Browning Evaluation of Ready-to-Eat Apples as Affected by Modified Atmosphere Packaging

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The color and polyphenol oxidase (PPO) activity of fresh-cut Golden delicious apples were evaluated throughout cold storage under modified atmospheres. The shelf life of cut apples was extended to several weeks, especially when an initial atmosphere of 90.5% N_2 + 7% CO_2 + 2.5% O_2 and plastic pouches of 30 $cm^3/cm^2 \cdot bar \cdot 24$ h were used. Under these conditions, a maximum 62% PPO activity depletion was observed. In all cases, the faster the initial PPO activity decays, the less the color changes. A fractional conversion first-order model was proposed for predicting color changes in minimally processed apples. Browning was better described through lightness (L*) (k_L = 0.017 − 0.07 day^{-1}) and color difference (ΔE*) values ($k_{\Delta E}$ = 0.015 − 0.073 day^{-1}), which fitted the model with enough accuracy.

INTRODUCTION

The market demand for minimally processed fruits and vegetables has undergone an important rise during recent years because of busy lifestyles, increasing purchasing power, and increasingly health-conscious consumers (*1*). Therefore, they request fresh-like processed products with quality attributes (such as appearance, texture, and flavor) similar to those of the raw produce (*2*). Minimal processing has been defined as a combination of procedures, such as washing, sorting, trimming, peeling, and slicing or chopping, that do not affect the fresh-like quality of the food (*3*).

Nevertheless, because the tissular integrity of fruits is more easily altered during processing, ready-to-use commodities are more perishable than the original materials (*4*). Thus, fresh-cut fruits are more difficult to produce than other minimally processed products. Their shelf life is affected by many factors, including cultivar, stage of ripeness at cutting, and storage atmosphere or temperature (*5*). Fresh-cut apples (slices, wedges, or cubes) are potential lightly processed products and, although they have scarcely been produced commercially, fruit marketers have shown a great interest in their development. From a microbiological point of view, their shelf life has been estimated in the range of 2−3 weeks (*6*). However, much more information is needed about quality losses in these products.

Mechanical stress during processing results in cellular delocalization of enzymes and their substrates, leading to biochemical deteriorations such as enzymatic browning, off-flavors, and texture breakdown (*7*). Enzymatic browning of apples is caused by the action of polyphenol oxidase (PPO), which catalyses oxidation of phenolic compounds containing two *o*-dihydroxy groups to the corresponding *o*-quinone (*8*). Because thermal treatments are not suitable to process this sort of product, several methods have been tried to inhibit PPO activity and avoid browning of fruits. Ascorbic acid has been long applied in combination with organic acids and calcium salts to prevent enzymatic browning and maintain firmness of fruits (*9*). On the other hand, exclusion of oxygen (*10*), addition of chemicals (*11−13*), or refrigeration (*11, 14*) may contribute to enhancing the shelf life of fresh-cut apples. Although these techniques are not as effective as thermal treatments in terms of microbiological safety (*15*), a proper combination of preserving factors may become an excellent way to extend the original quality of these commodities (*16, 17*).

Among the chemical agents used in the past to avoid enzymatic browning of fresh-cut fruits, sulfites have been the most widespread. However, their use has been restricted because of consumer concerns over their harmful effects. Hence, several antibrowning chemicals such as ascorbic acid or 4-hexylresorcinol have shown to be good alternatives to sulfites in fresh-cut apples (*9, 11, 13*). Nicoli et al. (*18*) have proposed the use of a modified atmosphere (80% N_2/20% CO_2) to preserve apple slices from oxidative browning. Other authors (*19, 20*) have suggested methods based on the application of edible coatings to reduce browning and moisture loss of fresh-cut apples. Osmotic dehydration has been combined with the use of preservatives in order to enhance the preservation of color of apple cuts as well (*21*). Nevertheless, as far as we know, no work has been carried out to study the influence of different modified atmospheres and plastic permeability on browning and

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Table 1. Physicochemical Properties of Non-Processed Fresh-Cut Apple Cubes[a]

a_w	0.9855 ± 0.0007
pH	3.87 ± 0.19
soluble solids (°Brix)	14.4 ± 0.4
total acidity (g citric acid/100 g)	0.34 ± 0.15
Formol index (mL NaOH/100 g)	4.0 ± 1.4
dry content (%)	14.65 ± 0.04
moisture content (%)	85.35 ± 0.04
ashes (%)	2.1 ± 0.4
protein (%)	0.325 ± 0.015
color parameters	
L^*	83.3 ± 0.4
a^*	−1.1 ± 0.6
b^*	13.27 ± 0.04
firmness (N)	9.17 ± 0.15

[a] Mean ± S.E.

