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Antioxidant Content of Fresh-Cut Pears Stored in High-O_2 Active Packages Compared with Conventional Low-O_2 Active and Passive Modified Atmosphere Packaging

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The antioxidant content of fresh-cut 'Flor de Invierno' (*Pyrus communis* L.) pears dipped into a 0.75% w/v *N*-acetylcysteine + 0.75% w/v glutathione solution and packaged under 70 kPa of O_2 atmospheres (HOA) was evaluated as an alternative to actively modified low-O_2 atmosphere (LOA) and passively modified atmospheres (PA). Changes in color, vitamin C, individual phenolic compounds, and antioxidant activity of fresh-cut pears as well as in O_2, CO_2, and ethylene headspace concentrations inside the packages were assessed for 14 days at 4 °C. Not only did the use of antioxidants prevent browning and reduce ethylene production of fresh-cut pears, but also their application under LOA best maintained vitamin C, chlorogenic acid, and antioxidant capacity compared with HOA. The results show that the use of glutathione and *N*-acetylcysteine enhanced the formation of phenylpropanoids in fresh-cut pears stored under LOA.

KEYWORDS: Minimal processing; *N*-acetylcysteine; glutathione; ethylene; color; phenolic compounds; vitamin C; antioxidant capacity

INTRODUCTION

Antioxidant constituents of fruits and vegetables have been widely reported to have beneficial effects on the maintenance of health and the prevention of cancer and cardiovascular diseases (*1, 2*). 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 radicals and other deleterious species such as lipid peroxides (*3*). Pears are fruits with a low antioxidant capacity compared to pigmented fruits (*4*). In this fruit, the contribution of phenolic compounds to antioxidant capacity has been reported to be much greater than that of vitamin C (*5*).

'Flor de Invierno' pear (*Pyrus communis* L.) is a winter pear variety with a big fruit, yellowish green skin and a firm, sweet, crispy, juicy, and white flesh (*6*). Apart from its interesting organoleptic attributes, it shows an excellent physiological response to minimal processing, especially when processed at optimal ripeness state (*7*).

Browning of cut surfaces is one of the greatest hurdles to the commercial marketing of fresh-cut pears. The destruction of fruit cellular compartmentation allows the oxidation of phenolic compounds by polyphenol oxidase (PPO), thus decreasing the nutritional content of fresh-cut commodities (*8*). In addition, several factors such as physical damage, the presence of O_2, extended storage, low relative humidity, high temperatures, or chilling injury may promote vitamin C losses with subsequent browning if ascorbic acid falls below a threshold level (*9*).

Natural thiol-containing compounds with antioxidant properties, such *N*-acetylcysteine and glutathione, have been reported to prevent browning in 'Flor de Invierno' pears (*10*). Thiol-containing antibrowning additives react with *o*-quinones formed during the initial phase of enzymatic browning reactions, yielding colorless adducts or reducing them back to *o*-diphenols (*11*). Low-O_2 and elevated-CO_2 atmospheres can also reduce surface browning. This decrease in the browning phenomena is accompanied by several physiological effects such as a decrease in respiration rates and a delay in the climacteric onset of the rise in ethylene (*12*). Day (*13*) hypothesized that high O_2 concentrations may cause substrate inhibition of PPO or, alternatively, high levels of colorless quinones formed may cause feedback inhibition of the enzyme. However, the results achieved in this field are often controversial, but it is agreed that modified atmosphere packaging alone cannot effectively prevent fresh-cut fruit browning. Gorny et al. (*14*) found that low-O_2 (0.25 or 0.5 kPa), elevated-CO_2 (air enriched with 5, 10 or 20 kPa CO_2), or high-O_2 (40, 60 or 80 kPa) active

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Table 1. Physicochemical Characteristics of 'Flor de Invierno' Pear before Processing[a]

soluble solids (°Brix)	13.92 ± 0.05
total acidity (g of citric acid/100 g)	0.31 ± 0.07
pH	4.3 ± 0.5
pulp color	
*L**	73.0
*a**	−1.4
*b**	10.4
firmness (N)	43.3 ± 0.7

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

atmospheres alone did not effectively prevent surface browning of fresh-cut pear slices.

Studies on the influence of modified atmosphere packaging and the use of antioxidant compounds on the shelf life of fresh-cut pears have mainly focused on the sensory quality of the commodity (*14–17*). Knowledge about the impact of dipping treatments and packaging conditions on antioxidant properties of fresh-cut pears is still incomplete, especially in regard to high-O_2 active packaging. Therefore, the present work aims to assess the combined effect of an *N*-acetylcysteine + glutathione dip and packaging under high-O_2 atmospheres (HOA) on color, vitamin C, phenolic compounds, and antioxidant capacity of fresh-cut 'Flor de Invierno' pears, as well as to compare the results with those obtained under conventional low-O_2 atmospheres (LOA) and passively modified atmospheres (PA). Ethylene, O_2, and CO_2 headspace concentrations were also evaluated during 14 days of storage at 4 °C.

MATERIALS AND METHODS

Reagents. *N*-Acetylcysteine, glutathione, metaphosphoric acid, and DL-1,4-dithiothreitol (DTT) were purchased from Acros Organics (Fair Lawn, NJ); 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azinobis(3-ethylbenzthiozoline)-6-sulfonic acid (ABTS), 4-hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid (Trolox), sulfuric acid, methanol, hydrogen chloride (HCl), potassium persulfate ($K_2S_2O_8$), formic acid (HCOOH), and acetonitrile were obtained from Sharlau Chemie, S.A. (Barcelona, Spain); chlorogenic acid, (−)-epicatechin, ferulic acid, *p*-coumaric acid, sinapic acid, and quercetin were purchased from Sigma Chemical Co. (St. Louis, MO).

Plant Material. Pears (*P. communis* L. cv. Flor de Invierno) were harvested at commercial maturity from the same orchard (Lleida, Spain) in mid-October. Immediately after harvesting, pears were placed in a refrigerated chamber (4 °C) for 1 month. The ripeness state was characterized prior to processing by measuring the maximum force necessary to penetrate the flesh of a whole fruit with an 8 mm diameter probe (Mechanical Fruit Firmness Tester, QA Supplies, LLC, Norfolk, VA). Firmness and other physicochemical characteristics of pear flesh (10 fruits) are shown in **Table 1**: soluble solids content (Atajo RX-100 refractometer; Atago Co., Tokyo, Japan), total acidity (AOAC 2000), pH (Crison 2001 pH-meter; Crison Instruments SA, Alella, Barcelona, Spain), and color (Minolta CR-400 chroma meter; Konica Minolta Sensing, Inc., Osaka, Japan).

