

	Journal of Horticulture and Postharvest Research Journal Homepage: https://www.jhpr.birjand.ac.ir	 University of Birjand
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Enhancing postharvest quality and antioxidant enzyme activities in Camarosa strawberries by chlorogenic acid treatment during cold storage

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ARTICLE INFO

Original Article

Article history:

Received 18 July 2025

Revised 24 December 2025

Accepted 6 January 2026

DOI: 10.22077/jhpr.2026.9777.1550

P-ISSN: 2588-4883

E-ISSN: 2588-6169

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A B S T R A C T

Purpose: Strawberries are highly perishable fruits with a very limited postharvest shelf life due to their soft texture and high susceptibility to decay. This study aimed to explore an eco-friendly and effective method for maintaining postharvest quality by investigating the effects of chlorogenic acid (CGA) on the physiological and biochemical characteristics of Camarosa strawberries during cold storage. **Research Method:** Freshly harvested strawberries were treated with different concentrations of CGA (25, 50, 75, and 100 μM) and stored at 4 °C for 16 days. Fruit quality indicators, including firmness, decay rate, antioxidant capacity, enzymatic activities, and oxidative stress markers were evaluated at regular intervals. **Findings:** CGA treatment, especially at 75 and 100 μM , significantly preserved fruit firmness, delayed decay symptoms, and maintained higher levels of total phenols, flavonoids, and proline. It also reduced the accumulation of malondialdehyde, electrolyte leakage, and hydrogen peroxide. Moreover, key antioxidant enzymes, including catalase, superoxide dismutase, and peroxidase, displayed higher activity in treated fruits, indicating improved oxidative stress tolerance. These combined effects contributed to prolonged postharvest life and better preservation of nutritional and sensory qualities. **Research limitations:** The study was limited to one cultivar under controlled storage; further research under commercial conditions is suggested. **Originality/Value:** This work highlights the potential of chlorogenic acid as a natural, safe, and cost-effective postharvest treatment to improve storage stability and fruit quality, offering promising implications for sustainable horticultural practices.

Keywords:

Antioxidant capacity, Decay, Phenolic compounds, Proline, Shelf life

INTRODUCTION

Strawberries (*Fragaria × ananassa* Duch. cv. Camarosa) are highly popular fruits, cherished not only for their delightful taste but also for their numerous health benefits. As non-climacteric fruits, strawberries must be harvested at full ripeness. However, their soft texture and rapid loss of firmness make them highly susceptible to fungal infections, leading to a swift decline in quality post-harvest. The short shelf life of strawberries is primarily attributed to postharvest maturation, senescence, and mechanical injuries incurred during harvest (Zhu & Zhou, 2007). The biochemical and physiological characteristics of strawberries change during storage, making them prone to postharvest deterioration and diseases (Bahmani et al., 2022). Improper storage temperatures significantly accelerate weight loss, decay, and fruit softening (Seeram et al., 2006). Consequently, fresh strawberries commonly experience substantial physiological weight loss and firmness reduction during storage, which markedly restricts their commercial shelf life (Meighani & Roozkhosh, 2024).

Several techniques have been developed to enhance the postharvest quality of strawberries. Among these, low-temperature storage is widely used to maintain the quality of nutraceuticals by decreasing respiration rates and reducing physiological disorders (Aghdam & Bodbodak, 2014). However, due to their perishable nature, strawberries do not fare well when stored at low temperatures without additional treatments. Alternative postharvest storage techniques have been explored to extend the shelf life of strawberries, including modified atmosphere packaging (Geyer et al., 2018), gamma radiation (Filho et al., 2018), osmotic treatments (Jiang et al., 2021), chemical applications (Shu et al., 2020b), hypobaric storage (Hashmi et al., 2016), and edible coatings (Qamar et al., 2018; Quintana et al., 2021).

In recent years, greater emphasis has been placed on natural and bio-based postharvest approaches as safer alternatives to synthetic preservatives. Edible coatings enriched with bioactive compounds have been shown to effectively reduce weight loss and decay while maintaining firmness, antioxidant capacity, and nutritional quality of strawberries during cold storage (Bahmani et al., 2024; Elabd & Saleh, 2025). Likewise, naturally occurring bioactive compounds have received increasing attention for their role in preserving strawberry quality. Plant-derived extracts and polysaccharide-based coatings, particularly when combined with nanotechnology, have demonstrated improved efficiency in suppressing fungal decay and delaying senescence (Zamandi et al., 2025; Carhuavilca-Paredes et al., 2025). Among these compounds, polyamines such as putrescine have been reported to maintain fruit firmness, reduce physiological weight loss, and enhance antioxidant enzyme activity during strawberry storage. Moreover, chitosan–putrescine nanocomposite systems have shown enhanced performance in preserving overall fruit quality and reducing postharvest decay under cold storage conditions (Bahmani et al., 2024).

Chlorogenic acid (CGA) is one of the predominant phenolic compounds naturally present in a wide range of crops, including tomatoes, apples, pineapples, blueberries, and coffee (Duarte et al., 2010; Upadhyay & Mohan Rao, 2013). CGA has garnered considerable attention due to its diverse biological activities, including potent antioxidant, anticancer, and antidiabetic properties (Santana-Gálvez et al., 2017). Moreover, CGA has exhibited antimicrobial properties. For instance, Martínez et al. (2017) stated that CGA entirely suppressed spore germination and mycelial growth of several plant pathogenic fungi by altering fungal membrane permeability, underscoring CGA potential as a biological fungicide. Jiao et al. (2018) observed that CGA decreased *Penicillium expansum* infection in peaches by triggering the fruit's immune response. Similarly, postharvest treatment of CGA on kiwifruit enhanced defense-related enzyme activity and helped preserve fruit quality throughout storage (Zhang et al., 2020).

