

EFFECTS OF WATER DEFICIT DURING MATURATION ON AMINO ACIDS AND JUJUBE FRUIT EATING QUALITY

Jacinta Collado-González¹, Zulma N. Cruz², Sonia Medina¹, Carmen D. Mellisho³, Pedro Rodríguez², Alejandro Galindo³, Isabel Egea⁴, Félix Romojaro⁴, Federico Ferreres¹, Arturo Torrecillas^{3*}, Angel Gil-Izquierdo^{1*}

¹Department of Food Science and Technology, CEBAS-CSIC, P.O. Box 164, Espinardo, 30100, Murcia, Spain

²Department of Physiology and Biochemistry, Instituto Nacional de Ciencias Agrícolas (INCA), Ctra. De Tapaste, km 3.5, San José de Las Lajas, Mayabeque, Cuba

³Department of Irrigation, CEBAS-CSIC, P.O. Box 164, Espinardo, E-30100 Murcia, Spain

⁴Department of Biology of the Stress and Plant Pathology, CEBAS-CSIC, P.O. Box 164, Espinardo, E-30100 Murcia, Spain

atorrecillas@cebas.csic.es, angelgil@cebas.csic.es

Jujube yield and fruit characteristics can be clearly modified by water deficit imposed during fruit maturation. One essential (cystine (Cys-cys)) and seven non-essential (4-hydroxyproline (p-Hyp), α -amino adipic acid (AADA), ornithine (Orn), β -aminoisobutyric acid (BAIB), α -amino-n-butyric acid (AABA), cystathionine (Cysta), and homocystine (Hcys-cys)) amino acids were identified for the first time. Fruits from plants exposed to moderate water deficit during the maturation stage (T1) initiated the ripening phase earlier than control (T0) fruits and had an improved eating quality. Fruits subjected to severe water deficit (T2) showed changes in their physical characteristics and reached a more advanced degree of ripening than T0 and T1 fruits, with not only most of the fruit chemical characteristics that determine taste being improved but also the nutritional value. The decrease in the asparagine (Asn) content of the fruit as a result of severe water deficit is a positive aspect, which prevents acrylamide formation during heat-processing of the fruit.

Keywords: ethylene emission; fruit quality; plant water relations; fruit respiration; amino acids

ВЛИЈАНИЕ НА НЕДОСТИГОТ НА ВОДА ЗА ВРЕМЕ НА СОЗРЕВАЊЕ ВРЗ АМИНОКИСЕЛИНИТЕ И ХРАНЛИВИОТ КВАЛИТЕТ НА *ZIZYPHUS JUJUBA*

Приносот и карактеристиките на *Zizyphus jujuba* очигледно можат да се модифицираат со наметнат недостиг на вода при созревањето. За прв пат се идентификувани една есенцијална (Cys-cys) и седум неесенцијални аминокиселини: 4-хидроксипролин (p-Нур), α -аминоадипинска киселина (AADA), орнитин (orn), β -аминоизобутерна киселина (BAIB), α -амино-n-бутерна киселина (AABA), цистатионин (Cysta) и хомоцистин (Hcys-cys). Овошките изложени на умерен недостиг на вода за време на созревањето (T1) побрзо започнувале со фазата на созревање во однос на овошките од контролната група (T0) и имале подобар прехранбен квалитет. Овошките изложени на голем недостиг на вода (T2) покажале промени во физичките карактеристики и достигнувале понапреден степен на созревање од овошките од T0 и T1, и тоа не само со подобрени хемиски карактеристики кои го подобруваат вкусот, но и со подобрена нутрициска вредност. Намалувањето на содржината на аспарагин (Asp) во овошките како резултат на голем недостиг на вода има позитивна страна затоа што го спречува образувањето на акриламид за време на топлинската обработка на овошјето.

Клучни зборови: емисија на етилен; квалитет на овошје; однос на вода и растенија; респирација кај растенија; аминокиселини

1. INTRODUCTION

Jujube or Chinese jujube tree (*Zizyphus jujuba* Mill.) is a native fruit tree of China, where it has been cultivated for more than 5000 years, and yields about 450,000 tons annually in that country alone [1]. Although species of the *Zizyphus* genus are considered as minor crops, they are an integral part of the culture and way of life of millions of Asian and African people and are considered as crops with substantial growth potential by the International Centre for Underutilized Crops in Southampton, U.K. [2].

Z. jujuba is admired for its wide adaptation, easy management, early bearing, and the nutritional value of its fruit, which is eaten fresh, dried, or processed into confectionary. It is a nutritionally valuable fruit for the variety of essential nutrients it contains such as carbohydrates, crude fibre, crude protein, flavonoids, and several essential minerals and vitamins [3–7]. Moreover, jujube fruits have been shown to possess biological activities associated with the antiproliferation of cancer cells [8], the alleviation of brain nerve disorder [9], regulation of immune function [10], antimicrobial and antiviral properties [11], and the reduction of blood triglyceride levels [12]. It has analeptic, palliative, and antitumor uses [7, 13]. Nevertheless, Choi *et al.* [4, 6] recently identified high levels of free asparagine (Asn), which is the major precursor of acrylamide, a potentially toxic compound formed during the heat-processing of plant foods [14, 15].

From the agronomic point of view, *Z. jujuba* is a very interesting crop because it is able to withstand severe drought during the growing season. Cruz *et al.* [16] showed that the jujube tree is able to maintain leaf turgor and productivity under severe water deprivation thanks to two simultaneous and complementary mechanisms: stomatal regulation and active osmotic adjustment. Despite the increasing water shortage in the areas considered most suitable for jujube tree cultivation, hardly any reports exist on the effect of irrigation and its relationship with the jujube fruit yield and quality. An exception is the work of Cui *et al.* [17], who showed that regulated deficit irrigation improves fruit quality and water use efficiency. It is well known that the free amino acids present in some fruits contribute to their taste and to defense mechanisms against water deficit. Clarifying the changes in amino acid contents is therefore an im-

portant step in elucidating the response of jujube fruits to water deficit.

For these reasons, the aim of the present paper was to ascertain whether water deficit during the fruit maturation period is a useful tool for the management of some important quality attributes in jujube fruits. For this, fully irrigated trees were compared with deficit-irrigated trees and trees submitted to a period of non-irrigation during fruit maturation.

2. EXPERIMENTAL

The experiment was carried out in 2011 at a farm near the town of Albatera (Alicante, Spain) (38° 12' N, 0° 51' W). The plant material consisted of 7-year-old jujube trees (*Zizyphus jujuba* Mill. cv. Grande de Albatera), planted at 2 m × 6 m. The soil of the orchard is a Torrifluent with a sandy loam texture, very low electrical conductivity (109 µS/cm, 1:10 w:v), high lime content (57 %), very low organic matter content (0.3 %), and low exchangeable potassium (40 mg/kg) and available phosphorus (20 mg/kg). The irrigation water had an electrical conductivity of between 1.7 and 2.2 dS/m and a Cl⁻ concentration ranging from 36 to 48 mg/l.

The climate of the area is strictly Mediterranean, with mild winters, low annual rainfall, and hot, dry summers. During the experimental period, the average daily maximum and minimum air temperatures were 32 and 22 °C, respectively, while the mean daily air vapour pressure deficit (VPD_m) [18] ranged from 1.25 to 3.25 kPa and the reference crop evapotranspiration (ET₀) [18] was 189 mm. The total rainfall was negligible (1.8 mm on day of the year, DOY, 221).

