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The role of peroxidase on the antioxidant potential of fresh-cut 'Piel de Sapo' melon packaged under different modified atmospheres

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Abstract

The effects of different initial in-package O_2 and CO_2 concentrations (2.5 kPa O_2 + 7 kPa CO_2, 10 kPa O_2 + 7 kPa CO_2, 21 kPa O_2, 30 kPa O_2 and 70 kPa O_2) on peroxidase activity, vitamin C content, total phenolics and antioxidant capacity of fresh-cut 'Piel de Sapo' melon have been investigated for 14 days at 4 °C. The radical scavenging activity of fresh-cut melon strongly increased after 9 days storage related to a synthesis of phenolic compounds, especially under 2.5 kPa O_2 + 7 kPa CO_2 atmospheres. Low O_2 levels best maintained vitamin C and phenolic content during the storage. However, stressful too-low O_2 and high CO_2 levels induced an important increase of peroxidase activity under 2.5 kPa O_2 + 7 kPa CO_2 atmosphere, which was directly related to changes of vitamin C throughout storage. Therefore, 70 kPa O_2 atmospheres are proposed to prevent anaerobic conditions during storage of fresh-cut melon and thus, reduce wounding stress and deteriorative changes related to high peroxidase activity in tissue.

Keywords: Fresh-cut melon; Modified atmosphere packaging; Phenolic compounds; Peroxidase; Vitamin C; Antioxidant capacity

1. Introduction

Consumption of fruits and vegetables is associated with a lowered risk of cancer, diabetes, cardiovascular and neurological diseases (Del Caro, Piga, Vacca, & Agabbio, 2004; Kaur & Kapoor, 2001). This protection is due to the presence of antioxidant constituents in fruits and vegetables such as vitamins and phenolic compounds. Their biological properties result from their capacity of decreasing oxidative damage and sequestering reactive oxygen species (ROS), which could initiate cascade reactions that result in the production of hydroxyl radical and other destructive species such as lipid peroxides (Lurie, 2003).

'Piel de Sapo' melon (*Cucumis melo* L.) is a Spanish cultivar, whose fruits are oval shaped with reticular greenish color skin and white sweet flesh. Melon antioxidant properties are mainly due to its high amounts of beta carotenoids of 4700 μg/100 g (Souci, Fachmann, & Kraut, 2000) and moderate vitamin C content of 25–42 mg/100 g (Li, Yao, Yang, & Li, 2006; Moreiras, Carvajal, Cabrera, & Cuadrado, 2001).

Due to cutting operations, a great number of cells are disrupted, which causes the release of enzymes and their substrates and promotes the increase of oxidative enzyme-catalysed processes. Wounding and physiological stress may stimulate peroxidase (POD), whose activity in fresh-cut cantaloupe melon could be a response to increased oxidative stress in the cut fruit (Lamikanra & Watson, 2001). POD can oxidize both mono- and di-phenols in the presence of small amounts of hydrogen peroxide, which is frequently the oxidizing agent, although, in some cases, oxygen may also activate the enzyme (Robinson, 1991). POD is involved in multiple deteriorative changes affecting flavor, texture, color and nutrition in processed fruits and vegetables. It appears to be a relationship between residual POD activity and the development of off-flavors and off-odors in foods (Bett-Garber, Lamikanra, Lester, Ingram, & Watson, 2005).

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Reduced O_2 and high CO_2 levels have been proved to effectively control enzymatic browning, firmness and decay of fresh-cut fruits (Soliva-Fortuny & Martín-Belloso, 2003). However, problems associated with the development of off-odors, physiological and microbiological decay, browning and softening may appear when O_2 level is too low and CO_2 is accumulated in packages (Allende, Luo, McEvoy, Artés, & Wang, 2004). Thus, high O_2 atmospheres ($\geqslant$70 kPa O_2) have been suggested as an effective method to inhibit the growth of microorganisms and prevent undesired anoxic fermentation (Allende et al., 2004; Amanitidou, Smid, & Gorris, 1999; Jacxsens, Devlieghere, Van der Steen, & Debevere, 2001; Van der Steen, Jacxsens, Devlieghere, & Debevere, 2001). Previous works have been focused on assessing the effects of different modified atmosphere packaging systems on visual, sensory and microbial quality of fresh-cut fruits but their impact on nutritional and antioxidant properties has been neglected up to date and scarce information is available, especially in fresh-cut melon. Therefore, the purpose of the present work was to study the effect of modified atmosphere packaging (MAP) on vitamin C, phenolics and antioxidant activity as affected by peroxidase activity in fresh-cut 'Piel de Sapo' melon.

