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# Moringa Oleifera as a Substitute of Nitrogen (N) in Nata De Coco Production

*Payung* Layuk*, *Elisa* Winanda, *Joula* Sondakh, and *Meivie* Lintang
National Research and Innovation Agency. Indonesia

**Abstract.** Nata de coco is a fermented product by Acetobacter xylium. The formation of nata requires a source of Carbon (C) and Nitrogen (N). The nitrogen used in the production such as Urea or Ammonium Sulfate (ZA) causes some people to feel reluctant to consume nata de coco. One of the natural protein sources that have high nitrogen content is Moringa Leaf (Moringa oleifera). The research aims to determine the appropriate concentration of Moringa leaf extract to obtain nata de coco with good physical properties and favored by consumers. The research method used was completely randomized design consisting of four replications and two treatments. The treatment consisted of concentration of Moringa leaf extract and Amonium Zulfat (ZA), which were concentrated as follows 2.5%, 5%, 7.5 % and 10%. Analysis was carried out on the yield, thickness, crude fiber, and organoleptic tests for the best treatment (color, aroma, taste and texture) from 20 semi-trained panelists. The results showed the treatment with 10% of Moringa leaves concentration and Amonium Zulfat (ZA) were the best treatment with yield of 85.11%, thickness 0.66 cm and crude fiber 17.26% and the organoleptic test score were 3-4 (neutral-like)

## 1 Introduction

Nata de coco is a cellulose compound *(dietary fiber)*, which is produced from coconut water through a fermentation process involving the microbe Acetobacter xylinum. These bacteria will convert glucose into cellulose. This interwoven cellulose is what makes nata a white solid sheet. Nata is a food that has a gel-like shape with a chewy texture, solid, white colored, and slightly transparent. Nata is usually used as a dessert or as canned food mixed with fresh fruit [1].

Coconut water used as raw material in the production of nata de coco is a by-product of copra that has not been used optimally. Coconut water already contains Carbon and Nitrogen but only available in insufficient quantities therefore it needs to be added for the growth of *Acetobacter xylinum* [2,3]. *Acetobacter xylinum* will form nata if it is grown in coconut water which has been enriched with carbon (C) and nitrogen (N) through a controlled process. If the ratio of C and N is set optimally and the process is well controlled, then all liquids will turn into nata without leaving any residue (Zerro residual substrate). The addition of urea will produce a higher yield which means that *Acetobacter xylinum* requires a nitrogen source for

* Corresponding author: playuk21@gmail.com

cellulose biosynthesis. The nitrogen sources used in the production of nata de coco generally use inorganic nitrogen sources which cause many pros and cons, especially related to food safety issues when this product is consumed [5]. The carbon source added is generally sugar (sucrose) while the nitrogen source is in the form of urea and ammonium sulfate (ZA) [5,6,7,8]. *Acetobacter xylinum* bacteria which are aerobic will grow optimally at a temperature of 28ºC at a pH of 3.5 to 7.5 [9,10]. The use of sugar in nata will affect the fermentation process because sugar is a carbon source for nata and some of the sugar used will be synthesized into cellulose and acid [11]. Sugar levels that are too high in fermentation can slow down the metabolism of *Acetobacter bacteria*, this occurs because the growth of microorganisms is influenced by the concentration of solutes in the solution [12]. The growth of nata-forming bacteria requires a medium acidity level (pH 4.3), carbon and nitrogen source, starter concentration and fermentation time of 7-10 days if the fermentation is too long it will also affect the taste [13].

The source of nitrogen in nata fermentation is one of the important factors as nutrients for the growth of *Acetobacter xylinum*. The inorganic nitrogen source used in general are urea and ZA because it is easy to obtain and inexpensive. However, it becomes an issue because the material is intended for plants not for food products. Utilization of organic compounds as a nitrogen source has not been widely carried out. Nitrogen is needed to activate the extracellular enzymes of the *Acetobacter xylinum*. Researchers are looking for an alternative source of Nitrogren as a substitute for inorganic nitrogen in the production of nata de coco. The results of the study [14] showed that organic nitrogen such as tofu liquid waste, mung bean sprouts and *Azolla microphylla* could be an alternative to inorganic nitrogen sources (urea, ZA) which are commonly used in the production of nata de coco. These materials can be ascertained to be more environmentally friendly because they are organic material, do not cause harmful residues are easy to make and have been proven to produce high quality nata de coco [5].

