Physico-chemical properties of 'Phoenix' jujube fruit (*Ziziphus jujuba* Mill.)

J. Reche[1], F. Hernández[1], M.S. Almansa[2], S. García[2], P. Legua[1] and A. Amorós[2,a]

[1]Plant Sciences and Microbiology Department, Miguel Hernández University, Research Group in Plant Production and Technology, Ctra. de Beniel, km 3.2, E-03312 Orihuela, Alicante, Spain; [2]Applied Biology Department, Miguel Hernández University, Ctra. de Beniel, Km 3.2, E-03312 Orihuela, Alicante, Spain.

Abstract

Nutritionists recommend fruits and vegetables containing large amounts of phytochemicals because of their health benefits. Epidemiological and nutritional studies suggested that the higher one's fruit and vegetables' consumption, the lower the incidence of chronic diseases. Jujube fruits contain high levels of vitamins, carotenoids, phenolic compounds and antioxidant activities so they are considered healthy foods. Nevertheless, jujube is considered as an underutilized species and is less known than other fruits so their varieties have not been investigated in depth in Spain. In this work, a traditional cultivar of jujube, 'Phoenix', cultivated in Spain, has been studied. It is called 'Phoenix' because they have an elongated and narrow shape, similar to a date fruit. The fruits of this cultivar have a weight of 9.17 g with an equatorial diameter of 23.90 mm and a height of 39.59 mm. Its stone has a weight of 0.36 g, a diameter of 6.81 mm and a height of 26.97 mm, which has a pulp yield of 95.12%. In commercial ripening it has a total chlorophyll content of 0.37 mg 100 g^{-1} fresh weight and a total carotenoid content of 0.19 mg eq β-carotene 100 g^{-1} fresh weight. External colour corresponds to the following reflection parameters: 72.10 (L*), -0.20 (a*) and 34.68 (b*), a Chroma index of 34.94 and a Hue angle of 90.29. The protein content is 0.615 mg g^{-1} fresh weight, soluble solids are high with 24.07 °Brix, and the content of sucrose, glucose and fructose in percentage of 6.51, 3.02 and 4.84, respectively. Succinic acid is the major organic acid (0.82%), followed by ascorbic acid (0.36%), citric acid (0.33%), and malic acid (0.26%). Therefore, the commercial 'Phoenix' cultivar is of medium size, yellowish and has a slightly greenish colour, it is sweet and has low acidity. It is very suitable for fresh consumption.

Keywords: colour, chlorophylls, carotenoids, sugars, organic acids

INTRODUCTION

The origin of the azufaifo or jujube is from China, where it is widely used in its traditional medicine. It extends to regions of Asia, America and the Mediterranean, in zones of hot summers and cold winters. It is a crop that resists drought very well, so it is a species of great interest in the Spanish southeast. The consumption of jujube fresh, dry or preserved, has a high nutritional power and great interest in traditional Chinese medicine. Fresh jujube has pharmacological use for its anti-inflammatory and analgesic power. In addition to seeds, they also have sedative and hypnotic properties (Jiang et al., 2007) and to combat insomnia and anxiety. It is also characterized by its medicinal properties for treating fertility and diabetes (Ambasta, 1986; Erenmemisoglu et al., 1995).

Currently there is a high interest in healthy food consumption, with nutritious products and with high antioxidant properties, looking for replacement of antioxidants with natural sources (Termentzi et al., 2006). These natural sources have a protective effect on the diet, because of their content in carotenoids, vitamins, antioxidants and phenolic compounds, in addition to other phytochemical compounds (Lampe, 1999; Scalbert and Williamson, 2000). The usual consumption of fruits and vegetables is considered to be able to prevent diseases (Joseph et al., 1999; Surh, 2003). Jujube fruit is a natural source of these compounds.

[a]E-mail: aamoros@umh.es

Jujube paste, mash, syrup and confectionery are consumed to improve health and maintain a good state of health. Previous studies have revealed that jujube has several components, triterpenic acids, amino acids, flavonoids, cerebrosides (Pawlowska et al., 2009), phenolic acids, mineral constituents (Li et al., 2007) and polysaccharides.

The fruits mature between August and September, but there are some cultivars like that of this study, 'Phoenix', which ripens throughout the month of October. In other countries like India, they usually mature between February and April.

Therefore, the objective of this study is to evaluate such composition, as well as nutritional power and quantify organic acids, sugars and protein of 'Phoenix' jujube.