2. Materials and methods

2.1. Sample preparation

'Piel de Sapo' melons (*Cucumis melo* L.) harvested in Valencia (Spain) were stored in a ventilated room at 10 °C prior to processing. Melons at commercial ripeness were sanitized in a 200 ppm NaClO solution for 2 min, rinsed with tap water, and dried by hand. The fruits were sliced and cut to obtain trapezoidal sections. At this stage a physicochemical characterization was carried out (Table 1). Calcium dips have been proved to be effective as firming agents in fresh-cut melon (Luna-Guzmán, Cantwell, & Barreto, 1999; Luna-Guzmán & Barrett, 2000). Thus, melon pieces were dipped for 1 min in a solution of 0.5% w/v calcium chloride at a product: solution ratio of 1:2. Once the excess of water was completely drained, 100 g of fruit were packaged in polypropylene trays. The trays were sealed using a digitally controlled compensated vacuum ILPRA Food Pack Basic V/6 system (ILPRA Systems. CP., Vigevono, Italy). The modification of package atmosphere was carried out by flushing a mixture of 2.5 kPa O_2 + 7 kPa CO_2, 10 kPa O_2 + 7 kPa CO_2, 21 kPa O_2, 30 kPa O_2 or 70 kPa O_2 in a ratio product: gas mixture of 1:2. The O_2 and CO_2 permeance of the sealing film were 110 and 500 $cm^3\ m^{-2}\ day^{-1}\ bar^{-1}$ at 23 °C and 0% RH, respectively (ILPRA Systems España, S.L. Mataró, Spain). The trays were stored at 4 ± 1 °C in darkness for 14 days. A pair of trays was taken at each sampling time and two replicates analyses were carried out for each tray.

Table 1
Physicochemical characteristics of unprocessed melon

Soluble solids (°Brix)	12.45 ± 0.7
Total acidity (g citric acid/100 g)	0.276 ± 0.005
pH	5.71 ± 0.05
Total phenolic compounds (mg gallic acid/100 g)	15.4 ± 2.3
Total vitamin C (mg vitamin C/100 g)	45.2 ± 3.5
Pulp color	
L^*	73.8 ± 3.9
a^*	−3.11 ± 0.22
b^*	19.21 ± 1.9
Firmness (N)	3.3 ± 0.7

Values are the mean of three independent determinations ± standard deviation.

2.2. Total phenolic compounds

The amount of total phenolic compounds in fresh-cut melon was determined according to the Folin-Ciocalteu procedure (Singleton, Orthofer, & Lamuela-Raventos, 1999) with some modifications. Fresh-cut melon was ground and centrifuged at 22100*g* for 15 min at 4 °C with a centrifuge AVANTI™ J-25 (Beckman Instruments Inc., Fullerton, CA, USA) and then, filtered through a Whatman no.1 filter. An aliquot of 0.5 ml of the supernatant was added to 0.5 ml of Folin-Ciocalteu solution. After 3 min, 10 ml of saturated sodium carbonate solution were added and brought up to 25 ml with distilled water. The absorbance of the blue color that developed was read at 725 nm after 1 h in darkness conditions. Concentrations were determined by comparing the absorbance of the samples with standards. Results were expressed as milligrams of gallic acid in 100 g of fresh-cut melon.

2.3. Peroxidase extraction

Peroxidase enzyme was extracted as described by Elez-Martínez, Aguiló-Aguayo, and Martín-Belloso (2006). A portion of 50 g of processed melon was blended and mixed with 50 g of 0.2 M l^{-1} sodium phosphate buffer (pH = 6.5). The homogenate was centrifuged at 6000*g* for 15 min at 4 °C with a centrifuge AVANTI™ J-25 (Beckman Instruments Inc., Fullerton, CA, USA). The resulting supernatant was collected and filtered through a Whatman no. 1 paper.

2.4. Peroxidase assay

Peroxidase activity was assayed spectrophotometrically in a buffer consisting of 0.02 M Na_2HPO_4 and 0.08 M NaH_2PO_4, 20 mM guaiacol, 4 mM H_20_2, enzyme extract (150 μl), pH 6 in a total volume of 3 ml (Civello, Martínez, Chaves, & Anon, 1995). The changes in absorbance due to oxidation of guaiacol at 470 nm and 25 °C were recorded using a CECIL CE 2021 spectrophotometer (Cecil Instruments Ltd, Cambridge, UK). The absorbance of each replicate was measured in triplicate. The initial reaction rate was estimated from the linear portion of the plotted curve.

One unit of POD activity was defined as a change in absorbance at 470 nm min^{-1} ml^{-1} of enzymatic extract. Activity values were given as a percentage of the activity at the beginning of storage (RA, %).

