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Research Paper

Influence of ultrasonic treatment on the quality of jujube (*Zizyphus mauritiana* Lamk.) extract cultivars

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Abstract. In the present study, effect of ultrasonic treatment (80% amplitude level (400 W power), 24 kHz frequency, 5 min, 20°C) on the total phenolics and flavonoids, vitamin C contents, radical scavenging capacity (DPPH), total antioxidant activity (TAC), reducing power, total plate count (TPC), yeast and mould (Y & M) of water soluble extracts of five seasonal cultivars of Jujube, were investigated. The physical and chemical characteristics of Jujube cultivars were also monitored in the study. It was observed that ultrasonic treatment significantly ($P < 0.05$) improved the total phenolics and flavonoids, vitamin C contents, DPPH radical scavenging capacity, TAC and reducing power of water soluble extracts of Jujube cultivars. Moreover, significant ($P < 0.05$) reduction of TPC, Y & M was found after ultrasonic treatment. The results suggest that ultrasonic treatment may be applied to improve the nutritional value especially the antioxidant potential of jujube cultivars.

Keywords: Jujube cultivars, ultrasonic, total phenolics, antioxidant potential, microbial inactivation.

INTRODUCTION

The ber or jujube (*Ziziphus* spp.), belongs to family Rhamnaceae, is native to South and Central Asia as well as China and mainly distributed in the tropical and subtropical regions of the world (Azam-Ali, 2006; Tripathi, 2014). It is found in various shapes, colors, sizes and tastes all over the world. It is consumed in Pakistan as fresh fruit and rarely in the form of jam. As this fruit contains biologically active compounds such as vitamin C, triterpenic acids, phenolics, flavonoids, and polysaccharides; therefore, it has been in use as a fruit and remedy (Preeti and Tripathi, 2014). This tiny fruit may have therapeutic uses for allergies, constipation, vitamin A and C deficiencies, depression etc. as reported therein (Lamien-Meda, 2008). Moreover, it has also been reported that this fruit has antifungal, antibacterial, anticancer (Melanoma cells), anti-inflammatory, antiulcer, antioxidant, immunostimulant and wound healing properties (Preeti and Tripathi, 2014).

Antioxidants are paramount to biologists and clinicians because they have been reported in protecting the human body against damages caused by reactive free radicals generated in atherosclerosis, cancer, ischemic heart disease, Parkinson's disease, Alzheimer's disease and even in aging process (Aruoma, 2003; Hemati *et al*., 2010). Currently, some studies have been reported regarding phenolics, flavonoids and antioxidant capacity of some cultivars of jujube from China (Zhao *et al.*, 2014), India (Krishna and Parashar, 2013), Turkey (Kamiloglu *et al.*, 2009) and France (Zozio *et al.*, 2014). As nutritional quality and antioxidant potential of fruits depend on different climatic conditions, soil characteristics and geographical locations (Avais *et al.*, 2011; Gull *et al.*, 2012; Lee and Kader, 2000), it was enticing to conduct research on jujube regarding its antioxidant activity locally grown in Pakistan.

Usually, thermal processing of foods decreases the freshness of foods, thereby decreasing its organoleptic quality and may deteriorate nutrition. As the consumers

Gola (Desi) Pak white (Seb) Karela (Katha) Ghor (Sindhi) Umran-11 (Faisalabadi)

Figure 1. Jujube cultivars showing their assigned names and local names in brackets.

are becoming more health conscious and demand nutritious foods with minimal processing, that is why; non-thermal technologies (alternative to thermal processing) such as pulse electric field, osmotic dehydration, irradiation, membrane filtration, ozone treatment, high pressure, have been proven to be less destructive regarding nutrition of foods (Khandpur and Gogate, 2015; Rawson *et al.*, 2011). Food processing by the use of ultrasonic treatment (non-thermal technology) is considered to be advantageous in minimizing flavor loss, enhancing quality, reducing chemical and physical hazards (Ercan and Soysal, 2011). Moreover, it has also been proven to retain nutritional value and microbiological safety in orange juice (Valero *et al.*, 2007), strawberry juice (Tiwari *et al.*, 2009), red grape juice (Tiwari *et al.*, 2010), guava juice (Cheng *et al.*, 2007), apple (Valavanidis *et al.*, 2009), tomato juice (Wu et al., 2008), apple juices (Abid *et al.*, 2014), carrot (Jabbar *et al.*, 2014), pomegranate juices (Alighourchi *et al.*, 2013) and strawberry (Dubrovic *et al.*, 2011).

People in Pakistan have little information regarding its health promoting effects, that is why, this fruit is underutilized. To the best of our knowledge, no study has been conducted to investigate the influence of ultrasonic treatment on the antioxidant potential of jujube cultivars.

Hence, on the basis of interesting results of the aforementioned studies regarding antioxidant activity of jujube belonging to different countries as well as effect of ultrasonic treatment of different fruit juices on antioxidant potential, the main objective of the current study was to evaluate the influence of ultrasonic treatment on the total phenolics and flavonoids, vitamin C contents and antioxidant activity of the extracts from five different cultivars of jujube locally grown in Pakistan.

MATERIALS AND METHODS

DPPH (2, 2-diphenyl-1-picrylhydrazyl), catechin and gallic acid were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). Folin-Ciocalteu reagent was purchased from VWR Prolabo Chemicals, France. HPLC grade methanol was purchased from RCI Labscan Ltd., Thailand. Sodium phosphate, aluminum trichloride ($AlCl_3$) and sodium carbonate were purchased from Daejung Co. Ltd., Korea. Calcium carbonate was purchased from BDH Laboratory Supplies, England. All other chemicals were of analytical grade.

