ISSN 2709–3662 (Print)
ISSN 2709–3670 (Online)
https://doi.org/10.52587/JAF060108
Journal of Agriculture and Food
2025, Volume 6, No.1, pp. 103-117

Controlled atmosphere (CA) storage of apples

Irrum Babu, Sidra Rashid*, Hafiz Usama Noor, Rabiya Zulfiqar, Hammad Naeem, Muhammad Hamdan Rashid, Rabia Kanwal, Zarina Yasmin, Bareera Shafique, Muhammad Abrar

Abstract

Controlled Atmosphere (CA) storage is a widely used technique to extend the shelf life of agricultural and horticultural produce. The impact of low oxygen (O_2) levels (1%, 2%, and 3%) in combination with 1% carbon dioxide (CO_2) on the quality attributes of apples during 180 days of storage at 0°C ± 2°C and 85-95% relative humidity was studied. Results demonstrated that CA storage significantly reduced the percentage weight loss compared to the controlled treatment, indicating the effectiveness of low-oxygen conditions in minimizing physiological deterioration. While a general decrease in total phenols was observed across all treatments, CA conditions were more effective in preserving these compounds. Notably, low oxygen atmospheres significantly retarded the decline in antioxidant activity, suggesting that CA can help maintain the balance of reactive oxygen species and delay oxidative senescence. The results exhibit the benefits of CA storage in extending the shelf life and maintaining the nutritional value of apples. The optimal storage conditions identified in this research can be valuable for the postharvest industry to improve the quality and marketability of apples.

Keywords: Atmosphere, Post-harvest, Total antioxidant, Total Phenolic Contents
Article History: 29th January **Received:** 2025; **Revised:** 9th April, 2025; **Accepted:** 10th April, 2025

Post Harvest Research Centre, Ayub Agriculture Research Institute, Faisalabad, Pakistan;
Corresponding email: sidra87313@gmail.com

Introduction

Apple (*Pyrus malus*) is a fruit of temperate climates and belongs to the family Rosaceae. Apple is a climate-sensitive crop and the fourth most widely produced fruit. Apples are among the most widely used fruits, both for direct consumption and as a raw material for the preparation of various food products. Fresh apples are a rich source of bioactive components, including phenolic compounds, ascorbic acid, and particularly flavanols, such as proanthocyanidins and catechins. They also contain free amino acids and fatty acids, which play significant roles in human health and the preservation of fruits (Yeung et al., 2021). The apple fruit composition is 84.7% water, 0.4g proteins, 0.8 g fiber, 0.3 g ash, 13.9 g carbohydrates, 0.3 g lipid, and vitamin C 8 mg/100 g. Different minerals in apples include iron (480 µg/100g), potassium (145 mg/100g), calcium (7 mg /100g), sodium (0.3 mg/100g), Phosphorus (12 mg), Iodine (2 µg), and magnesium (6 mg/100g) (Francini et al., 2021).

Apple, being a good source of food and nutrition, is in high demand throughout the year and is therefore primarily stored in large quantities. The months of August and September are ideal for apple harvest, and the central portion of the produce is typically delivered to local stores or markets for sale and consumption. However, the price point is relatively low during these months (Zhang et al., 2020). Proper storage is necessary to obtain a reasonable price and to supply this fruit during the off-season. Apple, being a climacteric fruit, has its quality deteriorate quickly after harvest owing to the respiration process as well as ethylene production. Besides quantitative losses, the loss of quality and texture is also a serious issue in stored apples. According to Hussain et al. (2020) and Shah et al. (2002), about 17% of apples produced are lost during postharvest operations in Balochistan, while 28% losses in apple fruit with cold storage for about 22 weeks were reported by Ilyas et al. (2007). Postharvest damages may also be influenced by production area, market distance, and the type of transportation (Ilyas et al., 2007).

Apple is difficult to store for an extended period due to its vulnerability to texture deterioration and rapid ripening. Different methods have been employed to extend the storage life of apple fruit, utilizing various approaches at both the pre- and post-harvest phases. The current global drive for healthier food has led to an increase in demand for convenient and fresh food products with high nutritional value and free from additives. On the other hand, most hazardous to human health and have residual effects, besides being pricey. Consequently, all these aspects have led to research for harmless and more competitive alternatives (Gudkovsky et al., 2021).

CA storage is the most frequently active technique. Several studies have revealed the beneficial effects of Controlled Atmosphere (CA) and Modified Atmosphere Packaging (MAP) on the postharvest management of fruits and vegetables. CA is a sealed arrangement in which different kinds of gases are regulated from their standard atmospheric pressure. The amount of oxygen (O_2) is reduced, and the level of carbon dioxide (CO_2) is raised. As a result of the altered composition of air, various physiological processes, such as ethylene production, senescence, and respiration rate, are reduced while maintaining quality (Klein et al., 2020).

Apples, like many fruits, thrive in a carefully controlled environment during storage. Cold stores, often employing Controlled Atmosphere (CA) or Ultra Low Oxygen (ULO) techniques, are considered ideal. These methods enable extended storage, sometimes for up to a year, at temperatures between 0 °C and 5°C, depending on the apple variety, where high humidity (90-95%) is crucial to minimize moisture loss. CA/ULO storage involves reducing oxygen levels and increasing carbon dioxide, creating an atmosphere that slows down the natural ripening process. Apples, when properly chilled, produce minimal ethylene, a plant hormone that triggers and enhances ripening. However, even a small amount of ethylene can initiate ripening in nearby or closely stored apples. ULO storage significantly reduces ethylene production, making the apples less susceptible to the ripening hormone's effects. This careful control of the storage environment ensures that the apples maintain their quality and freshness for extended periods of storage.

