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Effect of High-Oxygen Atmospheres on the Antioxidant Potential of Fresh-Cut Tomatoes

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The effect of different initial in-package O_2 and CO_2 concentrations (2.5 kPa of O_2 + 5 kPa of CO_2, 10 kPa of O_2 + 5 kPa of CO_2, and 21, 60, and 80 kPa of O_2) on the antioxidant potential of fresh-cut tomatoes was investigated. Changes in individual phenolic compounds, individual carotenoids, vitamin C, and antioxidant capacity as well as in O_2, CO_2, and ethylene headspace concentrations inside packages were assessed for 21 days at 4 °C. High-oxygen and passive atmospheres induced higher production of carotenoids and phenolic compounds. The degradation of the initial content of vitamin C was highly promoted by the presence of oxygen. Lower hydrophilic antioxidant capacity (DPPH assay) was obtained in tomato slices stored under 80 kPa of O_2, whereas the antioxidant capacity of the lipophilic fraction was enhanced with oxygen availability inside headspace packages. Therefore, 2.5 kPa of O_2 + 5 kPa of CO_2 atmospheres not only reduced the formation of carotenoids but also maintained vitamin C in fresh-cut tomatoes.

KEYWORDS: Fresh-cut tomato; minimal processing; antioxidant potential; carotenoids; phenolic compounds; vitamin C

INTRODUCTION

Epidemiological studies have suggested that the consumption of tomato reduces the risk of some types of cancer and heart diseases (*1*). This beneficial effect is believed to be due, at least partially, to the action of tomato antioxidant compounds, which could reduce oxidative damage in the body. Tomatoes are the main dietary source of lycopene, a carotenoid with high oxygen-radical scavenging and quenching capacities, and β-carotene, which is the main carotenoid with provitamin A activity (*2*). However, carotenoids are highly unsaturated compounds with an extensive conjugated double-bond system and are therefore susceptible to oxidation, isomerization, and other chemical changes during processing and storage (*3*). Other antioxidant compounds such as phenolics confer health-promoting effects to tomato and derived products. Phenolics possess reducing character, capacity to sequester reactive oxygen species (ROS) and several electrophiles such as metallic ions, tendency to self-oxidation, and capacity to modulate the activity of some cell enzymes (*4*). On the other hand, tomatoes have a high concentration of vitamin C. Experimental studies have shown that vitamin C plays an important role in human health, including effects on the immune system and the risk of Alzheimer's disease (*5*).

Fresh-cut fruits are generally more perishable than whole fruits because they have been subjected to physiological stresses caused by physical damage or wounding. Slicing operations induce a rapid rise in CO_2 and ethylene production associated with the wounding response, which can reduce the quality of tomato slices (*6*). However, the content of some bioactive compounds such as flavonoids can increase as a result of mechanical damage to the fruit (*7*). Modified atmosphere packaging (MAP) effectively extends the shelf life of fresh-cut tomato. In this way, Artés et al. (*8*) observed that the shelf life of tomato slices could be maintained for 10 days at 2 °C under both passive and active MAP conditions (7.5% O_2 and 0% CO_2). Furthermore, Aguayo et al. (*9*) obtained fresh-cut tomatoes with good quality, appearance, and texture when storing them at 5 °C during 14 days in MAP conditions (3 kPa of O_2 + 4 kPa of CO_2, with N_2 as balance gas). However, problems associated with the development of off-odors, physiological and microbial decay, browning, and softening may appear when the O_2 level is too low and CO_2 is accumulated in packages (*10*). Thus, high-O_2 atmospheres have been suggested as an effective method to inhibit the growth of microorganisms and prevent undesired anoxic fermentation (*11*). Previous works have focused on assessing the effects of different modified atmosphere packaging systems on the quality of fresh-cut fruits (*12*). However, the impact of high-O_2 atmospheres on the nutritional and antioxidant properties of fresh-cut tomato has been neglected to date. Therefore, the aim of the present work was to determine the effect of high-O_2 atmospheres on individual carotenoids, phenolics, vitamin C, and antioxidant capacity of fresh-cut tomato, as well as to compare the results with those obtained under conventional low-O_2 and passive atmospheres (ca. 21 kPa of O_2). Ethylene, O_2, and CO_2 headspace concentrations were also evaluated during 14 days of storage at 4 °C.

MATERIALS AND METHODS

Reagents. Metaphosphoric acid and DL-1,4-dithiotreitol (DTT) were purchased from Acros Organics (Fair Lawn, NJ); Celite, magnesium carbonate, tetrahydrofuran (THF), methylene chloride, anhydrous

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sodium sulfate, methanol, hexane, acetonitrile, ascorbic acid, sulfuric acid, chlorydric acid, and acetic acid were obtained from Scharlau Chemie, SA (Barcelona, Spain). *tert*-Butylhydroquinone, β-apo-8′-carotenal, chlorogenic acid, caffeic acid, *p*-coumaric acid, ferulic acid, kaempferol, quercetin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azinobis-(3-ethylbenzothiazoline)-6-sulfonate ($ABTS^+$), and 6-methoxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich Co. (St. Louis, MO). Lycopene, lutein, neurosporene, γ-carotene, δ-carotene, β-carotene, and phytofluene were obtained from Carotene Nature GmbH (Lupsingen, Switzerland).

Quality Characterization. Tomatoes (*Lycopersicon esculentum* Mill. cv. Bola) harvested in Alicante (Spain) were purchased from a local supermarket (Lleida, Spain) at commercial maturity and stored at 4 ± 1 °C. pH (Crison 2001 pH-meter; Crison Instruments SA, Alella, Barcelona, Spain), titratable acidity, soluble solids content (Atago RX-1000 refractometer; Atago Co. Ltd., Tokyo, Japan), color (spectrophotocolorimeter Minolta CR-400; Konica Minolta Sensing, Inc., Osaka, Japan), and firmness (Mechanical Fruit Firmness Tester, QA Supplies, LLC, Norfolk, VA) were determined. Analytical characteristics of whole tomatoes were as follows: pH 4.30 ± 0.21; titratable acidity = 0.51 ± 0.2; soluble solids = 5.9 ± 0.1 °Brix; color $L^* = 50.0 \pm 2.6$, $a^* = 13.9 \pm 2.1$, and $b^* = 16.4 \pm 1.3$; firmness = 48.4 ± 3.7 N.

