Influence of modified atmosphere and ethylene levels on quality attributes of fresh tomatoes (*Lycopersicon esculentum* Mill.)

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Abstract

Controlling storage atmosphere is a key factor for delaying postharvest fruit quality loss. The objective of this study is to evaluate its influence on physico-chemical, sensorial and nutritional quality attributes of two tomato fruit cultivars (Delizia and Pitenza) that respectively have a short- and long-storage life. To that end, the effect of two types of bags with different gas permeability, combined or not with an ethylene sorbent, on tomato organoleptic and nutritional properties were compared during fruit storage at 13 °C. CO₂ and O₂ were critical factors for controlling tomato postharvest behaviour. Weight loss, firmness, color and visual quality were only affected by bag permeability just as total sugar content and acidity for Pitenza tomatoes. (*trans*)-2-Hexenal also appears to be related with CO₂ and O₂ levels. Lycopene, total phenols (TP) and ascorbic acid (AA) contents were also affected by the packaging form and the storage length. Ethylene removal in combination with MAP led to a higher content in TP and AA in the short-life tomato cultivar.

Key words: ascorbic acid, ethylene, lycopene, modified atmosphere, phenolic compounds, quality.

Chemical compounds cited in this article:

Lycopene (PubChem CID: 446925); L-ascorbic acid (PubChem CID: 23667548)

1. Introduction

Tomato fruit (*Solanum lycopersicum* L.) is widely cultivated and consumed around the world as a fresh or processed product. Tomato is considered a climacteric fruit in which ripening is accompanied by an increase in respiration and ethylene production, which entails quality loss through physicochemical changes such as softening and color evolution (Akbulut, Akbulut, Seniz & Eris, 2007).

Modified atmosphere packaging (MAP) is particularly useful for storing perishable crops. MAP can reduce the respiration rate and metabolic activity, humidity loss and microbial growth during commercial handling (Kader & Watkins, 2000). The advantages of MAP depend on the variety, ripening stage as well as temperature and storage length. MAP achieves significant benefits when plant products are maintained under optimal temperature, humidity and atmosphere composition conditions (Zhuang, Barth, & Cisneros-Zevallos, 2014). When the O₂ and CO₂ levels are not adequate, an irregular maturation can occur, causing the appearance of undesired flavors and odors, physiological disorders as well as increased decay caused by pathogenic fungi.

The importance of ethylene in fruit ripening and its effects on quality of perishable crops are also well known (Yang & Hoffman, 1984; Lelièvre, Latché, Jones, Bouzayen & Pech, 1997; Salveit, 1999). These effects depend on the cultivar, tissue sensitivity to ethylene, exposure duration, ethylene concentration as well as atmospheric composition and temperature (Salveit, 1999). Since ethylene may accelerate ripening and senescence, it is necessary to minimize the exposure to ethylene for climacteric fruits like tomato during fruit postharvest handling and storage. This is especially important in those cultivars with a short-storage life.

Ethylene biosynthesis and action can be blocked by chemical compounds acting in the pathway of ethylene biosynthesis or at the hormone receptor level (Yang & Hoffman,

1984; Bregoli et al., 2002). Furthermore, the use of ethylene sorbents reduces its concentration in surrounding fruit and vegetables in storage areas and different studies are being conducted aimed at obtaining active materials (Keller, Ducamp, Robert & Keller, 2013).

In tomato fruit, the efficacy of 1-methylcyclopropene (1-MCP) as an inhibitor of ethylene action delaying the ripening-related processes has been evidenced (Guillén, Castillo, Zapata, Martínez-Romero, Valero & Serrano, 2006). In fact, it has been demonstrated that 1-MCP reduces both ethylene production and respiration rate and in turn retards fruit ripening (Guillén, Castillo, Zapata, Martínez-Romero, Serrano & Valero, 2007).

On the other hand, it has been reported that an adsorbent-catalyst device including activated carbon-1% Pd leads to preservation of tomato postharvest quality by reducing the ethylene concentration in storage areas (Martínez-Romero, Guillén, Castillo, Zapata, Valero & Serrano, 2009). When carbon-1% Pd was used in combination with MAP it also exerted a positive effect on the postharvest quality of tomato, decreasing the weight loss as well as slowing down the firmness loss and color evolution. Previous results also showed that volatile compounds were reduced in MAP-packages, an effect that was enhanced by adding the ethylene adsorbent material (Bailén, Guillén, Castillo, Serrano, Valero & Martínez-Romero, 2006). In addition to these results, more studies are required in order to find the most appropriate conservation atmosphere for prolonging the shelf life of more perishable fruit and it would also be relevant to know the influence that CO₂, O₂ and C₂H₄ level exert on the nutritional quality of the packaged products.

