ORIGINAL ARTICLE

The Effect of Nanotechnology on Nutrient Characteristic of Catfish (*Clarias gariepinus*) Flour: Ball Milling Method

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ABSTRACT

Introduction: Nanonization is a process nanotechnology to manipulate the size of particles to increase the bioavailability of the nutrition content in the body, especially the bioactive component. Ball milling is a simple technology to manipulate particles, including catfish flour. This study aimed to analyze the effect of the nanonization using ball miller on the nutrient properties of the catfish flour. **Methods:** This was an experimental study by trial and error to find the duration of the milling or grinding process using a ball miller (capacity: 500 grams) to get a particle size of the flour. Before and after the milling process, the proximate properties (water, ash, protein, fat, carbohydrate content) and amino acid profile were measured and analyzed the differences using paired sample t-test. **Results:** The catfish flour could reach the nano-size after 3 hours of milling with 120 rpm of speed. The size of catfish flour before milling was 1.012 nm, and after 3 hours of milling was 692,6 nm. The nutrient characteristics were significantly different, it reduced water and protein content but increased ash content. Moreover, the milling process increases the amino acid profile, especially essential amino acids. **Conclusion:** Ball miller can be used to change the particle size of catfish flour to nano size, it did affect the nutrient content.

Keywords: Ball milling, Catfish, Flour, Grinding, Nanonization

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INTRODUCTION

In the last few decades, nanotechnology has become one of the areas that have received great attention. One of them is in the food and nutrition sector. Several previous studies have shown that a nutrient with nanoparticle size has better utilization, absorption, and bioavailability than common-size nutrients (1-4). Nanotechnology is one type that focuses on the nanosize (1-100 nm), either particle or system. Nanotechnology includes everything in nanometer size and includes all processes of manipulating a particle size or system into nano size (5-6).

Ball milling is one of the processes in nanotechnology used in manipulating the size of a particle into a nanometer size (7). In principle, ball milling is a milling process that utilizes the rotational movement of a hollow cylinder or tube filled with milling balls. Milling balls that are usually used are made of steel, stainless steel, ceramic, or rubber. Due to its reputation as an economical and ecologically benign process, ball milling is frequently employed in the industrial sector (8). The benefits of the ball milling technology, according to Gorrasi and Sorrentinz (9) include cost-effectiveness, dependability, the convenience of use, consistent outcomes due to energy and speed control, and application in both wet and dry circumstances. However, there are some possible disadvantages, including contamination, noise, lengthy processing time, and the resulting uneven form of nanomaterials.

Catfish is a variety of freshwater fish that is found mainly in tropical waters. Catfish has a characteristic grey or silver color with a roughly cylindrical body, a largemouth, spines in front of the dorsal and/or pectoral fins, no scales, and barbels on the head (10). The African catfish is a type of catfish that is widely cultivated in Indonesia (Clarias gariepinus). African catfish are larger than most catfish (11). According to a study conducted by Oladipo and Bankole (12), the nutritional content of catfish is 17,50% protein, 6,55% fat, 1,02% crude fiber, and 70,35% water. Another study conducted in Indonesia shows that African catfish (Clarias gariepinus) contains 17,09% protein and 2,75% fat so it is categorized as a high-protein food with moderate fat content (13).

This study attempted to determine the impact of the ball milling process on nutrient characteristics., such as the macronutrient and amino acid profile of catfish (Clarias gariepinus) flour. The nanomaterials produced from this study are expected to increase the utilization of catfish flour nutrient content. Since the smaller the particle size, the larger the surface area of the particle. The smaller particle would make it easier to react with digestive enzymes and is more easily metabolized by the body (15). Furthermore, it can increase the use of local food to optimize the treatment of nutritional problems in Indonesia through functional food.

MATERIALS AND METHODS

Materials

The catfish flour used in this study is the body part of catfish (Clarias gariepinus) flour produced by PT Carmelitha Lestari.

