

## Role of Extraction Conditions in the Recovery of Some Phytochemical Compounds of the Jujube Fruit

N. Shams Najafabadi<sup>1</sup>, M. A. Sahari<sup>1\*</sup>, M. Barzegar<sup>1</sup>, and Z. Hamidi Esfahani<sup>1</sup>

### ABSTRACT

In this study, the combined effects of various experimental parameters (solvent concentration, extraction temperature, pH, extraction time, and light conditions) on the recovery of phytochemical compounds from the jujube (*Ziziphus jujuba* var *vulgaris*) fruit were investigated in a batch system using a 2<sup>5</sup> full-factorial design. The independent variables were coded at two levels and their actual values were selected based on the results of single-factor experiments. Total Phenolic Content (TPC), Total Monomeric Anthocyanin Content (TMAC), and vitamin C content values were used for the determination of phytochemical compound content in jujube extract. The results showed that pH, extraction temperature, and solvent concentration were the most significant ( $P < 0.05$ ) factors affecting the TPC, TMAC, and vitamin C content. The optimal extraction conditions of phytochemical compounds were found to be as follows: ethanol concentration of 60%, pH of 3, extraction time of 180 min, extraction temperature of 25°C, and absence of light. In the optimized conditions, the maximum experimental values for TPC, TMAC, and vitamin C content were 164.51 mg gallic-acid equivalents per gram of dry weight (mg GAE g<sup>-1</sup> DW), 52.94 mg cy-3-glu 100 g<sup>-1</sup> DW, and 137.12 mg L-AA 100 g<sup>-1</sup> DW, respectively. The high content of phytochemical compounds in the jujube extract indicates that jujube extract might be considered as a potential source of nutraceuticals in the future.

**Keywords:** Herbal medicines, Nutraceuticals, *Ziziphus jujuba* var *vulgaris*.

### INTRODUCTION

The jujube plant (*Ziziphus jujuba* Mill.) from the Rhamnaceae family is extensively grown throughout the tropical and subtropical areas of the world, especially in Asia, the Americas, and the Mediterranean region (Kou *et al.*, 2015). Due to its multi-functionality and nutritional benefit in the human diet, the jujube fruit has gained popularity in recent years. This fruit is rich in vitamin C and other biochemicals, particularly phenolic compound, flavonoids, anthocyanins, and organic acids, which have been reported to reduce disease risk (Wu *et al.*, 2012; Gao *et al.*, 2011). In general, malic, citric, succinic and ascorbic acids

were found to be the most predominant organic acids in the jujube fruit. Moreover, cyanidin-3, 5-diglucoside was determined as the major anthocyanin in jujube extract (Shams Najafabadi *et al.*, 2017a). Diseases have been treated with herbal medicines throughout the duration of human civilization. Even today, plant materials continue to play a major role in primary healthcare and some plants have been shown to be potential sources of new anti-microbial agents (Nazni and Mythili, 2013).

Extraction is the most important process in recovering phytochemical compounds from the plant material (González and González, 2010; Shams Najafabadi *et al.*, 2017b). A few factors, including extraction methods,

<sup>1</sup> Department of Food Science and Technology, Faculty of Agriculture, Tarbiat Modares University, Tehran, Islamic Republic of Iran.

\*Corresponding author. e-mail: sahari@modares.ac.ir

type of solvent, sample-solvent ratio, temperature, pH, extraction time, etc., influence the rate of extraction and quality of extracted phytochemical compounds (Dorta *et al.*, 2012). The optimization of extraction conditions can be achieved by either empirical or statistical methods and is essential for the commercial application of the phytochemical compound extraction process (Hismath *et al.*, 2011). Extraction procedures depend on plant materials, which have different matrices and unique properties in terms of structure and composition (related to species, varieties, ripening stages, etc.). Therefore, considerable caution should be exercised when using procedures that have been developed for specific plant tissue types and phytochemical extraction conditions should be optimized for each plant material (González and González, 2010; Chew *et al.*, 2011).

There are very few studies on the optimization of extraction conditions of phytochemical compounds from the jujube (*Ziziphus jujuba* var *vulgaris*) fruit. Therefore, the objective of the present study was to investigate the best extraction conditions to maximize the recovery of phytochemical compounds from the jujube fruit using  $2^5$  full-factorial design.

## MATERIALS AND METHODS

### Plant Materials

About 10 kg of sweet-mature jujube fruit (*Ziziphus jujuba* var *vulgaris*) was obtained from South Khorasan Agricultural and Natural Resources Research and Training Center, Iran. The pieces of fruit were washed in cold tap water and their stones were manually removed. Then, they were dried in a hot-air oven (Memmert, Germany) at 40°C for eight hours. The initial moisture content of jujube fruit was found to be 47.51%, which was reduced to 3.46% by drying. Then, using a mill, a fine powder was obtained (Moulinex, Type DPA1,

CMMF 800W, France), which was passed through a 30-mesh sieve.

### Preparation of Extracts

A total of 1 g of powdered jujube fruit was extracted with 20 mL of extraction solvent in a 100 mL conical glass flask. The conical flask was sealed with parafilm and wrapped with aluminum foil to prevent solvent loss and exposure to light. Moreover, 0.05% HCl was used to adjust the pH of the extracts. The mixture was then shaken in a shaking machine (IKA®, KS 4000i Control, India) at a constant speed of 180 rpm at the required temperature throughout the extraction process. After the extraction process was completed, the jujube fruit extract was filtered through Whatman Filter No.1 in order to obtain a clear extract. Subsequently, the filtrate was subjected to analysis without overnight storage. All extraction processes were done in duplicate and all analyses on each sample were done in triplicate.