In this study, we aim to create carbon dioxide (CO2)-enriched and oxygen-depleted environments (CA) for the production of marketable apples with high nutritional quality. This study specifically examined the effects of varying oxygen (O2) and carbon dioxide (CO2) levels on storage behavior and nutritional attributes. Period, weight loss, firmness, acidity, sugars, ascorbic acid, and color of stored apple at 0^0C and 85–90 % RH. Moreover, Figure 1 illustrates the overall process flow. Firstly, pre-storage preparations (mentioned in the Materials and Methods section) were performed, followed by CA storage, in which the optimal temperature, relative humidity (RH), and gas composition were adjusted (depending on the apple variety) to attain the maximum storage period required to maintain the quality parameters of the apple. During the last day of storage, post-evaluation was performed.

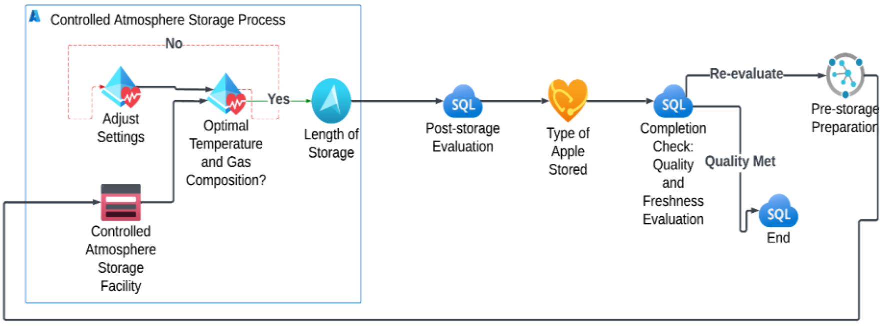

Figure 1. Scheme of CA Storage Study (SQL: Structured Query Language)

Materials and Methods

The study was conducted at the Post-Harvest Research Center (PHRC), AARI, Faisalabad. Apples (Variety: Red Delicious) were obtained from Murree Hill Fruit Research Station. Healthy, evenly sized, and lacking any visible external damage, the fruits were collected and moved to the PHRC laboratory, which features an air-blast cooling unit. Pre-cooled apples were washed with an antifungal solution of thiabendazole at 200 ppm and then immersed in a 3% Calcium Chloride (CaCl2) solution for 3 minutes, respectively (Jackson et al., 2003).

Fruit Storage

After the surface water had dried, the apples were divided into four groups. The controlled group was stored in a cold store at 0 ºC in a natural atmosphere. At the same time, the other three treatments were stored in CA chambers at a low temperature of 0 °C ± 2 °C and 85-95% relative humidity (RH), with oxygen (O2) levels of 1%, 2%, and 3%, and a constant carbon dioxide (CO2) concentration of 1% for all three treatments. At 15-day storage intervals, physical and chemical analyses of the fruits were conducted, and data on firmness percentage, weight loss, total soluble solids, color, acidity, pH, phenolic content, and reducing sugars were recorded.

Fruit Firmness, Total soluble solid (TSS), pH, Acidity

A penetrometer (PCE-FM 200) with an 8 mm plunger was used to measure flesh firmness on two opposite sides of each fruit, and the results were expressed in kg (Rab et al., 2012). Total soluble solids content (SSC) was determined using a digital refractometer (DR301-95 (A.KRUSS OPTRONIC, Germany) at room temperature. The extracted juice sample was thoroughly shaken using a shaker. The juice sample was placed (dropped) on a completely dry and clean prism of a refractometer, and the reading was recorded directly in °Brix.

pH of well-homogenized fruit juice was measured with the help of a digital pH meter (Model: IQ 150). The digital pH meter was first standardized with the buffer solutions of pH 4 and 7.

The acidity of apple juice samples was measured with the help of an acidity meter (model GMK-835N). A small amount of sample was placed on the sensor of the acidity meter. The acidity of the samples was measured in percentage according to the method outlined in AOAC (2006).

Weight Loss of Fruit

Fruit samples of known weight were weighed on a weighing scale (Sartorius AG, Germany, model: GM1501) after a 15-day storage interval. The loss in weight during the entire storage period was stated as the weight loss of each sample. The difference between the final and initial weights was attributed to the weight loss that occurred throughout the entire storage period (Gundewadi et al., 2018).

$$\text{Weight loss\% } (\%) = \text{period}/\text{Fresh} \times 100$$

Ascorbic acid, Phenolic Content, Total antioxidant

The amount of ascorbic acid in apple juice was determined by the titration method by using 2,6 2,6-dichlorophenol indophenol dye and quantified in mg of ascorbic acid per 100 g weight (AOAC, 2000).

$$\text{Ascorbic acid (mg/100 g of pulp)} = \frac{\text{Titer} \times \text{Volume make up} \times \text{Dye factor} \times 100}{\text{Aliquot} \times \text{sample weight}}$$

The Folin-Ciocalteu method was employed to determine the total phenolic content, expressed as milligrams of gallic acid equivalents (GAE) per 100 g of fresh weight (Singleton et al., 1999).

Total antioxidant activity was measured using the CUPRAC method in apples, following the method described by Apak et al. (2004), as 1 mol Trolox g^{-1} fresh weight.

Statistical analysis

The trial was conducted as a completely randomized design (CRD) with three replicates, and the averaged data were analyzed using CRD factorial ANOVA in the statistical software Statistix 8.1 (Heumann & Shalabh, 2016).

Results and Discussion

Weight loss

The weight loss after 180 days of storage was less than 7% for all CA-stored fruit compared to 9.6% in control fruit. The mean values of the treatments (as given in Table 1) indicated that the minimum weight loss was observed in T3 (1.02%) when stored at a low oxygen level (2%) and high CO2 (1%), while the maximum weight loss (4.63%) was in the controlled treatment stored at normal cold store conditions.

