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Impact of exogenous GABA treatments on Decay, Senescence, and Antioxidant Capacity in Strawberry Fruit

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Abstract

γ-Aminobutyric acid (GABA), a plant growth regulator and bioactive compound, plays an important role in fruits. The presented study aimed to investigate the impact of applying γ-aminobutyric acid (GABA) externally on strawberry fruit's quality and postharvest shelf-life. To determine the most effective GABA concentration, strawberries were treated with 0, 2, 5, and 10 mmol. L^{-1} GABA for five minutes, followed by storage at 90% relative humidity at 4 °C for 12 days. The findings revealed that the use of GABA in concentrations of 5 mM have a significant effect on reducing fruit decay and weight loss. Moreover, treatment with 5 or 10 mmol. L^{-1} of GABA noticeably delayed senescence of strawberry fruit. GABA treatments at 5 or 10 mmol. L^{-1} resulted in a significant decrease in the accumulation of hydrogen peroxide (H_2O_2) and Malondialdehyde (MDA), while it promoted the accumulation of phenols and flavonoids, which ultimately enhanced the antioxidant capacity. The study also demonstrated that exogenous GABA treatment positively influences the concentration of endogenous GABA and total anthocyanin content. Ascorbic acid content was not significantly affected by GABA treatment. Overall, the optimal GABA concentration for enhancing strawberry quality and extending the postharvest shelf-life of the fruit was determined to be 5 mmol. L^{-1} .

Keywords: Antioxidant capacity, Ascorbic acid, γ-aminobutyric acid, phenolic compound Strawberry.

1. Introduction

Strawberry fruit, classified as a type of berry, is commonly enjoyed in its fresh form or used in various processed products. It boasts a wealth of nutrients, including glucose, vitamins, minerals, and bioactive compounds such as carotenoids, ascorbic acid, folate, and phenolic compounds. Many of these compounds serve as natural antioxidants, enhancing the nutritional value and quality of the fruit (Fan et al., 2009).

These compounds collectively and synergistically contribute to human health by promoting wellness and preventing diseases (Pombo, Rosli, Martínez, & Civello, 2011). Strawberry fruit is considered as a non-climacteric fruit, and for optimal marketing quality, it is crucial to harvest them when they are fully ripe (Ali, Hatamnia, Malekzadeh, Sayyari, & Aghdam, 2023; C. Lee, Lee, & Lee, 2022; Promyou, Raruang, & Chen, 2023). Strawberry fruit is highly perishable due to its characteristics such as high respiration rate, low mechanical strength, and susceptibility to pathogen attacks. As a result, undesirable changes can occur during postharvest handling, including drying, loss of flesh smoothness, and mechanical damage (Alaei & Mahna, 2023). Therefore, there is an urgent need to reduce decay and increase the preservation post-harvesting strawberries, and until now, numerous treatments have been used post-harvesting such as covering by chitosan, covering with calcium, salicylic acid treatment, UV-C radiation, and ultrasonic treatment in order postharvest preservation of the strawberry fruit. However, some of these methods are not logical from commercial aspect regarding customers' lower priority or need for confirmation of their effectiveness (Li et al., 2021; Malekzadeh, 2024; Malekzadeh, Hatamnia, & Tiznado-Hernández, 2023; Pombo et al., 2011). The conventional approach to controlling postharvest decay and spoilage in strawberries often involves the use of various fungicides. However, these methods come with potential risks to human health and the environment. Consequently, there is a need to explore and evaluate new, effective methods for enhancing fruit storage that minimize these risks (C. Lee et al., 2022).

GABA, or γ-aminobutyric acid, is a natural occurring substance found in various fruits and vegetables, including tomatoes (Deewatthanawong, Rowell, & Watkins, 2010), apples (Cheng et al., 2023), peaches (Wu et al., 2023), bananas (Y. Wang, Luo, Huang, Yang, Gao, & Du, 2014), and grape (Asgarian, Karimi, Ghabooli, & Maleki, 2022). It acts as a non-protein amino acid and serves for multiple purposes. GABA functions as a signal molecule, and enhances plants' resistance to both biotic and abiotic stresses (Cheng et al., 2023). Additionally, it acts as a potent scavenger of reactive oxygen species (ROS) and possesses direct antioxidant properties. Recently, researchers have followed experiments involving the application of exogenous GABA treatment (Cheng et al., 2023; X. Lee, Tan, & Cheng, 2022; Malekzadeh, Khosravi-Nejad, Hatamnia, & Sheikhakbari Mehr, 2017).

