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IMPACT OF ENRICHED CO_2 IN MODIFIED ATMOSPHERE PACKAGING ON THE PRESERVATION OF "PALMARITAS" STRAWBERRIES AND THE EXTENSION OF THEIR SHELF LIFE

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ABSTRACT

This study evaluated the effect of modified atmospheres (MAPs) with low oxygen levels (2.5%) and high carbon dioxide levels (from 5 to 20%) on strawberries. During 12 days of storage at 4°C, various quality and chemical content parameters were evaluated. These including decay, color, weight loss, firmness, total soluble solids, total polyphenols, anthocyanins, and flavonoids, as well as antioxidant capacity and individual phenolic *compounds* (quercetin, caffeic acid, gallic acid, (-) epicatechin, and p-coumaric acid). Results showed that high CO_2 levels had a positive impact on strawberry preservation: weight loss was reduced, anthocyanin content increased, decay and softening were significantly reduced, and vitamin C content was maintained. At the same time, these high concentrations of CO_2 did not affect sugar content, color, antioxidant capacity, total phenolic compounds, or total flavonoid compounds of strawberries. A storage time of 12 days at 4°C, under MAP with an initial gas condition of 2.5% O_2 + 15% CO_2 + 82.5% N_2 or 2.5% O_2 + 20% CO_2 + 77.5% N_2, was found to be ideal for preserving the quality of fresh strawberries.

Keywords: Strawberry, modified atmosphere packaging, quality, phenol compounds, shelf life

INTRODUCTION

Red fruits are an important agricultural product in Morocco, where they were grown across a total area of 8,403 hectares in the 2020/2021 season. In fact, strawberries occupied 42% of this country's total cultivated agricultural surface at that time. Overall production accounts for 148,000 tons and has been increasing in recent years (**MAPMDREF, 2021**). Strawberries are consumed in their natural form or used as ingredients in various processed food products.
The fruit's nutritional value is remarkable as it contains a high amount of vitamin C, anthocyanins, and flavones, all of which are known to provide high antioxidant activities (**Guerrero-Chaves *et al.*, 2015**). However, strawberries' high perishability, soft texture, and susceptibility to decay make them difficult to transport without proper refrigeration and preservation methods.
Several factors contribute to the deterioration of strawberries, including microbiological, biological, chemical, and biochemical reactions, along with a series of further mechanical, physical, physiological, and psychological factors. Additionally, strawberries' high rates of respiration and transpiration impose limitations on their storage potential and make them susceptible to fungal spoilage, particularly due to *Botrytis cinerea* (**Cagnon *et al.*, 2013**).
Several techniques are available to protect strawberries. Cold storage is the most commonly used method to keep them fresh. MAP and fungicides designated as cold supplements are also used to extend their commercial life (**Brandenburg and Zagory, 2009**).
The use of MAP combined with cold has proven its effectiveness in preserving certain vegetables and fruits (**Matar, 2021; Barrios *et al.*, 2014**). Applying optimal gas composition can help in reducing the product's respiration rate, prevent senescence and fermentation, and consequently extend its shelf life (**Oliveira *et al.*, 2015; Kahramanoğlu, 2019**).
Several studies have shown that exposing strawberries to a CO_2-enriched atmosphere can delay senescence and prevent fungal decay, leading to a longer shelf life (**Dong *et al.*, 2014; Hyang-Lan *et al.*, 2021**).
The preservation of strawberries by MAP has been previously investigated, and quality parameters have been assessed. However, to the best of our knowledge, the impact of MAP with a low level of O_2 and high levels of CO_2 combined with cold storage on antioxidant activity, phenolic content, and quality attributes of "Palmaritas" strawberries produced under the edaphic and climatic conditions of Morocco has not yet been investigated. This variety has seen widespread cultivation in Morocco, particularly in the northern regions, due to its ability to consistently deliver excellent flavor throughout the entire season. Moreover, it offers superior shelf life and is excellently suited for long distance shipping markets.
The main goal of this study was to determine the effect of MAP combined with cold storage on the quality parameters of strawberries, their antioxidant activity, and their chemical content, especially vitamin C, polyphenols, anthocyanins, and flavonoids.

MATERIAL AND METHODS

Origin of strawberries used in this study: Our research was conducted on cultivated strawberries (Fragaria x ananassa Duch.) of the "Palmaritas" variety obtained directly from the experimental fruit and vegetable growing research station at Larache, belonging to the National Institute of Agronomic Research of Tangier, located at (N 35°8'39,97608, W 6°9'4,10616). The strawberries, harvested at three quarters colored and full red colored, were transported to our laboratory in transparent polypropylene (PP) packages with a capacity of 500 g each, and kept under refrigeration at a temperature of 4°C. Only uniform and undamaged strawberries were selected, free from any signs of deterioration. They were washed with tap water and left to drain at ambient temperature for 10 min on absorbent material before use.

Experimental Design

Each package constituted by Polyethylene tray (11x12x5 cm), contained approximately 50 to 100 g of selected strawberries. This tray was covered by plastic bags for MAP. A wholly randomized factorial experimental design (5 x 4) with three repetitions was applied. Experimental treatments featured five distinct levels of gas composition (Tab 1) associated with four sample dates (0, 4, 8, 12 days).

Table 1 Gas composition for experimental treatments

Gaz composition	O_2 (%)	CO_2 (%)	N_2 (%)
Control	21.0	0.03	78.9

MAP1	2.5	5.00	92.5
MAP2	2.5	10.00	87.5
MAP3	2.5	15.00	82.5
MAP4	2.5	20.00	77.5

MAP was created using a gas mixer (KM100-200_3M, Germany) and a vacuum packaging machine (Electrolux EVP, type EVP45G, Italy). Following the injection of the modified atmosphere, the packaged fresh strawberries were kept at a temperature of 4 ± 0.5°C and a relative humidity of 85 ± 5% for 12 days.

Weight loss

To determine weight loss, the strawberries were weighed with an electric balance (Kern ABJ220-4NM, Germany) every four days. Three trays of fruit per treatment were weighed, and the same trays were consistently used throughout the experiment. In order to measure weight loss, the following equation was used:

$$\% \, Weight\ loss = [(W1 - W2)/W1]x100$$

where W1 = initial weight of the strawberries and W2= final weight after a specified duration.

Quantity estimation of rotted strawberries

During the storage period, decay was visually assessed by examining the fruit for any visible mold growth. Any strawberries with such symptoms were considered to have decayed, and the percentage of decayed strawberries was calculated.

