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## Quality retention of fresh-cut pepper as affected by atmosphere gas composition and ripening stage



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#### ARTICLE INFO

Article history:
Received 28 April 2014
Received in revised form
19 August 2014
Accepted 20 August 2014
Available online 6 September 2014

Keywords: Capsicum annuum L. Microbial growth Texture Color Storage

#### ABSTRACT

The responses of fresh-cut (FC) vegetables to  $CO_2$  and  $O_2$  levels depend on their ripening stage and degree of processing. In this work we evaluated the effect of storage under different  $CO_2$  (2.5; 5; 10 and 15 kPa) and  $O_2$  (2.5 and 5 kPa) combinations or air on quality retention of FC green and red pepper. Atmospheres with 15 kPa  $CO_2$  caused physiological injury at both ripening stages. Red pepper strips were less tolerant to  $CO_2$  enrichment within the range 5–10 kPa. Ripe FC peppers were also more sensitive to  $O_2$  reductions below 5 kPa. Marked benefits were obtained at both ripening stages with 5 kPa  $O_2 + 5$  kPa  $CO_2$ . CA-stored strips showed lower spoilage and dehydration and ion leakage. Storage under 5 kPa  $O_2 + 5$  kPa  $CO_2$  was highly effective to maintain the firmness and resistance to bending of the strips. The selected CA caused no alterations in color, acidity, sugars and antioxidants and was effective to maintain lower respiration rate. CA maintained lower counts of mesophilic bacteria, yeasts and molds in red ripe strips.

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## 1. Introduction

Sweet peppers are together with tomato the most popular Solanaceous fruit marketed worldwide (Howard, Hernandez-Brenes, & Villalon, 2000; Marín, Ferreres, Tomás-Barberán, & Gil, 2004). They are consumed at green and red ripe stages either cooked in sauces and prepared foods or minimally processed in salads and snacks (Tadesse, Hewett, Nichols, & Fisher, 2002). Fresh-cut (FC) products are one of the fastest growing segments of the vegetable industry (Clement, 2004). By saving preparation time and reducing wastage, minimal processing improves commodity use convenience (Oms-Oliu et al., 2010). However, processing causes a number of physical and physiological changes which increase perishability (Corbo, Speranza, Campaniello, D'Amato, & Sinigaglia, 2010; Watada, Ko, & Minott, 1996). FC products consequently require a tightly adjusted postharvest management. Proper

management is a sine qua non condition, but even under recommended storage temperatures, deteriorative changes are extremely rapid (Rojas-Graü, Oms-Oliu, Soliva-Fortuny, & Martín-Belloso, 2009; Weichmann, 1986). Modified atmosphere packaging has been recommended for some FC products (Gorny, 2001). Optimal storage atmosphere conditions depend on the type of commodity as well as on its developmental stage and processing degree (Oms-Oliu, Aguiló-Aguayo, Soliva-Fortuny, & Martín-Belloso, 2009). Modified atmosphere packing (MAP) did not result in marked improvement in quality retention of whole peppers (Akbudak, 2008; Koide & Shi, 2007; Saltveit, 1997). However, the results reported in the literature are quite variable. Hypo-oxygenic atmospheres ranged from lack of any benefit (Mercado, Valpuesta, Reid, & Cantwell, 1995) to high decay control (Luo & Mikitzel, 1996). Previous studies evaluating the efficacy of MAP and the effects of CO2 on FC pepper also showed wide variability. González-Aguilar, Ayala-Zavala, Ruiz-Cruz, Acedo-Félix, and Díaz-Cinco (2004) reported that atmospheres reaching 10 kPa CO<sub>2</sub> and 2 kPa O<sub>2</sub> maintained quality for 21 days, whereas in other studies marked deterioration was observed already after 12 days (López-Gálvez, El-Bassuoni, Nie, & Cantwell, 1997). El-Bassuoni and Cantwell (1994) found increased softening and electrolyte leakage in green FC pepper stored under 10 kPa

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CO<sub>2</sub>. In contrast no damage was observed under similar atmospheres by González-Aguilar et al. (2004). Most works conducted to date on MAP of FC peppers have tested one ripening stage and a single gas combination (Howard & Hernandez-Brenes, 1997; Pilon, Oetterer, Gallo, & Stopo, 2006). Moreover, the effects of O<sub>2</sub> on quality maintenance of FC pepper have also received little attention. In this work we evaluated the influence of atmosphere gas composition and ripening stage on quality retention of freshcut red and green pepper.

