El ácido oxálico como herramienta pre-cosecha para mantener la calidad poscosecha de alcachofa (*Cynara scolymus* L.)

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Resumen

Hoy en día los tratamientos precosecha con compuestos vegetales exógenos son socialmente aceptados para mejorar la producción de alimentos de origen vegetal, mantener su calidad poscosecha, y mejorar su funcionalidad. Por ello, el objetivo de este trabajo fue evaluar el efecto de la aplicación precosecha de ácido oxálico (OA) sobre la producción, parámetros de calidad, respiración, y bioactividad de la alcachofa (*Cynara scolymus* L.) durante su almacenamiento poscosecha. Con respecto a los resultados, se pudo observar que el tratamiento con OA no afectó a la producción y calidad de las alcachofas, aunque si se contabilizaron más alcachofas de primera clase en las tratadas con OA, así como una mayor firmeza el día de la recolección. Además, las alcachofas tratadas con OA experimentaron retrasos en su tasa de respiración, lo que favorece el retraso de la senescencia típico del almacenamiento de este tipo de verduras. Así mismo, las alcachofas tratadas también mostraron mayor actividad antioxidante hidrosoluble y mayor concentración de compuestos fitoquímicos, medidos como compuestos fenólicos. Por lo tanto, se puede concluir que el tratamiento precosecha con OA puede mejorar algunos factores cruciales en la alcachofa, como el retraso de la tasa de respiración, el aumento de la actividad antioxidante y del contenido de compuestos bioactivos, siendo una herramienta de origen natural, útil para mejorar ciertas propiedades demostradas como beneficiosas para la salud de los consumidores.

Palabras clave: Ácido oxálico, tratamiento precosecha, alcachofa, compuestos fenólicos

Abstract

Oxalic acid as preharvest tool to keep post-harvest quality and bioactivity of artichoke (*Cynara scolymus* L.). The objective of this work was to evaluate the effect of oxalic acid (OA) preharvest treatment on the artichoke on (*Cynara scolymus* L.) development by determining head weight, number of artichokes harvested by plant, and yield (g/plant) at the first harvest date. In addition, artichokes were stored for 21 days at 2 ºC and quality parameters (weight loss, firmness, and color), respiration rate, antioxidant activity and bioactive compounds (phenolics), measured by Folin Ciocalteau and HPLC-DAD-ESI/MSn were analyzed. Oxalic Acid treatment increased the percentage of first class artichokes though no significant differences between artichokes from control and those from OA-treated plants were found in during the developmental process. However, OA-treatment reduced the respiration rate of artichokes and led to higher total hydrosoluble antioxidant activity and greater amounts of total phenolics and hydroxycinnamics and luteolins concentration both at harvest and during cold storage. Thus, it can be concluded that OA preharvest treatment can decrease some crucial factors in artichoke, like respiration rate, leading to a delay in the postharvest senescence process

and enhance antioxidant activity, and phytochemicals content, being a natural and useful tool to improve the reported health-beneficial properties of artichokes consumption.

Keywords: Oxalic acid, preharvest treatment, artichoke, quality, phenolics.

Introduction

Artichoke (*Cynara scolymus* L.) is an ancient herbaceous perennial plant, originated from the Mediterranean areas of North Africa and nowadays widely grown around the world, with Italy and Spain being the main producer countries (FAOSTAT, 2012). Furthermore, the consumer's demand for artichokes has increased recently because of their proved reputation as health food. In fact, numerous research studies have raised evidences about the antioxidant properties and health beneficial effects of artichokes (de Falco et al., 2015). These effects have been attributed to their phytochemical composition, especially to the polyphenolic fraction, mainly composed of mono- and dicaffeoylquinic acids and flavonoids (de Falco et al., 2015). The actions of these phenolic antioxidants include radical scavenging and inhibition of the production of reactive species derived from normal metabolism of cells. Hence, antioxidants may prevent damage to lipids, proteins and nucleic acids and consequent cellular damage and therefore, many of the chronic diseases of the occidental world before commented (Del Rio et al., 2013; de Falco et al., 2015).

