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Effect of modified atmosphere packaging on the physiological and functional characteristics of Spanish jujube (*Ziziphus jujuba* Mill.) cv 'Phoenix' during cold storage

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ABSTRACT

Jujube fruits cv 'Phoenix' were stored in modified atmosphere packaging (MAP) using a polyester (12 µm)-polypropylene (60 µm) film at 5 °C and 90% RH during 49 days. Jujube fruits stored without packaging and in normal air and same temperature and RH served as control. The atmosphere composition at the steady state was at 35 days with 14.50 kPa O_2 and 3.86 kPa CO_2. The atmosphere packaging showed an almost zero amount of ethylene during all storage days. The jujubes at MAP have been very effective as they presented the same appearance throughout the 49 days of storage. On the other hand, the fruits in control showed a wrinkled and non-commercial appearance at day 21. Treatment with MAP caused a significant delay in the ripening of the fruit after harvest. It caused less weight loss, more firmer and more intense color. Improved total carotenoids, total phenols, hydrophilic-total antioxidant activity (H-TAA), lipophilic-total antioxidant activity (L-TAA). Meanwhile, the maturity index (MI) was reduced compared with control jujubes.

1. Introduction

Jujube fruits are widely consumed in Asian countries as a food and food additive due to its high nutritional value (Almansa et al., 2016). Different parts of the jujube plant are used as pharmacological agents (Jiang et al., 2007). There are many jujube cultivars, each one with different physico-chemical, physiological and functional characteristics. Most studies have been done on Asian cultivars; however, the Spanish cultivars are still very poorly studied (Reche et al., 2019). The 'Phoenix' cultivar is characterized by an elongated appearance, is very sweet with a total soluble solids content of 24°Brix at commercial maturity (Reche et al., 2018). This cultivar is usually harvested mid-October, and thus considered as late-cultivar. Then, the extension of the postharvest period is very interesting for expanding the commercial time for consuming raw jujubes in Spain.

The jujube is a non-climacteric fruit (Almansa et al., 2016; Zhang et al., 2018), but at room temperature spoils quickly, especially by dehydration and increments in brown index and rot rate (Wu et al., 2016). On these way the storage life of jujube fruits is extremely short and the rapid perishability of the fruits is the main postharvest problem (Siddiq and Uebersax, 2012). So it would be very interesting to study the postharvest behavior of these fruits to increase their shelf life.

The decrease in storage temperatures reduces the metabolic activity of the fruits, which extends its postharvest life. These temperatures can be between -2 and 10 °C but temperatures of −1 °C or lower for a long time produce chilling injury (Wu et al., 2016). MAP is a method of extending the shelf-life of fresh produce. The technology involves the modification of the air inside the package with a beneficial mixture of elevated carbon dioxide and reduced oxygen. It is achieved by the natural interplay between the respiration of the produce and the transfer of gases through the packing material. The effectiveness of MAP on extending shelf-life is dependent on several factors, such as the type of produce, gas mixture, storage temperature, packing material and hygiene during handling (Valero and Serrano, 2010). MAP technology has already been used successfully in other fruits such as peach and nectarines (Akbudak and Eris, 2004; loquat (Amorós et al., 2008); plum (Díaz-Mula et al., 2011a); grape (Martínez-Romero et al., 2003); mango (Pesis et al., 2000), pomegranate (Selcuk and Erkan, 2014), among others. The MAP has been little studied in jujubes. Only one article has been published that showed the efficacy of MAP in decreasing weight loss and delayed the reduction of sulphur firmness by 20 days (Lu et al., 2014). On the other hand, jujubes have higher total

