

## **Application of the Response Surface Methodology for the Optimization of the Aqueous Enzymatic Extraction of *Pistacia Khinjuk* Oil**

**M. Ahmadi<sup>a\*</sup>, Gh. Zahedi<sup>b</sup>, F. Karimi<sup>a</sup>**

<sup>a</sup> Chemical Engineering Department, Faculty of Engineering, Razi University, Kermanshah, Iran.

<sup>b</sup> Process Systems Engineering Centre (PROSPECT), Faculty of Chemical Engineering, University Teknologi Malaysia, UTM Skudai, 81310 Johor Bahru, Johor, Malaysia.

Received 25 September 2012; Accepted 27 December 2012

**ABSTRACT:** Aqueous enzymatic extraction of oil from pistacia khinjuk was performed using cellulase. The central composite design was used to optimize the parameters that are significant to the process. The influence of three regressors on the percentage of oil recovery from seed was evaluated using second-order polynomial multiple regression model. Analysis of variance showed a high coefficient of determination ( $R^2$ ) value of 0.99 indicating a satisfactory adjustment of the regression model to the experimental data. The positive sign for the coefficient of the interaction between pH and reaction time on the response indicated that a simultaneous increase in the pH and the reaction time led to an increase in oil recovery percentage. The optimum condition are as follows: temperature of 60 °C, pH of 9, reaction time of 20 h and oil extraction recovery of 74.93%.

**Keywords:** Cellulase, Central Composite, Design and Response Surface Methodology, Enzymatic Extraction, *Pistacia khinjuk*, Seed Oil.

### **Introduction**

*Pistacia* is a genus of flowering plants belonging to the family Anacardiaceae (Shuraki and Sedgley, 1994), that comprises 11 species (Zohary, 1996). Among them, *Pistacia vera* L., *Pistacia atlantica* subsp. *mutica* (Fisch. & C. A. Mey.) Rech. f. (*Pistacia mutica*), and *Pistacia khinjuk* Stocks, are the native species to Iran (Razavi, 2006), that only *P. vera* has economical importance and its cultivation, as a traditional nut crop, extends to the dry land areas of the country. *P. vera* and *P. khinjuk* are the most primitive species and also postulated that *P. khinjuk* was directly

descended from *P. vera* (Zohary, 1996) as a bridge to other *Pistacia* species

Iran is the world's largest producer of *Pistacia* spp., with over 44% of the world production. Most of the production is from orchards that account for 53% of world planted, but there are a few places, such as in the Zagros Mountains, where wild pistachio persists in natural and extensively managed (i.e., semi-natural) stands (Razavi, 2006). They are the most important types of pistachio and for this reason, Iran is known as the origin of pistachios. Therefore the *Pistacia khinjuk* seed would be as a novel source of the plant oil for the pharmaceutical industries. The oil obtained from *P. khinjuk* seed showed an antihelminthic effect against

\*Corresponding Author: [m\\_ahmadi@razi.ac.ir](mailto:m_ahmadi@razi.ac.ir)

protoscoleces of *E. granulosus* and anti-echinococcal activity. The oil from plant seeds is conventionally extracted either by mechanical pressing or solvent extraction (Mani *et al.*, 2007; Shojaei *et al.*, 2011). Mechanical pressing is a very inefficient process with low oil recovery.

In spite of its higher efficiency in the range of 90–98%, solvent extraction (SE) suffers a poor quality protein in oil cake (meal), and it requires high investment and energy requirements. The commercial solvent for SE process is hexane that is listed among the hazardous air pollutants associated with neurological and respiratory disorders with prolonged exposure (the International Standard Organization permits only 50 ppm residual hexane in the oil seed meal)(Sharma *et al.*, 2001). Hence, there is a need to explore a safe and efficient oil extraction alternative process.

