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Postharvest gaseous ozone treatment enhances quality parameters and delays softening in Cantaloupe melon during storage at 6°C

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POSTHARVEST GASEOUS OZONE TREATMENT ENHANCES QUALITY PARAMETERS AND DELAYS SOFTENING IN CANTALOUPE MELON DURING STORAGE AT 6°C

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Running title: Quality of Melon Stored in an Ozone Atmosphere

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ABSTRACT

BACKGROUND: This trial shows postharvest gaseous ozone treatment to evaluate the quality parameters and cell wall enzymes during the Cantaloupe melon cv *Caldeo* storage at 6°C for 13 days.

RESULTS: The fruits were kept in cold storage and treated with 0.15 ppm gaseous ozone during the day and 0.3 ppm overnight; the control fruits (CK) are stored in normal atmosphere. The firmness was higher and ethylene concentration was significantly lower on treated fruits compared to CK (untreated). During storage, microbial counts showed a lower content in both ozone and CK fruits and from 9 days the ozone sample showed a decrease in mesophilic content Additionally, the total carotenoids had a tendency to be higher, without significant differences between the two theses . The same trend was observed for the ascorbic acid, the colour, the total SSC and the acidity contents. Finally, the ozone treatment reduced the activities of the cell wall enzymes *α*-arabinopyranosidase, *β*-galactopyranosidase and polygalactorunase starting from the 3rd day of storage. Pectin methyl esterase activity does not seem to be affected by the gas treatment.

CONCLUSION: gaseous ozone treatment during cold storage was effective in decreasing ethylene production and delaying fruit softening in Cantaloupe melon by extending the quality maintenance.

Keywords: *Cucumis melo* L, O_3, quality, cold storage, softening, cell wall enzymes.

INTRODUCTION

Cantaloupe melon is a climacteric fruit with physiological and biochemical changes related to fruit ripening, such as fruit skin colour and softening. Its identifying characteristics are its round shape, the presence of the netting with or without suture, the colour of its skin (under the netting), which ranges from green to yellow, and its orange and juicy firm pulp with a high Brix. Cantaloupe cv Caldeo is a commercial hybrid melon with extended shelf life (E.S.L.), up to 7-10 days, and good overripe resistance..

O_3 is a naturally occurring gas in the atmosphere generated by the passage of oxygen gas through a high-voltage electrical discharge or by ultraviolet light irradiation.[1] It can be applied either as a gas or dissolved in water. For commercial use, ozone must be produced on site and it is classified as GRAS (generally recognised as safe) for food contact applications in the USA. In 2001, the Food and Drug Administration (FDA, 2001)[2] approved the application of ozone as a direct food additive for the treatment, storage, and processing of foods in gaseous and aqueous phases. The product of ozone degradation is oxygen; therefore, it leaves no residues on treated commodities. New techniques for reducing undesired microbial contamination, spoilage and decay, as well as for maintaining the product's visual, textural and nutritional quality, are required in all steps of the production and distribution chain.[3]

During the postharvest period, O_3 can be applied to fresh produce as a pre-storage treatment, during storage or transportation[4]. Most studies[5-7] have been focused on the antimicrobial efficacy of ozone treatment, although some recent studies have also examined the effects of ozone on quality related attributes for fruits and vegetables.[8,9] Ozone enrichment of stored foodstuffs provide odour control and extend storage life by destroying the ripening/senescence promoting hormone ethylene.[10,11]

Ong *et al*.[12] exposed papaya fruit to gaseous ozone (0, 1.5, 2.5, 3.5 and 5.0 ppm) for 96 h prior to ambient air storage of 25 ± 3°C with 70 ± 5% RH for another 10 days. Fruits treated with up to 3.5 ppm of ozone had a lower respiration rate and delayed ripening compared to the control, while higher ozone concentration (>3.5 ppm) produced more ethylene and caused injury to fruit tissue.

In kiwifruit ozone application blocked ethylene production,[13] delayed ripening, and stimulated antioxidant and anti-radical activities of fruits by suppressing a set of candidate kiwifruit proteins related to ripening that are sensitive to carbonylation.[14]

Exposure to ozone significantly increased quality attributes of tomatoes such as sugars, antioxidative status and aroma whilst maintaining fruit firmness.[15] In some studies on apples,

pears[10] and grapes,[16,17] no change due to exposure to gaseous ozone was found. Other studies, on the other hand, have shown a better preservation of the firmness of cucumbers (40 ppb)[10] and kiwis (300 ppb)[14] with a continuous ozone treatment, and of strawberries exposed to gaseous ozone at 1.5 ppm for 3 days,[18] which delayed their softening process. Laureano *et al.*[19] reported that postharvest gaseous ozone treatments lead to an increase in skin hardness in all the grape varieties studied, but the hardening degree is variety-dependent.

In general, nutritional and quality parameters of vegetables (such as respiration rate, ethylene emission, weight loss, colour, glucose, fructose and sucrose content, organic acids, and vitamin C contents) were maintained or were increased following ozone exposure.[20,21]

To date there are very few bibliographical works that deal with melons that have been cut and washed with ozonized water[22,23] while there is only one by Selma et al.[24] on whole and cut melons in which samples were exposed for 30 min to 5000, 10000 and 20000 ppm ozone for 30 min under 90–95% RH and 11°C, followed by storage at 5°C for up to 7 days. In this trial ozone treatment greatly reduced initial counts of all microbial groups tested and no impact of ozone treatment on visual quality, aroma, firmness and soluble solids content after treatment and storage were observed.

Therefore, our research sought to examine the possibility of using gaseous ozone treatment to extend the shelf-life of melons, and specifically to preserve all the qualitative characteristics of the fruit.

