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Improvement of flavonoid content of climbing swamp fern by fermentation with *Lactobacillus plantarum* using response surface method-central composite design

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Abstract

Climbing swamp fern fronds commonly found in daily Indonesian diet, especially in Kalimantan. It has a lot of beneficial properties for health, among others, due to its phytochemical content. Fermentation using lactic acid bacteria may increase beneficial phytochemical content, since the bacteria metabolize and modify the chemical composition of the fronds. Optimization of flavonoid content of fermented climbing swamp fern fronds is presented in this work. Fronds of climbing swamp fern was purchased from local farmers in Kubu Raya district, West Kalimantan. The fronds were fermented using *Lactobacillus plantarum* ATCC 8014 with varying sugar, salt and time of fermentation as designed using response surface method – central composite design (RSM-CCD). The fermented fronds were then dried, grinded, macerated and evaporated to get crude extract. The flavonoid content of the crude extract then determined using aluminium chloride assay. The results of the present study indicate that quadratic effect of fermentation time ($p = 0.028$) and interaction between salt concentration and fermentation time ($p = 0.016$) are significantly affect the flavonoid content. According to the RSM-CCD result the highest flavonoid content can reach 38.4 mg QE/g extract and can be achieved when the sugar, salt and fermentation time were set to 7.4%, 7.4% and 168.5 hours, respectively. Validation of the optimum condition show that experimental results give flavonoid content of 38.54 ± 1.57 mg QE/g extract. Optimization of flavonoid content of fermented climbing swamp fern fronds indicate a good agreement between mathematical model and experimental results. The flavonoid content increased 4.5 fold compared to center point condition when the frond were fermented at optimum condition.

Keywords: Climbing swamp fern fronds, fermentation, lactic acid bacteria, *Lactobacillus plantarum*, response surface method.

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1. Introduction

Climbing swamp fern can be easily found in forest around Kalimantan area and its frond is commonly found as part of daily vegetable diets in local dish [1]. Traditional knowledge claimed that it has many benefits, including treatment of anemia, increase breast milk production and antiaging agent [2]. The frond also reported has antibacterial properties [3].

Various chemical components can be found in the consumed fern parts. Anti-anemia and breast milk promoting properties is mainly related to the present of iron in the frond [4]. Main components of the fern that has antioxidants properties are flavonoids and phenolic compounds [5,6]. Other than that, it has some important nutrition component including protein, fatty acid, and also various vitamins including ascorbic acid, β-carotene, and folic acid [6,7].

Due to its high water content, like other vegetables in general, frond of climbing swamp fern tend to deteriorate quickly. Fermentation using lactic acid bacteria can be performed to improve shelf life of the vegetables [8]. This method is commonly found in various fermented vegetables products, such as kimchi, sauerkraut, etc. [9].

Fermentation process may change the nutritional composition and phytochemical component of the vegetables due to active metabolism of microorganisms [10,11]. The fermentation of vegetables can be spontaneous or with addition of exogenous microorganisms [12,13]. Lactic acid bacteria are commonly used for fermentation of various fermented vegetables product, and also found as main bacteria in microbial population of spontaneous fermentation process [14]. In the present study, fronds of climbing swamp fern were fermented using *Lactobacillus plantarum* ATCC 8014 as exogenous bacteria with addition of sugar and salt.

The experiment was performed to determine the optimum condition to get the highest flavonoid content by adjusting sugar, salt and fermentation time with response surface method-central composite design approach.

2. Materials and methods

2.1 Materials

Fronds of climbing fern were purchased from farmers in Rasau Jaya Umum III Village, Kubu Raya District, West Kalimantan Province. Salt and sugar used in the present study was purchased in traditional market in Kemuning market, Pontianak, West Kalimantan. All chemical used are analytical grade, unless otherwise stated.

2.2 Optimization of Fermentation

RSM-CCD was used to optimize flavonoid content of fermented fronds of climbing swamp fern. The optimized parameters were salt concentration, sugar concentration and time of fermentation with various levels as presented in Table 1. The fermentation was performed in a plastic container. As much as 100 g of the fronds was put into a container followed by addition of salt and sugar according to the concentration presented in Table 1. Then, 5 mL of 10^7 cell/mL *L. plantarum* culture was added. The fermentation was performed at room temperature for the designated time (Table 1). Total of 20 experiments (Table 2) were performed and total flavonoid content of each experiment was measured.

