Title: FACTORS AFFECTING FERULIC ACID PRODUCTION FROM BANANA STEM WASTE BY FULL FACTORIAL DESIGN (FFD)

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Significant of Study to Industry: Anti-oxidant such as ferulic acid is desired and demanded in the food and pharmaceutical industries. The demanded material was not easy to get due to the nature of the process involved and limited feedstock for the production. The intricate process to obtain the product, including via extraction or hydrolysis, also becomes a major stumbling block that needs to be demolished for future growth. This study implement the use of low-cost process with enzymatic hydrolysis from soil culture to acquire the ferulic acid that profoundly reduce the gap of achieving a better process design. Furthermore, the use of a statistical tool to generate a great predictor model was applied in the study. The aim is to have a good mathematical model that can be applied in the future works, especially with the demands of a current industry that embracing the Industrial Revolution 4.0.

Abstract

There are countless attempts on applying banana stem waste (BSW) as a feedstock for renewable energy, the materials are also known to be excellent substrate for various bioproducts. Ferulic acid happens to be one of the bioproducts that can be produced from BSW recognized to be great anti-oxidant compound and desired by pharmaceutical and food industries around the globe. This study employed enzymatic hydrolysis of feruloyl-polysaccharide from banana stem waste (BSW) by soil mixed culture (SMC) to produce ferulic acid (FA) using 25 full factorial design (FFD) to investigate the effect and interaction of these five factors affecting FA production: fermentation temperature (A; ^{o}C), agitation (B; rpm), water-to-BSW ratio (C;v/v), substrate-to-inoculums ratio (D;v/v), and inoculation time (E; days). The linear model was well fitted at R2=0.8019 with factors contribution percentages in the order of E > C > A > D > B. Inoculation time had 27.37% contribution indicating the importance of cell growth activities. The interaction of DE was highest since the SMC needs sufficient time for substrate utilization to get a high FA yield. The most FA output produced was 1.2187 mg FA/g BSW with parameters at ambient temperature, 150 rpm agitation, 1:1 water-to-BSW ratio, 1:1 substrate-to-inoculums ratio, and one day of inoculation. The hydrolysis process applied in this study found to be affected by various factors yet could be great option for production of ferulic acid. Meanwhile, BSW is proven feasible and great for producing ferulic acid naturally.

Keywords: Ferulic acid; Banana stem waste; Full factorial design; Soil mixed culture

1. Introduction

Ferulic acid (FA) has numerous physiologic functions, including anti-microbial, anti-oxidant, and anti-cancer activities, which are used in many applications, particularly the food, cosmetic,

and pharmaceutical industries [1]. FA may potentially prevent coronary vessel disease, increase sperm vitality [2], and lower serum cholesterol [3]. Ferulic acid found to be available in lignocellulosic materials comprised of cellulose, hemicelluloses, and lignin [4]. Due to the low cost and high availability, some lignocellulosic materials such as Eucalyptus wood, barley bran, corncobs, corn leaves, and oat fiber have been investigated as alternative phenolic acid sources [5].

The banana stem waste is available year-round found to be a potential lignocellulosic source for ferulic acid production. Every 60 kg of banana harvested is estimated to produce 200 kilograms of discarded waste stems [6]. In Malaysia alone, banana plantations comprise an estimated 34,000 ha [7]. Banana stems regarded as waste due to activity by the farmers. It usually left at the banana plantation after harvesting and allowed to degrade naturally. This practice seems wasteful because banana stems were discovered to be a suitable substrate, especially with abundant amounts available in tropical countries.

The one factor at-a-time method is conventionally used to screen and optimize parameters. Unfortunately, this method is instead time-consuming and generates misleading factor interactions for new raw materials and processes. A statistical approach that varies all of the factors simultaneously could estimate the combined effect of the selected variables and their significance. Full factorial design (FFD) used in this study is based on the statistics fundamental principle, randomization, replication, and duplication. It simplifies the screening process by statistically assessing the interactions between multiple factors over a range of values. Previously, FFD was successfully employed to extract FA from maize bran [8], brewer's spent grain [1], and paddy straw [9].

