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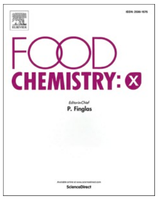

Application of γ-aminobutyric acid improves the postharvest marketability of strawberry by maintaining fruit quality and enhancing antioxidant system

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ABSTRACT

The capability of 5, 10, 15 mM γ-aminobutyric acid (GABA) to improve the postharvest quality and antioxidant system of strawberry was evaluated in this study. The application of GABA had no effect on fruit skin color and firmness. The weight loss in fruits treated with 10 mM GABA was significantly lower than the control. GABA treatments resulted in higher levels of total soluble sugar, titratable acid, SOD and CAT activities with 10 mM being the most significant effect. Specifically, 10 mM GABA significantly induced the accumulation of fructose, oxalic acid, and succinic acid. Besides, GABA application increased the content of total anthocyanins and total flavonoids, and DPPH radical scavenging activity in fruits. The GABA-treated fruits especially at 5 mM and 10 mM displayed less ROS and MDA. These data suggested that application of 10 mM GABA might be a promising strategy to improve the postharvest marketability of strawberry.

1. Introduction

Strawberry is one of the most widely cultivated fruit crops in the world. It is highly favored by customers for its health-promoting properties with bioactive compounds (vitamins, minerals, and flavonoids, etc.), besides excellent taste, and unique flavor. However, strawberry fruits are very perishable, being susceptible to water loss, mechanical damage, and pathogen attack during postharvest storage, which gives a rise to enormous economic losses for the strawberry industry (Zhang et al., 2022). The two commonly used preservation techniques to reduce postharvest decay and prolong shelf life of strawberries are low temperature treatment and fungicide application (Liu et al., 2021). However, low temperature treatment is not sustainable due to energy consumption, high expense, and chilling injuries (Saleem et al., 2021). The use of fungicides can leave residue in fresh produce and cause potential risks to environment and human health (Wang et al., 2021). Hence, it is necessary to develop and apply safe and healthy preservation agents to maintain the quality and extend the shelf life of strawberry fruit.

γ-aminobutyric acid (GABA) is a four-carbon, non-proteinogenic amino acid, and ubiquitously present in plants and animals, which has been gaining much attention due to its health-promoting function (Nikmaram et al., 2017). In higher plants, γ-aminobutyric acid is mainly metabolized via the GABA shunt, a short pathway involved in the activity of evolutionary-conserved enzymes that bypass two steps of the tricarboxylic acid (TCA) cycle. GABA function as a metabolite and signal molecule in regulating stress tolerance, growth, and development of plant (Khan et al., 2021; Kinnersley & Turano, 2000; Li, Dou, Zhang, & Wu, 2021). In recent years, GABA is considered as a safe compound and widely used to maintain fresh produce postharvest quality during storage. Multiple lines of evidence have shown that GABA treatments decrease decay rate, coordinate C/N metabolism, mitigate oxidative damage to improve the storage performance of fruits (Asgarian, Karimi, Ghabooli, & Maleki, 2022; Rastegar, Khankahdani, & Rahimzadeh,

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Abbreviations: ROS, reactive oxygen species; MDA, malondialdehyde; SOD, superoxide dismutase; CAT, catalase; DPPH, 1,1-Diphenyl-2-picryl-hydrazyl; FRAP, ferric reducing antioxidant power.

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2020; Sheng et al., 2017). However, there have been no reports about the effects of GABA treatments on strawberry postharvest production.

In this study, we investigated the influence of exogenous γ-aminobutyric acid on outer appearance, inner quality, and antioxidant system of strawberries during storage, aiming to provide an alternative strategy to reinforce stress tolerance, improve storage quality, and prolong shelf life of postharvest strawberry fruit.

2. Materials and methods

2.1. Chemical reagents

The main chemicals used in this experiment included γ-aminobutyric acid (AR, Macklin, Shanghai, China), anthrone (AR, Kefeng, Shanghai, China), ethyl acetate (AR, Xilong, Guangdong, China), Sodium hydroxide (AR, Keshi, Chengdu, China), sucrose (standard products, Yuanye, Shanghai, China), glucose (standard products, Yuanye, Shanghai, China), fructose (standard products, Yuanye, Shanghai, China), oxalic acid (standard products, Yuanye, Shanghai, China), succinic acid (standard products, Yuanye, Shanghai, China), quinic acid (standard products, Yuanye, Shanghai, China), shikimic acid (standard products, Yuanye, Shanghai, China), citric acid (standard products, Yuanye, Shanghai, China), malic acid (standard products, Yuanye, Shanghai, China), acetonitrile (GR, Knowles, Chengdu, China), quercetin (AR, Solarbio, Beijing, China), gallic acid (AR, Keshi, Chengdu, China), Sodium nitrite (AR, Keshi, Chengdu, China), Nitro blue tetrazolium (BR, Yuanye, Shanghai, China), 1,1-Diphenyl-2-picryl-hydrazyl (BR, Yuanye, Shanghai, China), 2,3,5-triphenyltetrazolium chloride (AR, Solarbio, Beijing, China).

2.2. Plant material and experiment design

Commercially mature fruits of strawberry (*Fragaria* × *ananassa* Duch, 'Benihoppe') were harvested from a local farm in Chengdu, PR China on 15 January 2022, and delivered to the laboratory within one hour. Only 150 fruits of uniform color and size and without any visible defects were selected to perform postharvest treatments in this experiment. Subsequently, 30 fruits were stored for analysis on the harvest day (0 d), while other 120 fruits were randomly segmented into four groups and immersed in 0 (control), 5, 10, 15 mM GABA solution for 15 min. Fruits were put into multi-cell plastic tray after air-drying and stored at 4 °C with 85 % relative humidity for 12 days. Each treatment included 3 replicates with 10 fruits per replicate.

2.3. Sensory evaluation

A generic descriptive analysis method was used to characterize strawberry sensory qualities. A panel of eight assessors were trained to evaluate the two attributes, namely, color and overall visual quality, which were assigned a score between 0 and 5, as described by Pang et al. (2020). Sensory evaluation was performed after 12 days of storage. Subsequently, the strawberries were sampled to determine other indicators.

2.4. Determination of skin color, firmness, and weight loss

The external color of strawberries was determined using a CR-400 chromometer (Konica Minolta, Japan) to obtain a* (redness), b* (yellowness) and L* (brightness) values, according to Jiang et al. (2023) method. Firmness was evaluated using a WDGY-4 digital fruit sclerometer (Beijing, China) and defined as newton (N). The weight loss was computed as the percentage (%) of decrease in weight that weight on each sampling day relative to the original weight.

2.5. Determination of soluble sugar and titratable acidity

Ground fruit tissue (0.2 g) was homogenized with 2 mL of distilled water. After centrifugation, 1 mL of clear solution was diluted 10-fold for further analysis. The measurement of soluble sugar was conducted using the protocol outlined by Jiang et al. (2023). Titratable acidity was measured titrimetrically with 0.1 M sodium hydroxide (NaOH) and expressed as percentage citric acid equivalents. The sugar-acid ratio was determined according to the formula: sugar-acid ratio = soluble sugar/ titratable acidity.

2.6. Determination of soluble sugar and organic acid components

The components of soluble sugars and organic acids were extracted as previously described with some modifications (Liu, Zhang, & Zhao, 2013). Fruit sample was pulverized in liquid N_2. One gram of fine powder was homogenized in 5 mL ultrapure water. After ultrasound-assisted extraction in ice-water bath for 30 min and centrifugation at 10,000 g for 10 min, the clear liquid was separated from the residue. The residue was repeated the extraction with 4 mL ultrapure water. The resulting clear liquid was diluted to 10 mL in volumetric flask and filtered using a 0.22 μm syringe filter for high-performance liquid chromatography (HPLC) analysis.

