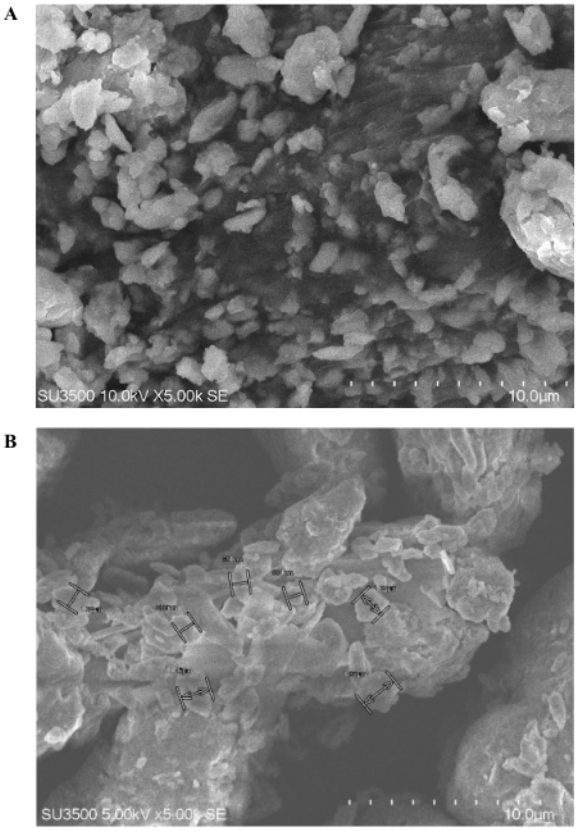



**Figure 2**. Microscopic photo of catfish bone flour: (A) machine and (B) traditional.

**Table 2**. Catfish bone nutritional content.

| Parameter | Test Results | |
|---|---|---|
| | Catfish Bone Flour (traditional) | Catfish Bone Flour (machine) |
| Water content (%) | 4.51 | 5.63 |
| Ash content (%) | 60.36 | 44.10 |
| Energy (kcal/100 g) | 185.97 | 257.88 |
| Protein (%) | 20.22 | 36.25 |
| Lipid (%) | 9.09 | 11.36 |
| Carbohydrate (%) | 5.82 | 2.66 |
| Calcium (mg/100 g) | 19,458.13 (19.4%) | 15,901.40 (15.9%) |
| Phosphorus (mg/kg) | 104,211.01 (10.4%) | 92,949.89 (9.3%) |

### 3.3.2. Ash content

The percentage of ash content in the traditional method is 60.36%, while the machine method shows a lower figure of 44.1%. Research by Bechtel et al. (2019) presented an ash content of 53.95%. Tuna fish bones revealed an ash content of 44% (Abbey et al., 2016). Differences in ash content in fishbone can be affected by the mineral composition of each ingredient. Bone mineral content in fish bones is influenced by the nutritional components of each species of fish when searching for food in aquatic habitats (Sumarto et al., 2021). Ash content shows leftover inorganic substances from the combustion of organic material. Bone contains living cells and an intracellular matrix in the form of mineral salts. The mineral consists of 80% calcium phosphate, and the rest is calcium carbonate and magnesium phosphate (Taufiq & Fadlila, 2021).

### 3.3.3. Energy content

The energy content of catfish bone flour with the traditional method is 185.97 kcal/100 g, while the machine method is 257.88 kcal/100 g, which is higher. Research by Abbey et al. (2016) analyzing the flour content of tuna fish bones showed an energy content of 242.5 kcal/100 g.

### 3.3.4. Protein content

Catfish bones with the traditional method contain 20.22% protein, while the machine method is 36.25% higher. This result shows that the protein content is sufficient when compared to other research showing that tuna fish bones contain 28.66% protein (Abbey et al., 2016). The protein content of catfish bone flour is 19.47% (Rosidi et al., 2021). Protein from tilapia fish bones is 14.81% (Hemung, 2013) using modern machine methods. This decrease in protein content is thought to be caused by protein denaturation caused by high heating temperatures. Through flocculation, the protein structure will be damaged. This process is the initial stage of denaturation. Analysis of the denaturation temperature range of a product and the optimal selection of thermal processing parameters are important for fish raw materials because they have an impact on the quality, stability, and functionality of the final product (Strzelczak et al., 2021).