Recently, the application of natural and eco-friendly compounds as preharvest treatments to delay ripening and senescence and preserve fruit and vegetable quality has received considerable attention. One of these naturally occurring compounds recently evaluated is oxalic acid (OA), which have been demonstrated to retard the postharvest ripening process and to maintain quality and functional properties in sweet cherry, kiwi and peach when applied as preharvest treatment (Martínez-Esplá et al., 2014; Zhu et al., 2016; Razavi and Hajilou, 2016). Moreover, the effect of oxalic acid (OA) post-harvest treatment on the overall quality of artichokes during storage at 20 ºC has been recently evaluated, showing that OA treatment might be a promising method for delaying postharvest deterioration and maintaining the overall quality of artichokes (Ruíz-Jiménez et al., 2014).

Thus, the aim of this study was to evaluate the effect of preharvest application of oxalic acid (OA) on artichoke development on plant, quality parameters (weight loss, firmness and color), respiration rate, antioxidant activity and phenolic compounds (measured by Folin Ciocalteu method and HPLC-DAD-ESI/MSn) on artichoke (*Cynara scolymus* L.) heads at harvest and along 21 days of storage.

Materials and methods

Plant material and treatments. The experiment was performed with artichoke 'Blanca de Tudela' cultivar during the developmental cycle in the 2015 autumn-winter period, in a commercial plot located at Bigastro (Alicante, Spain), by using 12 rows (grouped in 3 replicates of 4 rows each one) of 32 plants for each treatment (control and OA 2 mM). Freshly prepared OA 2 mM solution (containing 0.5% of Tween-20) was foliarly sprayed with a mechanical mist sprayer and repeated at 3 dates of the growth cycle: T1 (45 days before harvesting), T2 (24 days before harvesting) and T3 (3 days before harvesting), by using 30 L in each application.

Artichoke production and storage experiment design. Artichokes were harvested at commercial development stage, being the first date of harvesting, December 18, 2015. Weight and number of artichokes harvested from each row were recorded and number of

artichokes per plant, yield per plant and mean artichoke weight were calculated. After that, artichokes were separated as first (without visual defects and diameter higher than 80 mm) and second class (with slight visual defects and/or diameter lower than 80 mm) and the percentage of each category was evaluated. Results are expressed as mean ± SE. First class artichokes from the 3 rows of each replicate were combined and around 6 kg of per treatment and replicate were transported to the laboratory in 2 hours. Once in the lab, 4 lots of 3 artichokes were sampled per each replicate and treatment, they were labeled and weighted, and then stored at 2 ºC and 85% of R.H. (relative humidity). After 0, 7, 14 and 21 days of cold storage one lot of each replicate and treatment was taken at random for analytical determinations.

Quality parameters: Weight loss, firmness and color. Weight loss (%) was determined by weighing artichoke lots at day 0 and after each sampling date and expressed as a percentage with respect to weight at day 0. Firmness was measured twice independently in each artichoke heads of each replicate using a TX-XT2i texture analyzer (Stable Microsystems, Godalming, UK) according to Ruíz-Jiménez et al. (2014) and results were expressed as the force-deformation ratio (N mm-1). External color was determined at 3 points around the equatorial external surface of each artichoke head by using a Minolta colorimeter (CRC200, Minolta Camera Co., Japan), using the CIELab coordinates. Results are expressed as mean ± SE.

Respiration rate. Respiration rate was measured in refrigeration by placing each artichoke lot or replicate in 3.3 L jars for 30 min. Samples of 1 mL from the jar atmosphere were withdrawn and used for injection into GC-TCD for CO_2 quantification in duplicate, according to a previous report (Ruíz-Jiménez et al., 2014), and respiration rate was expressed as mg of CO_2 kg-1 h-1. Results are expressed as mean ± SE.

Total phenolic compounds extraction and quantification. For each artichoke head, the greenest external bracts were removed and then one 2-mm slice of the edible fraction (internal bracts and receptacle) from each of the 3 artichokes of each replicate was taken, cut in small pieces and combined to obtain a homogeneous sample of each replicate for phenolics quantification and identification. The same procedure was performed for obtaining samples for antioxidant activity determination, and in both cases extraction was performed immediately. Samples were crushed with water/methanol (2:8) containing 2 mM NaF (to inactivate polyphenol oxidase activity and prevent phenolic degradation) and centrifuged at 10,000 g for 15 min at 4 °C. Total phenolics were measured in duplicate by using the Folin-Ciocalteu reagent and concentration expressed as milligrams of gallic acid equivalent per 100 g of fresh weight (Valero et al., 2011).