The chromatographic separation of soluble sugars and organic acids employed the method of Zheng, Zhang, Quan, Zheng, and Xi (2016) with some modifications. The soluble sugar in the samples was analyzed using an Agilent 1260 system with a refractive index detector (RID) and a CNW NH2-RP column (250 × 4.6 mm, 5 μm). The temperature of detector and column was respectively set as 40 °C and 30 °C. The mobile phase consisted of 80 % acetonitrile plus 20 % water (v/v) with a flow rate of 0.5 mL min^{-1} and injection volume of 20 μL. The organic acid was measured by an Agilent 1260 instrument. Chromatographic separation was conducted with a Silgreen C18 column (250 × 4.6 mm, 5 μm) at 25 °C and a variable wavelength detector (VWD) at 210 mm. The mobile phase was 0.5 % (w/v) ammonium dihydrogen phosphate (pH 2.7) and acetonitrile (99:1, v/v) with flow rate of 1.0 mL min^{-1}. The injection volume was 10 μL.

2.7. Determination of AsA, TAC, TPC, and TFC

Sample powder (0.4 g) was suspended with 2 mL 5 % (w/v) trichloroacetic acid, and then spined at 8000 g for 20 min at 4 °C. The upper phase was pooled and used to measure ascorbic acid (AsA) content according to the method of Jiang et al. (2023). AsA content was read at 534 nm and expressed as g AsA kg^{-1} FW. Each sample (1.0 g) was weighed out and dissolved in 10 mL extraction solvent consisting of methanol, acetone, deionized water and glacial acetic acid (4:4:2:1, v/v/v/v). After centrifugation, total anthocyanin content (TAC) in the clear solution was quantified according to the pH differential method (Jiang et al., 2023) and expressed as g pelargonidin 3-glucoside equivalent kg^{-1} FW. One gram of finely ground sample was extracted with 5 mL 80 % (v/v) acetone and shaken well at 10 min intervals. After one hour, the supernatant was separated by centrifugation for 10 min at 5000 g for total phenolic content (TPC) and total flavonoid content (TFC) measurement. TFC and TPC were respectively evaluated using aluminium chloride colorimetric assay and Folin–Ciocalteu method as described by Jiang et al. (2023) and calculated as g quercetin equivalent kg^{-1} FW and g gallic acid equivalent kg^{-1} FW.

2.8. Determination of ROS and MDA

Fine powder of each sample (0.1 g) was extracted with 1 mL of cold phosphate buffer (50 mM, pH 7.8, 1 % PVP). The suspension was spined at 8000 g for 20 min at 4 °C. Production rate of superoxide anion ($O_2^{\bar{}}$) was determined by detecting the formation of nitrite from hydroxylamine in the presence of superoxide anion according to the method of

Jiang et al. (2023). Sodium nitrite was used to construct calibration curve for calculation of production rate of O_2^{-} and the result was expressed as nmol min^{-1} g^{-1} FW. Fruit tissue (0.1 g) was extracted with 1 mL acetone and centrifuged for 10 min at 4 °C and 10,000 g. Then clear solution was collected to determine the H_2O_2 content with a commercial kit (G0112F, Suzhou Grace Biotechnology Co. Ltd., Suzhou, China) according to the user's manual. The result was expressed as μmol g^{-1} FW. Fruit sample (0.1 g) was homogenized with 1 mL of 10 % trichloroacetic acid (TCA). The mixture was spined at 10,000 g and 4 °C for 10 min. The supernatant was used to detect MDA content according to the protocol described by Jiang et al. (2023). The MDA content was expressed as expressed as μmol kg^{-1} FW.

2.9. Determination of SOD, CAT, DPPH and FRAP

For SOD activity measurement, 0.1 g of fruit sample was homogenized in 1 mL of precooled phosphate buffer (100 mM, pH 7.0, 1 mM EDTA, 4 % PVPP). After centrifugation at 10,000 g and 4 °C for 10 min, the upper phase was remained, and SOD activity was assayed with the method of Zhang et al. (2022). To determine CAT activity, 0.1 g of fruit

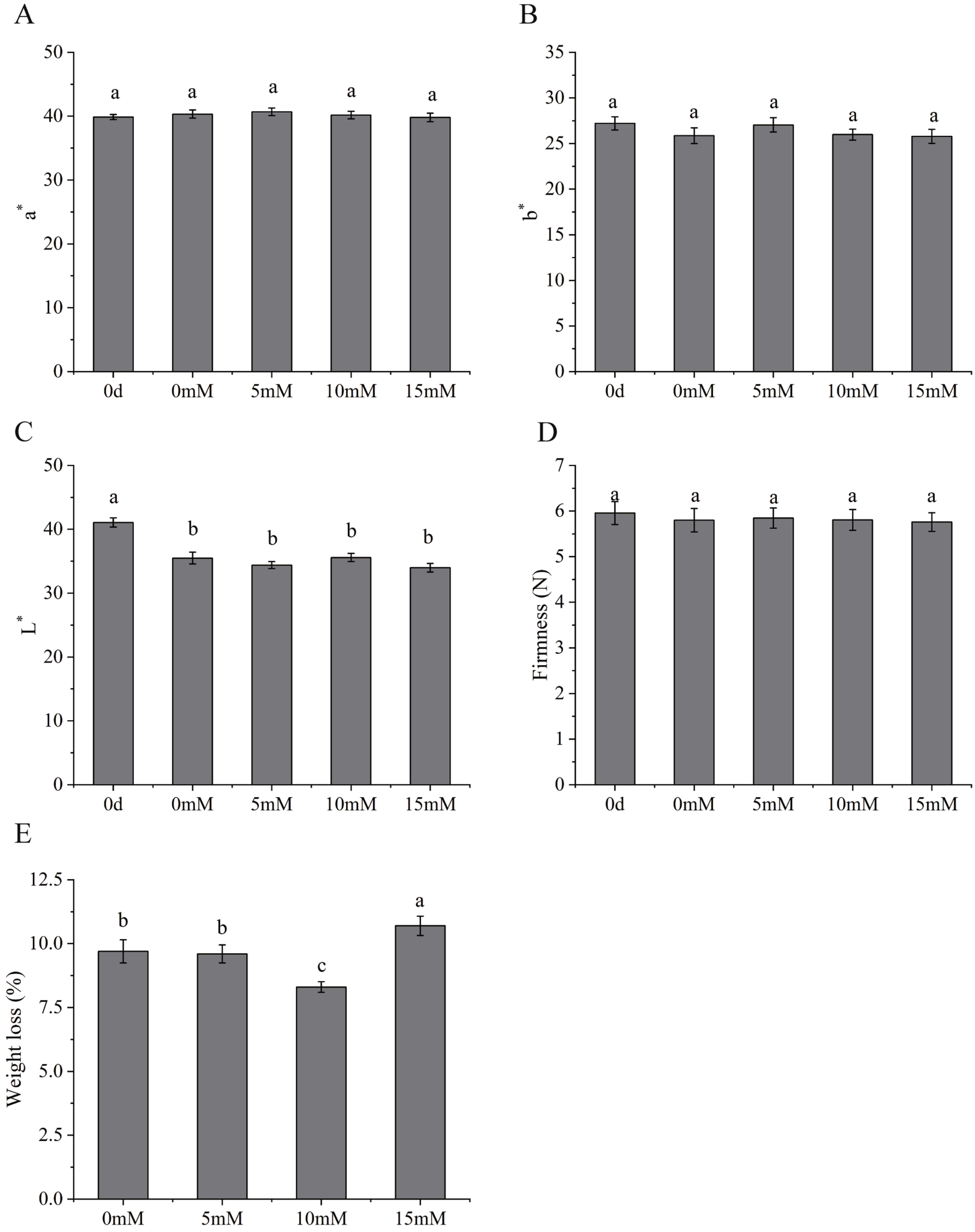

Fig. 1. Skin color (A-C), firmness (D) and weight loss (E) of strawberries after postharvest GABA treatments. The parameter values presented in each figure are indicated as mean ± standard error (n = 3). Different letters denote statistically differences between different treatments (Duncan test, $P \leq 0.05$).

sample was homogenized in 1 mL extraction medium. The clear solution was obtained after centrifugation at 10,000 g and 4 °C for 10 min. The level of CAT activity was measured with a commercial kit (G0106F, Suzhou Grace Biotechnology Co. Ltd., Suzhou, China) according to the user's manual. For total antioxidant activities (DPPH and FRAP) measurement, 0.1 g of fruit tissue was added into the ice-cold 80 % ethanol. After homogenate was spined at 10,000 g and 4 °C for 10 min, the supernatant was collected for further analysis. The extract was respectively mixed with fresh DPPH solution and working FRAP reagent and measure the total antioxidant activities according to the method outlined by Zhang et al. (2022).