Sample Preparation. 'Flor de Invierno' pears were sanitized by immersion for 2 min in water containing 200 ppm of free chlorine, rinsed with tap water (10–15 °C) for 3–5 min, and drained. Processing operations were carried out at room temperature (20 °C). Pears were peeled, and the core tissue was completely removed with a pear peeler and coring device. The remaining tissue was cut manually into 6 cm wedges. Pear wedges were dipped for 1 min in aqueous solutions (4 °C) of 0.75% w/v *N*-acetylcysteine + 0.75% w/v glutathione. The concentrations of the antibrowning agents were chosen in accordance with previous studies (*10*). Sterile distilled water was used as the control treatment. Once the excess of water was completely air drained, 100 g of pear wedges was packaged in polypropylene trays (173 × 129 × 50 mm). The trays were sealed with a 64 μm thick polypropylene film with O_2 and CO_2 permeances of 110 and 500 $cm^3\ m^{-2}\ day^{-1}\ bar^{-1}$ at 23 °C and 0% relative humidity, respectively (ILPRA Systems España, S.L., Mataró, Spain). In addition, the film water vapor permeance was 3 $g \cdot m^{-2}\ day^{-1}$ at 38 °C and 90% relative humidity according to the manufacturer's information. Active modification of package atmospheres was carried out before the trays were sealed by flushing a mixture of 2.5 kPa of O_2 + 7 kPa of CO_2 (balance N_2) (LOA) or 70 kPa of O_2 (balance N_2) (HOA) using a digitally controlled compensated vacuum ILPRA Food Pack Basic V/6 system (ILPRA Systems CP, Vigevono, Italy). For passive modification of package atmospheres (PA), the trays were sealed without flushing any gas mixture. The relationship between the amount of product and the injected gas mixture was 1:2 v/v. Eighty-four packages were stored at 4 ± 1 °C in darkness. Initial analyses were determined within the next 4 h after packaging, thus corresponding to values at time 0.

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 5Å column (4 m × 0.35 mm, df = 10 μm) at 60 °C and 100 kPa, whereas a sample of 0.33 μL was injected to a Pora-PLOT Q column (10 m × 0.32 mm, df = 10 μm) at 75 °C and 200 kPa for CO_2 and ethylene determinations. Two trays were taken at each time to perform the analysis, and two readings were carried out for each package.

Vitamin C Content. The determination of vitamin C concentrations in fresh-cut pear was performed by HPLC-UV. The extraction procedure and the chromatographic conditions were based on a previous study carried out by Odriozola-Serrano et al. (*18*). 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 L^{-1} of DTT. The mixture was stirred and centrifuged at 22100*g* for 15 min at 4 °C (Centrifuge AVANTI J-25, Beckman Instruments Inc., Fullerton, CA). The supernatant was vaccum-filtered through Whatman no. 1 paper. The sample was 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) working at 245 nm. Duplicates of 20 μL of each extract were injected into a reverse-phase C18 Spherisorb ODS2 (5μm) stainless steel column (250 mm × 4.6 mm) (Waters), used as stationary phase. A 0.01% sulfuric acid solution 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 pear. Two trays were taken at each sampling time to perform replicate analyses throughout 14 days of storage.

Phenolic Compounds. A high-performance liquid chromatography (HPLC) method was used for the analysis of individual phenolic compounds. The extraction was carried out following the method validated by Hertog et al. (*19*). A sample of 0.50 g of freeze-dried pear tissue was carefully mixed with 40 mL of 62.5% aqueous methanol (2g L^{-1} TBHQ) and 10 mL of 6 M HCl. After refluxing at 90 °C for 2 h with regular swirling, the extract was allowed to cool, then made up to 100 mL with methanol, and finally sonicated for 5 min. The extract was then passed through a 0.45μm 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 in a reverse-phase C18 Spherisorb ODS2 (5 μm) stainless steel column (4.6 mm × 250 mm) operating at room temperature with a flow rate of 1 mL min^{-1}. A gradient elution was used 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 from 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 phenolic components were quantified by comparison with external standards of phenolic compounds such as

chlorogenic acid, (−)-epicatechin, ferulic acid, *p*-coumaric acid, sinapic acid, and quercetin. Results were expressed as milligrams of phenolic compounds in 100 g of fresh-cut pear. Two trays were taken at each sampling time to perform replicate analyses throughout 14 days of storage.

Antioxidant Capacity. The antioxidant capacity of fresh-cut pear was analyzed using two independent methods.

The determination of free radical scavenging effect of antioxidants on 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$) radical was carried out according to the procedure described by Elez-Martínez and Martín-Belloso (*20*). The ABTS assay, based on the ability of the antioxidants to scavenge the blue-green radical cation 2,2′-azinobis(3-ethylbenzothiazoline)-6-sulfonate (ABTS$^{\bullet+}$) was conducted according to the method described by Re et al. (*21*) with some modifications. Pear samples were centrifuged at 22100*g* for 15 min at 4 °C (Centrifuge AVANTI J-25, Beckman Instruments Inc.) and filtered through Whatman no. 1 paper. Aliquots of 0.01 mL of the supernatant were mixed with 3.9 mL of methanolic DPPH or ABTS$^{\bullet+}$ solutions and 0.09 mL of distilled water. The homogenate was shaken vigorously and kept in darkness for 30 min. The absorption of the samples was measured with a CECIL CE 2021 spectrophotometer (Cecil Instruments Ltd., Cambridge, U.K.) at 515 nm for DPPH assay or at 734 nm for ABTS assay.

The percentage of inhibition of the absorbance 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 by using a regression equation between the Trolox concentration and the percent inhibition, and results were expressed as milligrams of Trolox equivalent in 100 g of fresh-cut pear. Two trays were taken at each sampling time to perform replicate analyses throughout 14 days of storage.

