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Optimisation of Omega 3 Rich Oil Extraction from Elateriospermum Tapos Seed by Microwave Assisted Aqueous Enzymatic Extraction

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Microwave assisted aqueous enzymatic extraction (MAAEE) of Elateriospermum Tapos Seed (ETS) was performed in this study. This is a novel method which can reduce the extraction time and solvent consumption. The Elateriospermum Tapos oil (ETO) yield was optimised by using a central composite design (CCD) at condition parameters of microwave power (110 - 1,100 W), extraction time (30 - 120 s), enzyme cocktail concentration (1 - 5 %) and particle size (0.5 - 1.5 mm). The effect of each parameter was investigated by Response Surface Methodology (RSM). The optimal oil extraction yield was obtained at 110 W microwave power, 30 s extraction time, 1 % enzyme cocktail concentration and 0.5 mm particle size. Under these conditions, the maximum value obtained for the oil extraction yield was 46.12 ± 1.48 % recovery which was in good agreement with the predicted value (43.87 %). Thus, MAAEE could speed up extraction process as compared to Soxhlet which required 6 h extraction time to achieve similar amount of extraction yield.

1. Introduction

Elateriospermum tapos seed (ETS), locally known as Perah seed, is a monoecious conopy from the Euphorbiaceae family species situated in Southeast Asian tropical rainforest, which includes the Peninsular Malaysia, Peninsular Thailand, Brunei, Sumatra, Java and Borneo (Lim, 2012). This ETS is one of the underutilised local seed in Malaysia and it was reported to have high nutritional values, including high content of protein (59.32 %) (Husin et al., 2013), carbohydrate (25.36 %) and oil (38.59 - 57.5 %) (Tan et al., 2014). In addition, ETO contains rich essential fatty acids including linoleic acid (31.76 %) and linolenic acid (17.14 %) (Yong and Salimon, 2006). Essential fatty acids, especially omega 3 (linolenic acid) and omega 6 (linoleic acid), are crucial for human due to the fact that they cannot be synthesised by the human body.
Organic solvent (mainly hexane) is frequently utilised to extract oils from plant seeds. However, regulatory problems associated with the use and disposal of organic solvent have undesirable effects on the oil quality, cost and the environment as well as being harmful for human health (Li et al., 2013). For these reasons, alternative green and economical extraction methods are required to replace the usage of organic solvent in oil extraction. Nowadays, microwave assisted aqueous enzymatic extraction (MAAEE) has been proven to be efficient for oil extraction from various seeds such as Isatis indigotica seed (Gai et al., 2013a), pumpkin seed (Jiao et al., 2014), Forsythia suspense seed (Gai et al., 2013b) and yellow horn seed (Li et al., 2013). This is due to the fact that the MAAEE technology is safe, environmentally friendly, cheap and able to accelerate the extraction yield. Association of the hydrolytic enzymes, including cellulase and pectinase, are commonly utilised to hydrolyse and degrade the cell wall thus improving the release of intracellular content (Zhang et al., 2013). Based on previous and current literatures, it can be presumed that the MAAEE method has not yet been employed for ETS oil extraction. This study aimed to extract ETS oil using the MAAEE, optimise the condition towards a higher extraction yield by central composite design (CCD) and determine the influence of the each parameter towards the yield.

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2. Materials and Methods

2.1 Plant Material

The ETS was obtained from a local farm in Kuala Lipis, Pahang, Malaysia. The ETS was botanically identified by Dr. Shamsul Khamis (Biodiversity Unit, Institute of Bioscience, University Putra Malaysia, Malaysia) with specimen voucher number SK2782/15. The seeds were manually cleaned and flooded with tap water in order to separate the extraneous matters. The shells were removed afterwards. The cleaned seeds were grinded using a laboratory grinder MX-898 M (Panasonic, Malaysia) and sieved by Retschsiever (Retsch, Germany) to a desired particle size and then stored at -20 °C in airtight bags until further use.

