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Siti Nur Jannah, Dwi Handayani, Susiana Purwantisari, Mohamad Endy Yulianto, Rizka Amalia, and Indah Hartati

Application of Response Surface Methodology to The Optimization of Coco Vinegar Production as Antioxidant and Antidiabetes Through Bubble Biofermentation Process

Siti Nur Jannah[1, a)], Dwi Handayani[2, b)], Susiana Purwantisari[1, c)], Mohamad Endy Yulianto[2, d)], Rizka Amalia[2, e)] and Indah Hartati[3, f)]

[1]Department Biology, Faculty of Science and Mathematic, Diponegoro University, Semarang-Indonesia
[2]Industrial Chemical Engineering, Vocational School, Diponegoro University, Semarang-Indonesia
[3]Chemical Engineering, Faculty of Engineering, Wahid Hasyim University, Semarang-Indonesia

[a)] Corresponding author: nurjannah.suroso@gmail.com
[b)] dwihandayanimt@gmail.com
[c)] susiana_purwantisari@yahoo.co.id
[d)] endy_y@yahoo.com
[e)] Corresponding author: riz.chemicalia@gmail.com
[f)] hartatiprasetyo@gmail.com

Abstract. An effort to increase the added value of coconut water through the production of coco vinegar powder as an antioxidant and antidiabetic by fermentation have the advantage of being easier and cheaper than the chemical processes. The development of bubble biofermentation is an applicative technique in increasing production scale as well as the quality of coco-vinegar. It is because of the simple design, the easy flow and stirring, the uniform retention time in biofermentor, the wider contact area with lower energy, the increasing mass transfer and allowing large capacity. This research aimed to optimize the operation conditions of coco vinegar production through the bubbling biofermentation process. The process parameters studied were fermented time (18-22 hours), oxygen flowrate (0.1-0.3) and percent of inoculum (12-18%). Research showed that the modeled 52% adequate to predict the bubble biofermentation studied in this research (R^2=0.52). The percent of the inoculum was the most influencing variable for bubble biofermentation process. The bubble bioferementation process was optimum at a fermentation time of 23.364 h, oxygen flowrate of 3 and percent inoculum of 20.045%.

Keywords: coconut water, bubbling biofermentation, coco vinegar, RSM

INTRODUCTION

Young coconut fruit is one of the tropical plant products which consists of components of fruit flesh and coconut water that can be consumed. The carbohydrate (sugar) content of coconut water ranges from 1.7 to 2.6%, so it can be processed into vinegar product, which is derivative fermented products [1]. Vinegar containing 4% acetic acid, glycerol alcohol, esters, reducing sugar, pentoses, salts, and other substances.

The bioactive components formed after fermentation allows vinegar from coconut water (coco-vinegar) to be developed into functional beverage products. Previous researchs explain that vinegar contains functional compounds which are benefit for human health such as organic acids, antioxidants, amino acids and peptide compounds [2]. Antioxidants contained in several types of vinegar, polyphenol components: catechins and epicatechins can significantly reduce oxidative stress[3]. Some studies explain that vinegar can be functioned as an anti-diabetic. It was proven that vinegar can significantly reduce the blood glucose in diabetic rats [2].

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Vinegar production methods can range from traditional methods to complex methods. The tradiotional methods are performed by employing wood casks and surface culture, meanwhile the latest method is performed in a submerged fermentor [4]. Many technical devices and types of biofermentor have been developed to improve the industrial production of vinegar. Generally, these improvements increase the speed of the transformation of ethanol into acetic acid in the presence of acetic acid bacteria[5]. Fermentation for producing cocovinegar is more profitable process because it is easy and inexpensive. The conventional fermentation process does not guarantee the stability of the product quality because its conditions cannot be controlled. Therefore we need a technology that can control the environmental conditions during the fermentation process.The development of bubbling biofermentation is an applicative technique for increasing the scale of production and the quality of coco-vinegar. Bubble biofermentator have a simple design, fixed part, controlled flow and agitation, uniform space time, larger contact area, lower energy input, larger capacity and higher mass transfer rate.

This research aims to produce healthy coco-vinegar drinks through bubbling biofermentation process using microbes: *Sacchaomices cerevisiae* and *Acetobacter aceti*. The application of Response Surface Methodology (RSM) to the optimization of coco vinegar production through bubble biofermentation process was studied in this research and presented in this paper.

MATERIALS AND METHODS

Materials and Apparatus

The main raw materials for producing vinegar are alcohol, acetobacter bacteria, oxygen, and some herbs and fruits as a flavor. In this research, the alcohol for vinegar production is made from fermented coconut water [6]. The coconut water was supplied by a local supplier from Semarang, Central Java, Indonesia. The apparatus used in this research was a bubble biofermentor.

