Jurnal Teknologi

Full Paper

A FACTORIAL ANALYSIS STUDY ON FACTORS CONTRIBUTION TO FERULIC ACID PRODUCTION FROM OIL PALM FROND WASTE

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Article history
Received
4 February 2016
Received in revised form
27 June 2016
Accepted
15 September 2016

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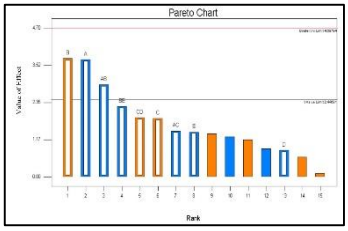
Graphical abstract



Abstract

Enzymatic hydrolysis of fibre-pressed oil palm frond (FPOPF) was done using mix culture for the production of ferulic acid. Mix culture was prepared by acclimatizing the soil obtained from palm oil plantation with FPOPF substrate for 30 days. The substrate had a ratio of FPOPF to water of 1:10. Design Expert 7.1 was used to aid the experimental design. A half fractional two-level factorial analysis with five factors was selected for the experimental design resulting in a total of 16 runs. The factors controlled were temperature (A; 26°C and 40°C), pH value (B; 5 and 9), agitation speed (C; 0 and 150 rpm), inoculum percentage (D; 2% and 10%), and response time (E; 1 and 3 days). The result obtained showed that the experimental design model was significant with a coefficient of determination value of 0.8978. Two factors that contributed the most to the process were temperature and pH value. This model was proved to be repeatable with a validation test percentage error at 4.15% to 6.83%.

Keywords: Oil palm frond, ferulic acid, factorial design, experimental design, mix culture

Abstrak

Proses hidrolisis berenzim terhadap sabut pelepah sawit (FPOPF) telah dijalankan dengan menggunakan kultur bercampur bagi menghasilkan asid ferulik. Kultur bercampur telah disediakan dengan mengaklimatisasi tanah dari ladang kelapa sawit bersama-sama substrat FPOPF selama 30 hari. Substrat yang digunakan mempunyai nisbah FPOPF kepada air sebanyak 1:10. Perisian Design Expert 7.1 telah digunakan bagi menghasilkan rekabentuk eksperimen. Analisis rekabentuk berfaktor pada pecahan separa bersama lima faktor telah dipilih sebagai rekabentuk eksperimen dengan nilai ujikaji sebanyak 16. Faktor-faktor yang dikawal ialah suhu (A; 26°C dan 40°C), pH (B; 5 dan 9), kelajuan pengadukan (C; 0 dan 150 rpm), peratusan inokulum (D; 2% dan 10%) dan tempoh masa kajian (E; 1 dan 3 hari). Hasil kajian menunjukkan bahawa model rekabentuk eksperimen adalah ketara dengan nilai pekali penentuan sebanyak 0.8978. Dua faktor yang banyak menyumbang kepada proses ini ialah suhu dan pH. Hasil ujikaji terbukti mampu diulang melalui ujian pengesahan dengan ralat dari 4.15% hingga 6.83%.

Kata kunci: Pelepah kelapa sawit, asid ferulik, rekabentuk berfaktor, rekabentuk eksperimen, kultur campuran

1.0 INTRODUCTION

Ferulic acid (FA) is an organic acid that possesses a variety of physiological properties including antioxidant, antimicrobial, anti-inflammatory, anti-thrombosis, and anti-cancer activities. With these properties and its low toxicity, ferulic acid has been widely used in pharmaceutical industries [1]. Some of the applications of ferulic acid include being a raw material for production of vanillin and preservatives, a crosslinking agent for the preparation of food gels and edible films, and an ingredient in sports foods and skin protection agents [2, 3, 4, 5].

Oil palm frond (OPF) is an oil palm biomass which is produced as byproduct of the palm oil industry [6]. OPF has the potential as an alternative and renewable source for the production of value-added products [7,8]. However, OPF is commonly used as substitute for grass in forage and fodder by the local industry [9]. With the right application of the OPF, other raw materials that have relatively higher production costs can be overlooked. Moreover, the dependence on food-based raw material such as sugar cane, tapioca starch, and soybean can be reduced. Meanwhile, fibre-pressed oil palm frond (FPOPF) is a biomass recovered after the removal of oil palm frond juice. Known for its high content of feruloyl-polysaccharide, the FPOPF stands as one of the substrates for ferulic acid production via biological treatment.