Sample Preparation. Tomatoes were sanitized in a 200 μL L^{-1} NaClO solution for 2 min, rinsed with tap water, and dried by hand prior to cutting operations. The fruits were cut into 7 mm thick slices from the stem-end portion perpendicularly to the long axis of the fruit with a commercial slicing machine (Food Slicer-6128; Toastmaster Corp., Menominee, MI). Five tomato slices (100 g) were packaged in each polypropylene tray. The active modification of package atmosphere was carried out by flushing a mixture of 2.5 kPa of O_2 + 5 kPa of CO_2, 10 kPa of O_2 + 5 kPa of CO_2, 60 kPa of O_2, and 80 kPa of O_2 balance with N_2, before the trays were sealed, using a digitally controlled compensated vacuum packaging machine (ILPRA Food Pack Basic V/6, ILPRA Systems, CP, Vigevono, Italia). For a passive modification of package atmospheres (PA), the trays were sealed without fluxing gas mixture. The relationship between the amount of product and the gas headspace was 1:2 (v/v). The O_2 and CO_2 permeance of the 64 μm thick polypropylene sealing film were 5.2419×10^{-13} mol of O_2 m^{-2} s^{-1} Pa^{-1} and 2.3825×10^{-12} mol of CO_2 m^{-2} s^{-1} Pa^{-1} at 23 °C and 0% RH, respectively (ILPRA Systems España, S.L., Mataró, Spain). The packages were stored at 4 ± 1 °C in darkness until random withdrawal for analysis. Three trays were taken at each time to perform the analysis, and two readings were carried out for each package.

Headspace Gas Analysis. The gas composition of the package headspace was determined with a gas analyzer (Micro-GC CP 2002, 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 O_2 concentrations was carried out by injecting a sample of 0.25 μL to a CP-Molsieve 5 Å packed column (4 m × 0.32 mm, d.f. = 10 μm) at 55 °C and 90 kPa, whereas a sample of 0.33 μL was injected to a Pora-PLOT Q column (10 m × 0.32 mm, d.f. = 10 μm) at 75 °C and 140 kPa for carbon dioxide and ethylene quantification.

Determination of Carotenoids. The extraction method was based on a procedure proposed by Tonucci et al. (*13*). First, 2.5 g of magnesium carbonate and 2.5 g of Celite, used as filter aid, were added to 25 g of tomato with 0.5 mg of internal standard (β-apo-8′-carotenal). The mixture was blended for 20 min in an Omni Mixer with 25 mL of tetrahydrofuran (THF) and then filtered through Whatman No. 1 filter paper using a Büchner funnel. The solid material was extracted two or three more times until it was devoid of red/orange color. The THF extracts were combined, and the volume was reduced by about two-thirds under vacuum at 35 °C with a rotary evaporator. Fractions of the combined extract were portioned into 250 mL of methylene chloride and 150 mL of saturated sodium chloride aqueous solution in a separatory funnel. The water layer was washed with methylene chloride until carotenoids were completely removed. The methylene chloride layer containing carotenoids was dried over anhydrous sodium sulfate and filtered through Whatman No. 42 filter paper. The volume of the filtrate was reduced under vacuum to approximately 25 mL and brought up to 50 mL with methylene chloride. Then the extracts were passed through a Millipore 0.45 μm membrane and injected into the HPLC system. Conditions for the HPLC separations were those reported by Khachik et al. (*14*). The HPLC system was equipped with a 600 Controller and a 486 Absorbance Detector (Waters, Milford, MA) working at 470 nm. Samples were introduced into a reverse-phase C18 Spherisorb ODS2 (5 μm) stainless steel column (4.6 mm × 250 mm) through a manual injector equipped with a sample loop (20 μL). The flow rate was 0.7 mL min^{-1} at room temperature. An isocratic elution of 85% acetonitrile/10% methanol/3% methylene chloride/2% hexane was maintained from 0 to 10 min, followed by a linear gradient to 45% acetonitrile/10% methanol/23% methylene chloride/22% hexane from 10 to 40 min. At the end of the gradient, the column was equilibrated under the initial conditions for 20 min. Carotenoids were quantified by comparison with external standards of lycopene, lutein, neurosporene, γ-carotene, δ-carotene, β-carotene, and phytofluene. Results were expressed as milligrams of carotenoids per kilogram of tomato.

Vitamin A. Vitamin A values were expressed as retinol equivalents (RE) (*15*). To calculate RE on the basis of carotenoids, the following conversion was employed: RE = (μg of β-carotene/6) + (μg of γ-carotene/12).

