Optimize the extraction of phenolic compounds of jujube (*Ziziphus Jujube*) using ultrasound-assisted extraction method

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***Abstract*— Ultrasound-assisted extraction method was applied for phenolic compounds extraction from jujube by the simultaneous maximization of the yield in the total phenolics using the response surface methodology. A Box-Behnken was used to investigate the effects of four independent variables, namely time (20-50 min), temperature (20-50 ˚C), sound intensity (60-100%) and solvent composition (40-80%) on the dependent variables (amount of total phenolic content and antioxidant activity). A second-order polynomial model was used to describe the experimental data regarding the total phenolics. Correlation coefficient (R^2) of the model for total phenolic content was 0.98. Optimal conditions for total phenolic content were temperature 48^{0}C, 72% solvent composition, sound intensity 96% and 48 min. In optimal conditions, the total phenolic content 15.8 mg gallic acid equivalent /g dry matter was predicted by the model. Under optimized conditions the experimental values agreed with the values predicted by models.**

Keywords*— *Jujube; phenolic compounds; ultrasound-assisted extraction; response surface methodology

I. INTRODUCTION

Jujube (Ziziphus spp.), also known as ''ber'', belongs to the Rhamnaceae family and contains approximately 40 species. Jujube is distributed mainly in tropical and subtropical parts of the world [1]. Jujube has been commonly used as a drug in traditional Chinese medicine as an analeptic, palliative, antibechic and has also been commonly used as food, food additive and flavorant for thousands of years [2-3]. Some phenolic compounds, such as chlorogenic acid, caffeic acid, catechin, epicatechin and rutin, were isolated from jujube [4].

Phenolic compounds are secondary metabolites that are derivates of the pentose phosphate, shikimate and phenylpropanoid pathways in plant. Phenolic compounds exhibit a wide range of physiological properties, such as anti-allergenic, anti-artherogenic, anti-inflammatory, anti-microbial, antioxidant, anti-thrombotic, cardioprotective and vasodiolatory effects [5-7].

The beneficial effects derived from phenolic compounds have been attributed to their antioxidant activity. Phenolic compounds could be a major determinant of antioxidant potentials of foods, and could be a natural source of antioxidants [8]. The research on phenolic compounds has been growing lately because of the increasing worldwide demand for phenolic compounds and its increasing application in the food industry [9]. Extraction is the first step in the isolation of phenolic compounds from plant materials. Traditional methods, such as soxhlet extraction, which have been used for many decades, are very time-consuming and require relatively large quantities of solvents. There is an increasing demand for new extraction techniques with shortened extraction time, reduced organic solvent consumption, and increased pollution prevention. Novel extraction methods including ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), supercritical fluid extraction (SFE) and accelerated solvent extraction (ASE) are fast and efficient for extracting chemicals from solid plant matrixes [10].

Each vegetable material has its unique properties in terms of phenolic extraction. Thus, it is important to

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develop an optimal extraction method. Classical optimization studies use the one-factor-at-a-time approach; in which only one factor is variable at a time while all others are kept constant. This approach is time-consuming and expensive. In addition, possible interaction effects between variables cannot be evaluated and misleading conclusions may be drawn. The response surface methodology (RSM) can overcome these difficulties, since it allows accounting for possible interaction effects between variables. If adequately used, this powerful tool can provide the optimal conditions that improve a process [11].

The objective of this study was as follow: to determine the optimal ultrasound-assisted extraction (UAE) conditions for phenolic compound extraction from jujube by maximization of the yield in the total phenolics (TP).

II. MATERIALS AND METHODS

A. Plant material and chemicals

Samples were dried and ground, and then a fraction that was sieved through a 10-mesh sieve and retained on a 40-mesh sieve was selected and stored in a freezer at −20 °C until extraction. All chemicals were of analytical grade and obtained from Merck (Darmstadt, Germany) and used without further purification

B. Chemicals

All chemicals were of analytical grade and obtained from Merck (Darmstadt, Germany), Sigma-Aldrich Company Ltd. (Gillingham, UK) and used without further purification.

C. Ultrasound-assisted extraction

UAE was applied by means of a high intensity ultrasound probe system of 200 W and 24 kHz (model UP 200H, Dr.Hielscher GmbH, Germany) with a horn fitted of micro tip: 2 mm (S_2) which was immersed in a water bath in which a precipitate glass with the sample was placed (internal dimensions: 280:195:135 mm). Amplitude of ultrasonic vibrations was 100% of nominal power (maximum amplitude of 260 μm) and acoustic power of 0.171402 W and intensity of 21.8346 W/cm^2 .The ultrasonic intensity was determined calorimetrically by measuring the time–temperature increase of the suspension under adiabatic conditions [12]. The UAE procedure was used for the extraction of TP from samples according to the experimental design (for optimization).