### 3.3.5. Lipid content

The lipid content of catfish bone flour showed 9.09% in the traditional method, while the machine method showed a higher value of 11.36%. Research by Rosidi et al. (2021) discovered that *Clarias gariepinus* fish bones contain 3.56% lipid, which is significantly higher than *Sardinella fimbriata*, which is only

0.77%. Additionally, tuna fish bones contain 11.3% lipid (Abbey et al., 2016). The lipid content in boiled catfish bones is 2.11%, while in smoked fish bones, it is 1.95% (Adeniji et al., 2015). Catfish (*Clarias gariepinus*) is a fatty fish, and the content of fish bones correlates with body fat. In line with the research by Umar et al. (2018), the production process uses the heating method using firewood, which has a lower lipid content than using a machine. Firewood produces more heat, and this is a result of a higher differential temperature.

### 3.3.6. Carbohydrate content

The carbohydrate content of catfish bone flour with the traditional method was 5.82% higher than the carbohydrate content of catfish bone flour with the machine method. Research by Ferazuma et al. (2011) conducted the production of catfish bone flour as an ingredient in the formula for making crackers, showing a carbohydrate content of 15.03% with modern machine methods. Other research identified the nutritional content of bone flour in several species of catfish (Sumarto et al., 2021). The result showed carbohydrate content of *Pangasius hypophthalmus* 3.93%, *Clarias batrachus* 6.25%, *Hemibagrus mumurus* 4.16%, and *Paraplotosus albilabris* 6.21% with the production process using machines. The results of the calculation of carbohydrate content using the *by difference* method with calculations involving the water content, protein ash content, and lipid content of the food source.

### 3.3.7. Calcium content

The calcium content of catfish bone flour is 19,458.13 mg/100 g in the traditional method is higher, while it is 15,901.4 mg/100 g in the machine method (Table 3). This calcium content is higher than other research, which showed calcium content of 13,184.3 mg/100 g in the skeleton of tuna fish and 15,469.3 mg/100 g in the gills of tuna (Abbey et al., 2016). Using only boiling as a solvent, Bechtel et al. (2019) found 21.27% Ca (dry wt basis) in channel catfish bone fractions. Other researchers have reported 18.07% Ca in hake bone flour (Flammini et al., 2016) and 23.69% Ca in silk carp bone flour (Yin et al., 2016) using a similar method. The difference in calcium levels is due to differences in species, sex, biological cycle, and body parts, in addition to environmental factors such as season, fish habitat, and the number of available nutrients.

**Table 3**. Catfish bone nutritional value: literature review.

| Product | Parameter |
|---|---|
| | Calcium (%) |
| **Fishbone flour** | |
| *Clarias gariepinus* (Traditional method) | 19.4 |
| *Clarias gariepinus* (Machine method) | 15.9 |
| **Fishbone flour (article)** | |
| *Clarias batrachus* [11] | 21.27 |
| *Sardinella fimbriata* [30] | 4.96 (µg/g) |
| *Clarias gariepinus* | 4.79 (µg/g) |
| *Atlantic salmon* [37] | 24.92 |
| *Baltic cod backbones* | 27.79 |
| *Trichiurus savala* [38] | 27.81 |
| *Sardinella longiceps* | 32.73 |
| *Sardinella fimbriata* [39] | 31.98 |
| *Sardiella gibbose* | 28.34 |
| *Sardiella albella* | 26.02 |
| **Nano-calcium from fishbone (article)** | |
| *Snakehead Fish Bone* [24] | 39.8 |