Given its antifungal properties and additional benefits, CGA is hypothesized to be effective in managing postharvest diseases in strawberries. In light of the growing interest in natural bioactive compounds for postharvest preservation, CGA is hypothesized to represent a promising eco-friendly strategy for improving postharvest quality and disease management in strawberries. This study investigates the enhancement of postharvest quality and enzymatic antioxidant levels of strawberries through the external application of chlorogenic acid during cold storage.

MATERIALS AND METHODS

Fruit materials and treatment

The experiment was conducted using *Fragaria × ananassa* cv. Camarosa strawberries, grown during the 2020 cultivation season in a greenhouse located in Zanjan, Zanjan Province, Iran. Uniform and healthy fruits, free from visible defects or mechanical damage, were harvested at commercial maturity (when approximately 75% of the surface exhibited red coloration) (Hernández-Muñoz et al., 2006), and immediately transferred to the laboratory. The strawberries were allowed to air-dry at room temperature (20 °C) for 10 minutes prior to the application of chlorogenic acid. The fruits were then randomly divided into five groups, each containing 240 fruits per treatment, with three replications (80 fruits per replication). They were wrapped in cloth mesh and completely immersed in chlorogenic acid solutions (25, 50, 75, and 100 µM) or deionized water (as a control) at 20 °C for 5 minutes. After treatment, the strawberries were removed and then placed on filter paper at room temperature (20 °C) for 30 minutes to remove any residual surface solution. Both treated and control fruits were subsequently packed into transparent, collapsible polyethylene terephthalate (PET) containers, each containing 4 to 5 perforations (7–8 mm in diameter), designed to help maintain the internal gas composition during storage. The containers were stored in refrigerated conditions at 4 °C and 90% relative humidity for 16 days. Twenty strawberries were randomly selected from cold storage for each replication. Both control and treated fruits were sampled every four days (days 4, 8, 12, and 16) and then stored at 20 °C for 24 hours (shelf-life) for physiological and biochemical analyses.

Decay incidence

The incidence of fruit decay was evaluated by calculating the percentage of decayed fruits relative to the total number of fruits per replicate (Aghdam & Fard, 2017).

Fruit firmness

Fruit firmness was measured using a texture analyzer (Model FT011, Facchini srl, Alfonsine, RA, Italy) equipped with a 5 mm diameter cylindrical probe. Measurements were taken on opposite sides of each fruit, and the average firmness was reported in newtons (N).

Total soluble solids (TSS) and titratable acidity (TA)

TSS was measured using a digital refractometer (Model PAL-1; Atago Co., Tokyo, Japan). Homogenized strawberry samples were filtered, and a small volume of the filtrate was placed on the refractometer prism. TSS content was recorded in degrees Brix on days 4, 8, 12, and 16 of storage. Titratable acidity was determined by titrating 10 mL of freshly extracted juice with 0.1 N NaOH until the pH reached 8.1. The results were expressed as the percentage of citric acid equivalent, following the method described by Hernández-Muñoz et al. (2006)

Proline, Malondialdehyde (MDA), Electrolyte leakage (EL), and hydrogen peroxide (H₂O₂) contents

Proline content was determined according to Sánchez et al. (2001). Fresh tissue (1 g) was homogenized in 3% sulfosalicylic acid and centrifuged at 6000 rpm for 10 min. Two milliliters of the supernatant were reacted with acid ninhydrin and glacial acetic acid, incubated at 100 °C for 1 h, and then extracted with toluene. Absorbance was measured at 520 nm, and proline concentration was calculated from a standard curve and expressed as $\mu\text{mol g}^{-1}$ FW.

Malondialdehyde (MDA), used as an indicator of lipid peroxidation, was quantified according to the thiobarbituric acid (TBA) reaction described by Liu et al. (2018). Approximately 1 g of tissue was extracted in 5 mL of 10% trichloroacetic acid (TCA) and centrifuged at 10,000 rpm for 15 min. Two milliliters of the supernatant were mixed with an equal volume of 10% TCA containing 0.6% TBA. The mixture was heated at 100 °C for 20 min, cooled immediately in ice, and centrifuged at 6000 rpm for 10 min. Absorbance of the supernatant was recorded at 532 and 600 nm, and MDA concentration was calculated using the following formula (1):

$$\text{MDA} = (A_{532} - A_{600}) \times W \times V / 155 \times 1000 \quad (1)$$

Where A_{532} and A_{600} are the absorbances at 532 nm and 600 nm, respectively; W is the sample weight, and V is the TCA volume.

Electrolyte leakage (EL) was determined according to the method of Promyou et al. (2012). Fruit discs were rinsed with distilled water and immersed in 10 mL of deionized water at 3°C for 1 h. The initial conductivity (C1) of the bathing solution was measured using a conductivity meter (Model SG3-FK2, China). Samples were subsequently autoclaved at 121 °C for 20 min, cooled to room temperature, and the final conductivity (C2) was recorded (2).

$$\text{Electrolyte leakage was expressed as EL (\%)} = (C1 / C2) \times 100 \quad (2)$$

Hydrogen peroxide (H₂O₂) was measured following Ma et al. (2016). One gram of fresh tissue was homogenized with 5 mL of 1% TCA and centrifuged at 12,000 rpm for 15 min at 4°C. A 0.5 mL portion of the supernatant was mixed with 0.5 mL of 10 mM potassium phosphate buffer (pH 7.0) and 1 mL of 1 M potassium iodide (KI). After 10 min of incubation in the dark at room temperature, absorbance was measured at 390 nm. H₂O₂ concentration was calculated from a standard curve and expressed as $\mu\text{mol g}^{-1}$ FW.

