Indonesian Food Science and Technology Journal
IFSTJ : Vol 4 no 1 December 2020
ISSN : 2615-367X

Optimization Of Glucose and Nitrogen Consentrations In Nata de Citrus Culture System From Calamansi Orange Juice

Nita Anggreani#*, Ahmad Jais#, Bambang Gonggo Murcitro*, Anggun Maulidia#, Egi Dia Safitri#

Department of Medical Laboratory Technology, Akademi Analis Kesehatan Harapan Bangsa (AAK-HB), Jl. Depati Payung Negara Padang Kemiling, Bengkulu, 38213, Indonesia
* Department of Soil Science, Faculty of Agriculture, Universitas Bengkulu, Jl. W.R.Supratman Kandang Limun, Bengkulu, 38371A, Indonesia
*email : nita0220048203@aakharapanbangsa.ac.id

Submission date : 16 October 2020 Published date : 31 December 2020

Abstract—**Calamansi orange is a superior fruit that is being promoted for planting in Bengkulu Province. Calamansi citrus preparations have only a few variations, generally made into calamansi orange syrup. This research was conducted to optimize the nata fermentation medium based on glucose and nitrogen concentrations. This study used a completely randomized design (CRD) with two factors. The first factor was the variation of glucose in the medium by diluting orange juice 0 ; 0.5; 1; 1.5 and 2 times. The second factor was the variation of nitrogen in the medium obtained by adding 0.6; 0.7 and 0.8% Ammonium Sulfate. The results were analyzed using ANOVA and the significantly different variables were further analyzed using second order orthogonal polynomials method. The optimum results were obtained at a glucose concentration of 1.76% (due to 1.65 times dilution of calamansi orange juice 1.65 times) and a nitrogen concentration of 0.17% (due to 0.8% ammonium sulfate addition).**

Keywords—**Calamansi; Glucose; Nitrogen; Nata de Citrus**

I. Introduction

Calamansi orange (*Citrofortunella microcarpa*) is one of the fruits that is being promoted for planting in Bengkulu Province. Until 2017, calamansi oranges have been planted in an area of 39 hectares [1]. This orange tastes very sour so it is less desirable if eaten directly. Therefore, this orange is processed beforehand so that it is comfortable to consume. Processed calamansi oranges are still limited. Currently, Kalamansi orange juice is only processed into syrup. Kalamansi orange syrup is now widely consumed by the public and is used as souvenirs for tourists. The potential of Kalamansi citrus fruit should be further developed so that it can become a variety of useful products and can increase regional income.

Research that has been done on processed products from calamansi oranges include calamansi orange syrup [2,3], calamansi orange marmalade [4], essential oils from the by-products of calamansi orange [5,6], and calamansi orange candy. Other processed products need to be researched and developed. Besides being made as syrup, orange juice has the potential to be processed into nata products called nata de citrus [9-11]. Nata is bacterial cellulose with a sturdy consistency and slightly chewy texture. Nata from citrus juice includes those prepared from lemon [9], grapefruit [10], and from rejected Berastagi orange [11].

Acetobacter xylinum bacteria are cellulose-producing bacteria. In their metabolism, these bacteria need nutrients. The nutrients needed by *Acetobacter xylinum* include C, H, N, and minerals in a controlled process. The fermentation medium usually contains only a part of the source of the nutrients needed because it lacks the nutrients it needs to be added from outside. Sucrose, glucose, fructose, and flour can be added as a carbon source. As a nitrogen source, you can add urea, ammonium sulfate, and yeast (yeast) extract [12]. Sugar concentration, pH, and ammonium sulfate concentration are important factors affecting moisture content, hardness, and other physical properties of nata de coco [13].

This study aimed to optimize the glucose concentration (from the Calamansi orange juice dilution) and the nitrogen concentration (from the addition of ammonium sulfate) in the fermentation medium which produces the best quality of nata de citrus.

II. Material and Methods

This research was conducted in July - September 2020 at the Bacteriology Laboratory of the Harapan Bengkulu Health Analyst Academy.

