The effect of fortification *Moringa oleifera* leaves powder on calcium content in otak-otak products of *Clarias* sp.

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**Abstract.** *Clarias* sp. is one of the most common types of freshwater fish. The utilization of *Clarias* sp. is only limited to fresh consumption, whereas the nutritional content of *Clarias* sp. is potential as a source of nutrition. High protein and low-fat content make *Clarias* sp. can be processed into diversification of traditional food such as otak-otak products. The otak-otak products can be used as functional food, but there is a weakness i.e. the low of Calcium of otak-otak products. This study aims to determine (1) fortification of *Moringa oleifera* leaves on organoleptic and (2) mineral content of Calcium the otak-otak products. The method used is an experimental method with a simple Completely Randomized Design. Independent variables of this study were concentration of *Moringa oleifera* leaves powder 4%, 8% and 12% while dependent variable was Calcium content. The results showed that fortification of *Moringa oleifera* leaves powder had a significant effect on organoleptic aroma, taste, texture, appearance and Calcium content of the otak-otak products. The best fortification of *Moringa oleifera* leaves powder is 4% with the results of aroma of 41.00; taste 37.25; texture 38.00 and appearance of 38.05. Calcium content of otak-otak products reached 3.09±0.099 mg.

**1. Introduction**

Catfish (*Clarias* sp.) is one type of the potential freshwater fish commodity. The total production of catfish reached 841.75 thousand tons in 2017 and increased in 2018 which reached 1.81 million tons [1]. Catfish can be cultivated on limited land and water sources with high stocking densities like all types of feed and relatively easy cultivation techniques [2]. In addition, this fish also has a larger body shape with more meat content. However, the use of catfish is only limited to fresh consumption. Whereas catfish contains 17.09% protein, 2.75% fat, 78.05% water, 1.25% ash, and contains carbohydrates as much as 0.86% [3]. With a high protein content and low-fat content, catfish can be processed into diversified products in the food sector such as fish balls, fish dumplings, fish sausages, and otak-otak products of catfish.

Otak-otak products of fish is one of the diversified fishery products originating from the Sumatera Island to develop to other areas which is processed from fresh fish meat. To obtain quality otak-otak products of fish, a combination of the right raw materials and additives is needed. Additional ingredients added in the processing of otak-otak products of fish are tapioca flour, as well as spices such as sugar, salt, shallots, garlic, pepper, and coconut milk. Otak-otak products of fish are easy to obtain with good taste at low prices.
and easy processing, so they are liked by various circles of society [4]. Sofyan and Karim [5] reported that, otak-otak products of fish contain fairly low content of calcium, ranging from 0.24% from processed snakehead fish, 0.27% from processed mackerel, and 0.19% from processed milkfish. Apriyatcha and Priandy [6] said that, the calcium content of processed otak-otak products of fish per 100 g of raw material is 2.6 mg. Therefore, the development of otak-otak products of fish can be used as a food product that can be consumed by the community by adding nutritional or nutritional elements in its processing, one of which is the mineral calcium.

Calcium is the most abundant mineral in the body and about 99% of the total calcium is in hard tissues, namely bones and teeth and the rest in the blood and body cells. Humans need 1000-1500 mg of calcium per day, but the average Indonesian only consumes 300 mg of calcium per day. If this continues, there will be a calcium deficiency in the body which can cause osteoporosis or bone loss which is characterized by loss of bone density [7]. This encourages consumers to consume products or foods with sufficient calcium content. Sources of calcium apart from animals such as fish, shrimp, and other processed products, can also be obtained from plants or green vegetables such as spinach, mustard greens, watercress, cassava leaves, and Moringa sp. leaves. The calcium content per 100 g of ingredients in spinach is 267 mg, mustard greens are 220 mg, watercress is 182 mg, and cassava leaves are 165 mg [8]. The calcium mineral content in Moringa sp. leaves contains as much as 440 mg of calcium per 100 g of material [9]. Therefore, Moringa sp. leaves can be used as one of the ingredients for products fortification to increase its calcium content.

