Characteristic of Nano-Calcium Bone from a Different Species of Catfish (*Pangasius hypophthalmus*, *Clarias batrachus*, *Hemibagrus nemurus* and *Paraplotosus albilabris*)

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Abstract. Fishbone contains mineral components that can potentially be exploited to improve the nutrition of food products. This research was aimed to determine the quality characteristics of nano-calcium bone from a different species of catfish. This research used an experimental method by producing nano-calcium from other types of catfish (*Pangasius hypophthalmus*, *Clarias batrachus*, *Hemibagrus nemurus*, and *Paraplotosus albilabris*) using 1M NaOH. The production of nano-calcium from the bone of different species of catfish produce a white fishbone nano-calcium color, with the whiteness degree of 90.7% (*P. hypophthalmus*); 88.9% (*C. batrachus*), 89.1% (*H. nemurus*), and 88.4% (*P. albilabris*). The moisture content in catfish bone nano-calcium in *P. hypophthalmus*, *C. batrachus*, *H. nemurus*, and *P. albilabris* was relatively similar, ranging from 5.71%-6.12%. Calcium content of catfish bone nano-calcium very high in *P. hypophthalmus*, *C. batrachus*, *H. nemurus*, and *P. albilabris* were 87.4%; 89.6%; 89.1%, and 86.1% respectively. While the phosphorus level in *P. hypophthalmus* fishbone was 11.2%, *C. batrachus* fishbone was 9.4%, *H. nemurus* fishbone was 9.8%, and *P. albilabris* fishbone was 12.7%. The particle size of nano-calcium in *P. hypophthalmus* fishbone was 248-672 nm, in *H. nemurus* fishbone 206-594 nm, and nano-calcium in *C. batrachus* fishbone was 135-573 nm.

1. Introduction

Fishery potential in Riau Province is quite significant, both marine fisheries and freshwater fisheries. The potential of catfish species production like *P. hypophthalmus*, *C. batrachus*, *H. nemurus*, and *P. albilabris* are relatively huge. Fish production of Riau province from public waters, ponds, and cages in 2015 for *P. hypophthalmus* were 28,365 tons, 15,804.4 tons for *C. batrachus*, *H. nemurus* was 2,575.71 tons, and *P. albilabris* was 7,835.5 tons [1].

The utilization of fishery's potential is through consumptions in the form of fresh or processed products. *P. hypophthalmus*, *C. batrachus*, *H. nemurus*, and *P. albilabris* are widely consumed fish by the public. Fish processing production in Riau is mostly in the form of the smoked fish industry, meatballs, nuggets, chips, and fish fillet. These processes generate fishery waste or byproducts. Fishing industry wastes such as bones, head, skin, tail, and internal fish organs have not been fully utilized and the number of waste levels continues to increase as the fisheries industry, household consumption, and scale of fish raw material processing industry develop. Waste of the fisheries industry and household consumption can be used as an additive for food products. One of the potential waste of fishery products that can be developed is the waste of fishbones.

Fish processing generally produces 15-30% fishbone waste with 60-70% mineral content. The higher bone waste potential generated, Fishbones have a lot of potential such as a source of halal gelatin [2], the potential to generate a source of calcium is also getting more significant. The level of calcium and phosphorus in the fishbone is high (2% or 20 g/kg, dry base) so that the fishbone can be used as a source of natural calcium to meet daily calcium intake.

Various studies on the utilization of fishbone as a source of natural calcium have been conducted, including the analysis of bioavailability and application potential. Some of the examples are the
extraction of calcium from the tuna bones with protein hydrolysis method using autoclaving time parameter and frequency of boiling [3]; *P. hypophthalmus* fishbone powder as a source of calcium and phosphorus in the biscuits production [4]; red snapper bone powder in soya milk [5]; and salmon, snapper, and catfish bone powder in bread products [6].

In these studies, fishbone powder has the potential to become a fortificant ingredient in food products to enrich the nutritional value. Calcium from the bones has to be converted into simpler forms before the fortification process; therefore, it can be ingested in the form of smaller particle size. Calcium generally available in micro (µ) size, and supposedly only 50% of the total calcium consumption is absorbed during the metabolism process [7] and one of the alternatives to maximize the increase of calcium absorption is by making it in the nano-calcium form [8]. Nano-calcium is calcium produced using nanotechnology to form calcium in a tiny size (a nanometer, nm). Nano-calcium is a very efficient predigestion mineral entering the body's cells due to its super small size and can quickly enter the receptor for it to be absorbed by the body quickly and thoroughly. Nanotechnology is the design, characterization, production, and application of structures, devices, and systems by controlling shape and size at the nanometer scale [9].

