

# Improving the oil yield of Iranian *Jatropha curcas* seeds by optimising ultrasound-assisted ethanolic extraction process: a response surface method

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## RESEARCH ARTICLE

### Abstract

An ultrasound-assisted solvent extraction (UASE) procedure has been optimised to speed up oil extraction from *Jatropha curcas* L. seeds by response surface methodology-Box-Behnken design (RSM-BBD). The preliminary study to determine the best solvent for extracting oil from *J. curcas* containing more than 60% oil revealed that ethanol had the highest efficiency among chloroform, acetone, *n*-hexane and methanol. Three independent variables including UASE time (10-60 min), UASE temperature (30-60 °C) and ratio of ethanol to sample (15:45, v/w) were investigated to achieve the highest oil yield. RSM-BBD demonstrated that a second-order polynomial model can adequately be developed with a high R<sup>2</sup> (0.9952) using multiple linear regression analysis. The linear effect of ethanol to sample ratio had the most significant ( $P < 0.05$ ) effect on yield value. The most suitable combination of variables for higher yield increase (61.94%) was UASE time of 51.69 min, UASE temperature of 44.57 °C and a ratio of ethanol to the sample of 44.78 (v/w). At this optimal condition, the yield increased to 62.12%, which was close to the amount predicted by the model which was nearly 2.5× more than the obtained yield in the Soxhlet extraction procedure (25.63%). Gas chromatography-mass spectrometry of the oils extracted under optimal conditions showed that the most dominant fatty acids were oleic (26.15%), linoelic (24.21%), palmitic (21.09%) and stearic (6.28%) acids, respectively.

**Keywords:** fatty acids analysis, *Jatropha curcas* seed, oil extraction, optimisation, ultrasound-assisted extraction

## 1. Introduction

*Jatropha curcas* L. is a genus of flowering plants in the spurge family, *Euphorbiaceae*, which is native to the American tropics, most likely Mexico and Central America. The name is derived from the Greek words *ιατρός* (iatros), meaning 'physician', and *τροφή* (trophe), meaning 'nutrition'. It contains approximately 170 species of succulent plants, shrubs and trees (some are deciduous, like *Jatropha curcas*). Most of these plants are native to the Americas, with 66 species found in the Old World (Goula, 2013). Mature plants produce separate male and female flowers. It is a perennial shrub or small tree native to South and Central America but is cultivated in many tropical regions, such as Africa and Asia. It also thrives in poor, stony soils and under adverse climatic conditions (Bhasabutra and Sutiponpeibun,

1982). This plant is used as a live fence and for erosion control in many parts of the world.

The seed contains 40-60% viscous oil known as *curcas* oil (Lin *et al.*, 2003; Tang *et al.*, 2007). It is considered as a potential source of non-edible fuels along with its different medicinal properties. *J. curcas* is a promising feedstock of oil for biodiesel production. The oil of *J. curcas* seeds is also incorporated in cosmetics and soap production. In addition, the seed kernel meal remaining after extracting oil is rich in nutrients and is used as an organic fertiliser (Makkar *et al.*, 2008).

High-intensity ultrasound is a novel technology to improve extraction process of lipophilic/hydrophobic compounds from plant/animal materials (Fairbanks, 2001). High-intensity ultrasonication can accelerate heat and mass

transport in a variety of food process operations and has been successfully used to improve drying, mixing, homogenisation and extraction (Li *et al.*, 2004). Vinatoru (2001) pointed out that use of ultrasound by disrupting cell walls of raw plant tissues can facilitate the release of extractable compounds and enhance mass transport of solvent from the continuous phase into plant cells. Zhang *et al.* (2008) by conducting ultrasound-assisted extraction (UAE) of oil from flaxseeds reported that UAE can be an effective and indeed feasible method for the production of the plant oils.

Recently, use of response surface methodology (RSM) is popular to optimise technical parameters principally because of its advantages to other approaches required optimising a process, such as a decrease of time and expenses as well as a saving in the consumption of reagents and materials (Gharibzahedi *et al.*, 2014). Several variables with RSM are examined simultaneously with a minimum number of trials, according to experimental designs, which enables the discovery of interactions between variables (Gharibzahedi *et al.*, 2013a). Since no specific study has been conducted on the optimisation of UAE of *J. curcas* oil in order to maximise its yield, in this study a simple and efficient UAE technique was developed for extraction of *J. curcas* seeds of oils prior to analysis of fatty acids by gas chromatography-mass spectrometry (GC-MS). Some important parameters involved in UAE including time, temperature and ratio of solvent to sample were optimised according to the experimental design methodology based on a RSM in term of Box-Behnken design (BBD).

## 2. Materials and methods

### Raw materials

Seeds of *J. curcas* L. were collected from a botanical garden located in Bandar Abbas city (Hormozgan province, Iran) during May-June 2012. The seeds were dried in a dark place at room temperature for 48 h. They were then ground in a blender to produce fine powders with an average particle size of 0.4 mm.

