Application of Nano Technology on Liquid Food Formula Containing Catfish (*Clarias gariepinus*) Flour and *Moringa oleifera* Leaves Flour

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Abstract: Nanotechnology can manipulate the size of the particles into nanometers (1 – 1000 nm). Studies show that the benefits of nanotechnology in increasing the bioavailability of nutrients have been proven, but their effect on nutrient content has not been widely studied. This study aims to analyze the influence of nanotechnology on the nutritional and amino acid contents of liquid food formula containing catfish flour and Moringa leaves flour. The nanoparticles making process of catfish flour and Moringa leaves flour was carried out using the ball-milling method. Liquid food formula containing catfish flour and Moringa leaves flour is made by a combination of vacuum drying and dry mixing methods. Macronutrient content was obtained from the proximate analysis, mineral content was obtained from ICP-OES analysis, and amino acid content was obtained from UPLC analysis. Statistical analysis was performed using the ANOVA test to determine the difference in the nutritional content of nano liquid food formula and non-nano liquid food formula. The water, Fe, Zn, Se, and Cu content of non-nano liquid food formula was significantly higher than that of nano liquid food formula, whereas the carbohydrate content of nano liquid food formula was significantly higher ($p < 0,05$). Nano liquid food formula had significantly higher amino acid content of serine, glutamic acid, valine, alanine, lysine, aspartic acid, leucine, cysteine, methionine, and tryptophan, while the amino acid content of phenylalanine, arginine, glycine, tyrosine, threonine, and histidine was significantly higher in the non-nano liquid food formula ($p < 0,05$). The nanotechnology process gives different effects on the characteristics of nutrients, both in non-nano and nano instant liquid food containing catfish flour and Moringa leaves flour.

1 INTRODUCTION

The US National Nanotechnology states that nanotechnology is related to nanometer-sized particles or systems (generally 1-100 nm). Nanotechnology includes the process of manipulating a system or functional material such as molecules or atoms into nanoscale (Riehemann *et al*, 2009; Srinivas *et al*, 2010). The discovery of nanotechnology has a great influence in the field of food and nutrition.

The nano size of a nutrient will affect the surface area to increase the absorption and bioavailability of nutrients (Wajda, 2007; Pereira, 2014; Fathi *et al*, 2012; Sonkari *et al*, 2012). Moreover, nanotechnology can also affect the physical and chemical characteristics of food (He and Hwang, 2016). In this study, nanotechnology was applied in the making process of Moringa leaves flour and catfish flour as the basic ingredients for making liquid food formulas.

Moringa (*Moringa oleifera*) is a plant that grows in tropical and subtropical countries. Moringa is

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known to have many health benefits, so it is widely used as medicine, functional food, and nutraceutical (Sreelatha and Padma, 2009; Rodriguez-Perez *et al*, 2015; Nascimento *et al*, 2017; Saucedo-Pompa *et al*, 2018). One part of Moringa that is widely used is the leaves. Moringa leaves are known to be high in antioxidants (Oka *et al*, 2016), protein, and essential amino acids (Sanchez-Machado *et al*, 2010; Castillo-Lopez *et al*, 2017; Nascimento *et al*, 2017). Other than that, Moringa leaves also contain high levels of bioactive compounds such as phenolics and flavonoids (Ferreira *et al*, 2008; Sreelatha and Padma, 2009; Devisetti *et al*, 2015; Rodriguez-Perez *et al*, 2015; Nascimento *et al*, 2017; Saucedo-Pompa *et al*, 2018; Paramo-Calderon *et al*, 2019). The high content of bioactive compounds makes Moringa leaves a potential ingredient in the treatment of inflammation and infection (Sreelatha and Padma, 2009).

