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Optimization of The Aqueous Enzymatic Extraction (AEE) of Rice Bran Oil With Cellulase Using Response Surface Methodology

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Abstract

Rice Bran Oil (RBO) is an oil extracted from rice bran with unsaturated fat content according to World Health Organization (WHO) standards. The enzymatic extraction process of rice bran for oil extraction using cellulase enzymes is considered effective because it is capable of producing oil. Parameters of temperature and incubation time have an influence on the yield of oil produced. So that research is needed regarding the optimum conditions of the enzymatic extraction process including temperature and incubation time on the yield and levels of FFA RBO.Software Design Expert was used in this study to optimize RBO extraction with cellulase using the Response Surface Methodology (RSM) method in the Central Composite Design (CCD) factorial experimental design. The selected independent variables consisted of incubation temperatures, namely 35, 50, and 65 ^{0}C and incubation time for 2, 3, and 4 hours. The results show that the RBO yield is 1.7% and the minimum target for FFA levels is 8.4% at a temperature of 51.5 ^{0}C with an incubation time of 4 hours. Processing data with Design Expert software produces an analysis of ANOVA experimental data. Incubation time has a significant level ($p<0.05$) on RBO yield and incubation temperature has a significant level ($p<0.05$) on RBO FFA.

INTRODUCTION

In Indonesia, rice made from rice plants is a basic diet, yet 10% of it is wasted as bran, (DGFC, 2020; Sharif et al., 2014) which can be harmful to the environment if not managed (Azis et al., 2014; Begum et al., 2015; Sharif et al., 2014). Meanwhile, the nutritional content of rice bran is 65% per grain (Begum et al., 2015; Loypimai et al., 2015) with chemical composition consists of 12-22% oil, 11-17% protein, 6-14% fiber, 10-15% water and 8-17% ash and nutrients (Garba et al., 2019; Sharif et al., 2014). RBO can be obtained through conventional methods with solvents (Loypimai et al., 2015) as well as modern extractions such as *Microwave Assisted Extraction* (MAE) (Pandey & Shrivastava, 2018) and *Aqueous Enzymatic Extraction* (AEE) (Mounika et al., 2020). Unfortunately, the negative impact caused by solvents is that they are toxic to humans and the environment (Fraterrigo Garofalo et al., 2021; Karthika, 2020). AEE is one of the most recent extraction techniques, which uses water and enzymes to hydrolyze and dissolve cell walls to release oil from rice bran (Garba et al., 2019; Souza et al., 2019).

The use of enzymes and extraction processes that operate under atmospheric conditions has a good impact on oil yields because they do not damage the nutritional content therein (Vallabha et al., 2015). Cellulase, an enzyme that

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can break down the cellulose in rice bran cell walls, is one of the enzymes utilized in RBO extraction. (Giovannoni et al., 2020). While the factors that influence this process are incubation time and temperature (Mounika et al., 2020; Mwaurah et al., 2020) because temperature can result in a decrease in the amount of yield due to protein denaturation, denaturation of enzymes, darkening of the color of the oil, and degradation of bioactive components (González & Muñoz, 2017; Kuddus, 2018). Meanwhile, time has an effect on perfect extraction results, although in some cases it can reduce the quality of the oil (González & Muñoz, 2017; Kuddus, 2018)

Determination of optimal operating conditions is necessary in order to produce an effective and efficient process. The response surface methodology (RSM) is used to design experiments with the aim of minimizing the number of trials and developing mathematical models to predict responses (Chelladurai et al., 2020) so that it can make it easier to find the optimum area (Kuddus, 2018). Based on the type of design, there are Central Composite Design (CCD) and Box-Behnken Design (BBD), where CCD allows for experiments with only 2 factorials, while BBD requires three-level factorials to build a model(Bhattacharya, 2021). Therefore, this study aims to determine the optimum parameters of the AEE process with the independent variables of incubation temperature and incubation time with yield percentage and free fatty acid (FFA) responses using RSM with a CCD design.

MATERIALS AND METHODS

Materials

Bran by-product of rice milling is obtained from the Muntilan area, Central Java Province, Indonesia. Cellulase Enzyme (Novozyme) was purchased from Agrotekno Consultant, Yogyakarta, Indonesia. Chemicals in HCl (37% purity, Merck) and NaOH (99% purity, Merck) was purchased from PT Hepilab Sukses Bersama, Semarang, Indonesia.