### Experiment Design

The experimental design for this study was divided into two major parts. First, the influence of different independent variables (e.g. solvent concentration, pH, extraction time, extraction temperature, and light conditions) was investigated using single-factor experiments to select the significant variables and to determine a preliminary range for each variable. Second, the optimization of these five variables for the extraction of Total Phenolic Content (TPC), Total Monomeric Anthocyanin Content (TMAC), and vitamin C content (L-AA) from the jujube fruit was studied using a  $2^5$  full-factorial design.

### Single-Factor Experiments

(a) Ethanol and water were selected as extraction solvent in the present study. The

samples were extracted with ethanol concentration ranging from 20 to 100% by fixing the pH, extraction time, and extraction temperature at 5, 90 minutes, and 25°C, respectively. The best solvent concentration was selected according to the value of TPC (mg GAE g<sup>-1</sup> DW).

(b) Using the best extraction solvent selected through the first single-factor experiment, by fixing extraction time (90 minutes) and extraction temperature (25°C), samples were extracted with pH ranging from 1.5 to 7. The extraction procedure is described in the solvent extraction section. The best pH was selected according to the value of TPC (mg GAE g<sup>-1</sup> DW).

(c) Samples were extracted using the best solvent concentration and the best pH value determined by the first two single-factor experiments. The extraction procedure was repeated, as described in the section on single-factor experiments, by varying the extraction time from 30 to 360 minutes while keeping the extraction temperature constant at 25°C. The best extraction time was selected according to the value of TPC (mg GAE g<sup>-1</sup> DW).

(d) Using the best solvent concentration and the best pH selected in the aforementioned single-factor experiments, samples were extracted at various extraction temperatures ranging from 25 to 55°C at the optimum time determined in the single-factor experiment mentioned in section (c). The extraction procedure was repeated, as described in the solvent extraction section. The best extraction temperature was selected according to the values of TPC (mg GAE g<sup>-1</sup> DW). Based on the results of the single-factor experiment, ranges of the four factors (solvent concentration, pH, extraction time,

and extraction temperature) were determined for the 2<sup>5</sup> full-factorial design. The results of the single-factor experiments were analyzed using SPSS software (Ver. 20; SPSS Inst., Cary, NC, USA). All data are shown as the mean±Standard Deviation (SD) of three replicates. Means were compared by the one-way Analysis Of Variance (ANOVA), followed by Duncan's test (P< 0.05).

### Full-Factorial Experiments

In order to evaluate the effect of the interaction of the various independent parameters (solvent concentration, pH, extraction time, extraction temperature, and light conditions) on TPC, TMAC, and vitamin C content recovery, a full-factorial design with five parameters at two levels was applied, as shown in Table 1. The design consisted of 32 experimental points, including full 2<sup>5</sup> factorial points and two replicates at the center point. The experiments were carried out in random order to avoid any systematic error in the experimental data. Each experiment was repeated three times. The statistical software Design Expert (Version 7.0.0, Stat-Ease, Inc., Minneapolis, MN, USA) was used to analyze the experiment data.

### Extract Characterization

TPC was determined using a spectrophotometer (carry 60, Agilent, Us) by the Folin–Ciocalteu colorimetric method (Yang *et al.*, 2010). In brief, 20 µL of aqueous-ethanolic extract was mixed first with 1.58 mL of distilled water and then with 100 µL of Folin–Ciocalteu reagent, and 300 µL of

**Table 1.** Levels and units of parameters in full-factorial design.

| No run | Parameters            | Unit | Level 1  | Level 2 |
|--------|-----------------------|------|----------|---------|
| 1      | pH                    | -    | 3        | 5       |
| 2      | Solvent concentration | %    | 40       | 60      |
| 3      | Time                  | Min  | 90       | 180     |
| 4      | Temperature           | °C   | 25       | 35      |
| 5      | Light conditions      | -    | Presence | Absence |

saturated  $\text{Na}_2\text{CO}_3$  (20%) was added. After the mixture stood for 30 minutes at  $40^\circ\text{C}$ , the absorbance was measured to be 765 nm. The standard curve of the absorbance of gallic acid was used and the results were reported as mg gallic-acid equivalents per gram of dry weight of the extract ( $\text{mg GAE g}^{-1}\text{ DW}$ ).