Color Attributes. The color of fresh-cut pears was determined with a Minolta CR-400 chromameter (Konica Minolta Sensing, Inc., Osaka, Japan), equipped with a D65 light source and the observer at 10°. Two trays were taken at each time to perform the analysis, and five fruit pieces were evaluated for each package. Three readings were obtained for each replicate by changing the position of the pear wedges in the optical glass cell to get uniform color measurements. Color values of CIE L^* (lightness), a^* (red-green), and b^* (yellow-blue) were measured through reflectance values. Hue angle ($h°$) was calculated by eq 1.

$$h = \arctan\frac{b^*}{a^*} \quad (1)$$

Data Analysis. Statistical analysis was performed using Statgraphics plus v. 5.1 software (Manugistics, Inc., Rockville, MD). Data were analyzed by multifactor analysis of variance, and a Duncan multiple-range test was applied to determine differences among means, with a significance level of 0.05. Principal component analysis (PCA) was carried out 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 that 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 the PC.

RESULTS AND DISCUSSION

Changes in Headspace Gas Composition. Figure 1 shows the variation of headspace gas partial pressures during storage of fresh-cut pears packaged under different modified atmospheres. As expected, a decrease in the O_2 concentrations throughout storage together with an increase in the CO_2 concentrations was observed with respect to initial in-package conditions. Headspace O_2 and CO_2 concentrations were not significantly influenced by the dipping treatment ($P \geq 0.05$). The amount of O_2 inside HOA packages decreased continuously but remained >50 kPa for 14 days, whereas the accumulation of CO_2 exceeded 15 kPa, which was also observed in PA packages (**Figure 1a**,**b**). Previous works showed that the

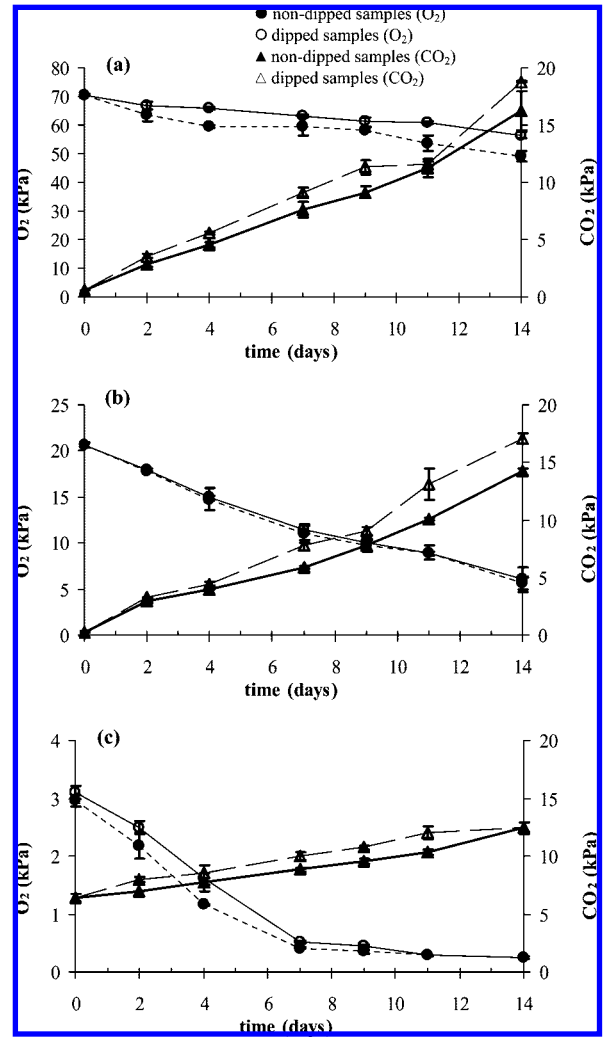

Figure 1. O_2 and CO_2 headspace composition in packages of fresh-cut 'Flor de Invierno' pears dipped into a 0.75 w/v *N*-acetylcysteine + 0.75% w/v glutathione solution and stored under HOA (**a**), PA (**b**), and LOA (**c**) during 14 days at 4 °C. Values are mean ± standard deviation.

application of HOA for fresh-cut 'Flor de Invierno' pears did not significantly reduce respiration rates compared with PA or LOA (*22, 23*). Superatmospheric O_2 levels may stimulate, have no effect on, or reduce respiration rates, depending on the commodity, maturity and ripeness stage, O_2 concentration, time, and storage temperature, as well as CO_2 and ethylene concentrations (*24*). A rapid decrease in the O_2 concentrations was observed in fresh-cut pear LOA packages, reaching concentrations below 1 kPa after 1 week (**Figure 1c**). Rojas-Graü et al. (*25*) also reported a rapid depletion of headspace O_2 concentrations in fresh-cut 'Fuji' apple packages stored under LOA compared with a progressive decrease under PA, whereas the use of *N*-acetylcysteine or ascorbic acid did not significantly affect O_2 consumption rates. The package headspace of fresh-cut pears stored under LOA exhibited the lowest CO_2 accumulation compared with other packaging atmospheres, thus reaching concentrations below 15 kPa after 2 weeks of storage (**Figure 1c**). Consistently, CO_2 production of fresh-cut 'Fuji' apples was significantly lower in samples stored under LOA than under PA (*25*). Gorny et al. (*26*) found that low O_2 (0.25%) and elevated CO_2 (20%) acted synergistically to decrease the respiration of fresh-cut peach slices.

Ethylene concentrations in the package headspace atmospheres were significantly influenced ($P < 0.05$) by storage

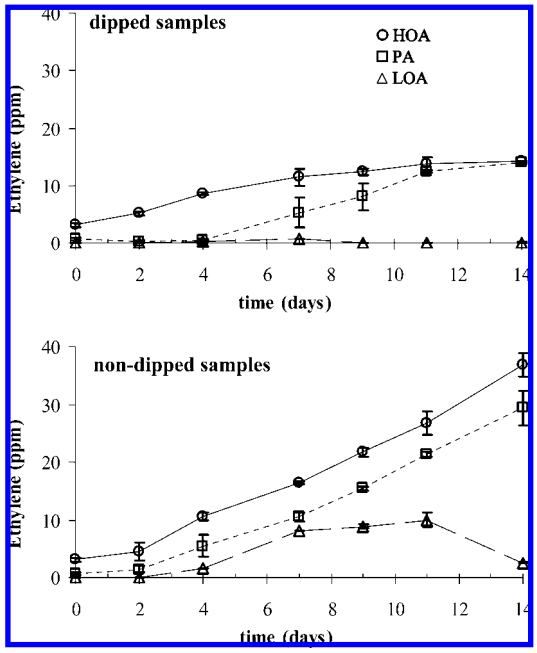

Figure 2. Ethylene headspace concentrations in packages of fresh-cut 'Flor de Invierno' pears dipped into a 0.75 w/v *N*-acetylcysteine + 0.75% w/v glutathione solution and packaged under HOA, PA, and LOA during 14 days at 4 °C. Values are mean ± standard deviation.

