

CHARACTERISTICS OF NATA FROM CARICA (*Carica Pubescens*) EXTRACT WITH DIFFERENT CONCENTRATION OF ACETOBACTER XYLINUM AND FERMENTATION TIME

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ABSTRACT

Nata de carica is a product of the innovation of fermentation of carica fruit (*Carica pubescens*) with the use of *Acetobacter xylinum* as a culture. The objective of this study was to analyze the impact of *Acetobacter xylinum* starter concentrations and fermentation time on the physical characteristics (chewiness, color, thickness, yield), chemical (crude fiber content, water content) and organoleptic (descriptive test) of nata de carica. The experimental design was used factorial design with two factors, *Acetobacter xylinum* starter concentration (10%, 15% and 20%) and fermentation time (7 days and 14 days). The results showed that there was a relationship between thickness, yield, chewiness and crude fiber, the thicker the nata, the greater the yield, chewiness and crude fiber. The best results of nata de carica treatment was treatment with 20% *Acetobacter xylinum* concentration and 14 days of fermentation time with a thickness value of 6.29 mm, a yield value of 41.04%, a texture value (efficiency) of 521.27 g/mm, crude fiber value of 1.05%, a color value of L* 50.36, a value of a*-1.87, a b* - 3.93 and water content of 98.86%. The results of the research can be concluded that the starter concentration and the fermentation time influence the physical and chemical properties of the nata de carica produced.

Keywords: Carica, Characteristics, Fermentation, Nata, Starter

INTRODUCTION

Nata is a product of fermentation by *Acetobacter xylinum* bacteria in media containing sugar, an acidic environment and requires a nitrogen source for its growth activity. Nata processing can use ingredients from coconut water, pineapple juice, tomato juice and other fruits that contain lots of sugar, protein, vitamins and require nitrogen sources for their activities. Some nata can be produced from certain materials so that the name is adjusted to the name of the material, for example nata de coco is made from coconut water, nata de molasses from molasses, nata de soy from tofu liquid waste, nata de citrus from sour oranges, nata de pina from pineapple, and so on. (Mulawi & Kristina, 2019). Based on the definition of nata, nata fermentation can be developed with different raw materials, one of which is by using carica fruit juice. Carica fruit contains vitamins, proteins, minerals, carbohydrates and sugars needed by *Acetobacter xylinum* to form nata so that it has the potential to be developed as a source of nata raw material.

Acetobacter xylinum is a bacterium used in nata production that plays a role in cellulose production. The presence of a starter in making nata is an important requirement because it plays a role in increasing the number of *Acetobacter xylinum* colonies to produce

enzymes to form nata (Putri *et al.*, 2021). The use of different starters is done to maximize nata production so as to produce the desired characteristics of nata products. (Nurdin, 2023).. According to research Marlinda and Hartati (2019) stated that the optimum condition of *Acetobacter xylinum* to produce maximum nata de banana with the addition of *Acetobacter xylinum* as much as 30% resulted in a thickness of 2.77 cm, yield of 51%, and water content of 85.8%. The length of nata fermentation is also an important factor in determining the quality of nata produced during fermentation. This is done to determine the right fermentation time to produce good quality nata products. According to research Aulia *et al.* (2020) The optimal conditions for making guava juice nata are at a sugar concentration of 5%, pH 3 and a fermentation time of 14 days with a yield of 60.18%, carbohydrate content of 7.25%, protein content of 0.11%, sucrose content of 1.96%, and water content of 97.20%.

For this reason, research is needed on the effect of different concentrations of *Acetobacter xylinum* starter and the length of fermentation in the process of making nata products from carica fruit juice raw materials and analyzing the effect on the physical, chemical, and organoleptic characteristics of carica fruit juice nata.

METHODOLOGY

Material

The materials used in this study include raw materials and analytical materials. The raw materials for making nata de carica are perfectly ripe carica fruit from the Dieng area, *Acetobacter xylinum* starter, granulated sugar, vinegar, ZA (Zavelzure ammoniak or Ammonium sulfate), and distilled water. Chemical analysis materials used are distilled water, H₂SO₄, NaOH, 70% alcohol, filter paper.

Tools

The tools used in this study include *stainless steel* knives, *stainless steel* taxis, cutting boards, analytical scales (*ohaus*), blenders, measuring cups, 500 ml volumetric flasks, glasses, spoons, *stainless steel* pots, gas stoves (rinnai), spatulas, plastic trays, labels, incubators, plastic wrap and thermometers, while the equipment used for analysis includes a caliper, drying oven, desiccators, tongs, aluminium cups, WR-10 *chromameter*, *Texture Profile Analysis TXT 32*.

