The effect of addition sucrose concentrations toward weight of Nata DE Lontar (*Borassus flabellifer*) Linn

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Abstract. Making nata made from older *Borassus flabellifer* wine (namely Nata de Lontar), it needs addition sucrose to produce nata with the optimum weight. The research aimed to know (1) The effect of addition sucrose with different concentrations on older palm wine toward the weight of “nata de lontar” (*Borassus flabellifer*) (2) The concentration of sucrose is the most influential part to produce optimum weight of “nata de lontar”. This study belonged to an true experimental research with posttest only control group design. The independent variable in this research was the addition of sucrose with different concentrations of 6%, 9%, and 12%. The dependent variable in this study was the weight of nata. The results were analyzed using one-way anova and then followed by a test of Least Significant Difference at the 5% significance level. The results of the experiment show (1) There have been a weight difference “nata de lontar” from bacterial fermentation in older palm wine *Borassus flabellifer* Linn medium with the addition of different concentrations of sucrose with p-value less than < 0.05. (2) Addition 12% sucrose concentration to the medium has influenced the formation of nata optimum results with average 46.13 grams.

1. Introduction

Nata comes from the Spanish word meaning cream. Nata is solid, white and transparent of colors and chewy [1]. Nata is health food product, using cereals, legumes, sweet corn, unpolished rice, mung bean and nata de coco as sources of dietary fiber [2]. Nata fermentation by acetic acid bacteria *Acetobacter xylinum* to produce cellulose [3].

Nata is a fermented product made of high sugar solution using *Acetobacter xylinum*. Enzyme from these bacteria converted sugar in fermentation media into a million of cellulose fibril (fiber) which will become white or transparent and compact. Fiber as supplement was high in insoluble fiber, the results seem to imply that the insoluble fiber in the supplement was responsible for the reduction in serum triglyceride of the hyperlipidemic subjects and blood cholesterol (TC) [2]. Cellulose produced by *Acetobacter xylinum* is named bacterial cellulose (BC) or microbial cellulose [4]. A cellulose derivative using the bacterial cellulose nata de coco has also been synthesized [5].

Carboxymethyl cellulose (CMC) has been widely used in edible coatings for fresh fruits and vegetables [6]; [7] The derivative is herein referred to as carboxymethylnata (CMN). The CMN
coatings reduced the rate of ripening of bell pepper fruits. [8] and [9] investigated the use of edible natural biodegradable coatings to replace commercial synthetic waxes as coatings for citrus fruits.

Under a microscope, the nata looks like irregular fibril mass resembling threads or cotton. Generally, nata is made by using coconut water known as nata de coco. Nata can also be made from other materials containing sugar such as pineapple, tomato, cocoa [1] and palm wine from Borrasus flabellifer Linn.

Palm plant (Borrasus flabellifer Linn.) is used by human, whether used for food, building materials, household furniture and handicrafts. Borrasus flabellifer is one type of palm that grows in coastal areas in dry climates, such as in Buleleng and Karangasem Bali Indonesia. Papyrus plant (Borrasus flabellifer Linn) cultivated by Bali’s farmers as a shade plant whose leaves are used as craft materials such as fans, hats, mats and to write media of traditional words. Whereas wood of palm plant can be used as building materials, tools and materials hand-crafted. Palm fruit can be used as a mixture of iced drinks or eaten fresh. Palm flower containing sap which can be consumed as a fresh beverage or through processed into brown sugar.

In Buleleng regency the province of Bali of Indonesia, precisely in the village of Abasan, district Tejakula. Palm (Borrasus flabellifer Linn.) sap is sold to manufacturers of brown sugar and palm wine. Best quality sap used as raw material for making palm wine and brown sugar are freshly harvested sap. Palm wine that is old with age more than one day (older palm wine) result palm wine value decrease. This happens because the sugar in palm wine decreases every day and the level of acidity increases. Circumstances sap acid can not generally be used as a basic ingredient of making palm wine and brown sugar. In an effort to improve the utilization of older palm wine, research must be done to produce a older palm wine processing technology to produce new products that have higher economic value. One of them is nata.