Collection of jujube cultivars

Five cultivars of *Zizyphus mauritiana* Lamk., namely Gola (Desi), Pak white (Seb), Karela (Katha), Ghor (Sindhi) and Umran-11 (Faisalabadi) (Figure 1), were purchased from the local market of Sargodha, Pakistan. They were identified from the Citrus Research Institute, Horticulture division, Sargodha, Pakistan. Initially, all the fruits had green color at the time of collection and started to turn yellow and ultimately brown. All the fruits were stored at 4°C before making extracts.

Physical analysis of jujube fruits

Samples (fifteen fruits) of each cultivar were taken to measure physical characteristics according to Ezz *et al.* (2011) and Saran *et al.* (2006). Fruit weight (g) and seed weight (g) were determined using a digital calibrated analytical balance, whereas fruit length (cm), fruit diameter (cm), seed length (cm) and seed width (cm) were measured using a digital vernier caliper. Fruit volume (cm^3) was measured with water displacement method. Pulp (%) and pulp:stone ratio were also calculated.

Chemical analysis of jujube fruits

The moisture, ash, total soluble solids (TSS), total sugars, reducing sugars and non-reducing sugars, pH and titratable acidity of jujube fruit were determined according to AOAC (2003). The TSS-Acid ratio, which indicates the ripeness (Maturity Index) of fruits was also calculated (Lacey *et al.*, 2009).

Preparation of jujube extracts and their ultrasonic treatments

The preparation of jujube extracts and their ultrasonic

treatments were carried out by following procedure as described by Cansino *et al.* (2013) with some modifications. Fruits (immediately after turning brown) from each jujube cultivar were selected on the basis of uniform shape and color. All the fruits were washed thoroughly with tap water so that all the dirt was removed. A 500 g (15 to 200 fruits depending upon variations in weight of each cultivar) of each jujube cultivar was taken and destoned. Then the pulp portions with peel were sliced into very small pieces. To make homogeneous mixture, after homogeneous mixing of pieces of each cultivar, 200 g of each was separately taken into a domestic blender and 600 g of distilled water was added into the blender. Blending was done until the mixture was completely homogenous. After removing all the contents from the blender, the mixture was first stirred for 1 h having magnetic stirrer inside and then filtered through a muslin cloth. Out of filtered mixture, one part (150 mL) was given ultrasonic treatment (24 kHz Frequency, 80% amplitude level (320 W power, pulse duration 5 Sec on and 5 Sec off, 5 min at 20°C) by using an ultrasonic processor (UP400S, Hielscher Ultrasonics GmbH Hielscher USA, Inc.) with 400 W power, 3 mm diameter titanium probe (inserted up to 2 inch inside the sample), and the other part (150 ml) was considered control which was not given ultrasonic treatment.

The ultrasonic treatment was performed in the dark so as to avoid light interference. In our preliminary trials of antioxidant activity assays and vitamin C contents, optimization of different parameters such as amplitude (%), duty cycle, time and temperature was carried out and the maximum antioxidant activity was observed at the above mentioned adjustments of ultrasonic processor. The pulse durations 5 s each on and off with a duty cycle (50%) was observed to be more suitable for better results compared to without on and off adjustment. During ultrasonic treatment, it was observed that the temperature within the sample was increased up to 70-75°C. By increasing the temperature far away from 20°C, samples showed decreased antioxidant activity and decreased vitamin C contents. Therefore, temperature was maintained at 20°C by automatic circulation of cold water through 500 mL jacketed vessel (7.6 cm ID×9.3 cm OD×13.5 cm Depth×14.9 cm Height). By increasing the time of ultrasonic treatment beyond 5 min, vitamin C contents as well as total phenolics and antioxidant activity were decreased. Therefore the time of ultrasonic treatment was adjusted up to 5 min. All the samples (with and without ultrasonic treatment) were centrifuged at 4,000 rpm for 20 min. The clear supernatant was filtered with Whatman 1. All the samples of each jujube cultivars were prepared in the same manner in duplicate. Then, from each duplicate filtrate (extracts) triplicates were taken for all measurements and analysis. The main purpose of preparing extracts was to have a pronounced effect of ultrasonic treatment on the antioxidant potential of jujube cultivars as ultrasonic performs well in liquid samples rather than semi-solid. The jujube extracts actually mimic juice of the fruits and most of the phytochemicals may be expected in the extracts. Ultrasonic treatment may have a great interest in liberation of more antioxidant compounds from the cells, thereby manifesting more antioxidant potential of fruit extracts. The aliquots of extracts of all jujube cultivars were stored at -18°C in air tight pre-sterilized media bottles for further analysis. After thawing, for all the analysis regarding antioxidant potential, the extracts of all jujube cultivars were filtered through 0.45 µm filters.

Microbiological evaluation of jujube extracts

Microbiological evaluation of ultrasonic treated and untreated extracts of jujube cultivars regarding total plate count (TPC) and yeast & mold (Y & M) counts, was carried out using the method as described by Khandpur and Gogate (2015).

Phytochemical analysis of jujube extracts

The extract of each jujube cultivar was also evaluated qualitatively for the presence of flavonoids, flavonoid aglycones, flavonoid glycosides, phenols, tannins, saponins, steroids, terpenoids and glycosides by the following methods as described by Okala *et al*. (2014), with some modifications, who also followed methods as described in Trease and Evans, (1989) and El-Olemyl *et al.* (1994).