Fishbone of catfish species (*P. hypophthalmus*, *C. batrachus*, *H. nemurus*, and *P. albilabris*) has potential as a major source of calcium and nano-calcium. In producing nano-calcium from the fishbones of each species of catfish, the quality characteristics of nano-calcium have to be discovered. This study aimed to get the quality characteristics of bone powder and nano-calcium from the bone of catfish species as fortification ingredients of nutritious food.

2. **Methodology**

2.1. Materials dan Methods

The primary material of this research was derived from catfish bones (*P. hypophthalmus*, *C. batrachus*, *H. nemurus*, and *P. albilabris*), and other supporting materials used for analysis.

The method used in this research was the experimental method, by producing fishbone powder and nano-calcium from fishbones of different species of catfish (*P. hypophthalmus*, *C. batrachus*, *H. nemurus*, and *P. albilabris*) in three repeats.

2.2. Research Stages

The stages of the research are:

1) Production of fishbone powder was made in reference to [10] with the following modifications:
   a. Sorting process and retrieval of fishbone.
   b. Fishbones boiled at a temperature of ± 80°C for 15 minutes, this boiling process aimed to facilitate the separation of the parts attached to the bone such as meat or blood.
   c. Drain and wash the fishbone in running water to separate the meat attached to the bones.
   d. Make fishbones tender by using heating system pressure (pressure cooker) for 1 hour.
   e. Cut out fishbones into smaller sizes (3-5 cm) to help the crushing process of the fishbones.
   f. Fishbones are soaked in a solution of 1.5M NaOH for 2 hours. The purpose is to eliminate and decrease the fat contained in the fishbones.
   g. Drain the fishbones that have been soaked and washed again with flowing clean water to eliminate or rinse the remaining NaOH solution from sticking in the fishbones.
   h. Repeat draining the fishbone, and then dry them by using an oven with temperature 55-60°C for 48 hours.
   i. Crushed the dried fishbone using a blender, and then filtered using an 80 mesh size, so that it becomes a smooth and homogenous fishbone powder.

2) Production of nano-calcium from fishbone was referring [11] with some following modification:
   a. Fishbone powder (*P. hypophthalmus*, *C. batrachus*, *H. nemurus*, and *P. albilabris*) in 80 mesh size
   b. Soak the fishbone powder (extraction) using 1M NaOH solution with 100°C using a magnetic stirrer stirring system for 1 hour.
c. After that, cool down the extract at room temperature, then, perform the filtration process and neutralization using distilled water to obtain neutral pH.

d. From the filtration, the solids obtained is dried out using an oven in 45-50°C for 48 hours.

e. Filtration process using a 120 mesh size to obtain a homogenous powder

3) Nano-calcium powder from fishbone (catfish) is obtained.

2.3. Observation Analysis

The parameter used in this research includes observation of powder colors and whiteness degree (%), yield [12], proximate analysis [12], calcium content [13], Scanning Electron Microscope (SEM) analysis [14], and Particle Size Analyzer (PSA) [15]. Data analysis was done using statistical analysis (ANOVA) in SPSS Software 26,0 version at a 95% confidence level (p<0,05).

3. Results and Discussion

3.1. Fishbone Characteristics

The characteristics of catfish bone powder produced in the research (P.hypophthalmus, C.batrachus, H.nemurus, and P.albilabris) are presented in Table 1.

| Characteristic         | P.hypophthalmus | C.batrachus | H.nemurus | P. albilabris |
|------------------------|-----------------|-------------|-----------|--------------|
| Fishbone powder color  | Dominantly white and light beige | Dominantly white and light beige | Dominantly white and light beige | White with a light beige |
| Whiteness degree (%)   | 85.6±0.59 b     | 83.3±0.71 a | 83.8±0.66 a | 82.5±0.67 a  |
| Moisture (%)           | 6.18±0.32 a     | 5.82±0.38 a | 5.95±0.41 a | 5.68±0.26 a  |
| Ash (%)                | 58.42±0.75 b    | 56.85±0.84 a | 56.23±0.77 a | 58.37±0.78 b |
| Protein (%)            | 24.39±0.38 a    | 24.66±0.31 b | 24.52±0.36 b | 24.71±0.29 b |
| Fat (%)                | 7.18±0.27 b     | 6.42±0.22 b | 6.75±0.29 b | 4.93±0.13 a  |
| Carbohydrate (%)       | 3.93±0.11 a     | 6.25±0.14 b | 4.16±0.18 b | 6.21±0.15 b  |
| Calcium (%)            | 51.5±0.78 b     | 56.8±0.81 c | 56.1±0.76 c | 48.1±0.74 a  |
| Phosphorus (%)         | 34.7±0.82 b     | 31.0±0.73 a | 32.0±0.69 a | 35.4±0.78 c  |