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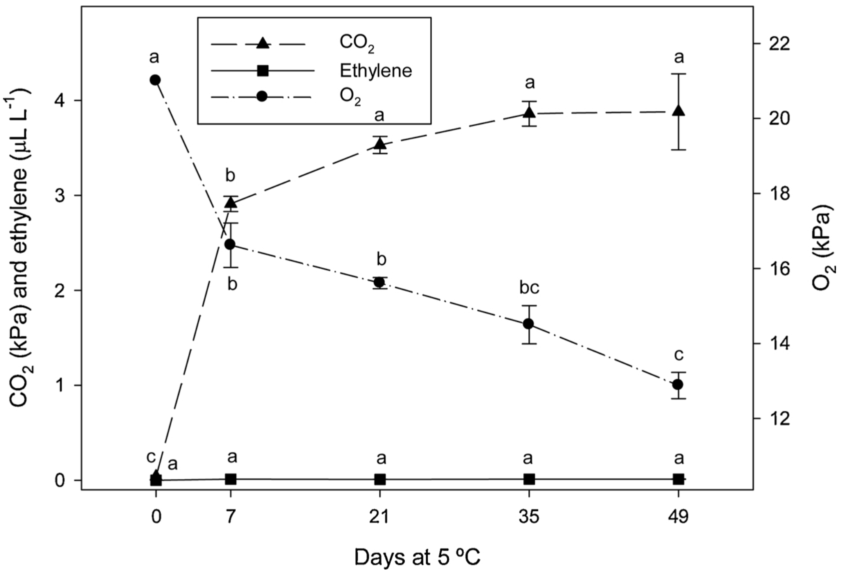

Fig. 1. Changes in O_2, CO_2 and ethylene concentration inside MAP packages containing jujube fruits 'Phoenix' cultivar during storage at 5 °C. Data are the mean ± SE (n = 6). Least significant differences (LSDs) test at 95% confidence level are shown. Different letters indicate significant differences ($p < 0.05$) during each storage time.

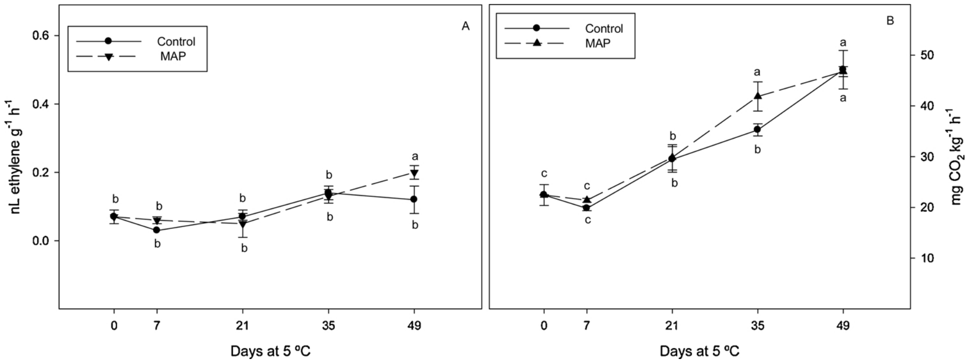

Fig. 2. Ethylene and CO_2 production rates during storage at 5 °C of jujube fruits 'Phoenix' cultivar under control and MAP conditions. Data are the mean ± SE (n = 6). Least significant differences (LSDs) test at 95% confidence level are shown. Different letters indicate significant differences ($p < 0.05$) during each storage time and treatment.

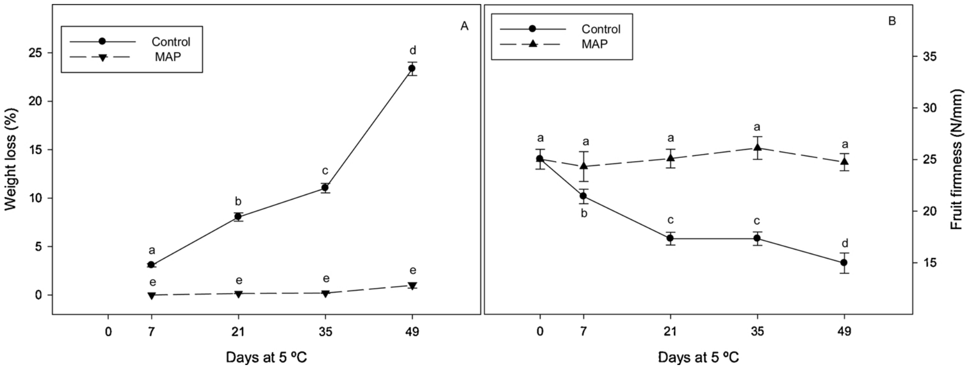

Fig. 3. Weight loss and fruit firmness changes during storage at 5 °C of jujube fruits 'Phoenix' cultivar under control and MAP conditions. Data are the mean ± SE (n = 45). Least significant differences (LSDs) test at 95% confidence level are shown. Different letters indicate significant differences ($p < 0.05$) during each storage time and treatment.

phenol contents as compared to other fruits, such as persimmon, pomegranates, and apples (Fu et al., 2011). In this sense, it is worthy to study the evolution of bioactive compounds and antioxidant activity under MAP packages. For this reason, the objective of this work was to study, for the first time, a MAP study with cv 'Phoenix' Spanish jujubes stored at lower temperature to extend their postharvest life. The evolution of physicochemical and phytochemical parameters and bioactive compounds of the control and MAP jujubes stored at 5 °C were studied.

