Fortification of Nano Calcium of Freshwater Mussel 
(*Pilsbryoconcha* sp.) Shell on Cookies Towards Proximate Composition and Calcium Content

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**Abstract.** This research was aimed to determine the effect of nano calcium of freshwater mussel (*Pilsbryoconcha* sp.) shell on cookies towards proximate composition and calcium content. Cookies were prepared by fortified with nano calcium of freshwater mussel shell consist of 4 levels of treatment: 0%, 0.5%, 1%, and 1.5%. Cookies were evaluated for proximate analysis (moisture, ash, protein, fat, and carbohydrate content), calcium, phosphorus content, and consumer acceptance. Findings it was observed that fortification of nano calcium of freshwater mussel shells significantly increased the calcium (Ca) and phosphorus (P) content of cookies when compared to the control treatment, with the highest value of calcium (6.91%) and phosphorus (1.36%) of 1.5% fortification treatment. Meanwhile, based on consumer acceptance that 1% fortification of nano calcium of freshwater mussel shell on cookies was most preferred with calcium content 5.97%, phosphorus content 1.24%, and proximate content of moisture, ash, protein, fat, and carbohydrate content was 3.26%, 2.26%, 10.12%, 29.32%, and 55.33%, respectively.

1. **Introduction**

Freshwater mussel (*Pilsbryoconcha* sp.) is one of the fishery products originating from the Sungai Paku village waters. So far, it has been used as a side dish for people around the river and a source of protein for fish feed. However, use is limited to meat only, while the use on the shell has not been carried out [1]. Wasted mussel shell will certainly be a serious problem that needs attention so that it does not become a source of environmental pollution and a source of disease for the surrounding community. Waste of shell from freshwater mussel of Sungai Paku water is contained high valuable resource, mainly calcium content 61.39%, followed by other nutrients such as water 0.41%, protein, 3.44%, 0.18% fat, 93.01% ash, 2.97% carbohydrates and 1.80% crude fiber [2]. Calcium plays an important role in helping to fulfill the nutrition of children and adults, especially for bone growth and osteoporosis. Moreover, calcium also plays a role in muscle contraction, teeth, nerve impulse, and blood clotting [3], 99% of the body’s calcium supply is stored in the bones and teeth The high amount of nutrients in the mussel shell, especially the calcium content, it means that the shell of freshwater mussel from Sungai Paku water is one of waste that has functional value but has not been utilized.

Common calcium that is consumed at the moment it is in the form of micro calcium, which is the magnitude absorption by the body, is only 50%, causing a deficiency. Calcium is small in size or it is called ‘nano’. which is applied to the product food will make the calcium more easily absorbed by the body. Nanotechnology is the design, characterization, production, and application of structures, devices, and systems by controlling shape and size at scale nanometer. According to Qhatan, 2017 [4] Nano is the metric unit of the nanometer (nm). Like the millimeter, the nanometer is defined as a scale of length, having no special physical meaning. Specifically, it is equivalent to one billionth of one meter (i.e., 1 nm = 10^-9 m). Therefore nano calcium can be absorbed almost 100% by the body.

To anticipate the abundance of waste, further processing is undertaken so that the waste can be utilized. One of the efforts made is a fortification of nano calcium of freshwater mussel shell on cookies production. Cookies are one type of food that can be consumed by anyone, due to it is made
from soft, crunchy dough, and the texture looks less dense. So far, cookies that are usually consumed only contain macronutrients, namely carbohydrates, proteins, fats, and very little or no micronutrients such as minerals. While the average calcium the body needs per day which Recommended Dietary Allowance (mg/day) for 1-3 years 700, 4-8 years old 1000, 9-18 years old 1300, 19-50 and 51–70 years old males 1000, 51–70 years old females and >70 years old 1200, 14–18 years old, pregnant/lactating 1300, 19–50 years old, pregnant/lactating 1000 [5]. Therefore, cookies need to be increased in calcium content through fortification with nano calcium of freshwater mussel shell.