Making nata derived from wine raw materials, has not received much attention. This is caused by the lack of public knowledge in the use of wine into nata. Nata is a product of the bacterial Acetobacter aceti subspecies xylinum fermentation of a mixture material and glacial acetic acid were added to adjust pH to pH 3-4. A decrease in pH of the medium that passes through the pH optimum fermentation process causes disruption and disintegration of re nata cellulose into glucose that can be oxidized again to acetic acid. Sucrose passing optimum levels, causing a lot of sugar that is converted into acid. Nata sheet was harvested after 25 days [10].

Based on that explanation, then do research “The Effect of addition sucrose concentrations on older palm wine toward weight nata de lontar (Borassus flabellifer Linn)”

2. Methods

2.1 Research design
The Research design was used is a true experimental research, with the posttest only control group design has been using a Comletely Randomized Design have been using simple random technique. The number of samples that have been used followed the formula \( t(r-1) \geq 20 \) [11] where \( t \) is the treatment, \( r \) is the replication. The treatments that have been used different concentrations of sucrose 6%, concentration sucrose 9% and concentration sucrose 12%.

2.2 Population and sample
Population that has been used is older palm wine with volume 1500 ml is derived from 3 strands mature in 1 palm plant of Borassus flabellifer Linn. The study sample was using older palm wine with a volume of 1200 ml and the sample was divided into 24 samples. Each sample containing 50 ml volume of older palm wine.

2.3 Materials and Equipment
Older palm wine, sucrose, Diammonium phosphate (DAP), Zwavelzure Ammoniac (ZA), Alcohol 70% concentration, glacial acetic acid, starter (mother liquor) Acetobacter xylinum, aquadest, cotton, and
aluminium foil. And equipment were: petri dish, filter, bekker glasses, trays, paper, erlenmeyer, pipet, spatula, analytical balance, autoclave, hot plate, latex for closed of bottles, bunsen, and glasses volume.

2.4 Data Collection technique
2.4.1 “Nata de Lontar” Production
Older palm wine that has been aged for about 3 days of tapping filtered and put into erlenmeyer respectively of the volume of 50 ml and then boiled in hot plate. Each older palm wine has been added ZA fertilizer as much as 0.2 g/50 ml of older palm wine, DAP 0.3 g/50 ml of older palm wine, 0.1 ml of glacial acetic acid/50 ml and sucrose according to treatment that is 0 g/50 ml, 6%= 3 g/50 ml, 9%= 4.5g/50 ml and 12%= 6 g/50 ml then boil. Hot older palm wine solution is removed and put into each petri dish. The petri dish is placed on trays and then was done closing the tray using paper. Cool solution approximately 1 hour, paper cover is opened and each solution was added to a solution of 8 ml starter. Trays that had been given starter has been closed with the paper. The next solution is incubated at room temperature (about 28°C).

2.4.2 Data Analysis technique
The data that is heavy nata fermented taken on day 5 were measured using analytical balance. Data analysis technique using one-way anova for hypothesis testing. If in the analysis there is a meaningful difference, then do a further LSD (Least Significance Difference) at 5% significance level.

3. Results and Discussion
3.1 Results
Weight nata fermentation result are shown in Table 1. The optimal weight of nata that has been produced is at a concentration of 12%. The lowest nata weight was produced at a concentration of 6%. Increased nata weight has occurred, after administration of increased sucrose to the solution.

| Treatment | Weight of nata per 50 ml older palm wine (gr) |
|-----------|-----------------------------------------------|
|           | 6%    | 9%    | 12%   |
| 1         | 41.95 | 45.20 | 46.92 |
| 2         | 38.34 | 43.26 | 44.03 |
| 3         | 42.13 | 45.45 | 48.46 |
| 4         | 36.75 | 43.90 | 45.56 |
| 5         | 40.13 | 44.23 | 44.16 |
| 6         | 40.65 | 43.46 | 45.28 |
| 7         | 38.20 | 44.19 | 47.94 |
| 8         | 41.23 | 42.05 | 46.65 |
| Total     | 319.38| 351.74| 369.00|
| Average   | 39.92 | 43.98 | 46.13 |
Differences in treatment as result of fermented older palm wine illustrated in Figure 1.