D. Determination of total phenolics

The TP was determined by the Folin-Ciocalteu colorimetric method [13]. For this, 100μl of extract having concentration of 2000 ppm was poured in test tube and then 500 μl of diluted solution of Folin-Ciocalteu reagent (1:10 ratio) was added. One minute later, 1.5 ml of 20% sodium carbonate was added and stirred. The mixture contained in the test tube was kept for 2h in darkness and room temperature and after that, absorption rate of the solution in wave length of 760nm was determined. Measurements were carried out in triplicate and calculations were based on a calibration curve obtained with gallic acid (Sigma, St. Louis, MO). TPC were expressed as mg of gallic acid equivalents (GAE) per g of dry matter.

E. Experimental design

Optimization of extraction conditions of phenolics from jujube was carried out using RSM [14]. Box-Behnken design consisting of twenty eight experimental runs was employed including four replicates at the center point. The all runs were carried out in triplicate. The effects of unexplained variability in the observed response due to extraneous factors were minimized by randomizing the order of experiments. The design variables were the extraction temperature (X_1, °C), concentration ($X_{2,}$ %), sound intensity ($X_{3,\ \%}$) and time ($X_{4,}$ min) while dependent variable was TP.

The response surface regression (RSREG) procedure of statistical analysis system (SAS) and Design-Expert 7 software were used to analyze the experimental data. Experimental data were fitted to a second-order polynomial model and regression coefficients obtained. The generalized second-order polynomial model used in the response surface analysis was as follows:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{\substack{i=1 \\ i<j}}^{k-1} \sum_{j=2}^{k} \beta_{ji} X_i X_j$$

Where β_0, β_i, β_{ii}, and β_{ij} are the regression coefficients for intercept, linear, quadratic and interaction terms, respectively, and X_i, and X_j are the independent variables. The Design-Expert 7 software was used to generate response surfaces and contour plots while holding a variable constant in the second-order polynomial model.

III. RESULTS AND DISCUSSIONS

A. Fitting the model

The three factors and lower, middle and upper design points for RSM in coded and actual/uncoded values are shown in Table 1.

Table 1. Independent variables and their coded and actual values used for optimization.

Independent variable	Symbol	Coded level		
		-1	0	+1
Temperature	X1	20	35	50
Solvent composition	X2	40	60	80
Intensity	X3	60	80	100
Time	X4	20	35	50

The experimental design employed with the observed data for all runs are reported in Table 2.

Multiple regression equations were generated as a function of the independent variables and their coefficients. The results of ANOVA of the independent variables for the quadratic polynomial model of UAE are shown in Table 3.

The model was adequate and explained most of the variability for method. The factors of temperature, solvent composition, intensity and time showed significant effects on the extraction of TP ($p<0.01$) (Table 3).

Table 2. The experimental design employed with the observed data

Runs	Temperature	Solvent composition	Intensity	Time	Total phenolic
1	50	60	100	35	16.43273
2	35	80	100	35	17.20545
3	35	80	80	50	15.02364
4	35	60	60	50	10.02364
5	35	60	80	35	11.34182
6	35	80	60	35	11.18345
7	50	60	60	35	10.41455
8	20	40	80	35	8.869091
9	35	60	100	20	12.20545
10	20	60	60	35	8.805455
11	35	40	60	35	6.669091
12	35	60	80	35	11.30545
13	35	60	80	35	11.28727
14	20	60	80	20	9.687273
15	35	60	80	35	11.35091
16	50	80	80	35	15.96406
17	50	60	80	50	14.42085
18	20	60	100	35	12.03927
19	35	40	80	20	9.450909
20	35	40	80	50	7.711121
21	35	80	80	20	12.25091
22	35	60	80	35	11.36
23	35	60	60	20	9.16
24	20	80	80	35	11.92364
25	50	60	80	20	11.46909
26	20	60	80	50	11.11455
27	35	60	100	50	15.82364
28	50	40	80	35	10.91142

The estimated effects of each variable as well as their interactions on extracted phenolic compounds are shown in Table 3. Multiple linear regressions using the second-order polynomial model (Eq. (1)) were performed on the results of Table 3.

