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Physical and Chemical Properties Nata de Cascara on The Different Treatment of Fermentation's Time with SCOBY (Symbiotic Culture of Bacteria and Yeast)

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Abstract

Cascara, the dried outer pulp and skin of the coffee cherry is usually discarded as waste. However, there is a growing interest in reutilizing this coffee cherry by-product as a unique product. This study aims to determine the effect of fermentation time on the physical and chemical properties of Nata de Cascara Arabica with a SCOBY starter. The material used in this research was Arabica cascara coffee as the raw material for making kombucha. The research method was designed as a Completely Randomized Design (CRD) with fermentation time as the factor, conducted in five repetitions. Fermentation periods of 8, 12, 16, and 20 days were analyzed for various physical and chemical parameters, including SCOBY's thickness, yield, color, moisture content, and crude fiber. The data on the results of physical and chemical properties were analyzed using Analysis of Variance (ANOVA) at a 95% confidence level. If differences were found, the Duncan test was applied. The results showed that longer fermentation times significantly affected ($p<0.05$) moisture content and color (Redness), while thickness, yield, color (lightness), and crude fiber content increased. The best treatment for making Nata de Cascara Arabica is fermentation for 20 days.

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Introduction

Cascara is a derivative product from the dry peel of coffee cherries and can be processed in such a way that it can be brewed as it is brewing tea in general (Sastra and Bawono, 2018). Cascara which is brewed into tea has a distinctive sour taste and aroma like fruits (Nafisah and Widyaningsih, 2019). The fermentation of the physical and chemical properties of Nata de Cascara Arabica can be expected from the best old fermentation recommendations that can create Nata de Cascara Arabica with good quality. Fresh cascara contains 6.11% crude protein, crude fiber 18.69%, tannins 2.47%, caffeine 1.36%, lignin 52.59%, fat 1.07%, ash 9.45%, calcium 0.23%, phosphorus 0.02%. Cascara also contains several vitamins, including vitamins C and E which are very good for the skin and human body (Takwa et al., 2018). Cascara can also be processed from various types of coffee skins, such as the Robusta and Arabica types, which have red berries.

SCOBY is a microorganism used in kombucha tea fermentation which will produce secondary products in the form of nata. Nata is a mixture of Acetobacter bacteria with yeast which are generally *Brettanomyces bruxellensis* species, *Candida stellata*, *Schizosaccharomyces pombe*, *Torulaspora delbrueckii*, and *Zygosaccharomy bailii* (Mousavi et al., 2018). SCOBY consists of several bacteria and yeast. Stevia is a sugar product derived from leaf extraction by utilizing dissolved chemical substances. The sweetness level in stevia sugar is 2.5 times compared to sucrose (Tuna et al., 2015). The sweet taste of stevia leaves comes from the glycoside content which consists of 2 main components namely stevioside and rebaudioside (Yulianti *et al.,* 2014). Stevia is a type of sweetener that has no calories at all (Ghazi *et al,* 2013).

Nata in the form of a thick membrane containing 35-62% cellulose, turbid white, and chewy as a result of fermentation of nata-forming bacteria, namely

Acetobacter xylinum. Nata is a synthetic cellulose formed from anabolic fermentation processes in liquid media, to produce complex cellulose compounds from the formation of simple compounds (sugar) (Lempang, 2006). Nata can be used as a functional food for dietary purposes, and to improve the digestive process because it is a source of food fiber, it also functions in overcoming the problem of excess cholesterol and all its effects and plays a role in the prevention of colon cancer (Naufalin and Wibowo, 2004). The process of making nata is influenced by several factors related to the growth conditions of *Acetobacter xylinum* which plays an important role in the fermentation process into nata. The growth of *Acetobacter xylinum* is influenced by factors including pH, temperature, nitrogen sources, and carbon sources (Rizal *et al.,* 2013). Therefore, this research aims to determine the effect of different fermentation times on the physical and chemical properties of Nata de Cascara Arabica with SCOBY Starter.

