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Indian apple quality characteristics as affected by controlled atmosphere storage

Usman Ali[1], Rohit Maurya[1], Santanu Basu[2,3], Koushik Mazumder[1]*

*[1]National Agri-Food Biotechnology Institute, Sector-81 (Knowledge City), S.A.S. Nagar, Mohali-140306, Punjab, India. [2]Dr. S.S. Bhatnagar University Institute of Chemical Engineering and Technology (SSBUICET), Punjab University, Chandigarh, 160014, India. [3]Department of Molecular Science, BioCenter, Swedish University of Agricultural Sciences, P.O. Box 7015, SE-750 07 Uppsala, Sweden. *E-mail: koushik@nabi.res.in, kmazumder78@gmail.com*

Abstract

The effects of controlled atmosphere (CA) storage on postharvest qualities of apple (*Royal Delicious*) were investigated. In comparison to the postharvest quality of fresh apple (0 day) a significant change in the fruit qualities such as respiration rate, firmness, ripening, color, low molecular weight sugar, enzyme activity and concentrations of volatile compounds were observed during CA storage. After 90 days CA storage, apples showed a significant increase in respiration rate (58.7%), ripening index (13.9%) and reduction in firmness (35.8%) compared to fresh apples. During 90 days CA storage, the apple aroma volatile compounds showed a significant increase in their total concentrations from 151.2 µg/kg to 280.7 µg/kg compared to initial storage, followed by a remarkable decrease to 141.8µg/kg during 180 days of storage. Therefore, the study enables us to conclude that the prolonged CA storage of apples significantly reduced the fruit quality.

Key words: Apple, postharvest storage, controlled atmosphere storage, shelf life

Introduction

Apples (*Malus x domestica Borkh*) are among the most consumed fresh fruits globally. Approximately 64.6 million tons of fruits are consumed annually (Mditshwa *et al.,* 2018). During 2019-20, the total production of apples in India was 2783000 Metric Tons (MT) (Statistics, 2020). Apples are a rich source of several bioactive compounds such as vitamins, phenolic compounds, antioxidants and organic acids (Mditshwa *et al.,* 2018), which positively affect human health (Radenkovs and Juhnevica-Radenkova, 2018). Among India's commercially produced apple varieties Royal Delicious is one of the most important varieties known for its taste, nutritional and sensory attributes. In India, J and K, Himachal Pradesh and Uttrakhand were the major apple producers during 2017-18, with 1808,330 MT, 446,570 MT and 58,660 MT, respectively (Statistics, 2018). Lack of harvesting, packaging, storage, and transportation facilities are the major cause of apple's high postharvest losses (12.3%) (Statistics, 2018). The consumers are more concerned about the nutritional quality of fruits, which depends upon the pre-harvest climatic conditions, harvesting maturity, postharvest regime and fruit response to the storage (Radenkovs and Juhnevica-Radenkova, 2018).

Due to being perishable, apples are more prone to postharvest storage disorder during extended storage. Therefore, to reduce the losses in apples during postharvest storage and increase the window period for marketability, apples are stored in controlled atmosphere storage at around 0°C for extended storage (Mditshwa *et al.,* 2018). However, several studies have revealed that the extended controlled atmosphere storage of apples also reduced the fruit quality (Mditshwa *et al.,* 2018; Raffo *et al.,* 2009). Further, longer the controlled atmosphere storage duration causes a higher reduction of volatiles, depending on the storage duration and composition (O_2 and CO_2 level) (Raffo *et al.,* 2009). The decline in fruit quality during long-term storage greatly affects consumer satisfaction. However, to our knowledge, no detailed study has been reported on the effect of controlled atmosphere storage on the postharvest quality of an important Indian apple cultivar (Royal Delicious). Therefore, the objective of the present study was to investigate the effect of CA storage on postharvest quality attributes of the apple (Royal Delicious). The study will be significantly helpful for determining the range of CA storage time and ensuring the best quality for the Royal Delicious variety of apples in the supply chain.