The storage of apples in low-oxygen and high-CO2 environments significantly influenced their weight loss and overall quality. Research indicated that controlled atmospheres can effectively reduce weight loss by slowing down respiration and ethylene production, which are critical factors in fruit ripening and decay. Therefore, during CA storage, weight loss was effectively reduced because balancing gas concentrations and temperature prevented physiological disorders (Watkins et al., 2012). As Park et al. (2018) indicated in their research, CA storage minimized apple weight loss by maintaining high humidity and controlling temperature, thereby reducing the vapor pressure deficit and enhancing storage conditions compared to traditional cold storage methods. While low oxygen levels induce anaerobic respiration, resulting in the accumulation of byproducts such as ethanol and lactate, which can lead to cellular damage and weight loss (Cukrov et al., 2016). In favor of this study, the results of Weber et al. (2013) indicate that weight loss is reduced when fruit is stored in a controlled atmosphere (CA). The study focused on 'Maxi Gala' apples, which were stored in a combination of 0.8 kPa O2 and 2.0 kPa CO2 at 1°C, resulting in a weight loss of only 3.5%. As well, storing 'Royal Gala' apples at 0.4 kPa O2 and 1.2 kPa CO2 resulted in higher flesh firmness and a lower percentage of weight loss compared to higher oxygen levels (Berghetti et al., 2021).

Table 1. The weight loss percentage of Apples stored under a controlled atmosphere

Treatment	Days to storage												
	0	**15**	**30**	**45**	**60**	**75**	**90**	**105**	**120**	**135**	**150**	**165**	**180**
T_1	0.00 ±0.00l	2.66 ±0.03S	6.28 ±0.06F	9.66 ±0.1A	1.76 ±0.02Y	3.38 ±0.03P	5.84 ±0.06G	0.20 ±0.00k	0.68 ±0.01f	2.16 ±0.02W	0.00 ±0.00l	0.88 ±0.01c	3.58 ±0.04O
T_2	0.62 ±0.01fg	3.56 ±0.04O	7.53 ±0.08D	0.00 ±0.0l	2.26 ±0.02V	3.98 ±0.04N	6.96 ±0.07E	0.26 ±0jk	0.76 ±0.01e	2.34 ±0.02U	0.28 ±0ij	1.86 ±0.02X	3.96 ±0.04N
T_3	0.78 ±0.01de	4.82 ±0.05J	8.33 ±0.08C	0.34 ±0i	2.44 ±0.02T	4.36 ±0.04M	0.00 ±0.0l	0.48 ±0h	1.13 ±0.01b	3.42 ±0.03P	0.42 ±0h	2.28 ±0.02UV	4.58 ±0.05L
T_4	1.42 ±0.01Z	5.73 ±0.06H	8.77 ±0.09B	0.84 ±0.01cd	2.94 ±0.03Q	4.68 ±0.05K	0.00 ±0.0l	0.56 ±0.01g	1.24 ±0.01a	0±0l	0.56 ±0.01g	2.84 ±0.03R	4.98 ±0.05I

Mean values are statistically insignificant, having similar alphabet LSD value, $P \geq 0.01$

Firmness

The firmness of apples varied due to the storage atmosphere, and after 180 days of storage, a significant change in fruit firmness was observed, with averages of 4.26, 4.43, 5.36, and 4.86 from T1 to T4, respectively. CA storage maintained the firmness of all three treatments; however, the best results were observed in T3, which had a 1:2 ratio of CO2 and O2. (Figure 2).

Firmness is a key determinant in consumer selection, with firmer apples often being favored for their perceived freshness (Su et al., 2024). Apples with higher firmness tend to have a longer shelf life, reducing waste and increasing profitability for producers (Hu et al., 2020). The firmness loss of apples during storage is a multifaceted issue influenced by various factors, including temperature, water loss, and the composition of their cell walls. Water loss significantly contributed to the decline in firmness, resulting in undesirable physical changes, such as skin shriveling (Hasan et al., 2024). The composition of cell wall sugars also affects texture, with specific cultivars exhibiting rapid texture loss due to changes in galactose and galacturonic acid levels (Liu et al., 2023). The study by Stevanović (2022) depicted that CA storage significantly reduced the metabolic activities of apples, resulting in lower respiration and transpiration rates. This resulted in less water loss and better firmness, maintaining a higher quality compared to apples stored in regular air conditions. In addition, another study (Babu et al., 2022) found that lowering the oxygen level during CA storage from 20.8% to 1-2% decreased the respiration rate, which ultimately helped reduce firmness loss.

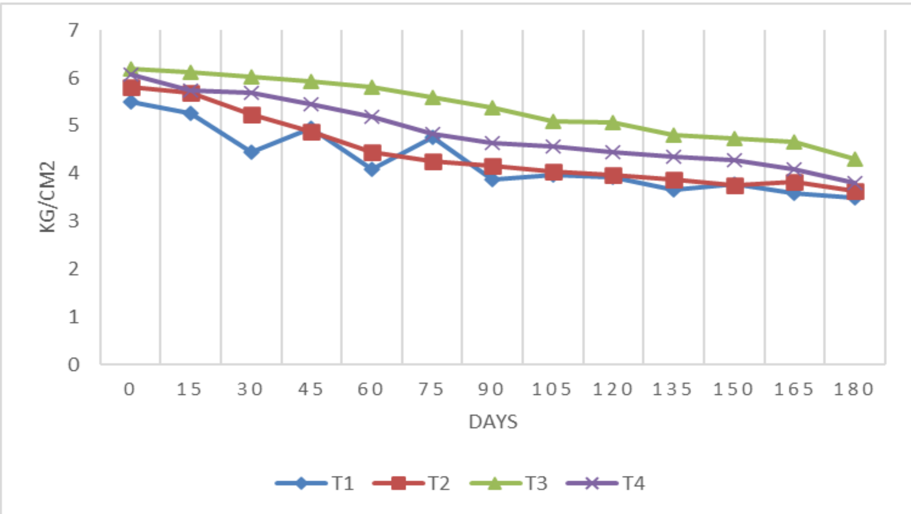

Figure 2. Firmness of apples throughout the storage period

Total Soluble Solids (TSS)

During the 180-day storage period, the total soluble solids (TSS) of all four treatments increased; however, a significant difference ($p < 0.05$) was observed between the treatments. CA storage resulted in a lesser change in TSS compared to standard cold storage, as shown in Table 2. The observed mean value of the control treatment was 16.29, whereas for T3 (O_2 2% and CO_2 1%), the average value was 13.27, which was lower than that of other CA-treated samples.