Table 2. Changes in L^* and $h°$ Values on Fresh-Cut 'Flor de Invierno' Pears Stored under HOA, LOA, and PA during 14 Days at 4 °C[a]

	LOA		PA		HOA	
days	D	C	D	C	D	C
			L^*			
0	73.8 a	70.6 a	73.7 a	71.9 a	72.6 a	70.2 a
2	74.1 a	70.4 a	74.2 a	71.3 ab	72.1 ab	70.8 a
4	73.9 a	69.9 a	74.4 a	71.2 ab	71.2 bc	70.6 a
7	74.2 a	68.9 a	74.9 a	70.3 bc	70.9 c	69.3 b
9	74.1 a	67.1b	74.2 a	69.5 c	70.5 c	66.8 c
11	74.4 a	66.3 b	73.2 a	69.4 c	70.5 c	66.1 cd
14	74.1 a	66.7 b	73.9 a	69.7 c	70.5 c	65.6 c
			$h°$			
0	102.4 a	86.1 c	100.6 a	95.3 a	101.2 a	97.9 a
2	101.4 a	86.6 bc	100.0 a	94.5 a	100.4 a	94.1b
4	100.0 a	88.1 a	99.3 a	92.0b	99.5 a	88.6 c
7	100.4 a	87.6 ab	99.8 a	85.9 c	100.6 a	88.2 c
9	103.3 a	88.0 a	98.3 ab	84.4 cd	99.2 a	85.4 d
11	101.8 a	88.2 a	96.3 b	84.2 cd	99.1 a	85.1 d
14	100.9 a	88.0 a	95.8 b	83.5 d	99.8 a	84.8 d

[a] Values within a column followed by the same letter indicate that mean values are not significantly different by Duncan's multiple-range test ($P < 0.05$). HOA, 70 kPa of O_2; PA, passive atmosphere; LOA, 2.5 kPa of O_2 + 7 kPa of CO_2; L^*, lightness; $h°$, hue angle; D, samples dipped into 0.75 w/v *N*-acetylcysteine + 0.75% w/v glutathione; C: samples dipped into distilled water.

atmosphere and dipping treatments. The application of a dip consisting of *N*-acetylcysteine and glutathione decreased ethylene production of fresh-cut pears (**Figure 2**). Rojas-Graü et al. (*25*) also found reduced ethylene accumulation in packages of fresh-cut 'Fuji' apples dipped into *N*-acetylcysteine (1% w/v) solution and stored under LOA or PA compared with the use of ascorbic acid. The action mechanism of thiol-containing compounds on ripening process and ethylene inhibition is not clear. However, Frenkel (*27*) observed that the onset of ripening is influenced by a decline of sulfhydryl gradient in fruit. These authors demonstrated that SH compounds such as cysteine or dithiothreitol could retard ripening of 'Bartlett' pears.

Table 3. Changes in a^* and b^* Values on Fresh-Cut 'Flor de Invierno' Pears Stored under HOA, LOA, and PA during 14 Days at 4 °C[a]

	LOA		PA		HOA	
days	D	C	D	C	D	C
			a^*			
0	−2.3 a	0.9 a	−1.9 ab	−1.4 a	−1.9 a	−1.7 a
2	−2.3 a	0.8 a	−1.9 ab	−1.2 a	−1.9 a	−1.0 b
4	−2.1 a	0.5 a	−2.0 a	−0.5 b	−1.7 a	0.4 c
7	−2.1 a	0.6 a	−2.0 a	1.3 c	−1.9 a	0.6 d
9	−2.8 a	0.5 a	−1.8 abc	2.1 d	−1.7 a	1.5 e
11	−2.4 a	0.5 a	−1.4 bc	2.2 d	−1.8 a	1.7 ef
14	−2.2 a	0.6 a	−1.3 c	2.4 d	−1.9 a	1.8 f
			b^*			
0	10.2 a	12.5 a	9.9 a	15.0 a	9.7 a	12.5 a
2	11.4 b	13.1 ab	10.8 ab	15.1 a	10.3 ab	14.3 b
4	11.9 b	14.1 bc	12.4 b	15.8 a	10.5 ab	15.9 c
7	11.4 b	13.9 abc	11.7 bc	18.4 b	10.0 a	19.4 d
9	11.7 b	14.8 cd	12.2 b	21.3 c	10.4 ab	19.3 d
11	11.4 b	16.0 d	12.6 b	21.9 c	11.0 b	19.5 d
14	11.5 b	16.1 d	12.8 b	21.0 c	11.2 b	19.5 d

[a] Values within a column followed by the same letter indicate that mean values are not significantly different by Duncan's multiple-range test ($P < 0.05$). HOA, 70 kPa of O_2; PA, passive atmosphere; LOA, 2.5 kPa of O_2 + 7 kPa of CO_2; D, samples dipped into 0.75 w/v *N*-acetylcysteine + 0.75% w/v glutathione; C, samples dipped into distilled water.

Ethylene production was reduced or completely inhibited in 'Flor de Invierno' pear wedges stored under LOA, whereas the headspace concentrations in HOA and PA packages containing nondipped samples reached maximum values of 30–40 ppm after 14 days (**Figure 2**). Rojas-Graü et al. (*25*) also reported lower ethylene production in fresh-cut 'Fuji' apples under LOA than under PA. Consistently, Soliva-Fortuny et al. (*28*) reported an almost complete inhibition of ethylene production in fresh-cut 'Conference' pears stored under the absence of O_2 or LOA, given that O_2 is required for ethylene synthesis. According to these authors, low O_2 concentrations combined with elevated amounts of CO_2 may act synergistically to inhibit ethylene production. The inhibition of ethylene under anaerobic or low O_2 concentrations has been reported by many authors, suggesting that O_2 participates in the conversion of 1-aminocyclopropane-1-carboxylic acid (ACC) to ethylene (*29*). Therefore, high-O_2 atmospheres may dramatically promote the biosynthesis of ethylene due to an excessive amount of O_2. ACC oxidase follows an ordered binding mechanism in which it first binds to O_2 and then to ACC. However, when the O_2 concentration was increased from 21 to 100 kPa, there was little influence on the apparent K_m for ACC (*30*).