2.5. Vitamin C content

The determination of the vitamin C concentration in fresh-cut melon was performed by HPLC–UV. The extraction procedure and the chromatographic conditions were based on a previous study carried out by Odriozola-Serrano, Hernández-Jover, and Martín-Belloso (2007). A portion of 25 g of fruit was added to 25 ml of a solution containing 45 g of metaphosphoric acid and 7.2 g of DL-1,4-dithiothreitol (DTT) per liter. The mixture was stirred and centrifuged at 22100*g* for 15 min at 4 °C with a centrifuge AVANTI™ J-25 (Beckman Instruments Inc., Fullerton, CA, USA). The supernatant was vaccum-filtered through Whatman no. 1 paper. The sample were passed through a millipore 0.45 μm membrane and injected into the HPLC system.

The HPLC system was equipped with a 600 Controller and a 486 Absorbance Detector (Waters, Milford, MA, USA) working at 245 nm. Duplicates of 20 μl of each extract were injected into a reverse-phase C18 Spherisorb® ODS2 (5 μm) stainless steel column (250 mm × 4.6 mm) (Waters, Milford, MA, USA), used as stationary phase. A 0.01% solution of sulphuric acid adjusted to pH 2.6 was used as the mobile phase. The flow rate was fixed at 1 ml min^{-1} at room temperature. Results were expressed as milligrams of vitamin C in 100 g of fresh-cut melon.

2.6. Antioxidant capacity

The antioxidant capacity of fresh-cut melon was studied through the determination of free radical-scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, according to the procedure described by Elez-Martínez and Martín-Belloso (2007). Samples of melon were centrifuged at 6000*g* for 15 min at 4 °C with a centrifuge AVANTI™ J-25 (Beckman Instruments Inc., Fullerton, CA, USA) and filtered through a Whatman no.1 paper. Aliquots of 0.01 ml of the supernatant were mixed with 3.9 ml of methanolic DPPH of 0.025 g l^{-1} and 0.090 ml of distilled water. The homogenate was shaken vigorously and kept in darkness for 30 min. The absorption of the samples was measured on a CECIL CE 2021 spectrophotometer (Cecil Instruments Ltd, Cambridge, UK) at 515 nm against blank of methanol without DPPH. Results were expressed as a percentage decrease with respect to the absorption value of a reference DPPH solution.

2.7. Headspace gases analysis

The gas composition of the package headspace was analyzed with a micro-GC CP 2002 gas analyzer (Chrompack International, Middelburg, The Netherlands) equipped with a thermal conductivity detector. An aliquot of 1.7 ml was automatically withdrawn through an adhesive rubber septum with a sampling needle directly connected to the injection system. The determination of the oxygen concentration was carried out by injecting a sample of 0.25 μl to a CP-Molsieve 5 Å column (4 m × 0.35 mm, df = 10 μm) at 60 °C and 100 kPa whereas 0.33 μl portion was injected to a Pora-PLOT Q column (10 m × 0.32 mm, df = 10 μm) at 75 °C and 200 kPa for carbon dioxide. Initial gas compositions were determined within the next 15 min after packaging, thus corresponding to the values at time 0 shown in Table 2. Two trays were taken at each sampling time to perform the analysis and two readings were carried out for each package.

2.8. Statistical analysis

Significance of the results and statistical differences were analyzed using the Statgraphics plus v.5.1 software (Manugistics, Inc., Rockville, MA, USA). Data were analyzed by multifactor analysis of variance. Duncan multiple-range test was applied to determine differences among means, with a level of significance of 0.05. Principal component analysis (PCA) was conducted on data to obtain an overview of correlations among variables. PCA is a multivariate statistical technique based on the calculation of linear combinations between the variables that explain the most variance of the data. As a result, data can be reduced to a set of new variables called principal components (PCs). The correlation matrix is used to standardize the variables which are not measured on the same scale. The loadings of the PC define the direction of greatest variability and the score values represent the projection of each object onto PC.

3. Results and discussion

3.1. POD activity of fresh-cut melon

POD activity of fresh-cut 'Piel de Sapo' melon was related to the availability of O_2 inside packages (Fig. 1). Enzymatic activity in fresh-cut melon packaged under initial 2.5 kPa O_2 + 7 kPa CO_2 conditions was maintained or even increased throughout storage, whereas under initial 70 kPa O_2 atmospheres it decreased progressively reaching 56% inactivation at 14 days storage. POD activity in samples stored under other packaging conditions decreased steadily throughout storage, depending on the packaging atmosphere. Hence, the higher the O_2 availability, the greater the decrease in POD activity throughout storage. The increase of POD activity under 2.5 kPa O_2 + 7 kPa CO_2 atmosphere after one week of storage may be a response to membrane damage because of the stress caused by too-low oxygen and too-high carbon dioxide levels (Lester, 2003). On the other hand, the observed reduction in POD activity under 70 kPa O_2 atmospheres could be related to a decrease in tissue damage. The application of

Table 2
Changes of O_2 and CO_2 concentrations in packages of fresh-cut 'Piel de Sapo' melon stored under modified atmosphere during 14 days at 4 °C