Research by Castillo-Lopez *et al* (2017) stated that Moringa leaves contain 36,83% protein, 8,16% fat, 6,56% ash, 3,79% water, 41,29% carbohydrates, and crude fiber 3,37%. Furthermore, Moringa leaves also contain essential amino acids such as histidine, tyrosine, methionine, phenylalanine, isoleucine, leucine, and lysine. Moringa leaves can be consumed fresh or processed and can be made in the form of flour. Moringa leaves flour can be used as an alternative material with a high content of bioactive compounds to improve the nutritional quality of food products (Paramo-Calderon *et al*, 2019). According to research by Devisetti *et al* (2015), Moringa leaves flour contains 28,9% protein, 4,8% fat, 7,9% ash, 27,2% carbohydrates, and 31,2% dietary fiber.

African catfish (*Clarias gariepinus*) is a type of catfish with a larger size than common catfish. Catfish is one type of freshwater fish high in protein content (Chan M and Chan G, 2009). Catfish can be processed into flour to increase shelf life and increase utilization. Mervina *et al* (2012) showed that catfish body flour contains 63,83% protein, 10,83% fat, and 20,51% carbohydrates.

In this study, Moringa leaves flour and catfish flour are processed into high protein liquid food. Liquid food is food with a liquid to a thick consistency that is given orally or parenterally. Usually, liquid food is given to someone who has chewing, swallowing, and digesting food problems due to certain physiological conditions (Almatsier 2004). Previous studies have developed liquid food products by adding catfish flour to improve protein quality (Huda 2014; Wibisono 2015; Faidaturrosyida 2017) and adding Moringa leaves flour to increase minerals content (Marta 2017). The combination of Moringa leaves flour and catfish flour is expected to improve nutritional quality of liquid food.

The novelty in this study is the application of nanotechnology to liquid food ingredients, namely catfish flour and Moringa leaves flour. The use of nanotechnology in basic ingredients is expected to increase the absorption of nutrients and bioactive compounds contained in these materials. The objective of this study was to examine the differences in the chemical characteristics of liquid food containing Moringa leaves flour and catfish flour without and with nanotechnology.

2 MATERIALS AND METHODS

2.1 Nano Flour Manufacturing Process

The manufacturing process of nano Moringa leaves flour and nano catfish flour is carried out at Research Center of Nanoscience and Nanotechnology ITB. The ball-milling method was the type of nanotechnology used in the manufacturing process of nano Moringa leaves flour and nano catfish flour. This process uses an instrument called a ball-miller were in this study using the Prolabo ball-miller. The consideration in choosing this method were because the particle that will be resized was a flour, and it was a dry particle.

As much as 500 g of catfish flour was put into a porcelain tube and then put the porcelain balls into the tube. Turn on the porcelain tube rotation process at a speed of 120 rpm. Catfish flour milled for 3 hours. For Moringa leaves flour, the milling process was alike. But the amount of flour put into the porcelain tube is 250 g. Moringa leaves flour milling process is 6 hours. That time was determined through a trial error during the research.

2.2 Liquid Food Production

The liquid food production is carried out at the SEAFAST Center IPB and Laboratory of Department Nutrition IPB. The liquid food formula applied in this study was developed from a previous study by Marta (2017). The ingredients used in the production of liquid food, both non-nano and nano, are presented in Table 1.

Table 1: Liquid Food Formula Ingredients for 1 L.

Ingredient	Unit	Quantity
Catfish flour	g	45
Soya flour	g	24
Moringa leaves flour	g	18
Egg white flour	g	18
Full cream milk flour	g	48
Skimmed milk flour	g	60
Catfish oil	g	7,5
Olive oil	g	12
Salt	g	1,5
Refined sugar	g	44
Pandan flavor	ml	2
Water	ml	800
Lemon juice	ml	10
Mineral Mix		
Selenium (Se)	mg	404,58
Zinc (Zn)	mg	161,23

The first step in making liquid food is to mix soya flour, egg white flour, catfish oil, olive oil, salt, and water until an emulsion is formed. After that, boil the emulsion with Moringa leaves flour, catfish flour, pandan flavor, and lemon juice until it boils. The results of the boiling process will produce materials in liquid form.

