

# Passive modified atmosphere affects the quality of minimally processed escarole

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## Abstract

This study aimed to evaluate the quality changes of the minimally processed escarole under passive modified atmosphere, packaged in different flexible plastic packages, which included polyvinyl chloride (PVC) stretch film, low-density polyethylene (LDPE) bag, polypropylene (PP) bag, and bi-oriented polypropylene (BOPP) bag, during storage for 20 days at 0°C and 90%–95% RH. The atmosphere of 16% O<sub>2</sub> and 3% CO<sub>2</sub> formed in the PVC overwrap package provided the lowest browning index and the best conservation of ascorbic acid, chlorophyll, and carotenoids. During the experiment, no differences in phenolic compounds and polyphenol oxidase activity among the treatments were observed, while the activity of peroxidase showed peaks in different analysis days. Weight loss of all samples did not exceed 1%. The minimally processed escarole showed sensitivity to high CO<sub>2</sub> concentrations. Thus, a simple PVC stretch film provided the best visual and nutritional preservation of the minimally processed escarole.

## Practical applications

Escarole is one of the most consumed leafy vegetable as a minimally processed product, however, there is no information about its quality changes associated with the package. This produce is widely commercialized in the same package of other leafy vegetables. This research has focused on application of passive modified atmosphere technology for quality preservation of escarole during storage, in order to indicate the most adequate package for its quality and nutritional conservation. The results implied that the quality of minimally processed escarole is better-maintained using PVC stretch film, which is quite different from the usual plastic bags used for minimally processed leafy vegetables. This information is quite interesting at processor and market levels in order to standardize the packaging of this product and prolong its visual and nutritional quality.

## 1 | INTRODUCTION

Escarole (*Cichorium endive* var. *Latifolia* L.) is a leafy vegetable largely consumed cooked or as salad in Europe, Western Asia, and part of America. It is considered a rich source of bioactive compounds,

such as phenolic compounds and carotenoids, which have antioxidant effects, preventing degenerative diseases in the human body (Azevedo-Meleiro & Rodriguez-Amaya, 2005; Feltrim, Cecílio Filho, Rezende, & Barbosa, 2008; Mascherpa, Carazzone, Marrubini, Gazzani, & Papetti, 2012; Tiveron et al., 2012).

The market for minimally processed products (MPP) is one of the fastest growing segments on the food sector, due to the high consumer demand for fresh and healthy foods ready to eat or with easy preparation (Cozzolino et al., 2016). Due to the peeling and cutting steps, the fresh cut products are highly perishable, hence they are commercialized under refrigeration to ensure security and proper shelf life for commercialization. Additionally to low temperature, others techniques such as modified atmosphere (MAP) has been used to increase the shelf life of these products, reducing water loss and retarding the growth of microorganisms (Esturk, Ayhan, & Gokkurt, 2014; Mantilla, Mano, Vital, & Franco, 2010).

Modified atmosphere packaging (MAP) technique aims to control in-pack  $O_2$  and  $CO_2$  concentrations though the exchanging of this gases between outside atmosphere and the headspace inside the MPP package, which is formed naturally by the vegetables (Rai, Kaur, & Patil, 2011). The most critical factor to obtain the desired MAP is the package specification, especially the ones related to barrier properties. If a package with very low oxygen transmission rate is used in MPP with high respiratory rate, it can result in a reduction of the internal level of  $O_2$ , leading to anaerobic respiration, loss of quality, and increasing the risk of contamination by anaerobic pathogens (Chinsirikul et al., 2014).

On the other hand, high  $CO_2$  content may cause injuries, such as necrosis, taste losses, unpleasant odor development, acceleration of nutritional loss, and degradation of plant tissues (Hodges & Toivonen, 2008; Poubol & Izumi, 2005). Passive modification of the atmosphere is a simple application and low-cost technique for the conservation of MPP (Jiang, Joyce, & Terry, 2001).

The quality of the MPP involves a number of attractive attributes to the consumer, such as appearance, texture, flavor and nutritional value. However, these characteristics are affected during storage. It could occur leaf yellowing or darkening, browning on the cut-off points, and physiological disorders. These symptoms can be reduced or alleviated, depending on the atmosphere composition within the package (Manolopoulou, Lambrinos, Chatzis, Xanthopoulos, & Aravantinos, 2010; Martínez-sánchez, Tudela, Luna, Allende, & Gil, 2011).