Determination of Phenolic Compounds. A high-performance liquid chromatography method (HPLC) was used for the analysis of individual phenolic compounds. The extraction was carried out following the method validated by Hertog et al. (*16*). A sample of 0.5 g of previously freeze-dried tomato tissue was carefully mixed with 40 mL of 62.5% methanol (2 g/L of *tert*-butylhydroquinone) and 10 mL of 6 M HCl. After refluxing at 90 °C for 2 h with regular swirling, the extract was cooled and subsequently made up to 100 mL with methanol and sonicated for 5 min. The extract was then passed through a 0.45 μm filter prior to injection. The HPLC system was equipped with a 600 Controller and a diode array detector (Waters), which was set to scan from 200 to 600 nm. Separations were performed on a reverse-phase C18 Spherisorb ODS2 (5 μm) stainless steel column (4.6 mm × 250 mm) at room temperature with a flow rate of 1 mL min^{-1}. A gradient elution was employed with a solvent mixture of 2.5% HCOOH in water (solvent A) and 2.5% HCOOH in acetonitrile (solvent B) as follows: linear gradient from 5 to 13% B, 0−15 min; linear gradient from 13 to 15% B, 15−20 min; linear gradient from 15 to 30% B, 20−25 min; isocratic elution 30% B, 25−28 min; linear gradient from 30 to 45% B, 28−32 min; isocratic elution 45% B, 32−35 min; linear gradient 45 to 90% B, 35−40 min; isocratic elution 90% B, 40−45 min; linear gradient to reach the initial conditions after 5 min; post-time 10 min before the next injection. Individual phenolics were quantified by comparison with external standards of chlorogenic acid, caffeic acid, *p*-coumaric acid, ferulic acid, kaempferol, and quercetin. The results were expressed as milligrams of phenolic compounds per kilogram of tomato.

Determination of Vitamin C. The extraction procedure was based on a previously validated method (*17*). A portion of 25 g of fruit was added to 25 mL of a 4.5% metaphosphoric solution with 7.2 g L^{-1} DTT as reducing agent. The mixture was crushed, homogenized, and centrifuged at 22100*g* for 15 min at 4 °C. The supernatant was vacuum-filtered through Whatman No. 1 paper. The sample was then passed through a Millipore 0.45 μm membrane and injected into the HPLC system. A Waters 600E multisolvent delivery system was used for the analysis. Samples were introduced onto the column via a manual injector equipped with a sample loop (20 μL). Separation of ascorbic acid was performed using a reverse-phase C18 Spherisorb ODS2 (5 μm) stainless steel column (4.6 mm × 250 cm). The mobile phase was a 0.01% solution of sulfuric acid adjusted to pH 2.6. The flow rate was fixed at 1.0 mL min^{-1}. Detection was performed with a 486 Absorbance Detector (Waters) set at 245 nm. Vitamin C was quantified throughout a calibration curve built with pure ascorbic acid standards, and results were expressed as milligrams of vitamin C per kilogram of fresh-cut tomato.

Determination of Antioxidant Capacity. Antioxidant capacity was studied through the evaluation of the free radical scavenging effect on the $DPPH^•$ radical according to the procedure described by Odriozola-Serrano et al. (*18*). In addition, the ABTS assay, based on the ability of the antioxidants to scavenge the blue-green radical cation $ABTS^+$ was conducted according to the method described by Re et al. (*19*). Two fractions (hydrophilic and lipophilic) were prepared from fresh-cut tomatoes and used in the antioxidant assay following the method proposed by Lenucci et al. (*20*). About 5 g of sample was mixed with 25 mL of absolute methanol or hexane and then were centrifuged at 6000*g*

for 15 min at 4 °C (Centrifuge Medigifer; Select, Barcelona, Spain) to obtain the hydrophilic and lipophilic extracts, respectively. Aliquots of 0.1 mL of the hydrophilic or lipophilic supernatants were mixed with 3.9 mL of methanolic DPPH or ABTS solutions. The homogenate was shaken vigorously and kept in darkness for 60 min. Absorption of the samples was measured with a spectrophotometer (CECIL CE 2021; Cecil Instruments Ltd., Cambridge, U.K.) at 515 nm for the DPPH assay or at 734 nm for the ABTS assay. The percentage of inhibition of the radicals ($DPPH^{\bullet}$ and $ABTS^{+}$) was calculated and plotted as a function of the concentration of Trolox for the standard reference data. The final DPPH and ABTS values were calculated using a regression equation between the Trolox concentration and the percentage of DPPH or ABTS inhibition. Results were expressed as millimoles of Trolox equivalents per kilogram of fresh-cut tomato.

Statistical Analysis. Statistical analysis was performed using the Statgraphics Plus v.5.1 Windows package (Manugistics, Inc., Rockville, MD). Data were analyzed by multifactor analysis of variance, and a Duncan multiple-range test was employed to find differences among means, with a level of significance of 0.05. Principal component analysis (PCA) was carried out to obtain relationships among variables. The loadings plot was used to summarize the main relationship between different variables themselves. Variables that appear close together in this plot correlated positively. On the other hand, the score plot was carried out to represent the projection of each sample into PC, defining different groups.

RESULTS AND DISCUSSION

Changes in Carotenoid Profile. Changes in total and individual carotenoids of fresh-cut tomatoes stored under different initial in-package conditions are shown in **Table 1**. Initial carotenoid content in fresh-cut tomatoes ranged from 113.3 to 116.6 mg kg^{-1}. A marked decrease in total carotenoids over time (21 days) was observed in samples stored under 2.5 kPa of O_2+5 kPa of CO_2 or 10 kPa of O_2+5 kPa of CO_2. This trend may be explained by the fact that modified atmospheres including either reduced O_2 or elevated CO_2 are generally considered to inhibit the biosynthesis of carotenoids (*21*). In contrast, exposure to ≥60 kPa of O_2 induced a higher production of carotenoids, which may be related to the oxidative stress induced by high ethylene content inside packages (**Figure 1c**). Consistently, other authors (*25*) have reported that ripening of tomatoes is accelerated under 40−50 kPa of O_2 compared with air. The presence of O_2 rapidly induces enzymatic activities related to the ethylene biosynthetic pathway, resulting in a large burst of ethylene synthesis. This enhancement of ethylene promotes physiological changes in climacteric fruits such as the initiation of ripening in tomatoes (*22*).