Strawberry firmness

To assess the firmness of the strawberries, a TA1 texture analyzer (Ametek Lloyd Instruments Ltd, UK) was used, with a crosshead speed of 1mm/s and a penetration depth of 10mm. The resulting force-distance curve provided the textural parameter for firmness, which was expressed in N. Firmness was evaluated by measuring the maximal force necessary to penetrate the fruit sample; each result was the mean of three measurements.

Strawberry color

It was determined using a Chroma Meter CR-300 apparatus (Konica Minolta, Japan). The measurement included a* (redness), b* (yellowness), and L∗ (lightness). Hue angle (H) (editorial angle of color; range from 0º to 360º) and chroma value (C) (color saturation; range from 0 to 60) were obtained through calculations based on the equations:

$$C = (a^2 + b^2)\frac{1}{2}, \text{and } H = arctan\,(b*/a*)$$

Total soluble solids (TSS)

After analyzing firmness and color, strawberries were homogenized in a grinder (Moulinex Picadora, France). TSS percentage was assessed in the juice of ground strawberries using a Refractomer Digital C Brix 0-45% (Refractometer, France) at 20°C. To measure the Brix degree of the strawberry juice, the lens was covered with a drop of the juice, and the reading was recorded. Calibration was performed utilizing deionized water, and the lens was washed carefully between each sample reading and the next.

Total phenolic content (TPC)

It was assessed following the procedure described by **Siriwoharn *et al.* (2004)**. Standard curve preparation: The calibration curve (CC) was plotted by preparing cascade dilutions from a stock solution of gallic acid. To measure TPC, the sample (0.5 mL) with distilled water (7.5 mL) and Folin-Ciocalteu reagent (phenol Oxford, India) (0.5 mL) are mixed, while a blank was prepared with 0.5 mL of distilled water. The tubes were immersed in a water bath (Memert GFL, Germany) at 40°C for 20 minutes, then removed and refrigerated in an ice bath for 3 minutes. Subsequently, 3 mL of solution at 20% sodium carbonate was added to the tube and agitated in vortex. The tubes were placed in a water bath at 40°C for 20 minutes, then cooled in an ice bath for 3 minutes. Absorbance was determined using a UV spectrophotometer with a wavelength of 755 nm (Evolution Thermo Scientific, China). The TPC was determined by calculating the gallic acid equivalent (GAE) amount within 10-400 ppm concentration range using a gallic acid calibration curve with an R^2 value of 0.9986. The TPC was calculated using the following formula:

$$TPC\,[mg\ GAE\ /100g\ FW] = ((AEch - b) * V * F)/(a * Q))$$

where AEch = sample absorbance at 755 nm, b = y-intercept of the CC, a = the slope of the CC, V = extract volume, F = dilution factor, and Q = quantity of homogenized fresh strawberry in g.

Ascorbic acid (AA)

AA assessment was conducted according to **AOAC (2000)**. The extracts were first homogenized in 10 mL of an aqueous solution containing 1% metaphosphoric acid (Pan Reac Applichem, Spain). The homogenized extracts were then titrated with 2,6-dichlorophenol-indophenol (Flulka, India) solution (9.32×10^{-4} mol /L) until a stable pink color persisted for 30 seconds. Titration results were expressed in milligrams per 100 g of fresh weight (fw).

Total anthocyanin content (TAC)

TAC was assessed with the pH differential method per the AOAC procedure described in **Lee *et al.* (2005)**. Sample absorbance was recorded at 700 nm and 510 nm in two buffers with pH 4.5 (0.4 M sodium acetate) and pH 1.0 (0.025 M potassium chloride), utilizing a 201 UV-Vis Evolution spectrophotometer (Thermo Scientific, China). Pigment concentration was calculated and expressed as mg of pelargonidin-3-glucoside equivalents per g of fresh weight using the following equation:

$$\text{Total Anthocyanes [mg Pg-3-glu/g FW]} = \frac{(A * M * DF * V * 1000)}{(\varepsilon * d * Q)}$$

where A = (Ab510 nm − Ab700 nm) at pH 1.0; (Ab510 nm − Ab700 nm) at pH 4.5; MW (molecular weight) = 433.2 449.2 g/mol; DF = factor of dilution; 1 = cuvette pathlength in cm; d = width of the cuvette [cm]; V = extract volume; Q = quantity of extract [g]; ε = 31,600 L/cm/mol, molar extinction coefficient for pelargonidin-3-Glucoside. 10^3: factor to convert g to mg.

Total flavonoid content (TFC)

The measurement of TFC was conducted using the colorimetry assay developed by **Zhishen *et al.* (1999)**. One (1) mL of strawberry extracts were added with 7575 µL of 5% $NaNO_2$ solution (Fluka, Germany); after 5 minutes, 1 mL of 2% (w/v) $AlCl_3$ solution (PanReac Applichem, Spain) was added. Following a 6-minute incubation at ambient temperature, the mixture was supplemented with 0.5 mL of 1 mol/L NaOH (Sigma Aldrich, Sweden). A volume of 10 mL was achieved by adding distilled water, and the resulting absorbance was recorded at 410 nm against a white background. Total flavonoid content was calculated by reference to the CC of quercetin: the TFC in the samples was expressed in mg of quercetin equivalent per 100 g of fresh weight (mg QE/100 g fw). The measurements were carried out three times to ensure accuracy.

Antioxidant capacity

The technique described by **Brand-Williams *et al.* (1995)** was used to evaluate the samples' antioxidant capacity. This involved measuring their capacity to scavenge the free radical of 2,2-Diphenyl-1-Picrylhydrazil (DPPH) (Sigma-Aldrich, India). A 0.1 mM of DPPH and standard solutions of Trolox/MeOH (Sigma-Aldrich, France) at different concentrations (1-1.5-2-3 and 4 mM) were prepared. After preparing the solutions, 0.1 mL of each solution was mixed with 10 mL of the DPPH/MeOH solution. After a 30-minute incubation period, the absorbance was measured at 517 nm.
Measurements were performed three times, and the outcomes were evaluated from a standard Trolox curve (WWW Chemical, Belgium) within a concentration range of 0.1-0.5 mM. The samples' antioxidant capacity was reported as µmol Trolox equivalents per g of fresh weight (fw), using the following equation:

$$Antioxidant\ activity\ (\mu mol\ TE/g\ fw) = (As - b)\ x\ V/(\ a\ x\ Q)$$

where As = sample absorbance; a = slope of the CC; b = y-intercept of the CC; Q = amount of homogenized strawberry (g); V = volume of the extract [L].

