Physic-chemical characteristic of nata de coco

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Abstract. The fermentation technology for processing nata de coco can be used by using coconut water waste sources and the use of Acetobacter xylinum bacteria. The purpose of this study is to analyze the physico-chemical quality of nata de coco produced by using a combination of sources of raw materials and their concentration on the quality of the nata de coco produced. The methodology for making the basic formula used 5 grams of nitrogen, ammonium sulfate and acetic acid in coconut water. The treatment used is in the form of using a variety of carbon sources in the form of granulated sugar, pineapple honey, tapioca and molasses with variations in concentrations of 3, 6 and 9%. The data obtained is then analyzed using IBM SPSS Statistics 23. Software. Based on the results of analysis, the results show that the different uses of source types and carbon concentrations significantly affect physical characteristics such as thickness, weight, residual water volume, color, hardness, frequency and value of TPT from nata de coco produced. The highest levels of crude fiber and cellulose were obtained from the treatment of 3% tapioca flour carbon sources of 0.68% and 0.78%. SEM observations of the treatment of 3% tapioca flour with a magnification of 10,000 times the actual size with cellulose tape sizes ranging from 120.8 nm to 155.5 nm with irregular shapes and uneven surface texture. The results of the FTIR spectrum of cellulose nata de coco treatment of 3% tapioca flour there was a group absorption peak at wave number 3392.17 cm⁻¹, vibration was at wave number 1402.55 cm⁻¹, 1052.86 cm⁻¹, 1110.02 cm⁻¹ 1629.47 cm⁻¹.

1. Introduction

The technology of processing nata de coco is the process of utilizing coconut water waste by adding several sources of carbon and nitrogen and acids to provide optimal environmental conditions for the growth of acetobacter xylinum. Nata products can be processed from several main raw material sources which are quite varied, mango; dragon fruit; apple; banana; salaca; pineapple [1-6].

Nata de coco is generally used as a food product and processed food. However, in line with the development of research activities, the use of nata de coco and its derivative products is growing. Nata de coco and cellulose can be used not only for food products, but also used for the detection of sugar levels in urine; biofilm; bioplastics; or as a metal adsorbent [7-10].

Recently, nata can be processed by combining several uses of carbon and nitrogen sources with several concentrations of their use. The use of toge extract can also be used to produce nata as the results of research from [11]. The use of other microbial isolates has also begun to be investigated to produce optimal nata products [12].

The use of the type of raw material, its concentration of additional materials, microbes and the age of the starter used can affect the quality of the nata produced. Based on the results of the data generated, it can be used for further applications. The purpose of this study to analyze the physico-chemical quality
of nata de coco produced by using a combination of sources of raw materials and their concentration on the quality of the nata de coco produced.

2. Materials and Methods
This study began with the process of making nata de coco with two types of treatment and then fermented for 7 days. In the first treatment the fixed components used were coconut water, acetic acid, nitrogen source ZA / Zwavelzuur Ammonia 5% and variations in the source of carbon used were sugar (GP), honey pineapple juice (NM), tapioca flour (TT) and molasses (MS) with concentrations of 3%, 6% and 9% respectively.

Analysis of physical characteristics carried out were the nata thickness test, yield, nata weight measurement, residual water volume measurement, color measurement, texture measurement, total dissolved solids (TPT) measurement and microscopic analysis (SEM). This characteristic analysis is carried out after nata de coco is harvested. Analysis of the chemical characteristics of nata de coco included pH measurement, water content analysis, ash content analysis, cellulose content analysis and crude fiber content analysis, and infrared spectrophotometry (FTIR).

All types of treatment and concentration of carbon sources and nitrogen experimental designs used in this study were Completely Randomized Design (CRD). This CRD analysis used 7 treatments for carbon sources and 6 treatments for nitrogen sources with 2 replications each. The observational data were then analyzed using variance analysis (ANOVA) with the help of using IBM SPSS Statistics 23 software to determine the effect of type treatment and concentration of carbon sources

3. Results and Discussion
Characteristics of nata de coco products can be observed from their physical and chemical parameters. The physical parameters observed included yield, nata weight measurement, residual water volume measurement, color measurement, texture measurement, total dissolved solids (TDS) measurement and microscopic analysis (SEM). The chemical quality parameters observed included: pH measurement, water content analysis, ash content analysis, cellulose content analysis and crude fiber content analysis, and infrared spectrophotometry (FTIR).

