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Nata De Coco Production with Addition of Anthocyanin from Mangosteen Peel Extract

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Abstract - *Mangosteen peel is rich in antioxidant compounds which has beneficial for human health and this potential is being utilized by the community. Mangosteen peel extract contains anthocyanin compounds. Anthocyanin is considered as active compounds whose consumption is associated with beneficial health effects. Nata de coco has many benefits for health, especially digestion, because of its high fiber content, low calories, and does not contain cholesterol. This research examines the optimum operating conditions of the nata de coco production process with the addition of mangosteen peel anthocyanin extract and the characteristics of the resulting nata de coco. This research study was carried out with various variables including: The ratio between mangosteen peel extract and coconut water (1:19 and 9:1 (v/v)), fermentation time 10 and 13 days), and Acetobacter xylinium volume (2,5% and 10%). During the research, an analysis of the yield of mangosteen peel extract was carried out with a result of 36.25% and the total anthocyanin content in the extract was identified using the pH difference method and spectrometry, showing a result of 2.9%. The ratio of mangosteen peel extract to coconut water of 1:19 (v/v%) and a volume of Acetobacter xylinium of 10% was the best product, showing a total anthocyanin content of 57.48%. The organoleptic test results showed a good response according to SNI. Thickness, weight and pH have also been evaluated, showing results of 1.6 cm, 86 grams respectively and acidic properties at pH 4. However, the crude fiber content has been evaluated, resulting in a low content of 2.5%. Therefore, the potential for anthocyanin extract to be added to Nata de coco needs to be reviewed so that it becomes a superior product in accordance with \ other standards\.*

Keywords: *acetobacter xylinium, anthocyanin, mangosteen peel, MAE, nata de coco*
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INTRODUCTION

Mangosteen is a fruit that is widely consumed by people in Indonesia. Based on data from the Statistic Indonesia 2019 mangosteen fruit production in Indonesia reached 322.414 tons in 2020. Mangosteen peel is the largest part of the mangosteen fruit around 70-75%. Mangosteen peel is rich of antioxidant and beneficial for human health and this potential is being utilized by the community (Miryanti *et al*., 2011). Antioxidants are compounds that can inhibit and prevent the formation of new free radicals (Zahro *et al*., 2019). Antioxidants are needed to prevent oxidative stress, which plays an important role in the etiology of various degenerative diseases (Werdhasari, 2014). Based on data from the International Agency for Research on Cancer (IARC) in 2012 there were at least 14.067.894 new cases and 8.201.575 people died from cancer. Lack of antioxidants is one of the triggers. Due to the urgency of this problem, many products have been developed that contain antioxidants which are good for health and cancer prevention.

Mangosteen peel extract contains anthocyanin compounds. Anthocyanin is a group of phenolic compounds that have thermopile properties so that the extract drying method is operated at low temperatures (Pustiari *et al*., 2015). Anthocyanin are considered as active compound whose consumption is associated with beneficial health effects. Anthocyanins can reduce the risk caused by oxidative compounds. Anthocyanins have recently been suggested as potential compounds that play a role in the treatment of cardiovascular disease, type 2 diabetes and cancer (Herfayati *et al*., 2020).

Previous study conducted by Farida (2015), anthocyanin levels from mangosteen peel extraction using the MAE method reached an optimum point in 10 minutes and decreased in 15 minutes. The highest anthocyanin content was found in the ratio of ingredients: 2% citric acid solvent 1:20 (w/v). In a study conducted by Permadi *et al*., (2012) regarding the extraction of mangosteen peel using the MAE method, it was also found that the optimum length of time was 10 minutes, the feed: solvent ratio was 1:20 (w/v) with a power of 399 watt. The ratio of material: solvent in the two studies above is the reference for the ratio used in this study. The MAE method was chosen because based on research by Cardoso *et al*, (2014) the results of MAE extraction were higher than conventional extraction at equivalent time and temperature levels.

Nata de coco is a food product made from fermented coconut water by the bacterium *Acetobacter xylinum*. Currently, many nata have been created from various raw materials, for example from pineapple juice called nata de pina. There is even nata made from tempeh waste called nata de soya. But the most popular is nata de coco, which is nata made from fermented coconut water. Apart from coconut water, the process of making nata de coco also requires acid as a pH regulator of the media as well as carbon and nitrogen sources. Carbon and nitrogen sources are needed so that nata yields are optimal (Nuraini, 2015).

Most of the nutrients in nata de coco is in the form of cellulose fiber, which is better known as bacterial cellulose. Nata de coco has many benefits for health, especially digestion, because of its high fiber content, low calories and does not contain cholesterol (Cashew, 2016). According to LIPI's Biology Research and Development Center, the nutritional content of nata de coco per 100 grams of nata contains 80% water, 20 grams carbohydrates, 146 cal calories, 20 grams fat, 12 mg calcium, 2 mg phosphorus and 0.5 mg Fe (Sulistiyana, 2020).