To provide greater information, the aim of this paper was to study the effect of packages with different permeability to O₂, CO₂ and ethylene alone, or in combination with an ethylene sorbent, on tomato quality, including physico-chemical and sensory attributes,

as well as important nutritional aspects including the contents of lycopene, the most abundant carotenoid in tomato fruit, ascorbic acid and total phenols. To know whether such effects depend on the variety, two tomato cultivars showing different storage lives were employed in the study.

2. Materials and methods

2.1. Plant material

The influence of the packaging system was studied in two tomato (*Lycopersicon esculentum* Mill.) cultivars, Delizia (Raf type) and Pitenza with a short- and long-storage life, respectively. The tomato fruit were grown in greenhouses under Mediterranean climate conditions located in Nijar (Nijar, Almería, Spain). Each tomato cultivar was harvested at the corresponding commercial maturity; breaker for Delizia and red for Pitenza tomatoes (USDA, 1999). The fruits were delivered to the laboratory and sorted for uniformity according to their shape, size and color.

2.2. Storage conditions

Randomly chosen fruits, eight in the case of Pitenza and five for Delizia (ca. 1 kg) cultivars were packaged in polypropylene bags (0.025 mm thickness) of 270 × 350 mm size as a replicate. In order to evaluate the effect of different conservation atmospheres on postharvest performance of tomato fruits, non-perforated bags of polypropylene (PP), as well as bags including 4 perforations by a syringe needle (22G ~ 0.7 mm diameter), were employed. Moreover, the ethylene concentration inside the bags was reduced by introducing a commercial ethylene sorbent which was kindly supplied by Keepfresh (Madrid, Spain). It consisted of a sachet containing 2.30 g of active material (KMnO₄ impregnated on a porous material, the composition was not provided by the supplier).

This experimental design was used to evaluate four packaging systems: PP and perforated PP (PPpf) bags without the ethylene sorbent, and the same samples with the active material, labelled as PPA (non-perforated + sorbent) and PPpfA (perforated + sorbent). In parallel, unpackaged tomatoes were stored as control samples. Permeance values as well as details related to the determination of gas permeation rates through the bags are provided in Supplementary material section.

For each determination, three biological replicates per treatment, cultivar and sampling period were used. The quality parameters were analysed at the beginning of the experiment and after 5 and 9 days for Delizia, and after 8, and 16 days for Pitenza tomatoes, in accordance with the tomatoes shelf life and preliminary experiments. All fruits were stored at $13\text{ }^{\circ}\text{C} \pm 0.5\text{ }^{\circ}\text{C}$ and 65 % RH. Before the analysis, the packages were opened and the fruit kept in air atmosphere for 48 h at $20\text{ }^{\circ}\text{C} \pm 0.5\text{ }^{\circ}\text{C}$ and 65 % RH to simulate shelf-life (SL).

2.3. Gas composition

Gas samples were analyzed after 3 and 7 days for Delizia (short-storage life cultivar), and after 6, and 14 days for Pitenza (long-storage life) tomatoes, immediately before opening the bags and transferring the tomatoes at $20\text{ }^{\circ}\text{C}$.

To determine the O_2 and CO_2 contents inside the bags, 15 mL of the headspace atmosphere was withdrawn using a gas syringe and injected into a Checkmaster 2000 analyzer (Lippke, Germany). To facilitate sample extraction a silicone septum was provided on the bag surface. Results were expressed as % O_2 and % CO_2 inside the bags.

The ethylene analysis was performed by injecting 1 mL samples from the bags in a gas chromatograph. The gas chromatograph (6850 Series II, Agilent Technologies, USA) was equipped with a flame ionization detector (FID) and an Ultrabond

semicapillar column (Restek, Teknokroma, Barcelona) of 30 m length and 530 μm i.d. Oven and injector temperatures were 100 and 80 $^{\circ}\text{C}$, respectively. Helium was used as a carrier gas at a flow rate of 3.2 mL min^{-1} . Results were expressed as $\mu\text{L L}^{-1}$ ethylene inside the bags.

Rates of carbon dioxide (CO_2) and ethylene (C_2H_4) produced by the fruits were also determined. Three replicate biological samples were analysed per cultivar. In each replicate, three fruits were incubated in 2 L sealed glass jars at 13 $^{\circ}\text{C}$ for 2 h. One mL of gas was withdrawn from the headspace of the jars and injected in the gas chromatograph either for CO_2 or for ethylene analysis. Ethylene analysis was carried out as indicated above and the CO_2 analysis was performed in a gas chromatograph (5890 Series II, Hewlett Packard HP, USA) equipped with a Chemosorb 102 80/100 mesh column (Supelco, Teknokroma, Barcelona) of 0.30 m length, 3.2 mm in diameter and a thermal conductivity detector (TCD). Oven and injector temperatures were 100 and 80 $^{\circ}\text{C}$, respectively. Results were expressed as $\text{mL kg}^{-1} \text{ h}^{-1}$ and $\text{nmol kg}^{-1} \text{ s}^{-1}$ for CO_2 and ethylene production respectively.

