



Evaluation on Physicochemical Properties and Antioxidant Capacity of Two Iranian Jujube (*Ziziphus jujuba* Mill.) Cultivars

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Article History: Received: 25 September 2018 /Accepted in revised form: 07 November 2018

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Abstract

Jujube (*Ziziphus jujuba* Mill.) is an important medicinal plant in Iran. Jujube fruit contains amino acids, polysaccharides and microelements. Ripened jujube fruits were collected at fully mature stage from two research collection of jujube at Khaf and Birjand centers in south Khorasan province, Iran, early in September, 2015. The physicochemical properties and antioxidant capacity were investigated. The antioxidant activity was determined by free radicals (DPPH) scavenging method. The results were showed a significant difference between evaluated physicochemical parameters. The acidity and pH of Khaf jujube were 0.72%, 4.37 and the acidity and pH of Birjand jujube were 0.37%, 4.65; respectively. Protein, fat, fruit moisture and vitamin C in Khaf jujube (3.92%, 0.72%, 0.75% and 137.59 mg/100gr fresh weight) were more than Birjand jujube (3.24%, 0.4%, 0.06% and 125.11 mg/100gr fresh weight). Birjand jujube had more reducing sugars percent (35.93 %). Seed's moisture, 100-grain weight and the antioxidant activity of Birjand jujube (0.156%, 151.03 mg, Ec_{50} = 0.1704 mg/ml) was higher than Khaf sample (0.11, 103.06, Ec_{50} = 0.3135 mg/ml). The viscosities of two jujube varieties were in shear thinning fluids category. The viscosity of Khaf jujube sample was higher than Birjand jujube in different durations and shear rates. The transparency of the color of the Birjand sample (18.21%) was higher than Khaf jujube (17.67%). Overall, based on sensory evaluation, physicochemical and consistency properties of Khaf jujube were more suitable than Birjand jujube while the antioxidant activity of Birjand jujube was higher than Khaf sample.

Keywords: Antioxidant Capacity, Consistency, Iranian Jujube Fruit, Physicochemical Properties

Introduction

Chinese jujube (*Ziziphus jujuba* Mill.) is belonging to the Rhamnaceae family and is indigenous to China and has been cultivated since 4000 years ago [1]. In traditional Chinese medicine, jujube fruits are mainly used to treatment of some diseases such as tumors and cardiovascular diseases related to the production of radical species resulting from

oxidative stress, commonly consumed in fresh and dried forms [2]. Jujube is one of the most valuable medicinal plants, which grows in South-Khorasan province, Iran, as the major producer of jujube in Iran [3]. Jujube fruits can be eaten as fresh, dried, or candied. Compared with other fruits, fresh jujube is lower in water content, but higher in soluble solids, phenolics and ascorbic acid [4]. In addition, jujube fruit contains amino acids, organic

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acids, polysaccharides, and microelements especially high potassium and iron content [1]. Also, it has been reported that more than 98% of jujube production in Iran belongs to South Khorasan province [5].

Recently, the high antioxidant activity of the extracts from different parts of jujube fruit such as peel, pulp and seeds has been reported. This antioxidant activity has been attributed to the high level of phenolic compounds [6, 7]. Vitamin C, as an antioxidant, is one of the most important nutritional quality factors in many horticultural crops and has many biological activities in the human body [8].

In previous researches were done to consider the influence of various jujube varieties on their antioxidant activity [9]. Koley *et al.*, (2016) evaluated 12 commercial cultivars of Indian jujube (*Zizyphus mauritiana* Lamk) that has been widely used in traditional medicine for treating various kinds of diseases [10]. In this study, their ascorbic acid (AA), total phenolics (TPH), flavonoids (TF), and total antioxidant activity (AOX) were measured. Results indicate that Indian jujube is a good source of ascorbic acid and total phenol compounds ranging from 19.54 to 99.49 mg/100 g and 172 to 328.6 mg GAE/100 g, respectively.