#### 2. Materials and methods

### 2.1. Plant material

Bell peppers (*Capsicum annuum* L.) at green and red stages were purchased at the Mercado Abastecedor do Porto, Porto, Portugal and immediately transported to the laboratory. Fruit was washed with water containing 100 mg  $L^{-1}$  sodium hypochlorite and adjusted to pH 6.5 with hydrochloric acid, for 3 min. The fruit peduncles, placenta and seeds were removed, and the pericarp was cut into 5  $\times$  1 cm strips and rapidly cooled to 5  $\pm$  0.5 °C.

# 2.2. Experimental setup and selection of optimal atmosphere composition for green and red pepper strips

A gas mixer (MAP Mix 9000, PBI Dansensor, Denmark), coupled to an external buffer tank was used to adjust the desired concentration of  $O_2$ ,  $CO_2$  and  $N_2$ . The following gas combinations were prepared with  $N_2$  used as a balance gas:

```
i. Air (control)
ii. 2.5 kPa CO<sub>2</sub>; 2.5 kPa O<sub>2</sub>
iii. 5 kPa CO<sub>2</sub>; 2.5 kPa O<sub>2</sub>
iv. 10 kPa CO<sub>2</sub>; 2.5 kPa O<sub>2</sub>
v. 15 kPa CO<sub>2</sub>; 2.5 kPa O<sub>2</sub>
vi. 2.5 kPa CO<sub>2</sub>; 5 kPa O<sub>2</sub>
vii. 5 kPa CO<sub>2</sub>; 5 kPa O<sub>2</sub>
viii. 10 kPa CO<sub>2</sub>; 5 kPa O<sub>2</sub>
ix. 15 kPa CO<sub>2</sub>; 5 kPa O<sub>2</sub>
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Prior to flushing each gas mixture was bubbled through water, to increase the relative humidity (RH). The RH inside the jars was recorded with a (Rotronic HygroPalm HP21, Switzerland) and was in all cases between 85 and 93%. One hundred and 50 g of green or red bell pepper strips were placed in 1.9 L glass sealed jars containing two ball valves. Three replicates were prepared for each ripening stage, and gas mixture. The jars were connected to a gas circulation system (5 mL min<sup>-1</sup>) and the fruit was stored for 7 and 10 days at 5 °C in darkness with the gas mixtures indicated above. The headspace from each jar was daily monitored with an O<sub>2</sub>/CO<sub>2</sub> gas analyzer (Check Mate 9900, PBI Dansensor, Denmark). After 0, 7 and 10 days samples were taken and fruit quality was visually evaluated on individual pepper strips, based on the incidence of soft rots and molds, dehydration and softening symptoms by using an intensity scale (0 = excellent; 1 = good; 2 = acceptable; 3 = poor). Strips decayed or having extensive softening were classified as poor. Samples with moderate softening or dehydration but without decay, were categorized as acceptable. Strips showing no marked softening and slight dehydration in the cut surfaces were considered good. Excellent strips showed no visual symptoms of decay or dehydration and remained firm. The deterioration index (DI) was calculated as follows: DI =  $\sum$  (Injury level × Number of fruit strips in this level)/ Total number of strips.

## 2.3. Effect of the selected atmosphere on green and red pepper strips quality

Fresh-cut green and red peppers were prepared as described in section 2.1 and stored in a) air (control) or b) under 5 kPa  $O_2 + 5$  cor 12 days. Five jars containing 30 pepper strips were prepared for each treatment, ripening stage and storage time. The whole experiment was repeated twice. Samples were taken prior to storage after 7 and 12 days and immediately evaluated or otherwise frozen in liquid  $O_2 + 0$ 0 cuntil analysis.

## 2.3.1. Respiration rate

Pepper strips weighing approximately 150 g were placed in hermetically sealed jars. Samples from the headspace were withdrawn through a silicon septum located on each jar with a syringe, at the beginning of the incubation period and after 1 h to allow  $CO_2$  accumulation. Gas samples were evaluated with a gas analyzer (Check Mate 9900  $O_2/CO_2$ ; PBI Dansensor; Denmark). Four jars were analyzed for each storage time, ripening stage and gas treatment. Results were expressed as mg  $CO_2$  kg<sup>-1</sup> h<sup>-1</sup>.