2.4. Physico-chemical and sensorial quality

Tomato surface color was analysed by measuring Hue angle (h°) with a Minolta chromameter (CM – 3500d, Konica Minolta Sensing, Inc., Japan) at three different points located in the equatorial area. Fruit firmness was determined using a Texture analyser (TA-XT-Plus, Stable Micro System, Surrey, UK) equipped with two flat plates. The force (N) required to cause a deformation of 3 % in the fruit at a speed of 25 mm min^{-1} was registered. Color and texture were measured in all fruits included in the different replicates. Then, every tomato in the replicate was liquefied in a commercial Turmix blender (Moulinex, Barcelona, Spain), the homogenised sample was filtered through gauze, and then titratable acidity (TA) and soluble solids content (SSC) were

measured. The AT was expressed as % citric acid after titration with NaOH 0.1 N (AOAC, 1984) and the SSC was measured with an ATAGO/X-1000 digital refractometer (Tokyo, Japan) and expressed as °Brix. Homogenised samples were immediately frozen at -80 °C for further analysis.

A panel of eight trained panelists evaluated visual quality as previously described (Artés, Conesa, Hernández & Gil, 1999). Three number codes were used to name samples in order to avoid the identification of the applied treatments. A 9-point scale was used (9 = excellent, 7 = very good, 5 = good, limit of marketability, 3 = fair, limit of usability and 1 = poor, inedible).

2.5. C6 aldehydes volatile compounds

Twenty-four hours prior to the analysis, blended samples were transferred from the freezer to a refrigeration chamber at 5°C. Five millilitres of NaCl (1% w/v) were added to 5 g of tomato samples in a hermetic vial where the free headspace was 33 mL. The mixture was stirred for 40 min at 37 °C and the compounds were extracted by headspace mode using an SPME device (Supelco, Bellefonte, PA) with an 85 µm Carboxen/PDMS coating. After extraction the SPME device was inserted in an HP 5890 series II gas chromatograph equipped with an HP 5972 mass-selective detector. The compounds adsorbed by the fibre were desorbed in the injection port of the GC-MS for 10 min at 210 °C with the purge valve off (splitless mode). The compounds were separated in a 30 m, 0.32 mm, 0.25 µm TRB-5MS capillary column (Teknokroma, Barcelona, Spain) with the following chromatographic conditions: He as the carrier gas, splitless injection, 210 °C injector temperature, 5 min at 40 °C, first heating ramp to 60 °C at 3 °C/min, second heating ramp to 200 °C at 10 °C/min, and 5 min at 200 °C. Hexanal and (trans)-2-hexenal were identified by comparison with mass spectra from the library database

(NIST 98), and by comparison with authentic standards. Calibration curves were constructed by the method of standard addition.

2.6. Constituents of interest in nutrition

Lycopene, the major carotenoid in tomato fruit, as well as ascorbic acid and total phenolic compounds, considered the largest contributors to the antioxidant capacity of tomato fruit (Takeoka, Dao, Flessa, Gillespie, Jewell & Huebner, 2001), were also analyzed. Three replicate samples per cultivar, treatment and sampling day were used.

Lycopene content was determined according to the methodology proposed by Fish, Perkins-Veazie & Collins (2002) with minor modifications. Tomato puree (0.4 g) was weighed and 5 mL of 0.05 % (w/v) BHT in acetone, 5 mL of 95% ethanol and 10 mL of hexane were added. After 15 min of shaking, 3 mL of deionized water were added and the samples shaken for another 5 min. Absorbance of the hexane layer was measured at 503 nm versus a blank of hexane and results were expressed as mg per 100 g fresh weight (FW).

The *total phenols (TP)* content in tomato fruit was determined according to the Folin-Ciocalteu (FC) method (Mazur, Nes, Wold, Remberg & Aaby, 2014) with slight modifications. The tomato extract (0.2 mL) was mixed with an FC reagent (1.0 mL). After 3 min, 7 % sodium carbonate (0.8 mL) was added; the mixture was incubated for 60 min and the absorbance was measured at 765 nm. The TP content was expressed as mg gallic acid equivalents (GAE) per 100 g FW.

Ascorbic acid (AA) was determined according to the method described by Zhang, Liu, Zhang, Zhan & Wang (2014) with minor modifications. Samples of 1 g of tomato puree were homogenized in 8 mL oxalic acid solution (1% w/v) using an Ultra Turrax (T25, Janke & Kunkel, Germany) and then centrifuged at 5000 rpm for 15 min at 4 °C. The supernatant was analyzed in a 1200 Agilent HPLC using a reversed phase Phenomenex

Gemini 5u C6 Phenyl 110A column (4.6 by 150 mm, 5 μ m particle size) and 0.1 % oxalic acid solution as mobile phase at a flow rate of 0.5 mL/min. Ascorbic acid was measured at 243 nm, quantified by external calibration and the results were expressed as mg per 100 g FW.