Measurement of ascorbic acid content

Ascorbic acid concentration was quantified via KI titration. The endpoint was reached when the fruit extract darkened to a stable blue. The volume of iodine solution used in the titration was measured, and ascorbic acid content was calculated using a standard curve and expressed as mg per 100 g of fresh weight (Saini, 2001).

Measurement of total phenolics, flavonoids, and antioxidant capacity

The levels of total phenolics, flavonoids, and antioxidant activity were assessed following the method described by Pineli et al. (2011). For extraction, 1 g of strawberry tissue was homogenized in 8 mL of 80% methanol, and the mixture was centrifuged at 8,000 rpm for 10 minutes.

Total phenolic content was determined using the Folin–Ciocalteu method, and absorbance was measured at 720 nm after 30 minutes of reaction. Results were expressed as mg gallic acid equivalents (GAE) per 100 g fresh weight.

The total flavonoid concentration was measured using a colorimetric method. Results were expressed as milligrams of rutin equivalents (RE) per 100 grams of fresh weight.

The antioxidant capacity of the samples was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. The DPPH radical scavenging activity was then calculated using the following formula (3):

$$\text{DPPH scavenging activity (\%)} = \frac{(A_c - A_s)}{A_c} \times 100 \quad (3)$$

Where A_c is the absorbance of the control (without extract), and A_s is the absorbance of the sample containing the extract.

Enzyme assay

Extraction of samples

Enzymatic extraction was performed under cold conditions (4 °C) to preserve enzyme activity. Fresh fruit tissue (1 g) was homogenized in 3 mL of ice-cold 50 mM potassium phosphate buffer (KH_2PO_4 , pH 7.8) containing 0.2 mM disodium EDTA (Na_2EDTA) and 2% (w/v) polyvinylpyrrolidone (PVP). The homogenate was subsequently centrifuged at 14,000 rpm for 20 minutes at 4 °C. The resulting supernatant was collected and used for the determination of superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) enzyme activities (Chen et al., 2011).

SOD activity

SOD activity was determined according to Chen et al. (2011). SOD activity was expressed as units per gram of fresh weight ($\text{U g}^{-1} \text{FW}$).

CAT activity

CAT activity was determined following the procedure of Ali et al. (2016). CAT activity was expressed as units per gram of fresh weight ($\text{U g}^{-1} \text{FW}$).

POD activity

POD activity was measured using the method described by Liu et al. (2005). POD activity was reported as units per gram of fresh weight ($\text{U g}^{-1} \text{FW}$).

Statistical analysis

The study was carried out using a factorial arrangement based on a completely randomized design (CRD) with three replications. To determine statistically significant differences among treatment means, Duncan's multiple range test was conducted at a significance threshold of $p \leq 0.05$. The analysis of variance was carried out using SPSS software (version 18.0). All data were reported as mean values $\pm$ standard error (SE)

RESULTS

Fruit firmness, TA, and TSS

Fruit firmness decreased across all treatments; however, CGA-treated fruits softened more slowly. Untreated fruits lost 76.4% of their firmness, while fruits treated with 75 and 100 μM CGA lost 38.9% and 41% of their firmness, respectively, after 16 days of storage (Fig. 1a). A

similar trend was observed for TA. After 16 days of storage, the TA of control fruits was lower than that of CGA-treated fruits, although TA levels were statistically similar across all treatments after 4 days of storage (Fig. 1b). TSS levels did not change significantly during storage, although there was a slight increase, with control fruits exhibiting higher TSS than treated fruits (Fig. 1c).