PPO activity of fresh-cut apples throughout storage time.

The aims of this work were to evaluate the influence of using different packaging materials and atmosphere conditions on the PPO activity and color changes that occurred in minimally processed apples during storage, and to kinetically model those evolutions. The study was also focussed on determining any possible correlations among color variables and PPO activity changes.

MATERIALS AND METHODS

Sample Preparation. Apples (Golden Delicious) at commercial maturity were purchased from a local market. A portion of 32 kg from a total amount of 250 kg was processed, according to military standard sampling procedures (*22*). The fruits were cleaned, peeled, cored, and cut into 1-cm thick cubes. At this stage, a characterization was carried out according to AOAC (*23*) procedures (Table 1). Apple pieces were dipped for 1 min in a 1% (w/v) L-ascorbic acid and a 0.5% (w/v) calcium chloride solution in a product/solution ratio of 1:2. After drainage of the excess of solution, ca. 100 g of apple cubes were packaged in plastic bags (CRYOVAC Europe, Grace S. A. Sant Boi de Llobregat, Barcelona, Spain) of low oxygen permeability (LOP, 15 cm³/cm² · 24 h · bar at 23 °C, 0% RH) or medium oxygen permeability (MOP, 30 cm³/cm² · 24 h · bar at 23 °C, 0% RH) under different atmosphere conditions (100% N_2 or 90.5% N_2 + 7% CO_2 + 2.5% O_2) and sealed by an EGAR VAC Basic-9 digital compensated vacuum machine (Egarvac S. C. P., Terrassa, Barcelona, Spain). The relation between the amount of product and the injected gas mixture was 1:2. Bags were stored at 4 ± 1 °C in darkness until analysis up to 90 days. Two bags were taken at each time to perform the analysis and two replicates were carried out for each bag.

Determination of PPO Activity. *Enzyme Extraction.* PPO was isolated from 50 g of apple pieces, which were blended and dipped in a McIlvaine buffer solution (1:1) at pH = 6.5. This buffer contained 1 M NaCl (Riedel-de-Haën AG, Seelze, Germany) and 5% polyvinylpolypyrrolidone (Sigma-Aldrich Chemie, Steinheim, Germany). The homogenate was stirred 5 min and centrifuged at 12000 rpm for 30 min at 4 °C (Centrifuge AVANTI J-25, Beckman Instruments Inc., Fullerton, CA). The supernatant solution was collected and filtered through Whatman No. 1 paper. The resulting solution constituted the enzymatic extract.

PPO Activity Measurement. Enzymatic activity was determined spectrophotometrically, placing 3 mL of 0.05 M cathecol (Sigma-Aldrich, Steinheim, Germany) and 75 μL of extract in a 1-cm path cuvette. Assays were carried out while monitoring color change with a Cecil CE 1010 spectrophotometer (Cecil Instruments Ltd., Cambridge, UK). One unit of PPO activity was defined as a change in absorbance at 400 nm per min and mL of enzymatic extract. The initial reaction rate was estimated from the linear portion of the plotted curve and was expressed as relative activity (RA, %), calculated using eq 1:

$$RA = 100 \cdot \frac{A}{A_0} \qquad (1)$$

where A and A_0 are the current and the initial PPO activities, respectively.

Color Measurement. The CIELAB coordinates (L*, a*, b*) of minimally processed apple pieces were directly read with a spectrophotocolorimeter Color-Eye 3000 (Macbeth-Kollmorgen Inst. Corp., Newburgh, NY) with a D_{75} light source and the observer at 10°. Hue (H*), chroma difference (ΔC*), and total color difference (ΔE*) were calculated from the former variables.