2.10. *Statistical analyses*

A completely randomized design (CRD) with three biological replicates was employed in this study. IBM SPSS Statistics 23.0 (SPSS Inc., Chicago, IL, USA) was applied to analyze the data via one-way analysis of (ANOVA) and Duncan's multiple range test. Statistical level of significance was $P \leq 0.05$. All diagrams were plotted with Origin2020 (MicroCal Software Inc., Northampton, MA, USA).

3. Results and discussion

3.1. *Effect of exogenous GABA on sensory property, skin color, firmness, and weight loss*

Skin color was a pivotal visual indicator of maturity and postharvest lifecycle for fresh horticultural crops (Zhang, Jiang, Cao, & Jiang, 2021). Moreover, it generally reflected the changes in chilling injury (CI) index (Elbagoury, Turoop, Runo, & Sila, 2021). In our study, the values of a* and b* had no significant change during storage, while the L* value decreased significantly after 12 days of storage, but no significant difference was found in L* value between GABA treatments and the control (Fig. 1A-C). Moreover, the color score in the sensory evaluation of strawberries showed no significant difference for any of the treatments given (Table. S1). These findings indicated that exogenous GABA did not affect fruit peel color, in line with the investigation of Cheng et al. (2023). Contrary to our results, some reports have demonstrated that the use of GABA could maintain the skin color and retard the chilling injury-induced color change of fresh produce during storage. Wang et al. (2014) found that the peel lightness (L* value) and hue angle (h° value) declined more slowly after the banana was treated with 20 mM GABA. Fan et al. (2022) observed that 1 mM GABA treatment inhibited the decline of hue angle and delayed de-greening of Chinese olives. Ngaffo Mekontso, Duan, Cisse, Chen, and Xu (2021) reported that the carambola fruit treated with 2.5 mM exogenous GABA under cold storage displayed higher L* value and less severe ribs-edge browning, a typical CI symptom. Shang, Cao, Yang, Cai, and Zheng (2011) showed that exogenous GABA had a significant effect on reducing the peach fruit internal browning, and 5 mM GABA was the optimal. Notably, the overall visual quality of strawberries treated with GABA was better than that of control, with the highest score in 10 mM GABA-treated group (Table S1). This result may be due to the influence of GABA on keeping antioxidant activity and preventing the quality deterioration in stored fruits.

The firmness in strawberry was slightly decreased after storage, but no significant difference was observed between GABA treatments and control (Fig. 1D). The experiment conducted by Al Shoffe, Nock, Zhang, and Watkins (2021) showed that pre- and post-harvest GABA application had few significant effects on apple firmness. However, the decrease of kiwifruit firmness was significantly inhibited by 10 mM GABA treatment (Liu et al., 2023). Cornelian cherry fruits treated with 5 mM GABA maintained firmness which may arise from lower cell wall degrading enzymes (Aghdam, Kakavand, Rabiei, Zaare-Nahandi, & Razavi, 2019).

Weight loss of fresh fruit during storage was mainly related to respiration and moisture evaporation through the fruit skin. The thin skin of strawberries allowed a fast water loss, which gave a rise to shriveling, compromised appearance and disorder (Hernandez-Munoz, Almenar, Del Valle, Velez, & Gavara, 2008). It has been reported that GABA could inhibit fruit weight loss by maintaining membrane integrity and reducing respiration rate (Saeedi, Mirdehghan, Nazoori, Esmaeilizadeh, & Saba, 2022; Wang et al., 2014). At the end of the storage, a decline in strawberry weight was observed in all treatments. Compared to the control, 5 mM and 10 mM GABA treatments suppressed the loss of fruit weight, with the most significant effect from 10 mM GABA application. However, weight loss in 15 mM GABA treatment was higher than the control, reaching 10.7 % (Fig. 1E). In pistachio fruit, 10 mM GABA treatment reduced the weight loss during cold storage, but not significantly (Saeedi et al., 2022). In Chinese olives, the lower weight loss was found in all GABA-treated groups, and 1 mM exogenous GABA was most effective (Fan et al., 2022). These findings suggested that the optimal of GABA is variable in diverse plants and an inappropriate concentration might lead to an opposite effect.

3.2. *Changes of soluble sugar components under exogenous GABA treatment*

The amount and composition of soluble sugar largely impact fruit quality, and play multiple roles as energy substance, signal molecule and osmotic regulator (Zhang et al., 2023). The content of soluble sugar was increased after 12 days of storage in all treatments. It was noteworthy that 5 mM and 10 mM exogenous GABA significantly induced the accumulation of soluble sugar compared to the control, but 15 mM exogenous GABA had no better effect than the control (Fig. 2A). The glucose, fructose and sucrose are the predominate sugars in strawberry; glucose and fructose present in similar amount (Akšić et al., 2019). The analysis of sugar components showed that fructose content in samples at day 12 under 5 mM and 10 mM GABA treatment was 1.29- and 1.35- fold higher than that at 0 d, respectively, and 1.23– and 1.28-fold higher than the control, whereas no significant difference was found among 0 d, control, and 15 mM GABA treatment (Fig. 2B). The glucose content was higher in 5 mM and 15 mM GABA treatments than 0 d and control. The application of 10 mM GABA slightly increased the glucose content compared to control and 0 d, but the differences were not statistically significant. Meanwhile, no significant difference was observed in glucose content between 5 mM and 10 mM GABA treatments (Fig. 2C). The sucrose content experienced a significant decline during storage. The application of different concentration of GABA had no effect on sucrose accumulation (Fig. 2D). Our results were similar to the outcomes of the study in GABA-treated grapes, where higher levels of soluble sugar (glucose, fructose and sucrose) and acidity were detected after 60 days storage at 1 °C (Asgarian et al., 2022). There is growing evidence that a high level of soluble sugar contributes to maintain fruit quality, delay fruit senescence, and enhance chilling tolerance during storage (Zhang et al., 2023). It has been reported that GABA treatments delay the decrease of soluble sugar and acidity in fruit during storage probably by inhibiting the respiration rate, since sugars and organic acids are the main substrates of respiration (Asgarian et al., 2022; Han et al., 2018; C. Li et al., 2021).

3.3. *Changes of organic acid components under exogenous GABA treatment*

With the increase of storage time, the content of organic acids reduced in strawberries (Koyuncu & Dilmaçünal, 2010), as documented in current study. After 12 days of storage, the content of titratable acid markedly decreased in 0 mM GABA group (control), but significantly increased in GABA treatment samples, and 10 mM exogenous GABA showed the strongest effect (Fig. 3A). Still, the sugar-acid ratio in postharvest strawberries was significantly higher than the fresh strawberries with no treatment (0 d) (Fig. S1). It has been demonstrated that