## 2.3.2. Mass loss and soft rots

Five groups of 30 pepper strips were weighed at the beginning of the experiment, and during storage. Mass loss was calculated as  $100 \times (W_i - W_f)/W_i$ , where  $W_i$  and  $W_f$  represented the initial and final sample mass, respectively. Soft rots were visually evaluated on individual strips by using an intensity scale (0 = no soft rots; 1 = incipient soft rots; 2 = moderate soft rots; 3 = severe soft rots). A soft rot index was calculated as:  $SRI = \sum (Injury \ level \times Number \ of fruit strips in this level)/Total number of strips.$ 

## 2.3.3. Color

Surface color was measured on the outer side of the strips with a chroma meter (Model CR-400, Minolta, Osaka, Japan) to obtain CIE  $L^*$ ;  $a^*$ ;  $b^*$  values. The hue angle was calculated as  $180 - \mathrm{tg}^{-1}$   $b^*/a^*$  and  $\mathrm{tg}^{-1}$   $b^*/a^*$  for green and red peppers respectively. Sixty measurements were done for each gas treatment, ripening stage, and storage time.

## 2.3.4. Texture

Texture was evaluated by two different assays using an INSTRON texture analyser (Model 2519-101, INSTRON, USA) with a 10 N load cell. For bending tests, bell pepper strips (5 cm  $\times$  1 cm and 4 mm thick) were horizontally held (1 cm from each end). A probe with circular flat tip (6 mm diameter) was used to displace the middle of the strips at a speed of 7.5 mm s<sup>-1</sup> and the force required for bending (15 mm) was determined. The resistance to deformation was calculated as the slope of the force/time curves. Results were expressed in N s<sup>-1</sup>. Puncture tests were performed on the inner side of the pepper strips by compressing the fruit tissue 2 mm in the middle of the strip, at a rate of 2 mm s<sup>-1</sup> with a 1 mm diameter probe and recording the maximum force developed during the test. Results were expressed in Newton (N). For both assays thirty pepper strips were randomly selected from each jar and evaluated for each gas treatment, ripening stage and storage time.

## 2.3.5. Sugars, pH and acidity

Frozen pulp tissue was processed in a refrigerated mill and 2 g of the resulting powder were extracted with 10 mL of ethanol. The mixture was centrifuged (MPW-350R, Poland) at  $9000 \times g$  for 10 min at 4 °C. Three independent extractions were done for each storage time. Total sugars content was measured using the phenol—sulfuric acid assay (Southgate, 1976) with D-glucose as a standard at 490 nm in a spectrophotometer (Spectronic GENESYS 6,

Thermo Fisher Scientific, MA, USA). Results were expressed as grams of glucose per kilogram of fresh fruit.

For acidity and pH measurements 10 g of fruit were processed in a mill (Multi-mill attached to a Kenwood Major Titanium KM023, Germany) and added to 100 mL of water. Fruit pH was measured potentiometrically and acidity was determined titrimetrically with (NaOH 0.1 mol  $\rm L^{-1}$ ) until pH 8.2. Three measurements were done for each gas treatment and storage time. Results were expressed as  $\rm [H^+]$  mmol per liter.

## 2.3.6. Electrolyte leakage

Two pepper strips of each sample were incubated at 23 °C in 25 mL of distilled water. During incubation, samples were agitated in a shaker (Barnstead, MaxQ SHKA 4000, Iowa, USA) at 100 rpm. The electrical conductivity of the bathing solution was measured before the samples were immersed ( $C_i$ ) and after 5 min ( $C_f$ ) of incubation using a conductivity meter (Eutech, CyberScan CON 510, Singapore). Samples were then homogenized in an Ultraturrax (IKA-Werke, T25 Basic, Germany), centrifuged at  $9000 \times g$ ; 10 min and the conductivity of the supernatant ( $C_T$ ) was measured as previously described. Electrolyte leakage was calculated as  $100 \times (C_f - C_i)/C_T$ 

## 2.3.7. Extractable juice

Three pepper strips, randomly selected from different jars, were compressed against a weighed filter paper  $(W_i)$  (1 kg, 30 s). The strips were removed and the filter paper was weighed  $(W_f)$ . The extracted juice was calculated by determining the weight gain of the filter paper  $(W_f-W_i)$ , and expressed in gram per kilogram of fresh weight. Three replicates were done for each gas treatment and storage time.