Aqueous enzymatic extractions could serve as a potential alternative in oil industries due to high specificity and low operating temperatures. These are the reasons that make the enzyme process more economical for oil extraction processes (Rosenthal *et al.*, 1996). The enzymes break down the cell structure of plants. The cell wall of plants consists mainly of pectic substances, cellulose, hemicelluloses, lignin and protein, whereas lipid bodies are enveloped in a lipoprotein layer. Enzymes like cellulase, hemicellulase and pectinase break down the cell, while proteases permeabilize the liposome membrane and facilitate oil release from the oil body (Fullbrook, 1983; Rosenthal *et al.*, 1996). Aqueous enzymatic oil extraction is based on simultaneous isolation of oil and protein from the oil seed by dispersing finely ground seed in water and separating the dispersion by centrifugation into oil, solid, and aqueous phases. Dobozi *et al.* reported treatment of mustard seeds with cellulolytic enzymes resulted in an increase (20–30%) in oil yield (Dobozi *et al.*, 1988). Optimization of the

enzymatic treatment during aqueous oil extraction with cellulases from sunflower seeds has been reported by Sineiro *et al.* (Sineiro *et al.*, 1998). Latifa *et al.* reported oil and protein extraction from sesame seeds during an enzyme-assisted aqueous extraction process (Latif and Anwar, 2011). Extraction of oil from watermelon seeds by aqueous enzymatic extraction method has been studied by Sui *et al.* and obtained the optimum parameters from single-factor experiments and response surface methodology (Xiaonan *et al.*, 2011). Extraction of oil from peanut with a ultrasonic-assisted aqueous enzymatic extraction by response surface method has been studied (Li *et al.*, 2011a). Najafian *et al.* found that oil extraction from olive can be enhanced by enzymatic hydrolysis and demonstrated that pre extraction enzyme digestion increases cellular degradation and significantly increases oil recovery upon extraction (Najafian *et al.*, 2009). An aqueous enzymatic extraction of peanut oil and protein has been studied by Jian *et al.* (Jiang *et al.*, 2010). Hadj-Taieb *et al.* studied the effect of enzymatic formulation on Tunisian olive oil extraction yields (Hadj-Taieb *et al.*, 2012). Optimization of the aqueous enzymatic extraction of pine kernel oil by response surface methodology and extraction of olive oil using enzymatic formulations during malaxation has also been reported (Yang *et al.*, 2011).

Previous studies have not been carried out on the aqueous enzymatic extraction of oil from *Pistacia khinjuk*. This study was conducted to optimize the process parameters (temperature, reaction time and pH) of enzymatic extraction of oil from the *Pistacia khinjuk* by cellulase with RSM, and to find the optimum operating conditions that maximize the oil recovery.

## Materials and Methods

*Pistacia Khinjuk* seeds were purchased from the local market in Iran. The seeds

wrapped in plastic bags and stored at 4°C until use. Seeds were ground and screened to select the fraction size. All the chemicals used were from Merck (Darmstadt, Germany) or Sigma-Aldrich (Buchs, Switzerland) Chemical Companies. Cellulase preparation from *Aspergillus niger* was obtained from Sigma Chemical Company.

#### - Aqueous extraction of Pistacia Khinjuk

Pistacia Khinjuk was dispersed in distilled water to prepare a slurry at a ratio of 1:6 w/v using a flask. The pH of slurry was adjusted to the desired value with 0.1 N NaOH or 0.1 N HCl, and was stirred by a magnetic stirrer at 250 rpm for 30 min. The enzymes were added at different concentrations, and the samples were incubated at different temperatures, times, with controlled speed of mixing. The samples were then incubated at constant temperatures. A shaker-incubator (DK-S1060, DAIKI SCIENCE CO.) was used for temperature-controlled shaking of the sample solutions, followed by centrifugation (10000g, 30 °C) for 20 min (MIKRO 200, HETTICH) yielding three distinct phases (i) an oil phase, (ii) creamy phase and (iii) aqueous phase. The upper oil layer was separated and weighed. Oil recovery was expressed relative to that obtained by Soxhlet extraction with hexane.

$$\% \text{ oil recovery} = \frac{\text{weight of oil extracted} \times 100}{\text{total weight of oil estimated by soxhlet method}}$$

The total amount of extracted oil was determined with Soxhlet apparatus following the standard AOAC standard procedure (Horowitz, 1984). All experiments were repeated three times to render mistakes during experiments.