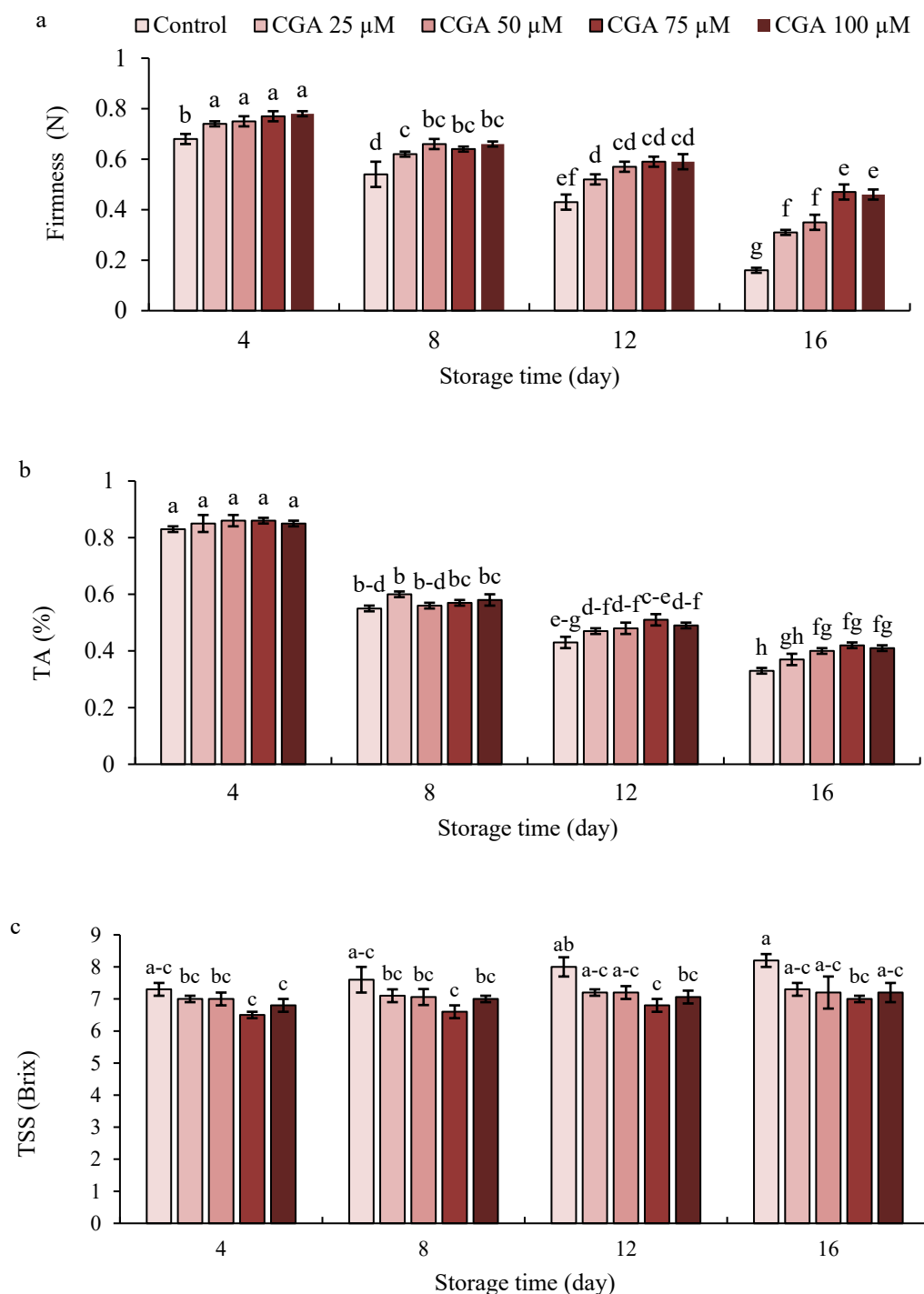


Fig. 1. Effect of CGA on firmness (a), TA (b), and TSS (c) of strawberry fruit during storage. The vertical bars represent the means $\pm$ SE (Duncan's multiple range test, $p < 0.05$, $n=3$).

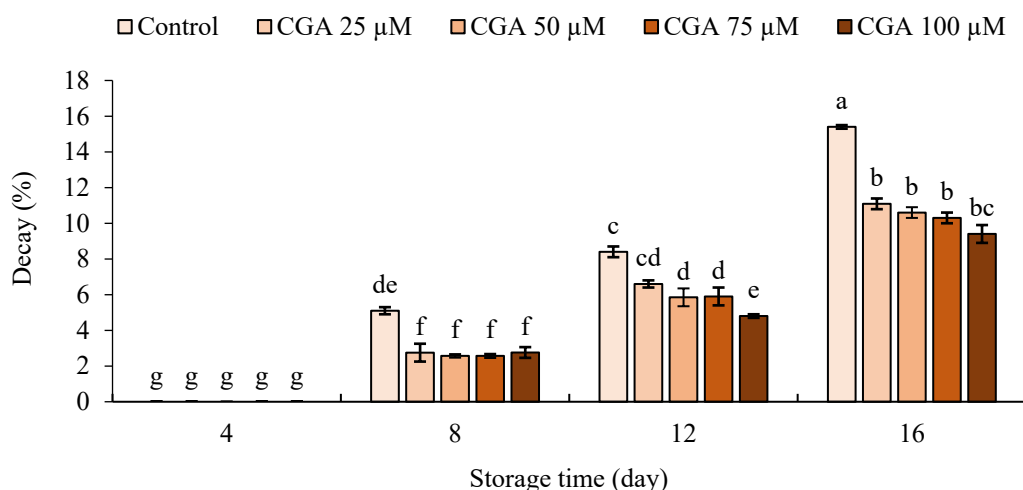


Fig. 2. Effect of CGA on decay of strawberry fruit during storage. The vertical bars represent the means $\pm$ SE (Duncan's multiple range test, $p < 0.05$, $n=3$).

Decay incidence

Decay incidence increased over the storage period. After 16 days, CGA-treated fruits exhibited approximately 28-39% less decay compared to control fruits, with 100 μ M CGA showing the greatest reduction in decay symptoms (Fig. 2).

Ascorbic acid

Ascorbic acid levels declined in all treatments; however, fruits treated with 75 μ M and 100 μ M CGA exhibited a slower decrease than the control and lower CGA doses. After 16 days of storage, ascorbic acid content in fruits treated with 25, 50, 75, and 100 μ M CGA was 36.4%, 33%, 55.9%, and 51.9% higher, respectively, than in control fruits (Fig. 3).

