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FULL PAPER

The quality of nata de coco from sawarna and mapanget coconut varieties to the time of storing coconut water

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Abstract

This study was aimed to find out the type of coconut water and its storage time in order to get the best of nata de coco quality. The design of experiments conducted in this study nested random design 2 factors. The main factor coconut varieties consisting of two levels: palm fruit varieties sawarna and the coconut fruit varieties mapanget, the storage time of coconut water is nested in palm varieties which consists of 5 levels i.e. 0, 6, 12, 18 and 24 hrs. Each treatment was repeated 3 times so that the total of all 30 samples. The results showed that the nata de coco best coconut water obtained from fresh. However, it demonstrated that coconut water stored for 6 hrs of varieties of sawarna, the quality of nata de coco generated no difference from nata de coco coconut water varieties of fresh mapanget (0 hrs of storage) and reducing sugar in coconut water varieties sawarna is an important factor as the carbon source for *Acetobacter xylinum*. Thus, in order to get nata de coco with the best quality, coconut water of sawarna varieties which is still fresh or has been stored for 6 hrs can be used.

1. Introduction

Coconut water as a product of coconut fruits processed can be used as raw materials for making nata (Hamad *et al*., 2011; Lestari *et al*., 2014). Nata raw material is also easily obtained, available at any time and there are in each area. Indonesia has the largest land oil plants in the world with a total area of 3.82 million hectares with a production of 15.9 billion grains of coconuts per year which are mostly used to meet domestic needs (Rukmana and Yudirachman, 2016). This suggests that the use of coconuts by Indonesian society is very high, which means coconut water waste generated is also high.

The utilization of coconut water as raw material for making nata de coco so far is still derived from a mixture of various coconut varieties. Nutrient content, especially sugar in coconuts, is different for each variety. Varieties that are easily obtained, especially in Indonesia, are coconut varieties, especially coconut varieties in Sawarna (DSA) and coconut in Mapanget (DMT) (Rukmana and Yudirachman, 2016). This potential is one of the supporters of the continued supply of nata de coco raw materials.

DMT and DSA varieties have the same pH of 5.5 and total DMT solids are 5.95% higher than DSA of 5.69%. DSA reduction sugar by 3.95% is greater than DMT by 3.11%, whereas DMT potassium content is greater, ie 280 mg/100 g compared to DSA of 249.7 mg/100 g. DSA varieties with DMT have the same sodium content of 51 mg/100 g, while the vitamin C content of DMT is greater (2.46 mg/100 g) when compared to DSA (2.24%). Calcium content of DMT is greater, which is 40 mg/100 g when compared to DSA of 35.3 mg/100mL (Runtuwu *et al*., 2011)

Nata de coco fiber in the form of cellulose at 2.5% (Hidayat *et al*., 2006; Gea *et al*., 2010; Ma *et al.*, 2012). In addition to the high fiber content, this product has other advantages that the manufacturing process is easy and does not require a long time. This differs from the fruits and vegetables that require a long time to be consumed as a source of fiber.

Workmanship for using coconut water from a mixture of different varieties of coconuts while the use of coconut water from one variety to the manufacture of this product has not been done. The content of nutrients, especially sugar in the coconut fruit is different for each variety. Sugar in the making of this beverage products plays a very important because cellulose fiber is formed during the fermentation of sugars derived from (Jung *et al.*, 2010; Castro *et al*., 2011; Anas *et al*., 2012; Sunagawa *et al*., 2013).

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Coconut water use in the manufacture of these products is generally nata maker cannot use fresh coconut water directly due to various factors such as the distance between the source of raw materials to the place of production, transportation constraints and limitations of the production site. Factors that cause coconut water should be kept by the manufacturer before it is processed further nata.

During storage, fermented coconut water will be carried out by the natural microbes that may cause deterioration in chemical nutrients, especially in sugar because sugar is the best carbon source for microbial growth (Naufalin and Wibowo, 2004; Zeng *et al*., 2011; Alwi *et al*., 2015). Decreased levels of this sugar will affect the quality of the resulting cellulose fibers. Research on the use of coconut water that has undergone a process of storage as a raw material of nata de coco has not been conducted so far. This study was aimed to investigate the type of coconut water and its storage time to obtain the best quality of nata de coco.