Materials and methods

Materials

The used materials were cascara Arabica, starter SCOBY, stevia, distilled water, 0.1 N NaOH solution, water, an indicator of PP, a solution of methanol, H_2SO_4 0.3 N, and 1.5 N. The used equipment was a beaker, measuring cup, analytical balance, drop pipette, volume pipette, test tube, stainless pan, stir bar, spoon, plastic, rubber band, thermometer, glass container, plastic container, plastic cup, tissue, vortex, Erlenmeyer, burette, gas stove, filter cloth, and calipers.

Method

The study was conducted from December 2019 to February 2020 in the Laboratory of Food Engineering and Agricultural Products and the Laboratory of Chemistry and Food Nutrition, Faculty of Animal Husbandry and Agriculture and Integrated Laboratory of Diponegoro University, Semarang. The analysis included water content, color, thickness, yield, and crude fiber content.

The Production of Arabica Coffee Cascara Tea

The production of Cascara tea solution referred to the method carried out by Yuwanti et al. (2018), namely Cascara Arabica Coffee weighed 25 grams (5%) and 500 ml of water was heated to 90° C. The Arabica Coffee Cascara was dissolved in water and waited for 5 minutes while stirring to form the Arabica Coffee Cascara tea solution. Cascara pulp was filtered and the tea solution was placed in a glass container/bottle. The tea solution was cooled to room temperature.

The Production of Kombucha Cascara Arabica Coffee

The production of Kombucha cascara was based on the method from Nurhayati et al. (2018). In brief, the Arabica cascara tea solution was mixed with Stevia by 10% (w/v) and then stirred evenly. The Arabica cascara tea was then put in a glass container that had been sterilized correctly and then added with SCOBY and acidified Kombucha until the solution reached pH 4. The Kombucha was fermented for 8, 12, 16, and 20 days. Kombucha cascara, which had been fermented, was then filtered to separate the Kombucha cascara and Nata layer. Kombucha cascara solution was then pasteurized at 70 ° C for 10 minutes.

Thickness Measurement

Thickness measurement was referred to the method of Awwaly et al., (2011). The nata was cut to a size of 2x2 cm and soaked in water for 2 days. After the soaking process, nata was then boiled at 100° C for 10 minutes and drained to dry. The thickness of nata (mm) was measured with calipers from at different sides.

Yield Quantification

The yield measurement was referred to the method of Iryandi *et al.,* (2014). The yield calculation was stated in the formulation as follows:

$$\text{Yield} = \frac{\text{Weight nata (g)}}{\text{Total weight of liquid medium used (ml)}} \text{ x } 100\%$$

Color Measurement

The color measurement was referred to the method of Saputra and Hidaiyanti (2015) by using the Digital Color Meter application. Nata was cut into cubes with a size of 2x2 cm and then measured for the L * a * b value Putro *et al.,* (2015). L (lightness) is the level of brightness, the higher the L value the brighter the color, and the lower the darker the color L. A * (redness) value if positive indicated red, and if negative indicated green. While the value of b * (yellowness) if positive showed yellow, and if negative showed blue color.

Moisture Content Measurement

Moisture content measurement was referred to the method of Shagti (2017). Calculation of moisture content was first carried out with oven the porcelain cup for 1 hour at a temperature of 105°C. After that the cup was put into the desiccator for 15 minutes and then weighed (A). The sample was weighed as much as 2 g in a dried cup (B). The cup containing the sample was then oven for 5 hours at 105°C. After 5 hours the cup was put into the desiccator for 15 minutes then weighed (C). This step was repeated until a constant weight was achieved. Moisture content can be calculated using the following formula:

$$\text{Moisture Content} = \frac{B-C}{B-A} \text{X } 100\%$$

Where:

A = Weight of the empty cup (g)

B = Cup weight + initial sample (g)

C = Cup weight + sample after roasting (g)

Crude Fiber Determination

The calculation of crude fiber content was conducted following the methods outlined by Putranto and Taofik (2017). Initially, 1 g of the sample was placed in a glass beaker and treated with 50 ml of 0.3N H2SO4 solution. The mixture was boiled using an upright cooler for 30 minutes and then allowed to cool for 15 minutes. Subsequently, 25 ml of 1.5N NaOH solution was added, and the mixture was boiled again for 30 minutes and cooled for another 15 minutes. The filtration process was carried out by sequentially passing 50 ml of hot distilled water, a boiled sample, 50 ml of 0.3N H_2SO_4, another 50 ml of hot distilled water, and finally, 25 ml of C or 95% alcohol, equal to the volume of the sample, through filter paper. The residue left on the filter paper was then dried by aeration.