Determination of total phenolics and total flavonoids of jujube extracts

The total phenolic contents were determined by spectrophotometric method with some modifications (Singleton *et al.*, 1999). Gallic acid was used as a standard and the results of total phenolics were expressed as mg of gallic acid equivalents (GAE) per 100 g of fresh weight (FW) sample. Flavonoid contents were determined by the described spectrophotometric method of Jia *et al.* (1999). Catechin was used as a standard and the results were expressed as mg of (+)-catechin equivalent (CE) per 100 g of FW. All determinations were carried out in triplicates and the experiments were run in duplicate.

Determination of DPPH antioxidant capacity, free radical scavenging activity (RSA) and total antioxidant capacity (TAC) of jujube extracts

The capability of the jujube extracts to scavenge 2, 2-diphenyl-1-picrylhydrazyl radical ($DPPH^{\cdot}$) was determined by the spectrophotometric method of Yi *et al.*

(2008) with some modifications. Following equation was used to calculate the percentage inhibition of radical scavenging activity (RSA%): where A (control) is the absorbance of the control and A (sample) is the absorbance of the sample. Ethanol was used as a blank. The DPPH radical scavenging activity was also expressed as a µmol equivalent of Trolox/g of FW by following the aforementioned assay. All determinations were carried out in triplicates and the experiments were done in duplicate.

For the determination of total antioxidant capacity (TAC), the extracts were analyzed by using the spectrophotometric method described by Prieto *et al.* (1999). Antioxidant activity was calculated by using ascorbic acid standard calibration curve and the results were expressed as mg ascorbic acid equivalent (AAE)/100 g of FW. All determinations were carried out in triplicates and the experiments were done in duplicate.

Determination of vitamin C and reducing power ability of jujube extracts

Vitamin C (ascorbic acid) contents of jujube extracts before and after ultrasonic treatment were determined by 2,6-dichlorophenol-indophenol ($C_{12}H_6C_{l2}NNaO_2.2H_2O$) titration method (AOAC, 2003). All of the above determinations were performed in triplicates for each of jujube cultivar. The reducing power of extracts of all jujube cultivars was determined using the spectrophotometric method as described by Hegazy and Ibrahium (2012) with some modifications. The absorbance of the sample mixture was measured using spectrophotometer at 700 nm. Reducing power ability was calculated using ascorbic acid standard calibration curves and the results were expressed as mg ascorbic acid equivalent (AAE)/100 g of FW. The increased absorbance of the sample mixture depicted increased reducing power. All the determinations were carried out in triplicates and the experiments were done in duplicate.

Statistical analysis

Statistical analysis was performed using Minitab statistical software version 16 (Minitab Inc., State College, PA, USA). Completely randomized design (CRD) was performed with one-way ANOVA and significant differences between mean values of all the data were determined by Tukey's pair-wise comparison test ($P < 0.05$). The normality assumptions were found to be satisfied through Shapiro–Wilk test.

RESULTS AND DISCUSSION

Physical characteristics of jujube cultivars

Table 1 shows the physical characteristics of jujube cultivars. The fruit weight (g) of all jujube (*Zizyphus mauritiana* Lamk.) cultivars varied significantly ($P < 0.05$) from each others. Among the jujube cultivars, Pak white (Seb) had the maximum fruit weight (28.96 g), seed weight (1.26 g), fruit diameter (3.63 cm) and volume (30.13 cm^3) followed by Karela (Katha). As the Karela (Katha) cultivar had the maximum fruit length (5.13 cm), therefore the seed length (2.33 cm) of that cultivar was also noted as the maximum compared to other cultivars. Pak white (Seb) cultivar was noted to have the highest seed width (0.97 cm) compared to others cultivars. Generally, pulp (%) of Pak white (Seb), Karela (Katha) and Umran-11 (Faisalabadi) was high, however, Karela (Katha) had maximum pulp (82.87%) compared to the other cultivars. Pulp:stone ratio depicts the edible to non-edible ratio. Pulp:stone ratio of Karela (Katha) (25.51) was also maximum followed by Pak white (Seb) (18.81), due to presence of more pulp (%) as the seed weight (g) is very less compared to fruit weight (g).

Chemical characteristics of jujube cultivars

Chemical characteristics of all the investigated jujube cultivars have been presented in Table 2. All the jujube cultivars had a moisture content > 80%, but Pak white (Seb) showed the highest moisture (%) content. Ash (%) content of all the jujube cultivars varied significantly ($P < 0.05$) from each other. Protein (%) and fat (%) contents of all jujube cultivars were very low. The pH of Pak white (Seb) varied significantly ($P < 0.05$) from all other cultivars. The TSS (%) and acidity (%) of jujube cultivars ranged 9.15 to 11.38 and 0.57 to 1.11 respectively. The results of the present study concerning moisture (%), ash (%), protein (%), fat (%), acidity (%), total sugar (%), TSS (%) and pH are in agreement with the results obtained by jujube grown in Bangladesh (Burhan Uddin and Hussain, 2012) and Sri Lanka (Ketipearachchi *et al.*, 2015). The acidity and TSS/Acidity have an inverse relationship. Even though, TSS/Acidity shows maturity index; however, it also depicts the taste profile of the fruits. The TSS/Acidity of Umran-11 (Faisalabadi) is the maximum (50.42) compared to others which means this cultivar is the sweetest among all the other cultivars. The low values of TSS/Acidity were shown by Pak white (Seb) and Ghor (Sindhi) which means those cultivars have some sort of tart taste compared to others. All the jujube cultivars showed higher reducing sugar content (%) compared to their non-reducing sugar content (%).