Material and methods

Apples (Royal Delicious) with commercial maturity, uniform size, and shape were harvested from an apple orchard (Kinnaur, Himachal Pradesh, India) and supplied by Godwin Agro Products Limited, an apple storage industry located at Lalru, Mohali, Punjab, India. The freshly harvested fruits were packed in commercially used multilayer corrugated cardboard boxes and transported to the laboratory within 24 h after harvesting.

All the other chemicals were procured from Sigma-Aldrich Chemicals (USA).

Experiment design: The apples were sanitized for 2 min with 100 ppm hypochlorite solution and rinsed with deionized water. Further, the apples were dried at room temperature, and 15 apples were taken for the determination of postharvest qualities of apples at 0 day. The rest of the samples were packed in boxes.

The apple boxes were stored in the controlled atmosphere (CA) storage under refrigerated conditions at 0±1°C and 98% relative humidity (RH), with 1.5-2% and 1% O_2 and CO_2 concentration respectively (Awad and Jager, 2003). Subsequently, 15 apples were selected for analysis at storage intervals of 90, 135, and 180 days, each corresponding to the opening of the controlled atmosphere storage chamber. The samples were transported to the laboratory within 1 h after being taken out from the storage chamber and used to determine apples' postharvest quality parameters after CA storage.

Determination of respiration rate: The respiration rate was determined as described earlier (Ali *et al.,* 2019). The respiration rate was determined by using regression concentrations slop of CO_2 against the time and the respiration rate is expressed as mL CO_2/kg.h.

Colour: The colour parameters (L*, a* and b*) were obtained using a Hunter lab colorimeter (Color Flex EZ, USA) from the different sides of the apple to bring uniformity in the colour characteristics. The colour parameters (a* and b*) were further used to determine the hue angle (Rojas-Grau *et al.,* 2007) calculated as:

Hue angle= arctangent (b* / a*)

Fruit firmness: The texture measurement of apples was determined as described earlier (Ali *et al.,* 2019; Sahraei Khosh Gardesh *et al.,* 2016). The fruits were peeled at the penetration point and used for firmness determination.

Ripening Index: The juice's Total Soluble Solids (TSS) was measured with a digital refractometer and reported as % (°Brix). Fruit juice acidity was assessed through titration, presented as g malic acid equivalent per 100 g-1 fresh weight (Guillén *et al.,* 2013). The TSS to Total Acidity (TA) ratio was employed to calculate the ripening index (RI) (Guillén *et al.,* 2013).

Determination of organic acids and sugars: The core tissues of the apple were removed and juice was extracted using the kitchen juicer (HL7715/00, Philips). The apple juice was centrifuged and the supernatant was filtered through a 0.2µm nylon filter. The filtered supernatant was used for the determination of organic acids and sugars by HPLC (Agilent Technologies, USA) system by using Hi-Plex H, 300x7.7mm column (Agilent Technologies, USA) connected with refractive index (RI) detector (Petkovsek *et al.,* 2007).

The quantifications of sugars and acids were carried out by using the external HPLC-grade standards. All the data analysis was performed by Open Lab software.

Enzyme activities: The enzyme polyphenol-oxidase (PPO) and the peroxidase (POD) were extracted as described earlier (Ali *et al.,* 2019).

Determination of PPO and POD activity: PPO and POD activity were obtained as described earlier (Ali *et al.,* 2019). The enzyme activity was presented as an alteration of the sample's absorbance/minute/gram fresh weight (Ali *et al.,* 2019).

Volatile compounds determination: A GC (Agilent Technologies 7890, USA) instrument coupled with mass spectrometer (MS) detectors where SPME was used for the identification of volatile components, as described earlier (Ali *et al.,* 2019). The NIST mass spectra library was used for volatile compounds identification and the retention time of the sample was correlated with standard. Regression equations were used to calculate concentrations by injecting each standard in five different concentrations to obtain a calibration curve.

Statistical analysis: The data were analyzed using analytical software (Prism Graph Pad 7) for analysis of variance (ANOVA) and results were presented as mean ±SE. Tukey's multiple comparison tests were used to compare the mean values at a significant level ($P < 0.05$).