As a member of the *Musaceae* family, the palm oil falls under the monocotyledons (*Liliopsida*), a plant group that includes grasses and orchids. In the cell walls of these monocotyledons, ferulic acid (3-methoxy-4-hydroxycinnamic acid) exists as the main phenolic acid. On common occurrence, ferulic acids are linked by ester bonds with hemicellulose chains. These hemicellulose chains mostly consist of arabinose residues and they also polymerize with lignin through ether bonds [10]. Being the precursors of coniferyl alcohol and sinapyl alcohol, substituted cinnamic acids such as ferulic acid are known to be available in all plants. Ferulic acid can be found in the plant cell wall. The crosslinks with polysaccharides, such as arabinoxylans in grasses, pectin in spinach and sugar beet, and xyloglucans in bamboo, are making the release of ferulic acid from polysaccharides a difficult task. For the purpose of breaking the crosslink and the releasing ferulic acid from the plant cell walls, two methods were developed [11].

Chemical hydrolysis *via* alkaline hydrolysis is one of the methods to release ferulic acid from polysaccharides. The simplicity of the method is one of the reasons that it is often used to determine the content of ferulic acid in bran [12]. The same study claimed that the release of ferulic acid in a short time at high alkaline concentration and high temperature was one of the special traits of this method. However, the purification of ferulic acid from the hydrolysate has its difficulties as there were many other components present. The other method on releasing ferulic acid is the enzymatic hydrolysis method. This method has a higher chance in producing ferulic acid without furfural [13]. Enzymatic hydrolysis focuses on using feruloyl esterase (FAE) to release ferulic acid from polysaccharide. There are extensive researches on the preparation of ferulic acid using FAEs which some of them involving the combination with polysaccharide hydrolases. However, researchers have yet to find this method practical on a commercial scale due to the high cost in enzyme production by microorganisms and the long reaction time required to hydrolyse the bound ferulic acid.

The aim of this work was to determine the main effects of five factors that affecting the ferulic acid production *via* enzymatic hydrolysis using mix culture by fractional two-level factorial method. This study is crucial for the optimisation stage later because each main effect has the tendency to affect one another as the experiment runs.

2.0 METHODOLOGY

The OPFs were obtained from the palm oil plantation located at Lepar Hilir, Kuantan. These OPFs were pressed first for their juice and sap by following a method described earlier by Zahari *et al.* [14]. The fibre-pressed oil palm fronds (FPOPFs) were kept in freezer for storage purposes. For the preparation of standard calibration curve, ferulic acid 99% was purchased from Sigma Aldrich (Malaysia). Acetonitrile, the mobile phase in high performance liquid chromatography (HPLC) analysis, was purchased from Fisher Scientific (Malaysia).

2.1 Mix Culture Preparation

The mix culture was prepared by mixing FPOPF substrate with the soil obtained from the palm oil plantation. FPOPF substrate had a 1:10 ratio of FPOPF weight to water volume (g/mL). This ratio was selected after several trial and error experiments. The soil used was first sieved before filling up to 1/6 of the 4 L batch bioreactor. The bioreactor was left to acclimatize for 30 days where each day, 120 mL of FPOPF substrate was added to the bioreactor. Upon reaching the 30th day, the acclimatized mix culture was ready for enzymatic hydrolysis in 250 mL conical flasks. From the mix culture identification, a significant quantity of *Aspergillus niger* was detected. *A. niger* has been used widely in enzymatic hydrolysis due to its ability to produce FAE [5]. The acclimatized mix culture acted as the source of the enzyme.