Therefore, the purpose of this study is to evaluate the effects of different concentrations of exogenous GABA treatment on postharvest, chilling injury, and biochemical metabolism in strawberry fruits during storage time.

2. Material and methods

2.1. Treatment of Strawberry fruit

The fresh strawberry fruit after ripeness (75% of the stage of getting red) was harvested from Iran's greenhouse from a commercial aspect. The healthy fruit is chosen among constant size, without contamination, and without disease or damage, and is immediately transferred to the laboratory. The strawberry fruit is put under water before treatment and then, quickly immersed in the solution at 20 °C for 5 minutes. For GABA treatment, 960 fruits were selected and divided into 4 pieces (240 fruits in each piece) with three repetitions (80 fruits in each repetition). The four solutions for treatment were control (distilled water), G2 (2 mM of GABA), G5 (5 mM GABA), and G10 (10 mM GABA). After immersion, the fruits got dried at room temperature for 60 minutes. Then, all fruits were maintained for 12 days at a temperature of 4 °C and 90 % RH. From each of the repetitions, 20 fruits were randomly chosen for measurements of antioxidant capacity, and next, the rest of the fruits were frozen in liquid nitrogen and they are kept at 80 °C.

2.2. Determination of Weight Loss, H_2O_2 and malondialdehyde content

Weight loss of the samples was calculated using a digital scale during the storage period and expressed as a percentage, following the method described by Malekzadeh (2015). The measurement of lipid peroxidation was conducted using the 2-thiobarbituric acid (TBA) reaction (Zhao et al., 2022). The concentration of H_2O_2 was determined following the method described by Chen. (Chen, Jia, & Yue, 2010).

2.3. The assessment of antioxidant capacity

2.3.1. Measurement of DPPH. scavenging capacity

DPPH radical scavenging (%) capacity was assayed following to Sirivibulkovit et al., (Sirivibulkovit, Nouanthavong, & Sameenoi, 2018).

2.3.2. Measurement of ABTS•+ scavenging capacity assay

To determine the antioxidant capacity of strawberry fruit in the presence of ABTS radical cation ($ABTS^{•+}$), the analysis was carried out following the method described by Toor and Savage, Savage (Toor & Savage, 2005).

2.4. Measurement of phenolic and flavonoid accumulations

Phenols and flavonoid accumulation were measured according to the method of Toor and Savage (Toor et al., 2005).

2.5. Measurement of total anthocyanin and ascorbic acid content

The total anthocyanin contents of the samples were evaluated by employing the pH-differential method as outlined in the described procedure by Orak (Orak, 2007).

2.6. Measurement of endogenous GABA content

GABA concentration was assayed by the method described by Zhang et al. (G. Zhang & Bown, 1997).

Data analysis

The data analysis was performed using SPSS version 25. Two-way analysis of variance (ANOVA) was performed on the data, and subsequent post-hoc multiple comparisons were carried out. The threshold for determining statistical significance was established at a significance level of $P < 0.05$. The results were presented as the mean ± standard error of triplicate experiments, indicating the average value and the variability among the three replicates. Data manipulation was performed using Microsoft Excel, while GraphPad Prism 8 software was used for graphing the results.

3. Results and Discussion

3.1. Effect of GABA on Weight Loss percentage, H2O2 and MDA content in Strawberry

Figure 1.A, illustrates the weight loss of strawberries treated with different concentrations of GABA over a 12-day storage period. As depicted, all treatments, including the control group (untreated), exhibited weight loss over time. However, the strawberries treated with 5 mM GABA demonstrated the least amount of weight loss at the conclusion of the storage period (day 12), while the control group showed the most substantial weight loss. The strawberries treated with 2 mM and 10 mM GABA also experienced weight loss, but to a moderate degree. Collectively, the results suggest that GABA treatment can mitigate weight loss in strawberries during storage, with the 5 mM concentration of GABA appearing to be more effective than the other concentrations tested.