2.3 Determination of Flavonoid Content

Flavonoid content was determined using method described by Dewi et al. [15]. Briefly, 500 µL of sample was mixed with 0.1 mL 10% w/v $AlCl_3$ solution, 1.5 mL methanol, 2.8 mL distilled water and 0.1 mL 1 M potassium acetate in a test tube. The mixture was then homogenized and incubated for 30 minutes for in dark room at room temperature. The absorbance of the mixture at 415 was then recorded. The total flavonoid content was indicated as mg quercetin equivalent per 1 g sample (mg QE/g).

3. Results and Discussions

Flavonoids are considered as one of components that has contribution for antioxidant activity [16,17]. Therefore, total flavonoid content is one of important factor to be determined in a food product which considered as functional food. Fermentation has been applied to increase a particular chemical component of a food product, such as flavor compounds [10], phenolic compounds [11], and other bioactive compound that contribute to antioxidant properties [18]. In the present study, flavonoid content of fermented climbing swamp fern was optimized to get a high result using response surface method approach. The result of the present study is presented on Table 1. The Anova results is presented in Table 4, while the mathematical equation obtained is presented in equation (1). According to the Anova results, only quadratic effect of fermentation time (p=0.028) and interaction between salt concentration and fermentation time (p=0.016) has significant effect on flavonoid content. Equation (1) can be used to predict flavonoid content with varying salt concentration, sugar concentration and fermentation time. A contour plot from the experiment results is presented on Figure 1. The contour plot indicate that the flavonoid content obtained in the present study is quite low, as indicated by the lowest flavonoid content at the center of the contour plot.

Table 1: Levels of parameters optimized in the present study

Parameters	Unit	-1.63	-1	0	+1	+1.63
Salt Concentration	%w/w	0.64	2	4	6	7.36
Sugar Concentration	%w/w	0.64	2	4	6	7.36
Time	hours	47.5	72	108	144	168.5

Table 2: Central composite design for the optimization of flavonoid content of fermented frond of climbing swamp fern

Standard Order	Salt Concentration (%)	Sugar Concentration (%)	Time (h)
1	2	2	72
2	6	2	72
3	2	6	72
4	6	6	72
5	2	2	144
6	6	2	144
7	2	6	144
8	6	6	144
9	0.64	4	108
10	7.36	4	108
11	4	0.64	108
12	4	7.36	108
13	4	4	47.5
14	4	4	168.5
15	4	4	108
16	4	4	108
17	4	4	108
18	4	4	108
19	4	4	108
20	4	4	108

Table 3: Experimental results of RSM-CCD

Standard Order	Salt Concentration (%)	Sugar Concentration (%)	Time (h)	Flavonoid Content (mg QE/g)
1	2	2	72	19.5
2	6	2	72	7.5
3	2	6	72	12.1
4	6	6	72	8.7
5	2	2	144	9.6
6	6	2	144	15.6
7	2	6	144	10.9
8	6	6	144	19
9	0.64	4	108	5.1
10	7.36	4	108	8.1
11	4	0.64	108	3.6
12	4	7.36	108	7.6
13	4	4	47.5	6.7
14	4	4	168.5	19
15	4	4	108	7.8
16	4	4	108	11
17	4	4	108	9.9
18	4	4	108	9.5
19	4	4	108	4.7
20	4	4	108	8.9

Table 4: Anova table of RSM CCD. Response Surface Regression: Flavonoid versus Salt concentration (%), Sugar concentration (%), and Fermentation time (hours)

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	9	286.483	31.831	2.45	0.090
Linear	3	60.378	20.126	1.55	0.262
X1	1	1.027	1.027	0.08	0.784
X2	1	2.001	2.001	0.15	0.703
X3	1	57.350	57.350	4.41	0.062
Square	3	88.162	29.387	2.26	0.144
X1*X1	1	0.809	0.809	0.06	0.808
X2*X2	1	0.196	0.196	0.02	0.905
X3*X3	1	86.270	86.270	6.64	0.028
2-Way Interaction	3	137.944	45.981	3.54	0.056
5.	1	14.311	14.311	1.10	0.319
X1*X3	1	108.781	108.781	8.37	0.016
X2*X3	1	14.851	14.851	1.14	0.310
Error	10	129.925	12.992		
Lack-of-Fit	5	105.731	21.146	4.37	0.066
Pure Error	5	24.193	4.839		
Total	19	416.408			