The calcium content of catfish bone flour was higher in the traditional method, which is 19.4%, while the machine method showed 15.9%. Research by Bechtel et al. (2019) showed that the calcium content of *Clarias batrachus* fish bone flour was higher, namely, 21.27%, using a machine with enzymatic addition treatment. Other research revealed that *Sardinella fimbriata* fish bone flour has a calcium content of 4.96 (µg/g) with a modern machine production process, while *Clarias gariepinus* fish bone contains 4.79 (µg/g) calcium (Rosidi et al., 2021). Atlantic salmon bone flour contains calcium levels of 24.92% and the Baltic cod backbone of 27.79% using modern machines with the addition of chemicals (Bubel et al., 2015).

Research by Logesh et al. (2012) identified the bone calcium levels of *Sardinella longiceps* (oil sardine) and *Trichiurus savala* (ribbon fish) through two treatments. In treatment I, bones were soaked in 0.5 mol/L sodium hydroxide for 20 h and then in 40% (v/v) ethanol solution for 15 h. In treatment II, bones were soaked in 1.0 mol/L sodium hydroxide for 30 h and then in 60% (v/v) ethanol solution for 10 h. The results showed that the calcium levels were significantly different. The bones of *Sardinella longiceps* in treatments I and II contained 26.39 and 32.72% calcium, while the bones of *Trichiurus savala* fish contained 19.33 and 27.81% calcium. In the bones of *Sardinella fimbriata*, *Sardinella albella*, and *Sardinella gibbose* fish with the same treatment as the previous study, calcium levels were 31.98, 28.34, and 26.02%, respectively (Xavier Eugien et al., 2014). Additionally, research by Muryati et al. (2019) identified the nanocalcium content of snakehead fish bone, which showed 39.8% results through calcination at a temperature of 900°C and preparation by the ball milling method.

### 3.3.8. Phosphorus content

Phosphorus results showed 104,211.01 mg/100 g in the traditional method and 92,949.89 mg/100 g in the machine method. Tuna fish bones contain 1,010.2 mg/100 g of phosphorus, while tuna fish gills produce 1,071.8 mg/g of phosphorus (Abbey et al., 2016). Nevertheless, catfish bone flour, through an enzymatic process, showed 8.12% for 5 min and 9.24% for 30 min (Bechtel et al., 2019).

## *3.4. Study of calcium absorption rate of catfish flour in experimental animals*

Fishbone is composed of approximately 30% collagen and 60–70% inorganic compounds such as calcium, phosphorus, magnesium, iodine, and selenium (Välimaa et al., 2019). Fishbone naturally contains calcium and phosphorus in the right ratio of approximately 2:1 from hydroxyapatite, which is considered the most bioavailable form of calcium. In addition to calcium, phosphorus used by the fertilizer and

chemical industries can be insulated and recovered from fish bones (Välimaa et al., 2019). The soluble calcium concentration correlated with total solubility, with the highest soluble calcium found at pH 3 and the lowest at pH 7 (Busca et al., 2021). At higher pH, calcium solubility was very low in all cases. *Micromesistius poutassou* bone flour exhibited the highest soluble ion concentration (158.78 mg/L) at pH 3 at room temperature. The solubility was inversely associated with pH, with all flours exhibiting not more than 10% solubility at pH 7 and approximately 40% solubility at pH 3. However, the temperature did not play a significant role in the solubility profile of fish bone flour, even though tuna fish bone flour is processed using modern machines (Busca et al., 2021).

The calcium absorption test in experimental animals was carried out on both samples, namely, samples using the machine and traditional methods, by observing the time intervals for 30 min, 1 h, 2 h, 4 h, and 24 h. Table 4 shows the results of the absorption test. There was a gradual increase over time until after 2 h, which was the highest absorption peak, then a gradual decrease until 24 h (Figure 3). The peak time interval at 2 h post-administration of fish bone flour showed the maximum concentration of calcium absorption. Furthermore, there is a decrease because there is no more absorption and elimination.

