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Capability of oxygen-scavenger sachets and modified atmosphere packaging to extend fresh barberry shelf life

Razieh Niazmand^{1*}  and Samira Yeganehzad²

Abstract

Background: Barberry has long been used as an herbal remedy since ancient times which is found throughout temperate and subtropical regions of the world. Given the short harvesting season and limited shelf life of the barberry, we evaluated the possibility of using modified atmosphere packaging and oxygen-scavenger sachets to increase its storage period. For this purpose, the physicochemical characterization (antioxidant activity, anthocyanin, phenolic compounds, and ascorbic acid content, acidity, firmness, color, and decay incident) of fresh barberry samples packaged within different atmospheres was investigated over 4 weeks of storage at 4 and 25 °C. The barberries were packaged with low-density polyethylene/polyester (LDPE/PET) films under natural atmosphere (C), N₂ gas (N), vacuum (V), or in the presence of an oxygen scavenger (OS).

Results: The results revealed that with increased storage period, the O₂ and CO₂ levels inside the packages decreased and increased, respectively. The antioxidant activity and amounts of anthocyanin, phenolic compounds, and ascorbic acid all decreased with increasing storage period. Among the studied atmospheres, the OS and, subsequently, V packages were most capable of maintaining the quality of fresh barberries, with the decay incidence being approximately 30 times lower inside these packages relative to the control. Increasing the storage temperature accelerated the intensity of chemical changes and decay across all samples.

Conclusion: The barberries inside the OS packages stored at 4 °C (and even at 25 °C) still had good sensory properties in terms of chemical properties, texture, and color after 4 weeks. Fortunately, it seems that this packaging technology makes the exportation and delayed consumption of the fresh barberry possible by maintaining its quality.

Keywords: Postharvest quality, Vacuum, Nitrogen atmosphere, Storage, shelf life

Background

The consumer interest in fresh, long-life, health-promoting food products has grown given the establishment of the modern lifestyle and the natural desire for consuming a healthy diet. A growth has thus been observed in the popularity of fresh fruits and vegetables, which have desirable flavors besides their nutritional characteristics [1]. Micronutrients (e.g., ascorbic acid or vitamin

C), tocopherols (vitamin E), and phytochemicals (e.g., carotenoids; phenolic compounds) are available in fruits, making them a dominating aspect of dietary recommendations for a healthy diet [2]. The risk of developing a variety of chronic diseases is minimized with increased fruit intake owing to the presence of numerous bioactive compounds as well as the absence of synthetic additives [3] as well as the absence of synthetic additives [4].

Following harvesting, fruit quality deteriorates secondary to reactions that take place within living tissues involving chemicals and enzymes [1]. This makes the preservation of fruit quality until reaching the consumer

*Correspondence: r.niazmand@rifst.ac.ir

¹ Department of Food Chemistry, Research Institute of Food Science and Technology, Mashhad, Iran

Full list of author information is available at the end of the article

extremely vital, with various postharvest treatments having been developed for this purpose [5].

Modified atmosphere packaging (MAP) and active packaging (AP) are two recent evolutions in the field of food preservation, with the former being the focus of many investigations on the postharvest preservation of both fruits and vegetables. MAP involves the use of film packaging to trap a product within modified levels of oxygen and carbon dioxide. Respiration and gas transfer are the key processes responsible for compositional changes that occur within the packaged atmosphere of a natural product [5]. Oliveira et al. [6] reviewed how foodborne pathogens are affected by MAP conditions applied for the preservation of fresh-cut fruits and vegetables. Fagundes et al. [7] looked at inducing a delay in cherry tomato ripening using MAP involving 5% O₂ + 5% CO₂ + balanced N₂ inside multilayer plastic bags. Rodriguez and Zoffoli, [8] arrived at the most suitable partial pressures of CO₂/O₂ for preserving 'Brigitta' blueberry fruit within MAP. Villalobos et al. [9] examined the quality of fig aroma subjected to cold storage with various types of passive MAP.

Oxygen induces food spoilage via the oxidation of constituents (e.g., lipids and vitamins) and the promotion of microbial growth. The incorporation of oxygen-scavenging agents into packaging films can facilitate the continuous elimination of oxygen molecules derived from either the food product or the outer environment [10]. While the residual oxygen present in the headspace can merely be reduced to a potentially destructive level of 0.5–2 vol% via vacuum packaging or MAP, films containing oxygen-scavenging agents can extend product life by minimizing the oxygen level to 0.1 vol% [11]. The impact that such oxygen-scavenging materials have on product quality has been explored in several studies. Charles et al. [12] reported that the browning of fresh endives was controlled by the use of an oxygen scavenger, which decreased the transient period to 50%. Aday and Caner [13] demonstrated that such agents could impede strawberry metabolism and mold growth, reducing product decay by over 50% and more efficiently maintaining the quality relative to the control.

Berberis vulgaris, commonly known as barberry, is a shrub that grows tart, red berries. The largest amount of seedless barberry grow in Iran and is used as fresh fruit or food additive [14]. The quality of the *B. vulgaris* crop is adversely affected by water activity, microbial growth, enzyme activity, and pigment alterations secondary to oxidation. Considering the fact that the barberry fruit is only harvested between October and November, postharvest preservation is critical. After harvesting, phenolase and polyphenolase enzymes are released and induce browning by affecting the anthocyanin pigments, damaging both barberry quality and appearance [15].

Hence, the postharvest life of the *B. vulgaris* fruit is relatively limited, and many processes affect the quality of the product during its ripening. Extending the shelf life of fresh barberry has thus been a continuous challenge to mankind.

To our knowledge, the MAP of fresh barberries has not previously been examined. Thus, this work intended to study the quality changes in fresh barberries packaged in the presence of an oxygen scavenger, nitrogen, or vacuum during refrigerated or shelf storage.

Materials and methods

Materials

Fresh barberries (*Berberis vulgaris*) were collected from an orchard of the Research Institute of Food Science and Technology (Mashhad, Khorasan Razavi, Iran) in October. The foliage was removed and the barberry seeds were kept in a refrigerator until packaging. A bilayer plastic film (45 µm thickness) made of low-density polyethylene (LDPE) and polyester (PET, internal layer with a thickness of 12 µm) was procured from Apaden Plast Factory (Mashhad, Razavi Khorasan, Iran). Oxygen-scavenger sachets (capacity of 3000 ml) were supplied from Basteh Raz Salamat Paya Co. (Tehran, Iran). All chemicals and organic solvents were of analytical grade, having been obtained from either Merck (Darmstadt, Germany) or Sigma-Aldrich Chemical (St. Louis, MO, USA).

Barberry packaging

Approximately 70 ± 2 g of fresh barberries were placed in sterilized bags made of LDPE/PET films. The oxygen permeability and the water vapor transmission rate (Brugger model, Feinmechanik, Germany) of films were 0.248 g/m² d and 125 cm³/m² d bar, respectively at 25 °C. Packaging was done under four different atmospheres:

1. Control (C): atmospheric air.
2. N₂-enriched atmosphere (N): air eliminated and replaced with N₂ gas (>90% N₂) using a MAP machine (Henkelman, A200 Gerhard, Netherlands).
3. Vacuum (V): 90% of air eliminated with a MAP machine and there were 10% atmosphere air inside the packages. The composition of remained air inside the packs after packaging was as 21.7% O₂, and <0.2% CO₂.
4. Oxygen scavenger (OS): oxygen-scavenger sachets placed inside the package walls; atmospheric air (21.7% O₂ and <0.2% CO₂).

For each of these treatments, 16 packages of barberries were made, and three were evaluated per treatment on a weekly basis until the end of the storage period [28 days].