2. Materials and methods

2.1 Raw material

The materials used in this study were the coconut water from coconut varieties sawarna (DSA) and varieties mapanget (DSM) enough old, obtained from Dampit village of Malang. Old age criteria from coconut husk are dark brown color, flesh thickness of 1.5 cm and coconut milk can be taken. Figure 1 shows the epidermis color and thickness of coconut flesh between Sawarna (DSA) and Mapanget (DMT) varieties. *Acetobacter xylinum* obtained from the Microbiology Laboratory of the UB, sucrose, glucose, extracts of yeast, peptone bacto, glacial acetic acid, Na_2HPO_4, $MgSO_47H_2O$, gelatin, H_2SO_4, NaOH, Aquadest, K_2SO_4 and $CaCO_3$.

Figure 1. Color the epidermis as well as the thickness of the coconut meat DSA and DMT

2.2 Breeding Acetobacter xylinum in starter

Starter namely strains *A. xylinum* cultured in the medium. Making the starter is done by growing a pure strain into the coconut water from coconut varieties sawarna and the coconut fruit varieties mapanget were enriched using nutrients in the form of sucrose and ammonium sulfate and pH of the media was made into a 4 by adding glacial acetic acid. The number of *A. xylinum* that will be inoculated into the fermentation medium uniform i.e. 2 x 10^7 cells/mL. To reach the cell number is calculated directly using hemocytometer (Oliveira *et al*., 2015; Sulistyani *et al*., 2016)

2.3 Nata de coco production

Good coconut water from coconut varieties sawarna and varieties mapanget is boiled at 100°C for 15 mins. Further thereto are added nutrients in the form of sucrose 2% and 0.06% ammonium sulfate number then boiled again. After the media poured into fermentation tanks and closed using parchment paper and further straining cloth is tied using a rubber band. Media were allowed to cool for 12 hrs after it was made into a media pH 4 by adding glacial acetic acid into 20 mL of media. Starter inoculation *A. xylinum* into the media so that it ferments further incubated for 14 days.

2.4 Experimental design

The experimental design used in this study is a randomized design nested 2 factors. The first factor coconut varieties consisting of two levels, namely Vr1 = varieties sawarna (DSA), Vr2 = varieties mapanget (DMT). While the second factor old coconut water storage which consist of 5 level including P1 = 0 hr, P2 = 6 hrs, P3 = 12 hrs = 18 hrs P4, P5 = 24 hrs. The second factor is nested on the first factor, each treatment was repeated 3 times, so we obtained 30 samples.

2.5 The observation of nata de coco

Observation of pH in the nata de coco fermentation media was carried out every day for 14 days (Brooks *et al*., 2013), cell development of *A. xylinum* methods Total Plate Count/ TPC (AOAC, 2002) and the thickness of nata (Gayathry, 2015). Whereas, the weight parameters (Gayathry, 2015) and the total fiber content of nata de coco (McCleary, 2014) were observed on day 14 after harvesting.

2.6 Data analysis

Data obtained during the study were analyzed using Analysis of Variance (ANOVA). If there is a real difference then a further test by using Honestly Significant Difference (HSD) at α = 5% is conducted (Hanafi, 2012; Kumalaningsih, 2012).

3. Results and discussion

3.1 pH

Different varieties of coconut and coconut water storage time significantly affect the condition of l growth *A. xylinum* in the process of nata pellicle formation. The stages of the formation of nata takes place during the fermentation process. During the fermentation process,