2. Materials and methods

2.1. Plant material and experimental design

The jujubes were harvested from a commercial farm with 21-years-old jujube trees (latitude 38°10′22,29″N x longitude 0°51′36,138″W, 19 m above sea level) in Albatera (Alicante, Spain). Trees were trained as a vase and spaced 4 m × 4 m. The jujube fruits under study belonged to cv 'Phoenix'. Nine hundred fruits from nine trees (100 fruits per tree) were hand-harvested at commercial ripening stage (above 15°Brix). Fruits were immediately transported (30 min), under cold conditions (at 5 °C), to the laboratory. Then, 405 fruits were selected based on homogeneous colour and size, and absence of visual defects, and distributed at random into 27 lots of 15 fruit. Three lots were used to determine physicochemical properties at harvest (day 0). The remaining lots were individually deposited in polypropylene baskets. 12 lots were left in the refrigeration chambers without film, which would serve as control, while the other 12 lots were used for MAP treatment. These 12 baskets were thermos-sealed on top with the film with a total

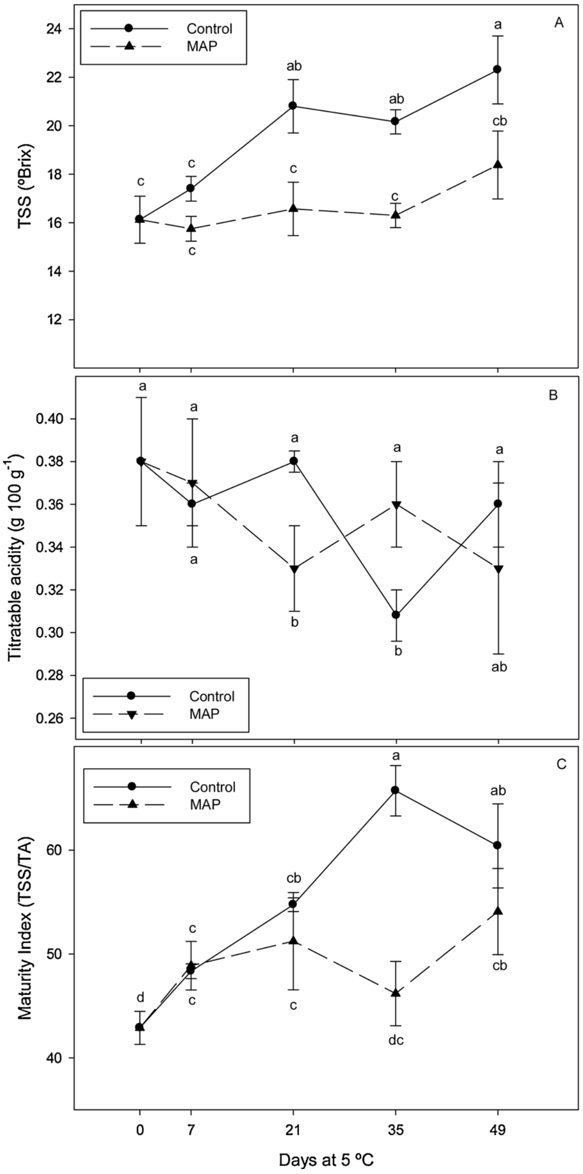

Fig. 4. Total Soluble Solids (TSS) (A), Total Acidity (B) and Maturity Index (C) changes during storage at 5 °C of jujube fruits 'Phoenix' cultivar under control and MAP conditions. Data are the mean ± SE (n = 6). Least significant differences (LSDs) test at 95% confidence level are shown. Different letters indicate significant differences ($p < 0.05$) during each storage time and treatment.

area of 336 cm^2, 14 cm x 24 cm. The film was composed of polyester (12 µm)-polypropylene (60 µm) (Amcor Flexibles, Barcelona, Spain) with permeability to O_2 = 75 mL O_2 m^{-2} day^{-1} atm^{-1}, CO_2 = 350 mL CO_2 m_2 day^{-1} atm^{-1} and water vapour = 75 mL H_2O mL m^{-2} atm^{-1}. All baskets, control and MAP, were stored at 5 °C and 90% RH. Four samples were taken at 7, 21, 35 and 49 days after collection. In each sampling, 3 control lots and 3 MAP lots were taken in which all analyzes below mentioned were carried out.