Preparation for Material And Reagents

Fresh rice bran was pretreated using a 30 mesh filter and stabilized at -18^{0}C until it was used (Mounika et al., 2020; Zigoneanu et al., 2008). Before being used for the extraction process, the rice bran was baked for 20 minutes at 110^{0}C.

Preparation of RBO Extraction

A modification from Xu et al. (2020) was used to perform the AEE method on rice bran. As much as 50 grams of dry rice bran is put into a 500 mL beaker glass then added 300 ml of distilled water accompanied by stirring then heated at 90^{0}C for 5 minutes. After completion, the solution is cooled to room temperature and then neutralized using 2N NaOH or 2N HCl. Then the solution was added with 66.4 ml of cellulase enzyme and homogenized. After that, the mixture was incubated at the temperature and time determined from the simulation results with the Design Expert software. After the incubation process ended, the mixture was centrifuged for 20 minutes at 6000 rpm (Ohaus brand type FC5706 230V, America) and the top layer, in the form of an emulsion, was taken using a spatula and dropper, then they were stored in a container and put in the freezer for 24 hours. Next, the top layer was heated at 40^{0}C for 3 hours and centrifuged at 4000 rpm for 20 minutes. The top layer (oil) was taken using a micropipette (Dragon Lab brand, made in China) (Li et al., 2017) then the yield was calculated (Eq. 1) (Mounika et al., 2020).

$$yield(\%) = \frac{m_1(g) - m_0\,(g)}{m_b(g)}\, x\ 100\% \qquad (1)$$

Where, m_1 is the mass of oil and the container, m_0 is the mass of container and m_b is the mass of bran.

Determination of Free Fatty Acid (FFA) Levels

The titration method is used to analyze FFA levels in RBO (Noureen et al., 2021). RBO weights of as much as 0.5 g and 95% ethanol that neutralized to pH 7 with 0.1 N KOH were taken in 5 ml and put into a 25 ml Erlenmeyer whose empty weight was known. The Erlenmeyer containing the solution was heated to boil on the hotplate, then three drops of phenolphthalein (PP) indicator with a concentration of 1% (v/v) was added and titrated with 0.1 N KOH until formed pink solutions. The requirement for KOH solution was neatly recorded, and the free fatty acid content was calculated using the (American Oil Chemists' Society (AOCS), 2009) Ca 5a-40 method using Eq. (2).

$$\%\,FFA = \frac{V \times N \times 28.2}{m} \qquad (2)$$

where V = required volume of KOH (ml), N = normality of KOH solution, m = sample mass (grams), and 28.2 = molecular weight of oleic acid (g/mol).

Experimental Design

RSM was used to establish the minimum requirements for FFA and the maximum circumstances for attaining the yield, while the Central Composite Design (CCD) matrix was employed.. The independent variables used used in this study were incubation temperature (A) and incubation time (B) (Table 1).

The CCD uses 13 experiments where five central points have been used to estimate the statistical error of the experiment. Software Design-Expert 13 trial version used to code and integrate each level per factor.

Statistical Analysis of Optimal Conditions

Statistical analysis calculations such as determination coefficient (R^2), adjusted determination of coefficient (Ra^2), sum of square (SS), dan predicted determination of coefficient (Rp^2) was used for experimental data to evaluate the acceptability of various models. The regression coefficient of each polynomial model was analyzed by analysis of variance (ANOVA). All terms in the model are tested with a significance F value at a probability level (p) of less than 0.05. After the effect of the independent variables on the response is described by means of polynomial equations analyzed, then the optimization process is carried out using the Derringer methodology (Maran et al., 2013). Numerical optimization with this technique obtains one or more objectives, both in process and response variables. The possible goals resulting from this technique are maximizing, minimizing, within range, setting to the correct value (factors only), target, and none (response only).

RESULTS AND DISCUSSION

To evaluate the effect of independent variables (temperature and incubation time) and find the optimum operating conditions on the response (yield and percent FFA) desired. Variables and experimental designs using CCD are presented in Table 2.

