COMBINED EFFECT OF OPERATIONAL VARIABLES ON AQUEOUS ENZYMATIC OIL EXTRACTION FROM PALM PRESSED FIBRE

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ABSTRACT

The extraction of residual oil from palm pressed fibre (PPF) via aqueous enzymatic oil extraction (AEOE) process was studied based on the different operational variables. An application of the response surface methodology (RSM) and Box-Behnken Factorial Design by using a statistical package was used to develop a process design and modelling of the AEOE process. Ultrasonication was applied as a pre-treatment before the aqueous enzymatic oil extraction (AEOE) process, resulting in a both significant increase in yield and a reduction in process time. The use of Celluclast® 1.5L FG in AEOE gave a 58.91% oil yield at pH 5.6 in 5 hr at 50°C. Incorporating the ultrasonication at 485 W for 15 min prior to the additional of Celluclast® 1.5L FG increased the oil yield to 60.96% and reduced the extraction time to 3 hr. The effect of Celluclast® 1.5L FG on oil yields and glucose production from PPF combined with other processed parameters such as hydrolysis time, X_1, enzyme to fibre ratio, X_2 and liquid to fibre ratio, X_3 were also determined. The results of analysis of variance (ANOVA) indicated that in both models, oil extraction (Y_1) and glucose production (Y_2) were statistically significant (oil extraction, $p<0.01$ and glucose production, $p<0.0001$). Post-ANOVA showed that hydrolysis time, X_1 (p-value <0.0001) and the enzyme to fibre ratio, X_2 (p-value 0.0038) had a significant ($p<0.05$) effect on the oil extraction, Y_1; whereas all the independent variables such as hydrolysis time, X_1 (p-value <0.0001), enzyme to fibre ratio, X_2 (p-value 0.0002) and liquid fibre ratio, X_3 (p-value 0.0279) had a significant effect ($p<0.05$) on glucose production, Y_2. The observation confirms the results that the combination of the ultrasonication step as a pre-treatment and AEOE using Celluclast® 1.5L FG increases the oil extractability at reduced time.

Keywords: palm pressed fibre, aqueous enzymatic oil extraction.

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INTRODUCTION

Malaysia is currently one of the biggest exporters of palm oil in the international market. Based on the *Malaysian Oil Palm Statistics 2011*, a total of 92.9 million tonnes of fresh fruit bunch (FFB) were processed to produce about 18.9 million tonnes of crude palm oil (CPO). In the process of the oil extraction, 12.5 million tonnes of palm pressed fibres (PPF) were generated. The PPF and shell are burnt as fuel in the boiler to raise steam, and the residual

boiler ash and clinker are used for surfacing of estate roads (Gurmit, 1994). According to Kirkaldy and Susanto (1976), PPF contains, on a dry weight basis, approximately 40% cellulose, 21% lignin, 24% pentosan and 5% ash. It has been found that oil extracted from PPF is a rich source of carotenoids, vitamin E (tocopherols and tocotrienols) and sterols (Choo *et al*., 1996). Oil recovered from PPF has been reported to contain about 3.7% phospholipids consisting mainly of phosphatidylcholine and phosphatidyethanolamine (Goh *et al*., 1982). Many applications can be produced from PPF oil such as pharmaceuticals, food and cosmetics, nutraceuticals (for phytonutrient-riched fibre oil) and fibre composite, pulp and paper and adsorbents (for de-oiled fibre) (Choo *et al*., 2003).

A few technical papers have reported that solvent extraction followed by aqueous extraction is a commercial method of oils and fats extraction from fruits and oil seeds. According to Dominguez *et al*. (1996), solvent extraction of oil from seeds is the most efficient extraction technique. During the extraction process, the oil diffuses and is extracted through the solvent, while the protein remains in the meal with the fibres and carbohydrates. For practical and economic reasons, n-hexane is normally used as the extraction solvent, and furthermore it is also cheap and abundant. It was reported by Chin and Tan (1976) that solvent extraction minimises the effluent disposal problem. While n-hexane is broadly accepted as the most efficient solvent for oil extraction, its flammability, explosiveness, mild toxicity and environmental impact on the other hand, are concerns for the industry (Cheng and Lavente, 2002). As such, research has focused on the development of alternatives to hexane as the extracting solvent. The least expensive, safest and therefore most desirable solvent is water. Alternative extraction processes with biorenewable solvents have been revised; among them, the most extensively studied is aqueous extraction, leading to high quality of oil and a detoxified protein product (Lawhon *et al*., 1981; Hagenmaier *et al*., 1974; Rhee *et al*., 1972). The aqueous processing of oil-bearing materials eliminates the potential hazards of explosion and fire; eliminates the negative environmental impact due to the emission of organic solvent, and does not leave toxic or undesirable solvent residues in the resulting food product (Cheng and Levente, 2002). However, the main limitations of the aqueous process appear to be the demulsification requirement to recover oil when emulsions are formed and the treatment of the resulting aqueous effluent (Rosenthal *et al*., 1996). With the objective of improving the yield of aqueous processes, enzymes have been used to facilitate oil release.