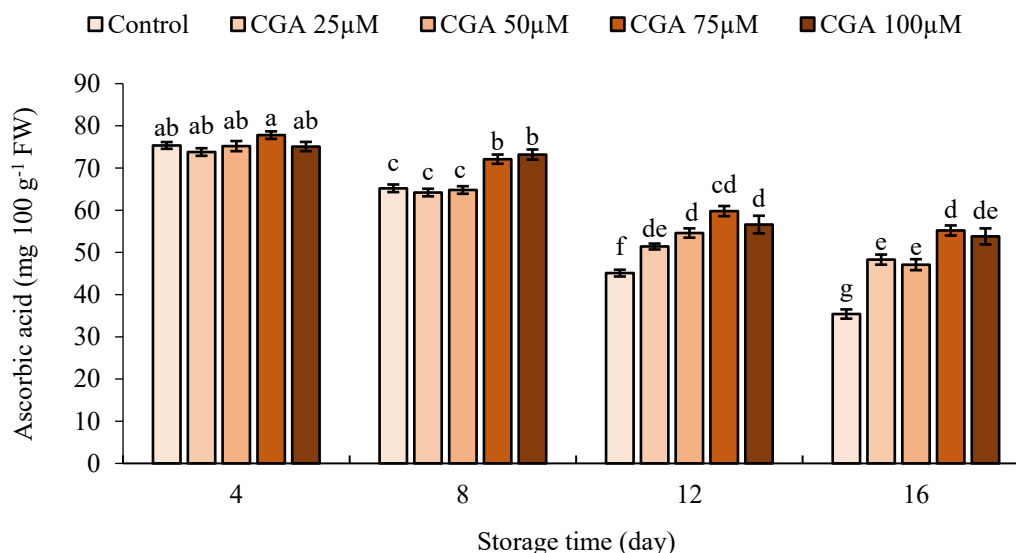


Fig. 3. Effect of CGA on ascorbic acid of strawberry fruit during storage. The vertical bars represent the means $\pm$ SE (Duncan's multiple range test, $p < 0.05$, $n=3$).

Total phenol, total flavonoid, and antioxidant capacity

Total phenol, total flavonoid, and antioxidant capacity decreased with increasing storage time, regardless of treatment. Untreated fruits had lower levels of total phenol, total flavonoid, and antioxidant capacity compared to CGA-treated fruits at all storage times. After 16 days, the total phenol content in fruits treated with 25, 50, 75, and 100 μM CGA was 26.6%, 37.7%, 39%, and 38.1% higher, respectively, than in untreated fruits (Fig. 4a). Total flavonoid content increased by 28.7%, 68.3%, 71.6%, and 74.9%, respectively (Fig. 4b), and total antioxidant capacity increased by 28%, 29.6%, 48.2%, and 48.6%, respectively (Fig. 4c).

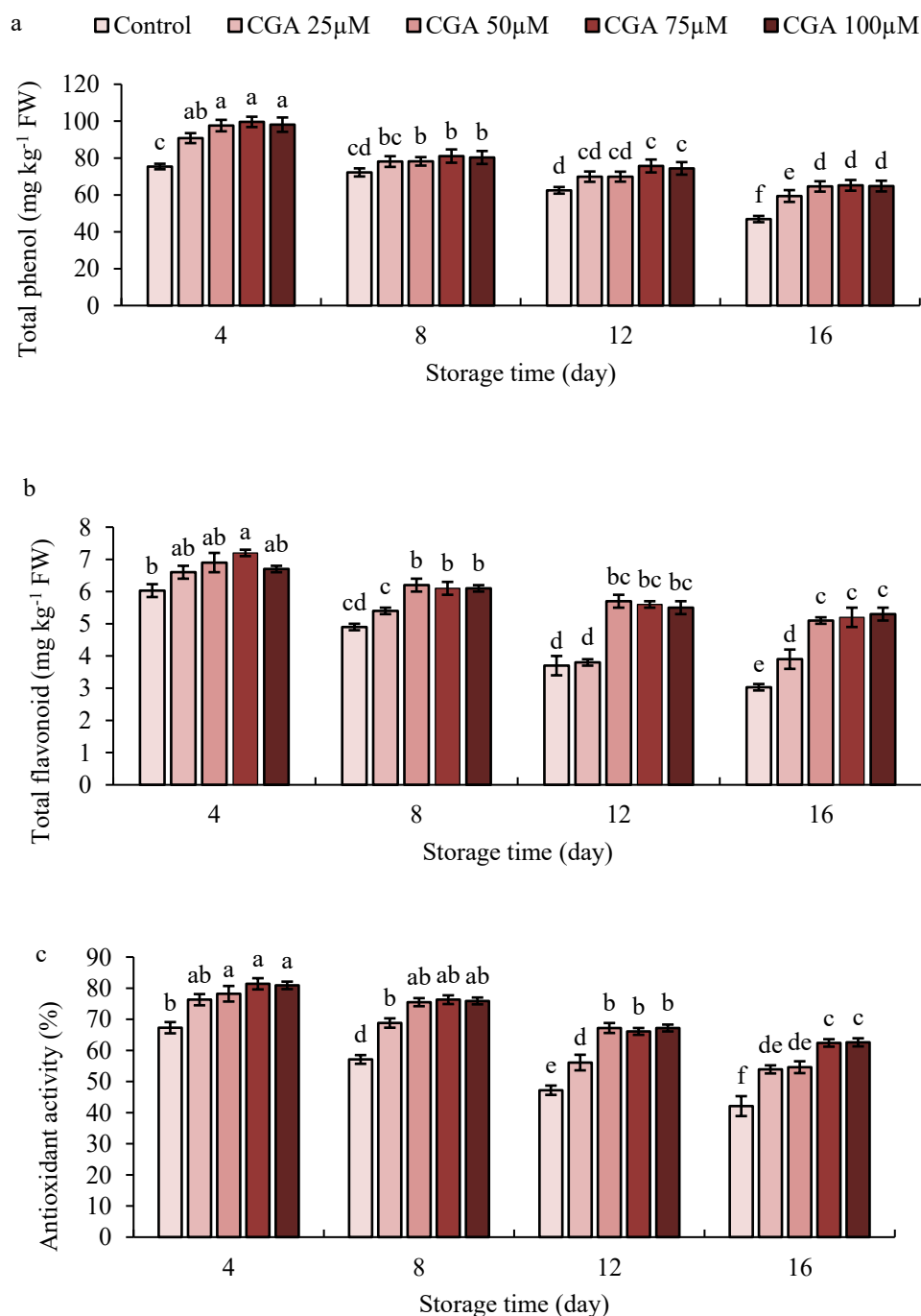


Fig. 4. Effect of CGA on total phenol (a), total flavonoid (b), and antioxidant (c) of strawberry fruit during storage. The vertical bars represent the means $\pm$ SE (Duncan's multiple range test, $p < 0.05$, $n=3$).