Identification of phenolic compounds by HPLC-DAD-ESI/MSn and quantification by RP-HPLC-DAD. For identification and quantification of individual phenolics the same extraction as above was used. Regarding HPLC system, previously reported procedure was used (Gironés-Vilaplana et al., 2013). The equipment consisted of a binary pump (model G1376A), an autosampler with automatic and refrigerated injector (model G1377), an in-line degasser (model G1379B), and a photodiode array detector (model G1315D). The HPLC system was controlled by the ChemStation for LC 3D Systems software (Agilent, Rev. B.01.03 SRD, nov. 2006). The mass detector was the Bruker HCT Ultra ion trap spectrometer, equipped with an electrospray ionization interface, and controlled by Esquire Control software (vers. 6.1. No. 92.0, Bruker Daltoniks, Gmbh, Germany). The ionization conditions were 350 °C and 4 kV, for capillary temperature and voltage, respectively. The nebulizer pressure and nitrogen flow rate were 65.0 psi and 11 L/min, respectively. The full-scan mass covered the range of m/z from 100 to 1200. Collision-induced fragmentation experiments were performed in

the ion trap using helium as the collision gas, with voltage ramping cycles from 0.3 to 2 V.

Individual phenolics quantification was performed in duplicate in each sample by using a HPLC-DAD system with the same conditions that were used for phenolics identification. Different phenolics were characterized by chromatographic comparison with analytical standards as well as quantified by the absorbance of their corresponding peaks. Luteolins were quantified as quercetin 3-O-rutinoside at 360 nm, and hydroxycinnamic acids as 5-O-caffeoylquinic acid at 320 nm. Results are expressed as mg 100 g-1 and are the mean ± SE.

Antioxidant activity. Total antioxidant activity (TAA) of hydrophilic (H-TAA) and lipophilic (L-TAA) extracts was quantified as described in Valero et al. (2011) Results are expressed as mg of Trolox equivalent 100 g-1 and are the mean ± SE.

Statistical analysis. Results are expressed as mean ± SE of three replicates. In field results a t-Student test was performed to find significant differences at $P<0.05$ between treatments. In storage experiments data for the analytical determinations were subjected to analysis of variance (ANOVA), using HSD Duncan's test to examine if differences were significant at $P<0.05$. All analyses were performed with SPSS software package v. 12.0 for Windows.

Results and discussion

Field results. No significant differences were observed in the number of artichokes harvested by plant, weight of artichoke heads or plant yield (Table 1). Thus, OA treatment did not affect artichoke developmental process on plant. However, a significant increase was noted in percentage of first class artichokes due to OA treatment, (20.23% vs. 12.80%, in OA and control, respectively). This effect was attributed to a decrease on visual defects as a consequence of OA treatment, since no effect of this treatment was observed in head size.

Respiration rate. At harvest, respiration rate of the artichoke heads was significantly lower in those from OA-treated plants than in control ($P<0.05$, Table 2). This respiration rate decreased when artichokes were stored at 2 ºC in both control and OA treated ones, as a consequence of cold storage, but a significant increase in respiration rate was observed in control artichokes from day 7 to day 21, while no significant changes occurred in OA-treated ones ($P<0.05$, data not shown). This effect of preharvest OA treatment on decreasing respiration rate at harvest would indicate an effect of OA on reducing cell metabolism rate during artichoke development on plant, which, in turn, would be responsible for delay senescence process during storage. In this sense, postharvest OA treatments also reduced respiration rate in fruits such as pomegranate, sweet cherry and banana, leading to a delay in the postharvest ripening process (Sayyari et al., 2010; Valero et al., 2011). Moreover, postharvest dipping OA treatment of artichokes also decreased respiration rate along storage (Ruíz-Jiménez et al., 2014). However, this is the first time that this reduction is demonstrated through OA preharvest application, which would account for a lower deterioration rate, since in this commodity, with such a high respiration rate, it is widely accepted that respiration rate is inversely correlated with its shelf life (Gil-Izquierdo et al., 2002; Ruíz-Jiménez et al., 2014).