3.1. Yield Analysis
Analysis of nata de coco yield was carried out after 7 days. The yield of nata de coco was measured by the gravimetric method and expressed in weight per volume of the liquid medium used. Based on the results of the analysis of the yield of nata de coco with the type treatment and concentration of the use of several carbon sources, the results obtained as shown in Figure 1 below.

The highest yield of nata de coco in the first treatment used a variety of carbon sources and concentrations with the highest average yield value for the use of 9% sugar (GP 9) of 22.1% and the lowest average value of nata de coco yield in the treatment molasses 6% (MS 6) of 13.33%.

3.2. Results of Physical Parameter Analysis
The physical quality characteristics of nata de coco using different types and concentrations of carbon sources include thickness, weight, residual water volume and the value of TDS as shown in Table 1 below. Based on the results of statistical analysis, the thickness of the data, the weight of the data, the volume of residual water and the value of TPT with different types and concentrations produced significantly different values significantly at the 95% confidence level. This shows that the type and concentration of carbon sources can affect the parameters of thickness, weight, volume of residual water, and TPT produced.
Figure 1. Histogram of the Yield Value of Nata de Coco With Various Carbon Sources.

Table 1. Characteristics of thickness, nata weight, residual water volume and TPT value.

| Substrate | Thickness (mm) | Weight (gram) | Water Residual Volume (mL) | TDS (°Brix) |
|-----------|---------------|--------------|---------------------------|-------------|
| GP 3      | 11.5500\textsuperscript{a} | 162.8450\textsuperscript{ab} | 850.00\textsuperscript{abc} | 7.150\textsuperscript{cd} |
| GP 6      | 13.7250\textsuperscript{abc} | 183.8450\textsuperscript{bcd} | 810.00\textsuperscript{a} | 10.200\textsuperscript{e} |
| GP 9      | 15.5000\textsuperscript{bc} | 220.0200\textsuperscript{cd} | 790.00\textsuperscript{a} | 10.050\textsuperscript{e} |
| NM 3      | 11.6750\textsuperscript{a} | 150.0700\textsuperscript{ab} | 830.00\textsuperscript{abc} | 4.750\textsuperscript{a} |
| NM 6      | 14.1250\textsuperscript{abc} | 195.1700\textsuperscript{bcd} | 795.00\textsuperscript{a} | 5.600\textsuperscript{b} |
| NM 9      | 15.8750\textsuperscript{c} | 221.8150\textsuperscript{d} | 820.00\textsuperscript{ab} | 5.150\textsuperscript{ab} |
| TT 3      | 13.7000\textsuperscript{abc} | 192.8250\textsuperscript{bcd} | 855.00\textsuperscript{abc} | 6.700\textsuperscript{c} |
| TT 6      | 13.8500\textsuperscript{abc} | 179.2150\textsuperscript{abcd} | 850.00\textsuperscript{abc} | 6.550\textsuperscript{c} |
| TT 9      | 12.2500\textsuperscript{ab} | 191.8950\textsuperscript{bcd} | 890.00\textsuperscript{bc} | 6.550\textsuperscript{c} |
| MS 3      | 13.2250\textsuperscript{abc} | 170.7650\textsuperscript{abc} | 900.00\textsuperscript{c} | 6.850\textsuperscript{c} |
| MS 6      | 10.7000\textsuperscript{a} | 133.3400\textsuperscript{a} | 860.00\textsuperscript{ab} | 7.750\textsuperscript{d} |
| MS 9      | 13.8250\textsuperscript{abc} | 194.6000\textsuperscript{bcd} | 840.00\textsuperscript{abc} | 10.500\textsuperscript{e} |

Numbers followed by the same letter show significantly different levels of 95% confidence interval.

The results of measuring the thickness of nata de coco on an average variety of carbon sources and concentrations ranged from 10.70 mm to 15.875 mm. The highest nata thickness and nata weight were obtained from NM (Honey Pineapple) with a concentration of 9% namely 15.875 mm and 221.815 grams. Nata thickness is the result of the metabolism of the bacterium *Acetobacter xylinum* which can be used as a parameter to determine the growth and ability of these bacteria to convert nutrients into cellulose [13]. Cellulose produced by the bacterium *Acetobacter xylinum* will bind to one another to form a layer of nata which continues to thicken.