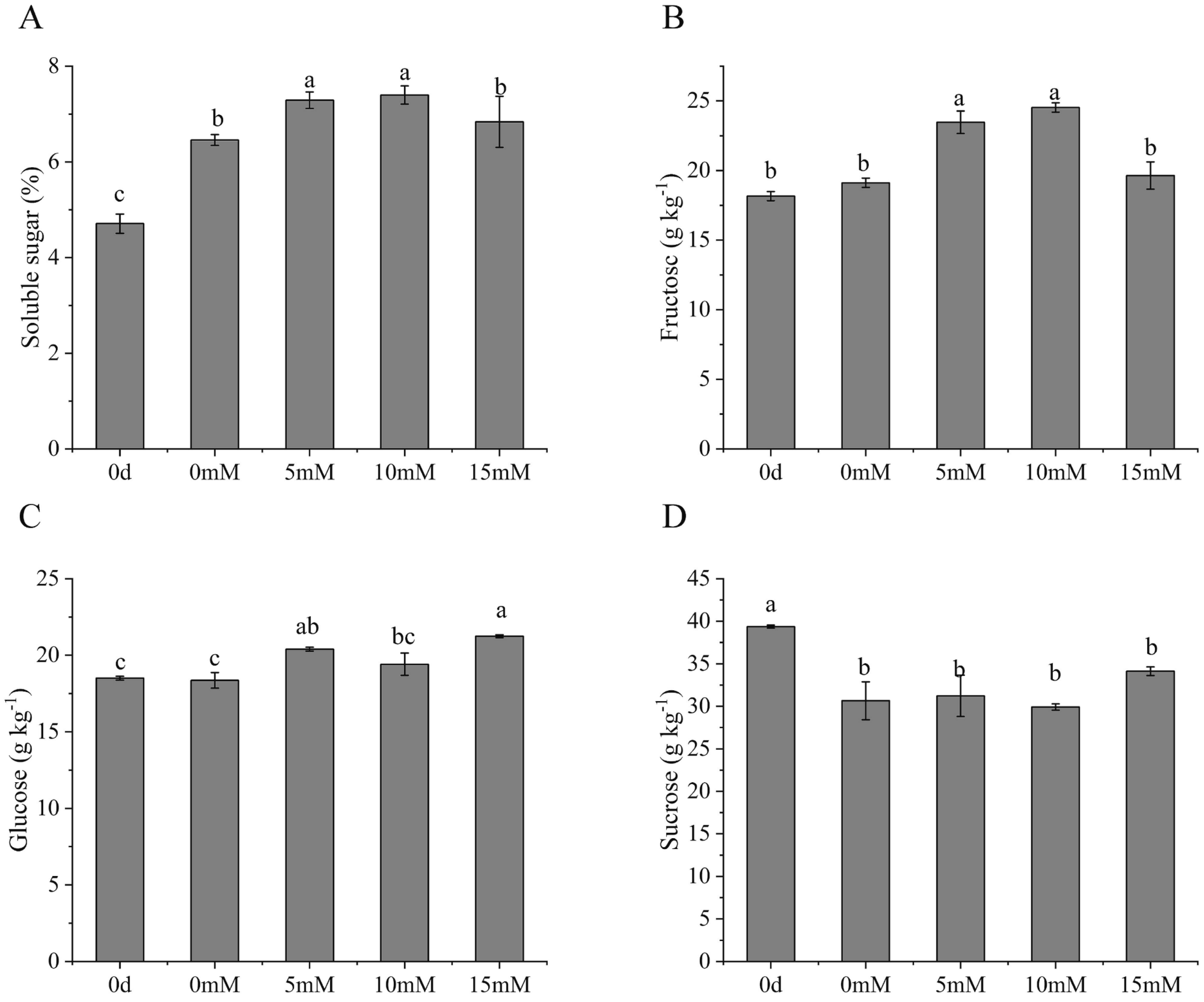

Fig. 2. Soluble sugar contents of strawberries after postharvest GABA treatments. (A) total soluble sugar, (B) fructose, (C) glucose, (D) sucrose. The parameter values presented in each figure are indicated as mean ± standard error (n = 3). Different letters denote statistically differences between different treatments (Duncan test, P ≤ 0.05).

retaining higher levels of both organic acids and soluble sugars in GABA-treated fruits was important since retaining sugar-acid ratio can maintain fruit postharvest quality during cold storage (Asgarian et al., 2022; Shang et al., 2011). HPLC analysis showed that oxalic acid content in the control group had a significant decrease compared to the 0 d, while the fruits treated with GABA increased to some extent, with the most significant increase at 10 mM GABA treatment (Fig. 3B). It was noticeable that 10 mM exogenous GABA also significantly induced the accumulation of succinic acid. However, the content of succinic acid in fruits treated with 5 mM and 15 mM GABA had no significant difference with that of control and 0 d (Fig. 3C). No significant difference was observed in all treatments with respect to contents of quinic acid, shikimic acid, and citric acid (Fig. 3D-F). The malic acid content was significantly decreased after 12 days of storage, and effect of GABA treatments was like that in control (Fig. 3G). It could be shown that GABA treatments, particularly 10 mM GABA application, might upregulate titratable acid content by promoting oxalic acid and succinic acid accumulation in postharvest strawberries. Consistent with our results, a previous study found that 10 mM GABA treatment greatly delayed the loss of titratable acidity in postharvest apples by accelerating malate, succinate and oxalate biosynthesis and inhibiting respiratory metabolism (Han et al., 2018). However, Cheng et al. (2023) investigated the influence of pre-harvest GABA sprays on the harvest indices of apples and found the fruit sprayed with GABA had lower acidity than that in control. Asgarian et al. (2022) proposed that exogenous GABA affect the production of organic acid, probably owing to the association of GABA with the tricarboxylic acid cycle (TCA) cycle.

3.4. Changes of AsA, TAC, TPC, and TFC under exogenous GABA treatment

Ascorbic acid (AsA) is a natural antioxidant that exists in fruit and vegetable, which is generally decreased after prolonged storage (Asgarian et al., 2022; Barbagallo, Chisari, & Patané, 2012; García-Pastor et al., 2020). Tavarini, Degl'Innocenti, Remorini, Massai, and Guidi (2008) demonstrated that the ascorbic acid in fruit after storage could be higher than that in fruit at harvest time, which was closely associated with harvest time. In current study, the content of AsA in all treatments dramatically increased after 12 days of storage, but no significant difference in AsA was found between GABA treatments and control (Fig. 4A). Some research groups have documented that fruit treated with GABA retained ascorbic acid at higher level (Aghdam, Razavi, & Karamneghad, 2016; Asgarian et al., 2022). The evidential increases were shown in total anthocyanin content (TAC) in the GABA-treated and control samples compared to 0 d. However, only 15 mM GABA-treated fruits displayed markedly higher content of total