2.2 Two-level Factorial Analysis Experimental Setup

The enzymatic hydrolysis was performed in 250 mL conical flasks. To identify the factors that affect the

ferulic acid production, several factors were obtained through preliminary and literature study. Five factors, temperature (A), pH value (B), reaction time (C), agitation rate (D) and inoculum to substrate volume ratio (E) were screened during this process. The inoculum in this method stands for the mix culture used. The substrate was first sterilised by autoclave before being used for this experiment. The pH value (B) of the sample was controlled with the addition of sodium hydroxide (pH 12). The amount of NaOH added were varied to gain the pH value of the sample at pH 5 and pH 9. The inoculum was added to the sample at respective percentage ratio of 2% and 10% of inoculum to substrate. Incubator shaker (INFORS HT Ecotron) was used to control temperature (A) at 26°C and 40°C, and agitation speed (D) of 0 and 150 rpm. The reaction time (C) was controlled by removing the samples from the incubator at specific time, 1 and 3 days.

With the aid of Design Expert 7.1, the factor analysis method suitable for these parameters was the two-level factorial design with five factors. Two-level factorial analysis at half fraction was applied to this research resulting in 16 runs in total. Each run was replicated three times. Table 1 shows the condition and value of the high-level and low-level for each factor.

Table 1 Level for each factor for screening process

Factor	Symbol	a = -1	a = +1	Unit
Temperature	A	26	40	°C
pH value	B	5	9	pH
Agitation Speed	C	0	150	rpm
Time	D	1	3	days
Inoculum Percentage	E	2	10	%

2.3 HPLC Analysis

The HPLC analysis was performed according to Kareparamban *et al.* [15] with minor modifications. The method was first developed for the estimation of ferulic acid from herbal preparation. The analysis was carried out using Agilent 1100 series with a C-18 column. The mobile phase used had a ratio of acetonitrile to acetic acid (10%) of 20:80 v/v. The flow rate was kept at 1.0 mL/min with a wavelength of detection at 319 nm. Each sample was injected to the column at the volume of 20 µL. During the analysis the column oven temperature was maintained at 30°C.

3.0 RESULTS AND DISCUSSION

Screening of five factors were performed with the assistance of Design Expert software and analysed for their effect on the production of ferulic acid using mix culture *via* enzymatic hydrolysis. The experimental design running sequence and the response obtained (Y) are shown in Table 2. From the two-level factorial analysis, each of the factors contributing to the process was analysed by Design Expert. The percentage contribution of each factor to the process is shown on Table 3.

3.1 Main Effect Analysis

From Table 3, pH (B) stood as the factor with the highest percentage contribution to the process at 23.76%, followed by temperature (A) at 23.34%. As for agitation rate (C), time (D) and inoculum percentage (E), these three factors showed low values of percentage contribution at 5.77%, 1.20% and 3.39%, respectively.

Table 3 Percentage contribution of each main factor

Factor	Percentage Contribution (%)
A	23.34
B	23.76
C	5.77
D	1.20
E	3.39

The main effect also can be represented by the pareto chart of the process as in Figure 1. In terms of effect value, factor B gave the highest value followed by factor A. Factor C comes in as the third highest value of effect with factor E at the fourth. Factor D has the lowest value of effect to the process.

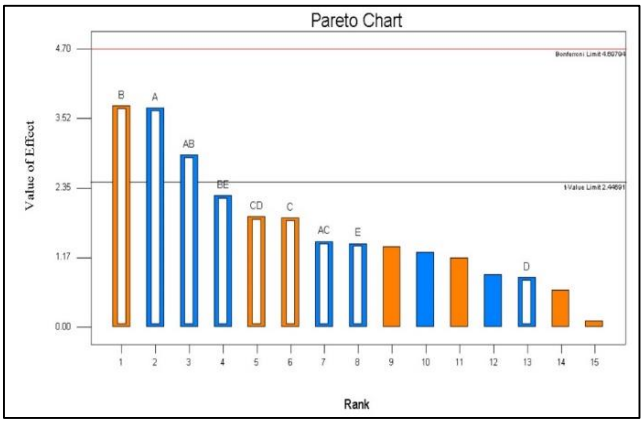


Figure 1 Pareto chart for each factors with their value effect to the process

Ferulic acid production process is crucially affected by FAE. Similar to other enzymes, pH value

and temperature play a critical role in inducing FAE relative activity. The optimum value of these factors differed from one FAE to another as FAE itself has a wide range of substrates and sources [16]. A study on the temperature and pH profiles of multiple FAEs [17] discovered that three FAEs in *Cellulosilyticum ruminicola* has an optimum pH range from 6 to 9 and temperature range from 35 to 40°C. This shows that both pH value and temperature affect ferulic acid production.