15 jujube cultivars late in their maturation were analyzed in the red stage for bioactive compounds; including total phenolics, total flavonoids, and ascorbic acid. The antioxidant activity was evaluated using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH). The results indicated that the contents of bioactive compounds and antioxidant capacities vary between different jujube cultivars. Correlation analysis indicated that the ascorbic acid and polyphenols were 2 main components responsible for the antioxidant activity of jujubes [11].

Gao and colleagues (2011) showed physical, chemical and antioxidant properties of various varieties of jujube in China through some experiments [12]. The results showed that a statistically significant difference exists between varieties of studied jujubes [12]. The species is the main indicative agent of antioxidant activity that was affected by the levels of phenolic compounds, ascorbic acid and another antioxidant compounds, respectively [13]. Kamiloglu (2009), studied antioxidant activity and phenolic compounds of methanol extracts of 15 native jujube cultivars of Turkey, and found that there was significant difference between the antioxidant activities of

different species [14]. Zhang *et al.*, (2010) considered the antioxidant capacity of three varieties of jujube in China and they showed that these Chinese jujube cultivars have higher antioxidant capacity [2]. Wang *et al.*, (2012) showed that the nanostructure properties in pectin and composition of neutral sugars may have a significant effect on the physicochemical properties of the jujube during maturation period [15].

The main objectives of this study therefore were to evaluate of the physicochemical properties and antioxidant capacity of two jujube clones in south Khorasan province, Iran were investigated.

Materials and methods

Materials

Ripened Jujube fruits (*Zizyphus jujuba* Mill.) were collected at fully mature stage (red color) separately from two Research Collection of Jujube at Khaf and Birjand centers in South Khorasan province, Iran, early in September, 2015. All trees were similar regarding age (10 years) and orchard management. South Khorasan has a cold desert climate with hot summers and a significant difference between day and night temperatures.

Fruits were hand harvested and then transported to the post-harvest laboratory within a day and stored at 4 ± 1 °C for 2 days for more evaluation and processing.

Chemicals used in this study were ethanol, methanol, concentrated hydrochloric acid, N-hexane, 0.1 Normal sodium hydroxide, potassium hydroxide, phenolphthalein, indicator methylene blue, indicator diphenylpicryl hydrazine, concentrated sulfuric acid, copper sulfate, potassium sulfate, saturated lead acetate, Fehling solution A and B, activated carbon, DPPH indicator (1 and 1-diphenyl-2-picryl hydrazil) invert sugar standard solution. All chemicals had high purity and food grade and were purchased from Merck (Germany), Sigma-Aldrich, Dr. Mojalali (Iran) companies.

Physical Analysis

Jujube puree

The Jujube fruits soaked and boiled in a certain amount of water (The amount of water consumed was the equal to the weight of the fruit). After softening the fruit tissue, it was passed through of a 1 mm sieve and then separating the flesh, skin,

and seeds. The Jujube seeds were washed to remove any adhering Jujube flesh. Finally the seeds dried in the oven at 25 °C for 24 h. The oil of the seeds was extracted by solvent extraction. Then concentrated Jujube section was stored at -18 °C for further analysis.

Fruits Weigh

The weight of 100 jujube fruits was randomly determined by scale with precision of 0.01 gram.

Fruit and Seed Moisture

The moisture content of fruit and seed samples were determined by drying 5 g of sample in hot air oven at 105 C until constant weight [16].

Chemical Analysis

Titrateable Acidity (TA)

Titrateable acidity (TA) was measured by titration with 0.1 N NaOH solution to reach the end point of action in pH 8.1. It expressed as percentage of citric acid [17].

pH

It measured by pH meter device made in Switzerland, (Metrohm-model 691).

Fat

It measured by Soxhlet method according using AOAC method No. 963.15 [18].

Seed Oil

Jujube seed oil was extracted with N-hexane solvent (at a ratio of 3: 1 (W/V)) [19].

Protein

It was performed by Kjeldahl method [20].

Reducing sugar, total sugar and sucrose

They were measured by Lin-Aynon method [21].