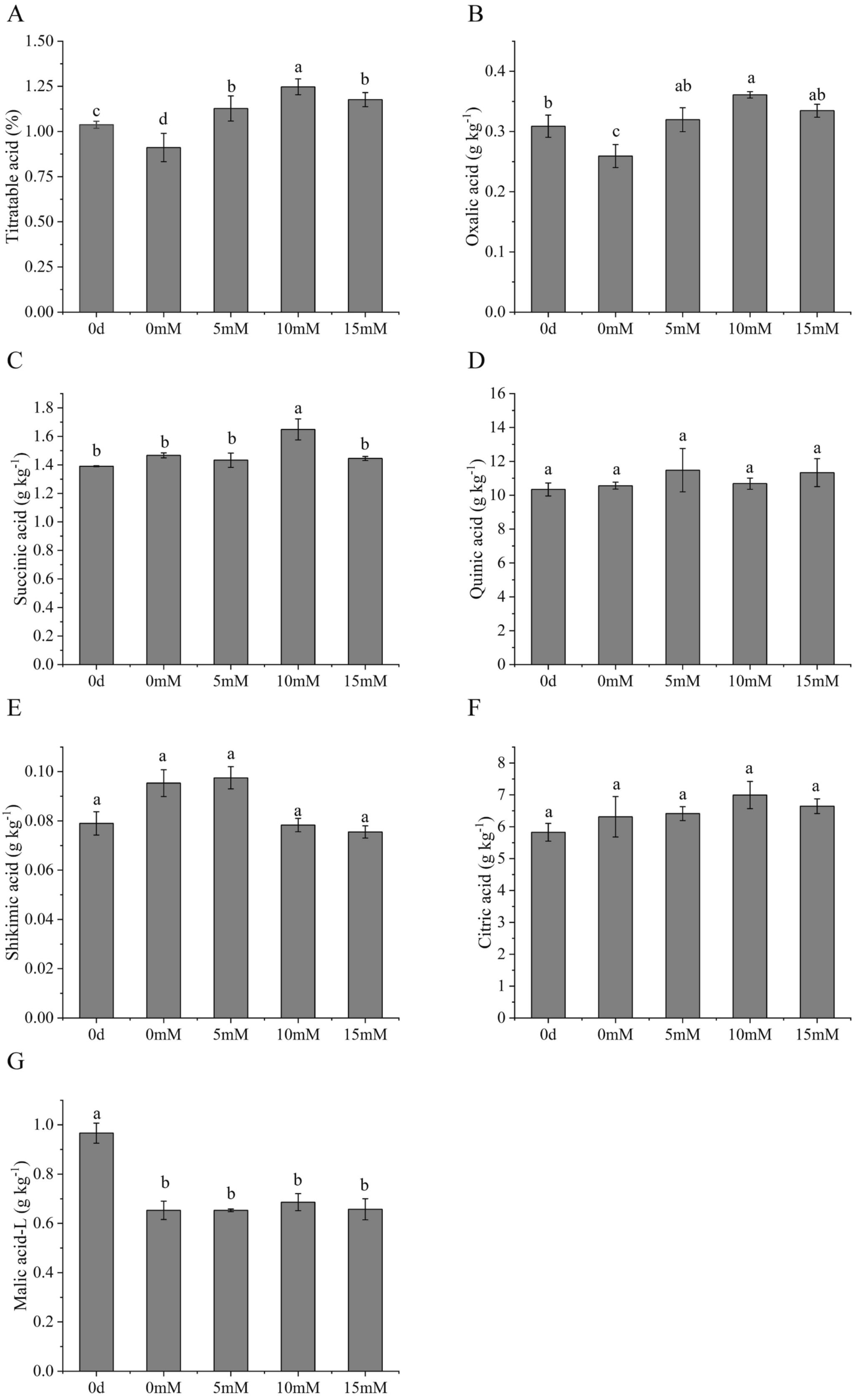

Fig. 3. Organic acid contents of strawberries after postharvest GABA treatments. (A) titratable acid, (B) oxalic acid, (C) succinic acid, (D) quinic acid, (E) shikimic acid, (F) citric acid, (G) malic acid. The parameter values presented in each figure are indicated as mean ± standard error (n = 3). Different letters denote statistically differences between different treatments (Duncan test, $P \leq 0.05$).

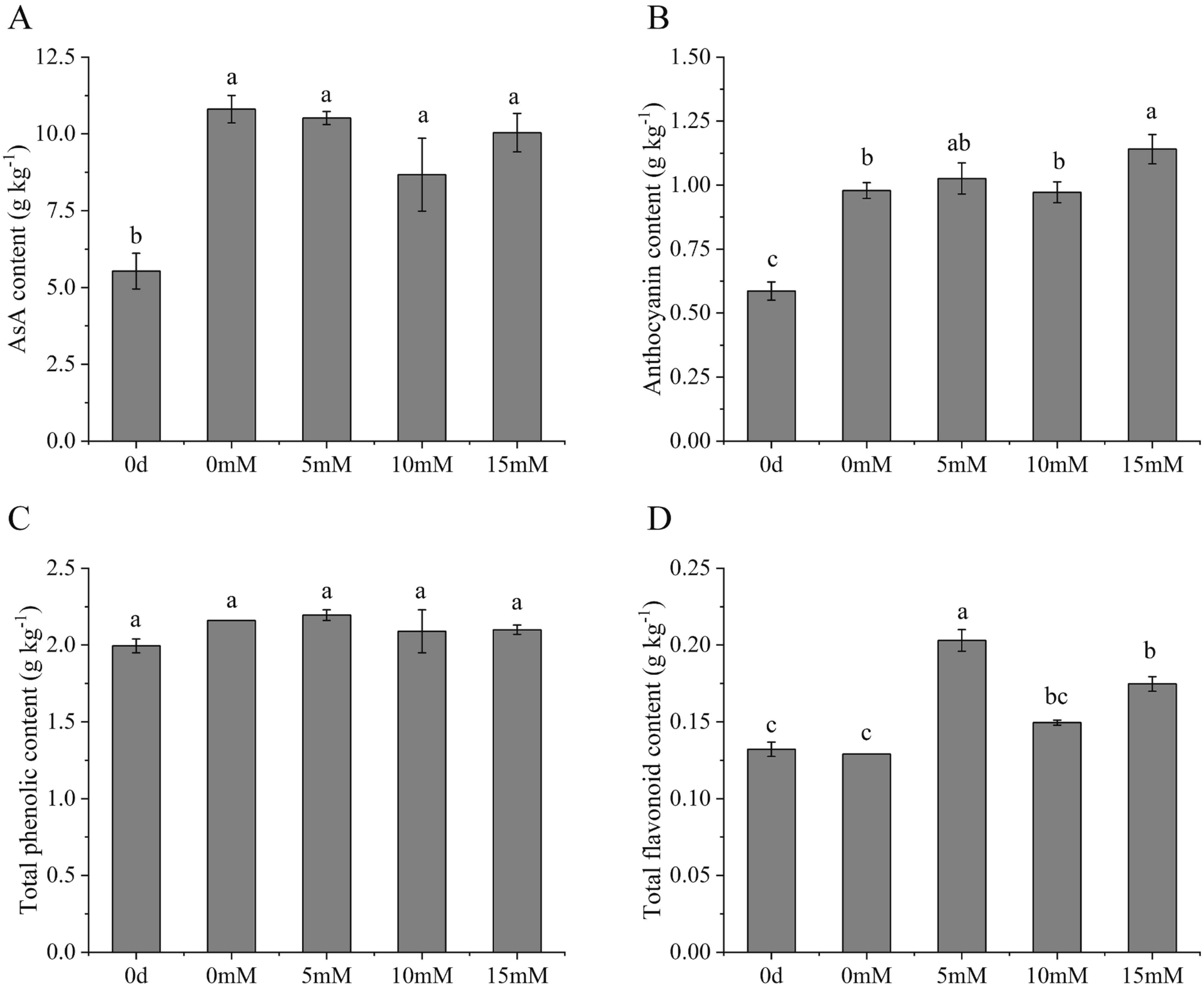

Fig. 4. Ascorbic acid (A), total anthocyanins (B), total phenolics (C), and total flavonoids (D) of strawberries after postharvest GABA treatments. The parameter values presented in each figure are indicated as mean ± standard error (n = 3). Different letters denote statistically differences between different treatments (Duncan test, $P \leq 0.05$).

anthocyanins than that of control (Fig. 4B). In pistachio, 10 mM GABA treatment effectively inhibited the degradation of total chlorophyll in kernel and total anthocyanins in kernel skin with the progress of storage period (Saeedi et al., 2022). There was no noticeable change in total phenolic content (TPC) regardless of storage conditions (Fig. 4C). It was documented that preharvest application of different concentration of GABA progressively increased total phenolics and anthocyanins in pomegranate fruit during storage (Lorente-Mento et al., 2023). The total flavonoid content (TFC) in the control was comparable to that of 0 d. All GABA-treated fruits had higher total flavonoid contents and were increased by 53.85 %, 15.38 %, and 30.77 % compared to the control, respectively (Fig. 4D). Rastegar et al. (2020) proposed that the mechanism of GABA in inducing accumulation of TAC, TPC and TFC during storage may be owing to stimulating related enzyme activities involved in phenylpropanoid pathway.

3.5. *Effect of exogenous GABA on O_2^- production, H_2O_2 and MDA content*

The O_2^- generation rate in samples under all storage conditions was significantly higher than that at 0 d. GABA-treated fruit exhibited approximately production rate to the control (Fig. 5A). The accumulation of H_2O_2 was remarkably enhanced in the control and GABA-treated fruit at the end of storage compared to 0 d. The GABA-treated fruit had significantly lower H_2O_2 content than those of the control, which was 10.32 %, 11.90 % and 4.37 % lower, respectively, in 5 mM, 10 mM and 15 mM GABA treatments (Fig. 5B). As expected, the MDA content was boosted noticeably after storage. The MDA level in the GABA-treated fruit were lower than that in the control, and the lowest content of MDA was obtained in 5 mM GABA treatment, decreased by 15.61 % compared to the control (Fig. 5C). ROS (O_2^-, H_2O_2, etc.) accumulation are induced when the fresh produce undergoes various changes during storage, which gives rise to membrane degradation with MDA production. It has been demonstrated that the application of exogenous GABA can enhance the tolerance of plants against many kinds of abiotic and biotic stresses by suppressing ROS accumulation. In the present study, the content of H_2O_2 and MDA was lower in GABA-treated fruits, suggesting that GABA could effectively relieve oxidative stress in strawberry fruits during storage, in accordance with the argument in other fruits (Liu, Ma, Liu, Liu, & Wang, 2023).

