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Research Article

Characteristics of Nile Tilapia (*Oreochromis niloticus*) Fish balls with The Addition of Asian Sea Bass (*Lates calcarifer*) Bones Bio-calcium

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Abstract
Nile tilapia has high protein content so it can be processed into fish balls. Fish balls are a processed fish product that is high in protein but low in calcium. Asian sea bass (*Lates calcarifer*) bones are a type of fishery by-product that can be utilized by processing them into a source of calcium. It is known that the average human daily calcium requirement is 800 mg - 1300 mg per day. The addition of bio-calcium from Asian sea bass fish bones was carried out to increase the nutritional value of calcium in fish balls and meet consumers' calcium intake. The research aimed to assign the effect of Asian sea bass bone bio-calcium (FPB) addition on the physical, chemical, and sensory characteristics of Nile tilapia fish balls. The research method used was laboratory experimental with a Completely Randomized Design (CRD). The treatment of the current study was the addition of FPB at different concentrations (0; 3; 5 and 7%). The research analysis carried out included gel strength, calcium, protein, water, fat, ash contents and sensory characteristics. The research showed that the addition of FPB had significant effect on gel strength, calcium, protein, water, fat, ash contents and sensory characteristics ($P<0.05$). FPB at a concentration of 3% showed the best treatment of hedonic value with gel strength of 1889.757 g.cm, calcium content of 1095.1 mg/100g, protein content of 13.23%, moisture content of 72.28%, fat content of 1.39% and ash content of 2.43%. The addition of fishbone bio-calcium at proper concentration increases the texture and calcium content of Nile tilapia fish balls without any sensory interference. The fishbone bio-calcium could be a cheap source of calcium for the food industry to provide high protein and calcium food including fish ball for society to overcome calcium deficiency.

Keywords: Asian sea bass bio-calcium, fish bone, Nile tilapia, fish balls

1. Introduction

Nile tilapia (*Oreochromis niloticus*) is one of the fisheries products that has high economic value in Indonesia. The production and processing of tilapia increase continually as well as it is consumed frequently by the people. Ministry of Marine Affairs and Fisheries Republic of Indonesia reported that the latest Nile tilapia production data in Indonesia in 2021 achieved of 1.35 million tons with a value of IDR 33.62 trillion. This amount increased by 9.63% compared to the previous year which was 1.23 million tons with a value of IDR 29.19 trillion [1]. Nile tilapia can be processed into several products including fish meatballs, sausages, etc. Meatballs is generally produced using beef and chicken as the main raw materials in processing. However, fish meat can be produced for meatballs, and it is usually called as fish balls. Fish balls is one of diversification in processing fishery products.

One of the fish species usually used as raw material for making fish balls is Nile tilapia. This fish is commonly cultivated in Indonesia, hence it is easy to find. Nile tilapia fish has thick white flesh, which is suitable for processing into food including fish balls. Fish balls produced from fish flesh are known to be rich in protein, but low in other nutrients such as calcium [2].

The human body requires calcium over all other minerals. Calcium is a nutrient that is crucial to the body's mineral composition. In general, calcium has two roles in the human body: it aids in the development of teeth and bones and controls bodily biological processes. Although the need for calcium increases during growing, it remains necessary until adulthood. When new bone forms during bone formation, the old bone is concurrently eliminated [3]. Bio-calcium is calcium in powder form with a micron size ranging from 10-50 microns produced from living creatures such as fish bones. According to Luu and Nguyen [4], bones are one of the parts of the fish that contain mineral with the higher calcium content. Because the main components of fish bones are calcium, phosphorus and carbonate, fish bones are rich in calcium which humans need. Asian Sea bass (*Lates calcarifer)* produced as a fish fillet generates solid waste such as bone, skin, head, etc. [5] which could be environmental matter. The solid waste including bone could be processed as bio-calcium to reduce that problem. Fish bones contain very high concentration of calcium depending on the type of fish. The calcium content in bio-calcium made from Asian Sea bass bones treated using high pressure heating was 35.9% [5]. Fish bone bio-calcium could be fortified to escalate the nutritional value of processed food products, especially calcium. Previous study showed that fish bone flour fortification at proper concentration could increase calcium content of food products without sensorial interference. Siangchin et al. [6] reported that bread fortified with Asian sea bass bone powder 5% could increase calcium content of bread from 22.58 mg/100 g to 535,3 mg/100 g. Mayonnaise added with bio-calcium from Asian sea bass bone at concentration of 2.5% upgraded calcium content by 54 times without sensorial interferences [7]. Butter cookies added with knife-fish bone flour at 6% had calcium content of 2.5% [8]. A'yun et al. [9] reported that catfish meatballs added with 10% catfish (*Clarias sp)* bone powder was preferred by panelists compared to those added with higher concentration. However, chemical characteristics of fish ball added with fishbone powder in that study was not available. Thus, the addition of bio-calcium powder affected the characteristics of the resulting product. Adding too much fish bone bio-calcium into food product could cause a gritty taste in the product which is less favorable [7]. According to Fong-in et al. [10], the more fish bone meal added to the mixture, the more pronounced the chalky/sandy taste will be observed. The research aimed to elaborate the impact of Asian Sea bass bone bio-calcium powder (FPB) at different concentrations on physical, chemical and sensory attribute of Nile tilapia fish balls.