Days	2.5 kPa O_2 + 7 kPa CO_2		10 kPa O_2 + 7 kPa CO_2		21 kPa O_2		30 kPa O_2		70 kPa O_2	
	O_2 (kPa)	CO_2 (kPa)	O_2 (kPa)	CO_2 (kPa)	O_2 (kPa)	CO_2 (kPa)	O_2 (kPa)	CO_2 (kPa)	O_2 (kPa)	CO_2 (kPa)
0	2.99 ± 0.08a	6.41 ± 0.07a	10.31 ± 0.19a	6.60 ± 0.11a	20.82 ± 0.05a	0.09 ± 0.01a	30.45 ± 0.19a	0.5 ± 0.7a	71.6 ± 0.4a	0.17 ± 0.3a
2	2.645 ± 0.014b	7.52 ± 0.03b	10.06 ± 0.05ab	7.6 ± 0.4ab	18.77 ± 0.25b	2.47 ± 0.18b	29.4 ± 0.9ab	3.9 ± 0.3b	65.0 ± 1.0b	3.1 ± 0.3b
4	2.2 ± 0.3c	8.9 ± 0.5c	9.91 ± 0.04ab	8.0 ± 0.5b	17.32 ± 0.05c	3.4 ± 0.6c	29.3 ± 0.4ab	5.0 ± 0.6bc	63.7 ± 0.9bc	4.9 ± 1.3c
7	1.120 ± 0.007d	9.75 ± 0.07cd	9.53 ± 0.07b	10.4 ± 0.6c	15.89 ± 1.19d	4.9 ± 0.3d	28.73 ± 0.09bc	5.9 ± 0.3c	62.0 ± 1.3bc	7.0 ± 0.3d
9	0.89 ± 0.09de	9.90 ± 0.07d	9.4 ± 0.3b	11.0 ± 0.9cd	16.1 ± 0.3d	6.75 ± 0.14e	28.11 ± 0.03bc	8.03 ± 0.22d	61 ± 5bc	7.3 ± 0.4de
11	0.730 ± 0.014e	11.7 ± 0.9e	8.8 ± 0.3c	11.45 ± 0.18cd	15.2 ± 0.3d	8.0 ± 0.5f	27.27 ± 1.18c	9.37 ± 0.25e	60.1 ± 0.4bc	8.42 ± 0.15f
14	0.35 ± 0.04f	12.50 ± 0.14e	7.1 ± 0.5d	12.0 ± 0.8d	13.45 ± 0.17e	10.2 ± 0.5g	25.4 ± 0.6d	11.8 ± 0.7f	58.1 ± 2.4c	9.90 ± 0.25g

Data are the means of four replications. Different letters in the same column indicate that mean values are significantly different by Duncan's multiple-range test ($p < 0.05$).

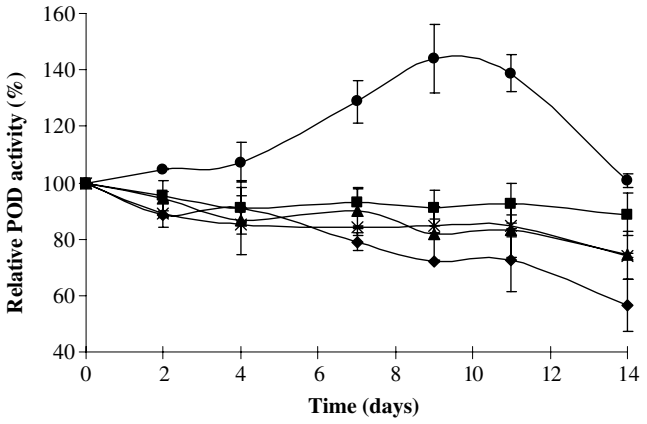

Fig. 1. Evolution of relative POD activity (%) of fresh-cut 'Piel de Sapo' melon stored under modified atmosphere during 14 days at 4 °C. Atmosphere conditions: ● 2.5 kPa O_2 + 7 kPa CO_2, ■ 10 kPa O_2 + 7 kPa CO_2, ▲ 21 kPa O_2, × 30 kPa O_2, ◆ 70 kPa O_2. Data shown are mean ± standard deviation.

high O_2 treatments has been reported to prevent oxidative injury of fresh produce (Allende et al., 2004; Amanatidou, Slump, Gorris, & Smid, 2000; Lu & Toivonen, 2000). The oxidative stress is associated with the accumulation of ROS such as superoxide (O_2^-) and H_2O_2 (hydrogen peroxide), which are particularly reactive and can cause lipid peroxidation and a subsequent loss of membrane integrity. Cantos, Tudela, Gil, and Espín (2002) reported that the possible limiting substrate for the POD reaction could be H_2O_2 and thus, the application of high oxygen atmospheres to prevent the oxidative stress may explain the decrease in POD activity.