Proline, MDA, EL, and H₂O₂

Proline content increased during storage in all treatments, with CGA-treated fruits exhibiting higher proline levels than untreated fruits. There was no statistically significant difference in proline levels among CGA-treated fruits throughout storage (Fig. 5a). MDA levels increased during storage in all treatments, but untreated fruits had higher MDA levels than CGA-treated fruits. The lowest MDA levels were observed in fruits treated with 50, 75, and 100 μ M CGA at 8, 12, and 16 days after storage (Fig. 5b).

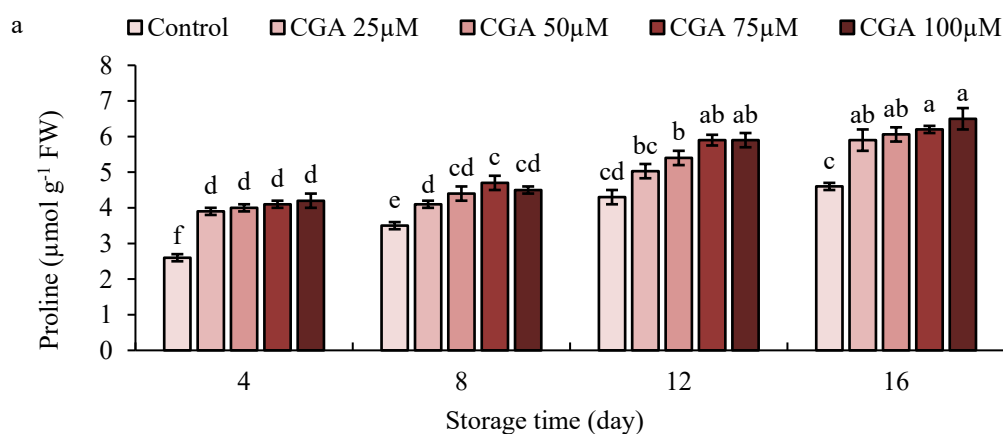
EL increased with storage time in all treatments, with untreated fruits showing higher EL levels than treated fruits. However, there was no significant difference in EL levels among different CGA treatments (Fig. 5c). H₂O₂ content increased with storage time in all treatments. After 16 days, the lowest H₂O₂ content was recorded in fruits treated with 75 and 100 μ M CGA, followed by fruits treated with 25 and 50 μ M CGA (Fig. 5d).

Antioxidant enzyme activity

CAT activity increased in all treatments up to 8 days of storage, after which it decreased to the lowest level at 16 days. CAT activity was higher in CGA-treated fruits compared to untreated fruits, with 75 and 100 μ M CGA treatments showing the highest stimulation of CAT activity at 16 days (Fig. 6a).

SOD activity also increased up to 12 days of storage and then decreased at 16 days. SOD activity was higher in CGA-treated fruits compared to untreated fruits, with all CGA levels showing a similar stimulatory effect on SOD activity (Fig. 6b).

POD activity increased until 8 days of storage and then decreased to the lowest level at 16 days. No significant difference in POD activity was observed between fruits with and without CGA treatment (Fig. 6c).