The same letter on each line indicates not significantly (p<0.05)

Based on the result of the experiment using statistical analysis (ANOVA), different species catfish bone powder significantly affects the characteristics of the whiteness degree, ash, protein, fat, carbohydrates, calcium, and phosphorus content in different species of catfish bone (p<0.05), whereas on the characteristics of the moisture of fishbone powder did not show a significant effect (p<0.05).

The color of the fishbone meat produced from each species of catfish was dominantly white and light beige for P.hypophthalmus, the same goes for C.batrachus and H.nemurus, which was dominantly white and light beige, and for the P.albilabris, the color was white with a light beige. In catfish of P.hypophthalmus, C.batrachus, H.nemurus, and P.albilabris, it affected the color of fishbone powder produced.

The moisture in P.hypophthalmus fishbone powder is 6.18% but varies in C.batrachus, H.nemurus, and P.albilabris bone powder which was relatively similar (5.82% and 5.68%). Based on the experiment, the moisture in P.hypophthalmus, C.batrachus, and P.albilabris still meet the Indonesian National Standards. The moisture of an ingredient in the production of fishbone powder can also be influenced by the physical and chemical content of the raw materials of the fishbones and also can be influenced by the drying process. The moisture in all treatments was not significantly different. The moisture of these results still meets the criteria of fishbone powder based on the Indonesian National Standards No. 01-3158-1992 that requiring maximum moisture of 8% for bone powder. The moisture of the calcium powder soaked by A. bilimbi in this study showed no significant difference[16]. The moisture of these results was closer to the research by[3] which reported the moisture of the fishbone powder by hydrolysation method was 5.6%. It is still higher than the study
conducted by [17] on Chitala chitala (Belida) fishbone powder with moisture ranging from 3.12% to 3.99%. Products that have lower moisture will have longer durability during storage.

The ash content of P. hypophthalmus fishbones powder was relatively similar to P. albilabris fishbones powder ash content, while the ash content of C. batrachus and H. nemurus fishbone powder were lower than those two. Differences in ash content on fishbones can be affected by the mineral composition of each ingredient of fishbones. Bone mineral content in fishbone is influenced by nutritional components of each species of fish in searching for food in aquatic habitats.

Ash content analysis aims to determine the total ash content and the content of each mineral found in fishbone powder. The ash content in food indicates the amount of inorganic material that remains after the organic material destructed [18].

The ash content result of this research was higher than the ash content obtained by [19] in her research on P. hypophthalmus fishbone powder of 33.5%. Meanwhile, [3] on the Thunnus spp bone powder and [20] on O. niloticus fishbone powder. The ash content obtained was 84.22% and 75.83% respectively. The mineral contributes to the high ash content in bone powder. Bones contain living cells and intracellular matrix in the form of mineral salts.

The protein content of catfish bone powder particularly in C. batrachus, H. nemurus, and P. albilabris, were relatively similar, while P. hypophthalmus fishbone powder was different with slightly higher protein content. A protein component found in fishbones depends on the composition of nutrients that make fishbone formation. Types of fishbone that are hard and cartilage have different components for each species fishbone. The protein content of these results is much higher than the results of several studies with a slightly different method, among others [3] that was 0.48%-1.29%; [21] 0.21% and [17] 0.26%-14.25%. The protein contents of this research were still lower than the [19], which is 33.5%.

The protein content of fishbone powder extracted by A. bilimbi is high compared to extraction with acid (HCl) and base (NaOH). That high protein content could also benefit from the absorption of calcium in the intestinal mucosa. It transports calcium through the gut cells through diffusion using a calcium-binding protein that leads erythrocyte cytoplasm to the base membrane [3]. That the lysine and arginine amino acids could help the absorption of calcium in the gut [22].