Ascorbic Acid

High performance liquid chromatography device which was equipped with UV-VIS detector (knauer, Made in Germany) was used for determining Ascorbic acid. Separation was performed by Eurospher column (250× 6.4×5, 18 °C) with injection volume of 20 ml and the column temperature was fixed at 25 °C. The phosphate buffer with pH=2.8 was used as solvent with flow rate of 0.7 ml/ min [22].

Viscosity

To measure the viscosity of jujube concentrate, Brookfield viscometer Model ULTRA Dv- III was used. Spindle (RV3) was used for this test. Jujube concentrate brix was 35 and apparent viscosity of the samples was measured in ambient temperature of 25°C (room temperature).

Textural Analysis

Lloyd texture analyzer (model TA+, e U SA) was used for measurement [23].

Color

The color of the concentrate jujube sample was determined by Image J software, the image segmentation, converting RGB image file to Lab file and finally color space analysis were performed.

Color space of Lab consists of three components: L* brightness of image between zero (equal to black) and 100 (equal to full reflection of light), amounts of a* value is unlimited and equal to red color until green color, amounts of b* value is unlimited equal to yellow color until blue color.

Antioxidant Capacity

The capacity of scavenging of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical was monitored according to the method reported by Singh *et al.*, 2002. 0.1 ml the Jujube concentrate at various concentrations was respectively added to 0.49 ml of Methanol and 0.39 ml of DPPH methanol solution (4 mg/100 ml). Then, the mixtures were vortexed vigorously and allowed to stand in the dark for 60 min. Finally, the absorbance of these mixtures was measured by using a spectrophotometer at 512 nm. The sample concentration providing 50 % of radical scavenging activity (EC₅₀) was obtained through interpolation of linear regression analysis. The lower EC₅₀ indicates higher radical scavenging activity.

Radicals scavenging power was chosen as an indicator of antioxidant activity [19]. The radical scavenging activity was calculated from the following equation

$$\% \text{ DPPH scavenging} = \left(\frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \right) \times 100$$

After providing the graph of DPPH scavenging percent against antioxidant compounds, suitable mathematical model was fitted on data points, and then the concentration which antioxidant compounds of concentrate were able to scavenge 50% of free radicals was calculated as EC₅₀. It

should be noted that the EC₅₀ decreases by increasing scavenging power [19].

Statistical Analysis

Data was reported as mean \pm standard deviation. Each data is mean of 3 replications. They were analyzed by "SAS" software. Analysis of variance (ANOVA) accompanied with Duncan test were conducted to identify the significant difference between samples ($p < 0.05$).

Results and Discussion

Physical Properties

Analysis results of some physical properties of the two Jujubes concentrate samples (Birjand and Khaf) are summarized in Table 1.

Jujube Concentrate Weight

The results showed that different weight of concentrate with certain dissolved matter percentage (Brix 35) was produced. Khaf clone with 875.072 grams per each 500 gram of jujube fruit had higher concentrate production in comparison with Birjand clone. The difference may be due to the plant species, climatic conditions and its impact on the physical and chemical properties of fruit. According to a research on 5 species of

Chinese jujube by Gao *et al* in 2011, the difference in concentrate production efficiency results showed in coherence in ripening rate of jujubes with complete maturation degree that was influenced by species and could be due to the ability of adaptation to different climatic conditions and soil properties [12].

Weight of 100 Jujube Fruits

Results of variance analysis showed that the average weight of studied jujubes, with 95% insurance, was significantly different. The main reasons for the variation in weight of the fruits are species and environmental conditions [12].

Moisture

The results of variance analysis showed that the amounts of moisture in the fruits and seeds had significant difference with 95% confidence level. Moisture is not an inherent index of fruit and is dependent on several factors such as fruit harvesting time, storage conditions and consequences of drying method and type of packaging. Similar studies carried out by Gao and colleagues on the species in China in 2011 showed that the amounts of moisture in different species varied from 78% to 83% [12].