HPLC determination

Extraction

Phenolic compounds were extracted following the protocol described in **Kajdžanoska *et al.* (2011)** with certain modifications. 5 g of the sample was added to 100 mL of methanol and stirred one hour at 40 °C. After centrifugation (Hettich Universal 320 R, Germany) at 4500rpm for 30 minutes at 4°C, the supernatant was recovered, and the insoluble plant material was subjected to two extractions with 10 mL of methanol. Methanol was then removed from the pooled extracts using a rotary-evaporator (BUCHI Rotavapor R200, Germany) at a temperature below 40°C for 45 minutes. The sample was then diluted with 10 parts methanol-water (80/20) and filtered through a 0.45 µm filter (Branchia, France) before injection. The extracts were stored at -50°C until injection into the HPLC system.
Standards and reagents
The polyphenol standards used in this study, namely p-Coumaric acid, fumaric acid, (-) epicatechin, quercetin, gallic acid, and caffeic acid, were obtained from Sigma-Aldrich (USA) and Merck (Germany). Acetonitrile (HPLC-grade) was obtained from Sigma-Aldrich (USA), while ultra-pure water was collected from a Milli-Q system (Millipore, Milford, MA, USA). Formic acid with a purity greater than 99% was supplied by WWR Chemical, England.

All polyphenolic standards in this study had a purity level greater than 95%. Stock solutions of polyphenols were prepared at a concentration of 1 g/L by dissolving the specific amount of each compound in methanol. These stock solutions were stored at 4°C until use.

Devices and operating conditions

Phenolic acid contents were identified and quantified using a high-performance liquid chromatography (HPLC) system from Shimadzu, Kyoto, Japan. The system consisted of an LC-20AD pump, a liquid chromatography SIL-20A auto-sampler, and an SPD-M20A diode array detector (DAD).
The analytes were separated using a Varian CP30713 Microsorb 100 C18 column (250 × 4.6 mm, 5 µm) at a temperature of 25 °C. The HPLC system operated in gradient mode at a flow rate of 0.8 mL/min, with a mixture of acetonitrile (for HPLC, for UV, ≥99.9% (GC)) (A) and formic acid/deionized water (5/95%) (B). The solution was additionally degassed by ultra-sonication before use. The gradient program used for the separation was: 0 min A: 5%, 15 min A: 15%, 6 min A: 33%, 8 min A: 50%, 5 min A: 5%. After each run, the column was equilibrated for an additional 3 minutes with A: 5%.
The analytical validation parameters were calculated using the data obtained from standard injections, as per the methods described by an **ICH Q2B Guideline (1997)**. The limit of detection (LOD) was determined by assessing the minimum level at which the external standard could be detected. This was achieved by employing the following formula:

$$LOD = 3.3\, Sb / a$$

where: a: slope of calibration line, Sb: standard deviation in intercept.
The limit of quantification (LOQ) was determined using the following formula:

$$LOQ = 10\, Sb / a$$

Statistical analysis

Statistical analysis was carried out by applying analysis of variance (ANOVA) with General Linear Model (GLM) procedure in SAS (Version 9.2000). The pairwise differences between least-square means were evaluated by Tukey's HSD test. Differences were considered significant when $p \leq 0.05$. This method was used to evaluate the effect of gas concentration doses and storage time on the parameters under investigation at a significance level of $p \leq 0.05$.

RESULTS AND DISCUSSION

Weight loss

Due to respiration, strawberries gradually lost weight during storage at 4°C. Table 2 presents the weight loss results of strawberries stored at 4°C under various MAP treatments: significant differences were observed. As the table shows, the primary effect of MAP treatments with lower O_2 and high CO_2 is the reduction of the extent of weight loss during storage.
The strawberries packaged with MAP treatments showed minimal weight loss during storage: approximately 0.9% to 0.18% on Day 12. In contrast, on the same day, we found the weight of control packaged strawberries greatly reduced by 7.45%. The weight loss of strawberries stored in MAP was found to be well under the acceptable marketability limit of 3% to 6% for soft fruits.
According to **Chitravathi *et al.* (2015)**, MAP treatments with a lower level of O_2 can result in a reduced respiration rate in fresh produce. **Barrios *et al.* (2014)** observed a comparable phenomenon, noting that reduced levels of O_2 led to a down-regulation of the respiration rate of strawberries. **Ma *et al.* (2017)** showed that slowing down the respiration rate can be advantageous in prolonging these fruits' post-harvest storage period.

Total soluble solids (TSS)

Dong *et al.* (2014) reported that saccharides constitute more than 80% of the TSS found in strawberry fruit, followed by acids, soluble pigments, pectin, and other compounds. This indicates that the saccharinity of the fruit is the primary determinant of its TSS, making the latter one of the most influential factors in assessing the fruit's overall value. During storage, TSS levels significantly decreased in all treated samples. Table 2 shows that on Day 4, the MAP-treated fruit had lower TSS levels than the control; however, during storage, no substantial changes were noted in TSS regarding the percentage of CO_2. The decrease in TSS content during storage may be due to the physiological processes of maturation, which use sugar as substrate in the respiration process. **Gil *et al.* (1997)** reported similar findings, noting a decrease in sugar content in both CO_2-treated and untreated samples.
TSS are closely associated with fruit weight loss. Due to their higher level of water loss, fruits may contain elevated levels of dry matter content. As a result, even though MAP may provide a more suitable environment for preventing dry matter loss, the percentage of dry matter in treated fruits may remain comparable to that of untreated samples due to the higher weight loss observed in the latter. According to **Petriccione *et al.* (2015)**, fruits packaged under MAP may exhibit higher TSS levels than unbagged fruit. Our findings align with a previous study by **Taleb (2017)**, who also observed a decrease in TSS levels of strawberries after cold storage, with or without MAP.