#### - Experimental design

As shown in Table 1, a CCD in the form of the  $2^3$  full factorial design was used, in

which three independent variables were converted to dimensionless ones ( $x_1, x_2, x_3$ ), with the coded values at 3 levels: -1, 0, +1. The arrangement of CCD as shown in Table 2 was in such a way that allows the development of the appropriate empirical equations (second order polynomial multiple regression equations) (Lapin, 1997; Tomaino *et al.*, 2010):

The predicted response ( $y$ ) was therefore correlated to the set of regression coefficients ( $\beta$ ): the intercept ( $\beta_0$ ), linear ( $\beta_1, \beta_2, \beta_3$ ), interaction ( $\beta_{12}, \beta_{13}, \beta_{23}$ ) and quadratic coefficients ( $\beta_{11}, \beta_{22}, \beta_{33}$ ). The "Design expert" (Trial version 5) was used for regression and graphical analyses of the obtained data.

$$y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 \quad (1)$$

## Results and Discussion

### - Effect of hydrolysis time on oil recovery

Enzymatic extraction of oil from Pistacia Khinjuk seeds were subjected to different durations of incubation (4-24hr). The effect of different incubation period on the recovery oil from Pistacia seed is given in Fig.1. The results show that the oil recovery increased with prolonged enzymatic extraction time and after 16 hours the increase was not significant. Oil recovery of 70% was observed within the first 16 hours of incubation with slight increase of 1.3% afterwards. Similar observations have been reported in literature (Li *et al.*, 2012; Sharma *et al.*, 2001; Yang *et al.*, 2011). It has also been reported that the optimal duration of the enzymatic extraction process was about 18 h (Sharma *et al.*, 2001). Sharma *et al* showed that the minimum incubation time to achieve maximum oil recovery from rice bran by enzyme-assisted aqueous extraction was about 18 h (Li *et al.*, 2012).

Table 1. Independent variables and their levels for the central composite design used in the present study

Variable	Symbol	Coded variable levels		
		-1	0	1
Temperature	$x_1$	40	50	60
pH	$x_2$	4	6.5	9
Extraction time	$x_3$	4	12	20

Table 2. Arrangement of the Central Composite Design for the three independent variables used in the present study along with the theoretically predicted values for the four response variables. The actual values are also given in the parenthesis

Experiments no.	Temperature ( $x_1$ )	pH ( $x_2$ )	Reaction time ( $x_3$ )	oil recovery	
				Experimental	Predicted
1	-1	-1	-1	16.10	16.23
2	1	-1	-1	20.05	21.09
3	-1	1	-1	19.69	21.06
4	1	1	-1	24.91	25.92
5	-1	-1	1	24.52	24.34
6	1	-1	1	29.94	29.20
7	-1	1	1	69.94	69.65
8	1	1	1	74.93	74.51
9	-1	0	0	29.35	29.43
10	1	0	0	35.11	34.29
11	0	-1	0	28.01	28.41
12	0	1	0	54.95	53.48
13	0	0	-1	15.30	11.99
14	0	0	1	38.03	40.34
15	0	0	0	30.22	31.86
16	0	0	0	31.08	31.86

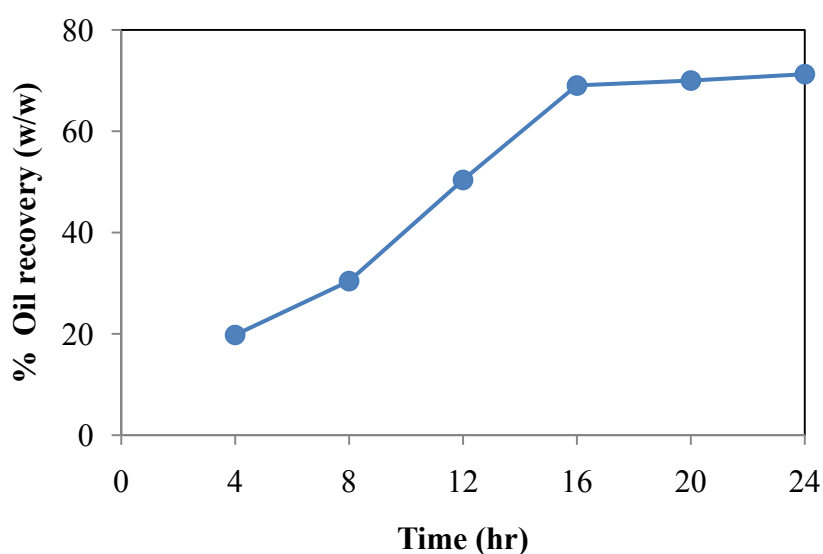


Fig. 1. Effect of incubation time on the oil recovery by enzymatic aqueous extraction of Pistacia khinjuk (pH 8, 50°C, 80rpm)

#### - Influence of pH on oil recovery

In order to assess the effect of pH on oil recovery, the enzymatic extraction was carried out at different pH values in the range of 4-9 by adding the desired amount of 0.1N HCl or 0.1 N NaOH into the slurry. The mixture was then incubated overnight at 50°C with constant mixing at 80 rpm. As shown in Figure 2 the oil recovery with aqueous enzymatic extraction by cellulase

increased along with the increase in pH. Sharma et al reported that the optimum value of pH to achieve maximum oil recovery from peanut seeds by protease was in the range of 7-10 (Sharma *et al.*, 2001). similar result was reported by Jian et al that found optimum pH of 9.5 for aqueous enzymatic extraction of peanut oil (Jiang *et al.*, 2010).