A. Material

The research materials used included calamansi orange, luff schoorl reagent, sodium thiosulfate, starch indicator, ammonium sulfate, sucrose, calcium carbonate (pH regulator), aquades, and *Acetobacter xylinum* bacteria. The tools used include knives, slicing mats, filters, beakers, glass stirrers, measuring cups, trays, analytical scales, spatulas, watch glasses, paper covers, rubber, ovens, micrometers, and desiccators.*B. Methods*

This study used a Completely Randomized Design (CRD) with two factors. The first factor was the glucose concentration with five levels which varied according to the orange juice dilution system as much as 0 (no dilution); 0.5 ; 1 ; 1.5 and 2 times. The second factor is the nitrogen concentration of three levels according to the variation of ammonium sulfate concentration in the medium of 0.6; 0.7 and 0.8%. Each treatment was repeated three times.

Observations made in this study included variables of wet weight (g), thickness (mm), yield (%), and pH of the remaining medium.

The glucose levels of Calamansi orange juice were analyzed using the Luff-Schoorl method according to SNI-01-2891-1992.

The stages in the fermentation of nata de citrus are presented in Figure 1.

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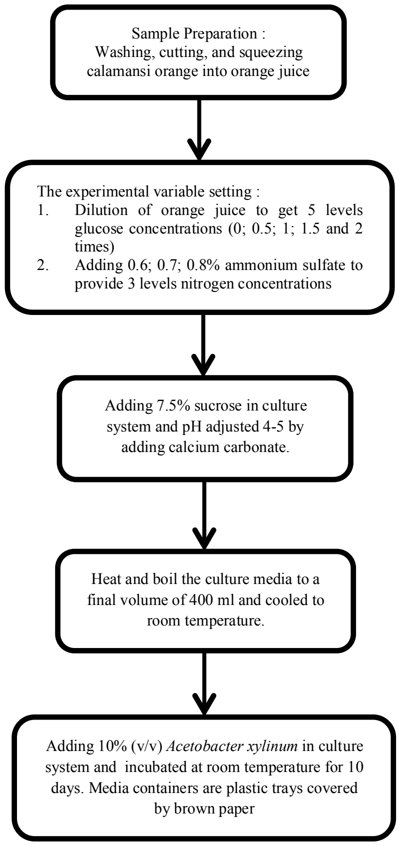

Figure 1. Scheme to prepare nata de citrus from calamansi orange.

On day 10, the nata de citrus formed was analyzed, which included wet weight (gr), thickness (mm), yield (%), pH of the remaining medium, and water content (%). Previously, the nata was washed and cleaned of impurities and then drained for further analysis.

Wet weight was analyzed by weighing nata de citrus using a scale. The thickness was measured using a micrometer, the yield was calculated using the formula:

$$\text{Yield} = \frac{\mathit{The\ Wet\ weight\ of\ nata\ de\ citrus}}{\mathit{Initial\ Medium\ Weight}}\ x\ 100\%$$

pH was measured using a pH meter. Moisture content was measured by means of gravimetry using an oven.

All data were analyzed using Analysis of Variance (ANOVA) at the 5% confidence level. If The F count ≥ F table implying that the interaction of both treatments was significantly different, the next step was to use the Polynomial Orthogonal method to determine the optimum glucose and nitrogen concentrations.

Orthogonal polynomials are one of the approaches used to find the most suitable regression equation to draw the response pattern (trend) of observation variables to quantitative treatment at the same interval [14,15].

III. RESULT AND DISCUSSION

The results of the glucose analysis in calamansi orange juice using the luff schoorl method are shown in the data in Table 1 at 0 times dilution. The glucose level at the dilution level was then calculated by the dilution formula ($V_1M_1 = V_2M_2$).

TABLE I
GLUCOSE CONCENTRATION OF CALAMANSI ORANGE JUICE

Dilution (times)	Glucose Concentration mg/100 ml
0	2.9
0.5	1.93
1	1.45
1.5	1.16
2	0.97

The pH of kalamansi orange juice was measured between 3.2 -3.5. Calcium carbonate added to the culture medium serves to adjust the degree of acidity (pH) [16]. Calcium carbonate is a neutralizer of the resulting organic acids in the fermentation process, so that the pH of the culture medium can bemaintained [17]. In this study, the pH could be adjusted up to 4-4.5 by calcium carbonate.