Research related to calcium absorption *in vitro* using the $CaCO_2$ cell line model showed a 40% of the amount of calcium absorbed and the total calcium absorbed (>150 mg/kg).

**Table 4**. Average calcium levels in experimental animals with time interval.

| Time | Code | |
|---|---|---|
| | TTM | TTB |
| Pre | 12.65 ± 1.32 | 11.85 ± 0.38 |
| Post | | |
| 30 min | 20.81 ± 2.62 | 19.33 ± 2.70 |
| 1 h | 20.33 ± 2.64 | 19.12 ± 0.72 |
| 2 h | 23.54 ± 1.27 | 21.35 ± 1.60 |
| 4 h | 19.21 ± 1.50 | 18.32 ± 3.93 |
| 24 h | 16.57 ± 1.45 | 13.65 ± 3.21 |

TTM: Fishbone flour (machine); TTB: Fishbone flour (traditional).

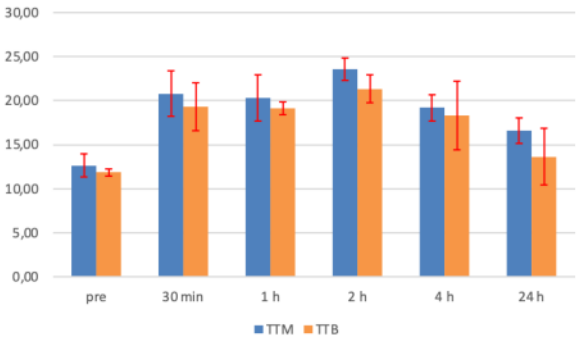



**Figure 3**. Average calcium absorption between time intervals.

The calcium solubility of fish bone flour increases significantly as the particle size decreases, which is due to an increase in surface area and the breakdown of the collagen matrix. Therefore, decreasing the particle size of fish bones becomes an alternative to increasing calcium absorption (Yin et al., 2016). Calcium absorption is higher in the machine method due to the smaller nanoparticle size, so the absorption turns out more perfectly.

The advantage of this research is the comparison of two methods of making fish bone flour into calcium preparations of nanoparticle size. The first method is a traditional one that is easy to do and can be adapted by the general public because it does not require special tools. In the second method, special tools are used to support the process of producing fish bone flour, which is expected to produce a superior product. Another advantage is that in this study, an absorption test was included to see the absorption of calcium in the body through experimental animals, which was accompanied by time intervals so that changes in the absorption value were depicted. The drawback of this study is that only one type of fish is used. Because it does not compare the various variations of catfish, the data displayed may be limited.

## 4. Conclusion

This study showed that fishbone flour with a machine-based production method was superior in particle size and in accordance with the nanoparticle criteria. Moreover, the value of protein content was higher. In addition, based on the results of the absorption tests in experimental animals, the average value of calcium content was also higher, thus indicating optimal absorption capacity. In contrast, in the fishbone flour of the traditional method, the calcium content was higher but the particle size was larger, and it was not included in nanoparticles but microparticles, so the absorption results were lower. In conclusion, the nanoparticle size was able to increase the absorption of calcium gradually until the absorption peak at 2 h post-intervention compared to the microparticle size.

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## Conflict of Interest

The authors declare no conflict of interest.

## References

Abbey, L., Glover-Amengor, M., Atikpo, M. O., Atter, A., & Toppe, J. (2016). Nutritional content of fish powder from low value fish and fish byproducts. *Food Science Nutrition*, *5*(3), 374-379. https://doi.org/10.1002/fsn3.402

Adeniji, A. R., Ayoade, O., Akinwande, B. O., & Oludare, O. (2015). Nutritional Evaluation of Boiled and Smoked Catfish (Clarias gariepinus) using Different Parts (Bone, Skin and flesh). *International Journal of Innovative Research in Science, Engineering and Technology*, *4*(10), 10465-10469. https://doi.org/10.15680/IJIRSET.2015.0410127

Association of Official Analytical Chemist (AOAC). (1990). *Official Methods of Analysis* (15th ed.). Association of Official Analytical Chemist. v. 2.