Mathematical Models and Statistical Analysis. Experimental data were fitted to a fractional conversion model (eq 2 by nonlinear regression procedures of the Statgraphics Plus v. 3.1 program (Statistical Graphics Co., Rockville, MD):

$$\frac{C - C_f}{C - C_0} = \exp(-k \cdot t) \qquad (2)$$

where C is the measured color parameter, k is the first-order kinetic constant, t is the time of storage, C_f is the final equilibrium value and C_0 is the intercept of the curve.

Analysis of variance of the fitting and confidence intervals at $p = 0.05$ were carried out using the same statistical package. All estimated parameters are given with their respective confidence intervals.

An analysis of covariance procedure was used to decide whether significant differences existed among apple cubes stored under the several conditions. The least significant difference (LSD) test was employed to determine differences among results. Furthermore, data were explored by multivariate methods by means of principal components analysis (PCA) to evaluate any relationship among the studied variables. The aim of using PCA was to be able to decide whether focusing the study on a certain parameter would be enough to assess color degradation of ready-to-eat apples.

RESULTS AND DISCUSSION

Changes in PPO Activity. PPO activity of minimally processed Golden Delicious apples decreased exponentially from the early days after processing (Figure 1). In previous studies it was shown that, in nonprocessed fruits, PPO activity remained relatively constant during the first 3 months of controlled-atmosphere storage (*25*, 26) or decreased slightly depending on the composition of the storage atmosphere (*27*).

The values of RA (%) vs storage time were adjusted to a fractional conversion first-order kinetic model to describe the inactivation of PPO after processing. The regression parameters of the model ($p = 0.05$) are displayed in Table 2, which shows high determination coefficients (R^2) and a good fitting to the experimental data. Kinetic constants varied from 0.032 to 0.07 day⁻¹ depending on the preservation conditions. Thus, enzymatic activity in fresh-cut apples packaged in the LOP material decreased with similar velocity in both cases and remained at constant values of 60−69% of the initial RA since the sixth week of study (Figure 1). The kind of packaging material affected significantly the evolution of PPO activity of fresh-cut apples during their storage ($p < 0.05$). Hence, fresh-cut apples packaged in the MOP plastic bags exhibited greater but steadier inhibitions ($k = 0.032 - 0.04$ day⁻¹).

Rather than the film permeability, the packaging atmosphere composition was the most significant factor affecting the PPO activity depletion in ready-to-eat apples ($p < 0.0001$). More intense reductions of enzymatic activity (38−61% of the initial RA at the end of

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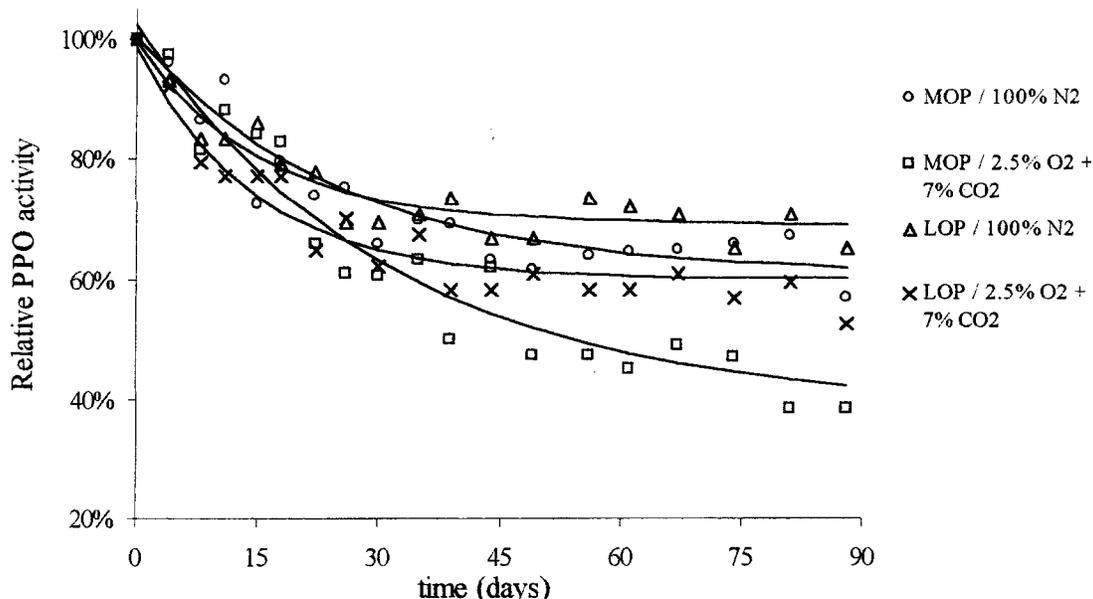
Figure 1. Evolution of the PPO activity of fresh-cut apples throughout storage at 4 °C. MOP: Medium oxygen permeability (30 $cm^3/cm^2 \cdot 24$ h $\cdot$ bar at 23 °C, 0% RH). LOP: Low oxygen permeability (15 $cm^3/cm^2 \cdot 24$ h $\cdot$ bar at 23 °C, 0% RH).