Note: X1 = Salt concentration (%), X2 = Sugar concentration (%), X3 = Fermentation time (hours)

Flavonoid content (mg QE/g) = 59.2 - 7.21 X1 - 2.96 X2 - 0.631 X3 + 0.059 X1*X1 - 0.029 X2*X2 + 0.001888 X3*X3 + 0.334 X1*X2 + 0.0512 X1*X3 + 0.0189 X2*X3 … (Equation 1)

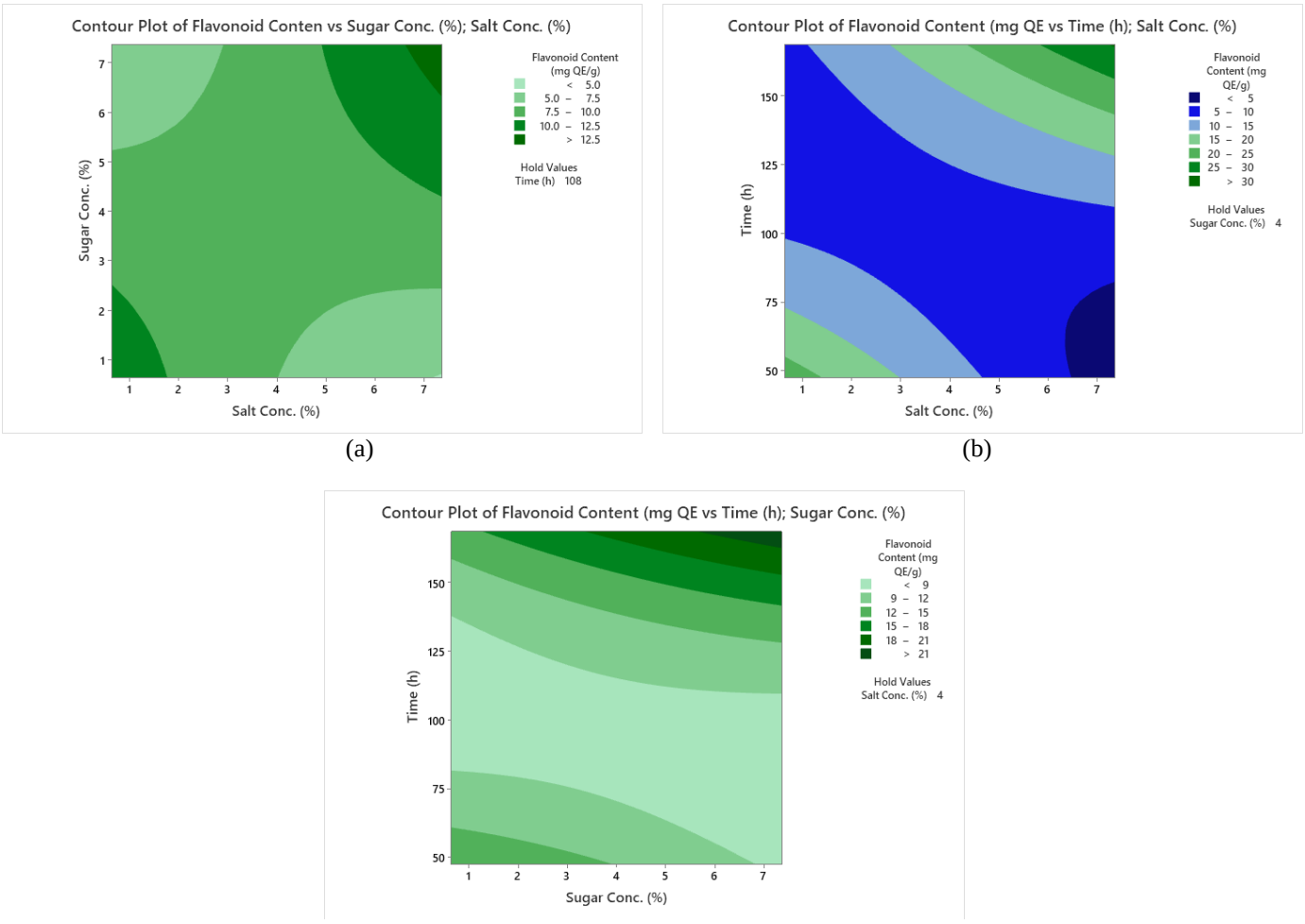

Figure 1: Contour plot showing the effect of (a) salt concentration and sugar concentration (b) salt concentration and fermentation time and (c) sugar concentration and fermentation time on flavonoid content of fermented climbing swamp fern.