Rot rate

The strawberry fruit was susceptible to both mechanical damage and microbial infections, the latter including *Botrytis cinerea* and *Rhizopus nigricans*. The rot rate is considered the primary factor that limits the shelf life of strawberries (**Wszelaki and Mitcham, 2003**). In contrast, strawberries that were kept in modified atmosphere packaging (MAP) containing 10% CO_2 exhibited a decay rate of 14% after being stored for four days, and the rate maintained itself at 25% until the end of the experiment with CO_2 levels of 15%, 20%, and 5%, respectively (Tab 2).
Increasing CO_2 concentration to 15% and 20% slowed down rotting. At those concentration levels, the percentage of rotten fruit was low. The treatments with 5%, 15%, and 20% CO_2 were effective in controlling fruit rot. The high concentration of CO_2 in the MAP treatments was efficient in preventing the growth of aerobic microorganisms; the MAP group demonstrated better performance in this regard.
The deterioration of the control group could be due to the presence of the pathogenic fungus *Botrytis cinerea*, which causes gray mold; it can infect leaves and floral parts at the field stage, and can remain present during storage. The results obtained for fruits kept under modified atmosphere can be explained by referring to **Chambroy *et al.* (1993)**, who noted a significant reduction in the development of *B. cinerea* in strawberries when exposed to CO_2 levels ranging from 10% to 20%.
Our results are in accordance with those of **Hyang-Lan *et al.* (2021)**, who, in a series of *B. cinerea* inoculation experiments, demonstrated the reduction of decay in strawberry fruit by high CO_2 treatment. Their results indicated that a high CO_2 treatment had a reliable decay-suppressing effect.

Textural analysis

The firmness values of strawberries stored in MAP and under control conditions are depicted in Table 2. A significant variation between control and MAP was observed from the 4th day of storage. Along storage time, the decrease in firmness of the control samples and the MAP samples stored under 5% CO_2 was significant as compared to the samples stored under 10%, 15%, and 20% CO_2.
Strawberry firmness was associated with higher CO_2 levels. By the end, the firmness values of MAP strawberries for 10% to 20% CO_2 were 2.21 to 2.98 N, while for the control and 5% CO_2 samples, they corresponded to 1.41 to 1.51 N.
Fruit firmness is a crucial aspect in determining the quality of strawberries. Along the storage period, a pectin degradation process occurs within the middle cell layers, leading to gradual hydrolyzation into soluble pectin acids and eventually into saccharides. As observed, the firmness of strawberries decreased during storage, whether with or without MAP, as noted by **Dong *et al.* (2014)**. These changes in texture (gradual rot) are probably due to cell wall degradation.
Our findings align with those of **Anami *et al.* (2020)**, who reported that strawberry texture can be preserved by high partial pressures of CO_2 and low partial pressures of O_2 from the onset of storage, thereby maintaining their overall quality. Several studies have reported that strawberries kept in CO_2-enriched atmospheres exhibit firmer texture than those kept in air (**Pelayo *et al.*, 2003; Wszelaki and Mitcham, 2003**). According to **Harker et al. (2000)**, an increase of 60% in cell-to-cell adhesion was observed in CO_2-enriched treatments due to changes in the pH of the apoplast.
Plant cell walls are mainly composed of pectin, cellulose, and hemicellulose, which play an important role in determining fruit texture (**Broxterman and Schols, 2018**). In strawberries, ripening is accompanied by cell wall degradation, which results in the breakdown of hemicellulose and the increased solubilization of pectin (**Rosli *et al.*, 2004**).

Color

Fruit color is the most crucial factor for consumers; any alterations in external color are significant. The strawberries stored in MAP and the control samples showed no significant differences in color parameters, including lightness, chroma, and hue angle (Tab 2). Throughout the storage period, the control group exhibited a decrease in L* value on Day 12. The same tendency was observed for chroma and hue angle.
It can be concluded that MAP treatments did not have a significant impact on the color indices of strawberries. **Hyang *et al.* (2021)** obtained similar findings, as they reported that the application of a high-CO_2 treatment did not impact the skin color of strawberries.

Vitamin C content

The most important nutrient in strawberries is vitamin C; however, it is easily lost through oxidation. Conversely, as a reducing substance, vitamin C has the potential

Table 2 Mean (± standard deviation) of various parameters of strawberries during storage at 4 °C in air or CO_2-enriched air. These parameters include weight loss (WL), total soluble solids (TSS), rot rate (RR), firmness, lightness (L*), hue angle, chroma, vitamin C (VitC), total polyphenol content (TPC), total flavonoid content (TFC), total anthocyanin content (TAC) and antioxidant capacity (AC).