Headspace gas inside the packages

To monitor the gas concentrations (O_2 , CO_2 , and N_2) inside the packages, a portable gas analyzer (AGC Map-Pak Headspace Gas Analyser, Ireland) was used before opening the packages at each evaluation point. Calibration was done using atmospheric air. Values were expressed as percentages of gases composition in the headspace of packages. It is worthy to mention that the in vacuum packages, the changes of remained air composition were reported.

Physical analyses

Weight loss measurement

The barberry packages were weighed initially and then weekly throughout storage. Equation 1 was used to determine the weight loss (WL) percentage:

$$WL = 100 \times (W_i - W_f) / W_i, \quad (1)$$

where the initial and eventual sample weights are denoted by W_i and W_f , respectively [3].

Color measurement

Sample photographs were scanned using a desktop scanner (Canon Scan 8400F). A 3×2 cm segment of each image (JPEG format; 400 dpi) was selected using Adobe Photoshop (Adobe Inc., USA). After reducing the noise, the pixel parameters were converted to the L^* and a^* parameters using Image J (1.40 g) software [16].

Firmness

The barberry firmness was determined using the 3-mm flat probe of the TA.XT-PLUS Texture Analyzer (Stable Micro Systems Ltd., UK). Compression was applied at 0.5 mm/s until reaching 2 mm; we noted the maximum force (Newton) that developed. For each storage time and temperature, 20 measurements were made. The results were expressed in Newton [3].

Chemical and phytochemical analyses

For the determination of moisture content, an oven (Memmert, Germany) was used to dry the samples at $105^\circ C$ until reaching a stable weight [17]. Titratable acidity was measured according to the AOAC (2005). To determine the ascorbic acid content (mg/100 g), metaphosphoric acid extraction of 2,6-dichlorophenol indophenol dye was performed ahead of spectrophotometry at 500 nm according to the method of Shivembe and Ojinnaka [18]. To assess the total anthocyanin content (TAC), the pH differential procedure of the AOAC was followed, as described by Brito et al. [19]. Buffers

with adjusted pH values of 1.0 (potassium chloride) and 4.5 (sodium acetate) were utilized for the evaluation of absorption at 510 and 700 nm, respectively. The level of pigment (mg cyanidin 3-glucoside equivalents per 100 g) was determined using Eqs. 2 and 3:

$$A = (A_{510} - A_{700})pH_1 - (A_{510} - A_{700})pH_{4.5}, \quad (2)$$

$$TAC = (A \times MW \times DF \times 100) / (\epsilon \times L). \quad (3)$$

In these equations, MW denotes the molecular weight (449.2 g/mol); DF represents the dilution factor; L is the cuvette path length in cm; and ϵ denotes the molar extinction coefficient for cyanidin 3-O- β -D-glucoside (26,900 L/mol. cm).

A blender was utilized to homogenize the barberries, and a cheese cloth was used to filter the blended samples. The Folin–Ciocalteu procedure described by Ahmadian-Kouchaksaraie and Niazmand [20] was slightly modified and followed to determine the content of total phenolic compounds (TPC) in the barberry extracts. First, the diluted extract (1.5 ml; twofold dilution using distilled water) was added to distilled water (3.16 ml) and Folin–Ciocalteu reagent (200 μ l). After mixing for 3 min, 20% Na_2CO_3 (50 μ l) was also added. The mixture absorbance was evaluated at 763 nm with a UV–Vis spectrophotometer (Shimadzu UV160A, Japan) following 2 h of incubation at $25^\circ C$ in the dark. The calibration curve was plotted using different gallic acid concentrations. The TPC was reported as mg of gallic acid equivalents (GAE) per 100 g.

For the evaluation of antioxidant properties, we slightly modified the procedure of Soleimanifar et al. [21] and assessed the ability of the constituents to sequester the stable DPPH (2,2-diphenyl-1-picrylhydrazyl) radical. First, the diluted extract (100 μ l) was mixed with the DPPH solution (3.9 ml; methanol solution of DPPH with absorbance between 0.9 and 1). Then, the mixture was shaken vigorously and the reaction was allowed at $25^\circ C$ in the dark until reaching a steady-state, before measuring the absorbance at 517 nm. The spectrophotometer was set to zero using methanol. Equation 4 was employed to calculate the DPPH radical scavenging capacity percentage ($RSC_{DPPH} \%$):

$$RSC_{DPPH} \% = \left(\frac{A_{Control} - A_{Sample}}{A_{Control}} \right) \times 100. \quad (4)$$

In this equation, $A_{control}$ and A_{sample} denote the absorbance of the control and extract, respectively.

The procedure described by Ahmadian-Kouchaksaraie et al. [22] was slightly modified and employed to conduct the FRAP assay. This method is based on the ability of antioxidants to reduce the ferric tripyridyltriazine

complex, generating its colored, ferrous compound. Acetate buffer (300 mM; pH=3.6), 2,4,6-tripyridyl-s-triazine (TPTZ; 10 mM) solution in HCl (40 mM), and $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (20 mM) solution were employed as the stock solutions. A fresh working solution was prepared using acetate buffer (25 ml), TPTZ solution (2.5 ml), and $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ solution (2.5 ml). After mixing, this “FRAP” solution was incubated for half an hour at 37 °C. Then, the barberry extracts (150 μl) were added to the fresh FRAP solution (3 ml); this mixture was left in the dark for half an hour. The absorption was then read at 593 nm, with various concentrations of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ being used in the preparation of the calibration curve.

Decay incidence

Visual inspection was used to evaluate the decay incidence (DI) at 7, 14, 21 and 28 days of storage. To this end, the fruits that had superficial mold lesions were counted and noted, while those that lacked any sign of decay were regarded as being healthy. Equation 5 was used to calculate the DI in each package [13]:

$$\text{DI (\%)} = (\text{infected barberries} \times 100) / \text{total barberries.} \quad (5)$$

Statistical analysis

In this study, all experiments were performed in three replications. The analysis of the data was done using one-way ANOVA based on a completely randomized, simple design and using Minitab software version 16.2.4. The comparison of means was made using Tukey's test at a 95% confidence level ($P < 0.05$). The plots were drawn using Microsoft Excel 2013 software.

Results and discussion

Headspace gas composition inside packages

The variations in O_2 and CO_2 levels within the headspace of packages during storage at 4 and 25 °C are shown in Figs. 1 and 2, respectively. The results revealed that the effect of atmosphere type on O_2 and CO_2 levels during storage was significant ($P < 0.05$).

Generally, with increasing storage period, the O_2 levels inside the packages decreased, while the CO_2 levels increased. These changes can be attributed to barberry respiration, chemical interactions, and microbial activities as well as gas diffusion through the LDPE/PET film. Our findings are consistent with those of Candir et al. [23] and Koort et al. [24], where it was reported that O_2 and CO_2 levels inside the packages decreased and increased to various degrees, respectively, depending on the fruit and film types.

The O_2 level inside the OS packages remained minimal (less than one percent) throughout storage, indicating the

efficiency of the OS in O_2 absorption. This reduced the intensity of oxidative and microbial reactions as well as fermentation, meaning that the CO_2 concentration also stayed low.

It is also important to note that in the vacuum packaging, although the volume of air was reduced initially, the gaseous composition remained constant. As the storage period increased, depending on the chemical and microbial interactions, the gas composition changed and the O_2 level inside the package decreased. Some reports explain that atmospheres containing low levels of O_2 (1 to 5%) and high concentrations of CO_2 (5 to 10%) may prolong product life by limiting the conversion of 1-amino-cyclopropane-1-carboxylic acid to ethylene [25].

