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Effect of *cultivar x ozone treatment* interaction on the total polyphenol content and antioxidant activity of globe artichoke

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Abstract

Two globe artichoke cultivars (*Violet de Provence* and *Apollo*) were harvested at an experimental field in Sicily, immediately washed with ozonised water and stored in: i) normal atmosphere; ii) ozone-enriched atmosphere for 3 days and for the last 4 days in normal atmosphere; iii) ozone-enriched atmosphere for 7 days. A control (samples unwashed and stored at room temperature) was also investigated. The effect of *cultivar x ozone treatment* interaction on water content losses, total polyphenols content and antioxidant activity was evaluated after 0, 3 and 7 days of storage. Washing with ozonised water and storage under O_3-enriched atmosphere allowed higher water retention compared with the control, especially for *Violet de Provence*. After 3 days of storage in ozone-enriched atmosphere, on average of cultivars, the total polyphenols content and antioxidant activity increased by 11.7% and 5.5%, respectively. By contrast, after further 4 days of storage in ozonised atmosphere, *Apollo* and *Violet de Provence* displayed a significant reduction in their level of total polyphenols and aantioxidant activity. The exposure of globe artichoke heads to an ozone-enriched atmosphere should not exceed 3 days aimed at preserving their high nutritional value, with special emphasis on total polyphenols content.

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Introduction

Over the years, major attention has been focused on food safety and quality due to the increasing cases of foodborne diseases. Among the technologies adopted, ozonation has recently proposed to extend the shelf-life of fruits and vegetables, ensuring the reduction of their microbial load (Zhang *et al*., 2005). Due to its strong oxidising capacity and spontaneous decomposition to non-toxic products, ozone (O_3) is, at the same time, an effective and safe disinfectant which has been increasingly recommended in the horticultural industry as alternative to chlorine-based agents (Kim *et al*., 1999; Zhang *et al*., 2005). However, ozone, applied as gas or ozonated water, may affect quality in relation to the sensitivity of the vegetable (Skog and Chu, 2001). For globe artichoke [*Cynara cardunculus* var. *scolymus* (L.) Fiori], which is very perishable and sensitive to water content loss, ozone application during postharvest may reduce the microbial population and nutritional quality loss of the produce, on condition that specific postharvest treatments are considered for each cultivar (Restuccia *et al*., 2014). However, the effects of ozone application on the quality of globe artichoke deserves further investigation also with the aim of preserving the level of antioxidant compounds. Indeed, globe artichoke heads are an excellent source of polyphenols (Lombardo *et al*., 2012, 2013; Pandino *et al*., 2011, 2012a, 2012b), which play important roles: i) as scavengers against the harmful free radicals derived from the oxidative stress and implicated in several disorders; ii) on the product sensory characteristics, such as colour, bitterness and aroma; iii) on the processing suitability of the heads, being substrates for oxidative enzymatic and non-enzymatic browning reactions (Lattanzio *et al*., 1994). Therefore, in the present work the effect of postharvest treatments with ozone on water content loss, total polyphenols content and antioxidant activity of globe artichoke whole heads was studied. In particular, we investigated how the interruption of ozone insufflation in cooling chamber could influence product shelf-life.

Materials and methods

Plant material, experimental field and management practices

Experimental field trial was conducted during the 2012-2013 growing season at a farm located in Ramacca (37° 23 N, 14° 41 E, 144 200 m asl) in the Catania Plain (Sicily, Italy), which is a typical area for the globe artichoke cultivation. The local climate is semiarid-Mediterranean, with mild winters and hot-rainless summers. Two globe artichoke cultivars were studied: *Violet de Provence*, a reflowering multiclone cultivar actually widespread in all the Mediterranean Basin, producing elongated

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green heads with purple shades, and *Apollo*, a *Romanesco* type cultivar, characterised by spherical shaped heads with deep violet bracts. These cultivars were arranged in a randomised block experimental design with four replicates, consisting of 50 plants per plot. Semi-dormant offshoots (*ovoli*) were manually planted in August, adopting a planting density of 1.0 plant m^2. A typical fertilisation programme (200 kg N, 80 kg P_2O_5 and 100 kg K_2O per ha) was applied. Drip irrigation was carried out during the summer, when the accumulated daily evaporation (measured from an unscreened class *A-Pan* evaporimeter near the crop) reached 35 mm. Weed and pest control followed standard commercial practice. Lateral shoots were removed twice in November and mid-February, having only one shoot per plant.