After drying, the filter paper was folded, placed in a porcelain cup, and placed in an oven for 6 hours. After 6 hours, the porcelain cups containing the samples were left in a desiccator for 15 minutes and then weighed (Y). Subsequently, the sample was electrically heated to 600°C for 6 hours until it turned into white ash. The level of crude fiber in the sample can be calculated using the following formula:

$$\text{Fiber Content} = \frac{\text{Y} - \text{Z} - \text{A}}{\text{X}} \text{ X } 100\%$$

Where:
Y = Weight of filter paper after oven
Z = Weight of the filtered and heated sample
A = Weight of the cup + ash
X = Initial sample weight

Data Analysis

Data on moisture content, color, thickness, yield, and crude fiber content were analyzed using Analysis of Variance (ANOVA) with a 95% confidence level, then followed by Duncan Multiple Range Test (DMRT) to determine the differences between the treatments given. The entire analysis process was carried out using the SPSS 22.0 for Windows application.

Results and Discussion

Thickness

Table 1 demonstrates a significant effect of fermentation time ($p < 0.05$) on the thickness of Nata de Cascara. Prolonged fermentation in Nata de Cascara leads to an increase in thickness. The longer the fermentation period, the thicker the nata formed. This finding aligns with the results of Awwaly et al. (2011), who stated that extended fermentation results in cellulosic thickening and a chewy texture of nata. This occurs due to the continuous availability of adequate nutrition, allowing bacteria to maintain high metabolism and reproduction rates. As a result, *A. xylinum* secretes cellulose, which binds to form layers, steadily thickening due to the ongoing metabolic processes of *A. xylinum*. This observation is further supported by Iryandi et al. (2014), who noted that the duration of fermentation influences *A. xylinum*, leading to the production of cellulose threads that contribute to the thicker formation of nata.

Table 1. Moisture Content, Color, Thickness, and Yield of Nata de Cascara Arabica

Parameter	Treatment			
	T 1	T 2	T 3	T 4
Moisture Content (%)	99.50 ± 0.00 d	98.73 ± 0.25 c	98.33 ± 0.26 b	97.82 ± 0.27 a
Color (L)	54.28 ± 4.26 b	29.56 ± 13.15 a	59.04 ± 12.77 b	68.78 ± 12.02 b
Color (a)	2.90 ± 0.60 a	8.84 ± 4.50 b	5.58 ± 2.99 ab	2.28 ± 2.31 a
Thickness (%)	0.11 ± 0.01 a	0.36 ± 0.03 b	0.48 ± 0.05 c	0.62 ± 0.11 d
Yield (%)	1.65 ± 0.93 a	3.80 ± 0.21 b	4.84 ± 0.80 b	7.51 ± 1.13 c

* Different superscripts show a significant difference ($p < 0.05$).

Yield

Based on Table 1 it seems that the fermentation time has a significant effect ($p < 0.05$) on the thickness of Nata de Cascara on the yield value of Nata de Cascara. The longer the time of fermentation of Nata de Cascara will make the yield value increases. Cellulose growth also affects the number and age of starter cultures, where a good starter is to be healthy and active, available in sufficient quantities, in a suitable morphological form, free from contamination, and its ability to form nata products. This is consistent with the opinion of Lempang (2006) who states that the best starter in the formation of nata is 11 days old because at that time the rate of pellicle formation on the surface of the liquid media in the incubation bottle is very fast which shows the amount and activity of bacteria is very high. Starter decreases if the age of the culture gets older, the results of nata are forming due to the fermentation media which contains an old starter which is very easy to experiences contamination so as to produce thin nata. This is supported by the opinion of Awwaly et al. (2011) which states that the best number of starters in making nata is 10-20% to produce maximum nata thickness.