TMAC was estimated by the pH-differential method (Cheok *et al.*, 2013). Two buffer systems were used: 0.025M potassium chloride buffer (pH 1.0) and 0.4 M sodium acetate buffer (pH 4.5). A total of 200  $\mu\text{L}$  of the aqueous-ethanolic extract was mixed with 1.8 mL of either potassium chloride or sodium acetate buffer. The absorbance values of samples were found to be 510 and 700 nm using the following equation:

$$A = (A_{510\text{ nm}} - A_{700\text{ nm}}) \text{pH}_{1.0} - (A_{510\text{ nm}} - A_{700\text{ nm}}) \text{pH}_{4.5} \quad (1)$$

The results were reported as mg of cyaniding-3- glucoside equivalents per 100 g dry weight of extract ( $\text{mg cy-3-g } 100\text{ g}^{-1}\text{ DW}$ ) using a molar absorptive coefficient ( $\epsilon$ ) of  $26,900\text{ L mol}^{-1}\text{ cm}^{-1}$ , Molecular Weight (MW) of  $449.2\text{ g mol}^{-1}$ , Dilution Factor (DF), Absorption value (A), and the cell path Length (L) of 1 cm in the following equation:

$$\text{Monomeric anthocyanin pigment (mg L}^{-1}\text{)} = A \times \text{MW} \times \text{DF} \times 1000 / (\epsilon \times L) \quad (2)$$

Vitamin C content was determined by the 2,6 dichlorophenol indophenol titration method. A sample solution equivalent to 0.2 mg ascorbic acid  $\text{mL}^{-1}$  was prepared in water containing 3% (w/v) metaphosphoric acid. It was titrated against standard 2,6 dichlorophenol indophenol solution of 0.5 mg  $\text{mL}^{-1}$  concentration until the pink color developed completely (Nazni and Mythili, 2013). The results were reported as mg L-ascorbic acid equivalents per 100 g dry weight of the extract ( $\text{mg L-AA } 100\text{ g}^{-1}\text{ DW}$ ).

## RESULTS AND DISCUSSION

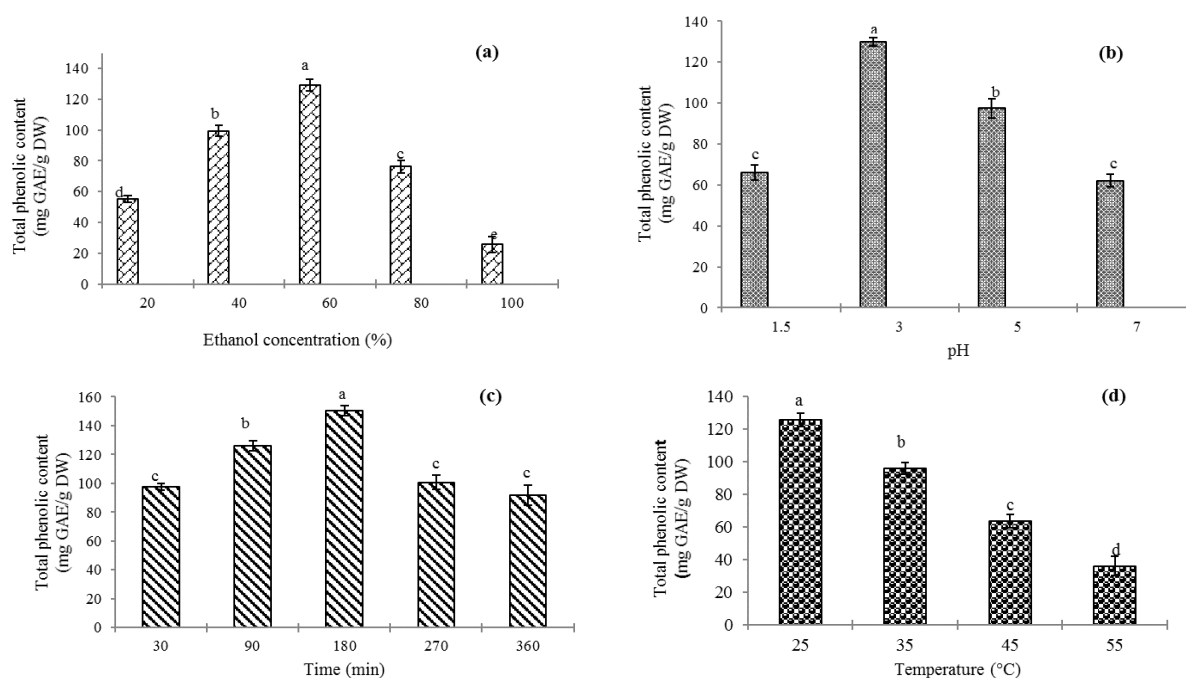
### Extraction Solvent Concentration Evaluation

The selection of extraction solvent is critical for the complex food samples as it

determines the amount and type of phenolic compounds that can be extracted. Aqueous alcohols—particularly ethanol, acetone, and methanol—are most commonly employed in phenolic extraction from botanical materials (González-Montelongo *et al.*, 2010). Ethanol and water were selected as extraction solvents in this study because they are safer and less toxic than other organic solvents (Chew *et al.*, 2011). Figure 1 (a) shows that ethanol concentration has a significant effect ( $P < 0.05$ ) on the TPC of the jujube extract. TPC increases with the increase in the ethanol concentration up to 60% ( $129.21\text{ mg GAE g}^{-1}\text{ DW}$ ), followed by a reduction, until it reaches a minimum of  $25.67\text{ mg GAE g}^{-1}\text{ DW}$  at 100%. Hence, we propose that most of the phenolic compounds in the jujube fruit have a moderately polar characteristic. Similarly, Chew *et al.* (2011) revealed that maximum amount of total phenolics in *Centella asiatica* extracts was obtained at about 60% ethanol, followed by a decrease with further increase in concentration. Uma *et al.* (2010) also report that increasing the acetone concentration beyond 60% dramatically reduces the amount of phenolics extracted from henna (*Lawsonia inermis*) leaves. A remarkable drop in TPC at 100% ethanol indicates that absolute solvents do not ensure a good recovery of phenolic compounds as compared to aqueous ethanol. Thus, moderate ethanol concentration values of 40 and 60%, respectively, were selected as the lower and upper levels to be employed in full-factorial optimization.