Nano Flour Manufacturing Process

The manufacturing process of nano flour was conducted in the Research Center of Nanoscience and Nanotechnology, Bandung Institute of Technology. Nano catfish flour in this study was processed through a ball milling process. The instrument used in this nano flour manufacturing process is a Prolabo ball miller. Catfish flour was ground using milling balls which are put into a porcelain tube. A total of 500 grams of catfish flour and several milling balls were put into a porcelain tube, then rotated the porcelain tube at a speed of 120 rpm for 3 hours. The duration of the milling process is determined by trial and error during the study based on the modification of the Zhang method (16).

Chemical Analysis

1. Proximate

The macronutrient content of catfish flour was determined using proximate analysis, which included water, ash, protein, fat, and carbohydrate content. It was conducted at Balai Besar Industri Agro (BBIA) and Saraswanti Indo Genetech. SNI 01-2891-1992 was used as the proximate analysis guidelines for water content (point 5.1), ash content (point 6.1), protein content (point 7.1), and fat content (point 8). The gravimetric method was used to determine fat content and the Kjeldahl method was used to determine protein content. Meanwhile, the carbohydrate content was determined using the by difference method, as described in SNI 01-3775-2006 appendix point B.6.

2. Ultra-Performance Liquid Chromatography (UPLC)

The Amino acid profiles of catfish flour were analyzed using the UPLC at the SIG Laboratory, Bogor, Indonesia. The flour was weighed as much as 1 gram, put into a 20 ml headspace vial, and added HCl solution to hydrolyze the sample protein. Afterward, the sample was transferred to a 50 ml volumetric flask to be added aquabides up to the marked line and homogenized to become a filtrate. The filtrate was collected using a 0,2 µm syringe filter and added an internal standard of amino acid, then derivatization of the sample. The sample was ready to be injected into the UPLC system. The amino acid profile of the sample was obtained from the interpretation using a ratio of the analyte compared with the standard internal area. The formula used is as follows:

$$AA = \frac{(L_1/L_2) \times (C\ std/10^6) \times BM \times Va \times fp}{W\ sample\ or\ V\ sample}$$

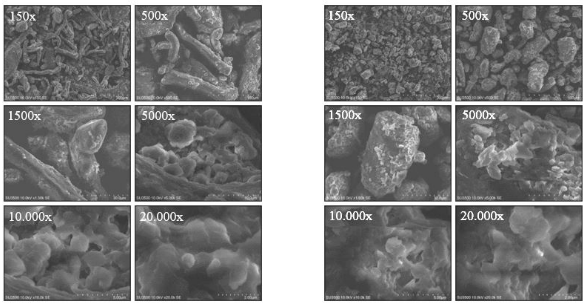
AA: amino acids content (mg/kg)
L_1: area of amino acids analyte
L_2: area of standard analyte
C std: concentration of amino acids standard solution (pmol/µl)
BM: molecular weight of amino acids
Va: final sample volume (µl)
fp: dilution factor
W sample: sample weight (g)
V sample: sample pipetting volume (ml)

Statistical Analysis

Univariate and bivariate analyses were used in this study's statistical analysis. To describe the average and standard deviation of the data, a univariate analysis was used. The difference in nutritional content of catfish flour before and after the ball milling process was determined using bivariate analysis and the paired sample t-test.

RESULT

Nanotechnology is a technology related to particles or systems with a nano-size, usually 1-100 nm (5,6). The size of catfish flour before milling was 1.012 nm in this study, and after 3 hours of milling was 692,6 nm, as measured by Scanning Electron Microscope (SEM). The particle before milling has a larger size than the particle after milling (presented in Figure 1). The nutritional content of catfish flour showed in Table I. The water content before milling was 7,23 % and decrease significantly after milling become 5,71%. As well as the protein content, it decreased from 67,54% to 61,02%. However, ash content increased from 10,91% to 17,74%.