### Chemical and reagents

All solvents and other chemicals used for chromatography analysis and the sample preparation were of analytical grade. Reagent grade water was collected from a Milli-Q water purification system (Millipore, Bedford, MA, USA). Chloroform, *n*-hexane, methanol, ethanol and acetone with the purity higher than 99% were purchased from Merck Chemical Co. (Darmstadt, Germany). A mixture of standard fatty acid methyl esters (FAMES) in the range of C<sub>4</sub> to C<sub>24</sub> including saturated, mono-unsaturated, and polyunsaturated FAMES were purchased from Sigma-

Aldrich (Sigma-Aldrich Chemical Co., St. Louis, MO, USA) and used for the preparation of GC calibration standards.

### Conventional and ultrasonic-assisted extraction procedures

Crude oil was determined by the Soxhlet method. The oil was obtained by exhaustively extracting 5.0 g of each sample in a Soxhlet apparatus using *n*-hexane (boiling point range 55-60 °C) as the extractant (Gharibzahedi *et al.*, 2012a). For ultrasonic-assisted extraction of *J. curcas* oil, 5.0 g of seed powder was charged into suitable glass vessels and then the exact volume of extracting solvent was added. The vessels were sealed tightly with aluminium foil to prevent moisture absorption of the surrounding air by the used solvent. The ultrasonic probe (ultrasonic Power Sonic 505; Hwashin Technology, Daegu, Republic of Korea) was directly inserted into the mixture. The samples were extracted under continuous ultrasonic frequency at 40 kHz and power output of 20 W. A BBD was used for the optimisation of experimental parameters in UAE (Table 1). In the next step, the obtained mixture was filtered through a Whatman No. 4 filter paper (Sigma-Aldrich, York, UK). The residue was re-extracted two more times and the filtrates from the three extraction stages were combined and the solvent was removed under vacuum at 40 °C by a rotary evaporator (model VV2000; Heidolph, Schwabach, Germany). The obtained oil was re-dissolved with *n*-hexane before filtration through a layer of anhydrous sodium sulfate placed over a filter paper in a funnel, and the hydrophobic solvent subsequently removed. In the next step, the oil was weighed and transferred into 15 ml sample vials, gently flushed with nitrogen gas, capped and stored at -18 °C until analysis.

### Yield calculation

The yield of *J. curcas* was determined according to the following formula:

$$\text{Yield (\%)} = \frac{m_e}{m_t} \times 100 \quad (1)$$

Where  $m_e$  and  $m_t$ , respectively, are the mass (g) of oil extracted from the sample and the mass (0.5 g) of *J. curcas* seed powder.

### Methylation of fatty acids

Esterification of extracted fatty acids is a necessary step prior to GC-MS analysis. After the extraction, 10 ml of methanol and 2 ml of 2% sulfuric acid were poured in a round bottomed flask, refluxed for 1 h at 70 °C and poured into an extracting funnel. 10 ml of *n*-hexane and 10 ml distilled water to remove oil and FAME residues were then added to solution in a separating funnel and shaken for

**Table 1.** Experimental design and results for the of extraction yield from *Jatropha curcas* seeds by ultrasound-assisted extraction (UAE) technique.

Run	Independent variables			Oil yield (%)	
	UAE time (min)	UAE temperature (°C)	Ethanol/sample ratio	Experimental <sup>1</sup>	Predicted
1	10	30	30	18.52±1.23	17.62
2	60	30	30	53.86±0.45	52.79
3	10	60	30	33.90±0.78	34.97
4	60	60	30	35.72±0.63	36.62
5	10	45	15	10.38±0.42	10.57
6	60	45	15	23.58±0.79	23.95
7	10	45	45	37.86±1.02	37.50
8	60	45	45	61.12±1.41	60.93
9	35	30	15	21.64±0.23	22.35
10	35	60	15	30.54±0.51	29.28
11	35	30	45	59.38±0.39	60.64
12	35	60	45	55.60±0.37	54.89
13	35	45	30	42.94±0.18	44.48
14	35	45	30	43.79±0.82	44.48
15	35	45	30	46.70±0.64	44.48

<sup>1</sup> Mean ± standard deviation (n=3).

2 min. The organic layer consisting of analytes in *n*-hexane was separated for GC-MS analysis.

### Gas chromatographic analysis

In order analyse and quantify the different fatty acids, a 30 m × 0.22 mm, 0.25 µm film thickness fused-silica capillary column DB-23 connected to a GC system (6890N; Agilent Technologies, Michigan, DE, USA) equipped with a MS detector (5975, model EI; Agilent Technologies) was used. 1.0 µl of each sample was injected into the GC column at a split ratio of 50:1. Nitrogen as the carrier gas was used at a constant flow of 1.0 ml/min. The temperatures of injector and detector, respectively, were 250 and 280 °C (Zhang *et al.*, 2008). The column temperature programme was as follows: initial column temperature: 42 °C for 30 s, 42 to 192 °C at 25 °C/min, 192 to 204 °C at 3 °C/min, 204 to 228 °C at 8 °C/min, hold for 4 min at 228 °C, 240 °C at 4 °C/min, hold for final 2 min. Calibration curves of peak area and concentration were established by injecting reference FAME samples of known concentrations into the GC-MS.