This study aims to determine the effect of fermentation time, concentration ratio of mangosteen peel extract to coconut water, and volume of *Acetobacter xylinum* on the characteristics of the result of nata de coco.

MATERIALS AND METHOD

Materials

The materials used were mangosteen peel, citric acid, distilled water, *Acetobacter xylinum*, coconut water and sugar.

Extraction Anthocyanin from Mangosteen Peel

A total of 800 grams of mangosteen peel was dried in the sun for 12 hours, pulverized using a blender and sieved using a 60-mesh sieve, then stir with magnetic stirrer for 15 minutes. Extraction of anthocyanins from mangosteen peel was carried out using the MAE (Microwave Assisted Extraction) method with 2% distilled water – citric acid as a solvent, the ratio of ingredients to solvent was 1:20 (w/v), 160 watt of power, and 10 minutes of time. The anthocyanin extract from mangosteen peel was then weighed and measured to determine the yield and total anthocyanin content value.

Analysis Method

The mangosteen peel extract then mixed with pre-boiled coconut water at 100 ºC. This experiment used factorial design experiment to determine the effects of multiple variables on a response with the ratio of mangosteen peel extract to coconut water (1:19 and 9:11 (v/v)), the volume of *Acetobacter xylinum* (2.5% and 10%), and fermentation time (10 and 13 days).

Total Anthocyanin Content (TAC) of Mangosteen Peel Extract

The total anthocyanin content of each sample was evaluated by spectrophotometric methods. The samples had been squeezed, and then the absorbance was measured using a UV - Vis spectrophotometer. Total Anthocyanin levels were determined by eq. (1) and pH differences.

$$TAC = \frac{A}{\varepsilon x l} xMWxDFx\frac{V}{Wt}x100\% \quad (1)$$

Crude Fiber Content of Nata De Coco

Analysis of crude fiber content was evaluated using the potentiometric method. 2 grams of each sample was put into an Erlenmeyer flask and 200 ml of boiled 0.255 N H2SO4 solution was added, then heated for 30 minutes. The suspension is filtered with filter paper, the residue remaining in the Erlenmeyer is washed with boiled distilled water until it is no longer acidic. The material was dried with filter paper at 110oC until the weight was constant. Cool in a desiccator and weigh.

Organolaptic

Organoleptic testing was carried out on samples that gave the best results from testing Total Anthocyanin Content (TAC) and crude fiber content. 25 panelists were prepared to rate the samples on a 5-point hedonic scale for the texture, color and smell used (1 = very soft, not bright, rancid aroma; 2 = soft, not bright, slightly rancid aroma, 3 = slightly soft, bright, to have a fruity aroma; 4 = somewhat firm, somewhat

attractively bright, fruity; 5 = firm, attractively bright, very fruity).

Thickness, Weight, and pH

The best sample was taken from the previous test. Thickness (cm) was measured using a digital caliper. Weight (gr) using a digital balance and determining pH using pH paper.

RESULTS AND DISCUSSION

Yield of Mangosteen Peel Extract

Research conducted by Farida, *et al* (2015) on extracting anthocyanins from mangosteen peel with citric acid solvent ratios of 1:10, 1:20, and 1:30 resulted in yields ranging from 25.39% to 37.92% at 5-10 minutes. Meanwhile, in this study of optimization of quality characteristics of antioxidant nata de coco with the addition of anthocyanins from mangosteen fruit peel extract, the yield of mangosteen fruit peel extract was 36.25% under the operating conditions of the ratio of ingredients to citric acid solvent 1:20 (b/v), P = 200 W, and t = 10 minutes. The amount of solvent used affects the yield value produced, where the more solvent used, the greater the yield of the resulting extract. The number of solvent results in sufficient components of extracted compounds along with impurities or unwanted substances are also extracted. The length of extraction time also affects the yield, the longer the extraction time, the greater the yield (Farida *et al*, 2015).

Total Anthocyanin Content of Mangosteen Peel Extract

Anthocyanins in mangosteen peel were extracted using the Microwave Assisted Extraction (MAE) method with a ratio of ingredients: citric acid of 2% 1:20 (w/v), 200 watts of power for 10 minutes. Determination of Total Anthocyanin Content (TAC) was carried out using the pH difference method and a spectrophotometer. Research on mangosteen peel extraction conducted by Pustiari, *et al* (2019) used a mixed solvent of 70% ethanol - 3% citric acid, resulting in a TAC value of 12.25%. While in this study the total anthocyanin content produced by extract was 2.992% using 2% citric acid solvent. The difference in TAC values was influenced by the type of solvent used, where the solvent composition of the solvent mixture 70% ethanol - 3% citric acid produces extracts with a large number of anthocyanins (Pustiari *et al*, 2019).