## 2.3.8. Phenolic compounds and hydroxycinnamic acid-derivatives

Frozen fruit pulp was ground in a mill with liquid N<sub>2</sub> (Multi-mill attached to a Kenwood Major Titanium KM023, Germany) and 2 g of the resulting powder were poured in 5 mL of cool ethanol. Samples were then centrifuged at  $9000 \times g$  for 15 min at 4 °C. The supernatant was collected and the pellet was re-extracted with 5 mL ethanol and centrifuged as described above. The supernatants were combined to make a final volume of 10 mL. The determination of phenolic compounds was performed according to Obied, Allen, Bedgood, Prenzler, and Robards (2005). Briefly, 1 mL of each fruit extract was mixed with 1 mL of an ethanolic solution containing 27 mmol/L HCl. Subsequently 8 mL an ethanolic solution containing 0.55 mol/L HCl were added to each test tube and the absorbance of the solutions was measured at 280 nm and 320 nm to evaluate total phenolics and hydroxycinnamic acid derivatives respectively. Corresponding standard curves were prepared using ethanolic solutions of gallic acid, and caffeic acid respectively. Results were expressed in mg per kilogram of fresh fruit.

## 2.3.9. Antioxidant capacity

The antioxidant capacity was measured by the phosphomolybdenum method according to Prieto, Pineda, and Aguilar (1999). Briefly, one mL of fruit extract was combined with 1 mL of reagent solution (0.6 mol  $\rm L^{-1}$  sulfuric acid, 28 mmol  $\rm L^{-1}$  sodium phosphate, and 4 mmol  $\rm L^{-1}$  ammonium molybdate). The tubes were incubated at 95 °C for 90 min. The absorbance of the solution was measured at 695 nm against a blank sample. A standard curve with ascorbic acid was prepared and total antioxidant capacity was expressed as mmol of ascorbic acid per kilogram.

## 2.3.10. Microbiological assays

Approximately 25 g of pepper strips were put into two sterilized beakers containing 225 mL of tryptone saline solution (1 g  $\rm L^{-1}$ 

**Table 1** Deterioration index (0 - excellent, to 3 - poor) in green and red ripe bell pepper strips stored at 5  $^{\circ}$ C in air (control) and under different controlled atmospheres (CA): 2.5 or 5 kPa  $O_2$  and 2.5; 5; 10 or 15 kPa  $O_2$  for 7 or 10 days.<sup>a</sup>

| Time at 5 °C (d) | CA composition (O <sub>2</sub> /CO <sub>2</sub> ) (kPa) | Red           | Green        |
|------------------|---|---------------|--------------|
|                  | 21/0  | 0.4hijk       | 1.3ef        |
|                  | 2.5/2.5   | 0.1jk         | 0.2jk        |
|                  | 2.5/5   | 0.1jk         | 0.2jk        |
|                  | 2.5/10  | 0.2jk         | 0.2jk        |
|                  | 2.5/15  | 0.2jk         | 1.5de        |
|                  | 5/2.5   | 0k            | 0.2 <i>i</i> |
|                  | 5/5   | 0k            | 0.1jk        |
|                  | 5/10  | 0k            | 0.3jk        |
|                  | 5/15  | 0.2jk         | 1.6de        |
| 10               | 21/0  | 2.8 <i>a</i>  | 2.7 <i>a</i> |
|                  | 2.5/2.5   | 1.4de         | 1.0fg        |
|                  | 2.5/5   | 1.7cd         | 0.7gh        |
|                  | 2.5/10  | 2.1 <i>bc</i> | 0.4hijk      |
|                  | 2.5/15  | 2.1 <i>bc</i> | 1.8bcd       |
|                  | 5/2.5   | 0.3jk         | 1.6de        |
|                  | 5/5   | 0.5hij        | 0.7ghi       |
|                  | 5/10  | 0.7ghi        | 0.4ijk       |
|                  | 5/15  | 1.5de         | 2.2b         |
| Pooled SD        | 0.1   |               |              |

<sup>&</sup>lt;sup>a</sup> Values followed by different letters indicate differences on a Fisher test at a level of significance of  $\alpha \leq 0.05$  (n=3).

tryptone and 5 g L<sup>-1</sup> NaCl). Samples were stirred for 10 min and from each beaker a series of decimal dilutions was prepared. One mL from appropriate dilutions was poured in triplicate in plate count agar (PCA) and yeast extract, glucose, chloramphenicol agar (YGC), media for bacteria and yeast and molds counts respectively. The plates were incubated at 30 °C for aerobic mesophilic bacteria and at 20 °C for molds and yeast. Results were expressed as log of colony forming units (CFU) per gram of fresh fruit.

## 2.4. Statistical analyses

Results were analyzed by ANOVA with the SAS software package (SAS, 2001). The model assumptions of homogeneity of variance and normality were probed by means of Levene's and Shapiro–Wilk's tests, respectively. Variance (var) and degree of freedom (df) of each treatment, time of storage, state of maturity and parameter analyzed was calculated. Pooled standard deviation (Pooled SD) for all measurement physical determination was calculated. Means were then compared by a Fisher test at  $\alpha \leq 0.05$ .