2.7. Statistical analysis

The analysis of variance (ANOVA) at $P \leq 0.05$ was performed and when significant differences were observed, Tukey's Multiple Range Test was applied using PASW Statistics 18 for Windows (SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Gas composition inside the packages

O₂ and CO₂ concentrations in the packages containing tomatoes (cvs Delizia and Pitenza), together with the respiration rate evolution for the two cultivars, are shown in Figure 1. As expected, the O₂ concentration inside all packages decreased (Figure 1A) and the CO₂ increased (Figure 1B) as a consequence of product respiration. Such an effect was more marked in bags containing Delizia tomatoes as a consequence of the higher respiration rate exhibited by this tomato cultivar (Figure 1C). Similar O₂ (%) values were found the two sampling days for both cultivars, which indicates that an oxygen steady state was already achieved the first day of analysis. However, the CO₂ concentration continued evolving in PP bags. The CO₂ % values found at the end of fruit storage were around 8 % for Delizia tomatoes regardless of the MAP system used. However, significant differences were found between PP and PPpf films for Pitenza tomatoes (Figure 1B). The increase of permeability due to the presence of perforations in the packages (Supplementary material) allowed for the reaching of the highest O₂ equilibrium concentration inside the bags and likewise the lowest CO₂ accumulation when comparing with the continuous PP film (Figure 1A, B). The effect of perforations

was more relevant on the oxygen concentration since the exchange rate of this gas increased 5-fold while that of CO₂ only doubled.

It is important to notice that, unlike previous results (Bailén et al., 2006), no influence of the ethylene sorbent on O₂ and CO₂ concentrations inside the bags was observed in this study since the significant differences found in the atmospheric composition inside the packages were only associated with film permeability.

Ethylene accumulation was considerably higher in Delizia than in Pitenza tomatoes bags (Figure 2A), which is explained by the higher (ca. 10-fold higher) ethylene production of Delizia tomatoes (Figure 2A).

The decrease of ethylene inside the Delizia bags along the storage period may be due to the initial decrease in ethylene production as well as to the effect that the deficiency of O₂ and the CO₂ accumulation have on the synthesis of ethylene. In fact, it is known that the oxidation of 1-aminocyclopropane-1-carboxylic acid (ACC), the final stage of ethylene biosynthesis, requires the presence of O₂ as well as a low concentration of CO₂ (Salveit, 1999; Sozzi, Trinchero & Fraschina, 1999). The Pitenza cultivar produced low and constant ethylene levels in comparison with the Delizia fruit. The fact that the ethylene did not decrease under long-term storage in the Pitenza tomato bags leads to the assumption that the O₂ and CO₂ levels generated in the bags had little effect on ethylene biosynthesis in the colored Pitenza cultivar.

As shown in Figure 2, the selected sorbent drastically reduced the ethylene content inside the bags, which has allowed us to further discover the effect that this gas, in combination with AM, exerts on fruit quality.

3.2. *Effect of packaging on physico-chemical and sensorial quality*

The notable effect of the packaging on the physico-chemical and sensorial quality of stored fruits is shown in Table 1. These quality attributes were affected by the cultivar, packaging and the storage time (Table 2).

For Delizia tomatoes, the highest weight loss (5.22 %) was found in control (unpackaged) samples after 9 days. After this period, weight loss was ca. 3 % in packaged tomatoes. Decreasing the ethylene content inside the packages did not affect the weight loss, which is in accordance with results found by Sabir & Agar (2011) when using 1-MCP to inhibit ethylene action. Similar results were found in Pitenza samples. Therefore, results indicate that the weight loss reduction is mainly a consequence of the water vapour accumulation within the plastic bags during storage as other authors previously indicated (Linke & Geyer, 2002; Akbudak, Akbudak, Seniz & Eris, 2012).

Fruit firmness decreased during storage (Table 1). The initial firmness values were close to 12 N for Delizia and 5 N for Pitenza tomatoes. The higher decline of firmness was observed in Delizia tomatoes, a fact that might be related to major changes in fruit maturation of this cultivar harvested at the breaker stage as opposed to Pitenza fruits that are harvested when they are fully red. However, softening of fruits of both cultivars was considerably reduced by packaging, independently of the presence or not of perforations as well as of the ethylene sorbent. This result indicates that the modified atmosphere and the high humidity reached inside the packages slow down the process of ripening linked to the evolution of this quality parameter. In fact, it has been proven that the modified atmosphere decreases the activity of the pectinesterase and poligacturonase enzymes involved in the cell wall degradation (Akbudak et al., 2007; 2012). Other authors found that firmness loss of tomatoes preserved under MAP may be reduced by using an ethylene sorbent or 1-MCP (Opiyo & Ying, 2005; Bailén et al.,

2006; Martínez-Romero et al., 2009). Therefore, results reinforce the idea suggested by Johnston, Hewett, Hertog, & Harker (2002) who concluded that there is a complex relationship between fruit softening and ethylene.