Days	Sample	**WL (%)**	**TSS %**	**RR (%)**	**Firmness (N)**	**L***	**h angle (deg)**	**Chroma**	**Vit C (mg / 100 g fw)**	**TPC (mg GAE/100g fw)**	**TFC (mg QE/100 g fw)**	**TAC (µg Pg-3-glu/g fw)**	**AC (µmol Trolox /g)**
0	**Control**	0±0^{Ab}	8.56±0.35^{Aa}	0±0^{Aa}	3±0.18^{Aa}	45.56±1.6^{Aa}	42.09±1.3^{Aa}	45.76±1.69^{Aa}	57.14±0^{Aa}	277±7.70^{Aa}	45.33±2.46^{Aa}	97.81±2.972^{Ac}	185.03±0.42^{Aa}
	Control	5.39±0.73^{Aa}	8.16±0.64^{Aab}	0.25±0.00^{Ab}	1.77±0.20^{Ab}	47.87±0.96^{ABa}	42.8±0.33^{Aa}	47.68±3.68^{Aa}	35.23±7.18^{Ab}	157.66±6.73^{Ab}	29.50±5.67^{Ab}	97.45±3.85^{BCd}	191.12±1.99^{Aa}
	CO_2: 5%	0.90±0.02^{Ba}	7.80±0.36^{Aa}	0.00±0.00^{Ba}	3.12±0.55^{Aa}	42.35±1.11^{Ba}	37.21±0.60^{Ba}	46.71±3.67^{Aa}	25.92±6.41^{Ab}	186.51±9.29^{Ab}	29.50±1.32^{Ab}	86.70±5,26^{BAb}	190.52±6.58^{Aa}
4	**CO_2:10%**	0.09±0.01^{Bab}	7.20±0.36^{Ab}	0.00±0.00^{Bb}	2.83±0.59^{Aa}	51.78±4.30^{Aa}	44.28±2.29^{Aa}	42.95±5.97^{Aa}	30.15±2.74^{Ab}	151.89±8.00^{Ab}	18.33±8.83^{Abc}	66.70±3,84^{Cd}	189.25±7.98^{Aa}
	CO_2:15%	0.11±0.10^{Ba}	7.33±0.51^{Ab}	0.00±0.00^{Ba}	2.73±0.70^{Aa}	45.76±2.15^{Aba}	41.63±1.05^{ABa}	47.04±2.56^{Aa}	38.09±16.49^{Aab}	174.08±39.15^{Ab}	31.33±0.28^{Aab}	74.00±6,92^{BAc}	191.36±2.71^{Aa}
	CO_2:20%	0.11±0.04^{Bab}	8.20±0.30^{Aab}	0.00±0.00^{Ba}	3.02±0.70^{Aa}	41.38±4.01^{Ba}	38.11±2.70^{ABa}	44.86±0.62^{Aa}	38.09±8.24^{Ab}	178.43±6.54^{Ab}	29.16±8.25^{Ab}	95.98±7.02^{Ac}	181.11±0.55^{Aa}
	Control	6.52±0.01^{Ac}	7.73±0.15^{Aab}	1.00±0.00^{Ac}	1.66±0.55^{ABb}	43.48±2.47^{Aa}	43.92±2.62^{Aa}	46.41±1.21^{Aa}	21.92±7.59^{Ab}	157.13±6.15^{Ab}	27.66±1.75^{Ab}	175.56±02.9^{Ba}	186.17±4.49^{Ba}
	CO_2: 5%	0.43±0.02^{Bab}	7.43±0.56^{Ab}	0.00±0.00^{Ba}	1.65±0.29^{Bb}	44.10±2.85^{Aa}	44.50±3.78^{Aa}	47.68±2.97^{Aa}	13.15±0.00^{Ac}	168.18±30.67^{Ab}	29.33±6.80^{Ab}	154.47±16.04^{CBa}	188.77±0.18^{Aba}
8	**CO_2:10%**	0.02±0.005^{Bab}	7.23±0.15^{Ab}	0.16±0.14^{Bb}	2.53±0.41^{Aba}	46.66±2.54^{Aa}	42.99±1.62^{Aa}	45.78±1.19^{Aa}	21.92±7.59^{Abc}	160.61±14.97^{Ab}	30.16±3.21^{Ab}	127.37±7.34^{Db}	186.96±3.49^{Ba}
	CO_2:15%	0.07±0.04^{Ba}	7.26±0.11^{Ab}	0.00±0.00^{Ba}	2.24±0.39^{Aba}	45.72±0.03^{Aa}	42.05±2.24^{Aa}	46.88±4.14^{Aa}	35.08±15.19^{Aab}	164.20±12.72^{Ab}	39.00±14.29^{Aa}	149.02±5.42^{CDb}	188.41±2.19^{Aba}
	CO_2:20%	0.11±0.07^{Bab}	7.43±0.25^{Ac}	0.00±0.00^{Ba}	2.70±0.21^{Aa}	47.06±3.36^{Aa}	42.14±4.17^{Aa}	45.07±1.95^{Aa}	35.08±7.59^{Ab}	150.23±18.34^{Abc}	23.33±3.61^{Ab}	202.67±9.06^{Aa}	194.38±0.54^{ABa}
	Control	7.45±0.28^{Ac}	7.50±0.26^{Ab}	1.00±0.00^{Ac}	1.51±0.06^{Ab}	37.95±2.53^{Ab}	36.80±3.23^{Ab}	38.35±3.50^{Ab}	22.82±7.9^{Ab}	121.00±8.27^{Ac}	9.50±2.00^{Ac}	143.78±4.75^{Ba}	102.51±24.94^{Ab}
	CO_2: 5%	0.07±0.05^{Bab}	7.50±0.20^{Ab}	0.25±0.25^{Ba}	1.41±0.43^{Ab}	43.23±3.70^{Aa}	42.16±2.81^{Aa}	46.47±1.24^{Aa}	12.82±0.00^{Ac}	144.00±10.17^{Ab}	15.83±8.80^{Ab}	162.6±5.42^{ABa}	78.60±34.83^{Ab}
12	**CO_2:10%**	0.13±0.09^{Ba}	7.50±0.40^{Ab}	0.58±0.14^{ABa}	2.46±0.94^{Aa}	47.44±3.21^{Aa}	40.30±2.30^{Aa}	41.46±6.52^{Aa}	177.72±9.60^{Aa}	145.74±16.02^{Ab}	11.16±2.75^{Ac}	177.72±9.60^{Aa}	131.36±36.73^{Ab}
	CO_2:15%	0.11±0.005^{Ba}	7.30±0.52^{Ab}	0.25±0.43^{Ba}	2.98±1.18^{Aa}	45.56±7.94^{Aa}	39.33±3.8Aa	42.13±2.72^{Aa}	192.00±22.30^{Aa}	154.07±11.69^{Ab}	17.00±0.50^{Ab}	192.00±22.30^{Aa}	93.33±17.24^{Ab}
	CO_2:20%	0.18±0.046^{Ba}	7.80±0.10^{Abc}	0.25±0.00^{Bb}	2.21±0.8^{Aa}	46.29±4.00^{Aa}	42.26±3.37^{Aa}	42.13±1.72^{Aa}	162.29±7.48^{ABb}	135.61±22.31^{Ac}	6.16±2.08^{Ac}	162.29±7.48^{ABb}	108.79±23.33^{Ab}

Data are expressed as mean ± SE of triplicate assays.
Lower case letters indicate significant differences ($p < 0.05$) among days within the same treatment. Capital letters indicate significant differences ($p < 0.05$) among treatments within the same day

to eliminate free radicals produced during the typical metabolic process of fruits and vegetables. It can protect cells from damage and decelerate the rate of fruit senescence. As a consequence, the assessment of strawberry quality often includes vitamin C content as a significant parameter (**Lu *et al.*, 2022**). In our study, vitamin C significantly decreased along storage in both the control and the MAP treatments. The maximum vitamin C content was recorded on day 0 (57.14 mg /100 g fw), and the minimum was recorded on Day 12 at 5% CO_2 (12 mg/100 g) (Tab 2). As storage time increased, the amount of ascorbic acid in the strawberries decreased, which could be attributed to oxidation and irreversible conversion of ascorbic acid to dehydro-ascorbic acid catalyzed by the enzyme ascorbinase (**Panda *et al.*, 2016**). Vitamin C concentration decreased notably in strawberries stored under normal atmospheric conditions and low CO_2 levels, as opposed to those stored in MAPs with higher levels of CO_2. Several studies have reported that fruit and vegetable vitamin C content can be preserved by applying storage in CO_2-enriched atmospheres (**Perez and Sanz, 2001**)

Our findings indicated that at the end of experimentation, the concentration of vitamin C was greater in strawberries stored in MAPs than in those stored under regular atmospheric conditions. Our investigation of vitamin C content proved that MAPs with high levels of CO_2 (15% and 20%) protected the vitamin C content better than normal atmospheric conditions.