At a concentration of 3% the use of tapioca starch (TT) carbon sources had the highest weight compared to the media of granulated sugar, honey pineapple juice and molasses. Based on the results of measurements of the volume of residual water the average media of nata de coco on various variations of carbon sources and concentrations ranging from 790 ml to 900 ml.
Other physical properties parameters of the nata de coco products observed include color (L) and texture in the form of hardness and elasticity. The results of statistical analysis of these parameters are as shown in Table 2 below.

**Table 2. Color (L), hardness and elasticity result analysis**

| Substrate | Lightness (L) | Hardness (N) | Elasticity (mJ) |
|-----------|---------------|--------------|-----------------|
| GP 3      | 55.3100<sup>de</sup> | 4.4115<sup>ab</sup> | 5.9950<sup>c</sup> |
| GP 6      | 46.4450<sup>c</sup> | 11.6730<sup>ab</sup> | 2.1050<sup>abc</sup> |
| GP 9      | 56.9050<sup>e</sup> | 13.8425<sup>b</sup> | 5.4900<sup>bc</sup> |
| NM 3      | 56.9500<sup>e</sup> | 14.3660<sup>b</sup> | 1.6450<sup>b</sup> |
| NM 6      | 48.7800<sup>cd</sup> | 5.4355<sup>b</sup> | 1.6050<sup>ab</sup> |
| NM 9      | 48.4500<sup>cd</sup> | 7.8690<sup>ab</sup> | 1.2650<sup>ab</sup> |
| TT 3      | 58.9600<sup>e</sup> | 12.8850<sup>ab</sup> | 3.0000<sup>abc</sup> |
| TT 6      | 62.5600<sup>e</sup> | 6.8775<sup>ab</sup> | 0.8050<sup>a</sup> |
| TT 9      | 55.9850<sup>e</sup> | 8.6890<sup>ab</sup> | 0.8550<sup>a</sup> |
| MS 3      | 38.7700<sup>b</sup> | 2.6895<sup>a</sup> | 4.1150<sup>abc</sup> |
| MS 6      | 30.7600<sup>a</sup> | 2.3550<sup>a</sup> | 1.1900<sup>a</sup> |
| MS 9      | 28.0300<sup>a</sup> | 7.9595<sup>ab</sup> | 3.0100<sup>abc</sup> |

Numbers followed by the same letter show significantly different levels of 95% confidence interval

The results of ANOVA analysis on statistical data for color (L) or lightness parameters, hardness and elasticity showed significantly different significantly with the treatment of the use of different types and concentrations of carbon sources. According to Edria et al [14], nata color is influenced by the interaction of sucrose with nutrients in the raw material of water that can, the more interaction between the two, the nata color tends to be less bright (less white). Measurements made will produce a value denoted by L. The value of L indicates the brightness, namely the chromatic color value 0 means black until the maximum value of 100 means white. Based on the results of the analysis, the colors of nata de coco produced 28.03 to 62.56. The higher the L value, the brighter the color of the nata de coco produced.

Physical properties can affect the quality of food and the level of consumer acceptance of food. In terms of appearance, nata has a high aesthetic value, the appearance of the white is rather clear, the texture is thick and the aroma is fresh [15]. Testing of nata de coco textures aims to test the value of hardness cycle (hardness) and chewiness (elasticity). Hardness cycle (hardness) is determined from the maximum force at pressure or first compression which is expressed in units of N.

According to Darmajana [16], nata hardness can be influenced by the thickness of the nata, also the compactness between the nata layers formed by bacteria and the formation of cellulose formed. The mineral content contained in the nata media also determines the level of violence[17]. Whereas according to Widy [18], nitrogen-containing compounds can reduce the level of nata hardness. This is presumably because the formation of a bond between the nitrogen component and the precursor polysaccharide that exists causes the polymer structure to be looser and more elastic. Characteristics of nata de coco that are not too hard but have a chewy texture are also a texture orientation that is quite desired. The addition of carbon sources in the form of 3% tapioca flour produces sufficient quality of hardness with a fairly thick texture.
3.3. Microstructure Analysis
To find out the microstructure profile of the fiber and polymer forms of cellulose produced, it can be done using a SEM (Scanning Electrone Microscope) tool. The results of the microstructural shape analysis of nata de coco control compared to nata de coco using the addition of tapioca by 3% as shown in Figure 2 below.

