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Application of *Acetobacter Xylinum* in The Production of *Nata De Legen* From Palmyra Palm Neera

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Abstract. Legen (neera) is produced from palmyra palm (*Borassus flabellifer* Linn.) neera that favored local people, but storability of legen not durable, this palmyra neera fermentation is relatively quick so it doesn't have high selling value. A technology is needed in the form fermentation to produce *nata de legen* using *Acetobacter xylinum*. *Nata de legen* is made by fermentation using legen medium with the addition of sucrose and food grade ZA. *Nata de legen* production was carried out on 15 medium samples which were repeated twice. *Nata de legen* post-harvest processing is carried out for bleaching and softening using strong base solutions. The results showed that the 15 medium samples succeeded in producing *nata de legen* with white turbid morphology, chewy, fibrous texture, incubation period 7-9 days, thickness of nata ranging between 1.05-1.55 cm, weight between 500-700 g, the surface is smooth or wavy and smells acid. The results of post-harvest processing produce white *nata de legen* and nata fibers easily removed.

1. Introduction

Palmyra Palm (*Borassus flabellifer*) is native to the Indian subcontinent and Southeast Asia, including Indonesia [1]. Almost all parts of palm trees can be used. Primary product of palmyra palm is nira obtained from tapping flowers, which can be drunk directly or processed into sugar, and secondary product of palmyra palm is used for craft materials [2]. All of these products are of economic value, both for farmers and sellers. The problems experienced by farmers and sellers are legen (palmyra neera) and Palmyra fruit don't last long and spoil quickly, if they are not sold within a few days they will be spoiled and thrown away. This causes loss to farmers and sellers. Application of technology is needed as an effort to use legen into another food products that have more selling value and could increase profit for farmers and sellers. The technology chosen is applying fermentation to produce nata de legen. In general, the production of nata is done by direct inoculation into liquid medium. Immobilization of cells is a technique used to trap the cells into a matrix. The use of immobilized cells for the production of nata is one alternative to the product resulted in a cell-free nata [3]. Nata is a bacterial cellulose formed from bacterial activity [4], with *Acetobacter xylinum* metabolizes glucose and converts it into bacterial cellulose that has unique properties including high purity, crystallinity and mechanical strength [5]. Cellulose products produced by aerobic bacteria (*Acetobacter xylinum*) get much attention because of their unique physicochemical properties compared to plant cellulose, including their application in the food industry [6]. Legen that has been processed into nata de legen has high selling value and potential so that it can improve the status of the surrounding community to become a business center for nata de



legen with skilled and creative producers in processing nata de legen into a variety of food and beverage products.

The process of making nata de legen from palmyra is an effort to increase the economic value of legen, which so far has only been used to make legen drinks, which not everyone likes, because of its high alcohol content. Nata is a processed food product good for health for its high fiber which is useful to help digestive process of food. It also has high economic value and profitable because the production process requires simple ingredients and low cost. Nutrient content of palmyra neera consists of water (87.66%), sugar (12.04%), protein (0.36%), fat (0.36%), and ash (0.21%). The result of processing isotonic drinks with a combination technique obtained sugar content of 10.4-16.3%, pH 4.0-4.3, as well as the smell and taste of the preferred category until very preferred [7]. Different results on mesocarp of Palmyra fruit showed a water content of 77.31%, total fat 0.11%, ash 1.43%, tannin 0.08%, total carotenoids 8324.6 μg / 100 g, carotene 6217.48 μg / 100 g [8]. Another technique for preserving nata is using the drying method. Nata samples were dried using three physical drying methods such as oven, tray dryer or freeze dryer until it achieved 3-5% moisture content [9].

The problem experienced by legen farmers and sellers is that it performs natural fermentation in a short time which causes destructive legen, sour taste, and the smell of alcohol so that it does not sell and will eventually be discarded. This causes losses for farmers and sellers. One effort to reduce losses from damaged legen is to process it into other products, such as liquid or solid palm sugar which has a long production process and uses a lot of fuel but has a low selling value. Therefore, other technologies are needed to utilize legen which is spoiled into other food products that have a higher selling value and increase profits for farmers and sellers. The technology applied to increase legen value is conventional biotechnology in the form of fermentation to process legen into nata de legen using *Acetobacter xylinum*. The aim of this research was to study legen fermentation technology into nata de legen using the *Acetobacter xylinum*.