An inverse relationship prevailed between the pressure of O_2 and the temperature inside the LDPE packages, such that the pressure decreased with increased temperature. This indicates that the activation energy for film permeability to O_2 was less than the activation energy of the fruit. The stability of O_2 and CO_2 levels is affected by the permeability of the film and the type of fruit, the latter being due to the fact that various fruits have different respiration rates. There are also some conflicting reports suggesting that the respiratory rate is not affected by CO_2 levels below 20 kPa [24].

It seems that the O_2 and CO_2 changes inside the packages were more pronounced at 25 °C than at 4 °C, which can be due to augmented chemical reaction rates at the higher temperature as well as a more favorable temperature for microbial growth. Giuggioli et al. [26] confirmed that the intensity of changes in O_2 and CO_2 levels inside fresh blueberry packages was greatly affected by the temperature, such that 3 days of storage at 20 °C resulted in 70% greater changes relative to 15 days of storage at 1 °C.

Weight loss

The preservation of a fruit's net weight until it reaches the consumer is a vital aspect of marketing. In addition to quantity, weight loss impacts texture, appearance, and nutritional quality.

The results show that the atmosphere type had a significant effect on the weight loss of the various barberry packages during 28 days of storage at 4 and 25 °C (Table 1). The comparison of means revealed that the weight loss significantly increased with extended storage periods at both temperatures ($P < 0.05$). This drop was noticeably more at the higher temperature in all studied packages, which is expectable due to temperature-dependent augmentation of respiration rate, transpiration rate, and microbial activity. Contrary to our findings, Wani et al. [27] found that the weight loss of

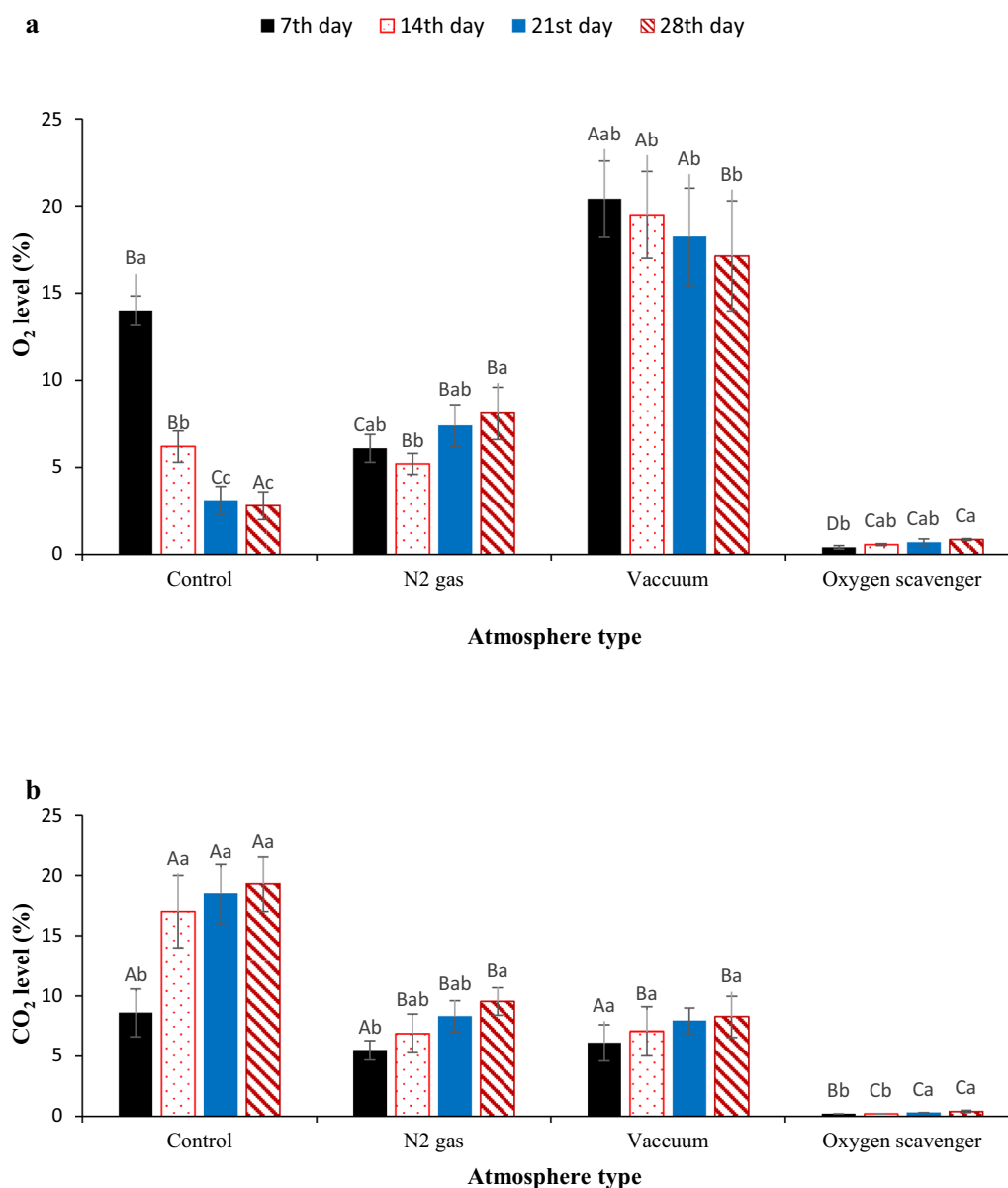


Fig. 1 Changes of **a** O₂ and **b** CO₂ levels inside the barberry packages under different atmospheres during storage at 4 °C. Error bars show standard deviation (SD). Non-similar uppercase and lowercase letters in each column indicate a significant difference among atmosphere type and storage period, respectively, according to the Tukey test ($P < 0.05$)

cherries did not differ significantly between storage at room and refrigerator temperatures.

The results show that at 4 °C, after 7 days, weight loss was less in the OS packages than in the control. With increasing storage period, weight loss in the OS packages occurred slower relative to the other samples, such that after 4 weeks of storage at 4 °C, the OS packages had the least weight loss (0.94%), with the V packages coming second in this regard (1.3%). This trend was replicated in packages stored at 25 °C, but there was no significant

difference in weight loss among the OS, V, N and C packages ($P > 0.05$).

Due to the low permeability of LDPE film to vapor, the air inside a package becomes saturated then super-saturated with water vapor, leading to condensation; this vastly impacts the product's shelf life. Weight loss also can occur due to evaporation and cross-respiration (producing water and heat). Moreover, respiration induces weight loss through the consumption of carbon atoms. Minimized weight loss at lower temperatures can be