Table 2. Parameter Values of the Fractional Conversion Model Used to Describe the Evolution of Relative PPO Activity (%) in Fresh-Cut Apples throughout 90 days of Storage at 4 °C[a]

plastic	atmosphere	RA_0	RA_f	k_{PPO} (day^{-1})	R^2
MOP	100% N_2	100 ± 10	61 ± 9	0.04 ± 0.03	0.7922
MOP	2.5% O_2 + 7% CO_2	102 ± 10	38 ± 13	0.032 ± 0.018	0.9071
LOP	100% N_2	100 ± 7	69 ± 4	0.07 ± 0.04	0.8337
LOP	2.5% O_2 + 7% CO_2	99 ± 7	60 ± 5	0.07 ± 0.04	0.8143

[a] MOP, medium oxygen permeability (30 $cm^3/cm^2 \cdot 24$ h $\cdot$ bar at 23 °C, 0% RH). LOP, low oxygen permeability (15 $cm^3/cm^2 \cdot 24$ h $\cdot$ bar at 23 °C, 0% RH). $p \leq 0.05$. RA_0 and RA_f = initial and stabilization values, respectively, defined by the assayed model. k_{PPO} = kinetic constant. R^2 = determination coefficient. Mean ± S.E.

the storage period) were induced by the presence of 7% CO_2 in the packaging atmosphere (Table 2). In this regard, Barrett et al. (*27*) attributed the shifts observed under controlled-atmosphere storage in the PPO activity of whole Golden Delicious apples to the effect of high CO_2 concentrations. Other authors have related changes in the metabolism of phenolic compounds induced by high CO_2 concentrations in other minimally processed produce (*28*). No comparable studies on PPO activity in apple pieces have been carried out.

Color Changes. Lightness of ready-to-eat apples decreased exponentially from 83.2 ± 0.5 to 79.6 ± 1.3 during 90 days of refrigerated storage (Figure 2). An ANOVA test did not show significant differences ($p \leq$ 0.05) in any of the studied conditions until at least 11 days of storage. L* initial values of fresh-cut apples were maintained during 30 days without any significant decrease when packaged in LOP bags under 100% N_2. Nicoli et al. (*18*) observed that an 80% N_2/20% CO_2 atmosphere preserved minimally processed apples (dipped in ethanol or cysteine solutions) from enzymatic browning reactions for at least 9 days of refrigerated storage. McHugh & Senesi (*20*) reported no significant changes in L* values in wrapped fresh-cut apples during 12 days of storage under noncontrolled atmospheres.

The intensity of browning depended on the factors that were combined to extend the shelf life of fresh-cut apples. Apple cubes packaged under different modified atmospheres were more efficiently preserved from browning when O_2 was initially absent. The initial atmosphere composition was the factor that showed more influence ($p < 0.0001$) on the evolution of lightness. On the

contrary, the permeability of the packaging material did not affect significantly the browning reactions that took place in the apple tissue. Besides, the interaction between composition of the packaging atmosphere and the permeability of the plastic material was highly significant ($p < 0.0001$), leading to less color losses when 100% N_2 and the use of LOP bags were combined.