2. Materials and Methods

2.1. Materials

The raw material used in the current study was fresh Nile tilapia (*Oreochromis niloticus)* (each weight of 250±20 g) obtained from the fish market in Semarang Central Java Indonesia and transported to the laboratory using a cool box containing ice to maintain the freshness of the fish. Other components, including tapioca flour and more spices, were purchased from supermarket in Semarang. The fish was then washed and kept in the freezer (-20°C) before used.

2.2. FPB Production

Fish bone bio-calcium powder (FPB) was produced as follow the method of Wijayanti et al. [5]. Asian sea bass bone was cut into size of 4-5 cm using a sawing machine. Fish bones are boiled for 30 min at 100°. The boiled samples are then washed using running water to clean any sticking meat. Cleaned samples were placed in the Duran bottle and then added with distilled water using a ratio of 1:4 (w/v) and high pressure heated for 90 min at 121℃. Autoclaved samples were dried at 50℃ for 48 h using an electric oven and crushed with a multifunction grinder to obtain a rough powder. The rough powder was soaked in hexane with a ratio of 1:10 (w/v) and mixed using an overhead stirrer at speed of 150 rpm for 60 min at room temperature (RT, 28-30℃). After draining the hexane, the sample was left at room temperature. Following that, the defatted powder was stirred at room temperature for 60 min while being bleached with 2.50% (v/v) hydrogen peroxide (H_2O_2) at a sample/solvent ratio of 1:10 (w/v). After rinsing the bleached powder with tap water for fifteen minutes, it was dried for twenty-four hours at 50°C. Next, 25 grinding balls (20 mm in diameter) were used in a ball mill machine to grind the dry powder (200 g) for 2.50 hours at a speed of 200 rpm. Fine powder bio-calcium (FPB) was obtained by screening the resultant powder through a sieving machine that used a 75 µm sieve. The Laser Particle Size Analyzer yielded an FPB particle size of 19.72 ± 4.25 µm.

2.3. Nile Tilapia Fish balls production with addition of FPB

The Nile tilapia fish balls was made as per method of Wijayanti et al. [11], which had been modified with the addition of bio-calcium. The Nile tilapia fish was washed with running water, then the filleting process was carried out to remove the meat and then grind it. Fish flesh (800 g) was ground then added with other ingredient such as tapioca flour 15% of the weight of the fish meat, salt (1.5%), garlic (1%), pepper (0,5%), flavoring (0.2%) and ice cubes (10%) then stirred and mixed with FPB at different concentrations 0(K); 3%(A); 5%(B) and 7%(C) until homogeny for 2 min. After that, the dough was taken and shaped into a ball weighing around 3-5 grams. The boiling process was done at 100°C for 20 min until the fish balls floated on the surface which indicated that the fish balls were cooked. After that, draining was carried out for 15 minutes and were then packed using polyethylene plastic and chilled stored before analysis.

2.5 Gel strength

Gel strength analysis was carried out using the Texture Analyzer model TATX2 [12]. The Tilapia fish balls were balanced and placed precisely on a stainless-steel plate directly under the probe. Test was done at room temperature. The spherical probe at a diameter 5 mm with a speed of 60 mm/minute was carried out for gel strength analysis. The gel strength value was obtained by the formula:

Gel strength (g.cm) = Hardness (g) x Deformation (cm).

2.6. Calcium content determination

The determination of calcium content used was done using ICP-OES. Firstly, sample should be destructed into small size. The next step was to put 1 gram of each destruction sample into a 100 mL beaker, then added 3 mL of AquaRegia (a mixture of HNO3 and concentrated HCL) with a ratio of 3:1, then heat it on a hot plate at 80°C for 30 minutes until the sample was dissolves, then the sample was taken and cooled, then demineralized water was added until the volume increases to 25 ml, the sample had been destroyed and formed a solution, then filtered with filter paper, the clear filtrate was ready to be tested with ICP-OES [13]