A potential new technology that may improve extraction of oils from plants is the high intensity ultrasound, which is an application of high intensity and high frequency sound waves that physically interact with materials (Luque-Garcia and Luque de Castro, 2003). According to Vinatoru (2001), ultrasound has been suggested to disrupt plant cell walls thereby facilitating the release of extractable compound and enhance mass transport of solvent from the plant cells. The use of ultrasound in food processing has also been extensively studied. Shah *et al*. (2005) utilised ultrasound as a pre-treatment before aqueous enzymatic oil extraction (AEOE) to extract oil from *Jatropha curcas* L. seed kernel. Szentmihalyi *et al*. (2002) compared ultrasound based extraction with traditional Soxhlet extraction of rosehip oil from waste hip seeds. In addition, Sharma and Gupta (2004) had also found that the yields from almond, apricot seeds and rice bran were enhanced upon ultrasonic pre-irradiation, when a novel extraction process with a three-phase partitioning was used for oil extraction. According to Mason *et al*. (1996), the mechanical effect of ultrasound provides a greater penetration of solvents into cellular materials and improves mass transfer. Therefore, there is an additional benefit for the use of power ultrasound in the extraction processes which results from the disruption of biological cell walls to facilitate the release of content.

In oil palm fruit, the oil globule is located inside the plant cell linked with proteins and a wide variety of carbohydrates (*i.e.* cellulose, hemicelluloses, pectin and starch). It was reported by Dominguez *et al.* (1996) and Rosenthal *et al*. (1996) that an enzyme system can be used to degrade the insoluble cell wall components to increase the permeability of the oil in aqueous extraction. Based on the mechanical rupturing, the AEOE method that utilises enzymes (*i.e*. cellulases, hemicellulase, pectinase, amylases and proteases) would assist to degrade the cell walls, lipid bodies and break down carbohydrates (Badr and Sitohy, 1992; Bhatnagar and Johari, 1987; Lanzani *et al*., 1975). The enzyme-assisted extraction is a new biological route in the extraction of oils and fats and is attracting a lot of attention from researchers due to its mild conditions, low energy costs and good quality of the protein and oil product (Dominguez *et al*., 1994; Caragay, 1983). Some researchers conducted their research in this novel oil extraction method with soyabean (Rosenthal *et al*., 2001; Dominguez *et al*., 1995a), peanut (Sharma *et al*., 2002), mandarin peels (Mishra *et al*., 2005), *Jatropha curcas* L. seed kernel (Shah *et al*., 2005), almond and apricot seeds (Sharma and Gupta, 2006) as well as sunflower kernels (Dominguez *et al*., 1995b). With the demand for increasing the oil extraction rate (OER) of the palm oil and with the current scenario of using the green technology process, a comprehensive study on the AEOE on PPF was carried out and is discussed in this article.

The aim of this study was to develop a process flow of AEOE for oil recovery from the PPF by incorporating it with mechanical extraction; and to investigate the effect of operational variables (*i.e.* hydrolysis time, enzyme to fibre ratio and liquid to fibre ratio) on the efficiency of the enzymatic action during aqueous processing on PPF. This was followed by an optimisation of the aqueous enzymatic oil extraction by response surface methodology (RSM).

MATERIALS AND METHODS

Materials

The fresh PPF was obtained from the Palm Oil Mill Technology Centre (POMTEC), Labu, Negeri Sembilan, Malaysia. The cellulase was from *Trichoderma reesei* by Novo Nordisk, Denmark and commercially named Celluclast® 1.5L FG and has a declared activity of 700 EGU g^{-1} (EGU = Endo-Glucanase Units).

Pre-treatment and Aqueous Enzymatic Extraction of Oil from Palm Pressed Fibre (PPF)

The general process flow chart for AEOE of PPF in the laboratory is shown in *Figure 1*. The experimentation on AEOE was carried out based on the following operational variables; enzymatic extraction treatment time or hydrolysis time (60-300 min), enzymes activity (50-200 EGU g^{-1}), liquid volume (100 - 250 ml), temperature (35°C - 60°C), pH (4.0-6.0), speed of orbital shaker (50 - 150 rpm), period of ultrasonication (5-20 min) and fibre surface area (course fibre *vs*. fine fibre).