This research examines the use of organic nitrogen from natural ingredients to answer the problem of the underutilized coconut water which is still considered waste and food safety related to the use of urea and ZA as a source of nitrogen in the production of nata de coco. One of the natural ingredients that are getting a lot of attention at this time is Moringa (*Moringa Oleifera*) because it contains quite high nutrition, especially its protein content. There are several nicknames for Moringa trees including The Miracle Tree, Tree for Life, and Amazing Tree. The nutritional composition of fresh Moringa leaves contain 94% water, 22.7% protein, 4.65% fat, 51.66% carbohydrate, 7.92% fiber and 350-550 mg potassium. Dried Moringa leaves contain 4.09% water, 28.44% protein, 2.74% fat, 7.95% ash content, 57.01% carbohydrate, 12.63% fiber,1600-2200 mg potassium and 307.30 kcal/100g energy [15]. By looking at the high protein content Moringa Oleifera it can be used as a source of Nitrogen in the production of nata de coco. This research aims to determine the appropriate concentration of Moringa leaf extract to obtain nata de coco with good physical properties and is favored by consumers. It is hoped that this research can be one of the solutions that can replace inorganic materials such as urea and ZA which have been controversial due to the food safety.

## 2 Materials and methods

### 2.1 Time and place

The research was carried out from February to July 2021 at the Laboratory of North Sulawesi Assessment Institute for Agricultural Technology (BPTP).

## 2.2 Materials and tools

The ingredients used are coconut water, Moringa leaf extract, food grade ammonium sulfate (ZA), glacial acid (dexi), granulated sugar, *Acetobacter xylinum* bacterial liquid. The tools used are a plastic tray with a size 23 x 17.5 x 35 cm, a boiler, gas stove, filter cloth, tampi, opaque paper, rubber, jar bottles, scales, measuring cup, cutting board, knife, caliper, oven and tools for analysis using SPSS.

## 2.3 The procedure for producing moringa leaf extract

Moringa leaf extract was obtained from the yards of residents' houses by cutting the stems of the Moringa tree, then the Moringa leaves were separated from the leaf stalks, washed with clean water, and then immersed in hot water at $90^0$C for 1 minute. Then it was blended with the addition of distilled water in a ratio of 2:1. Then filtered with a filter cloth and with a cotton swab remove the particles present in the extract material. Moringa extract is then sterilized by cooking until it boils for 1 hour. Moringa extract is then put into a bottle and stored in the refrigerator to be ready to be applied.

## 2.4 The procedure of making nata de coco

Plastic trays and paper archives are sterilized in the oven. Coconut water is filtered, put in a saucepan, and then cooked until it boils. Then added sugar 3% w/v, glacial acid 1% w/v and Moringa extract. Moringa extract and ZA was added in various concentrations (2.5%, 5%, 7.5%, 10%). After all the ingredients are well mixed, add the coconut water until it boils, 300 ml of coconut water is put into a sterilized plastic tray and then covered with paper and tied with rubber then refrigerated for one night. Fermentation weights were recorded and labeled. The seed inoculation stage *(kultur)* as much as 10% w/v of nata seed (stater) was added to each treatment at a temperature of 28ºC – 30ºC. The treatment was repeated four times. Fermentation was carried out at a temperature of 28ºC - 30ºC for 8 days. The finished nata is removed from the mold, then the thickness is weighed, and the results are recorded. Nata de coco sheets were washed with running water then soaked for 3 days and the water was changed every day. The nata de coco sheets are cut into pieces and then boiled to preserve and remove the sour smell and taste. Nata de coco was then sampled for crude fiber and organoleptic analysis.

## 2.5 Experimental design and data analysis

The research design used was completely randomized design (CRD) with 2 treatments and 4 replications, namely the concentration of Moringa extract and ZA respectively (2.5%, 5%, 7.5% and 10% w/v). The parameters observed were yields using the procedure [16], thickness of nata de coco [5], total fiber content of nata de coco [17] and organoleptic test [18]. Organoleptic tests were carried out on nata de coco products using a hedonic scale on color, aroma, taste, texture and overall value, the results of which were expressed on a scale of 1-5 where 1 (disliked), dislike slightly (2), neutral (3) liked (4) and really like (5). The research data were analyzed using SPSS Analysis of Variance (ANOVA). To find out the difference between treatments, it was continued with Duncan's test at a level of α 0,05 [19,20].