2.4. Protein Content

The Kjeldahl method of AOAC [14] is used in protein content analysis. After homogenizing a 2 g sample of fish balls, the sample was placed inside a folded piece of paper and placed inside the destruction flask. Two catalyst tablets were added in the destruction flask and then 15 mL of concentrated H_2SO_4 (95–97%) and 3 mL of H_2SO_2 were mixed in the acid room. Further, the samples were destructed at 410 °C for 2 h until the samples were clear. The destructed samples were allowed to cool at ambient temperature. To the flask, a total of 50–75 mL of distilled water and 25 mL of 4% H_3BO_3 were added. Prior to the distillation process, 50–75 mL of sodium hydroxide–thiosulfate solution were added to the flask containing destructed samples. The distillates were collected in a 250 mL Erlenmeyer flask and were titrated using 0.1 N HCl until the solution's color turned gray and the titrant volume were recorded. A blank solution was prepared as same as the procedure of sample solutions. The formula below can be used to determine protein content.:

$$\text{protein (\%)} = \frac{(\text{ml HCL sample} - \text{ml HCL blank}) \times \text{N HCL} \times 14{,}007 \times 100 \times 6.25}{\text{mg sample}}$$

2.7. Moisture content analysis

The AOAC method was followed in carrying out the moisture content analysis [14]. Before being weighed, the empty cup was prepared, dried in the oven for an hour, and chilled in a desiccator for thirty minutes. A gram (1 g) of the sample was taken, put in the cup, and heated in an oven set to 105°C for 24 hours. After 30 minutes of cooling in a desiccator, the object was weighed once more. The following formula was used to calculate the concentration of moisture content (wet weight) of the data.:

$$\text{Moisture content (\%)} = \frac{sample\ weight\ (g) - weight\ of\ saucer\ and\ sample\ (g)}{sample\ weight\ (g) - weight\ of\ saucer\ (g)} \times 100\%$$

2.8. Fat content analysis

After weighing the sample in total to 1-2 g (W1) on filter paper and placing it in a Soxhlet tube, the fat flask, which had been weighed at its fixed weight (W2), was also placed in the tube. Following its insertion into the extractor chamber, the Soxhlet tube was flushed with 250 milliliters of petroleum ether. A Soxhlet distillation equipment was used to house the extraction tube, and it was distilled for six hours. After the solvent was distilled, it was collected in the extractor chamber. The solvent was then extracted to prevent it from being added back to the fat flask, and the flask was dried at 105°C in an oven before being cooled in a desiccator until the weight remained constant (W3) [14]. The following formula was used to calculate the fat content:

$$\text{Fat content (\%)} = \frac{W3\ (g) - w2\ (g)}{W1\ (g)} \times 100\%$$

2.9. Ash content analysis

The porcelain cup was dried for thirty minutes at a temperature of 105°C. After the porcelain cup had been in a desiccator for thirty minutes, it was weighed (A) and a sample weighing four to five grams was added. (B). After being burned on an electric stove until it was smokeless, the porcelain cup was put in a muffle furnace set at 550°C until it had completely ashed. After the cup was cooled in a desiccator, it was weighed (C). The following formula was used to calculate the ash content [14]:

$$\text{Ash content (\%)} = \frac{C\ (g) - A\ (g)}{B\ (g) - A\ (g)} \times 100\%$$

2.10. Hedonic analysis

Based on Indonesian Standard [15], which provides directions for organoleptic or sensory testing on fisheries products, hedonic analysis was performed. The hedonic test is a technique for gauging consumer preference for a product by employing a rating sheet that often includes attributes like texture, taste, scent, and appearance. The panelists' degree of preference is the basis for the test's evaluation. The chosen quality range determines the like level. Hedonic values were used in the hedonic test; the scale runs from 1 to 9 and represents the following values: 1 represents very strongly dislike, 5 represents neutral, 7 represents like, and 9 represents very strongly like.

2.10. Data Analysis

A completely randomized design (CRD) was employed for the experiment. Analysis of variance (ANOVA) was used to assess the data for each sample, and Tukey HSD was used for multiple comparisons. The statistical analysis was performed using SPSS version 17.0 (International Business Machines Corporation, USA) and all parametric data were collected in triplicate.

3. Results and Discussions

3.1. Gel Strength

The gel strength of Nile tilapia fish balls with the addition FPB at varying concentrations are presented in Figure 1.The addition of bio-calcium had a significant effect on gel strength of Nile tilapia fish balls ($P<0.05$).

The gel strength without the addition of FPB or control (K) was 1433.87 g.cm. Nile tilapia fish balls with the addition of 3% FPB had higher gel strength compared to control fish balls by 31.79%. Fish balls added with 5% FPB showed that the gel strength increased by 104% from the control (K) and fish balls added with of 7% FPB had an increase of 93% from the control (K).

The results showed that the gel strength in this study increased with the addition of FPB when compared to the control. The gel strength with the addition of 7% FPB decreased slightly when compared to meatballs with the addition of 5% FPB. This showed that the gel strength value of Nile tilapia fish balls had reached its optimum at 5% FPB. The addition of FPB up to 5% could increase the gel strength. The increase of gel strength of fish balls with addition FPB up to 5% in this study might be due to the calcium ion of FPB which enhanced activity of endogenous enzyme TGase to increase the crosslink of myosin heavy chain (MHC) in the protein of fish balls resulting in high breaking force [16].