The increase in TSS during apple storage can be attributed to several biochemical and physical changes that occur over time. As apples ripen, starches are converted to sugars, resulting in higher total soluble solids (TSS) levels (Kassebi et al., 2022). During CA storage, the rate of metabolic activity was slowed due to the gaseous composition, as compared to cold storage.

In support of this statement, the results of Majidi et al. (2014) research are presented. As Majidi et al. (2014) stated, the utilization of a gas mixture consisting of 5 kPa O2 and 3 kPa CO2 significantly retarded the ripening process compared to air in cold storage. Similarly, Stevanović's (2022) study found that apples stored in controlled atmosphere (CA) exhibited significantly lower changes in total soluble solids (TSS) compared to those stored in regular air, indicating better preservation of TSS during the storage period. The research by Butkeviciute et al. (2022) found that prolonged storage under controlled atmosphere (CA) conditions resulted in a more stable composition of phenolic compounds and antioxidant activity, which in turn affected total soluble solids (TSS).

Table 2. Total soluble salts (TSS) of Apples stored under a controlled atmosphere

Treatment	Days to storage												
	0	**15**	**30**	**45**	**60**	**75**	**90**	**105**	**120**	**135**	**150**	**165**	**180**
T_1	12.68±0.13Zab	15.2±0.15LM	17.4±0.17F	19.74±0.2A	14.3±0.14QR	16.4±0.16J	17.78±0.18E	12.54±0.13bc	13.24±0.13WX	14.02±0.14ST	12.64±0.13abc	13.82±0.14TU	14.7±0.15OP
T_2	13.2±0.13WX	15.88±0.16K	18.24±0.18D	12.68±0.13Zab	14.9±0.15NO	16.68±0.17HI	18.32±0.18D	12.62±0.13bc	13.34±0.13WX	14.14±0.14RS	12.86±0.13Za	14.18±0.14RS	15.06±0.15MN
T_3	13.8±0.14TU	16.5±0.16IJ	18.68±0.19C	13.12±0.13XY	15.16±0.15LM	17.1±0.17G	12.44±0.12c	12.88±0.13YZ	13.62±0.14UV	14.24±0.14RS	13.42±0.13VW	14.3±0.14QR	15.24±0.15LM
T_4	14.54±0.15PQ	16.8±0.17H	19.1±0.19B	13.8±0.14TU	15.38±0.15L	17.36±0.17F	12.48±0.12bc	13.12±0.13XY	13.86±0.14TU	12.54±0.13bc	13.68±0.14UV	14.56±0.15P	15.34±0.15L

Acidity

The mean values for acidity of T1, T2, T3, and T4 during the storage period were observed as 0.63, 0.82, 1.42, and 1.34, respectively. The results indicated that T3 performed well. At the same time, the higher acidity value decreased in the control compared to the CA stored samples (as shown in Table 3).

The decrease in acidity level during cold storage can be attributed to several biochemical processes that occur under low-temperature conditions. Cold storage affects the metabolism of organic acids, leading to their degradation and a subsequent decline in fruit acidity. The study by Shu et al. (2024) demonstrated that lower temperatures reduced respiration rates; however, prolonged storage still resulted in significant loss of organic acids in apples. At the same time, CA helped maintain the acidity content because modification of the atmosphere around the fruit reduced acid degradation. In the same context, Stevanović's (2022) research analyzed various quality parameters, including acidity, and found that apples stored in a controlled atmosphere exhibited significantly lower changes in quality compared to those stored in regular air, indicating better preservation of fruit acidity during storage.

Table 3. Acidity of Apples stored under a controlled atmosphere

Treatment	Days to storage												
	0	**15**	**30**	**45**	**60**	**75**	**90**	**105**	**120**	**135**	**150**	**165**	**180**
T_1	1.08 ±0.01R	0.68 ±0.01a	0.55 ±0.01d	0.47 ±0.00e	1.11 ±0.01Q	0.74 ±0.01X	0.43 ±0f	1.51 ±0.02B	1.42 ±0.01F	1.33 ±0.01J	1.47 ±0.01D	1.35 ±0.01I	1.27 ±0.01LM
T_2	0.94 ±0.01S	0.62 ±0.01b	0.4 ±0.00g	1.3± 0.01K	0.91 ±0.01T	0.7± 0.01Z	0.37 ±0.00h	1.49 ±0.01C	1.4± 0.01G	1.3± 0.01K	1.44 ±0.01E	1.34 ±0.01IJ	1.22 ±0.01N
T_3	0.89 ±0.01U	0.70 ±0.01Z	0.42 ±0.00f	1.26 ±0.01M	0.87 ±0.01V	0.57 ±0.01c	1.55 ±0.02A	1.47 ±0.01D	1.37 ±0.01H	1.28 ±0.01L	1.42 ±0.01F	1.3± 0.01K	1.21 ±0.01N
T_4	0.72 ±0.01Y	0.61 ±0.01b	0.15 ±0.00i	1.15 ±0.01P	0.76 ±0.01W	0.48 ±0.00e	1.52 ±0.02B	1.44 ±0.01E	1.35 ±0.01I	1.51 ±0.02B	1.4± 0.01G	1.3± 0.01K	1.18 ±0.01O

Vitamin C

The mean values of Vitamin C for T_1, T_2, T_3, and T4 on the first day of research were 23.7, 21.9, 25.6, and 22.3, respectively. On the last day of the experiment, the vitamin C mean values were 7.9, 10.8, 18.6, and 12.2, respectively. These values (as shown in Table 4) indicate a significant difference ($p < 0.05$) between treatments and storage days. There was a lesser decrease in Vitamin C in samples stored in CA compared to the control. The decrease in values during storage is due to prolonged storage, as vitamin C is susceptible to degradation under these conditions. Research by Budiarto et al. (2024) indicated that as storage time increased, vitamin C levels consistently declined. Similar to cold storage, the vitamin C level decreased more in CA than in cold storage. In support of this, a study by Randhawa et al. (2020) found that vitamin C levels decreased during cold storage due to its instability, which was exacerbated by time and temperature. Higher temperatures accelerated degradation, resulting in significant losses, as observed in orange juice stored at varying temperatures over 40 days. Moreover, the study by Hussain et al. (2017) indicated that lower temperatures in CA storage reduced the respiratory rate and slowed oxidation processes, leading to less conversion of ascorbic acid to dehydroascorbic acid and, consequently, higher retention of vitamin C compared to cold storage.