Quality parameters: Weight loss, firmness and color. Weight loss of artichokes increased significantly during cold storage ($P<0.05$), reaching values of 28.4 ± 0.8% and 28.7 ± 0.6% for control and OA-treated ones, respectively, at the end of storage, which are mainly due to transpiration rate. However, after 14 days of storage weight loss was significantly lower in OA-treated artichokes than in controls. Firmness (N mm-1) values

were higher in artichokes from plants treated with OA than in controls at day 0 (Table 2). Firmness decreased sharply during the first week of storage, although significant higher values were also found in OA-treated samples at this time and since then similar values were obtained from control and OA-treated artichokes.

Regarding color CIELAB parameters, at harvest were: L* = 56.60±0.54, a* = -13.10±0.34, b* = 29.81±0.32, Chroma = 32.59±0.33 and Hue = 113.70±0.58 for control artichokes; and L* = 55.33±0.46, a* = -13.94±0.18, b* = 29.74±0.28, Chroma = 32.86±0.26 and Hue = 115.14±0.37 for OA-treated artichokes. A significant decrease in color Chroma index was observed after 14 days of storage in control artichokes, while these changes started after 21 days in OA-treated ones (data not shown). This Chroma index reduction was mainly due to browning of the leaf tips attributed to polyphenol oxidase activity (Cefola et al., 2012), which was delayed by OA preharvest treatment.

Taking into account all these quality parameters (weight loss, firmness and CIELAB color), the maximum storage time was 14 days for artichokes, so the phytochemical analysis was performed in samples of day 0 and day 14 of storage.

Phytochemical composition. Methanolic extracts of artichokes revealed a wide range of hydroxycinnamic acids (HA) derivatives and luteolin derivatives (data not shown). Quantification of individual phenolics at days 0 and 14, showed that OA treatment led to artichokes with significantly higher ($P<0.05$) content of total hydroxycinnamic acids and of total luteolins as compared with those of control ones (Table 2). This effect was mainly due to the OA induced increase on 3, 5-dicaffeoylquinic acid and luteolin 7-O-glucuronide, the major hydroxycinnamic acid and the major luteonin, respectively. In general, an increase of total hydroxycinnamic acids and total luteolins content as well as in individual compounds was observed from day 0 to 14, probably due to the concentration caused by the weight loss, which is mainly water.

Most of phenolic compounds found in the present research have been previously reported in artichokes (Abu-Reidah et al., 2013; Garbetta et al., 2014). Taking into account the increase in these phytochemicals as a result of OA treatments and the health beneficial effect attributed to phenolic compounds, and especially to luteolins, in cancer, cardiovascular diseases and neurological disorders (Del Rio et al., 2013; de Falco et al., 2015; Nabavi et al., 2015; Yang et al., 2015), preharvest OA treatment would lead to increase the health beneficial effect of artichoke consumption.

Total phenolics content and total antioxidant activity. Total phenolic content at harvest was also significantly enhanced as a consequence of OA treatments and its concentration increased along storage time (data not shown). This increase was around 50 and 30% in control and OA-treated artichokes, respectively, after 14 days of storage and, as commented previously for individual phenolic increases along storage, could be probably due to the concentration caused by the weight loss. However, after 14 days of storage weight loss was ~15%, so a real increase in phenolics cannot be discarded. Total phenolic content and total hydroxycinnamic acids content were strongly correlated ($r=0.957^{**}$, $P<0.05$) taking into account data of both treatments and sampling dates. Nonetheless, total phenolic content values must be interpreted with caution since Folin Ciocalteau reagent can react, not only with phenolics, but also with a variety of non-phenolic reducing compounds including tertiary aliphatic amines, amino acids (tryptophan), hydroxylamine, hydrazine, certain purines, and other organic and inorganic reducing agents leading to an overestimation of the phenolics content (Ikawa et al., 2003). Moreover, in this case, total phenolic content was lower than total phenolics measured by HPLC-DAD, due to different phenolics may have various responses to the Folin-Ciocalteu's reagent, presenting lower absorption resulting in underestimations of

compounds too (Ikawa et al., 2003). Nevertheless, it is interesting to point out that both, total phenolic compounds measured by Folin Ciocalteau reagent and total phenolic compounds calculated as the sum of individual phenolics measured by by HPLC-DAD-ESI/MSn were increased by OA treatment of artichoke plants.