#### 3. Results and discussion

# 3.1. Determination of optimal atmosphere composition for fresh-cut peppers

Juice exudate, softening and decay were the main deterioration symptoms observed during storage. Increasing CO<sub>2</sub> in the range 2.5–10 kPa reduced visual quality loss in green fruit, but was not beneficial in red peppers (Table 1). Levels of 5 kPa CO<sub>2</sub> were tolerated by both green and ripe fruits and no evidence supported that red peppers are more tolerant to high CO<sub>2</sub> as previously reported (Mercado et al., 1995). Indeed, 10 kPa of CO<sub>2</sub> reduced the benefits obtained and should not be recommended for ripe fruit. Results after 10 days of storage suggested that regardless of the ripening stage, atmospheres with 15 kPa CO<sub>2</sub> resulted in fruit physiological injury.

The effect of  $O_2$  levels on the storage atmosphere of FC peppers has received almost no attention to date and most studies performed in CA-MAP of minimally processed peppers have evaluated a single  $O_2$  concentration. The outcome of changing  $O_2$  partial

pressure is not clear in whole pepper. In some cases reducing  $O_2$  resulted in no benefits (Mercado et al., 1995), while other studies marked decay control was observed below 3 kPa (Luo & Mikitzel, 1996). Conesa, Verlinden, Artés-Hernández, Nicolaï, and Artés (2007) found that storage of FC peppers in 3 kPa  $O_2$  induced fermentation. However, in the present work we did not detect off-flavors even at the lowest  $O_2$  level. The responses to low  $O_2$  varied depending on the ripening stage. No marked differences between 2.5 and 5 kPa of  $O_2$  were recorded in unripe peppers. In contrast, greater benefits were found in red fruit with 5 kPa  $O_2$  (Table 1). To further characterize the CA-induced changes in the nutritional, physical, chemical and microbiological storage we selected the atmospheres with 5 kPa  $O_2$  + 5 kPa  $CO_2$ .

## 3.2. Effect of the selected atmosphere on quality retention of green and red pepper strips

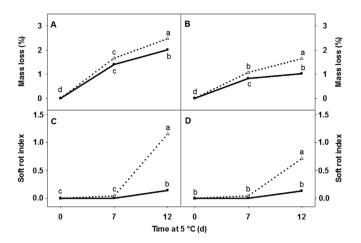
## 3.2.1. Mass loss and soft rots

Previous studies have shown that whole red peppers are less susceptible to dehydration than green fruit. This is likely due to full cuticle development and higher wax deposition in ripe fruit (Díaz-Pérez, Muy-Rangel, & Mascorro, 2007). In contrast to what occurs in whole fruit FC red fruit showed higher susceptibility to dehydration than unripe peppers. CA-storage reduced mass loss at both ripening stages (Fig. 1A and B). Green peppers strips stored in CA showed lower mass loss than the control already at day 7. After 12 days of storage, the dehydration of control red and green peppers was 20% and 40% higher than that of CA-stored strips.

Green peppers showed lower susceptibility to soft rots than red fruit. After 12 days green and red CA-stored fruit showed the 5 and 8 fold lower soft rot index than the controls (Fig. 1C and D). Storage under 5 kPa  $CO_2$  and 5 kPa  $O_2$  markedly reduced soft rots and dehydration of pepper strips at both green and red stages. This indicates that FC pepper strips could significantly benefit from CA storage.

#### 3.2.2. Respiration rate, extractable juice and electrolyte leakage

Immediately after processing the respiration rates were 11 and 8 mg kg<sup>-1</sup> h<sup>-1</sup> for red and green peppers (Table 2). After 1 day the respiration of CA stored green and red pepper slices was lower than that of the controls. Similar results were reported in low O<sub>2</sub> CA by Conesa et al. (2007). Reduced respiration rate of FC pepper may be caused by a drop in the intracellular pH (Bown, 1985). In control red fruit the RR increased steadily during storage. The respiration rate of green peppers showed no variation during the first week of storage, but rapidly increased afterward. After 12 days the respiration rate of fruit held in CA was three to four folds lower than that of the control. Similar results were found in other CA-stored commodities (Escalona, Verlinden, Geysen, & Nicolaï, 2006).