To maintain and store liquid food quality before the chemical characteristic analysis is done, a vacuum drying process is carried out so that liquid food is obtained in the form of instant powder. Vacuum drying is carried out at temperature 50 ºC. The time required for vacuum drying 1 L of liquid food is 30 minutes to convert liquid food products into flakes form. The flakes form is then ground until a smooth texture like flour is obtained.

The last stage is a dry mixing process to mix skimmed milk flour, full cream milk flour, refined sugar, and mineral mix (Se and Zn) with product results from the vacuum drying process. The dry mixing process is carried out for 5 minutes per 1 kg instant liquid food powder.

2.3 Chemical Characteristic Analysis

The chemical characteristic analysis of non-nano and nano liquid food is carried out at Balai Besar Industri Agro (BBIA) Laboratory and Saraswati Indo Genetech (SIG) Laboratory. The analyzes performed in this study included proximate analysis, Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES), and Ultra Performance Liquid Chromatography (UPLC).

2.3.1 Proximate Analysis

Proximate analysis was conducted to examine the water, ash, fat, protein, and carbohydrate content of liquid food, both non-nano and nano. The water content was analyzed according to SNI 01-28911992 point 5.1, the ash content was analyzed according to SNI 01-2891-1992 point 6.1, the fat content was analyzed based on the Gravimetric method according to SNI 01-2891-1992 point 8, the protein content was analyzed based on the Kjeldahl method according to SNI 01- 2891-1992 point 7.1, and the carbohydrate content was analyzed based on the by difference method according to SNI 01-3775-2006 Appendix point 8. The results of the proximate analysis are presented in percent units.

2.3.2 Minerals Analysis

ICP-OES analysis was conducted to examine the minerals, namely iron (Fe), zinc (Zn), selenium (Se), copper (Cu) of liquid food. The results of the ICP-OES analysis are presented in mg/kg units. Sample preparation by mixing 0,5-1 grams of liquid food samples with 10 ml of concentrated HNO_3 in a vessel. Hereafter is the solution destruction using microwave digestion (ramp to 150 ºC for 10 minutes and hold at 150 ºC for 15 minutes). Move the destructed product to a 50 ml volumetric flask and then add 0,5 ml of the internal standard yttrium 100 mg/L. Dilute with aquabides to the mark, then homogenize and filter with filter paper.

Examine the sample solution in the ICP-OES system at a wavelength of 238,2 nm for Fe, 213,9 nm for Zn, 196 nm for Se, and 327,4 nm for Cu. After was the results obtained, interpret the results using a standard calibration curve with the equation:

$$Y = bx + a \quad (1)$$

with the following formula:

$$TE = \frac{(\text{Int sample} - a) / b \times V \times fp}{\text{W sample or V sample}} \quad (2)$$

TE : trace element contents (mg/kg)
Int sample : sample intensity ICP-OES
a : intercept standard calibration curve
b : slope standard calibration curve
fp : sample dilution factor
V : final sample flask volume (ml)
W sample : sample weight (g)
V sample : sample pipetting volume (ml)

2.3.3 Amino Acids Profile Analysis

UPLC analysis was conducted to examine the amino acids profile of liquid food. The results of the UPLC analysis are presented in mg/kg units. UPLC analysis begins with the preparation of 1 point concentration of standard amino acids using internal standards. After that, sample preparation by weighing 1 gram of sample into a 20 ml headspace vial then hydrolysis with HCl solution. The hydrolyzed sample was then transferred to a 50 ml volumetric flask. Add aquabides to the mark and then homogenize. Filter the solution with a 0,2 m syringe filter and collect the resulting filtrate. Add standard internals and then derivatization of the sample. Next, inject the solution into the UPLC system.

