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Original Article

Variation of Bioactive Compounds and Antioxidant Activity of Jujube (*Ziziphus jujuba)* Fruits at Different Stages of Ripening

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Abstract

Fruits of two jujube (*Ziziphus jujuba* Mill.) cultivars were collected at four stages of ripening and analyzed in terms of chemical compounds. The cultivars were grown under the climatic conditions of the Sub-Carpathian area Ramnicu-Valcea in Romania (45°6′17″N, 24°22′32″E). Total phenols content, total flavonoids content, organic acids from jujube fruits and related antioxidant activities were investigated during the ripening stages. The antioxidant activity of alcoholic extracts was investigated by the 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method. Total phenolic content was determined by using the Folin-Ciocalteu assay, while determination of flavonoids was done by aluminium nitrate colorimetric method, and organic acids were separated by reversed phase chromatography. The results obtained have indicated significant differences ($p < 0.05$) found in bioactive compounds and antioxidant activity, depending on cultivars and ripening stage. Total phenolic content ranged from 1634.44 to 472.38 mg GAE/100g in methanolic extracts of the two varieties studied, while total flavonoids content ranged from 19.9 to 48.5 mg QE/100 g. As regards the influence of ripening stage, the results show that the highest antioxidant activity was recorded in the stage of white maturity. The impact of maturity stage was significant on organic acid distribution of jujube extracts, and ascorbic acid is the major organic acid found in jujube fruits, followed by malic acid and lactic acid. Jujube fruits are rich in bioactive compounds and can be a complement in human's healthy eating.

Keywords: antioxidant activities, jujube, organic acid, total flavonoids content, total phenols content

Introduction

Ziziphus jujuba is a fruit tree species native in Central Asia where is cultivated since ancient times and from where it was expanded to Asia Minor, Europe and America. In Romania, the Chinese date palms can be found in semi-spontaneous status in Dobrogea region (Ciocarlan, 2000), located between the Danube and the Black Sea; there are several places where the Chinese date palms exist in naturalized populations (Stanica, 2008). This fruit tree has been growing for several hundred years in Ostrov and Jurilovca villages and is called by local people "Dobrogea's olive tree" (Stanica, 2016). In the southern part of Romania there are appropriate natural conditions for Chinese jujube cultivation, the trees are extremely drought resistant and this species can be cultivated in integrated and organic farms and in small private gardens (Stanica, 2009). According to literature, Chinese date palm has a complex chemical composition that is useful to human health. The main biologically active components are vitamin C, phenolics, flavonoids, triterpenic acids, and polysaccharides (Gao *et al.*, 2013). Twelve flavonoids and nine phenolic acids were identified in ber (*Ziziphus mauritiana*) extract by LC–DAD–MS analysis made by Memon *et al.* (2013). Investigations conducted by Li *et al.* (2007) showed that Chinese jujube contained 80.86-85.63% carbohydrate, 57.61-77.93% reducing sugar, 0.57-2.79% soluble fibre, 5.24-7.18% insoluble fibre, 4.75-6.86% protein, 0.37-1.02% lipid, 17.38-22.52% moisture and 2.26-3.01% ash. Two phenolic acids (chlorogenic acid and caffeic acid) and three flavonoids (catechin, epicatechin and rutin) were determined by high-performance liquid chromatography in extracts of dried Chinese jujube fruit by Hudina *et al.* (2008). A total of 25 polyphenolic compounds were

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identified and classified as 10 flavan-3-ols, 13 flavonols, 1 flavanone, and 1 dihydrochalcone by Wojdylo *et al*. (2016) in four Spanish jujube cultivars. Jujube has a long history of usage as a fruit and remedy. Recent phytochemical studies of jujube fruits have shed some light on their biological effects, such as the anticancer, anti-inflammatory, antiobesity, immunostimulating, antioxidant, hepatoprotective, and gastrointestinal protective activities and inhibition of foam cell formation in macrophages (Gao *et al*., 2013; Abedini *et al*., 2016; Rajopadhye and Upadhye, 2016; Keerthi *et al*., 2016). In this paper, total phenols content, total flavonoids content, organic acid from jujube fruits and related antioxidant activities were investigated during the ripening stages in two jujube varieties grown under the climatic conditions of the Sub-Carpathian area Ramnicu-Valcea, Romania.