Browning kinetics of minimally processed apples can be described by L* through a first-order fractional conversion model. L* values were fitted to eq 2 (Table 3), and were described likewise in most of the tested samples. K values ranged from 0.017 to 0.07 day^{-1}. It may be stated that kinetic constants are strongly influenced by the combination of the studied factors. Thus, when MOP plastic and presence of O_2 as well as LOP bags and absence of O_2 were set, L* changes took place up to four times more slowly than in other cases (Table 3). However, stabilization values (L_f^*) did not significantly differ according to the diverse tested conditions (78.0−79.9). Therefore, it can be deduced that the processing affects only the rate of browning during the storage of ready-to-eat apples and has little influence over the substrates or products of such reactions.

Changes in a* values have previously been used in monitoring enzymatic browning at cut apple surfaces (*20*). In the present study, neither a* values of apples (−1.2 ± 1.0) nor the blue-yellow chromatism (b*) (12.3 ± 2.3) shifted significantly throughout 90 days of storage ($p \leq 0.05$), which is evidence of the little color changes achieved under the tested conditions. Moreover, hue (H*) and chroma difference (ΔC^*) did not significantly
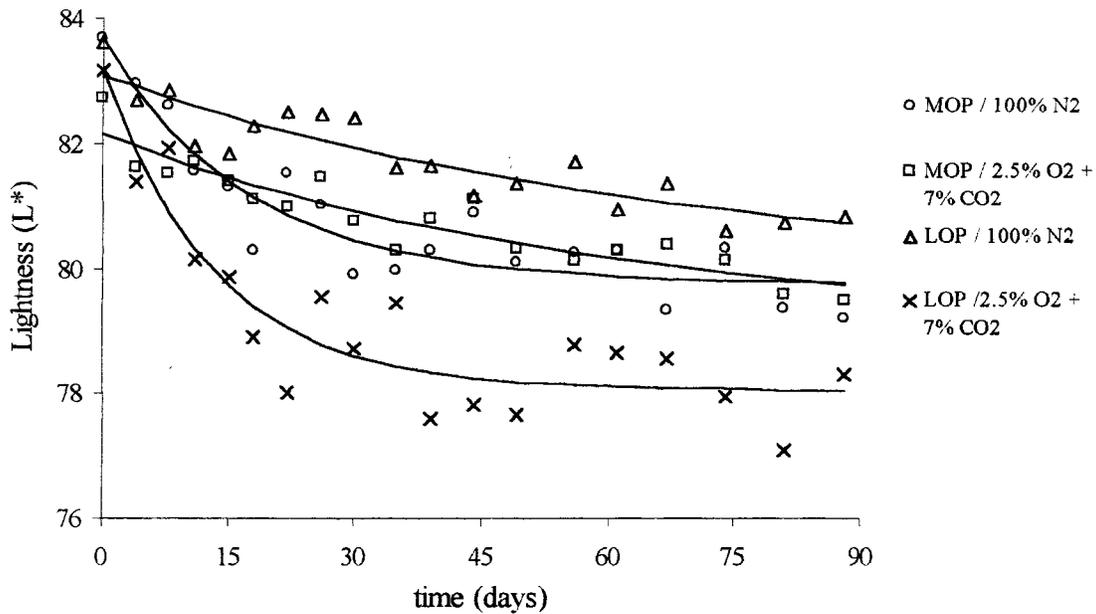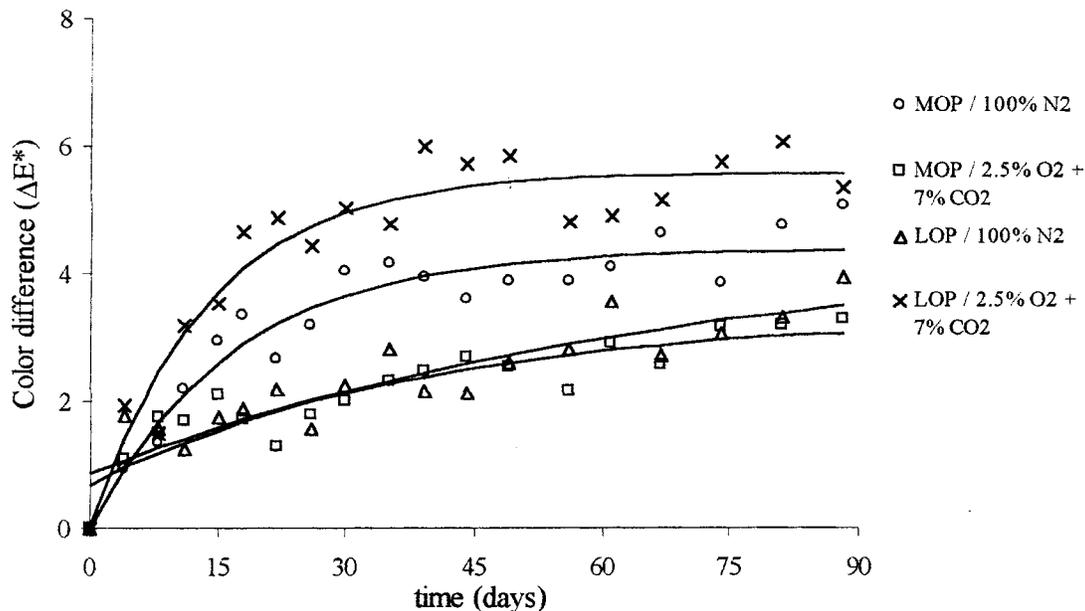
Figure 2. Evolution of lightness (L*) in fresh-cut apples throughout storage at 4 °C. MOP: Medium oxygen permeability (30 $cm^3/cm^2 \cdot$ 24 h $\cdot$ bar at 23 °C, 0% RH). LOP: Low oxygen permeability (15 $cm^3/cm^2 \cdot$ 24 h $\cdot$ bar at 23 °C, 0% RH).