MATERIALS AND METHODS

Experimental procedure and treatment

Cantaloupe (*Cucumis melo* L. var. *cantalupensis*, cv. Caldeo) were grown with no chemicals and were harvested 30 days after pollination from a farm in Central Italy in July 2016. After the washing for two minutes in chlorinated water solution (150 mg L^{-1} sodium hypochlorite at pH 6.5) and the commercial preparatory steps for the market, the fruits were taken to the laboratory in appropriate thermal boxes, and a low temperature (6.0 ± 1.0°C) was maintained throughout transportation. Melons were selected by size (20.0 ± 5.0 cm diameter), weight (1600 ± 180 g) and external skin colour (55% yellowness), and absence of defects. The fruits were divided into two groups of 60 each and, respectively:

i) 60 control fruits (CK) subjected to cold storage in normal atmosphere at 6.0 ± 2.0°C and 90 ± 5.0% RH;

ii) 60 fruits treated continuously with gaseous ozone 0.15 ppm day and 0.3 ppm overnight (O_3) in cold storage at 6° C and 90% RH.

The ozone was supplied by an ozone generator (C32-AG, Industrie De Nora Spa, Milan, Italy) with a nominal production capacity of 32 g O_3 h^{-1}.

During the 13 days of the experiment, five samples were collected (0, 3, 6, 9 and 13 days).

Physical and physiological analyses

Fruits at 6°C were permitted to reach room temperature before undergoing each test.

The soluble solid content (°Brix) of eight melon fruits from each treatment and sampling time were measured using a digital refractometer (Atago CO. Ltd., Tokyo, Japan).

The colour was assessed with a CM-2600d colorimeter (Konica Minolta Inc., Ramsey, NY) set at SCE (specular component excluded) measuring CIELAB coordinates L, a, and b. The reference parameter used in this trial was the value b (yellowness).

Flesh firmness was determined with a digital penetrometer model 53205 (TR Turoni&Co., Forlì, Italy) provided with an 8-mm tip; results are expressed as the maximum force (N) required the 7-mm probe to penetrate the melon pulp.

To achieve a more significant result on texture, firmness was measured also by the Universal testing machine (model 4301 Instron Inc., Canton, MS, USA): cantaloupe melon slices (two cm thick) were placed over the steel plate and compressed with a flat probe (8 mm diameter) using four different parts of the internal tissue. The load was fixed at 10 N and when tissue reached this resistance value the bar was quickly raised. Tissue firmness was expressed as 'deformation to 10 N'. Bar speed was set at 25 mm min^{-1}.

Oxygen, carbon dioxide and ethylene production was measured by placing 10 melon fruits inside a plastic jar (three jars per treatment, vol. 50 L), tightly capped with a lid, for 1 h at 20°C. Sampling for gas production was performed continuously from the same jars until the end of the storage period. Oxygen and carbon dioxide partial pressures were monitored with an Oxycarb analyser (Isolcell, Bolzano, Italy). The ethylene partial pressure was measured with a gas chromatograph (Fractovap 4200, Carlo Erba, Milan, Italy) equipped with a flame ionisation detector (FID) and a 1.5m long activated alumina column (80/100 mesh). A nitrogen carrier flow of 20 mL min^{-1} was used. Ethylene measurement was performed isothermally at 100 °C.

Chemical analyses

For chemical and enzymatic analyses, all reagents not indicated in the text were supplied by Sigma–Aldrich (Milan, Italy).

For the following chemical analyses, three fruits from each treatment were used. As for the physical and physiological analyses, in order to have a significant sample, knowing the

variability of melon flesh, four different parts of the melon flesh were taken and homogenized together.

The total acid content (TA) was determined after titrating 10 mL of flesh juice with 0.1 M NaOH, and the results were expressed as mg citric acid per 100 grams F.W.

The ascorbic acid content (AA) was determined according to the method of Malik and Singh,[25] with some modifications. The melon fruits (3 g) were homogenised using 6 mL of 16% (v v^{-1}) metaphosphoric acid solution containing 0.18% (w v^{-1}) ethylene-diamine tetracetic acid (disodium salt EDTA). The homogenate was centrifuged at 10000g for 20 min (Beckman JA-21 centrifuge), filtered and collected. The assay mixture contained 400 µL of extract, 200 µL of 3% metaphosphoric acid and 200 µL of diluted Folin's reagent (1/5, *v*/*v*) in a final volume of 2 mL. After incubation for 10 min, the absorbance was measured at 760 nm using a 25 UV-Vis spectrophotometer (Perkin Elmer Instruments Ltd., Seer Green, Beaconsfield, U.K.). The ascorbic acid content was expressed as milligrams of ascorbic acid per 100 g fresh weight.

Total carotenoids (mg Kg^{-1} *β*-carotene) were extracted using a solution of acetone with 20% (v v^{-1}) water.[26] After 30 min in the dark, the samples were centrifuged at 13000g for 15 min at 5°C and subsequently analysed by a 25 UV-Vis spectrophotometer.

Enzymatic assays

Glycosidase α-L-arabinopyranosidase (α-ara, EC 3.2.1.55) and β-D-galactopyranosidase (β-gal, EC 3.2.1.23) extraction was conducted using 3 g of melon fruit dissolved in 6 mL of 50 mM Na-acetate buffer (pH 5.5) with the addition of 1.4 mM NaCl, 0.2% (w v^{-1}) cysteine, and 1% (w v^{-1}) PEG 3350; the solution was homogenised with UltraTurrax for 1 min on ice, and then centrifuged at 39800 g for 30 min at 4°C.[27] A volume of 2.5 mL of supernatant was filtered through a PD10 Sephadex G25 column (GE Healthcare Europe GmbH, Milan, Italy) equilibrated at 4°C with Na-acetate buffer, and the enzyme was recovered using 3.5 mL of Na-acetate buffer. The assay mixture consisted of 100 µL of enzyme extract in 900 µL of corresponding p-nitrophenol derivatives of specific substrate. Final concentrations in 50 mM sodium acetate buffer were: 10 mM α-L-arabinopyranoside (pH 4.7) and 2 mM β-D-galactopyranoside (pH 4.3). The reaction mixture was incubated at 37°C for 60 min. To terminate the reaction, 5 mL of 0.1 M Na_2CO_3 was added; the p-nitrophenol formed was determined by measuring the absorbance at a wavelength of 405 nm. Enzyme activities were expressed as µmoles of p-nitrophenol formed per min per g of F.W.