### Experimental design and statistical analysis

The experimental design, regression analysis of the experimental data and quadratic model building was developed using software Design-Expert (trial version 8.1.6; Stat-Ease Inc., Minneapolis, MN, USA). A BBD was employed to identify relationships between the response

functions and the extraction variables, as well as to determine those conditions that optimised the UAE of *J. curcas* seeds oil. The influence of three process parameters namely UASE time (10-60 min;  $X_1$ ), UASE temperature (30-60 °C;  $X_2$ ) and ratio of ethanol to sample (15:45, v/w;  $X_3$ ) on the oil yield value ( $Y$ ) of *J. curcas* seeds was studied using the BBD. For statistical calculations, the relation between the coded and experimental values is described by Equation 2:

$$x_i = \frac{X_i - X_0}{\Delta X} \quad (2)$$

Where  $x_i$ , is the coded value of the variable,  $X_i$  the experimental value of the variable,  $X_0$  the experimental value of  $X_i$  at the centre point, and  $\Delta X$  the step change value of the variables.

The generalised regression second-order polynomial model proposed for predicting the oil yield is given as:

$$Y = \beta_{k0} + \sum_{i=1}^4 \beta_{ki} X_i + \sum_{i=1}^4 \beta_{kii} X_i^2 + \sum_{i<j=2}^4 \beta_{kij} X_i X_j \quad (3)$$

Where  $Y$  is the predicted response (oil yield),  $\beta_{k0}$ ,  $\beta_{ki}$ ,  $\beta_{kii}$  and  $\beta_{kij}$  represent the regression coefficients, and  $X_i$ ,  $X_j$  are the coded independent factors.

The quality of the fit of polynomial model was expressed by the coefficient of determination  $R^2$ , adjusted  $R^2$  ( $R^2_{adj}$ )

and adequate precision (ADP) in Equations 4-7, respectively (Gharibzahedi *et al.*, 2012b):

$$R^2 = 1 - \frac{SS_{\text{residual}}}{SS_{\text{model}} + SS_{\text{residual}}} \quad (4)$$

$$R^2_{\text{adj}} = 1 - \frac{SS_{\text{residual}} / DF_{\text{residual}}}{(SS_{\text{model}} + SS_{\text{residual}}) / (DF_{\text{model}} + DF_{\text{residual}})} \quad (5)$$

$$ADP = \frac{\max(\bar{y}) - \min(\bar{y})}{\sqrt{\bar{V}(\bar{y})}} \quad (6)$$

$$\bar{V}(\bar{y}) = \frac{1}{n} \sum_{i=1}^4 V(\bar{y}) = \frac{p\sigma^2}{n} \quad (7)$$

In Equations 4-7, SS is the sum of squares, DF is the degrees of freedom,  $\bar{y}$  is the predicted value, p is the number of model parameters,  $\sigma^2$  is the residual mean square from analysis of variance (ANOVA) table, and n is the number of experiments.

ANOVA procedure for determining the best extracting solvent followed by Duncan's test using SPSS 13 (SPSS Inc., Chicago, IL, USA) software was applied to determine the significant difference ( $P < 0.05$ ) between treatment means.

### 3. Results and discussion

#### Selection of the best extraction solvent

The proper extraction solvents with good solubility towards the analytes and high efficiency of oil extraction were considered for the extraction. Extractions with chloroform, ethanol, acetone, *n*-hexane and methanol were examined. Results showed that ethanol had the highest efficiency to extract oil from *J. curcas* seeds and therefore selected as the extraction solvent in UAE treatments (Figure 1). Ethanol is a suitable candidate to study as an extraction solvent since its cost is very low and it may be produced from a large variety of biological materials using simple technologies. Furthermore, although flammable (flash point = 8.9 °C; ignition temperature = 425 °C), this alcohol

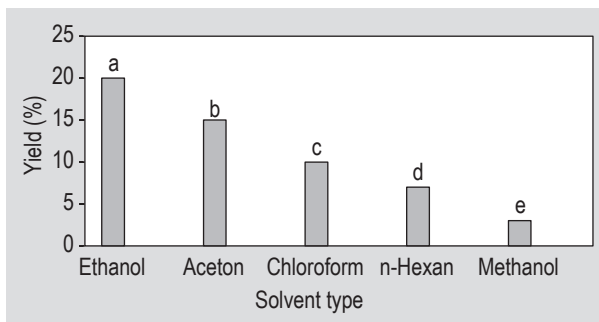


Figure 1. Effect of the different solvents on the yield of oil extracted from *Jatropha curcas* seeds. Bars with different letters (a-e) are significantly different ( $P < 0.05$ ).

is recognised as non-toxic and has less handling risks than hexane (flash point = -23 °C; ignition temperature = 225 °C). The application of ethanol as an extraction solvent can also avoid eventual toxicity problems of meals for animal feedstuff (Ferreira-Dias *et al.*, 2003; Rittner, 1992). Using ethanol as the extracting solvent could be beneficial if the plant pigments and other polar bioactive compounds are of interest for producing an oil's green colour (Balasubramanian *et al.*, 2011).