Based on *Figure 1*, 10 g PPF was weighed into a 250 ml conical flask and about 100-250 ml of 0.05 M citrate buffer (pH 4.0-6.0) was added and the mixture was put inside the Brasonic® Ultrasonic Cleaner Model 5510 for ultrasonic pre-treatment step at 485W with different operation times, varying from 5 to 20 min. Following that, the sample flask was capped and put inside the MaxQ® Mini 4450 Incubated Shaker and the temperature was increased gradually to the required temperature. Subsequently, enzymes were added to the sample and the shaker was set at different operation speeds varying from 50 to 150 rpm, and at different allocated periods of 1 to 5 hr. After completion, the treated fibre was separated from the liquid phase and washed by using 1 litre of hot water to remove the free oil that was still attached on the treated PPF. Finally, the treated fibre was dried for 5 hr at 103°C before being subjected to the oil extractor for quantifying the residual oil content. The liquid phase was analysed for glucose content.

Extraction of the Residual Oil from Enzymatic Treated Palm Pressed Fibre (PPF)

The percentage of enzymatic extracted PPF oil recovery was determined according to the oil retained in PPF after enzymatic treatment. The oil extraction yield was calculated as difference between the total oil content in the fresh PPF and the residual oil in the treated PPF which was measured by Soxhlet oil extractor with petroleum ether. The residue of PPF

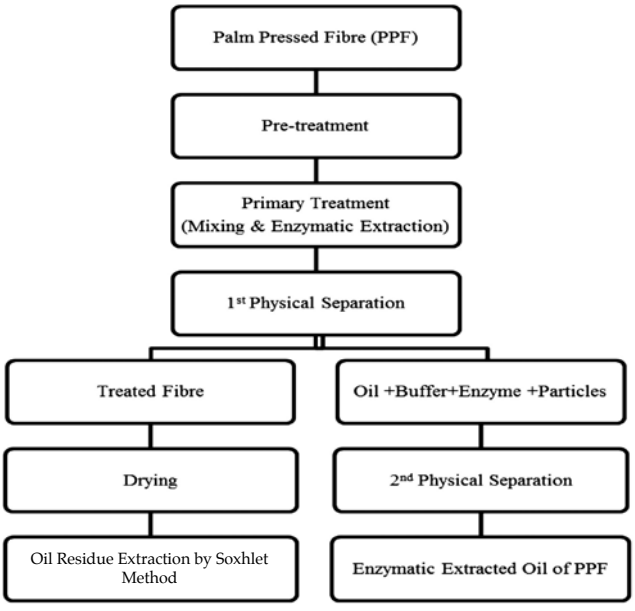


Figure 1. The process flow for the aqueous enzymatic oil extraction of palm press fibre (PPF).

oil extraction by solvent extractor was carried out at 110°C, with the period of immersion, washing and recovery of 50 min, 35 min and 30 min, respectively. The percentage of extracted oil could be calculated by Equations (1) to (3).

$$\text{Total oil content in fresh fibre (\%)} = \frac{\text{weight of oil in fresh fibre (g)}}{\text{weight of fresh fibre (g)}} \times 100$$

Equation (1)

$$\text{Residual oil (\%)} = \frac{\text{weight of oil residue in enzymatic treated fibre (g)}}{\text{weight of enzymatic treated fibre (g)}} \times 100$$

Equation (2)

$$\text{Enzymatic extracted oil (\%)} = \frac{\text{(total oil in fresh fibre) - (residual oil)}}{\text{total oil in fresh fibre}} \times 100$$

Equation (3)

Glucose Content Analysis

The analysis of the glucose content in the liquid phase was to investigate the occurrence of the reaction between cellulose and cellulase enzyme. The glucose concentration was determined by HPLC using Waters 2410 Refractive Index Detector and the column was a Waters, Sugar-Pak 1 Column 6.5 mm i.d x 300 mm length. The mobile phase was 100% deionised water at a flow rate of 0.6 ml min^{-1} and temperature of 70°C.

Experimental Design, Evaluation and Statistical Analysis

The RSM was used to predict the optimum condition for the enzymatic hydrolysis treatment in order to obtain the maximum extraction of residual oil, based on the operational variables such as hydrolysis time, enzyme to fibre ratio and liquid to fibre ratio. The experiment and assessment measured data were evaluated based on a Box-Behnken, fractional factorial design for three variables (Montgomery, 1997) and analysed to RSM using the statistical package, Design Expert version 6.0.4 (Statease Inc., Minneapolis, USA) for formulating the statistical model for AEOE process.

Three independent and two dependent variables were used in the aqueous processing of PPF with enzyme as the processing aid. The independent variables were x_1 (extraction-treatment time, min), x_2 (enzyme to fibre ratio, EGU/g/g) and x_3 (liquid to fibre ratio, ml g^{-1}). The objective functions were Y_1 (oil extraction yield, % of the total extractable oil determined by Soxhlet) and Y_2 (glucose production, g/100 ml sample) (*Table 2*). A total of 17 runs needed for this design were performed in random order, including five replicates of the centre region to generate a quadratic response model (Myers and Montgomery, 2002; Alvarez and Canet, 1999; Alvarez *et al.*, 1999).