Effect of ultrasonic treatment on total plate counts (TPC) and yeast and mold (Y & M) of jujube extracts

Figure 2 shows the TPC and Y & M of the extracts from jujube cultivars before and after ultrasonic treatment. A significant ($P < 0.05$) reduction in TPC and Y & M were observed after ultrasonic treatment of the extracts of

Table 1. Physical characteristics of jujube cultivars.

Properties	Jujube cultivars				
	Gola (Desi)	Pak white (Seb)	Karela (Katha)	Ghor (Sindhi)	Umran-11 (Faisalabadi)
Fruit weight (g)	2.31 ± 0.09^{E}	28.96 ± 0.49^{A}	23.17 ± 0.33^{B}	3.37 ± 0.07^{D}	6.70 ± 0.14^{C}
Fruit length (cm)	1.40 ± 0.01^{E}	4.17 ± 0.06^{B}	5.13 ± 0.15^{A}	1.93 ± 0.06^{D}	3.11 ± 0.05^{C}
Fruit diameter (cm)	1.41 ± 0.02^{E}	3.63 ± 0.05^{A}	2.95 ± 0.05^{B}	1.74 ± 0.04^{D}	1.91 ± 0.07^{C}
Fruit volume (cm^3)	1.66 ± 0.06^{E}	30.13 ± 0.23^{A}	23.06 ± 0.05^{B}	3.10 ± 0.08^{D}	5.72 ± 0.08^{C}
Seed weight (g)	0.54 ± 0.02^{C}	1.26 ± 0.03^{A}	0.75 ± 0.03^{B}	0.56 ± 0.02^{C}	0.33 ± 0.02^{D}
Seed length (cm)	0.81 ± 0.01^{E}	2.03 ± 0.02^{B}	2.33 ± 0.03^{A}	1.39 ± 0.02^{D}	1.87 ± 0.02^{C}
Seed width (cm)	0.82 ± 0.01^{B}	0.97 ± 0.02^{A}	0.72 ± 0.02^{C}	0.85 ± 0.01^{B}	0.53 ± 0.01^{D}
Pulp (%)	70.59 ± 1.08^{B}	81.82 ± 1.59^{A}	82.87 ± 0.58^{A}	71.90 ± 3.17^{B}	78.95 ± 1.06^{A}
Pulp: Stone ratio	2.99 ± 0.06^{E}	18.81 ± 0.10^{B}	25.51 ± 0.69^{A}	4.31 ± 0.39^{D}	16.22 ± 0.63^{C}

Means with different letters in the same row (A-E) are significantly different ($P < 0.05$) from each other.

Table 2. Chemical characteristics of jujube cultivars.

Properties	Jujube Cultivars				
	Gola (Desi)	Pak white (Seb)	Karela (Katha)	Ghor (Sindhi)	Umran-11 (Faisalabadi)
Moisture (%)	82.03 ± 1.25^{B}	87.26 ± 0.81^{A}	85.38 ± 0.75^{A}	81.08 ± 0.97^{B}	85.70 ± 0.94^{A}
Ash (%)	0.91 ± 0.03^{A}	0.44 ± 0.36^{D}	0.53 ± 0.03^{C}	0.74 ± 0.05^{B}	0.61 ± 0.03^{C}
Protein (%)	0.87 ± 0.15^{B}	1.30 ± 0.10^{A}	1.06 ± 0.21^{AB}	0.90 ± 0.10^{B}	1.30 ± 0.10^{A}
Fat (%)	0.28 ± 0.02^{B}	0.41 ± 0.01^{A}	0.22 ± 0.01^{C}	0.42 ± 0.02^{A}	0.32 ± 0.01^{B}
pH	4.51 ± 0.07^{B}	4.79 ± 0.06^{A}	4.62 ± 0.03^{B}	4.59 ± 0.03^{B}	4.51 ± 0.05^{B}
TSS (%)	11.38 ± 0.42^{A}	10.09 ± 0.35^{BC}	9.15 ± 0.18^{D}	10.57 ± 0.33^{AB}	9.52 ± 0.25^{CD}
Acidity (%)	0.28 ± 0.01^{B}	0.37 ± 0.02^{A}	0.22 ± 0.01^{C}	0.34 ± 0.02^{A}	0.19 ± 0.02^{C}
TSS/acidity	40.21 ± 2.02^{B}	27.24 ± 1.15^{C}	42.29 ± 2.60^{AB}	31.49 ± 2.30^{C}	50.42 ± 5.56^{A}
Total sugars (%)	8.75 ± 0.53^{A}	7.83 ± 0.64^{AB}	7.34 ± 0.18^{B}	8.23 ± 0.22^{AB}	7.78 ± 0.19^{AB}
Reducing sugars (%)	6.69 ± 0.23^{A}	6.04 ± 0.07^{B}	5.72 ± 0.12^{BC}	5.54 ± 0.09^{C}	5.93 ± 0.06^{B}
Non-reducing sugars (%)	2.06 ± 0.67^{A}	1.78 ± 0.62^{A}	1.62 ± 0.15^{A}	2.69 ± 0.28^{A}	1.85 ± 0.18^{A}