![Figure 2. SEM results of Nata surface at 10,000 times magnification treatment control (a) tapioca flour 3% (b).](image)

The results of SEM observations on various samples showed the presence of cellulose fibril fibers on the surface of the sample. In control media using 10,000 times the size of glucose measured, it can be estimated that the fiber surface layer in the control with an irregular layer shape such as irregular threads and can be estimated the size of the cellulose tape ranges from 71.49 nm to 151.9 nm (Figure 2a) While the results of SEM observations of the treatment of 3% tapioca flour with an increase of 10,000 times the actual size with the size of cellulose tape ranged from 120.8 nm to 155.5 nm with irregular shapes and uneven surface texture and formed sharp and blunt angles (Figure 2b). Based on the results of the analysis, it can be seen that the existence of differences in carbon sources can affect the quality of the cellulose fibrils formed.

3.4. Analysis of Chemical Characteristics
Chemical characteristics were analyzed to determine the acidity, water content, ash content, and coarse and cellulose content found in the nata de coco products produced. The results of the chemical characteristics of the nata de coco products produced by varying the type and concentration of the carbon source are obtained as shown in Table 3 below.

Based on the results of chemical quality analysis, it was shown that the use of different types and concentrations of carbon sources produced different pH, water content, ash content, crude fiber content and cellulose content. The results of the analysis of the average cellulose nata de coco levels in various carbon sources and concentrations ranged from 0.38% to 0.78%. The value of cellulose nata de coco content from various carbon sources and concentrations with the highest average cellulose content was treated with 3% tapioca flour (TT 3) of 0.78% and the lowest average cellulose content in honey pineapple juice treatment 3% (NM 3) of 0.38%.

Cellulose is a natural polymer in the form of carbohydrates (polysaccharides) that have white fiber, are insoluble in water and organic solvents. Cellulose obtained from the fermentation process is a type of microbial polysaccharide which is suspended by cellulose fibers produced by Acetobacter xylinum. The method of analyzing cellulose content used is the Chesson method which is used to measure cellulose content.

Based on the results of the FTIR spectrum, showed the results of the cellulose spectrum of nata de coco treatment of 3% tapioca flour there was an absorption peak OH stretching group at wave number 3392.17 cm⁻¹, vibrations found at wave numbers 1402.55 cm⁻¹ showed vibration -CH₂ - bending, vibration in wave number 1052.86 cm⁻¹ shows CO stretching group, vibration at wave number 1110.02 cm⁻¹ shows COC stretching vibration and at wave number 1629.47 cm⁻¹ there is carbonyl group or C = O stretching.
Table 3. The chemical characteristic results of nata de coco.