The actual and coded levels of the independent variables used in the experimental design are shown in *Table 1*. The quadratic model for predicting the optimal point is expressed according to Equation (4).

$$Y_k = b_0 + \sum_{i=1}^{3} b_i X_i + \sum_{i=1}^{3} b_{ii} X_i^2 + \sum_{i<j} b_{ij} X_i X_j$$

Equation (4)

where Y_k is the predicted response [Y_1 = oil yield (%); Y_2 = glucose production (g/100 ml)]. Coefficients b_0, b_i and b_{ii} represent the constant, linear and quadratic effects and b_{ij} represent the interaction effect. The X_1, X_2 and X_3, coded variables corresponding to the hydrolysis time, enzyme to fibre ratio and liquid to fibre ratio. Annotated analysis of variance (ANOVA) was chosen for model fitting and adequacy [model represented by Equation (4)].

RESULTS AND DISCUSSION

A Combination of Ultrasonic Pre-treatment and AEOE for Oil Recovery from PPF

Ultrasonication is a processing aid in the extraction of organic compound contained in the body of plants and seeds (Cho *et al.*, 2006; Shah *et al.*, 2005; Sharma and Gupta, 2004; Luque-Garcia and Luque de Castro, 2004; Vinatoru, 2001; Mason *et al.*, 1996) and the application of this process may improve extraction of oil in PPF. From *Figure 2*, the use of only cellulase in AEOE gave 52.3% oil yield from PPF at pH 5.6 in 3 hr at 50°C. However, the use of ultrasonic pre-irradiation at 485W for 15 min prior

TABLE 1. THE EXPERIMENTAL RANGE AND LEVELS OF INDEPENDENT VARIABLES

Variables	Range and levels		
	-1	0	1
Hydrolysis time (minute), X_1	60.00	180.00	300.00
Enzyme to fibre ratio (EGU/g/g), X_2	5.00	13.00	20.00
Liquid to fibre ratio (ml g^{-1}), X_3	10.00	17.50	25.00

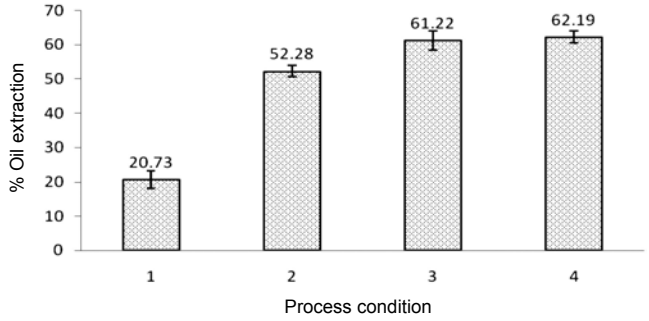


Figure 2. The effect of varying pre-treatment process conditions on aqueous enzymatic oil extraction (AEOE) from palm pressed fibre at 3 hr of incubation. Different pre-treatment processes when the mixture was incubated are: (1) Control – it was run in which no enzyme was added, (2) Celluclast® 1.5L FG only, (3) 15 min of ultrasonication followed by AEOE by Celluclast® 1.5L FG, (4) 15 min of ultrasonication followed by AEOE by Celluclast® 1.5L FG and Viscozyme® L.

to the enzymatic reaction increased the oil yield to 61.2%. On the other hand, *Figure 3* shows that with the ultrasonication pre-treatment step, the process time for AEOE can be reduced without any decrease in oil yields.

In addition, the use of ultrasonic at 485W for 15 min followed by AEOE gave a yield of 60.96% and reduced the extraction time to 3 hr. Moreover, in 4 hr of AEOE process without pre-treatment of ultrasonic, about 56.09% of oil was recovered from PPF. Thus, ultrasonic pre-irradiation step reduced time required to extract oil from PPF and hence can improve the OER upon AEOE. This enhancement in oil extraction was due to the collapse of cavitation bubbles during ultrasonic pre-irradation, which released an enormous amount of energy. This was expected to damage cell walls and result in better contact between the extraction medium and oil bodies (Sharma and Gupta, 2006).

