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Quality Effect of Ozone Treatment for the Red Raspberries Storage

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The improvement of the storage solutions and the infrastructure is a significant opportunity to the management of the post - harvest fruit losses and the maintenance of the quality in the supply chain. The environmental issue and the sustainability of the applied processes are the key of the succesful for the innovation of the food system; the sanification of the storage environment could represent a good opportunity to achieve this objective. The focus of this work was to evaluate the efficacy of the ozone (O_3) such environmental sanitization system to limit both the quantitative and the qualitative losses of the raspberry fruits. Fruits exposed to the O_3 constant concentration of 500 ppb (S1) and to the O_3 concentration between 200 and 50 ppb (S2) were compared with fruits maintained in the normal atmosphere (control). The efficacy of the S1 and S2 treatments were evaluated measuring along the storage time the fruits weight losses (%), the total soluble solids content (SSC), the pulp firmness, the acidity content, the skin colour and the most important nutraceutical compounds. The fruits were stored at 1±1°C in a cold room held at 90–95% RH for 3, 6, 9 and 13 days. After each time at low temperature the same berries were held in shelf life for different additional days (respectively +6, +3, +9 and +2) at 20±1°C to simulate retailer conditions. All the qualitative and nutraceutical traits were maintained to the exposure of O_3. Fruits stored with the S2 treatment showed the highest sensorial profile.

1. Introduction

Nowadays to promote and market fruits in a competitive way it's necessary to develop and to apply processes and strategies within of the environmental and sustainability issue.
With improvements in storage technologies and the increase in global trade, today's consumer expects to find fresh berries available for purchase outside of the local harvest season.
Red soft fruits such as raspberry have became much appreciated and demanded by consumers owing to their wholesome and healthy image. Although they are rich in nutritional compounds are sensitive to water loss and they are highly perishable so, the storage time is restricted to few days. The raspberry fruits if stored at 0–0.5°C and 90–95% relative humidity (RH) can be maintained in normal atmospheric (A.N.) conditions for 5–7 days (Salunkhe and Desai, 1984).
Techniques that have already been tested successfully include modified atmosphere packaging (MAP (Agar and Streif, 1996) and controlled atmosphere (CA) (Haffner et al., 2002) but the use of gaseous ozone (O_3) could be a promising solution to extend the shelf life of these perishable fruits because it's safer than other commonly used gases such as CO_2, N_2, and O_2. Due the lowest threshold limit value–short term exposure limit (TLV-LTEL) value the O_3 has been recommended as a generally recognized as safe (GRAS) disinfectant or sanitizer for foods in the United States (Graham, 1997). Thanks to the disinfecting abilities O_3 prevents formation on storage walls, wooden boxes and containers of various colonies of microorganisms, including especially steady against low temperatures.
Previous studies showed the use of the gas to inactivate pathogens through oxidization and the

decomposition to nontoxic products (Khadre et al., 2001) and to increase the shelf life of berries fruits but the efficacy of the treatments on the quality attributes of the fruits are contradictory (Perez et al., 1999). O_3 treatment of 0.35 ppm at 20 °C for 4 days was inefective to prevent fungal decay in strawberries cv Camarosa (Perez et al.,1999), while Nadas et al., 2003 reported that an atmosphere containing O_3 in 1.5 ppm exhibited reduced decay, weight loss and fruit softening. However discoloration of some fruits and losses in the volatile aroma content and the effect on the nutraceutical compound can be occurred (Nadas et al., 2003). After 3 days of storage at 2°C the vitamin C content of ozone-treated strawberries was three times bigger that of control fruits but the anthocyanin content was significantly lower than untreated fruits. Song et al., 2003 reported the limited effect of the O_3 fumigation on the marketability of the blueberry cv Coville. Blackberries exposed to 0.1 and 0.3 ppm of for 12 days at 2 °C exhibited a significant reduction in *Botrytis cinerea* spoilage without any adverse effects on fruit quality (Barth et al., 1995). However, when cranberries were stored in ozonated conditions for 5 weeks a significant increase in rot and overall quality loss were observed. The efficacy of ozone must be individually assessed for each commodity and species taking into account its recommended post-harvest handling and storage practices (Perez et al., 1999). A difference in the way in which each fruit responded to the O_3 treatments varied greatly and was most likely due to the shape of the fruit, location or presence of seeds and drupelets, as well as the distribution of cells on the fruit or within the crevices.

