HW 28

OPTIMIZATION OF FERULIC ACID PRODUCTION FROM OIL PALM FROND WASTE VIA ENZYMATIC HYDROLYSIS USING SOIL CULTURE

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ABSTRACT An enzymatic hydrolysis of fibre-pressed oil palm frond (FPOPF) was carried out using soil culture for ferulic acid production. The soil obtained from palm oil plantation was acclimatized with FPOPF substrate for 30 days before being used as soil culture. FPOPF substrate was prepared with a 1:10 ratio of FPOPF to water. By using Design Expert 7, a central composite design with two factors was selected as the experimental design resulting a total of 13 runs. Two factors studied were temperature (24°C to 28°C) with 26°C as centre point, and agitation rate (130 rpm to 170 rpm) with centre points at 150 rpm. The ANOVA showed that the experimental design model was significant. The coefficient of determination value was 0.8873, indicating that the probability this model was obtained through noise was 11.27%. The optimum condition obtained was temperature at 26°C and agitation rate at 150 rpm with a response of 205.724 mg FA/kg OPF. The optimization was repeatable with a small error of 8.41% from validation test.

Keywords: Ferulic acid; optimization; oil palm frond

1. INTRODUCTION

Ferulic acid (FA) is an organic acid that has been used widely in pharmaceutical industries for its multiple physiological properties including antioxidant, antimicrobial, anti-inflammatory, anti-thrombosis, and anti-cancer activities [1]. FA applications include being the source for vanillin and preservatives production, thin film for food packaging, food supplement and skin care products [2, 3, 4, 5]. Oil palm frond (OPF) is the leaf and the branch part of the oil palm tree. In Malaysia, the availability of OPF is quite high as it is a by-product of the palm oil industry [6]. OPF has the potential as an alternative source for value-added products [7,8]. The fibre-pressed oil palm frond (FPOPF) is obtained after pressing the juice of OPF. The fibre from that process is used for this study. Enzymatic hydrolysis has a higher possibility in producing FA without furfural compared to the conventional chemical hydrolysis [9]. Enzymatic hydrolysis mainly focuses on utilizing the reaction caused by feruloyl esterase (FAE) to release FA from polysaccharide. The objective of this study was to determine the optimum condition for FA production from FPOPF via enzymatic hydrolysis. Two factors were controlled for the optimization process.

2. MATERIALS AND METHOD

Raw OPFs were obtained from a palm oil plantation located at Lepar Hilir, Kuantan. OPFs were first pressed to remove the sap within the fibres by following a method described by Zahari et al. [10]. FPOPF were stored in freezer to avoid any contamination. FA 99% was purchased from Sigma Aldrich (Malaysia). As for the mobile phase used in high performance liquid chromatography (HPLC) analysis, Acetonitrile was purchased from Fisher Scientific (Malaysia).

2.1 Soil culture preparation

The soil culture was prepared by mixing the soil from palm oil plantation together with FPOPF substrates. FPOPF substrates were prepared by mixing dried FPOPF with water at a 1:10 ratio of FPOPF weight to water volume (g/mL). The soil was poured into a 4 L batch bioreactor up to 1/6 of the volume. 120 mL of FPOPF substrates were added daily into the bioreactor for the next 30 days. On the 30th day, the soil culture was acclimatized and was available for enzymatic hydrolysis. The soil culture identification process indicated that a significant quantity of *Aspergillus niger* was

detected. *A. niger* has the ability to produce FAE [5]. The soil culture is proven to be the source of FAE for enzymatic hydrolysis.

2.2 Central composite design experimental setup

Enzymatic hydrolysis was carried out in 250 mL conical flasks. From a previous screening process, the conditions for other factors such as; pH value, soil culture to substrate volume ratio and reaction time were fixed at pH 9, 2% and 24 hours respectively. Substrates were autoclaved for sterilization before being used. The pH value was controlled by the addition of sodium hydroxide (pH 12). The conical flasks were placed in incubator shakers (INFORS HT Ecotron) in an attempt to control temperature and agitation speed. The temperature was controlled at 24°C, 25°C, 26°C, 27°C and 28°C while the agitation speed was controlled at 130 rpm, 140 rpm, 150 rpm, 160 rpm and 170 rpm. After 24 hours, the samples were removed from the incubator and were kept in freezer. The central composite design was made possible with the aid of Design Expert version 7 software. The design used for the optimization for process was a central composite design with two factors, 5 centre points and one response. There were 13 runs in this design where each run was repeated three times for replication. The properties of each runs and its response can be found in table 1.

2.3 HPLC analysis

The analysis for FA production was done by using the HPLC. The method for analysis was carried out with reference from Kareparamban et al. [11] and a small adjustment. HPLC Agilent 1100 series with a C-18 column was used for the analysis. The mobile phase used in this analysis was prepared with a ratio of acetonitrile to acetic acid (10%) at 20:80 v/v. The wavelength detection, flow rate, injection volume and column oven temperature were fixed at 319 nm, 1.0 mL/min, 20 μL and 30°C respectively.