Changes in Color Attributes. L^* and $h°$ values can be used as indicators of browning of fresh-cut 'Flor de Invierno' pears (*10*). In addition, changes in a^* and b^* values have previously been used in monitoring pear surface color (*16, 17*). **Tables 2** and **3** show color quality of fresh-cut 'Flor de Invierno' pears throughout storage. Modified atmospheres alone were not effective in controlling enzymatic browning of fresh-cut 'Flor de Invierno' pears. These findings agree with those reported by Gorny et al. (*14*) on fresh-cut 'Bartlett' pear slices packaged under low O_2 (0.25 or 0.5 kPa), elevated CO_2 (air enriched with 5, 10, or 20 kPa CO_2), or superatmospheric O_2 (40, 60, or 80 kPa) levels. As a consequence of mechanical operations during processing, nondipped pear wedges developed browning rapidly thereafter. The use of *N*-acetylcysteine and glutathione prevented cut surface browning under all packaging conditions ($P < 0.05$), although their antioxidant effect seems to decrease under HOA. On the other hand, L^* and $h°$ initial values of dipped pears packaged under LOA

were maintained during the time without significant decrease (**Table 2**). A dip of *N*-acetylcysteine + glutathione was also shown to reduce changes in a^* and b^* values during storage, especially under initial LOA (**Table 3**). The antibrowning effect of thiol-containing compounds was also reported by Rojas-Graü et al. (*25*) in fresh-cut 'Fuji' apples dipped into 1% *N*-acetylcysteine and stored under LOA or PA. Although LOA in combination with dips of ascorbic acid and calcium chloride has proved to maintain the overall sensory quality of fresh-cut 'Conference' pears for 3 weeks, low quantities of O_2 combined with high CO_2 concentrations were detrimental to flavor perception and caused massive production of fermentative metabolites beyond 3 weeks (*28*).

Vitamin C. Figure 3 shows changes in vitamin C concentrations of fresh-cut 'Flor de Invierno' pears throughout storage. As reported by Gil et al. (*6*) in fruits such as mango, strawberry, and watermelon, processing operations do not seem to have a significant effect on the loss of vitamin C in fresh-cut pears. Initial vitamin C after processing may be due to packaging conditions rather than to processing operations (**Figure 3**). Initial vitamin C concentrations [3.3–4.6 mg 100 g^{-1} of fresh weight (fw)] were shown to be significantly affected by the dipping treatment and packaging atmospheres during storage ($P < 0.05$). As expected, ascorbic acid oxidation was greatly favored by the presence of O_2. According to Soliva-Fortuny et al. (*31*), the vitamin C content of fresh-cut 'Conference' pears was kept almost constant throughout storage in the absence of O_2. Information about the effects of high O_2 concentrations on antioxidant content of fresh produce is scarce. Day et al. (*32*) reported that high-O_2 modified atmosphere packaging did not preferentially decrease ascorbic acid content in prepared lettuce. However, our results show that HOA led to the greatest decrease in vitamin C content. Vitamin C concentrations of nondipped pear wedges stored under HOA could have fallen below the threshold of acceptability, corresponding to 50% of initial ascorbic acid content (<2 mg kg^{-1}), beyond 9 days of storage. Physiological disorders related to browning appeared in 'Conference' pears when ascorbic acid decreased below 2 mg kg^{-1} (*9*). Although the main factor affecting vitamin C degradation is the initial availability of O_2, nondipped samples stored under LOA underwent a substantial loss of vitamin C after 1 week of storage, when anoxic conditions could have been reached inside the packages. According to Tudela et al. (*33*), high CO_2 levels could increase vitamin C loss by accelerating ascorbate peroxidase-catalyzed oxidation processes. In fact, previous studies showed an important increase in peroxidase activity of fresh-cut 'Piel de Sapo' melon stored under LOA (*7*). On the other hand, a dip of *N*-acetylcysteine + glutathione maintained vitamin C content of fresh-cut pears stored under LOA. In fruit tissue, glutathione and ascorbic acid can form a redox couple that is involved in the regeneration of ascorbic acid (*34*).

Phenolic Compounds. An initial phenolic content of 17–18 mg 100 g^{-1} of fw was observed in fresh-cut 'Flor de Invierno' pears (**Table 4**). Chlorogenic acid, a hydroxycinnamic acid derivative, constituted the main phenolic in fresh-cut 'Flor de Invierno' pears. Chlorogenic acid concentrations were significantly affected by the dipping treatment and storage atmosphere ($P < 0.05$). Fresh-cut pears stored under HOA underwent a substantial loss of chlorogenic acid throughout storage. High-O_2 atmospheres induced the loss of certain phenolic compounds in fresh-cut prepared lettuce

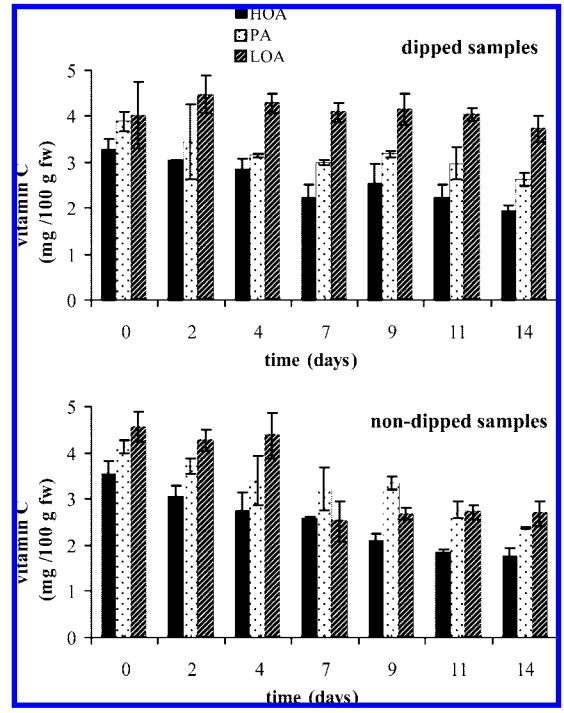

Figure 3. Vitamin C content of fresh-cut 'Flor de Invierno' pears dipped into a 0.75 w/v *N*-acetylcysteine + 0.75% w/v glutathione solution and stored under HOA, PA, and LOA during 14 days at 4 °C. Values are mean ± standard deviation.

in comparison with air or low-O_2 modified atmosphere (*32*). Cocci et al. (*35*) also reported that the O_2 availability in the package headspace of fresh-cut apples stored under air packaging could have led to a stronger degradation of phenolic compounds than under 5 kPa of O_2 + 5 kPa of CO_2 atmospheres. This phenomenon could be due to the fast oxidation of phenolic compounds on the cut surface, directly in contact with the O_2 in the package headspace. Enzymatic oxidation of chlorogenic acid via polyphenol oxidase (PPO) has been associated with pear browning (*5*). Our results show that the chlorogenic acid content was higher in fresh-cut pears dipped into *N*-acetylcysteine + glutathione solution than in nondipped samples packaged under LOA and PA (**Table 4**). Thus, the application of antioxidants may reduce chlorogenic acid degradation, which is known to happen much more quickly than for other fruit phenolics such as catechin and proanthocyanidins (*36*).

