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Optimization of ultrasound-assisted extraction of watermelon seed oil using response surface methodology

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Abstract
Watermelon seed oil is a very important component of the watermelon seeds and has many useful functions to human health. In this paper, ultrasound-assisted extraction of crude oil from watermelon seeds was investigated using response surface methodology (RSM). The Box–Behnken experimental design was applied, using n-hexane as a solvent. The effects of some operating parameters such as extraction time (60 to 90 min), temperature (40 to 60 ºC) and solvent to seed ratio (8 to 12 mL g^{-1}) on the yield of watermelon oil has been investigated. The results indicate that all three parameters showed a significant effect on the oil extraction (p<0.05). The optimum extraction parameters were as follows: extraction time, 80 min; extraction temperature, 55 ºC; and solvent to seed ratio, 11 mL g^{-1}. Under these conditions, the oil recovery was 40.10 %, which well matches with the predicted value. The results show that the ultrasound-assisted extraction may be an effective method for oil extraction from watermelon seeds.

Keywords: Watermelon, ultrasound-assisted, oil, n-hexane

1. Introduction

In recent years, more attention has been focused on the utilization of food processing by-products and wastes. Noticeably such utilization would contribute to maximizing the available resources and result in the production of various new products for better utilization (Tlili *et al*., 2011) [11]. In this aspect, watermelon (*Citrullus lanatus*) is one of the major fruits grown in the warmer parts of the world. It is a rich source of vitamins (A, B, C and E), mineral salts (K, Mg, Ca and Fe), specific free amino acids (citrulline and arginine), and carotenoids. Watermelon biomass is majorly composed of pulp, seeds and rind. The pulp or juice from watermelon is used for human consumption while rind and seeds are major wastes. The rind is mainly used for extraction of pectin whereas; seeds are a potential source of protein and lipids (Musa, 2012; Eladawy and Taha, 2001) [8, 2].

Watermelon seeds are recognized as an important source of many functional components and are generally used for herbal, therapeutic, as well as clinical application. Its oil is a highly suitable softening agent in natural baby care formulations and light body emulsions due to its stable shelf life and moisturizing capabilities (Tlili *et al.*, 2011) [11]. It is being used as a cooking oil and as a food additive in many Western countries. Furthermore, watermelon seed oil has strong antioxidant properties and has been recognized for several health benefits such as prevention of chronic diseases like diabetes, cardiovascular diseases, improving gut health, as well as overweight treatment. Thus, it offers the large potential to use these waste seeds for the production of oil and functional ingredients.

Traditionally, watermelon seed oil is being extracted using mechanical press extraction and solvent extraction methods (Yeboah, 2007) [13]. Press extraction method consumes more energy and often-associated low yield. Similarly, solvent extraction often takes longer time for extraction and organic solvents used for extraction can be harmful to human and environment (Baboli and Kordi, 2010) [1]. Moreover, Watermelon oil is thermally unstable and may degrade at higher temperatures. Hence, an improved or better extraction technique is desirable.

Many improved techniques can be used for oil extraction, among which, ultrasound-assisted extraction (UAE) is more efficient method since high oil yields are obtained from the plant matrix (Zhang *et al*., 2008) [15]. In addition to these, in this extraction method, the time required is less and low volume of solvent is enough for efficient extraction (Hemwimol *et al.*, 2006) [3]. All these benefits are due to cavitation bubbles generated by the ultrasound, which disrupts the surface of the solid matrix and enhances mass transfer through diffusion (Lazic and Veljkovic, 2007) [5]. This method had shown good efficiency in obtaining vegetable oils from various sources. However, there seem to be no report about ultrasound-assisted extraction oil from watermelon. Thus, in this study, the ultrasonic extraction parameters such as extraction time, extraction temperature, and solvent/sample ratio, were optimized with the Response surface methodology (RSM) employing a three-parameter, three-level Box–Behnken design (BBD).

Materials and Methods

Material

Fresh watermelon fruits were purchased from local fruit market, Tenali, Andhra Pradesh. The fruits were sliced open using a clean stainless steel laboratory knife, and the seeds were collected manually from the fruit. n-hexane used in the extraction was of analytical grade.