Moringa is one type of tropical plant that can grow and develop in Indonesia. This plant can grow in tropical to subtropical areas and on all types of soil. All parts of the Moringa plant from leaves, flowers, stems, fruit, seeds, skin to roots have various beneficial properties. M. Oleifera leaves has been used as an alternative food source to combat malnutrition, especially among children and infants [10]. M. Oleifera leaves are reported to contain substantial amounts of vitamin A, C and E [11]. The leaves of M. Oleifera have also been found to contain appreciable amounts of total phenols, proteins, calcium, potassium, magnesium, iron, manganese and copper [11]. Fresh Moringa leaves per 100 g contain 440.0 mg of calcium, 0.7 mg of iron, 0.9 g of fiber, and 6.7 grams of protein [9]. However, Moringa leaves have no selling value even though they contain various scientifically proven nutrients. The high nutritional content of Moringa leaves allows it to be used as an ingredient that can be added in various preparations to increase its nutritional value or commonly referred to as fortification. One of them is processed into Moringa leaves powder to make it more durable in storage and can increase the effectiveness in processing. Based on this, it is necessary to analyze the calcium content and sensory evaluation on the otak-otak products of catfish (Clarias sp.) with the addition of Moringa leaves powder to increase the nutritional content especially calcium, thus it can be used as a functional food. The purpose of this study was to develop diversification of otak-otak products of catfish (Clarias sp.) with the fortification of Moringa oleifera leaves powder as a source of calcium.

2. Materials and Methods

The procedure of this research was carried out through two stages, pilot and main study.

2.1 Pilot study

Pilot study is used to determine the best concentration of Moringa leaves powder preferred by participants in making otak-otak products of catfish which will be used as a reference in the main research. Pilot study will begin with the proximate testing and calcium content in Moringa leaves powder obtained from online stores. Furthermore, sensory evaluation using the hedonic test was carried out to get the best treatment preferred by the participants. The participants used were semi-trained from 20 students of Universitas Brawijaya. This is following of Khatimah et al. [12], which state that sensory evaluation through hedonic tests can be carried out with 20 semi-trained participants from among students. Based on the results of research by Winnarko and Mulyani [13], the addition of the best Moringa leaves powder in skipjack nugget
products is 10%. Therefore, the results of this study were used as a reference in determining the concentration in the pilot study with the range of addition of *Moringa* leaves powder to otak-otak products of catfish of 4%, 8%, and 12%. The process of making otak-otak products of catfish can be seen in Figure 1. The formulations of pilot study modified by [14] in the making of otak-otak products of catfish can be seen in Table 1.

**Table 1.** Formulation for pilot study of making otak-otak products of catfish

| Ingredients                      | Formula |
|----------------------------------|---------|
|                                  | F1 (4%) | F2 (8%) | F3 (12%) |
| *Clarias* sp. (g)                | 100     | 100     | 100      |
| Tapioca flour (g)                | 30      | 30      | 30       |
| *M. oleifera* leaves Powder (g)  | 4       | 8       | 12       |
| Onion (g)                        | 10      | 10      | 10       |
| Garlic (g)                       | 5       | 5       | 5        |
| Sugar (g)                        | 4       | 4       | 4        |
| Salt (g)                         | 4       | 4       | 4        |
| Pepper (g)                       | 1       | 1       | 1        |
| Flavoring (g)                    | 1       | 1       | 1        |
| Galangal (g)                     | 2       | 2       | 2        |
| Leek (g)                         | 4       | 4       | 4        |
| Egg                              | 1       | 1       | 1        |
| Coconut Milk (g)                 | 25      | 25      | 25       |
| Ice (g)                          | 10      | 10      | 10       |
Figure 1. The processing of making otak-otak products of catfish on pilot study

2.2 Main study

The main study was conducted with the aim of obtaining the best concentration of otak-otak products of catfish (Clarias sp.) with the addition of Moringa leaves powder. The results of the best concentration of Moringa leaves powder from the pilot study were used as the basis for the main study. For the main study, the test parameter is to be carried out in the analysis of calcium content. The procedure for making otak-otak products of catfish with the addition of Moringa leaves powder can be seen in Figure 2. The formulations of main study modified by Alam et al. [14] in the making of otak-otak products of catfish can be seen in Table 2.
Table 2. Formulation for main study of making otak-otak products of catfish

| Ingredients            | Formula |
|------------------------|---------|
|                        | F1 (2%) | F2 (4%) | F3 (6%) |
| Clarias sp. (g)        | 100     | 100     | 100     |
| Tapioca flour (g)      | 30      | 30      | 30      |
| M. oleifera Powder (g) | 4       | 8       | 12      |
| Onion (g)              | 10      | 10      | 10      |
| Garlic (g)             | 5       | 5       | 5       |
| Sugar (g)              | 4       | 4       | 4       |
| Salt (g)               | 4       | 4       | 4       |
| Pepper (g)             | 1       | 1       | 1       |
| Flavoring (g)          | 1       | 1       | 1       |
| Galangal (g)           | 2       | 2       | 2       |
| Leek (g)               | 4       | 4       | 4       |
| Egg                    | 1       | 1       | 1       |
| Coconut Milk (g)       | 25      | 25      | 25      |
| Ice (g)                | 10      | 10      | 10      |