$$TP\ (\text{mg GAE/g d.m.}) = 128.2796 + 35.93552{*}X1 + 55.89339{*}X2 + 58.56421{*}X3 + 22.47135{*}X4 + 10.81161{*}X1^2 + 18.06986{*}X1X2 + 23.51455{*}X1X3 + 11.686{*}X1X4 + 6.359642{*}X2^2 + 20.81889{*}X2X3 + 26.37871{*}X2X4 + 14.56718{*}X3^2 + 21.1577{*}X3X4 \quad (1)$$

Regression coefficients for intercept, linear, quadratic, and interaction terms are coded values. Good fits were achieved and most of the responses' variability was explained by the model, the coefficients of multiple determination (R^2) being 98.31 for TP. The closer the value of R^2 to unity, the better the empirical models fits the actual data. On the other hand, the smaller the value of R^2 the less relevance the dependent variables in the model have in explaining the behavior of variations [15-16].

Table 3. Regression coefficients of predicted quadratic polynomial models for total phenolic (TP)

Coefficient	Value
β_0	128.2796***
Linear	
β_1	35.93552***
β_2	55.89339***
β_3	58.56421***
β_4	22.47135***
Quadratic	
β_{11}	10.81161***
β_{22}	6.359642***
β_{33}	14.56718***
β_{44}	-0.11019ns
Crossproduct	
β_{12}	0.060233***
β_{13}	0.078382***
β_{14}	0.051938***
β_{23}	0.052047***
β_{24}	0.087929***
β_{34}	0.070526***
R^{2a}	98.31

[a] Coefficient of multipledetermination., * Significant at 5%., ** Significant at 1%. *** Significant at 0.1%.

Analysis of variance (ANOVA) showed that the selected quadratic models adequately represented the data obtained for TP in relation to the average response. The lack of fit testing was used to verify the adequacy of the fit [14]. ANOVA for the lack of fit test did not show inadequacy of the model with regard to TP ($p > 0.05$), indicating that the model could adequately fit the experimental data.

B. Analysis of response surfaces

The effect of different extraction conditions on the TP are reported (Table 3) by the coefficient of the second order polynomials. Response surface were used to illustrate the effect of extraction temperature, solvent composition, intensity and time on the responses.

Fig. 1 shows the response surface for the effect of the independent variables on the TP. As shown in Table 3, the linear, quadratic and interaction effects of temperature ($p < 0.001$) were positive, which explained the observed nature of the curve as shown in Fig. 1. Other factors that contribute to the TP include the linear effects ($p < 0.001$) of solvent composition, intensity and time its interaction effects ($p < 0.001$) together (Table 3). The TP was linearly related to the extraction time and the quadratic term was found to be not significant, which resulted in a linear increase in yield with time (Fig. 1). Fig.1 indicates that a long time, a high extraction temperature, high intensity and high solvent composition of ethanol were necessary for high TP. Therefore, the optimum area of temperature, solvent composition, sound intensity and time for the desired TP were 40-50 ^{0}C, 70-80%, 85-100% and 35-50 min, respectively.

Table 4. Comparison between predicted and experimental values of the response variable in UAE method.

Method	Independent variables				Predicted value	Observed value
	T(°C)	Solvent composition (%)	Intensity(%)	Time(min)		
UAE	48	72	96	48	15.8	16.0

Some studies have demonstrated that UAE could be used as a useful method for extraction of organic compounds [9, 17-18]. Sound waves can create bubbles in a liquid and produce negative pressure. The bubbles form, grow, and finally collapse. Close to a solid boundary, cavity collapse is asymmetric and produces high-speed jets of liquid. The liquid jets have strong impact on the solid surface. Therefore, it can increase extraction rate [19]. Also, ultrasound waves can increase mass transfer of some compounds because of increasing cell wall permeability [20].

C. Determination and Experimental Validation of the Optimal Conditions

In order to verify the predictive capacity of the model, an optimum condition was determined using the simple method and the maximum desirability for the phenolic contents extraction, (Table 4). The measured values lay within a 95% mean confidence interval of the predicted value for TP for the UAE. These results confirm the predictability of the model for the extraction of phenolics from jujube in the employed experimental conditions. From a technological point of view, other conditions giving results close to those obtained for the optimum are desirable. This is particularly important when there are some drawbacks related to the process, such as the use of a high quantity of solvent, or problems with the degradation of phenolics at high temperatures. In Fig. 1 the darkest regions could be explored for this purpose.