Association of Official Analytical Chemist (AOAC). (2000). Moisture in Malt Gravimetric Method. (935.29). In AOAC (Ed.), *Official Methods of Analysis* (17th ed.). Association of Official Analytical Chemist.

Bechtel, P. J., Watson, M. A., Lea, J. M., Bett-Garber, K. L., & Bland, J. M. (2019). Properties of bone from Catfish heads and frames. *Food Science and Nutrition*, *7*(4), 1396-1405. https://doi.org/10.1002/fsn3.974

Bubel, F., Dobrzański, Z., Bykowski, P. J., Chojnacka, K., Opaliński, S., & Trziszka, T. (2015). Production of calcium preparations by technology of saltwater fish by product processing. *Open Chemistry*, *13*(1), 1333-1340. https://doi.org/10.1515/chem-2015-0146

Busca, K., Wu, S., Miao, S., Govindan, A., Strain, C. R., O'Donnell, S. T., Whooley, J., Gite, S., Paul Ross, R., & Stanton, C. (2021). An in vitro study to assess bioaccessibility and bioavailability of calcium from blue whiting (Micromesistius poutassou) fish bone powder. *Irish Journal of Agricultural and Food Research*. https://doi.org/10.15212/ijafr-2020-0140

Cámara-Martos, F., & Amaro-López, M. A. (2002). Influence of dietary factors on calcium bioavailability: A brief review. *Biological Trace Element Research*, *89*, 43-52. https://doi.org/10.1385/BTER:89:1:43

Cormick, G., & Belizán, J. M. (2019). Calcium intake and health. *Nutrients*, *11*(7), 1606. https://doi.org/10.3390/nu11071606

Cormick, G., Zhang, N. N., Andrade, S. P., Quiroga, M. J., di Marco, I., Porta, A., Althabe, F., & Belizán, J. M. (2014). Gaps between calcium recommendations to prevent pre-eclampsia and current intakes in one hospital in Argentina. *BMC Research Notes*, *7*, 920. https://doi.org/10.1186/1756-0500-7-920

Deniz, D. Y., Kahraman, M., Erdem Kuruca, S., Suleymanoglu, M., & Gungor, A. (2015). 4-Vinylbenzene Boronic Acid-Hydroxy Apatite/Polyvinyl Alcohol Based Nanofiber Scaffold Synthesized by UV-Activated Reactive Electrospinning. *International Journal of Polymeric Materials and Polymeric Biomaterials*, *64*(14), 727-732. https://doi.org/10.1080/00914037.2014.1002130

European Commission. (2011). *Commission Recommendation Number 2011/696/EU on the definition of nanomaterial*. European Commission.

Ferazuma, H., Marliyati, S. A., & Amalia, L. (2011). Substitution of Dumbo Catfish Head Flour (Clarias Gariepinus Sp) to Increase Calcium Content of Crackers. *Journal of Nutrition Food*, *6*(1), 18-27.

Flammini, L., Martuzzi, F., Vivo, V., Ghirri, A., Salomi, E., Bignetti, E., & Barocelli, E. (2016). Hake fish bone as a calcium source for efficient bone mineralization. *International Journal of Food Sciences and Nutrition*, *67*(3), 265-273. https://doi.org/10.3109/09637486.2016.1150434

Greiner, R. (2009). Current and projected applications of nanotechnology in the food sector. *Nutrire*, *34*(1), 243-260.