MATERIALS AND METHODS

Plant material and sample processing

Fruits of *Ziziphus jujube* Mill. 'Phoenix' were collected in August 2015 from a commercial farm located in San "Isidro" (latitude 38°10'22,29"N; longitude 0°51'36,138"W, 19 m a.s.l.), Alicante, southeastern Spain. Jujube trees were 20 years old, trained as a vase, spaced at 4×4 m, were grown with homogeneus conditions of irrigation, fertilization, and pest control. Thirty fruits from 5 trees (6 fruits tree^{-1}) were hand-harvested at physiological maturity, and immediately transported under ventilated conditions to the laboratory for the analyses of the physical and chemical properties.

Physical and chemical determinations

Once in the laboratory, the jujube fruits were immediately processed. In each one of the fruits (30 fruits), the following parameters were measured: equatorial and polar diameters (mm) and fruit height (mm) using a digital caliper (model CD-15 DC; Mitutoyo (UK) Ltd., Telford, UK), with 0.01 mm accuracy; fruit weight (g) was measured using a digital scale Sartorius (model BL-600, Madrid, Spain), with an accuracy of 0.01 g. Colour measurement was made in the peel of fruit on two opposite faces at the equatorial zone. Colour was assessed according to the Commission Internationale de l'Éclairage (CIE) and expressed as L* (brightness or lightness; 0 = black, 100 = white), a* (-a* = green, +a*=red) and b* (-b* = blue, +b* = yellow). These values were then used to calculate Hue angle degree [H° = arctang (b*/a*)], where 0° = red-purple; 90° = yellow, 180° = bluish-green and 270° = blue, and Chroma [$C=(a^{*2} + b^{*2})^{1/2}$], indicative of the intensity or colour saturation. Colour was measured using a Minolta C-300 Chroma Meter (Minolta Corp., Osaka, Japan) coupled to a Minolta DP-301 data processor.

Then, the fruits were cut in half, the stone was carefully removed and the pulp was hand-squeezed using a commercial kitchen juicer. The freshly squeezed juices were centrifuged at 10000 g for 20 min (Sigma 3-18K, Germany) and they were kept in a freezer at a temperature of -80°C until analysis. Weight, diameter and length of stones were also measured.

For the chlorophylls extraction, 3 g of sample were homogenized with 80% acetone in a hand mortar and sea sand was added to aid the extraction of chlorophylls. The supernatant was centrifuged and collected, which was measured at two absorbances, 664 and 647 nm in the spectrophotometer. The process was performed at low temperatures.

For carotenoids, 5 g of jujube sample were prepared with 1 mM acetate tris buffer at pH 6, $CaCl_2$ and ethyl acetate. Along with sea sand in a mortar it was crushed and the sample was homogenized. The green supernatant was then centrifuged and collected to measure the absorbance at the spectrophotometer at 450 nm. The process was performed in cold to avoid erroneous values.

For the determination of proteins, the method of Bradford (1976) was used, using bovine serum albumin (BSA) as the calibration standard. The Bio-Rad protein colourimetric assay was used to measure the total protein concentration based on the colour change of the Coomassie G-250 bright blue dye that binds to amino acid residues and allows the detection of small concentrations of protein.

Determination of total soluble solids, sugars and organic acids

Total soluble solids (TSS) were measured using an Atago digital refractometer (model N-20; Atago, Bellevue, Wash., USA) at 20°C with values being expressed as °Brix.

Organic acids and sugars profile were quantified according to Carbonell-Barrachina et al. (2012). Briefly, 1 mL aliquot of centrifuged juice was passed through a 0.45-µm Millipore filter and then injected into a Hewlett-Packard series 1100 (Wilmington Del., USA) high-performance liquid chromatography (HPLC). The elution buffer consisted of 0.1% phosphoric acid with a flow rate of 0.5 mL min^{-1}. Organic acids were isolated using a Supelco column (Supelcogel TM C-610H column 30 cm × 7.8 mm) and Supelguard (5 cm × 4.6 mm, Supelco, Inc., Bellefonte, PA) and absorbance was measured at 210 nm using a diode-array detector (DAD). These same HPLC conditions (elution buffer, flow rate and column) were used for the analysis of sugars. The detection was conducted using a refractive index detector (RID). Standards of organic acids (oxalic, citric, tartaric, malic, quinic, shikimic, succinic and fumaric acids) and sugars (glucose, fructose, sucrose and sorbitol) were obtained from Sigma (Poole, Dorset, UK). Calibration curves were used for the quantification of organic acids, showing good linearity ($r^2 \geq 0.999$). Results for both organic acids and sugars were expressed as concentrations g 100 mL^{-1} of fresh weight (fw). Sugars and organic acids were determined in triplicate.