Methods

Experiment Design

The experiments are designed by using Central Composite Design. The independent variables of bubble biofermentation process were X1, X2, and X3 fermentation time, oxygen flowrate and percentage of inoculum respectively. Each variable to be optimized was coded at five levels: - α, -1, 0, +1 and + α. This gives a range of these variables of bubble biofermentation process (Table-1).

TABLE 1. Central Composite Design of the optimization process of coco vinegar production through bubble biofermentation process

Independent variables	Coded variable levels				
	-α	-1	0	+1	**+α**
Fermentation time (hour)	16.63641	18	20	22	23.36359
Oxygen Flowrate (L/min)	0.031821	1	0.2	0.3	0.368179
Percentage of Inoculum (ml)	9.95462	12	15	18	20.04538

Fermentation Procedure

Acetobacter aceti inoculum preparation

13 g of Nutrient Broth/NB was dissolved in 1 L of hot aquades and sterilized at 121 °C for 15 minutes. Two ose of 48 hours of *Acetobacter aceti* culture was inoculated in 10 ml of liquid medium and aseptically incubated at 37 °C for 48 hours. 10 mL of the culture was placed in an Erlenmeyer having 100 mL of liquid media and incubated at 37 °C for 48 hours. Then the culture was inoculated in 1000 ml of liquid media and incubated at 37 °C for 36 hours. The inoculum was then used for the fermentation process.

Coco Vinegar Fermentation

10 liters of sterillized coconut water was loaded into the feed tank. Sugar solution, ethanol and inoculum of were added to the feed tank. The fermentation time was varied between 18-22 hours, the oxygen flowrate was varied between 0.1-0.3 L/min and the inoculum added was varied btween 12-18%. The reaction mixture was then injected to the fermentor column. The samples taken and analyzed for its acetic acid concentration.

RESULTS AND DISCUSSION

Response surface methodology (RSM) have been used in many researchs for optimizing various process among others: optimization of pressurized liquid water extraction of curcumin [7], ash reduction from low-grade coal [8], rice husk lignin extraction [9], fiber reinforcement [10], etc.

RSM is an empirical statistical technique employed for multiple regressions analysis. RSM used multivariable-quantitative data to solve multivariable equation simultaneously. In this research, a central composite design has employed the response, namely the acetic acid concentration [7]. The independent variables of bubble biofermentation process were X1, X2, and X3 fermentation time (hour), oxygen flowrate (L/min), and percentage of inoculum (%), respectively. The data obtained by carrying out the experiment according to central composite design were analyzed by Statistica 8. The data obtained were tabulated on Table 2.

TABLE 2. Experimental data on the production of vinegar from coconut water hrough bubble biofermentation process

Fermentation Time (hour)	Oxygen Flowrate	Percentage of Inoculum	Acetic Acid Concentration (%v/v)
18	1	12	0.185
18	0.1	18	0.317
18	0.3	12	0.428
18	0.3	18	0.249
22	0.1	12	0.189
22	0.1	18	0.317
22	3	12	0.249
22	0.3	18	0.428
16.63641	0.2	15	0.72322
23.36359	0.2	15	0.94858
20	0.031821	15	0.263266
20	0.368179	15	0.281934
20	0.2	9.95462	0.2136
20	0.2	20.04538	0.374723
20	0.2	15	0.8359
20	0.2	15	0.8359

Process parameters studied consisted of fermentation time, oxygen flow rate and percentage of inoculum. The results showed that the linear and quadratic fermentation process time had a positive effect on the acetic acid concentration of the product. The interaction effect between the fermentation process time and oxygen flow rate and the interaction between the fermentation process time and the percentage of inoculum also has a positive effect on the acetic acid concentration of coco vinegar products. While the oxygen flow rate, percentage of inoculum, and interaction between oxygen flow rate and percentage of inoculum have a negative effect on the acquisition of acetic acid levels in the product (Table 2). The correlation between the levels of acetic acid in coco vinegar products obtained through the fermentation process using bubble reactors with process parameters which include fermentation time, oxygen flow rate and percentage of inoculum are given according to equation 1.

$$Y= -0{,}2246 +2{,}5866X_1 + 0{,}1443X_1X_1 -4{,}9191X_2 - 3{,}6548X_2X_2 -2{,}0053X_3 -0{,}2387X_3X_3+ 2{,}8101X_1 X_2 +0{,}0222 X_1X_3 - 2{,}2313X_2X_3 \quad (1)$$

where X1 represents the fermentation process time, X2 represents the oxygen flow rate and X3 represents the inoculum percentage.