Guéguen, L., & Pointillart, A. (2000). The Bioavailability of Dietary Calcium. *Journal of the American College of Nutrition*, *19*(Suppl. 2), 119S-136S. https://doi.org/10.1080/07315724.2000.10718083

Hemung, B. (2013). Properties of Tilapia Bone Powder and Its Calcium Bioavailability Based on Transglutaminase Assay. *International Journal of Bioscience, Biochemistry and Bioinformatics*, *3*(4), 306-309. https://doi.org/10.17706/IJBBB.2013.V3.219

Herpandi, N. H., & Adzitey, F. (2011). Fish bone and scale as a potential source of halal gelatin. *Journal of Fisheries and Aquatic Science*, *6*(4), 379-389. https://doi.org/10.3923/jfas.2011.379.389

Hunt, C. D., & Johnson, L. A. K. (2007). Calcium requirements: New estimations for men and women by cross-sectional statistical analyses of calcium balance data from metabolic studies. *American Journal of Clinical Nutrition*, *86*(4), 1054-1063. https://doi.org/10.1093/ajcn/86.4.1054

Ikhsan, M., Muhsin, & Patang. (2016). The effect of drying temperature variations on the quality of dumbo catfish (Clarias gariepinus). *Journal of Agricultural Technology Education*, *2*(2), 114-122. https://doi.org/10.26858/jptp.v2i2.5166

Lee, Y. K., Jung, S. K., Chang, Y. H., & Kwak, H. S. (2017). Highly bioavailable nanocalcium from oyster shell for preventing osteoporosis in rats. *International Journal of Food Sciences and Nutrition*, *68*(8), 931-640. https://doi.org/10.1080/09637486.2017.1307948

Li, X., Chen, G., Le, Z., Li, X., Nie, P., Liu, X., Xu, P., Wu, H. B., Liu, Z., Lu, Y. (2019). Well-Dispersed Phosphorus Nanocrystals within Carbon via High-Energy Mechanical Milling for High Performance Lithium Storage. *Nano Energy*, 59, 464-471. https://doi.org/10.1016/j.nanoen.2019.02.061

Logesh, A. R., Pravinkumar, M., Raffi, S. M., & Kalaiselvam, M. (2012). Calcium and phosphorus determination in bones of low value fishes, Sardinella longiceps (Valenciennes) and Trichiurus savala (Cuvier), from Parangipettai, Southeast Coast of India. *Asian Pacific Journal of Tropical Disease*, *2*(Suppl. 1), S254-S256. https://doi.org/10.1016/S2222-1808(12)60160-1

Mosaddegh, E., & Hassankhani, A. (2014). Preparation and characterization of nano-CaO based on eggshell waste: Novel and green catalytic approach to highly efficient synthesis of pyrano [4, 3-b] pyrans. *Chinese Journal of Catalysis*, *35*(3), 351-356. https://doi.org/10.1016/s1872-2067(12)60755-4

Muryati, M., Hariani, P. L., & Said, M. (2019). Preparation and Characterization Nanoparticle Calcium Oxide from Snakehead Fish Bone using Ball Milling Method. *Indonesian Journal of Fundamental and Applied Chemistry*, *4*(3), 111-115. https://doi.org/10.24845/IJFAC.V4.I3.111

Nwanna, L. C., Kolahsa, M., Eisenreich, R., & Schwarz, F. J. (2008). Pre-treatment of dietary plant feedstuffs with phytase and its effect on growth and mineral concentration in common carp (Cyprinus carpio L.). *Journal of Animal Physiology and Animal Nutrition*, *92*(6), 677-682. https://doi.org/10.1111/j.1439-0396.2007.00764.x

Nwanna, L. C., & Schwarz, F. J. (2007). Effect of supplemental phytase on growth, phosphorus digestibility and bone mineralization of common carp (Cyprinus carpio L). *Aquaculture Research*, *38*(10), 1037-1044. https://doi.org/10.1111/j.1365-2109.2007.01752.x

Park, H. S., Jeon, B. J., Ahn, J., & Kwak, H. S. (2007). Effects of nanocalcium supplemented milk on bone calcium metabolism in ovariectomized rats. *Asian-Australasian Journal of Animal Sciences*, *20*(8), 1266-1271. https://doi.org/10.5713/ajas.2007.1266