RESULTS AND DISCUSSION

The physical measures of ‘Phoenix’ jujube fruits and its stones are listed in Table 1. Its average weight is of 9.17±0.35 g. This fruit is lighter than the Spanish ‘GAL’ and ‘DAT’ cultivars (Hernández et al., 2016) as well as the Chinese cultivars (Gao et al., 2011), but it weighs more than the Spanish ‘MSI’ and ‘PSI’ cultivars. It has an equatorial diameter of 23.90±0.49 mm, smaller than most Spanish cultivars. Although ‘Phoenix’ is a smaller sized fruit, its height reaches almost the same level as the registered in ‘GAL’ cultivar, which fruits are reported to be of the larger sized ones (Hernández et al., 2015), concluding that ‘Phoenix’ has an almond shape. Its stone has a weight of 0.36±0.02 g, a diameter of 6.81±0.10 mm, and a height of 26.97±0.36 mm.

Table 1. Physical measures of fruits and stones of ‘Phoenix’.

	Fruits				Stones		
	Weight (g)	Equatorial diameter (mm)	Polar diameter (mm)	Height (mm)	Weight (g)	Diameter (mm)	Height (mm)
Phoenix	9.17±0.35	23.9±0.49	23.87±0.38	39.59±0.36	0.36±0.02	6.81±0.10	26.97±0.36

The data are the average ± SE of 30 jujube fruits.

For the ‘JG’ cultivar (Almansa et al., 2016) the weight obtained was very high value compared to ‘Isidro’ that is almost double, 23.4±1.2 g, which supposes that it has larger size, since from the equatorial and polar diameter to the height there is a big difference. In contrast, for the same study, the ‘JM’ cultivar has an average weight of 8.5±0.4 g. This value is closer to the one that was obtained, and fruit measurements are also closer.

The colour parameters of ‘Phoenix’ are reported in Table 2. Its lightness has a positive L* value of 72.10±0.32 similar to the Spanish cultivars (Hernández et al., 2016). The negative a* parameter -0.20±0.21 is a lot higher than in the other cultivars (Hernández et al., 2016), which means that ‘Phoenix’ jujube fruit is less green than in the other cases. Compared with the b* parameters measured by Hernández et al. (2015), this cultivar is less yellow than the other ones. Both values of Chroma index 34.94±0.9 and Hue angle 90.29±0.42 are lower than values reported by Hernández et al. (2016), so the colour of ‘Phoenix’ is a bit duller and less yellow. For ‘JM’ and ‘JG’ jujube, the colour parameters are similar to ‘Isidro’ for L and b, whereas for a* the values are slightly higher, which suggests that ‘Phoenix’ is greener (Almansa et al., 2016).

Table 2. Colour by reflection of 'Phoenix' jujube fruits.

	L*	a*	b*	C	H°
Phoenix	72.10±0.32	-0.20±0.21	34.86±0.19	34.94±0.90	90.29±0.42

The data are the average ± SE of 30 jujube fruits.

All the chlorophylls and carotenoide values are rather low in this study, with exception of the proteins. These values are listed in Table 3. The total chlorophylls amounts were 0.37±0.02 mg 100 g^{-1} fw, lower than the value obtained by Qiuping and Wenshui (2007) for jujube fruits preserved in the first days. The chlorophyll content *a* and *b* in 'Phoenix' is much higher than those obtained for 'Mallacy' and 'Bambawy' cultivars in Iraq (Al-Niami et al., 1992).

Table 3. Chlorophylls and carotenoids of 'Phoenix' jujube fruits.

	Total chlorophylls (mg 100 g^{-1} fw)	Chlorophyll a (mg 100 g^{-1} fw)	Chlorophyll b (mg 100 g^{-1} fw)	Total carotenoids (mg eq β-carotene 100 g^{-1} fw)	Proteins (mg g^{-1} fw)
Phoenix	1.85±0.12	1.15±0.09	0.71±0.04	0.19±0.02	0.61±0.01

The data are the average ± SE of 3 jujube fruits juices.

The rate for chlorophyll *a* was 0.23±0.02 mg 100 g^{-1} fw and for cholorphyll *b* was 0.14±0.01 mg 100 g^{-1} fw. Total carotenoids content was 0.19±0.02 mg eq β-carotenone 100 g^{-1} fw, a very low value compared to other cultivars, for example the jujube fruits analysed by Sun et al. (2011) in Chinese cultivars.

'Phoenix' registered a slightly higher protein content of 0.615±0.017 mg g^{-1} fw, this value is within the normal range. Protein content of 'Phoenix' jujube is a value that is within the range of Chinese jujube cultivars (Li et al., 2005). 'Phoenix' showed a protein content similar to that obtained by Almansa et al. (2016) for the Spanish cultivar 'JM'. In the same study, the protein content of 'JG' was obtained, which presented slightly higher values but within a normal range for jujube.