Scanning electron microscopy (SEM) was conducted in order to observe the surface morphology of fibre due to different treatment on PPF. As shown in *Figure 4a*, SEM data indicated that ultrasound facilitated development of micro fractures and disruptions of cell wall of PPF. In addition, on pre-irradiation for 15 min, many pores appeared at the surface of fibre. In this study, the yield of oil from PPF was enhanced by AEOE when an ultrasonication step was used as a pre-treatment. In fact, cavitation bubble collapse is known to produce an ultrasonic jet, which acts as a solvent micropump that can force the extraction medium to reach out to the interior of the cellular structure (Albu *et al.*, 2004). *Figure 4b* shows the surface morphology of enzymatic treated PPF without 15 min of ultrasonic pre-treatment. It shows relatively loose structure compared to the fresh PPF, which has a parallel ridges structure (*Figure 4c*). In this case, the cellulase enzyme breaks down the glycosidic bond of the cellulose leading to the damage the cell wall. As observed by Sun and Cheng (2004), the enzymes attack the cellulose of the fibre progressively which being the primary wall is the first target. The enzyme peels off the cellulose, which results in the formation of protruding fibrils. In summary, the combination of the ultrasonic pre-treatment and AEOE could enhance the oil recovery from PPF, as observed in the graph plotted of *Figure 3*.

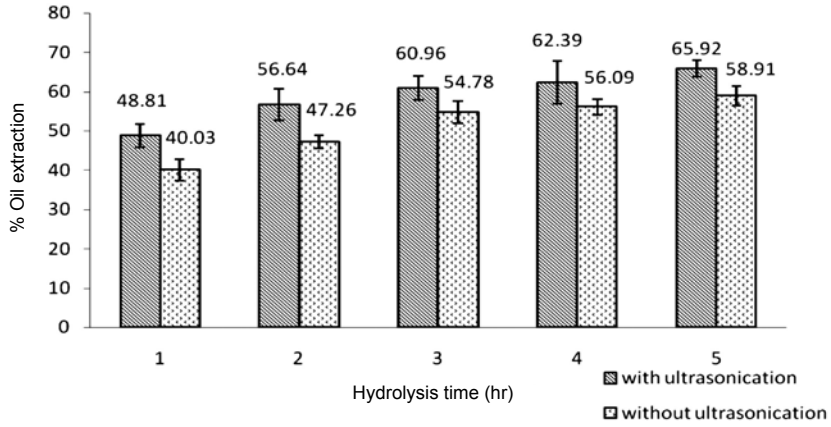
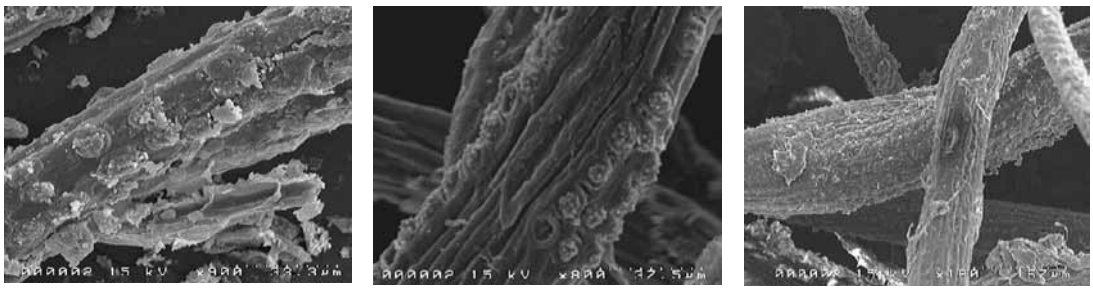


Figure 3. The effect of using ultrasonication as a pre-treatment before aqueous enzymatic oil extraction from palm pressed fibre.

TABLE 2. BOX-BEHNKEN EXPERIMENTAL DESIGN FOR THE INDEPENDENT VARIABLES (actual and coded levels) WITH DETERMINED DEPENDENT OIL EXTRACTION AND GLUCOSE PRODUCTION

	Independent variables						Responses (dependent variables)	
	Actual level			Coded level				
Experiment No.	Time, x_1 (min)	Enzyme-to-fibre ratio, x_2 (EGU/g/g)	Liquid-to-fibre ratio, x_3 (ml g^{-1})	Time, X_1 (min)	Enzyme-to-fibre ratio, X_2 (EGU/g/g)	Liquid-to-fibre ratio, X_3 (ml g^{-1})	Oil yield Y_1 (%)	Glucose production Y_2 (g/100 ml)
1	180	5.0	10.0	0	-1	-1	54.392	0.333
2	180	5.0	25.0	0	-1	1	54.105	0.293
3	300	20.0	17.5	1	1	0	62.165	1.612
4	300	13.0	25.0	1	0	1	61.274	1.074
5*	180	13.0	17.5	0	0	0	59.232	0.603
6	180	20.0	25.0	0	1	1	59.566	0.650
7	180	20.0	10.0	0	1	-1	60.743	0.689
8	60	5.0	17.5	-1	-1	0	47.409	0.094
9	300	13.0	10.0	1	0	-1	61.354	1.547
10*	180	13.0	17.5	0	0	0	58.221	0.605
11	60	13.0	10.0	-1	0	-1	51.408	0.215
12	60	13.0	25.0	-1	0	1	51.177	0.173
13*	180	13.0	17.5	0	0	0	56.195	0.588
14	300	5.0	17.5	1	-1	0	61.058	0.983
15*	180	13.0	17.5	0	0	0	56.081	0.563
16*	180	13.0	17.5	0	0	0	54.498	0.453
17	60	20.0	17.5	-1	1	0	53.524	0.224

Note: *Replication of centre region.