### Extraction pH Evaluation

Concerning the recovery of phenolic compounds, pH can act according to different mechanisms and plays a significant role in the extraction procedure. The pH modification (addition of acid to the extraction media) is frequent in the case of polyphenol recovery and offers some advantages such as increased phenol stability, increased dissolution of phenolic



**Figure 1.** Effect of: (a) Ethanol concentration; (b) pH; (c) Extraction time, and (d) Extraction temperature on TPC of jujube extract. Values are mean $\pm$ SD (n= 3). Values marked by different letters are significantly different (P< 0.05).

compounds, increased disintegration of cell walls, facilitation of phenolic compounds solubilization, and diffusion from the plant material (Friedman and Jürgens, 2000). Figure 1 (b) indicates that pH up to 3 causes a significant increase in the TPC, but a significant (P< 0.05) decrease is observed in this parameter with the increase of pH up to 7. The jujube extracts obtained with pH 3 and 5 had the highest TPC (129.89 and 97.56 mg GAE g<sup>-1</sup> DW, respectively) among all samples. Based on the present research findings, pH 3 and 5 values were selected for full-factorial optimization.

### Extraction Time Evaluation

Extraction time is a crucial factor for minimizing energy-usage and cost of the extraction process. The extraction time ranges from a few minutes to up to 24 hours (Lee *et al.*, 2005). In this study, the range of extraction time was designed on the basis of practical and economical aspects. Figure 1 (c) shows that an increase in extraction time

from 30 to 180 minutes is accompanied by a small increase in TPC from 97.76 to 150.51 mg GAE g<sup>-1</sup> DW. Beyond 180 minutes, further increase in process duration did not significantly (P> 0.05) increase the TPC. This phenomenon can be explained by Fick's second law of diffusion, which states that the final equilibrium is attained between the solution concentrations in the solid matrix and solvent after a particular duration. Hence, an excessive extraction time is not useful for extracting more phenolic compounds (Silva *et al.*, 2007). Furthermore, a prolonged extraction process may lead to phenolics oxidation due to exposure to light and oxygen. Taking into account these facts, extraction time periods of 90 and 180 minutes were selected for full-factorial optimization.

### Extraction Temperature Evaluation

The selection of an appropriate extraction temperature was the final step in a series of single-factor experiments. The TPC of

jujube extract decreased slightly when extraction temperature was increased from 25 to 55°C, as shown in Figure 1 (d). This result is in accordance with the study by Chan *et al.* (2009), who reports that temperature shows significant ( $P < 0.05$ ) effect on the TPC of neem leaves in an acetone aqueous system. In general, increasing the temperature beyond certain values may encourage the concurrent decomposition of the phenolic compounds that had already been mobilized at lower temperature, or even the breakdown of phenolics still remaining in the plant matrix. Additionally, high temperature may encourage solvent loss through vaporization and increase the cost of the extraction process from the industrial point of view (Chew *et al.*, 2011). Therefore, moderate extraction temperatures of 25 and 35°C, respectively, were chosen as the lower and upper levels to be applied in full-factorial optimization.

### Full-Factorial Experiments

Based on the observations from single-factor experiments, the minimum and maximum levels of solvent concentration, pH, extraction time, and extraction temperature that influences the TPC were chosen (Table 1). In the present investigation, the  $2^5$  full-factorial experimental design was formulated to optimize the extraction parameters and examine the interaction of different associated parameters responsible for obtaining high levels of TPC, TMAC, and vitamin C content in jujube extract (Table 2).

#### Effects of Extraction Factors on TPC of Jujube Extract

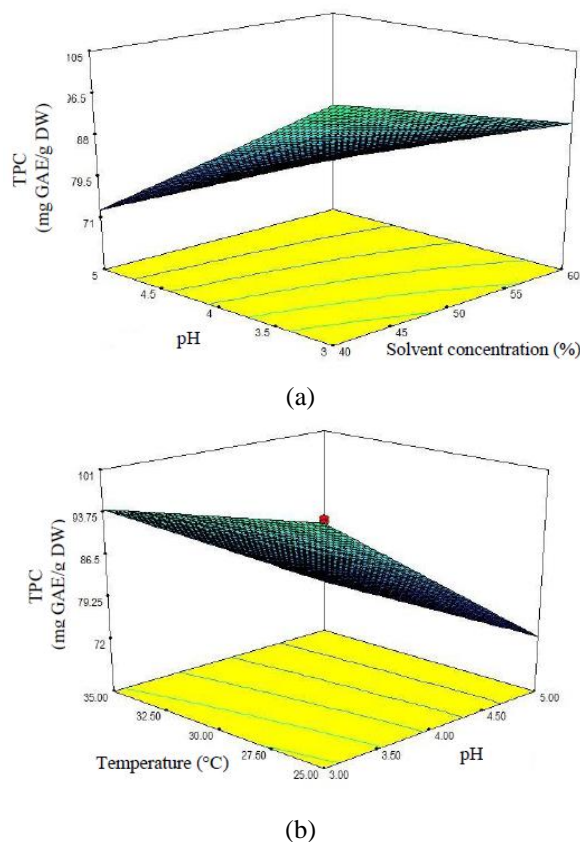
The TPC values of the jujube extract were determined by 32 experimental runs generated by the central composite design, ranging from 70.16 to 164.51 mg GAE g<sup>-1</sup>