Wijayanti et al. [16] reported that bio-calcium from Asian sea bass bone added in the surimi gel up to 10% augmented its breaking force up to 108%. However, the decrease on gel strength of fish balls occurred due to the high presence of FPB resulting the decrease of myofibril protein content, hence it yielded a lower gel strength.

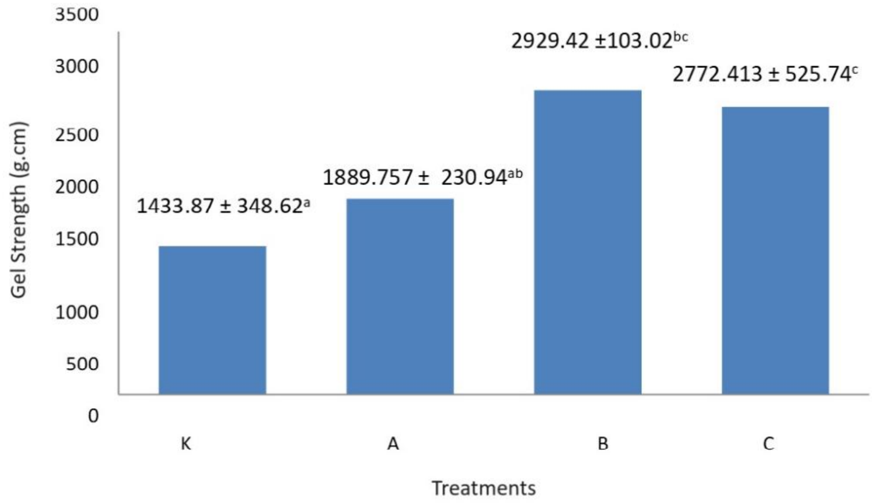

Figure 1. Gel strength Nile Tilapia Fish Balls added with FPB at different concentrations. K: Nile tilapia without FPB; A: Nile tilapia with 3%FPB; B: Nile tilapia with 5% FPB; C: Nile tilapia with 7% FPB. Different superscript letters indicate significant differences (P < 0.05).

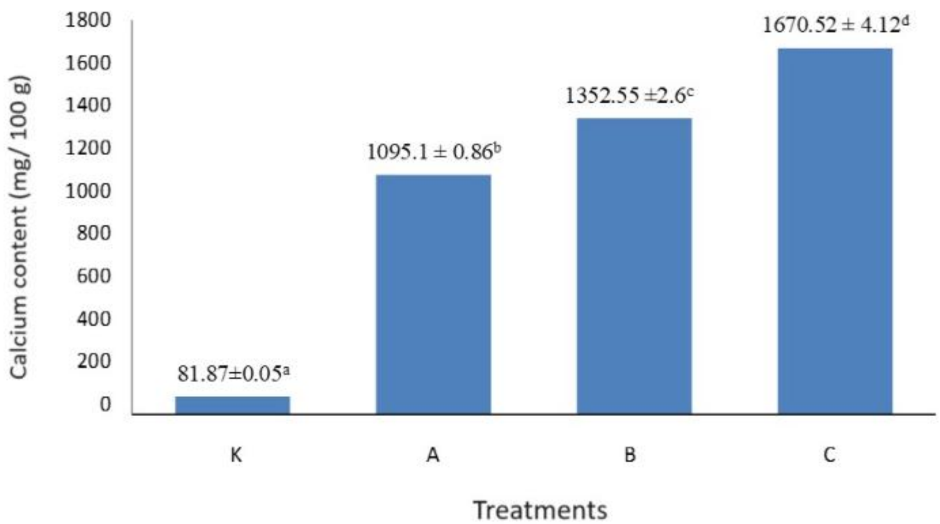

Figure 2. Calcium content of Nile Tilapia Fish Balls added with FPB at different concentrations. K: Nile tilapia without FPB; A: Nile tilapia with 3%FPB; B: Nile tilapia with 5% FPB; C: Nile tilapia with 7% FPB. Different superscript letters denote significant differences (P < 0.05).

According to Sun et al. [17], myofibril protein is required to produce gel products; this protein is crucial to the coagulation and gel formation processes.. However, the protein contained in FPB has undergone several processing processes, resulting in the denatured protein, which causes the protein in the FPB to be unable to form a gel or prevent the formation of a gel between the meat and tapioca flour. The strength of the gel in fish balls can be influenced by several factors. One factor is the ingredients used in the process of making the meatballs. Apart from that, the processes carried out in making fish balls, such as washing the fish meat and cooking, can affect the formation of the gel and the strength of the resulting gel. According to

Somjid et al. [18]), an increase in gel strength can also be caused by the loss of several components in fish flesh during washing which can inhibit the gel formation process.