There are limited information on the use of different packaging and the adequate balance of headspace atmosphere composition on minimally processed escarole. Thus, the aim of this study was to evaluate the effects of passive modified atmosphere on the visual, physiological, biochemical, and nutritional aspects of minimally processed escarole.

## 2 | MATERIAL AND METHODS

### 2.1 | Plant Material and Experimental Setup

Escarole (*Cichorium endive* var. *Latifolia* L. cv *Amazonas Gigante*) were obtained from conventional farm located in Piracicaba (São Paulo, Brazil) and immediately transported to the Physiology and Biochemistry Postharvest Laboratory of the University of São Paulo, under refrigerated conditions. The material were standardized according to its size, color and absence of mechanical damage. The selected escaroles were washed with tap water and then cut in the base of the head to separate the leaves, which have passed through the new selection. The washed leaves were transferred to a cold room at 15°C and sanitized by immersion in sodium hypochlorite solution (200 mg/L) at 5°C for 10 min. After sanitization, whole leaves were manually cut into strips with a stainless steel knife. The slices were again sanitized for 5 min and centrifuged for 1.5 min in domestic centrifuge (Arno, São Paulo, SP, Brazil) with average angular velocity of 760 x g to remove the excess water. After cutting, 150 g of escarole were packed in different plastic films: low-density polyethylene (LDPE) bag, the most common package used for MPP in Brazil; polypropylene (PP) bag; polypropylene bi-oriented (BOPP) bag and PVC (polyvinyl chloride) stretch film. The first three packs had dimensions of 21 x 24 cm and were heat sealed. For the third treatment with the PVC film, the leaves were placed in polystyrene trays (21 x 14.5 x 1.5 cm) which were wrapped with the stretch film. All samples were stored at 0°C and 90%–95% RH for 20 days. Analyses were performed on day 0, after processing and then following every four days until the 20th day of storage. Table 1 shows the package film properties concerned to  $O_2$  and water vapor permeabilities.

### 2.2 | Experimental design and statistical analysis

The experimental design was completely randomized in a factorial scheme 4 x 6, with four treatments and six periods of analysis, including time zero (after processing). Three replicates were used for weight loss, physical, chemical, and gas analysis and triplicates for pigment analysis, enzyme activity and total phenolic compounds. The results were submitted to analysis of variance (ANOVA), with

**TABLE 1** Thicknesses, oxygen, and water vapor transmission rates of the selected films

Film	Thickness ( $\mu m$ )	$O_2$ TR at 23°C ( $mL m^{-2} d^{-1}$ )	WVTR at 38°C and 90% RH ( $g water m^{-2} d^{-1}$ )
PVC	14	5.000	361
PP	30	2.927	5.72
LDPE	30	6.270	5.71
BOPP	30	1.396	4.17

Notes.  $O_2$  TR: Oxigen transmission rate; WVTR: Water vapor transmission rate; LDPE: low-density polyethylene; PP: polypropylene; BOPP: polypropylene bi-oriented; PVC: polyvinyl chloride.

averages compared by Tukey test ( $p \leq 0.01 \leq 0.05$ ). The data were submitted to the Pearson correlation coefficient ( $r$ ), were considered significant correlations between variables, values  $>0.700$ . Statistical analyzes were performed using the statistical software Statistical Analysis System Model 9.3 (SAS, 2011).

### 2.3 | Atmosphere composition inside the packages

The concentrations of gases in the headspace of the packages were monitored using a gas analyzer CheckMate 9900 O<sub>2</sub>/CO<sub>2</sub> PBI Dansensor (Minneapolis, MN, USA). The gas samples were taken via syringe (hypodermic needle) coupled silicone septa previously set in individual containers. The values were expressed as percentage in volume (v/v). The Day 0 gas analysis was performed 1 hr after sealing the packages, which were maintained at experimental storage conditions.

### 2.4 | Browning Index, Total Chlorophyll and Carotenoids Content

For browning index (BI) determination were used 3 replicates, analyzing 10 pieces of minimally processed escarole strips from each replicate. The BI was based on the proportion of leaf area affected following rating scale from 0 (no browning) to 3 (severe browning). BI was calculated by the formula:  $IE = \Sigma$  (browning note  $\times$  percentage of the affected area corresponding to the sample) according to Pen and Jiang (2003). Samples with IE higher than 2 were considered unmarketable. For quantification of total chlorophyll and total carotenoids content, 0.25 g of sample were mixed with a 80% acetone solution, and centrifuged at 10,000  $\times$  g for 10 min at 4°C. The supernatant was used for the measurement of pigments by means of a spectrophotometer (Biochrom, model Libra S22) at wavelengths of 663, 646 and 470 nm for determination of chlorophylls a, b, and carotenoids, respectively, from which the values were calculated the total values of chlorophyll and carotenoids. The formulas used were described by Lichtenthaler (1987). The results were expressed as milligrams of the pigment per 100 g fresh weight (100 mg/g FW).