Head harvest, postharvest treatments and sampling

At least 300 disease-free heads for each cultivar were harvested at the end of March, at marketable stage (Mauromicale and Ierna, 2000), and directly transported from the experimental field to *Violetto Ramacchese Cooperativa Agricola*, an industrial company located in Ramacca (Eastern Sicily, Italy), kept at room temperature and processed within 24 h. Briefly, about 60 heads, for each postharvest treatment, were prepared by removing the leaves and cutting the floral stems to 5 cm in length and packaged into perforated plastic boxes according to Italian commercial practices. Sixty unwashed heads were stored at room temperature throughout the experiments (control), whereas the remaining were immersed in ozonised tap water (for 5 min), drained and stored in a dark cooling chamber (with an internal volume of 165 m^3, maintained at 4±1°C and 90% of relative humidity) under the following conditions: i) in normal atmosphere; ii) in an ozone-enriched atmosphere for 3 days and in normal atmosphere for the last 4 days; iii) in an ozone-enriched atmosphere for 7 days. A more detailed description of both the system of washing with ozonised water and storage under O_3-enriched atmosphere, provided by SAIM Service s.r.l. (Latina, Italy), was already reported by Restuccia *et al*. (2014). Sub-samples (15 heads) of each postharvest treatment and cultivar were transported to University of Catania laboratories under refrigerated conditions for subsequent evaluation of antioxidants content on the day of processing and after 3 and 7 days of storage.

Water content losses determination

At each sampling, five globe artichoke heads, for each cultivar and postharvest treatment were selected, weighted and then oven-dried at 65°C (Binder, Milan, Italy) until constant weight, in order to determine the water content (WC) and to compute its losses (WCL), expressed as % of the initial value, throughout the experiments.

Chemical analyses

Five whole heads per postharvest treatment, cultivar and storage time were freeze-dried (Christ freeze drier, Osterode am Harz, Germany) and stored at 20°C until chemical analyses. The total polyphenol content (TPC) was determined according to Restuccia *et al*. (2014), using the Folin-Ciocalteu assay and chlorogenic acid as standard. The antioxidant activity (AA) was evaluated as percentage inhibition of 1,1-diphenyl-2-picrylhydrazyl radical (Brand-Williams *et al*., 1995).

All the reagents and solvents, of analytical or high-performance liquid chromatography grade, were purchased from Sigma-Aldrich (Milan, Italy). All the chemical analyses were performed in triplicate.

Statistical analysis

Bartlett's test was used to test the homoscedasticity, following which the data from each storage time were subjected to analysis of variance (ANOVA) as a factorial combination of *postharvest treatment (4) x cultivar (2)*. Means were separated by Duncan's multiple range test, when the *F*-test was significant. Percent values were transformed to arcsin √x (Bliss transformation) prior to analysis and then subjected to ANOVA; untransformed data were reported and discussed. Statistical analysis was performed by the statistical package SPSS® Statistics 13.0 (Armonk, NY, USA).

Results

The initial value for WC, TPC and AA are reported in Table 1. The unwashed heads stored for 7 days at room temperature achieved a significantly higher WCL (2.4% and 2.6% for *Apollo* and *Violet de Provence*, respectively) than that reported for the other postharvest treatments (from 0.8% to 1.4%; Figure 1A). After 3 or 7 days of cold storage, independently from storage conditions and cultivar, samples washed in ozonated water resulted in lower WCL as compared to the control (Figure 1A). Moreover, after 3 days of cold storage in ozone-enriched atmosphere, TPC increased by 13.6% and 9.8% in *Apollo* and *Violet de Provence* heads, respectively. The ozone injection for further 4 days significantly reduced (11% and 18.9%, respectively for *Apollo* and *Violet de Provence*) the TPC compared with the same batch stored for 3 days. The interruption of ozone injection in the cooling chamber preserved the TPC compared with the injection of the gas up to the 7th day of storage (32.9 *vs* 27.8 g kg^1 of dry matter, on average of cultivar). Finally, after both 3 and 7 days of storage the control achieved the lowest TPC (Figure 1B). A similar trend was observed for the antioxidant activity (Figure 1C). In particular, for both cultivars, the interruption of ozone insufflation in the cooling chamber after 3 days ensured a higher AA than the extended injection of this gas for 7 days. The unwashed heads stored at room temperature showed the lowest AA for both *Apollo* and *Violet de Provence* (61.6% and 58.7%, respectively) (Figure 1C). Finally, a positive correlation across the postharvest treatments was found between TPC and AA, after both 3 days (r=0.739**) and 7 days (r=0.756***) of cold storage (*data not shown*).

Discussion

In the present work, we investigated the effect of postharvest treatments with an ozone-enriched atmosphere (for 3 or 7 days) on water content losses, total polyphenols content and antioxidant activity of two globe artichoke cultivars widely cropped. In particular, the effect of ozone application on TPC is relevant since these substances play a significant role in the detoxification process *versus* the reactive oxygen species (ROS), which could derive from ozone decomposition (Alothaman *et al*., 2010). The observed increase in the TPC of the whole head up to the 3rd day of storage may be due to the activation of the phenylalanine ammo-

Table 1. Water content, total polyphenols content and antioxidant activity of whole globe artichoke heads as affected by cultivar × ozone treatment interaction.