According to commercial color requirements, Pitenza tomatoes were harvested with an h° close to 48 (red) and Delizia tomatoes with an h° close to 90 (breaker stage) (Table 1). The use of the ethylene absorbent did not influence this quality parameter; it was only affected by bag permeability. Packaging delayed color evolution and, as expected, this effect was more evident in fruits of the less mature cultivar (Delizia) in which the packaging markedly delayed the development of red color. The modified atmosphere reached inside the PP bags (PP and PPA) was more effective in delaying the color change than the atmosphere reached in the perforated packages (PPpf and PPpfA).

In spite of the fact that the color evolution may be ethylene-dependent or independent (Abdi, McGlasson, Holford, Williams & Mizrahi, 1998), it has been shown that ethylene sorbents and 1-MCP in combination with MAP may retard the color evolution in tomato fruit (Opiyo & Ying, 2005; Bailén et al., 2006; Sabir & Agar, 2011). Our results show that the effect of packaging reducing O_2 levels is more relevant than drastically reducing ethylene within the bags. This might then be explained by the relevance of adequate O_2 and CO_2 levels in chlorophyll degradation (Streif & Bangerth, 1976) and also because of the fact that endogenous ethylene levels might be high enough to initiate or maintain the mechanism related to color evolution. As Salveit (1999) indicated, once the ripening of climacteric fruit has started, the internal ethylene concentration quickly increases to saturation levels and the reduction of external ethylene concentration has no effect on retarding the processes involved in fruit ripening.

The above results reflect the positive effect that the MAP exerts on fruit weight loss, firmness and color, physical parameters crucial in the purchase decision. It also shows that the combination of MAP and exogenous ethylene in the bag did not significantly affect such a positive effect. Nevertheless, it has to be considered that changes induced by MAP might be related to the influence of CO₂ and O₂ levels on the action of the ethylene produced by the fruit (Yang and Hoffman, 1984).

Unlike the significant influence that MAP exerts on the physical quality parameters, only small differences have been found when comparing the SSC and TA of the packaged and unpackaged fruit (see Table 1). Differences were only temporally found in Pitenza tomatoes, which were harvested at a more mature stage (Table 2). In agreement with results found in the Delizia cultivar, Sabir & Agar (2011) did not find significant differences in the SSC in untreated tomato fruits or tomato fruits treated with 1-MCP and stored in MAP. Likewise, the same authors did not observe any influence of preservation atmosphere on the acidity of tomatoes (cv. Perla) harvested at two maturity stages (Sabir & Agar, 2011). The ethylene sorbent did not alter TA and SSC content (Table 1), which agrees with previous results by Bailén et al. (2006) when using active carbon in MAP. Our results agree with data from Salveit (1999) indicating that SSC and TA are quality parameters whose evolution is less inhibited by ethylene removal.

As visual appearance and firmness have been described as the main traits available to the consumer for assessing fruit quality when buying tomato (Grierson & Kader, 1986), the effect of packaging on fruit appearance was also evaluated (Table 1). Delizia and Pitenza tomatoes exhibited an excellent appearance upon arrival to the laboratory and their visual quality declined during storage. Packaged tomatoes exhibited better visual appearance, although no effect was induced by altering permeability of the bags

or by using the ethylene sorbent. The packaging allowed for the preservation of a good fruit appearance (≥ 5) even after 9 and 18 days of storage for Delizia and Pitenza tomatoes, respectively, which results in a substantial increase of the shelf life of tomato fruit compared to unpackaged samples which at that time were already not suitable for commercialization.

3.3. Effect of packaging on C6 aldehydes volatile compounds

C6 aldehydes, volatile molecules derived from lipoxygenase (LOX) activity, are recognised as important factors in determining the flavour quality of the fruit (Buttery, Teranishi & Ling, 1987). In fact, Ortiz-Serrano & Gil (2010) denoted from the odour units logarithms ($\log U$) that hexanal and *trans*-2-hexenal should contribute to tomato aroma at any stage of ripening, especially hexanal.

The high content of *trans*-2-hexenal observed in this study was in the range previously published by Ortiz-Serrano & Gil (2010) who considered that the concentration assigned to *trans*-2-hexenal was in part enhanced due to the isomerization of *cis*-3-hexenal. Significant differences were found in the hexanal and (*trans*)-2-hexenal levels for Delizia and Pitenza tomatoes, these were considerably higher in the case of Pitenza samples. As shown in Table 2, the concentration of these aldehydes was also affected by the fruit packaging and by the storage length.