Onion, garlic, galangal, scallions mashed

Catfish (Clarias sp.)
Cleaning the Fish
Fillet and Skinless
Weighed the white meat of catfish 100 g
Puree with ice using a blender
Dough making
Mix until the dough is evenly mixed
Wrapped in banana leaves
Steamed at 50°C
Otak-otak Products of Catfish
Calcium Content and Proximate Analysis

Figure 2. The processing of making otak-otak products of catfish on main study
The test parameters carried out in main study were chemical include calcium analysis. Chemical analysis was carried out by proximate test which included protein, carbohydrate, fat, water, and ash content.

2.3 Sensory evaluation
Sensory evaluation is an assessment of products quality by using an organoleptic test formula as a tool or instrument. This study using the hedonic test. The hedonic test is a sensory analysis test that aims to determine differences in the quality of several products through assessment and is used to determine the content of participants preference for products being tested. This content of preference is commonly referred to as the hedonic scale [15]. This test is carried out on a hedonic scale between 1 to 4, where 1 = very dislike, 2 = dislike, 3 = like and 4 = like very much. This hedonic test was carried out by semi-trained participants from 30 students of Universitas Brawijaya. This is following the research conducted by Bunta et al. [16], which used 30 semi-trained participants on sensory evaluation through hedonic tests.

2.4 Calcium content
Analysis of calcium content can be done using the AAS (Atomic Absorption Spectrophotometer) instrument. The first step was to take 5 g of the sample in the form of ash, then put it in a 250 mL measuring cup and add 10 mL of concentrated HNO₃, then heated on a hot plate at 35°C in a fume hood for 1 hour until the smoke turned white and the extract turned clear. Then cooled, if it has cooled then added 1 mL of HCl and 20 mL of distilled water and filtered with Whatman 41 filter paper in a 50 mL volumetric flask. The filtrate obtained was then diluted with distilled water to 50 mL in a volumetric flask. Furthermore, calcium analysis was carried out using AAS with a wavelength of 422.7 nm using a Ca cathode lamp. To determine calcium content can use the formula:

\[
\text{Calcium (\%) } = \frac{\text{Concentration Sample (mg L}^{-1}\times \text{V. sample (L)}}{\text{Sample Weight (g)}} \times \text{Dilution Factor}
\]

2.5 Statical analysis
Parameters were analyzed by ANOVA (Analysis of Variance) with criteria for accepting or rejecting statistical hypotheses. If the results are significantly different, then Duncan's Multiple Range Test (DMRT) will be continued. The sensory evaluation was analyzed using Kruskal-Wallis. To get the best treatment of all parameters, it was analyzed using the de Garmo method.

3. Result and Discussion
3.1 Pilot study
3.1.1 Chemical characteristics of moringa leave powder
Moringa leaves powder can be used as a substitute or fortification in the development of food products and can be used to add nutritional value. In addition, the use of Moringa leaves powder is also used to extend the shelf life and improve products quality. Moringa leaves powder is obtained through several processing processes, by sorting, washing, drying, and milling. The color of Moringa leaves powder is a bright yellowish-green caused by the drying process in the sun, so it cannot cause excessive enzymatic reactions [17]. The results obtained show that Moringa oleifera leaves contain nutritious compounds. The nutritional content contained in 100 g dried Moringa leaves is 12.5 g fiber, 2185 mg calcium, 25.6 mg iron, 225 mg phosphorus, 29.4 g protein, 41.2 g carbohydrates, and 5.2 g fat [18]. The proximate composition of Moringa leaves powder per 100 g can be seen in Table 3.
Table 3. Proximate composition of *Moringa oleifera* leaves powder

| No  | Proximate Composition | Value (%) | Comparison |
|-----|-----------------------|-----------|------------|
| 1.  | Calcium (mg)          | 1935.0*   | 2003.0**   |
| 2.  | Protein (g)           | 23.73*    | 27.1**     |
| 3.  | Carbohydrate (g)      | 52.55*    | 38.2**     |
| 4.  | Fat (g)               | 6.90*     | 2.3**      |
| 5.  | Water (g)             | 5.27*     | 7.5***     |
| 6.  | Ash (g)               | 11.55*    | 7.95***    |

Sources:
*) Laboratorium Gizi Fakultas Kesehatan Masyarakat Universitas Airlangga
**) Widowati *et al*. [9]
***) Aminah *et al*. [26]