Three irrigation treatments were considered, in which irrigation was carried out daily and during the night using a drip irrigation system with one lateral pipe per tree row. Control plants (treatment T₀) were irrigated to ensure non-limiting soil water conditions (112 % ET₀), T₁ plants were irrigated according to the criteria used by the farmer (64 % ET₀), and T₂ plants were irrigated as in T₀ but irrigation was withheld for 36 days (from DOY 202 to 238). The total water amounts applied during the measurement period were 213 and 122 mm for treatments T₀ and T₁, respectively.

2.1. Chemicals

The AQC reagent was purchased from Chemos GmbH (Regenstauf, Germany). The Bis-Tris

reagent and all amino acid standards (histidine (His), 1-methyl-histidine (Met-His), 4-hydroxyproline (p-Hyp), asparagine (Asn), phosphoethanolamine (PEA), arginine (Arg), glutamine (Gln), serine (Ser), glycine (Gly), ethanolamine (EA), aspartic acid (Asp), citrulline (Cit), glutamic acid (Glu), threonine (Thr), alanine (Ala), γ -Amino-n-butyric acid (GABA), α -Aminoadipic acid (AADA), proline (Pro), Ornithine (Orn), β -Aminoisobutyric acid (BAIB), α -Amino-n-butyric acid (AABA), lysine (Lys), Cystine (Cys-cys), cystathionine (Cysta), tyrosine (Tyr), valine (Val), Methionine (Met), homocystine (Hcys-cys), leucine (Leu), isoleucine (Ile), tryptophan (Trp), and phenylalanine (Phe)) were obtained from Sigma Aldrich (Madrid, Spain). Acetonitrile and methanol (both of LC-MS grade), sulfuric acid, nitric acid, perchloric acid, formic acid, ammonium acetate, ascorbic acid, and sodium hydroxide were obtained from Panreac Química S.A. (Barcelona, Spain), and metaphosphoric acid was from Merck (Darmstadt, Germany). Boric acid was bought from Probus (Badalona, Spain), while arabinose, glucose, sucrose, malic acid, citric acid, oxalic acid, and calcium disodium EDTA were purchased from Sigma (Steinheim, Germany). The Milli-Q water used was produced by an Elix® 3 Millipore water purification system (Molsheim, France).

2.2. Plant water status

The predawn leaf water potential (Ψ_{pd}) was measured in mature leaves located on the south facing side, from the middle third of the tree (two leaves per tree and four trees per treatment), using a pressure chamber (model 3005, Soil Moisture Equipment Co., Santa Barbara, CA, USA). The midday (12 h solar time) stem water potential (Ψ_{stem}) was measured in a similar number and type of leaves as Ψ_{pd} : leaves were enclosed in a small, black plastic bag covered with aluminum foil for at least 2 h before the measurements in the pressure chamber [19].

Midday gas exchange in attached leaves, leaf conductance (g_{lmd}), and net photosynthesis (P_{nmd}), were measured with a field-portable, closed gas-exchange photosynthesis system (LI-6400, LICOR Inc., Lincoln, NE, USA), in a similar type of leaf as used for the Ψ_1 measurements. Two measurements were taken on four trees per treatment.

2.3. Fruit weight, firmness, and color

Jujube fruits were harvested in their commercial ripening state (the S7 stage of growth, according to Choi *et al.* [4]) and immediately transported to the laboratory, on 27 August (DOY 239).

The fruits were divided into the edible portion (peel and pulp) and pit (shell and seed), and the average weight, moisture content, firmness, peel and flesh color, total soluble solids, ethylene emission, and respiratory rate were immediately measured in each fresh edible portion sample. Aliquots of all samples were stored at -20 °C and freeze-dried before other analyses.

Average fruit weight was estimated by weighing 25 fruits per replicate. For the moisture content, samples were oven-dried at 105 °C to constant weight (AOAC, 2000). The flesh firmness was determined as the load needed to break the flesh after removal of the peel in the equatorial zone, according to Egea *et al.* [20], using a Penefel-type penetrometer (Copa-Technology, Tarascon, France) equipped with a rod 8 mm in diameter.

The external and internal color were measured by reflectance with a Minolta CR 2000 colorimeter (Osaka, Japan) and the results were expressed in the CIELAB L^* , a^* , b^* system. The mean values of the lightness (L^*), red-greenness (a^*), and blue-yellowness (b^*), parameters for each fruit were calculated. The objective color was calculated as chromaticity or chroma ($C^* = (a^{*2} + b^{*2})^{1/2}$) and hue angle ($H^\circ = \arctan(b^*/a^*)$) [21].

2.4. Fruit ethylene emission and respiratory rate

Ethylene and CO₂ production were determined by placing 16 fruits in a hermetically sealed container, of known volume and equipped with a silicone septum. After 2 h, 1 ml of the internal atmosphere of the container was extracted with a syringe for the determination of ethylene. The ethylene was quantified in a Hewlett-Packard HP5890 (Bristol, United Kingdom), equipped with a flame ionization detector and a stainless-steel column (3 m \times 3.2 mm diameter) packed with 80/100 mesh activated alumina. The quantification was carried out following calibration, point-by-point, with an external standard [22]. The CO₂ quantification was performed directly in the head space of the container, 2 h after closure, using a bench-top headspace gas analyzer (PBI Dansensor, CheckMate 9900).

2.5. Fruit total soluble solids, vitamin C, sugar, organic acids, and bioelements

Total soluble solids (TSS) were determined by measuring the refractive index of the juice extracted from the fruit, with a digital refractometer (Atago, Tokyo, Japan). Titratable acidity (TA) was determined by potentiometric titration with 0.1N NaOH at pH 8.1 [23]. The ripening index was estimated as the ratio between TSS and TA.

Vitamin C was determined according to Egea *et al.* [23]. The lyophilized powder of the edible portion (0.5 g) was diluted with 10 ml of cold 50 ml/metaphosphoric acid, making up the homogenized mixture to 50 ml with the same solvent. The final solution was kept on ice in darkness for 30 min and then centrifuged at 20 000 g for 25 min. Then, the supernatant was passed through a C18 Plus Sep-Pack cartridge and a 0.2- μ m filter. Quantification was carried out with a high-performance liquid chromatograph (Shimadzu LC-10Atvp, Kyoto, Japan), using a thermostated ion exchange column (ION-300) at 30 °C with isocratic elution. The absorbance at 254 nm was recorded with a UV-visible detector. A standard curve in the concentration range 10–100 mg/kg ascorbic acid was used.

To determine the sugar and organic acid contents, lyophilized powder of the fruit edible portion (0.5 g) was diluted with Milli-Q water (50 ml) and then centrifuged (1200 g) at 4 °C for 30 minutes. After centrifugation, the supernatant was filtered through a Durapore 0.45 μ m HV filter (Millipore Corporation, USA) and then passed through a C18 Plus Sep-Pack cartridge (Waters Corporation, Massachusetts, USA). Quantifications were carried out by a Shimadzu LC-10Atvp HPLC (Kyoto, Japan), using a thermostated ion-exchange column (ION-300, Teknochroma) at 30 °C, with isocratic elution of the mobile phase (2.5 mM H₂SO₄). Two detectors were connected in tandem: a Shimadzu Refractive Index Detector, to detect the sugars, and a Shimadzu UV-Vis Detector for the spectrophotometric detection of the organic acids. The detection wavelength was 210 nm for oxalic acid and 230 nm for citric and malic acids. The sugar and organic acid quantifications were performed by means of calibration curves for each compound prepared with solutions made from standards of each organic acid and sugar [24].