The total hydrosoluble antioxidant activity of the hydrophilic extracts (H-TAA) was significantly higher in artichokes from plants treated with OA than in control artichokes (Table 2) either at harvest and along storage and no significant changes were observed along storage. Antioxidant activity was also measured in the lipophilic extracts (L-TAA) showing values ca. 30 mg 100 g-1 at harvest without significant differences between control and treated artichokes, or changes along storage time (data not shown). Thus, as reported previously (Ruíz-Jiménez et al., 2014), the major compounds responsible for artichoke antioxidant properties are hydrophilic compounds.

This high antioxidant activity of artichokes has been previously reported, and attributed to their phenolic compounds (Cefola et al., 2012). However, in this work no significant correlations between antioxidant activity and the different phytochemicals groups were found. Thus, other compounds not reported here can be responsible of this antioxidant activity, such as ascorbic acid, a hydrophilic antioxidant found at relatively high concentration in artichoke (Gil-Izquierdo et al., 2002). In addition, the combination of phytochemicals and their synergistic mechanisms in the fruit matrix could be also highly responsible for the antioxidant results here presented, as has been reported in others vegetable food products (Gironés-Vilaplana et al., 2013).

Conclusions

Overall results show that OA treatments of artichoke plants increased artichoke quality parameters at harvest, such as firmness and decreased the appearance of visual defects. Losses of quality parameters related with senescence were delayed in OA-treated artichokes, probably due to the decease on respiration rate induced by OA found at harvest and along storage, without affecting artichoke development on plant or plant yield. Moreover, OA preharvest application enhanced hydrosoluble antioxidant activity and total phenolic concentration at harvest. These effects were generally maintained along storage. Thus, given the proved health beneficial effects of phenolic compounds on a wide range of human diseases, the OA treatment of artichoke plants would lead to artichokes with higher beneficial effect, both at harvest and after 14 days of cold storage. Then, OA preharvest treatments of artichoke plants could be a natural and eco-friendly tool to improve artichoke quality and its health-beneficial properties, both at harvest and along a moderate storage period.

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Tables and Figures

Table 1 – Field parameters from control and oxalic acid (OA) treated plants at the first harvest date.

	Control	OA-treated
Number artichokes/plant	2.47 ± 0.16[a]	2.86 ± 0.12[a]
Yield of artichokes (g/plant)	331.09 ± 17.43[a]	343.40 ± 21.55 [a]
Weight of individual artichokes (g)	129.54 ± 6.78[a]	124.42 ± 7.81[a]
% 1st class	12.80 ± 1.53[a]	20.23 ± 1.5[b]
% 2nd class	87.20 ± 1.53[a]	79.77 ± 1.9[b]

Data are the mean ± SE of three replicates of four rows each one. Different letters in a row show significant differences ($P < 0.05$) between treatments according to Duncan's test.

Table 2 – Respiration rate, firmness, total hydroxycinnamic acids, total luteolins, Total Phenolic content (TPC), and hydrosoluble antioxidant activity (H-TAA) of control and OA-treated artichokes at harvest.

	Control	OA-treated
Respiration rate (mg CO_2/kg*h)	123.55 ± 9.18[a]	82.29 ± 7.36[b]
Firmness (N/mm)	3.37 ± 1.05[a]	5.32 ± 0.80 [b]
Total Hydroxycinnamic acids (mg/100g F.W.)	546.85 ± 25.43[a]	660.52 ± 15.83[b]
Total Luteolins (mg/100g F.W.)	33.45 ± 4.88[a]	55.54 ± 5.68[b]
TPC (Folin, mg gallic acid/100g F.W.)	309.52 ± 22.75[a]	386.06 ± 11.36[b]
H-TAA (mg Trolox/100 g F.W.)	291.47 ± 21.80[a]	414.89 ± 24.97[b]

Data are the mean ± SE of three replicates. Different letters in a row show significant differences ($P < 0.05$) between treatments according to Duncan's test.