2. Materials and Methods
The shell of freshwater mussel was obtained from Sungai Paku waters. The shell of freshwater mussel was formed into nano calcium and then analyzed the physical characteristics, chemical, and microscopy. The observation was conducted in the Fisheries product technology laboratory Universitas Riau and Research Center of Laboratory of Agricultural Postharvest Development IPB University. Microscopy analyses were conducted in the laboratory Mechanical Engineering of Sepuluh November Institute of Technology.

2.1. Materials and Equipment
The material used for nano calcium production was a freshwater mussel shell, 1 N HCl, 3 N NaOH, and distilled water. The tools used in this study include hammer mills, mortar, jars, thermometers, ovens, furnaces, filter paper, pH paper and scales, 80 mesh sieves, hot plate, centrifuge, as well as proximate analysis tools, Atomic Absorption Spectrophotometer (AAS), Scanning Electron Microscopy (SEM) and Particle Size Analyzer (PSA).

The materials used for cookie production are wheat flour, nano calcium of freshwater mussel shell, refined sugar, vanilla, baking powder, eggs, butter, and powdered milk. The materials for chemical analysis are nitric acid, H₂O₂, catalyst, citric acid, vanadate molybdate reagent, H₂SO₄, PP Indicator, NaOH, H₃BO₃, indicator (methyl red-blue), 0.1 N HCl, and distilled water. The tools for making cookies are gas stoves, ovens, 80 mesh sieves, blenders, mixers, rollers, analytical scales, and tools for chemical analysis are measuring cups, Erlenmeyer, tubes, measuring pipettes, analytical scales, Kjeldahl flasks, fat flasks, volumetric flask, hot plate, soxhlet, desiccator, Whatman filter paper, distillator, porcelain cup, kiln, and score sheet.

2.2. Methods
This research begins by producing and analyzing nano calcium from freshwater mussel shells of Sungai Paku waters. Furthermore, the nano calcium that has been obtained was applied to cookie production. The experimental method was used in this research with fortified of nano calcium on cookies production with 4 level treatments: 0%, 0.5%, 1%, and 1.5% from the weight of wheat flour. The parameter analysis was measured for proximate analyses (moisture, protein, fat, ash, and carbohydrate content) [7], calcium [8], phosphorus content, and consumer acceptance [9]. The ingredients formulation of cookies with fortified nano calcium of freshwater mussel shell can be seen in Table 1.
Table 1 The formulation of cookies with fortified nano calcium of freshwater mussel shell (g)

| No. | Ingredients                                      | 0%  | 0.5% | 1%  | 1.5% |
|-----|--------------------------------------------------|-----|------|-----|------|
| 1   | Wheat flour                                      | 350 | 350  | 350 | 350  |
| 2   | Nano calcium of freshwater mussel shell          | 0   | 1.75 | 3.5 | 5.25 |
| 3   | Refined sugar                                    | 100 | 100  | 100 | 100  |
| 4   | Vanilla                                          | 0.5 | 0.5  | 0.5 | 0.5  |
| 5   | Baking powder                                    | 0.5 | 0.5  | 0.5 | 0.5  |
| 6   | Eggs                                             | 2   | 2    | 2   | 2    |
| 7   | Margarine                                        | 200 | 200  | 200 | 200  |
| 8   | Butter                                           | 50  | 50   | 50  | 50   |
| 9   | Powdered milk                                    | 50  | 50   | 50  | 50   |

2.3. Nano calcium Production
The flour of freshwater mussel shell was obtained according to Dian, 2020 [2]. Furthermore, the shell flour that has been formed is made of nano calcium according to Fernandez method [6]: Mussel shell flour was extracted with 1N HCl for 3 days and heated for 1 hour at 90°C. The extraction results are filtered with filter paper so that the filtrate is obtained. The next step is precipitation with 3N NaOH solution with a ratio of 1:5. Then the white mineral precipitate is separated from the filtrate. The white precipitate obtained is then washed using distilled water until the pH is neutral, The white precipitate was dried using an oven at 60°C for 24 hours. Then do the grinding and mortar, after that it is done for 3 hours with a temperature of 100°C. Then refined using a mortar. The samples were then kiln at 600°C for 6 hours to remove organic compounds. The last step is powdered nano calcium powder using a mortar and analyzed. Nano calcium was analyzed for whiteness degree using Kett Digital Whiteness-meter, proximate analyses (AOAC, 2005) [7], calcium content using AAS method, microscopy analyses using SEM, and particle size using PSA.