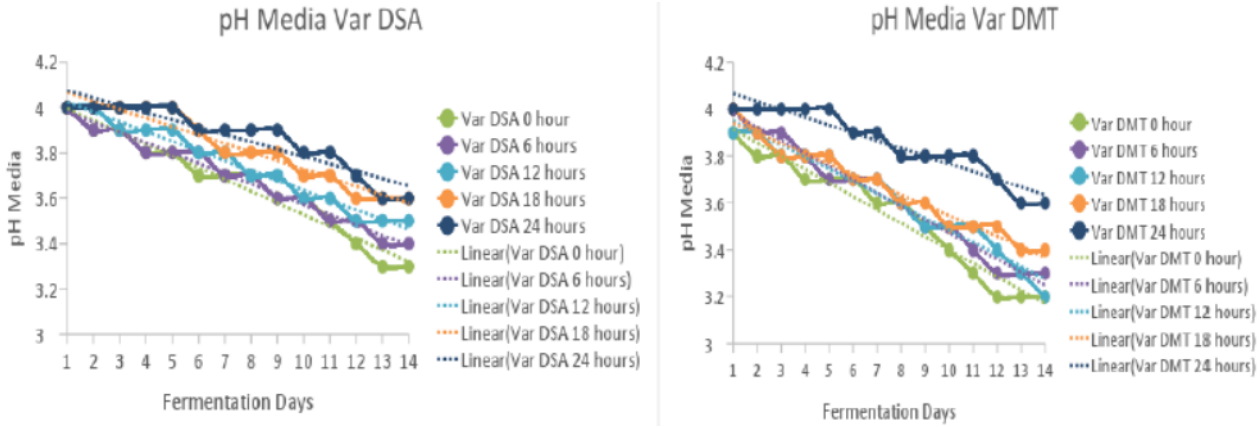

Figure 2. Graphic of growing media of pH *A. xylinum* during fermentation (14 days) on DSA and DMT on different time coconut water storage

the pH of the media as one of the conditions for the growth of *A. xylinum* had decreased since the first days of fermentation. It can be seen in Figure 2.

Varieties of media pH DSA and DMT are significantly different. DMT varieties pH is more acidic than varieties DSA though, during fermentation, a decrease in pH between the two varieties is likely to be similar. Different coconut water storage time significantly gave an effect on the condition of the media pH. The pH of fresh coconut water varieties DMT (0 hrs of storage) gives the average pH more acidic than other treatments do. The longer the storage time of coconut water, growing media pH rise although still under acidic conditions. Fresh coconut water (0 hrs of storage) had a pH ranging from 3.20 to 3.33 while coconut water that had been stored for more than 6 hrs of storage (6, 12, 18 and 24 hrs of storage) had a higher pH than the pH of fresh coconut water. This shows that the longer the storage time of coconut water, the more increasing the pH of the media. This typical pH conditions may affect the growth of *A. xylinum* as the pH of the media is one of the requirements that must be met in order that *A. xylinum* can grow well in the process of nata pellicle formation.

During the 14-day fermentation, the pH of the growing medium of *A. xylinum* decreased gradually, increasingly acidic. This condition caused the activity of the *A. xylinum* increased so nata pellicle formation process could run well. These conditions will produce nata de coco with good quality. Lestari *et al*. (2014) stated that *A. xylinum* can grow well in a medium with some condition that sufficient nutrients mainly containing a carbon source, a source of nitrogen, minerals, vitamins and media pH is acidic pH. In the process of nata pellicle formation, pH is necessary for the growth of *A. xylinum* which is ranging between 3-4 (Castro *et al*., 2011; Gea *et al*., 2011). During fermentation, the pH of the growing medium of coconut varieties that vary with a different time storage, media pH coconut water as a medium for growing *A. xylinum* is still under acidic conditions (ranging in Figure 2) so that the condition is still supporting activity of *A. xylinum* in the process of formation of pellicle nata.

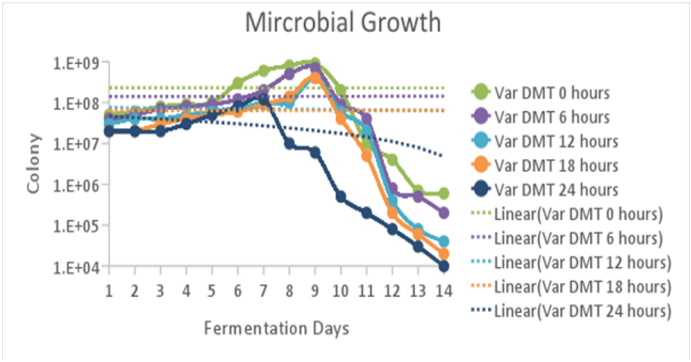

Figure 3. Graphic of growth of *A. xylinum* during fermentation (14 days) in coconut water media variety mapanget (DMT) with different coconut water storage time

3.2 Growth of Acetobacter xylinum

Graphic of the growth of *A. xylinum* for nata fermentation pellicle formation can be seen in Figure 3.