For Delizia cultivar, the control samples showed an increase in hexanal and (*trans*)-2-hexenal the first day of sampling while a considerable loss of these volatile compounds was observed at the end of the storage period (Table 1). The concentration of these two compounds increased in the control samples at the beginning of MAP storage. However, it is important to remark that at the end of the storage period the level of both volatile compounds were higher in packaged samples, non-existing statistical

differences associated with bags permeability or with the presence of the ethylene sorbent inside the packages.

The volatile hexanal increased during storage of Pitenza tomato fruits. The hexanal and (*trans*)-2-hexenal contents were always higher in packaged than in unpackaged tomato samples. After 9 days of storage, the highest hexanal levels were found in tomatoes preserved in PP bags. This sampling day we observed that the incorporation of the ethylene sorbent led to a decrease in hexanal. The (*trans*)-2-hexenal concentration obtained for unpackaged Pitenza tomatoes was lower than that found at harvest and no influence of ethylene concentration was evidenced in this volatile. However, its content was affected by the O₂ and CO₂ levels in the headspace, with a slightly higher concentration in tomatoes preserved in perforated bags (PPpf and PPpfA).

We want to emphasize that only the final balance of volatiles will determine the final fruit aroma. However, from the achieved results and considering that mainly C6 aldehydes/alcohols are the major volatiles giving tomato its fresh aroma (Buttery, 1993) we could envisage that an optimal packaging of the fruit (adequate conservation atmosphere and storage length) could positively affect the fruit aroma when comparing with unpacked samples storage over the same period.

3.4. Effect of packaging on compounds of interest in nutrition

As shown in Table 2, lycopene, ascorbic acid (AA) and total phenols (TP) content were influenced by the different parameters that were study. At the beginning of the experiment the highest lycopene content was found in Pitenza tomatoes, in agreement with their red coloration at harvest. As expected, an accumulation of this carotene was observed throughout the storage in both cultivars, (Figure 3). Major differences in lycopene content observed in stored Delizia tomatoes were related to the O₂ and CO₂ levels inside the bags. In fact, control samples had the higher lycopene level followed

by tomatoes packages in perforated bags while the lowest lycopene accumulation was observed in fruit kept in PP bags (Figure 3). Furthermore, taking into account that no significant differences in CO₂ levels were found in the four MAP samples at day 7 (Figure 1B), it appears that O₂ concentration has the greatest effect on the lycopene accumulation. For Pitenza fruit, the effect of packaging was only noticeable after a long storage time when higher lycopene content was observed for unpackaged fruit. No significant differences were observed between packaging systems which indicates that the O₂ present inside the PP bags (ca. 6 %) was still high enough to further affect the synthesis and accumulation of this compound. In spite of the results found by Martínez–Romero et al (2009), no influence of exogenous ethylene content was appreciable in the lycopene content. As Streif & Bangerth (1976) noted, lycopene synthesis is affected by ethylene even at low O₂ level. However, the high CO₂ level inside packages may inhibit exogenous ethylene action which would lead to the conclusion that O₂ and CO₂ levels have an important effect on the lycopene content in tomato fruits.

MAP had a positive effect on increasing TP content in tomatoes of the Delizia cultivar (Figure 4). Results from this study revealed that high CO₂ accumulation might be involved in the higher TP content of Delizia tomatoes stored under MAP conditions. It has been reported that CO₂ may induce an increase in the activity of the enzyme phenylalanine ammonia-lyase (PAL), a key enzyme at the entry point of phenolic biosynthesis in lettuce (Ke & Salveit, 1989). As far as we know, the effect of CO₂ on the induction of PAL activity in tomato fruit remains unknown. Although PPpf bags showed lower CO₂ accumulation at the beginning of the experiment (Figure 1) and higher TP content at the end of the storage (D2) (Figure 4) than PP bags, it is possible that CO₂ accumulation under both conditions were high enough to increase PAL activity and hence TP. Reducing ethylene levels in the bags led to an increase in

phenolic content in the Delizia cultivar (Figure 4). Since ethylene increases PAL activity in different crops such as lettuce (Ke & Salveit, 1989) and citrus fruits (Lafuente, Zacarias, Martínez-Téllez, Sanchez-Ballesta & Dupille, 2001), other enzymes involved in the complex network of the phenylpropanoid metabolism pathway might participate in the changes found in the present work. Furthermore, Heredia & Cisneros-Zevallos (2009) previously observed that wounded tomatoes did not apparently respond to exogenous ethylene in the phenolic content which indicated that this stress does not affect tomato tissue at the gene level. Comparison of results found in the PP and PPpf films also indicated that the presence of very low O₂ levels in PP films (Figure 1) may influence TP biosynthesis. The effect of packaging on TP content in fruits of the Pitenza cultivar was different since these postharvest factors barely altered TP levels. This different behaviour might be related in part to the cultivar and its tolerance of O₂ and CO₂ levels. Likewise, it has to be noted that CO₂ accumulation was about half as high in Pitenza than in Delizia tomato bags (Figure 1), which further suggest the link between CO₂ accumulation and TP.