#### Model fitting and adequacy checking

As considered in Table 2, the constructed model was highly significant ( $P < 0.0001$ ). The value of lack of fit for the model was 0.6245 (Table 2), which implied that the lack of fit was not significant relative to the pure error (Gharibzahedi *et al.*, 2013b). By using multiple regression analysis on the experimental data, the response variable and the test variables were related by the following second-order polynomial equation:

$$Y = 44.48 + 9.25 X_1 + 15.98 X_3 - 8.77 X_1^2 - 2.48 X_3^2 - 8.36 X_1 X_2 + 2.52 X_1 X_3 - 3.17 X_2 X_3 \quad (8)$$

The  $R^2$  of 0.9952 was showed by ANOVA of the quadratic regression model, indicating that only 0.48% of the total variations were not explained by the model. The high  $R^2_{\text{adj}}$  value (0.9866) also confirmed that the model was highly significant. Commonly, a high  $R^2$  shows that the variation was accounted and that the data fitted satisfactorily to the second-order polynomial equation. However, a large value of  $R^2$  does not always imply that the regression model is a good one.  $R^2_{\text{adj}}$  is a modification of  $R^2$  that adjusts for the number of explanatory terms in a model. Unlike  $R^2$ , the  $R^2_{\text{adj}}$  increases only if the new term improves the model more than would be expected by chance (Gharibzahedi *et al.*, 2015). At the same time, a very low value 4.69 of the critical value (CV) clearly indicated a very high degree of precision and a good deal of reliability of the experimental values. As a general rule, the CV should not be greater than 10% (Gharibzahedi *et al.*, 2014). ADP measures the signal-to-noise ratio, with a ratio greater than 4 being desirable. For the proposed model, this value was 34.25, suggesting very good signal-to noise ratio (Gharibzahedi *et al.*, 2012b). Figure 2 shows that the second-order polynomial regression model was in good agreement with the experimental results. In this figure, each of the observed values is compared to the predicted value calculated from the model. Figure 2 proves that the model cover the experimental range of studies sufficiently.

To ensure that the fitted model provides an adequate approximation to the real system, it is necessary to check it statistically. Unless the model reveals an adequate fit, proceeding with the investigation and optimisation of the fitted response surface likely give poor or misleading results

Table 2. ANOVA of the experimental results of the response surface methodology-Box-Behnken design.<sup>1</sup>

Source	Sum of squares	DF	Mean of squares	F-value	P-value <sup>2</sup>
Model	3,363.90	9	373.77	115.25	<0.0001**
X <sub>1</sub> (UAE time, min)	677.49	1	677.49	208.91	<0.0001**
X <sub>2</sub> (UAE temperature, °C)	0.70	1	0.70	0.21	ns
X <sub>3</sub> (ethanol/sample ratio)	2,042.24	1	2,042.24	629.73	<0.0001**
X <sub>11</sub>	283.72	1	283.72	87.48	0.0002**
X <sub>22</sub>	0.16	1	0.16	0.051	ns
X <sub>33</sub>	22.63	1	22.63	6.98	0.0459*
X <sub>12</sub>	280.90	1	280.90	86.62	0.0002**
X <sub>13</sub>	25.30	1	25.30	7.80	0.0383*
X <sub>23</sub>	40.20	1	40.20	12.39	0.0169*
Residual	16.22	5	3.24		
Lack of fit	8.44	3	2.81		0.6245 <sup>ns</sup>
Pure error	7.78	2	3.89		
Core total	3380.12	14			

R<sup>2</sup>=0.9952; R<sup>2</sup><sub>adj</sub>=0.9866; CV=4.69; ADP=34.25

<sup>1</sup> DF = degree of freedom; UAE = ultrasound-assisted extraction; R<sup>2</sup> = coefficient of determination; R<sup>2</sup><sub>adj</sub> = adjusted R<sup>2</sup>; CV = critical value; ADP = adequate precision.

<sup>2</sup> \*P<0.05; \*\*P<0.01.

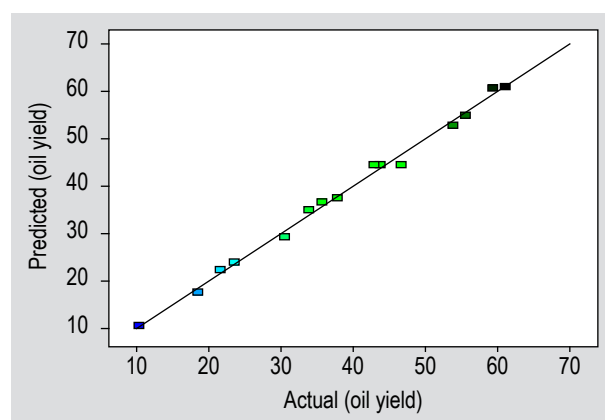


Figure 2. Comparison between predicted and actual values of oil yield extracted from *Jatropha curcas* seeds using ultrasound-assisted extraction method.