### 2.5 | Ascorbic acid content

The extract for analysis was prepared by homogenized 30 g of the sample with 10 mL of distilled water. This mixture was filtered to obtain the liquid extract. Ascorbic acid content was determined by titration of a 10 mL aliquot of the extract diluted in 50 mL of oxalic acid (10%) with DCFI indicator (indofenol-sodium 2,6-dichlorophenol) until color changed. The results were expressed in mg of ascorbic acid per 100 g fresh weight (100 mg/g FW) (Carvalho et al., 1990).

### 2.6 | Total phenolic compounds and polyphenol oxidase (PPO) and peroxidase (POD) activity

The total phenolic compounds (TPC) were determined according to the methodology of Singleton and Rossi (1965), with adaptations. The extract was prepared by milling 1 g of sample, added

to 9 mL ethanol and centrifuged at 15,000 xg at 4°C for 20 min. For the measurement sample were mixed 0.3 mL of the plant extract with 0.75 mL of Folin-Ciocalteu 10%; 1.20 mL of water and 0.75 mL of 4% sodium carbonate, and incubated in the dark for 2 hr. The TPC analysis was performed in spectrophotometer (Biochrom, model Libra S22) at 765 nm in triplicate. The calculation of total phenolic compounds was carried out by drawing the standard curve with gallic acid. The results were expressed in mg of gallic acid equivalents per 100 grams of fresh sample (100 mg GAE g<sup>-1</sup> FW). The extract used for enzyme analysis was elaborated adapting the method used by Zhan, Fontana, Tibaldi, and Nicola (2009): 0.5 g of frozen leaves were added to 12 mL of 50 mM sodium phosphate buffer (pH 7.0) (on ice), and subsequently centrifuged at 20,000 x g for 20 min 4°C. The enzyme activity was carried out by spectrophotometry. To analyze the activity of polyphenol oxidase (EC. 1.10.3.1 PPO) was followed the methodology proposed by Degl'Innocenti, Guidi, Pardossi, and Tognoni (2005) and modified by Zhan et al. (2009). The analysis was performed by reading at 480 nm of 0.1 mL of the enzyme extract incubated with 1.9 mL of 25 mM catechol in quartz cuvettes. After a minute of the first reading, a new reading was performed. It was considered as an enzymatic unit PPO, the minimum difference in absorbance of 0.001 per minute between readings. The results were expressed as PPO units per mg protein (U mg<sup>-1</sup> protein). For peroxidase activity (EC 1.11.1.7 POD), reading followed the recommendations of Degl'Innocenti et al. (2005): the sample contained 0.16 mL of the extract incubated with 0.004 mL of distilled water, 0.2 mL of 35 mM hydrogen peroxide and 1.6 mL of 10 mM guaiacol. Readings were taken immediately after the addition of guaiacol and after 1 min at a wavelength of 470 nm. Were considered as a POD unit, the minimum increase in absorbance of 0.001 per minute. The results were expressed in micromoles of guaiacol oxidized per minute per mg protein (mmol guaiacol min<sup>-1</sup> mg<sup>-1</sup> protein). Protein analysis was performed by the method of Bradford (1976) using bovine serum albumin as standard.

### 2.7 | Weight loss

The weight loss was determined by the difference of the initial weight of the samples with the weight values obtained at each experimental evaluation period. The results were expressed in percentage of mass loss.

## 3 | RESULTS

### 3.1 | Atmosphere composition inside the package

There were significant differences ( $p < 0.01$ ) in the gas headspace composition of both O<sub>2</sub> (Figure 1a) and CO<sub>2</sub> (Figure 1b) concentrations. Initially, as the atmosphere modification started taking place, a rapid decrease in the percentage of O<sub>2</sub> and, consequently, CO<sub>2</sub> increasing were observed for most of the tested films, except for

the PVC, which achieved and maintained an equilibrium atmosphere (16% O<sub>2</sub> and 3% CO<sub>2</sub>) during the storage.