(−)-Epicatechin and quercetin contents increased significantly throughout storage of fresh-cut 'Flor de Invierno' pears (**Table 4**). (−)-Epicatechin is the flavan-3-ol compound found in fresh-cut 'Flor de Invierno' pears at concentrations of 1.8–2.0 mg 100 g^{-1} of fw at day 0, whereas the initial concentrations of the flavonol compound, quercetin, were about 0.18–0.2 mg 100 g^{-1} of fw. The increase in their content during the storage period could be directly associated with a physiological response to stress conditions. Physiological stress may stimulate phenylalanine ammonia-lyase (PAL, EC 4.3.1.5) activity with a consequent further production of phenylpropanoids (*37*). The PAL activation of the phenylpropanoid metabolism could be elicited through induced reactive oxygen species (*38*). A substantial increase of (−)-epicatechin and quercetin content was observed in nondipped samples stored under HOA or PA. However, production of epicatechin as a wounding response seems to be triggered in dipped pear wedges stored under LOA.

Table 4. Phenolic Content of Fresh-Cut 'Flor De Invierno' Pears Stored under HOA, LOA, and PA during 14 Fays at 4 °C[a]

	chlorogenic acid		epicatechin		ferulic acid		*p*-coumaric acid		sinapic acid		quercetin		total phenolics	
days	D	C	D	C	D	C	D	C	D	C	D	C	D	C
							LOA							
0	17.4 a	16.8 a	2.0 d	1.9 bc	0.9 a	0.8 a	0.6 a	0.7 a	0.04 e	0.03 c	0.2 b	0.2 d	21.3 bc	20.5 a
2	16.7 b	15.3 b	2.1 d	2.0 bc	0.9 a	0.6 b	0.4 c	0.4 d	0.05 d	0.05 b	0.2 b	0.2 d	20.4 d	18.5 b
4	14.5 d	15.3 b	2.6 c	1.8 bc	0.9 a	0.5 c	0.5 b	0.4 d	0.05 d	0.06 a	0.2 b	0.2 d	18.7 e	18.3 bc
7	15.8 c	13.0 c	3.4 bc	1.7 c	0.6 b	0.3 e	0.5 b	0.4 d	0.06 bc	0.06 a	0.2 b	0.3 c	20.6 cd	15.9 cd
9	17.2 ab	12.5 c	3.3 bc	2.1 ab	0.5 c	0.6 b	0.5 b	0.5 c	0.06 c	0.06 a	0.4 a	0.5 a	21.9 ab	16.2 c
11	16.7 b	12.3 c	3.6 b	2.0 bc	0.4 d	0.4 d	0.4 c	0.4 d	0.07 b	0.06 a	0.4 a	0.5 a	21.7 b	15.7 d
14	17.4 a	11.9 c	4.5 a	2.4 a	0.2 e	0.6 b	0.1 d	0.6 b	0.08 a	0.04 b	0.4 a	0.4 b	22.7 a	16.1 c
							PA							
0	17.7 ab	18.0 a	1.9 b	1.9 d	0.9 a	0.9 a	0.6 a	0.6 a	0.04 c	0.03 d	0.2 b	0.2 c	21.3 a	21.6 a
2	17.0 cd	16.3 b	1.9 b	2.2 c	0.6 c	0.3 e	0.4 c	0.5 b	0.06 ab	0.04 c	0.2 b	0.2 c	20.3 c	19.5 b
4	17.6 ab	14.5 c	1.8 b	2.3 c	0.6 c	0.4 d	0.5 b	0.4 c	0.07 a	0.05 ab	0.2 b	0.2 c	20.8 abc	18.0 c
7	18.0 a	13.2 cd	1.9 b	2.3 c	0.6 c	0.5 c	0.4 c	0.4 c	0.06 ab	0.05 ab	0.2 b	0.4 b	21.2 ab	16.9 cd
9	17.2 bc	12.4 de	1.9 b	2.7 b	0.7 b	0.5 c	0.4 c	0.4 c	0.06 ab	0.05 ab	0.4 a	0.5 a	20.8 abc	16.6 cd
11	16.9 cd	11.1 ef	2.3 a	2.9 b	0.7 b	0.4 d	0.5 b	0.5 b	0.06 ab	0.06 a	0.4 a	0.5 a	21.0 ab	15.6 d
14	16.5 d	10.6 f	2.3 a	3.7 a	0.7 b	0.6 b	0.6 a	0.6 a	0.04 c	0.04 c	0.4 a	0.5 a	20.6 bc	15.9 d
							HOA							
0	18.5 a	17.3 a	1.8 b	2.0 c	0.9 a	0.8 a	0.6 a	0.7 a	0.03 d	0.04 bc	0.2 c	0.2 c	22.0 a	21.1 a
2	15.5 bc	14.5 b	1.7 b	3.0 b	0.7 c	0.5 d	0.4 c	0.4 c	0.05 bc	0.06 a	0.2 c	0.3 b	18.6 c	18.8 b
4	14.3 d	14.4 b	1.8 b	3.0 b	0.8 b	0.6 c	0.4 c	0.4 c	0.05 bc	0.06 a	0.2 c	0.3 b	17.6 d	18.8 b
7	14.8 cd	13.7 b	2.0 ab	2.8 b	0.4 e	0.4 e	0.4 c	0.4 c	0.06 ab	0.06 a	0.3 b	0.5 a	17.9 d	17.9 c
9	16.0 b	12.7 bc	1.7 b	2.9 b	0.5 d	0.5 d	0.4 c	0.4 c	0.06 ab	0.07 a	0.4 a	0.5 a	19.2 b	17.1 c
11	11.0 e	10.1 cd	2.2 a	2.9 b	0.5 d	0.7 b	0.5 b	0.5 b	0.06 ab	0.05 b	0.4 a	0.5 a	14.7 e	14.8 d
14	11.1 e	9.5 d	2.2 a	3.9 a	0.4 e	0.7 b	0.5 bb	0.5 b	0.07 a	0.03 d	0.4 a	0.5 a	14.6 e	15.2 d

[a] Values within a column followed by the same letter indicate that mean values are not significantly different by Duncan's multiple-range test ($P < 0.05$). HOA, 70 kPa of O_2; PA, passive atmosphere; LOA, 2.5 kPa of O_2 + 7 kPa of CO_2; D, samples dipped into 0.75 w/v *N*-acetylcysteine + 0.75% w/v glutathione; C, samples dipped into distilled water; total phenolics quantified by HPLC (values are the result of the sum of each component).