DW (Table 2). The minimum and maximum values of the TPC of jujube extract were observed in Run 3 and Run 20, respectively. The P-values (Table 3) were used as a tool for checking the significance of each parameter, which in turn might indicate the interaction patterns between the variables (Hou and Chen, 2008). ANOVA indicated that all parameters had significant ( $P < 0.05$ ) effects on the TPC of jujube extract (Table 3). Among all the five extraction parameters studied, pH, solvent concentration, and extraction temperature were found to play the most critical role in the extraction of phenolic compounds from jujube fruit. Figure 2 illustrates three-dimensional plots by presenting the response as a function of two factors and keeping the other constant at its middle level. Each figure reveals the effects of the selected parameters on TPC. Figure 2 (a) depicts a higher recovery of phenolic content at pH 3 and ethanol concentration of 60%. Both pH and solvent concentration showed significant ( $P < 0.05$ ) effects on TPC (Table 3). The increase in pH up to 5 during extraction resulted in the simultaneous decrease in TPC. Moreover, the TPC value gradually mounted with the increase in ethanol concentration and achieved an optimum value at about 60%, before it began to decrease. Figure 2 (b) denotes the effects of the interaction of the pH and extraction temperature parameters on TPC. Extraction temperature has a significant ( $P < 0.05$ ) influence on TPC (Table 3). At the lower and upper levels of pH, the increase in extraction temperature up to 35°C leads to the deceleration of phenolic compound extraction. However, the extraction of phenolic compounds was observed to be positively influenced by the synergism between pH and extraction temperature ( $P < 0.05$ ). This implies that the extraction is largely favored in two cases: low extraction temperature in the presence of low pH and high extraction temperature in the presence of high pH. From the industrial point of view, low pH with low extraction temperature would be more suitable, as high extraction temperature

**Table 2.** Experimental results for responses (TPC, TMAC, and vitamin C content) according to 2<sup>5</sup> full-factorial design.<sup>a</sup>

| No run          | Solvent concentration (%) | pH | Temperature (°C) | Time (min) | Light conditions | TPC (mg GAE.g <sup>-1</sup> DW) | TMAC (mg cy-3-glu 100 g <sup>-1</sup> DW) | Vitamin C (mg L-AA 100 g <sup>-1</sup> DW) |
|-----------------|---------------------------|----|------------------|------------|------------------|---------------------------------|---|--|
| 1               | 40                        | 5  | 35               | 90         | A                | 78.21±3.31                      | 28.59±1.65                                | 75.25±3.87                                 |
| 2               | 40                        | 3  | 35               | 180        | P                | 80.34±4.45                      | 35.21±2.11                                | 95.51±4.66                                 |
| 3               | 40                        | 5  | 35               | 180        | P                | 70.16±3.21                      | 25.33±1.09                                | 65.53±2.88                                 |
| 4               | 60                        | 3  | 25               | 90         | A                | 135.41±6.89                     | 48.11±2.44                                | 132.24±5.92                                |
| 5               | 60                        | 5  | 25               | 180        | P                | 71.52±3.55                      | 25.12±1.31                                | 67.33±2.98                                 |
| 6               | 60                        | 5  | 35               | 180        | A                | 79.12±3.01                      | 29.54±1.47                                | 78.38±3.58                                 |
| 7               | 60                        | 5  | 35               | 90         | P                | 70.31±3.06                      | 24.95±1.15                                | 64.91±2.55                                 |
| 8               | 40                        | 5  | 25               | 180        | A                | 78.21±3.66                      | 28.22±1.43                                | 75.76±3.23                                 |
| 9               | 40                        | 5  | 35               | 90         | P                | 70.52±2.98                      | 24.11±1.62                                | 63.61±2.48                                 |
| 10              | 60                        | 5  | 25               | 90         | A                | 79.58±3.86                      | 29.13±1.33                                | 79.86±3.32                                 |
| 11              | 60                        | 3  | 25               | 180        | P                | 80.52±4.12                      | 35.21±2.65                                | 95.54±4.98                                 |
| 12              | 40                        | 3  | 35               | 90         | A                | 95.81±4.57                      | 44.29±2.21                                | 106.92±5.11                                |
| 13              | 40                        | 3  | 25               | 180        | A                | 100.11±4.88                     | 45.81±2.48                                | 109.98±5.27                                |
| 14              | 60                        | 3  | 35               | 180        | A                | 132.22±5.45                     | 47.12±2.44                                | 125.13±5.67                                |
| 15              | 40                        | 3  | 25               | 90         | P                | 98.52±4.11                      | 37.93±2.31                                | 99.13±4.77                                 |
| 16              | 60                        | 3  | 35               | 90         | P                | 79.12±3.61                      | 34.44±2.36                                | 91.35±4.34                                 |
| 17              | 60                        | 3  | 35               | 90         | A                | 135.91±5.21                     | 47.01±2.46                                | 124.99±5.18                                |
| 18              | 60                        | 5  | 35               | 90         | A                | 75.19±3.01                      | 27.01±1.24                                | 73.51±3.02                                 |
| 19              | 40                        | 3  | 35               | 90         | P                | 90.23±4.22                      | 41.01±1.85                                | 99.53±4.56                                 |
| 20 <sup>b</sup> | 60                        | 3  | 25               | 180        | A                | 164.51±6.67                     | 52.94±2.69                                | 137.12±5.46                                |
| 21              | 60                        | 5  | 25               | 180        | A                | 76.91±3.86                      | 28.01±1.56                                | 74.51±3.27                                 |
| 22              | 40                        | 5  | 25               | 180        | P                | 73.02±2.91                      | 25.10±1.31                                | 68.22±2.88                                 |
| 23              | 40                        | 3  | 25               | 90         | A                | 106.25±5.34                     | 48.94±1.99                                | 107.22±4.87                                |
| 24              | 60                        | 3  | 35               | 180        | P                | 127.22±5.31                     | 44.51±1.67                                | 118.17±5.88                                |
| 25              | 60                        | 5  | 35               | 180        | P                | 74.15±2.36                      | 26.22±1.44                                | 71.32±2.49                                 |
| 26              | 40                        | 3  | 25               | 180        | P                | 93.25±3.88                      | 40.21±0.97                                | 102.32±4.61                                |
| 27              | 60                        | 3  | 25               | 90         | P                | 130.02±5.12                     | 45.11±1.33                                | 125.01±5.41                                |
| 28              | 60                        | 5  | 25               | 90         | P                | 74.05±2.46                      | 26.12±1.77                                | 72.32±2.56                                 |
| 29              | 40                        | 5  | 35               | 180        | A                | 75.31±2.77                      | 28.44±1.56                                | 72.81±2.67                                 |
| 30              | 40                        | 5  | 35               | 90         | P                | 73.01±2.69                      | 25.10±1.61                                | 68.35±2.48                                 |
| 31              | 50                        | 4  | 30               | 135        | A                | 98.55±3.45                      | 33.12±1.66                                | 85.32±2.31                                 |
| 32              | 50                        | 4  | 30               | 135        | P                | 92.14±2.99                      | 30.22±1.09                                | 78.43±2.99                                 |
| 33              | 40                        | 5  | 25               | 90         | A                | 78.92±2.81                      | 29.99±1.24                                | 70.88±2.36                                 |
| 34              | 40                        | 3  | 35               | 180        | A                | 88.20±3.15                      | 40.95±0.99                                | 98.23±4.45                                 |