Total phenolic contents (TPC)

Along the storage period, TPC decreased significantly in both control and MAP treatments. In the course of 12 days storage, the control group experienced a TPC reduction of the order of roughly 56%, while the 5%, 10%, 15%, and 20% CO groups experienced decreases of about 48%, 47%, 44%, and 54%, respectively (Tab 2). Our results are in agreement with the research conducted by **Taleb (2017)**. Throughout the experimental storage period, we observed significant TPC variations in all the MAP treatments and the control group. Our findings indicate that neither method (cold or modified atmosphere) was able to prevent a decrease in phenolic compounds, as evidenced by the reduction observed in the samples. This coincides with the conclusions drawn by **Klopotek *et al.* (2005).**

Total flavonoid contents (TFC)

The corresponding TFC values were in the range of 4.5 to 0.7 mg quercetin per 100g fw of strawberries and yielded significant differences ($p \leq 0.05$) throughout the entire storage time. TFC exhibited a general decrease during storage, with a reduction of approximately 65%, 75%, and 85% of control for 4 days, 8 days, and 12 days, respectively. Among all treatments, no significant differences were detected in TFC (Tab 2). After a 12-day storage period at 10°C, **Shin *et al.* (2008)** discovered a reduction in TFC levels in strawberries; they also noted a rise in water loss and a decline in anthocyanin content during the same period. **Nunes *et al.* (2005)** observed that the collapse of the cell membrane due to water loss facilitated the interaction between polyphenol oxidases and polyphenols/flavonoids. This interaction ultimately led to a reduction in TPC and TFC.

Total anthocyanin content (TAC)

Total anthocyanin content (TAC) in our experiment ranged between 74 and 202 µg g^{-1} fw. No significant differences in TAC were noted among the various treatments throughout the storage period. However, a slight decrease in TAC was observed after 4 days of storage in MAP, followed by an increase in TAC up to the end of experimentation (Tab 2).

Kanellis *et al.* (2009) observed that subjecting strawberries to high CO_2 treatments (ranging from 10% to 40%) resulted in a decrease in TAC. This reduction was related to a decrease in the activity of the uridine diphosphate (UDP) flavonoid glycosyltransferase (UFGT) enzyme and to a decline in anthocyanin stability attributed to pH alterations.

At the end of their experimentation, the MAP treatment showed a higher increase in total anthocyanin content compared to the control group (100% versus 80% increase) (Tab 2). These findings demonstrate that anthocyanin pigments continue to be synthesized during storage, even at cold storage temperatures: the highest increase in this pigment was observed in the MAP treatment. Moreover, these findings are consistent with the results reported by **Pelayo *et al.* (2003)**, who observed similar trends. **Li *et al.* (2019)** also reported a delay of four days in the synthesis of anthocyanins in strawberry fruit during 20% CO_2 treatment.

Altogether, however, the impact of enriched CO_2 on anthocyanin synthesis in strawberries has yielded contradictory findings. **Shin *et al.* (2008)** reported that strawberries stored in air had higher levels of anthocyanins than those stored in CO_2, while **Blanch *et al.* (2012)** noted that the accumulation of anthocyanins in strawberries treated with 20% CO_2 did not decrease. Additionally, **Gil *et al.* (1997)** observed that while CO_2 had negligible effects on the anthocyanin levels in the external tissues of strawberries, it had a significant impact on the anthocyanin content in the internal tissues.

Antioxidant capacity

The changes in the antioxidant activity of "Palmaritas" strawberries stored in MAP at varying levels of CO_2, as measured through the DPPH method, are presented in Table 2. Strawberries had an initial antioxidant activity of 185.03 ± 0.415 µmol Trolox g-1 of fw. In general, no significant variation was noted among treatments throughout the entire storage period. The antioxidant capacity of strawberries stored in various MAPs remained stable for up to 8 days before declining by approximately 44% at the end of the storage period.

Changes in antioxidant activity over time can be attributed to fluctuations in the levels of specific health-related compounds present in the strawberries. According to **Chu *et al.* (2000)**, the antioxidant capacity of fruits is affected by various compounds such as flavonoids, phenolic acids, amino acids, ascorbic acid, tocopherols, and pigments.

HPLC analysis

Many studies investigating the influence of MAP on the nutraceutical quality of foods have primarily focused on total phenols or flavonoids, thereby overlooking the significance of the specific chemical structure of phenolic compounds. Factors such as hydroxylation, glycosylation, and polymerization levels play an important role in determining the efficacy of these compounds (**Tsao, 2010**). The characteristics mentioned above not only affect the antioxidant and metal-chelating properties of the compounds but also exert a significant impact on their bioaccessibility and bioavailability (**Kumar and Pandey, 2013**).

Identification and quantification of individual polyphenols in strawberry

Multiple chemical classes of polyphenols have been discovered in strawberries, including flavonoids, phenolic acids, lignans, stilbenes, tannins, and coumarins (**Abountiolas, 2016**). In this investigation, various phenolic compounds were identified in the "Palmaritas" variety using external standard at different wavelengths (Table 3).

Detection and quantification limits

Table 3 shows the LOQ and LOD values (in µg/mL). The method's sensitivity is closely associated with the magnitudes of the two limits. The LOQ values obtained were lower than those of all the phenolic compounds analyzed in the strawberry samples, thereby demonstrating the method's reliability. The LODs for all compounds lay within the range of 0.7 to 9.0 µg/mL, which is equivalent to 1.4-18.7 µg/g in fresh strawberry samples. The LOQs ranged from 2.3 to 27.0 µg/mL, which is equivalent to 4.6-54.2 µg/g in fresh strawberry samples.

Calibration and analytical sensitivity

Table 3 presents the calibration sensibility values: the slope of the calibration line indicates the degree to which the response changed with a one-unit change in analyte concentration. The highest value was observed for p-Coumaric acid, followed by gallic acid.

High-performance liquid chromatograms of phenolic compounds in strawberries at different wavelengths are shown in Figure 1.

Table 3 Wavelength (λ), regression equation (Y= slop*concentration + B), retention time (RT), regression coefficient R^2, limit of detection (LOD), and limit of quantification (LOQ) during HPLC-UV analyses of polyphenol standards.