3.2 Calcium content

Calcium content of Nile Tilapia fish balls added with FBP at different concentrations is displayed at Figure 2. Different concentration of FPB had varying impact on calcium levels of Nile Tilapia fish balls ($P<0.05$). The calcium content increased with the increment concentration of FPB added due to the high calcium content of fish bone bio-calcium. Benjakul et al [19] reported that fish bones made into powder have a high calcium mineral content, so they can be used as a form of food that is acceptable to consumers. As calcium is the primary component of fish bones, they are extremely rich in this mineral from a nutritional and dietary standpoint. Nile tilapia fish balls with the addition FPB of 3% increased calcium levels by 12.38 times compared to the control. The addition of 5% FPB increased by 15.52 times compared to the control and the addition of 7% increased by 19.40 times compared to the control fish balls. This result indicated that bio-calcium addition had effect on calcium content in fish meatballs. The addition of 7% bio-calcium resulted in the highest calcium content in fish balls. The more bio-calcium that is added to the tilapia fish meatball processing process, the higher the calcium level contained in the fish meatballs. This is in accordance with the research by Nawas et al. [20], which stated that the calcium content of fried snack increased as the rise of concentration of fish bone powder used. This condition showed that fish bones powder has a high calcium content so they can increase calcium levels in fish balls.

The better concentrations of bio-calcium addition in fish balls were 3% and 5%. This was because at these concentrations, the calcium content in fish balls were 1095 mg/100g and 1352 mg/100g at a concentration of FPB 3% and 5%, respectively. These values are fulfilled the daily calcium requirement around 1000-1300 mg according to the growth phase in humans. Hence, concentration of 5% FPB added in the fish balls in current study could be recommended to meet the daily calcium requirement.

According to Aspray [21], the recommended daily calcium requirement is 600-1400 mg. The highest need is in the children and elderly phase, 1,400 mg until the child is 18 years old. This is related to optimizing growth in children. However, at concentration 7% FPB, the calcium content of fish balls was too high compared to acceptable daily intake. Different calcium content of fortified food with fish bone flour (bio-calcium) were observed by several studies due to difference concentration added, different species of fish bone used and different bio-calcium processing methods.

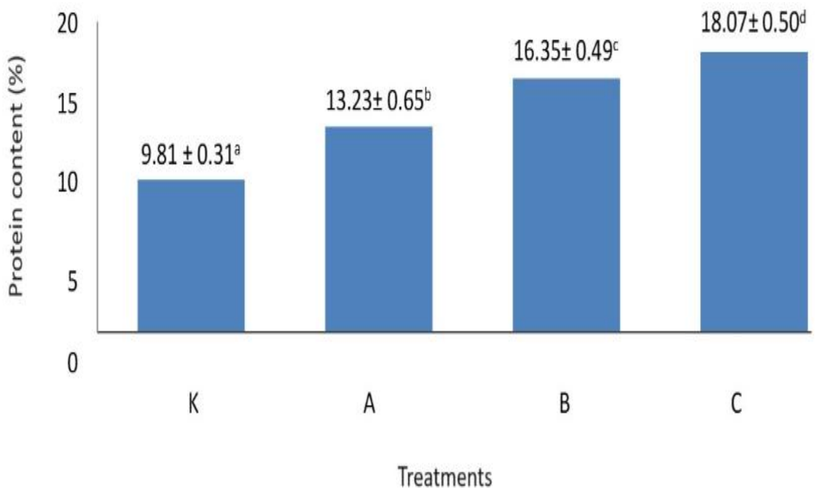

Figure 3. Protein content of Nile Tilapia Fish Balls added with FPB at different concentrations. K: Nile tilapia without FPB; A: Nile tilapia with 3%FPB; B: Nile tilapia with 5% FPB; C: Nile tilapia with 7% FPB. Different superscript letters denote significant differences ($P < 0.05$).

Addition 5% Asian sea bass bone flour to the bread increased the calcium content to 535.3 mg/100 g [6]. Mayonnaise added with 7.5% ultrasonicated Asian sea bass bio-calcium contained calcium of 1740 mg/100 g [7]. Butter cookies added with 6% knife-fish bone flour at 6% had calcium content of 2500 mg/100 g [8]. Higher calcium content (6700 mg/100 g) was observed in surimi gel fortified with 8% bio-calcium from Asian sea bass bone [16]. The calcium added in the food should be considered as calcium requirement. Taking more over 2500 mg of calcium per day can have a negative impact on the body's mineral equilibrium which could cause ureterolithiasis related to hypercalcemia [22].