Figure 1. Graphic Heavy “nata de lontar” *Borrasus flabellifer* Linn

The observation results of nata color has shown the color is muddy brown as Figure 2 below.

Figure 2. Nata production.

3.2 Data analysis
Data were analyzed using one-way anova with the stages of normality test using Kolmogorov-Smirnov, homogeneity data using Levene test and hypothesis test using one-way anova shown in Table 2.

| Group | Average | Deviation Standard | F-value | p-value |
|-------|---------|--------------------|---------|---------|
| 6%    | 39.923  | 1.957              | 30.837  | 0.000   |
| 9%    | 43.968  | 1.087              |         |         |
| 12%   | 46.125  | 1.645              |         |         |
Based on Kolmogorov-Smirnov test results have shown the value of p (probability) is more than 0.05 shows the distribution of the data follow a normal curve distribution. Homogeneity test showed p-value greater than 0.05, the data shows homogenous distribution of the data.

Results of hypothesis testing showed p-value less than 0.05 have demonstrated an nata weight difference between treatments. Hypothesis testing continued with LSD test to analyze treatment significantly and the level of significance shown in Table 3.

Table 3. Results LSD testing using Post Hoc Test at 5% significance levels

| Group          | Different | Value p(Sig.) |
|----------------|-----------|---------------|
| 6% with 9%     | -4.04500* | 0.000         |
| 6% with 12%    | -6.20250* | 0.000         |
| 9% with 6%     | 4.04500*  | 0.000         |
| 9% with 12%    | -2.15750* | 0.014         |
| 12% with 6%    | 6.20250*  | 0.000         |
| 12% with 9%    | 2.15750*  | 0.014         |

3.3 Discussion

Nata be made from various types of materials that contain sugar, protein, minerals [12]. One nata besides the manufacture of coconut water is older palm wine *Borrasus flabellifer* Linn. *Borrasus flabellifer* Linn reinforced by [13] stating that nata fermentation by using raw material produces nata is still low, ranging from 27.36 to 60.32% or average 34.31%. Low nata is due to the fermentation medium vulnerable to contamination by fungi. Nata fermenting bacteria is *Acetobacter xylinum* as the growing medium contains water, protein, fat, carbohydrate, ash and some minerals to nourish the growth of bacteria. Nutrients are added to the growth medium of bacteria are sucrose as a carbon source, ZA source of nitrogen, DAP is source of phosphate.

Under a microscope, the nata looks like irregular fibril mass resembling threads or cotton [1].

![Figure 3. Irregular fibrill mass of nata under microscope](source)

Source : [14]

*Acetobacter xylinum* bacteria use sucrose as a source of energy and carbon sources into extracellular cellulose so that the cells of bacteria trapped in a cellulose fiber layer.
The longer incubation supplies nutrients begin to decrease so that it will increase competition between bacterial cells to sustain life [16]. Based on statistical analysis that shows the influence of the addition of different concentrations of sucrose by weight ratio nata de lontar with statistical F-value = 30.837 and significance of 0.000. Figures significance less than < 0.05 shows there is significant contribution to the weight variation of sucrose concentration nata de lontar. This was confirmed by [17] that has proven different concentrations of sucrose in making nata of squash has given significantly different effect on weight nata at best is 0.76 cm thick. Sucrose has a heavy influence on nata also made by [18] who have proved that the addition of sucrose was significantly affected nata weight of liquid tofu waste with the highest nata weight 23.79 grams with addition of 10 grams per 200 ml of tofu whey.