3.6. *Effect of exogenous GABA on antioxidant enzymes and total antioxidant capacity*

Antioxidant enzymes play a pivotal part in alleviating or preventing the damage derived from ROS. The SOD and CAT activities significantly decreased under control and GABA treatments compared to 0 d. The

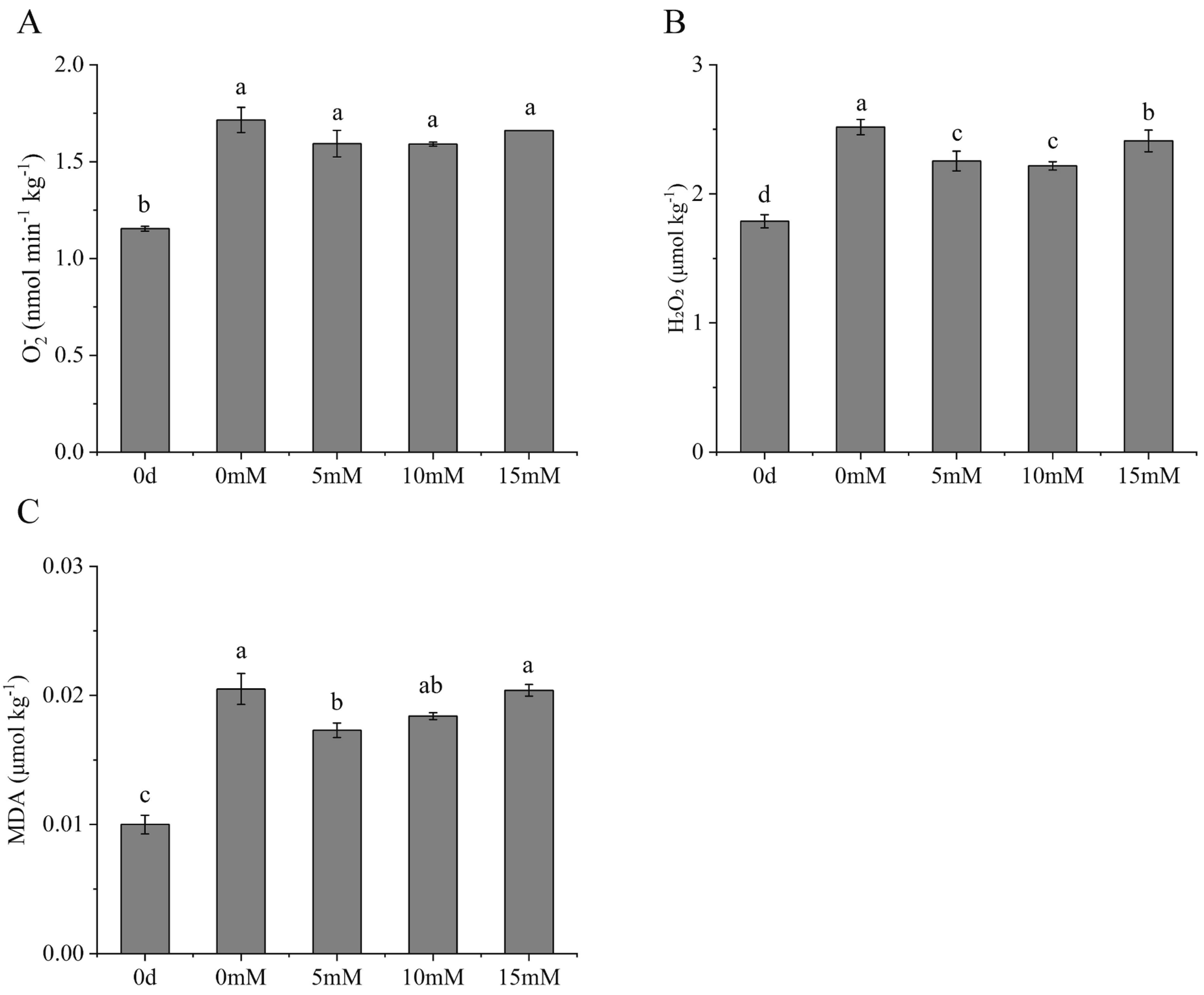

Fig. 5. ROS and MDA contents of strawberries after postharvest GABA treatments. (A) O_2^{-} production rate, (B) H_2O_2 content, (C) MDA content. The parameter values presented in each figure are indicated as mean ± standard error (n = 3). Different letters denote statistically differences between different treatments (Duncan test, $P \leq 0.05$).

activity levels of the two enzymes in GABA-treated samples was higher than those of control. The highest activity level of SOD and CAT was observed in 10 mM GABA treatment, followed by 5 mM and 15 mM GABA treatments (Fig. 6A, B). Interestingly, the total antioxidant activities (DPPH and FRAP) were higher under GABA-treated and control samples than those of 0 d (Fig. 6C, D). Compared to the control, the GABA treatments more effectively increased the DPPH radical scavenging activity, with the most significant effect in 10 mM and 15 mM GABA-treated fruit (Fig. 6C). FRAP values in GABA-treated fruit were approximately equivalent to the control and there was no significant difference (Fig. 6D). In Mango, postharvest application of GABA enhanced DPPH scavenging capacity and activities of CAT and POD during storage, thereby prolonging the fruit postharvest life (Rastegar et al., 2020). In pear, GABA-induced resistance against *P. expansum* in harvested fruit was associated with the enhancement of activities or gene expression of various defense-related enzymes (Yu et al., 2014). In banana, GABA treatment significantly relieved fruit chilling injuring by increasing antioxidant system and promoting proline accumulation (Wang et al., 2014), as reported in peach (Shang et al., 2011) and cucumber (Malekzadeh, Khosravi-Nejad, Hatamnia, & Sheikhakbari Mehr, 2017). These findings have shown that GABA has a remarkable scavenging ability of reactive oxygen species through improving antioxidant system in postharvest fruit.

4. Conclusion

The results of this investigation revealed that application of exogenous GABA could influence the postharvest quality attributes of strawberry. The higher contents of total soluble sugar, titratable acid, total anthocyanins, and total flavonoids, as well as the lower contents of ROS and MDA were observed in GABA-treated fruits. Moreover, GABA treatments enhanced antioxidant capacity by increasing the activities of SOD and CAT, and DPPH radical scavenging activity in strawberries. In general, 10 mM GABA was considered to be optimal concentration to improve storability of strawberry especially due to its excellent performance of increasing sugar and acid contents and decreasing weight loss rate. However, the molecular mechanism underlying GABA-induced postharvest changes in strawberry needs further study.

CRediT authorship contribution statement

Yunting Zhang: Conceptualization, Funding acquisition, Writing – original draft. **Bangyu Lin:** Data curation, Writing – original draft. **Guohao Tang:** Data curation, Methodology, Visualization. **Yan Chen:** Data curation. **Meiyi Deng:** Investigation. **Yuanxiu Lin:** Software. **Mengyao Li:** Software. **Wen He:** Visualization. **Yan Wang:** Data curation. **Yong Zhang:** Formal analysis. **Ya Luo:** Formal analysis. **Qing Chen:** Writing – review & editing. **Xiaorong Wang:** Writing – review &