<sup>a</sup> Data are given as mean±SD (n= 3). Light conditions: P= Presence of light, A= Absence of light. <sup>b</sup> Optimum conditions recovery maximum TPC, TMAC, and vitamin C content from jujube fruit using acidified ethanol aqueous solvent. No. run 31 and 32 were central point in full-factorial experiments.



**Figure 2.** Interaction three-dimensional plots for TPC recovery: (a) pH and solvent concentration, and (b) pH and extraction temperature.

would render the extraction procedure uneconomical. The extraction time had the least effect on the TPC of jujube extract among all parameters (Table 3). The present results are in accordance with the results obtained by Ruenroengklin *et al.* (2009) in their study of the extraction of phenolic compounds from the litchi fruit.

It must also be kept in mind that certain complications arise when recovering phytochemical compounds from plant by-products, due to their high enzyme activity. However, the process of drying the plant by-product before extraction, immediately immersing the by-product in methanol, and using an acid extraction medium protects the material from oxidation (Chew *et al.*, 2011). The pH of the water can determine the degree of solubility of water-soluble

compounds and influence the possible solubilization of the hydrolysable fraction. Other factors such as extraction temperature and time, the liquid-to-solid ratio, and the cultivar also affect extraction with solvents (Ng *et al.*, 2012).

#### Effects of Extraction Factors on TMAC of Jujube Extract

According to the analysis of results in Table 2, TMAC recovered from jujube extract reaches a maximum at 60% solvent concentration (v/v), pH of 3, extraction temperature of 25°C, extraction time of 180 minutes, and absence of light ( $52.94 \pm 2.69$  mg cy-3-glu  $100 \text{ g}^{-1}$  DW). ANOVA was applied to highlight the effects of different parameters on



the TMAC extraction process. Table 3 shows the ANOVA of five independent parameters for TMAC extraction. The larger is the magnitude of F-value, the smaller is the P-value, and the more significant is the corresponding parameter. From the ANOVA given in Table 3, it can be observed that the pH, extraction temperature, solvent concentration, and light conditions significantly ( $P < 0.05$ ) affect the TMAC of the extract. The most influential independent factor that affects TMAC is pH. The interactions between these five factors are also significant. The interactions between extraction temperature and pH have more significant results compared to other combination factors (Figure 3(a)). Moreover, the interactions between pH and solvent concentration also have a prominent effect on the TMAC of jujube fruit extract [Figure 3 (b)].