Table 1 Physical properties of Birjand and Khaf. Jujubes

Sample	Concentrate Weight	Fruit Weight (g/100 fruits)	Fruit moisture	Seed moisture
Birjand	847	151.03 \pm 4.77 a*	0.06 \pm 0.01 b	0.15 \pm 0.00 a
Khaf	875.07	103.06 \pm 4.64 b	0.75 \pm 0.01 a	0.11 \pm 0.00 b

Numbers are average of standard deviation, Mean \pm SD (n=3)

*Different lower case superscript letters in the column indicate significant difference ($P < 0.05$)

Table 2 Chemical properties of Birjand and Khaf Jujubes

Parameters	Birjand	Khaf
Acidity	0.37 \pm 0.06 b*	0.72 \pm 0.09 a
pH	4.65 \pm 0.02 a	4.37 \pm 0.02 b
Fat	0.40 \pm 6.79 b	0.72 \pm 1.35 a
Protein	3.24 \pm 0.05 b	3.92 \pm 0.02 a
Total sugar	43 \pm 4.81 a	34.13 \pm 3.69 a
Reducing sugar	35.93 \pm 0.76 a	28.93 \pm 4.61 b
Sucrose	6.85 \pm 0.69 a	5.20 \pm 0.46 a
Ascorbic Acid	125.11 \pm 1.75 b	137.59 \pm 0.81a

Numbers are average of standard deviation, Mean \pm SD (n=3)

*Different lower case superscript letters in the column indicate significant difference ($P < 0.05$)

Chemical Properties

Analysis results, titratable acidity (TA), PH, fat, protein, total sugar, reducing sugar, sucrose content and vitamin C of two Jujube concentrate samples (Birjand and Khaf) are presented in Table 2.

Titratable Acidity(TA)

The statistical results showed titratable acidity of concentrate was significantly different from each other with 95% confidence level. According to data obtained, it was determined that Khaf sample had higher acidity. In a research conducted on 5 species of Chinese jujube, titratable acidity percent was variable from 0.2 to 0.47 that had lower acidity in comparison with tested jujubes in this study [12].

pH

The results obtained from the mean of PH in jujube concentrate showed a significant difference with 95% confidence level (table 2). Burhanudin *et al.*, (2012) studied, 23 Chinese jujube samples were used for preparing jam and pickles, pH values ranged from 2.5-3.5 [24]. Wang *et al.*, (2012) investigated the effect of ripening on physico-chemical properties of Zhanhuva and Huvanjoa jujube. The result showed that the fruits pH was reduced during the ripening period (from 4.93 to 4.63 and 4.95 to 4.78) [15].

Fat

The average of total fat in jujube samples was significantly different (95%) (table 2). The results of this study were similar to the results of different Chinese jujube species measured from 0.37 to 1.02 [1].

Seed Oil

Seed oil extracted from two varieties of Khaf and Birjand jujube were 3.18 and 1.87 gr/100 gr respectively. In a study conducted in 2015 by Basiri, the efficiency of oil extraction from pomegranate seeds was reported 17.33 percent. The results were much higher in comparison with present study [19].

Protein

Statistical results showed significantly different within the 95% confidence interval (table 2). The Iranian samples in comparison with Chinese jujube species had lower protein. Li *et al* in 2007 showed the protein content of 5 species of Chinese jujube varied from 4.75 to 6.86 %.

Sugars

Statistical results showed that the amount of total sugar and sucrose in the extract of Khaf and Birjand jujube was not significantly different but reducing sugars in the concentrate had significant difference with 95% confidence level (table 2). Wu *et al.*, (2012) during a research on a pear shape jujube species investigated that main sugars in this species of jujube are fructose, glucose and sucrose that their quantity increases by ripening and again reduces during storage [25]. In another research on several species of jujube in the Loess Plateau of China the total sugar of them was reported between 9.8 to 14.7 percent [12]. In a research carried out by Li and colleagues in 2007 on the nutritional properties of several species of Chinese jujube, the amount of reducing sugar in dried matter varied from 57.61 to 77.93 percent that was significantly higher in comparison with considered jujubes in present study [1]. In another research, anti-diabetics effect of jujube extract was identified. Alcohol extract of jujube fruit effectively reduced glucose and triglyceride level of diabetic rats' serum in doses of 0.5, 1, 1.5 gram in comparison with control [26].