Dried peel and flesh samples from the fruit moisture content determination were finely crushed in an agate mortar prior to determination of the mineral concentrations. The peel and flesh N concentrations were measured using a Thermo-Finnigan 1112 EA elemental analyzer (Thermo-Finnigan, Milan, Italy). The peel and flesh powders were subjected to nitro-perchloric acid attack (5:3, *v/v*) in a microwave oven (CEM Mars Xpress, NC, USA), reaching 200 °C in 20 min and holding this temperature for 2 h. The P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, and B concentrations of the arils were quantified by inductively coupled plasma emission optical spectrometry (Iris Intrepid II, Thermo Electron Corporation, Franklin, USA).

2.6. Amino acids

Individual amino acids (authentic standards) were prepared by dissolving each amine in Bis-Tris (pH 6.5). Calibration standards were generated by diluting the stock solutions to 1, 0.5, 0.25, 0.12, 0.06, and 0.03 mM. In the case of amino thiols, calibration standards were produced by diluting the stock solutions to 2, 1, 0.5, 0.25, 0.12, and 0.06 μ M in Milli-Q water.

Free amino acids were determined by following a method described previously [25]. Briefly, 20 mg of the fine powdered edible portion or pit of the jujube fruits were homogenized with 500 μ l of extraction buffer (MeOH / water, 1:1, *v/v*) using an ultra turrax (IKA, T10, Germany), for 30 seconds on ice. The samples were then incubated on dry ice for 5 minutes. The homogenates were sonicated in an ultrasound bath for 1 minute followed by centrifugation (Eppendorf centrifuge 5804 R, Hamburg, Germany) for 10 minutes at 17900 g, at 4 °C. The supernatants were transferred to limited volume vials and the precipitates were re-extracted with 500 μ l of extraction buffer, homogenized, incubated on ice, and centrifuged again. All supernatants were combined. The extracts were immediately derivatized.

The derivatization of amino acids and amino thiols was accomplished by following the Waters AccQ TagTM Ultra UHPLC amino analysis procedures, as described by Nagumo *et al.* [26] and Salazar *et al.* [25]. Briefly, 350 μ l of borate derivatization buffer (0.2 M sodium borate, pH 8.8, with 5 mM calcium disodium EDTA), 50 μ l of amino acid standard or jujube extract, and 100 μ l of reconstituted AQC (10 mM AQC dry powder in acetonitrile) were placed in a 2-ml propylene vial [27, 28]. This solution was vortexed for several seconds, allowed to stand for 1 minute at room temperature, and then heated in a heating block for 10 minutes at 55 °C. After removing the vial from the heating block, the sample was injected into a UHPLC-MS/MS.

Amino acids and thiols were analyzed by reverse phase UHPLC as reported by Nagumo *et al.* [26] and Salazar *et al.* [25], with slight modifications. Briefly, chromatographic separation was carried out on an AccQ Tag Ultra BEH column (2.1 \times 100 mm, 1.7 μ m) (Waters Corp., Ireland). Two types of eluent were used for gradient separation. Mobile phase A consisted of 50 ml of an aqueous solution (acetonitrile, formic acid, and 5 mM ammonium acetate in water) (10 : 6 : 84, *v/v/v*) diluted with 950 ml of Milli-Q water. Mobile phase B was a mixture of acetonitrile and formic

acid (99.9 : 0.1, v/v). The injection volume was 20 μl and the elution was performed at a flow rate of 0.5 $\text{ml}/\text{min}^{-1}$. The gradient profile was: 99.9 % A at 0–0.5 min, 90.9 % A at 5.7 min, 78.8 % A at 7.7 min, 40.4 % A at 8–10 min, 10 % A at 10.01–12.00 min, and 99.9 % A at 12.01–14.00 min. These compounds were identified using a UHPLC system coupled to a 6460 tandem mass spectrometer (Agilent Technologies, Waldbronn, Germany). Data acquisition and processing were performed using MassHunter software version B.04.00, from Agilent Technologies. The MS analysis was applied in the multiple reaction monitoring (MRM) mode, which was performed using the positive ionization mode. The MS parameters fragmentor (ion optics; capillary exit voltage) and collision energy were optimized for each analyte (Table 6). The allocation of these parameters, along with preferential MRM transition of the corresponding analytes, generated the most abundant product ions. The MRM transition used for each derivatized amino acid/thiol corresponded, in most cases, to the AMQ moiety (171^+) – which results from the collision-induced cleavage at the ureide bond of the AMQ adduct of each amino acid/thiol [25, 29]. The working conditions for the MS parameters of the electrospray source were as follows: gas flow, 9 l/min; nebulizer, 40 psi; capillary voltage, 4000 V; nozzle voltage, 1000 V; gas temperature, 325 $^{\circ}\text{C}$; sheath gas tem-

perature, 390 $^{\circ}\text{C}$; and jetstream gas flow, 11 l/min. The acquisition time was 12 min for each sample.

2.7. Statistical design and analysis

The experiment had a completely randomized design with four replications, each consisting of three adjacent tree rows; each row had 11 trees. Physiological measurements were taken on the inner tree of the central row of each replicate, these trees being healthy, uniform, and very similar in appearance, while the other trees served as border trees. The data were processed using SPSS software version 19 for Windows [30]. One-way analysis of variance was carried out and mean values were compared by Tukey's multiple range test. All means were compared at the 0.05 level of significance. Values for each replicate were averaged before the mean and the standard deviation of each treatment were calculated.

3. RESULTS AND DISCUSSION

3.1. Plant water status

Significant differences between the irrigation treatments were noted in the Ψ_{pd} and Ψ_{stem} values from DOY 209 to 238 (Figures 1B and 1D).

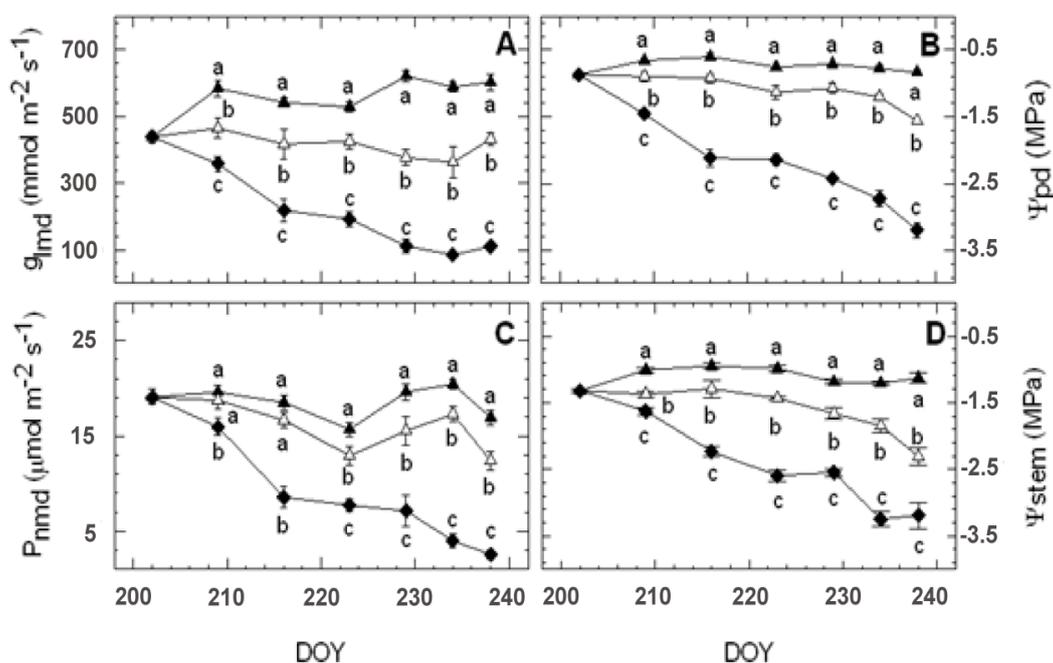


Fig. 1. Midday leaf conductance (g_{lmd} , A), predawn leaf water potential (Ψ_{pd} , B), midday net photosynthesis (P_{nmd} , C), and midday stem water potential (Ψ_{stem} , D) values (mean \pm SE, not shown when smaller than symbols, $n = 4$), for jujube plants in treatments T0, T1, and T2 during the experimental period. Different letters on data points at each date indicate significant differences according to Tukey's test ($P \leq 0.05$).