2.2. Gas composition inside the packages

Firstly, the gas composition of each MAP sample was analysed. The CO_2 and O_2 concentrations of each package were quantified in duplicate by extracting 1 mL of headspace atmosphere using an airtight syringe. Subsequently, the following were injected into a gas chromatograph GC 14B (Shimadzu, Tokyo, Japan) equipped with a thermal conductivity detector (TCD), with the characteristics explained in Díaz-Mula et al. (2011a). Results were expressed as kPa CO_2 and kPa O_2 inside the baskets (n = 6).

The ethylene content inside the packages was determined for quantification (in duplicate) of 1 mL of the atmosphere that was withdrawn with a gas syringe and injected into a Shimadzu TM GC-2010 gas chromatograph (Kyoto, Japan), with the characteristics explained in Díaz-Mula, et al. (2011a). Results were expressed as µL L^{-1} into the baskets (n = 6).

2.3. Respiration rate and ethylene production of fruits

Secondly, for each sampling date, the packs were opened and the jujubes were placed in a 750 mL glass jar hermetically sealed with a rubber cap for 30 min to measure the production of cold temperature ethylene (5 °C). For ethylene quantification (in duplicate) 1 mL of the atmosphere was withdrawn with a gas syringe and it was injected into the gas chromatograph as explained in the previous section. Results were the mean ± SE of determinations for six replicate and expressed as nL g^{-1} h^{-1}. The rate of respiration of CO_2 emission was quantified in a similar way, injecting 1 mL of the glass jar atmosphere into the gas chromatograph GC 14B described above. Results were expressed as mg kg^{-1} h^{-1} and were the mean ± SE. For day 0, respiration and ethylene rates were measured at 20 °C. These parameters were also measured in the control fruits.

Subsequently the parameters involved in points 2.4 and 2.5 were analyzed for all samples, control and MAPs in each time storage.

2.4. Fruit quality parameters

The fruits of each lot were weighed using a digital balance (model BL-600; Sartorius, Madrid, Spain) to calculate the weight loss. Fruit firmness was measured with a texturometer (TX-XT2i Texture Analyzer, Stable Microsystems, UK) with a force that achieved a 3% deformation of the jujube diameter. Results were expressed as the force-deformation (N mm^{-1}) and were the mean ± SE (n = 45). Jujube peel color was assessed at two equidistant points of the equatorial region of individual fruit in each 45 fruits using a Minolta colorimeter CR200 model using D65 illuminant (Minolta Camera Co., Japan). The result were expressed using the CIE L*a*b* system. The fruit was then cut into pieces and mixed to have a homogeneous juice sample for each replica. The total soluble solids (TSS) and titratable acidity (TA) were determined in duplicate. The TSS concentration was determined with a digital refractometer Atago Pocket PAL-1 (Atago Co. Ltd., Tokyo, Japan) at 20 °C, and expressed as °Brix. TA (g of malic acid equivalent per 100 g^{-1} fresh weight) was determined by automatic titration (TitraLab AT1000 series, Hach) with 0.1 N NaOH up to pH 8.1, using 1 mL of diluted juice in 30 mL distilled H_2O. The maturity index (MI) was calculated as the ratio between the TSS and the titratable acidity.

2.5. Phytochemical parameters

Vitamin C was measured by titration with iodine in acid medium with a titrant HachTitraLab AT 1000 series.

The method of Tomás-Barberán et al. (2001) was used for total phenolic extraction by using water: methanol (2:8) containing 2 mM NaF. The phenolic content was quantified using the Folin-Ciocalteu reagent and results (mean ± SE) were expressed as mg gallic acid equivalent g^{-1} fresh weight.