2.2 Reagents

Cellulase from Aspergillus niger brand Tokyo Chemical Industry was obtained from Scienfield Expertise PLT, Selangor, Malaysia , pectinase from Aspergillus Niger (1.02 U/mg) and proteinase from Aspergillus Melleus (≥ 3 units/mg solid) of the brand Sigma Aldrich was purchased from Tay Scientific Instruments Sdn. Bhd, Johor, Malaysia. Hexane analytical and GC grade brand Qrec were obtained from Syarikat Pustaka Elit, Johor, Malaysia and chemical brand Merck from VNK Sdn Bhd. Johor, Malaysia.

2.3 Soxhlet Extraction

In the Soxhlet extraction (SE) process, 5 g of ETS (grinded at 0.5 mm particle size) were placed into each thimble. Analytical grade hexane was used as a solvent (150 mL). This process was carried out by refluxing each sample for 6 h on a heating mantle. After the elapsed time, a rotary evaporator was used to evaporate the solvents. The SE extraction produced 48.99 ± 0.79 % of oil per 100 g of seeds, which was set at 100 % oil recovery in the comparison with the MAAEE in this study. Next, the samples were collected and preserved at -20 °C in sealed bottles for further analysis.

2.4 Microwave Assisted Aqueous Enzymatic Extraction

Total yield from ETS was extracted using a domestic microwave oven (2,450 MHz, Sharp Model, Malaysia). 5 g of grinded ETS and enzyme cocktail (cellulose: pectinase: proteinase) ratios of 1.4 : 1.7 : 1.4 were accurately weighed and placed into a flask (250 mL) with 1 : 5 solid to solvent ratio (based on preliminary experiment). Aqueous based solvent was used as a solvent in MAAEE extraction Subsequently, the MAAEE extraction was conducted according to the design of experiment (DOE) at the following conditions; 110 - 1,100 W microwave power, 30 - 120 s extraction time, 1 - 5 % enzyme cocktail concentration and 0.5 - 1.5 mm particle size with a total of 30 experiment runs (Table 1). After extraction, the obtained solution was centrifuged at 10,000 rpm for 15 min, and oil phase was withdrawn using a micropipette. The oil was weighed and the extraction yield was expressed as the mass percentage as shown in Eq(1). Then the samples were collected and preserved at -20 °C in sealed bottles for further analysis.

$$\text{Total oil yield } (\%) = \frac{\text{oil yield obtained by MAAEE } (\%)}{\text{oil yield obtained by SE } (\%)} \times 100\ \% \quad (1)$$

2.5 Experiment Design and Statistical Analysis

The Response Surface Methodology (RSM) was applied to optimise the process parameters for MAAEE extraction yield of the ETO and discover the interaction of the parameters. A central composite design (CCD) was developed with a fractional three level/four factor experimental design and six replicates at the centre point by using software called Design Expert 6.0. The microwave power (A), extraction time (B), enzyme cocktail concentration (C), and particle size (D) were the independent parameters studied to optimise the oil yield (Y). The independent parameters were coded at three levels (-1, 0 and +1). The complete experimental design consisted of 30 experimental points (Table 1). The second order polynomial regression model was used to express Y as a function of the independent parameters, as shown in Eq(2) below:

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} + \sum \sum \beta_{ij} X_i X_j \quad (2)$$

Where Y is the total oil yields, β_0 is constant, β_i β_{ii} and β_{ij} are the linear, quadratic and interactive coefficient, respectively. X_i and X_j are the independent parameters level. The goodness of fitting of the model was evaluated by regression coefficient and the analysis of variance (ANOVA). The regression coefficient for each term combination of each process variable was calculated using the p-value generated by a t-test. The larger the magnitude of the t-test and smaller the P-value signifies a higher significant of the corresponding coefficient. The 3D- surface contour plot was generated to evaluate the interaction of each parameter.