Equation 1 represents the relationship between response and fermentation process variables on coco vinegar production. The suitability of model and data can be seen from the value of R^2. R^2 values range from 0 to 1. The closer to the value of 1, the equation of the model approaches the experimental data. The results of data analysis show that the R^2 value of the model is 0.52 (Table 3).

TABLE 3. The data of parameter effects and parameter interactions on the production of vinegar from coconut waterhrough bubble biofermentation process

	Effect Estimates; Var.:Kadar asam asetat; R-sqr=,52211; Adj:0, (Spreadsheet6) 3 factors, 1 Blocks, 16 Runs; MS Residual=,0790979 DV: Kadar asam asetat									
Factor	Effect	Std.Err.	t(6)	p	-95,% Cnf.Limt	+95,% Cnf.Limt	Coeff.	Std.Err. Coeff.	-95,% Cnf.Limt	+95,% Cnf.Limt
Mean/Interc.	-0,22467	1,08291	-0,20747	0,842505	-2,8745	2,42511	-0,22467	1,08291	-2,87446	2,42511
(1)Waktu fermentasi (jam)(L)	2,58660	2,244050	1,15264	0,292902	-2,9044	8,077602	1,29330	1,122028	-1,45221	4,03880
Waktu fermentasi (jam)(Q)	0,14432	0,168473	0,85664	0,424529	-0,2679	0,556560	0,07216	0,084237	-0,13396	0,278280
(2)Laju alir oksigen(L)	-4,91914	5,390900	-0,91249	0,396693	-18,1102	8,271914	-2,45957	2,695450	-9,05510	4,135957
Laju alir oksigen(Q)	-3,65488	3,851260	-0,94901	0,379257	-13,0786	5,768827	-1,82744	1,925633	-6,53930	2,884413
(3)Persentase inokulum(L)	-2,00532	3,13954	-0,63873	0,54659	-9,6875	5,676859	-1,00266	1,569770	-4,84375	2,838429
Persentase inokulum(Q)	-0,23875	0,168474	-1,41711	0,206223	-0,6510	0,173495	-0,11937	0,084237	-0,32549	0,086747
1L by 2L	2,81016	2,457313	1,14359	0,296360	-3,2027	8,822988	1,40508	1,228657	-1,60134	4,411494
1L by 3L	0,02227	0,279725	0,07961	0,939137	-0,6622	0,706730	0,01113	0,139862	-0,33110	0,353365
2L by 3L	-2,23139	3,514098	-0,63498	0,548873	-10,8301	6,367290	-1,11570	1,757049	-5,41504	3,183648

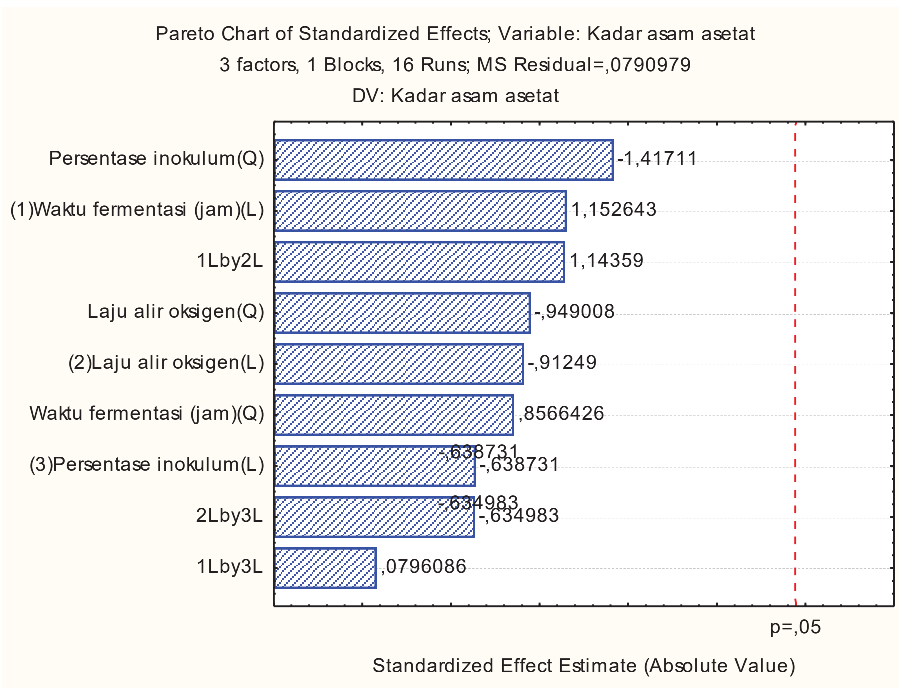

FIGURE 1. Pareto Chart of the Standardized Effect Estimate (Absolute Value)

Analysis of experimental data designed using Response Surface Methodology provide information about the most influential process variables. Figure 1 shows a pareto chart of the coco vinegar fermentation process. The results show that the percentage of inoculum (Q) is the most influential variable in the coco vinegar production process through the fermentation process using bubble reactors, then followed by the fermentation time (L) and the interaction between the fermentation process time and the oxygen flow rate. A contour plot graph between the fermentation time variables, oxygen flow rate and inoculum percentage are presented in Figure 2-4.