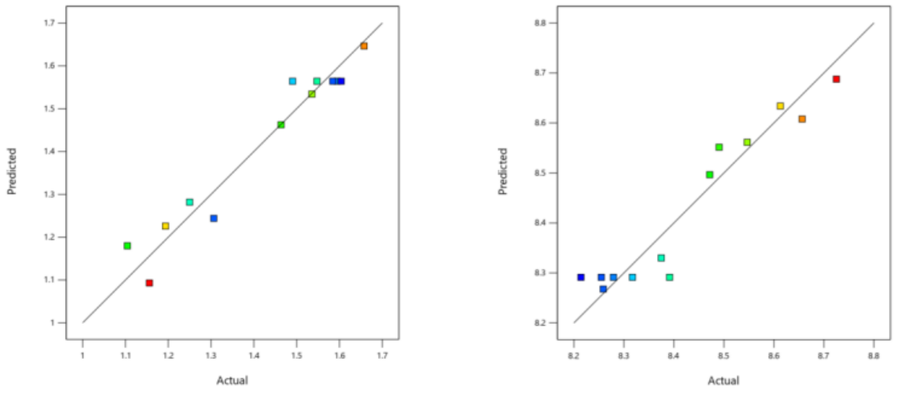
Percentage results *error* has the meaning of the relationship between variables to the response, *Mean Absolute Percentage Error* (MAPE) aims to measure the average percentage of error in a prediction model (Moreno et al., 2013), in other words MAPE is able to show the magnitude of the prediction data error with the actual acquisition. MAPE can be obtained by Eq. (3).

Table 1. Independent Variables Using CCD with Coding Level.

Factor	Unit	Minimum	Maximum	Lower Limit (-1)	Upper Limit (+1)
(A) Temperature	^0C	28.8	71.2	35	65
(B) Time	Hour	1.59	4.41	1	4

Table 2. CCD Experiment Design with Experimental Data and Predictions

Run	Temperature	Time	Yield (%)		FFA (%)		% *Error*	
	(^0C, A)	(Hour, B)	Experiment	Predicted	Experiment	Predicted	yield	FFA
1	50	4.41	1.6574	1.6460	8.6564	8.6078	0.6854	0.5619
2	50	1.59	1.1043	1.1797	8.4716	8.4965	6.8252	0.2938
3	71.2	3	1.1934	1.2256	8.6129	8.6338	2.6957	0.2429
4	35	4	1.4634	1.4622	8.4904	8.5515	0.0827	0.7193
5	65	4	1.5354	1.5339	8.5466	8.5613	0.0951	0.1718
6	35	2	1.3062	1.2437	8.2587	8.2678	4.7879	0.1098
7	50	3	1.5944	1.5640	8.2791	8.2913	1.9092	0.1474
8	50	3	1.5842	1.5640	8.2548	8.2913	1.2776	0.4422
9	50	3	1.6038	1.5640	8.2141	8.2913	2.4841	0.9398
10	65	2	1.1557	1.0929	8.7249	8.6876	5.4323	0.4276
11	50	3	1.5472	1.5640	8.3914	8.2913	1.0832	1.1929
12	50	3	1.4902	1.5640	8.3171	8.2913	4.9497	0.3102
13	28.8	3	1.2496	1.2814	8.3747	8.3300	2.5472	0.5333
					MAPE(%)		2.6812	0.4687



(a) (b)

Figure 1. Relationship between Actual Value (Experiment) and Predicted Value (a) Yield (b) FFA.

Table 3. Yield Optimization Model and FFA on RBO Extraction with Cellulase Enzyme

Model	Sequential p-value		Lack of Fit p-value		Adjusted Ra^2		Predicted Rp^2		Remarks
	yield	FFA	yield	FFA	yield	FFA	yield	FFA	
Linear	0.0332	0.1561	0.0083	0.0327	0.3928	0.1723	0.1364	-0.1713	
2FI	0.4886	0.1926	0.0069	0.0359	0.3622	0.2465	0.0716	-0.1700	
Quadratic	**0.0005**	**0.0014**	**0.2060**	**0.5563**	**0.9065**	**0.8528**	**0.7196**	**0.6876**	***Suggested***
Cubic	0.3972	0.3604	0.1234	0.6440	0.9095	0.8630	-0.2041	0.7020	***Aliased***

$$MAPE = \frac{1}{n}\sum_{t=1}^{n}\left|\frac{A_t - F_t}{A_t}\right| \times 100\% \qquad (3)$$

where A_t is the actual values data point t, F_t is forecast values at data point t, n is the number of data points. Table 2 shows the MAPE value of the yield response of 2.6812% and the FFA response of 0.4687%. Based on Moreno et al.(2013) MAPE is less than 10%, indicating that the value has an accurate relationship between the observed and the predicted value. They have illustrated by the linear line graph presented in Figure 1.

The model suitability has been observed against the experimental data to obtain the most suitable model for predicting response results. Four types of polynomial models, namely linear, interactive (2FI), quadratic, and cubic, are used to predict response variables of experimental data. Some parameters, like *sequential p-value, lack of fit p-value, adjusted* Ra^2, and *predicted* Rp^2 (Table 3), were used to conclude which model type is most suitable for optimization of percent yield and percent FFA of RBO.