3. Results and Discussion

3.1 Fitting the Mathematical Model

The experimental design and corresponding data for the ETO yield are presented in Table 1. The regression coefficient of the intercept, linear, quadratic and interaction parameters of the model were calculated using least square technique and presented in Table 2. It was shown that two linear parameters, microwave power (A) and particle size (D) as well as interaction AB and AD were highly significant at the level of $P < 0.05$, whereas linear parameters C and D, interaction AC, BC, BD and CD, and all the quadratic parameters were insignificant ($P > 0.05$). Consequently, the second order polynomial model used to express extraction yield ETO (Y) as Eq(3) of independent parameters are shown below:

$$\begin{aligned} Y = {} & 59.6493 - 0.0236A - 0.2079B + 0.4525C - 16.3024D + 9.9632 \times 10^{-6}A^2 + 1.2472 \times 10^{-3}B^2 \\ & - 6.5341 \times 10^{-3}C^2 - 2.9617D^2 + 5.9231 \times 10^{-5}AB - 7.6529 \times 10^{-4}AC + 6.2066 \\ & \times 10^{-3}AD + 1.5847 \times 10^{-3}BC - 0.0123BD - 0.1073CD \end{aligned} \quad (3)$$

Where Y is the ETO yield (%), A is the microwave power (W), B is the extraction time (min), C is the enzyme cocktail concentration (%) and D is particle size (mm).

Table 1: Experimental data on total yield

Std	Block	Microwave power (W)	Extraction time (s)	Enzyme cocktail concentration (%)	Particle size (mm)	Yield (%)	Predicted Value	Residual
1	Block 1	110	30	1	1.5	24.9094	24.5683	0.3411
2	Block 1	1,100	30	1	0.5	41.1829	39.2326	1.9502
3	Block 1	110	120	1	0.5	44.2847	44.8850	-0.6003
4	Block 1	1,100	120	1	1.5	25.7164	25.8254	-0.1090
5	Block 1	110	30	5	0.5	47.5837	47.8796	-0.2959
6	Block 1	1,100	30	5	1.5	21.3852	21.1899	0.1953
7	Block 1	110	120	5	1.5	20.8364	23.1916	-2.3552
8	Block 1	1,100	120	5	0.5	40.8932	41.6392	-0.7461
9	Block 1	550	75	3	1	29.3895	29.3513	0.0382
10	Block 1	550	75	3	1	30.9330	29.3513	1.5817
11	Block 2	110	30	1	0.5	42.2304	42.8207	-0.5903
12	Block 2	1,100	30	1	1.5	17.4695	19.5907	-2.1213
13	Block 2	110	120	1	1.5	18.8301	17.9914	0.8387
14	Block 2	1,100	120	1	0.5	39.3834	39.0404	0.3430
15	Block 2	110	30	5	1.5	21.7214	21.6640	0.0574
16	Block 2	1,100	30	5	0.5	33.2887	33.7269	-0.4382
17	Block 2	110	120	5	0.5	45.5021	42.9803	2.5217
18	Block 2	1,100	120	5	1.5	21.4517	20.4610	0.9907
19	Block 2	550	75	3	1	25.1690	25.5842	-0.4153
20	Block 2	550	75	3	1	24.3976	25.5842	-1.1867
21	Block 3	110	75	3	1	29.0818	28.9990	0.0828
22	Block 3	1,100	75	3	1	25.7749	25.8396	-0.0648
23	Block 3	550	30	3	1	28.0712	27.1697	0.9015
24	Block 3	550	120	3	1	26.9539	27.8374	-0.8835
25	Block 3	550	75	1	1	24.9761	25.0283	-0.0522
26	Block 3	550	75	5	1	24.9457	24.8756	0.0702
27	Block 3	550	75	3	0.5	31.9510	34.0953	-2.1443
28	Block 3	550	75	3	1.5	16.5423	14.3800	2.1623
29	Block 3	550	75	3	1	27.4370	24.9781	2.4590
30	Block 3	550	75	3	1	22.4472	24.9781	-2.5309

Based on the analysis of variance (ANOVA) for the experimental results given in Table 2, the model was shown to be precise and applicable due to the model F- value of 35.36, which implied that the model was significant ($P < 0.01$). There was only a 0.01 % chance that a "model F-value" of this stature was due to noise. A non-significant "lack of fit", where 0.8 F-value ($P > 0.05$), also confirmed the validity of the model. A desirable determination coefficient ($R2 = 0.9744$) was obtained, which implied that the sample variations of 97.44 % for the MAAEE efficiency of ETO yield were attributable to independent parameters, with only 3.56 %

of the total variation that was not explainable by the model. Comparison of the R2 adj with the R2 in Table 2 showed that the value did not differ greatly, indicating a good statistical model.