Means with different letters in the same row (A-D) are significantly different ($P < 0.05$) from each other.

jujube cultivars. The untreated extracts had TPC in the range of 2.84 to 3.28 (Log CFU/g) whereas ultrasonic treated extracts were found to have decreased contents in the range between 1.5 (Log CFU/g) and 1.85 (Log CFU/g). There was a log reduction of > 1.28 of TPC in all the treated samples, but the maximum reduction was observed in Gola (Desi) and Umran-11 (Faisalabadi). Similarly, the untreated extracts had Y & M in the range of 2.27 to 2.48 (Log CFU/g) whereas ultrasonic treated extracts were found to have decreased contents in the range between 0.95 (Log CFU/g) and 1.35 (Log CFU/g). In this way, there was a log reduction of >0.89 (Log CFU/g) of Y & M in all the treated extracts, but the highest reduction was found in Karela (Katha). The presented results are concurrent with the results of previous findings where the reduction of microbial load in Spinach juice and apple juice has also been observed after ultrasonic application (Abid *et al.*, 2013; Khandpur and Gogate, 2015). The decrease in microbial load might be due to destruction of microbial cells when ultrasonic treatment was applied (Patist and Bates, 2008). Acoustic pressure amplitude is related to the intensity of the ultrasonic waves and in this way, greater amplitude would result in faster and violent collapse of the microbial cells (Alzamora *et al.*, 2011). In the present study, amplitude was 80% (400 W power) which might be the cause of destruction of microbial cells more efficiently. Ultrasound waves cause conversion of electrical energy into mechanical energy. Cavitation bubbles result due to pressure gradient after propagation of ultrasonic energy in the liquid medium (Costa *et al.*, 2013). That reduction in microbial load in the present study depicts the safety of jujube extracts which ultimately may enhance the shelf life of jujube extracts without significant influence on the nutritional properties.

Phytochemicals in the extracts from jujube cultivars

Test for flavonoids, flavonoid aglycones, flavonoid glycosides, phenols, tannins, saponins, steroids, terpenoids and glycosides were positive for all the jujube

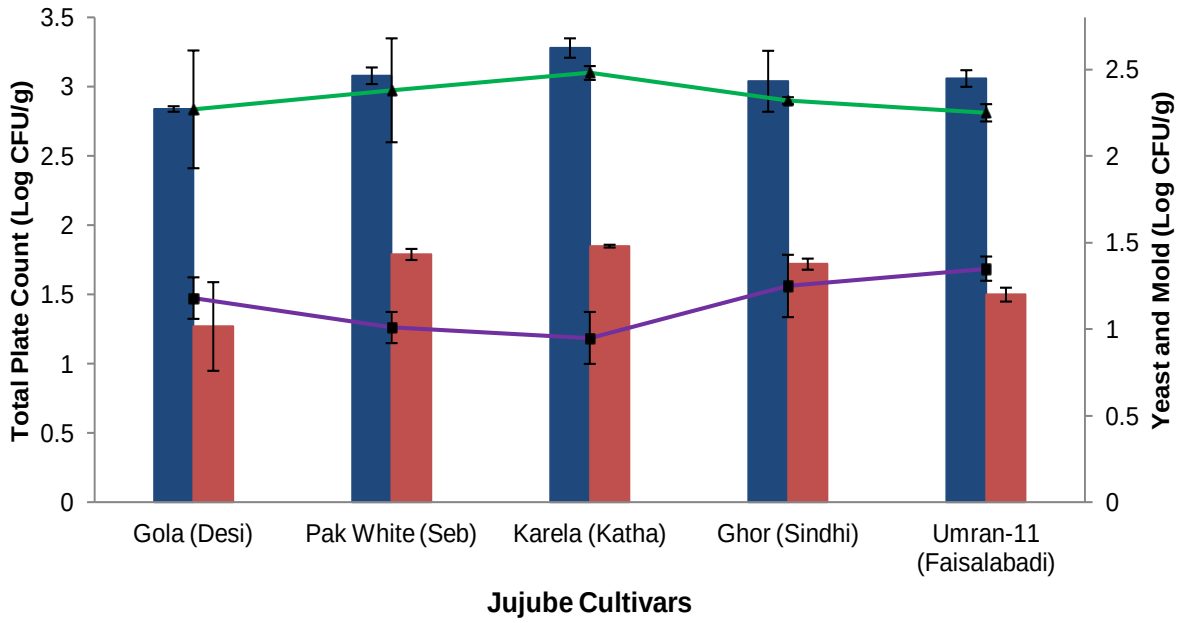

Figure 2. Effect of ultrasonic treatment on total plate counts (TPC) (blue bars showing untreated extracts and red bars showing ultrasonic treated extracts) and Yeast and Molds (green lines showing untreated extracts and purple lines showing ultrasonic treated extracts) of jujube extract cultivars. Statistical difference is noted as $P < 0.05$.

extract. All of the above mentioned phytochemicals detected in the present study have also been reported to be present in jujube fruit from Nigeria (Okala *et al.*, 2014) and India (Esteki and Urooj, 2012). Phytochemicals such as terpenoid, saponins, flavonoids, phenols, glycosides and tannins have been reported to have antioxidant activity (Dragland *et al.*, 2003; Cai *et al.*, 2004; Dai and Mumper, 2010; Koley *et al.*, 2011). Moreover, aforementioned phytochemicals have also been reported in Indian jujube (Das, 2012; Esteki and Urooj, 2012). On the basis of the identification of such phytochemicals in the present study, further quantitative estimation of phytochemicals was carried out.