As the storage progressed, the headspace atmosphere of LDPE and PP bags reached equilibrium at the 4th day, with a slight change on 20th day, when the product presented a higher degree of deterioration. These two packages had mean values of 12% O<sub>2</sub>, and 5% CO<sub>2</sub> for LDPE and 11% CO<sub>2</sub> for PP. The BOPP bags provided a rapid and sustained reduction of O<sub>2</sub> reaching 4% on the 20th day, while its CO<sub>2</sub> content reached and maintained 21% from the 8th day.

### 3.2 | Browning index, total chlorophyll and carotenoids content

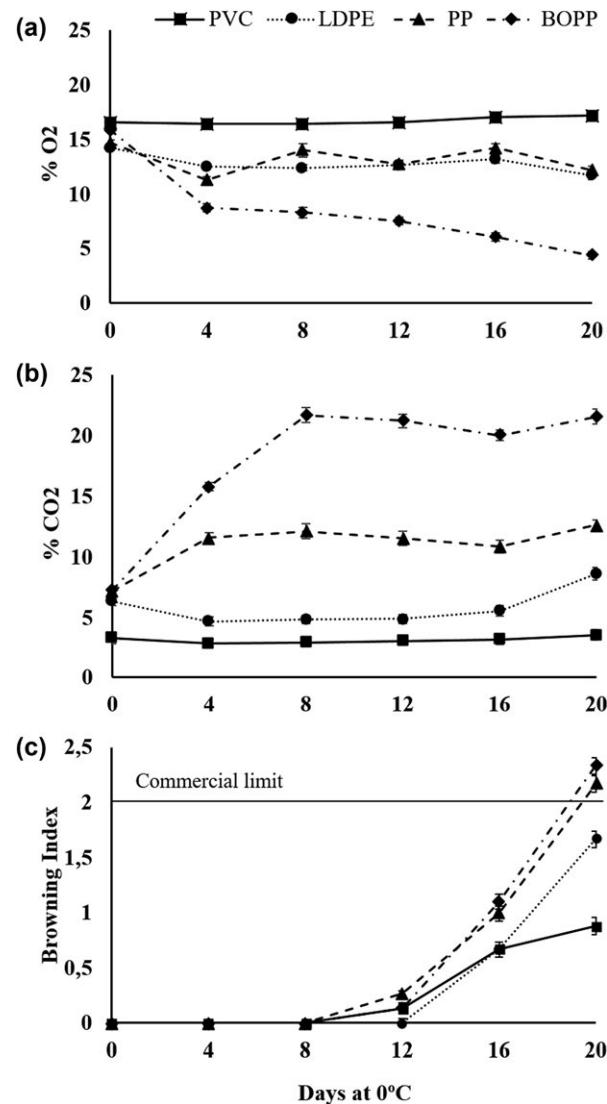
Browning index (BI), the total chlorophyll and carotenoids content were affected by treatments and storage according to the F test (Table 2). A slight increase in BI started at the 12th day in all the samples (Figure 1c). The samples packaged with PVC film had the lowest BI among all films. The samples packaged in PP and BOPP films exceeded the BI marketable limit on the last day of storage, and these samples showed largely darkened points on the leaves area surface. Total chlorophyll content (Figure 2a) and carotenoids (Figure 2b) decreased in all films tested. Among the treatments, PVC was more effective ( $p < 0.01$ ) in the conservation of these pigments in most part of storage. On the other hand, PP and BOPP provided the lowest values observed in samples. The initial chlorophyll and carotenoids values were 40.2 mg 100 g<sup>-1</sup> FW and 4.49 mg 100 g<sup>-1</sup> FW, respectively.

### 3.3 | Ascorbic acid

The content of ascorbic acid (AA) decreased gradually in all treatments (Figure 2c). Leaves packaged in PVC statistically differed ( $p < 0.01$ ) from the others at the 20th day, it retained the highest content of AA in most part of the storage. The lowest AA values were obtained for the samples packed in BOPP. The atmosphere formed inside PVC film provided a retention of 50% ascorbic acid at the last day of analysis, while the gas atmosphere of BOPP retained only 27% of the initial value. There was a positive correlation between the AA content and total chlorophyll ( $r = 0.944$ ), total carotenoids ( $r = 0.939$ ) and total phenolic compounds ( $r = 0.893$ ). That implies the strong influence that the AA has on these parameters and their relationship with the browning of tissues. Also, a negative correlation of AA with BI ( $r = -0.773$ ) was observed, indicating that the degradation of this acid cause higher darkening of the tissues. The initial AA values were from 25.50 mg 100 g<sup>-1</sup> FW to 7.15 mg 100 g<sup>-1</sup> FW in the BOPP samples at the last day of storage.