This study is the first to explore banana stem waste as a biomass source for ferulic acid production by enzymatic hydrolysis using soil mixed culture. Two-level FFD was statistically applied to assess production effects by the selected factors and while observing the interactions between these factors on the output.

2. Materials and Method

The following are the materials used and the methods involved in this research. No human and animals were harmed during the experiments.

2.1 Raw materials and chemicals

Banana stem waste (BSW) was used as the substrate, obtained banana plantation area from Kuantan, Pahang, specifically in the Gambang area. The stems were chopped into 2 cm × 4 cm and then stored in ambient room temperature. Before use, the BSW mixed with distilled water in a 1:1 v/v ratio. Ferulic acid (FA) 99% was purchased from Sigma Aldrich (Malaysia). Acetonitrile HPLC grade used as a mobile phase purchased from Fisher Scientific (Malaysia).

2.2 Mixed culture and inoculums

Soil sample for soil mixed culture (SMC) was obtained from banana plantation soil. It was collected using plastic pipes (polyvinyl chloride) under 10cm depth from the surface. Banana stem sludge (BSS) prepared as an inoculum source in this study by mixing obtained soil with the prepared substrate in 1:4 v/v ratios according to the inlet substrate per day for an entire one month. After that, it used as soil mixed culture (SMC) in the experiments.

2.3 Feruloyl polysaccharide analysis

The hydrolysis experiments were performed in a 250 ml conical flask on an incubator shaker. The reaction mixture was done by fermentation of banana stem waste (BSW) with soil mixed culture at 1:1 ratio (v/v) according to the conditions set by the experimental design. The enzymatic hydrolysis was stopped by immediate centrifugation at 10000 rpm for 5 min and chilled. The supernatants were separated to conduct the analytical assays.

2.4 Experimental design

The full factorial design requires fewer measurements than the classical one-at-a-time experiment to give the same precision. Design-Expert software (Stat-Ease, version 8.0.6) was used for the screening process. A two-level 2^5 full factorial design was employed involving five factors; fermentation temperature (A;^{o}C), agitation (B;rpm), water-to-banana stem waste ratio (C;v/v), substrate-to-inoculums ratio (D;v/v), and inoculation time (E;days).

Table 1. The value of level for each factor in FFD.

Variable	Symbol	Real value of levels		
		-1	0	+1
Temperature (°C)	A	ambient	-	35
Agitation (rpm)	B	no agitation	-	150
Water to banana stem waste ratio (v/v)	C	1	1.5	2
Substrate to inoculums ratio (v/v)	D	1	1.5	2
Time (day)	E	1	3	5

The yield of ferulic acid (mg FA/g BSW) was set as the dependent variable. A total of 44 sets of hydrolysis experiments, including center points, were carried out. The order of the running experiments was restrictedly randomized to eliminate possible bias [10]. Table 1 contains the list of ranges for each factor, which were selected by preferring mainly straightforward factors, which do not need the use of chemical treatments or complex fermentation preparation. In the experimental design, low and high factors coded as −1 and +1; the midpoint coded as 0 (for numerical factors). The standard approach to the analysis of the experimental design data is to evaluate a list of the main and interaction effects supported by an analysis of variance (ANOVA) following a linear regression model as in Eq.1, indicating which effects are significant [12].

$$\hat{y}_i = b_0 + \sum_{i=1}^{n} b_i X_i \quad \text{Eq. 1}$$

In Eq.1, $\hat{y}_i$ represents the value of the response or dependent variable, b_0 is the interception coefficient, b_i the linear coefficients, n the number of variables studied, and X_i represents the coded independent variables.

2.5 High Performance Liquid Chromatography (HPLC) Analysis

The amount of ferulic acid was measured by the HPLC system (Agilent, HP-1100) with a diode array detector (DAD) at 280 nm wavelength and Agilent Zorbaq SB-AQ C18 analytical column.

The condition of the column controlled at 30^{o}C. Acetonitrile and water (55%:45%) used as the mobile phase at 1 ml/min flow rate, and the volume of injection for every vial was 10 µl. A set of standard dilutions prepared at a ferulic acid concentration in the range of 0.1 to 0.5 g/l..