Results and discussion

Respiration rate: Characteristically, the apple is a climacteric fruit and the climacteric behaviour was clearly explained by the respiration rate of the fruit (Fig. 1A). The increase in the fruit respiration rate potentially affects the quality and shelf life of the fruits (Akdemir and Bal, 2020). The respiration rate of the fresh apple was 11.6 mLCO_2/kg.h during initial storage, which further significantly ($P < 0.05$) increased by 1.6-1.7 fold (18.4 -20.4 mlCO_2/kg.h) during 90-135 days of CA storage. A slight reduction in respiratory activity (19.5 mlCO_2/kg.h) was observed during 180 days of storage. The results were inconsistent with several studies suggesting the dramatic increase in the respiration rate of climacteric fruit during the initial stage and falling off again due to physiological breakdown (Lam and Wan, 1983).

Colour: Colour is an important quality attribute of apples because the colour of the apples affects the consumer's perception of ripeness and eating quality of fruits (Jonathan and Hewett, 1998). The change in the fruit colour is associated with fruit metabolism (Ali *et al.,* 2019). During initial CA storage, apple showed a reduced hue value of 29.4 (Fig. 1B). After 90 days of storage, the hue value significantly ($P < 0.05$) increased to 38.8. The maximum hue value of 41.7 was observed after 135 days of CA storage and started declining (26.7) during 180 days. The increase in the hue value might be associated with the changes in the background colour of the apple (Jonathan and Hewett, 1998). Further, a decline in the hue value during a later stage might be due to the breakdown of carotenoids (Lewinsohn *et al.,* 2005).

Firmness: The firmness of the fruits is an important quality attribute for their edible quality and market value. The apple's firmness decreases continuously during CA storage (Fig. 1C) as fruit ripening increases (Akdemir and Bal, 2020; Radenkovs and Juhnevica-Radenkova, 2018). Initially, the firmness of the fresh apple was 45.6 N. After 90 days of CA storage, a significant ($P < 0.05$) reduced firmness (35.9%) was observed. As time progressed, the firmness gradually decreased over the storage period. During 135-180 days of CA storage, a 41.5-41.9% reduction was observed in apple firmness compared to the fresh fruits. Similar results supporting our findings in previously reported studies (Akdemir and Bal, 2020) showed 30.59% firmness reduction in Granny Smith apple after 4 months of storage at 2°C with 90% relative humidity. Further, our results revealed that after 90 days of CA storage, apples' firmness was recorded below 36 N, which suggested poor consumer acceptability (Ali *et al.,* 2019).

Ripening Index: The ratio of TSS and TA was expressed as the apples' ripening index (RI) (Fig. 1D), showing an advance in ripening during extended CA storage. After 90 days of storage, a significant increase (13.9%) in the apples' RI was observed compared to the fresh apples and increased continuously during