# 3 Results and discussion

## 3.1 The yield of nata de coco

The addition of Moringa extract in coconut water aims to increase the content of vitamins and nitrogen so that the resulting nata de coco is more optimal. With the use of ZA previously reported that the ideal composition with the addition of N produces nata de coco with good characteristics [21]. Moringa leaves are rich in nutrients, especially protein 22.7 – 28.44% as a source of nitrogen, which is expected to replace chemicals such as urea or food grade ZA as a natural source of nitrogen that is easy to obtain and inexpensive. The results of the analysis showed that the higher the concentration of nitrogen added, the higher the yield of nata de coco. The same thing was reported [22,23] which showed that the addition of nitrogen concentration could increase the amount of polysaccharide formed, but too high a concentration resulted in a decrease in yield and a decrease in whiteness index in the nata de coco produced. The results of the BNT test obtained in this study showed that the yield of nata de coco using Moringa leaf extract was not significantly different from using ZA at a concentration of 2.5%, 5% and 7.5% except for a concentration of 10%. The high yield was due to incubation for 8 days at an optimal temperature of 26-28°C for the growth of Acetobacter xylinum bacteria to form solid cellulose which would become nata sheets with a certain thickness [4]. The highest yield was obtained by adding 10% concentrations of Moringa extract and ZA 79.95% and 89.50%, respectively (Figure 1).

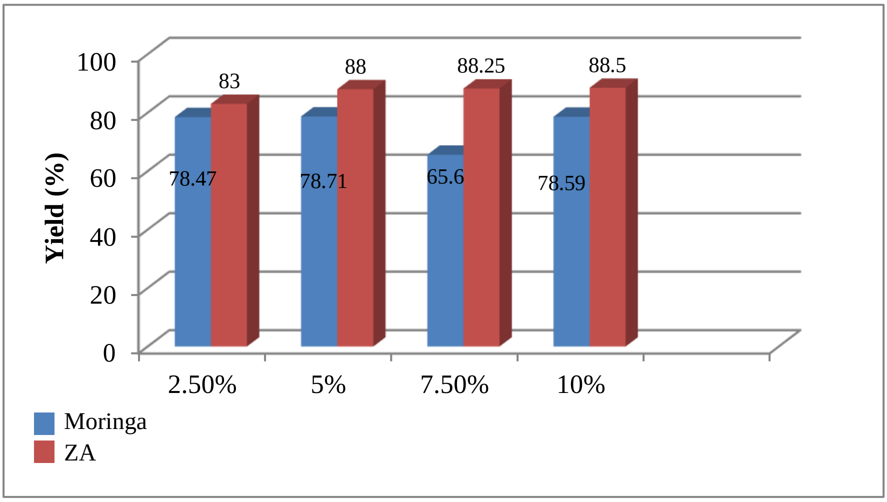


**Fig.1.** Histogram of the effect of nitrogen concentration on the yield of nata de coco.

## 3.2 The Thickness of nata de coco

The thickness of nata de coco was measured using a digital caliper by taking samples at ten points. The density of nata produced after the fermentation process is due to bacteria producing enzymes capable of breaking down glucose into cellulose in the form of polysaccharide threads. Polysaccharide threads will form a matrix that thickened and condensed and is called nata. Thickness is influenced by variations in substrate, material composition, environmental conditions, and the ability of *Acetobacter xylinum* to produce cellulose. Incubation time will affect the formation of cellulose which will be thicker and stronger, but fermentation that is too long will make *Acetobacter xylinum* bacteria experience a death phase due to running out of nutrients, causing cells to lose a lot of spare energy [24].

In Figure 2, it can be seen that the higher the concentration of nitrogen source added, the thicker the nata de coco produced. The results of the BNT test on the thickness of nata showed that the concentrations of 2.5%, 5%, 7.5% were not significantly different but significantly different from the 10% concentration of both Moringa extract and ZA. The highest thickness was obtained with the addition of 10% Moringa extract which was 0.66 cm and 0.68 cm for ZA. These results are consistent with the study that the use of sprout extract had a significant effect on the formation of cellulose and nata thickness [25]. Research stated that sprout extract as a nitrogen source could produce better quality of nata [26]. This means that Moringa leaf extract can replace ZA nitrogen or ammonium sulfate [5,6,7,8].