3. Results

The overall result of FFD experiments is presented in Table 2. A high yield of ferulic acid obtained was 1.2187 mg FA/g BSW. It was achieved within the parameters' range, which was at ambient temperature, 150 rpm agitation, 1:1 (v/v) water-to-banana stem waste (BSW) ratio, 1:1 (v/v) substrate-to-inoculums ratio, and one day. It indicates that the range selected was suitable for this study. Discussions will focus thoroughly on the main effects and their interactions..

Table 2. Experimental data for FFD of ferulic acid production

Std	Run	A	B	C	D	E	Ferulic acid yield (mg/g)		Std	Run	A	B	C	D	E	Ferulic acid yield (mg/g)
39	1	ambient	150	1.5	1.5	3	0.8114		2	23	35	no agitation	1	1	1	0.9666
20	2	35	150	1	1	5	0.6366		31	24	ambient	150	2	2	5	0.5686
21	3	ambient	no agitation	2	1	5	0.6101		41	25	ambient	no agitation	1.5	1.5	3	0.6300
15	4	ambient	150	2	2	1	0.6073		33	26	ambient	no agitation	1.5	1.5	3	0.6062
19	5	ambient	150	1	1	5	0.6507		17	27	ambient	no agitation	1	1	5	0.6204
9	6	ambient	no agitation	1	2	1	0.8161		12	28	35	150	1	2	1	0.7619
18	7	35	no agitation	1	1	5	0.6176		1	29	ambient	no agitation	1	1	1	0.8164
32	8	35	150	2	2	5	0.6793		37	30	ambient	no agitation	1.5	1.5	3	0.5818
24	9	35	150	2	1	5	0.5702		5	31	ambient	no agitation	2	1	1	0.6818
22	10	35	no agitation	2	1	5	0.6674		35	32	ambient	150	1.5	1.5	3	0.6268
28	11	35	150	1	2	5	0.7009		27	33	ambient	150	1	2	5	0.6471
6	12	35	no agitation	2	1	1	0.7992		30	34	35	no agitation	2	2	5	0.6547
13	13	ambient	no agitation	2	2	1	0.6379		34	35	35	no agitation	1.5	1.5	3	0.9123
11	14	ambient	150	1	2	1	0.7561		3	36	ambient	150	1	1	1	1.2187
36	15	35	150	1.5	1.5	3	0.8477		4	37	35	150	1	1	1	0.8736
25	16	ambient	no agitation	1	2	5	0.8459		7	38	ambient	150	2	1	1	0.8223
40	17	35	150	1.5	1.5	3	0.9097		42	39	35	no agitation	1.5	1.5	3	0.8736
23	18	ambient	150	2	1	5	0.6032		16	40	35	150	2	2	1	0.8139
38	19	35	no agitation	1.5	1.5	3	0.8685		8	41	35	150	2	1	1	0.7537
10	20	35	no agitation	1	2	1	0.8885		14	42	35	no agitation	2	2	1	0.8295
44	21	35	150	1.5	1.5	3	0.7985		43	43	ambient	150	1.5	1.5	3	0.8782
29	22	ambient	no agitation	2	2	5	0.5817		26	44	35	no agitation	1	2	5	0.6268

4. Discussions

4.1 Analysis of variance (ANOVA)

Only contribution above 1% was selected except for main factors in order to construct a significant model as shown in Fig. 1. The linear regression equation was as in Eq. 2.

FA = 0.7425 + 0.325 A + 0.0092 B - 0.0488 C - 0.0154 D - 0.0863 E - 0.0255 AB + 0.0298 AC + 0.0198 AD - 0.0217 BD + 0.0232 `CE + 0.0359 DE + 0.0325 ABD + 0.0294 ABE - 0.0191 ADE + 0.0201 BCD + 0.0181 BDE - 0.0233 CDE - 0.0220 ABCE Eq. 2

In Eq. 2, FA represents ferulic acid concentration, A is temperature, B is agitation, C is water-to-banana stem waste ratio, D is substrate-to-inoculums ratio, E is time, AB, AC, AD, BD, CE, DE, ABD, ABE, ADE, BCD, BDE, CDE, and ABCE are the interactions involved in the model.