Pectin methyl esterase activity (PME, EC 3.1.1.11) was assayed using Hagerman and Austin's procedure[28] with modifications for use in melon. Extraction was performed on three sets of 5

g of melon fruit, homogenised with UltraTurrax for 1 min on ice, in 5 mL of 0.2 M phosphate buffer at pH 7.5 with the addition of 1 mM EDTA and 8% NaCl. The homogenate was centrifuged at 39800g for 30 min at 4°C. Then the supernatant was filtered through a Sephadex G25 column.

For the assay, 600 µL of extract incubated with 150 µL of 0.01% bromothymol blue and 2 mL of 0.5% pectin citrus (w v^{-1}) in 2 mM phosphate buffer (pH 7.5) were used. A spectrophotometric reading was taken at 620 nm for 6 min. Enzyme activity is expressed as µmoles galacturonic acid per min per g of F.W.

For polygalactorunase (PG, EC 3.2.1.15) extraction, the same procedure was used as for PME but with 0.5 M NaCl and 2% PEG (polyethylene glycol). The procedure of Lohani *et al.*[29] adapted to melon, was used for the assay: 400 µL of extract were incubated with 1.2 mL of 0.2 M sodium acetate (pH 4.5) buffer, and 1.2 mL of 0.7% polygalacturonic acid was added. The sample was incubated in a water bath at 37°C for 60 min, then boiled (100°C for 5 min) after the addition of 200 µL of 0.1% of 3,5 dinitrosalicylic acid (DNS) prepared in 30% potassium sodium tartrate and 0.4 M NaOH. PG activity reading was taken at 540 nm. Enzyme activity is expressed in µmoles galacturonic acid per g of F.W.

Microbiological analyses

This experiment was assayed using the Ukuku *et al.*[30] procedure.

Forty-two rind plugs 25 mm in diameter per melon samples with a surface area of ≃ 4.9 cm^2 were cut at random from the whole melon surfaces. Flesh adhering to the rind plugs was trimmed off using a sterilised stainless steel knife. The rind plugs were homogenised in 90 mL of sterile 0.1% peptone water using a commercial blender (International PBI, Milan, Italy) for 2 min. Decimal dilutions of the sample were made with 0.1% peptone water (BBL, Difco), and 0.1 mL portions were plated in duplicate on Plate Count Agar (PCA; BBL, Difco), which were incubated at 30°C for 72 h for mesophilic aerobic bacteria; and plates of Potato Dextrose Agar (BBL, Difco) acidified with 10% tartaric acid (PDAA) to pH 3.5, which were incubated at 25°C for 5 days for enumeration of yeast and moulds.

Statistical analysis

All chemical and biochemical values are the results of three replicate samples (±SE). Analysis of variance (ANOVA) was performed on the obtained data, and Tukey's test was performed to identify significant differences between samples at $P < 0.05$. The statistical analyses were performed using Sigma plot 13 (Trial version, Systat Software, San Jose, CA, USA).

RESULTS AND DISCUSSION

Quality parameters

Table 1 shows the values measured on cantaloupe melon samples during cold storage at 6°C as regards the colour, the TSS content and some chemical parameters (titratable acidity, ascorbic acid and total carotenoids) involved in qualitative characterisation of the fruit.

The colour change was assessed by studying, in particular, the b* parameter (yellowness) of the melon pulp, which showed no significant differences between the two experimental conditions, with values of 29.9 recorded at the start of the test and that reached 30.5 - 31.5 after 13 days. The L* and a* values also showed no difference in results between the two conditions (data not shown). A uniform trend was observed after the gas treatment of tomato fruits with 10 µL g^{-1} of O_3 also by Rodoni et al.[21] while Botondi et al.[22], instead, found that washing melon cubes with ozonized water induced a gradual decrease of colour (parameters L* and C*) during storage.

The TSS content of the melon samples remained constant over time in both of the conditions (CK and O_3) until day 6 (values around 10.5 °Brix), after which the samples stored in a normal atmosphere showed a downward trend until day 13 (CK = 8.7 and O_3 = 10.1 °Brix), probably attributable to an initial ageing of the CK samples, with a partial degradation of their sugars. Studies carried out on papaya fruits treated with O_3 at different concentrations[31] and on kiwis[32] treated with O_3 and stored at low temperatures did not differ as to TSS content, compared to the control samples.

No significantly different values were found between the treatments performed as regards TA (Table 1). Alì et al.[31] however observed that in papayas the content of organic acids decreases after treatments with O_3 at 1.5 and 5 ppm; however, other studies on strawberries and citrus fruits[33,34] provided data that was consistent between the O_3 treated condition and the control condition.

As to the analysis of the total carotenoid content of the samples treated with gaseous O_3, although it presented a tendentially higher trend during storage (from 18.6 at the start of the test to 28.2 mg kg^{-1} FW after 13 days), it was not significantly different with respect to the control samples (from 18.6 to 23.0 mg kg^{-1} FW). Other studies reported similar results: continuous ozone exposure at 1 ppm for 6 days had no effect on β-carotene and lycopene content in tomatoes[15] and ozone exposure at 2 or 4 ppm for 2 min had no effect on lettuce.[35]

As regards the AA levels present in melon samples (Table 1), no significant differences in results were found. The values measured at the start of the test, 38.7 mg 100 g^{-1} FW, remained almost constant until the end of the test (38.4 mg 100 g^{-1} FW for the CK and 36.5 mg 100 g^{-1}

FW for the O_3 sample). Likewise, an increase in AA content was observed in kiwi fruit treated with gaseous ozone at 0.3 ppm and stored at 0°C for 3 months[14] and in strawberries[33] treated with gaseous ozone at 0.3 and 0.35 ppm. On the other hand, AA content was reduced when strawberries were exposed to gaseous ozone at 5000 mg L^{-1} for 6 days,[36] probably due to the high concentration used.

Physiological and technological analyses

As we observed in the trial (data not shown), most studies that used low doses of ozone have revealed that ozone treatment did not result in a higher "stress" respiration rate (tomatoes[37] and peach[38]).