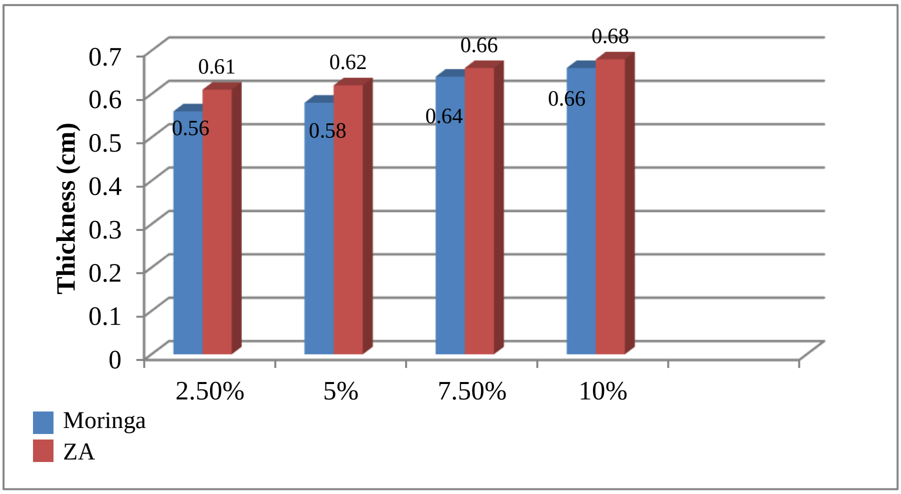


**Fig. 2.** Histogram of the effect of nitrogen source concentration on the thickness of nata de coco.

### 3.3 The crude fiber of nata de coco

In general, during the process of forming the nata layer, bacterial cells will be trapped in the nata cellulose layer. The more the number of cells trapped in the nata layer, the thickness, weight and crude fiber content decreased because the trapped bacterial cells could not metabolize properly. This is in accordance with the opinion [3] which said that during the formation of the nata layer, the number of *Acetobacter xylinum* cells in the fermentation decreased because many bacterial cells were trapped in the nata cellulose layer. In Figure 3, it can be seen that the crude fiber content of nata de coco using Moringa leaf extract is higher than ZA. The higher the Moringa extract added, the higher the crude fiber produced. This is because Moringa leaves as a natural ingredient contain around 7.92% - 12.63% crude fiber which can increase the concentration of fiber in nata de coco [15]. The results of the Ducan test α 0.05 the concentration of Moringa extract at 2.5%, 5% and 7.5% treatments were not significantly different, but the 10% concentration was significantly different. The results of statistical analysis showed no differences between treatments for the use of ZA. So, it can be said that the crude fiber of nata de coco using ZA comes from coconut water which has been changed to cellulose with the help of *Acetobacter xylinum* bacteria. As a food source of fiber, the use of Moringa leaf extract can be recommended as a source of nitrogen in the production of nata de coco.

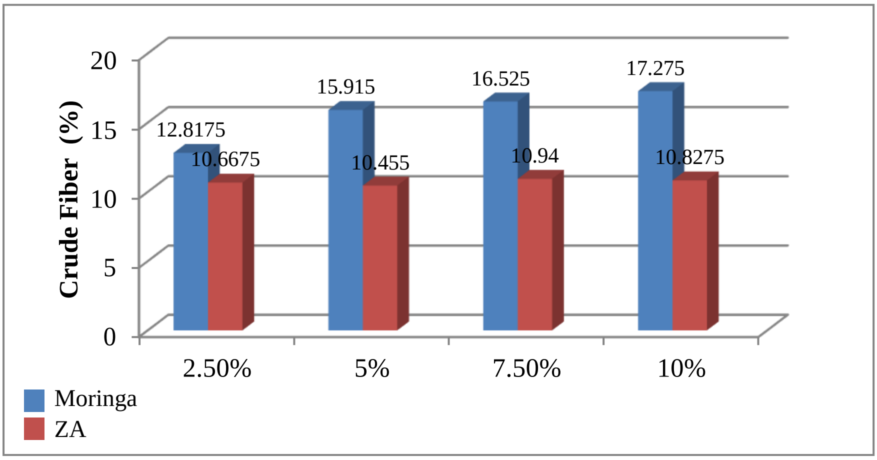


**Fig. 3.** Histogram of the effect of nitrogen source concentration on the crude fiber of nata de coco.

### 3.4 Organoleptic Test of Nata de Coco

Organoleptic test was carried out on the best treatment which was the use of 10% Moringa extract and 10% ZA. In Figure 4, it can be seen that the organoleptic value of the use of ZA is higher than the use of Moringa extract. However, when viewed as a whole, the scores given by the panelists were in the range of likes to really like (3.5 – 4.2).