**Fig. 1.** Mass loss (A, B) and soft rot index (C, D) in red (A,C) and green (B,D) pepper strips stored at 5 °C in air (control,  $-\Delta$ –) or under 5 kPa O<sub>2</sub> and 5 kPa CO<sub>2</sub>, ( $-\Phi$ –) for 7 or 12 days. Values followed by different letters indicate differences based on a Fisher test at a level of significance of  $\alpha \le 0.05$  (n = 5).

The extent of tissue damage was also assessed through the evaluation of fruit electrolyte leakage and tissue extractable juice (Table 2). Storage in CA prevented the increase of electrolyte leakage in both green and red peppers strips. After 7 days at 5 °C, air-stored fruit already showed higher electrolyte leakage than peppers maintained in CA. The differences were even more dramatic at the end of the storage period. Extractable juice increased markedly at the last sampling date in the control but remained unchanged in CA-stored pepper strips, suggesting an improved maintenance of tissue integrity.

## 3.2.3. Texture

No significant differences in firmness were found between green and red pepper strips at day 0 (Table 2). However, unripe peppers softened less than red strips during storage. Howard and Hernandez-Brenes (1997) reported moderate textural changes in stored FC jalapeño. After 12 days, CA-stored green and red peppers remained firmer than the corresponding controls. The bending resistance of the green pepper strips before storage was higher than that of the red strips. After 7 days control red peppers showed lower bending resistance than fruit in CA. At day 12 also green peppers under 5 kPa CO<sub>2</sub> + 5 kPa O<sub>2</sub> showed higher (*ca.* 40%) bending resistance than air-stored fruit (Table 2). Texture loss of peppers has been mainly related to dehydration since wall metabolism is more restricted than in other commodities (Jen & Robinson, 1984; Toivonen & Brummell, 2008). Bending resistance correlated better with mass loss than with softening.

**Table 2**Extractable juice, electrolyte leakage, respiration rate, firmness and bending resistance of red and green pepper strips stored at 5 °C in air (control) or under 5 kPa O<sub>2</sub> and 5 kPa CO<sub>2</sub> (CA) for 7 or 12 days.<sup>a</sup>

| Storage time at 5 °C (d) |         | Extractable juice (g kg <sup>-1</sup> ) |              | Electrolyte leakage (%) |              | Respiration rate (mg kg <sup>-1</sup> h <sup>-1</sup> ) |              | Firmness (N) |        | Bending resistance (N s <sup>-1</sup> ) |             |
|--------------------------|---------|---|--------------|-------------------------|--------------|---|--------------|--------------|--------|---|-------------|
|                          |         | Red                                     | Green        | Red                     | Green        | Red   | Green        | Red          | Green  | Red                                     | Green       |
| 0                        | -       | 1.9b                                    | 2.8b         | 7de                     | 7de          | 11cde   | 8de          | 3.1 <i>c</i> | 3.6a   | 7d                                      | 16a         |
| 1                        | Control | _                                       | _            | _                       | _            | 15 <i>bc</i>  | 10 <i>de</i> | _            | _      | _                                       | _           |
|                          | CA      | _                                       | _            | _                       | _            | 7e  | 5e           | _            | _      | _                                       | _           |
| 7                        | Control | 2.6b                                    | 4.7 <i>b</i> | 12 <i>c</i>             | 8de          | 21 <i>b</i>   | 12cd         | 2.6d         | 3.5 ab | 6d                                      | 14b         |
|                          | CA      | 2.2b                                    | 1.6 <i>b</i> | 10 <i>cd</i>            | 5e           | 11 <i>d</i>   | 6e           | 2.6d         | 3.5 ab | 7d                                      | 15 ab       |
| 12                       | Control | 23.6a                                   | 27.6a        | 20a                     | 17 <i>b</i>  | 35a   | 35a          | 2.4 <i>e</i> | 3.3b   | 6d                                      | 10 <i>c</i> |
| CA                       | CA      | 4.7b                                    | 6.4b         | 8de                     | 10 <i>cd</i> | 9cde  | 14bcd        | 2.8d         | 3.6a   | 7d                                      | 14b         |
| Pooled SD                |         | 0.5                                     |              | 2                       |              | 2   |              | 0.4          |        | 2                                       |             |

<sup>&</sup>lt;sup>a</sup> Values followed by different letters indicate significant differences on a Fisher test at a level of significance of  $\alpha \le 0.05$  (n = 75 for firmness and bending resistance, n = 4 for respiration rate and n = 3 for extractable juice, electrolyte leakage).