Lamikanra and Watson (2000) suggested that relatively high POD activity levels could substantially limit the commercial shelf-life of 'Cantaloupe' melon. Thus, levels of residual enzymatic activity under 70 kPa O_2 atmospheres, lower than those found using other conditions, could contribute to reduce deteriorative quality changes of fresh-cut 'Piel de Sapo' melon. However, other metabolic processes related to quality maintenance of the commodity could take place under high O_2 atmospheres. For instance, fresh-cut 'Piel de Sapo' melon produced more ethylene under 70 kPa O_2 than under 2.5 kPa O_2 + 7 kPa CO_2 or 21 kPa O_2 storage atmospheres (Oms-Oliu, Raybaudi-Massilia Martínez, Soliva-Fortuny, & Martín-Belloso, 2007). In addition, the exposure to elevated O_2 levels may have different effects on respiration, production of fermentative metabolites and sensory quality, depending on the commodity, O_2 concentrations, storage time and temperature.

3.2. Vitamin C content

Table 1 reported a moderate vitamin C concentration of unprocessed 'Piel de Sapo' melon (41.7–48.7 mg 100 g^{-1} fw). However, vitamin C depleted due to minimal processing and initial concentrations detected in fresh-cut melon at day 0 were lower than that of unprocessed fruit.

During the preparatory steps of minimal processing, the natural protection of fruit (the peel) is removed and hence, they become highly susceptible to oxidation. Fig. 2a shows that initial values of vitamin C detected in fresh-cut melon stored under a 2.5 kPa O_2 + 7 kPa CO_2 atmosphere were quite higher than the amount observed under packaging conditions which contained higher initial O_2 concentrations. As expected, ascorbic acid oxidation was greatly favored by the presence of oxygen and thus, a marked decrease in vitamin C content was observed in samples stored under 10 kPa O_2 + 7 kPa CO_2, air, 30 and 70 kPa O_2 atmospheres at day 0. The strongest depletion in vitamin C was detected in fresh-cut melon packaged under $\geqslant$30 kPa O_2 atmospheres, reaching the lowest content after 14 days of storage.

A marked decrease in vitamin C content was observed in samples stored under 2.5 kPa O_2 + 7 kPa CO_2 atmosphere from the beginning of storage despite the restriction in O_2 concentrations inside packages. This initial storage atmosphere induced too-low O_2 and high CO_2 concentrations in packages of fresh-cut melon during storage which could contribute to high oxidative stress in fresh-cut melon and, in turn, an increase in peroxidase activity and vitamin C oxidation. Lamikanra and Watson (2000) demonstrated the relative affinity of cantaloupe POD for ascorbic acid over guaiacol, and the ability of ascorbic acid in dip solutions to prevent the oxidation of carotenoids in cut cantaloupe melon pieces stored at 4 °C. High CO_2 levels could also provoke a cytoplasm acidification with the consequent impairment of mitochondrial function that could result in oxidative damage, which could be overcame by ascorbate peroxidase, enzyme that catalyses ascorbic acid oxidation (Pinto, Lentheric, Vendrell, & Larrigaudiere, 2001; Tudela, Espín, & Gil, 2002).

3.3. Total phenolic compounds

Unprocessed 'Piel de Sapo' melon contained small amounts of phenolic compounds (15.4–20 mg gallic acid 100 g^{-1} fw) (Table 1). Although no data are available related to total phenolic compounds in 'Piel de Sapo' melon, Lamikanra and Watson (2001) reported a low content in 'Cantaloupe' melon (5.16 mg gallic acid 100 g^{-1} fw). Fig. 2b shows the evolution of total phenolic compounds of fresh-cut 'Piel de Sapo' melon stored under different atmospheres. The initial phenolic content was maintained or slightly decreased for 4–7 days, but then the values rose, reaching a maximum accumulation at 9 days storage. Beyond this day, the phenolic content decreased and reached final values about 9.2–11.1 mg gallic acid 100 g^{-1} fw in all packaging conditions. A 2.5 kPa O_2 + 7 kPa CO_2 atmosphere induced a higher production of phenolic compounds compared to other atmosphere conditions, which may be related to an enhanced oxidative stress induced by too-low O_2 and high CO_2 concentration inside packages (Fig. 2b). Accumulation of phenolic compounds is a physiological response to infections or injuries (Amanatidou et al., 2000). Wounding may stimulate phenylalanine ammonia lyase (PAL, E.C. 4.3.1.5) activity during minimal processing with consequent further production of the phenolic compounds (Saltveit, 1997). The PAL activation of the phenylpropanoid metabolism could be elicited through induced reactive oxygen species (Reyes, Villarreal, & Cisneros-Zevallos, 2006).