The significance of the model was analysed by the analysis of variance (ANOVA), as shown in Table 3.The *F*-value and *P*-value were 5.6212 and <0.0001, respectively, signifies that the estimated model fits the experimental data reasonably [11]. The value of R^2 obtained was 0.8019. The previous study stated that R^2 value above 0.6 is considerably accepted [12-13]. Adjusted R^2 and predicted R^2 values were 0.6592 and 0.5246, respectively. It justified that, if the model is significant, lack of fit not significant, and adjusted and predicted R^2 values are within 0.2 of each other, the model provides good predictions for average outcomes [14].

Table 3. Analysis of variance (ANOVA) of feruloyl polysaccharide hydrolysis from BSW using soil mixed culture.

Source	Sum of Squares	Mean Square	F Value	p-value Prob > F

Model	0.6455	0.0359	5.6212	<0.0001 significant
A	0.0466	0.0466	7.3040	0.0122
B	0.0037	0.0037	0.5789	0.4538
C	0.0764	0.0764	11.969	0.0020
D	0.0076	0.0076	1.1873	0.2863
E	0.2384	0.2384	37.376	< 0.0001
AB	0.0285	0.0285	4.4723	0.0446
AC	0.0284	0.0284	4.4558	0.0450
AD	0.0125	0.0125	1.9648	0.1733
BD	0.0151	0.0151	2.3697	0.1363
CE	0.0172	0.0172	2.6943	0.1132
DE	0.0413	0.0413	6.4759	0.0175
ABD	0.0339	0.0339	5.3144	0.0297
ABE	0.0277	0.0277	4.3461	0.0475
ADE	0.0117	0.0117	1.8321	0.1880
BCD	0.0129	0.0129	2.0348	0.1661
BDE	0.0105	0.0105	1.6412	0.2119
CDE	0.0174	0.0174	2.7276	0.1111
ABCE	0.0155	0.0155	2.4363	0.1311
Residual	0.1595	0.0064		
Lack of Fit	0.1170	0.0069	1.2980	0.3667 not significant
Pure Error	0.0424	0.0053		
Cor Total	0.8049			
R^2	0.8019			
Adj R^2	0.6592			
Pred R^2	0.5246			

4.2 Main effects contribution

The percentage contribution of main factors and selected interactions were shown in Figure 1. It is observed that factor time, E, had the highest significant effect, followed by water-to-BSW ratio, C, temperature, A, substrate-to-inoculums ratio, D, and lastly factor agitation, B. The time of inoculation found to be the most crucial factor, with a 27.37% contribution. The process needs a suitable period to enter stages of inoculums growth and consumption of substrate (metabolism).

The pattern has been reported by other previous studies where they stated that contact time between the mixtures is crucial to ensure the increased yield of product [15-16].

Term	Stdized Effects	Sum of Squares	% Contribution
Intercept			
A-Temperature	0.065	0.047	5.35
B-Agitation	0.018	3.693E-003	0.42
C-Water to Banana Stem ratio	-0.083	0.076	8.76
D-Substrate to Inoculum ratio	-0.026	7.574E-003	0.87
E-Time	-0.15	0.24	27.37
AB	-0.051	0.029	3.27
AC	0.051	0.028	3.26
AD	0.034	0.013	1.44
AE	-0.019	2.895E-003	0.33
BC	-5.704E-003	2.603E-004	0.030
BD	-0.037	0.015	1.74
BE	-0.021	3.604E-003	0.41
CD	0.014	1.547E-003	0.18
CE	0.040	0.017	1.97
DE	0.061	0.041	4.74
ABC	4.806E-003	1.848E-004	0.021
ABD	0.056	0.034	3.89
ABE	0.050	0.028	3.18
ACD	0.024	4.611E-003	0.53
ACE	-0.011	9.407E-004	0.11
ADE	-0.033	0.012	1.34
BCD	0.034	0.013	1.49
BCE	3.546E-003	1.006E-004	0.012
BDE	0.031	0.010	1.20
CDE	-0.040	0.017	2.00
ABCD	-0.024	4.599E-003	0.53
ABCE	-0.038	0.016	1.78
ABDE	-0.014	1.480E-003	0.17
ACDE	0.014	1.677E-003	0.19
BCDE	-4.080E-003	1.331E-004	0.015
ABCDE	4.473E-003	1.601E-004	0.018
Curvature		0.16	18.50
Lack Of Fit		0.000	0.000
Pure Error		0.042	4.87

Figure 1. The percentage contribution of each main factors and their interaction throughout screening experiments in FFD.