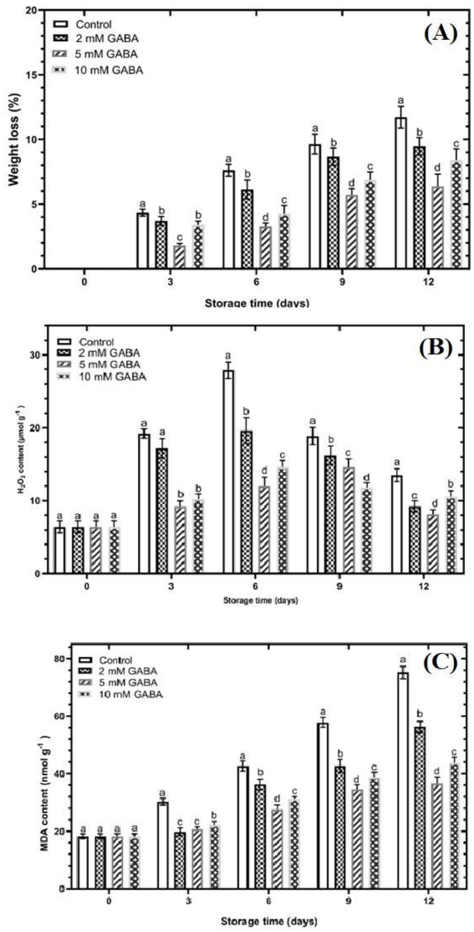

Figure 1- Effect of GABA treatment on A. Weight Loss. B. H_2O_2 content, C. MDA content of strawberry fruit during storage. Values are presented as means ± SD (standard error). Different letters in the same storage period indicate significant differences ($P < 0.05$).

As it has been shown in Figure 1B, in the control fruit, the accumulation of H_2O_2 increased quickly and then decreased during storage time. Nevertheless, during the storage period, H_2O_2 accumulation in fruits treated with 5 mmol. L^{-1} of GABA was lower than other treatments. After 12 days of storage with the treatment of 5 and 10 mmol. L^{-1} of GABA, the H_2O_2 accumulation was reduced 39.5 % and 31.7 %, respectively, compared to the control fruits.

As it has been shown in Figure 1C, during the storage period the content of MDA in untreated and exogenous GABA-treated strawberry fruit has constantly increased. Nonetheless, during the whole storage period, its amount in 5 or 10 mM was significantly reduced in comparison to untreated fruits and as well, in the concentration of 2 mmol of L^{-1} GABA ($P < 0.05$). In comparison to untreated fruit, MDA content in fruits decreased by 51.43 % and 41.82 % at the end of storage time.

3.2. Effect of GABA Treatment on phenol and Flavonoid Content in Strawberry fruit

The change of phenol and flavonoid content in strawberry fruit has been shown in Figure 2. During the storage time of strawberry fruit at 4 ˚C in both control and GABA-treated samples, an upward trend has been observed in the accumulation of phenol and flavonoid. Strawberry fruit with 5 and 10 mmol. L^{-1} of GABA showed a considerable level of phenol and flavonoid accumulation from day 9 to 12 ($P < 0.05$) compared to the untreated sample.

As shown in Figure 2A, the total phenolic content in strawberry fruit is influenced by both GABA treatment and storage time. Over time, fruits treated with 2-, 5- and 10-mM GABA showed a significant increase in phenolic content compared to the control group. On days 6, 9 and 12, the total phenolic content in fruits treated with 5 mM GABA was significantly higher than in all other groups. By day 12, significant differences in phenolic content were evident among all treatments, demonstrating the positive effect of GABA on maintaining the phenolic quality of strawberry fruits during the storage period.