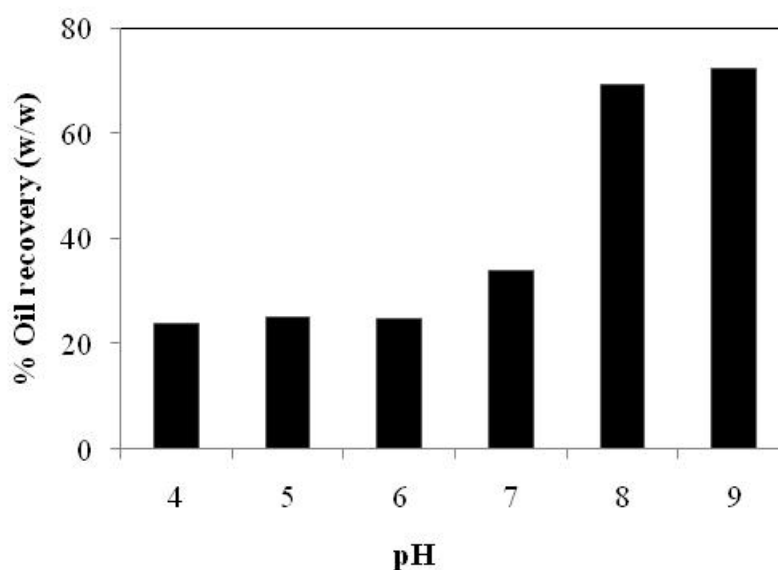


Fig. 2. Effect of pH on the enzymatic oil extraction from Pistacia khinjuk. The enzyme mixture was then incubated overnight at 50°C with constant mixing at 80 rpm

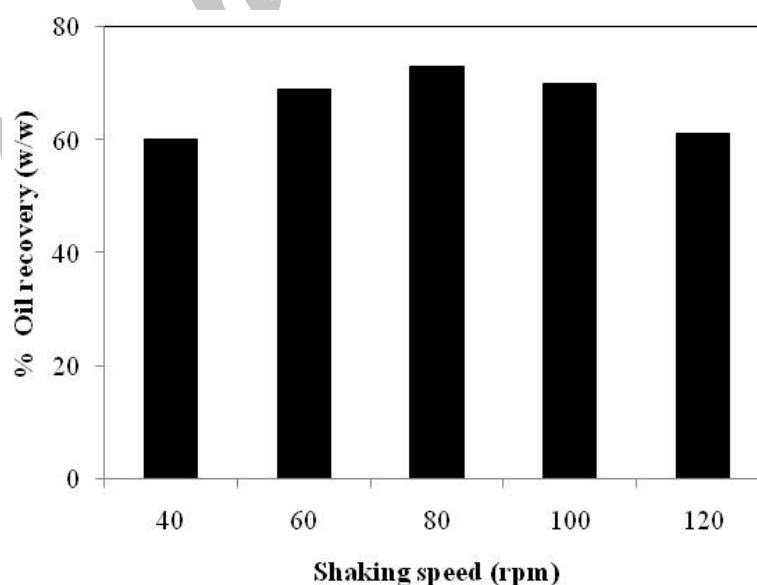


Fig. 3. Effect of mixing speed on the enzymatic oil extraction from Pistacia khinjuk by aqueous Enzyme Extraction (pH 8, overnight incubation at 50°C)

- Effect of mixing speed on enzymatic oil recovery

Mixing speed has been identified as another parameter that could significantly affect the oil recovery via enzymatic extraction. The effect of mixing speed on oil recovery from *P. Khinjak* was studied in the range of 40-120 rpm. Figure 3 shows the effect of mixing speed on the oil recovery. Increasing the mixing speed from 40 to 80 rpm led to an increase in the oil recovery. It was observed that the oil obtained at 80 rpm was clear, whereas increased more than 80 rpm oil recovery was reduced. Increasing the speed led to the formation of a clearly visible emulsion of the oil layer at the top of decanter. Sharma *et al* also found decreasing in mixing speed led to a decrease in oil recovery from peanut seeds (Sharma *et al.*, 2002).