Table 2: Regression coefficients and ANOVA results

Source	Sum of Squares	DF	Mean Square	Value	Prob > F	
Block	238.2729	2	119.1364			
Model	1,947.845	14	139.1318	35.36255	< 0.0001	significant
A	44.91663	1	44.91663	11.41628	0.0049	significant
B	2.006209	1	2.006209	0.50991	0.4878	not significant
C	0.105012	1	0.105012	0.02669	0.8727	not significant
D	1,749.12	1	1,749.12	444.5667	< 0.0001	significant
A^2	15.12838	1	15.12838	3.845118	0.0717	not significant
B^2	16.19075	1	16.19075	4.115138	0.0635	not significant
C^2	0.001734	1	0.001734	0.000441	0.9836	not significant
D^2	1.391649	1	1.391649	0.35371	0.5622	not significant
AB	27.85208	1	27.85208	7.079051	0.0196	significant
AC	9.184308	1	9.184308	2.334338	0.1505	not significant
AD	37.75529	1	37.75529	9.596108	0.0085	significant
BC	0.325467	1	0.325467	0.082723	0.7782	not significant
BD	1.2258	1	1.2258	0.311557	0.5862	not significant
CD	0.184199	1	0.184199	0.046817	0.8321	not significant
Residual	51.14769	13	3.934438			
Lack of Fit	37.20966	10	3.720966	0.800895	0.6565	not significant
R^2	0.9744			R^2 adj	0.9469	

3.2 Optimisation of the Experimental Condition by Response Surface Methodology (RSM) Analysis

3.2.1 Effect of microwave Power

The microwave power had a significant effect on the extraction process. As in the three dimensional responses surface profile in Figure 1 (a), at varying microwave power and microwave exposure time with constant particle size and enzyme cocktail concentration, the extraction yield was favourable at low microwave power with short extraction time. At longer extraction time (120 s), the yield was high at lower and high microwave power but low at moderate power. The extraction yield would improve with the increase of the microwave power at shorter extraction time, but high power can cause poor extraction yield due to the degradation of thermally sensitive compounds (Abert-Vian et al., 2013). In Figure 1(b), at varying enzyme cocktail concentration and microwave power with constant extraction time and particle size, the difference concentrations of enzyme cocktail did not contribute significant effects to the extraction yield. However, at high concentration of enzyme cocktail, the ETO yield was increased at low microwave power, and then decreased at high microwave power. This might be due to the denaturation of the enzyme by the high microwave power and the fact that the exposure of the extraction time was not enough to hydrolyse the cell wall of ETS. According to a study conducted by Jiao et al. (2014) in extraction of pumpkin seed, higher microwave power led to localised temperature increment, leading to denaturing of the enzyme. In Figure 1 (c), at varying particle size and microwave power with fixed extraction time and concentration enzyme cocktail, the level of microwave power did not give any significant effect on the ETO yield even with different particle sizes. Low microwave power improved the extraction yield. In view of the ETO extraction, low microwave power with shorter extraction duration and small particle size contributed to higher extraction yields.

3.2.2 Effect of Extraction Time

The extraction time did not give a positive effect to the extraction ETO yield. The range of extraction time may have not been enough for the extraction process. The same goes to the interaction of extraction time towards varying enzyme cocktail concentration and particle size, as shown in Figure 1(d) and Figure 1(e). However, short extraction time (30 s) as in Figure 1(a), tended to give better extraction yield at low microwave power (110 W)., the extraction yield can be increased with longer extraction time, however this increment was found to be very minimal (Abert-Vian et al., 2013). The decrease of extraction yield with further extraction time might be due to the depletion of the substrates and/or product inhibition of enzymes (Jiao et al., 2014).