### 3.4 | Total Phenolic compounds content and Activity of PPO and POD

The total phenolic compounds (TPC) (Figure 3a) decreased during storage regardless of the treatments. However, some variations



**FIGURE 1** Changes in %O<sub>2</sub> (a), and %CO<sub>2</sub> (b) concentrations inside packages and Browning Index (c) in minimally processed escarole during storage at 0°C. Notes. Values are the mean of three replicates. Vertical bars represent the standard error of the mean ( $n = 3$ )

were observed. There were a peak increased on the TPC of PVC samples that differ significantly ( $p < 0.01$ ) only on the 4th day. In the 20th day, the PP and BOPP samples showed higher TPC than the others. In addition to the relationship with ascorbic acid, a positive correlation was observed between TPC and total chlorophyll content ( $r = 0.877$ ) and total carotenoids ( $r = 0.885$ ). The initial values were from 164.30 mg GAE 100 g<sup>-1</sup> FW and the lowest value were 53.43 mg GAE 100 g<sup>-1</sup> FW in the LDPE films at the last day of storage.

No significant effect of films, storage duration and their interaction on the PPO activity was found (Table 2). The mean of PPO activity was 0.27 U mg<sup>-1</sup> protein. The POD activity (Figure 3b) in the leaves showed significant differences ( $p < 0.05$ ) between the films during storage. The initial values of the POD activity was 0.37  $\mu$ mol guaiacol min<sup>-1</sup> mg<sup>-1</sup> protein. There were peaks in the POD activity in

Quality parameters	Storage duration	Film	Storage duration x Film
Carbon dioxide concentration (CO <sub>2</sub> %) <sup>2</sup>	**	**	**
Oxygen concentration (O <sub>2</sub> %)	**	**	**
Ascorbic acid (AA mg 100 g <sup>-1</sup> FW) <sup>3</sup>	**	**	**
Total Chlorophyll (mg 100 g <sup>-1</sup> FW) <sup>4</sup>	**	**	**
Total Carotenoids (mg 100 g <sup>-1</sup> FW) <sup>5</sup>	**	**	**
Total phenolic compound (mg GAE 100 g <sup>-1</sup> FW) <sup>6</sup>	**	**	**
Browning Index <sup>7</sup>	**	ns	ns
Weight loss (%) <sup>8</sup>	**	**	**
PPO activity (U mg <sup>-1</sup> protein)	ns	ns	ns
POD activity (μmol guaiacol min <sup>-1</sup> mg <sup>-1</sup> protein) <sup>9</sup>	**	ns	**

Notes. The results were obtained from the average of three repetitions. \*\*:  $p \leq 0.01$ ; NS: not significant. 1 = transformed data, lambda value: 1<sup>2</sup>; 2 =  $x^{-0.5}$ ; 3 =  $x^{0.5}$ ; 4 =  $x^0$ ; 5 =  $x^0$ ; 6 =  $x^1$ ; 7 =  $x^{-2}$ ; 8 =  $x^0$ ; 9 =  $x^0$

the leaves packed in PVC, BOPP and LDPE at different periods. The higher activity was observed in the BOPP samples on the 20th day (0.81 μmol guaiacol min<sup>-1</sup> mg<sup>-1</sup> protein).

### 3.5 | Weight loss

At the end of storage, the weight loss was less than 1%. The highest weight loss (Figure 3c) were 0.79% and 0.52% achieved in PVC and LDPE packages respectively, that differed ( $p < 0.01$ ) from the others at 12th day of storage.

## 4 | DISCUSSION

Observing the results, it could be imply that MP escarole has sensibility to high CO<sub>2</sub> environments. The exceeded BI from PP and BOPP samples could be explain by the high CO<sub>2</sub> concentration inside these films, which also promoted undesirable odors in the product, symptom also reported in broccoli under high CO<sub>2</sub> conditions (>20%) in the package (Lucera et al., 2011). Although some authors recommend higher proportion of CO<sub>2</sub> then O<sub>2</sub> inside the packages to preserve the quality of most minimally processed leafy vegetables (Barth et al., 1993; Kaji, Ueno & Osajima, 1993), the balance of these gases must be manipulate in order to avoid CO<sub>2</sub> damage or anaerobic respiration.