3.3. Protein content

Protein content of Nile tilapia fish balls added with FPB at different concentration is shown in Figure 3. The addition of FPB into fish balls at different concentrations had a significantly impact on the protein content of Nile tilapia fish balls (P<0.05). There was a significant difference between the addition of FPB at concentrations of 3, 5, and 7% and the control (K). The protein content of fish balls rose as the concentration of FPB rose. Without the inclusion of FPB (K), the average protein level of fish balls was 9.81%. When 3% FPB was added to fish balls, the protein content rose by 35% over the control group (K). When 5% FPB was added to Nile tilapia fish balls, the protein content increased by 67% compared to the control. The protein content of fish balls with the addition of 7% FPB also increased by 84% in comparison with the control. The more bio-calcium added, the higher protein content value was observed. This was due to the calcium content of FPB used from fish bones, where fish bones are also a source of protein. According to Benjakul and Karnjanapratum [23], protein levels can be influenced by the addition of fish bone bio-calcium, because fish bones are known to have a high protein level. However, the level of protein also depends on the amount of ingredients added and can also be influenced by the moisture content. According to Wijayanti et.al [5], the protein content of Asian sea bass bones was 25-28% and the protein content of its bio-calcium was 5-7%. The increase in protein levels of fish balls added with FPB is in accordance with research by Sitepu [24] that showed the protein content of cookies increased from 13.31 to 25.88% due to high protein content of tuna bone flour added in the formulation.

3.4. Moisture content

Moisture content of Nile tilapia fish balls added with FPB at varying concentrations is presented in Figure 4. Different concentration of FPB gave a significant impact on moisture content of fish balls (P<0.05). The higher FPB added, the lower moisture content was observed in the resulting fish ball. The moisture content of fish balls without and with addition of FPB were quite high (71.19-73.11%).

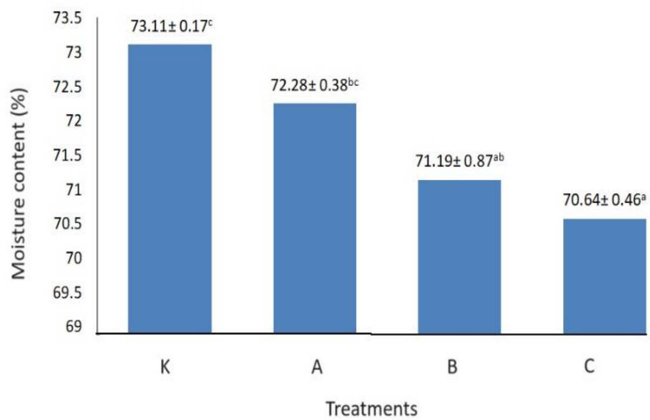

Figure 4. Moisture content of Nile Tilapia Fish Balls added with FPB at different concentrations. K: Nile tilapia without FPB; A: Nile tilapia with 3%FPB; B: Nile tilapia with 5% FPB; C: Nile tilapia with 7% FPB. Different superscript letters denote significant differences ($P < 0.05$)

With the addition of FPB, the moisture content of the fish balls in this study was marginally higher than the maximum of 70% specified in SNI Fish Balls 7266:2017 for moisture content. As the concentration of FPB addition increased, the moisture content of Nile tilapia fish balls decreased. The addition of bio-calcium can result in an increase in Ca^{2+} particles which can bind OH particles contained in water molecules (H_2O). Moreover, the addition FPB into fish ball also increased the solid mater in the fish balls dough, hence the moisture content decreased. Wijayanti et al. [7] showed that moisture content of mayonnaise lowered when it was added with bio-calcium from Asian sea bass bone. Asian sea bass bio-calcium produced by different methods contained a low moisture content (3.81-7.05%) [5]. Hence, the higher the concentration of FPB given, the lower the moisture content in the fish balls was observed. Other study showed similar trend of moisture content when fish bone bio-calcium was added in the whole wheat cracker resulting a lower moisture content (1.36%) compared to control (2.94%) [23]. The moisture content in fish balls affects product quality and is affected by various things, including the raw materials and processing process. According to Zambrano et al. [25], the parameters that determine the quality of a food product are decided by various factors and one of them is the moisture content which also defines the level of durability of the food or food product. The high moisture content was also observed in fish ball marketed in Malaysia from 73.0-88.71% [26]. The cooking process by boiling is also a factor that can influence the moisture content of the product.

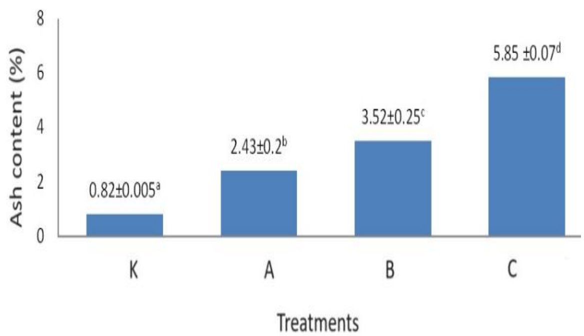

Figure 5. Fat content of Nile Tilapia Fish Balls added with FPB at different concentrations. K: Nile tilapia without FPB; A: Nile tilapia with 3%FPB; B: Nile tilapia with 5% FPB; C: Nile tilapia with 7% FPB. Different superscript letters denote significant differences ($P < 0.05$).