Materials and Methods

Samples

In this research the fruit composition of two jujube cultivars ('Ya Tsao', 'Ta-Jan Tsao') was analyzed. The fruits come from the experimental orchard of University of Craiova (SCDP Valcea), located in Sub-Carpathian area in Oltenia Region, Romania (45°6′17″N, 24°22′32″E). Jujube samples were harvested in September 2016 at four edible stages of maturity that were determined based on the surface color, from white maturity (yellow skin color = stage 1) to red maturity (having 100% red surface area = stage 4). Jujubes were picked up randomly from different parts of several trees of the same species and were free from visible blemishes and diseases. After harvest, the fruits were immediately stored at -20 °C ± 0.2 °C until further use.

Analytical methods

The jujube samples were evaluated on the basis of their dry matter at 105 °C and soluble solids content. Soluble solids were measured in the juice pressed from the whole fruit. Soluble solids concentration was measured using a digital refractometer and the results were expressed as percentages.

Extraction procedure

Pulp and whole fruit tissue (50g) from each cultivar have been finely chopped and then homogenized (using an IKA T-25 ULTRA-TURRAX mixer-homogenizer). Two extracts have been made: an aqueous extract used to quantify the organic acids and ascorbic acid, and a methanolic extract for extracting polyphenolic compounds and estimating their antioxidant properties. The extraction procedure was as follows: 2 g of product have been mixed with 20 mL water / 20 mL methanol, with 2% hydrochloric acid and put in sealed polyethylene flasks; and then were kept for 60 minutes at a temperature of 30 °C under the ultrasounds action.

Chemicals and reagents

Folin-Ciocalteu reagent (2N, Sigma-Aldrich), gallic acid (Sigma-Aldrich), anhydrous sodium carbonate (Sigma-Aldrich), methanol (Merck), 2,2-diphenyl-1-picrylhydrazyl (DPPH; Merck), ascorbic acid (Merck), quercetin (Merck), aluminium nitrate (Sigma-Aldrich), potassium acetate (Sigma-Aldrich), malic acid (Sigma-Aldrich), tartaric acid (Sigma-Aldrich), oxalic acid (Sigma-Aldrich), ascorbic acid (Sigma-Aldrich), and lactic acid (Sigma-Aldrich) were used in this experiment.

Determination of total phenol content

Total phenolics content in the extracts were colorimetrically determined with Folin-Ciocalteu reagent by using the method described by Cosmulescu *et al*. (2015). The absorbance of mixture was measured at 765 nm using an Evolution 600 UV/VIS spectrophotometer (Thermo Scientific, USA). Gallic acid was used to prepare the standard curve (0-250 mg/L). Gallic acid was used as reference standard and results were expressed as gallic acid equivalents in milligrams per 100 g (mg GAE/100g).

Total flavonoids content

Determination of flavonoids was done by aluminium nitrate colorimetric method described by Cosmulescu *et al*. (2015). Quercetin was used for preparing the standard curve (0-100 mg/L). The results were expressed as milligrams of quercetin equivalents per 100 g (mg QE/100g).

Anti-oxidant activity

The capacity of scavenging of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical was monitored according to the method reported by Cosmulescu *et al*. (2015). Anti-oxidant capacity was expressed in mg ascorbic acid equivalents per 100g (mg acid ascorbic /100g).

Organic acid content

The organic acids in the sample test solution were separated by reversed phase chromatography on a 250 mm × 4.6 mm i.d., 5 μm particle Hypersil Gold aQ Analytical Column, of which they were detected by absorbance and quantified with external calibration graphs. The HPLC analysis was performed with a Surveyor Thermo Electron system comprising a vacuum degasser, Surveyor Plus LCPMPP pump, Surveyor Plus ASP autosampler and a diode array detector with 5 cm flow cell by using the method described by Nour *et al*. (2010). Integration, data storage and processing were performed by Chrom Quest 4.2 software. Organic acid content was expressed in mg per liter (mg /L).