The ratio of water-to-BSW was second most important contributed 8.76%. Water is known as a universal solvent and very important in the biological system. It has a solvent function for

organisms and cells to dissolve nutrients and scavenges wastes or metabolites. Water also stabilizes the structure of molecules and cells. It has been stated by in other research that insufficient water during fermentation can cause poor diffusion of solutes and gas. It can disrupt cell metabolism due to the lack of substrates in or near the cell [17]. Factor temperature had a 5.35% contribution became the third-highest significant factor for the process. It is vital as enzyme activities depend on the surrounding medium. Because of this, the most suitable temperature is essential to ensure the enzyme is at its best possible condition. This behavior also proved by the previous study where they investigate the temperature effect on the enzyme activation [18].

The substrate-to-inoculums ratio had a lesser contribution, which is 0.87%. The different feeding amounts of the substrate could affect the consumption rate of the process. Because of this, the enzymatic reaction could decline or increase hence affecting the production of FA [19]. Factor agitation was the least contributed effect with only 0.42%. The agitation helps the mixture to disperse efficiently to increase their contact area and ensure full coverage during the reaction. Al-Zuhair et al. (2003) also stated in their work that a large interfacial area allowed the enzyme to penetrate the interface at higher agitation speeds. It could enhance the hydrolysis and increase the production of FA.

4.3 Interaction of factors

In this two-level factorial design, only two-way interaction factors will be discussed. Among them, the two highest contributions (based on Fig. 1) were the interaction between substrate-to-inoculums ratio and time with 4.74% and the interaction between temperature and agitation with 3.27%.

Figure 2 shows the plot of a high and low amount of substrate-to-inoculums ratio (D) at a different time (E). It indicates that a more extended time FA increased with the amount of substrate, whereas for a short period, the yield of FA slightly decreased when the substrate amount increased. Due to this behavior, the process needs a suitable ratio of substrate-to-inoculums since time is required for the inoculums to consume substrate. It can also be related to the limiting substrate phenomenon and inoculums' growth rate.

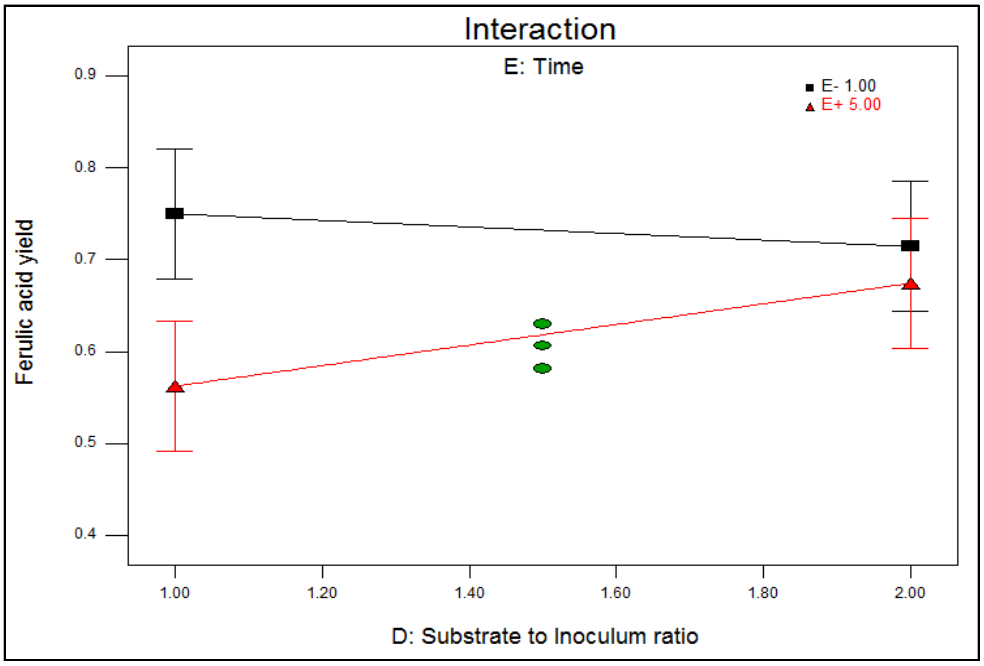

Figure 2. Plot of interaction between substrate to inoculums ratio (D) and time (E).