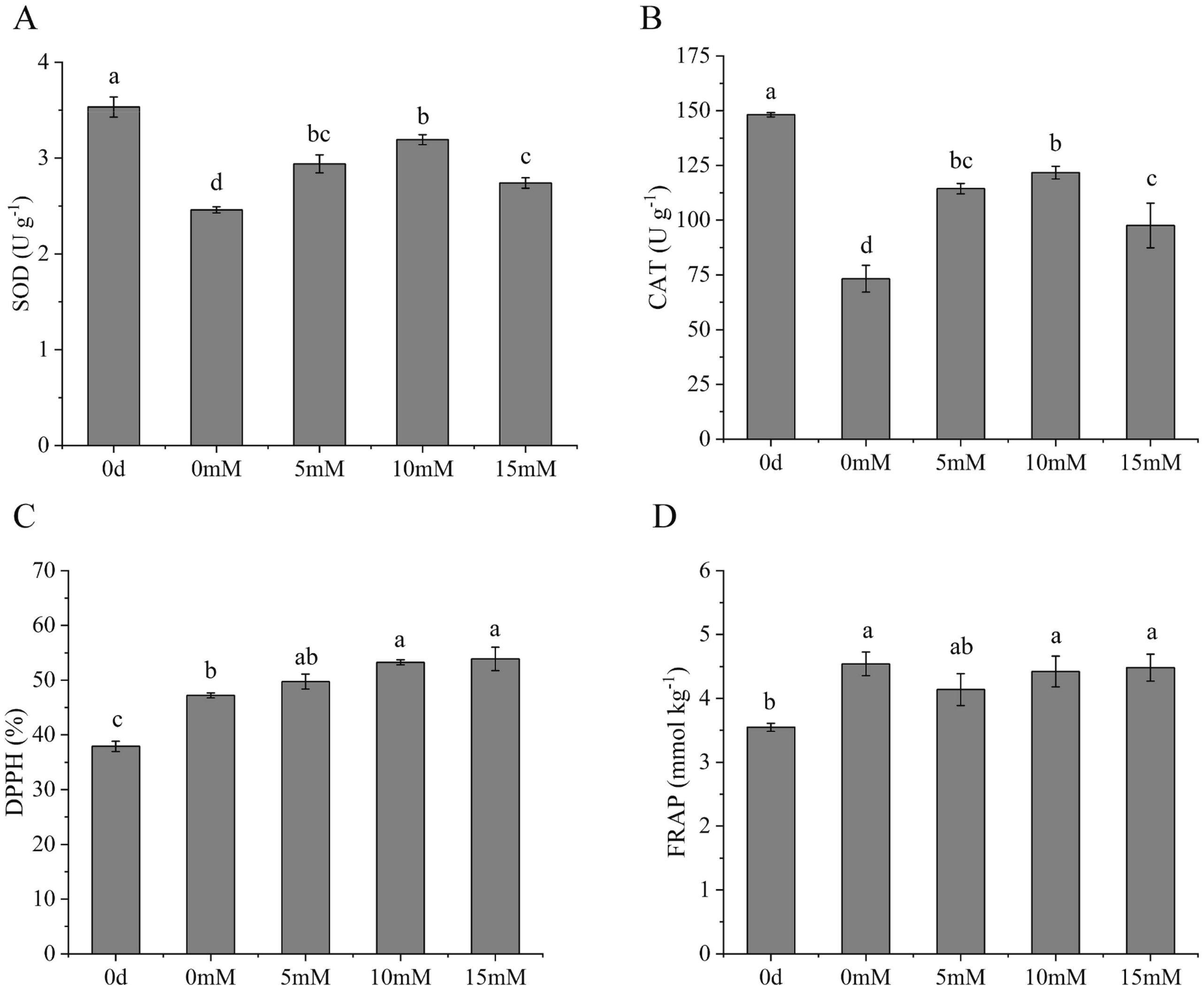

Fig. 6. Antioxidant enzymes and total antioxidant capacity of strawberries after postharvest GABA treatments. (A) SOD activities, (B) CAT activities, (C) DPPH, (D) FRAP. The parameter values presented in each figure are indicated as mean ± standard error (n = 3). Different letters denote statistically differences between different treatments (Duncan test, $P \leq 0.05$).

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101252.

References

Aghdam, M. S., Kakavand, F., Rabiei, V., Zaare-Nahandi, F., & Razavi, F. (2019). γ-Aminobutyric acid and nitric oxide treatments preserve sensory and nutritional quality of cornelian cherry fruits during postharvest cold storage by delaying softening and enhancing phenols accumulation. *Scientia Horticulturae, 246*, 812–817. https://doi.org/10.1016/j.scienta.2018.11.064

Aghdam, M. S., Razavi, F., & Karamneghad, F. (2016). Maintaining the postharvest nutritional quality of peach fruits by γ-aminobutyric acid. *Iranian Journal of Plant Physiology, 5*(4), 1457–1463. https://doi.org/10.30495/ijpp.2015.539673

Akšić, M. F., Tosti, T., Sredojević, M., Milivojević, J., Meland, M., & Natić, M. (2019). Comparison of sugar profile between leaves and fruits of blueberry and strawberry cultivars grown in organic and integrated production system. *Plants, 8*(7), 205. https://doi.org/10.3390/plants8070205

Al Shoffe, Y., Nock, J. F., Zhang, Y., & Watkins, C. B. (2021). Pre-and post-harvest γ-aminobutyric acid application in relation to fruit quality and physiological disorder development in 'honeycrisp'apples. *Scientia Horticulturae, 289*, Article 110431. https://doi.org/10.1016/j.scienta.2021.110431

Asgarian, Z. S., Karimi, R., Ghabooli, M., & Maleki, M. (2022). Biochemical changes and quality characterization of cold-stored 'sahebi'grape in response to postharvest application of GABA. *Food chemistry, 373*, Article 131401. https://doi.org/10.1016/j.foodchem.2021.131401

Barbagallo, R. N., Chisari, M., & Patané, C. (2012). Polyphenol oxidase, total phenolics and ascorbic acid changes during storage of minimally processed 'California wonder'and 'quadrato D'Asti'sweet peppers. *LWT-Food Science and Technology, 49*(2), 192–196. https://doi.org/10.1016/j.lwt.2012.06.023

Cheng, P., Yue, Q., Zhang, Y., Zhao, S., Khan, A., Yang, X., et al. (2023). Application of γ-aminobutyric acid (GABA) improves fruit quality and rootstock drought tolerance in apple. *Journal of Plant Physiology, 280*, Article 153890. https://doi.org/10.1016/j.jplph.2022.153890

Elbagoury, M. M., Turoop, L., Runo, S., & Sila, D. N. (2021). Regulatory influences of methyl jasmonate and calcium chloride on chilling injury of banana fruit during cold storage and ripening. *Food Science & Nutrition, 9*(2), 929–942. https://doi.org/10.1002/fsn3.2058

Fan, Z., Lin, B., Lin, H., Lin, M., Chen, J., & Lin, Y. (2022). γ-Aminobutyric acid treatment reduces chilling injury and improves quality maintenance of cold-stored chinese olive fruit. *Food Chemistry: X, 13*, Article 100208. https://doi.org/10.1016/j.fochx.2022.100208

García-Pastor, M. E., Zapata, P. J., Castillo, S., Martínez-Romero, D., Guillén, F., Valero, D., et al. (2020). The effects of salicylic acid and its derivatives on increasing pomegranate fruit quality and bioactive compounds at harvest and during storage. *Frontiers in Plant Science, 11*, 668. https://doi.org/10.3389/fpls.2020.00668

Han, S., Nan, Y., Qu, W., He, Y., Ban, Q., Lv, Y., et al. (2018). Exogenous γ-aminobutyric acid treatment that contributes to regulation of malate metabolism and ethylene synthesis in apple fruit during storage. *Journal of agricultural and food chemistry, 66* (51), 13473–13482. https://doi.org/10.1021/acs.jafc.8b04674

Hernandez-Munoz, P., Almenar, E., Del Valle, V., Velez, D., & Gavara, R. (2008). Effect of chitosan coating combined with postharvest calcium treatment on strawberry (Fragaria× ananassa) quality during refrigerated storage. *Food Chemistry, 110*(2), 428–435. https://doi.org/10.1016/j.foodchem.2008.02.020

Jiang, L., Chen, X., Gu, X., Deng, M., Li, X., Zhou, A., et al. (2023). Light quality and sucrose-regulated detached ripening of strawberry with possible involvement of abscisic acid and auxin signaling. *International Journal of Molecular Sciences, 24*(6), 5681. https://doi.org/10.3390/ijms24065681

Khan, M. I. R., Jalil, S. U., Chopra, P., Chhillar, H., Ferrante, A., Khan, N. A., et al. (2021). Role of GABA in plant growth, development and senescence. *Plant Gene, 26*, Article 100283. https://doi.org/10.1016/j.plgene.2021.100283

Kinnersley, A. M., & Turano, F. J. (2000). Gamma aminobutyric acid (GABA) and plant responses to stress. *Critical Reviews in Plant Sciences, 19*(6), 479–509. https://doi.org/10.1080/07352680091139277