**Table 3**Surface color (*Hue*), lightness (*L*\*), sugars, acidity, pH, antioxidant capacity, total phenols and hydroxycinnamic acids (HCA) in red and green pepper strips stored at 5 °C in air (control) or under 5 kPa O<sub>2</sub> and 5 kPa CO<sub>2</sub> (CA) for 7 or 12 days.<sup>a</sup>

|   |         | Time at 5 °C (d) |               |              |               |              |             | Pooled SD |
|---|---------|------------------|---------------|--------------|---------------|--------------|-------------|-----------|
|   |         | 0                |               | 7            |               | 12           |             |           |
|   |         | Red              | Green         | Red          | Green         | Red          | Green       |           |
| L*  | Control | 28f              | 31cd          | 29e          | 34a           | 30de         | 34a         | 3         |
|   | CA      | 28f              | 31 <i>cd</i>  | 29 <i>e</i>  | 34 <i>a</i>   | 32 <i>bc</i> | 33 ab       |           |
| Hue   | Control | 32 <i>b</i>      | 126a          | 30 <i>c</i>  | 124a          | 31 <i>c</i>  | 125a        | 3         |
|   | CA      | 32 <i>b</i>      | 126a          | 30 <i>c</i>  | 124a          | 30 <i>c</i>  | 126a        |           |
| Sugars (g kg <sup>-1</sup> )                      | Control | 39a              | 21 <i>b</i>   | 37a          | 21 <i>b</i>   | 40a          | 20 <i>b</i> | 3         |
|   | CA      | 39a              | 21 <i>b</i>   | 41a          | 21 <i>b</i>   | 40a          | 19 <i>b</i> |           |
| Acidity ([H <sup>+</sup> ] mmol L <sup>-1</sup> ) | Control | 28 <i>b</i>      | 11 <i>d</i>   | 29 <i>b</i>  | 11 <i>d</i>   | 25 <i>c</i>  | 13 <i>d</i> | 1         |
|   | CA      | 28 <i>b</i>      | 11 <i>d</i>   | 33a          | 11 <i>d</i>   | 24 <i>c</i>  | 12 <i>d</i> |           |
| pH  | Control | 5.25e            | 5.81 <i>b</i> | 5.21e        | 5.62 <i>c</i> | 5.30e        | 5.86ab      | 0.08      |
|   | CA      | 5.25e            | 5.81 <i>b</i> | 5.00f        | 5.72bc        | 5.43d        | 6.01a       |           |
| Antioxidants (mmol kg <sup>-1</sup> )             | Control | 7.3a             | 4.3b          | 6.9a         | 4.1 <i>b</i>  | 6.7a         | 4.6b        | 0.5       |
| , ,   | CA      | 7.3a             | 4.3b          | 6.7 <i>a</i> | 3.7 <i>b</i>  | 7.5a         | 4.6b        |           |
| Total phenols (mg kg <sup>-1</sup> )              | Control | 193a             | 122e          | 176bc        | 145d          | 177abc       | 178ab       | 10        |
|   | CA      | 193a             | 122e          | 158 <i>d</i> | 149d          | 160cd        | 158d        |           |
| HCA (mg kg <sup>-1</sup> )                        | Control | 106ab            | 84 <i>c</i>   | 95abc        | 90 <i>bc</i>  | 99abc        | 111a        | 6         |
|   | CA      | 106ab            | 84c           | 85c          | 93 <i>bc</i>  | 82d          | 100abc      |           |

<sup>&</sup>lt;sup>a</sup> Values followed by different letters indicate differences on a Fisher test at a level of significance of  $\alpha \le 0.05$  (n = 60 for  $L^*$  and Hue; n = 3 for sugars, acidity, pH, antioxidants, total phenols and HCA).

## 3.2.4. Color, sugars, acidity, antioxidant capacity and phenolic compounds

Fruit lightness ( $L^*$ ) increased during storage of ripe pepper strips (Table 3) while the hue decreased indicating continued surface reddening. No change in surface color was detected in green fruit. Sugar content was 40 g/L in red fruit and 20 g/L in green peppers. Acidity was approximately three fold higher in red peppers, and tissue pH values were 5.2 and 5.8 for red and green fruit respectively. Sugars, pH and acidity were not markedly affected by the storage atmosphere. The total antioxidant activity was 60% higher in red peppers than in green fruit. The main hydrophilic antioxidant in pepper is ascorbic acid, the concentration of which increases during ripening in most cultivars (Howard et al., 2000). No marked changes in total antioxidant capacity occurred during storage or in response to CA. Total phenolics (TP) were 193 and 122 mg kg<sup>-1</sup> in red and green strips respectively (Table 3). Hydroxycinnamic acids (HCAs) represented 50-70% of TP and in agreement with Howard et al. (2000), were also higher in ripe fruit. After 12 days of storage, both TP and HCAs were lower in CA-stored red strips than in the controls. Tissue damage and senescence has been associated with increased accumulation of phenolic compounds (Beltrán, Selma, Martín, & Gil, 2005; Mateos, Ke, Cantwell, & Kader, 1993).