(Li *et al.*, 2007). Since the residuals from the least squares fit play a key role in judging model adequacy (Myers and Montgomery, 2002), the authors investigated the normality assumption by constructing a normal probability plot of the residuals (Figure 3A). Results showed that the normality assumption was satisfied as the residual plot approximated along a straight line. This result was also depicted by plotting residuals vs the predicted response (Figure 3B).

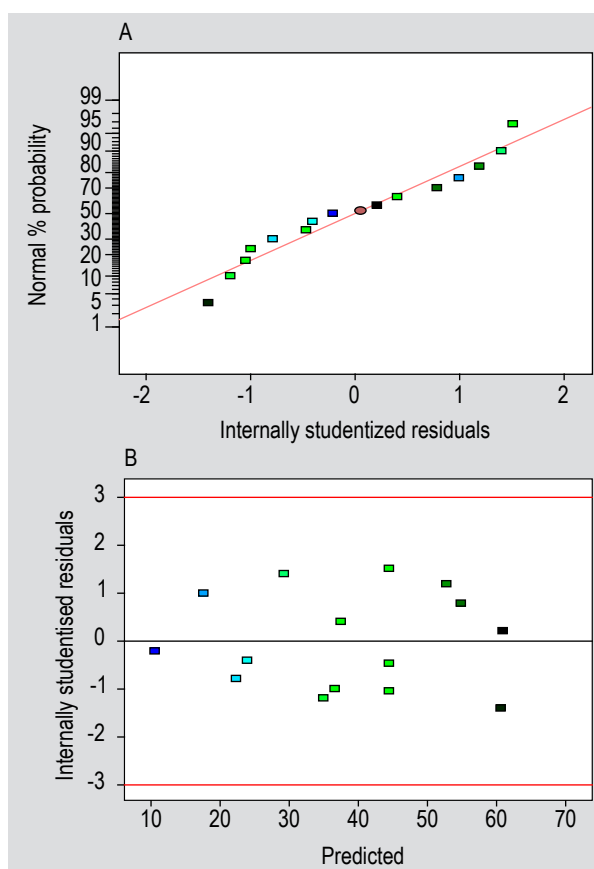
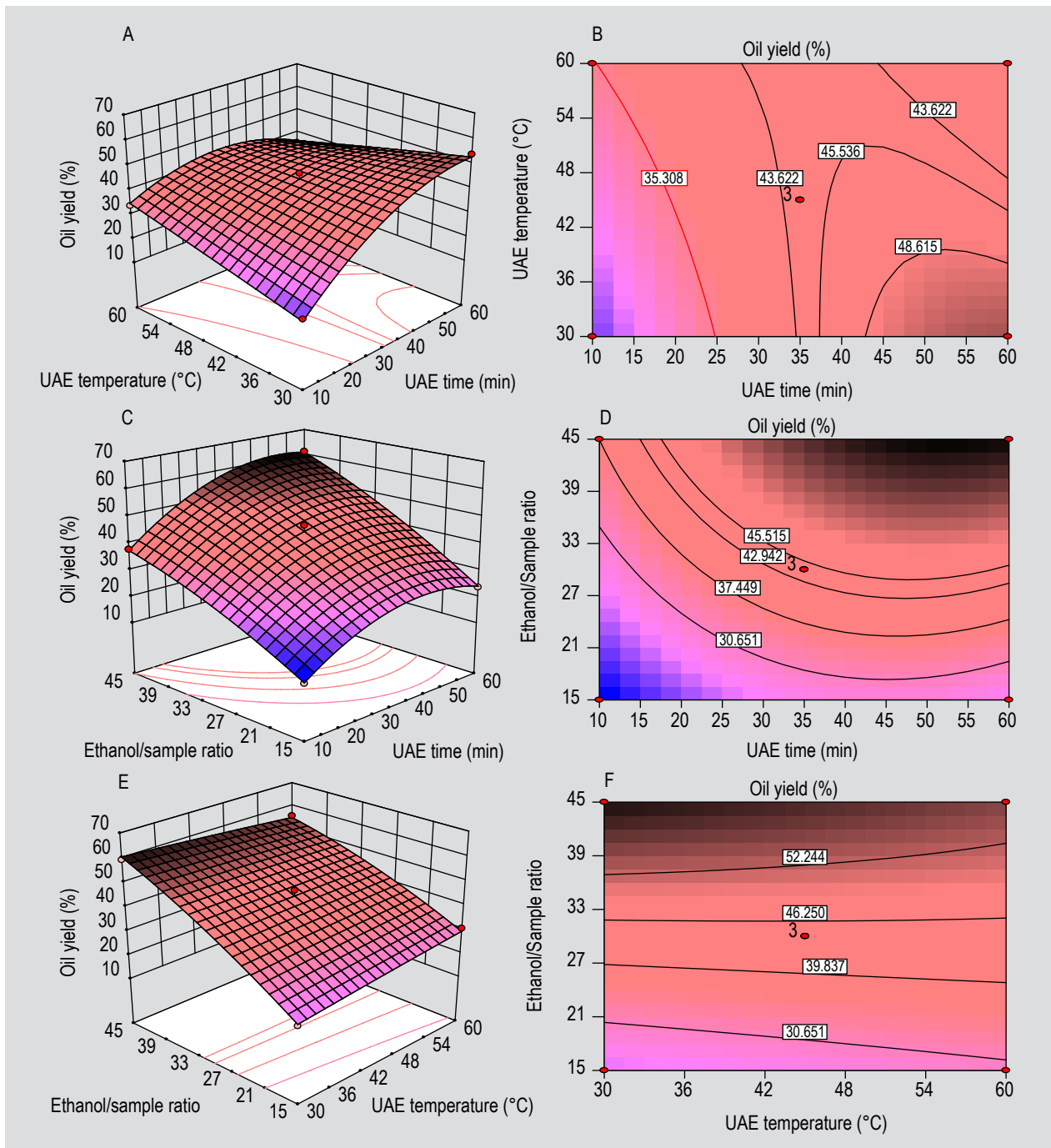