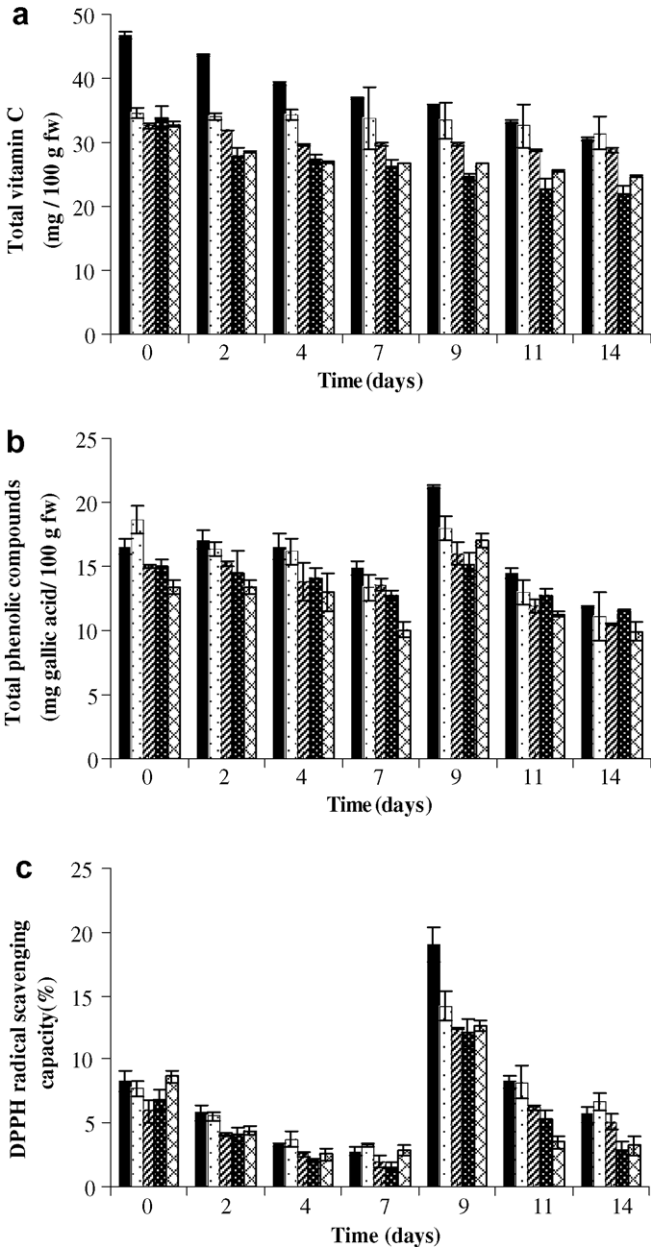

Fig. 2. Total vitamin C, total phenolic compounds and DPPH radical scavenging capacity of fresh-cut 'Piel de Sapo' melon stored under modified atmosphere during 14 days at 4 °C. Atmosphere conditions: ■ 2.5 kPa O_2 + 7 kPa CO_2, ⬚ 10 kPa O_2 + 7 kPa CO_2, ▨ 21 kPa O_2, ▩ 30 kPa O_2, ⊠ 70 kPa O_2. Data shown are mean ± standard deviation.

Phenolic content was best maintained in fresh-cut 'Piel de Sapo' melon stored under 2.5 kPa O_2 + 7 kPa CO_2 and 10 kPa O_2 + 7 kPa CO_2 atmospheres for 4 days. On the other hand, melon initially packaged under 21, 30

and 70 kPa O_2 atmospheres exhibited lower phenolic content throughout storage, especially under 70 kPa O_2 atmosphere. These conditions induced a strong depletion of phenolic content at day 0 compared to 2.5 kPa O_2 + 7 kPa CO_2 and 10 kPa O_2 + 7 kPa CO_2 atmospheres (Fig. 2b). Thus, the phenolic content of just-processed fresh-cut melon packaged under 2.5 kPa O_2 + 7 kPa CO_2 and 10 kPa O_2 + 7 kPa CO_2 atmospheres was about 16–20 mg gallic acid 100 g^{-1} fw whereas concentration was about 13 mg gallic acid 100 g^{-1} fw under 70 kPa O_2. Cocci, Rocculi, Romani, and Dalla Rosa (2006) reported that the oxygen availability in the package headspace of fresh-cut apples stored under 21 kPa O_2 could have led to a stronger degradation of the functional compounds such as phenolic compounds.