The result of consumer preference test for nata de coco's color of Moringa extract is lower than ZA. This is due to the color of the Moringa extract which affects nata de coco to be greenish so that the panelists gave a lower value. Color is a visualization of a product that is immediately visible compared to other variables, so color will directly affect the perception of panelists [27]. The results showed that the addition of nitrogen concentration could increase the number of polysaccharides formed, but too high a concentration could result in a decrease in yield and a decrease in whiteness index in the nata de coco produced [22,23]. The decrease in whiteness index affects the level of consumer acceptance of the product. Aroma is a component of odor caused by a product that is identified by the human senses. Aroma is a key variable, because in general, consumers' taste for food products is largely determined by aroma. In Figure 4, it can be seen that the aroma of nata de coco using Moringa extract is lower than the use of ZA, but the results of Duncan's test α 0.05% did not show a significant difference between treatments with concentrations of 2.5%, 5% and 7.5% except 10%. This is because the high aroma of Moringa extract (10%) can affect the value of the aroma of nata de coco produced. The aroma value ranges from neutral to like (3-4). Likewise with the texture of nata de coco, the results of the preference test show that consumers give a value from neutral to like (3 – 4). The results of Duncan's test 0.5% in the two treatments using Moringa extract, and ZA did not show a significant difference. In general, the resulting nata de coco product has a chewy texture and can be chewed well. Overall, consumers prefer nata de coco products that use ZA because the color is brighter and the aroma is similar to the commercial nata de coco, which is popular in the market, Inaco products. From the results of organoleptic tests, the use of Moringa extract can be used as a substitute of Nitrogen sources such as Urea, NPK, ZA, and can be accepted by consumers with a neutral to like value (3 - 4), but still needs improvement on color and socialization to consumers that protein-rich Moringa leaves can be used as a source of organic nitrogen in the production of nata de coco as a fiber-rich product.

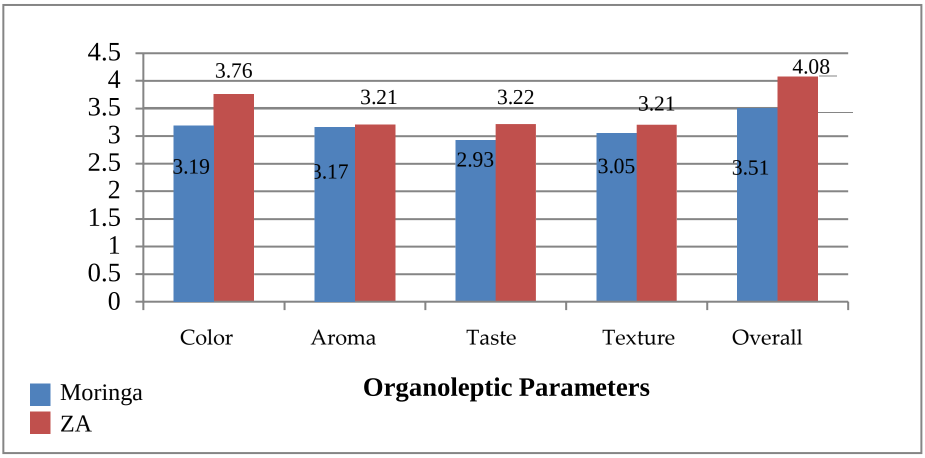


**Fig. 4.** Histogram of organoleptic values of nata de coco with Moringa extract and ZA

## 4 Conclusions

Moringa leaves (*Moringa Oleifera*) as a nitrogen source in the production of nata de coco produced nata de coco with a quality that was not significantly different from the use of ZA. Judging from the benefits of Nata de coco as a dessert that is rich in fiber, the best treatment is the use of 10% Moringa extract with yield of 78.59%, thickness 0.66 cm and crude fiber 17.26% and the highest organoleptic value is like (3, 5- 4) both in terms of color, aroma, texture and overall value.

Judging from the physical, chemical (fiber) properties and organoleptic tests, it is suggested that Moringa extract can be used as a substitute of chemicals nitrogen such as ZA in the production of nata de coco. However, color improvement is still needed and socialization to consumers that protein-rich Moringa leaves can be used as a source of organic nitrogen that is safe for consumption in the production of fiber-rich nata de coco. In addition, the costs incurred are lower than the use of inorganic materials. As an illustration of the business by processing 100 liters of coconut water, the profit for Moringa extract is IDR. 437,000 and ZA is IDR. 433,000, with a B/C ratio of 1.20 and 1.18, respectively.

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