Based on Table 3, the quadratic model is the most suitable model and is recommended for optimizing the yield and FFA of rice bran oil extraction. Further modeling of experimental data cannot be done using the linear and 2FI models. The cubic model is expressed by *aliased* which means this model cannot be used because it is not accurate (Khelifa et al., 2021).

Equation Model for Yield Percentage and FFA Content

The empirical relationship shown between the quadratic model and the interaction between variables will be transformed into the second-order polynomial equation. The final equation for yield optimization is shown by Eq. (4). Meanwhile, for the optimization Eq. for FFA content is shown in Eq. (5).

$$Yield\ (\%) = -0.713401 + 0.05655A + 0.432801B + 0.003708AB - 0.000690A2 - 0.075555B2 \qquad (4)$$

$$FFA\ (\%) = 9.02298 - 0.014701A - 0.401465B - 0.006833AB + 0.000424A^2 + 0.130413B^2 \qquad (5)$$

where A is temperature and B is time.

Statistical Analysis for Yield and FFA content

The ANOVA regression model for predicting the percent yield and FFA RBO levels

Table 4. ANOVA Regression Model for RBO Yield Percentage Prediction.

Source	Actual Coefficient	Sum of Squares	df	Mean Square	F-value	p-value	Remarks
Model	- 0.713401	0.4223	5	0.0845	24.27	0.0003	significant
A-Temperature	0.056550	0.0031	1	0.0031	0.8965	0.3753	
B-Time	0.432801	0.2175	1	0.2175	62.50	< 0.0001	
AB	0.003708	0.0124	1	0.0124	3.56	0.1013	
A^2	-0.000690	0.1676	1	0.1676	48.17	0.0002	
B^2	-0.075555	0.0397	1	0.0397	11.41	0.0118	
Residual		0.0244	7	0.0035			
Lack of Fit		0.0157	3	0.0052	2.42	0.2060	not significant
Pure Error		0.0086	4	0.0022			
Cor Total		0.4466	12				
Adeq Prec	13.8019						
R^2	0.9455						

Table 5. ANOVA Regression Model for RBO FFA Prediction

Source	Actual Coefficient	Sum of Squares	df	Mean Square	F-value	p-value	Remarks
Model	9.02298	0.3084	5	0.0617	14.91	0.0013	significant
A-Temperature	-0.014701	0.0923	1	0.0923	22.31	0.0022	
B-Time	-0.401465	0.0124	1	0.0124	2.99	0.1273	
AB	-0.006833	0.0420	1	0.0420	10.16	0.0153	
A^2	0.000424	0.0632	1	0.0632	15.27	0.0058	
B^2	0.130413	0.1183	1	0.1183	28.59	0.0011	
Residual		0.0290	7	0.0041			
Lack of Fit		0.0108	3	0.0036	0.7971	0.5563	not significant
Pure Error		0.0181	4	0.0045			
Cor Total		0.3374	12				
Adeq Prec	9.6069						
R^2	0.9142						

using the cellulase enzyme is presented in Tables 4 and 5.

Based on the results of experimental data analysis with ANOVA which can be seen in Table 4. states if the model has value *F-value* 24.27 with *p-value* 0.0003 (0.03%) which indicates that the model is significant because of the value *p-value* less than 0.05 (5%) so that the model has a significant influence. Score *p-value* more than 0.05 is considered insignificant, in this case the time model term (B), factor squared of temperature (A^2) and the time squared factor (B^2) is significant to the model and temperature (A) and the interaction between time and temperature (AB) is not significant to the model.

Score *Adeq precision* is a measure of the signal to noise ratio (S/N), where the expected ratio is > 4 (Singh et al., 2015). This model is value *Adeq prec* of 13.8019 which indicates that the model is feasible to use. In Table 4, the inconsistency and the lack of fit were not significant in comparison to the pure error, as indicated by the F-value of 0.1109. The model has focused on ideal fitness; therefore, a non-significant of fit is beneficial (Bayuo et al., 2019).

Table 5 shows that the results of the experimental data analysis using ANOVA to predict the percent FFA RBO can be seen that the model has value *F-value* 14.91 with *p-value* 0.0013 (0.13%) which shows that the model is significant because it is less than 5%. Model terms A, AB, A^2, and B^2 are significant models. This indicates that time (B) is not significant to the model. Score *adeq precision* is a measure of the signal-to-interference ratio, where the expected ratio is greater than 4 (Singh et al., 2015). This model gains value *adeq* *precision* of 9.6069 which indicates that the model is feasible to use.

