CHANGES IN CHEMICAL COMPOSITION, TOTAL PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITIES OF JUJUBE (*Ziziphus jujuba* Mill.) FRUITS AT DIFFERENT MATURATION STAGES

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Abstract. Jujube (*Ziziphus jujuba* Mill.) fruit is an important medicinal plant in Turkey. Several characteristics of jujube fruit harvested at four maturation stages were evaluated. The maturation stages were classified by degree (%) of dark color formation on the fruit surface [Stage (S1), 1–10% (S2), 11–50% (S3), 51–100% (S4)]. Fruit weight, width, length, stone weight, flesh/stone ratio, total soluble solids (TSS), titratable acidity (TA), organic acids and specific sugars were determined. Total phenolic content (TPC) was measured along with antioxidant activity (AOC), determined by Ferric Reducing Ability of Plasma (FRAP) and Trolox Equivalent Antioxidant Capacity (TEAC). Results of the study showed that TSS increased from 12.8% to 18.3% from the S1 to the S4 fruit stage, respectively. The S3 had the highest TPC (6518 mg GAE·kg fw). The highest AOC were recovered from S2 (TEAC; 74.4 µmol TE·g fw, FRAP; 50.9 µmol TE·g fw), followed by S3 (TEAC; 63.6 µmol TE·g fw, FRAP; 37.6 µmol TE·g fw). The main organic acid was citric acid as determined by the HPLC method. Fructose content tended to be more stable than glucose or sucrose.

Key words: Jujube, TEAC, FRAP, phenolic, organic sugar

INTRODUCTION

Jujube (*Ziziphus jujuba* Mill.) is a species long-cultivated in the Mediterranean region and southern and eastern Asia, South China, and currently, jujube trees can be found in the flora of South and in some regions of North Italy, and west and south Turkey. This plant grows in areas with Mediterranean climate [Kirkbride et al. 2006, Ercisli et al. 2007, Ecevit et al. 2008]. Among the wild growing edible fruits, jujube is the most widespread in Turkey and has served as a source of food and medicine for years

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[Gültekin 2007, Ecevit et al. 2008, Kamiloğlu et al. 2009]. Recently, jujube plantations have been established as an alternative fruit crop for Turkey. Consumers are finding out about foods that can be classified as functional foods that are rich in health properties due to increased communication. Fruit quality is defined as the degree of excellence or superiority of a combination of attributes, properties or characteristics that give each commodity value as human food [Kader 1999]. Phytochemicals, which act as antioxidants, are increasingly being shown to help to optimize human health by neutralizing harmful free radicals in the body. These antioxidants reduce oxidative damage by cells that can lead to cancer, heart disease, and other degenerative diseases [Prior 2003]. Studies have shown potential neuroprotective effects of plant origin antioxidants such as flavonoid compounds against alcohol-induced injury [Antonio and Druse 2008]. Jujube, widely distributed in Turkey, has gained wide attention in native herbal medicine for treatment of a broad range of disorders. Chemical analyses of jujube fruit have shown high levels of flavonoids, [Pawlowska et al. 2009], total phenolics and antioxidant activity [Li et al. 2007, Kamiloğlu et al. 2009]. Recently, semi-mature fruit are also being consumed as fresh or processed products. Currently, there is little information available on the effect of maturity stages of jujube fruit on phytochemical and antioxidant properties. Most of current literature is focused on the chemical properties of mature jujube. The objective of this study was to determine the fruit characteristics, phytochemical accumulation, antioxidant activity and sugars of jujube at four different maturity stages.

MATERIALS AND METHODS

Plant material. The Jujube fruits were harvested from an orchard established in Antakya, Hatay, Turkey. Fruits were hand harvested at approximately 15 day intervals to get fruit in four different maturity stages from the beginning of July to mid-August 2010. The maturation stages were classified by degree (%) of dark color formation on the fruit surface [(Stage (S1), 1–10% (S2), 11–50% (S3), 51–100% (S4)]. Fruit were transferred from the field to the laboratory within the same day for physical and phytochemical analysis.

Physical measurements

Measurements were done by means of precision analytical scales and digital micrometer calipers. Flesh to stone ratio was interpreted as a share of the mesocarp compared with fruit weight, expressed in percentage. The fruit color was measured using a Minolta portable Chroma meter (Minolta, Model CR-400) which provided CIE L*, a* and b* values. Chroma and hº values were also calculated according to Sacks and Shaw [1994]. Then, about 100 g of fruit with three replicates for each maturity stages were frozen at -20°C. At the time of analysis, fruits were thawed and homogenized in a standard food blender. Slurries were used to determine TSS contents by refractometery (Atago, Pal-1) and TA (expressed as % citric acid) was determined by titrating with 0.1 N NaOH to an endpoint of pH 8.10 [Perkins-Veazie and Collins 1993]. Chemical analysis was completed within two weeks of storage.