3.1.2 The best concentration of fortification *moringa* leaves powder

Pilot study was conducted using 3 treatments fortification of *Moringa* leaves powder 4%, 8%, and 12% of the weight of catfish. The concentration of *Moringa* leaves powder in the making of otak-otak products of catfish refers to the results of Winnarko and Mulyani [13] research, the concentration of adding *Moringa* leaves flour at 5%, 10%, and 15% in fish nuggets. To determine the best concentration of fortification of *Moringa* leaves powder, and sensory evaluation using the hedonic test was carried out by 20 participants. The results of the sensory evaluation were processed using Kruskal-Wallis with SPSS 25. Based on the results of the Kruskal-Wallis statistical analysis in the pilot study, the results were significantly different (*P* < 0.05) for the acceptability of appearance, taste, aroma, and texture. Meanwhile, the highest mean rank of acceptability was 4% of the fortification of *Moringa* leaves powder with the appearance 38.05, aroma 41.00, taste 37.25, and texture 38.00. Thus, it can be concluded that the best fortification of *Moringa* leaves powder with concentrations 4%. The best concentration in the pilot study is used as a reference for the main study. The pilot study for appearance acceptability graph can be seen in Figure 3.

![Figure 3. Graph of appearance acceptability (F1: 4%; F2: 8%; F3: 12%)](image-url)
means it is less favored by the participants. The higher concentration of fortification of *Moringa* leaves powder, the more the appearance of the resulting moss-green color. The pilot study for aroma acceptability graph can be seen in Figure 4.

**Figure 4.** Graph of aroma acceptability (F1: 4%; F2: 8%; F3: 12%)

Based on the results of the Kruskal-Wallis test, it can be analyzed that the fortification treatment of *Moringa* leaves powder has a significant effect (P-value < 0.05) on the aroma acceptability of otak-otak products of catfish in the pilot study. The highest average value of aroma acceptability 2.45 was found in the F1 (4% *Moringa* leaves powder fortification concentration). While the lowest average value 1.5 was found in the F3 (12% concentration of *Moringa* leaves powder fortification). The graph showed that the higher concentration of fortification of *Moringa* leaves powder, the lower the resulting graph, which means it is less favored by the participants. The higher concentration of fortification of *Moringa* leaves powder, the more unpleasant the aroma will be. The pilot study for taste acceptability graph can be seen in Figure 5.

**Figure 5.** Graph of taste acceptability (F1: 4%; F2: 8%; F3: 12%)

Based on the results of the Kruskal-Wallis test, it can be analyzed that the fortification treatment of *Moringa* leaves powder has a significant effect (P-value < 0.05) on the taste acceptability of otak-otak products of catfish in the pilot study. The highest average value of taste acceptability 2.45 was found in the F1 (4% *Moringa* leaves powder fortification concentration). While the lowest average value was found in the F3 (12% concentration of *Moringa* leaves powder fortification). The graph showed that the higher concentration of fortification of *Moringa* leaves powder, the lower the resulting graph, which means it is less favored by the participants. The higher concentration of fortification of *Moringa* leaves powder, the more bitter the taste produced. The pilot study for texture acceptability graph can be seen in Figure 6.
Based on the results of the Kruskal-Wallis test, it was analyzed that the fortification treatment of *Moringa* leaves powder had a significant effect (P-value < 0.05) on the texture acceptability of otak-otak products of catfish in the pilot study. The highest average value of texture acceptability 2.9 was found in the F1 (4% *Moringa* leaves powder fortification concentration). While the lowest average value was found in the F3 (12% concentration of *Moringa* leaves powder fortification). The graph showed that the higher concentration of fortification of *Moringa* leaves powder, the lower the resulting graph, which means it is less favored by the participants. The higher concentration of *Moringa* leaves powder fortification, the denser of texture.

Fortification of *Moringa* leaves powder can affect the sensory and acceptability on otak-otak products of catfish (*Clarias* sp.). Igbabul *et al.* [19] reported a wider increase in acceptability of cookies fortified with *Moringa* leaves powder. The improved acceptability of *Moringa* substituted otak-otak products of catfish may be due to versed phenolic and bioactive compounds in *Moringa* leaves which combine with other substances in the ingredients to form an aromatic complex with pleasant sensory and acceptability.