Table 5. Changes in Antioxidant Capacity of Fresh-Cut 'Flor De Invierno' Pears Stored under HOA, PA, and LOA during 14 Days at 4 °C[a]

	LOA		PA		HOA	
days	D	C	D	C	D	C
			DPPH Assay			
0	21.0 a	17.6 a	16.4 a	14.3 a	20.9 a	15.8 a
2	14.0 bc	10.2 b	15.5 a	11.5 b	15.4 b	9.6 b
4	15.2 b	10.6 b	12.0 b	11.9 b	12.9 c	9.4 bc
7	14.7 bc	9.5 b	12.0 b	9.1 c	11.9 cd	6.6 d
9	13.7 bc	9.6 b	11.2 bc	9.1 c	11.8 cd	5.9 d
11	13.6 bc	10.0 b	11.0 bc	8.9 c	10.4 de	5.7 d
14	11.4 c	9.9 b	10.0 c	9.0 c	9.6 e	7.1 cd
			ABTS Assay			
0	20.3 a	18.0 a	17.7 a	15.7 a	20.0 a	14.8 a
2	15.1 b	11.5 b	16.0 ab	12.1 b	14.6 b	10.4 b
4	16.1 b	10.0 b	14.1 b	10.7 bc	12.7 bc	9.6 bc
7	15.8 b	11.0 b	10.9 c	9.1 cd	10.9 c	7.2 cd
9	14.6 b	10.6 b	10.2 c	8.2 d	11.3 c	7.4 cd
11	15.5 b	10.7 b	10.7 c	7.9 d	11.9 c	5.8 d
14	13.0 b	10.1 b	9.8 c	8.3 cd	10.2 c	5.6 d

[a] Values within a column followed by the same letter indicate that mean values are not significantly different by Duncan's multiple-range test ($P < 0.05$). HOA, 70 kPa of O_2; PA, passive atmosphere; LOA, 2.5 kPa of O_2 + 7 kPa of CO_2; D, samples dipped into 0.75 w/v *N*-acetylcysteine + 0.75% w/v glutathione; C, samples dipped into distilled water.

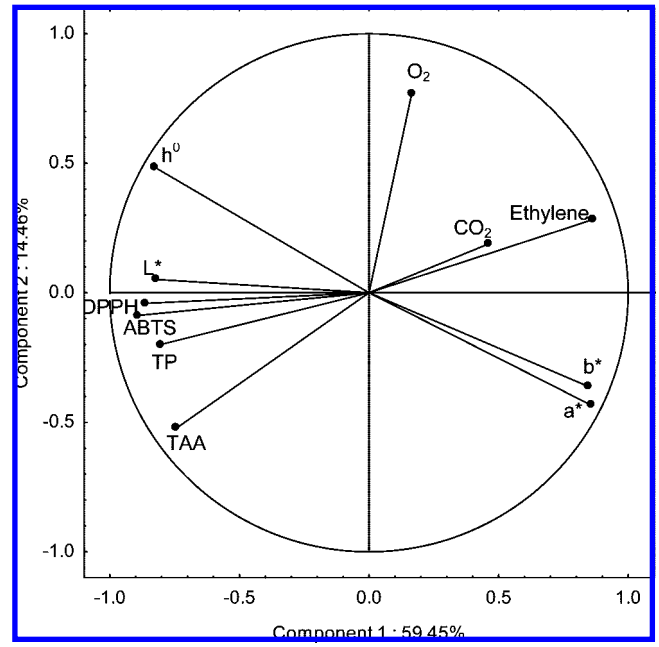

Figure 4. PCA plot of fresh-cut 'Flor de Invierno' pear dipped into a 0.75 w/v *N*-acetylcysteine + 0.75% w/v glutathione solution and stored under HOA, PA, and LOA during 14 days at 4 °C.

Changes in other phenolic compounds found at minor initial concentrations in fresh-cut 'Flor de Invierno' pears such as *p*-coumaric acid (0.5–0.7 mg 100 g^{-1} of fw), ferulic acid (0.8–0.9 mg 100 g^{-1} of fw), and sinapic acid (0.03–0.04 mg 100 g^{-1} of fw) are shown in **Table 4**. Fresh-cut 'Flor de Invierno' pears packaged under LOA, PA, or HOA underwent a substantial depletion of *p*-coumaric and ferulic acid during storage period, which may be a consequence of their conversion to sinapic acid. The *p*-coumaric acid is formed in plant products via the action of PAL due to the phenylpropanoid metabolism. This compound may be hydroxylated further in positions 3 and 5 and possibly methylated via *O*-methyl transferase with *S*-adenosylmethionine as methyl donor, leading to the formation of caffeic, ferulic, and eventually sinapic acid (*39*). A dip of *N*-acetylcysteine + glutathione solution did not significantly affect the phenolic production under PA or HOA. However, dipped pear wedges initially packaged under LOA exhibited the most important accumulation of sinaptic acid and a decrease in *p*-coumaric acid and ferulic acid contents ($P < 0.05$). Glutathione and even *N*-acetylcysteine as a precursor of glutathione may be