Ranjan, R., Sawal, R. K., Ranjan, A., & Patil, N. (2019). Comparison of calcium absorption from nano- and micro-sized calcium salts using everted gut sac technique. *Indian Journal of Animal Science*, *89*(3), 337-339. https://doi.org/10.56093/ijans.v89i3.88101

Rosidi, W. N. A. T. M., Arshad, N. M., & Mohtar, N. F. (2021). Characterization of sardinella fimbriata and clarias gariepinus bones. *Biodiversitas*, *22*(4), 1621-1626. https://doi.org/10.13057/biodiv/d220405

Ross, A. C., Taylor, C. L., Yatkine, A. L., Valle, H. B. (2011). *Dietary Reference Intakes for Calcium and Vitamin D*. National Academies Press.

Sembok, W. Z. (2013). *Botany and Plant Physiology (GTN3300)*. Universiti Malaysia Terengganu.

Strzelczak, A., Balejko, J., Szymczak, M., & Witczak, A. (2021). Effect of protein denaturation temperature on rheological properties of baltic herring (Clupea harengus membras) muscle tissue. *Foods*, *10*(4), 829. https://doi.org/10.3390/foods10040829

Sumarto, Desmelati, Sari, N. I., Angraini, R. M., & Arieska, L. (2021). Characteristic of Nano-Calcium Bone from a Different Species of Catfish (Pangasius hypophthalmus, Clarias batrachus, Hemibagrus nemurus and Paraplotosus albilabris). *IOP Conference Series: Earth and Environmental Science*, *695*, 012055. https://doi.org/10.1088/1755-1315/695/1/012055

Taufiq, N., & Fadlila, R. N. (2021). Production of Calcium (Ca) Nano Particles from Waste Catfish Bone (Pangasius sp) Using Ultrasound-Assisted Solvent Extraction Method. *Al-Kimia*, *9*(1), 9-15. https://doi.org/10.24252/al-kimia.v9i1.16390

Umar, F., Oyeroand, J. O., & Ibrahim, S. U. (2018). Comparative Nutritional Composition of African Catfish (Clarias gariepinus) smoked with melon shell briquettes and firewood. *Journal of Fish Life Science*, *3*(1), 39-43.

U.S. Department of Agriculture. (2019). *Food Data Central*. U.S. Department of Agriculture.

Välimaa, A. L., Mäkinen, S., Mattila, P., Marnila, P., Pihlanto, A., Mäki, M., & Hiidenhovi, J. (2019). Fish and fish side streams are valuable sources of high-value components. *Food Quality and Safety*, *3*(4), 209-226. https://doi.org/10.1093/fqsafe/fyz024

Xavier Eugien, L., Pravinkumar, M., Logesh, A. R., Viswanathan, C., & Raffi, S. M. (2014). Estimation of calcium and phosphorus in bones of three low value fishes Sardinella fimbriata, Sardinella albella and Sardinella gibbosa from Muttom, Southeast Coast of India. *International Journal of Current Research*, *3*(2), 43-46.

Yin, T., Du, H., Zhang, J., & Xiong, S. (2016). Preparation and Characterization of Ultrafine Fish Bone Powder. *Journal of Aquatic Food Product Technology*, *25*(7), 1045-1055. https://doi.org/10.1080/10498850.2015.1010128

Yin, T., Park, J. W., & Xiong, S. (2015). Physicochemical properties of nano fish bone prepared by wet media milling. *LWT – Food Science and Technology*, *64*(1), 367-373. https://doi.org/10.1016/j.lwt.2015.06.007

Zhu, W., He, W., Wang, W., Bu, Y., Li, X., Li, J., & Zhang, Y. (2021). Effects of thermoultrasonic treatment on characteristics of micro-nano particles and flavor in Greenland halibut bone soup. *Ultrasonics Sonochemistry*, *79*, 105785. https://doi.org/10.1016/j.ultsonch.2021.105785

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