Effect of ultrasonic treatment on total phenolics and total flavonoids of jujube extracts

Results regarding the effect of ultrasonic treatment on total phenolics and total flavonoids have been presented in Table 3. The phenolic acids may act as free radical acceptors and chain breaker compounds (Wanasundara *et al.*, 1997). Gola (Desi) was found to have maximum phenolics before and after ultrasonic treatment. Many phenolic compounds such as Protocatechuic acid, *p*-hydroxybenzoic acid, Chlorogenic acid, Vanillic acid, Caffeic acid, Vanillin, *p*-Coumaric acid, Ferulic acid, *o*-Coumaric acid have been identified in Gola (Desi) jujube by Memon *et al.* (2012). The highest content of phenolics in Gola (Desi) jujube cultivar in the present study might be due to the presence of aforementioned phenolic compounds. Moreover, in general, some more studies have also reported that jujube contains phenolic compounds such as *p*-hydroxybenzoic acid, Chlorogenic acid, Vanillic acid, Caffeic acid, *p*-Coumaric acid, Ferulic acid, *o*-Coumaric acid, Rutin, Quercetin, Phlorizin, Catechol, Gallic acid, Catechin, Epicatechin, (Zhao *et al.*, 2014; Muchuweti *et al.*, 2005). The present results of phenolic contents of Gola (94.13 mg GAE/g of FW) and Ghor (87.25 mg GAE/g of FW) (without ultrasonic treatment) were almost similar to those observed in jujube (94 mg GAE/mL jujube extracts) grown in India (Islam *et al.*, 2015). In another study conducted in India (Koley *et al.*, 2011), it was observed that Gola, Umran and Seb had higher (more than twice) phenolic contents than aforementioned investigated jujube cultivars in the present study. In the present study, even though the increase in phenolics was slight in all ultrasonic treated jujube extracts, but that increase was significant ($P < 0.05$). The ultrasonic treated jujube extract had phenolics in the range 85.79 to 116.3 (mg GAE/100 g of FW), whilst untreated extracts (control) had lower contents 65.75 to 94.13 (mg GAE/100 g of FW) of phenolics. The increased liberation of cell wall bound phenolics may be caused by ultrasonic treatment. Our results are concurrent with the results obtained by Alighourchi *et al.* (2013) who also observed increased contents of phenolics in the pomegranate juice after ultrasonic application.

In the present study, the flavonoid contents of Gola were also considerable. Memon *et al.* (2012) reported naringenin, a major flavonoid identified in Gola Lemai variety of *Ziziphus mauritiana*. The flavonoid contents of

Table 3. Effect of ultrasonic treatment on the total phenolics, total flavonoids, DPPH, RSA (%), total antioxidant capacity, vitamin C and reducing power of jujube extract cultivars.

Jujube cultivars	Treatment	Total phenolics[1]	Total flavonoids[2]	DPPH[3]	RSA (%)[4]	TAC[5]	Vitamin C[6]	Reducing power[7]
Gola (Desi)	Control[a]	94.13 ± 4.34^{a}	57.19 ± 7.48^{a}	1.38 ± 0.05^{cd}	35.20 ± 1.90^{b}	255.51 ± 11.87^{b}	51.65 ± 2.45^{c}	107.05 ± 12.13^{b}
	Ultrasonic	116.30 ± 8.84^{A}	70.35 ± 4.38^{AB}	2.96 ± 0.08^{A}	53.54 ± 2.78^{B}	476.24 ± 9.05^{B}	63.03 ± 1.91^{C}	152.24 ± 10.02^{B}
Pak white (Seb)	Control[a]	73.30 ± 3.11^{c}	25.81 ± 6.50^{b}	1.69 ± 0.06^{a}	47.25 ± 1.41^{b}	268.20 ± 8.63^{a}	63.88 ± 2.58^{b}	124.21 ± 9.01^{a}
	Ultrasonic	87.67 ± 6.18^{C}	41.31 ± 5.08^{C}	2.02 ± 0.06^{D}	56.08 ± 2.30^{A}	498.52 ± 12.82^{A}	83.80 ± 2.06^{A}	138.51 ± 7.14^{B}
Karela (Katha)	Control[a]	65.75 ± 3.10^{d}	31.78 ± 6.29^{b}	1.58 ± 0.04^{b}	44.45 ± 1.66^{b}	242.53 ± 8.46^{c}	69.42 ± 2.17^{a}	96.35 ± 6.05^{bc}
	Ultrasonic	85.79 ± 6.27^{C}	41.61 ± 6.05^{BC}	2.19 ± 0.06^{C}	59.70 ± 2.03^{AB}	419.57 ± 14.41^{D}	84.80 ± 2.68^{A}	169.31 ± 15.60^{A}
Ghor (Sindhi)	Control[a]	87.25 ± 4.58^{b}	61.72 ± 5.79^{a}	1.45 ± 0.04^{c}	40.18 ± 1.59^{a}	276.14 ± 10.36^{a}	63.64 ± 2.02^{b}	89.76 ± 6.18^{c}
	Ultrasonic	109.08 ± 7.42^{B}	72.05 ± 4.59^{A}	2.14 ± 0.10^{C}	58.35 ± 2.04^{C}	440.28 ± 9.11^{C}	75.35 ± 2.72^{B}	137.19 ± 5.40^{B}
Umran-11 (Faisalabadi)	Control[a]	83.96 ± 2.99^{b}	51.84 ± 7.43^{a}	1.36 ± 0.03^{d}	39.17 ± 1.35^{a}	237.55 ± 6.84^{c}	41.60 ± 2.07^{d}	95.59 ± 4.84b^{c}
	Ultrasonic	106.34 ± 7.20^{B}	62.50 ± 5.9^{B}	2.29 ± 0.07^{B}	63.73 ± 3.18^{C}	420.57 ± 6.40^{D}	52.97 ± 1.90^{D}	119.52 ± 7.03^{C}