3.2.3 Effect of Enzyme Cocktail Concentration

Treatment with enzyme cocktail can enhance the extraction yield due to the fact that enzymes can hydrolyse the structure of polysaccharides of the cell walls and protein associated with the lipid bodies to enhance oil

release (Gai et al., 2013a). Based on the results from this study, the concentration of the enzyme cocktail gave a negative effect on the increment of the extraction ETO at difference extraction times and particle sizes, as shown in Figure 1(d) and Figure 1(f). However, at fixed particle size and extraction with different microwave power (Figure 1(b)), high enzyme cocktail concentration tends to enhance the extraction yield at low microwave power and then decrease at high power. The high power was parallel with the increasing of temperature. In this study, the at microwave power 1,100 W, the temperature was obtained in the range 80 - 95 °C. Low extraction ETO yield was obtained due to the enzyme cocktail being denatured at high microwave power.

3.2.4 Effect of Particle Size

The particle size of the ETS had a significant positive effect on the extraction yield. The results showed that the finer the particle size, the higher the yield that was extracted. This may be due to the fact that the finer particle size has a large surface area which allows easy contact with solvent, which could then increase the extraction yield. In addition, finer particle size can allow deeper penetration of microwave (Abert-Vian et al., 2013). As in Figure 1(c), with different levels of microwave power at constant enzyme cocktail concentration and extraction time, the smallest particle size (0.05 mm) gave the highest yield at low power, though subsequently, the yield tends to decrease at high microwave power. Conversely, at 1.5 mm particle size, the difference of microwave power gave a negative effect on the changes of the extraction oil yield. In Figure 1(e) and Figure 1(f), for the interaction factor between particle size and extraction time and enzyme cocktail concentration, differences in exposure time and concentration enzyme cocktail did not have any significant effect on the extraction yield as the other parameters were kept constant.

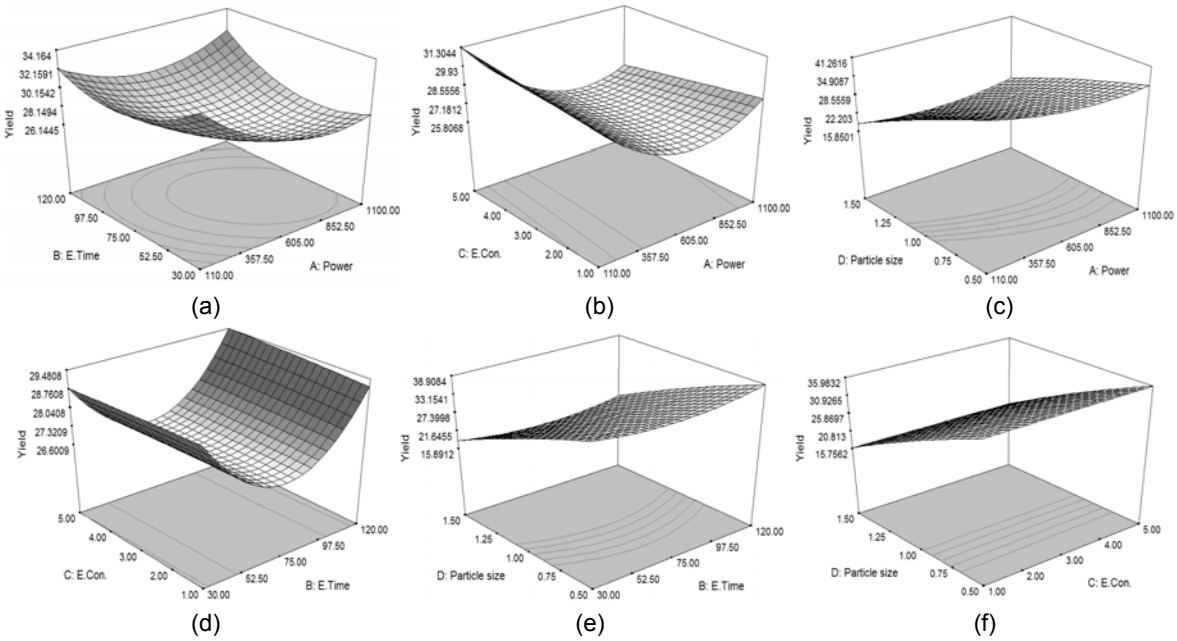

Figure 1: Response surface plot and interaction graph showing effect of the extraction parameters on the extraction yield of ETO