Figure 3. (A) Normal probability of internally studentised residuals. (B) plot of internally studentised residuals vs predicted response.

**Analysis of influence of variables on the oil yield**

Table 2 shows that the linear and quadratic effects of UAE time ( $P < 0.0001$  and  $P < 0.001$ , respectively) and of ethanol/powdered seed ratio ( $P < 0.0001$  and  $P < 0.05$ , respectively) were significant on the oil yield. However, the linear and quadratic effects of UAE temperature were not significant ( $P < 0.05$ ). All the present interactions including UAE time and UAE temperature ( $P < 0.001$ ), UAE time and ethanol/

powdered seed ratio ( $P < 0.05$ ), and UAE temperature and ethanol/powdered seed ratio ( $P < 0.05$ ) were significant on the response variable (Table 2). The most significant ( $P < 0.05$ ) effect on the oil yield was shown to be the linear effect of ethanol/sample ratio followed by the linear and quadratic effects of UAE time and interaction effect of UAE time and UAE temperature (Table 2, Equation 8). As shown in Figure 4, the variation in the oil yield value of *J. curcas* seeds could be explained as a nonlinear function of three



**Figure 4.** Response surface (A, C and E) and contour (B, D and F) plots showing the significant ( $P < 0.05$ ) interaction effects on the variation of oil yield extracted from *Jatropha curcas* seeds using ultrasound-assisted extraction (UAE) method.

independent variables. An increase in two factors of UAE time and ratio of ethanol/sample led to an enhancement in oil yield obtained from *J. curcas* seeds. Increased diffusivity of the solvent into cells and enhanced desorption of the oil from the cells can explain for the enhancement of the extraction yield by increasing the solvent/sample ratio (Luque-Garcia and Luque de Castro, 2004). Similar results were found for the UAE of saikosaponins from a traditional Chinese medicine named 'chai hu bupleurum' (Zhao *et al.*, 2007) and UAE of oil from flaxseed Zhang *et al.* (2008). It seems that ultrasonic waves could disrupt the cell walls, thus larger contact area between solvent and material during higher times can lead to enhance the release of oil from plant structures by disruption of the inner cell walls (Zhang *et al.*, 2008). Balachandran *et al.* (2006) reported that the ultrasound continued to be effective even during the later extraction stage through an enhancement to the internal diffusivity, although the effects were smaller. A significant decrease in the oil yield was observed by increasing UAE temperature from 45 to 60 °C. This fact can be attributed to the decreased surface tension by the increasing UAE temperature affecting the bubble formation and collapse (Gharibzahedi *et al.*, 2015; Luque-Garcia *et al.*, 2004). At higher temperature, the bubbles may be easily collapsed thus reducing the intensity of the mass transfer enhancement (Zhang *et al.*, 2008). Ultrasound technique generally creates a few cavitation bubbles because of a high acoustic cavitation threshold. However, the bubbles burst with greater force, which increased cell tissue disruption during extraction process (Vilkhu *et al.* 2008). Decrease of the oil yield at higher temperatures can also be due to the increased vapor pressure of solvent and its influence on the occurrence and the intensity of acoustic cavitation (Gharibzahedi *et al.*, 2013c). More bubbles were created at the higher vapor pressure, but they collapsed with less

intensity owing to a smaller pressure difference between inside and outside of bubbles (Hromádková *et al.*, 1999).