3.4. Antioxidant capacity

Fig. 2c shows the evolution of the antioxidant capacity of fresh-cut 'Piel de Sapo' melon through the DPPH radical scavenging method. Antioxidant capacity of fresh-cut melon decreased during the first 7 days of storage, then underwent a marked increase at 9 days storage and finally continued to decrease beyond that time under all packaging atmospheres. A similar change pattern has been reported for some cultivars of fresh-cut citrus segments stored under 21 kPa O_2, where the antioxidant activity decreased at the beginning of the storage period and later increased, after 12 days of storage (Del Caro et al., 2004). At 9 days storage, the increase in antioxidant capacity of fresh-cut melon may be related to an increase in total phenolic compounds (Fig. 2b and c). The most marked increase in phenolic compounds was observed in fresh-cut 'Piel de Sapo' melon stored under 2.5 kPa O_2 + 7 kPa CO_2 atmosphere (Fig. 2b). It has been proved that activity of the phenylpropanoid pathway increases under stressful conditions and phenolic compounds are synthesized and accumulated (Kang & Saltveit, 2002).

Low O_2 atmospheres, 2.5 kPa O_2 + 7 kPa CO_2 and 10 kPa O_2 + 7 kPa CO_2, induced an enhanced antioxidant capacity of fresh-cut melon throughout storage compared to 21, 30 and 70 kPa O_2 atmospheres (Fig. 2c). After 14 days of storage, the radical scavenging capacity was greater in samples stored under low O_2, while those stored under 70 kPa O_2 levels showed the lowest antioxidant capacity. According to Martínez-Sánchez, Marín, Llorach, Ferreres, and Gil (2006), 21 kPa O_2 induced a particularly marked decrease in antioxidant capacity of leaves of wild rocket compared to samples stored under 5 kPa O_2 + 5 kPa CO_2 or 5 kPa O_2 + 10 kPa CO_2 atmospheres, which could be due to losses in some antioxidant constituents and total phenolic compounds.

In our study, a decrease in phenolic compounds and vitamin C content during the first 7 days of storage led to a decrease in antioxidant capacity. In addition, the increase in antioxidant activity was probably due to the synthesis of phenolic compounds due to the induced stress metabolism. Thus, the evolution of total phenolic compounds and vitamin C content showed a significant correlation with the antioxidant capacity throughout storage. Other authors reported a correlation between antioxidant capacity and ascorbic acid content of citrus fruit segments, confirming that ascorbic acid is the main antioxidant in fruits of the *Citrus* genus (Del Caro et al., 2004). However, changes in the antioxidant properties of fresh-cut mandarin were not entirely related to ascorbic acid concentration (Piga, Agabbio, Gambella, & Nicoli, 2002). Antioxidant capacity of fruits and vegetables is known to depend on a wide number of compounds (Viña & Chaves, 2003). In this regard, Chu, Chang, and Hsu (2000) have indicated that several phytochemicals, such as flavonoids, phenolic acids, aminoacids, ascorbic acid, tocopherols and pigments, might contribute to the total antioxidant activity.

3.5. Principal component analysis

In order to globally interpret the results for fresh-cut Piel de Sapo' melon packaged under different conditions, principal component analysis (PCA) was used. PCA was performed on all samples and variables (antioxidant capacity, POD activity, total phenolic compounds, vitamin C, O_2 and CO_2 in-package concentrations) to obtain an overview of the samples. This multivariate technique processed the main information on a limited number of variables. Three principal components (PC1, PC2 and PC3) were calculated. They account for 83.5% of the total variation of the data set (Fig. 3). The most important component (PC1) explained 45.48% of the total variance. This variation was mainly described by the variables total phenolic compounds, vitamin C, POD activity and headspace O_2 concentration. PC2 explained 21.3% of the total variance and it is mainly composed of CO_2 concentration. Finally, PC3 explained only 16.71% of variance and this variation was mostly described by antioxidant capacity. Fig. 3a and b shows the loading plots of PC1 vs. PC2 and PC3, respectively. Variables that appear close together in this plot correlated positively. As can be seen in Fig. 3a, there is a close relationship between POD activity, vitamin C and total phenolic compounds, demonstrating a relative affinity of POD enzyme for both substrates. In addition, CO_2 accumulation inside packages does not seem to affect bioactive compounds (total phenolic compounds and vitamin C), antioxidant capacity and POD activity of fresh-cut 'Piel de Sapo' melon. As expected, a good correlation was observed between vitamin C and O_2 concentration inside packages (Fig. 3b). Thus, the lower the O_2 concentration, the greater the vitamin C content. The radical scavenging capacity and total phenolic compounds were found to be similarly loaded on PC3, which indicated that both total phenolics and antioxidant capacity are closely related. By the contrary, antioxidant capacity did not appear to be well correlated with vitamin C content (Fig. 3b).