With regard to the ethylene trend during their storage, it was found that there was an inhibitory effect of the gaseous ozone treatment (Fig. 1A). The values of the initial samples were around 19 µL Kg^{-1} h^{-1} and, starting from day 6, the CK exhibited a sharp increase until it reached a maximum value after 13 days, at 83.5 µL Kg^{-1} h^{-1} of ethylene. The samples treated with O_3 remained significantly lower, with values that stayed constant with respect to the initial ones (around 16-18 µL Kg^{-1} h^{-1}). It has been observed that ozone can be used in storage rooms to oxidise ethylene in the air and to reduce postharvest decay,[10] delaying the ripening and senescence process and in this way extending the shelf-life of fruits and vegetables. The efficiency of ozone in ethylene removal, however, was found to be significantly increased at higher doses of ozone or in continuous ozone supply at above 1 ppm[11]. In kiwifruits stored with 0.3 µL L^{-1} gaseous O_3 at 0°C, upon transfer to 20°C after 2 and 4 months of storage the control fruit exhibited a typical climacteric rise in ethylene production, as opposed to ozone treated fruit, which consistently showed basal levels of ethylene production.[13]

Fig. 1B and 1C show that the firmness of the melons stored at 6°C has highlighted that the samples treated with gaseous ozone substantially kept well over time, compared with those of control conditions, which steadily softened. Except for the values measured on day 9 likely due to sample variability, athe data showed significant differences in all the samples taken both in the tests carried out using the penetration meter and using the Instron instrument. The percentage differences between the O_3 condition values with respect to those of the CK condition in the various samplings ($P \leq 0.05$) ranged respectively between the lowest value of 16% on day 9 and a peak of 33% on days 3 and 13 in the assessments made using the penetration meter and between 10% on day 9 and 30% at the end of the test in the assessments carried out using the Instron instrument. The delayed softening of ozone-treated fruit was correlated with the inhibition of ethylene production.[39] A number of authors have

studied the effect of ozone on texture maintenance: some of these found no effect on textural changes in cantaloupe[40] and apple;[10] on the other hand, several studies found texture maintenance in kiwifruit continuously exposed to ozone at 300 ppb,[14] in papaya exposed to ozone at 1.5–3.5 ppm for 4 days,[31] and in tomatoes treated with gaseous ozone at 10 ppm for 10 min,[21] where softening of the fruit, associated with ripening, was delayed in ozone-exposed samples. In other studies on carrots[41] it has been found that treatment with ozonized water caused delayed tissue toughening due to reduced lignification of cell walls.

Cell wall enzyme activities

To investigate the effect of ozone on wall softening, we analysed the changes in cell wall modifying enzymes associated with degradation of pectins: α-ara, β-gal, PG and PME activities. These enzymes have been reported as being key enzymes involved in fruit softening.[42] Ethylene enhances softening during the ripening of climacteric fruits such as peach and Charentais melon by increasing the activities of cell wall hydrolytic enzymes.[43,44]

Cantaloupe Charentais melon represent a model fruit for understanding the molecular events involved in the softening process. Pech *et al.*[45] observed that transgenic suppression of ethylene production has been a powerful tool to discriminate between ethylene-dependent and independent softening events. Antisense ACC oxidase melons exhibit strong reduction of softening, but significant residual softening still persists indicating the presence of an ethylene-independent component in flesh softening. A range of cell wall-related genes polygalacturonases, xyloglucan endotransglycosylase/hydrolases, expansin and β-galactosidases demonstrated that specific genes of each families could be categorized as totally ethylene-dependent, totally ethylene-independent or partially ethylene-dependent.[44] These data suggest the presence of cell wall ethylene regulated genes contributing to the ethylene-dependent softening and genes that are not regulated by ethylene contributing to the ethylene-independent component of softening.[44,45]

Recent studies of kiwifruits[13] showed that the key enzymes of ethylene biosynthesis ACC synthase and ACC oxidase remained at very low levels during ripening in fruit exposed to ozone, suggesting a mechanism by which ozone controls kiwifruit ripening.

For both glycosidases α-ara and β-gal, the kinetics we found showed values higher than those of the control samples compared to those treated with ozone from day 6 of storage and up to the end of the test (Fig. 2A and 2B). α-ara and β-gal degraded cell wall arabino-galactans and galactans, respectively, and the side chains of some pectin backbones, and this degradation resulted in increased pectin solubility and yield cell wall polysaccharides more accessible to

other pectin-degrading enzymes.[46] The softening that occurs during the ripening of many fruits is attributed to the dissolution of the components of the cell wall, in particular of the pectic polysaccharides present in it. In our experiments, the inhibitory action of ozone on these glycosidases slows down the solubilisation process of neutral sugars of the cell wall, contributing to maintaining the fruit's firmness.

PME hydrolysed cell wall pectin methyl esters made pectin susceptible to degradation by PG. PG played an important role in the disassembly of pectin by hydrolysing the α-1,4-glycosidic bonds between galacturonic acid residues in galacturonans.

In our experimentation, the slowing of degradative glycosidases enzymes determined by treatment with ozone combined with similar effects shown in Fig. 2C by the PG kinetics curve, which acts on the fraction of the pectic chains of the cell wall. In particular, it showed significantly different values for treatments with O_3 with respect to the CK in the tests carried out on days 3 and 13: the highest figures were those of the O_3 samples (1.73 and 1.76 μmol g^{-1} FW min^{-1}) while the CK values were respectively 1.01 and 1.26 μmol g^{-1} FW min^{-1}. In the samples taken on days 6 and 9, the PG activity curves of the two samples (O_3 and CK) showed similar values. This last remark may be dependent on the variability of sampling.

Lastly, the analysis of the enzymatic activity of the PME of the melons appeared to not be influenced by the gaseous ozone treatment compared to control samples (Fig. 2D), with values that were found to be rather consistent and uniform in the two conditions during their storage for 13 days.

In winegrapes also Botondi *et al.*[9] studied PME and PG activities in shock ozone gaseous treatments (18h, 1.5 g h^{-1}) and long treatments (4h each day, 0.5 g h^{-1}) prior to or during withering, and they showed a decline of PME activity in all samples (O_3 and CK) and of PG activity only in untreated berries after dehydration. Rodoni *et al.*[21] conducted analyses of the cell wall and found a decreased activity of PME (50% decrease) after 9 days of storage in tomatoes fumigated with ozone at 10 μL L^{-1} for 10 min compared to the untreated sample. These authors suggested that delayed fruit softening might be due to reduced solubilisation and depolymerisation of pectin polysaccharides.