| Substrate | pH Value | Moisture Content | Ash Content | Crude Fiber Content | Cellulose Content |
|-----------|----------|------------------|-------------|----------------------|-------------------|
| GP 3      | 3.7350<sup>b</sup> | 87.1300<sup>ab</sup> | 0.5000<sup>a</sup> | 0.2850<sup>ab</sup> | 0.5000<sup>abc</sup> |
| GP 6      | 3.8200<sup>c</sup> | 88.9500<sup>abcd</sup> | 0.6800<sup>a</sup> | 0.3300<sup>abc</sup> | 0.6250<sup>abcd</sup> |
| GP 9      | 4.0050<sup>d</sup> | 93.4250<sup>de</sup> | 0.5000<sup>a</sup> | 0.5400<sup>cd</sup> | 0.7350<sup>ad</sup> |
| NM 3      | 3.6400<sup>a</sup> | 86.8800<sup>ab</sup> | 0.9750<sup>a</sup> | 0.2650<sup>ab</sup> | 0.3800<sup>a</sup> |
| NM 6      | 3.8650<sup>c</sup> | 88.3400<sup>abc</sup> | 0.9600<sup>a</sup> | 0.3400<sup>ab</sup> | 0.4600<sup>b</sup> |
| NM 9      | 4.0050<sup>d</sup> | 90.6450<sup>abcd</sup> | 0.6300<sup>a</sup> | 0.4100<sup>bc</sup> | 0.5200<sup>abc</sup> |
| TT 3      | 3.8250<sup>c</sup> | 96.3450<sup>e</sup> | 0.6500<sup>a</sup> | 0.6800<sup>d</sup> | 0.7750<sup>d</sup> |
| TT 6      | 3.7050<sup>ab</sup> | 95.3850<sup>e</sup> | 0.8150<sup>a</sup> | 0.4250<sup>bc</sup> | 0.5200<sup>abc</sup> |
| TT 9      | 4.3200<sup>f</sup> | 95.5600<sup>e</sup> | 0.6850<sup>a</sup> | 0.3650<sup>abc</sup> | 0.6700<sup>cde</sup> |
| MS 3      | 4.0950<sup>e</sup> | 87.4000<sup>abc</sup> | 0.7200<sup>e</sup> | 0.1700<sup>ab</sup> | 0.5500<sup>abcd</sup> |
| MS 6      | 4.2950<sup>f</sup> | 85.7700<sup>a</sup> | 0.6900<sup>e</sup> | 0.2200<sup>ab</sup> | 0.5150<sup>abc</sup> |
| MS 9      | 4.4100<sup>g</sup> | 91.8750<sup>cde</sup> | 0.8100<sup>e</sup> | 0.2300<sup>ab</sup> | 0.4700<sup>b</sup> |

Numbers followed by the same letter show significantly different levels of 95% confidence interval.

3.5. Infrared Spectrum Results (FTI-IR)

Analysis of the FTIR spectrum is an analysis to find out the bonding group that is present in the cellulose sequence that forms sheets of nata de coco. Based on the results of the analysis, it is expected that the functional groups and bonds can be identified from the results of the FTIR spectral spectrum produced.

According to Marchessault and Sundararajan[19], IR spectra of pure cellulose give peaks at 3350 cm<sup>-1</sup> where peaks between 3400 cm<sup>-1</sup> - 3500 cm<sup>-1</sup> indicate -OH stretching, 2800 cm<sup>-1</sup> - 2900 cm<sup>-1</sup> indicating CH stretching, 1160 cm<sup>-1</sup> indicated COC stretching, and 1035 cm<sup>-1</sup> - 1060 cm<sup>-1</sup> indicated CO stretching. In the cellulose fingerprint area giving a peak of around 1400 cm<sup>-1</sup> indicates the presence of -CH2-bending. According to Silverstein et al. [20], the absorption peak of cellulose nata de coco is the -OH stretching group (stretching) which occurs at wave number 3245 cm<sup>-1</sup> and C-O stretching group (stretching) at wave number 1061 cm<sup>-1</sup>. Uptake of this group shows the presence of glycoside bonds and C-O bonds on the cellulose ring.
According to Rahmidar et al. [21] cellulose FTIR spectrum at wavy number 3777.36 cm⁻¹ appears the -OH stretching function group, at wave number 1637.56 cm⁻¹ shows the existence of functional group C = O stretching, then at wave number 1112.92 cm⁻¹ there is a COC stretching group and there is a CO stretching group at wave number 1056.99 cm⁻¹. Furthermore, in bacterial cellulose (nata), there were vibrations at wave number 3400.01 cm⁻¹ which showed the presence of -OH stretching alcohol, vibrations at wave number 1110.20 cm⁻¹ which showed the presence of COC β-1,4-glycosidic.

4. Conclusion

Based on the analysis of the physico-chemical characteristics, the highest thickness and weight of nata de coco is shown in the treatment of carbon sources of 9% pineapple honey juice of 15.9 mm with a weight of 221.82 g. The highest crude fiber content was obtained from the treatment of carbon sources of 3% tapioca flour 0.68%. The highest cellulose content was obtained from the 3% tapioca carbon source of 0.78%. Some physical and chemical quality parameters can be used as further research material. Further studies on nata de coco can be made to influence nutritional and vitamin values and techno-economic analysis for the development of optimal production scales.

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