Ascorbic Acid

Vitamin C levels in two Khaf and Birjand mass was measured by high performance liquid chromatography method, the results were summarized in Table 2. The statistical results showed that the average of ascorbic acid in jujubes was significantly different with 95% confidence level. A research was conducted by Melendez and his colleagues in 2004 on several samples of orange juice and ultra-freeze orange juice samples by HPLC method for measuring vitamin C at wave lengths of 210, 214, 230 nm. Vitamin C had an average of 546.26, 360.85 ppm [22]. Another research about this matter showed the level of jujube vitamin C in china in 2012 during ripening period. The result showed that vitamin C level during primary stage of jujube ripening (green color) had the highest level (310.32 mg/100gr) and during ripening this amount was reduced (199.58) [25]. Wojdylo and his colleagues (2016) studied on ascorbic acid content of 4 Spanish jujube fruits cultivars [27]. The content of L-ascorbic acid was very high and took values in the range of 387-555 mg/100 g fresh weight (fw). These differences were related to the type and variety of jujube, the geographical and climatic conditions of the jujubes

Viscosity

The results showed that the mean viscosity of two samples is significantly different with 95% of confidence level. It was necessary to determine the fluid viscosity in different temperature, time and shear stress because the viscosity is the criteria of measuring flow. The results of the viscosity measurements at different shear stresses and torques are given in Table 3. These results show that the viscosity of the Khaf sample is much higher than Birjand. Jujube extracts are included in Shear thinning fluids category. In a study conducted in 1998 by Stauffer and Brookfield (Mc-Michel) method, the chocolate flow behavior and viscosity were determined and showed that viscosity of chocolate, like jujube concentrate decreases with increasing shear rate [28]. Tamborrino *et al.*, (2012) studied reologic properties of olive paste by Brookfield rheometer. They found that visual viscosity increases by gradual increase of torque to a certain level and after that decreases [29].

Tissue Parameters Assessment (TPA)

TPA obtained results from TPA test on Birjand and Khaf extracts given in Table 4. Santini *et al.*, (2011) studied on quality parameters of grape extract and tissue properties of grape. The results were similar to jujube concentrate samples in present study [21].

Color Measurement

The results are presented in Table 5. The color index average in two experimented masses is compared in table 3. Variance analysis results in jujube concentrate showed that color index L^* with 95% confidence level was significantly different and in color indexes a^* and b^* with the same confidence level no significant difference was seen. In a study in 2012 conducted by wu *et al.*, in China, the level of color in several species of jujube during ripening period was measured in the same way

[25]. According to the results of study in immature jujubes value of factor a^* was -11.31 in Huvanjoa specie, -11.08 in Zhanhuva specie and according to plotted specification of this indicator, green color was shown in samples. After ripening of jujube fruit positive values obtained by measuring a^* factor 16.75 in Huvanjoa species, 19.76 in Zhanhuva Species, which indicates the loss of chlorophyll pigment (green) and red coloring in jujube shell [25].

Antioxidant Properties of Jujube Concentrate (DPPH Free Radical Scavenging Activity)

To measure the antioxidant power, it is necessary to prepare different concentrations of two evaluation jujube extracts. The results showed that all studied extracts had free radical scavenging power, this property increased with increasing of concentration of extractive effective compounds. The concentration in which half of the free radicals were scavenged (EC_{50}) in methanol extract of Khaf jujube was 0.3115 (mg/ml) on average and in methanol extracts of Birjand jujube EC_{50} was 0.1718 (mg/ml) on average, these results indicate that free radical scavenging power in Birjand sample is significantly higher than Khaf sample, because it can scavenge half of free radicals in lower concentrations. The results show that methanol solvent has a significant ability to extract more free radicals scavenging compounds of jujubes. Extracting solvent significantly affected antioxidant activity of fruits. Ranking in the antioxidant activity of extracts varied depending on the polarity of solvent and the method used to extract bioactive compounds [30]. Kallithraka and colleagues (1995) studied the effect of different solvents in extraction of various antioxidant compounds in grape seed [6]. They found that methanol causes the maximum extraction of phenolic compounds (Pro-cianidines and kateshines) from the grape seed. The mean of free radicals scavenging through the jujube extracts EC_{50} are given in Fig. 1.