2.4. Cookies Production
350 grams of wheat flour, 100 grams of sugar, 0.5 grams of vanilla, weighed and mixed until homogeneous. Adding 200 grams of margarine, 50 grams of butter, 0.5 grams of baking powder, 50 grams of milk powder, and 2 eggs and then made dough. Four doughs are made with the addition of the nano calcium of freshwater mussel shell (0.0.5, 1, and 1.5%) respectively. The dough is then thinned with a roller until the thickness reaches 1 mm, printed, and then rolled into a light brownish yellow color. Furthermore, cookies were analyzed for proximate content, calcium, phosphorus, and consumer acceptance.

2.5. Data Analysis
The data of nano calcium obtained were analyzed with descriptive analyses, and data cookies with fortified nano calcium were analyzed using analysis of variance (ANOVA) at 95% confidence level.

3. Results and Discussions
3.1. Physical characteristics
Based on the physical characteristics of nano calcium from freshwater mussel shell contained 31.76 % yield, 12.42% absorption, and 65.73% whiteness degree. It means the nano calcium of freshwater mussel shell from Sungai Paku was whiter than freshwater shell flour [2]. The obtained nano calcium produced more yield compared to the nano calcium from dried vannamei shrimp shells (13.92%) [12]. Meanwhile, based on the whiteness degree value obtained, it is still relatively low because of other components [13]. The whiteness degree of the nano calcium is influenced by its constituent mineral components. The main component of the nano calcium is calcium which has a white color, therefore the whiteness of the nano calcium must also be high [14].
3.2. Proximate and calcium content of nano calcium

Based on Table 2 showed that the ash content of nano calcium from a freshwater mussel shell was 95.07%. The ash content of nano calcium from freshwater mussel shell was higher than the ash content of nano calcium from vannamei shrimp shell (75.08%) and sea urchins shell (80.14%) [14] [15]. The high and low ash content of a material identified high or low mineral content contained in a material [16]. Furthermore, nano calcium from a freshwater mussel shell contained a calcium content of 58.04%. The calcium content almost the same as nano calcium produced from the oyster shell (56.77%) and crab shell (51.27%) [17] [18]. Calcium obtained from shellfish can be obtained from the aquatic environment, both that is absorbed or diffused directly by the shell from the water. Therefore, it is physically very hard shells [17].

| Parameter       | Average value (%) |
|-----------------|-------------------|
| Ash             | 95.07             |
| Moisture        | 0.45              |
| Protein         | 0.14              |
| Fat             | 0.29              |
| Carbohydrate    | 4.05              |
| Crude fiber     | 0.00              |
| Calcium         | 58.04             |
| Phosphorus      | 16.84             |

3.3. Morphology and particle size of nano calcium

The morphology of particle nano calcium of freshwater mussel shell was observed using SEM with a magnification of 20,000 times can be seen in figure 1. The particle size of this nano calcium was 1050 nm with 100% intensity. Nanoparticles are particles measuring 10 – 1000 nm [19]. Nanoparticles with a very small size, have better solubility compared to ordinary drugs in the body [20].

![Figure 1. The morphology of nano calcium particle of freshwater mussel shell](image)

Based on Figure 1 showed that freshwater mussel shell of nano calcium looks very distinctive with a homogeneous shape and particle size in the form of vaterite type crystals. The morphology of nano calcium of freshwater mussel shell was the same as the morphology of nano calcium from oyster shell,
namely the form of crystals of vaterite and a little aragonite and the morphology of nano calcium from crab shell in the form of vaterite crystals [17] [21]. Calcium crystals had different types of phases consisted of calcite, aragonite, and vaterite [22]. Calcite has rhombohedral crystals, scalenohedral cubes, and prismatic crystals. Aragonite is in the form of a cluster and discrete needle-like, while vaterite is a sphere [23].