Phenolic compound	λ (nm)	slope	B	RT (min)	R^2	LOD (µg/mL)	LOQ (µg/mL)
Gallic acid	280	$2.67\ 10^7$	-52832	4.58±0,13	0.999	1.436	4.353
Caffeic acid	320	$1.43\ 10^8$	-264408	12.24±0,17	0.994	2.312	7.008
(-) Epicatechin	276	$2.61\ 10^7$	-160049	15.03±0,20	0,999	9.236	27.989
P-Coumaric acid	320	$1.73\ 10^8$	-570370	17.55±0,24	0.992	2.597	7.871
Quercitin	276	$6.21\ 10^7$	-107208	30.08±0,15	0.998	0.766	2.322

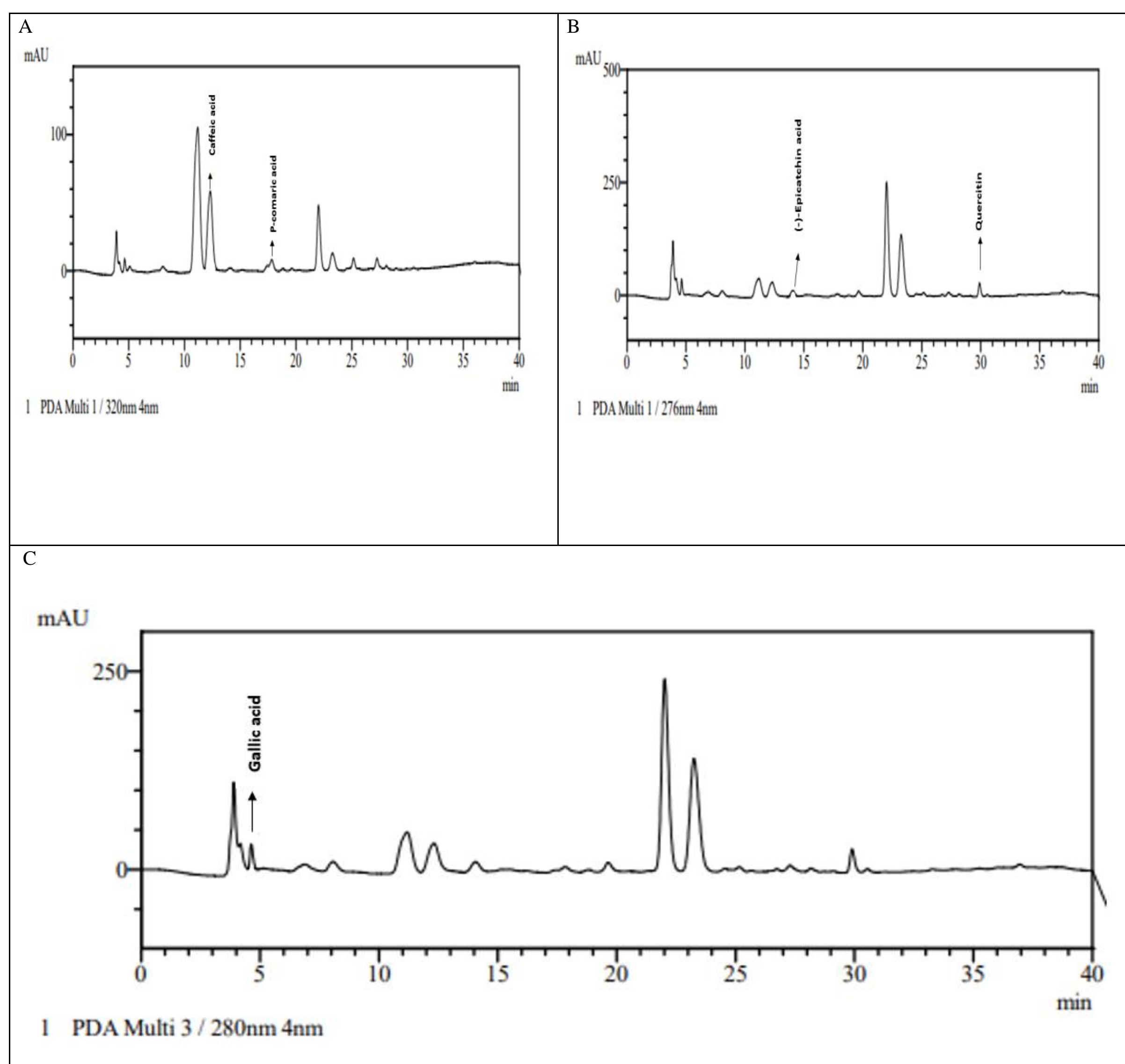

Figure 1 HPLC chromatogram of simultaneous separation of phenolic acids and flavonoids. (A) caffeic acid and p-coumaric acid. (B) (-) epicatechin and quercitin. (C) gallic acid.

Quantification of individual polyphenols in strawberry

Epicatechin was found to be the main phenolic compound (3.21 ± 0.34 mg/100 g) in strawberries, followed by gallic acid (2.54 ± 0.62 mg /100 g), caffeic acid (2.26 ± 0.42 mg/100 g), quercetin (1.82 ± 0.13 mg /100 g), and p-coumaric acid (0.78 ± 0.02 mg /100 g). Figure 2 shows the variation in the content of phenolic compounds in fresh strawberries during storage under different atmospheric conditions.

Quercetin

As shown in figure 2A, the control group had a concentration of 1.82 ± 0.13 mg/100 g fw. **Cordenunsi *et al.* (2005)** reported that the quantity of quercetin present in three different strawberry cultivars ranged from 3.9 to 6.8 mg/100 g fw. The amount of quercetin in strawberries during storage showed a slight decrease after 4 days, followed by a slight increase after 8 days, and then a decrease again after 12 days of storage. At the end of experimentation, a significant degradation of quercetin was observed in the control group, in contrast with the samples stored in MAP. **Gil *et al.* (1997)** noted an elevation in flavonol levels during refrigerated storage of strawberries.

Gallic acid

In the current study, the values for gallic acid content varied between 0.62 ± 0.005 and 3.74 ± 0.09 mg/100 g fw. The control showed a significant rise in gallic acid content for the duration of 4 and 8 days, followed by a decrease in 12 days at 4 °C (Fig. 2B). **Yamagishi (2006)** suggested that the synthesis of polyphenols, including gallic acid, in strawberries may be stimulated by cold storage conditions, which is consistent with our finding. The presence of gallic acid in strawberries can be beneficial, as it is a potent antioxidant that may have health benefits for consumers. Furthermore, the enrichment of gallic acid in strawberries can increase their commercial value as a functional food product, similarly to the enrichments frequently performed in lettuce, tomato, watermelon, and sweet potato (**Rivero *et al.*, 2001**). Our results suggest that the MAPs were not successful in preventing the degradation of gallic acid during storage.

p-Coumaric acid

The evolution of p-Coumaric acid content during the storage period was significant. The control samples showed a slight decrease in p-coumaric acid content throughout the storage period at 4 °C. In contrast, the samples stored in MAP exhibited a slight decrease in p-coumaric acid content at the onset, followed by an increase on the 8th day and a slight decline at the final storage time (Fig. 2C). Beyond four days of storage, MAP performed better at preventing the degradation of p-Coumaric acid as compared to the control samples. No similar results exist in the literature to compare to our finding.