Surface plots of responseBy charting the three-dimensional (3D) and two-dimensional (2D) picture surface curves of the two independent variables while leaving the other variables at the central (0) level, the interactive effect of the process variable AEE on RBO with cellulase enzymes has been further investigated. Figures 2 and 3 depict the 3D surface and 2D contour plots of the responses (percentage yield and FFA) to the interactions between the factors. The color of the contour plot represents response levels, with lower, medium, and better-optimized interaction regions indicated by blue to green to reddish tones.

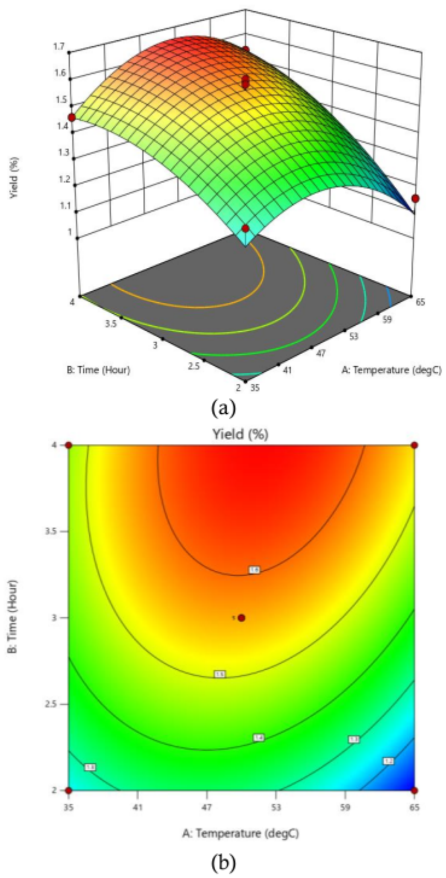
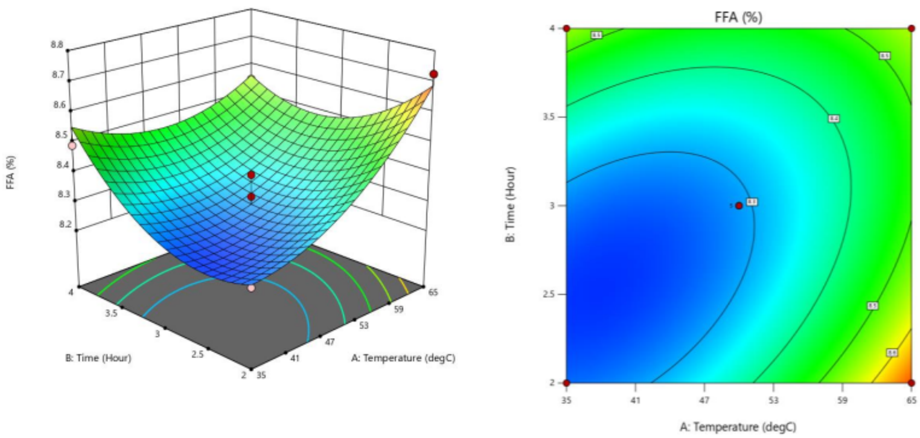


Figure 2. Plot shows the interactive effect of Temperature and Time in (a) 3D surface and (b) 2D contour on Yield RBO.

Figure 2 shows that if the incubation temperature does not have a significant effect on the yield of RBO produced it is consistent with the data presented in Table 4. This is justified by Figure 4 where at the highest incubation temperature the yield produced is relatively low. The reaction surface plot's bright green area denoted a region with little impact on yield value. It was found that the ideal yield area occurred during test time (3–4 hours) and the incubation temperature ranges between 40 and 60°C, where the yield value was higher with a range of input variable levels. This phenomenon occurs because the longer the incubation time, the longer the enzymes come into contact with the rice bran so that more and more hydrolyzed oil(González & Muñoz, 2017; Mounika et al., 2020). An incubation time that is too long can cause the quality of the oil to decrease and requires a lot of energy (Karthika, 2020; Qian et al., 2021) so that the optimum incubation time is between 1.4 – 3 hours (González & Muñoz, 2017).

The yield increased at 37-50°C incubation temperature, then decreased as the temperature increased because the cellulase enzyme can work optimally to hydrolyze at 50°C (Mat Yusoff et al., 2015; Xu et al., 2020). So, the yield of RBO is relatively highly produced because the cellulase enzymes work at their optimum conditions. A temperature rises above 600°C can decrease the yield of RBO due to the enzymes experiencing denaturation or inactivity so that the performance of the enzymes decreases. (Gokhale et al., 2013; Kuddus, 2018).