Table 4. Vitamin C content of Apples stored under a controlled atmosphere

Treatment	Storage Days												
	0	**15**	**30**	**45**	**60**	**75**	**90**	**105**	**120**	**135**	**150**	**165**	**180**
T_1	23.7±0.24E	19.86±0.2Q	15.26±0.15Z	7.9±0.08g	20.6±0.21NO	18.24±0.18U	13.64±0.14b	25.12±0.25BC	23.7±0.24E	21.02±0.21LM	22.86±0.23G	19.4±0.19RS	16.8±0.17W
T_2	21.88±	18.54±0.	13.5±0	21.89±0.22I	19.92±0.2Q	17.36±0.17V	10.8±0.11e	24.86±0.25C	23.38±0	20.3±0.2OP	21.56±0.	19.62±0.	16.7±0.1

	0.22I	19TU	.14b						.23F		22JK	2QR	7WX
T_3	20.28±0.2P	17.64±0.18V	11.24±0.11d	21.12±0.21L	19.22±0.19S	16.44±0.16XY	25.64±0.26A	24.44±0.24D	22.76±0.23G	18.6±0.19T	21.44±0.21K	18.72±0.19T	14.24±0.14a
T_4	19.24±0.19S	16.2±0.16Y	10.3±0.1f	20.78±0.21MN	18.7±0.19T	15.2±0.15Z	25.26±0.25B	24.2±0.24D	21.8±0.22IJ	22.34±0.22H	20.72±0.21MN	17.54±0.18V	12.22±0.12c

Total phenols

The result of this research depicted that the total phenolic content varied among all treatments in the following order: $T_3 > T_4 > T_2 > T_1$. An overall decline in total phenols was observed in all treatments; however, the drop under CA was less pronounced compared to storage in normal air. However, the decline was most rapid and significant in the control treatment, whereas the slightest difference was recorded for T3 throughout the storage days (as shown in Table 5).

The study by Dong et al. (2022) highlights the continuous decline of total phenolic content (TPC) in yellow peaches during shelf life, primarily due to polyphenol oxidation facilitated by polyphenol oxidase. CA storage has been shown to significantly enhance TPC retention, thereby mitigating oxidative damage from free radicals. Moreover, TPC decreases progressively through the ripening stages, with significant reductions noted from the unripe to the semi-ripe stages (Li et al., 2023). In favor of the CA storage study by Gracia et al. (2019), with controlled atmosphere (CA) storage conditions (e.g., 2% O2-10% CO2) effectively delaying ripening and maintaining fruit quality, including total phenolic content (TPC) levels.

Table 5. Total phenolic content of Apples stored under a controlled atmosphere

Treatment	Storage Days												
	0	**15**	**30**	**45**	**60**	**75**	**90**	**105**	**120**	**135**	**150**	**165**	**180**
T_1	203.5±2.04A	180.32±1.8DE	139.24±1.39F	77.32±0.77GHI	197.64±1.98JKL	182.44±1.82L	151.23±1.51M	214.26±2.14NO	200.08±2PQR	170.2±1.7S	215.5±2.16V	204.2±2.04X	181.2±1.81b
T_2	201.2±2.01AB	173.5±1.74DEF	118.2±1.18G	204.2±2.04HIJ	192.34±1.92JKL	177.52±1.78M	128.5±1.29M	211.8±2.12NO	198.1±1.98QR	173.42±1.73ST	212.68±2.13V	199.24±1.99Y	178.74±1.79c
T_3	192.62±1.93BC	162.34±1.62EF	103.2±1.03GH	202.24±2.02HIJ	190.36±1.9KL	168.2±1.68M	220.8±2.21N	209.78±2.1OP	192.06±1.92R	161.24±1.61TU	209.72±2.1VW	191.02±1.91Z	171.28±1.71d
T_4	184.64±1.85CD	154.6±1.55F	94.35±0.94GH	200.14±2IJR	186±1.86L	161.44±1.61M	218.76±2.19N	202.22±2.02PQ	184.55±1.85S	217.3±2.17U	205.63±2.06W	187.34±1.87a	158.32±1.58e

Total Antioxidant

A substantial decrease in antioxidant activity was observed in the control treatment stored at a low temperature. However, the apples kept in a low-oxygen atmosphere showed a significantly retarded decline in antioxidant activity (Figure 3). After 180 days of storage, the maximum retention of antioxidants was detected in T3 stored in a 3% oxygen environment after dipping in a 2% $CaCl_2$ solution.