The amino acid content of the sample was obtained from the interpretation using a ratio of the analyte area with the internal standard area. The formula used is as follows:

$$AA = \frac{(L_1/L_2) \times (C\ std/10^6) \times BM \times Va \times fp}{W\ sample\ or\ V\ sample} \quad (3)$$

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)

2.4 Statistical Analysis

Statistical data analysis was performed using SPSS version 23.0 for windows. Nutritional content data of liquid foods including protein, fat, carbohydrates, ash, water, minerals such as Fe, Zn, Se, Cu, and amino acids were analyzed using the one-way ANOVA test to determine the difference in the average of nutrient content between the non-nano liquid food product and nano liquid food product.

3 RESULT AND DISCUSSION

3.1 Proximate and Minerals

In this study, nanotechnology was applied to the raw ingredients, namely Moringa leaves flour and catfish flour. The liquid food examined in this study consisted of two formulas which are non-nano liquid food and nano liquid food. The analysis of the nutritional content of non-nano liquid foods and nano liquid foods is presented in Table 2 and Table 3.

Table 2: Proximate and Mineral Content of Instant Non-nano and Nano Liquid Food.

Nutrient	Non-Nano	Nano	*p value*
Water (%)	5,79	3,17	0,023*
Ash (%)	4,84	5,32	0,101
Fat (%)	13,99	11,70	0,452
Protein (%)	28,25	26,9	0,137
Carbohydrate (%)	46,15	52,9	0,048*
Fe (mg/kg)	7,39	2,33	0,003*
Zn (mg/kg)	3,45	< 0,004	0,016*
Se (mg/kg)	56,56	47,10	0,035*
Cu (mg/kg)	35,58	0,45	0,001*

Data are presented in mean ± SD. One-way ANOVA test. *significant ($p < 0,05$).

Previous research by Marta (2017) stated that the modified instant liquid food with catfish flour and Moringa leaves flour have 5,82% water, 3,74% ash, 4,09% fat, 15,69% protein, and 70,67% carbohydrates contents. Meanwhile, another study by Faidaturrosyida (2017) stated that the nutritional content of instant liquid food with the addition of catfish flour was 5,68% water, 3,71% ash, 9,27% fat, 15,41% protein, and 65,94% carbohydrates. The results in Table 2 show that the non-nano instant liquid food in this study had almost the same water content, higher ash, fat, and protein content and had lower carbohydrate content compared to the two studies.

In this study, the water content of non-nano liquid food was significantly higher than that of nano liquid food ($p < 0,05$). The studies by Shafi *et al* (2017) and Ahmed *et al* (2016) showed that the smaller the particle size of water chestnut flour, the lower the water content even though the difference is not significant. Lower water content in nano liquid food maybe due to the lower water content in the nano flour ingredients. The quality and nutritional content of a food product are greatly influenced by the quality and nutritional content of the ingredients used (Liu et al, 2018).

This is maybe due to the temperature during the manufacturing process of nano Moringa leaves flour and nano catfish flour using the ball-milling method. Schmidt *et al* (2016) and Takacs and McHenry (2006) state that the ball-milling process would cause a mechanochemical reaction that produced energy in the form of heat. This happens because of a mechanochemical reaction wherein the collision and friction between the milling balls in the system produce heat. This reaction will affect the sample's water content because the water content is susceptible to heat. It was discovered from those two studies that the longer the ball-milling process was carried out, the hotter the ball-milling system and milling balls used.

In contrast, nano-liquid food carbohydrate content was significantly higher than non-nano liquid food ($p < 0,05$) (Table 2). This means that the smaller particle size of Moringa leaves flour and catfish flour can increase the carbohydrate content of liquid food. A previous study conducted by Zucco *et al* (2011) shows that fine flour has a higher carbohydrate content than coarse flour in green lentil flour, navy bean flour, and Pinto bean flour. Carbohydrates consist of simple carbohydrates and complex carbohydrates. Leaves, one of which is Moringa leaves, is a type of ingredient that contains high amounts of complex carbohydrates, particularly fiber (Almatsier, 2010). The previous study on quinoa flour showed that the particle size of flour greatly affected the fiber content, both crude fiber and dietary fiber. Crude fiber and dietary fiber was increased significantly with the reduction of particle size of quinoa flour (Ahmed *et al*, 2018).