3. FINDINGS AND ARGUMENT

With a fit of a second order (quadratic) model for the full factorial CCD, the design consisted of 13 sets of experiments including 8 samples and 5 centre point. Five levels of variation of numeric factor were used in the experiments respectively. The five levels consisted of plus and minus alpha (axial point), plus and minus 1 (factorial points), and the centre point. Table 1 shows the value of each point for the respective factors and the responses. From the analysis of variance (ANOVA), the R^2 was discovered to be 0.8791. The Model F-value of 10.18 implies the model is significant. There is only a 0.41% chance that the model could occur due to noise.

Table 1: Yield of FA for each run

No	Temperature (°C)	Agitation speed (rpm)	Yield (mg FA/ kg OPF)
1	25	140	209.7175
2	27	140	183.6
3	26	160	168.9531
4	27	160	165.7292
5	24	150	145.8828
6	28	150	149.6126
7	26	130	131.7538
8	26	170	138.585
9	26	150	203.159
10	26	150	200.0238
11	26	150	205.7242
12	26	150	201.6246
13	26	150	202.4222

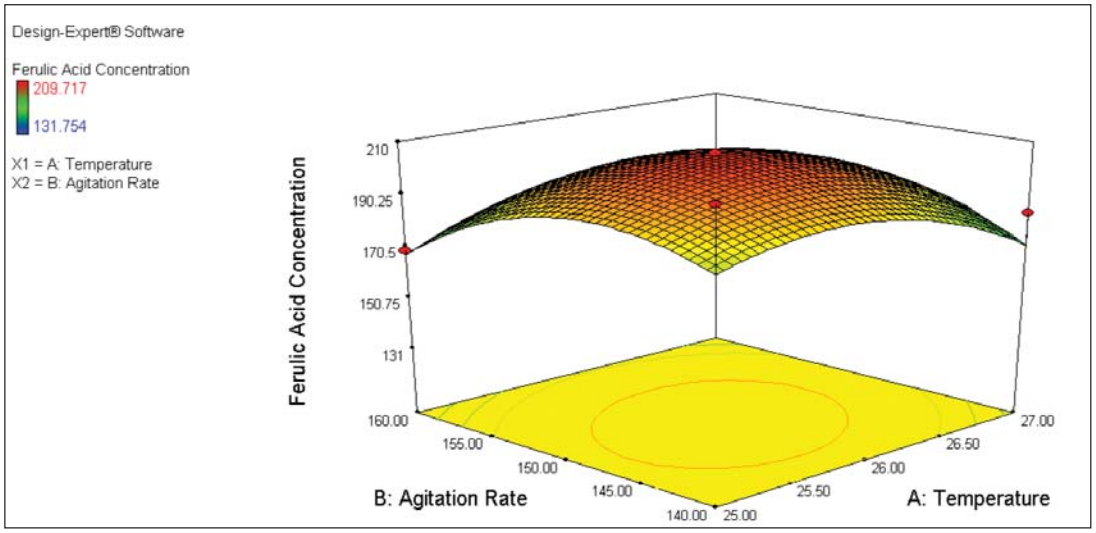

Figure 1 3D surface view of the model for optimizatio

The response surface plots unveiled an optimum point in the FA production process. The response surface plots showed plateau shape of contour. This proved that there exists a point where the yield is the highest for the process. The existence of the point with the highest value proved that optimum point was located in the studied range. Figure 1 shows the response surface plots of the process.

3.1 Result Validation Process for Optimization

A validation process for this model was conducted and is shown on table 2. Validation experiment showed an average error of 8.4131% hence proving that the experiment is repeatable. This proved that the optimization process was able to be carried out. From the central composite design, the optimum condition for FA production is temperature at 26°C and agitation speed at 150 rpm. From table 1, the optimum value of the process is at run 11 with 205.7242 mg FA/kg OPF.

Table 2: Validation process result and error value

Replicates	Temperature (°C)	Agitation (rpm)	Theoretical Value (mg FA/kg OPF)	Experimental Value (mg FA/kgOPF)	Percentage of Error (%)
1	26	150	205.7242	192.9798	6.20
2	26	150	205.7242	192.1649	6.60
3	26	150	205.7242	180.1041	12.45
				Average Error	8.41

4. CONCLUSION

Central composite design was able to determine the optimum condition for FA production from FPOPF via enzymatic hydrolysis using soil culture. Two factors were studied in a 13 runs of sample with 5 of them being the centre point. The optimum condition for temperature and agitation speed is 26°C and 150 rpm respectively yielding as high as 205.724 mg FA/kg OPF. The ANOVA of the model indicated that the coefficient of determination, R^2 is at 0.8791 proved that the model is significant to the experimental design. The response surface plot with a dome shape figure showed that the optimum point exists in the range of value studied. The validation test confirms that the optimization process is repeatable with an average error of 8.41%.

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