Research Design

This study used a two-factorial complete randomized design (CRD), namely the first factor of the amount of *Acetobacter xylinum* starter concentration with three levels, namely 10%, 15%, and 20% and the second factor is the length of fermentation time, namely 7 days and 14 days. Each treatment was replicated 3 times, resulting in 18 experimental units.

Research Stages

1. Method of Making Nata Seedlings (Starter)

The use of starter is a very important requirement that aims to increase the number of *Acetobacter xylinum* colonies that produce nata-forming enzymes. The process of making nata de coco starter starts with filtering coconut water as much as 1.5 liters and cooking coconut water until it boils (Majesty *et al.*, 2015). Then coconut water is added 500 grams of sugar and stirred until evenly distributed. Then storage is done into a closed bottle and fermentation is carried out for 7 days in a closed room. The results will form the top 2 layers and the bottom layer of liquid is the starter seed.

2. Preparation Stage of Carica Fruit Juice

The preparation stage starts with preparing the fruit juice (Sultan & lahming, 2022). The carica fruit to be used must be sorted first. In this study, the carica fruit used was ripe with the characteristics of the yellowed fruit skin but the flesh was not too soft, then the fruit was peeled and washed with running water, then the fruit was split and separated the pulp from

the seeds, then the fruit was cut into cubes, then the carica fruit was crushed using a blender with water added 2: 1. The result of the crushing is filtered using a filter cloth until the juice is separated from the carica fruit.

3. Nata de Carica Preparation

Preparation of nata de carica (Modification of Mulawi & Kristina, 2019) Nata de carica is made by preparing 1000 ml of carica juice cooked by heating at 80 °C for 10 minutes in a saucepan then adding sugar, ammonium sulfate and vinegar. then poured into a clean or sterile tray as much as 400 ml of each container and then cooled to room temperature. Next, *Acetobacter xylinum* starter (10%, 15%, 20%) was added according to the treatment. Then fermented with a fermentation time of 7 days, 14 days in an incubator with a temperature of 31 °C.

The nata harvesting process is carried out after fermentation. The characteristics of nata that is ready to be harvested are no brownish water, cloudy white color, no mold and neither hard nor mushy and has a thickness comparable to the volume of the height of the carica juice poured on the tray. After the fermentation time according to the treatment, the nata that has been formed and ready to be harvested and then washed with clean water and soaked with water for 2 days. The soaking water was changed every day. On the second day after soaking, the nata sheets were boiled for 15 minutes and cut the nata sheets into squares then washed.

Methods

Data analysis was carried out using *analysis of variance* (ANOVA) with a significance level of 0.05 and continued with the Duncan test. data analysis was carried out using SPSS version 24.

Analysis Procedure

The analysis carried out on the research of nata de carica products, namely thickness (Term Sorong), yield, texture (*Texture Profil Analysis*), color (Chromameter WR-10), crude fiber (AOAC, 2005), moisture content (AOAC, 2005), organoleptic test (descriptive).

RESULTS AND DISCUSSION

The results of the research conducted obtained nata de carica products with the addition of *Acetobacter xylinum* starter concentrations of 10%, 15% and 20% with fermentation times of 7 days and 14 days. Characterization carried out on nata de carica products consists of thickness analysis, yield, texture, color, crude fiber, moisture content and organoleptic test (descriptive). The results of physical analysis of nata de carica products with each treatment can be seen in Table 1.

Table 1. Physical analysis of nata de carica

<i>Acetobacter xylinum</i> starter	Fermentation time	Thickness (mm)	Yield (%)	Texture (mm/g)
10%	7 days	1.88±0.20 ^a	13.48±0.47 ^a	53.19±16.55 ^a
	14 days	4.15±0.16 ^c	30.68±0.91 ^d	127.98±16.48 ^b
15%	7 days	2.33±0.29 ^b	15.07±0.33 ^b	109.92±14.65 ^{ab}
	14 days	4.86±0.18 ^d	33.84±0.82 ^e	209.46±34.14 ^c
20%	7 days	4.12±0.16 ^c	17.4±0.65 ^c	165.44±28.84 ^{bc}
	14 days	6.29 ±0.18 ^e	41.04±0.83 ^f	521.27±66.84 ^d

Different notations on the same figure indicate significantly different at a significant level (α) 5% by Duncan's test.