Figure 3 shows that the effect of incubation temperature has a significant effect on the resulting FFA value (Table 5). This is justified by Figure 5 where at the highest incubatiogn temperature the FFA produced is relatively high. This phenomenon is due to the extraction temperature increased 50-70°C promotes the hydrolysis and oxidation of triglycerides thereby increasing the FFA content (Mahesar et al., 2014; Di Pietro et al., 2020; Salimon et al., 2011; Tambunan et al., 2012). This phenomenon is matched with research by Karthika (2020). While the incubation time did not have a significant effect on the resulting FFA RBO. Where the longer the incubation time of the resulting FFA there is no significant change. This phenomenon is because the FFA value is influenced by the quality and variety of raw materials, conditions of taking, and handling of raw materials (Di Pietro et al., 2020). According to (Arora et al., 2015; Zaidel et al., 2019) FFA values for *crude* RBO 6-8% of fresh rice bran.



(a) (b)

Figure 3. Plot shows the interactive effect of Temperature and Time in (a) 3D surface and (b) 2D contour on FFA RBO.

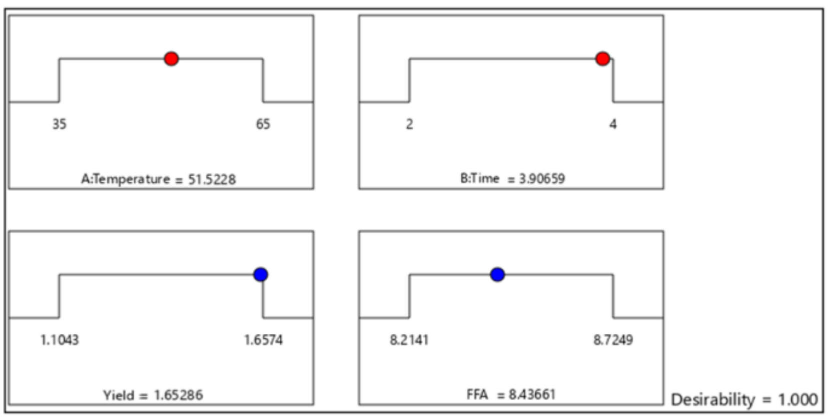


Figure 4. Optimization Results of Yield and FFA RBO

Optimization Results

The Derringer method is used to perform multi-response optimization which is characterized by the presence of the desirability function (Damayanti et al., 2021) which shows how fulfilled the value is at the optimum point. Based on the results of numerical optimization, it was obtained that the operating conditions for RBO extraction using cellulase enzymes with the hope that the maximum target was obtained were yields of 1.7% and the minimum target for FFA levels with a value of 8.4% in conditions temperature 51.5 ^{0}C with an incubation time of 4 hours which can be seen in Figure 4. Under these conditions the maximum yield has a value desirability ramp its 0.889. Desirability score close to 1 is the expected value (Rao & Murthy, 2018). But the goal of optimization is not just to search desirability equal to 1 but finds conditions that match expectations (Candioti et al., 2014).

CONCLUSION

Incubation time has a significant effect on the yield but does not have a significant effect on FFA RBO and incubation temperature has a significant effect on FFA but does not have a significant effect on RBO yield in the enzymatic extraction process of rice bran using cellulase enzymes. The optimization results show that the maximum yield response is 1.7% and the minimum target for FFA levels is 8.4% at a temperature of 51.5 ^{0}C with an incubation time of 3.9 hours.

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REFERENCES

American Oil Chemists' Society (AOCS). 2009. AOCS Official Method Ca 5a-40: Free fatty acids. AOCS.

Arora, R., Toor, A. P., Wanchoo, R. K. 2015. Esterification of high free fatty acid rice bran oil: Parametric and kinetic study. Chemical and Biochemical Engineering Quarterly. 29(4): 617–623.

Bayuo, J., Pelig-Ba, K. B., Abukari, M. A. 2019. Optimization of adsorption parameters for effective removal of lead(II) from. Physical Chemistry: An Indian Journal. 14(1): 1–25.

Bhattacharya, S. 2021. Central composite design for response surface methodology and its application in pharmacy. In Response surface methodology in engineering science. IntechOpen.