(a) (b)

Figure 1. Particle morphology of Catfish Flour (Scanning Electron Microscope) (a) catfish flour before milling and (b) catfish flour after milling. The catfish flour before milling has a larger size than after milling. The picture presents the results of measurements using SEM with 150x, 500x, 1500x, 5000x, 10.000x, 20.000x of magnification

Table I Catfish flour macronutrient content

Flour type	Water (%)	Ash (%)	Total Fat (%)	Protein (%)	Carbohydrate (%)
			Catfish flour		
Non-nano§	7,23 ± 0,02	10,91 ± 0,04	12,73± 0,05	67,54± 0,11	1,59 ± 0,19
Nano	5,71 ± 0,08	17,74 ± 0,01	12,76 ± 0,01	61,02 ± 0,16	2,78 ± 0,23
p value	0,023*	0,000*	0,205	0,011*	0,085

§Commercial product of catfish flour. Nano catfish flour resulting from the ball-milling process is presented in the mean ± standard deviation. Different test: Paired Sample T-Test. *significant p < 0.05.

Table II shows that the amino acid content of non-nano and nano catfish flour differs significantly (p < 0,05) for seven essential amino acids and six non-essential amino acids. Meanwhile, two other amino acids (isoleucine and alanine) did not differ significantly (p > 0,05), and three types of amino acids are not available (NA) in non-nano catfish flour products. When compared to non-nano catfish flour, nano catfish flour has a better composition and amino acid content. This is evident in nano catfish flour, which contains significantly more amino acids, both essential and non-essential.

Table II Amino acids profile of catfish flour before and after milling

Nutrient	Catfish flour		p value
	Before Milling§	After Milling	
Essential Amino Acids (ppm)			
Phenylalanine	28000 ± 156,89	34847,87 ± 230,91	0,015*
Isoleucine	27000 ± 130,11	27429,83 ± 142,88	0,147
Valin	27000 ± 212,18	29601,64 ± 210,02	0,036*
Lysine	41000 ± 317,12	45456,62 ± 307,63	0,031*
Leucine	41000 ± 230,91	49557,78 ± 169,54	0,009*
Threonine	25000 ± 220,24	37895,07 ± 207,82	0,007*

CONTINUE

Table II Amino acids profile of catfish flour before and after milling (CONT.)

Nutrient	Catfish flour		p value
	Before Milling§	After Milling	
Essential Amino Acids (ppm)			
Histidine	13000 ± 98,44	18121,40 ± 83,82	0,007*
Methionine	19000 ± 16,69	7599,58 ± 5,85	0,000*
Tryptophan	NA	4976,38 ± 6,56	NA
Non-Essential Amino Acids (ppm)			
Serine	24000 ± 178,72	32637,46 ± 195,90	0,010*
Glutamic acid	93000 ± 450,08	78988,97 ± 522,05	0,017*
Alanine	32000 ± 277,38	32266,40 ± 218,35	0,334
Arginine	34000 ± 80,44	41699,13 ± 89,08	0,005*
Glycine	18000 ± 168,51	41577,94 ± 260,48	0,005*
Aspartic acid	59000 ± 351,89	48828,25 ± 237,68	0,011*
Tyrosine	21000 ± 188,02	27791,04 ± 178,32	0,012*
Proline	NA	26115,41 ± 139,46	NA
Cystine	NA	19016,61 ± 13,60	NA

§Commercial product of catfish flour. Nano catfish flour resulting from the ball-milling process is presented in the mean ± standard deviation. NA: not available data. Different test: Paired Sample T-Test. *significant p < 0.05.

DISCUSSION

The smaller particle will increase the psycho chemistry characteristic, especially its utilization. However, the disadvantage of the milling process is the occurrence of physical collisions that can destroy molecules such as protein (17). Therefore, the number of the large molecule may decrease, but the utilization and the bioavailability can increase.