(a) (b) (c)

Figure 4. Scanning electron microscopy of: (a) ultrasonic pre-irradiated palm pressed fibre (PPF) after aqueous enzymatic oil extraction (AEOE), (b) non-irrediated PPF after AEOE and (c) non-irradiated fresh PPF without AEOE.

The Effect of Several Variables on Enzymatic Treatment

An enzymatic treatment on PPF with Celluclast® 1.5L FG was conducted with seven variables such as temperature, pH, enzyme activity, buffer volume, orbital shaker speed, ultrasonication period and fibre sizes. These experiments were performed to determine the optimum conditions for the oil extraction of PPF.

The enzyme-assisted oil extraction of PPF was carried out in temperatures ranging from 35°C to 60°C to obtain the suitable temperature for cellulase activities. From *Figure 5a*, it can be seen that cellulase reached its optimum activities at 45°C to 55°C by releasing about 61%-62% of the oil originally

contained in PPF. On the effect of pH, *Figure 5b*, shows that pH 5.6 gives the highest percentage of oil yield (64.81%). At this pH, cellulase reached its optimum activity to hydrolyse the PPF cell wall but if the pH is very high or very low, the enzyme can be denatured.

From the graph plotted in *Figure 5c*, it is seen that the percentage of oil yield increased with the enzyme activity. The treatment of PPF with the cellulase activity of 200 unit g^{-1} gives the highest percentage of oil extraction with the value of 60.46%. Beatriz *et al.* (2003) reported that higher enzyme activity lead to a higher yield of protein and oil extraction from the substrate. In this study, a different volume of the buffer solution was studied to obtain the ideal volume of buffer for cellulase activity needed to extract the PPF oil to the maximum. A 0.05M citrate buffer of pH 5.6 was used in this study. From the results obtained in *Figure 5d*, the highest oil yield of 53.08% was found in the enzymatic treatment with a 100 ml buffer solution. An ideal buffer quantity of 100 ml for an optimum oil yield in AEOE has been found to be consistent with Dominguez *et al*. (1995b) and Hagenmaier (1974).

On the effect of agitation, a high oil yield of 54.29% was recorded when the speed of the shaker was at 100 rpm as shown in *Figure 5e*. This would be the optimum speed. At a speed lower than that, the enzyme cannot hydrolyse the cell wall in an effective way, whereby higher agitation would cause foam formation. In addition, an ultrasonic period for different time intervals was conducted prior to the AEOE study and its efficiency on oil extraction from PPF is shown in *Figure 5f*. The graph in *Figure 5f* shows that the oil yield significantly increased with the period of ultrasonic pre-treatment. The highest oil yield of 55.65% was achieved when the period of ultrasonication was 20 min. The longer the period of ultrasonication, oil yield is expected to be enhanced significantly as ultrasonication may render the collapse of cavitations bubbles and release enormous amounts of energy.

The effect of the sizes of PPF on oil yield has also been investigated between the long fibre (length 20-30 mm) and the short fibre (length 2-5 mm). From the results obtained (*Figure 5g*), the treatment with the cellulase enzyme on fine PPF released about 72.06% of the oil originally contained in the fibre.