Meanwhile, the plot in Figure 3 illustrates the interaction between temperature, A, and agitation, B, where the rate of FA yield increased when there was no agitation during fermentation. Soil mixed culture was more affected by temperature rather than agitation. Temperature affected the enzyme activity where the mixed culture is in the condition that it is most compatible.

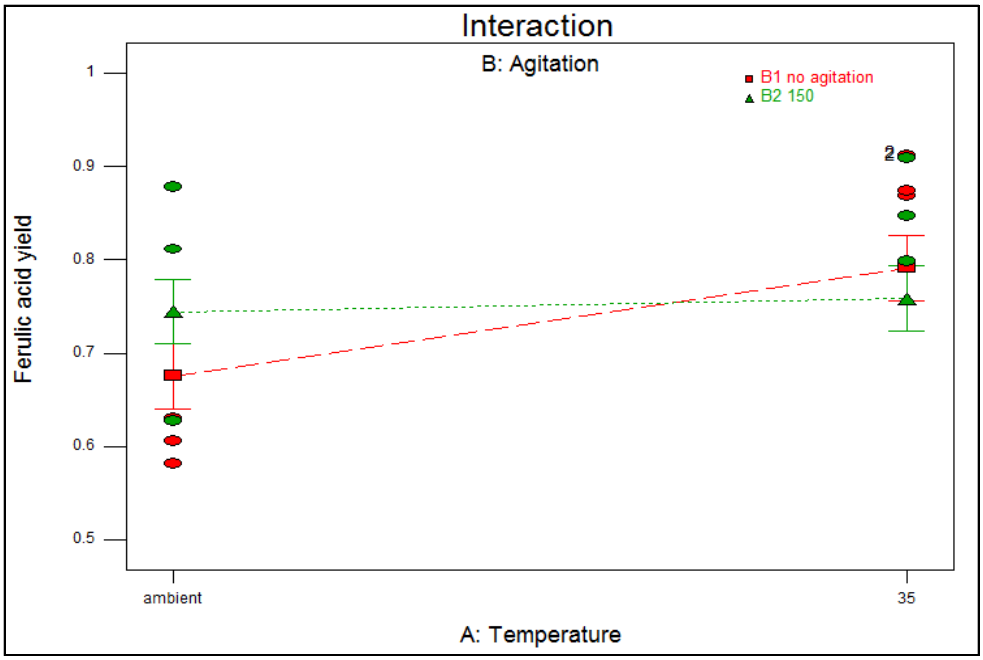

Figure 3. Plot of interaction between temperature (A) and agitation (B).

In other words, the optimized yield of FA could be produced without an unnecessary increase in temperature with the used of even slight agitation, hence reducing the cost of power consumed as well. But although agitation could also help increase the contact area, it could increase the air rate during the reaction. For this process, it can be concluded that the increase in air rate could inhibit the production of ferulic acid.

5. Conclusion

The production of ferulic acid from banana stem waste by soil mixed culture in this study revealed that the time of process was the most affected factor among other factors studied. The contribution for all factors following their significant order was 27.37% for a Time, 8.76% for water-to-BSW ratio, 5.35% for temperature, 0.87% for substrate-to-inoculums ratio, and 0.42% for agitation. Although these main factors play a significant role in the process, their interactions

proved to affect the reaction mechanism differently and could increase or inhibit the reaction. These data could be further used for future work on the study of FA production..

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References

[1] Mussatto, S. I., Dragone, G., & Roberto, I. C. (2007). Ferulic and p-coumaric acids extraction by alkaline hydrolysis of brewer's spent grain. Industrial Crops and Products, 25, 231–237.

[2] Saija, A., Tomaino, A., Trombetta, D., De Pasquale, A., Uccella, N., Barbuzzi, T., Paolino, D., & Bonina, F. (2000). In vitro and in vivo evaluation of caffeic and ferulic acids as topical photoprotective agents. International Journal of Pharmaceutics, 199(1), 39-47.