The graphic above shows that the different coconut varieties did not affect the growth of *A. xylinum* significantly during fermentation. Different coconut water storage time significantly gives effect on the growth of *A. xylinum*. The average growth of *A. xylinum* is the highest on the media which are still fresh coconut water (0 hrs of storage) in both the DSA and DMT varieties. The growth *A. xylinum* continued to increase from the first day until the day of fermentation to-9 fermentation. The highlight of the growth *A. xylinum* reached an average of the highest value on day 9 of the fermentation. Today the 10th until the 14th day of growth suffered a sharp decline. On the 14th day, nata de coco was harvested. This bacterium is an obligate aerobic bacterium that works to synthesize cellulose from sugar in the womb of a material (Alwi *et al*., 2015). The growth *A. xylinum* can run well on a medium with the requirements of sufficient nutrients, especially glucose (Jung *et al*., 2010).

Table 1.Variety effect of coconut and coconut water storage time on the quality of nata de coco

Treatment	Nata thickness (cm)	Nata Weight (g)	Nata crude fiber (%)	Sugar reduction (%)
DSA 0 hrs	1.87±0.03	246.67±5.77	2.01±0.05	3.25±0.01
DSA 6 hrs	1.62±0.03	210.00±10.00	1.91±0.05	3,14±0.01
DSA12 hrs	1.43±0.06	173.33±5.77	1.58±0.02	3.03±0.01
DSA 18 hrs	1.25±0.05	146.67±5.77	1.40±0.03	2.85±0.01
DSA 24 hrs	1.11±0.01	123.33±5.77	1.31±0.03	2.68±0.01
DMT 0 hrs	1.48±0.02	206.67±11.55	1.81±0.03	3.01±0.01
DMT 6 hrs	1.32±0.02	163.33±5.77	1.56±0.04	2.99±0.01
DMT 12 hrs	1.21±0.01	138.33±2.89	1.40±0.05	2.97±0.01
DMT18 hrs	1.17±0.02	113.33±5.77	1.30±0.07	2.49±0.01
DMT 24-hour	0.94±0.04	95.00±5.00	1.18±0.02	2.02±0.01

A. xylinum will continue to grow over the nutrients as long as the support growth is still available. It is visible on the first day until the 9th day of fermentation. The graphic of *A. xylinum* growth continued to increase since glucose (sugar) was much provided. On the 10th day of the fermentation, the growth of *A. xylinum* decline. This is possible because the nutrients necessary for the growth of *A. xylinum* has been reduced so that its growth is also slowing. Such conditions will certainly affect the quality of nata de coco generated. Wijayanti *et al*. (2016) suggested the formation stages nata pellicle layer in the form of cellulose hydrolysis process begins with the sucrose produces glucose and fructose using enzymes sucrase and invertase. When sucrose is in the growing medium has reduced the activity of *A. xylinum* also be decreased and nata produced is also unlikely to grow significantly when compared to the beginning of fermentation. That's why the common nata were harvested on the 14th day of fermentation.

3.3 Nata de coco quality

The quality observation parameter of nata de coco includes thickness, weight and crude fiber of nata. This can be seen in Table 1. Table 1 shows the varieties and coconut water storage time affects the quality of nata de coco generated. DSA varieties are better than DMT in improving the quality of nata de coco. This is because the reducing sugar content of DSA variety coconut water is higher than DMT (Runtunuwu *et al*., 2011). Coconut water containing sugar reduction in high levels has great potential as a medium for fermentation because it contains sugar which can serve as a fermentable sugar as well as a carbon source for the microbes (Yanuar and Sutrisno, 2015). In making nata de coco, sugar plays an important role as a source of carbon that can be changed by *A. xylinum* cellulose layer nata (Bhanthumnavin *et al*., 2016; Sainz *et al*., 2017).

Coconut water storage time also significantly affects the quality of nata de coco generated on all parameters nata observations in thickness, weight and crude fiber of nata. The use of fresh coconut water (0 hrs of storage) nata de coco provides the best quality when compared with coconut water that has been stored for 6, 12, 18 or 24 hrs. Nata de coco produced from coconut water stored in various storage periods with an interval of 6 hrs produces nata de coco with different qualities. The longer it is stored coconut water, nata de coco quality resulting in a decline in all parameters of observation in Figure 4.