The Ψ_{pd} and Ψ_{stem} values in T1 plants were lower than in T0 plants and higher than in T2 plants. In addition, the Ψ_{pd} values in T0 and T1 plants and Ψ_{stem} values in T0 plants remained constant during the experimental period, whereas the Ψ_{pd} and Ψ_{stem} values in T2 plants and Ψ_{stem} values in T1 plants showed a tendency to gradually decrease, reaching minimum values on DOY 238.

The midday leaf conductance (g_{lmd}) and net photosynthesis (P_{nmd}) values during the measurement period were high and nearly constant in T0 plants (Figures 1A and 1C). Moreover, the g_{lmd} values of T1 plants were also nearly constant, and intermediate between the T0 and T2 values (Figure 1A). However, the g_{lmd} and P_{nmd} values in T2 plants gradually decreased during the stress period, reaching minimum values on DOY 238.

Considering that Ψ_{pd} values depend on soil moisture levels [31, 32], the high and constant Ψ_{pd} values in T0 plants during the experiment indicate that these plants were not exposed to limiting soil water conditions (Figure 1B). Moreover, the existence of significant differences in the Ψ_{pd} , Ψ_{stem} , g_{lmd} , and P_{nmd} values among treatments point to a water deficit situation in T1 and T2 plants (Figure 1). The water deficit in the T1 plants can be considered as moderate because the Ψ_{pd} and Ψ_{stem} values in T1 plants reached minimum values of -1.56 and -2.28 MPa, respectively, while the g_{lmd} and P_{nmd} values were relatively high and constant, although lower than those in T0. At the end of the measurement period, the T2 plants showed very low Ψ_{pd} and Ψ_{stem} values (-3.20 and -3.14 MPa, respectively) (Figures 1B and D) and a considerable degree of stomatal regulation compared with the T1 plants (Figures 1A and C), indicating that treatment T2 induced a more severe water deficit.

3.2. Fruit weight, firmness and color

The total marketable jujube yield of the T1 plants was higher than that of the T2 plants and lower than for the T0 plants (Table 1). The fruit from T0 and T1 trees showed similar average weight, moisture content, and firmness. In contrast, T2 fruits showed the lowest average weight and moisture content, but the greatest firmness (Table 1).

Changes in fruit peel and flesh color were only observed in T2 fruits (Table 2). In this sense, the peel and flesh H^p values of T2 fruits were lower than those of T0 and T1 fruits, and the peel and flesh a^* and flesh b^* values of T2 fruits were higher than in the other treatments. The flesh L^* values of T2 fruits were decreased with respect to those of T0 and T1 fruits.

Fruit yield in the three irrigation treatments ($833 - 2500$ g m⁻² or $10 - 30$ kg tree⁻¹) (Table 1) can be considered as adequate according to the yields mentioned by Gao *et al.* [33] for different cultivars ($130 - 1800$ g m⁻²) and those obtained by Cui *et al.* [17], who considered the effect of different deficit irrigation levels during different growth stages ($14 - 24$ kg/tree). However, in contrast with our results, these authors indicated that low, moderate, or severe water deficit during the fruit maturation stage (stage III, early August – early September) had no significant effect on fruit yield and size but reduced the fruit water content. Unfortunately, Cui *et al.* [17] did not evaluate quantitatively the water status of the plants under severe water deficits and so our results cannot be compared with theirs.

Table 1

Effect of irrigation treatments on jujube yield (kg tree⁻¹), average fruit weight (FW, g), fruit moisture content (MC, %), and fruit firmness (FF, N)

Treatment	Yield	FW	MC	FF
T0	31.81a	30.23a	83.47a	64.00b
T1	21.36b	27.98a	79.66a	66.67b
T2	9.59c	14.40b	64.95b	82.33a

Means within a column that do not have a common letter are significantly different according to Tukey's test ($P \leq 0.05$).

The fruit size reduction observed in T2 fruits (Table 1) could be related to the results obtained recently (data not shown) in a very similar experiment by Cruz, who found that, in contrast with the axiom that expansive cell growth requires cell turgor, jujube fruit size in plants under severe water stress was reduced even though fruit turgor was maintained. This may have been due to enhancement of the cell elasticity mechanism (elastic adjustment), to maintain fruit turgor by reducing fruit cell size. The fact that jujube fruits under severe water deficit (T2) showed greater firmness could be related to the decreased moisture content in these fruits (Table 1), leading to a gummy and cork-like flesh structure. In this sense, Wu *et al.* [34] showed that *Z. Jujube* fruit firmness increased at the end of the ripening period (fruit surface 100% red) as a consequence of the lower fruit water content. This phenomenon has been observed in other fruits, such as plums, which became stiff, leading to an increase in the firmness values expressed as the load needed to break the flesh [35].

Changes in fruit peel and flesh color were evident in the T2 irrigation treatment (Table 2). The severe water stress induced changes in the fruit peel a^* and H^o values (from green towards a more intense red color). These changes were less

pronounced in the fruit flesh, which remained greener than the peel and showed a lower degree of luminosity while its colour was yellower than that of the T0 and T1 fruit flesh (Table 2).

Table 2

Effect of irrigation treatments on jujube fruit peel and flesh lightness (L^*), red-greenness (a^*), blue-yellowness (b^*), chroma (C^*), and hue angle (H^o) values

Treatment	L^*		a^*		b^*		C^*		H^o	
	Peel	Flesh	Peel	Flesh	Peel	Flesh	Peel	Flesh	Peel	Flesh
T0	78.02a	88.43a	-12.09b	-12.21b	45.04a	24.42b	46.64a	27.30a	105.04a	116.57a
T1	79.26a	88.17a	-11.18b	-12.13b	44.66a	25.17b	46.05a	27.94a	104.06a	115.73a
T2	67.55a	79.27b	3.04a	-9.91a	40.37a	27.16a	41.08a	28.91a	84.27b	110.02b

Means within a column that do not have a common letter are significantly different according to Tukey's test ($P \leq 0.05$).

3.3. Fruit ethylene emission, respiratory rate, and total soluble solids

Fruit ethylene emission was increased in T1 and T2 fruits with respect to the T0 fruits (Table 3). However, the fruit respiratory rate was highest in T1 fruits and lowest in T2 fruits, while T0 fruits

showed intermediate values (Table 3). Flesh TSS showed their minimum values in T0 fruits and were highest in T2 fruits, T1 fruits showing intermediate values. The TA values were unaffected by the irrigation treatment, but the TSS/TA ratio in T2 fruits was significantly higher than in T0 and T1 fruits (Table 3).