With regard to the individual carotenoids, lycopene was the main carotenoid found in fresh-cut tomatoes, accounting for 28% of the total carotenoid content. The initial lycopene content of tomato slices ranged from 31.4 to 33.7 mg kg^{-1} (**Table 1**). Other individual carotenoids that are lycopene precursors, such as phytofluene (5.0−5.1 mg kg^{-1}) and neurosporene (27.1−28.5 mg kg^{-1}), were also found in tomato slices. The increase in lycopene in fresh-cut tomatoes under ≥60 kPa of O_2 was greater over time than that observed under lower initial O_2 concentrations. On the contrary, neurosporene content decreased considerably throughout the storage period, leading to nondetectable levels at 14−21 days of storage in fresh-cut tomatoes stored under ≤10 kPa of O_2. The initial content of phytofluene was also decreased through the first 11 days of storage in fresh-cut tomatoes irrespective of the initial atmosphere, and then the values rose in tomato slices stored under high-O_2 atmospheres, reaching values of 4.1−4.2 mg kg^{-1} at the end of storage. Thus, initial atmospheres rich in oxygen might stimulate the transformation of some carotenoids into lycopene. Phytofluene undergoes a series of desaturation reactions, each of which creates a

Table 1. Carotenoid Content of Fresh-Cut Tomatoes Stored during 21 Days at 4 °C under Different Packaging Conditions[a]

		individual carotenoids (mg kg^{-1})							
	storage time (days)	lutein	lycopene	neurosporene	γ-carotene	δ-carotene	β-carotene	phytofluene	TC (mg kg^{-1})
T1	0	6.7b	33.7e	27.6a	20.0a	4.2a	19.3f	5.1a	116.6c
	7	10.1a	43.5c	6.9d	10.8d	3.3b	55.8d	2.6d	133.0a
	14	6.6b	47.7b	nd	4.3h	2.1c	54.5c	2.8d	118.0c
	21	7.1b	50.4a	nd	7.1g	1.9c	65.9a	4.1b	136.5a
T2	0	6.7d	32.3f	27.6a	18.6b	4.1a	19.0e	5.0a	113.3c
	7	13.5a	41.4d	6.7e	19.3a	3.2b	39.2bc	3.1d	126.4a
	14	4.6e	46.4b	8.3d	11.7bc	2.3c	37.2c	3.4cd	113.9c
	21	4.5e	50.0a	nd	11.0cd	2.2c	47.9a	4.2b	119.8b
T3	0	6.3d	31.4e	27.1a	20.1a	4.1a	19.4e	5.1a	113.5c
	7	13.5a	38.8b	12.9b	17.7b	3.1b	45.2b	3.0d	134.2a
	14	4.9e	44.9a	7.3c	10.8c	2.7b	38.9c	1.8f	111.3c
	21	4.3e	44.5a	7.6c	9.3d	1.9c	50.7a	2.1ef	120.4b
T4	0	6.5d	32.4c	28.5a	18.7b	4.3a	18.3f	5.0a	113.7a
	7	12.3a	27.5e	10.9d	13.9c	2.2e	37.3a	3.2c	107.3b
	14	10.4b	39.0a	9.8d	5.0f	1.8f	31.6c	2.1	99.7c
	21	11.1b	36.2b	4.5f	7.2e	2.2e	35.1b	2.5d	98.8c
T5	0	7.0d	32.2a	27.6a	20.5a	4.2a	19.7e	5.1a	116.3a
	7	13.8a	29.0b	11.1c	10.3b	2.3c	26.5a	3.2c	96.2c
	14	10.8bc	30.3ab	9.7b	5.1c	2.0c	22.8c	2.3d	83.0d
	21	14.5a	29.7ab	9.6b	4.2d	2.1c	20.8d	2.1d	83.0d

[a] Different letters in the same column for each in-package condition indicate significant differences among days ($p < 0.05$). TC, total carotenoids calculated by the sum of carotenoids determined by HPLC. T1, 80 kPa of O_2; T2, 60 kPa of O_2; T3, 21 kPa of O_2; T4, 10 kPa of O_2 + 5 kPa of CO_2; T5, 2.5 kPa of O_2 + 5 kPa of CO_2.