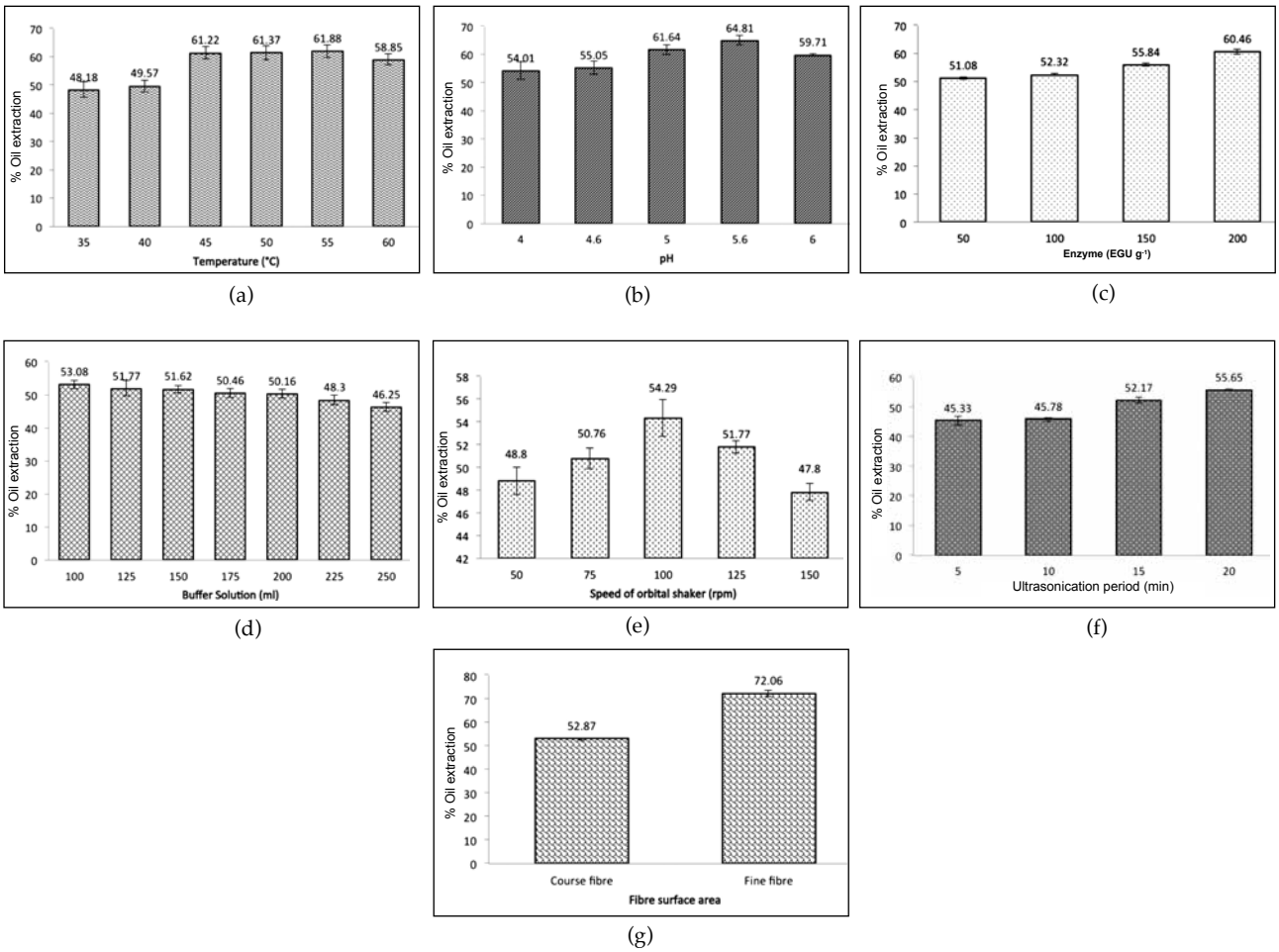


Figure 5. Efficiency of oil extraction from palm pressed fibre (PPF) by aqueous enzymatic oil extraction (AEOE) at different (a) temperature, (b) pH, (c) enzyme activity, (d) volume of buffer solution, (e) speed of mixing, (f) period of ultrasonication and (g) size of PPF.

This result is significantly high compared to the enzymatic treatment on course PPF, which extracts about 52.87% of the oil in the fibre. Philip *et al.* (1979) reported that the use of the physical treatment would reduce the size of the PPF. Grinding and milling increases the hydrolysis rate by increasing the surface area of reaction.

Response Surface Methodology (RSM)

The quadratic correlations between the hydrolysis time, enzyme to fibre ratio and liquid to fibre ratio and fitted model were given in *Table 3*.

The statistical significance of the quadratic model equation was evaluated by F-test ANOVA and results for all responses are summarised in *Table 4*. The F-value calculated for oil yield and glucose production models were recorded as 12.40 and 60.99. ANOVA showed that all the models were statistically significant. The glucose production model was highly significant with very low probability values ($p < 0.0001$), while the oil yield was found to be significant with a probability value of 0.0016. The R^2 value of oil yields and glucose production were quite high with the score of 0.9410 and 0.9874, indicating that the high proportion of variability was explained by the data and that the RSM model was adequate. Other than that, the lack of fit did not result in a significant F-value in the case of oil yield and glucose production, indicating that these models were sufficiently accurate for predicting those responses. In this study, the coefficient of variation (CV) was 12.06% for glucose production, while for oil yield CV was recorded to be 2.80%. The CV in the range of 0.52%-13.5% indicated good precision and reliability of the experiments carried out as suggested by Khuri and Cornell (1996), Kuehl (2000) and Ahmadi *et al*. (2005).

The estimated regression coefficient of the quadratic polynomial models for the response variables, the significance and standard error of each coefficient were determined by F-value and Prob > F which are listed in *Table 5*. The smaller the magnitude of the F-value, the more significant is the corresponding coefficient. Values of 'Prob > F' less than 0.05 indicate that model terms are significant. In this research, for Y_1; X_1 and X_2 are significant model terms. For Y_2; X_1, X_2, X_3, X_1^2, X_1X_2 and X_1X_3 are significant model terms.