Table 3

Effect of irrigation treatments on jujube fruit ethylene emission (C_2H_4 , nL/h g FW), respiratory rate (RR, CO_2 mg/h kg FW), total soluble solids (TSS, °Brix), titratable acidity (TA, % malic acid), and ripening index (TSS/TA)

Treatment	C_2H_4	RR	TSS	TA	TSS/TA
T0	0.027b	59.35b	15.47c	0.25a	61.31b
T1	0.143a	66.37a	20.20b	0.32a	62.56b
T2	0.118a	56.17c	26.83a	0.36a	77.53a

Means within a column that do not have a common letter are significantly different according to Tukey's test ($P \leq 0.05$).

Kader *et al.* [36] concluded that *Z. jujuba* fruits followed a non-climateric pattern. In contrast, Abbas and Saggar [37] and Abbas and Fandi [38], in *Z. mauritiana* fruits, and Al-Niami *et al.* [39], in *Z. spina-christi* fruits, showed a climacteric behaviour. Moreover, coinciding with these observations in closely related species, Zhang *et al.* [40] and Wang *et al.* [41] recently indicated that *Z. jujuba* fruits are climacteric. In this sense, the increase in ethylene emission and respiratory rate in T1 fruits reflect the fact that under moderate water deficit the fruits entered the ripening phase earlier than control (T0) fruits [39]. In addition, the increase in ethylene emission in T2 fruits – compared with T0 fruits – and the decrease in fruit res-

piratory rate in T2 fruits – compared with both T0 and T1 fruits – reflect the finding that under severe water deficit (T2) the fruits reach a higher degree of ripening [38].

3.4. Fruit vitamin C, sugar, organic acids and bioelements

The T2 fruit showed higher concentrations of vitamin C, sucrose, glucose, arabinose, malic acid, and oxalic acid and a lower citric acid content than T0 fruits (Table 4). These organic compounds showed similar values in T0 and T1 fruits, except sucrose and arabinose, which increased significantly in T1 fruits (Table 4).

Table 4

Effect of irrigation treatments on jujube fruit vitamin C (mg/100 g FW), sucrose (mg/100 g FW), glucose (mg/100 g FW), arabinose (mg/100 g FW), malic acid (MA, mg/100 g FW), citric acid (CA, mg/100 g FW), and oxalic acid (OA, mg/100 g FW)

Treatment	Vitamin C	Sucrose	Glucose	Arabinose	MA	CA	OA
T0	375.33b	8954.03b	4029.59b	3567.76c	520.34b	272.71a	34.46b
T1	380.65b	23808.80a	3833.23b	4368.44b	530.88b	284.76a	26.66b
T2	590.04a	18564.92a	8815.84a	9217.86a	715.37a	191.92b	57.12a

Means within a column that do not have a common letter are significantly different according to Tukey's test ($P \leq 0.05$).

The most abundant bioelements in the edible portion of jujube fruits were K, N, Ca, P, and Mg, followed, in descending order, by S, Na, B, Zn, Mn, Fe, and Cu (Table 5). Some changes in the nutrient content of the fruit edible portion were observed among the three irrigation treatments. In this respect, the mineral content of T1 fruits was very similar to that observed in T0 fruits, except that the K, P, and Cu contents were lower (Table 5). In contrast, the K, N, P, Mg, S, B, Mn, Fe, and Cu contents in the edible portion of T2 fruits were lower than in T0 fruits, whereas the Ca, Na and Zn levels were similar to those in T0 and T1 fruits (Table 5).

Under our experimental conditions, the jujube T0 fruits showed a vitamin C content (Table 4) similar to those found by Cui *et al.* [17] in fruits from cv. Lizao and by Gao *et al.* [42] in fruits from

10 promising jujube selections (Lingbaozao, Puai-sanhao, Fengmiguan, Junzao, Zaowangzao, Jinchangyihao, Taigumizao, Qingjianmuzao, and Jixianmuzao) and higher than those found by Li *et al.* [7] in fruits from five different *Z. jujuba* cultivars (Jinsixiaozao, Yazao, Jianzao, Junzao, and Sanbianhong). These values confirm that the jujube fruit is a rich source of ascorbic acid compared with other fruit species: according to the Food and Nutrition Board of the National Research Council [43], one 20-g portion of jujube fruit (a medium size fruit) could provide almost the entire daily vitamin C requirement of an adult. The increase in vitamin C content in fruits under severe water deficit (T2) (Table 4) is in line with the results of Cui *et al.* [17] and could have resulted from a concentration effect due to the decrease in fruit moisture content (Table 1).

Table 5

Effect of irrigation treatments on some mineral nutrient contents (mg/100g DW) in the edible portion (peel and flesh) of jujube fruit

Treatment	K	N	Ca	P	Mg	S	Na	B	Zn	Mn	Fe	Cu
T0	1052a	839a	66a	54a	49a	24a	6a	1.61a	0.70a	0.68a	0.51a	0.23a
T1	864b	749a	52a	45b	41ab	21a	5a	1.69a	0.61a	0.63ab	0.50ab	0.16b
T2	630c	587b	57a	39b	35b	13b	4a	1.10b	0.57a	0.51b	0.32b	0.06c

Means within a column that do not have a common letter are significantly different according to Tukey's test ($P \leq 0.05$).

In contrast with the findings of other authors [7], the sugars detected in the Grande de Albaterra cultivar fruits were mainly sucrose, glucose, and arabinose, along with traces of fructose. The presence of arabinose, one of the major neutral sugars in insoluble and soluble dietary fiber, suggests another favourable nutritional aspect of these fruits. High amounts of arabinose could be due to neutral arabinogalactan type polysaccharides or also the pectic type, which are linked to galacturonic acid residues [44]. The fact that the arabinose content of T2 fruits was higher than that of T0 and

T1 fruits agrees with the effect of water deficit on the cell wall of the date palm fruit observed by Gribaa *et al.* [45]. These authors showed that the arabinose content was significantly higher in the pectin-enriched extracts from fruits under non-irrigated conditions and suggested that this could be involved in maintaining the hydration of the cell and cell wall flexibility under water deficit.

Moderate water deficit (T1) increased the sucrose and arabinose contents, as reflected in the higher TSS levels (Tables 3 and 4), indicating that moderate water deficit during fruit maturation im-

proves jujube fruit eating quality because the fruits taste sweeter. Severe water deficit (T2) improved not only the fruit taste but also the nutritional value, because these fruits showed increases in sucrose, glucose, arabinose, vitamin C, malic acid, and oxalic acid, but also a decrease in citric acid (Table 4).

The overall level of bioelements in the edible portion of jujube fruits (Table 5) differed slightly with respect to those found in five different cultivars by Li *et al.* [7]. For example, K was the predominant bioelement in both cases but we observed levels higher than those in cultivar Yazao, the richest source of K among the Chinese jujube cultivars studied by Li *et al.* [7]. Also, the Zn contents were above those reported by Favier *et al.* [46] and Li *et al.* [7], although the Ca content was similar to that reported by Favier *et al.* [46] and that found in cultivar Jinsixiaozao by Li *et al.* [7]. The Mg and Na contents were within the range found by Favier *et al.* [46] and Li *et al.* [7], although P and the micronutrients Cu, Fe, and Mn showed values clearly lower than those reported by Li *et al.* [7]. These differences could be ascribed to the cultivar's efficiency for nutrient uptake, the edaphoclimatic conditions, or cultural practices [47]. Severe water deficit (T2) induced decreases in the K, N, P, Mg, S, B, Mn, Fe, and Cu contents in the edible portion of the fruits, compared with T0, while moderate water deficit (T1) induced no changes in the fruit content of most of the bioelements, except K, P, and Cu, which were lower than in T0 fruits (Table 5). These findings agree with the idea that drought restricts not only water acquisition but also nutrient uptake [20, 21, 48].