Total antioxidant activity (TAA) was quantified as described by Arnao et al. (2001). This methodology allows the determination of TAA due to both hydrophilic (H-TAA) and lipophilic (L-TAA) compounds in the same extraction using 50 mM phosphate buffer pH 7.8 and ethyl acetate. The upper fraction was used for L-TAA while the lower fraction for H-TAA quantification using the enzymatic system composed of the chromophore 2,2´-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid)

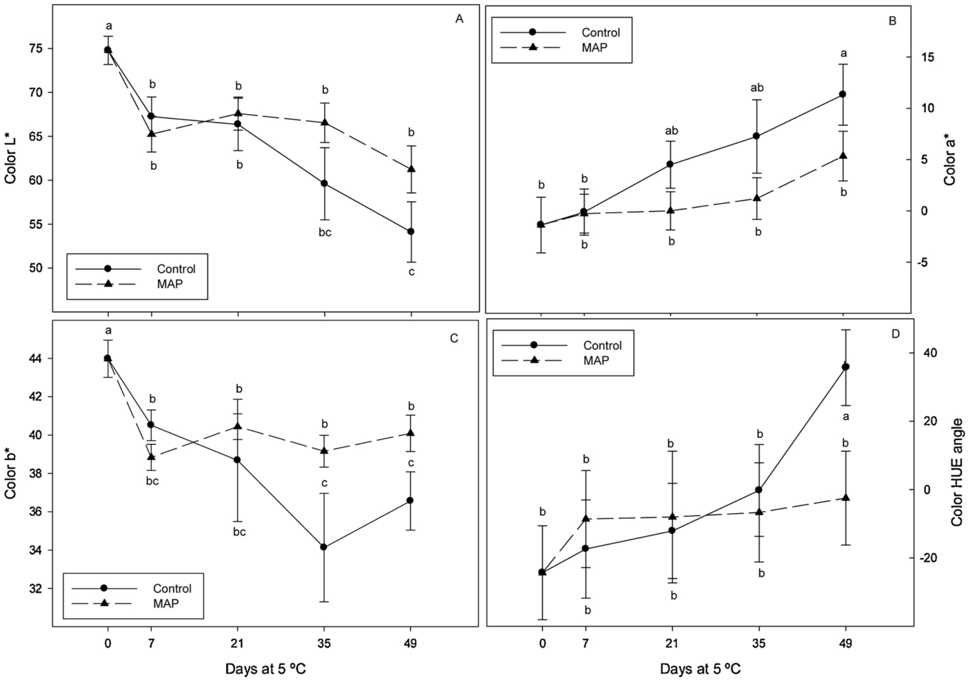

Fig. 5. Color parameters L* (A), a* (B), b* (C) and HUE angle (D) changes during storage at 5 °C of jujube fruits 'Phoenix' cultivar under control and MAP conditions. Data are the mean ± SE (n = 90). Least significant differences (LSDs) test at 95% confidence level are shown. Different letters indicate significant differences ($p < 0.05$) during each storage time and treatment.

diammonium salt (ABTS), horseradish peroxidase enzyme (HRP) and its oxidant substrate (hydrogen peroxide). The reaction was monitored at 730 nm until a stable absorbance was obtained using a UNICAM Helios spectrophotometer (Cambridge, UK). After that, a suitable amount of jujube fruit extract was added and the observed decrease in absorbance was determined. A calibration curve was performed with Trolox as standard antioxidant for both H-TAA and L-TAA. The results were expressed as mg Trolox equivalent 100 g^{-1} fw. Total carotenoids were estimated in the lipophilic extract (Arnao et al., 2001). The absorbance was measured at 450 nm in a UNICAM Helios- spectrophotometer (Cambridge, UK). The result were expressed as mg of β-carotene equivalent 100 g^{-1} fresh weight, taking into account the $\varepsilon_{cm}^{1\%} = 2560$ and the results were the mean ± SE.

2.6. Statistical analysis

Statistical analyses were performed using the software package SPSS 18.0 for Windows (SPSS Science, Chicago, IL, USA). A basic descriptive statistical analysis was followed by an analysis of variance test (ANOVA) for mean comparisons. The method used to discriminate among the means (multiple range test) was Fisher's LSD (Least Significant Difference) procedure at a 95.0% confidence level.