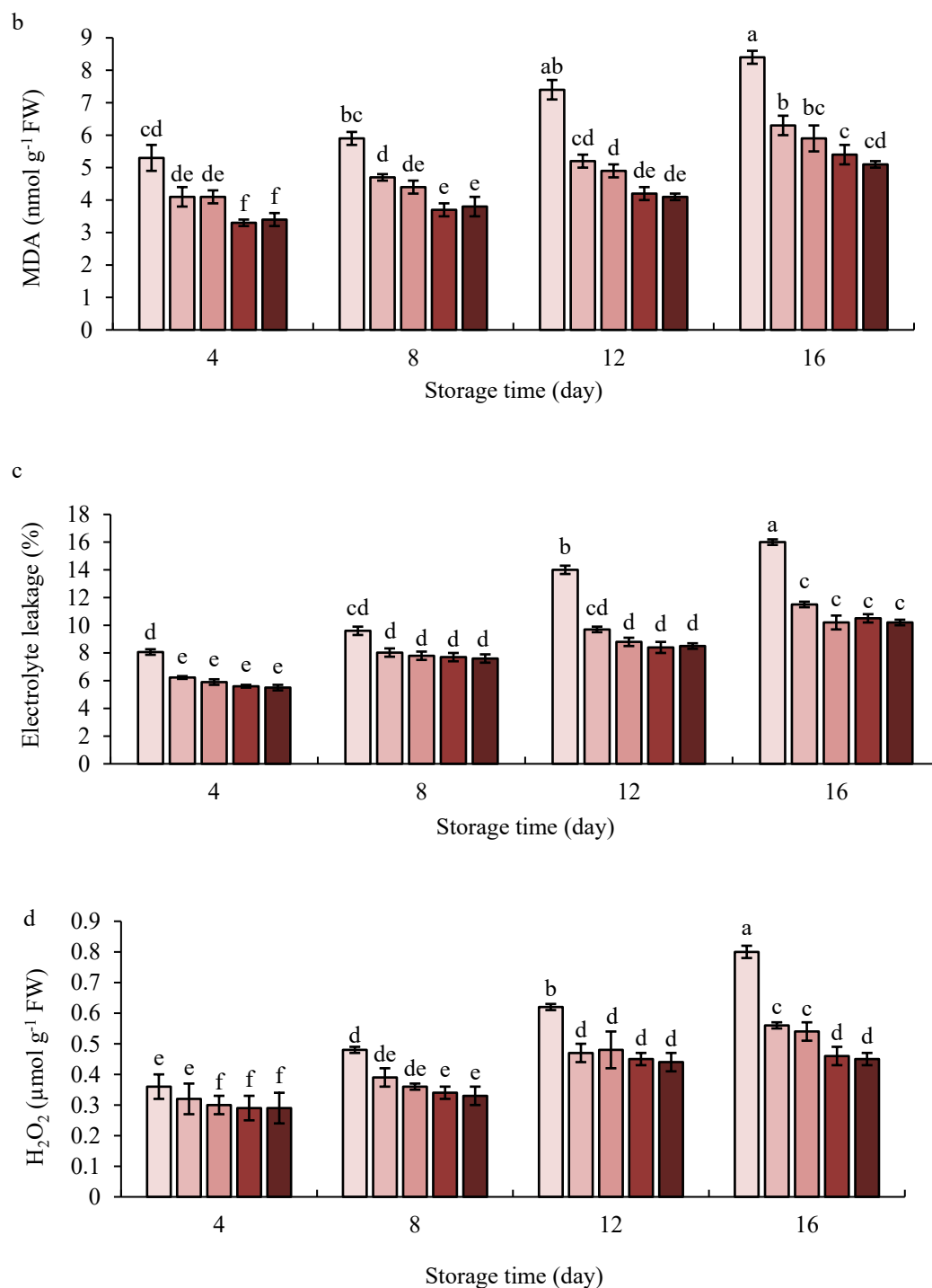


Fig. 5. Effect of CGA on proline (a), MDA (b), EL (c), and H₂O₂ (d) of strawberry fruit during storage. The vertical bars represent the means $\pm$ SE (Duncan's multiple range test, $p < 0.05$, $n=3$).

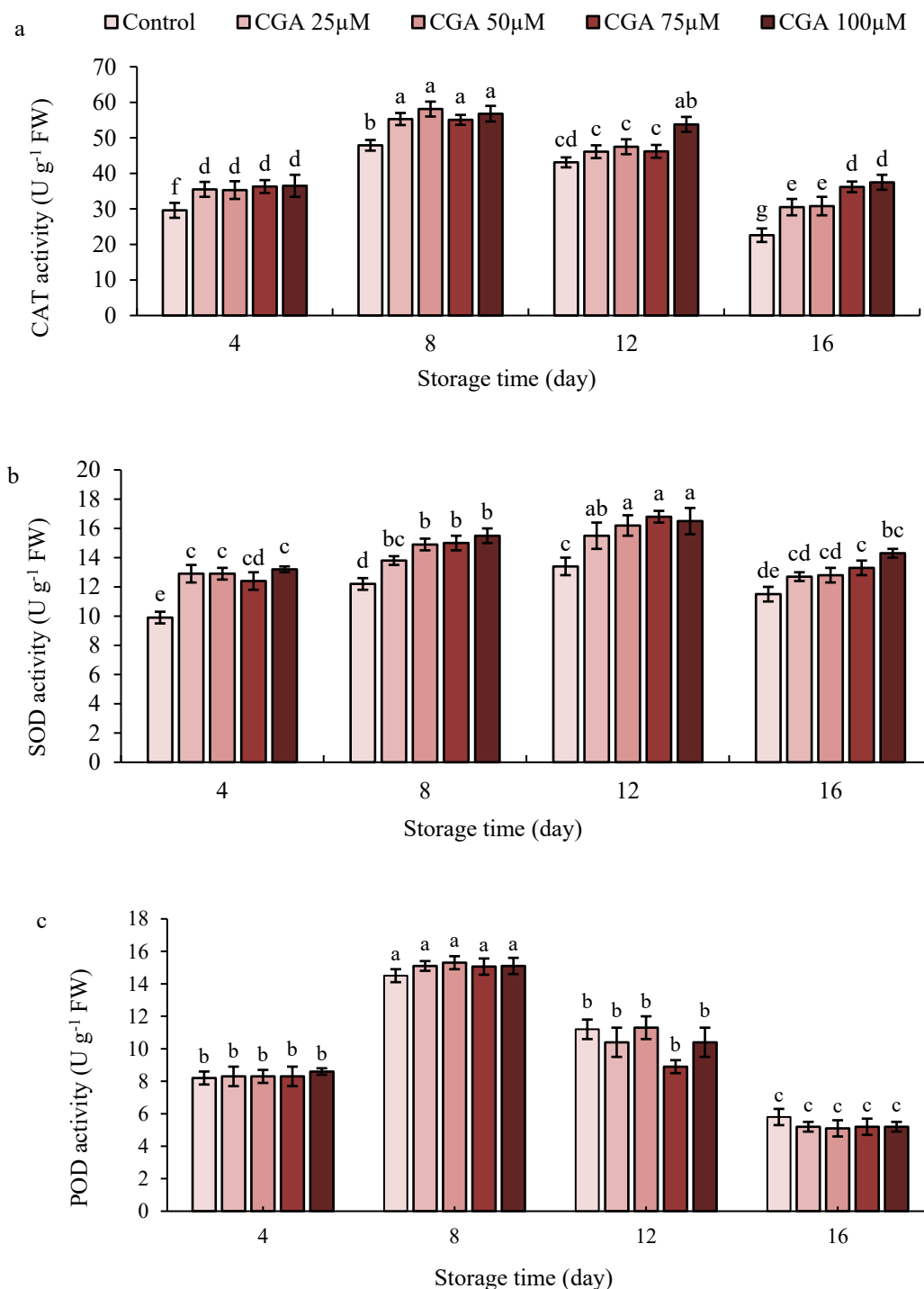


Fig. 6. Effect of CGA on CAT (a), SOD (b), and POD (c) of strawberry fruit during storage. The vertical bars represent the means $\pm$ SE (Duncan's multiple range test, $p < 0.05$, $n=3$).