- Central composite design

In this work, the relationship between percentage of oil recovery and three controllable factors (namely temperature, pH and extraction time) were studied. A CCD is shown in Table 3 allows the development of mathematical equations where each response variable  $y$  is assessed as a function of temperature ( $x_1$ ), pH ( $x_2$ ) and extraction time ( $x_3$ ) and calculated as the sum of a constant, three first-order effects (terms in  $x_1$ ,  $x_2$  and  $x_3$ ), three interaction effects (terms in  $x_1x_2$ ,  $x_1x_3$  and  $x_2x_3$ ) and three second-order effects ( $x_1^2$ ,  $x_2^2$  and  $x_3^2$ ) as expressed in Eq. (1). The results obtained were then analyzed by ANOVA to assess the "goodness of fit". The models for oil recovery is significant as determined via the  $F$ -test at the 5% confidence level ( $\text{Prob} > F < 0.05$ ). Only the statistically significant terms were included in the model.  $\beta_{12}$ ,  $\beta_{13}$  and  $\beta_{11}$  coefficient were non-significant. The following final empirical model (equations in terms of actual parameters for the regressors) was used to quantitatively describe the effects of temperature, pH and

extraction time on the characterization of aqueous enzymatic oil extraction:

$$\begin{aligned} \% \text{ oil recovery} = & 53.92 + 0.253X_1 \\ & - 20.07X_2 + 0.574X_3 \\ & + 0.512X_2X_3 + 1.458X_2^2 \\ & - 0.089X_3^2 \end{aligned}$$

The coefficient of multiple determinations ( $R^2$ ) is 0.994 that indicate a satisfactory adjustment of the quadratic model to the experimental data. Therefore, the empirical equations are adequate to represent the relationship between the variables.

As shown in Table 4, the coefficient of variance (CV) has been found to be 5.11 %. The CV is (the ratio of the standard error of estimate to the mean value of the observed response) is a measure of reproducibility of the model and as a general rule a model can be considered reasonably reproducible if its CV is less than 10% (Vining, 2003). This model has high  $R^2$  value and shows no lack of fit. By applying diagnostic plots such as normal probability plot of residual, plot of residuals versus predicted values independence and randomness of the residuals were satisfied. The adequate precision value is a measure of the "signal to noise ratio" and was found to be 54.76, that indicates an adequate signal (see Table 4). A ratio  $>4$  is desirable (Vining, 2003). The predicted models can be used to navigate the space defined by the CCD.

The relative contribution of each factor to the dependent variable (oil recovery) was directly measured by the respective coefficient in the fitted model. A positive sign for the coefficients ( $\beta_1$ ,  $\beta_2$  and  $\beta_3$ ) in the fitted models indicates that the level of oil recovery increased with increase in the levels of factors. The greatest coefficient ( $\beta_3 = 14.13$ ) in the fitted model reveals the high sensitivity of the time in enzymatic extraction. On the other hand, the lowest  $\beta_1$ s was obtained that indicates that the oil recovery is not significantly affected by the temperature.