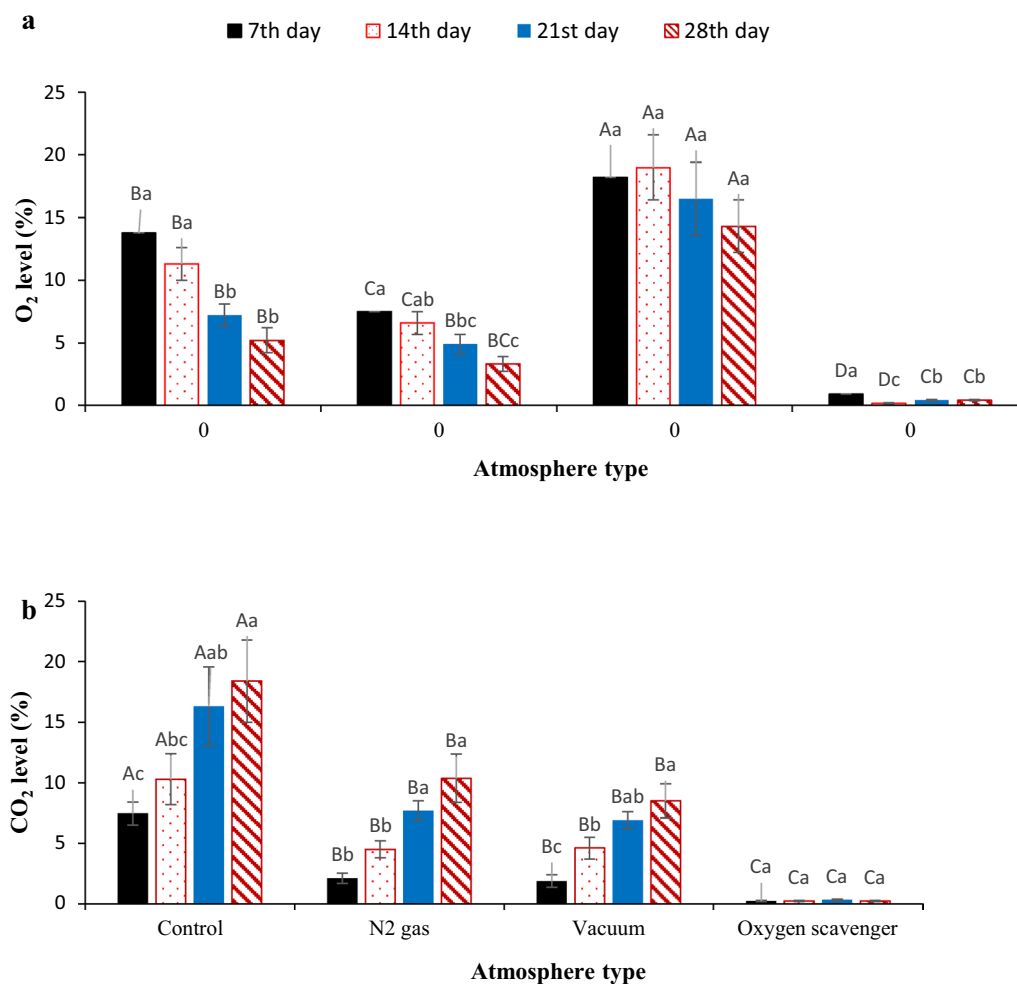


Fig. 2 Changes of **a** O₂ and **b** CO₂ levels inside the barberry packages under different atmospheres during storage at 25 °C. Error bars show standard deviation (SD). Non-similar uppercase and lowercase letters in each column indicate a significant difference among atmosphere type and storage period, respectively, according to the Tukey test ($P < 0.05$)

Table 1 Weight loss* changes (%) in barberry packages under different atmospheres during storage at 4 and 25 °C

Atmosphere type	Temperature	Storage day			
		7	14	21	2
4 °C	Control	0.75 ± 0.25Ac	1.99 ± 0.09Ab	2.41 ± 0.12Aa	2.41 ± 0.02Aa
	N ₂ gas	0.84 ± 0.02Ab	1.27 ± 0.10Bab	1.83 ± 0.26Ba	2.05 ± 0.61ABa
	Vacuum	0.81 ± 0.05Ac	0.93 ± 0.13Cbc	1.13 ± 0.13Cab	1.38 ± 0.10BCa
	Oxygen scavenger	0.25 ± 0.03Bc	0.35 ± 0.02Dc	0.65 ± 0.02Db	0.94 ± 0.15Ca
25 °C	Control	1.40 ± 0.29Bc	4.10 ± 0.62ABb	6.24 ± 0.78Aab	7.64 ± 1.66Aa
	N ₂ gas	2.87 ± 0.24Ad	5.11 ± 0.60Ac	7.06 ± 0.42Ab	8.80 ± 0.80Aa
	Vacuum	1.31 ± 0.29Bc	3.27 ± 0.32Bb	5.82 ± 0.90Aa	6.79 ± 0.79ABa
	Oxygen scavenger	0.79 ± 0.07Bc	2.93 ± 0.55Bb	3.03 ± 0.53Bb	4.63 ± 0.85Ba

* Mean ± standard deviation (SD)

Non-similar uppercase and lowercase letters indicate a significant difference in columns and rows, respectively, for each temperature according to Tukey test ($P < 0.05$)

attributed to delayed physiological processes such as respiration and transpiration [25].

Color changes

The color of a food product is a vital factor that affects its marketability and acceptance among consumers. To measure the color of the barberries inside the packages, the parameters of L^* and a^* were evaluated, indicating the brightness and redness of the barberry fruits, respectively. The color of the barberries can be affected by the surface wax and anthocyanin content. Surface wax affects the brightness index, such that increased surface wax is accompanied by greater brightness.

The changes of the L^* and a^* parameters of the barberries during storage at 4 and 25 °C are shown in Table 2. The results reveal that the effect of atmosphere type on the color parameters of barberries inside the packages during storage at both temperatures was insignificant ($P > 0.05$). According to the results, with longer storage at both temperatures, the values of the L^* and a^* parameters for barberries packaged under different atmospheres decreased, but these decreases had no statistical significance ($P > 0.05$). This indicates that only a slight decrease occurred in the brightness and redness of the barberries during storage.

After 4 weeks, the L^* and a^* values were slightly higher for barberries stored at 4 °C than at 25 °C, indicating a more favorable color. It is noteworthy that the a^* value of barberries decreased significantly during storage, while the inverse occurred for the L^* parameter. In other words, it appears that the decrease in a^* coincided with an increase in L^* for the barberries during storage. These findings are in agreement with the findings of Duan et al.

[28], who revealed that blueberries stored under ambient atmosphere had higher L^* and b^* indices, but decreased redness (a^*) relative to those stored under modified atmosphere. The surface color of the product can be affected by the acidity, soluble solids, and anthocyanin content.

We found that following 4 weeks of storage, the highest redness was observed in the V packages at both temperatures. Candir et al. [23] demonstrated a significant decrease in the L^* parameter of pomegranates during 6 months of postharvest storage, but reported that the ones packaged under modified atmosphere remained brighter than the control.

Firmness changes

The changes in firmness of barberries packaged under the various atmospheres during storage at 4 and 25 °C are illustrated in Fig. 3. As seen, significant changes occurred in barberry firmness ($P < 0.05$). At both temperatures, with increased storage period, the firmness of the barberries packaged under all atmospheres generally decreased, leading to softer textures. However, this loss in firmness was insignificant in the V and OS packages, meaning that the texture of the barberries in these packages was preserved better relative to the control.

Radicals generated by aerobic respiration (e.g., superoxide and nitric oxide) weaken the cell wall and make pectin available to pectinase, leading to firmness loss in the postharvest period. Respiration is hindered by the modified atmosphere, stunting the production of radicals. In the presence of minute O_2 but high CO_2 levels, enzymes involved in tissue softening are activated less, maintaining the firmness of the product. This softening

Table 2 Changes of color parameters* in barberries packaged under different atmospheres during storage at 4 and 25 °C