All these considerations lead us to assume that for cantaloupe melon, as for other horticultural crops, ozone can thus perform a dual action on reducing the softening: i) by a direct action on the ethylene hormone, both chemically (oxidation) and metabolically (likely action exerted on ACS and ACO enzymes, precursors of ethylene) that slow the ripening process and by ii) a direct inhibition effect caused by the ozone molecule exerted on the

kinetics of the α-ara, β-gal and PG enzymes that slow the solubilisation of the polysaccharides of the cell walls.

In fact, there is clear evidence in literature that ozone may affect both ripening[14] and enzymes, e.g. through signalling molecules.[47]

Microbiological analyses

The results of the analysis of total mesophilic aerobes showed a trend that remained constant (values around 3.0 log cfu/cm^2) during storage in the control samples, probably influenced by the action of the low temperature. The samples treated with ozone showed a decrease of bacterial growth from day 9, with a significant decrease as it progressed from 3.1 log cfu/cm^2 at the start of the test to 1.6 and 1.7 log cfu/cm^2 respectively after 9 and 13 days of storage. To explain what we have found, we may assume that the gaseous ozone treatment at a relatively low concentration, as used in our experiment, did not exert a direct immediate biocidal action on the cell walls of the bacteria present, but rather a delayed inhibiting action, probably induced by the continuous ozone treatment, with likely selective effects on the duplication and growth of the microbial populations present. The efficiency of ozone treatment in reducing microbial counts on fresh produce depends on the dose of ozone being used (dose–ozone concentration × time of exposure) and on initial microbial counts/inoculum.[48,49]

Lastly, as regards the presence of yeasts and moulds, no differences were found between the experimental treatments, as their values remained constant during the storage around 1.0 log cfu/cm^2.

CONCLUSIONS

Ozone has a positive action on slowing softening of melons during storage at 6°C, and can be seen both by observing the data on the firmness and in the study of the kinetics of the PG, α-arabinopyranosidase, and β-galactopyranosidase enzymes.

The effect of the wall enzymes on preserving the firmness of the fruit seems to be exerted by limiting the production of ethylene in the samples treated with gaseous ozone.

This data, together with the physiological data of ethylene, make it possible to affirm that the use of gaseous ozone technology (0.15 - 0.3 ppm) prolongs storage at 6°C of *Caldeo* cantaloupe melon, without negative effects on the qualitative parameters studied.

Lastly, the ozone treatment performs a partial action on limiting the microbial growth of total mesophiles, exerted only in the last days of storage.

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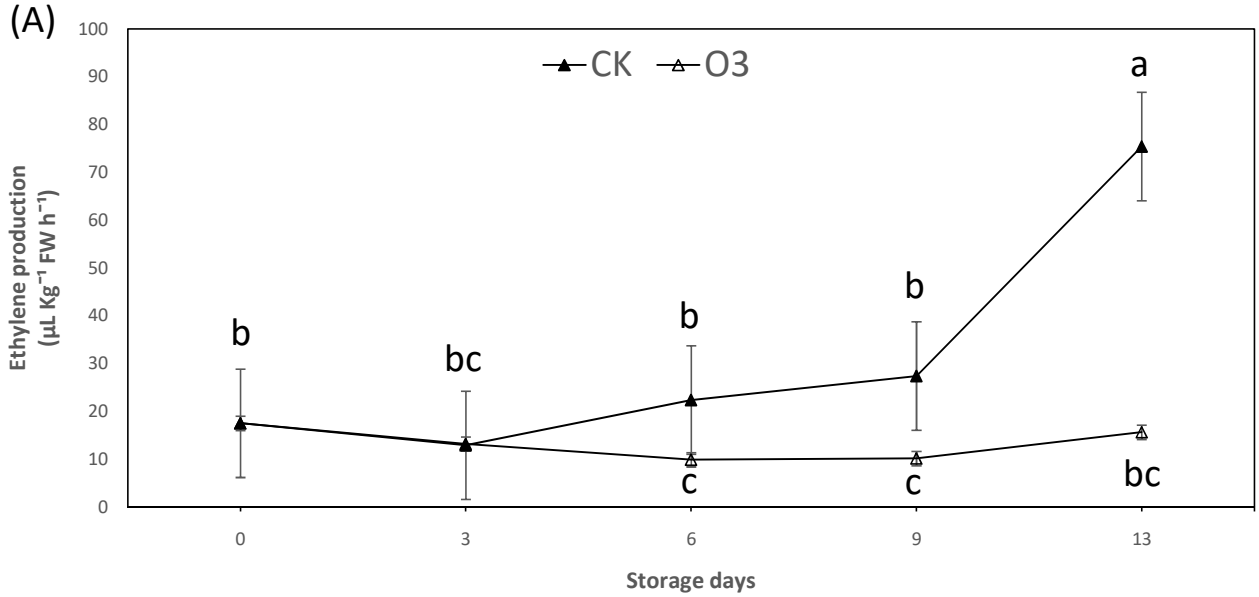
Captions