1. Thickness analysis

Based on Table 1, the results of thickness measurements on nata de carica show that the addition of *Acetobacter xylinum* starter 10%, 15% and 20% with fermentation duration of 7 days and 14 days shows significantly different results between treatments with each other. The addition of *Acetobacter xylinum* starter and the length of fermentation gave different mean values. The highest average value is in the treatment of 14 days of fermentation with a starter concentration of *Acetobacter xylinum* 20%, the results obtained are 6.29 mm. while the lowest results are in the treatment of 7 days of fermentation with a starter concentration of *Acetobacter xylinum* 10%, the results obtained are 1.88 mm. These results indicate that the higher the concentration of starter given, the thickness of the nata increases. *Acetobacter xylinum* requires time for the adaptation phase for 1 day, then growth increases (logarithmic phase) until the 5th and 7th days indicated by the thicker nata formed (Putri et al., 2013).

2. Yield analysis

Based on Table 1, the results of yield measurements on nata de carica show that the addition of starter *Acetobacter xylinum* 10%, 15% and 20% with a fermentation time of 7 days and 14 days shows significantly different results between treatments with each other. The highest average value of nata yield is with the treatment of the 14th day of fermentation with the addition of 20% *Acetobacter xylinum* starter concentration, which is 41.04%. While the lowest average nata yield was the 7th day fermentation length treatment with the addition of 10% *Acetobacter xylinum* starter, which was 13.48%.

Nata yield is influenced by the weight of nata and the thickness of nata produced after fermentation. The increase in nata weight is influenced by the cellulose sheets formed on the fermentation medium. The longer the fermentation time, the heavier the nata formed, so that the nata yield also increases. Different fermentation lengths produced different cellulose levels, the longer the 14th day of fermentation, the higher the cellulose content of the nata, so that the nata de carica is heavier and the yield increases. Yield is influenced by variations in substrate, material composition, environmental conditions, and the ability of *Acetobacter xylinum* to produce cellulose. (Marlinda & Hartati, 2019).

3. Texture Analysis (chewiness)

Based on Table 1, the results of the measurement of elasticity in nata de carica show that the addition of starter *Acetobacter xylinum* 10%, 15% and 20% with a fermentation time of 7 days and 14 days shows significantly different results between treatments with each other. Based on Table 1, it can be seen that the highest result is with the addition of *Acetobacter xylinum* 20% starter concentration and fermentation duration of day 14 amounting to 521.27 g/mm. while the lowest result is with the addition of *Acetobacter xylinum* 10% starter concentration and fermentation duration of day 7 amounting to 53.19 g/mm. The results of this study are supported by Nurdin. (2023). That the longer the incubation time, the more cellulose will be produced and the chewy texture of the nata, because there are still sufficient nutrients available so that the bacteria continuously metabolize and reproduce at a high enough level. Cellulose monomers from the secretion of *Acetobacter xylinum* continue to bind with each other to form layers that continue to thicken as the metabolism of *Acetobacter xylinum* continues.

4. Nata De Carica Crude Fiber

The type of fiber in nata de carica is crude fiber. Crude fiber is the result of sugar breakdown in the fermentation medium by the activity of *Acetobacter xylinum* (Suzanni et al., 2020). The results of chemical analysis of nata de carica products are presented in Table 2. Based on Table 2, the results of crude fiber analysis on nata de carica show that the addition of starter *Acetobacter xylinum* 10%, 15% and 20% with fermentation duration of 7 days and 14 days shows significantly different results between treatments with each other. The highest average value of fiber content is found in nata de carica products with a fermentation time of day 14 and a concentration of 20% *Acetobacter xylinum* starter, which is 1.05%. While the

results of the average value of the lowest fiber content were found in nata de carica products with fermentation duration on day 7 and *Acetobacter xylinum* starter concentration of 10%, which was 0.79%.

The high percentage of crude fiber produced cannot be separated from the influence of the inoculated starter. The activity of *Acetobacter xylinum* bacteria in cellulose production depends on the ability of bacteria to break down sucrose into cellulose. The high value of fiber is influenced by the activity of bacteria. Bacteria will perform cellulose-producing activities influenced by nutrients in the fermentation medium. N source is a nutrient that affects the formation of fiber, the bond between cellulose will be more compact so that the cellulose layer becomes stronger. (Nurdyansyah & Widyastuti, 2017).

Table 2. Chemical analysis of Nata de carica

<i>Acetobacter xylinum</i> starter	Fermentation time	Crude fiber Content (%)	Water content (%)
10%	7 days	0.78±0.01 ^a	98.60±0.04 ^b
	14 days	0.85±0.01 ^b	98.89±0.02 ^c
15%	7 days	0.89±0.01 ^b	98.68±0.02 ^a
	14 days	0.95±0.01 ^c	98.87±0.03 ^c
20%	7 days	0.98±0.01 ^c	98.69±0.04 ^a
	14 days	1.05±0.04 ^d	98.86±0.02 ^b

Note: Different notations in the same figure indicate significantly different at a significant level (α) 5% by Duncan's test.