Crude Fiber Content of Nata De Coco

Anthocyanins that have been extracted are then tested for fiber content. Crude fiber content test is carried out to determine the quality of nata de coco (Wahyuni, 2019). This analysis aims to determine the cellulose content as a source of dietary fiber produced by *Acetobacter xylinum* during the fermentation process (Mandey *et al*., 2020). The average results of crude fiber content in this nata de coco research ranged from 0.5%-8%.

Table 1. Crude Fiber Content With Factorial Design Experimental Results

Run	Ratio	Volume *Acetobacter xylinum* (%)	Day	Crude Fiber (%)	Result
1	1:19	2.5	10	8.0	3.625
2	9:11	2.5	10	5.0	0.5
3	1:19	10	10	0.5	-4.25
4	9:11	10	10	2.5	-0.5
5	1:19	2.5	13	2.5	-0.75
6	9:11	2.5	13	7.5	1
7	1:19	10	13	2.5	0.75
8	9:11	10	13	0.5	-3

Table 1 shows that in run 1 with the ratio of mangosteen peel extract : coconut water which is 1:19, the volume of *Acetobacter xylinum* is 2.5%, and the duration of fermentation is 10 days has the highest crude fiber content of 8%, while the lowest crude fiber content is in run 3 with the ratio of mangosteen peel extract: coconut water which is 1:19, volume of *Acetobacter xylinum* 10%, fermentation duration of 10 days and run 8 with the ratio of mangosteen peel extract: coconut water which is 9:11, volume of *Acetobacter xylinum* 10%, fermentation duration of 13 days with the same percentage of 0.5%.

. Theoretically, the reaction mechanism of Nata de coco fermentation with the bacterium Acetobacter xylinium had 3 stages. The first stage, hydrolysis of sucrose into fructose and glucose occurs with the enzyme sucrease. In the second stage, there was a reaction to change the intramolecular α-D-glucose to β-D-glucose used the isomerase enzyme found in the Acetobacter xylinium bacteria. This conversion process was caused by the glucose that plays a role in the formation of cellulose, namely glucose in the β form. In the third stage, an intermolecular reaction of glucose occurs through the binding of 1.4 βg lycoside. The final stage continues with the formation of cellulose polymerization by Acetobacter xylinium bacteria.

Pratiwi *et al*. (2005) stated that the greater the concentration of Acetobacter xylinum, the greater the number of bacteria, thus was positively correlated with the fiber content of nata. Meanwhile, in this study, the greater the volume of Acetobacter xylinum, the lower the fiber content value, as shown in Table 1. The average crude fiber content results at 10% volume was 0.25 - 5.0%, this is because the greater the volume of Acetobacter xylinum added to the The media causes the formation of metabolites in the form of acetic acid which can interfere with bacterial growth, and may be caused by increased osmotic pressure, causing cells to easily lyse so that the formation of cellulose was not optimal (Aulia *et al*., 2020).

Sulistiyana, (2020) explains that the fiber formed was the result of the breakdown of sugar in the fermentation medium by Acetobacter xylinum combined with fatty acids to form precursors in cell membranes, the precursors come out with enzymes that polymerize glucose into extracellular cellulose, therefore the longer the time fermentation means more extracellular cellulose was produced so the fiber content is higher. Meanwhile, in this study, the longer the fermentation time, the lower the fiber content produced, as shown in Table 1. The average crude fiber content for 10 days was 0.25 - 2.5%, this is because fermentation that is too long will cause Acetobacter xylinum bacteria experience a death phase because they run out of nutrients, causing the cells to lose a lot of fiber. Novia *et al*., (2021) and it is suspected that Acetobacter xylinum is less than optimal in synthesizing mangosteen peel extract into a cellulose matrix because of its low sugar content, the more the ratio of fruit peel extract. mangosteen, the lower the sugar contained in the fermentation media to form nata, the lower the sugar content will be. affects the lower extracellular cellulose which is formed from the breakdown of sugar (Novia *et al*., 2021).

Total Anthocyanin Content of Nata De Coco

Total Antocyanin Content test was carried out by squeezing nata de coco, after which the absorbance was measured using a uv-vis spectrophotometer and calculated using the TAC formulation.