IV. CONCLUSIONS

The high correlation of the model exhibited that: second-order polynomial model could be used to optimize extraction of phenolic compounds from jujube by UAE method for maximizing the yield of total phenolic compound. Intensity, solvent composition, temperature and time were found to be most effective in extracting phenolic compounds in UAE method, respectively. Hence, the optimum conditions for extraction of phenolics from jujube by UAE method were 48 ^{0}C, solvent composition of 72%, sound intensity of 96% and 48 min. Under optimized conditions the experimental values agreed with the values predicted by models.

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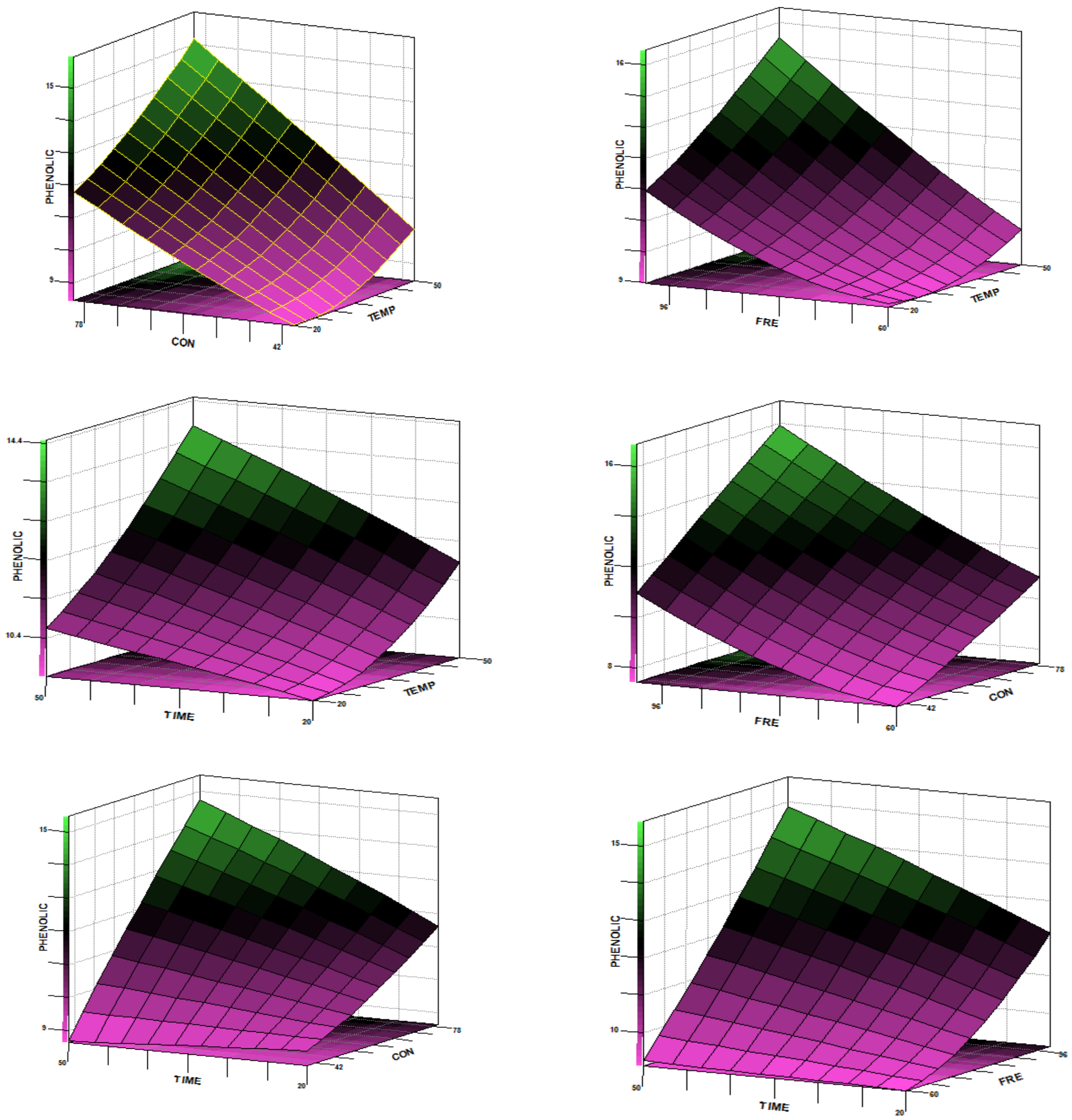

Figure 1. Response surface for the effects of extraction conditions on the extraction yield of phenolic compounds from jujube by UAE. The value of the missing independent variable in each plot kept at the center point.