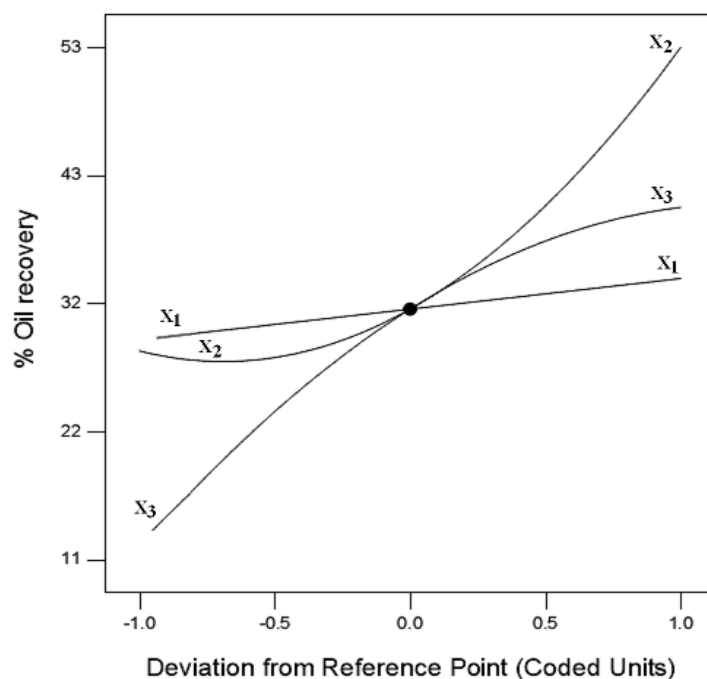


Fig. 4. Perturbation graph showing the effect of each of the independent variables on oil recovery while keeping other variables at their respective mid-point levels. (x<sub>1</sub>) Temperature, (x<sub>2</sub>) pH and (x<sub>3</sub>) Reaction time.

Table 3. Regression coefficients of the second order polynomial model for response variables

Factor	Estimated Coefficient	
	Coded Factors	Actual Factors
Constant	31.77**	54.23**
Linear		
Temperature (x <sub>1</sub> ) (°C)	2.53*	0.304*
pH (x <sub>2</sub> )	12.58**	-20.35**
Reaction time (x <sub>3</sub> ) (h)	14.13**	0.465**
Interaction		
x <sub>1</sub> ×x <sub>2</sub>	0.10	4.192
x <sub>1</sub> ×x <sub>3</sub>	0.15	1.936
x <sub>2</sub> ×x <sub>3</sub>	10.24**	0.512**
Quadratic		
Temperature (x <sub>1</sub> ) (°C)	-0.10	-1.011
pH (x <sub>2</sub> )	9.15**	1.464**
Reaction time (x <sub>3</sub> ) (h)	-5.67*	-0.089*
* p<0.05		
** p<0.0001		

Table 4. Analysis of variance (ANOVA) of RSM for enzymatic extraction

Source	SS <sup>a</sup>	DF <sup>b</sup>	MS <sup>c</sup>	F-value	Prob>F
Model	4734.8	6	789.13	263.04	< 0.0001
Residual	27	9	3.00		
Lack of Fit	26.63	8	3.33	9.00	
Pure Error	0.37	1	0.37		
Total	4761.77	15			
R <sup>2</sup>	0.994			Adj-R <sup>2</sup>	0.990
C.V%	5.11			PRESS	84.22

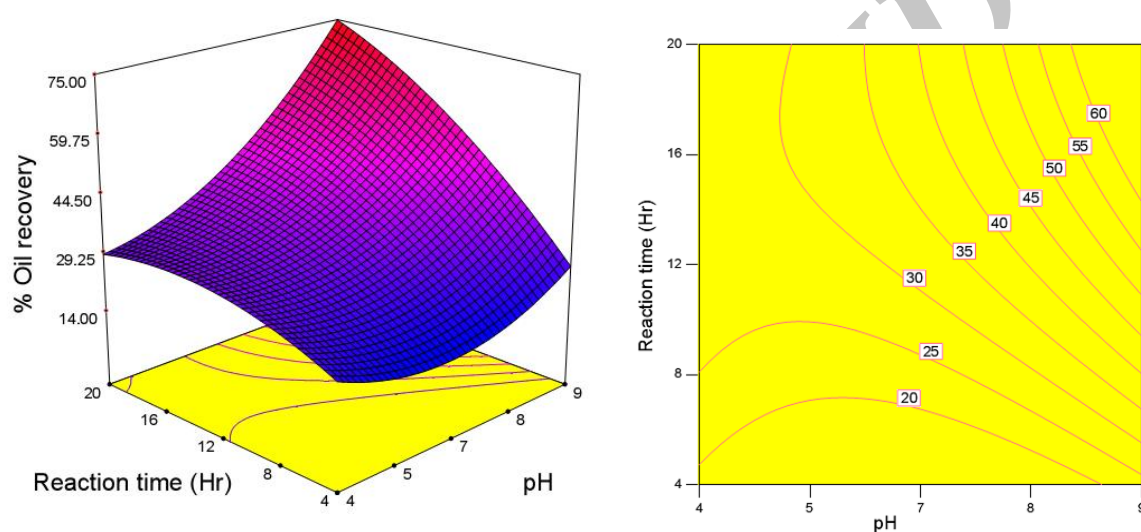
<sup>a</sup> SS, sum of squares<sup>b</sup> DF, degree of freedom<sup>c</sup> MS, mean square