Cultivar	WC (%)	TPC (g kg^{-1} DM)	AA (DPPH % inhibition)
Apollo	86.2a	28.0b	65.0^b
Violet de Provence	86.1^a	32.6^a	66.0^a

WC, water content; TPC, total polyphenols content; DM, dry matter; AA, antioxidant activity; DPPH, 1,1-diphenyl-2-picrylhydrazyl. ^{a,b}In each column values followed by different letters are significantly different at P≤0.05 by Duncan's multiple range test.

nia-lyase, the key enzyme in the polyphenols biosynthesis, during cold storage of globe artichoke (Lattanzio *et al*., 1994). In addition, the increased TPC may be caused by cell wall modification that occurred in plant tissues during ozone exposure and increased the polyphenols extractability through the release of some conjugated phenolic compounds present in the cell wall (Alothaman *et al*., 2010). This could also explain the increase in the antioxidant activity of the samples stored in O_3-enriched atmosphere. By contrast, a prolonged exposure (more than 3 days) to gaseous ozone caused, especially in *Violet de Provence*, a significant degradation of polyphenols, which may be partly attributed to the scavenging activity of these phytochemicals *versus* ROS (Alothaman *et al*., 2010). Nevertheless, the antioxidant activity for samples stored under O_3-enriched atmosphere was higher than the control and this indicates that the studied cultivars were able to control oxidative damage due to ozone exposure. In addition, water content losses were significantly reduced by ozone washing, independently from the storage conditions. Particularly, after both 3 and 7 days of cold storage, the rate of water content loss in *Violet de Provence* was lower in samples stored in ozone-enriched atmosphere. This is important since many vegetables undergo a deterioration in their turgidity as a result of a stress (as ozone is) induced in the tissues.

In conclusion, our findings demonstrated that storage in ozonised atmosphere up to 3 days might preserve the content of polyphenols and antioxidant activity of globe artichoke heads while maintaining product turgidity. The extension of quality retention, with special emphasis on water and total polyphenols content, could be important for globe artichoke heads destined to both fresh market and food industry. However, further studies are necessary to investigate other factors (*e.g.*, different exposure time to ozone, harvest time), which may improve quality of globe artichoke heads.

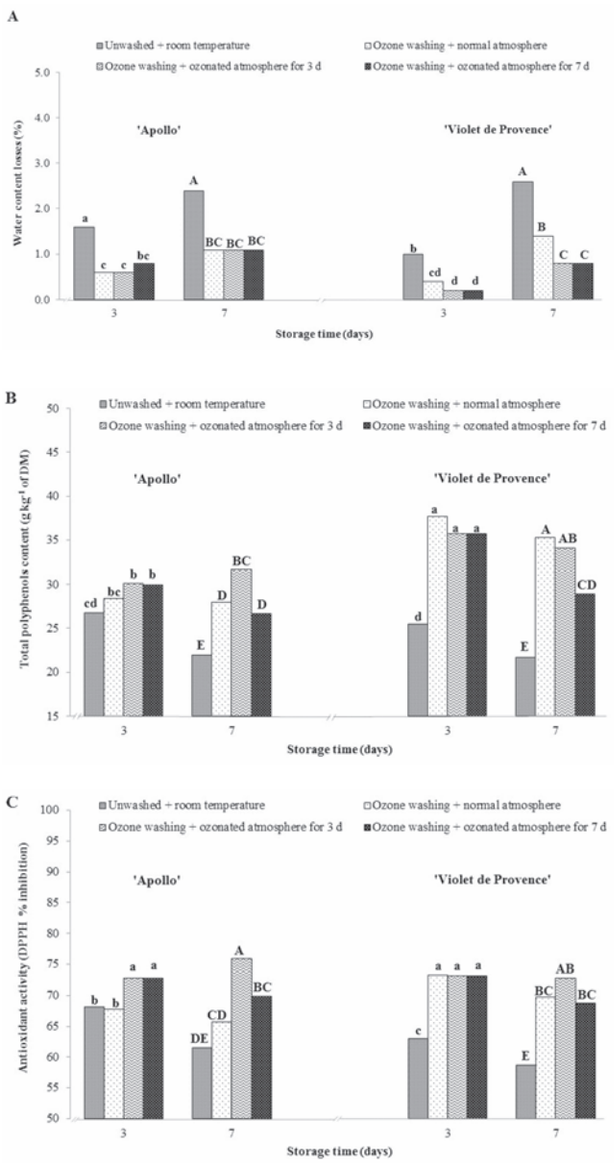

Figure 1. Effect of *cultivar* × *ozone treatment* interaction on water content (A), total polyphenol content (B) and antioxidant activity (C) of whole globe artichoke heads during cold storage. Values followed by different letters and within the same storage time are significantly different at P≤0.05 by a Duncan's multiple range test. DM, dry matter; DPPH, 1,1-diphenyl-2-picrylhydrazyl.

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