High CO<sub>2</sub> concentrations inside the package may cause physiological disorders, such as the occurrence of dark spots and tissue necrosis (Varoquaux & Wiley, 1994). This type of injury caused by high concentration of CO<sub>2</sub> (>10%) has been reported for other MPP such as butter lettuce, romaine lettuce and broccoli (Cefola et al., 2010; Kim et al., 2005; Martinez, Ares, & Lema, 2008; Varoquaux, Mazollier & Albagnac, 1996). The susceptibility to damage from CO<sub>2</sub> in the MP escarole can be compared to the same symptom observed

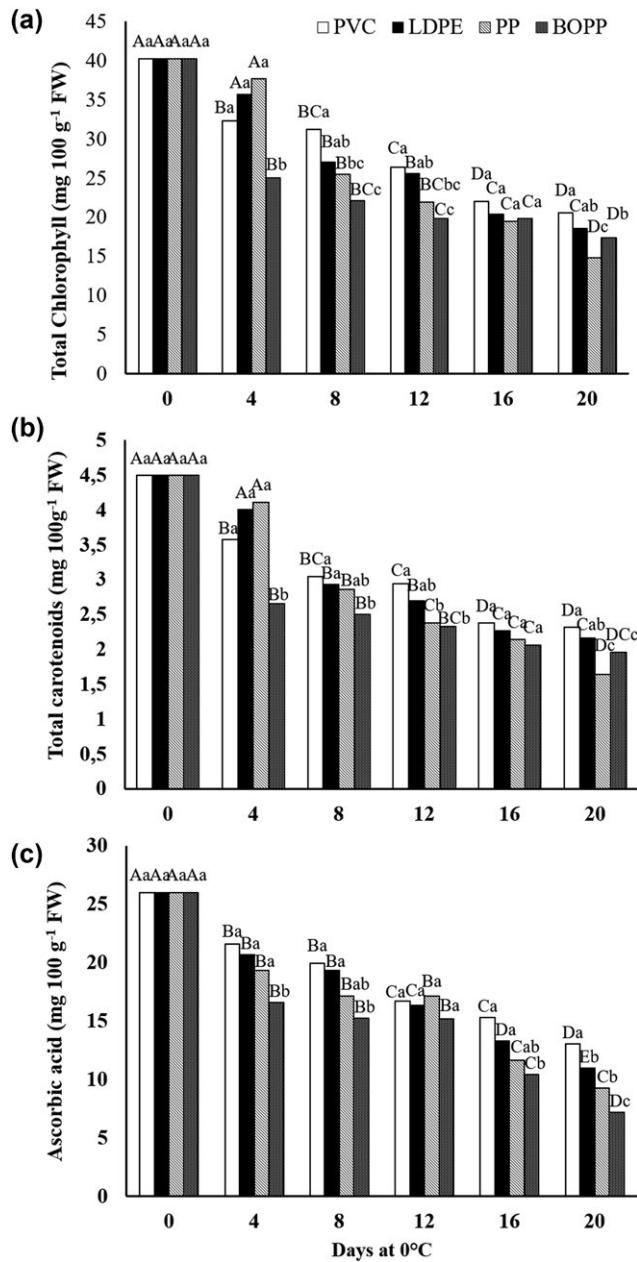
**TABLE 2** Effect of films, storage duration, and interaction (films x storage duration) on physiological aspects, nutritional and quality of escarole minimally processed stored at 0°C for 20 days

in butter lettuce MP, which showed dark spots on the surface and cutting areas under CO<sub>2</sub> atmosphere between 3% and 5% (Martinez et al., 2008).

The lowest chlorophyll values were observed in PP and BOPP due possibly to the high CO<sub>2</sub> content accumulated inside these packages, which could explain in part, the higher BI in these samples. High concentrations of CO<sub>2</sub> can reduce the intercellular pH and affect directly the degradation of chlorophyll. When the tissues become acid, it might occur pheophitization, process that comprises replacing the magnesium ion by hydrogen ions in the chlorophyll protein group, converting it into pheophytin, a brownish color compound that causes browning of tissues (Kirca, Yemis & Ozkan, 2006; Toivonen & Brummell, 2008).

The largest loss of AA was observed in the BOPP samples, probably due to the damage caused by high CO<sub>2</sub> concentrations, which could have stimulated the enzyme ascorbate peroxidase activity, that acts oxidizing AA, converting it into dehydroascorbic acid (DHA) (Lee & Kader, 2000). In leafy vegetables, CO<sub>2</sub> acts on the de-compartmentalization of these acids in chloroplasts (Asada, 1992). The AA content of broccoli MP also decreased when exposed to high CO<sub>2</sub> (10%) concentrations inside the package (Cefola et al., 2010).