The AA content in tomato of both cultivars (Figure 4) are in the range of published data (George, Kaur, Khurdiya, & Kapoor, 2004) and was higher in the Pitenza cultivar, which was harvested at a more mature stage. AA decreased in both cultivars after harvesting the fruit which is in agreement with previous data (Abushita, Hebshi, Daood & Biacs, 1997). Such a decrease has been explained by the fact that AA is used as a respiratory substrate or converted into sugar during ripening (Islam, Matsui & Yoshida, 1996). Therefore, the higher decrease in the Delizia cultivar might be associated with the higher respiration of this cultivar (Figure 1).

The initial AA decrease was sharply reduced by packaging in Delizia tomatoes (Figure 4, D1) while the AA loss in Pitenza cultivar was only reduced by using the

perforated bags. Such effects were lost after prolonged storage (D2). The inclusion of the ethylene sorbent was useful for reducing the decline in AA of Delizia tomatoes both in PP and PPpf bags after this period. However, reducing ethylene content did not alter AA levels in the Pitenza cultivar. In this cultivar, no significant differences were found between packages after 18 days (D2) and a notable increase in AA occurred in control samples.

Global results indicate then that, although packaging may aid to reduce the initial AA loss, a prolonged exposition to a modified atmosphere, which sharply reduces O₂ and increases CO₂ levels, would lead to lower AA content in tomato fruit. These results should be related to metabolic changes involved in AA biosynthesis or in AA stability (Holcroft & Kader, 1999). In fact, results from Pinto, Lentheric, Vendrell & Larrigaudiere (2001) suggested that high CO₂ levels cause acidification of the cytoplasm and this would alter the mitochondrial function and result in oxidative damage ending in vitamin C loss. Moreover, results indicate that reducing ethylene in the packages can positively affect this quality parameter although this effect can vary among tomato cultivars, which confirm previous findings by Martinez-Romero et al (2009). Although further studies have to be performed to clarify the mode of action of ethylene in MAP conditions, we found that, depending on the cultivar, ethylene removal can be favorable for the nutritional quality of tomato fruit.

4. Conclusions

In conclusion, global data indicate that in spite of the relevance of ethylene in tomato fruit maturity, CO₂ and O₂ are critical factors for controlling postharvest behaviour and quality of the fruit and that MAP is an important tool to better maintain its quality. The packaging forms used for the tomato preservation ensured the evaluation of four differentiated atmosphere compositions (O₂, CO₂, C₂H₄) for each cultivar. When

the ethylene sorbent was included in PP and PPpf bags no significant changes in CO₂ and O₂ levels were induced, which was suitable for attributing the influence of ethylene content on tomato fruit quality. Although removing ethylene from the bags did not affect most of the fruit quality attributes in packaged fruits, TP and AA contents were higher when the ethylene sorbent was used. Results also showed that the effect of the atmosphere composition on postharvest quality of tomatoes depends on the cultivar and length of storage. Although prolonged exposition to low O₂ and high CO₂ levels can be detrimental for fruit nutritional quality, global results show that an optimal MAP in combination with ethylene removal may suppose a good strategy to preserve and improve fruit quality attributes overall in short-life tomato cultivars.

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Figures caption

Figure 1. O₂ (A) and CO₂ (B) levels inside the tomato packages (cvs. Delizia and Pitenza) maintained at 13 °C. (C) Respiration rate (CO₂ production) of Delizia and Pitenza tomatoes during their storage at 13 °C. Values are means of three replicates ± standard deviation. Different letters for the same day of analysis mean significant difference at $P \leq 0.05$.

Figure 2. Ethylene levels inside the tomato packages (cvs. Delizia and Pitenza) maintained at 13 °C. (B) Ethylene production of Delizia and Pitenza tomatoes during their storage at 13 °C. Values are means of three replicates ± standard deviation. Different letters for the same day of analysis mean significant difference at $P \leq 0.05$.

Figure 3. Effect of packaging on lycopene content of tomato fruit (cvs. Delizia and Pitenza) during storage at 13 °C and then 48 h in air at 20°C (SL). For Delizia tomatoes D1= 5 and D2= 9 days while for Pitenza samples: D1= 8 and D2= 16 days. Values are means of three replicates ± standard deviation. Different letters for the same day of analysis mean significant difference at $P \leq 0.05$.