#### 3.2.5. Microbiological counts

CA storage prevented the increase in bacterial counts during storage in both green and red peppers strips (Table 4). CA also had

**Table 4** Aerobic mesophilic bacteria and molds and yeast in red and green pepper stripes stored at 5  $^{\circ}$ C and 90–95% RH in air (control) or under 5 kPa O<sub>2</sub> and 5 kPa O<sub>2</sub> (CA) during storage for 7 or 12 days.<sup>a</sup>

| Storage time at 5 °C (d) | Bacteria<br>(Log CFU g <sup>-1</sup> ) |              |                | Molds and yeast<br>(Log CFU g <sup>-1</sup> ) |               |                |                |                |
|--------------------------|--|--------------|----------------|---|---------------|----------------|----------------|----------------|
|                          | Red                                    |              | Green          |   | Red           |                | Green          |                |
|                          | Air                                    | CA           | Air            | CA  | Air           | CA             | Air            | CA             |
| 0                        | 3.9f                                   | 3.9f         | 3.9f           | 3.9f  | 3.4fg         | 3.4fg          | 3.3g           | 3.3g           |
| 7<br>12                  | 5.9c<br>7.3a                           | 4.3e<br>5.1d | 6.8ab<br>7.2ab | 5.4cd<br>6.6b                                 | 5.2de<br>7.2a | 3.7fg<br>4.4ef | 5.6cd<br>6.6ab | 4.7de<br>6.2bc |
| Pooled SD                | 0.3                                    |              |                |   | 0.2           |                |                |                |

<sup>&</sup>lt;sup>a</sup> Values followed by different letters indicate significant differences based on a Fisher test at a level of significance of  $\alpha \le 0.05$  (n=2).

some positive effects in controlling yeasts and molds in ripe fruit. In contrast, no differences were detected between CA- and air-stored green pepper strips. Overall, storage of FC peppers under 5 kPa  $CO_2 + 5$  kPa  $O_2$  might be useful to control microbial populations. The effects were more beneficial in the case of ripe strips. The effect of CA in bell peppers on microbial growth has not been studied in detail to date. In general for whole peppers it is accepted that moderate effects are observed in the control of deteriorative organisms (Akbudak, 2008; Koide & Shi, 2007; Saltveit, 1997). In the present study storage under 5 kPa  $CO_2 + 5$  kPa  $O_2$  reduced decay and bacterial multiplication. Luo and Mikitzel (1996) reported that decreasing O<sub>2</sub> alone was effective to decrease decay. Further work is needed to determine whether or not the lower bacterial population maintained in CA stored fruit resulted from a direct action of the storage atmosphere on the microorganisms or through an indirect effect on tissue integrity which would result in lower nutrients availability.

## 4. Conclusions

Although CA and MAP are not recommended for storage of whole green and red peppers, marked benefits could be obtained in FC bell peppers. Storage under 5 kPa  $O_2 + 5$  kPa  $CO_2$  was highly effective to reduce ion leakage, control soft rots, delay softening and maintain lower metabolic activity. CA stored red pepper strips also maintained in lower counts of aerobic mesophilic bacteria, yeasts and molds. Storage under 5 kPa  $O_2 + 5$  kPa  $CO_2$  resulted in no negative alterations in fruit color, sugars acidity or antioxidant capacity. Atmospheres with 15 kPa  $CO_2$  damaged both green and red fruit and should not be used for FC peppers. Although similar atmosphere combinations are usually recommended for FC peppers regardless of the ripening stage, results showed that red strips were less tolerant to  $CO_2$  enrichment in the range of 5-10 kPa  $CO_2$  and to  $CO_2$  reductions below 5 kPa. This may be useful for the development of MAP in the FC pepper industry.

## Acknowledgments

The authors thank the CONICET (PIP-0353) and the Agencia Nacional de Promoción Científica y Tecnológica (PICT 2009-0059) for financial support. Authors also acknowledge EuroTANGO II, EMACTION 2 Strand 1 Programme Doctoral Sandwich Mobility grant

given to L.M. Rodoni, and acknowledge Fundação para a Ciência e a Tecnologia for financial support through grant no. PEst-C/EQB/LA0006/2013.

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