The fat content of fishbone powder in each kind of fish is different, P. albilabris fishbone powder has lower fat than the C. batrachus, H. nemurus, and P. hypophthalmus bone powder. A high-fat content was found in P. hypophthalmus fishbone powder and allegedly, it is because the bones of catfish still contain the fat component that had not been adequately reduced during the weeding and heating process (boiling and presto steaming) and affected the content of catfish bone powder produced.

The fat content of fishbone powder in this research was relatively high. High-fat content in fishbone powder has to be limited to reduce the risk of rancidity and result in a more lasting bone powder. The standards of first quality fat bone powder to 3% at maximum and a maximum of 6% for the second quality. Fat content that meets the standards is P. albilabris fishbone powder of 4.93%. The C. batrachus and H. nemurus fishbone powder almost qualify the maximum fat content by the Indonesian National Standards. Fat content in this research is almost the same as [14] the research results standard for fat content in O. niloticus fishbone powder of 5.82%.

That previous studies have reported that fat is filling the bone, especially the main fishbones, which consists of many joints. Also, the fat can not be removed easily, because it is a complex bond, and it is difficult to be removed just by soaking the bone in an alkaline solution [20]. That the levels of fat in the bones are closely associated with the body fat of each species and usually large and mature fish are high in fat. Fish fatty acids are unsaturated fatty acids. The fatty acids in the fishbone powder were found to contain unsaturated fatty acids in almost 80% of the total species [23].

The carbohydrate content in P. hypophthalmus fishbone powder was lower compared to the carbohydrate content in C. batrachus, H. nemurus, and P. albilabris fishbone powder having relatively similar carbohydrate levels. Carbohydrates can contribute to the physical characteristics of the food. That carbohydrates also have an essential role in determining the characteristics of food ingredients.
such as flavor, color, scent, and texture of the food. Carbohydrates provide the food with a sweet taste, flavor, and distinctive shape as well as color. It also softens the texture [24].

Calcium is the fifth element and the highest cation in the body, which is 1.5%-2% of the whole body more than 99% of the calcium present in the cartilage and teeth. The remaining can be found in body fluids and soft tissue [25]. Calcium levels obtained in this study were slightly lower than the levels of calcium bone powder of *Thunnus* spp of 39.24% in [3] research results. That levels of calcium for fishbone powder set to 30% of weight for the first quality and 20% for the second quality. According to these standards, the *Chitala chitala* fishbone powder calcium levels belong to the first and second quality [26].

### 3.2. Characteristics of Fishbone Nano-Calcium

The characteristics of nano-calcium produced from the catfish bones is available in Table 2.

**Table 2. Characteristics of fishbone nano-calcium**

| Characteristics          | *P. hypophthalmus* | *C. batrachus* | *H. nemurus* | *P. albilabris* |
|--------------------------|--------------------|----------------|--------------|----------------|
| color of nano-calcium    | White              | White          | White        | White          |
| Whiteness degree (%)     | 90.7 ± 0.32        | 88.9 ± 0.38    | 89.1 ± 0.35  | 88.4 ± 0.44    |
| Moisture content (%)     | 6.12 ± 0.29        | 5.88 ± 0.26    | 5.96 ± 0.31  | 5.71 ± 0.21    |
| Calcium content (%)      | 87.4 ± 0.53        | 89.6 ± 0.56    | 89.1 ± 0.47  | 86.1 ± 0.48    |
| Phosphorus content (%)   | 11.2 ± 0.31        | 9.4 ± 0.38     | 9.8 ± 0.35   | 12.7 ± 0.29    |
| The particle size (nm)   | 248-672            | 135-573        | 206-594      | 130-467        |

The same letter on each line indicates not significantly (*p*<0.05);
*N*anocalcium extraction contains very small other minerals (*Fe, Zn, Mg, Na, K*).

Based on the result of the experiment using statistical analysis (ANOVA), different catfish bone powder (*P. hypophthalmus, C. batrachus, H. nemurus, and P. albilabris*) significantly affect the characteristics of yield, whiteness index, calcium, and phosphorus in the nano-calcium fishbone, while on the characteristics of the moisture of catfish bone nano-calcium showed no significant effect (*p*<0.05).