Table 2 Experimental design of factor screening process using 2^{5-1} factorial design and the response obtained

No	Variables					Response
	A	B	C	D	E	Y (mg FA / kg OPF)
1	-1	-1	-1	-1	+1	9.8857
2	+1	-1	-1	-1	-1	3.9868
3	-1	+1	-1	-1	-1	108.7659
4	+1	+1	-1	-1	+1	12.4540
5	-1	-1	+1	-1	-1	4.7254
6	+1	-1	+1	-1	+1	4.5355
7	-1	+1	+1	-1	+1	96.0523
8	+1	+1	+1	-1	-1	28.6467
9	-1	-1	-1	+1	-1	3.5989
10	+1	-1	-1	+1	+1	4.2481
11	-1	+1	-1	+1	+1	2.8176
12	+1	+1	-1	+1	-1	3.7947
13	-1	-1	+1	+1	+1	31.9888
14	+1	-1	+1	+1	-1	2.1085
15	-1	+1	+1	+1	-1	138.9518
16	+1	+1	+1	+1	+1	6.7992

3.2 Statistical Modeling and ANOVA

The ANOVA of this experimental design is shown in Table 4. The model F-Value at 5.86 implied that the model was significant. The Prob > F value of both factor A and B were lower than 0.5. This indicated that these two model terms were significant. However, the model terms for factor C, D and E were insignificant to the study. These factors may still affect the process however in a very minor way. This finding corresponds with the discussion in main effect contribution. The standard deviation, R^2, is 0.8978. This value implied that there was only a 10.22% chance that the data did not fit the experiment model.

From Table 5, the coefficient regression showed that the value of a_0 was higher than any other value. The existent of the design plateau was proved here as the other coefficient for the factors were lower than the intercepts. This plateau showed that the design had a maximum point which is likely to be the optimum point and possible for the optimisation process. With the values from Table 5, the model equation for ferulic acid production from FPOPF *via* enzymatic hydrolysis using mix culture treatment can be expressed by the equation below.

$$y = 28.96 - 20.64a_1 + 20.83a_2 + 10.27a_3 - 4.67a_4 - 7.86a_5 - 16.22a_1a_2 - 8.07a_1a_3 - 12.39a_2a_5 - 10.41a_3a_4$$

Table 5 Coefficient regression for linear equation regression for the 2^{5-1} factorial design for factor screening process

Factor	Coefficient Estimate
a_0	28.96
a_1	-20.64
a_2	20.83
a_3	10.27
a_4	-4.67
a_5	-7.86
a_1a_2	-16.22
a_1a_3	-8.07
a_2a_5	-12.39
a_3a_4	10.41

Table 4 Analysis of variance table (ANOVA) for factor screening process

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F
Model	26220.6467	9	2913.4052	5.86	0.0217
A-Temperature	6815.0352	1	6815.0352	13.70	0.0101
B-pH	6939.0675	1	6939.0675	13.95	0.0097
C-Agitation	1686.2673	1	1686.2673	3.39	0.1151
D-Time	349.1712	1	349.1712	0.70	0.4342
E-Percentage Inoculum	989.0629	1	989.0629	1.99	0.2081
AB	4211.1354	1	4211.1354	8.47	0.0270
AC	1040.7676	1	1040.7676	2.09	0.1981
BE	2457.0499	1	2457.0499	4.94	0.0679
CD	1733.0896		1733.0896	3.49	0.1112
Residual	2983.67	6	497.28		
Cor. Total	29204.31	15			
Std. Deviation	22.30				
Mean	28.96				
R^2	0.8978				
Adjusted R^2	0.7446				

Figure 2 shows the graph of predicted yield versus actual yield for each run that shows the yield distribution for each run in terms of predicted yield against actual yield. The straight line represents the point where the predicted value is equal to the actual value. The run at standard order 11 had the largest margin of difference as the predicted value was 32.982 mg FA/kg OPF while the actual value was 2.818 mg FA/kg OPF. This difference between predicted and actual value was one of the variances that contributed to the coefficient of determination value obtained in the ANOVA.