Oliveira et al. (18) stated that Brazilian catfish body flour contains 12% water, 3,95% ash, 76,16% protein, 7,72% fat, and 0,17% carbohydrates. In comparison, another study conducted in Indonesia by Agustia et al.2154 showed that catfish flour contained water, fat, protein, ash, and carbohydrates as much as 6,73%, 17,59%, 66,25%, 4,36%, and 4,86%, respectively. In this study, the non-nano catfish flour had almost the same water and protein content as Agustia et al. (19). Compared with the two previous studies, the fat and carbohydrate content were between the two studies, and the ash content was higher than the two studies. Based on the nutritional content of catfish flour in this study and those previous studies, catfish flour was classified as a food ingredient high in protein and fat content.

The high content of macronutrients in catfish flour shows that catfish flour used as an alternative ingredient is considered a functional food product. The nutritional quality of a functional food can increase, and it can overcome malnutrition problems in Indonesia. In this study, non-nano catfish flour contains significantly higher water and protein than nano catfish flour. In contrast, nano catfish flour had significantly higher ash content than non-nano catfish flour (Table I).

According to previous research, the nutritional content of a food ingredient, particularly flour, is greatly influenced by its particle size. According to Shafi et al. (20) and Ahmed et al. (21), decreasing flour particle size can significantly reduce water content. Another study (22) found that the water content of rye and barley flour was lower at smaller particle sizes. This assertion is consistent with the findings of this study on the water content of catfish flour. Temperature during the milling process may be to blame for the decreasing water content in nano catfish flour. During the milling process, milling balls in the ball milling method will collide and rub against each other. It will cause a mechanochemical reaction that releases energy in the form of heat and raises the temperature of the system. Increasing the system temperature causes the flour-water content to evaporate (24,25).

The ash content in food can be assumed as the amount of its mineral content. In this study, the ash content of the catfish flour significantly increased in the nano flour. This result is similar to the previous studies where mesquite (Prosopis alba) flour (26) and water chestnut flour with smaller particle sizes contained significantly higher ash. Other studies by Coda et al. (27) and Kim and Shin (28) showed no significant difference between the ash content of flours with different particle sizes. Still, there was a trend that flours with smaller particle sizes had higher ash content.

Aside from water and ash content, the protein content of catfish flour differed significantly between non-nano flour and nano flour (Table 1). The protein content of nano catfish flour was significantly lower than that of non-nano flour, according to the results of the analysis. This finding is consistent with previous research, which found that water chestnut flour (23) with smaller particle size contains significantly less protein and fat. Other studies on wheat flour by Hanif et al. (29) produced similar results, with the protein content of flour decreasing as the particle size of wheat flour decreased. A previous study found that catfish meat has a good amino acid composition because it contains all of the essential amino acids that the body requires, such as histidine, threonine, valine, methionine, leucine, isoleucine, phenylalanine, and lysine (14).

Some studies declare that materials with smaller particle sizes will have a larger surface area. It will affect the digestion, absorption, and bioavailability of these materials in the body and affect the solubility of these materials (1-4). Research conducted by Coda et al. (27) showed that the smaller the wheat bran fraction, the higher the amino acid content. That result is in line with several analysis results in this study, where the nutrients in catfish flour were significantly higher in nanoparticle size. However, in this study, glutamic acid and aspartic acid on non-nano flour were higher than in the nanoparticles. It may cause by the characteristic of those amino acids; both are polar amino acids and will destroy by the mechanical collision. The excess amount of these amino acids could increase free radicals and will harm the human body by cellular oxidative stress (30).

Many studies focused on the effect of nano-size on its absorption and bioavailability. However, the research regarding the impact of the milling process on the nutritional content (both macro and micronutrient) of the flour still needs to be elaborate, especially on different flour. Moreover, it will be complete if there is a study to find the effect of the milling duration on the nutritional content and explain its mechanism.

CONCLUSION

Catfish flour takes 3 hours to be processed in a ball miller to get nanoparticle size (692,6 nm). There is a differentiation in the nutrient content of the flour before and after the milling. The ash and water content increased, but the protein decreased.
In the amino acid content, the whole amino acid of catfish flour after milling increased, except for glutamic acid and aspartic acid.

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