Analytical procedures

Determination of total phenolic content (TPC). TPC was measured according to the procedure of Singleton and Rossi [1965]. For each replicate, a 3 g aliquot of the slurry was transferred to polypropylene tubes and extracted with 20 mL of extraction buffer containing acetone, water, and acetic acid (70:29.5:0.5 v/v) for two hours. After filtration, acetone was removed by rotary evaporation and the concentrated samples were brought to a final volume of 20 mL with de-ionized water. To determine TPC, 0.5 mL of each extract was combined with Folin-Ciocalteu's phenol reagent and water 1:1:20 (v/v) and incubated for eight minutes followed by the addition of 5 mL of 7% (w/v) sodium carbonate. After two hours, the absorbance was measured by an automated UV-vis spectrophotometer (Model T60U, PG Instruments) at 750 nm. Gallic acid was used as standard. The results were expressed as mg gallic acid equivalents in kg fresh weight basis (GAE·g fw).

Determination of total antioxidant capacity (AOC). AOC was estimated by two standard procedures FRAP and TEAC. FRAP was determined according to Benzie and Strain [1996]. The assay was conducted using three separate stock solutions containing 0.1 mol·L acetate buffer (pH 3.6), 10 mmol·L TPTZ [2,4,6-tris(2-pyridyl)-1,3,5-triazine] acidified with concentrated hydrochloric acid, and 20 mmol·L ferric chloride. These solutions were prepared and stored in the dark under refrigeration. Stock solutions were combined (10:1:1 v/v/v) to form the FRAP reagent just prior to analysis. For each assay, 2.95 mL of FRAP reagent and 50 µL of sample extract were mixed. After 10 minutes, the absorbance of the reaction mixture was determined at 593 nm on a spectrophotometer. For the standard TEAC assay, ABTS (2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) was dissolved in acetate buffer and prepared with potassium persulfate as described in Özgen et al. [2006]. The mixture was diluted in acidic medium of 20 mM sodium acetate buffer (pH 4.5) to an absorbance of 0.700 ±0.01 at 734 nm for longer stability. For the spectrophotometric assay, 2.95 mL of the ABTS + solution and 50 µL of fruit extract were mixed and incubated for 10 min and the absorbance was measured at 734 nm. Trolox was used as standard for both assays. The results were expressed as µmol Trolox equivalent in g fresh weight basis (TE·g fw).

Analysis of organic acid and sugars using HPLC

Fruit slurries (5 g) were diluted with purified water or meta-phosphoric acid (2.5%) solution for individual sugar and organic acid analysis, respectively. The homogenate was centrifuged at 6000 rpm for 5 min. Supernatants were filtered through a 0.45-µm membrane filter (Iwaki Glass) before HPLC analysis, and the mobile phase solvents were degassed before use. All samples and standards were injected three times each and mean values were used. The HPLC analysis was carried out using a Perkin Elmer HPLC system with Totalchrom Navigator 6.2.1 software, a pump and UV detector (Perkin Elmer Series-200) (Waltham, Massachusetts, USA). Separation and determination of organic acids was performed based on the method described by Shui and Liang [2002] and modified by Özgen et al. [2009a].

The separation was carried out on a SGE wakosil C18RS 5µm column (250 × 4.6 mm I.D.). Analysis of sugars was performed according to the method described by Bar-

tolome et al. [1995] using a refractive index (RI) detector (Perkin Elmer). The separation was carried out on a SGE SS Exsil amino column (250 × 4.6 mm I.D.).

Statistical analysis. Data were analyzed using SAS procedures and software [SAS 2005]. Means and standard deviations were obtained using TABULATE procedure. Analyses of variance were carried out by GLM procedure. The means were separated using least significant difference (LSD) method at P < 0.05 significance level.