5. Moisture Content Analysis

The treatment of the addition of *Acetobacter xylinum* starter concentration and fermentation duration did not significantly affect the water content. Based on Table 2, it can be seen that the highest water content is in the addition of 10% *Acetobacter xylinum* starter concentration and fermentation duration on day 14, which is 98.89%. While the lowest water content in the addition of *Acetobacter xylinum* starter concentration of 20% and fermentation duration of day 7 is 98.69%. The water content in a thin layer of nata results in a network that is not tightly formed and will bind large amounts of water so that nata is formed which has a greater water content. However, with the increase in the concentration of *Acetobacter xylinum*, the nata layer will be thicker so that the nata network is tighter and ultimately results in the water content of the nata decreasing. (Marlinda & Hartati, 2019).

6. Color Analysis

The color of nata de carica is measured using a chromameter with units of L*a*b. L is the level of brightness, the higher the L value, the brighter the color and the lower the L value, the darker the color. (Putriana & Aminah, 2013). The results of color analysis of each treatment of nata de carica products can be seen in Table 3.

Based on Table 3, the results of L* color measurements on nata de carica show that the addition of *Acetobacter xylinum* starter 10%, 15% and 20% with a fermentation time of 7 days and 14 days shows results that are not significantly different between treatments with each other. The results of the color analysis of L* nata de carica showed that during the fermentation process the L* value of nata de carica decreased. The results of measuring the color of L* nata de carica on the 7th day of fermentation until the 14th day decreased. The highest L* color of nata de carica is the 7th day of fermentation with the addition of 10% *Acetobacter xylinum* starter concentration, which is 60.2. While the lowest L* value of nata de carica is the treatment of the 14th day of fermentation with the addition of 20% *Acetobacter xylinum* starter concentration, which is 50.3. This is because the color is influenced by the thickness of the nata, the thicker the nata, the darker the color (cloudy), on the other hand, the thinner the nata, the lighter the color (white). In addition, the color produced in nata is influenced by the color of the raw material for making the nata. (Putriana & Aminah, 2013).

The value of a* is red (0 to 60) and green (0 to -60). Based on Table 3, the data of nata de carica color analysis results with the treatment of *Acetobacter xylinum* starter concentration and fermentation duration obtained results ranging from -2.34 to -0.46. The highest a* value is in the sample with the addition of 15% *Acetobacter xylinum* starter and 7 days fermentation time, while the lowest a* value is in the sample with the addition of 10% *Acetobacter xylinum* starter and 14 days fermentation time. The color of nata de carica produces a reddish color as the fermentation time increases. (Mustain et al., 2022)..

Based on the results of the analysis, the b* value is yellow (0 to 60) and blue (0 to -60). In Table 3, the data of nata de carica color analysis results with the treatment of *Acetobacter xylinum* starter concentration and fermentation duration obtained results ranging from -4.48 to 3.54. The highest b* value results are in the sample with the addition of starter *Acetobacter xylinum* 10% and fermentation duration of 7 days, while the lowest b* value is in the sample with the addition of starter *Acetobacter xylinum* 10% and fermentation duration of 14 days. The color of nata de carica produces a yellowish color as the fermentation time increases. (Romadhoni et al., 2023)..

Table 3. Color analysis of nata de carica

Acetobacter xylinum starter	Fermentation time	Color		
		L*	a*	b*
10%	7 days	60.20 ± 0.50 ^d	-1.02 ± 0.18 ^b	3.54 ± 1.46 ^d
	14 days	52.52 ± 0.50 ^b	-2.34 ± 0.25 ^a	-4.48 ± 0.52 ^a
15%	7 days	59.25 ± 1.80 ^d	-0.46 ± 0.06 ^b	0.83 ± 0.91 ^c
	14 days	51.53 ± 1.20 ^a	-1.94 ± 0.04 ^a	-3.75 ± 0.13 ^a
20%	7 days	55.88 ± 0.90 ^c	-0.69 ± 0.63 ^b	-0.93 ± 0.64 ^b
	14 days	50.30 ± 0.90 ^a	-1.87 ± 0.33 ^a	-3.93 ± 0.16 ^a

Note:

- Different notations in the same figure indicate significantly different at a significant level (α) 5% by Duncan's test.
- Color L: brightness level; color a*: red (0 to 60) and green (0 to -60); color b*: yellow (0 to 60) and blue (0 to -60).