Table 3. Parameter Values of the Fractional Conversion Model Used to Describe the Evolution of Lightness in Fresh-Cut Apples throughout 90 days of Storage at 4 °C[a]

plastic	atmosphere	L_0*	L_f*	k_L (day^{-1})	R^2
MOP	100% N$_2$	83.6 ± 0.8	79.8 ± 0.5	0.06 ± 0.03	0.8553
MOP	2.5% O$_2$ + 7% CO$_2$	82.2 ± 0.5	79.0 ± 2.0	0.017 ± 0.020	0.8550
LOP	100% N$_2$	83.0 ± 0.5	79.9 ± 3	0.02 ± 0.04	0.8118
LOP	2.5% O$_2$ + 7% CO$_2$	83.1 ± 1.2	78.0 ± 0.6	0.07 ± 0.04	0.8409

[a] MOP, medium oxygen permeability (30 $cm^3/cm^2 \cdot$ 24 h $\cdot$ bar at 23 °C, 0% RH). LOP, low oxygen permeability (15 $cm^3/cm^2 \cdot$ 24 h $\cdot$ bar at 23 °C, 0% RH). $p \leq$ 0.05. L_0* and L_f* = initial and stabilization values, respectively, defined by the assayed model. k_L = kinetic constant. R^2 = determination coefficient. Mean ± S.E.

Figure 3. Evolution of color difference ($\Delta E*$) in fresh-cut apples throughout storage at 4 °C. MOP: Medium oxygen permeability (30 $cm^3/cm^2 \cdot$ 24 h $\cdot$ bar at 23 °C, 0% RH). LOP: Low oxygen permeability (15 $cm^3/cm^2 \cdot$ 24 h $\cdot$ bar at 23 °C, 0% RH).

vary throughout the storage time. On the contrary, color difference ($\Delta E*$) reached values of 4.3 ± 1.0 CIE at 90 days of storage (Figure 3), because it is obtained not only from a* and b*, but also from L* changes. Thus, $\Delta E*$ kinetic constants were significantly similar ($p \leq$ 0.05) to those calculated for L* (Tables 3 and 4).