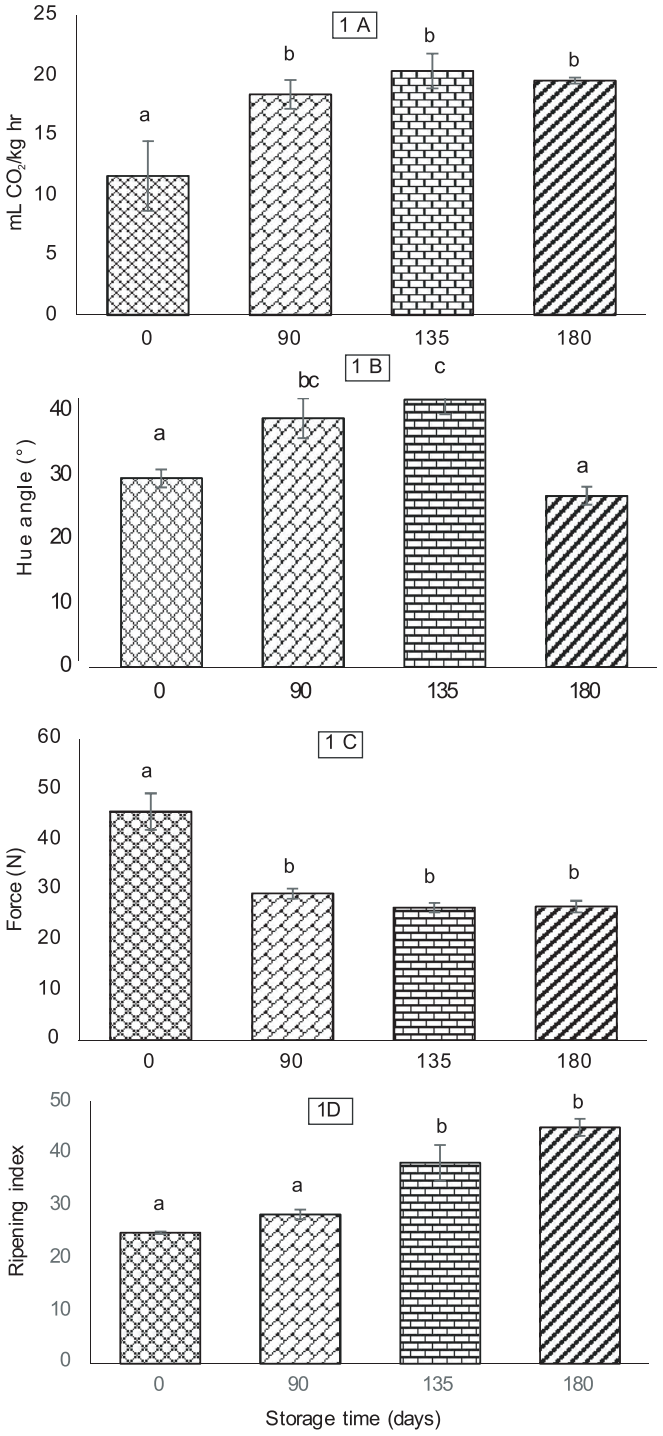

Fig. 1. Change in the respiration rate (A), color (hue angle) (B), firmness (C) and ripening index (D) of apple during CA storage.

storage. After 135 days of storage, fruits showed a significant ($P < 0.05$) increase (53.4%) in the RI followed by an 80.5% increase at the end of the CA storage (180 days) compared to the fresh apples. Moreover, the increase in the RI was due to the reduction in TA (Guillén *et al.,* 2013) during CA storage.

Sugars and organic acids content: The content of low molecular weight (LMW) carbohydrates (sucrose, glucose and fructose) were determined in fresh and apple stored under CA storage. A significant reduction in the LMW carbohydrates concentration was observed during CA storage (Fig. 2A). Initially, the sucrose concentration was 18.7 mg/ml in fresh apples (0 days). After 90 days of storage, a significant ($P< 0.05$) decrease (79.1%) in sucrose concentration was obtained (Suni *et al.,* 2000). Almost similar pattern of reduction was observed in the case of glucose and fructose. After 90 days CA storage, 78.4% and 65.9% reduction was observed in the concentration of glucose and fructose, respectively (Brizzolara *et al.,* 2020).

Tartaric and malic acids are the main organic acids in the apple. In fresh apples (0 day), tartaric and malic acid concentrations were 7.7 and 7.3 mg/ml respectively (Fig. 2B). The content of both tartaric and malic acid was found reduced continuously during CA storage. After 90 days of storage, 17.3% and 3.4% reduction in tartaric and malic acid concentrations were observed, respectively. The reduction in the acids concentration might be due to using acids as substrates in the metabolic processes as the respiration rate increased (Brizzolara *et al.,* 2020).