Figure 4. Effect of packaging on total phenols (TP) and ascorbic acid content of tomato fruit (cvs. Delizia and Pitenza) during storage at 13 °C and then 48 h in air at 20°C (SL). For Delizia tomatoes D1= 5 and D2= 9 days while for Pitenza samples: D1= 8 and D2= 16 days. Values are means of three replicates ± standard deviation. Different letters for the same day of analysis mean significant difference at $P \leq 0.05$.

Table 1

Table 1. Effect of the packaging on fruit quality attributes of Delizia and Pitenza tomatoes storage at 13 °C and then 48 h in air at 20 °C (SL).

		Weight Loss (%)		Firmness (N)		Color (<i>h</i> ^o)		TA (%)		SSC (° Brix)		Appearance (9-1)		hexanal (ng.g ⁻¹)		(E)-2-hexenal (ng.g ⁻¹)	
DELIZIA	Initial	—		12.65 ± 1.14		93.11 ± 3.97		0.49 ± 0.02		5.60 ± 0.66		8.28 ± 0.61		0.69 ± 0.16		0.43 ± 0.07	
	Days	5	9	5	9	5	9	5	9	5	9	5	9	5	9	5	9
	Control	3.65a	5.22a	6.37c	4.23c	55.45c	45.55c	0.47	0.49	5.50	5.82	5.58c	4.13c	0.88a	0.14c	0.70a	0.04b
	PP	2.80b	2.97b	8.47a	6.85a	79.33a	83.63a	0.47	0.51	5.89	5.76	7.08ab	6.92ab	0.67c	0.42a	0.19b	0.10a
	PPA	3.41ab	3.11b	7.95ab	6.46ab	80.05a	81.97a	0.47	0.52	5.50	6.11	7.50a	7.83a	0.68bc	0.43a	0.16b	0.11a
	PPpf	2.94b	2.89b	8.11ab	5.32bc	70.58b	66.70b	0.50	0.50	6.17	5.89	6.25bc	6.17b	0.74b	0.39a	0.25a	0.13a
	PPpfA	3.43ab	3.03b	7.24bc	5.71ab	75.30ab	68.77b	0.48	0.49	5.71	5.98	6.50b	6.42b	0.71b	0.33a	0.23a	0.08a
PITENZA	Initial	—		4.75 ± 0.46		48.12 ± 1.51		0.35 ± 0.02		3.97 + 0.31		8.08 ± 0.92		3.79 ± 0.62		0.91 ± 0.16	
	Days	9	18	9	18	9	18	9	18	9	18	9	18	9	18	9	18
	Control	5.58a	7.47a	3.80b	3.17b	43.92b	43.06c	0.25b	0.32	4.90	5.00a	6.50c	4.75c	4.48d	4.19d	0.56c	0.77c
	PP	2.51b	2.63c	4.87a	4.83a	45.48a	45.08a	0.33a	0.27	5.07	4.55b	7.33ab	7.17a	8.53a	6.37c	0.88b	1.22b
	PPA	2.89b	2.72bc	5.19a	4.72a	45.74a	45.35a	0.33a	0.30	5.30	4.60b	7.25a	7.25a	5.58c	9.10ab	0.80b	1.25b
	PPpf	2.13b	2.96bc	5.18a	4.57a	45.66a	44.10b	0.32a	0.29	5.27	4.55b	6.83b	6.50b	6.50b	9.44a	1.01a	1.50a
	PPpfA	2.50b	3.19b	5.06a	4.55a	45.41a	43.93b	0.33a	0.29	5.10	4.50b	6.92b	6.42b	5.39c	8.51b	1.04a	1.49a

Values are the mean ± standard deviation of three replicates.
Different letters for the same column indicate significant differences ($P \leq 0.05$).

Table 2

Table 2. Analysis of variance of the change in the atmospheric composition and quality parameters under study for Delizia and Pitenza tomatoes as affected by packaging (treatment) and storage time (time).

	Delizia			Pitenza			Cultivar	Cultivar x Treatment
	Treatment	Time	Treatment x time	Treatment	Time	Treatment x time		
O ₂ ^a	***	ns	ns	***	ns	ns	*	***
CO ₂ ^a	***	***	*	*	*	*	***	***
Ethylene ^a	***	***	***	***	***	***	***	***
Weight Loss	***	*	***	***	***	***	**	***
Firmness	***	***	***	***	***	ns	***	*
Color	***	*	***	***	***	*	***	***
SST	ns	ns	ns	***	***	***	***	***
Acidity	ns	ns	ns	***	*	**	***	***
Appearance	***	**	*	**	**	*	*	**
Hexanal	**	**	***	***	***	**	***	**
(trans)2-hexenal	***	**	**	***	**	ns	***	***
Lycopene	***	**	***	*	***	*	***	*
Ascorbic acid	***	***	***	***	***	***	***	**
Total phenols	***	***	*	*	*	**	***	***

ns, not significant
*, **, *** Significant differences at P ≤ 0.05, 0.01 and 0.001, respectively
^a concentration inside the bags

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