3. Results and discussion

3.1. Gas composition and ethylene production

The low storage temperature decreased jujube respiration. The O_2 concentration decreased to a concentration of 12.88 kPa and the CO_2 concentration increased to 3.6 kPa at day 49 of storage at 5 °C (Fig. 1) with significant differences. Therefore, a steady-state atmosphere was reached, as in other fruits like loquat (Amorós et al., 2008) or plum (Díaz-Mula et al., 2011a). These results are similar to those obtained by Jat et al. (2012) with Indian jujube fruit stored in MAP at room temperature. This is due to the effect of the low permeability of the film used together with the decrease in respiration due to the low storage temperature. As a result, the concentration of O_2 and CO_2 was slightly modificated. On the other hand, the accumulation of ethylene in the packages was very low at 5 °C, with concentration being 0.01 μL L^{-1} in all the samples inside the packages until day 49. The low ethylene emission rate of the jujube fruit is due to the fact that they are non-climacteric fruits (Almansa et al., 2016; Zhang et al., 2018), and to the decrease of metabolism at low temperatures. Indian jujube (*Z. mauritiana*), a climacteric specie (Abbas and Fandi, 2002), also decreased ethylene production when stored in MAP at room temperature (Jat et al., 2012). Any inhibitory effect on ethylene accumulation due to CO_2 concentration was observed. This may have due to low ethylene production rate of jujube fruit and the low CO_2 concentration reached inside packages. This rate could not be high enough to inhibit the biosynthesis of ethylene. However, in apricot MAP, ethylene production rate was inhibited, especially when CO_2 concentration inside packages was higher than 20 kPa (Pretel et al., 1993).

The jujubes in MAP and control maintained at 5 °C, showed low ethylene emission and a low respiratory level. There were no significant differences in ethylene during the 49 days of storage (about 0.1 nL ethylene g^{-1} h^{-1}) (Fig. 2A and B). This behavior of the fruits once removed from the package and measuring their respiration and ethylene emission was similar to their evolution of gas pressures within the packages. The maintenance of the fruit ethylene synthesis corroborated that they are a climateric fruits.

3.2. Fruit quality parameters

The MAP jujube fruit showed a weight loss very low during the 49 days of storage (Fig. 3A). However, when the jujubes were stored without film, the weight loss increased dramatically since 7 day of storage, and increased proportionally to storage days. Significant differences were shown and a weight loss of 23.3% was achieved within 49 days of storage. Therefore, the jujube MAPs were well hydrated at the end of the storage period, while the jujubes control showed wrinkles already in the third week of storage. This is a direct consequence of the film protective effect that causes a maintenance of the humidity of the fruits. This may have mainly been due to the effect of polyester-polypropylene film on increasing water vapor pressure around the fruit, and, in return, reduction of the transpiration rate (Amorós et al., 2008). This has been observed as a general effect of MAP on fruit and vegetables, and proven in jujubes (Lu et al., 2014), loquat (Amorós et al., 2008), apricot (Pretel et al., 1993), sweet cherry (Serrano et al., 2005), among others. This indicated that the film used in this work achieved the expected result for this parameter.

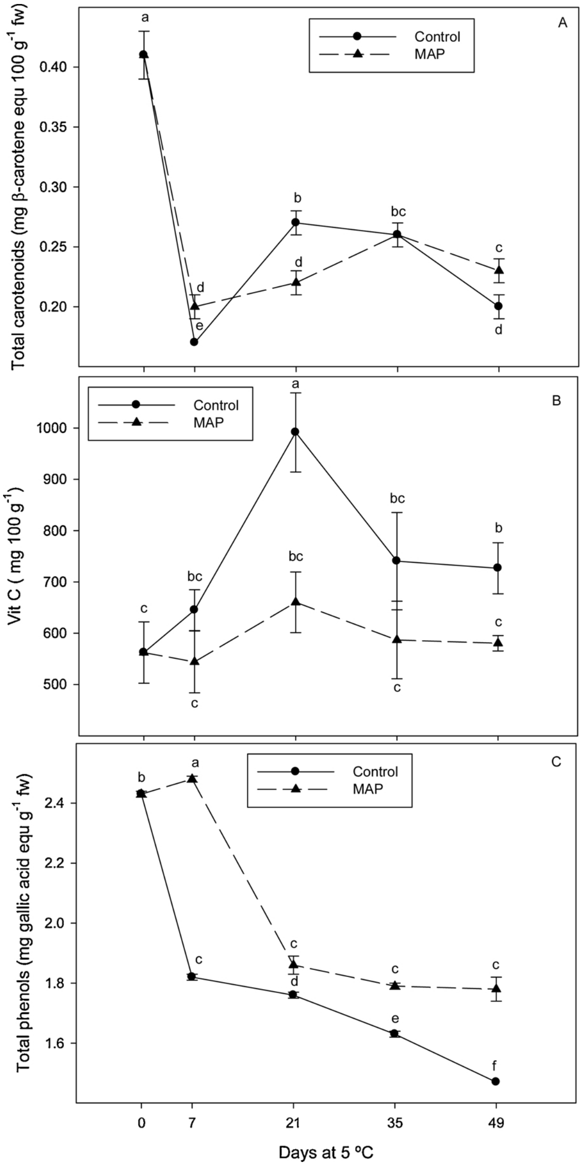

Fig. 6. Total carotenoids (A), Vitamin C (B) and total phenols contents (C) changes during storage at 5 °C of jujube fruits 'Phoenix' cultivar under control and MAP conditions. Data are the mean ± SE (n = 6). Least significant differences (LSDs) test at 95% confidence level are shown. Different letters indicate significant differences ($p < 0.05$) during each storage time and treatment.