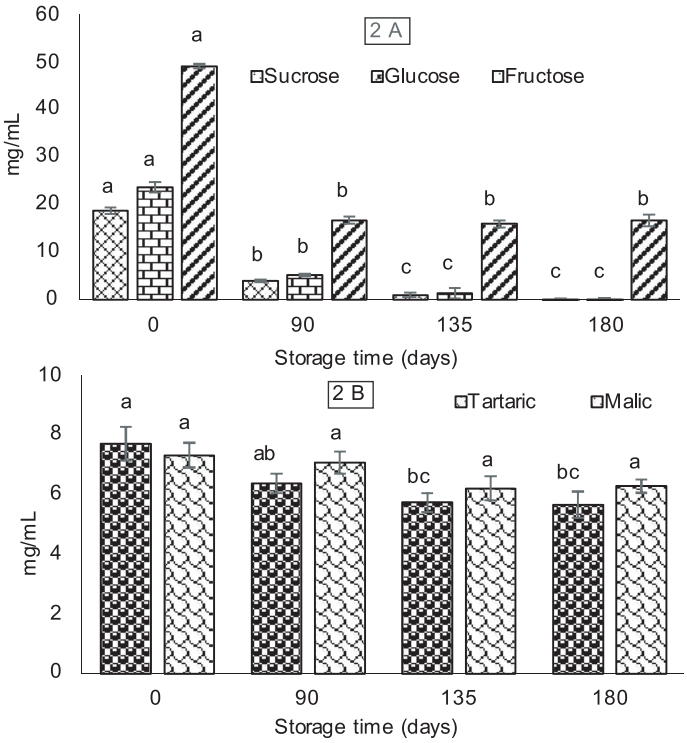

Fig. 2. Effect of CA storage on apple sugars (A) and organic acids (B).

Determination of enzyme activity

Peroxidase (POD) and Polyphenol (PPO) activity: The POD and PPO activity were measured in fresh and CA-stored apples (Fig. 3A and 3B). Fresh apples showed reduced enzymatic activity. However, after 90 days CA storage, a significant ($P < 0.05$) increase (3.3 fold) in the POD activity of apples was observed compared to fresh apples. Thereafter, enzymatic activity decreased slightly during storage for 135-180 days. Similarly, reduced PPO activity was found in fresh apples and increased significantly under CA storage. During 90-135 days of CA storage, a 2.0-3.3 fold increase in the PPO activity (Fig. 3B) was observed compared to the fresh apples (Ali *et al.,* 2019).

Volatile compounds: The GC-MS analysis of the volatile compounds isolated from homogenized pulp of fresh apple and after CA storage allowed to identify the 12 major volatile compounds including; 2 aldehydes (2-hexenal and hexanal), 4 butanoates (ethyl butanoate, ethyl 2-methyl butanoate, propyl butanoate and 2-methyl propyl butanoate), 1 acetates (hexyl