Fig. 5. Response surface and contour plots for the effect of reaction time and pH on the oil recovery, (Temperature=60 C)

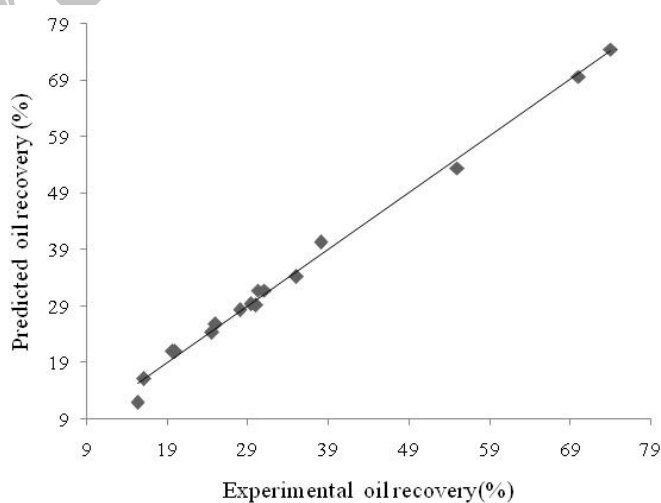


Fig. 6. Parity plot for the experimental and predicted value of oil recovery (%)



The perturbation plot, Fig. 4 shows the effects when all factors at the center point in the design space are compared. The perturbation plot assists the comparison of the effects of all factors at a particular point in the design space, when the factor curvature is sharper, the effect of factor is more important to the response. The plot was obtained for 50°C temperature, pH of 6.5 and reaction time of 12 hours. In order to gain a better understanding of the results, the predicted model is presented in Fig. 5 as the three-dimensional response surface plots contour plots. The effect of pH and reaction time on the oil recovery at fixed temperature at 60°C (the center point of this experiment) was shown in Fig 5. There was significant interaction between pH and reaction time. The pH has a slightly positive effect on the oil recovery. Similar result was also reported by Jiang et al. that found pH significantly affected the peanut oil recovery using enzymatic extraction (Jiang *et al.*, 2010). In addition, reaction time positively affected the oil recovery throughout the experiment. In the models for the oil recovery,  $x_3$  was identified as the major regressor variable affecting the responses (greatest coefficients,  $\beta_3=14.13$ ) and oil recovery increases almost linearly as reaction approaches its peak (at 20hr). Studies concerned with the oil recovery using enzymatic extraction indicated that there was significant interaction between temperature and reaction time. Generally, reaction time has a positive effect on the oil yield (Li et al., 2011b).

Fig. 6 shows that the response of the oil recovery was very sensitive to the reaction time, followed by pH, and finally, by temperature. For a model to be reliable, the response should be predicted with a reasonable accuracy by the model when compared with the experimental data. Fig.6 compares the experimental oil recovery with the predicted values obtained from the model. The figure indicated good agreements between the experimental and

predicted values of oil recovery. Second-order polynomial models obtained in this study were utilized for each response in order to determine the specified optimum conditions. By applying the method of desirability function, the optimum oil recovery condition was determined as follows: temperature of 60 °C, pH of 9 and a reaction time of 20 h. At this point, extraction of oil recovery was calculated at 74.65%.

### Conclusion

In this work the enzymatic Extraction of oil from *Pistacia khinjak* seed by cellulase was studied using CCD and RSM. The following conclusions could be made:

- There is a quadratic correlation between oil recovery and temperature. This is evidenced when oil recovery reaches its peak at the center point (60 °C) followed by a steady decrease afterward. With respect to pH, the oil recovery increased as the pH of aqueous solution was increased from 4 to 9.
- With respect to time, oil recovery increased as reaction time progressed from 4 to 20h.
- Optimum conditions for process could be achieved by setting temperature at 60 °C, pH at 9 and reaction time of 20 hours.

### References

- Dobozi, S., Halasz, A. & Kovacs, K. E. (1988). Enhancement of mustard oil yield by cellulolytic pretreatment. *Applied Microbiology Biotechnology* 29, 39-43.
- Fullbrook, P. (1983). Use of Enzymes in the Processing of Oilseeds. *Ibid* 60, 476-478.
- Hadj-Taieb, N., Grati, N., Ayadi, M., Attia, I., Bensalem, H. & Gargouri, A. (2012). Optimisation of olive oil extraction and minor compounds content of Tunisian olive oil using enzymatic formulations during malaxation. *Biochemical Engineering Journal* 62, 79-85.
- Horowitz, W. (1984). of Analysis of the Association of Official Analytical Chemists,

Washington, DC

Jiang, L., Hua, D., Wang, Z. & Xu, S. (2010). Aqueous enzymatic extraction of peanut oil and protein hydrolysates. *Food and Bioproducts Processing* 88, 233-238.