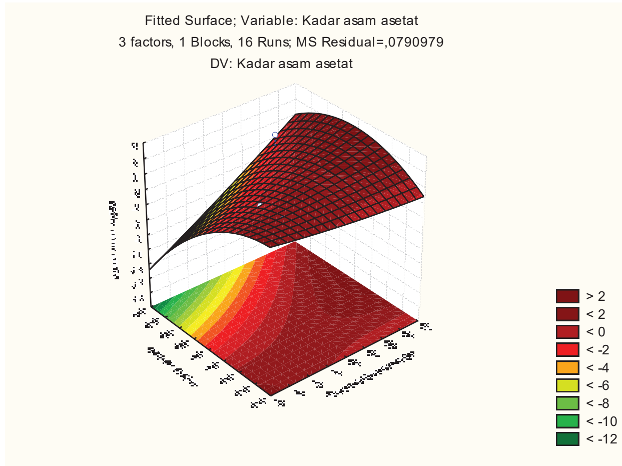

FIGURE 2. Contour plot between the fermentation time and the oxygen flow rate

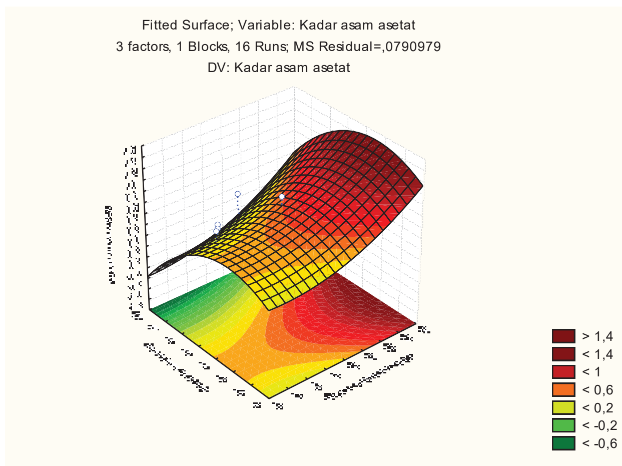

FIGURE 3. Contour plot between the fermentation time and the inoculum percentage

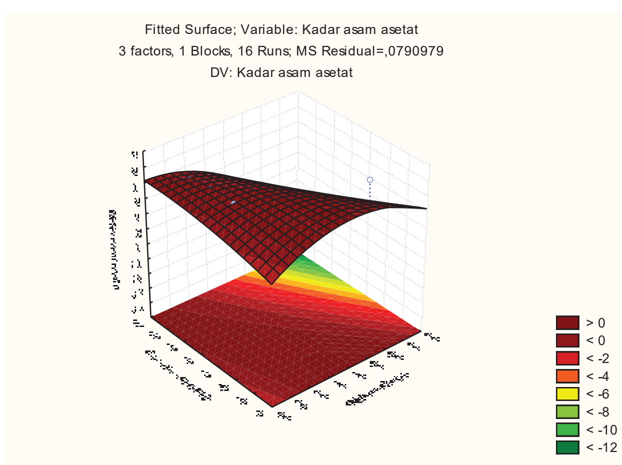

FIGURE 4. Contour plot between inoculum percentage and oxygen flow rate

The interaction between the fermentation time and oxygen flowrate (Figure 2) indicate that the increase in the oxygen rate does not increase the yield of acetic acid. Whereas the interaction between the fermentation time with percent inoculum (Figure 3) shows that the addition of inoculum can increase yield. Conversely, the interaction of the percentage of the noculum with the oxygen flow rate in Figure 4 shows that the addition of the inoculum does not always increase yield.

CONCLUSION

Research showed that the modeled 52% adequate to predict the bubble biofermentation studied in this research (R^2=0.52). The percent of the inoculum was the most influencing variable for bubble biofermentation process. The bubble bioferementation process was optimum at a fermentation time of 23.364 h, oxygen flowrate of 3 and percent inoculum of 20.045%.

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