[1]Total Phenolics (mg GAE/100 g of fresh weight (FW)), [2]Total Flavonoids (mg CE/100 g of FW), [3]2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) (µmol equivalent of Trolox/g of FW), [4]radical scavenging activity percentage (RSA%), [5]Total antioxidant capacity (mg ascorbic acid equivalent (AAE)/100 g of FW), [6]Vitamin C (mg/100 g of FW) and [7]Reducing power (mg AAE/100 g of FW), [a]Samples without ulotrasonic treatment; Means with different small letters (a-d) in the same column show significant ($P < 0.05$) differences between control samples whereas means with different capital letters (A-D) in the same column show significant ($P < 0.05$) differences between ultrasonic treated samples. Means of ultrasonic treated and untreated samples were all significantly ($P < 0.05$) different from each other in all the columns between each cultivar.

control samples (without ultrasonic treatment) of all jujube cultivars were in the range 25.81 to 61.72 (mg CE/100 g of FW). Our results regarding flavonoid contents were higher than some Indian jujube cultivars (Koley *et al*., 2011). There was a significant ($P < 0.05$) increase in flavonoid contents in all the extracts of jujube cultivars after ultrasonic treatment. The flavonoids ranged 41.31 to 72.05 (mg CE/100 g of FW) after ultrasonic treatment but Gola (Desi) and Ghor (Sindhi) were found to have maximum flavonoids both before (57.19 and 61.72 mg CE/100 g of FW) and after (70.35 and 72.05 mg CE/100 g of FW) ultrasonic treatment respectively. Pawlowska *et al.* (2009) identified ten flavonoids, that is, Quercetine 3-O-robinobioside; Quercetine 3-O-rutinoside; Quercetine 3-O-α-L-arabinosyl-(1→2)- α -L-rhamnoside; Quercetine 3-O-b-D-xylosyl-(1→2)-α-L-rhamnoside; Quercetine 3-O-β-D-galactoside; Quercetine 3-O-β-D-glucoside; 3',5'-DiC-β-D-glucosylphloretin; Quercetine 3-O-β-D-xylosyl-(1→2)-α-L-rhamnoside-4'-O-a-L-rhamnoside; Kaempferol 3-O-robinobioside and Kaempferol 3-Orutinoside, in jujube fruit. The presence of high concentration of flavonoids in the jujube included in the present study was confirmed due to presence of the above mentioned flavonoid compounds. Hydroxyl radicals generated through ultrasonic treatment may cause hydroxylation of aromatic ring of the phenolic compounds at the ortho-, meta- and para-positions. This phenomena has been suggested a reason for mounting the antioxidant properties of flavonoids extracted from food materials (Wanasundara *et al.*, 1997; Ashokkumar *et al.*, 2008).

Effect of ultrasonic treatment on DPPH antioxidant capacity and radical scavenging activity (RSA%) of jujube extracts

In this study, the increase in DPPH antioxidant capacity (µmol equivalent of Trolox/g of FW) of the extracts of all jujube cultivars was significant ($P < 0.05$) after ultrasonic treatment (Table 3). Antioxidants which were present in jujube cultivars might have an interaction with DPPH by transferring either electrons or hydrogen atoms to DPPH. In this way, the free radical character of DPPH (violet color) was neutralized (colorless). The samples (without ultrasonic treatment) had

slight variations in their DPPH capacity and ranged 1.36 to 1.69 µmol equivalent of Trolox/g of FW. The highest increase in DPPH antioxidant capacity was found in Gola (Desi) (from 1.38 to 2.96 µmol equivalent of Trolox/g of FW). Our results regarding DPPH antioxidant capacity were concomitant with the results of ultrasonic treated purple and Green Cactus Pear (*Opuntia ficus Indica*) Juice (Cansino *et al.*, 2013; Zafra-Rojas *et al.*, 2013). The RSA (%) of all jujube cultivars (without ultrasonic treatment) investigated in the present study ranged 35.20 to 47.25 (µmol equivalent of Trolox/g of FW) which were higher than the values observed in Indian jujube cultivars, that is, Gola, Seb and Umran (Koley *et al.*, 2011). The RSA (%) of ultrasonic treated extracts of jujube cultivars showed increased values compared to untreated extracts (Table 3). The maximum RSA (%) (63.73) was found in the extracts of Umran-11 (Faisalabadi) after ultrasonic treatment. It has been reported that DPPH radicals are scavenged by antioxidants such as phenolics and ascorbic acid contents, leading to increased antioxidant activity (Khandpur and Gogate, 2015; Kidmose and Martens, 1999). Therefore, it was supposed that ultrasonic treatment caused increased extraction of phenolic compounds or antioxidants from the extracts of jujube cultivars which caused increased DPPH radical scavenging capacity.