### Optimisation and verification of the model

Predicting the optimal level of independent variables to find maximum oil yield *J. curcas* seeds during UAE process were performed using numerical optimisation procedures. The overall optimum region to achieve the maximum oil yield was extraction time of 51.69 min, extraction temperature of 44.57 °C and ethanol/powdered seeds ratio of 44.78, v/w. The results of the optimisation in Figure 5 show that the corresponding predicted response value under the optimal conditions for oil yield was 61.94%. The yield maximum of oil produced experimentally was found to be  $62.12 \pm 1.09\%$ . Therefore, a good correlation between predicted and experimental values demonstrates the validity of the model used. The validation results revealed that there was no significant difference between experimental and predicted values ( $P > 0.05$ ), confirming the validity of the response model. On the other hands, the obtained yield in UAE method showed a significant 2.42-fold increase over the Soxhlet extraction procedure (25.63%). Zhang *et al.* (2008) also found that UAE procedure can significantly increase yield of flaxseed oil (84.9%) in comparison to the maceration extraction method (66.7%). Tian *et al.* (2013) also reported that the yield of pomegranate seed oil extracted by UAE (25.11%) was higher than by Soxhlet extraction (20.50%) and supercritical fluid extraction (15.72%). Generally, ultrasonic process improves liquid-liquid extraction by providing an intensified mass transfer between two immiscible phases and facilitated emulsification (Gharibzahedi *et al.* 2013a). However, Bimakr *et al.* (2012) showed that the crude oil yield extracted from winter melon (*Benincasa hispida*) seeds using UAE technique was lower than that

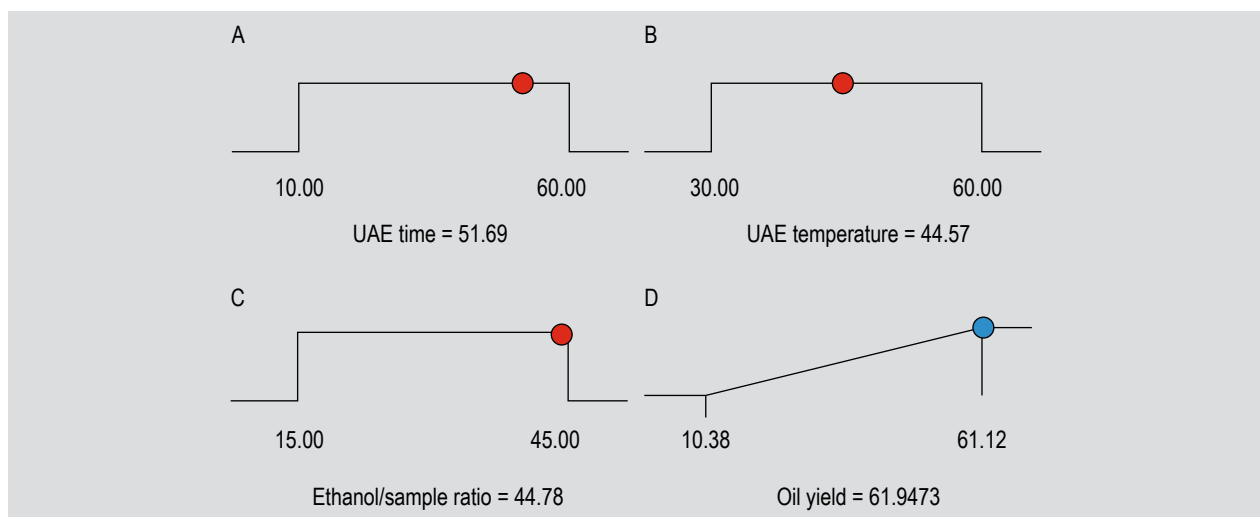


Figure 5. Schematic representation of the optimum values of factors, response, and the corresponding levels (UAE = ultrasound-assisted extraction).