In this study, fat, protein, and ash content did not differ significantly between non-nano liquid food and nano liquid food ($p > 0,05$). Like water and carbohydrates contents, the other nutritional content of liquid foods such as protein, fat, and ash may also be affected by the nutritional content of flour with different sizes. The results of previous studies showed that flour with a smaller particle size contains lower protein (Shafi *et al*, 2017; Ahmed *et al*, 2016; Hanif *et al*, 2014) and lower fat (Shafi *et al*, 2017; Ahmed *et al*, 2016), while the ash content (Kim and Shin 2014; Rodriguez *et al*, 2019) will increase as the particle size of flour decreases.

Mineral content (Fe, Zn, Se, Cu) of non-nano liquid food were significantly higher than nano liquid food ($p < 0,05$). The mineral content of the liquid food in this study may also be influenced by the mineral content of the ingredients used in the liquid food formula. Shafi *et al* (2017) stated that the particle size of water chestnut flour greatly affects the mineral content of flour. The results showed that the mineral content of water chestnut flour significantly decreased along with the decrease in the particle size of the flour. Another study by Rodriguez *et al* (2019) showed that the content of Cu mesquite (Prosopis alba) flour would decrease if the flour particle size decreased. However, the Fe and Zn contents tend not to be affected by the particle size of the mesquite flour. The trend differences in the mineral content of those two studies may be due to the ingredients that were used to make the flour. However, if observed from the results of this study and two previous studies by Shafi *et al* (2017) and Rodriguez *et al* (2019), it can be assumed that the smaller the particle size of flour or ingredient, the lower its mineral content. Further research is needed to determine the effect of particle size on the mineral content of ingredients, especially essential minerals so the use of nanotechnology in the food and nutrition sector can be optimized.

3.2 Amino Acids

Moringa leaves are known to have high amino acid content, especially essential amino acids (Sanchez-Machado *et al*, 2010; Castillo-Lopez *et al*, 2017; Nascimento *et al*, 2017). The amino acid profile analysis results showed that almost all amino acid content of non-nano liquid food and nano liquid food had significant differences ($p < 0,05$). From the 18 types of amino acids analyzed, only two types of amino acids do not have significant differences, i.e. isoleucine (Ile) and proline (Pro) (Table 3).

Table 3: Amino acid content of instant non-nano and nano liquid food (mg/kg).

Amino acids	Non-Nano	Nano	Difference (%)	*p value*
Essential				
Histidine (His)	13239,17 ± 146,14	8142,54 ± 61,67	-38,50	0,000*
Lysine (Lys)	17880,31 ± 68,66	27003,98 ± 290,15	51,03	0,001*
Leucine (Leu)	26303,22 ± 130,74	27440,49 ± 219,24	4,32	0,024*
Isoleucine (Ile)	15773,91 ± 54,86	16240,03 ± 176,82	2,96	0,071
Methionine (Met)	9690,72 ± 51,39	10651,07 ± 6,00	9,91	0,001*
Valine (Val)	17572,73 ± 70,89	18617,62 ± 61,77	5,95	0,004*
Threonine (Thr)	16200,85 ± 25,84	12802,65 ± 184,73	-20,98	0,007*
Phenylalanine (Phe)	24899,29 ± 170,63	15913,44 ± 46,36	-36,09	0,000*
Tryptophan (Trp)	2934,12 ± 12,57	3508,47 ± 23,84	19,57	0,001*
Non-Essential				
Alanine (Ala)	13064,39 ± 34,03	15352,45 ± 121,04	17,51	0,002*
Arginine (Arg)	20938,84 ± 89,02	14247,31 ± 130,01	-31,96	0,000*
Aspartic acid (Asp)	22519,61 ± 162,30	30766,96 ± 222,53	36,62	0,001*
Glutamic acid (Glu)	41543,44 ± 133,33	56648,93 ± 524,35	36,36	0,001*
Glycine (Gly)	12726,31 ± 72,44	11305,69 ± 87,58	-11,16	0,003*
Serine (Ser)	17414,62 ± 72,53	18029,26 ± 123,69	3,53	0,026*
Proline (Pro)	16185,55 ± 28,69	16282,95 ± 149,37	0,60	0,461
Cysteine (Cys)	1258,09 ± 14,14	2941,31 ± 0,95	133,79	0,000*
Tyrosine (Tyr)	15216,92 ± 80,53	8710,19 ± 62,81	-42,76	0,000*