Ultrasound-assisted extraction

The watermelon seeds were crushed using a household mixer. The crushed watermelon powder (20 g.) was mixed with n-hexane in a flask. For the ultrasound-assisted extraction experiments, a 150W, 20 kHz ultrasonic bath (Spinocotech, Sonica Ultra Sonicator) with temperature controller was used. During extraction, the temperature was controlled at a desired level within ±1 ◦C. After each extraction experiment, the extracts were filtered through the Whatman No. 1 filter paper under vacuum, and then the solution was collected and concentrated with a rotary evaporator (Heidolph instruments) to acquire the watermelon seed oil. The acquired oil was further dried in a vacuum dryer to remove the residual n-hexane.

Oil yield

The oil extraction was performed (in duplicate) using the process assisted by ultrasound (UAE), the oil yield was calculated from equation (1):

$$\text{Oil yield (\%)} = \left(\frac{\text{weight of extracted oil}}{\text{weight of seed}}\right) \times 100 \quad (1)$$

Experimental Design

To evaluate the influence of the experimental variables (time, temperature and solvent to seed ratio) and determine the optimum levels to maximize the oil yield, a Box-Behnken factorial experimental design with three levels was applied, including five runs at the central point, generated by Design Expert Ver. 7.1.0 statistical package. The levels employed for the independent variables are shown in Table 1. The full model used to describe the dependent or response variable (Y_r) involves the linear or main, interaction and curvature effects as shown in equation (2)

$$Y_r = b_0 + b_1A + b_2B + b_3C + b_4AB + b_5AC + b_6BC + b_7A^2 + b_8B^2 + b_9C^2 \quad (2)$$

Where, b_0, b_1, b_2, b_3. b_4, b_5, b_6, b_7, b_8 and b_9 are the regression coefficients; A, B and C are the coded values of the independent variables (time, temperature and solvent to seed ratio); and Y_r represents the response variable (oil yield percentage).

Statistical analysis

A second-order polynomial model was fitted to the mean values of the experimental results to get the regression equations. Analysis of variance (ANOVA) was carried out to find out the statistical significance of the model terms at a probability of 5%.

Table 1: The levels of different experimental variables in coded and actual forms for extraction watermelon oil

Independent variables	Symbols		Levels	
	Natural	Coded	Natural	Coded
Time (min)	t	A	60	-1
			90	0
			120	+1
Temperature (ºC)	T	B	40 °C	-1
			50 °C	0
			60 °C	+1
Solvent to seed ratio (mL/ g)	S	C	8	-1
			10	0
			12	+1

Results and discussion

Soxhlet extraction

Soxhlet extraction method resulted in an oil yield of 46.58 ± 0.15 %. As per published data, the average oil content of watermelon (Tak Shashi, 2016) [10] approximately matches our results. Table 2 presents the experimental combinations for ultrasound assisted watermelon oil extraction using a BBD and corresponding response data for the oil yield (OY %). Effects of the independent variables analyzed were shown in Table 3. The regression coefficients of intercept, linear, quadratic, and interaction terms of the model were calculated using the least square technique and are presented in Table 3. According to the statistical significance considered (p-value), it was clear that all the linear quadratic parameters were found to be significant (p<0.05), whereas, all the interaction effects except (Time and solvent to seed ratio) were insignificant (p<0.05). Time, temperature and solvent to seed ratio showed positive linear effects with the oil yield and thus, an increase in the magnitude of these variables results in an increase in the oil yield. With increasing extraction time and solvent ratio, the oil yield was also influenced significantly.

Table 2: Experimental conditions applied and oil yield obtained in experiment to assess the effects of the operating variables using a Box-Behnken design.

Run	A[a]	B[a]	C[a]	OY[b] (%)
1	60	40	10	33.9
2	90	40	10	34.2
3	60	60	10	34.3
4	90	60	10	37.4
5	60	50	8	33.6
6	90	50	8	32.3
7	60	50	12	33.7
8	90	50	12	38.6
9	75	40	8	34.2
10	75	60	8	34.8
11	75	40	12	35.2
12	75	60	12	37.9
13	75	50	10	39.1
14	75	50	10	40.9
15	75	50	10	39.2
16	75	50	10	39.8
17	75	50	10	38.9
[a] As in Table 1. [b] Oil yield obtained by Equation (1).				

The effects of exposure time on the OY do agree with similar studies for flaxseed oil (Zhang *et al.*, 2008) [15], tobacco seed oil (Lazic and Veljkovic, 2007) [5] and papaya seed oil (Samaram *et al.*, 2015) [9]. This result shows that higher yields of oil were obtained by exposing to more time because, more time allows washing of particle surface with the solvent, which facilitates easy access to oil molecules, followed by the second stage where oil extraction occurs by mass diffusion (Mello, 2015) [7].