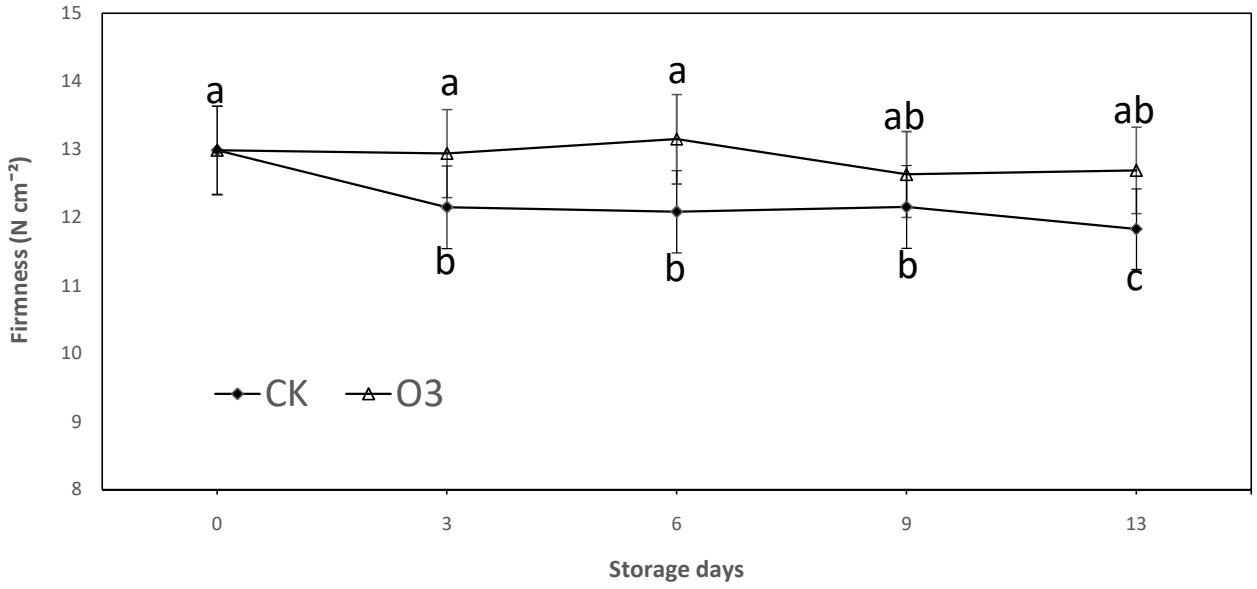
Figure 1. Ethylene production (A), penetrometer force (B) and firmness by Instron (C) of control (Ck) and O_3 melon fruit during cold storage at 6°C. Values are the mean (±SE) of three separate experiments. Vertical bars indicate standard error. Means followed by the same letter are not statistically different ($P < 0.05$).

Figure 2. α arabinopyranosidase (A), β galactopyranosidase (B), polygalactorunase (C) and pectin methyl esterase (D) activities of control (Ck) and O_3 melon fruit during cold storage at 6°C. Values are the mean (±SE) of three separate experiments. Vertical bars indicate standard error. Means followed by the same letter are not statistically different ($P < 0.05$).

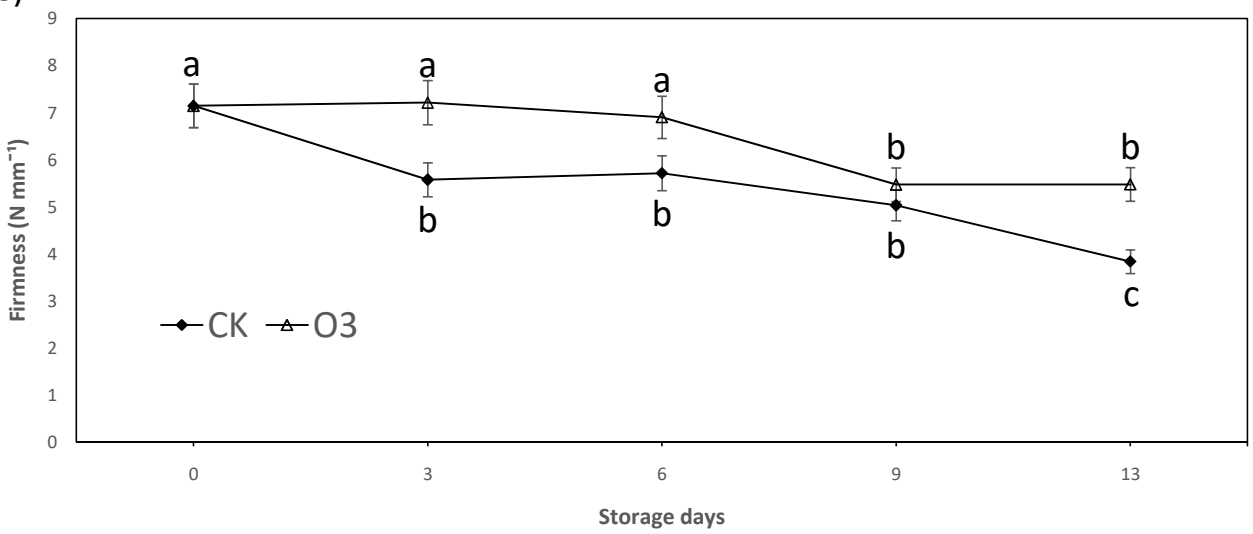
(A)


CK
O3
Ethylene production (μL Kg⁻¹ FW h⁻¹)
Storage days
0
3
6
9
13
0
10
20
30
40
50
60
70
80
90
100
b
bc
b
b
a
c
c
bc

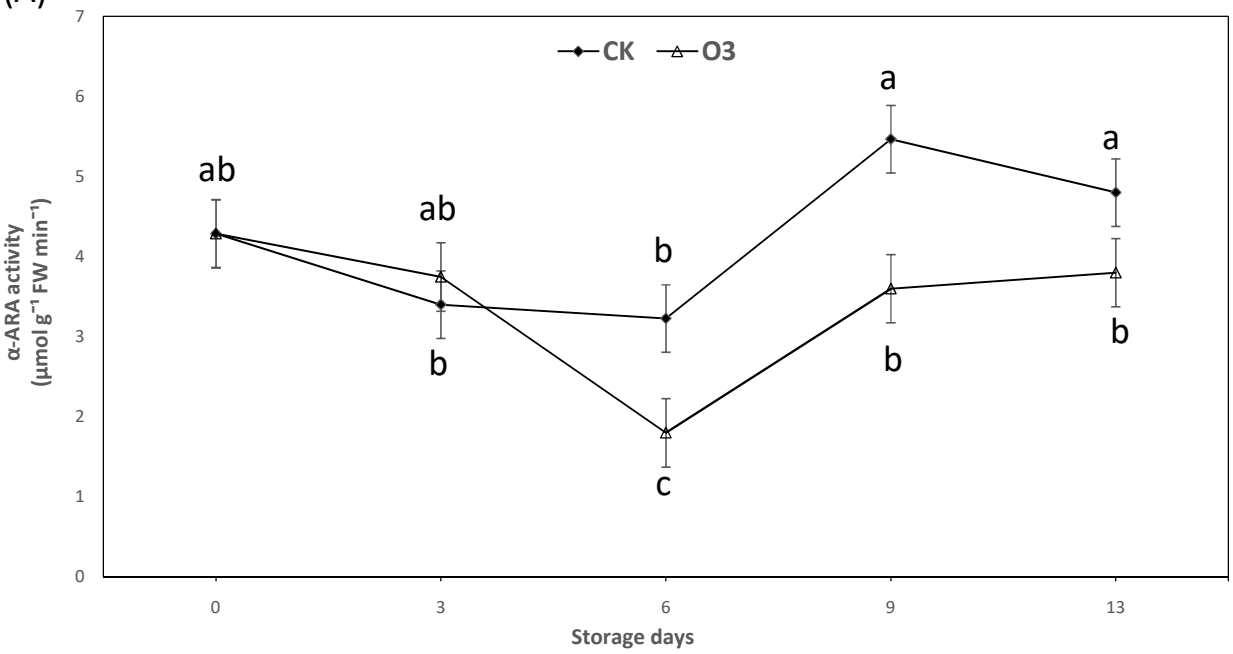
(B)