Atmosphere type	Temperature	Storage day							
		7		14		21		2	
		a*	L*	a*	L*	a*	L*	a*	L*
Harvesting day: L* = 44.8 ± 3.0 a* = 36.1 ± 2.3									
4 °C	Control	26.6 ± 1.1ABa	53.3 ± 1.7Aa	24.2 ± 1.5Ba	52.6 ± 3.0Aa	23.3 ± 2.7Aa	51.0 ± 0.8Aa	27.2 ± 1.3Aa	52.3 ± 2.0Aa
	N ₂ gas	29.1 ± 0.8ABa	52.7 ± 2.1Aa	26.2 ± 1.9ABa	52.6 ± 2.0Aa	26.7 ± 2.0Aa	52.4 ± 1.6Aa	25.8 ± 3.1Aa	51.5 ± 2.5Aa
	Vacuum	25.3 ± 2.4Ba	53.0 ± 2.3Aa	26.4 ± 0.7ABa	52.5 ± 1.4Aa	25.9 ± 2.9Aa	51.9 ± 2.9Aa	28.7 ± 1.9Aa	50.8 ± 1.6Aa
	Oxygen scavenger	27.5 ± 0.8Aa	53.7 ± 2.2Aa	28.0 ± 0.8Aa	53.3 ± 3.3Aa	26.3 ± 2.2Aa	52.7 ± 1.9Aa	28.3 ± 2.3Aa	51.5 ± 2.3Aa
25 °C	Control	26.4 ± 0.4Aa	54.9 ± 1.2Aa	24.3 ± 3.1Aa	53.3 ± 1.5Aa	20.9 ± 5.6Aa	52.4 ± 1.6Aa	25.8 ± 3.1Aa	51.8 ± 1.5Aa
	N ₂ gas	26.5 ± 3.0Aa	52.9 ± 0.8Aa	26.1 ± 1.2Aa	51.7 ± 3.2Aa	24.7 ± 0.6Aa	51.2 ± 3.1Aa	23.9 ± 1.5Aa	49.8 ± 1.9Aa
	Vacuum	28.3 ± 4.2Aa	53.0 ± 2.0Aa	26.0 ± 3.4AAa	52.7 ± 2.9Aa	24.5 ± 2.2Aa	49.8 ± 4.1Aa	27.2 ± 3.2Aa	49.1 ± 3.1Aa
	Oxygen scavenger	27.5 ± 3.5Aa	54.2 ± 2.2Aa	26.3 ± 1.9a	53.8 ± 1.0Aa	25.9 ± 2.6Aa	54.3 ± 1.8Aa	25.3 ± 2.3Aa	51.1 ± 3.1Aa

* Mean ± standard deviation (SD)

Non-similar uppercase and lowercase letters indicate a significant difference in columns and rows, respectively, for each temperature according to Tukey test ($P < 0.05$)

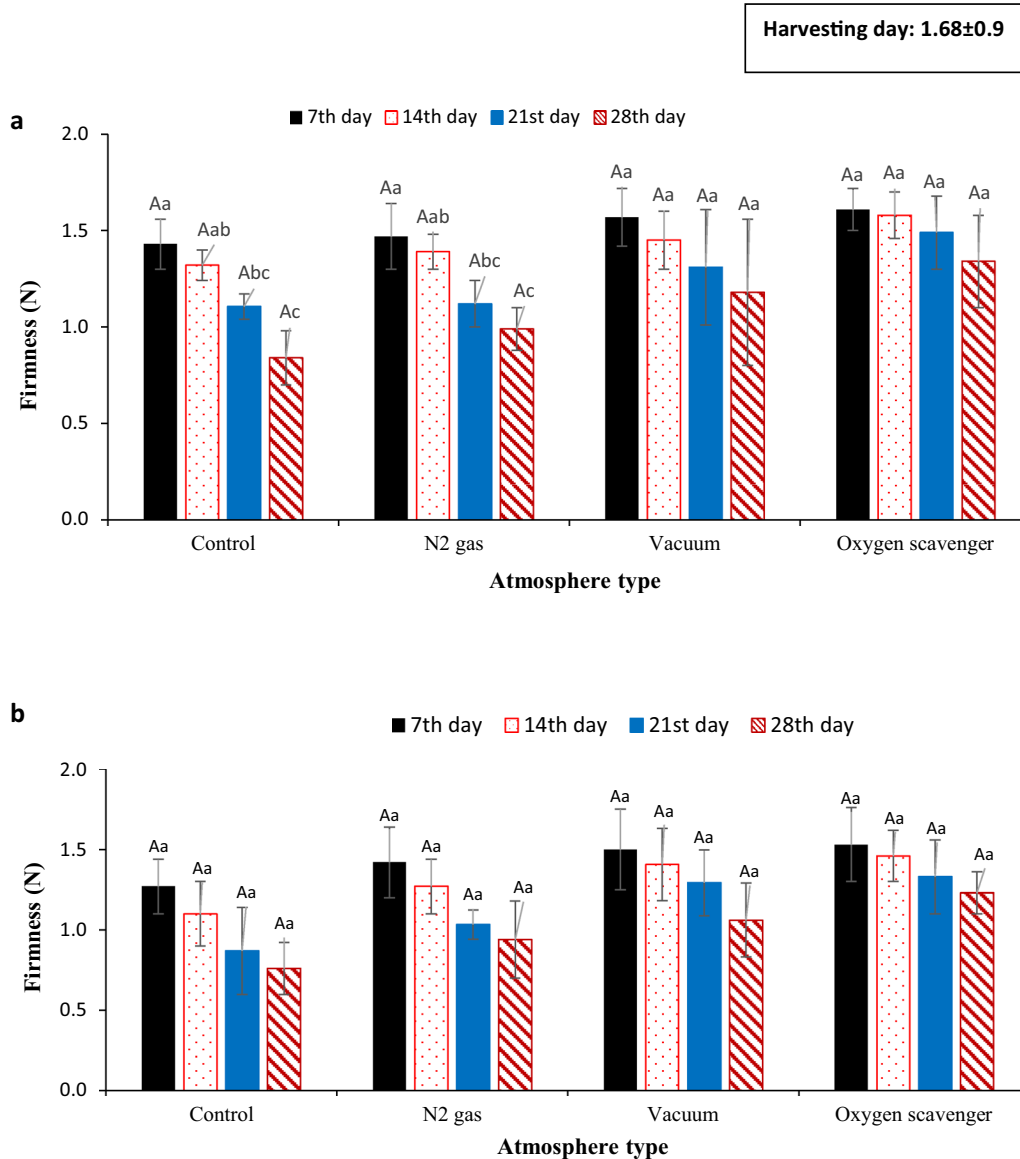


Fig. 3 Changes of barberry firmness under different packaged atmospheres during storage at **a** 4 °C and **b** 25 °C. Error bars show standard deviation (SD). Non-similar uppercase and lowercase letters in each column indicate a significant difference among atmosphere type and storage period, respectively, according to the Tukey test ($P < 0.05$)

is also reduced by storage at low temperatures, probably as a result of the decreased respiration and perspiration rates, as well as the induction of an inhibitory effect on the hydrolytic enzymes of the cell wall (e.g., polygalacturonase and pectin methyl esterase) and reduced ethylene production [25]. The polygalacturonase enzyme hydrolyzes the β -1-4 bond of galacturonic acid in the pectin chain, thereby reducing fruit firmness. Mendes et al. [29] described that the firmness of peaches packaged under modified atmosphere decreased.

After 4 weeks of refrigerated storage (4 °C), the highest firmness was observed in the OS and V packages, which exhibited 20.2 and 29.8% more firmness relative to the harvesting day, respectively, and 59.5 and 40.5% more firmness compared with the control on the same day (28th day), respectively. The same trend was observed at the temperature of 25 °C. The effect of storage atmosphere on firmness varies depending on packaging conditions and temperature. Vicente et al. [30] argued that tissue firmness or softness largely depends on hemi-cellulose

depolymerization, while Fava et al. [31] stated that flexibility or swelling depends on the regulation of internal turgor pressure mostly by cuticular wax, which is as an excellent barrier to excessive water loss. Firmness changes during storage also depend on the amount of water condensed at the surface of the product and water loss. Changes in tissue characteristics due to water loss are directly related to decreased turgor pressure, which may be associated with external periderm resistance or periderm removal for water vapor transfer [32].

Titratable acidity changes

The changes in the titratable acidity of barberry samples stored at 4 and 25 °C are exhibited in Tables 3 and 4, respectively. The results show that in the control sample, the acidity changes did not follow a certain trend, with the only significant decrease being identified after 28 days ($P < 0.05$). No significant change was observed in the acidity of the OS and N samples over the storage period ($P > 0.05$). Following 4 weeks of storage, the OS sample had the maximum acidity and the least change in this parameter relative to the harvesting day. It is worth noting that the acidity in the OS and V samples initially increased during the first 7 days of storage, but then gradually decreased. Respiration has been reported as the most important factor that can convert sugars into energy in the presence of O_2 , meaning that the fall in acidity after the initial rise could be related to the increase in O_2 concentration [32].