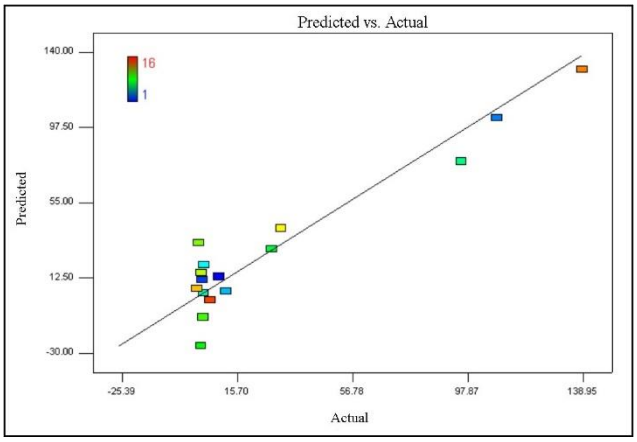


Figure 2 Graph of predicted yield versus actual yield obtained from Design Expert.

3.3 Data Validation Process

A validation run was carried out in order to determine whether the screening process was repeatable or not. This process was done by performing experiment of two points from the screening table. The value of the theoretical yield and the experimental yield were compared and their errors were calculated. The results obtained were tabulated in Table 6.

Table 6 The FA value obtained from validation run in comparison with the theoretical value from Design Expert 7.1

Point	FA yield (mg FA / kg OPF)	Theoretical yield (mg FA / kg OPF)	Percentage error
A	92.0661	96.0523	4.15%
B	109.9577	116.1680	6.83%

From Table 6, the percentage error of point A and point B were 4.15% and 6.83%, respectively. Point A was at the 7th run in the screening experimental table, while point B was the suggested point for best yield from Design Expert software. The factors condition for point B was temperature of 26°C, pH value of 9, agitation speed of 150 rpm, time of 1 day and the inoculum percentage of 2%. Each run was carried out with three replicates. This low percentage error signified that the validation process was successful hence proving that the screening process was repeatable.

4.0 CONCLUSION

The factorial analysis study was able to determine the factors that gave the most impactful effect to ferulic acid production in enzymatic hydrolysis of FPOPF using mix culture. Out of the five effects, two of them which are temperature (A) and pH value (B) were discovered to be affecting the process. Both of these factors contributed as high as 23.34% and 23.76% respectively. The presence of FAE in the process affects ferulic acid production. In order for the FAE to be presence at its most active state, the control of temperature and pH value is crucial in this study. With FAE being active, the yield of ferulic acid will increase.

The ANOVA of the model indicated that the coefficient of determination, R^2 is at 0.8978 which proved that the model is significant to the experimental design. The coefficient of regression for the model also showed that there exist a maximum point or design plateau hence proving that the design can be continued for optimisation process. This experiment proved to be repeatable as the validation experiment showed small percentage error ranged from 4.15% to 6.83%.

Acknowledgement

This research was made possible with the financial aid from the Ministry of Higher Education specifically research grant RDU131412 and RDU140338. A token of gratitude is reserved to the Faculty of Chemical and Natural Resources Engineering lab technical team.

References

[1] Gopalan, N., L. V. Rodríguez-Duran, G. Saucedo-Castaneda, and K. M. Nampoothiri. 2015. Review on Technological and Scientific Aspects of Feruloyl Esterases: A Versatile Enzyme for Biorefining of Biomass. *Bioresource Technology*. 193: 534-544.

[2] Gallage, N. J., E. H. Hansen, R. Kannangara, C. E. Olsen, M. S. Motawia, K. Jørgensen, I. Holmer, K. Hebelstrup, M. Grisoni, and B. L. Møller. 2014. Vanillin Formation from Ferulic Acid in Vanilla Planifolia is Catalysed by a Single Enzyme. *Nature Communications*. 5: 1-19.

[3] Barghini, P., D. Di Gioia, F. Fava, and M. Ruzzi. 2007. Vanillin Production using Metabolically Engineered Escherichia coli under Non-growing Conditions. *Microbial Cell Factories*. 6: 13.