2. Method

This research was conducted using a sample of 15 which was repeated twice each time, carried out in the resident's house of Shoberoh Village, Dalegan Village in natural conditions. This research was carried out through several stages, namely multiplication of the bacterium *Acetobacter xylinum*, making *nata de legen* and post-harvest processing from *nata de legen*. Making starter is done by breeding *A. xylinum* bacteria on siwalan or legen medium with the addition of sucrose (25 $\text{g}\cdot\text{L}^{-1}$) and foodgrade ZA (0.5 $\text{g}\cdot\text{L}^{-1}$) placed in a sterile bottle aerobically with an incubation of 7 days at room temperature (28-30 $^{\circ}\text{C}$). The next stage is the artificial *nata de legen* made on legen medium with the addition of sugar and foodgrade ZA with the same composition as the medium for making starter *Acetobacter xylinum*, the medium is boiled at 100 $^{\circ}\text{C}$ for 15 minutes then poured into as many sterile plastic trays 1 L/tray. The medium in the tray is left to cool and inoculated with the *A. xylinum* starter as much as 10%. Medium covered with plain brown paper. Medium that has been inoculated with starter bacteria is incubated at room temperature (28-30 $^{\circ}\text{C}$), for 7-9 days aerobically until *nata de legen* is formed. The third stage is the harvest of *nata de legen* and post-harvest processing to get the *nata de legen* ready for consumption. Nata harvest is done by nata separated from the rest of the medium and cleaned by the lowest layer by peeling. *Nata de legen* is washed with water until clean and cut into 1x1 cm pieces. Nata pieces are soaked with water for 3 days, every day the water is replaced. On the 4th day the nata was soaked in a strong base solution (NaOH) with a concentration of 0.5% for 24 hours. Then the nata is washed with water many times until the pH becomes neutral (pH 7). After the pH of nata neutral nata is boiled until boiling for 15 minutes. Nata is ready to be processed into food/beverage products.

3. Result and Discussion

The results of this study were obtained in 3 stages. Stage 1, making starter (multiplication) of *Acetobacter xylinum*. Stage 2, making *nata de legen* and stage 3 post-harvest processing. In production of starter (multiplication) of *Acetobacter xylinum* in 15 samples, each sample was repeated 2 times. The results showed that each sample of the medium in the bottle was inoculated with *A. xylinum* bacteria can grow well on legen medium with the yield of 0.9-1.2 cm at 7 days incubation (Figure 1),

and the medium shows a cloudy color. In the manufacture of this starter, no contaminated samples were found.

In the stage of making *nata de legen* which was also carried out on 15 samples which were repeated 2 times, obtained a good result which was obtained by *nata de legen* with a supple texture, whitish brown (cream) with a weight of 800-900 g.L⁻¹ media and only 1-1.5 cm in incubation 7-9 days (Table 1).

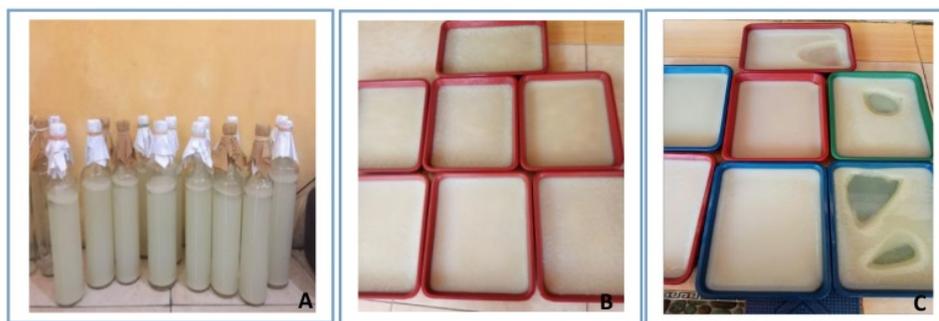


Figure 1. Starter *Nata De Legen* Containing *Acetobacter Xylinum* (A), *Nata De Legen* with A Smooth Surface (B), And Some Surfaces With Holes (C)

Result of research from *Acetobacter xylinum* application in the manufacture of starter and *nata de legen* from palmyra sap in Table 1. The application of *Acetobacter xylinum* multiplication or known as starter is carried out in the second stage, making *nata de legen*. According from the 15 samples that were repeated as many as two times, the results were shown in Table 1.

Table 1. Morphology of *Nata De Legen* Starter (*Acetobacter Xylinum*) And *Nata De Legen* Made from Legen

Sample	Starter morphology		<i>Nata de legen</i> / Liter medium						
	Solution	Thickness of nata (cm)	Texture	Fiber	Appearance	Thickness (cm)	Weight (g)	Smell	Surface
1	TW	1.0	Chewy	Fibrous	TW	1.25	643	Acid	Smooth
2	TW	0.9	Chewy	Fibrous	TW	1.05	500	Acid	Smooth
3	TW	1.2	Chewy	Fibrous	TW	1.45	700	Acid	Smooth
4	TW	0.9	Chewy	Fibrous	TW	1.35	686	Acid	Smooth
5	TW	0.9	Chewy	Fibrous	TW	1.20	629	Acid	Smooth
6	TW	0.9	Chewy	Fibrous	TW	1.30	657	Acid	Smooth
7	TW	1.2	Chewy	Fibrous	TW	1.50	700	Acid	Perforated
8	TW	1.0	Chewy	Fibrous	TW	1.25	643	Acid	Smooth
9	TW	1.1	Chewy	Fibrous	TW	1.25	657	Acid	Smooth
10	TW	1.0	Chewy	Fibrous	TW	1.45	700	Acid	Smooth
11	TW	1.2	Chewy	Fibrous	TW	1.50	700	Acid	Perforated
12	TW	1.0	Chewy	Fibrous	TW	1.50	700	Acid	Perforated
13	TW	0.9	Chewy	Fibrous	TW	1.10	609	Acid	Smooth
14	TW	0.9	Chewy	Fibrous	TW	1.25	643	Acid	Smooth
15	TW	0.9	Chewy	Fibrous	TW	1.25	643	Acid	Perforated