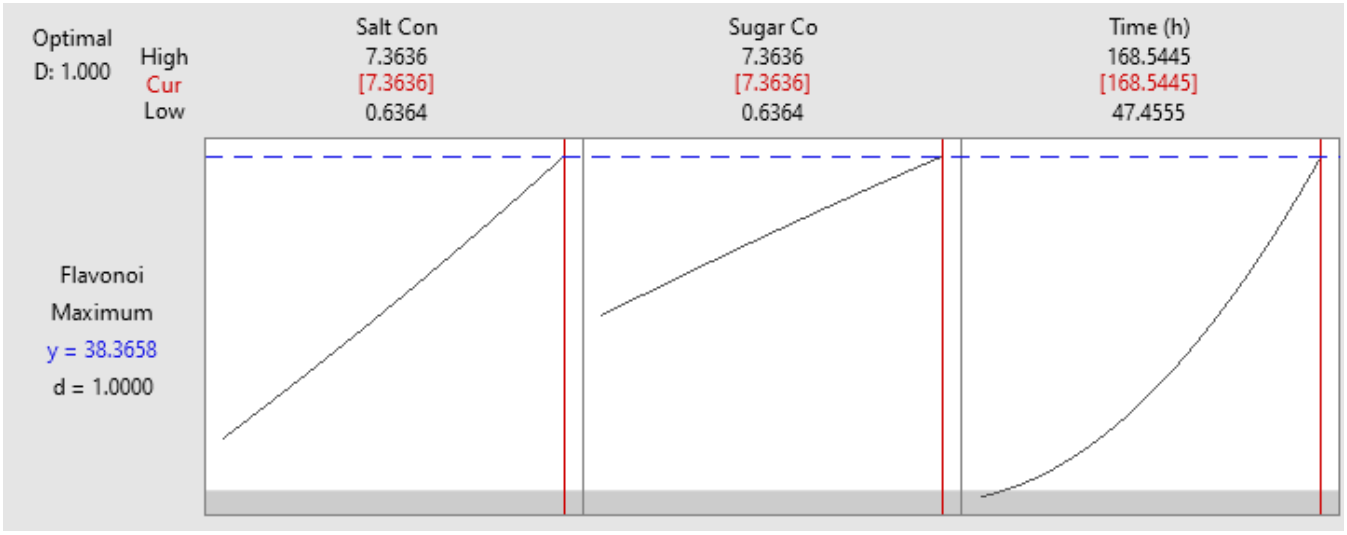

Figure 2: Response optimization plot of salt concentration, sugar concentration and fermentation time to get the highest flavonoid content of fermented climbing swamp fern.

Table 5: Validation of optimum condition

Condition	Salt Conc. (%)	Sugar Conc. (%)	Fermentation Time (h)	Flavonoid Content (mg QE/g)
Center Point	4	4	108	8.49
Optimum condition	7.4	7.4	168.5	38.36
Experiment 1	7.4	7.4	168.5	36.85
Experiment 2				39.96
Experiment 3				38.81
	Average			38.54±1.57

This bring us to predict that when higher content of flavonoid is desired, higher salt, sugar and fermentation time are required. From equation (1) one also can predict the flavonoid content of fermented climbing swamp fern. In the present study we set a maximum value for the flavonoid content and the predicted condition is presented on Figure 2. The predicted condition to get the highest flavonoid content for salt concentration, sugar concentration and fermentation time were 7.4%, 7.4% and 168.5 hours, respectively. At this condition the flavonoid content of the fermented fern can reach 38.36 mg QE/g. To validate this condition, a triplicate experiment was performed and the results is presented on Table 5. The experiment performed in the optimum condition give 38.54±1.57 mg QE/g, which is in a good agreement with the predicted value. Compared to center point, the flavonoid content increase about 4.5 fold.

4. Conclusion

Our experiment succeeded to improve flavonoid content of fermented climbing swamp fern. The optimum condition to get the highest flavonoid content was achieved at salt concentration, sugar concentration and fermentation time of 7.4%, 7.4% and 168.5 hours, respectively. At the optimum condition the flavonoid content reached 38.54±1.57 mg QE/g, which in a good agreement with the predicted value. Further experiment is required to investigate the relationship of flavonoid content and antioxidant properties of the fermented product.

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