Statistical analysis

Data have been subjected to analysis of variance (ANOVA) using Statgraphics Centurion XVI software (StatPoint Technologies, Warrenton, VA, USA). Differences were estimated with a multiple range test using the least significant difference (LSD) at $P < 0.05$.

Results and Discussion

Analytical methods

The results obtained have indicated significant differences ($p<0.05$) in dry matter content between ripening stage (Table 1). The dry matter contents of the

fruits from jujube increased with maturity stage of the two varieties. The minimum dry matter contents were found in the first harvest (white maturity). The same variation was recorded for soluble solids content. Dry matter total content and soluble solids content in jujube cultivars ranged from 17.92 to 33.2 % and 7 to 23%, respectively, depending on maturity stage. For comparison, Koley *et al.* (2016) have reported similar values of soluble solids content for 12 commercial genotypes of Indian jujube (10 to 19%).

Total phenols content

There are significant differences ($p<0.05$) in total phenolics content between cultivars and ripening stage (Table 2). Total phenolic content ranged from 475.3 to 1634.4 mg GAE/100 g in jujube fruits of the two cultivars studied. According to these results, total phenols content (mg GAE/100 g) of jujube was higher than that one found by Zhao (2014) in ethanolic extracts from seven cultivars of Chinese jujube (which ranged from 454.3 to 1298.9 GAE mg/100 g). A variation of total phenolics content was found by Li *et al*. (2007) in five cultivars of Chinese jujube (it ranged from 5.18 to 8.53 mg/g), and found also by Wojdylo *et al*. (2016) in Spanish jujube fruits (1442 to 3432 mg/100 g). All these results indicate that the content of total phenols is affected by cultivars, geographical conditions, horticultural factors, and more. The maturity stages had a significant influence in total phenolics content in jujube. The total phenolics content significantly decreased with the increase in maturity stages. In this present study the total phenolic content in jujube fruits decreased with maturity stage (2.6 times up to 3.1 times), which was in agreement with those reported previously for jujubes and other fruits. The same conclusion can be found in the study made by Wang *et al*. (2016) on jujube harvested in three edible stages of maturity. These results indicated that total phenolics content in jujube was clearly dependent on the ripening stage, and the highest concentrations were found within the more green stages. Wu *et al*. (2012) reported that un-coloring jujubes account as natural antioxidants with abundant proanthocyanidins.

Total flavonoids content

The results of flavonoids content, expressed as milligrams of quercetin equivalents per 100 g are presented in Table 2. Many significant differences ($p<0.05$) are observed in total flavonoids content between cultivars and ripening stage among cultivars. Total flavonoids content ranged from 26.7 to 48.5 mg QE/100 g in 'Ya Tsao' cultivar and between 19.9 and 34.6 in 'Ta-Jan Tsao' cultivar. The content of total flavonoids in genotypes of Indian jujube was also found to vary significantly, and the content ranged from 8.36 to 21.97 mg CE/100 g (Koley *et al*., 2016). The total flavonoids content of jujube fruits decreased with maturity stage (1.7-1.8 times). In the research made by Wang *et al.* (2016) the content of total flavonoid contents was significantly reduced by 6 folds from white maturity to red maturity stage.

Antioxidant capacity

In terms of antioxidant activity, the results are presented in Table 2, and they ranged from 1154.6 to 1661.4 mg ascorbic acid/100g. Antioxidant capacity, based on DPPH assay, is influenced by cultivar and ripening stage. Regarding the influence of ripening stage, the results show that the highest antioxidant activity was recorded in the stage of white maturity (stage 1). Similar results of decrease in DPPH radical scavenging activity along with the increasing maturity stage were also reported by others (Wang *et al*., 2016; Zozio *et al*., 2014).