15
14
13
12
11
10
9
8
Firmness (N cm⁻²)
a
a
a
ab
ab
b
b
b
c
CK
O3
0
3
6
9
13
Storage days

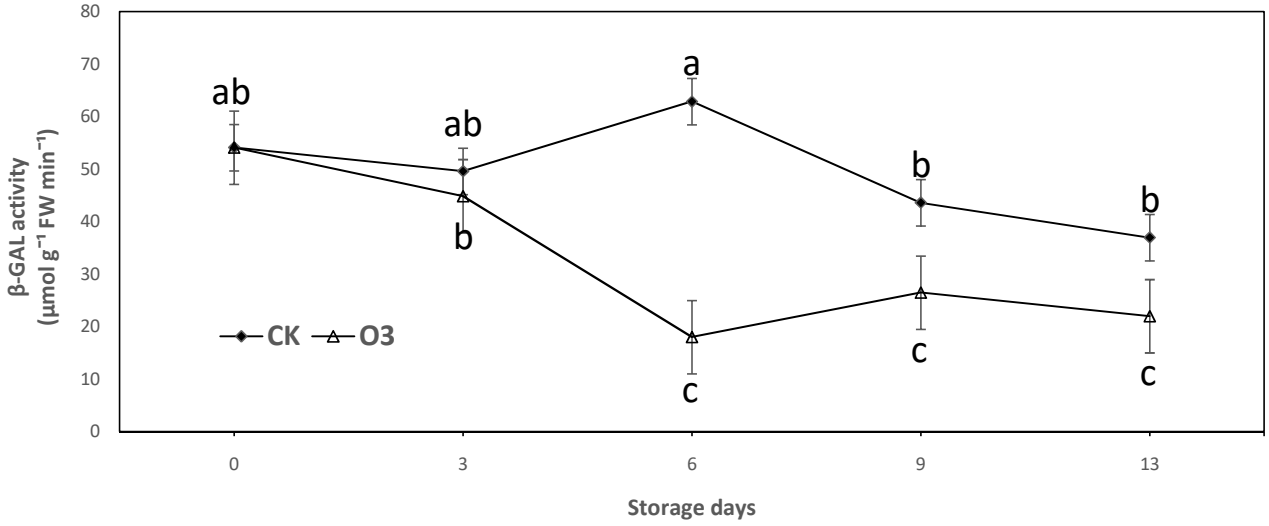
(C)



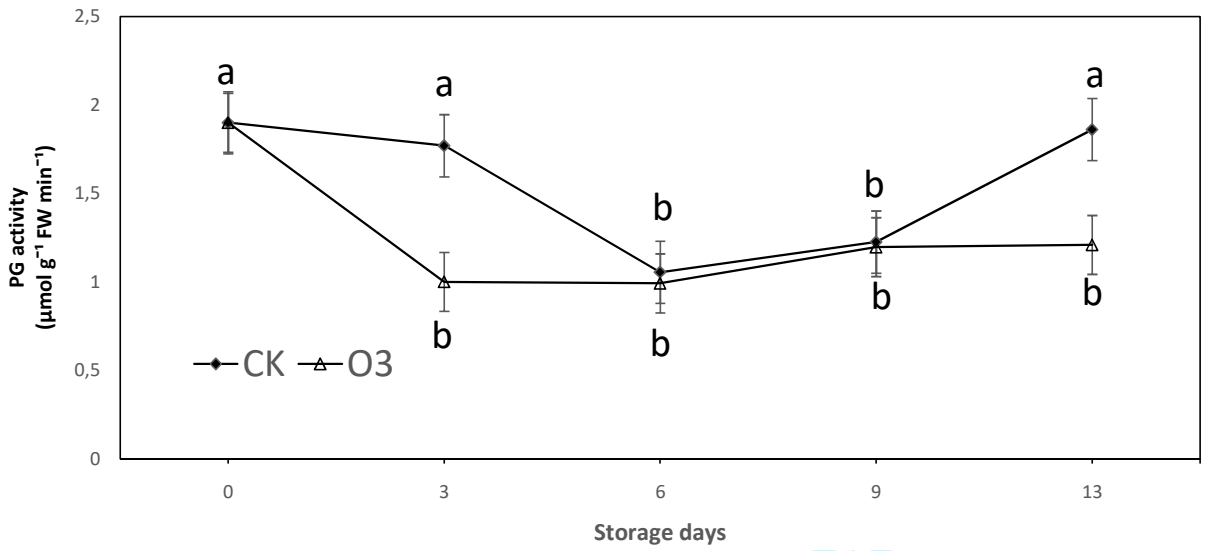
(A)


CK
O3
α-ARA activity (μmol g⁻¹ FW min⁻¹)
Storage days
0
3
6
9
13
ab
ab
b
a
a
b
b
b
c

(B)


β-GAL activity
(μmol g⁻¹ FW min⁻¹)
80
70
60
50
40
30
20
10
0
ab
ab
a
b
b
b
c
c
c
CK
O3
0
3
6
9
13
Storage days

(C)


2,5
2
1,5
1
0,5
0
PG activity (μmol g⁻¹ FW min⁻¹)
a
a
b
b
b
a
b
b
b
CK
O3
0
3
6
9
13
Storage days