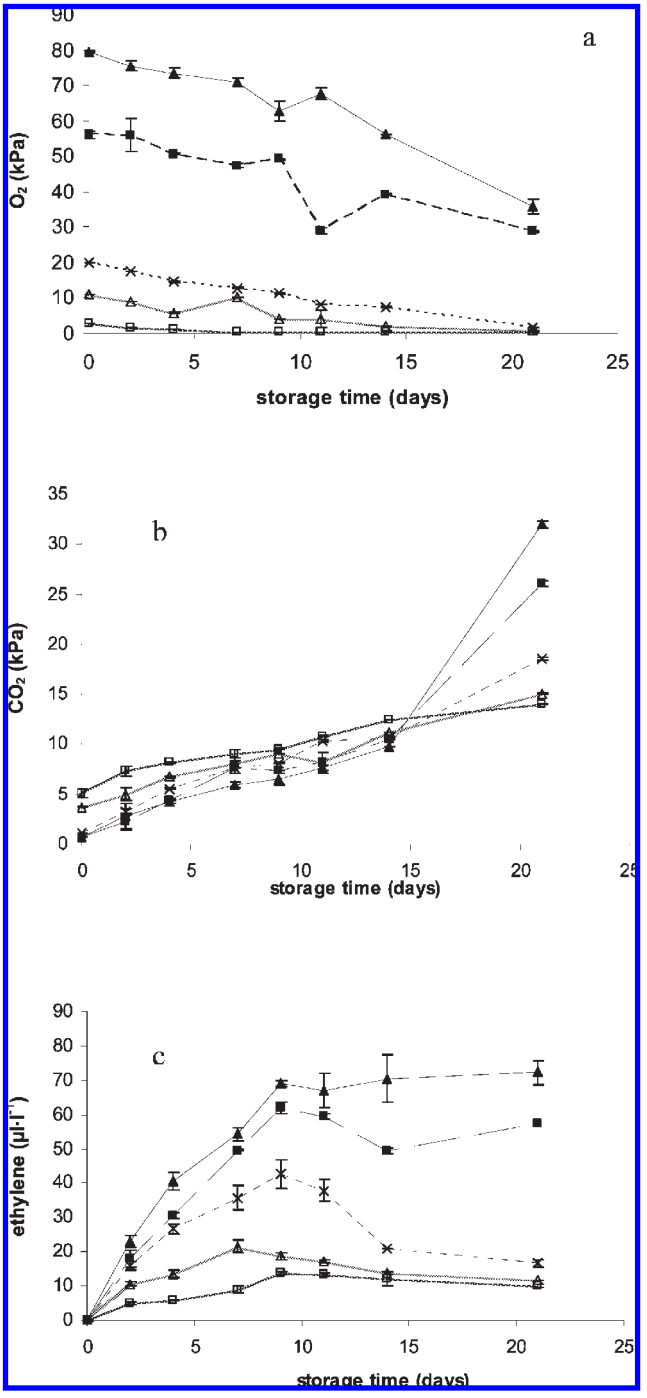

Figure 1. Oxygen (**a**), carbon dioxide (**b**), and ethylene (**c**) headspace composition in trays of fresh-cut tomato stored during 21 days at 4 °C under different packaging condition: (▲) 80 kPa of O_2; (■) 60 kPa of O_2; (×) 21 kPa of O_2; (△) 10 kPa of O_2 + 5 kPa of CO_2; (□) 2.5 kPa of O_2 + 7 kPa of CO_2. Data shown are mean ± standard deviation.

new double bond and extends the chromophore by two conjugated double bonds; the end product is lycopene, produced via the intermediate neurosporene (*23*). However, little is known about the mechanisms involved in carotenoid biosynthesis in plants. The biosynthetic enzymes are encoded by nuclear genes, and precursor proteins are post-translationally imported into plastids, where carotenoid biosynthesis occurs. Disruption of tissue by wounding, followed by exposure to various atmospheres, could promote the transcription of the genes or the transport of the mRNA related to the synthesis of carotenoids (*24*). β-Carotene and γ-carotene were found in tomato

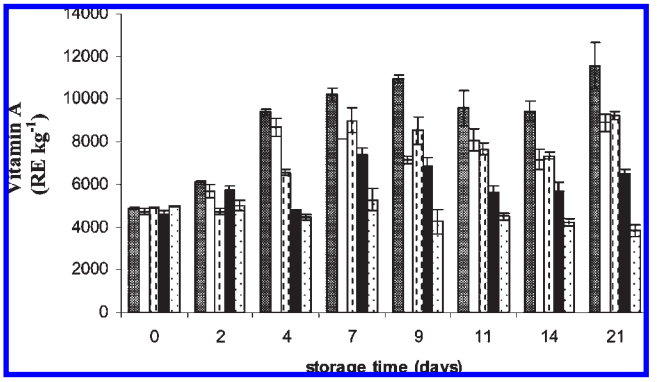

Figure 2. Vitamin A content of fresh-cut tomatoes stored during 21 days at 4 °C under different packaging conditions: (gray bars) 80 kPa of O_2; (white bars) 60 kPa of O_2; (dashed bars) 21 kPa of O_2; (black bars) 10 kPa of O_2 + 5 kPa of CO_2; (dotted bars) 2.5 kPa of O_2 + 5 kPa of CO_2. Data shown are mean ± standard deviation.

slices in amounts ranging from 18.3 to 19.7 mg kg^{-1} and from 18.6 to 20.5 mg kg^{-1}, respectively. Biochemical reactions related to the production of β-carotene seemed to increase in tomato slices stored under ≥10 kPa of O_2 atmospheres. As can be seen in **Table 1**, β-carotene content was higher in fresh-cut tomatoes under 80 kPa of O_2 than in other packaging conditions. Fresh-cut tomatoes underwent a substantial depletion of γ-carotenoid during the storage period in all of the studied samples, which may be a consequence of their conversion to β-carotene. Hence, γ-carotene may undergo cyclization to form six-membered rings at one end of the molecule, giving β-carotene as a product (*23*). Tomatoes have been found to be a rich source of vitamin A, which is related to the amounts of β-carotene and γ-carotene in the samples (*25*). The initial content of vitamin A in fresh-cut tomatoes was 4609−4995 RE kg^{-1}. The amount of vitamin A in tomato slices stored under ≥10 kPa of O_2 remarkably increased over time (**Figure 2**). On the basis of the present results, it seems that ethylene production and, in turn, oxygen availability have important effects on carotenoids with provitamin A activity. The greater increase of vitamin A observed in fresh-cut tomatoes could be associated with the acceleration of ripening and enhancement of senescence. Changes in other minor individual carotenoids such as lutein (6.3−7.0 mg kg^{-1}) and δ-carotene (4.1−4.3 mg kg^{-1}) are also shown in **Table 1**. The main losses of δ-carotene throughout the first 14 days of storage were found in fresh-cut tomatoes under high-O_2 atmospheres, whereas lutein was enhanced in tomato slices stored under these packaging conditions (**Table 1**).