The purpose of the present work was to evaluate the effects of two different ozone (O_3) treatments, applied as environmental sanitation tool, on the quality traits and the nutraceutical compounds of red raspberry cv Grandeur up to 15 days of storage.

2. Materials and methods

2.1 Plan material and experimental design

The tests were performed on everbearing variety of red raspberry (*Rubus idaeus* L.) cv Grandeur obtained at the red ripe stage of maturity on middle of September from a commercial orchard of the Agrifrutta Soc. Coop. SRL (Piedmont, Italy), where the experiment was carried out. Selected on the basis of uniform size, colour, and freedom from evident defects or diseases, the raspberries were randomly distributed in 150 g polyethylene terephthalate (PET) trays, with five replicates per treatment and analysis day. Raspberry fruits, exposed into the cold room (730 m^3 of volume) to two different flushing of O_3 (S1 and S2 treatment) were compared with control maintained under normal atmosphere (A.N.).

Fruits with the S1 and S2 treatment were maintained respectively under exposure to 500 ppb O_3 supplied by a generator (Biofresh, Northumberland) used in a continuous cycle and a constant flushing of 200 ppb (12 hours) and 50 ppb (12 h); both flushing were maintained up to the end of storage.

The fruits were stored at 1±1°C in a cold room held at 90–95% RH for 3, 6, 9 and 13 days. After each time at low temperature the same berries were held in shelf life for different additional days (respectively +6, +3, +9 and +2) at 20±1°C to simulate retailer conditions.

2.2. Measurements

The weight (water) loss of each raspberry trays was measured using an electronic balance (SE622, WVR, USA) with an accuracy of 0.01 g. Weight loss (%) was determined by comparing the weight of each tray on the sampling day with their initial weight determined on day 0.

Soluble solids (SSC) content was determined in the juice (from three trays randomly chosen for each treatment) with a digital refractometer Atago PR-101 (Atago, Japan) at 20°C and results were expressed as °Brix. The titratable acidity (TA) was measured using an automatic titrator (Titritino 702, Metrohm, Swiss). It was determined potentiometrically using 0.1 N NaOH to an end point of pH 8.1 in 5 mL of juice diluted in 50 mL of distilled water. Results were expressed as meq/L. Skin colour measurements were taken using a Minolta CR-400 chroma meter (Chroma Meter, Model CR-400, Minolta, Germany) with an 8 mm diameter measuring head and a C illuminant calibrated with manufacturer's standard white plate. Colour changes were quantified for 15 fruit each trays (three baskets were randomly chosen) in the L*, a* and b* colour space (Abbott, 1999). Hue angle (H◦) was then calculated as $H^\circ = \tan^{-1}(b^*/a^*)$, when a* and b* were >0 and $H^\circ = 180 + \tan^{-1}(b^*/a^*)$, when a* was <0 and b* was >0.

To determine the total anthocyanin content, the total phenolic content, and the total antioxidant capacity, extracts were obtained using 10 g of fruit added to 25 mL of extraction buffer (500 mL methanol, 23.8 mL

deionized water and 1.4 mL hydrochloric acid 37%). After 1 h in the dark at room temperature, the samples were thoroughly homogenized for a few minutes with an ultra turrax (IKA, Staufen, Germany) and centrifuged for 15 min at 3000 rpm. The supernatant obtained by centrifugation was collected and transferred into glass test tubes and stored at −20° C until analysis. The total anthocyanin content was quantified according to the pH differential method of Cheng and Breen (1991). Anthocyanins were estimated by their difference of absorbance at 510 and 700 nm in a buffer at pH 1.0 and pH 4.5, where Atot = (A515 – A700) pH 1.0 – (A515 – A700) pH 4.5. The results are expressed as milligrams of cyanidin-3-glucoside (C3G) equivalent per 100 g of fresh weight (fw). The total phenolic content was measured using a Folin-Ciocalteu reagent with gallic acid as a standard at 765 nm following the method of Slinkard and Singleton (1977). The results are expressed as milligrams of gallic acid equivalents (GAE) per 100 g of fresh weight (fw). Antioxidant activity was determined using the ferric reducing antioxidant power (FRAP) assay following the methods of Pellegrini et al. (2003), with some modifications. The antioxidant capacity of the dilute fruits extract was determined by its ability to reduce ferric iron to ferrous iron in a solution of 2,4,6-Tripyridyl-s-triazine (TPTZ) prepared in sodium acetate at pH 3.6. The reduction of iron in the TPTZ-ferric chloride solution (FRAP reagent) results in the formation of a blue-colored product (ferrous tripyridyltriazine complex), the absorbance of which was read spectrophotometrically at 595 nm 4 min after the addition of appropriately diluted fruits extracts or antioxidant standards to the FRAP reagent. The results are expressed as mmol Fe^{2+} per 1 kg of fresh fruits. All of these analyses were performed using the UV-Vis spectrophotometer 1600 PC VWR International.
In order to have additional parameters for evaluating the quality of fruits six panelists previously trained with commercial samples were invited to perform a sensory evaluation of the fruits. For that, 10 berries selected at random from each basket were presented to each panellist. The sensory descriptors used were flavour/taste, appearance and texture. During each session, the samples were presented in randomized order to the panelists, who judged the descriptors using a 9 point hedonic scale at room temperature (25±1°C), where 9= “like extremely,” 7 = “like moderately,” 5 = “neither like nor dislike,” 3 = “dislike moderately,” and 1 = “dislike extremely.” The scores below 3 indicated unacceptable samples.