RESULT AND DISCUSSION

Physical measurements. For the common assessment of horticultural maturity of many fruit crops such as jujubes, several pomological characteristics and maturation stages are commonly presented (tab. 1). In this study, during maturation and ripening there was a considerable increase in fruit weight. The highest increas (P < 0.05) among the different maturation stages. TSS increased from 12.8% to 18.3% from the S1 to the S4 fruit stage, respectively. TA also decreased from S1 to S4 and the maturation stages could be separated into two groups: S1 and S2 with 0.36% and 0.33%, respectively, and S3 and S4 have similar TA value of 0.41%.

Table 1. Means of several pomological characteristics of jujube fruits sampled at four maturation stages

Stage	Fruit weight (g)	Fruit width (mm)	Fruit length (mm)	Stone weight (g)	Flesh/stone ratio (%)	Soluble solid (%)	Acidity (%)
S1 (0% dark)	6.4 b	22.3 b	25.1 b	0.51 a	11.4 b	12.8 d	0.36 b
S2 (1–10% dark)	6.6 b	22.3 b	24.5 c	0.48 b	12.7 b	14.5 c	0.33 b
S3 (11–50% dark)	7.3 a	23.2 a	25.9 a	0.42 c	16.4 a	16.7 b	0.41 a
S4 (51–100% dark)	6.8 b	22.8 ab	25.7 a	0.39 c	16.5 a	18.3 a	0.41 a
Mean	6.7	22.6	25.3	0.45	14.3	15.6	0.38
$LSD_{0.05}$	0.5	0.6	0.4	0.03	1.8	1.2	0.04

Stages were represented by the proportion of the fruit skin that was darkly colored. Means in each column not followed by the same letter are significantly different at *P* < 0.05 according to the Least Significance Difference (LSD).

The color measurements of external and internal *L, a, b, Chroma* and *hue* are presented in Table 2. *L* indicates lightness, from 0 (black) to 100 (white): *a*, from -60 (green) to 60 (red): and *b*, from -60 (blue) to 60 (yellow). These characteristics in each of the maturation stages were statistically different (P < 0.05). External *a* changed from green to red color. This situation explains only the external color change associated with jujube maturity.

Analysis of sugars and organic acid using HPLC. The results of the sugar analyses and organic acid measurements are presented in Table 3. The average total sugar

Table 2. Means values of color parameters of jujube fruits sampled from four maturation stages

|	Stage	*L*	*a*	*b*	Chroma	hueº
External	S1 (0% dark)	73.2 a	-18.5 d	38.2	42.4 a	115.8 a
	S2 (1–10% dark)	72.1 a	-15.2 c	38.5	41.5 a	111.6 a
	S3 (11–50% dark)	66.0 b	-8.7 b	39.2	40.4 ab	99.0 b
	S4 (51–100% dark)	49.6 c	7.9 a	36.1	37.8 b	77.1 c
	Mean	65.2	-8.6	38	40.5	100.9
	LSD$_{0.05}$	3.3	2.6	ns	2.7	4.9
Internal	S1 (0% dark)	75.8 c	-16.5 b	28.9 a	33.3 ab	119.7 a
	S2 (1–10% dark)	75.2 bc	-16.7 b	29.5 a	33.9 a	119.4 a
	S3 (11–50% dark)	76.5 b	-15.6 a	28.6 a	32.6 b	118.6 b
	S4 (51–100% dark)	78.7 a	-15.1 a	27.4 b	31.3 c	118.9 b
	Mean	76.5	-16	28.6	32.8	119.2
	LSD$_{0.05}$	1	0.7	1	1.2	0.6

Stages were represented by the proportion of the fruit skin that was darkly colored. Means in each column not followed by the same letter are significantly different at $P < 0.05$ according to the Least Significance Difference (LSD).

Tab. 3. Mean values of total sugars and acids of jujube fruits sampled from for maturation stages

Stage	Sugars (g·100 g)				Acids (g·100 g)				
	fructose	glucose	sucrose	total	tartaric	malic	ascorbic	citric	total
S1 (0% dark)	5.2 a	8.1 a	1.5 d	14.8 b	0.04 a	0.12 c	0.06 c	0.31 a	0.54 a
S2 (1–10% dark)	4.1 b	6.4 b	2.2 c	12.7 c	0.02 b	0.09 d	0.08 b	0.19 b	0.38 d
S3 (11–50 dark)	4.2 b	6.6 b	10.3 b	21.1 a	0.01 c	0.15 b	0.10 a	0.19 b	0.45 c
S4 (51–100% dark)	4.0 b	6.2 b	11.6 a	21.8 a	0.02 b	0.17 a	0.10 a	0.20 c	0.48 b
Mean	4.4	6.8	6.4	17.6	0.02	0.13	0.08	0.22	0.46
LSD$_{0.05}$	0.3	0.7	0.4	1.26	0.01	0.02	0.04	0.11	0.15