3.2.5 Validation and Verification of Predictive Model

In order to further validate the reliability of the theoretical model prediction, the experiment (n = 3) that was performed at the optimised condition using microwave was as follows: 110 W microwave power, 30 s extraction time, 1 % enzyme cocktail concentration and 0.5 mm particle size. The ETO yield obtained from the actual experiment was 46.12 ± 1.48 % which indicated that the experiment was a good fit for the prediction value (43.87 %) by the regression model. The oil extraction condition determined through RSM was reliable and practical.

4. Conclusions

The study reported here is, to our knowledge, the first time that MAAEE technology has been employed and optimised for the oil extraction for ETS. The highest oil yield of 22.59 % was obtained when ETS was extracted at conditions of MAAEE 110 W microwave power, 30 s extraction time, 1 % cocktail enzyme and 0.5 mm particle size. The microwave power and particle size had given the most significant effect on the

extraction yield. This indicated that the MAAEE extraction is promising and is an environmental friendly extraction method for ETS as it is greener, faster, does not involve the use of organic solvents and gives an approximate of 50 % recovery of oil yield as compared to Soxhlet extraction.

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Reference

Abert-Vian M., Calcio-Gaudino E., Chemat F., Cintas P., Cravotto G., Fabiano-Tixier A. S., Paolo V., 2013, Microwave-assisted Extraction for Bioactive Compounds Theory, Eds. Chemat F. and Cravotto G., *Vasa*. New York Heidelberg Dordrecht London: Springer.

Gai Q.Y., Jiao J., Mu P.S., Wang W., Luo M., Li C.Y., Fu Y.J., 2013a, Microwave-assisted aqueous enzymatic extraction of oil from Isatis indigotica seeds and its evaluation of physicochemical properties, fatty acid compositions and antioxidant activities, Industrial Crops and Products 45, 303-311.

Gai Q.Y., Jiao J., Wei F.Y., Luo M., Wang W., Zu Y.G., Fu Y.J., 2013b, Enzyme-assisted aqueous extraction of oil from Forsythia suspense seed and its physicochemical property and antioxidant activity, Industrial Crops and Products 51, 274-278.

Husin N., Tan N.A.H., Muhamad I.I., Mohd N., 2013, Physicochemical and biochemical characteristics of the underutilized, Jurnal Teknologi 64, 57-61.

Jiao J., Li Z., Gai Q., Li X., Wei F., Fu Y., Ma W., 2014, Microwave-assisted aqueous enzymatic extraction of oil from pumpkin seeds and evaluation of its physicochemical properties, fatty acid compositions and antioxidant activities, Food Chemistry 147, 17-24.

Li J., Zu Y.G., Luo M., Gu C.B., Zhao C.J., Efferth T., Fu Y.J., 2013, Aqueous enzymatic process assisted by microwave extraction of oil from yellow horn (Xanthoceras sorbifolia Bunge) seed kernels and its quality evaluation, Food Chemistry 138, 2152–2158.

Lim T.K., 2012, Edible Medicinal and Non-Medicinal Plants, Springer, Dordrecht, Netherlands.

Tan N.A.H., Siddique B.M., Muhamad I.I., Kok F.S., 2014, The effect of solvents on the soxhlet extraction of omega 3 from perah oil, Jurnal Teknologi 67, 51-54.

Yong O.Y., Salimon J., 2006, Characteristics of Elateriospermum tapos seed oil as a new source of oilseed, Industrial Crops and Products 24 146-151.

Zhang G., Hu M., He L., Fu P., Wang L., Zhou J., 2013, Optimization of microwave-assisted enzymatic extraction of polyphenols from waste peanut shells and evaluation of its antioxidant and antibacterial activities in vitro, Food and Bioproducts Processing 9 158-168.