3.5. Fat content

Figure 5 shows the fat content of Nile tilapia fish balls that have been treated with FPB. The fat content was significantly affected ($P<0.05$) by the addition of FPB at various concentrations. Fish meatballs with a 3% FPB addition (A) had a 7% higher fat content than fish meatballs without the addition (K), which had a 1.38% fat content. Fish balls that had 5% FPB added (B) had a 97% higher fat content than fish balls that had 7% FPB added (K), while fish balls that had 7% FPB added had a 139% higher fat content than the control. The findings demonstrated that rising FPB concentrations also raised the fat content of Nile tilapia fish balls.

The increase of fat content of Nile tilapia fish balls might be due to the fat content in FPB. Wijayanti et al. [5] reported that FPB contained fat of 0.77%. Slight increase of fat content was also found in the surimi gel of threadfin bream when it was added with Asian sea bass bio-calcium due to fat content of the fish bone bio-calcium [16].

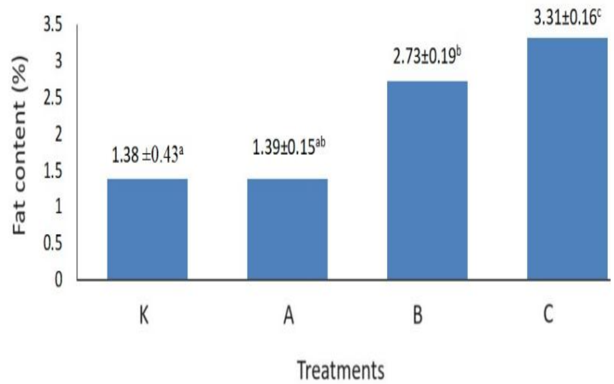

Figure 6. Ash content of Nile Tilapia Fish Balls added with FPB at different concentrations. K: Nile tilapia without FPB; A: Nile tilapia with 3%FPB; B: Nile tilapia with 5% FPB; C: Nile tilapia with 7% FPB. Different lowercase letters indicate significant differences ($P < 0.05$)

3.5. Ash content

Figure 6 illustrates the ash content of tilapia fish meatballs after adding FPB at various levels. The ash content of fish balls was significantly affected ($P<0.05$) by the addition of FPB at different doses. According to study findings, the average amount of ash in Nile tilapia fish balls without FPB (K) addition was 0.82%. The ash level of fish meatballs containing 3% FPB was 1.96 times higher than that of the control group (K). The amount of ash in fish balls increased by 3.3 times when 5% FPB was added, as opposed to the control (K). In comparison to the control, the ash content of fish meatballs that had 7% FPB added rose 6.2 times. As per the Indonesian National Standard (SNI), the treatment that included adding 3% of FPB to fish balls resulted in an ash content of 2.4%, meeting the minimum requirement. This result does not exceed the safe ash content limit for fish meatballs according to SNI. The ash level of Nile tilapia fish balls increased with the increase of

FPB concentrations added due to high ash content of FPB. Similar result had been reported that ash content of whole wheat cracker added with salmon frame bio-calcium increased from 3.99 to 8.54% in comparison with control [30].

According to Benjakul et al. [27], Based on dry weight, bio-calcium and calcined-bone powder contained high ash level. The ash contents of bio-calcium and calcined-bone powder were 72.20 and 99.75%, respectively. Fish bones are generally recognized to be mineral-rich. Determining the ash content can be important for several reasons, one of them being that it is a part of the proximate analysis for nutritional evaluation [28].

3.6. Hedonic value

Hedonic value of Nile tilapia fish balls is shown in table 1. Varying concentrations of FPB showed different impact on hedonic value of appearance, odor, taste, and texture (P<0.05). The hedonic value of appearance showed that Nile tilapia fish balls without FPB was similar with the appearance of Nile tilapia fish balls with 3% FPB. Both were the most liked by the panelist compared to fish ball added with 5% FPB (B) and those added with 7% FPB (C). Fish balls with no added FPB had average hedonic appearance value of 8.5 from the assessment of 30 panelists. The best appearance of fish meatballs with the addition FPB was achieved by fish balls with addition 3% FPB compared to 5% and 7% FPB. When the FPB increased up to 5%, the appearance decreased, hence the hedonic value lowered. The addition of FPB caused the appearance of the fish balls to become slightly yellowish in color, the surface smooth and rough depending on each treatment. Appearance is one of the parameters that can determine the level of panelist acceptance. There are two processes that may be used to encapsulate the entire process of deriving meaning from a product's appearance, including the panelist view on product such as color, form, and texture and/or a product's appearance might be characterized by a particular attribute due to the way that specific colors, materials, and other physical aspects are combined [29].