In nata fermentation, the bacteria *Acetobacter xylinum* can break down sugars into cellulose components. Cellulose formation begins the growth of bacteria marked by the turbidity in growth medium after 24 hours of incubation. After 36 – 48 hours of incubation are formed in the surface layer of translucent medium and gradually thickens to form a new layer over the conditions allow. Extra long variations in the treatment that fermentation also has had a heavy influence on nata as research conducted by [19] to have proven that different concentrations of sucrose and fermentation time nata de mango have significantly different effect on the weight of nata de mango. The treatment with addition 10% sucrose and fermentation 16 days have provided a high nata weight is 1.325 cm.

Whereas treatment with the addition of 6% sucrose concentration and fermentation time of 10 days give nata a low weight is 0.675 cm. [20] has got the addition of mass of sugar 7 grams up to 13 grams in 300 ml of boiling corn water for 2 weeks fermentation in making nata de corn obtained nata de corn with thickness that has been produced not significantly different. [10] has got different shape of cellulose production were used coconut water, coconut milk and skim used a spectrophotometer (Perkin Elmer), X-Ray Diffraction (X’Pert PRO PAN analytical), Thermogravimetric analyzer (Leco TGA 701 ), SEM-XRD (Hitachi S-3400 N) in nata de coco production.

The results the effect additional nutrition in making nata have also been supported by [21] who have proven that sugar and fermentation time had significant effect on the thickness of the producing nata de soya. From her research showed that the average thickness of nata was observed at the highest of nata giving treatment with the addition of sugar 100 grams/l which has been combined with long fermentation for 7 days, with the resulting thickness of 3.390 mm nata. While the thickness of the thinnest nata has been observed in the treatment with the addition of sugar 150 grams/l were combined with the length of fermentation 14 days with a thickness 2.403 mm nata. [22] has been done using pineapple in the manufacture of nata de pinna has got results the highest weight was reached in treatment B (additional sugar + ZA) has got results weight of nata 899 grams/5 L, followed by treatment A (without addition sugar and ZA) has got weight of nata 616.4 grams/5L, and C (stored for six months with the addition of nutrients both additional sugar +ZA) has got weight of nata 477.8
grams/5 L. The thickness of NP of the height and low as in treatment B (1.58 cm) followed by treatment A (1.24 cm) and C (0.88 cm), respectively.

The addition of organic nitrogen source derived from soybean germs has resulted in better nata weight compared with the addition of urea [1]. The addition of 20 ml greenbean germs extract/2000 ml solution was also done on nata de cassava research and has got results; average 5.4 grams nata/50 ml; 9.9 grams nata/ 50 ml; 15.9 grams nata/ 50 ml and 19.7 grams nata/50 ml in treatments 0%; 0.75%; 1.5%; 2.25%, respectively of sugar [23].

In the process of fermentation, Acetobacter xylinum bacteria cells will grow rapidly from the initial cell number by experiencing exponential growth until it reaches the maximum amount with a generation time of approximately 2 hours [24]. When the number of cells reached a certain amount, aggregate form began to appear and the number of cells in solution are not rising as long as nata aggregate formation, some bacteria enter the cell cavities between the aggregate so that the bacterial cells are not free in solution.

On culture growth, the availability of oxygen in the surface of the medium and in the presence of glucose will stimulate an increase in cell mass and increase the amount of cellulase is enzyme forming cellulose. During the formation of cellulose, the cavities are filled by water nata causing nata become thick and contains water as much as ±5-98% and 2-5% cellulose [24].

Based on these results research by measuring the weight of “nata de lontar” has obtained an average yield of successive 39.92 grams, 43.98 grams, and 46.13 grams with the addition of sucrose concentrations in a row 6%, 9% and 12% respectively. Data have shown that the addition of sugar concentration of 12% by weight nata optimum yield.

4. Conclusion
The results of research that has been done statistically obtained there have been a weight difference nata from bacterial fermentation in older palm wine Borassus flabellifer Linn medium with the addition of different concentrations of sucrose with p-value less than < 0.05. Addition 12% sucrose concentration to the medium has influenced the formation of nata optimum results with average 46.13 grams.
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