(D)

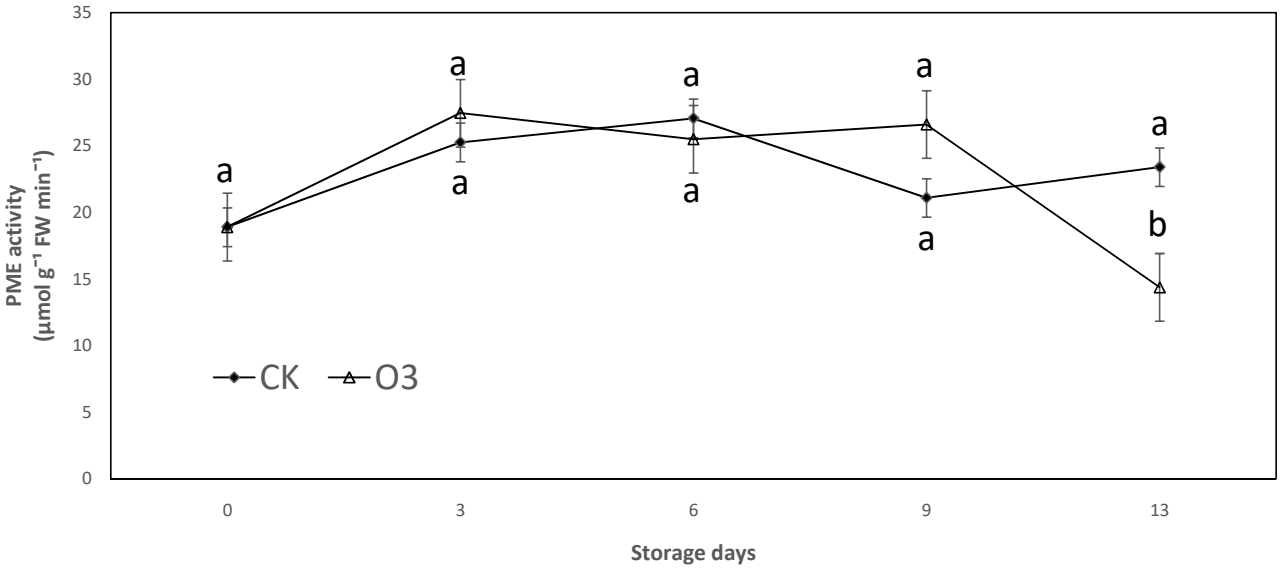

PME activity
(μmol g⁻¹ FW min⁻¹)
35
30
25
20
15
10
5
0
a
a
a
a
a
a
a
a
b
CK
O3
0
3
6
9
13
Storage days

Table 1

Changes in Ascorbic acid content (AA), β carotene, titratable acidity (TA), TSS and color of control (Ck) and O_3 melon fruit during cold storage at 6°C

	Day 0	Day 3		Day 6		Day 9		Day 13	
		CK	O_3	CK	O_3	CK	O_3	CK	O_3
AA (mg $100g^{-1}$ FW)	$38.7^{a}\pm2.5$	$35.5^{a}\pm2.3$	$38.1^{a}\pm1.9$	$39.05^{a}\pm2.9$	$41.05^{a}\pm2.7$	$37.1^{a}\pm2.0$	$38.9^{a}\pm1.7$	$36.4^{a}\pm2.3$	$38.5^{a}\pm1.8$
β carotene (mg Kg^{-1} FW)	$18.6^{ab}\pm2.4$	$15.5^{b}\pm5.1$	$22.7^{ab}\pm5.1$	$15.2^{b}\pm4.1$	$22.5^{ab}\pm4.7$	$22.6^{ab}\pm1.3$	$24.0^{ab}\pm1.3$	$23.5^{ab}\pm2.5$	$27.2^{a}\pm3.6$
TA (mg citric acid $100g^{-1}$ FW)	$42.4^{a}\pm4.2$	$50.2^{a}\pm4.8$	$44.3^{a}\pm3.8$	$43.8^{a}\pm3.5$	$44.1^{a}\pm3.4$	$47.6^{a}\pm4.8$	$47.1^{a}\pm4.8$	$42.0^{a}\pm3.3$	$47.6^{a}\pm3.0$
TSS (°Brix)	$10.5^{a}\pm0.4$	$10.7^{a}\pm0.5$	$11.5^{a}\pm0.5$	$10.5^{a}\pm0.5$	$11.3^{a}\pm0.5$	$9.5^{b}\pm0.6$	$11.6^{a}\pm0.8$	$8.7^{b}\pm0.6$	$10.1^{a}\pm0.4$
Color (*b value)	$29.9^{b}\pm0.8$	$36.3^{ab}\pm2.3$	$38.9^{a}\pm1.8$	$34.6^{a}\pm1.2$	$34.7^{a}\pm1.7$	$29.2^{b}\pm0.9$	$29.3^{b}\pm1.0$	$30.5^{ab}\pm1.5$	$32.5^{ab}\pm1.4$

Data are the mean (±SE) of analyses. Means, in the same group of analysis, followed by the same letter are not statistically different ($P < 0.05$).

Table 2
Mesophilic aerobes and yeasts and molds count (log CFU/cm^2) on the surface of untreated (CK) and treated (O_3) melon fruit during cold storage at 6 °C.

	Day 0	Day 3		Day 6		Day 9		Day 13	
		CK	**O3**	CK	**O3**	CK	**O3**	CK	**O3**
Mesophilic	3.1^{a}±0.2	3.0^{a}±0.1	3.0^{a}±0.3	2.9^{a}±0.2	2.7^{a}±0.2	3.1^{a} ±0.3	1.6^{b}±0.2	3.2^{a}±0.3	1.7^{b}±0.2
Yeasts and molds	1.1^{a}±0.2	0.8^{a}±0.1	1.1^{a}±0.1	1.2^{a}±0.2	0.9^{a}±0.1	1.1^{a}±0.3	0.8^{a}±0.2	1.2^{a}±0.3	1.0^{a}±0.2

Data are the mean (±SE) of three separate experiments. Means, in the same group of analysis, followed by the same letter are not statistically different ($P < 0.05$).