(-) Epicatechin

The MAP and control treatments yielded differences in terms of (-) epicatechin content. As shown in figure 2D, MAP (5-20 % CO_2) preserved the (-) epicatechin content in strawberries. **Oliveira *et al.* (2015)** conducted a study to investigate the impact of modified atmosphere on the preservation of polyphenols in pasteurized strawberry purees during storage, and they likewise noted that MAP did not affect (-) epicatechin.

Caffeic acid

In our experiment, the concentration of caffeic acid fluctuated between 2 and 5 mg per 100 g of fresh weight. No significant differences were detected in the concentration of caffeic acid between the control and the MAP treatments during

storage. However, the strawberries stored in MAP with 15% and 20% CO_2 showed better preservation of high levels of caffeic acid content along 8 and 12 days of storage (Fig. 2E). Little information is available elsewhere on the evolution of this compound under modified atmosphere in strawberries.

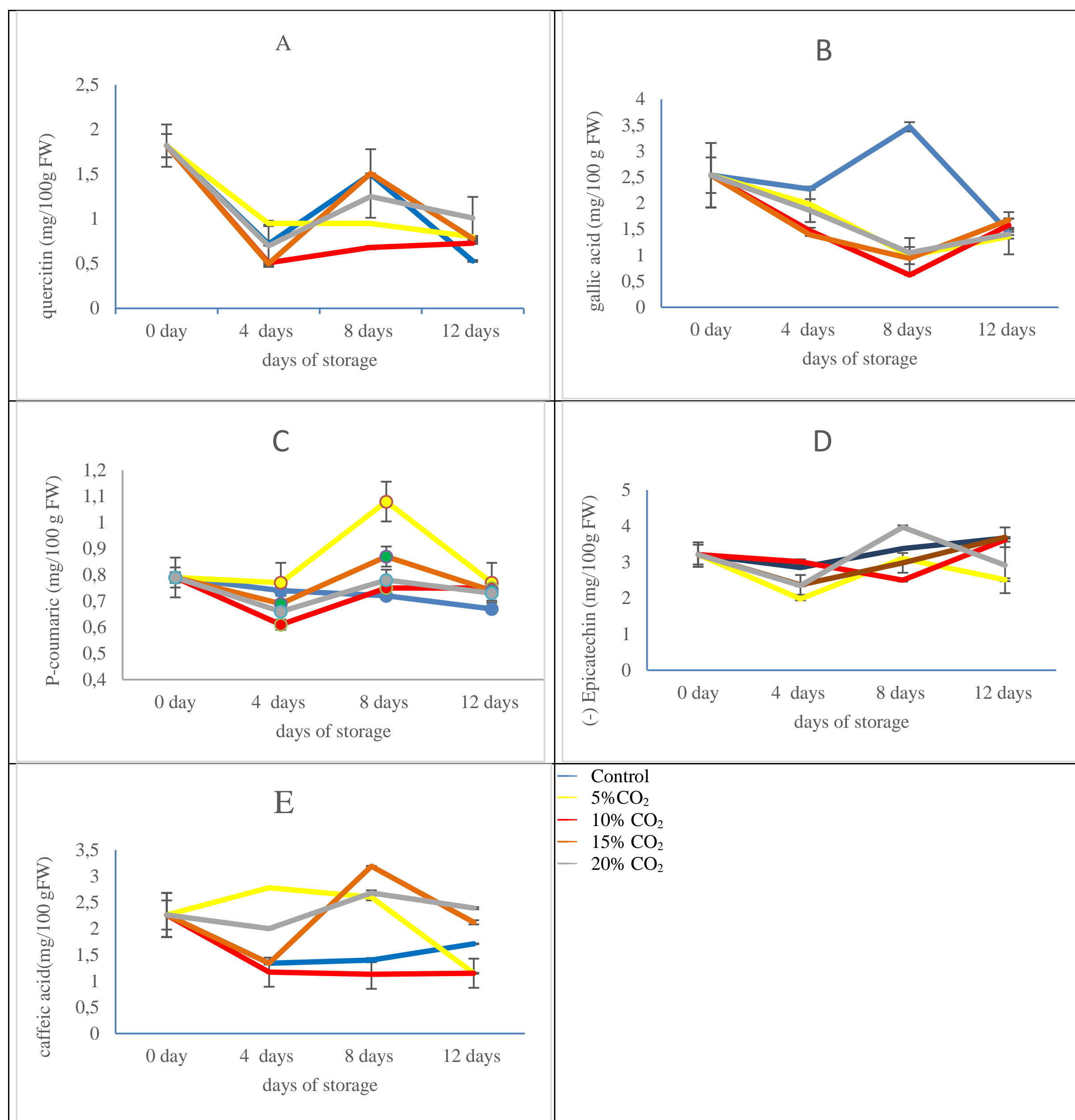

Figure 2 Effect of MAPs on polyphenol content of strawberries during storage at 4 °C. (A): quercetin, (B): gallic acid, (C): p-coumaric, (D): (-) epicatechin, (E): caffeic acid.

CONCLUSION

This study aimed to investigate the impact of MAP enriched with CO_2 and storage for 12 days at 4°C on the quality and chemical content of "Palmaritas" strawberries. Results indicated that MAP had a positive impact on the fruit, reducing weight loss, improving appearance, increasing anthocyanin content, and significantly reducing the rate of rotting and softening. MAP with a high level of CO_2 also helped to maintain vitamin C levels; TSS, color, antioxidant capacity, TPC, and TFC were not affected by the elevated level of CO_2 during storage. These results suggest that MAP in combination with refrigeration may represent a feasible approach to maintaining the quality of strawberry fruits. The optimal storage time for maintaining fruit quality was found to be 12 days at 4°C under MAPs with an initial gas mixture of 2.5% O_2 + 15% CO_2 + 82.5% N_2 or 2.5% O_2 + 20% CO_2 + 77.5% N_2. Moreover, when applying a combined technology, it is important to investigate sensory properties such as taste, odor, and texture of strawberries to ensure consumer acceptance.

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