Lapin, L. L. (1997). *Modern Engineering Statistics*. Wards worth Publishing Company, Belmont, CA, USA.

Latif, S. & Anwar, F. (2011). Aqueous enzymatic sesame oil and protein extraction. *Food Chemistry* 125, 679-684.

Li, F., Yang, L., Zhao, T., Zhao, J., Zou, Y., Zou, Y. & Wu, X. (2012). Optimization of enzymatic pretreatment for n-hexane extraction of oil from *Silybum marianum* seeds using response surface methodology. *Food and Bioproducts Processing* 90, 87-94.

Li, Y., Jiang, L., Sui, X., Qi, B. & Han, Z. (2011a). The study of ultrasonic-assisted aqueous enzymatic extraction of oil from peanut by response surface method. *Procedia Engineering* 15, 4653-4660.

Li, Y., Jiang, L., Sui, X. & Wang, S. (2011b). Optimization of the aqueous enzymatic extraction of pine kernel oil by response surface methodology. *Procedia Engineering* 15, 4641-4652.

Mani, S., Jaya, S. & Vadivambal, R. (2007). Optimization of Solvent Extraction of Moringa (*Moringa Oleifera*) Seed Kernel Oil Using Response Surface Methodology. *Food and Bioproducts Processing* 85, 328-335.

Najafian, L., Ghodsvali, A., Haddad Khodaparast, M. H. & Diosady, L. L. (2009). Aqueous extraction of virgin olive oil using industrial enzymes. *Food Research International* 42, 171-175.

Razavi, S. (2006). Pistachio production, Iran vs. the World. *Acta Hort* 726, 689-694.

Rosenthal, A., Pyle, D. L. & Niranjana, K. (1996). Aqueous and enzymatic processes for edible oil extraction. *Enzyme and Microbial Technology* 19, 402-420.

Sharma, A., Khare, S. K. & Gangodavilage, N. C. (2001). Enzyme-

Assisted Aqueous Extraction of Rice Bran Oil. *JAACS* 78, 817-821.

Sharma, A., Khare, S. K. & Gupta, M. N. (2002). Enzyme-Assisted Aqueous Extraction of Peanut Oil. *JAACS* 79, 215-218.

Shojaei, A., Javidnia, K. & Miri, R. (2011). Antioxidant and antimicrobial activity of ethanolic extract of pistacia khinjuk (*anacardiaceae*). *European Journal of Pharmacology* 668, Supplement 1, 43-44.

Shuraki, Y. D. & Sedgley, M. (1994). Effect of pistil age and pollen parent on pollen tube growth and fruit production of pistachio. *J. Hort. Sci* 69, 1019-1027.

Sineiro, J., Domínguez, H., Núñez, M. J. & Lema, J. M. (1998). Optimization of the enzymatic treatment during aqueous oil extraction from sunflower seeds. *Food Chemistry* 61, 467-474.

Tomaino, A., Martorana, M., Arcoraci, T., Monteleone, D., Giovino, C. & Saija, A. (2010). Antioxidant activity and phenolic profile of pistachio (*Pistacia vera* L., variety Bronte) seeds and skins. *Biochimie* 92, 1115-1122.

Vining, G. G. (2003). *Statistical Methods for Engineers*. Duxbury Press, An International Thomson Publishing, London 1V7AA

Xiaonan, S., Jiang, L., Li, Y. & Liu, S. (2011). The research on extracting oil from watermelon seeds by aqueous enzymatic extraction method. *Procedia Engineering* 15, 4673-4680.

Yang, L., Jiang, L., Sui, X. & Wang, S. (2011). Optimization of the aqueous enzymatic extraction of pine kernel oil by response surface methodology. *Procedia Engineering* 12, 4641-4652.

Zohary, D. (1996). The genus *Pistacia*, S. Padulosi, T. Caruso, E. Barone, Editors, Taxonomy, distribution, conservation and uses of *Pistacia* genetic resources. IPGRI, Palermo, Rome 1-11.