7. Nata de Carica Sensory Test

Sensory attribute characteristics were described by trained panelists using the focus group discussion method led by the panel leader. Assessment of sensory quality is used to determine the nature and description of a product based on its sensory properties. Parameters that can be identified by panelists include aroma, taste, texture and color presented in Table 4.

Table 4. Sensory test of nata de carica

Acrtobacter xylinum starter	Fermentation time	Parameters				
		Chewiness	Sour aroma	The scent of carica	Nata flavor	Color
10%	7 days	2.90±1.19 ^a	1.80±0.63 ^a	1.70±0.67 ^a	2.30±0.48 ^{ab}	3.10±0.56 ^a
	14 days	3.30±0.94 ^{ab}	1.70±0.67 ^a	2.00±0.81 ^a	2.20±0.91 ^{ab}	2.90±0.87 ^a
15%	7 days	3.30±0.40 ^{ab}	1.70±0.67 ^a	2.00±0.81 ^a	2.10±0.73 ^{ab}	2.60±0.60 ^a
	14 days	3.60±0.84 ^{ab}	1.70±0.67 ^a	1.90±0.73 ^a	2.10±0.73 ^{ab}	2.80±0.78 ^a
20%	7 days	3.20±1.03 ^{ab}	1.90±0.73 ^a	1.80±0.78 ^a	1.70±0.48 ^a	2.80±0.78 ^a
	14 days	3.90±0.73 ^b	1.70±0.67 ^a	1.90±0.73 ^a	2.60±1.07 ^b	2.70±1.49 ^a

Different notations in the same figure indicate significantly different at a significant level (α) 5% by Duncan's test.

Based on the results of the organoleptic test on the color of nata de carica in the treatment of *Acetobacter xylinum* starter concentration and fermentation duration can be seen in Table 4 based on the results of the panelists' assessment, the nata de carica obtained from the 6 treatments gave different values. The highest average value results in the treatment of adding 10% *Acetobacter xylinum* starter concentration with a fermentation time of day 7 which is 3.1, which is close to white.

Texture is one of the test parameters that aims to determine the level of chewiness of nata. Based on Table 4, the average result of the highest chewiness in the treatment of adding 20% *Acetobacter xylinum* starter and the 14th day of fermentation is 3.9, which is very chewy. This is because the length of fermentation of 14 days the results of the nata are somewhat thicker so that the texture felt by the panelists is chewy. The chewiness of nata is a measure that can be assessed by panelists. The chewiness of nata is influenced by the formation of cellulose. The activity of *Acetobacter xylinum* bacteria that use sugar for growth as an energy source is able to produce a layer of lender which will eventually become dense and form a chewy structure (Marlinda & Hartati, 2019).

Aroma is one of the parameters that determine the attractiveness or not of certain food products. Based on Table 4, the average result of the highest sour aroma is with the addition of 20% *Acetobacter xylinum* starter and the length of fermentation on day 14, which is 1.9, which is not sour. A good aroma for nata de carica is not sour. While the average aroma of carica is around 1.7-2. The addition of starter and the length of fermentation of nata de carica have no effect on the aroma of nata de carica. The non-acidic aroma of nata occurs because it passes through the harvest period with 1-2 days of soaking and after that the nata de carica is washed and then boiled for 10 minutes at a temperature of 100° C so that the sour aroma in nata de carica is lost during washing and boiling. (Salelatu & Rumahlatu, 2016). Taste is an organoleptic test parameter that involves the sense of tongue that will show 4 categories of taste, namely sweet, salty, bitter, and sour. Based on Table 4, the highest average result of nata flavor is with the addition of 20% *Acetobacter xylinum* starter concentration and day 14 fermentation time, which is 2.6, which is the taste of nata that is not too tasteless. Nata de carica with a good taste because the difference in fermentation time produces a good nata flavor that is relatively the same.

CONCLUSION

Based on the research data obtained, it can be concluded that the addition of *Acetobacter xylinum* starter concentration and fermentation duration affect the thickness, yield, texture (chewiness), crude fiber, but have no effect on water content and sensory tests of chewiness, sour aroma, carica aroma and nata taste. The best nata de carica treatment is the addition of 20% *Acetobacter xylinum* concentration and 14 days of fermentation with a thickness value of 6.29 mm, yield value of 41.04%, texture value (chewiness) of 521.27 g/mm, crude fiber value of 1.05%, color L* value of 50.36, a* value -1.87, b* value -3.93 and water content value of 98.86%. Descriptive organoleptic characteristics resulting from the chewiness parameter are very chewy, the color of nata is slightly whitish, the aroma of nata is not sour and the taste of nata is bland.

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