In barberries packaged under C and N atmospheres at 25 °C, the acidity gradually decreased until the 7th day of storage, before slightly increasing by day 14, then gradually decreasing again until the end of storage. Of course, it is important to note that these changes were not significant in most cases ($P > 0.05$). Generally, after 4 weeks, the lowest acidity was observed in sample C. After harvest and during storage, organic acids are converted to starch or sugar, leading to decreased titratable acidity [28]. Notably, changes in acidity can also affect the fruit taste.

Anthocyanin content changes

Barberry is a rich source of anthocyanins. Lack of oxygen, low pH, and low processing or storage temperature are the most important conditions that increase anthocyanin stability. So far, numerous studies have focused on the influence of temperature, processing, and storage duration on the stability of anthocyanins, but no definitive results have been obtained.

The content of anthocyanins in barberries packaged for 4 weeks under different atmospheres at 4 and 25 °C is portrayed in Tables 3 and 4, respectively. The results show that the effect of atmosphere type on anthocyanin content was significant at both temperatures ($P < 0.05$). At 4 °C, the anthocyanin content of the barberries in the control sample slightly increased during the first week of storage. Generally, with lengthened storage time, a decreasing trend was seen for anthocyanin content across

Table 3 Changes* of titratable acidity, anthocyanin and ascorbic acid contents in barberries packaged under different atmospheres during storage at 4 °C

Characteristics	Atmosphere type	Storage day			
		7	14	21	28
Harvesting day = 4.7 ± 0.13					
Acidity (%)	Control	4.08 ± 0.42Ba	3.51 ± 0.17Bab	3.89 ± 0.12Ba	3.24 ± 0.10Cb
	N ₂ gas	3.73 ± 0.08Ba	3.96 ± 0.20Ba	3.89 ± 0.21Ba	3.84 ± 0.17Ba
	Vacuum	4.89 ± 0.18Aa	4.69 ± 0.29Aab	4.55 ± 0.23Aab	4.20 ± 0.28Ab
	Oxygen scavenger	4.89 ± 0.25Aa	4.70 ± 0.19Aa	4.73 ± 0.27Aa	4.52 ± 0.17Aa
Harvesting day = 659.3 ± 6.3					
Anthocyanin (mg/100 g)	Control	688.4 ± 1.3Aa	622.6 ± 11.5Ab	611.0 ± 3.6Ab	548.4 ± 3.6Cc
	N ₂ gas	585.0 ± 2.3Cb	626.7 ± 22.6Aa	616.3 ± 9.4Aab	584.6 ± 15.2Bb
	Vacuum	599.3 ± 2.7Ba	596.4 ± 3.6Aa	535.5 ± 30.1Bb	497.7 ± 7.1Db
	Oxygen scavenger	607.6 ± 5.9Bc	686.3 ± 10.7Aa	647.3 ± 3.6Ab	632.7 ± 12.3Ab
Harvesting day = 7.28 ± 0.38					
Ascorbic acid (mg/100 g)	Control	5.87 ± 1.20Aa	4.94 ± 0.31Bab	4.09 ± 0.17Cb	3.74 ± 0.26Bb
	N ₂ gas	5.62 ± 0.59Aa	5.00 ± 0.18Bab	4.67 ± 0.33BCbc	4.01 ± 0.12Bc
	Vacuum	6.19 ± 0.31Aa	5.56 ± 0.35ABab	4.94 ± 0.25Bbc	4.29 ± 0.32Bc
	Oxygen scavenger	6.85 ± 0.50Aa	6.24 ± 0.38Aab	5.83 ± 0.28Ab	5.37 ± 0.34Ab

* Mean \pm standard deviation (SD)

Non-similar uppercase and lowercase letters indicate a significant difference in columns and rows, respectively, for each temperature according to Tukey test ($P < 0.05$)

Table 4 Changes* of titratable acidity, anthocyanin and ascorbic acid contents in barberries packaged under different atmospheres during storage at 25 °C

Characteristics	Atmosphere type	Storage day			
		7	14	21	28
Harvesting day = 4.7 ± 0.13					
Acidity (%)	Control	3.48 ± 0.20Bab	3.86 ± 0.14Aa	3.51 ± 0.17Bab	3.15 ± 0.13Ab
	N ₂ gas	3.69 ± 0.34Bab	3.95 ± 0.07Aa	3.53 ± 0.07Ba	3.46 ± 0.07Aa
	Vacuum	4.42 ± 0.20Aa	4.16 ± 0.46Aa	3.87 ± 0.46Aa	3.79 ± 0.70Aa
	Oxygen scavenger	4.56 ± 0.05Aa	4.33 ± 0.04Aa	3.94 ± 0.04Aa	3.85 ± 0.75Aa
Harvesting day = 659.3 ± 6.3					
Anthocyanin (mg/100 g)	Control	593.8 ± 25.9BCa	535.7 ± 23.1Bab	591.7 ± 22.4ABa	515.9 ± 34.7Bb
	N ₂ gas	557.2 ± 19.5Cb	647.3 ± 33.7Aa	537.0 ± 31.2Bb	585.1 ± 35.0ABab
	Vacuum	645.5 ± 6.6Aab	686.6 ± 40.2Aa	616.2 ± 25.7Ab	592.8 ± 22.2ABb
	Oxygen scavenger	624.8 ± 11.4ABa	672.4 ± 32.3Aa	635.2 ± 30.7Aa	608.9 ± 39.0Aa
Harvesting day = 7.28 ± 0.38					
Ascorbic acid (mg/100 g)	Control	5.67 ± 0.62Aa	3.35 ± 0.66Bb	2.43 ± 0.58Bb	2.32 ± 0.42Bb
	N ₂ gas	6.54 ± 0.84Aa	5.43 ± 0.90ABab	5.09 ± 0.91Aab	3.86 ± 0.59ABb
	Vacuum	6.41 ± 0.41Aa	5.96 ± 0.86Aab	5.19 ± 0.60Aab	4.18 ± 1.00ABb
	Oxygen scavenger	6.48 ± 0.81Aa	6.11 ± 0.81Aa	5.56 ± 0.67Aa	4.65 ± 1.10Aa

* Mean \pm standard deviation (SD)Non-similar uppercase and lowercase letters indicate a significant difference in columns and rows, respectively, for each temperature according to Tukey test ($P < 0.05$)

all packages and at both temperatures, which is consistent with the results of Dhinesh Kumar et al. [33]. However, the anthocyanin content in the N and OS packages increased significantly after 14 days of storage at 4 °C, but followed the same decreasing trend thereafter. Similarly, initial rises in anthocyanin content were seen in the C, N, V, and OS packages stored at 25 °C, but these changes were not statistically significant ($P < 0.05$). Correspondingly, Candir et al. [23] demonstrated that the anthocyanin content of pomegranates increased during the first 2 months of storage at 6 °C, but decreased thereafter until the 6th month. These researchers also reported that pomegranates stored under modified atmosphere packages had lower anthocyanin contents relative to the control.

The stability of anthocyanins is affected by their chemical nature, process temperatures, storage conditions, postharvesting operations, proximity to light and metals, and pH. Oxidative enzymes may be responsible for falls in anthocyanin content during storage. However, the synthesis of anthocyanins at low temperatures has also been reported. In addition, the synthesis or breakdown of anthocyanins is affected by the amounts of O₂ and CO₂ present [33].