Color

Table 1 indicates a significant effect ($p<0.05$) of fermentation time on the color of Nata de Cascara. The lightness value (L), ranging from 0 to 100, represents the appearance interval from dark to bright, where smaller values indicate a darker color, and larger values indicate a brighter color. During fermentation, the brightness value (L) of Nata de Cascara decreases. This

phenomenon is attributed to the thickness of Nata, wherein prolonged fermentation leads to increased thickness, resulting in a darker (turbid) color, whereas thinner Nata exhibits a lighter (white) color. This observation aligns with the findings of Putriana and Aminah (2013), who noted that thicker Nata absorbs more light, making it darker, while thinner Nata appears brighter due to lesser light absorption.

Additionally, the a* (redness) value indicates the red interval, with more positive values indicating a redder product and more negative values indicating a greener product. The a* value increases during fermentation, suggesting a shift towards a redder hue. This finding is consistent with the research by Putro et al. (2015), where a more positive a* value signifies a redder product, while a more negative value indicates a greener hue. Hence, accelerating the fermentation process can enhance the redness of Nata de Cascara.

Water Content

According to Table 1, the duration of fermentation has a significant effect ($p<0.05$) on the water content of Nata de Cascara. Extended fermentation leads to a decrease in the water content of Nata de Cascara due to the optimal growth of *A. xylinum*, facilitated by sufficient nutrition. The prolonged fermentation period allows *A. xylinum* to convert glucose into cellulose, resulting in thicker cellulose formation and denser cellulose tissue. As a consequence, the space available for water to be trapped within the cellulose structure diminishes, leading to a decrease in water content. This finding is in line with the research of Iskandar et al. (2010), which emphasizes that under optimal conditions, *A. xylinum* transforms glucose into cellulose, forming a thicker and denser cellulose structure with reduced trapped water. This observation is further supported by the insights of Novita et al. (2016), who state that the increased thickness of cellulose leads to stronger cellulose bonds, minimizing the available space for water entrapment and consequently lowering the overall water content in the resulting Nata de Cascara.

Rough Fiber Levels

Based on Illustration 1, it appears that the duration of fermentation has a significant effect ($p<0.05$). Nata de Cascara exhibits a high fiber content, a characteristic influenced by the nutrient content present in the raw material, namely cascara or coffee skin, which is rich in fiber and antioxidants. This aligns with the perspective of Judge and Setiawan (2014), who assert that elevated levels of fiber and antioxidants play a substantial role in the high crude fiber content of the final product.

Additionally, besides fermentation time, a substantial percentage of crude fiber is influenced by the nutrient content and pH in the medium, which is essential for the metabolic process of *A. xylinum*. This finding is consistent with the viewpoint of Hakim and Setiawan (2014), who state that the optimal growth conditions for *A. xylinum* involve a pH range of 4-5, a sugar concentration of 10-15%, a nitrogen content of 20.4-21%, and the presence of sufficient minerals and vitamins. These factors collectively contribute to the significant crude fiber content observed in Nata de Cascara.

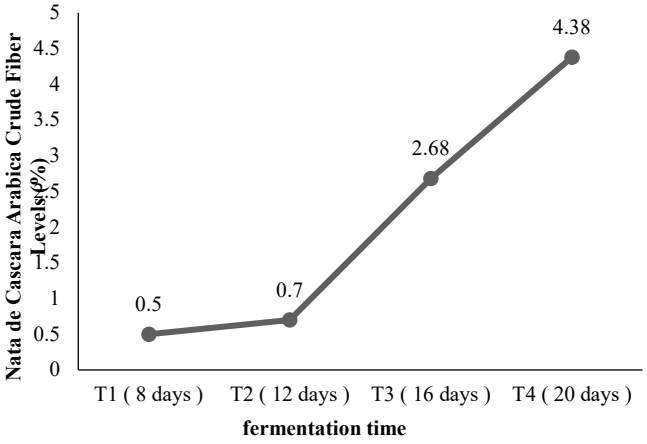

Figure1. Crude Fiber of Nata de Cascara Arabica

Conclusion

As the duration of fermentation increases, there is a noticeable decrease in water content and color (redness), while the thickness, yield, color (lightness), and crude fiber content show a clear increase. Based on these findings, the most favorable fermentation duration for producing Nata de Cascara is 20 days (T4). At this point, Nata de Cascara exhibits a water content of 97.82%, a yield of 7.51%, a thickness of 0.62%, a lightness value of 68.78%, a redness value of 2.28%, and a crude fiber content of 4.38%.

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