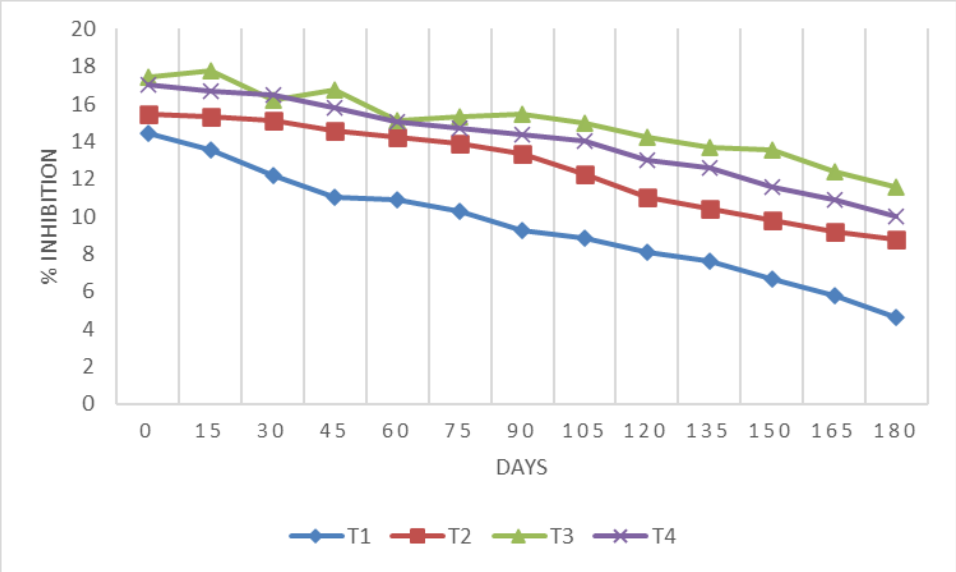

Figure 3. Antioxidant capacity is exhibited as a percentage inhibition throughout the storage period

The enzymatic antioxidant system controls the oxidative senescence in fruit. In this study, CA was capable of preserving the antioxidant activity in apples for an extended period, effectively maintaining the dynamic balance of reactive oxygen species (Luna et al., 2016). Saba and Sogvar (2016) also found that treatment with edible coatings was effective in resisting antioxidant reduction during the cold storage of apples. Consequently, CA can improve the internal defense ability and antioxidant level of apple tissue (Zhao et al., 2019). Furthermore, the accumulation of phenolic compounds is also associated with antioxidant capacity, which can estimate the maturity of fresh fruits and vegetables and help determine their antioxidant capacity (Guo et al., 2021; Ma et al., 2019). These results demonstrate that CA significantly delayed the loss of antioxidants, thereby decreasing the loss of the fruit to free radicals. These findings are in agreement with earlier studies on strawberries (Wang et al., 2003) and apples (Brackmann, et al., 1998). In this way, the shelf life of apples can be enhanced by reducing the incidence of decay due to the effect of controlled atmosphere, as CA influences the strengthening of cell walls and increases the ability of antioxidant enzymes (Buccheri et al., 2021).

Conclusion

By carefully controlling the conditions within the storage chambers, a significant extension in the shelf life of apples was achieved. Lowering oxygen levels while maintaining a 1% carbon dioxide level proved effective in minimizing weight loss and other quality parameters. This approach not only maintained the apples' good quality for longer but also helped retain vital nutrients, such as organic acids and phenols. A natural decline in antioxidants was observed; however, CA storage slowed this process, which is crucial for maintaining apples' appeal to consumers and reducing food waste. By utilizing the CA storage facility, local farmers and suppliers can expand their market reach and extend the distribution period, supporting the sustainability of the food supply chain by reducing the use of chemical preservation and optimizing the use of better storage techniques. It is essential to note that ideal CA conditions can vary depending on the apple variety and harvest time. As such, further research is essential to explore these techniques for optimal results. It can be suggested that CA storage is a novel technique for preserving the quality of apples. By understanding and optimizing these conditions, we can deliver fresher, more nutritious fruit to consumers while reducing our environmental impact.

Competing Interests

The authors declare that the research was conducted without any commercial or financial relationships that could be perceived as a potential conflict of interest.

Acknowledgement: Not applicable.

Funding: Not applicable.

Ethical statement: This article does not contain any studies involving human or Animal Subjects.

Code availability: Not applicable.

Consent to participate: All authors participated in this research study.

Consent for publication: All authors submitted consent to publish this research.

Data availability statement: The data presented in this study are available on request.

References

AOAC. (2000). Official Methods of Analysis. International 17th Edition, The Association of Official Analytical Chemists, Gaithersburg, MD, USA

AOAC. (2006). Official methods of analysis of AOAC International (18th ed.). *Arlington, VA. The Association of Official Analytical Chemists.*

Apak, R., Güçlü, K., Özyürek, M., & Karademir, S. E. (2004). Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. *Journal of Agricultural and Food Chemistry*, 52(26), 7970–7981. https://doi.org/10.1021/jf048741x

Babu, D. R., & Rao, K. N. (2022). Mathematical modeling of oxygen levels in a controlled atmosphere store for preservation of apple fruits. *Materials Today: Proceedings*, 68, 1472–1475. https://doi.org/10.1016/j.matpr.2022.07.095

Berghetti, M. R. P., Brackmann, A., Thewes, F. R., Schultz, E. E., Ludwig, V., Wendt, L. M., ... & Schmidt, S. F. P. (2020). Extremely low oxygen partial pressures for the storage of 'Royal Gala'apple. *Bragantia*, 79, 145–154. https://doi.org/10.1590/1678-4499.20190016

Brackmann, A., & Bortoluzzi, G. (1998). Influence of temperature, ca conditions and relative humidity on physiological disorders of 'fuji' apples. *Acta Horticulturae*, *464*, 369–374. https://doi.org/10.17660/actahortic.1998.464.56

Buccheri, M., Picchi, V., Grassi, M., Gandin, D., Bianchi, G., & Scalzo, R. L. (2021). Dynamic changes of antioxidants and fermentative metabolites in apple peel in relation to storage, controlled atmosphere, and initial low oxygen stress. *Scientia Horticulturae*, 288, 110312. https://doi.org/10.1016/j.scienta.2021.110312

Budiarto, R., Mubarok, S., Sholikin, M. M., Sari, D. N., Khalisha, A., Sari, S. L., & Adli, D. N. (2024). Vitamin C variation in citrus in response to genotypes, storage temperatures, and storage times: A systematic review and meta-analysis. *Heliyon*, 10(8), e29125. https://doi.org/10.1016/j.heliyon.2024.e29125