Changes in Phenolic Compound Profile. The sum of individual phenolics of fresh-cut tomatoes measured by HPLC was approximately 45.0−45.7 mg kg^{-1} (**Table 2**). Chlorogenic acid was the main hydroxycinnamic acid derivative in tomato slices, obtained in concentrations of 21.2−22.0 mg kg^{-1}. Tomato slices stored under ≥60 kPa of O_2 underwent the highest increase in chlorogenic acid through storage up to values of 26.7−30.4 mg kg^{-1} at 21 days compared to samples stored under other packaging conditions. In turn, chlorogenic acid was further increased in fresh-cut tomatoes stored under passive atmospheres in comparison with those stored under low-O_2 atmospheres during 11 days (**Table 2**). Consistently, Alasalvar et al. (*26*) reported that low O_2 and CO_2 levels prevent the accumulation of phenolic compounds in ready-to-eat shredded orange and purple carrots. Low O_2 levels can inhibit the biosynthesis of phenolic compounds in fresh-cut products, which are induced usually in response to cutting damage (*8*). Wounding may stimulate phenylalanine

Table 2. Phenolic Content of Fresh-Cut Tomatoes Stored during 21 Days at 4 °C under Different Packaging Conditions[a]

		individual phenolic compounds (mg kg^{-1})						
	storage time (days)	chlorogenic acid	ferulic acid	*p*-coumaric acid	caffeic acid	quercetin	kaempferol	SIP (mg kg^{-1})
T1	0	21.6d	4.0b	3.6a	2.5a	9.0e	4.3c	45.0c
	7	27.6b	3.5c	2.4a	1.8b	11.0d	6.1a	52.4b
	14	28.4b	4.1b	2.0a	nd	13.1b	6.2a	53.8b
	21	30.4a	4.2b	2.0b	nd	14.8a	6.2a	57.6a
T2	0	21.2e	4.2a	3.8a	2.7a	9.6e	4.2d	45.7c
	7	22.6d	4.1a	2.5c	2.0b	11.1c	5.6b	47.9b
	14	26.3ab	3.8bc	1.9d	nd	12.1b	5.5b	49.6ab
	21	26.7a	3.9b	1.9d	nd	12.9a	5.9a	51.3a
T3	0	22.0d	3.9c	3.6a	2.6a	9.6e	4.2d	45.7b
	7	26.3a	4.6a	2.5c	1.8c	11.1b	5.9ab	52.2a
	14	23.7c	4.2b	1.8d	nd	11.8ab	6.0ab	47.5b
	21	23.3c	4.3b	2.0d	nd	11.4b	5.7b	46.7b
T4	0	21.5b	4.2a	3.6a	2.7ab	9.4d	4.1c	45.1ab
	7	21.8b	3.7b	2.8b	2.8a	10.7b	5.7b	47.5a
	14	19.6c	4.0a	1.8c	1.7d	10.6b	5.2b	42.9b
	21	19.7c	4.0a	1.7c	1.7d	11.4a	5.2b	43.7b
T5	0	21.6c	4.1b	3.4a	2.7b	9.4d	4.0a	45.2b
	7	23.9a	4.1b	3.0b	2.4c	11.5a	4.2b	49.1a
	14	18.2d	3.7c	1.6c	1.7d	9.8c	4.2b	39.2c
	21	17.0e	3.9bc	1.6c	1.8d	9.3d	4.4b	38.0c

[a] Different letters in the same column for each in-package condition indicate significant differences among days ($p < 0.05$). SIP, sum of individual phenolic compounds determined by HPLC. T1, 80 kPa of O_2; T2, 60 kPa of O_2; T3, 21 kPa of O_2; T4, 10 kPa of O_2 + 5 kPa of CO_2; T5, 2.5 kPa of O_2 + 5 kPa of CO_2.

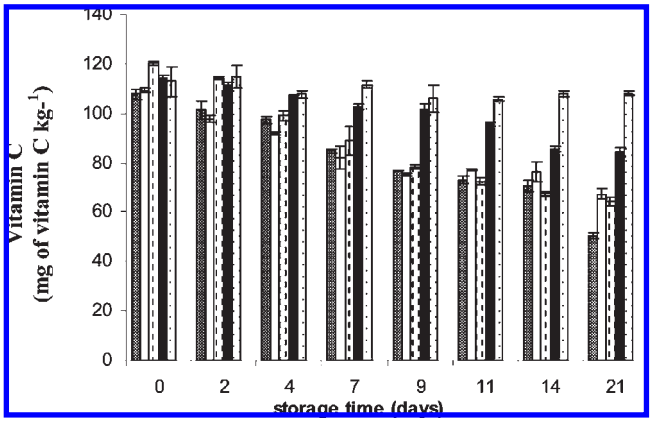

Figure 3. Vitamin C content of fresh-cut tomatoes stored during 21 days at 4 °C under different packaging conditions: (gray bars) 80 kPa O_2; (white bars) 60 kPa of O_2; (dashed bars) 21 kPa of O_2; (black bars) 10 kPa of O_2 + 5 kPa of CO_2; (dotted bars) 2.5 kPa of O_2 + 5 kPa of CO_2. Data shown are mean ± standard deviation.

ammonia-lyase activity (PAL) during minimal processing with consequent further production of phenolic compounds (*26*). The PAL activation of phenylpropanoid metabolisms could be elicited through induced reactive oxygen species (*27*). On the other hand, fresh-cut tomatoes have been found to be a rich source of flavonols such as quercetin and kaempferol. Initial quercetin concentrations in tomato slices were 9.0−9.6 mg kg^{-1}, whereas kaempferol was found at concentrations of 4.0−4.3 mg kg^{-1}. In this way, Martínez-Valverde et al. (*28*) reported quercetin and kaempferol concentrations in different tomato cultivars ranging between 7.2 and 43.6 mg kg^{-1} and between 1.2 and 2.0 mg kg^{-1}, respectively. Quercetin and kaempferol content increased significantly throughout storage irrespective of the initial packaging

Table 3. -Changes in Antioxidant Capacity of Fresh-Cut Tomatoes Stored during 21 Days at 4 °C at Different Initial In-Package Conditions[a]