Data are presented in mean ± SD. One-way ANOVA test. Difference in (-) means decreased its amino acids content in nano liquid food. *significant ($p < 0,05$).

Instant non-nano liquid food with Moringa leaves flour and catfish flour in this study contained higher amino acids than a similar study conducted by Kusharto *et al* (2018). The highest amino acid content of non-nano instant liquid food in this study was leucine (Leu), while in the previous study by Kusharto *et al* (2018) the highest amino acid content was in glutamic acid (Glu). Meanwhile, in both the previous and this study, amino acid cycteine (Cys) is the lowest amino acid content in the liquid food product.

In Table 3, non-nano liquid food significantly contains six amino acids, i.e. phenylalanine (Phe), arginine (Arg), glycine (Gly), tyrosine (Tyr), threonine (Thr), and histidine (His), which is higher than nano liquid food. Meanwhile, nano liquid food has ten amino acids content which is significantly higher than non-nano liquid food. These amino acids are serine (Ser), glutamic acid (Glu), valine (Val), alanine (Ala), lysine (Lys), aspartic acid (Asp), leucine (Leu), cysteine (Cys), methionine (Met), and tryptophan (Trp). It can be assumed that nano liquid food has a higher amino acid content than non-nano liquid food, both essential and non-essential amino acids.

The amino acid content of lysine in nano liquid food increased by 51,03% (9123,67 mg/kg) than its content in non-nano liquid food. The essential amino acid whose content decreased the most in nano liquid food was histidine, which decreased by 38,5% (5096,63 mg/kg). As for the non-essential amino acid content of nano liquid food, cysteine increased the most as much as 133,79% (1683,22 mg/kg) and tyrosine was the most decreased amino acid by 42,76% (6506,73 mg/kg). Liu *et al* (2018) state that the nutritional content of a food product is largely determined by the materials used in the production process.

Coda *et al* (2014) conducted a study related to the differences in the amino acid content of the wheat bran fraction with different particle sizes of 750, 400, 160, and 50 µm. The results showed that the highest amino acid content was in the particle size of 50-160 µm. It shows that the smaller the particle size, the higher the amino acid content of the wheat bran fraction. Amino acids glycine, cysteine, tyrosine, phenylalanine, histidine, lysine, and proline have the highest amino acid content at a particle size of 50 µm. Meanwhile, the other amino acids have the highest amino acid content at 160 µm.

4 CONCLUSIONS

Nanotechnology has different effects on nutrient characteristics of instant liquid food containing Moringa leaves flour and catfish flour, both nano and non-nano. Significant differences between the nutritional content of non-nano and nano instant liquid foods are found in water, carbohydrates, minerals such as Fe, Zn, Se, and Cu, and also almost all amino acids content. The results obtained in this study are influenced by the ingredients used in the instant liquid food production process, especially Moringa leaves flour and catfish flour. Several studies have shown that the particle size of flour greatly affects the nutritional content of various flours. Further research is needed regarding the effects of nanotechnology on Moringa leaves flour, catfish flour, and their products so that the utilization of local food can be more optimal.

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