The interaction between glucose and nitrogen concentrations contributed significantly to differences in the wet weight, the yield, or the thickness of the nata de citrus ($p \leq 0.05$). The interaction between glucose concentration and nitrogen concentration did not contribute significantly ($p > 0.05$) to the pH of the remaining medium and the water content.

With the orthogonal polynomial method, statistical analysis was carried out (all formula calculations calculated in excel) so that conclusions can be drawn about the most suitable order to show the observed variable response pattern to treatment [14,15].

The respon pattern of glucose and nitrogen concentration interaction on the wet weight of nata de citrus can be seen in**Figure 2**. The dilution of calamansi orange juice and the reduction in glucose concentration for the nitrogen concentration level of 0.13% or ammonium sulfate concentration of 0.6% formed a curve pattern linear decreasing. This shows that with the dilution and decrease in glucose concentration, the wet weight of nata de citrus will be lower. This line was not yet able to show the optimum result point.

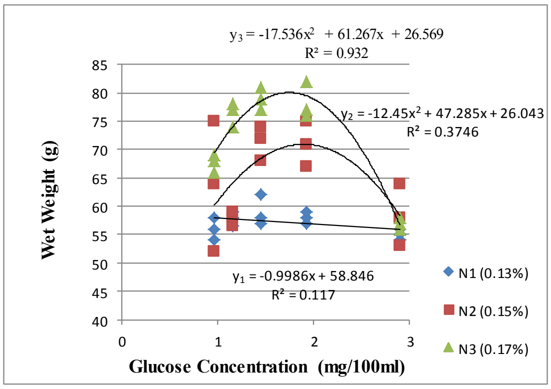

Figure 2. The Interaction Between Glucose Concentration and Nitrogen Concentration Against Wet Weight (gr) Nata de Citrus Kalamansi Orange

Different condition occured for the nitrogen concentration of 0.15% (ammonium sulfate 0.7%) and 0.17% (ammonium sulfate 0.8%) at the lower glucose concentration because the dilution produces a quadratic curve pattern (**Figure 2**). The fitting was good for nitrogen concentration 0.17% (R^2=0.932) and quite poor for 0.15% (R^2=0.3746).

The optimum wet weight of nata de citrus was produced at a nitrogen concentration of 0.17% (ammonium sulfate 0.8%). The glucose concentration that produces the most optimum wet weight of nata de citrus was the peak of the curve and could be calculated from the equation $y_3 = -17.536x^2 + 61.267x + 26.569$, then the optimum glucose concentration was obtained at a concentration of 1.75% or at a dilution of 1.66 times.

The respon pattern of glucose and nitrogen concentrations interaction on the thickness of nata de citrus can be seen in **Figure 3.** The dilution of Kalamansi orange juice and the reduction in glucose concentration for the nitrogen concentration level of 0.15% or the concentration of ammonium sulfate 0.7%, it formed a curve pattern linear decreasing. This shows that in this condition the tendency for the thickness of the nata de citrus to be lower with the dilution.

Different condition occured for the nitrogen concentration of 0.13% (ammonium sulfate 0.6%) and 0.17% (ammonium sulfate 0.8%) at the lower glucose concentration because the dilution produces a quadratic curve pattern. The fitting was good for both nitrogen concentration 0.13% and 0.17% as shown by high R^2 values.

The optimum thickness of nata de citrus was produced at a nitrogen concentration of 0.17% (ammonium sulfate 0.8%). The glucose concentration that produces the most optimum thickness of nata de citrus was the peak of the curve and could be calculated from the equation y3 = $-0.4501x^2 + 1.5902x + 0.6528$. The optimum glucose concentration point was obtained at a concentration of 1.77% or at a dilution of 1.64 times.