Table 3 Viscosity of Birjand and Khaf. jujubes

Cultivar	Torque (rpm)	Viscosity (centi-poise)
Birjand	25	620
	30	542.9
	50	476
Khaf	25	1700
	30	1414
	50	1178

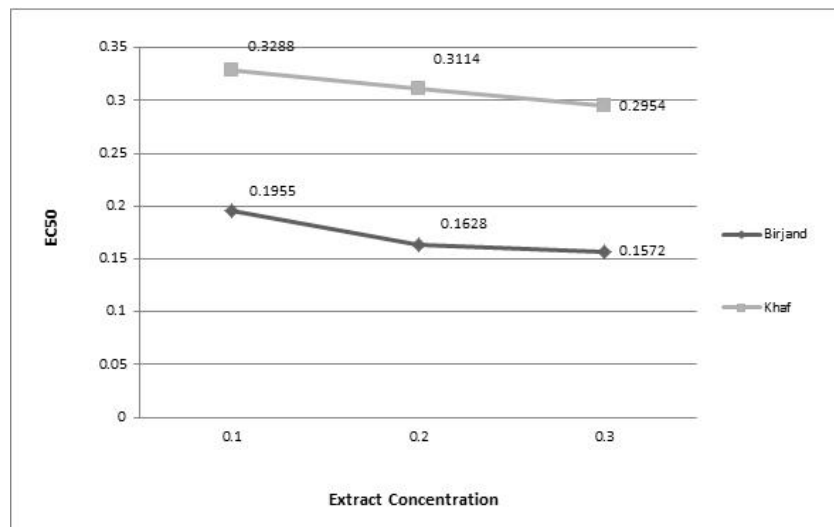
Table 4 Tissue profile analysis of Birjand and Khaf Jujubes

parameters	Birjand	Khaf
Sample height (mm)	27	27
Hardness1 (N)	0.04	0.04
Hardness2 (N)	0.04	0.04
Surface 1 (Nm)	⁵ - 9.90 E	0.00
Surface 2 (Nm)	⁵ - 5.26 E	⁵ -7.81 E
Hardness (N/m)	3.49	3.82
Adherence	0.53	0.64
Stretch ability (mm)	14.80	15.18
Elastic index	0.91	0.93
Gum condition (N)	0.02	0.03
Chewing ability (Nm)	0.00	0.00
Frangibility forced (Nm)	0.01	0.00
Adhesion force (N)	0.00 -	0.00-
Adhesiveness (Nm)	⁵ -1.23 E -	⁵ -0.05 E -

Table 5 Color indexes of Birjand and Khaf. jujubes

Samples	L*	a*	b*
Birjand	18.21± 0.12 a	2.27±0.32 a	3.53±0.10 a
Khaf	17.68± 0.21 b	2.60± 0.30 a	3.18 ± 0.22 a

Similar lower-case superscript letters show that data has not significant difference
Numbers are average of standard deviation

**Fig. 1** Comparison of EC₅₀ average in different methanol extracts

Conclusion

In this study, the physic-chemical properties and antioxidant power of two different samples of jujube in South Khorasan province in Iran were evaluated. The obtained data on two jujube clones were an attempt to find the industrial applications of this product. Jujube is a good source of antioxidant compounds and can be used to process

food products. The results of this study showed a significant difference in physiochemical parameters. Based on the data, Khaf jujube had more desirable sensory properties. The results on the viscosity of two jujube concentrates showed that they were included in shear thinning fluids category. Viscosity of Khaf jujube puree was higher than Birjand jujube in different durations and shear rates. Antioxidant power of Birjand cultivar was higher than another one. It seems that

the presence of antioxidant compounds such as phenolic compounds in Birjandjube was more. Overall, panelists confirmed sensory, texture, physicochemical and consistency properties in Khaf jube more suitable than Birjand sample but antioxidant ability of Birjand jube was better than Khaf jube.

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