Begum, A., Sarma, J., Borah, P., Moni Bhuyan, P., Saikia, R., Hussain Ahmed, T., Karki, S., Rupa Dowarah, J., Gupta, P., R Khanna, R., Rai, L., Lalhmangaihzuali, J., Goswami, A., Chowdhury, P. 2015. Microwave (MW) Energy in Enzyme Deactivation: Stabilization of Rice Bran from Few Widely Consumed Indigenous Rice Cultivars (Oryza sativa L.) from Eastern Himalayan Range. Current Nutrition & Food Science. 11(3): 240–245.

Candioti, L. V., De Zan, M. M., Cámara, M. S., Goicoechea, H. C. 2014. Experimental design and multiple response optimization. Using the desirability function in analytical methods development. Talanta. 124: 123–138.

Casas, M. P., González, H. D. 2017. Enzyme-assisted aqueous extraction processes. In Water Extraction of Bioactive Compounds. In Water Extraction of Bioactive Compounds From Plants to Drug Development. Elsevier.

Chelladurai, S. J. S., Murugan, K., Ray, A. P., Upadhyaya, M., Narasimharaj, V., Gnanasekaran, S. 2020. Optimization of process parameters using response surface methodology: A review. Materials Today: Proceedings. 37(2): 1301–1304.

Damayanti, A., Triwibowo, B., Megawati, M., Azhari, M., Fadriana, S. A. 2021. Optimization of Anthocyanin Extraction from Cockspur Coral (Erythrina Crista-Galli L.) Petals with Microwave-Assisted Extraction (MAE) using Response Surface Methodology. ASEAN Journal of Chemical Engineering. 21(2): 143.

Di Pietro, M. E., Mannu, A., Mele, A. 2020. NMR determination of free fatty acids in vegetable oils. Processes. 8(4): 410.

Fraterrigo, G., S., Tommasi, T., Fino, D. 2021. A short review of green extraction technologies for rice bran oil. Biomass Conversion and Biorefinery. 11(2): 569–587.

Garba, U., Singanusong, R., Jiamyangyuen, S., Thongsook, Ti. 2019. (PDF) Extraction and utilization of rice bran oil: A review extraction and utilization of rice bran oil: A review. Italian Journal of Fatty Substances. 96(3): 161–170.

Giovannoni, M., Gramegna, G., Benedetti, M., Mattei, B. 2020. Industrial Use of Cell Wall Degrading Enzymes: The Fine Line Between Production Strategy and Economic Feasibility. Frontiers in Bioengineering and Biotechnology. 8.

Gokhale, A. A., Lu, J., Lee, I. 2013. Immobilization of cellulase on magnetoresponsive graphene nano-supports. Journal of Molecular Catalysis. B, Enzymatic, Complete. 90: 76–86.

Karthika, M. S. 2020. Effect of Enzyme Assisted Aqueous Extraction and Ohmic Heating Treatment on Rice Bran Oil Recovery. Indian Journal of Pure & Applied Biosciences. 8(5): 177–184.

Khelifa, H., Bezazi, A., Boumediri, H., Garcia del Pino, G., Reis, P. N. B., Scarpa, F., & Dufresne, A. 2021. Mechanical characterization of mortar reinforced by date palm mesh fibers: Experimental and statistical analysis. Construction and Building Materials. 300: 124067.

Kuddus, M. 2018. Introduction to food enzymes. Enzymes in Food Biotechnology: Production, Applications, and Future Prospects. 1–18.

Li, P., Zhang, W., Han, X., Liu, J., Liu, Y., Gasmalla, M. A. A., Yang, R. 2017. Demulsification of oil-rich emulsion and characterization of protein hydrolysates from peanut cream emulsion of aqueous extraction processing. Journal of Food Engineering. 204: 64–72.

Loypimai, P., Moongngarm, A., Chottanom, P. 2015. Impact of stabilization and extraction methods on chemical quality and bioactive compounds of rice bran oil. Emirates Journal of Food and Agriculture. 27(11): 849–856.

Mahesar, S. A., Sherazi, S. T. H., Khaskheli, A. R., Kandhro, A. A., Uddin, S. 2014. Analytical approaches for the assessment of free fatty acids in oils and fats. Analytical Methods. 6(14): 4956–4963.

Maran, J. P., Manikandan, S., Thirugnanasambandham, K., Vigna Nivetha, C., Dinesh, R. 2013. Box–Behnken design based statistical modeling for ultrasound-assisted extraction of corn silk polysaccharide. Carbohydrate Polymers. 92(1): 604–611.