A principal components analysis (PCA) was used to explore relationships among data, thus reducing the amount of studied variables. Two principal components (PC1 and PC2) were calculated. They account for 73.95% of the variability in the original data (Figure 4). PC1 explains most of the variability due to the composition

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Table 4. Parameter Values of the Fractional Conversion Model Used to Describe the Evolution of Color Difference in Fresh-cut Apples throughout 90 days of Storage at 4 °C[a]

plastic	atmosphere	ΔE_0^*	ΔE_f^*	$k_{\Delta E}$ (day^{-1})	R^2
MOP	100% N_2	0.011 ± 0.8	4.4 ± 0.4	0.06 ± 0.03	0.7806
MOP	2.5% O_2 + 7% CO_2	0.9 ± 0.5	4.5 ± 2.5	0.015 ± 0.018	0.7280
LOP	100% N_2	0.7 ± 0.6	3.3 ± 1.1	0.026 ± 0.03	0.6150
LOP	2.5% O_2 + 7% CO_2	0.03 ± 0.9	5.6 ± 0.4	0.073 ± 0.03	0.8278

[a] MOP, medium oxygen permeability (30 cm^3/cm^2 · 24 h · bar at 23 °C, 0% RH). LOP, low oxygen permeability (15 cm^3/cm^2 · 24 h · bar at 23 °C, 0% RH). $p \leq 0.05$. ΔE_0^* and ΔE_f^* = initial and stabilization values, respectively, defined by the assayed model. $k_{\Delta E}$ = kinetic constant. R^2 = determination coefficient. Mean ± S.E.
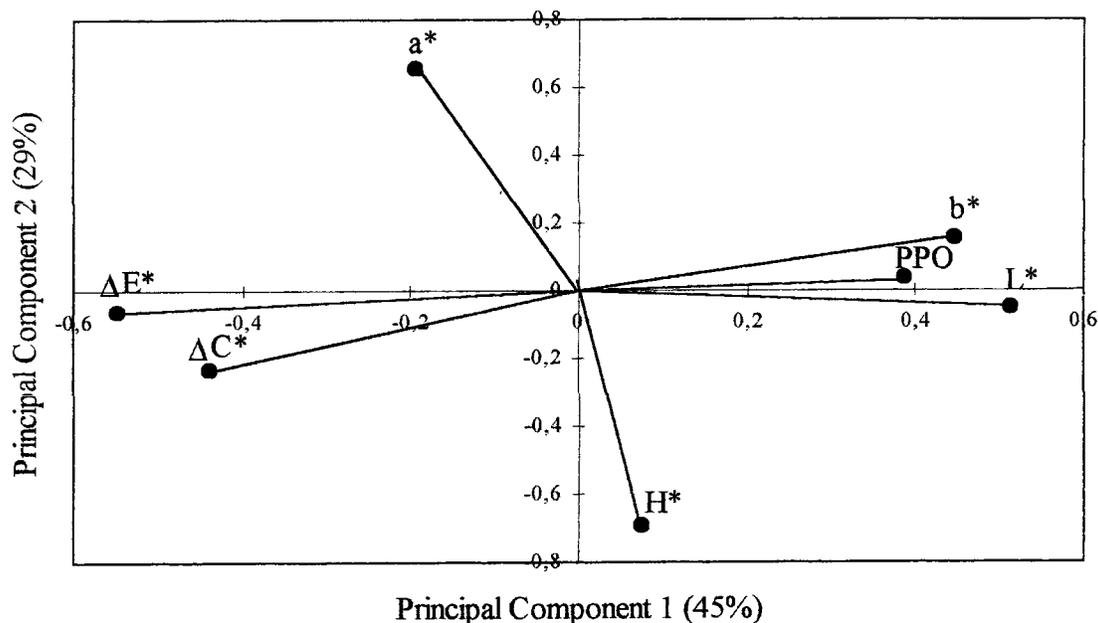
Figure 4. Principal component weights of the browning variables.

of the initial atmosphere. It summarizes most of the information given by L*, b*, and ΔE^*, which are strongly correlated with PPO activity values. These results show that both lightness and color difference are the parameters that were more affected by enzymatic browning pathways. On the other hand, PC2 is mainly composed by a* and H*, showing that these variables are weakly related to L*, b*, ΔE^*, and PPO activity.

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