CONCLUSION

This study has developed a process flow of AEOE for oil recovery from the PPF in a laboratory scale. The combination of the ultrasonication process as

TABLE 3. THE FITTED MODEL EQUATIONS

$$Y_1 = 56.85 + 5.29X_1 + 2.38X_2 - 0.22\ X_3 - 0.85\ X_1^2 + 0.046\ X_2^2 + 0.31\ X_3^2 - 1.25\ X_1\ X_2 + 0.038\ X_1\ X_3 - 0.22\ X_2\ X_3$$

$$Y_2 = 0.56 + 0.56X_1 + 0.18X_2 - 0.074X_3 - 0.21X_1^2 + 0.048X_2^2 + 0.024\ X_3^2 - 0.21X_1\ X_2 + 0.11X_1\ X_3 - 2.500 \times 10^{-4} X_2\ X_3$$

TABLE 4. ANALYSIS OF VARIANCE (ANOVA) FOR THE FITTED MODELS

Source	SS	DF	MS	F-value	Prob > F (P value)
a) Y_1 ($R^2 = 0.9410$)					
Model	279.52	9	31.06	12.4	0.0016
Residual	17.54	7	2.51	-	-
Lack of fit	3.43	3	1.14	0.32	0.8091
Pure error	14.11	4	3.53	-	-
Cor total	197.06	-	-	-	-
a) Y_2 ($R^2 = 0.9874$)					
Model	3.61	9	0.35	60.99	<0.0001
Residual	0.04	7	5.765×10^{-3}	-	-
Lack of fit	0.024	3	8.090×10^{-3}	2.1	0.2548
Pure error	0.016	4	4.022×10^{-3}	-	-
Cor total	3.2	-	-	-	-

Note: SS - sum of square; DF - degree of freedom; MS - mean square.

TABLE 5. REGRESSION COEFFICIENT OF THE FITTED MODEL FOR THE RESPONSE VARIABLES

Model term	Coefficient estimate	Standard error	F-value	Prob > F (p-value)
(a) For Y_1 Intercept	56.85	0.71		
X_1	5.29	0.56	89.42	<0.0001*
X_2	2.38	0.56	18.08	0.0038*
X_3	-0.22	0.56	0.16	0.7036
X_1^2	-0.85	0.77	1.22	0.3057
X_2^2	0.046	0.77	$3.545x10^{-3}$	0.9542
X_3^2	0.31	0.77	0.16	0.6996
X_1X_2	-1.25	0.79	2.5	0.1577
X_1X_3	0.038	0.79	$2.275x10^{-3}$	0.9633
X_2X_3	-0.22	0.79	0.079	0.7867
(a) For Y_2 Intercept	0.56	0.034		
X_1	0.56	0.027	441.02	<0.0001*
X_2	0.18	0.027	46.98	0.0002*
X_3	-0.074	0.027	7.65	0.0279*
X_1^2	0.21	0.037	33.27	0.0007*
X_2^2	-0.048	0.037	1.65	0.2394
X_3^2	-0.024	0.037	0.41	0.5443
X_1X_2	0.21	0.038	10.8	0.0134*
X_1X_3	-0.11	0.038	8.06	0.0251*
X_2X_3	$2.5x10^{-4}$	0.038	$4.336x10^{-5}$	0.9949

Note: *Highly significant at p-value less than 0.05 ($p<0.05$).

a pre-treatment and AEOE using Celluclast® 1.5L FG shows the increment in oil extractability of PPF and also reduces the time required for oil extraction. Several operational variables, *i.e*. temperature (°C), pH, enzyme activity (unit g^{-1}), buffer quantity (ml), speed of orbital shaker (rpm), ultrasonication period (min) and PPF sizes (mm) were evaluated for the batch AEOE experiments on PPF. The highest recovery of oil ranging from 53% to 73% was achieved with a process temperature of 55°C, pH of 5.6, 200 unit g^{-1} of enzyme, 100 ml of buffer solution, 100 rpm of orbital shaker speed and 20 min of ultrasonication process. The maximum oil yield of 72.06% was extracted from fine PPF as cellulase can easily attack the fibre wall and degrade the wall to expel out the oil inside the PPF. The second order polynomial model for oil extraction and glucose production formulated and developed using Design-Expert version 6.0.4 were found to be statistically significant at $P<0.005$ with $R^2=0.9410$ and $P<0.0001$ with $R^2= 0.9874$ respectively. There was a strong and significant effect of hydrolysis time, X_1 and enzyme to fibre ratio, X_2 on oil extraction. Whilst, for glucose production , hydrolysis time, X_1, enzyme to fibre ratio, X_2 and liquid to fibre ratio, X_3 are the significant model terms.

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