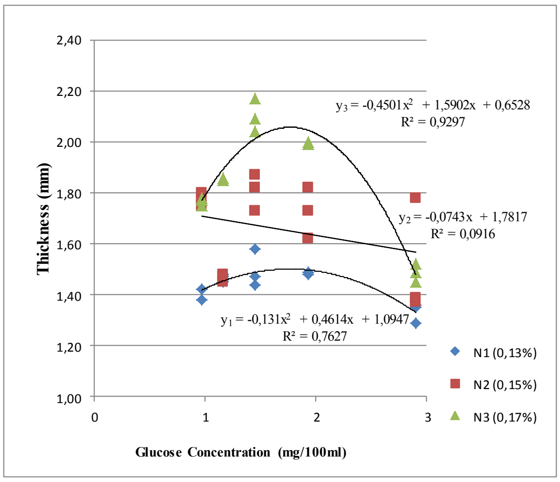

Figure 3. The Interaction Between Glucose Concentration and Nitrogen Concentration Against Thickness (mm) Nata de Citrus Kalamansi Orange

The respon pattern of glucose and nitrogen concentrations interaction toward the yield can be seen in **Figure 4**. The dilution of calamansi orange juice and the reduction in glucose concentration for the nitrogen concentration level of 0.13% or ammonium sulfate concentration of 0.6%, it formed a curve pattern linear decreasing. This shows that with the dilution and decrease in glucose concentration, the yield of nata de citrus is getting smaller.

Different condition occured for the nitrogen concentration level of 0.15% (ammonium sulfate 0.7%) and 0.17% (ammonium sulfate 0.8%) with lower glucose concentrations because the dilution produces a positive quadratic curve pattern. . The fitting was good for nitrogen concentration 0.17% (R^2=0.932) and quite poor for 0.15% (R^2=0.3663).

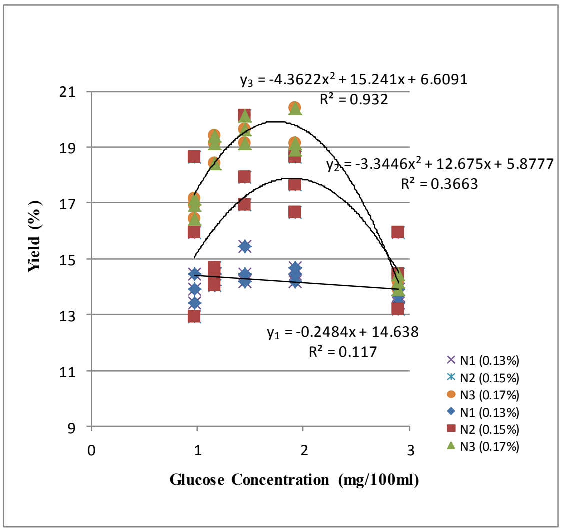

Figure 4. The Interaction Between Glucose Concentration and Nitrogen Concentration Against Yield (%) Nata de Citrus Kalamansi Orange

The optimum yield of nata de citrus was produced at a nitrogen concentration of 0.17% (ammonium sulfate 0.8%). The glucose concentration point that produces the most optimum yield of nata de citrus was the curve peak and could be calculated from the equation y3 = -4,3622x^2 + 15,241x + 6,6091. The optimum glucose concentration point was obtained at a concentration of 1.75% or at a dilution of 1.66 times.

The optimum average when viewed from the overall results of wet weight, thickness, and yield as described by means of the orthogonal polynomial plot above, the optimum concentration for glucose is 1.76% (Kalamansi orange juice dilution is 1.65 times) and nitrogen concentration is 0.17% (ammonium sulfate 0.8%).

When compared with previous studies, the results obtained are relevant. Ratnawati (2007) obtained the optimum results for glucose concentration in tamarind juice (*Citrus limon* L) at 1 times dilution [9]. This study did not use an orthogonal polynomial model in determining the optimum result, only according to experimental data. In this study, the optimum glucose concentration was also obtained at 1 times dilution (glucose 1.45 mg / 100 ml) as shown in Fig. 2–4.

Likewise, for the review of nitrogen concentration, the results obtained in this study are relevant. According to research by Lestariono (2001), the concentration of ammonium sulfate that produced the highest thickness and yield of nata de milko product was 0.8% ammonium sulfate [18].

IV. CONCLUSION

The optimum conditions for fermentation of nata de citrus from calamansi orange juice were obtained through predictive modelling. The calculated optimum conditions were at a glucose concentration of 1.76% (calamansi orange juice dilution was 1.65 times) and a nitrogen concentration of 0.17%. (ammonium sulfate addition of 0.8%).

ACKNOWLEDGMENT

We would like to thank the Directorate of Research and Community Service (DRPM), Deputy for Strengthening Research and Development, Ministry of Research and Technology / National Research and Innovation Agency for funding this research.

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