Butkeviciute, A., Viskelis, J., Liaudanskas, M., Viskelis, P., & Janulis, V. (2022). Impact of storage controlled atmosphere on the apple phenolic acids, flavonoids, and anthocyanins and antioxidant activity in vitro. *Plants*, 11(2), 201. https://doi.org/10.3390/plants11020201

Cukrov, D., Zermiani, M., Brizzolara, S., Cestaro, A., Licausi, F., Luchinat, C., ... & Tonutti, P. (2016). Extreme hypoxic conditions induce selective molecular responses and metabolic reset in detached apple fruit. *Frontiers in Plant Science*, 7, 146. https://doi.org/10.3389/fpls.2016.00146

Dong, X., He, Y., Yuan, C., Cheng, X., Li, G., Shan, Y., & Zhu, X. (2022). Controlled atmosphere improves the quality, antioxidant activity and phenolic content of yellow peach during the shelf life. *Antioxidants*, 11(11), 2278. https://doi.org/10.3390/antiox11112278

Francini, A., Fidalgo-Illesca, C., Raffaelli, A., & Sebastiani, L. (2021). Phenolics and mineral elements composition in underutilized apple varieties. *Horticulturae 2022*, 8(1), 40. https://doi.org/10.3390/horticulturae8010040

Gracia, A. P., Gimeno, D., Lasierra, B., Oria, R., & Venturini, M. E. (2017). Controlled atmosphere pallets to extend the shelf-life of Calanda peaches. In *VI International Conference Postharvest Unlimited 1256*, 623–630. https://doi.org/10.17660/ACTAHORTIC.2019.1256.89

Gudkovsky, V., Kozhina, L., Nazarov, Y., & Sutormina, A. (2021). Sustainability of apple fruits cv. Berkutovskoye to physiological diseases under different storage technologies. *In E3S Web of Conferences*, 254, 02025. EDP Sciences. https://doi.org/10.1051/e3sconf/202125402025

Gundewadi, G., Rudra, S. G., Sarkar, D. J., & Singh, D. (2018). Nanoemulsion based alginate organic coating for shelf life extension of okra. *Food Packaging and Shelf Life*, 18, 1–12. https://doi.org/10.1016/j.fpsl.2018.08.002

Guo, C., Bi, J., Li, X., Lyu, J., Xu, Y., & Hu, J. (2021). Investigation on the phenolic composition, related oxidation and antioxidant activity of thinned peach dried by different methods. *LWT*, 147, 111573. https://doi.org/10.1016/j.lwt.2021.111573

Hasan, M. U., Singh, Z., Shah, H. M. S., Kaur, J., & Woodward, A. (2024). Water Loss: A Postharvest Quality Marker in Apple Storage. *Food and Bioprocess Technology*, 1–26. https://doi.org/10.1007/s11947-023-03305-9

Heumann, C., & Shalabh, M. S. (2016). Introduction to statistics and data analysis. *Springer*. https://link.springer.com/book/10.1007/978-3-319-46162-5

Hu, Y., Han, Z., Sun, Y., Wang, S., Wang, T., Wang, Y., ... & Wu, T. (2020). ERF4 affects fruit firmness through TPL4 by reducing ethylene production. *The Plant Journal*, 103(3), 937-950. https://doi.org/10.1111/tpj.14884

Hussain, I., Rab, A., Khan, S. M., Naveed, K., Ali, S., Hussain, I., & Sajid, M. (2017). Response of ascorbic acid content and acidity to different storage temperatures and durations in oranges. *Pure and Applied Biology (PAB)*, 6(1), 304–309. https://dx.doi.org/10.19045/bspab.2017.60028

Hussain, S., Asi, M. R., Iqbal, M., Akhtar, M., Imran, M., & Ariño, A. (2020). Surveillance of patulin in apple, grapes, juices and value-added products for sale in Pakistan. *Foods*, 9(12), 1744. https://doi.org/10.3390/foods9121744

Ilyas, M. B., Ghazanfar, M. U., Khan, M. A., Khan, C. A., & Bhatti, M. A. R. (2007). Post harvest losses in apple and banana during transport and storage. *Pakistan Journal of Agricultural Sciences*, 44(3), 534-539.

Jackson, L. S., Beacham-Bowden, T., Keller, S. E., Adhikari, C., Taylor, K. T., Chirtel, S. J., & Merker, R. I. (2003). Apple quality, storage, and washing treatments affect patulin levels in apple cider. *Journal of food protection*, 66(4), 618–624. https://doi.org/10.4315/0362-028X-66.4.618

Kassebi, S., Farkas, C., Székely, L., Géczy, A., & Korzenszky, P. (2022). Late shelf life saturation of golden delicious apple parameters: TSS, weight, and colorimetry. *Applied Sciences*, 13(1), 159. https://doi.org/10.3390/app13010159

Klein, B., Falk, R. B., Thewes, F. R., de Oliveira Anese, R., dos Santos, I. D., Ribeiro, S. R., ... & Wagner, R. (2020). Dynamic controlled atmosphere: Effects on the chemical composition of cuticular wax of 'Cripps Pink'apples after long-term storage. *Postharvest Biology and Technology*, 164, 111170. https://doi.org/10.1016/j.postharvbio.2020.111170

Li, Y., Li, L., Zhang, X., Tian, J., Yan, J., Guo, L., ... & Yu, X. (2023). Differences in total phenolics, antioxidant activity and metabolic characteristics in peach fruits at different stages of ripening. *LWT*, 178, 114586. https://doi.org/10.1016/j.lwt.2023.114586

Liu, H., Lin, S., Zhang, M., Lv, Y., Ma, Y., Rao, J., & Zhu, Q. (2023). Changes in cell wall sugar neutral composition contribute to apple texture loss during storage among cultivars. *Horticulturae,* 9(3), 292. https://doi.org/10.3390/horticulturae9030292