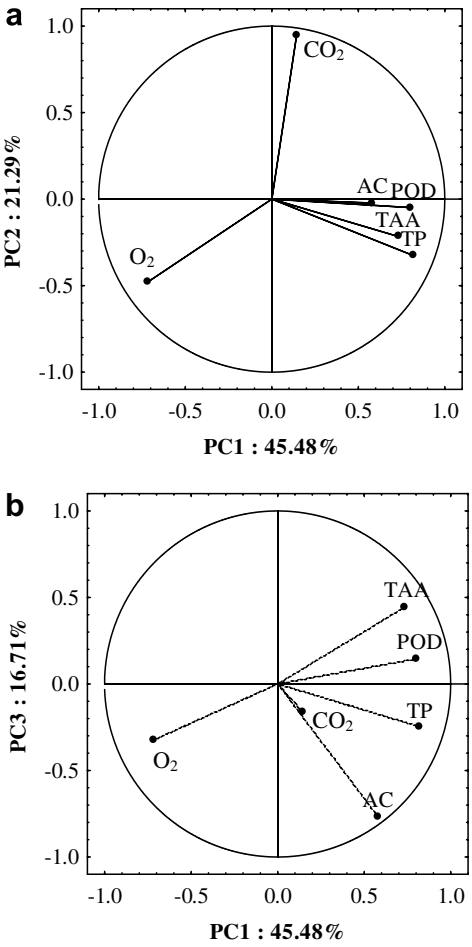

Fig. 3. PCA plot of fresh-cut 'Piel de Sapo' melon stored under modified atmosphere for 14 days at 4 °C: antioxidant capacity (AC), total vitamin C concentration (TAA), total phenolic compounds (TP), peroxidase activity (POD), oxygen concentration (O_2) and carbon dioxide concentration (CO_2).

The scores of PC1 vs. PC3 are plotted in Fig. 4 using different labels for the different packaging conditions. The main information in the data set was given by PC1, which explained 45.48% of total variance and described differences between samples packaged under low O_2 levels and high O_2 levels. It can be observed that a majority of the samples packaged under 2.5 kPa O_2 + 7 kPa CO_2 atmosphere are situated in the right part of the score plot whereas those samples packaged under 70 kPa O_2 were situated in the left part of the plot. Thus, fresh-cut 'Piel de Sapo' melon flushed with 2.5 kPa O_2 + 7 kPa CO_2 atmospheres scored higher on vitamin C and POD activity than packages contained higher O_2 concentrations. A minor portion of the total variation explained by PC3 (16.71%) described differences in antioxidant activity between samples stored under different atmosphere conditions. Therefore, it can be also deduced from Fig. 4 that samples corresponding to day 9, located at the bottom of the plot, are those with higher antioxidant capacity due to phenolic compounds synthesis.

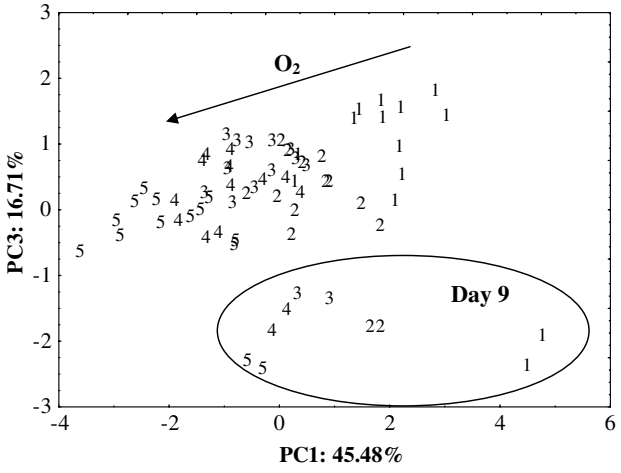

Fig. 4. Score plot of PC1 vs. PC3 of all sample labels for the different packaging conditions of fresh-cut 'Piel de Sapo' melon: 2.5 kPa O_2 + 7 kPa CO_2 (1), 10 kPa O_2 + 7 kPa CO_2 (2), 21 kPa O_2 (3), 30 kPa O_2 (4), 70 kPa O_2 (5).

4. Conclusion

The degradation of the initial content of vitamin C and total phenolic compounds was highly promoted by the presence of oxygen. Thus, low O_2 levels best maintained vitamin C and total phenolic compounds whereas CO_2 concentration did not seem to affect bioactive compounds. However, an enhanced oxidative stress induced by too-low O_2 and high CO_2 concentrations stimulated the synthesis of phenolic compounds and in turns, the increase of POD activity and loss of vitamin C. Total phenolic compounds and vitamin C content seem to be correlated to POD activity, suggesting that such compounds reacts as substrates of the enzyme. The results obtained in this study conducted with 'Piel de Sapo' melon suggest that 70 kPa O_2 storage atmospheres may decrease wounding stress and reduce deteriorative changes related to high POD activity in tissue. Eventually, phenolic compounds appeared to contribute in a greater manner than vitamin C to the antioxidant capacity of fresh-cut melon.

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