Stages were represented by the proportion of the fruit skin that was darkly colored. Means in each column not followed by the same letter are significantly different at $P < 0.05$ according to the Least Significance Difference (LSD)

amount was 17.6 g 100 g^{-1}. The S3 (21.1 g 100 g^{-1}) and S4 (21.8 g 100 g^{-1}) stages had the highest values. Fructose and glucose were found to be unchanging sugars in all maturation stages. Sucrose was very low in S1 and S2; however, in S3 and S4 it increased approximately 5–6 times. Fructose and glucose content tended to be more stable sucrose. The total organic acid value was the highest in the S1 (0.54 g 100 g^{-1}). The
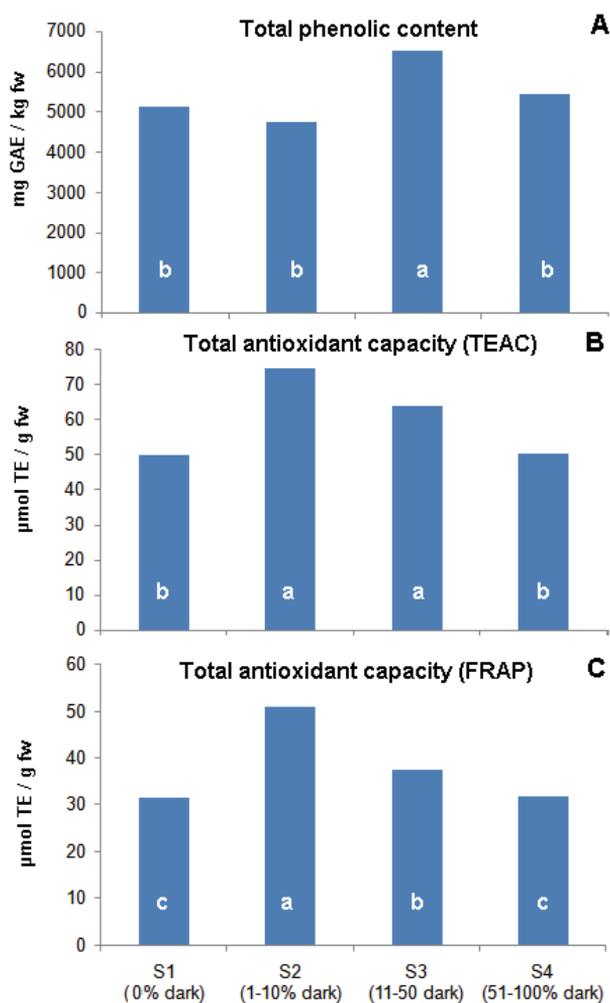
Stages were represented by the proportion of the fruit skin that was darkly colored. Means in each column not followed by the same letter are significantly different at *P* < 0.05 according to the Least Significance Difference (LSD).

Fig. 1. Means and mean separations for total phenolics and total antioxidant capacity determined by TEAC and FRAP of jujube fruits sampled from for maturation stages

main organic acid was citric acid (0.22 g 100 g^{-1}) followed by malic and ascorbic acids with mean value of 0.13 and 0.08 g 100 g^{-1} respectively. Malic acid concentrations (0.12 to 0.17 g 100 g^{-1}) seemed to increase while citric acid concentrations (0.31 to 0.20 g 100 g^{-1}) decreased as the fruit matured (tab. 3). All values in the various maturity stages were statistically significant (P < 0.05): however, they had different trends: the highest citric acid and tartaric acid content was measured in the S1, while the highest malic and ascorbic acid amount was recorded at the S4 stage.

Determination of total phenolic content and antioxidant capacity. The TPC and AOC were determined by two methods and the results are presented in Figure 1. TPC and AOC values varied significantly across the four maturation stages. S3 had the highest total phenolics content (6518 mg GAE·kg fw), followed by S4, S1 and S2 with mean values of 5451, 5138 and 4742 mg GAE·kg fw respectively. The highest antioxidant capacities were recovered from S2 (TEAC; 74.4 μmol TE·g fw, FRAP; 50.9 μmol TE·g fw) followed by S3 (TEAC; 63.6 μmol TE·g fw, FRAP; 37.6 μmol TE·g fw).