Other study showed that the yellowness color increased in surimi gel of threadfin bream when it was added with higher concentration of bio-calcium and decreased the hedonic value of appearance [16]. The higher hedonic value of odor was observed in fish ball control (K), however it was not different to fish ball with 3% FPB (A). The results showed that hedonic value of odor parameters were significant differences between the control (K) and fish balls with the addition of 5 and 7% FPB. Odor value of fish ball with 3% FPB was significant different to fish balls added with 7% FPB. Fish meatballs with the addition of bio-calcium have a product-specific odor due to the odor of FPB. The higher hedonic value of odor of fish balls treated with the addition of 3% FPB (7.86) was noticed compared to fish balls added with 5 and 7% FPB. The lower hedonic value of odor was observed by fish ball added with higher FPB especially at the 7% FPB compared to K sample (P<0.05).

Table 1. Hedonic value of Nile Tilapia Fish Balls added with FPB at different concentrations.

Treatments	Specifications			
	Appearance	Odor	Taste	Texture
K	$8.50±0.90^{b}$	$8.03±0.85^{b}$	$8.06±1.01^{c}$	$8.00±1.01^{b}$
A	$8.40±0.90^{b}$	$7.86±0.80^{b}$	$7.93±1.01^{bc}$	$7.93±1.01^{b}$
B	$7.27±0.69^{a}$	$7.40±0.97^{ab}$	$7.26±0.87^{ab}$	$7.33±0.76^{a}$
C	$6.80±0.61^{a}$	$7.06±0.42^{a}$	$6.60±0.96^{a}$	$6.20±0.99^{a}$

Note: Values represent means ± SD (n = 30).

Different superscript letters in the same column indicate significant differences (P < 0.05). K: Nile tilapia without FPB; A: Nile tilapia with 3%FPB; B: Nile tilapia with 5% FPB; C: Nile tilapia with 7% FPB.

Those might be due to unpleasant odor of FPB reduced specific odor of fish balls. However, fish ball with or without FPB were still preferred by panelists with score more than 7. The addition of FPB influenced the panelists' preference for the hedonic value of taste of Nile tilapia fish balls. The more concentration of FPB, the lower hedonic value of taste value given by the panelists. The higher value of taste on fish balls added with 3% FPB than those added with 5 and 7% FPB was found. It showed that the higher FPB concentrations caused the decrease of panelist preference on taste of Nile tilapia fish balls. However, fish balls added with 3% FPB had no different value with control (K). The hedonic taste of wheat cracker was similar when it was added with salmon bone bio-calcium in comparison with control and the score showed both were preferred by panelist with score more than 7 [30]. It is known that taste is an important aspect for food products because in general the food that is liked is food that has an appropriate taste. According to Sharif et al. [31], taste compounds are a determining factor in food quality that produce the sensation of bitter, sour, sweet, salty and aroma afterward. Aroma receptors in the nose and taste receptors in the mouth are primarily responsible for perceiving taste.

Different concentrations had varying impact on hedonic value of texture of Nile tilapia fish balls ($P<0.05$). The hedonic value texture decreased with the increased of FPB concentration added. The addition of FPB yielded harder texture of fish balls, hence the preference of panelist reduced when the concentration FPB increased. This result is coincidence with the gel strength shown in Figure 1. The texture of the meatballs added with 3% FPB was liked by the panelists compared to those added with 5 and 7% FPB. Nile tilapia fish balls added with 3% FPB had no different hedonic value of texture with control (K). According to Indonesian National Standard [15] for fish balls, the sensory value of fish balls must have a minimum score of 7, namely with the specifications of being dense, compact, and slightly chewy. The lower score of hedonic texture might be due to gritty texture as effect of the higher concentration of FPB, hence it was less liked by the panelists. Several studies showed that increasing the concentration of fish bone bio-calcium reduce the preference of panelist on texture of fortified food due to its sandy mouthfeel [7, 9, 10, 11, 16, 23, 30].

4. Conclusions

The addition of different concentration of Asian sea bass bone bio-calcium (FPB) had a significant impact ($p<0.05$) on the chemical (calcium, protein, water, fat, ash) and physical (gel strength, hedonic) quality of Nile tilapia fish balls. Concentration of FPB at 5% showed the best amount to obtain Nile tilapia fish ball with high calcium content and sensory value. The addition of FPB at proper concentration could increase calcium content without any sensory disturbance. The bioavailability of this bio-calcium should be tested for further study to ensure calcium absorption in the human body. However, the use fish bone bio-calcium as a calcium source in the food industry not only to provide high calcium product but also to reduce environmentally problem of fish industry waste.

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Data Availability statement

The data that were analyzed in the present article are available upon justifiable request to the corresponding author.

Conflicts of Interest

All authors declare that they have no conflicts of interest.

Authors Contribution

Meutthy Rahma Trisnawati, conducted the research, collected, and analyzed the data, and wrote the manuscript. Ima Wijayanti, supervised, wrote, edited, analyzed and reviewed. Putut Har Riyadi reviewed and edited.

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