[3] Ou, S., & Kwok, Kin-Chor. (2004). Ferulic acid: pharmaceutical functions, preparation and applications in foods. Journal of the Science of Food and Agriculture, 84:11, 1261–1269.

[4] Mussatto, S. I., & Teixeira, J. A. (2010). Lignocellulose as raw material in fermentation processes.

[5] Dimitrios, B. (2006). Sources of natural phenolic antioxidants, Trends in Food Science & Technology, 17, 505-512.

[6] Jamaluddin, M. F., Zainol, N., Salihon, J., & Abdul-Rahman, R. (2011). Factorial Analysis on Acetic Acid Production Using Banana Stem Waste. International Journal of Chemical and Environmental Engineering.

[7] Tock, J. Y., Lai, C. L., Lee, K. T., Tan, K. T., & Bhatia, S. (2010). Banana Biomass as Potential Renewable Energy Resource: A Malaysian Case Study. Renewable and Sustainable Energy Reviews, 14:2, 798 -805.

[8] Tilay, A., Bule, M., Kishenkumar, J., & Annapure, U. (2008). Preparation of ferulic acid from agricultural wastes: its improved extraction and purification. Journal of agricultural and food chemistry, 56:17, 7644-7648.

[9] Salleh, N. H. M., Daud, M. Z. M., Arbain, D., Ahmad, M. S., & Ismail, K. S. K. (2011). Optimization of alkaline hydrolysis of paddy straw for ferulic acid extraction. Industrial Crops and Products, 34:3, 1635-1640.

[10] Koohpaei, A. R., Shahtaheri, S. J., Ganjali, M. R., Forushani, A. R., & Golbabaei, F. (2008). Application of multivariate analysis to the screening of molecularly imprinted polymers (MIPs) for ametryn. Talanta, 75, 978–986.

[11] Zularisam, A. W., Ismail, A. F., Salim, M. R., Mimi, S., & Matsuura, T. (2009). Application of coagulation–ultrafiltration hybrid process for drinking water treatment: Optimization of operating conditions using experimental design. Separation and Purification Technology, 65, 193–210.

[12] Davies, M. P., Vern De Biasi, David P. (2004). Approaches to the rational design of molecularly imprinted polymers, Analytica Chimica Acta, 504, 7-14.

[13] Navarro-Villoslada F., Blanca S. V., Maria C. Moreno-Bondi. (2004). Application of multivariate analysis to the screening of molecularly imprinted polymers for bisphenol A. Analytica Chimica Acta, 504, 149–162.

[14] Stat-ease, Inc. 2011. Minneapolis, MN, USA, Design Expert Version 8.0.6.

[15] Yoon Sang-Hwal, Cui, L., Young-Mi, L., Sook-Hee, L., Sung-Hee, K., Myung-Suk, C., Weon-Taek, S., Jae-Kyung, Y., Jae-Yeon, K., & Seon-Won, K. (2005). Production of vanillin from ferulic acid using recombinant strains of Escherichia coli. Biotechnology and Bioprocess Engineering, 10:4, 378-384.

[16] Vaithanomsat, P., & Waraporn A. (2009). Feasibility Study on Vanillin Production from Jatropha curcas Stem Using Steam Explosion as a Pretreatment, World Academy of Science, Engineering and Technology, 29.

[17] Gervais, P., & Molin, P. (2003). The role of water in solid-state fermentation. Biochemical Engineering Journal, 13, 85-101.

[18] Szwajgier, D., & Jakubczyk, A. (2011). Production of extracellular ferulic acid esterases by lactobacillus strains using natural and synthetic carbon sources. Acta Scientiarum Polonorum. Technologia Alimentaria, 10(3), 287-302.

[19] Hodge, D. B., M. Nazmul, K., Daniel J. S., & James D. M. (2008). Soluble and insoluble solids contributions to high-solids enzymatic hydrolysis of lignocelluloses. Bioresource Technology, 99, 8940–8948

[20] Al-Zuhair, S., Masitah, H., & Ramachandran, K. B. (2003). Kinetics of the enzymatic hydrolysis of palm oil by lipase. Process Biochemistry, 38, 1155-1163