Anthocyanins are highly unstable and easily susceptible to degradation. Their color stability is strongly affected by pH, temperature, light, anthocyanin concentration, oxygen, enzymes, and other accompanying substances such as ascorbic acid, sugars, sulfites, copigments, and metallic ions, among others (Cavalcanti *et al.*,

2011). Evaluation of the factors that affect stability of anthocyanins indicates that pH is the most important extrinsic factor affecting anthocyanin degradation and a stabilizing agent commonly used in extraction processes (Castaneda-Ovando *et al.*, 2009). According to Tan *et al.* (2013) and Shi *et al.* (2003), heating might soften the cell membrane and weaken the bond between compounds, thus causing more cell compartments to spill out and transfer into the solvent. However, prolonged extraction time to temperature results in the decomposition of the desired product. Light affects anthocyanins in two different ways: light is essential for the biosynthesis of anthocyanins, but it also accelerates their degradation. Anthocyanins preserve their color much better when kept in the dark; the difference can be seen by comparing anthocyanin samples stored in light and in the dark at room temperature even for just 24 hours (Ruenroengklin *et al.*, 2008).

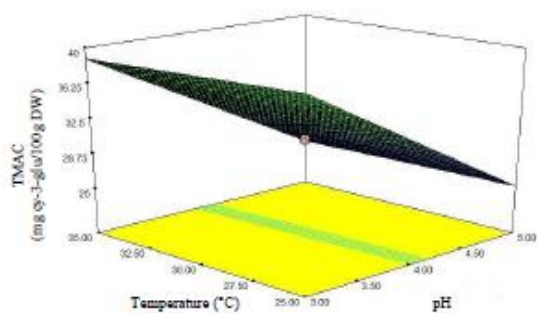
#### Effects of Extraction Factors on Vitamin C Content of Jujube Extract

The values of vitamin C content of jujube

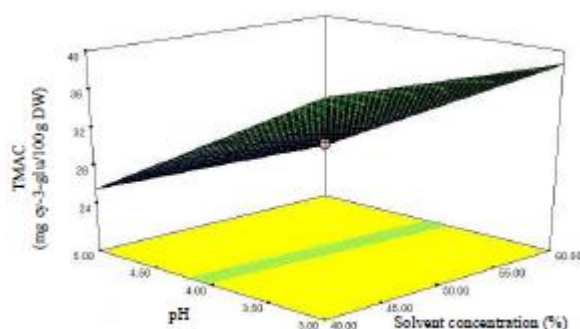
**Table 3.** ANOVA results for responses TPC, TMAC, and vitamin C content. <sup>a</sup>

| Responses              | Parameters                | DF | Mean square | F value  | P value  |
|------------------------|---------------------------|----|-------------|----------|----------|
| TPC <sup>a</sup>       | Solvent concentration (%) | 1  | 3469.50     | 26735.53 | < 0.0001 |
|                        | pH                        | 1  | 18031.12    | 39876.98 | < 0.0001 |
|                        | Temperature (°C)          | 1  | 3097.48     | 23868.75 | < 0.0001 |
|                        | Time (min)                | 1  | 2.64        | 20.66    | < 0.0001 |
|                        | Light conditions          | 1  | 451.88      | 348.14   | < 0.0001 |
| TMA <sup>b</sup>       | Solvent concentration (%) | 1  | 33.54       | 209.88   | < 0.0001 |
|                        | pH                        | 1  | 4097.76     | 25642.98 | < 0.0001 |
|                        | Temperature (°C)          | 1  | 495.79      | 3102.53  | < 0.0001 |
|                        | Time (min)                | 1  | 0.27        | 1.70     | < 0.2005 |
|                        | Light conditions          | 1  | 27.71       | 173.39   | < 0.0001 |
| Vitamin C <sup>c</sup> | Solvent concentration (%) | 1  | 1545.77     | 5512.24  | < 0.0001 |
|                        | pH                        | 1  | 24610.16    | 87760.36 | < 0.0001 |
|                        | Temperature (°C)          | 1  | 1831.09     | 6529.71  | < 0.0001 |
|                        | Time (min)                | 1  | 0.48        | 1.72     | < 0.1985 |
|                        | Light conditions          | 1  | 166.12      | 592.39   | < 0.0001 |

<sup>a</sup> DF= Degree of Freedom, P value < 0.05. <sup>a, b, c</sup> Values marked by different letters are significantly different ( $P < 0.05$ ).



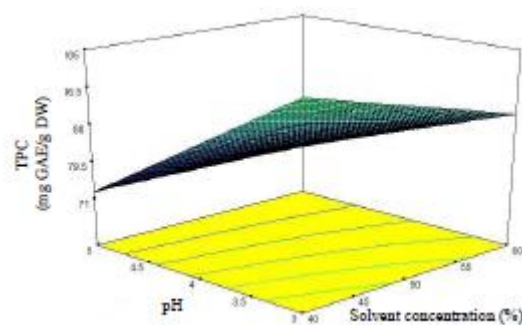
(a)



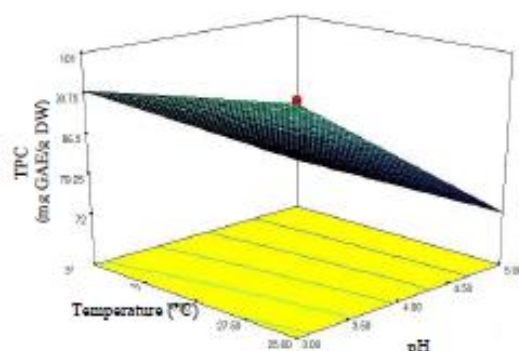
(b)