DISCUSSION

Non-climacteric fruits, such as strawberries, should be harvested at peak ripeness since they do not continue to ripen after being picked. The susceptibility of strawberries to mechanical and physical injuries is attributed to their soft texture and high water content, which create favorable conditions for microorganisms to enter the fruit. Postharvest fruit firmness, total soluble solids, titratable acidity, and respiration rate play crucial roles in assessing postharvest

fruit senescence, as highlighted by Zhang et al. (2020). This study showed that the application of CGA prevents the softening of strawberries and helps maintain their firmness (Fig. 1a). Water loss and the activity of extracellular pectolytic enzymes on pectin and other cell wall components are primary factors leading to decreased firmness (Jiang et al., 2019). Consistent with this study, Shuang et al. (2022) observed the positive effect of CGA on increasing the firmness of strawberries. Similarly, the average hardness in pears was improved with the use of CGA (Zhang et al., 2023). In the present study, CGA also caused an increase in TA (Fig. 1b), which aligns with the results of Shu et al. (2020a)

The results demonstrated that CGA effectively controlled postharvest decay in strawberries (Fig. 2). The effectiveness of CGA in controlling decay agents in fruits and vegetables has been reported in several studies. For instance, high levels of CGA in kiwifruit, tomato, and potato provided resistance against *Diaporthe* sp. (Zhang et al., 2020), *Alternaria alternata* (Wojciechowska et al., 2014), and *Pectobacterium atrosepticum* (Ngadze et al., 2012), respectively. Additionally, the application of CGA reduced decay in peach fruit caused by *P. expansum* (Jiao et al., 2018). Therefore, the antifungal properties of CGA are significant in slowing the decay process after harvesting horticultural plants.

Our results indicated that CGA treatment significantly increased total phenolics and flavonoid content in strawberries, thereby maintaining high antioxidant activities (Fig. 4a-c). CGA, a major phenolic compound within the hydroxycinnamic acid derivative family, is known for its ability to donate hydrogen and scavenge free radicals (Jiao et al., 2019). Other studies have also reported an increase in phenol and flavonoid levels with CGA application (Zhang et al., 2023). Consequently, the antioxidant properties of fruits during storage can be enhanced through the external application of CGA, as evidenced by other studies (Santana-Gálvez et al., 2017).

The elevated levels of proline observed in CGA-treated fruits (Fig. 5a) suggest that CGA enhances the fruit's physiological response to cold stress. A significant decline in MDA levels, a main product of lipid peroxidation, was also observed in CGA-treated fruits (Fig. 5b), indicating improved tolerance to low temperatures (Shu et al., 2020). EL in untreated fruits increased significantly, whereas CGA treatment reduced EL (Fig. 5c). Mei et al. (2020) documented that external CGA application effectively reduces membrane damage and lipid oxidation in apples. Our findings also indicated that CGA significantly decreased H₂O₂ levels in fruits (Fig. 5d). CGA is known to act as a direct scavenger of H₂O₂, thus preserving cell membrane integrity and maintaining postharvest quality (Zhang et al., 2020). Reduced H₂O₂ accumulation and electrolyte leakage may be associated with elevated flavonoid and phenolic content, enhanced antioxidant enzyme activity, and improved membrane integrity (Aghdam et al., 2025).

The findings demonstrated that CGA significantly enhanced the activity of key antioxidant enzymes, including CAT, SOD, and POD (Fig. 6a-c). These enzymes constitute an essential part of the plants enzymatic defense mechanism, responsible for neutralizing reactive oxygen species (ROS) and preserving fruit tissue integrity during postharvest storage (Asle-Mohammadi et al., 2024). The upregulation of antioxidant enzyme activity observed following CGA treatment is likely a response to elevated ROS levels, triggering a protective reaction by the host cells to alleviate oxidative stress (Zhang et al., 2020). Research indicates that CGA not only influences the activity of enzymes such as SOD, CAT, and glutathione reductase (GR), but also contributes to minimizing ROS-induced cellular damage (Wang et al., 2014). Moreover, exogenously applied CGA has been shown to affect gene expression related to antioxidant defense and phenolic metabolism (Mei et al., 2020). Consistent with these results, earlier studies have reported that CGA treatment can boost antioxidant enzyme activity in various fruits, including nectarine (Xi et al., 2017) and pear (Zhang et al., 2023).

CONCLUSION

The results indicated that fruits treated with chlorogenic acid (CGA) exhibited slower softening and reduced decay symptoms compared to untreated fruits. Treated fruits additionally showed a slower reduction in ascorbic acid content, along with increased levels of total phenolics, flavonoids, and antioxidant activity. Additionally, CGA-treated fruits had higher levels of proline and lower levels of malondialdehyde compared to untreated fruits during storage. CGA-treated fruits showed higher catalase and superoxide dismutase activities, particularly at later storage times, although peroxidase activity did not significantly differ between CGA-treated and untreated fruits throughout the storage period. Overall, the application of 75 and 100 μ M CGA was found to enhance the quality and biochemical properties of strawberries during storage.

Conflict of interest

The authors declare that there is no conflict of interest.

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