Note: TW = turbid white. Measurement of nata starter was carried out at the 7th day incubation and *nata de legen* measurements performed on the 6-9th day incubation (when the medium has not left)

The Result from 15 samples medium nata that were selected with *Acetobacter xylinum* with morphology of nata turbid white, chewy, fibrous, 7-9 days incubation period ranges from 1.05 to 1.55 cm, weighs between 500-700 g, smells acid, and no samples were contaminated. High fiber supplement mixture of nata was reported able to reduced lipid level of consumer [10]. No medium left after 9 days incubation. So almost all mediums change to *nata de legen*. Although there are some that have perforated but uncontaminated surface (Figure 2).



Figure 2. Post-harvest processing (box-shaped nata pieces) (A) and *nata de legen* bleaching (B)

At the post-harvest stage, processing of nata is carried out so that nata can be consumed. Unprocessed nata is still in sheet form, tastes sour and the fiber is difficult to remove. The nata formed after 9 days of incubation is in sheet form. This nata is immediately harvested and washed with water to remove the acid odor. Besides that the lowest layer of nata is also removed so that the remnants of the media become nonexistent. Nata subsequently cut into cubes or rectangles with a size according to taste (generally 1x1 cm) and nata then soaked in water until the color becomes whiter and the smell of acid reduced subs 3 days and each day the water is replaced prior to the next process is softening and bleaching.

Bleaching is done by soaking the nata in 0.5% caustic soda (NaOH) solution for 24 hours. Nata washed many times until the alkaline nature of the loss (held not slippery) and his own re-neutral pH (pH 7). Soft nata with features can be torn or loose fiber when bitten. The white and soft nata are then boiled and ready for the product.

Propagation results of *Acetobacter xylinum* on the legen medium showed that the starter had been formed with characteristics already formed white chewy mass on the surface of the media in the starter bottle and the medium liquid showed turbid white. The white chewy mass is called *nata de legen*, while the rather turbid liquid below it is called starter. This stater can be used to make *nata de legen* or make it stand again with the legen siwalan media. Siwalan Legen can be used as a medium for making nata because it has a composition similar to coconut water. According to [11], palmyra legen contains total sugar 10.93 (g/100 cc); Protein 0.35 (g/100 cc); Nitrogen 0.056 (g/100 cc); Phosphorus 0.14 (g/100 cc); Iron 0.4 (g/100 cc); Vitamin C 13.25 (mg/100 cc).

The high content of legen nutrition can be converted into nata. *Nata de legen* has a smooth, white and high-fiber texture. By using the bacterium *Acetobacter xylinum*, the liquid form of legen can be converted into cellulose fiber sheets that are chewy and can be used as a mixture of drinks or packaged foods. The thick sheet of cellulose was washed, boiled and cooked in sugar syrup for food applications such as desserts, fruit cocktails and jellies [12]. The results of the multiplication of bacteria *A. xylinum* / stater were then applied to the legen siwalan medium to make *nata de legen*. In this application the results obtained with the characteristics nata nata formed its thickness ranges between 1-1, 5 cm, b closely nata ranges from 5 to 700 g, surface texture of nata is smooth, some perforated with fiber regularly, supple, turbid white and smelled acid.

The morphology of the surface of the nata caused by the cover of the tray (brown paper) that is not neat so that it sticks to the media, the area with brown paper causes *A. xylinum* to not produce nata properly. However, the areas affected by the brown paper were not contaminated because the paper was still new (so it was still sterile) besides the medium which was high in acidity (pH 3) so that it could be a selector for the microbial environment or microbes that might be on the sticky brown paper. In post-harvest processing by soaking the pieces of nata in fresh water for 3 days with water replaced every day

and soaking in a strong base solution for 24 hours, it can whiten and squeeze the nata. Fibers of nata previously released when chewed. An increase in nata production has been reported in a way addition 12% sucrose concentration to the medium has influenced the formation of nata optimum results with average 46.13 g [13] and how to increase the feasibility of *nata de legen* production, it is necessary to increase the yield of nata production up to 90% [14].

4. Conclusion

Acetobacter xylinum which will be applied to the making of *nata de legen*, can be multiplied on the legen medium with the results of the starter liquid turbid white and the surface of the media formed nata with a thickness ranging between 0.9 -1.2 cm. *Nata de legen* produced from legen medium showed morphology of turbid white, supple, fibrous, incubation period of 7-9 days, with thickness of nata ranging from 1.05-1.55 cm, weight between 500-700 g, surface smooth or perforated and acidic. The results of post-harvest processing produce white *nata de legen* and fibers of nata easily removed.

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