**Figure 3.** Interaction three-dimensional plots for TMAC recovery: (a) Temperature and pH, and (b) pH and solvent concentration.

fruit extract varied between 63.61 and 137.12 mg L-AA 100 g<sup>-1</sup> DW, from Run 9 to Run 20, respectively (Table 2). The highest vitamin C recovery rate of 137.12 mg L-AA 100 g<sup>-1</sup> DW jujube powder was achieved with 60% solvent concentration (v/v), pH of 3, extraction temperature of 25°C, extraction time of 180 minutes, and absence of light (Table 2). Statistical analysis showed that all parameters in Table 3 except extraction time had a significant effect ( $P < 0.05$ ) on the vitamin C content of jujube extract. According to the parameters in Table 3, pH, extraction temperature, and solvent concentration play the most critical role in the recovery of vitamin C content. Increase in pH from 3 to 5 in jujube extract results in a significant ( $P < 0.05$ ) decrease in the vitamin C content (Figures 4-a and -b). The results also indicated that the extracted vitamin C content decreased by increasing



(a)



(b)

**Figure 4.** Interaction three-dimensional plots for vitamin C content recovery: (a) Temperature extraction and pH, and (b) pH and solvent concentration.

the extraction temperature up to 35°C (Figure 4-a). Figure 4b shows that the combined effects of pH and solvent extraction on the vitamin C content are significant ( $P < 0.05$ ).

Stability is a key problem of vitamin C analysis since this compound is very unstable in aqueous solutions (Phillips *et al.*, 2010). Apart from lowered temperature, pH could also play a role in stabilizing L-AA during the period of study since this molecule is well preserved in acid solutions. Generally, acidic pH ( $\approx 3$ ) is useful for sample preparations as it ensures sufficient stability and recovery of L-AA in the extracts. In these conditions, L-AA exhibits higher stability ( $\text{pH} < \text{pK}_a$ ) and the formation of its oxidation products is not favored (Spínola *et al.*, 2013).

## CONCLUSIONS

The present study confirms the advantages of the full-factorial design in optimizing the conditions for extraction of phytochemical compounds from jujube fruit. In conclusion, TPC, TMAC, and vitamin C content of the jujube extract were most significantly affected ( $P < 0.05$ ) by the pH level, followed by extraction temperature and solvent concentration. With the application of the full-factorial design, the interaction effects among the extraction factors can be assessed. The optimum conditions for the maximum recovery of phytochemical compounds were as follows: ethanol concentration of 60%; pH of 3; extraction time of 180 minutes; extraction temperature of 25°C; and absence of light. In the aforementioned conditions, the maximum amounts of 164.51 mg GAE g<sup>-1</sup> DW for TPC, 52.94 mg cy-3-glu 100 g<sup>-1</sup> DW for TMAC, and 137.12 mg L-AA 100 g<sup>-1</sup> DW for vitamin C content could be extracted from the jujube fruit. Further works may be carried out under the optimum conditions to elucidate the identity of the phenolic compounds and anthocyanins responsible for the antioxidant properties of the jujube fruit. Moreover, jujube extract can be an economically interesting phytochemical source for the nutraceutical and functional food market.

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## نقش شرایط استخراج در بازیافت برخی ترکیبات فیتوشیمیایی میوه عناب

ن. شمس نجف آبادی، م. ع. سحری، م. برزگر، و ز. حمیدی اصفهانی

## چکیده

در این تحقیق، اثرات ترکیبی متغیرهای مختلف (غلظت حلال، دمای استخراج، pH، زمان استخراج و شرایط نوری) بر بازیافت ترکیبات شیمیایی میوه عناب (*Ziziphus jujuba var vulgaris*) با استفاده از طرح آماری فاکتوریل کامل مورد بررسی قرار گرفت. قبل از آن متغیرهای مستقل در دو سطح کدگذاری شدند و مقادیر آنها با استفاده از روش یک فاکتور در هر زمان انتخاب گردید. محتوای فنول کل، محتوای آنتوسیانین کل و محتوای ویتامین ث به عنوان شاخص ترکیبات فیتوشیمیایی عصاره عناب مورد ارزیابی قرار گرفت. نتایج نشان داد که pH، دمای استخراج و غلظت حلال مهم‌ترین پارامترهای تاثیرگذار ( $p < 0.05$ ) بر محتوای فنول کل، آنتوسیانین کل و ویتامین ث عصاره عناب بود. شرایط بهینه استخراج ترکیبات فیتوشیمیایی شامل غلظت اتانول ۶۰٪، pH ۳، زمان استخراج ۱۸۰ دقیقه، دمای استخراج ۲۵°C و عدم حضور نور ارزیابی شد. تحت شرایط بهینه، بیشترین مقدار فنول کل، آنتوسیانین مونومری کل و ویتامین ث به ترتیب ۱۶۴/۵۱ میلی گرم گالیک اسید در گرم ماده خشک عصاره، ۵۲/۹۴ میلی گرم سیانیدین-۳-گلوکوزید در ۱۰۰ گرم ماده خشک عصاره و ۱۳۷/۱۲ میلی گرم L-آسکوربیک اسید در ۱۰۰ گرم ماده خشک عصاره گزارش گردید. محتوای بالای ترکیبات فیتوشیمیایی، حاکی از امکان استفاده عصاره عناب به عنوان یک منبع غذا-دارو در آینده می‌باشد.