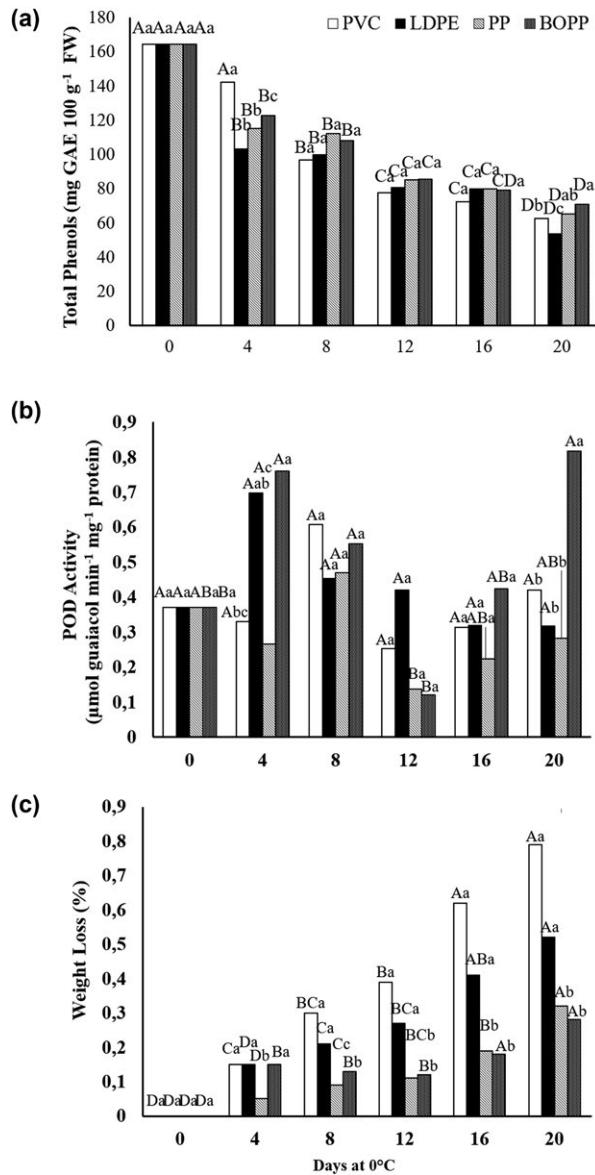
PVC film had better conserved the AA between all films studied, this can be explained by the low CO<sub>2</sub> concentrations in the packs (<6%) and the antioxidant action of endogenous AA and carotenoids, whose contents were higher in leaves packed in this film. Our results shows a positive correlation between those compounds in MP escarole. AA acts protecting the pigments against chemical and oxidative reactions, considering that this acid has a competitive action in the interactions between amides and carbonyl-amine in the enzyme active center that could result in the browning of tissue, in this process, AA is oxidized into DHA (Altunkaya & Gökm̄en, 2009). AA along with carotenoids have antioxidant activity in chloroplast structures, also



**FIGURE 2** Total Chlorophyll (a), total carotenoids content (b) and ascorbic acid content (c) in minimally processed escarole packaged in different films during storage at 0°C. Notes. The columns represent the average of three repetitions. Means followed by different uppercase letters within each day of storage and lowercase letters between treatments differ from each other by Tukey's test

maintaining the integrity of membranes (Schwartz & Von Elbe, 1983; Thompson, Legge & Barber, 1987).

The effect of gas concentration in phenolic content in minimally processed escarole is not yet elucidated. Although it is known that the TPC content increases during storage in MP lettuces due to damage caused by cutting (Martínez-sánchez et al. 2011), the gas content may influence the production of these compounds. The TPC in crisphead lettuce was reduced when exposed to 20% CO<sub>2</sub> due to decreased activity of phenylalanine ammonia-lyase (PAL)



**FIGURE 3** Total phenolic content (a), pod activity (b) and weight loss (c) in minimally processed escarole during storage at 0°C. Notes. The columns represent the average of three repetitions. Means followed by different uppercase letters within each day of storage and lowercase letters between treatments differ from each other by Tukey's test

(Mateos et al. 1993). Moreover, under low concentrations of O<sub>2</sub> (3%) in broccoli minimally processed, TPC remained unchanged for 17 days (Cefola et al., 2010). Reyes, Villarreal and Cisneros-Zevallos (2007) found that vegetables that have high AA content (5–60 mg 100 g<sup>-1</sup>) and initial TPC content between 60 and 200 mg 100 g<sup>-1</sup>, have its phenolic content decreased after cutting and during storage, as observed in cabbage. This may explain the decrease of TPC content in all treatments in escarole. The AA also has synergistic action with phenolic compounds as a reducing agent and preventing its levels reduction (Altunkaya & Gökmen, 2008). Thus, these compounds can reduce the loss of pigments reflecting in preserving color.