	DPPH Assay (mmol kg^{-1})									
storage time (days)	Hydrophilic fraction					Lipophilic fraction				
	T1	T2	T3	T4	T5	T1	T2	T3	T4	T5
0	3.16a	3.20a	3.26a	3.30a	3.30a	1.58d	1.62d	1.62b	1.49d	1.52b
2	3.19a	3.17a	3.33a	3.32a	3.26a	1.57d	1.66cd	1.62b	1.74a	1.66a
4	2.41b	2.34b	2.62b	2.74b	2.77b	1.71c	1.70c	1.63b	1.59bc	1.61a
7	2.27c	22.6c	2.39c	2.59c	2.68c	1.52d	1.63d	1.60b	1.54c	1.55b
9	2.31c	2.32b	2.26d	2.40d	2.68c	1.92a	1.89a	1.78a	1.59bc	1.51b
11	1.77d	1.83c	1.96e	2.28e	2.64c	1.73c	1.80b	1.66b	1.64b	1.38c
14	1.71d	1.85c	2.00e	2.31e	2.58d	1.84b	1.73c	1.58b	1.47d	1.36c
21	1.77d	1.71d	2.01e	2.16e	2.56d	1.80b	1.73c	1.59b	1.54c	1.33c
	ABTS Assay (mmol kg^{-1})									
storage time (days)	Hydrophilic fraction					Lipophilic fraction				
	T1	T2	T3	T4	T5	T1	T2	T3	T4	T5
0	2.15a	2.05a	1.97a	1.99a	2.09a	2.02a	2.00a	2.07a	2.02a	1.89a
2	2.04b	1.90b	2.00a	1.99a	1.90b	1.76b	1.75b	1.63b	1.62b	1.66b
4	1.75c	1.88b	1.80b	1.71b	1.78c	1.44c	1.65c	1.43c	1.54c	1.42c
7	1.80c	1.73c	1.68c	1.64c	1.68c	1.47c	1.42d	1.17d	1.29e	1.24d
9	1.70c	1.71c	1.55d	1.66c	1.75c	1.25d	1.06e	1.17d	1.26e	1.29d
11	1.05d	1.08d	0.92e	0.96d	0.93d	1.47c	1.05e	0.99e	1.31d	1.05e
14	1.00d	0.96d	0.81f	0.90d	0.93d	1.06e	0.93f	1.15d	0.92f	0.87f
21	0.83e	0.88e	0.76f	0.96d	0.83e	0.74f	0.76g	0.68f	0.84f	0.76g

[a] Different letters in the same column indicate significant differences among days ($p < 0.05$). T1, 80 kPa of O_2; T2, 60 kPa of O_2; T3, 21 kPa of O_2; T4, 10 kPa of O_2 + 5 kPa of CO_2; T5, 2.5 kPa of O_2 + 5 kPa of CO_2.

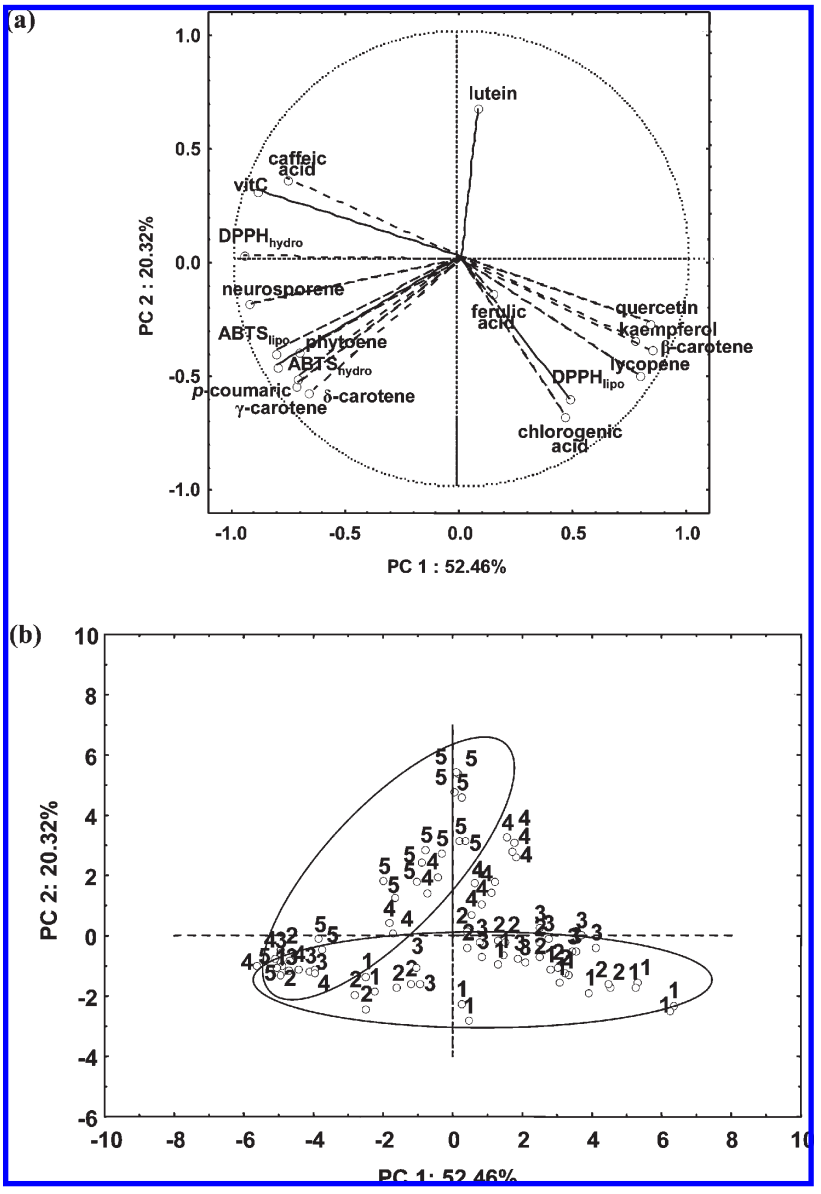

Figure 4. (**a**) Principal component and (**b**) score plot of fresh-cut tomatoes stored for 21 days at 4 °C under different packaging conditions: (1) 80 kPa of O_2; (2) 60 kPa of O_2; (3) 21 kPa of O_2; (4) 10 kPa of O_2 + 5 kPa of CO_2; (5) 2.5 kPa of O_2 + 5 kPa of CO_2. Two replicates were performed for each packaging condition and sampling time.