It is clear from previous reports that, higher temperature increases the vapour pressure of the solutes (Hemwimol *et al.*, 2006) [3] and decreases the viscosity and density of solvent (Q. A. Zhang *et al.*, 2009) [14]. Moreover, increase in temperature facilitate the disruption of the plant tissue and favours the collapse of cavitation bubbles (Samaram *et al.*, 2015) [9]. The combination of these effects favours the oil diffusion into the solvent (Hernández *et al.*, 2016) [4]. Therefore, increase in temperature contributed higher yields in the extraction of watermelon seed oil. Zhang *et al.*, (2008) [15] also observed similar effect for the ultrasound-assisted extraction of oil from flaxseed. However, an increase in temperature has no significant ($p > 0.05$) effect for longer extraction times, because extraction equilibrium can easily be reached at high temperatures (Yachmenev *et al.*, 2004) [12].

Table 3: ANOVA for oil yield by response surface quadratic model

Source	Coefficient Estimate[a]	Sum of Squares	df	F value	P value Prob > F
Intercept (b_0)	-77.73				
Model		144.22	9	26.87501	0.0001*
A(b_1)	1.17	6.13	1	12.97081	0.0087*
B(b_2)	1.30	5.95	1	12.60286	0.0093*
C(b_3)	6.60	13.78	1	29.18431	0.001*
AB(b_4)	0.004	1.96	1	4.150658	0.081**
AC(b_5)	0.051	9.61	1	20.35093	0.0028*
BC(b_6)	0.026	1.10	1	2.334745	0.1704**
$A^2(b_7)$	-0.012	33.07	1	70.03071	0.0001*
$B^2(b_8)$	-0.018	14.06	1	29.77913	0.0009*
$C^2(b_9)$	-0.556	20.89	1	44.24179	0.0003*
Lack of fit		0.68	3	0.34	0.7965**

The solvent to seed ratio (8 to 12) parameter has favoured the recovery of oil from watermelon seed since solvent increases the oil extraction due to the increased concentration gradient (Samaram *et al.*, 2015) [9]. The use of a high solvent to seed ratio could also enhance the diffusion through the reduction in the viscosity. It has been clear from many studies in the literature that extraction efficiency can be increased by using larger volumes of solvents and long extraction time (Hernández *et al.*, 2016) [4] This effect may be due to the increase in volume of solvent increases the solubility of oil in the solvent, which enhances oil extraction at higher temperatures. However, using large volumes of solvent is not economical (Lou *et al.*, 2010) [6].

Second-order polynomial model

The coefficients presented in Table 3 were re-estimated considering those for which the differences did not show statistical significance. Using the regression analysis, experimental parameters and response were correlated according to equation 2 was given below:

$$Y_{DT} = -77.73 + 1.17 + 1.30B + 6.60C + 0.004AB + 0.051AC + 0.026BC - 0.012A^2 - 0.018B^2 - 0.556C^2 \ldots (3)$$

Analysis of variance verified the validity of the predictive equation. The value for the correlation coefficient (0.98) and F regression test results showed that the model was able to represent the experimental data in the experimental range assessed. Fig.1 shows the strong correlation between the experimental data and the values predicted by the model.