3.5. Amino acids

Thirty-two free amino acids were found in the pit and the edible portion of jujube fruits, 11 of them essential and 21 non-essential (Tables 6–8). In this sense, two essential (Cys-cys and Met) and two non-essential (Cysta and Hcys-cys) amino acids fall within the definition of amino thiols since they have a sulfhydryl group.

The edible portion of T2 fruits showed significantly lower contents of total free and total essential free amino acids than that of T0 fruits (Table 7). While the edible portion of T1 fruits had a total free amino acid content similar to that of T0 fruits, the total essential free amino acid content was similar to that of T2 fruits and lower than that of T0 fruits (Table 7). Differences were observed among the responses of the free amino acids to T1 and T2 deficit irrigation. For example, while the levels of free amino acids in the edible portion of

T2 fruits had an overall tendency to decrease with respect to those in T0 fruits, Pro, Trp, and Cysta increased while His, Cys-cys, Met-his, BAIB, and Hcys-cys did not change. In the edible portion of T1 fruits, the levels of most of the free amino acids were similar to those in T0 fruits, except for 13 amino acids, 11 of which (Tyr, Leu + Ile, Phe, PEA, Gln, Asp, Cit, AADA, Orn, BAIB, and AABA) showed lower values and two (Cys-cys and Cysta) higher values (Table 7).

The effects of T1 and T2 on the concentrations of pit free amino acids were very different from those observed in the edible portion of the fruit (Tables 7 and 8). The total free amino acids and total essential free amino acids contents were similar for all three irrigation treatments. However, the responses of the amino acids to the T1 and T2 treatments differed greatly. For example, T2 decreased the levels of 12 amino acids (Thr, Lys, p-Hyp, Asn, Gln, Ser, Gly, Glu, Ala, GABA, Orn, and Hcys-cys), compared with T0, and increased the levels of 11 (Cys-cys, Met, Trp, Phe, PEA, Arg, EA, AADA, Pro, AABA, and Cysta), while eight amino acids (His, Tyr, Val, Leu + Ile, Met-his, Asp, Cit, and BAIB) showed similar levels in the T2 and T0 pits. As regards T1, 20 amino acids (His, Thr, Lys, Tyr, Leu + Ile, Trp, Phe, Met-his, p-Hyp, Asn, Arg, EA, Gly, Asp, Cit, Ala, AADA, BAIB, AABA, and Hcys-cys) showed levels similar to those of T0 pits, whereas five (Cys-cys, Met, PEA, Pro, and Cysta) showed higher concentrations and six (Val, Gln, Ser, Glu, GABA, and Orn) lower concentrations, compared with T0 pits (Table 8).

It is important to emphasize that the free amino acids identified in the edible portion (peel and flesh) and pit (shell and seed) of jujube fruits (Table 6) showed some differences with respect to those identified in three Korean cultivars (Boeumdeachu, Mechu, and Sanzoin) by Choi *et al.* [4, 6]. These authors identified cysteine (Cys) and hydroxylysine (Hyl), which were not found in the fruits of the Grande de Albaterra cultivar. However, to the best of our knowledge, this is the first time that the following have been identified in *Z. jujuba* fruits: one essential amino acid (Cys-cys) and seven non-essential amino acids (p-Hyp, AADA, Orn, BAIB, AABA, Cysta, and HCys-Cys) (Table 6). The presence of four amino thiols (Cys-cys, Met, Cysta and Hcys-cys) in jujube fruits can also be considered a valuable aspect, because biothiols are the most important antioxidants that protect cells from oxidative damage [49]. Also, Zagorchev *et al.* [50] indicated that the data available so far suggest that thiols play a central role in the abiotic stress tolerance of plants.

The levels of total amino acids in the edible portion of T0 fruit (Table 7) were similar to those found in the fruit pulp of the Boeum-deachu and Mechu cultivars [6]. The total essential amino acid content in the T0 fruit edible portion (Table 7) was also similar to that found in the fruit pulp of culti-

var Sanzo in [6]. However, the sums of the total and total essential amino acids in T0 pits (Table 7) were clearly lower than those found in the seeds of three Korean cultivars by Choi *et al.* [6], probably due to a dilution effect of the shell portion.

Table 6

MS parameters of the free amino acids identified in jujube fruits under UHPLC-QqQ-MS/MS conditions by the use of AQC derivatization [25, 26]

ID	Amino acid	Parent ion (m/z)	Daughter ion (m/z)	Fragmentor	Collision energy	t _R (min)
1	His	326.2	171	100	0	3.53
2	Met-his	340.2	171	100	10	3.61
3	p-Hyp	302.1	171	120	0	3.64
4	Asn	303.1	171	100	0	3.99
5	PEA	312.4	171	80	0	4.00
6	Arg	345.2	171	100	0	4.16
7	Gln	317.2	171	110	0	4.43
8	Ser	276.1	171	80	0	4.47
9	EA	232.1	171	100	0	4.48
10	Gly	246.1	171	90	0	4.72
11	Asp	304.1	171	140	0	5.13
12	Cit	340.2	171	100	10	5.20
13	Glu	318.1	171	110	0	5.39
14	Thr	290.1	171	120	0	5.66
15	Ala	260.2	171	100	0	6.12
16	GABA	274.1	171	90	0	6.18
17	AADA	332.3	171	110	0	6.64
18	Pro	286.2	171	90	0	6.69
19	Orn	473.2	171	110	5	7.09
20	BAIB	274.1	171	80	0	7.12
21	AABA	274.1	171	110	0	7.26
22	Lys	487.2	171	90	5	7.34
23	Cys-cys	581.6	171	90	0	7.38
24	Cysta	563.3	171	100	0	7.54
25	Tyr	352.2	171	90	0	7.88
26	Val	288.2	171	110	0	7.99
27	Met	320.2	171	90	0	8.00
28	Hcys-cys	609.4	171	130	0	8.32
29	Leu + Ileu	302.2	171	100	0	8.65
30	Trp	375.2	171	120	0	8.68
31	Phe	336.2	171	100	0	8.69

Abbreviations used: Histidine (His), 1-methyl-histidine (Met-His), 4-hydroxyproline (p-Hyp), asparagine (Asn), phosphoethanolamine (PEA), arginine (Arg), glutamine (Gln), serine (Ser), ethanolamine (EA), glycine (Gly), aspartic acid (Asp), citrulline (Cit), glutamic acid (Glu), threonine (Thr), alanine (Ala), γ -amino-*n*-butyric acid (GABA), α -amino adipic acid (AADA), proline (Pro), ornithine (Orn), β -aminoisobutyric acid (BAIB), α -amino-*n*-butyric acid (AABA), lysine (Lys), cystine (Cys-Cys), cystathionine (Cysta), tyrosine (Tyr), valine (Val), methionine (Met), homocystine (HCys-Cys), leucine (Leu), alloisoleucine (Ileu), tryptophan (Trp), phenylalanine (Phe).