Figure 4. Nata de coco from coconut water varieties DSA and DMT

The quality of nata de coco of fresh DSA variety (0 hrs of storage) is better than fresh DMT variety with the thickness of 1.87 cm, the weight of 246.67 g, the crude fiber of 2.01%, sugar reduction of 3.25% compared to storage treatment for 6.12 and 24 hrs. DSA varieties with a storage time of coconut water for 6 hrs, the quality of nata de coco is still better than fresh DMT varieties with a thickness of 1.62 cm, a weight of 210 g, the crude fiber of 1.91% and a sugar reduction of 3.14%. DSA varieties with 12 hrs long storage have better nata de coco quality compared to DMT, namely with a thickness of 1.43 cm, a weight of 173.33 g, the crude fiber of 1.58% and a sugar reduction of 3.034%. The quality of nata de coco in 18 hrs storage time, DSA variety was better than DMT variety with nata thickness difference of 0.08 cm, nata weight of 33.34 g, the crude fiber of 0.10% and sugar reduction of 0.36%. The quality of nata de coco in 24 hrs storage time, DSA variety is better than DMT variety with nata thickness difference of 0.16 cm, nata weight of 28.33 g, crude fiber of 0.13% and sugar reduction of 0.56%. This difference is one of them caused by the reducing sugar content of DSA which is higher than

DMT.

The stages of nata formation take place during the fermentation process. Stages of nata formation involve microorganisms, namely *A. xylinum*. This bacterium is an obligate aerobic bacterium that works by synthesizing cellulose from sugar in the content of an ingredient (Alwi *et al*., 2011). *A. xylinum* can grow well in a medium with several conditions, namely adequate nutrition, especially containing carbon (C), a source of nitrogen (N), minerals, vitamins, and media pH is an acidic pH (Lestari *et al*., 2014). The carbon element can be obtained from natural sugars contained in a material such as glucose, fructose and sucrose, so that the bacteria can work and can form a layer of nata (Pratiwi and Aryawati, 2012; Lempang, 2013). Wijayanti *et al*. (2016) stated the stages of the formation of a layer of nata pellicle in the form of cellulose begin with the hydrolysis of sucrose or starch which produces glucose and fructose using the enzyme sucrase and invertase enzymes.

Glucose or fructose that is formed is then converted into cellulose by *A. xylinum*. The formation of cellulose from glucose begins with the process of phosphorylation in glucose to glucose-6-phosphate which is catalyzed by the enzyme glucokinase. Furthermore, undergo an isomerization process which is catalyzed by the enzyme phosphoglucomutase to glucose-1-phosphate. After that, the formation of UDP-glucose is aided by the enzyme UDPG firophosphorylase and the formation of cellulose strings outside the cell with the help of the enzyme cellulose synthase (Anas *et al.,* 2012; Sunagawa *et al*., 2013). Sugar plays an important role in making nata de coco, because as a carbon source that can be converted by *A. xylinum* into a layer of cellulose nata (Bhanthumnavin *et al*., 2016; Sainz *et al*., 2017).

Most makers of nata de coco cannot immediately take advantage of fresh coconut water to be used as nata because of distance factor between the source of coconut water to the plant that is quite far, limited transportation or means of production, so sometimes coconut water is stored for several hrs before they were made into nata de coco, from the research that has been conducted, it is indicated that the best nata de coco produced was from fresh coconut water. However, it demonstrated that coconut water stored for 6 hrs of varieties of DSA, the quality of nata de coco generated no different with nata de coco coconut water varieties of fresh DMT (0 hrs of storage). This means that reducing the sugar content of coconut water varieties stored DSA for 6 hrs is still insufficient to meet the needs of a carbon source of *A. xylinum* the formation cellulose nata layer. Reducing sugar in coconut water varieties DSA is an important factor as the carbon source for *A. xylinum*. Although it kept for 6 hrs, the reduction of sugar levels is still sufficient for the microbial source of carbon for forming a layer of cellulose. Thus, to get nata de coco with the best quality can use coconut water varieties DSA is still fresh or has been stored for 6 hrs.

4. Conclusion

Good quality nata de coco is obtained from DSA variety coconut water with coconut water storage time for 6 hrs with a thickness of 1.62 cm, the weight of 210 g, the crude fiber of 1.91% and sugar reduction of 3.14%. The quality of nata de coco produced from DSA variety coconut water with a long storage time of coconut water for 6 hrs is better than the quality of nata de coco produced from fresh DMT variety (0 hrs of storage) coconut water which in this study was used as a control treatment.

Conflict of Interest

The authors declare no conflict of interest.

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