After 4 weeks of storage, the highest anthocyanin content was observed in the OS sample, which had 15.4 and 18% more anthocyanins relative to the C sample stored at 4 and 25 °C, respectively. Decreasing the availability of O₂ and thus increasing the CO₂ level reduces the biosynthesis of anthocyanins [32]. It has been reported that

modified atmospheres can prevent the biosynthesis of anthocyanins [24].

In general, the results of this study confirm that with increasing temperature, the rate of anthocyanin degradation is augmented, which can be correlated with a reduction in acidity as well as an increase in weight loss [26].

Ascorbic acid content changes

The results show that with the prolongation of the storage period, the ascorbic acid content in the barberries stored at both temperatures decreased (Tables 3 and 4), which is in agreement with the findings of Wani et al. [27]. Decreased ascorbic acid content may be related to a deficiency in its biosynthesis or its rapid decomposition under modified atmospheric conditions. It can also be ascribed to water loss, cell wall damage, and increased environmental temperature and humidity. Given that the compound has high sensitivity to the phenolase enzyme, temperature, light, oxygen, and pH [33], such reductions in ascorbic acid content during storage were expected.

A quick look at Tables 3 and 4 reveals that the atmosphere of the N, V, and OS packages more efficiently maintained the ascorbic acid content. Following 4 weeks of refrigerated storage (4 °C), the OS sample had the maximum level of ascorbic acid at 5.4 mg/100 g, which was 45.9% higher than the control. Modified atmospheres can reduce the oxidation of ascorbic acid to dehydroascorbic acid and thus help maintain it. The amount of ascorbic acid is also affected by genotypic differences, pre-harvest

climate conditions, and management methods during and after harvesting [25].

A similar result was observed at the higher temperature (25 °C), with barberries in the OS packages retaining the highest content of ascorbic acid (approximately twice that of the C samples) after 4 weeks of storage.

Changes in total phenolic compounds

Phenolic compounds are vital for cell and tissue protection as they act as direct antioxidants. The TPC content of barberries in the different packages over 4 weeks at 4 and 25 °C is given in Tables 5 and 6, respectively. The evaluation of changes in the C packages at both temperatures showed a decrease in TPC with increased storage time. This decrease was less initially at 25 °C than at 4 °C, but intensified toward the end of the 4 weeks; the final TPC content of the C sample was slightly higher at 4 °C than at 25 °C. The same was true for the other packages.

The amount of phenolic compounds is affected by genetics and the environment. Nonetheless, this content can also be affected by oxidization reactions that take place at the time of processing or storage, with stress conditions inducing a rise in TPC. The response to wound stress (stress of phytochemicals reducing) during minimal process operations is a well-known phenomenon. Generally, the process of oxidative regulation strengthens the system's dynamic balance by producing or inhibiting species of active oxygen. Firstly, the tissue responds to stress, leading to the production of active oxygen species

and activating the systems responsible for producing antioxidant compounds. If the stress level is high, the tissue may not be able to maintain the necessary balance, and the cells become damaged [29]. An increased TPC amount during refrigerated storage can also be related to the stimulation of enzymes involved in their biosynthesis during cold storage conditions [33].

The minimum changes in TPC at both temperatures were related to the OS packages, such that after four weeks, the TPC content was 32.7 and 23.2% higher than the C packages at 4 and 25 °C, respectively, decreasing by 8.1 and 17.2% relative to the harvesting day, respectively. The vacuum and nitrogen atmospheres were also able to retain the phenolic compounds well. It has been reported that the reduction of TPC during storage can be related to the decomposition of these compounds secondary to enzymatic activity, water loss, and changes in acidity and total soluble solids [33].

For the barberries stored at 25 °C, significant changes in TPC content were not observed after four weeks among the different packaging atmospheres ($P > 0.05$). Pinela et al. [1] stated that TPC content of green cress packaged under argon gas or natural atmosphere remained at its original level, while slight reductions were observed in those packaged under vacuum and nitrogen.

Antioxidant activity changes

Antioxidant activity was measured through the DPPH and FRAP methods. Tables 5 and 6 show the RSC_{DPPH}

Table 5 Changes* of total phenolic compounds and antioxidant activity in barberries packaged under different atmospheres during storage at 4 °C

Characteristics	Atmosphere type	Storage day			
		7	14	21	28
Harvesting day = 197.1 ± 4.4					
Phenolic compounds (mg/100 g)	Control	162.8 ± 2.1Ba	156.1 ± 1.4Ca	143.9 ± 1.9Ba	136.5 ± 11.0Ba
	N ₂ gas	151.3 ± 4.5Ca	159.7 ± 2.9BCa	148.2 ± 1.1Ba	145.1 ± 14.9Ba
	Vacuum	158.6 ± 3.6BCa	167.3 ± 2.7Ba	159.1 ± 11.1Ba	152.1 ± 11.9ABa
	Oxygen scavenger	191.6 ± 2.7Aa	186.6 ± 6.2Aa	190.4 ± 0.5Aa	181.2 ± 10.8Aa
Harvesting day = 72.9 ± 2.3					
RSC _{DPPH} (%)	Control	63.6 ± 6.7ABa	59.0 ± 3.7Bab	54.9 ± 3.6Abc	50.3 ± 3.0Bc
	N ₂ gas	67.1 ± 2.9Aa	66.3 ± 3.6Aa	51.2 ± 2.7Bb	49.8 ± 2.9Bb
	Vacuum	57.1 ± 3.8Ba	55.0 ± 4.1Ba	54.2 ± 2.8Aa	48.7 ± 3.1Bb
	Oxygen scavenger	60.3 ± 5.7ABa	58.6 ± 2.9Ba	56.4 ± 3.0Aa	59.2 ± 4.0Aa
Harvesting day = 99.4 ± 4.1					
FRAP (mmol/L)	Control	71.4 ± 4.6Ba	68.8 ± 5.1Bab	62.2 ± 3.8Bb	65.2 ± 8.3Bab
	N ₂ gas	71.1 ± 3.6Bab	73.3 ± 3.8Aa	71.7 ± 3.5Aab	69.3 ± 5.0ABb
	Vacuum	75.3 ± 4.1Aa	76.0 ± 3.4Aa	73.4 ± 6.0Aa	71.9 ± 4.1ABa
	Oxygen scavenger	71.5 ± 4.6Ba	72.6 ± 4.7Aa	75.2 ± 5.4Aa	76.3 ± 7.2Aa

* Mean \pm standard deviation (SD)

Non-similar uppercase and lowercase letters indicate a significant difference in columns and rows, respectively, for each temperature according to Tukey test ($P < 0.05$)

Table 6 Changes* of total phenolic compounds and antioxidant activity in barberries packaged under different atmospheres during storage at 25 °C