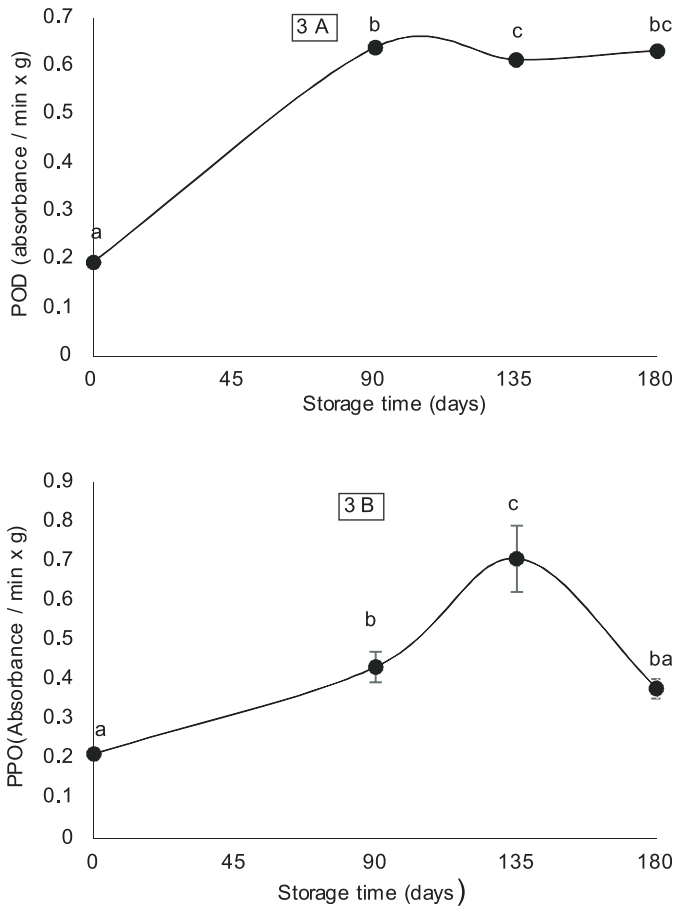

Fig. 3. Effect of CA storage on (A) peroxidase (POD) and (B) polyphenol oxidase (PPO) activity.

acetate), 4 hexanoates (ethyl hexanoate, hexyl hexanoate, butyl hexanoate, propyl hexanoate) and 1 sesquiterpene (α-farnesene) (Fig. 4). In addition to this 1-Hexanol, hexyl 2-methylbutanoate and ethyl octanoate were found as minor constituents. These volatile compounds were responsible for the apple aroma and provide characteristic fruity, sweet, green apple aroma and apple-like sensory responses (Ali *et al.,* 2019; Raffo *et al.,* 2009).

Initially, the fresh apples (0 day) showed reduced volatile compounds**.** In fresh apple, aldehydes and esters were in the range of 0.8-1.8 µg/kg and 0.1-6.8 µg/kg, respectively. The concentration of these volatile compounds increased significantly after 90 days of CA storage. The aldehydes and esters were in the range of 3.6-4.1 µg/kg and 1.1-49.7 µg/kg, respectively which suggested nearly 2.0-4.9 fold and 1.9-173.0 fold increase respectively in their concentrations compared to the fresh apples (0 day). The remarkable increase in the ester concentration during the storage period might be due to the predominant presence of precursors derived through lipoxygenase activity and lipid degradation (Ali *et al.,* 2019).

After 135 days CA storage, a significant reduction was observed in the concentrations of volatile compounds. The concentration of aldehydes and esters were in the range of 0.9-3.6 µg/kg and 0.6-45.6 µg/kg suggesting 12.4-74.2% and 8.2-62.3% reduction, respectively compared to 90-day storage. Further, during 180 days of CA storage, 41.7-65.5% and 23.0-69.0 % reduction in the concentration of aldehydes and esters were recorded. The reduction in the concentration of the volatile ester might be due to the ester hydrolyzation by the carboxylesterase and increased membrane fluidity resulting from the diffusion of volatiles in the environment (Ali *et al.,* 2019). The acyclic branched