This moisture maintenance of the MAP fruits is also observed to maintain the jujube firmness, which did not vary compared with respect to the control jujubes firmness of day 0 (Fig. 3B). However, control jujubes showed a significant decrease in the fruit firmness from day 7 of storage, which decreased proportionally to the stored days. This MAP effect was also corroborated by Lu et al. (2014) in MAP jujubes during 20 days of storage. Pretel et al. (1999) reported that low O_2 concentration is more effective at inhibiting fruit softening than high CO_2 concentration, conditions that were found in the atmosphere of the jujube MAP.

The MAP jujube maintained the TSS content about 16°Brix with respect to control jujube after 49 days of storage at 5 °C (Fig. 4A). While control jujubes significantlyenhanced their TSS from day 21 (20.2°Brix). The increase of this parameter being proportionally to the days stored, showing an increase in the maturation of control fruits with respect to the MAP jujubes. These TSS values are normal for this cultivar (Reche et al., 2018). The TA remained very similar in jujubes both control and MAP during the entire storage period. With values of TA close to 0.35 g 100 g^{-1} (Fig. 4B). This caused that the maturity index (MI) remained in the jujubes MAP about MI≅50, while in the controls it increased significantly from the day 21 of storage up to day 49 (Fig. 4C). Also, MAP peach and nectarine delayed the decrease in TA and the increase in TSS with respect to controls (Akbudak and Eris, 2004). This difference was also found in plums (Díaz-Mula et al., 2011a).

Jujube cv 'Phoenix' vary from green in immature stage to red and finally dark brown color in a ripening stage, as other Spanish jujube cultivars (Almansa et al., 2016). The jujube fruit external color varied significantly respect to day 0 during the storage cold period (Fig. 5). The jujubes were collected on day 0 with a slight green color (a* -1.37), with yellow background color (b* 44) that gave it a very high luminosity (L* 74.78). The color parameter values reported here were very similar to those previously reported in Spanish (Collado-González et al., 2014; Galindo et al., 2015; Almansa et al., 2016; Reche et al., 2018, 2019), and Chinese jujube cultivars (Wang et al., 2012). The MAP jujubes showed a color more similar to day 0 color than the controls, without significant differences in a* and HUE angle color. The yellow color measured by the coordinate b* is due to the content of carotenoids (Fig. 6A). There was a similar behavior of this figure and 5C, where the yellow color, and the total carotenoids, which decreased during the conservation were higher in the MAP jujubes, at the end of the conservation period. The decrease of the yellow color is parallel to the increase of the brown color of the jujubes when they mature. This decreased the luminosity (decrease of the L* parameter), of the fruit that was greater in the control jujubes than in the MAP (Fig. 5A). These effects can be attributed to a delay in the degradation of carotenoids induced by cold storage, and the effect of MAP at the end of the treatment. The delay in color change associated with the postharvest ripening process in MAP storage has also been shown in other fruits, such as mango (Pesis et al., 2000), table grape (Martínez-Romero et al., 2003) and loquat (Amorós et al., 2008) under MAP conditions. All these changes indicated that the fruits matured slightly during the cold conservation, less the MAP than the control.