The results of sensory evaluation indicate that the higher concentration of *Moringa* leaves powder added can reduce the level of acceptance and sensory. The acceptability of the sample can be attributed to the fact that the *Moringa* leaves powder are flavor retainer and helps to enrich the sensory properties of the otak-otak products of catfish (*Clarias* sp.). Bourekoua *et al.* [20] there was a significant decrease in the volume of the bread samples was added of 2.5% *Moringa* leaves powder. With the addition of 2.5% and 10% MLPs, there was a slight decrease in hardness and chewiness.

### 3.2 Main study

The main study refers to the best concentration obtained in the preliminary analysis, which is 4% so that the concentration range for fortification of *Moringa* leaves powder used in the main analysis is 0%, 2%, 4%, and 6%. The results of otak-otak products of catfish with the fortification of *Moringa* leaves powder can be seen in Figure 7.
3.2.1 Calcium content

Based on the ANOVA analysis, it was shown that the fortification of Moringa leaves powder had a significant effect (P-value < 0.05) on calcium content in otak-otak products of catfish. Furthermore, continued with Duncan's further test. Duncan's test results showed that there were significant differences between treatments. The highest calcium content 3.090 ± 0.099 was found in the F4 (8% fortification of Moringa leaves powder), while the lowest calcium content 0.013 ± 0.002 was found in the F1 (0% fortification of Moringa leaves powder).

Bolarinwa et al. [21] reported that the Moringa seed powder was added to bread, there was a significant increase in phosphorus, potassium, calcium and iron contents. Considering the results of calcium analysis, it can be concluded that the more addition of Moringa leaves powder can increase the calcium content in otak-otak products of catfish. Sengev et al. [22] also reported that there was a significant increase in the magnesium, calcium and beta-carotene content as the Moringa leaves powder concentration increased in the bread samples. The fortification of biscuits with sesame, soy butter, dried moringa leaves and coconut powder resulted in to a superior quality of biscuits in terms of its protein, minerals specially calcium, flavonoid content and organoleptic acceptability [23]. These calcium rich of otak-otak products of catfish (Clarias sp.) may help in solving many nutritional deficiencies and can add variety to the food industry.
3.2.2 Proximate Composition Otak-otak Products of Catfish

The results obtained from the proximate analysis are protein 12.73%, water 39.64%, lipid 5.29%, ash 2.27% and carbohydrate 40.07%. Bolarinwa et al. [21] reported that the Moringa seed powder was added to bread, there was a significant increase in protein, ash, fat, fiber, and vitamin A contents. Yetunde and Damilola [24] also reported that the substitution of Sorghum-Maize with Moringa seeds powder was found to increase the protein, ash, moisture, fat, carbohydrate and fiber contents of the “Ogi products”.

The quality requirements of otak-otak products of catfish are based on [25], the protein at least 5%, the maximum water 60%, maximum lipid 16%, and maximum ash 2%, and there is no limit on carbohydrate on otak-otak products of catfish according to SNI. It can be concluded that the otak-otak products of catfish with fortified Moringa leaves powder have complied with the Indonesian National Standard (SNI) except for the ash content. Since the ash content is closely related to minerals in a food ingredient, while otak-otak products of catfish in this study contain calcium which is a macro mineral with a fairly high content of 1.195 mg, so that it can increase the ash content. The higher ash content in a product the higher mineral content.

Table 4. Proximate composition otak-otak products of catfish with fortification of Moringa leaves powder

| Proximate Composition | Value  | SNI (2013) |
|-----------------------|--------|------------|
| Protein (%)           | 12.73* | Min. 5.0** |
| Water (%)             | 39.64* | Max. 60.0**|
| Fat (%)               | 5.29*  | Max. 16.0**|
| Ash (%)               | 2.27*  | Max. 2.0** |
| Carbohydrate (%)      | 40.07* | -          |
| Calcium (mg)          | 1.195* | -          |

Sources:
*) Laboratorium Gizi Fakultas Kesehatan Masyarakat Universitas Airlangga
**) SNI [25]

4. Conclusion

The findings of the study revealed that fortification of protein and mineral rich ingredients in the development of otak-otak products of catfish (Clarias sp.) resulted in improved proximate specially calcium content as nutritional and functional properties without adversely affecting their sensory acceptability. The best fortification of Moringa oleifera leaves powder is 4% with the results in the form of an aroma of 41.00; taste 37.25; texture 38.00; and appearance of 38.05. Calcium (Ca) content in otak-otak products of Clarias sp. reached 3.09±0.099 mg. Proximate Compositions protein 12.73%; fat 5.29%; carbohydrate 40.07%; water 39.6%; and ash 2.27%. These calcium rich of otak-otak products of catfish (Clarias sp.) may help in solving many nutritional deficiencies and can add variety to the food industry.

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