atmosphere. However, such an increase was the lowest in tomato slices stored under initial ≤10 kPa of O_2 atmospheres (**Table 2**). Zheng et al. (*29*) reported that high-O_2 atmospheres enhanced quercetin and kaempferol in blueberries in comparison with air. Quercetin in strawberry fruits stored under 60 and 100 kPa of O_2 was also better maintained than in fruits held in air after 14 days of storage (*30*). With regard to other phenolic acids, ferulic acid (3.9−4.2 mg kg^{-1}), *p*-coumaric acid (3.4−3.8 mg kg^{-1}), and caffeic acid (2.5−2.7 mg kg^{-1}) were found in minor quantities. As can be seen in **Table 2**, the caffeic acid content in tomato slices substantially depleted through the storage period, regardless of the initial packaging conditions. However, this phenolic acid was best maintained in fresh-cut tomatoes stored under low-O_2 atmospheres for 21 days, because a nondetectable amount of caffeic acid was found in tomatoes under ≥21 kPa of O_2 beyond 9−14 days. Fresh-cut tomatoes underwent a substantial depletion of *p*-coumaric acid during storage, which may be a consequence of its conversion to flavonols. *p*-Coumaric acid is formed in plant products via the action of PAL due to the phenylpropanoid metabolism. Biosynthesis of flavonols starts with the sequential addition of three molecules of malonyl-CoA to suitable hydroxycinnamic acid CoA esters such as *p*-coumaric acid, to form chalcones. The chalcones are then isomerized into (2*S*)-flavanones, which are converted into flavonols by hydroxylations and desaturations (*31*).

Changes in Vitamin C Content. Initial vitamin C contents of fresh-cut tomatoes ranged from 108 to 120 mg kg^{-1}. **Figure 3** shows changes in vitamin C concentrations of fresh-cut tomato through storage. As expected, vitamin C oxidation was greatly favored by the presence of oxygen. Soliva-Fortuny et al. (*32*) reported that the magnitude of vitamin C degradation can be related to the O_2 concentrations inside the packages. Hence, the higher amount of O_2 in the bag's headspace, the greater decrease in vitamin C content. A substantial variation of vitamin C over the storage time was observed in fresh-cut tomatoes stored under ≥10 kPa of O_2 atmospheres. Thus, the most dramatic decrease in vitamin C was detected in fresh-cut tomatoes stored under 80 kPa of O_2, reaching the lowest content

(50 mg kg^{-1}) after 21 days of storage at 5 °C. Fresh-cut tomatoes stored under initial 2.5 kPa of O_2 + 5 kPa of CO_2 atmosphere kept their initial vitamin C content (108−115 mg kg^{-1}) for 21 days. Consistently, Oms-Oliu et al. (*33*) reported that 70 kPa of O_2 atmospheres induced higher losses of vitamin C in fresh-cut pears in comparison to air and 2.5 kPa of O_2 + 5 kPa of CO_2 atmospheres.

Changes in Antioxidant Capacity. Antioxidant capacity of fresh-cut tomatoes measured through the DPPH and ABTS methods is shown in **Table 3**. With regard to the DPPH assay, the hydrophilic antioxidant capacity of fresh-cut tomatoes stored under 2.5 kPa of O_2 + 5 kPa of CO_2 was significantly higher than those of tomatoes stored under higher O_2 concentrations. According to ABTS values, nonsignificant differences in hydrophilic antioxidant capacity were observed among fresh-cut tomatoes packaged under different initial atmospheres. The highest antioxidant capacity of the lipophilic fraction was obtained in fresh-cut tomatoes stored under ≥60 kPa of O_2 irrespective of the assay carried out. Several methods have been used to evaluate the antioxidant profile of food products, and results may greatly vary depending on the experimental conditions and the specificity of the free radical used (*34*).

A principal components analysis (PCA) was used to determine relationships among antioxidant compounds. Two principal components (PC1 and PC2) were calculated. They account for 72.8% of the variability in the original data (**Figure 4a**). As can be seen in **Figure 4a**, there is a close relationship between DPPH hydrophilic values and vitamin C content. Therefore, hydrophilic antioxidant capacity of fresh-cut tomatoes determined through the DPPH assay could be mainly attributed to vitamin C rather than to phenolic compounds. The free radical DPPH is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors. The DPPH assay is not specific to any particular antioxidant component, thus applying to the overall antioxidant capacity of the sample (*35*). In addition, the statistical analysis reveals an outstanding correlation among the DPPH radical scavenging capacity of the lipophilic fraction, lycopene and β-carotene, whereas changes in phytofluene and neurosporene were well associated with the variation in the lipophilic ABTS antioxidant capacity (**Figure 4a**). The ABTS radical has been used to screen the relative radical-scavenging abilities of flavonoids and phenolics through their properties as electron- or H-donating agents (*36*). However, there was a slight relationship between flavonols, phenolic acid, and the hydrophilic antioxidant capacity determined through the ABTS radical. The score plot of PC1 versus PC2 from the full-data PCA model plotted in **Figure 4b** describes differences between fresh-cut tomatoes stored under different in-package conditions. It can be observed that most of the samples packaged under 2.5 kPa of O_2 + 5 kPa of CO_2 atmospheres are situated in the upper part of the plot, whereas samples packaged under 80 kPa of O_2 are located at the bottom (**Figure 4b**). Therefore, fresh-cut tomatoes stored under 80 kPa of O_2 atmospheres scored higher on flavonols, lycopene, β-carotene, chlorogenic acid, and DPPH values of the lipophilic fraction than those packaged under lower O_2 concentrations.

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