The color of nano-calcium fishbone generated from each species of fish by a direct observation found white as a dominant color, nevertheless, based on the assessment of whiteness index, each nano-calcium have different values, especially in nano-calcium from the bones of *P. hypophthalmus* having a higher whiteness index (90.7%) compared to the nano-calcium of *C. batrachus, H. nemurus*, and *P. albilabris*. Each species has a whiteness index of 88.9%, 89.1%, and 88.4% respectively.

The moisture of the *P. hypophthalmus, C. batrachus, and P. albilabris* fishbone nano-calcium have relatively similar moisture, which ranges from 5.71%-6.12%. Based on the result, the nano-calcium moisture of *P. hypophthalmus, C. batrachus, and P. albilabris* have already met the Indonesian National Standards which requires moisture ranges from 10%-12%. The moisture of an ingredient in the production of nano-calcium from the fishbones can also be influenced by the physical and chemical content of the raw materials of the fishbones, and also can be influenced by how the drying process is performed.

Different species of catfish bone calcium content have different levels, the highest levels of calcium in the nano-calcium is *C. batrachus* fishbone (89.6%), followed by nano-calcium of *P. hypophthalmus* fishbone 87.4% and the lowest calcium contained in *P. albilabris* fishbones nano-calcium. The highest phosphorus levels contained in the *P. albilabris* fishbone nano-calcium is 12.7%, whereas the nano-calcium of *P. hypophthalmus* fishbone is 11.2% and phosphorus content in the *C. batrachus* is 9.4%.

That the calcium levels content for fishbone powder is set for 30% for quality I and 20% for quality II. By referring to these standards, the calcium levels of *Chitala chitala* (Belida) fishbone powder belong to the first and second quality[26].
Figure 1. Morphology of nano-calcium particles on catfish bone (P.hypophthalmus, C.batrachus, H.nemurus, and P.albilabris).

The nano-calcium particle size formed from each kind of catfish bone (P.hypophthalmus, C.batrachus, H.nemurus, and P.albilabris) have a range of different particle size, P.albilabris has a finer nano-calcium particle size, which is 130-467 nm; compared with a particle size of P.hypophthalmus fishbone nano-calcium size, which is 248-672 nm, H.nemurus fishbone nano-calcium 206-594 nm and C.batrachus fishbone nano-calcium, 135-573 nm. Overall, the formation of calcium nanoparticles size on catfish bone (P.hypophthalmus, C.batrachus, H.nemurus, and P.albilabris) has successfully and following the standard size of nanoparticles are <1,000 nm. According to [27], the size of nanoparticles is in the range of 100-1,000 nm. The morphological form of the nano-calcium particle of catfish bone has a relatively similar crystal form of calcite (solid like a rock) (Figure 1).

The results of particle measurement using the Scanning Electron Microscope at 10,000x and 20,000x magnifications show that the shape of calcite (solid like a rock) can be found in the nano-calcium powder particle. According to [28], CaCO$_3$ crystal has three different crystal forms, namely calcite, aragonite, and vaterite. Calcite is as solid as a rock, the veterit has a flower shape (flower-like), while aragonite has a shape of a group of needles.

4. Conclusion

Based on the results of this study a conclusion can be drawn that the production of different species of catfish bone powder (P.hypophthalmus, C.batrachus, H.nemurus, and P.albilabris) significantly affect the characteristics of whiteness degree, ash, protein, fat, carbohydrates, calcium, and phosphorus of the fishbone, whereas the moisture of the catfish bone powder does not have a significant effect.

The characteristics of the nanocalcium from P.hypophthalmus fish bones are: white nanocalcium (90.7% whiteness degree), 6.12% moisture content, 87.4% calcium, 11.2% phosphorus, and 248-672 nm nanocalcium particle size. The characteristics of the nanocalcium from C. batrachus are white nanocalcium (88.9% whiteness degree), 5.88% moisture content, 89.6% calcium, 9.4% phosphorus, and 135-573 nm nanocalcium particle size. Nanocalcium characteristics of H.nemurus are white nanocalcium (89.1% whiteness degree), 5.96% moisture content, 89.1% calcium, 9.8% phosphorus, and 206-594 nm nanocalcium particle size. The characteristics of the nanocalcium from P.albilabris fishbones were white nanocalcium (88.4% whiteness degree), 5.71% moisture content, 86.1% calcium, 12.7% phosphorus, and 130-467 nm particle size of nanocalcium.

Calcium nanoparticle size (nano-calcium) formation in all catfish bones has been successful and is in accordance with nanoparticle size standards <1,000 nm.

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