[4] Plaggenborg, R., J. Overhage, A. Loos, J. A. C. Archer, P. Lessard, A. J. Sinskey, A. Steinbüchel, and H. Priefert. 2006. Potential of Rhodococcus Strains for Biotechnological Vanillin Production from Ferulic Acid and Eugenol. *Applied Microbiology and Biotechnology*. 72(4): 745-755.

[5] Lesage-Meessen, L., M. Delattre, M. Haon, J. F. Thibault, B. C. Ceccaldi, P. Brunerie, and M. Asther. 1996. A Two-step Bioconversion Process for Vanillin Production from Ferulic Acid combining Aspergillus niger and Pycnoporus cinnabarinus. *Journal of Biotechnology*. 50: 107-113.

[6] Wan Zahari, M., O. Abu Hassan, H. K. Wong, and J. B. Liang. 2003. Utilization of Oil Palm Frond - Based Diets for Beef and Dairy Production in Malaysia. *Asian-Australasian Journal of Animal Sciences*. 16: 625-634.

[7] Zahari, M. A. K. M., H. Ariffin, J. Salihon, M. N., Mokhtar, Y. Shirai, and M. A. Hassan. 2015. Case Study for a Palm Biomass Biorefinery Utilizing Renewable Non-food Sugars from Oil Palm Frond for the Production of Poly(3-hydroxybutyrate) Bioplastic. *Journal of Cleaner Production*. 87: 284-290.

[8] Zahari, M. A. K. M., S. S. S. S. Abdullah, A. M. Roslan, H. Ariffin, Y. Shirai, and M. A. Hassan. 2014. Efficient Utilization Of Oil Palm Frond For Bio-Based Products And Biorefinery. *Journal of Cleaner Production*. 65: 252-260.

[9] Hassan, O. A., M. Ishida, I. M. Shukri, and Z. A. Tajuddin. 1994. Oil-Palm Fronds As a Roughage Feed Source for Ruminants in Malaysia Oil-Palm Fronds As a Roughage. *Malaysia Agriculture Research and Development Institute (MARDI)*.

[10] Klepacka, J. and L. Fornal. 2006. Ferulic Acid and Its Position Among the Phenolic Compounds of Wheat. *Critical Reviews in Food Science and Nutrition*. 46: 639-647.

[11] Faulds, C. B. and G. Williamson. 1999. The Role of Hydroxycinnamates in the Plant Cell Wall. *Journal of the Science of Food and Agriculture*. 79: 393-395.

[12] Oosterveld, A., G. Beldman, and A. G. J. Voragen. 2000. Oxidative Cross-linking of Pectic Polysaccharides from Sugar Beet Pulp. *Carbohydrate Research*. 328: 199-207.

[13] Wu, M. K., Abokitse, S. Grosse, H. Leisch, and P. K. Lau. 2012. New Feruloyl Esterases to Access Phenolic Acids from Grass Biomass. *Applied Biochemistry and Biotechnology*. 168: 129-143.

[14] Zahari M. A. K. M., M. R. Zakaria, H. Ariffin, M. N. Mokhtar, J. Salihon, Y. Shirai, M. A. Hassan. 2012. Renewable Sugars From Oil Palm Frond Juice As An Alternative Novel Fermentation Feedstock For Value-Added Products. *Bioresour. Technol.* 110: 566-571.

[15] Kareparamban, J. A., P. H. Nikam, A. P. Jadhav, and V. J. Kadam. 2013. A Validated High-performance Liquid Chromatograhy Method for Estimation of Ferulic Acid in Asafoetida and Polyherbal Preparation. *Indian Journal of Pharmaceutical Sciences*. 75: 493-5.

[16] Esteban-Torres, M., I. Reverón, J. M. Mancheño, B. de Las Rivas, and R. Muñoz. 2013. Characterization of a Feruloyl Esterase from Lactobacillus plantarum. *Applied And Environmental Microbiology*. 79: 5130-6.

[17] Li, J. S. Cai, Y. Luo, and X. Dong. 2011. Three Feruloyl Esterases in Cellulosilyticum ruminicola H1 Act Synergistically To Hydrolyze Esterified Polysaccharides. *Applied and Environmental Microbiology*. 77: 6141-6147.