3. Results and discussion

The thin skin of fruits such as raspberries makes them susceptible to rapid water loss, resulting in shriveling and deterioration. The weight loss (water loss) parameter is particularly interesting to evaluate the success of storage and losses of 8-6 % affect the marketability of the fruits (Haffner et al., 2002). As expected the weight loss of the fruits due to the transpiration and the respiration increased over time and statistically significant differences were observed among all the treatments up to the end of storage. Control fruits and the S2 treatment showed similar weight losses content (respectively 5.30 % and 5.55%) for 3 days at 1±1°C followed by 6 days 20±1°C. Similar data were observed with the S1 treatment when raspberry fruits were stored for 6 days at 1±1°C followed by 3 days 20±1°C. Storing fruits up to 15 days weight losses limit to the marketability were observed for all the treatments (higher than 6 %); in fact independently from the exposure time to the different storage temperatures (9 and 13 days at 1±1°C followed respectively by 6 and 2 at 20±1°C) all samples have showed similar weight losses within range of 10.92% to 7.92 %.

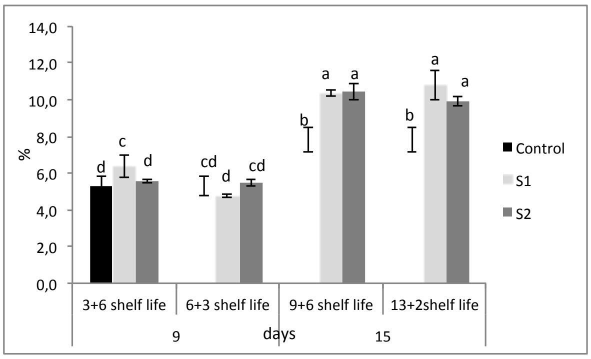


Figure1: Weight losses (%) in raspberry cv Grandeur

Results are expressed as means ± standard deviation. Values in the columns followed by different letters are significantly different at p ≤ 0.05 according to Tukey’s test

The soluble solids content (SSC) of the raspberries cv Grandeaur at harvest was 10.9° Brix; all treatments showed increases in the values during the storage time due the weight losses which greatly affects the concentration of the total sugars (Table 1) but no statistically significant differences were observed between treatments during the storage time unlike what was observed in other soft and perishable red fruits such as strawberries when the soluble solids levels steadily increased in ozoned fruits, reaching significantly higher levels than in controls after 7 days of storage (Skog and Chug, 2001).
The sweetness and acidity components affect the flavour and the taste of raspberry fruits, but this ratio (SSC/TA) remained nearly unchanged in all treatments (0.5) and during the storage period (0.6) (data not shown). Colour changes in the raspberries were significantly affected by the treatments especially after 15 days of storage with decreasing brightness (L) and increasing redness as indicated by decreasing chroma (C) and hue angle (h°) values. At the harvest time L value was of 42.1 (data not showed) and its decrease in reflects the darkening of fruits, probably due the accumulation of anthocyanins, and indicates that the ripening process is occurring in the fruits. Treatments S1 and S2 showed the highest L* values as a consequence of the O3 concentration showing after 15 days of storage similar L values (32.5 and 31.7 after 9+6 days of shelf life) and (37.6 and 36.1 after 13 + 2 days of shelf life). Raspberries stored with the S2 treatment has been maintained more vivid (higher chroma) during all the storage time. The h° value is directly related to the humidity during storage (Goncalves et al., 2007) and no statistically significant differences were observed between treatments.