The activity of PPO in this study followed the same kinetics occurring in minimally processed lettuce, which have peaks of activity in the first hours after processing and then decrease and remain stable during storage (Degl'innocenti et al., 2005; Mattos, Moretti & Yosino Da Silva, 2013). The POD activity peak on the 20th day of storage at BOPP may occurred due the higher levels of CO<sub>2</sub>. The activity of POD and PPO are related to the defense mechanisms of the vegetable under stress conditions (Sánchez et al., 2000; Zhang et al., 2011). Some authors had reported that the POD activity in different lettuce cultivars may increase or decrease during storage, due to the concentration of gases inside the package (Ke & Saltveit, 1989; Mattos et al., 2013). Our PPO activity values were in according to previous research for MP lettuce (Degl'innocenti et al., 2005; Zhan et al., 2012). The PPO and POD enzymes are directly related to enzymatic browning of tissues. The PPO catalyzes diphenols to the o-quinones in the presence of oxygen. The quinones passes though polimerization into brown pigmentation (Mayer, 1987). The POD has the same model of action on browning, but this enzyme uses hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) as a substrate instead of oxygen (Amiot et al., 1997; Robinson, 1991).

In our research, no significant correlations between the PPO and POD enzymes, the TPC content and BI were found, indicating that the browning in MP escarole may have been nonenzymatic. This may be due to the low activity of the enzymes, which do not oxidize phenolic compounds sufficiently to form quinones and subsequent initiate the browning process. In other studies, it has been suggested that resistance to enzymatic browning in MP leafy vegetables can be associated with high endogenous AA content (Bottino et al., 2009; Degl'innocenti et al., 2007; Landi et al., 2013). The AA can control the activity of enzymes by two mechanisms: reducing the pH of the cytosol of the cells or reducing quinones to their precursor forms of diphenols, during this process AA is converted to DHA (Nicolas et al., 1994; Vámos-vigyazo & Haard, 1981). The DHA content has been positively correlated with browning in lettuce (Heimdal et al., 1995). It is known that vegetables with high AA content are able to control effectively the accumulation of reactive oxygen species (ROS), such as H<sub>2</sub>O<sub>2</sub> (Cocetta et al., 2014; Reyes et al., 2007).

Previous work has shown that the content of phenolics, AA and the PPO and POD activity has no clear correlation with the browning in MP lettuce cultivars (Cantos, Espín, & Tomás-Barberá, 2001; Degl'innocenti et al., 2005, 2007). It can be inferred that the maintenance of higher AA levels had controlled the enzymatic browning in MP escarole. We can reinforce that hypothesis observing the negative correlation between the AA and the browning index ( $r = -0.773$ ), so we can highlight the PVC film, which was the most effective in preserving the AA content and hence, provided the lowest browning index in the product.

Although PVC has shown the highest weight loss at the end of storage, it was less than 1%, this might happened due to the high water vapor transmission of this package. On the contrary, BOPP bags had lower weight loss than the others at most storage period, probably because it had the lowest water vapor transmission rate,

which could have conserved the humidity within these samples. For MP broccoli, the maximum weight loss is 7% (Manolopoulou & Varzakas, 2011). The loss of weight is mainly caused by the loss of water from the vegetable respiration, this factor along with the vapor transmission rate of the film to water vapor as well as the storage temperature are the main parameters that affect the percentage of weight loss (Artés & Martínez, 1999). Data obtained in this study were similar to those observed by Manolopoulou et al. (2010) in minimally processed chicory.

## 5 | CONCLUSION

In conclusion, this research indicates that PVC stretch overwrap, which promoted concentrations of 16% oxygen and 3% carbon dioxide, provides better preservation of visual and nutritional quality of fresh cut escarole for up to 20 days at 0°C and 90%–95% UR. We also verified that minimally processed escarole has sensitivity to high CO<sub>2</sub> concentrations.

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