Mat Yusoff, M., Gordon, M. H., Niranjan, K. 2015. Aqueous enzyme assisted oil extraction from oilseeds and emulsion de-emulsifying methods: A review. Trends in Food Science & Technology. 41(1): 60–82.

Moreno, J. J. M., Palmer, P. A., Sesé, A. A., Cajal, B. B. 2013. Using the R-MAPE index as a resistant measure of forecast accuracy. Psicothema. 25(4): 500–506.

Mounika, E., Smith, Dr. D. Edukondalu, Dr. L., Raja, D. S. 2020. Quality parameters of ultrasound assisted aqueous enzymatic extraction of rice bran oil. International Journal of Chemical Studies. 8(5): 1765–1768.

Mwaurah, P. W., Kumar, S., Kumar, N., Attkan, A. K., Panghal, A., Singh, V. K., Garg, M. K. 2020. Novel oil extraction technologies: Process conditions, quality parameters, and optimization. Comprehensive Reviews in Food Science and Food Safety. 19(1): 3–20.

Noureen, H., Alam, S., Al Ayoubi, S., Qayyum, A., Sadiqi, S., Atiq, S., Naz, A., Bibi, Y., Ahmed, W., Khan, M. M., Sammi, S., Liaquat, M., Ahmad, S. 2021. Mechanism of rice bran lipase inhibition through fermentation activity of probiotic bacteria. Saudi Journal of Biological Sciences. 28(10): 5841–5848.

Pandey, R., Shrivastava, S. L. 2018. Comparative evaluation of rice bran oil obtained with two-step microwave assisted extraction and conventional solvent extraction. Journal of Food Engineering. 218: 106–114.

Rao, K. V., Murthy, P. B. G. S. N. 2018. Modeling and optimization of tool vibration and surface roughness in boring of steel using RSM, ANN and SVM. Journal of Intelligent Manufacturing. 29(7): 1533–1543.

Qian, J., Zhao, X., Zhao, C., Yang, H., Gou, L., Wang, W., Guo, H. 2021. Pretreatment Camellia Seeds by Protease and Application to Extraction of Camellia Oil. European Journal of Lipid Science and Technology. 123(1): 2000223.

Vallabha, V. S., Indira, T. N., Jyothi Lakshmi, A., Radha, C., Tiku, P. K. 2015. Enzymatic process of rice bran: a stabilized functional food with nutraceuticals and nutrients. Journal of Food Science and Technology. 52(12): 8252–8259.

Salimon, J., Abdullah, B. M., Salih, N. 2011. Hydrolysis optimization and characterization study of preparing fatty acids from Jatropha curcas seed oil. Chemistry Central Journal. 5(1): 1–9.

Sharif, M. K., Butt, M. S., Anjum, F. M., Khan, S. H. 2014. Rice Bran: A Novel Functional Ingredient. Critical Reviews in Food Science and Nutrition. 54(6): 807–816.

Singh, S., Singla, P., Arora, S. 2015. Statistical, Diagnostic And Response Surface Analysis of Nefopam Hydrochloride Nanospheres Using 35 Box-Behnken Design. International Journal of Pharmacy and Pharmaceutical Sciences. 7(10): 89–101.

Souza, T. S. P., Dias, F. F. G., Koblitz, M. G. B., Bell, M. L. N. de M.. 2019. Aqueous and Enzymatic Extraction of Oil and Protein from Almond Cake: A Comparative Study. Processes, 7(7): 472.

Tambunan, A. H., Situmorang, J. P., Silip, J. J., Joelianingsih, A., Araki, T. 2012. Yield and physicochemical properties of mechanically extracted crude Jatropha curcas L oil. Biomass and Bioenergy. 43: 12–17.

Xu, D., Hao, J., Wang, Z., Yang, H., Gao, Q., Ma, N., Wang, J., Ma, Y., Zhang, M.. 2020. Aqueous enzymatic extraction of oil from rice bran and its quality evaluation. Authorea Preprints.

Zaidel, D. N. A., Muhamad, I. I., Daud, N. S. M., Muttalib, N. A. A., Khairuddin, N., Lazim, N. A. M. 2019. Production of biodiesel from rice bran oil. Biomass, Biopolymer-Based Materials, and Bioenergy. pp. 409–447. Elsevier.

Zigoneanu, I. G., Williams, L., Xu, Z., Sabliov, C. M. 2008. Determination of antioxidant components in rice bran oil extracted by microwave-assisted method. Bioresource Technology. 99(11): 4910–4918.

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PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6

PAGE 7

PAGE 8

PAGE 9

PAGE 10