Table 1: Changes in soluble solids content (SSC), titratable acidity (TA) and colour of raspberry cv Grandeur

Treatments	**9 days**								**15 days**							
	3 + 6 sf				**6+ 3 sf**				**9+6 sf**				**13+2 sf**			
							°Brix									
Control	12.4	±	0.2	ns	12.4	±	0.2	a	11.7	±	0.2	ns	11.7	±	0.2	ns
S1	11.9	±	0.5	ns	11.5	±	0.4	b	12.0	±	0.2	ns	12.3	±	0.9	ns
S2	11.8	±	0.2	ns	11.8	±	0.9	b	11.8	±	0.5	ns	11.6	±	0.1	ns
							meq/L									
Control	17.0	±	0.6	ab	17.0	±	0.6	ns	16.8	±	0.5	ns	16.8	±	0.5	ns
S1	17.3	±	0.2	a	16.6	±	1.0	ns	14.3	±	2.1	ns	15.3	±	0.9	ns
S2	16.0	±	0.4	b	18.1	±	0.9	ns	16.1	±	0.9	ns	16.7	±	0.0	ns
							L									
Control	30.9	±	4.1	ns	30.9	±	4.1	ns	29.3	±	2.4	b	29.3	±	2.4	b
S1	31.0	±	3.3	ns	31.5	±	2.3	ns	32.5	±	2.2	a	37.6	±	5.6	a
S2	31.6	±	2.2	ns	31.4	±	2.5	ns	31.7	±	1.8	a	36.1	±	6.9	a
							CHROMA (*C)									
Control	42.9	±	6.8	ns	42.9	±	6.8	b	34.0	±	5.4	b	39.0	±	5.4	c
S1	43.1	±	6.4	ns	45.0	±	5.1	a	40.3	±	4.6	a	45.6	±	9.3	b
S2	44.8	±	4.9	ns	45.0	±	5.2	a	39.5	±	4.9	ab	49.4	±	5.4	a
							HUE (°h)									
Control	0.4	±	0.01	ns	0.4	±	0.01	ns	0.4	±	0.01	ns	0.4	±	0.1	ns
S1	0.4	±	0.02	ns	0.4	±	0.01	ns	0.4	±	0.02	ns	0.7	±	0.1	ns
S2	0.4	±	0.01	ns	0.4	±	0.01	ns	0.4	±	0.01	ns	0.6	±	0.1	ns

Results are expressed as means ± standard deviation. Values in the columns followed by different letters are significantly different at $p \leq 0.05$ according to Tukey's test

Previous studies have shown that raspberries are a good source of natural antioxidants phytochemicals that are believed to have significant biological activity although, this is the first report on changes in the cv Grandeur after storage using different O3 treatments. Cultivation conditions, ripeness, storage time and conditions are the main factors that affect the content of phenolic compounds and antioxidant capacity of berry fruits (Wang, 2003; Wang et al., 2009). Due to its high anthocyanin and polyphenol contents the cv Grandeur shows an high antioxidant potential in fresh fruits. Studies with black raspberries showed that storage of fruit at higher temperatures (up to 28° C) increased the level of bioactive compounds (such as anthocyanins) and antioxidant capacity, but the increase may have been due to moisture loss and metabolism of sugars (Tulio et al., 2006). In this study (Table 2), no losses of bioactive compounds during the storage time were detected and no statistically significantly differences were observed among treatments for each quality control. For all the storage time both control fruits than raspberries with the S1 and S2 treatments showed similar antioxidant capacity values in a range of 21.7 and 22.3 mmol Fe2+/kg.