3.4. Proximate of cookies
The result of the proximate and mineral content of cookies with fortified nano calcium of freshwater mussel was shown in table 3.

| Table 3 | The proximate and mineral content of cookies with fortified nano calcium of freshwater mussel shell (%) |
|---------|--------------------------------------------------------------------------------------------------|
| Parameters | 0% | 0.5% | 1% | 1.5% |
| Calcium | 4.25±0,18a | 5.16±0,21b | 5.97±0,22c | 6.91±0,43d |
| Phosphorus | 0.98±0,06a | 1.10±0,09b | 1.24±0,02c | 1.36±0,02d |
| Moisture | 3.70±0,11c | 3.45±0,13bc | 3.26±0,10b | 3.11±0,05a |
| Ash | 1.97±0,04a | 2.14±0,07ab | 2.26±0,09bc | 2.35±0,09c |
| Protein | 10.47±0,11b | 10.38±0,12b | 10.12±0,22ab | 9.80±0,22a |
| Carbohydrate | 52.95±0,57a | 53.83±0,22a | 55.33±0,47b | 58.12±0,77c |

Based on table 3, can be seen that the cookies with fortified 1.5% nano calcium of freshwater mussel were higher in calcium content and the ANAVA test showed that it was significantly different from 0, 0.5, 1, and 1.5% treatments. This result also saw that the higher of calcium concentration given, the higher the calcium value of these cookies.

The ash content of cookies from all treatments ranges from 1.97-2.35%, this value is still high, according to Indonesian National Standards (SNI), the maximum ash content in cookies is 1.5%, however, this result is comparable to Agustini et al., (2011) [10] where the ash content in cookies of scallop shell flour ranged from 1.93-2.32%. The high ash content in these cookies is also due to the very high ash content in nano calcium 95.07%.

The protein content of cookies in the range 9.80-10.47%, this value has fulfilled Indonesian national standards with a minimum protein cookie 9%. The same results were also shown by Hapsoro et al., 2017 [11] that which the average value of cookie protein content with the addition of crab shell flour ranged from 9.95 to 9.30%.

3.5. Consumer acceptance
The consumer acceptance of cookies with fortified nano calcium of freshwater mussel can be seen in table 4.

| Table 4 | The consumer acceptance of cookies with fortified nano calcium of freshwater mussel shell |
|---------|--------------------------------------------------------------------------------------------------|
| Parameters | 0% | 0.5% | 1% | 1.5% |
| Appearance | 7.57±0,08a | 7.64±0,06a | 7.71±0,12a | 7.50±0,08a |
| Colour | 7.54±0,08a | 7.58±0,11a | 7.68±0,15a | 7.43±0,02a |
| Aroma | 7.49±0,06a | 7.51±0,13a | 7.66±0,08a | 7.53±0,06a |
| Flavor | 7.41±0,08a | 7.49±0,01a | 7.53±0,03a | 7.43±0,05a |
| Texture | 7.26±0,06a | 7.19±0,03a | 7.12±0,13a | 7.07±0,08a |

Table 4 shows that all the treatments were favored by consumers with a value of more than 7. However, cookies with a 1% concentration of nano calcium of freshwater mussel were most favorable by consumer acceptance. Based on the ANOVA test, among treatment was not significantly different.
4. Conclusion
The fortification of nano calcium of freshwater mussel shells significantly increased the calcium content of cookies with the highest value of calcium 6.91% in 1.5% fortification treatment. Meanwhile, based on consumer acceptance that 1% fortification of nano calcium of freshwater mussel shell on cookies was most preferred with calcium content 5.97%, phosphorus 1.24%, moisture, ash, protein, fat, and carbohydrate content was 3.26%, 2.26%, 10.12%, 29.32%, and 55.33%, respectively. However, it needs to be tested further regarding the safety toxicity for absorption in the body.

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