Table 2. Total Anthocyanin Content With Factorial Design Experimental Results

Run	Ratio	Volume *Acetobacter xylinum* (%)	Day	TAC (%)	Result
1	1:19	2.5	10	7.48	22.06
2	9:11	2.5	10	36.29	-3.44
3	1:19	10	10	16.71	14.60
4	9:11	10	10	1.20	-12.01
5	1:19	2.5	13	13.48	13.29
6	9:11	2.5	13	1.80	-10.09
7	1:19	10	13	57.48	27.53
8	9:11	10	13	42.08	10.15

Table 2 shows that in run 7 with the ratio of mangosteen peel extract: coconut water (r) which is (1:19), the volume of *Acetobacter xylinum* 10%, and the length of fermentation 13 days has the highest TAC content of 57.486%. While the lowest TAC content is in the 4th run with the ratio of mangosteen peel extract: coconut water (r) which is (9:11), the volume of *Acetobacter xylinum* 10%, and the length of fermentation of 10 days which is 1.202%,

The main effect in this study was the volume of *Acetobacter xylinum* which is 14,60 where the higher the volume of *Acetobacter xylinum*, the higher the TAC level produced. While with an interaction effect of 27.534 where the longer the fermentation time and the more volume of *Acetobacter xylinum*, the higher the TAC content produced.

Putri, (2018) explained that the more volume of *Acetobacter xylinum*, the more acetic acid produced in the fermentation process, causing the media conditions to become acidic, the lower the pH or close to pH 1 in the medium, the higher the anthocyanin absorbance level, this situation causes the anthocyanin pigment to be in the form of flavylium or oxonium cations and the absorbance measurement will show the greater amount of anthocyanin.

The day of fermentation affects the TAC content in the nata. Kunci *et al*, (2008) explained that the longer the fermentation time, the more anthocyanins are trapped in the nata de coco.

Organoleptic

The best results of the crude fiber content test (run 1), the best results of the TAC test (run 7), and the best results of the fiber content test and TAC test (run 2) were then subjected to organoleptic analysis. Organoleptic tests include color, odor, and texture with an assessment range according to SNI, with range value 1 to 9.

Table 3. Organoleptic Results

Run	Color	Smell	Texture
1	6.23	3.93	6.43
2	6.2	4.7	6.16
7	5.9	5.2	6.4

Based on the results of organoleptic test analysis by 30 panelists, the experiment with the best color was obtained in run 1 with an organoleptic value of 6.23, the experiment with the best smell was obtained in run 7 with a value of 5.2, and the experiment with the best texture was obtained in run 1 with an organoleptic value of 6.43.

Thickness, Weight, and pH

The best results of the crude fiber content test (run 1), the best results of the TAC test (run 7), and the best results of the fiber content test and TAC test (run 2) were then analyzed for thickness, weight, and pH.

Table 4. Thickness, Weight, and pH Results

Run	Thickness (cm)	Weight (gr)	pH
1	1	65	4
2	0,9	47	3
7	1,6	86	4

The thickness is affected by the fermentation time of nata de coco. This was stated by Aulia *et al*., (2020) that the longer the fermentation period, the thickness will increase, which is optimal on day 13. In this study, the longer the fermentation time, the higher the thickness and weight values, the best was on run 7 with a ratio (1:19), *Acetobacter xylinum* volume 10%,

and 13 days of fermentation, namely 1.6 cm thick and 86-gram weight. According to Santosa *et al*., (2019) the greater the proportion of anthocyanin extract, the lower the thickness of the nata de coco produced. This is due to the presence of extracts in the content of coconut Rwhich can inhibit the growth of *Acetobacter xylinum* in the fermentation process.

The pH test experiments in run 1 and 7 produced a pH value of 4, while in experiment in run 2 produced a pH value of 3. According to Feri, (2007) the optimal conditions for forming nata with coconut water media occurred at pH 4.0, and nata formation only occurred at a pH between 3.5 – 7.5. This low pH occurs because during fermentation the bacteria *Acetobacter xylinum*, apart from forming cellulose, also breaks down sugars derived from extracts in the medium into organic acids in the metabolic process.

CONCLUSION

The total anthocyanin content (TAC) value of the mangosteen peel extract produced in this study was 2.992% and the yield of extract is 36,25%. The best value total anthocyanin content of nata de coco was 57,48%, and the best value of crude fiber content of nata de coco was 8%. The best results for crude fiber content and TAC test were then analyzed for organoleptic, thickness, weight and pH.

The experiment with the best color was obtained in the run 1 experiment with an organoleptic value of 6.23, the experiment with the best smell was obtained in the run 7 experiment with a value of 5.9, and the experiment with the best texture was obtained in the run 1 experiment with a value of 6,43.

The experiment with the best thickness was obtained in the run 7 experiment with a thickness of 1.6 cm nata de coco, the best weight was obtained in the run 7 experiment with a weight of 86 grams. Meanwhile, the pH is in the range of pH 3-4.

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