In order to increase the consumption of healthy foods, they must have an attractive taste. The combination and the ratio of sugars and organic acids have been related to flavor quality of fruits [Özgen et al. 2008]. As fruits approach maturity, the total sugars increase, mainly due to an increase in reducing sugars. Beadury [1992] suggested that perception of sweetness may be affected by others factors, such as TA. Celik et al. [2008] showed that TSS content in cranberry increased from 6 to 9.3% from the green to dark red stage. Similarly, Özgen et al. [2009b] found in TSS in *Arbutus andrachne* fruits increased from 16.0 to 20.6% from the green to red stages. Another study also showed that glucose and fructose were the main sugars present [Li et al. 2007].

Organic acids are also important for flavor. The predominant organic acid analyzed was citric acid (47.8%). The citric acid content seemed to decrease with fruit maturity while the malic acid content increased. Similar increase in malic acid was found in *Arbutus andrachne* fruits [Özgen et al. 2009b]. Many factors may affect fruit weight, TSS and TA of fruits including cultivar/genotype, altitude, environmental conditions [Kader 1999, Ercisli et al. 2007, Özgen et al. 2008, Sun et al. 2011].

The lowest AOC were determined at S1 and S4. Average AOC measurements were 59.5 and 37.9 μmol TE·g fw by TEAC and FRAP methods, respectively. TPC and AOC values varied significantly at the four maturation stages. TPC and AOC are reported to be closely associated with several factors including genotype, growing conditions, stage of maturity, fruit characteristics, size, storage temperature, postharvest durations and treatments [Kalt 2005, Özgen et al. 2008]. Blueberry fruit maturity had a significant effect on AOC and TPC, ripeness and fruit maturity interactions were significant [Connor et al. 2002]. Similar results were reported by Prior et al. [1998]. In another study done by Mainland et al. [2002] and Wang et al. [2013] the levels of AOC decreased from the ripe to the overripe stage.

CONCLUSIONS

In this study, we measured a number of fruit characteristics, along with phytochemical accumulation, antioxidant activity and sugar formation in jujube fruit at four different maturity stages. Significant variability was found for overall fruit characteristics tested in the study, phytochemical accumulation, antioxidant activity and types of sugars of jujube at four different maturity stages. Jujube fruits are a significant source of phenolic compounds. The present study indicated that the Jujube is rich source of phenolics and antioxidants, demonstrating its potential use as a functional foods. The S3 stage had highest TPC and S2 has the highest AOC. Therefore, to recover the highest anti-oxidant capacity, fruit should be harvested at the S2 and S3 stage.

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ZMIANY W SKŁADZIE CHEMICZNYM, CAŁKOWITEJ ZAWARTOŚCI FENOLI I AKTYWNOŚCI ANTYOKSYDACYJNEJ OWOCÓW GŁOŻYNY POSPOLITEJ (*Ziziphus jujuba* MILL.) NA RÓŻNYCH POZIOMACH DOJRZEWANIA

Streszczenie. Owoce głożyny pospolitej (*Ziziphus jujuba* Mill.) są ważną rośliną leczniczą w Turcji. Oceniono kilka cech głożyny zebranej w czterech fazach dojrzewania. Etapy dojrzewania oceniono według stopnia (%) tworzenia się ciemnego koloru na powierzchni owoców [etap (E1), 1–10% (E2), 11–50% (E3), 51–100% (E4)]. Określono wagę, długość i szerokość owoców, wagę pestek, stosunek miąższu do pestki, całkowitą zawartość rozpuszczalnych substancji stałych (TSS), kwasowość miareczkową (TA) oraz kwasy organiczne i cukry. Całkowitą zawartość fenoli (TPC) zmierzono razem z aktywnością antyoksydacyjną według metody FRAP i TEAC. Wyniki badania wykazały, że TSS zwiększył się, odpiwiednio, z 12,8% do 18,3% od etapu E1 do E4. E3 miał najwyższy TPC (6518 mg GAE·kg świeżej masy). Najwyższe AOC stwierdzono w E2 (TEAC; 74,4 μmol TE·g świeżej masy, FRAP; 50,9 μmol TE·g świeżej masy), następnie w E3 (TEAC; 63,6 μmol TE·g świeżej masy, FRAP; 37,6 μmol TE·g świeżej masy). Głównym kwasem organicznym był kwas cytrynowy określony metodą HPLC. Zawartość fruktozy była bardziej stabilna niż glukozy czy sacharozy.

Słowa kluczowe: głożyna pospolita, TEAC, FRAP, fenole, cukier organiczny