As Table 4 indicates, the content of total soluble solids in 'Phoenix' was quite high with a 24.07±0.34 °Brix, a higher value than the one obtained in Spanish cultivars (Hernández et al., 2015), which ranged from 14.6 to 18.4 °Brix, as well as in the Chinese 'Zanhuangzao' cultivar (Gao et al., 2011). 'Phoenix' had a sucrose percentage of 6.51±0.14, a glucose percentage of 3.02±0.10 and a fructose percentage of 4.84±0.09. As in Spanish cultivars, with exception of 'PSI' fruits, sucrose was the predominant sugar in jujube fruits, followed by fructose and last glucose. The total sugar content was the same as the one obtained in the 'DAT' cultivar, although lower than the Spanish 'GAL' and 'MSI' cultivars. It was still higher than those registered in the 'PSI' cultivar and most Chinese cultivars (Gao et al., 2011), with exception of 'Juanzo'. 'JM' had a sugar content similar to ours at the beginning of ripening, but with a more advanced ripening the sweetness increases considerably which means that it is a much sweeter fruit than the one analysed in this study (Almansa et al., 2016). 'JG' is more stable and shows values similar to those obtained by 'Phoenix' jujube.

Table 4. SST and sugars of 'Phoenix' jujube fruits.

	TSS (°Brix)	Sucrose (%)	Glucose (%)	Fructose (%)
Phoenix	24.07±0.34	6.51±0.14	3.02±0.10	4.84±0.09

The data are the average ± SE of 3 jujube fruits juices.

Organic acids like ascorbic acid, malic acid, citric acid and succinic acid, have been identified and quantified in the analysis of 'Phoenix' jujube juices.

The organic acids detected in 'Phoenix' were citric acid 0.33%, malic acid 0.26%, ascorbic acid 0.36% and succinic acid 0.82% (Table 5). The main organic acid was succinic followed by ascorbic and citric. The same results have been registered in 'MSI' and 'PSI' (Hernández et al., 2016). Percentages obtained in 'Phoenix' were much closer to those registered in 'DAT'. 'Phoenix' had low acidity compared to other cultivars.

Table 5. Organic acids contents of 'Phoenix' jujube fruits.

	Citric acid (%)	Malic acid (%)	Ascorbic acid (%)	Succinic acid (%)
Phoenix	0.33±0.04	0.26±0.01	0.36±0.06	0.82±0.02

The data are the average ± SE of 3 jujube fruits juices.

CONCLUSIONS

In view of the results obtained, 'Phoenix' jujube is yellowish green with low content in chlorophylls and carotenoids, more than the Chinese cultivars. It is a sweet fruit with a good protein content and low acidity.

ACKNOWLEDGEMENTS

The authors would like to thank the Ministry of Education Research, Culture and Sports of Generalitat Valenciana for funding to carry out this research (AICO-2016/015).

Literature cited

Al-Niami, J.H., Saggar, R.A.M., and Abbas, M.F. (1992). The physiology of ripening of jujube fruit (*Zizyphus spina-christi* (L) Wild). Sci. Hortic. (Amsterdam) *51* (*3-4*), 3–4, 303–308 https://doi.org/10.1016/0304-4238(92)90128-Y.

Almansa, S., Hernández, F., Legua, P., Nicolás-Almansa, M., and Amorós, A. (2016). Physico-chemical and physiological changes during fruit development and on-tree ripening of two Spanish jujube cultivars (*Ziziphus jujube* Mill.). J. Sci. Food Agric. *96* (*12*), 4098–4105 https://doi.org/10.1002/jsfa.7610.

Ambasta, S.P. (1986). Useful Plants of India. (New Delhi, India: CSIR, Publications and Information Directorate), pp.703.

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. *72* (*1-2*), 248–254 https://doi.org/10.1016/0003-2697(76)90527-3.

Carbonell-Barrachina, A.A., Calín-Sánchez, A., Bagatar, B., Hernández, F., Legua, P., Martínez-Font, R., and Melgarejo, P. (2012). Potential of Spanish sour-sweet pomegranates (cultivar C25) for the juice industry. Food Sci. Technol. Int. *18* (*2*), 129–138 https://doi.org/10.1177/1082013211414783.

Erenmemisoglu, A., Kelestimur, F., Koker, A.H., Ustun, H., Tekol, Y., and Ustdal, M. (1995). Hypoglycemic activity of *Ziziphus jujuba.* J. Pharm. Pharmacol. *47* (*1*), 72–74 https://doi.org/10.1111/j.2042-7158.1995.tb05737.x.