Effect of ultrasonic treatment on total antioxidant capacity (TAC) of jujube extracts

In the present study, the total antioxidant capacity (TAC) (mg AAE/100 g of FW) of the extracts of all jujube cultivars was significantly ($P < 0.05$) increased after ultrasonic treatment (Table 3). The TAC (mg AAE/100 g of FW) of extracts of jujube cultivars (without ultrasonic treatment) ranged from 242.53 to 276.14 (mg AAE/100 g of FW) whereas ultrasonic treated extracts were found to have TAC in the range between 419.47 and 498.52 (mg AAE/100 g of FW). The maximum TAC was found in Gola (Desi) (476.24 mg AAE/100 g of FW) and Pak White (Seb) (498.52 mg AAE/100 g of FW) after ultrasonic treatment. The increased TAC might be attributed to ultrasonic treatment because this technique increased liberation of bound antioxidants such as phenolics and ascorbic acid contents, leading to increased antioxidant activity (Khandpur and Gogate, 2015; Kidmose and Martens, 1999). Further, ultrasonic treatment may inactivate enzymes, for instance, polyphenol oxidases which are responsible for enzymatic browning (Jang *et al.*, 2009; Lopez *et al.*, 1994), leading to improved TAC values.

Effect of ultrasonic treatment on vitamin C contents and reducing power of jujube extracts

Vitamin C is considered an important nutrient, possessing antioxidant capacity, thereby protecting against free radicals (Esteve *et al.*, 2005). It also indicates nutritional quality of fruit juices (Bull *et al.*, 2004). Table 3 shows the effect of ultrasonic treatment on vitamin C contents of jujube cultivars. The vitamin C contents of untreated jujube extracts ranged from 41.60 to 69.42 (mg/100 g of FW). Among different jujube cultivars, Pak White and Karela showed maximum values. The results of the present study concerning vitamin C contents of samples (without ultrasonic treatment) of all jujube cultivars are in agreement with the results obtained by jujube grown in Bangladesh (Burhan Uddin and Hussain, 2012). The vitamin C contents of Indian jujube cultivar, that is, Gola (57.65 mg/100 g of FW) (Koley *et al.*, 2011) were almost similar to those observed of Gola (without ultrasonic treatment) in the present study (51.65 mg/100 g of FW) but Indian Seb and Umran cultivars were found to have lower contents (21.95 and 19.54 mg/100 g of FW respectively) (Koley *et al.*, 2011) compared to those observed in the above mentioned Pakistani cultivars. In the present study, the maximum vitamin C contents (69.64 mg/100 g) were shown by the samples (without ultrasonic treatment) of Karela cultivar which were lower than the jujube cultivars investigated in India (103.03 mg/100 g) and China (ranged from 192 to 359 mg/100 g) (Esteki and Urooj, 2012; Li *et al.*, 2007). The variations in vitamin C contents as reported by other researchers may be attributed to various factors such as maturity of the jujube fruits, cultivars used and geographical location. The vitamin C contents of ultrasonic treated extracts showed vitamin C contents between 52.97 and 84.80 (mg/100 g of FW). A significant ($P < 0.05$) increase in vitamin C was observed in the ultrasonic treated samples compared to untreated samples. The increase in vitamin C contents may be attributed to the removal of dissolved oxygen, destruction of the agents responsible for its degradation and production of cavitations during ultrasonic treatment (Cheng *et al.*, 2007).

The results of reducing power (mg ascorbic acid equivalent (AAE)/100 g of FW) have been presented in Table 3. The higher absorbance of the reaction mixture may indicate higher reducing ability of the biologically active compounds (possessing potent donating abilities) present in the extracts of jujube cultivars. The reducing power of the extracts of all jujube cultivars was significantly ($P < 0.05$) increased after ultrasonic treatment. The highest increase (169.31 mg AAE/100 g of FW) in reducing power was shown by the extracts of Karela (Katha) cultivar. The reducing power of extracts (without ultrasonic treatment) of jujube cultivars ranged from 89.76 to 124.21 mg AAE/100 g of FW whereas ultrasonic treated extracts were shown to have reducing power in the range between 119.52 to 169.31 mg AAE/100 g of FW. The increase in reducing power of jujube extracts might be due to the presence of appreciable concentrations of phenolics, flavonoids and vitamin C after ultrasonic treatment. The increased

reducing power results in higher antioxidant activity of jujube extracts.

The jujube fruit is underutilized and very cheap compared to costly fruits (apple, banana, strawberry, citrus, litchi and mango) possessing good antioxidant activity owing to presence of appreciable concentration of phytochemicals. Therefore, people in Pakistan should consume jujube due to its antioxidant activity. As the season as well as shelf life of this tiny fruit is very short, therefore, it should be converted into some valuable products such as jam, juices, syrups, pickles, etc. In addition, ultrasonic application may improve its nutrition regarding increase in phenolics, flavonoids, vitamin C. On the basis of our findings, it may be suggested that jujube fruits may be converted into nutritious and safe products after ultrasonic applications. Moreover, the increased antioxidant potential of jujube cultivars after ultrasonic treatment may be helpful for commercial as well as consumers health point of view as the technique maximizes the retention of nutrients.

CONCLUSIONS

On the basis of our results, it may be concluded that total phenolics, flavonoids, vitamin C were increased after ultrasonic treatment. Similarly, microbial load of jujube cultivars was decreased after ultrasonic treatment.

Hence, it may be suggested that ultrasonic treatment may improve the nutritional value of jujube which will be beneficial for consumer's health.

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