Koyuncu, M. A., & Dilmaçünal, T. (2010). Determination of vitamin C and organic acid changes in strawberry by HPLC during cold storage. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 38*(3), 95–98. https://doi.org/10.15835/nbha3834819

Li, C., Zhu, J., Sun, L., Cheng, Y., Hou, J., Fan, Y., et al. (2021). Exogenous γ-aminobutyric acid maintains fruit quality of apples through regulation of ethylene anabolism and polyamine metabolism. *Plant Physiology and Biochemistry, 169*, 92–101. https://doi.org/10.1016/j.plaphy.2021.11.008

Li, L., Dou, N., Zhang, H., & Wu, C. (2021). The versatile GABA in plants. *Plant signaling & behavior, 16*(3), 1862565. https://doi.org/10.1080/15592324.2020.1862565

Liu, C., Jin, T., Liu, W., Hao, W., Yan, L., & Zheng, L. (2021). Effects of hydroxyethyl cellulose and sodium alginate edible coating containing asparagus waste extract on postharvest quality of strawberry fruit. *LWT, 148*, Article 111770. https://doi.org/10.1016/j.lwt.2021.111770

Liu, Q., Li, X., Jin, S., Dong, W., Zhang, Y., Chen, W., et al. (2023). γ-Aminobutyric acid treatment induced chilling tolerance in postharvest kiwifruit (Actinidia chinensis cv. hongyang) via regulating ascorbic acid metabolism. *Food chemistry, 404*, Article 134661. https://doi.org/10.1016/j.foodchem.2022.134661

Liu, X., Ma, H., Liu, J., Liu, D., & Wang, C. (2023). The γ-aminobutyric acid (GABA) synthesis gene regulates the resistance to water Core-induced hypoxia stress for pear fruits. *Agronomy, 13*(4), 1062. https://doi.org/10.3390/agronomy13041062

Liu, Y., Zhang, X., & Zhao, Z. (2013). Effects of fruit bagging on anthocyanins, sugars, organic acids, and color properties of 'granny smith'and 'Golden delicious' during fruit maturation. *European Food Research and Technology, 236*, 329–339. https://doi.org/10.1007/s00217-012-1896-3

Lorente-Mento, J. M., Valero, D., Martínez-Romero, D., Badiche, F., Serrano, M., & Guillén, F. (2023). Preharvest multiple applications of GABA improve quality traits and antioxidant compounds of pomegranate fruit during storage. *Horticulturae, 9*(5), 534. https://doi.org/10.3390/horticulturae9050534

Malekzadeh, P., Khosravi-Nejad, F., Hatamnia, A. A., & Sheikhakbari Mehr, R. (2017). Impact of postharvest exogenous γ-aminobutyric acid treatment on cucumber fruit in response to chilling tolerance. *Physiology and Molecular Biology of Plants, 23*, 827–836. https://doi.org/10.1007/s12298-017-0475-2

Ngaffo Mekontso, F., Duan, W., Cisse, E. H. M., Chen, T., & Xu, X. (2021). Alleviation of postharvest chilling injury of carambola fruit by γ-aminobutyric acid: Physiological, biochemical, and structural characterization. *Frontiers in Nutrition, 8*, Article 752583. https://doi.org/10.3389/fnut.2021.752583

Nikmaram, N., Dar, B., Roohinejad, S., Koubaa, M., Barba, F. J., Greiner, R., et al. (2017). Recent advances in γ-aminobutyric acid (GABA) properties in pulses: An overview. *Journal of the Science of Food and Agriculture, 97*(9), 2681–2689. https://doi.org/10.1002/jsfa.8283

Pang, L., Wu, Y., Pan, Y., Ban, Z., Li, L., & Li, X. (2020). Insights into exogenous melatonin associated with phenylalanine metabolism in postharvest strawberry. *Postharvest Biology and Technology, 168*, Article 111244.

Rastegar, S., Khankahdani, H. H., & Rahimzadeh, M. (2020). Effect of γ-aminobutyric acid on the antioxidant system and biochemical changes of mango fruit during storage. *Journal of Food Measurement and Characterization, 14*(2), 778–789. https://doi.org/10.1007/s11694-019-00326-x

Saeedi, M., Mirdehghan, S. H., Nazoori, F., Esmaeilizadeh, M., & Saba, M. K. (2022). Impact of calcium and γ-aminobutyric acid (GABA) on qualitative attributes and shelf life characteristics of fresh in-hull pistachio during cold storage. *Postharvest Biology and Technology, 187*, Article 111863. https://doi.org/10.1016/j.postharvbio.2022.111863

Saleem, M. S., Anjum, M. A., Naz, S., Ali, S., Hussain, S., Azam, M., et al. (2021). Incorporation of ascorbic acid in chitosan-based edible coating improves postharvest quality and storability of strawberry fruits. *International Journal of Biological Macromolecules, 189*, 160–169. https://doi.org/10.1016/j.ijbiomac.2021.08.051

Shang, H., Cao, S., Yang, Z., Cai, Y., & Zheng, Y. (2011). Effect of exogenous γ-aminobutyric acid treatment on proline accumulation and chilling injury in peach fruit after long-term cold storage. *Journal of Agricultural and Food Chemistry, 59*(4), 1264–1268. https://doi.org/10.1021/jf104424z

Sheng, L., Shen, D., Luo, Y., Sun, X., Wang, J., Luo, T., et al. (2017). Exogenous γ-aminobutyric acid treatment affects citrate and amino acid accumulation to improve fruit quality and storage performance of postharvest citrus fruit. *Food Chemistry, 216*, 138–145. https://doi.org/10.1016/j.foodchem.2016.08.024

Tavarini, S., Degl'Innocenti, E., Remorini, D., Massai, R., & Guidi, L. (2008). Antioxidant capacity, ascorbic acid, total phenols and carotenoids changes during harvest and after storage of Hayward kiwifruit. *Food Chemistry, 107*(1), 282–288. https://doi.org/10.1016/j.foodchem.2007.08.015

Wang, F., Xiao, J., Zhang, Y., Li, R., Liu, L., & Deng, J. (2021). Biocontrol ability and action mechanism of bacillus halotolerans against Botrytis cinerea causing grey mould in postharvest strawberry fruit. *Postharvest Biology and Technology, 174*, Article 111456. https://doi.org/10.1016/j.postharvbio.2020.111456

Wang, Y., Luo, Z., Huang, X., Yang, K., Gao, S., & Du, R. (2014). Effect of exogenous γ-aminobutyric acid (GABA) treatment on chilling injury and antioxidant capacity in banana peel. *Scientia Horticulturae, 168*, 132–137. https://doi.org/10.1016/j.scienta.2014.01.022

Yu, C., Zeng, L., Sheng, K., Chen, F., Zhou, T., Zheng, X., et al. (2014). γ-Aminobutyric acid induces resistance against penicillium expansum by priming of defence responses in pear fruit. *Food chemistry, 159*, 29–37. https://doi.org/10.1016/j.foodchem.2014.03.011

Zhang, W., Jiang, H., Cao, J., & Jiang, W. (2021). Advances in biochemical mechanisms and control technologies to treat chilling injury in postharvest fruits and vegetables. *Trends in Food Science & Technology, 113*, 355–365. https://doi.org/10.1016/j.tifs.2021.05.009

Zhang, Y., Li, S., Deng, M., Gui, R., Liu, Y., Chen, X., et al. (2022). Blue light combined with salicylic acid treatment maintained the postharvest quality of strawberry fruit during refrigerated storage. *Food Chemistry: X, 15*, Article 100384. https://doi.org/10.1016/j.fochx.2022.100384

Zhang, Y., Tang, H., Lei, D., Zhao, B., Zhou, X., Yao, W., et al. (2023). Exogenous melatonin maintains postharvest quality in kiwiberry fruit by regulating sugar metabolism during cold storage. *LWT, 174*, Article 114385. https://doi.org/10.1016/j.lwt.2022.114385

Zheng, H., Zhang, Q., Quan, J., Zheng, Q., & Xi, W. (2016). Determination of sugars, organic acids, aroma components, and carotenoids in grapefruit pulps. *Food Chemistry, 205*, 112–121. https://doi.org/10.1016/j.foodchem.2016.03.007