Gao, Q.H., Wu, P.T., Liu, J.R., Wu, C.S., Parry, J.W., and Wang, M. (2011). Physico-chemical properties and antioxidant capacity of different jujube (*Ziziphus jujuba* Mill.) cultivars grown in loess plateau of China. Sci. Hortic. (Amsterdam) *130* (*1*), 67–72 https://doi.org/10.1016/j.scienta.2011.06.005.

Hernández, F., Legua, P., Melgarejo, P., Martínez, R., and Martínez, J.J. (2015). Phenological growth stages of jujube tree (*Ziziphus jujube*): codification and description according to the BBCH scale. Ann. Appl. Biol. *166* (*1*), 136–142 https://doi.org/10.1111/aab.12169.

Hernández, F., Noguera-Artiaga, L., Burló, F., Wojdyło, A., Carbonell-Barrachina, A.A., and Legua, P. (2016). Physico-chemical, nutritional, and volatile composition and sensory profile of Spanish jujube (*Ziziphus jujuba* Mill.) fruits. J. Sci. Food Agric. *96* (*8*), 2682–2691 https://doi.org/10.1002/jsfa.7386.

Jiang, J.G., Huang, X.J., Chen, J., and Lin, Q.S. (2007). Comparison of the sedative and hypnotic effects of flavonoids, saponins, and polysaccharides extracted from Semen *Ziziphus jujube.* Nat. Prod. Res. *21* (*4*), 310–320 https://doi.org/10.1080/14786410701192827.

Joseph, J.A., Shukitt-Hale, B., Denisova, N.A., Bielinski, D., Martin, A., Mcewen, J.J., and Bickford, P.C. (1999). Reversals of age-related declines in neuronal signal transduction, cognitive, and motor behavioral deficits with blueberry, spinach, or strawberry dietary supplementation. J. Neurosci. *19* (*18*), 8114–8121 https://doi.org/10.1523/JNEUROSCI.19-18-08114.1999.

Lampe, J.W. (1999). Health effects of vegetables and fruits: assessing mechanism of action in human experimental studies. Am. J. Clin. Nutr. *70* (*3*), 475s–490s https://doi.org/10.1093/ajcn/70.3.475s.

Li, J.-W., Ding, S.-D., and Ding, X.-L. (2005). Comparison of antioxidant capacities of extracts from five cultivars of Chinese jujube. Process Biochem. *40* (*11*), 3607–3613 https://doi.org/10.1016/j.procbio.2005.03.005.

Li, J., Fan, L., Ding, S., and Ding, X. (2007). Nutritional composition of five cultivars of Chinese jujube. Food Chem. *103* (*2*), 454–460 https://doi.org/10.1016/j.foodchem.2006.08.016.

Pawlowska, A., Camangi, F., Bader, A., and Braca, A. (2009). Flavonoids of *Zizyphus jujuba* L. and *Zizyphus spina-christi* (L.) Willd (*Rhamnaceae*) fruits. Food Chem. *112* (*4*), 858–862 https://doi.org/10.1016/j.foodchem.2008.06.053.

Qiuping, Z., and Wenshui, X. (2007). Effect of 1-methylcyclopropene and or chitosar coating treatments on storage life and quality maintenance of Indian jujube fruit. LWT *40* (*3*), 404–411 https://doi.org/10.1016/j.lwt.2006.01.003.

Scalbert, A., and Williamson, G. (2000). Dietary intake and bioavailability of polyphenols. J. Nutr. *130* (*8*), 2073S–2085S https://doi.org/10.1093/jn/130.8.2073S.

Sun, Y., Liang, Z., Shan, C., Viernstein, H., and Unger, F. (2011). Comprehensive evaluation of natural antioxidants and antioxidant potentials in *Ziziphus jujube* Mill. Var. spinosa (Bunge) Hu ex H.F. Chou fruits based on geographical origin by TOPSIS method. Food Chem. *124* (*4*), 1612–1619 https://doi.org/10.1016/j.foodchem.2010.08.026.

Surh, Y.J. (2003). Cancer chemoprevention with dietary phytochemicals. Nat. Rev. Cancer *3* (*10*), 768–780 https://doi.org/10.1038/nrc1189.

Termentzi, A., Kefalas, P., and Kokkalou, E. (2006). Antioxidant activities of various extracts and fractions of *Sorbus domestica* fruits at different maturity stages. Food Chem. *98* (*4*), 599–608 https://doi.org/10.1016/j.foodchem.2005.06.025.