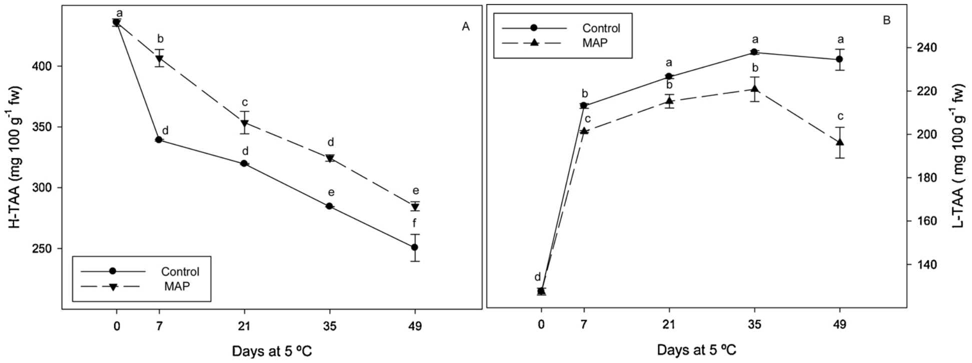

Fig. 7. H-TAA (A) and L-TAA (B) changes during storage at 5 °C of jujube fruits 'Phoenix' cultivar under control and MAP conditions. Data are the mean ± SE (n = 6). Least significant differences (LSDs) test at 95% confidence level are shown. Different letters indicate significant differences ($p < 0.05$) during each storage time and treatment.

3.3. Phytochemical parameters

The vitamin C content of the jujube fruit was very high, with 562.4 mg 100 g^{-1}, similar to those found by Collado-González et al. (2014). This content was increased by the effect of cold storage of the control fruits with a maximum on day 21 of storage, which subsequently decreases until day 49. The increase in this content may be due in part to the weight loss that occurred during the cold storage of the jujubes control and that does not occur in the jujubes MAP (Fig. 6B). From day 21 of storage there was a decrease in the vitamin C content probably due to its degradation due to aging of the fruits. However, in the MAP fruits there were no significant differences in the vitamin C content. The maintenance of the vitamin C content by MAP effect was also observed in other fruits such as loquat (Amorós et al., 2008).

The results obtained of the total phenols content of day 0 were in accordance with the obtained by Reche et al. (2018). During the cold storage period the jujube control total phenol content decreased significantly respect to day 0 (Fig. 6C). However, in the MAP jujubes this decrease was significantly delayed with respect to the jujubes control. This decrease during the 49 days of storage at 5 °C was 39.90% in the control fruits and 26.75% in the jujubes MAP with respect to the content of total phenols from day 0. Also, this decrease during the cold storage was not in agreement with other fruits results such as plum (Díaz-Mula et al., 2011b), sweet cherry (Serrano et al., 2009), peach and nectarine (Di Vaio et al., 2008), in which there was an increase in the total phenols content with cold storage. The delay in the degradation of total phenols in the MAP jujubes may be due to the fact that the decrease of O_2 and increase of CO_2 in the atmosphere of the package caused a delay in the ripening of the fruits. This atmosphere could reduce polyphenol oxidase (PPO) or peroxidase activities. These are the main enzymes responsible for the degradation of phenols, as Selcuk and Erkan (2014) observed in pomegranates.

H-TAA and L-TAA day 0 were similar to Reche et al. (2018) in this cultivar. Cold storage had a similar effect on the content of total phenols and H-TAA of the jujubes (Figs. 6C and 7A), since phenols are the main antioxidant compounds of jujubes (Choi et al., 2012). The jujube H-TAA decreased significantly respect to day 0. However, in the MAP jujube this decrease was significantly delayed with respect to the jujubes control. During the 49 days of cold storage the decrease was 42.52% in the control fruits and 34.67% in the jujubes MAP with respect to the content of total phenols from day 0. However, L-TAA presented the opposite behavior, since the increase of the cold storage period increased the L-TAA, and in greater quantity in the control than in the MAP jujubes, with significant differences (Fig. 7B). This behavior was the opposite in plums (Díaz-Mula et al., 2011b), since cold storage caused an increase in total phenols and H-AAT. While L-AAT decreased, although MAP caused delays effects, as in this study. In any case, both our results and those of Díaz-Mula et al. (2011b) suggest that MAP causes a delay in the maturation of the treated fruits.

4. Conclusion

The cold storage of jujube fruits (5 °C and 90% of RH) together with a MAP treatment achieved a significant delay of postharvest fruit ripening. This resulted in lower weight loss, lower IM, and greater firmness and color intensity. Interestingly, the content of total carotenoids, total phenols and H-TAA and L-TAA was greater than control jujubes. The package atmosphere was enriched in CO_2, and the content of O_2 decreased. The control jujubes had a non-commercial wrinkled aspect at day 21 of cold storage. While the MAP jujubes showed a normal visual aspect at day 49 of storage. Therefore, the film used increased the jujube shelf life more than twice as long as compared with the jujubes stored without this film (control jujubes).

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