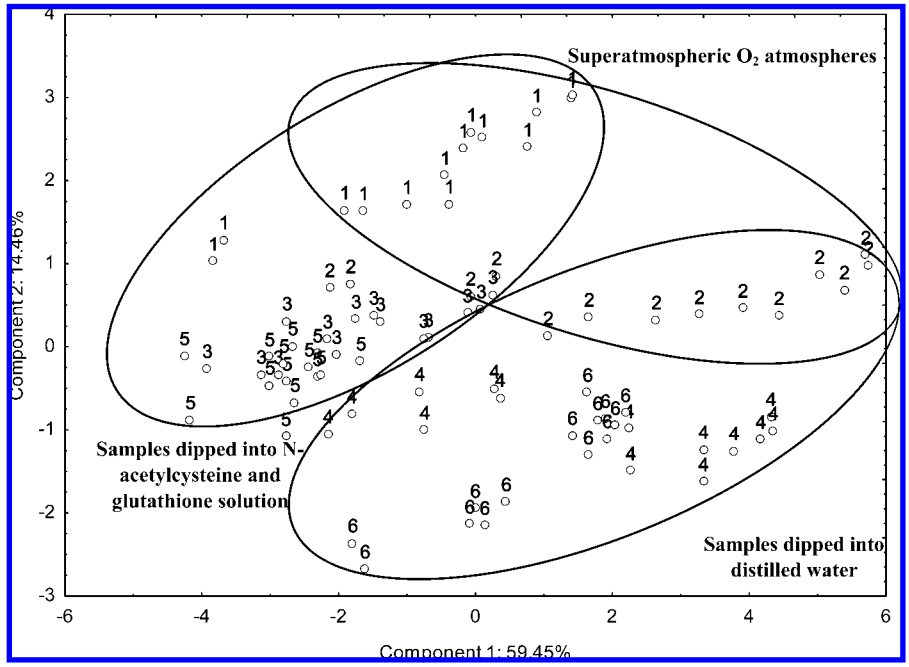

Figure 5. Score plot of PC1 versus PC2 of all sample labels for fresh-cut 'Flor de Invierno' packaged under HOA, LOA, or PA during 14 days at 4 °C. Samples dipped into a 0.75% w/v *N*-acetylcysteine + 0.75% w/v glutathione solution: 1 (HOA), 3 (PA), 5 (LOA). Samples dipped into distilled water: 2 (HOA), 4 (PA), 6 (LOA).

involved in the formation of phenylpropanoids under stress conditions. Ascorbate peroxidase-mediated conjugation of glutathione to *trans*-cinnamic and *p*-coumaric acids has been reported in plants under anoxia conditions (*40*).

Antioxidant Capacity. Table 5 shows the evolution of the antioxidant capacity of fresh-cut 'Flor de Invierno' pears through the DPPH and ABTS methods. Changes in antioxidant capacity were significantly affected by the dipping treatment and storage atmosphere ($P < 0.05$). The antioxidant capacity of fresh-cut pears packaged under LOA was significantly higher than those observed in pears stored under HOA and PA. Under superatmospheric O_2 levels, nondipped samples underwent a substantial loss in chlorogenic acid content (≥45%) and vitamin C (≥50%), which could account for the decrease in antioxidant capacity found in these samples beyond 9 days of storage (≥60%). The use of *N*-acetylcysteine + glutathione significantly enhanced the antioxidant capacity of fresh-cut pears under all storage conditions. The DPPH and ABTS values for dipped pear wedges after 14 days of storage were substantially higher than those for nondipped samples. According to DPPH and ABTS values, fresh-cut 'Flor de Invierno' pears dipped into *N*-acetylcysteine + glutathione and stored under LOA could best maintain their initial antioxidant capacity. Other authors have shown a substantial depletion in the antioxidant capacity of wild rocket leaves stored under atmospheric O_2 levels in comparison to samples stored under 5 kPa of O_2 + 5 kPa of CO_2 or 5 kPa of O_2 + 10 kPa of CO_2 atmospheres (*41*). Our findings show that thiol compounds such as *N*-acetylcysteine + glutathione not only play a relevant role in the prevention of enzymatic browning by reducing *o*-quinones to colorless phenol precursors but also can maintain antioxidant potential of fresh-cut pears.

PCA. A PCA was performed on all samples and variables (in-package gas concentrations, antioxidant capacity, total phenolic compounds, vitamin C, and color attributes) to obtain relationships among the studied parameters. The factor loadings of the analyzed compounds explained 73.91% of the total variation of the data. The two principal components, PC1 and PC2, explained 59.45 and 14.46% of the total variance, respectively. As can be seen in **Figure 4**, there is a close relationship between DPPH and ABTS values and total phenolic compounds, which, in turn, were highly correlated with L^* values. Thus, the antioxidant capacity of fresh-cut 'Flor de Invierno' pears could be mainly attributed to total phenolic content rather than to vitamin C concentrations. In a comparative study of six pear cultivars in terms of their phenolic and vitamin C contents, antioxidant capacity correlated well with the content of chlorogenic acid, the most common phenol found in pears (*5*). The scores of PC1 versus PC2 plotted in **Figure 5** describe differences between nondipped samples and those treated with *N*-acetylcysteine + glutathione. Thus, it can be observed that a majority of the dipped samples are situated in the left part of the score plot. These samples can be related to the highest values of L^*, $h°$, DPPH, ABTS, vitamin C, and total phenolic content. On the other hand, nondipped samples located in the right part of the plot can be related to high a^* and b^* values. The O_2 and CO_2 in-package concentrations did not seem to affect bioactive compounds and color attributes of fresh-cut 'Flor de Invierno' pears throughout the storage (**Figure 4**). Pear wedges stored under HOA scored the highest O_2 content as they are located in the upper part of the plot. Under superatmospheric O_2 levels, the nondipped samples were highly correlated with ethylene production (**Figure 5**).

Conclusions. A dip into a 0.75% w/v *N*-acetylcysteine + 0.75% w/v glutathione solution had an important influence on the reduction of enzymatic browning and ethylene production of fresh-cut pears stored under LOA, HOA, or PA. However, LOA better maintained antioxidant content of pear wedges compared with PA or HOA by preserving vitamin C, phenolic compounds, and antioxidant capacity of the commodity throughout storage. The antioxidant capacity of fresh-cut pears seems to be mainly attributed to phenolic compounds rather than to vitamin C content. In addition, our results suggest that glu-

tathione can be involved in the enhanced formation of phenylpropanoids under LOA.

ABBREVIATIONS USED

HOA, high-O_2 atmospheres; LOA, low-O_2 atmospheres; PA, passive atmospheres; DPPH, 2,2-diphenyl-1-picrylhydrazyl; ABTS, 2,2′-azinobis(3-ethylbenzothiazoline)-6-sulfonic acid; Trolox, 4-hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid; $h°$, hue angle; TAA, total vitamin C concentration; TP, total phenolic content.

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