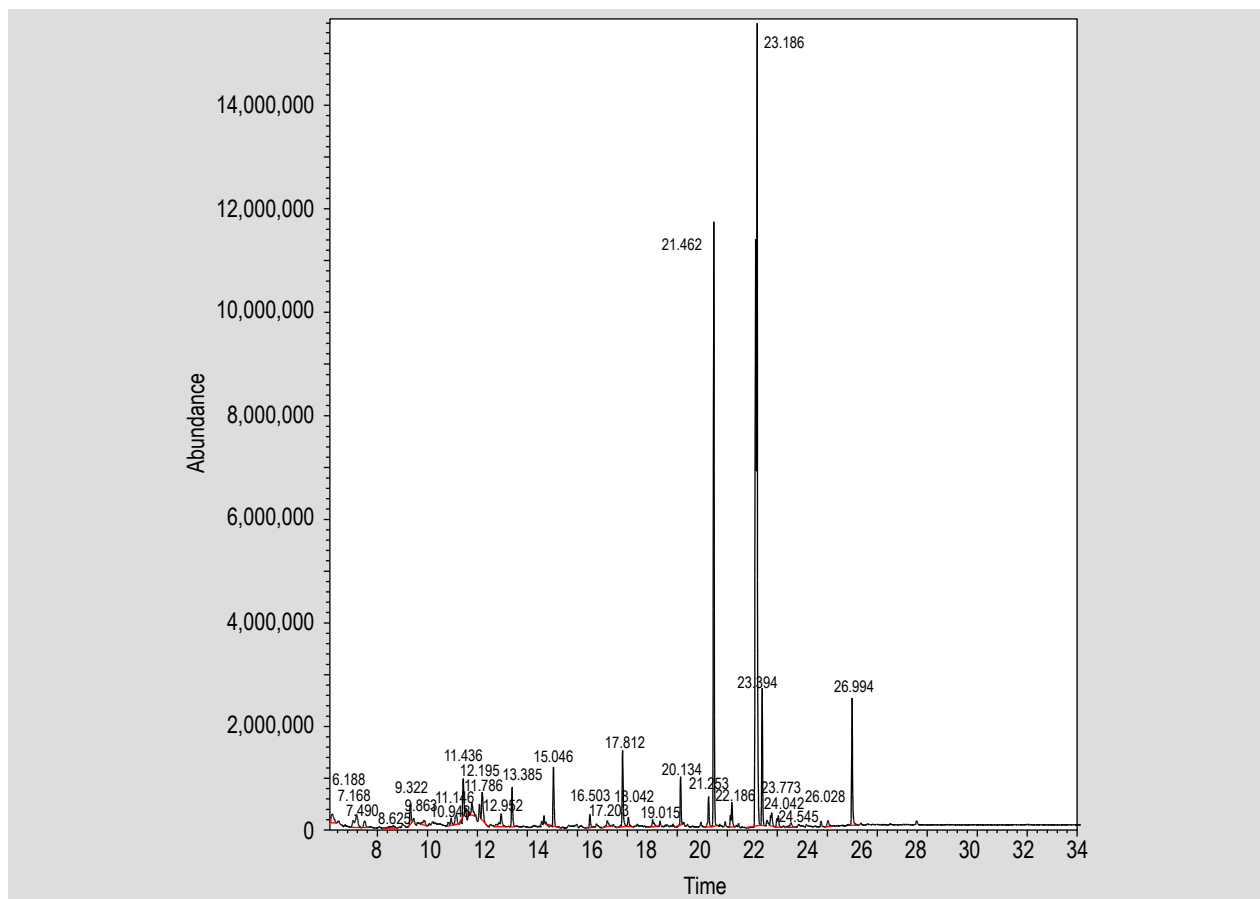
of the Soxhlet method, whereas antioxidant activity and total phenolic content of the oil obtained by UAE were noticeably higher than those of the Soxhlet extract. The fatty acids composition of *J. curcas* seeds oil extracted by UAE under the optimal conditions is represented in Table 3. Furthermore, fatty acids profile of this oil is also depicted in Figure 6. Oleic (26.15%), linoleic (24.21%), palmitic (21.09%) and stearic (6.28%) acids were the most dominant fatty acids present in the *J. curcas* oil. Gharibzahedi *et al.* (2013d) also reported that oleic acid was the dominant fatty acid in *Moringa peregrina* seed oil (77.9%). Other researchers showed that the linoleic acid was the dominant fatty acid in *J. curcas* oil (44.2-47.3%) and some *Labiatae* and *Umbelliferae* seed oils (Adebowale and Adedire 2006; Gubitz *et al.*, 1999; Matthäus *et al.*, 2015). Gubitz *et al.* (1999) and Adebowale and Adedire (2006) reported lower content palmitic acid contents of 14.1-15.3 and 11.3%, respectively in *J. curcas* oil than our results (19.68%).

#### 4. Conclusions

In the current study, a critical optimisation was conducted to maximise yield of *J. curcas* seed oil extracted by UASE with ethanol was the most suitable of four solvents evaluated

**Table 3. Fatty acids profile of fatty acids in oil extracted from *Jatropha curcas* seeds using the determined optimal conditions of ultrasound-assisted extraction process.**

Fatty acids	Area (%)
Decane	1.75
Undecane	1.07
1,1-dimethoxydodecane	0.67
Dodecane	1.46
Tridecane	1.89
Tetradecane	2.39
Pentadecane	0.58
Hexadecane	3.18
Heptadecane	0.62
Octadecane	1.72
Palmitoleic acid	4.05
Palmitic acid	21.09
Eicosane	1.33
Linoleic acid	24.21
Oleic acid	26.15
Stearic acid	6.28
Docosane	0.81
9,12-octadecadien-1-ol	0.46



**Figure 6. Partial gas chromatography (GC)-mass spectrometry chromatogram obtained from the oil extracted from Iranian *Jatropha curcas* seeds at optimal conditions. For details of the GC column and conditions see Section 2.**

for the extraction of oil. A RSM-BBD was subsequently employed with ethanol for experimental design and analysis of results of the oils extracted from *J. curcas* seeds using UAE technique. A satisfactory empirical model in term of second-order polynomial equation was developed for three parameters including UAE time and temperature and v/m ratio of ethanol to the sample to optimise the oil yield. An  $R^2$  value of 0.995 showed a good fit of the model with the experimental data. The optimal condition for the maximum oil yield (61.94%) was found at UAE time of 51.69 min, UAE temperature of 44.57 °C and ethanol/sample ratio of 44.78, v/w. The extracted oil under these conditions was contained high percentages of fatty acids of oleic, linoelic and palmitic. This study suggests that UAE with ethanol solvent can significantly improve yield of edible oils from plant sources during commercial extraction processes. Moreover, the produced biodiesel from *J. curcas* oil due to the high levels of unsaturated fatty acids especially oleic and linoleic acids has desirable good low temperature properties.

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