Luna, M. C., Tudela, J. A., Tomás-Barberán, F. A., & Gil, M. I. (2016). Modified atmosphere (MA) prevents browning of fresh-cut romaine lettuce through multi-target effects related to phenolic metabolism. *Postharvest Biology and Technology*, 119, 84–93. https://doi.org/10.1016/j.postharvbio.2016.05.001

Ma, Y., Ban, Q., Shi, J., Dong, T., Jiang, C. Z., & Wang, Q. (2019). 1-Methylcyclopropene (1-MCP), storage time, and shelf life and temperature affect phenolic compounds and antioxidant activity of 'Jonagold'apple. *Postharvest Biology and Technology*, 150, 71–79. https://doi.org/10.1016/j.postharvbio.2018.12.015

Majidi, H., Minaei, S., Almassi, M., & Mostofi, Y. (2014). Tomato quality in controlled atmosphere storage, modified atmosphere packaging and cold storage. *Journal of Food Science and Technology*, 51, 2155–2161. https://doi.org/10.1007/S13197-012-0721-0

Park, C., Park, S., Park, J., Choi, D., Kim, J., & Kim, Y. (2018). Prediction Method of Weight Loss through Measurement of Controlled Atmosphere (CA) Storage Environment. *International Journal of Food Engineering,* 4(3). https://doi.org/10.18178/IJFE.4.3.223-226

Rab, A., Khan, N., & Iqbal, I. (2012). Physico-Chemcial Quality of Apple Cv. Gala Must Fruit Stored at Low Temperature. *FUUAST Journal of Biology*, 2(1), 103–107.

Randhawa, M. A., Javed, M. S., Ahmad, Z., Amjad, A., Khan, A. A., Shah, F. U. H., & Filza, F. (2019). Amassing of Hydroxymethylfurfural, 2-Furfural and 5-Methyl furfural in orange (Citrus reticulata) juice during storage. *Food Science and Technology*, 40, 382–386. https://doi.org/10.1590/FST.41718

Saba, M. K., & Sogvar, O. B. (2016). Combination of carboxymethyl cellulose-based coatings with calcium and ascorbic acid impacts in browning and quality of fresh-cut apples. *LWT*- Stevanović, S., Marković, D., Milovančević, U., & Otović, M. (2022). Uticaj čuvanja u kontrolisanoj atmosferi na kvalitet plodova jabuke. *Zbornik Međunarodnog kongresa o procesnoj industriji–Procesing*, 35(1), 55–59. https://doi.org/10.24094/ptk.022.055

Shah, N. A., Khan, S., Kasi, M. A., & Khair, S. M. (2002). Post harvest and cold storage losses in apple of Balochistan. *Asian J. Plant Sci*, *1*(1), 65-66.

Shu, C., Liu, B., Zhao, H., Cui, K., & Jiang, W. (2024). Effect of Near-Freezing Temperature Storage on the Quality and Organic Acid Metabolism of Apple Fruit. *Agriculture*, 14(7), 1057. https://doi.org/10.3390/agriculture14071057

Singleton VL, Orthofer R, Lamuela-Raventos RM. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Foline Ciocalteau reagent. *Methods Enzymol*, 299(1), 152–178.

Stevanović, S. (2022). Effect of storage in controlled atmosphere on apple fruits quality. *Proceedings of the International Congress on Process Industry – Processing*, 35(1), 55-59. https://doi.org/10.24094/ptk.022.055

Su, Q., Yang, H., Li, X., Zhong, Y., Feng, Y., Li, H., ... & Zhao, Z. (2024). Upregulation of PECTATE LYASE5 by a NAC transcription factor promotes fruit softening in apple. *Plant Physiology*, 196(3), 1887–1907. https://doi.org/10.1093/plphys/kiae428

Tahir, I. (2006). *Control of pre-and postharvest factors to improve apple quality and storability* (No. 2006: 35).

Wang, S. Y., Bunce, J. A., & Maas, J. L. (2003). Elevated carbon dioxide increases contents of antioxidant compounds in field-grown strawberries. *Journal of Agricultural and Food Chemistry*, 51(15), 4315–4320. https://doi.org/10.1021/jf021172d

Watkins, C. B., & Nock, J. F. (2012). Controlled-atmosphere storage of 'Honeycrisp'apples. *HortScience*, 47(7), 886–892. https://doi.org/10.21273/HORTSCI.47.7.886

Weber, A., Brackmann, A., De Oliveira Anese, R., Both, V., & Pavanello, E. P. (2013). Atmosfera controlada para o armazenamento da maçã "Maxi Gala." *Ciência Agronômica/Revista Ciência Agronômica*, *44*(2), 294–301. https://doi.org/10.1590/s1806-66902013000200011

Yeung, A. W. K., Tzvetkov, N. T., El-Demerdash, A., Horbanczuk, O. K., Das, N., Pirgozliev, V., ... & Atanasov, A. G. (2021). Apple polyphenols in human and animal health. *Animal Science Papers and Reports*, 39(2), 105–118. https://hdl.handle.net/1822/73916

Zhang, Z., Igathinathane, C., Li, J., Cen, H., Lu, Y., & Flores, P. (2020). Technology progress in mechanical harvest of fresh market apples. *Computers and Electronics in Agriculture*, 175, 105606. https://doi.org/10.1016/j.compag.2020.105606

Zhao, H., Liu, B., Zhang, W., Cao, J., & Jiang, W. (2019). Enhancement of quality and antioxidant metabolism of sweet cherry fruit by near-freezing temperature storage. *Postharvest Biology and Technology*, 147, 113–122. https://doi.org/10.1016/j.postharvbio.2018.09.013

Citation

Babu, I., Rashid, S., Noor, H. U., Zulfiqar, R., Naeem, H., Rashid, M. H., Kanwal, R., Yasmin, Z., Shafique, B., & Abrar, M. (2025). Controlled atmosphere (CA) storage of apples. *Journal of Agriculture and Food*, 6(1), 103–117. https://doi.org/10.52587/JAF060105