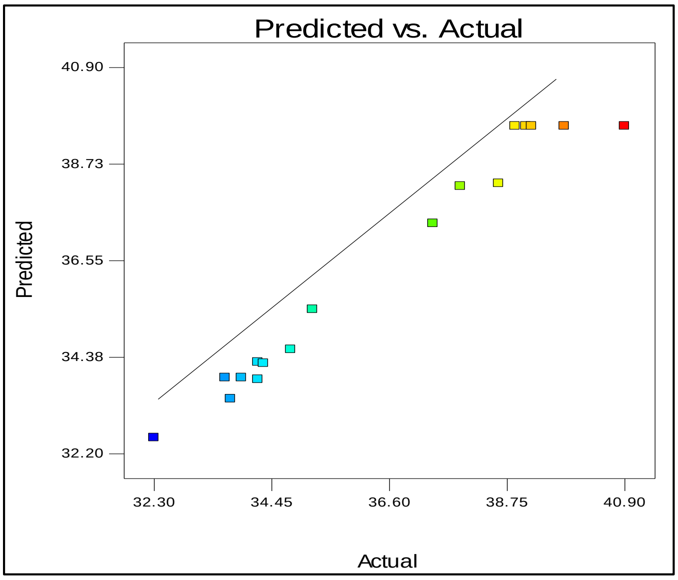
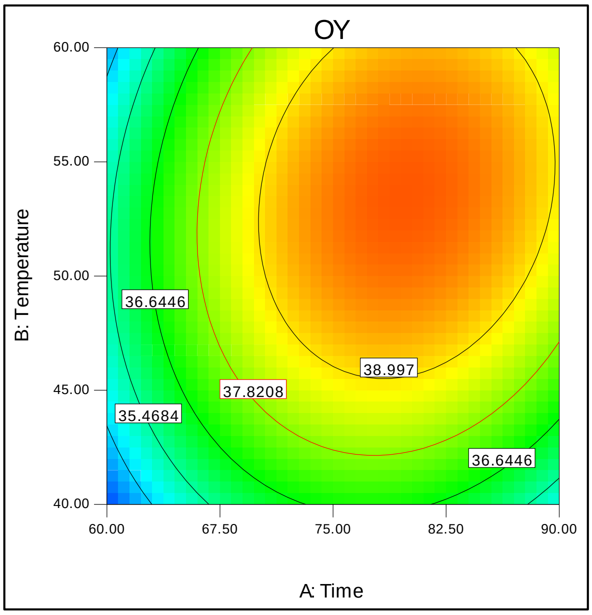


Fig 1: Correlation between the experimental and predicted oil yield (according to second-order polynomial model - Equation (3)) for UAE of watermelon seed oil

Optimization of extraction conditions

Second order polynomial model obtained in this study was utilized to obtain the optimized oil extraction parameters to meet out the above-stated criteria. The predicted maximum yield was 40.20%, obtained after 80 min, at 55 °C and with a seed to a solvent ratio of 11 mL g^{-1}. To verify the predictive capability of the model, experiments were conducted under the predicted condition, which resulted in 40.10 % of the oil. Using student t-test for averages, it was concluded that predicted value did not differ significantly (*p*>0.05). Fig. 2 shows the contour plot constructed according to Equation (3), which illustrates the correlation of independent variables with the variable response. Each contour plot is a function of two variables, keeping the third at this central point. Fig. 2 shows that the oil extraction is favoured in the combination of the higher levels of considered variables. It was observed that the effect of the use of a high amount of solvent for long extraction time is more pronounced, as already presented in Table 3.



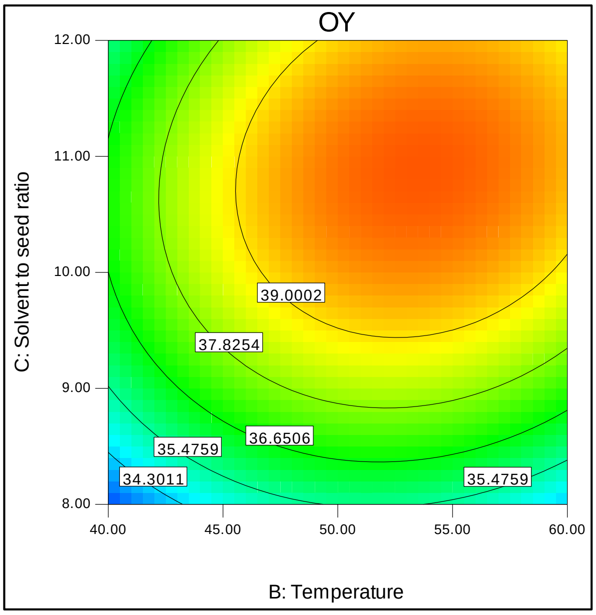


Fig 2: Contour plot for oil yield as a function of: a) time and temperature b) seed to solvent ratio and temperature (all obtained for the central condition).

Conclusion

In the present study, the ultrasound-assisted extraction of oil from the watermelon was performed with a three-parameter, three-level Box–Behnken design (BBD) based on the RSM. From the experimental results, it is clear that all three parameters showed a significant effect on the oil yield. From results, it is clear that the technique of ultrasound-assisted extraction efficiently and rapidly extracted watermelon seed oil. This is due to cavitation energy that could cause cell wall breakage of watermelon seeds. The maximum oil yield was obtained at 50 ºC with the extraction time of 75 min and solvent to seed ratio 10 mL g^{-1}.

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