Table 7

Effect of irrigation treatments (T0, T1, and T2) on the concentrations of free amino acids (g/kg DW) in the edible portion (peel and flesh) of jujube fruits

Amino acids	Treatment					
	T0		T1		T2	
<i>Essential</i>						
His	0.6±8.2E-02	a	0.4±4.8E-02	a	0.9±0.2	a
Thr	0.6±1.4E-02	a	0.5±4.0E-02	a	0.2±3.4E-02	b
Lys	0.1±2.1E-03	a	0.1±3.4E-03	a	6.0E-02±1.4E-03	b
Cys-cys	8E-05±1E-05	b	1.2E-04±1.0E-05	a	8.0E-05±1.0E-05	b
Tyr	1.4±0.2	a	0.7±5.3E-02	b	0.5±1.0E-02	b
Val	0.3±5E-03	a	0.2±1.3E-02	a	0.1±9.5E-03	b
Met	1.1E-03±1.0E-04	a	8.7E-04±4E-05	ab	4.5E-04±3E-05	b
Leu + Ile	0.1±4.6E-03	a	9.7E-02±5.0E-03	b	4.7E-02±5.2E-03	c
Trp	0.2±3.2E-02	b	0.2±1.1E-02	b	0.4±5.0E-02	a
Phe	5.1E-02±7.6E-03	a	2.4E-02±7.7E-04	b	1.8E-02±2.1E-03	b
<i>Non-essential</i>						
Met-his	6.0E-02±5.9E-03	a	7.0E-02±2.0E-02	a	5.0E-02±5.2E-03	a
p-Hyp	2.8±6.8E-02	a	2.3±0.1	a	1.5±0.2	b
Asn	37.9±1.4	a	33.6±3.3	a	14.0±3.0	b
PEA	1.2E-02±7.5E-04	a	7.7E-03±8.8E-04	b	6.2E-03±4.0E-04	b
Arg	4.0±0.3	a	3.4±0.3	a	1.4±0.2	b
Gln	1.1±1.8E-02	a	0.9±6.2E-02	b	0.3±2.9E-02	c
Ser	7.1±0.2	a	6.3±0.6	a	2.5±0.1	b
EA	1.4±2.6E-02	a	1.3±9.9E-03	a	1.0±2.3E-02	b
Gly	0.4±2.3E-02	a	0.4±1.8E-02	ab	0.2±3.4E-02	b
Asp	13.5±0.1	a	9.7±0.7	b	2.1±6.8E-02	c
Cit	5.8E-02±1.5E-03	a	3.8E-02±1.2E-04	b	2.0E-02±1.9E-03	c
Glu	6.3±0.4	a	6.4±0.4	a	1.3±0.3	b
Ala	0.9±4.3E-02	a	0.8±4.6E-02	a	0.2±8.1E-02	b
GABA	1.4±6.3E-02	a	1.3±6.3E-02	ab	1.0±6.3E-02	b
AADA	6.4E-02±3E-03	a	3.8E-02±2.7E-03	b	4.7E-02±3.5E-03	b
Pro	17.8±2.3	b	22.6±2.4	ab	26.9±2.0	a
Orn	0.3±1.0E-02	a	7.8E-02±8.0E-4	b	6.2E-02±5.3E-04	b
BAIB	8.3E-02±1.4E-03	a	5.5E-02±2.6E-03	b	6.4E-02±5.6E-03	ab
AABA	0.4±3.2E-03	a	0.3±7E-04	b	0.2±3.4E-02	b
Cysta	4.0E-03±2E-04	b	5.9E-03±4.1E-04	a	5.4E-03±1.3E-03	a
Hcys-cys	1.9E-04±2E-05	a	2.4E-04±2.0E-05	a	1.6E-04±4.0E-05	a
Total	99.3±5.3	a	92.0±7.5	a	55.2±6.5	b
Total essential	3.4±0.3	a	2.2±0.1	b	2.2±0.4	b

Means within a row followed by a different letter are significantly different according to Tukey's test ($P \leq 0.05$). Abbreviations as in Table 6.

Although the same amino acids were found in the fruit edible portion and pit, differences in the contribution of each free amino acid to the total free amino acids were evident (Tables 6–8). In the edible portion of T0 fruit, His, Thr, and Tyr constituted 77 % of the total essential amino acid content. However, in pits, which had much lower amino acid content than the edible portion, His,

Thr, and Tyr constituted only 42 % of the total essential amino acid content. Moreover, considering both essential and non-essential amino acids, Asn, Asp, Glu, and Pro constituted 76 % of total amino acids in the T0 fruit edible portion but only 59 % in pits (Tables 7 and 8).

Amino acids are the main transport forms of N in plants [51] and the irrigation treatments pro-

duced similar changes in the total N and total free amino acid contents in the edible part of the fruit (Tables 5 and 7). This suggests that the decrease in total free amino acids in T2 fruits could have been due to a decrease in the transport of amino acids into fruits under this severe water deficit [52]. Alternatively, or as a complementary possibility, the

decrease in total free amino acids in T2 fruits could have been caused by the partial inactivation of amino acid biosynthesis pathways in the fruit [52].

In addition to the fact that our results indicate high amounts of free amino acids in jujube fruits, it is important to emphasize the relatively high Asn content found in the edible portion of the

Table 8

Effect of irrigation treatments (T0, T1, and T2) on the concentrations of free amino acids (g/kg DW) in the pits (shell + seed) of jujube fruits