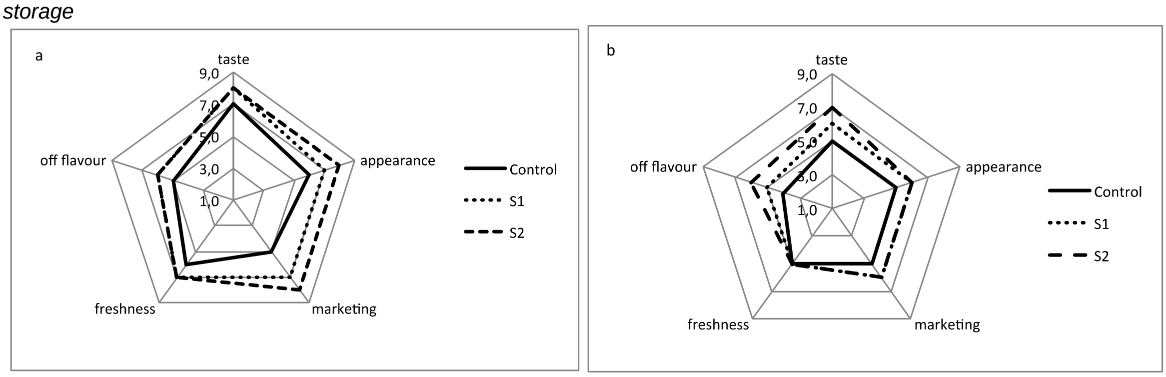
Table 2: Changes in nutraceutical compounds of raspberry cv Grandeur

	9 days						15 days					
	3 + 6 sf			6 + 3 sf			9 + 6 sf			13 +2 sf		
Total antioxidant capacity (mmol Fe^{2+}/kg)												
Control	21.8	± 2.1	ns NS	20.7	± 1.1	ns NS	21.7	± 1.5	ns NS	21.7	± 1.5	ns NS
S1	22.4	± 1.1	ns NS	22.2	± 0.9	ns NS	20.7	± 3.0	ns NS	21.6	± 1.6	ns NS
S2	21.2	± 0.2	ns NS	20.7	± 1.1	ns NS	21.7	± 2.6	ns NS	22.3	± 1.4	ns NS
Total anthocyanins (mg C3G/100g)												
Control	36.3	± 7.8	ns NS	36.3	± 7.8	ns NS	50.2	± 9.3	ns NS	50.2	± 2.3	ns NS
S1	31.0	± 7.2	ns NS	56.7	± 28.1	ns NS	39.1	± 10.2	ns NS	56.6	± 1.9	ns NS
S2	51.3	± 13.3	ns NS	39.8	± 5.8	ns NS	53.7	± 13.3	ns NS	35.0	± 9.1	ns NS
Total polyphenols (mgGAE/100 g)												
Control	266.6	± 31.7	ns NS	266.6	± 31.7	ns NS	258.3	± 43.7	ns NS	310.0	± 8.5	a NS
S1	194.1	± 60.8	ns NS	196.1	± 65.2	ns NS	261.3	± 28.8	ns NS	203.4	± 58.5	b NS
S2	192.2	± 28.2	ns B	212.5	± 28.9	ns B	315.1	± 33.0	ns A	246.8	± 53.4	ab AB

Results are expressed as means ± standard deviation. Values in the lines and in the column followed respectively by different uppercase and lowercase letters are significantly different at p ≤ 0.05 according to Tukey's test

The sensory quality of raspberry fruits at the end of 9 and 15 days of storage was evaluated by a sensory panel. The mean scores of sensory attributes (taste, appareance, marketing, freshness, off flavour), for berries stored with the S1 and S2 treatments and the control are summarized in Figure 2. As expected, the sensory scores of berries stored by O_3 were higher than control after 9 and 15 days of storage both. Raspberries control probably have suffered from anaerobic respiration, so the off-flavor of fruits have reduced the sensorial scores. Generally fruits stored with the S2 treatment were judged of better sensory quality than berries stored with the S1 strengthening the quality analysis.

Figure 2: Sensory evaluation of raspberry cv Grandeaur after 9 days (a) (6 + 3 sf) and 15 days (b) (13 +2 sf) of storage



4. Conclusions

Ozone generators may be of most use in cold room where fruits are stored and can be suggested as tool to improve the marketability of the raspberry fruits when they are maintained in shelf life conditions to the highest temperatures along all the supply chain. Depending on the needs of different storage times and shelf life to which raspberries are maintained, treatments S1 and S2 may also be used. The O_3 treatments were able to storage red fruits such as raspberry for a long time (15 days) maintaining unchanged the most important qualitative and nutraceutical traits of these perishable fruits. Among the different O_3 concentrations the S2 treatment (constant flushing of 200 ppb (12 hours) and 50 ppb (12 hours) showed the best performance ensuring the best visual appearance due the more attractive colour and the best control of off flavour.

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