Characteristics	Atmosphere type	Storage day			
		7	14	21	28
Harvesting day = 197.1 ± 4.4					
Phenolic compounds (mg/100 g)	Control	175.1 ± 2.7Ca	176.3 ± 2.6Aa	148.6 ± 3.1Ab	132.5 ± 6.1Ac
	N ₂ gas	194.3 ± 3.9Ba	164.0 ± 2.5Bb	159.0 ± 1.9Ab	154.2 ± 14.2Ab
	Vacuum	219.6 ± 3.2Aa	177.0 ± 3.6Ab	167.4 ± 17.0Ab	160.2 ± 15.2Ab
	Oxygen scavenger	180.2 ± 5.2Ca	181.4 ± 1.7Aa	171.4 ± 3.7Aa	163.3 ± 13.3Aa
Harvesting day = 72.9 ± 2.3					
RSC _{DPPH} (%)	Control	61.0 ± 5.5Aa	53.5 ± 6.0Aab	47.3 ± 4.5Aab	47.3 ± 4.5Aab
	N ₂ gas	64.2 ± 4.1Aa	59.2 ± 6.4Aab	51.5 ± 5.5Aab	51.5 ± 5.5Aab
	Vacuum	65.9 ± 6.4Aa	53.1 ± 7.8Aab	48.2 ± 3.3Ab	48.2 ± 3.3Ab
	Oxygen scavenger	59.8 ± 6.4Aa	59.3 ± 4.4Aa	52.6 ± 8.9Aa	52.6 ± 8.9Aa
Harvesting day = 99.4 ± 4.1					
FRAP (mmol/L)	Control	68.8 ± 2.0Aab	78.8 ± 10.1Aa	66.2 ± 7.8Aab	55.4 ± 9.9Ab
	N ₂ gas	75.5 ± 9.2Aa	84.9 ± 10.3Aa	71.4 ± 6.5Aa	62.4 ± 12.3Aa
	Vacuum	74.3 ± 9.5Aa	72.8 ± 8.7Aa	69.7 ± 15.2Aa	62.1 ± 3.0Aa
	Oxygen scavenger	75.8 ± 3.6Aa	78.7 ± 9.6Aa	70.4 ± 11.4Aa	72.5 ± 10.5Aa

* Mean \pm standard deviation (SD)Non-similar uppercase and lowercase letters indicate a significant difference in columns and rows, respectively, for each temperature according to Tukey test ($P < 0.05$)

and FRAP results for the barberries stored for four weeks under different packaged atmospheres at 4 and 25 °C, respectively. The initial shock after harvest was marked by a significant reduction in the antioxidant activity of barberries in the C packages, and the declining trend continued for the barberries packaged under all atmospheres. The RSC_{DPPH} of barberries stored under natural atmosphere (package C) at 4 °C decreased by 9.3, 13.9, 18, and 22.6%, in the first, second, third, and fourth weeks, respectively, while the corresponding decreases at 25 °C were 11.9, 19.4, 25.6 and 29.6%, respectively.

The reduction of antioxidant activity may also be attributed to the acceleration of TPC oxidation in the presence of oxygen. The presence of the OS was able to maintain the RSC_{DPPH} over the four weeks by 86.3 and 78.1% at 4 and 25 °C, respectively. The decomposition of color compounds and product aging may also be responsible for the drop in antioxidant activity [25].

The FRAP of barberries packaged under the natural atmosphere (sample C) at 4 °C decreased by 28.2, 30.8, 37.4, and 34.4% during weeks 1, 2, 3, and 4, respectively, while the corresponding decreases at 25 °C were 30.8, 20.7, 33.4 and 44.3%, respectively. The presence of the OS maintained the reducing power of iron in the barberries and thus FRAP did not show significant changes in these packages during storage at both temperatures. At the end of the 28th day, the FRAP of the barberries inside the OS packages stored at 4 and 25 °C increased by 23.2 and 27.1% relative to the harvesting day, respectively, while

the corresponding increases were by 17 and 30.9% in the C packages, respectively.

After 4 weeks of storage at both temperatures, the RSC_{DPPH} and FRAP of the barberries did not differ significantly between the V, N, and C packages ($P > 0.05$).

Decay incidence

Fungal decay was evaluated based on visual observations of molds. The results revealed that the atmosphere inside the packages clearly had a significant effect on fungal decay incidence (Table 7) during storage at 4 and 25 °C. As the storage period lengthened, increased fungal decay was seen in all packages other than the OS samples. Further, the rate of decay incidence was higher at 25 °C than at 4 °C, which is consistent with the results of Wani et al. [27].

Changing the atmosphere inside the packages during storage activates the fruit's defensive responses and increases disease resistance. When respiration decreases due to limited O₂ availability, a negative response may be observed to changes in the packaging atmosphere. Lower O₂ concentrations often cause fermentation. Furthermore, increasing the storage temperature elevated the rate of decay incidence. The condensation of water vapor inside the package can also facilitate further growth of microorganisms [32].

As shown in Table 7, barberries packaged in the presence of the OS did not undergo fungal decay during the storage period at both studied temperatures (except

Table 7 Decay incidence (%)^{*} in barberry packages during storage at 4 °C and 25 °C

Atmosphere type	Temperature	Storage day			
		7	14	21	28
4 °C	Control	2.0 ± 1.0Ac	2.3 ± 1.5ABc	20.7 ± 4.0Ab	29.0 ± 3.6Aa
	N ₂ gas	0.0 ± 0.0Bb	5.0 ± 3.0Ab	26.3 ± 5.5Aa	34.7 ± 4.5Aa
	Vacuum	0.0 ± 0.0Bb	0.0 ± 0.0Bb	0.7 ± 1.2Bb	10.3 ± 3.5Ba
	Oxygen scavenger	0.0 ± 0.0Ba	0.0 ± 0.0Ba	0.0 ± 0.0Ba	0.0 ± 0.0Ca
25 °C	Control	8.3 ± 1.5Ac	27.3 ± 2.5Ab	32.0 ± 4.6Aab	37.0 ± 3.0Aa
	N ₂ gas	5.0 ± 2.0Bc	20.0 ± 3.6Bb	28.0 ± 3.6Aa	32.0 ± 2.6Aa
	Vacuum	0.0 ± 0.0Cc	3.3 ± 1.2Cc	10.3 ± 2.1Bb	18.3 ± 3.5Ba
	Oxygen scavenger	0.0 ± 0.0Cb	0.0 ± 0.0Cb	1.0 ± 1.0Cab	1.7 ± 0.6Ca

^{*} Mean ± standard deviation (SD)

Non-similar uppercase and lowercase letters indicate a significant difference in columns and rows, respectively, for each temperature according to Tukey test ($P < 0.05$)

at days 21 and 28 at 25 °C), which can be attributed to very low levels of O₂ and CO₂ inside the packages. The packaging of barberries under vacuum also prevented the growth of fungi until the end of storage at 4 °C and until 21 days at 25 °C, with the rate of decay being less than 10%. It is worth noting, however, that the packages stored at 25 °C smelled sour after 4 weeks of storage.

In the N packages, after 2 weeks, suitable conditions for fungal growth prevailed and the rate of decay incidence was more than the corresponding C packages kept at each temperature. It has been reported that freshly harvested crops can respire under atmospheric conditions until the CO₂ concentration reaches a critical level of 10 to 15%; this minimum concentration is essential for preventing the growth of *Botrytis* species [24].

Conclusion

In this study, modified atmospheres were evaluated in terms of their impacts on the shelf life of fresh barberries packaged with PET/LDPE film for 4 weeks at 4 and 25 °C. The results showed that oxygen was the main cause of fungal decay in barberry packages, such that the packages containing the OS sachet has the least fungal decay as well as weight loss. Antioxidant activity and bioactive constituents including phenolic compounds, ascorbic acid, and anthocyanins were also better preserved in presence of OS relative to the natural atmosphere. The vacuum packaging was also suitable for prolonging the life of fresh barberries and preserving their quality. Furthermore, the results confirmed that temperature is a key factor involved in preserving the barberry fruit; increased temperature led to elevated rates of oxidative, chemical, and microbial reactions, thereby reducing the shelf life. In conclusion, it seems that the use of OS sachets or vacuum (as the second priority) in packaging combined

with storage at low temperatures entails the preservation of fresh barberries for a long period, facilitating delayed consumption and exportation to other cities and countries.

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Authors' contributions

RN wrote the proposal, participated in sample and data collections, analyzed the data, and wrote the paper. SY participated in sample collection and materials supply, and English editing of manuscript. Both authors read and approved the final manuscript.

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Author details

¹ Department of Food Chemistry, Research Institute of Food Science and Technology, Mashhad, Iran. ² Department of Food Processing, Research Institute of Food Science and Technology, Mashhad, Iran.

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