Amino acids	Treatment					
	T0		T1		T2	
<i>Essential</i>						
His	0.2 ± 7.2E-02	a	0.3 ± 3.0E-02	a	0.3 ± 2.7E-02	a
Thr	0.2 ± 6.5E-04	a	0.2 ± 9.1E-03	a	0.1 ± 5.6E-03	b
Lys	6.4E-02 ± 3.9E-03	a	6.5E-02 ± 3.1E-03	a	5.4E-02 ± 3.3E-04	b
Cys-cys	1.7E-04 ± 3.3E-06	b	2.0E-04 ± 2.0E-06	a	2.0E-04 ± 3.6E-06	a
Tyr	0.1 ± 7.2E-03	a	0.1 ± 1.2E-02	a	0.1 ± 1.4E-02	a
Val	6.3E-02 ± 2.1E-03	a	4.6E-02 ± 6.3E-04	b	6.6E-02 ± 3.3E-03	a
Met	3.4E-04 ± 1.1E-05	b	6.6E-04 ± 4.6E-05	a	6.6E-04 ± 8.4E-05	a
Leu + Ile	2.1E-02 ± 7.5E-04	a	2.1E-02 ± 1.8E-04	a	2.2E-02 ± 8.6E-04	a
Trp	0.5 ± 5.2E-02	b	0.4 ± 1.9E-02	b	0.6 ± 3.2E-02	a
Phe	6.7E-03 ± 6.6E-05	b	6.5E-03 ± 5.8E-05	b	8.2E-03 ± 1.1E-03	a
<i>Non-essential</i>						
Met-his	1.1E-02 ± 4.8E-04	ab	9.0E-03 ± 8.7E-04	b	1.2E-02 ± 7.5E-04	a
p-Hyp	1.1 ± 4.6E-02	a	1.1 ± 7.1E-02	a	0.7 ± 2.9E-02	b
Asn	6.5 ± 0.3	a	6.4 ± 0.9	a	3.9 ± 4.9E-02	b
PEA	8.8E-04 ± 2.9E-05	b	1.3E-03 ± 1.2E-04	a	1.8E-03 ± 9.4E-05	a
Arg	4.6 ± 4.1E-01	b	4.5 ± 2.6E-01	b	5.6 ± 0.3	a
Gln	0.3 ± 4.2E-03	a	0.2 ± 1.1E-02	b	0.2 ± 9.9E-04	b
Ser	1.0 ± 4.4E-02	a	0.7 ± 1.5E-02	b	0.5 ± 1.3E-02	c
EA	0.1 ± 6.0E-03	b	0.1 ± 1.9E-03	b	0.2 ± 1.9E-03	a
Gly	7.6E-02 ± 2.6E-03	a	8.1E-02 ± 3.7E-03	a	6.4E-02 ± 4.2E-03	b
Asp	1.7 ± 8.3E-02	a	1.5 ± 1.5E-01	a	1.6 ± 5.5E-02	a
Cit	1.3E-02 ± 1.3E-03	a	1.2E-02 ± 4.7E-04	a	1.3E-02 ± 1.4E-03	a
Glu	1.4 ± 4.2E-02	a	1.2 ± 6.8E-02	b	1.0 ± 0.2	b
Ala	0.4 ± 7.4E-03	a	0.4 ± 8.1E-03	a	0.3 ± 1.2E-02	b
GABA	0.3 ± 1.3E-02	a	0.2 ± 6.1E-03	b	0.2 ± 9.9E-03	b
AADA	1.2E-02 ± 1.9E-03	b	1.4E-02 ± 1.2E-03	ab	1.9E-02 ± 4.2E-04	a
Pro	3.1 ± 0.2	b	5.1 ± 0.8	a	6.7 ± 0.7	a
Orn	3.6E-02 ± 2.1E-03	a	2.7E-02 ± 1.4E-03	b	2.5E-02 ± 7.6E-04	b
BAIB	1.8E-03 ± 1.2E-04	ab	2.3E-03 ± 1.7E-04	a	1.5E-03 ± 2.2E-05	b
AABA	1.8E-03 ± 9.7E-05	b	2.0E-03 ± 2.2E-04	ab	1.0E-02 ± 6.4E-03	a
Cysta	5.4E-04 ± 3.5E-05	b	1.7E-03 ± 2.2E-04	a	2.0E-03 ± 2.1E-04	a
Hcys-cys	3.2E-04 ± 1.4E-05	a	3.1E-04 ± 7.8E-06	a	1.8E-04 ± 4.0E-06	b
Total	21.6 ± 1.3	a	22.7 ± 2.3	a	22.3 ± 1.5	a
Total essential	1.2 ± 7.4E-02	a	1.1 ± 7.5E-02	a	1.3 ± 8.4E-02	a

Means within a row followed by a different letter are significantly different according to Tukey's test ($P \leq 0.05$). Abbreviations as in Table 6.

fruit because Asn serves as a precursor of the potentially toxic acrylamide, which may be formed during the heat-processing of plant foods [15]. Recently, Choi *et al.* [4] showed that the Asn content of jujube fruit pulp decreased during the last stages of fruit growth, from 14.7 to 9.2 g/kg DW; representing 54.9 and 31.9 %, respectively, of the total free amino acid content. In this sense, at harvest we found slightly higher Asn levels in the edible portion of the Grande de Albaterra T0 fruits (37.9 g/kg DW) (Table 7), which contributed 38.2 % to the total free amino acid content. Despite the fact that most of the amino acids in both the edible portion and pits of jujube fruits showed a tendency to decrease as a result of water deficit, the decrease in the Asn content from 37.9 (T0) to 14.0 g/kg DW (T2) in the fruit edible portion and from 6.5 (T0) to 3.9 g/kg DW (T2) in the pits must be considered advantageous. In other words, severe water deficit during fruit maturation can be considered as a helpful tool to decrease the fruit Asn content, hence reducing the risk of formation of the potentially toxic acrylamide.

A common response to water deficit is the accumulation in plant tissues of some amino acids, mainly Pro (Tables 7 and 8), which may be used to achieve osmotic adjustment [53]. In this study, Pro was the most abundant amino acid in both the edible portion and pit. Indeed, in the T0 fruit edible portion Pro contributed 18 % to the total free amino acid content, whereas in T1 and T2 fruits this contribution was 25 and 49 %, respectively. Likewise, in the T0 pits Pro contributed 14 % to the total free amino acid content, whereas in T1 and T2 fruits this contribution was 23 and 30 %, respectively (Tables 7 and 8). These increases suggest that Pro could contribute to osmotic adjustment in water stressed jujube fruits. The role of Pro may not be confined to osmotic adjustment, because it also helps the cell to overcome water stress-induced oxidative stress [52, 53]. Whatever the case, it is important to indicate that the response of Pro in jujube fruits to water deficit was not as sensitive as expected, because it is well known that Pro levels in leaves increase in response to drought in direct proportion to the magnitude of the water deficit [54, 55].

4. CONCLUSIONS

In contrast to previous findings, a major conclusion of our study is that, under our experimental conditions, jujube fruit maturation was sensitive to water deficit, because the yield and fruit

size decreased and most of the chemical characteristics of the fruit were modified. To the best of our knowledge, this is the first time that one essential amino acid (Cys-cys) and seven non-essential amino acids (p-Hyp, AADA, Orn, BAIB, AABA, Cysta and HCys-Cys) have been identified in *Z. jujuba* fruits. The presence of four amino thiols (Cys-cys, Met, Cysta and Hcys-cys) underlines the functional character of these fruits.

Certain proportionality was observed in the response of jujube fruits to both deficit irrigation treatments. Fruits from plants exposed to moderate water deficit (T1) showed no change in fruit size, moisture content, firmness, or fruit peel and flesh color, but a decrease in fruit yield was accompanied by changes in ethylene emission, respiratory rate, and some fruit chemical characteristics, such as increases in the sucrose and arabinose contents, although no changes were found for most of the bioelements. This behavior indicates that T1 fruits entered the ripening phase earlier than control fruits and that moderate water deficit during fruit maturation improves jujube fruit eating quality because the fruits taste sweeter. Fruits from the more pronounced water deficit treatment (T2) showed decreased growth, resulting in smaller fruit size, lower moisture content and yield, and changes in firmness and peel and flesh color. Furthermore, changes in ethylene emission and the respiratory rate, likewise changes in chemical characteristics (increases of sugars and vitamin C and changes in organic acids), indicate that T2 fruits achieved a higher degree of ripening than T0 and T1 fruits, improving not only most of the fruit chemical characteristics that make up the taste but also the nutritional value.

The response of fruit amino acids to water deficit was not as sensitive as expected, since it did not show a direct relationship with the magnitude of the deficit. These results together with others, such as the decrease in fruit Asn content due to severe water deficit, may be the key to jujube fruit cultivation in arid and semiarid zones, due to a lower risk of acrylamide formation during fruit heat-processing.

Acknowledgements. The authors are grateful to the Projects AGL2010-19201-C04-01AGR and AGL 2011-23690 (CICYT), CSD 007-0063 (CONSOLIDER7 INGENIO 2010 "FUN-C-FOOD"), and CSIC 201170E041 (Spanish Ministry of Economy and Competitiveness), the Agencia Española de Cooperación Internacional para el Desarrollo (AECID) (A1/035430/11), the Fundación Séneca (04486/GERM/06), and the Ibero American Programme for Science, Technology and Development (CYTED), Action 112RT0460 CORNU-COPIA. ZNC, JCG, and AG were funded by a grant of the AECID, FPI and FPU Fellowship Programme, respectively, from the Spanish government.

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