Organic acid content

The amount of each organic acid found in jujube extracts is shown in Table 3. It was clear that the impact of maturity stage was significant ($p < 0.05$) on organic acid distribution of jujube extracts. As indicated by previous researchers (Wojdylo *et al*., 2016), ascorbic acid is the major organic acid found in jujube fruits, followed by malic acid and lactic acid. High content of ascorbic acid was recorded in red maturity stage in both varieties (319.2-321.9 mg/L). The same trend was registered for malic, lactic and tartaric acid. Oxalic acid was recorded in white maturity of fruits in

Table 1. Dry matter total content and soluble solids content of jujube fruits*

Maturity stage	Cultivar 'Ya Tsao'		Cultivar 'Ta-Jan Tsao'	
	Dry matter total content (%)	Soluble solids content (%)	Dry matter total content (%)	Soluble solids content (%)
Stage 1	17.92±0.03^{a}	7.0±0.08^{a}	16.39±0.12^{a}	9.0±0.12^{a}
Stage 2	21.35±0.13^{b}	9.5±0.11^{b}	18.56±0.16^{b}	13.5±0.08^{b}
Stage 3	22.21±0.08^{b}	10.5±0.07^{c}	19.39±0.09^{c}	15.0±0.09^{c}
Stage 4	33.20±0.12^{c}	20.5±0.13^{d}	32.31±0.04^{d}	23.0±0.13^{d}

*Data expressed as means ± SE of three samples analysed separately. Different superscript letters within the same column indicate significant differences ($P < 0.05$) among maturity stage

Table 2. Antioxidant activity, total phenolics content and total flavonoids content of jujube fruits*

Maturity stage	Cultivar 'Ya Tsao'			Cultivar 'Ta-Jan Tsao'		
	Total phenolic content (mg GAE/ 100 g)	Total flavonoid content (mg QE/100 g)	Antioxidant activity (mg acid ascorbic /100g)	Total phenolic content (mg GAE/ 100 g)	Total flavonoid content (mg QE/100 g)	Antioxidant activity (mg acid ascorbic /100g)
Stage 1	1643.4±67.8^{d}	48.5±1.9^{d}	1661.4±50.5^{c}	1269.6±45.1^{d}	34.6±1.2^{d}	1566.4±56.6^{c}
Stage 2	936.6±31.2^{c}	39.4±1.6^{c}	1348.9±46.2^{b}	704.8±23.9^{c}	29.9±1.3^{c}	1324.8±36.7^{b}
Stage 3	682.4±18.9^{b}	31.1±1.2^{b}	1267.1±38.7^{b}	557.2±17.2^{b}	25.7±0.8^{b}	1266.4±41.3^{b}
Stage 4	529.5±23.2^{a}	26.7±1.3^{a}	1164.7±41.3^{a}	475.3±16.6^{a}	19.9±0.7^{a}	1154.6±28.9^{a}

*Data expressed as means ± SE of three samples analysed separately. Different superscript letters within the same column indicate significant differences ($P < 0.05$) among maturity stage

Table 3. Organic acid contents of jujube extract*

Cultivar	Maturity stage	Oxalic (mg/L)	Tartaric (mg/L)	Malic (mg/L)	Lactic (mg/L)	Ascorbic (mg/L)
'Ya Tsao'	white maturity	92.3±3.2^b	30.6±1.4^a	106.1±4.2^a	105.1±3.8^a	280.4±12.2^a
	red maturity	45.3±1.8^a	35.6±1.3^b	268.2±9.8^b	163.9±6.1^b	321.9±14.3^b
'Ta-Jan Tsao'	white maturity	89.1±3.6^b	23.2±0.6^a	130.7±5.4^a	48.1±2.1^a	250.3±9.7^a
	red maturity	50.7±2.1^a	25.4±0.8^b	305.6±12.5^b	96.1±5.3^b	319.2±13.8^b

*Data expressed as means ± SE of three samples analysed separately. Different superscript letters within the same column indicate significant differences ($P < 0.05$) among maturity stage

both cultivars, the content ranging between 45.3 and 92.3 g/l. Gao *et al*. (2012) showed that ascorbic acid values were positively correlated with hardness in ten promising jujubes. The highest value in ascorbic acid of jujube fruits was recorded in harvested fruit at fully mature stage (637 mg/100g FW), by Moradinezhad *et al*. (2016).

Conclusions

The results presented in this study showed that total phenols content, total flavonoids content, organic acids of jujube fruits were all influenced by their developmental stage. Genotype too is influencing the composition of bioactive compounds in jujubes. *Zizyphus jujube* is good for direct consumption, and the fruit could be an important dietary source of natural antioxidants for prevention of diseases caused by oxidative stress.

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