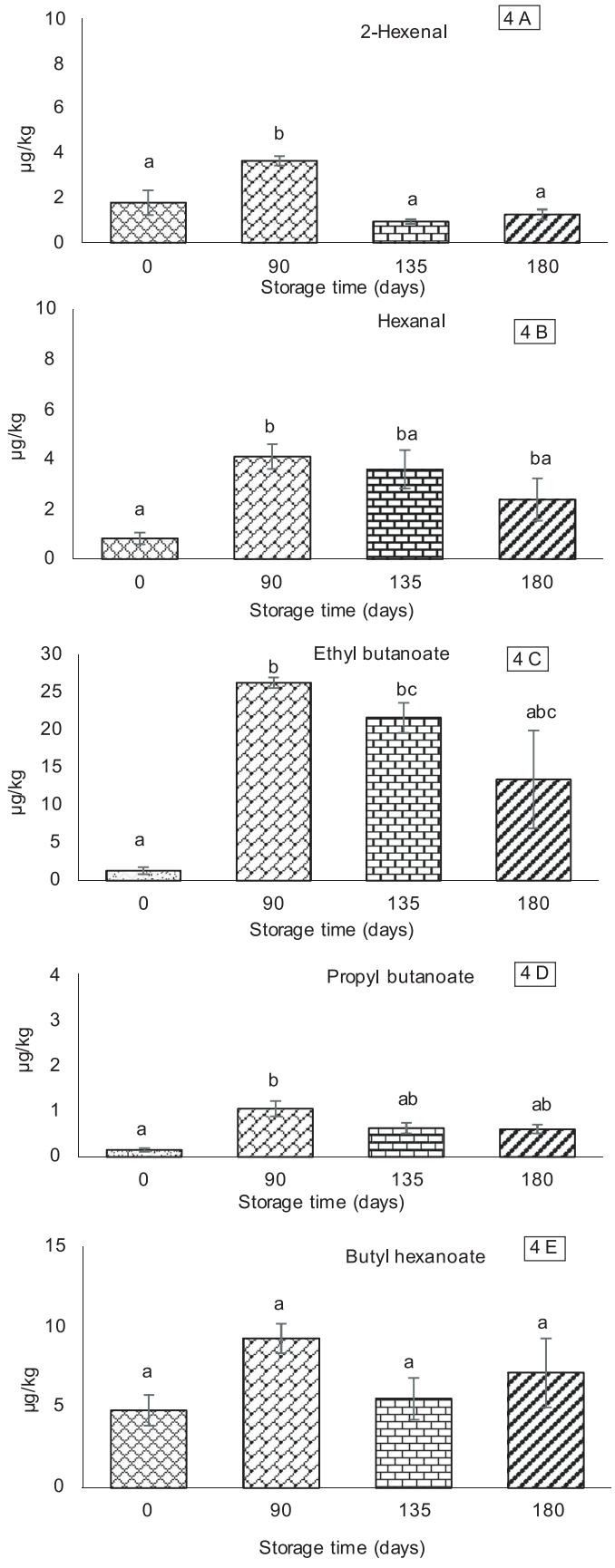

Fig. 4. Change in aroma volatile compounds (2-hexenal (A), hexanal (B), ethyl butanoate (C), propyl butanoate (D), butyl hexanoate (E) of apples during CA storage.

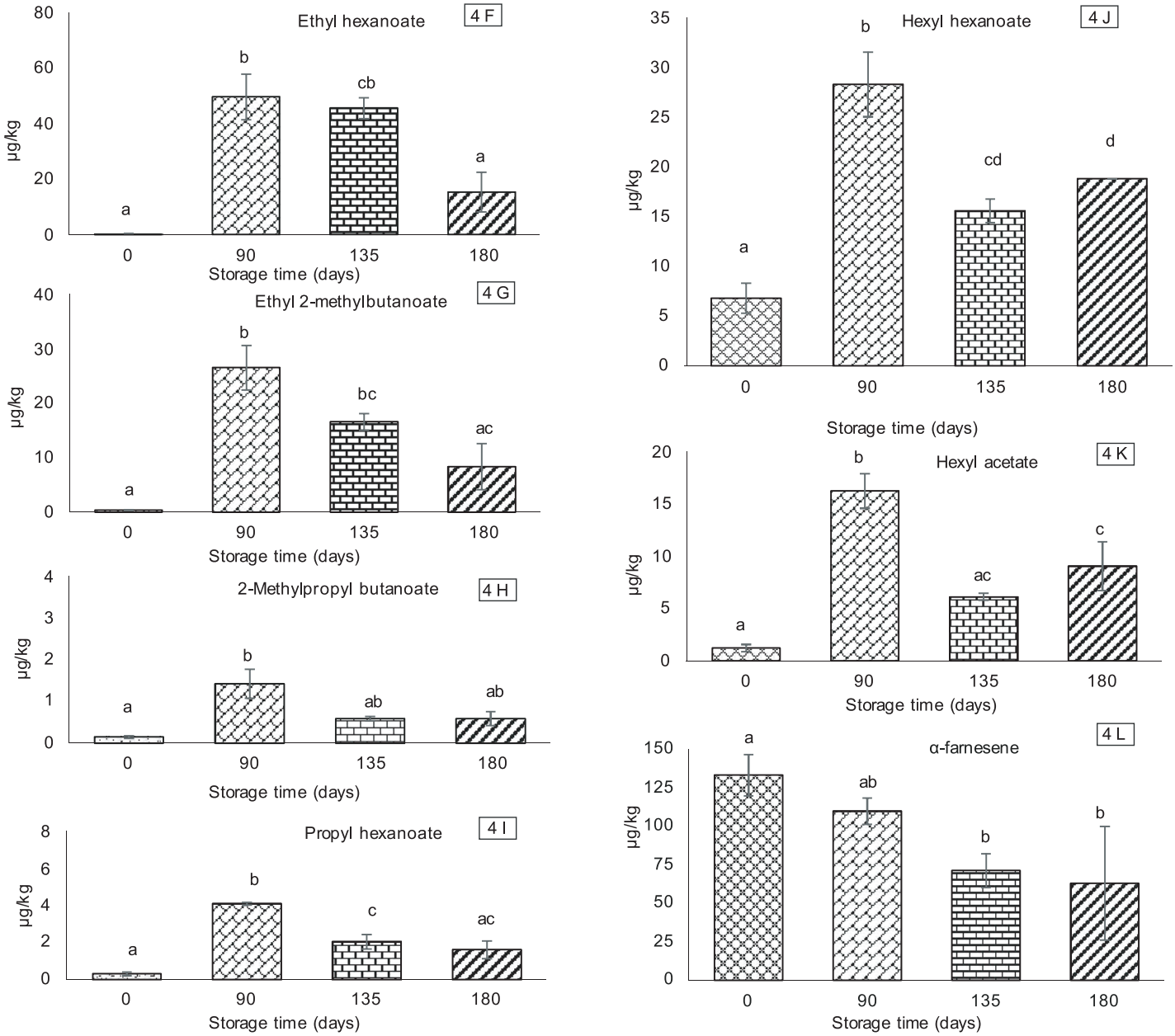

Fig. 4 contd.. Change in aroma volatile compounds (2-hexenal ethyl hexanoate (F), ethyl 2-methylbutanoate (G), 2-methylpropyl butanoate (H), propyl hexanoate (I), hexyl hexanoate (J), hexyl acetate (K) and α-farnesene (L) of apples during CA storage.

sesquiterpene (α-farnesene) is mostly associated with ripe apple and is predominantly synthesized in the fruit's epidermal and hypodermal cell layers (Ali *et al.,* 2019). In the present study, α-farnesene was found to be the only major volatile terpene compound (133.2 µg/kg) in fresh apples (0 days), which was inconsistent with the previous literature suggested high quality of α-farnesene emitted during harvesting (Ali *et al.,* 2019). The level of α-farnesene decreased significantly during CA storage. After 90 days of storage, the concentration of α-farnesene was 109.8 µg/kg, suggesting a 17.5% reduction compared to fresh apples. As the CA storage extended (135 days), the concentration of α-farnesene reduced to 71.2 µg/kg, suggesting a 46.5% reduction in concentration compared to initial storage. Finally, after 180 days of storage, 52.7% reduction in α-farnesene content was observed compared to initial storage. This was in agreement with a previous study showing that the production of α-farnesene in apples decreased after 2 months of storage (Ali *et al.,* 2019). The overall results suggested that the total volatile concentrations in apple under CA storage reached a maximum level of 280.7 µg/kg during 90 days, which decreased remarkably to 141.8-190.3 µg/kg during extended CA storage of 135-180 days.

The postharvest storage of apples under controlled atmosphere storage conditions increased the postharvest shelf life of fruits. However, the extended CA storage also affects apple quality parameters, significantly reducing low molecular weight sugars and volatile aroma compounds. After 90 days of CA storage, apples showed significant firmness reduction and increased enzymatic activity. The study concluded that the CA storage of apple effectively retained fruit quality for less than 90 days. The storage conditions can have a differential impact on the fruit's qualities. Therefore, the successful commercial development of CA storage will require a complete understanding of fruit quality changes with storage conditions. Further, additional postharvest management will be required to enhance the quality parameters of

apples during CA storage. However, detailed studies are needed to examine their effect on postharvest qualities of different apple cultivars.

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Conflict of interest: The authors declare that there is no conflict of interest.

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