The results indicated that the total flavonoid content in strawberry fruit is influenced by both GABA treatment and storage time (Figure 2B). Over time, a significant increase in flavonoid content was observed in fruits treated with 2 mM, 5 mM, and 10 mM GABA compared to the control group. Particularly on days 9 and 12, fruits treated with 10 mM GABA exhibited the highest flavonoid levels and showed significant differences compared to the other groups.

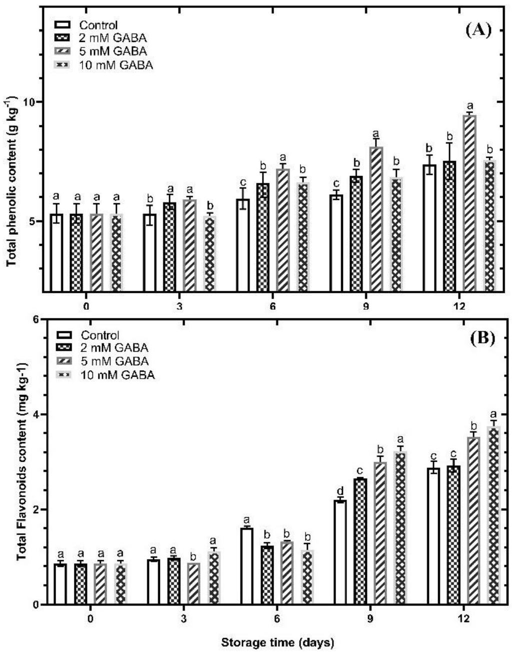

Figure 2- Effect of GABA treatment on A. Total Phenolic content. B. Total Flavonoids content of strawberry fruit during storage. Values are presented as means ± SD (standard error). Different letters in the same storage period indicate significant differences ($P < 0.05$).

3.3. Effect of GABA Treatment on Antioxidant Capacity in Strawberry

In this study, two methods have been used to review the effect of GABA treatment on antioxidant capacity in strawberry fruit during storage (Figure 3). The DPPH scavenging capacity in both samples of untreated fruits and those treated with GABA showed an increasing trend during the storage period. At the end of the storage period, DPPH scavenging capacity significantly increased in comparison to control group ($P < 0.05$). By 12 d of storage, treatment of 5 mmol L^{-1} showed the highest amount of DPPH scavenging capacity (93.16 %) (Figure 3A).

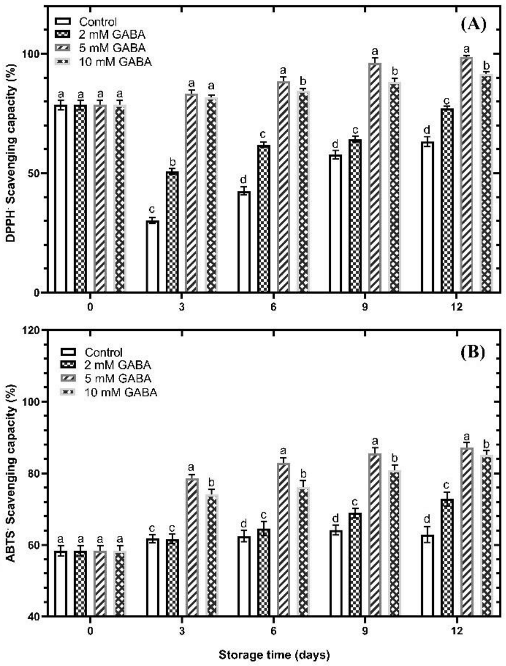

Figure 3- Effect of GABA treatment on A. DPPH radical scavenging capacity. B. ABTS scavenging capacity of strawberry fruit during storage. Values are presented as means ± SD (standard error). Different letters in the same storage period indicate significant differences (P < 0.05).

During the storage period, like the DPPH scavenging capacity, the $ABTS^{+}$ scavenging capacity showed an increasing trend in both the control group and the group treated with GABA. Compared to the control group, treatment of 5 and 10 mmol. L^{-1} of GABA showed a significant increase in ABTS scavenging capacity, 9 and 12 days after storage. At the end of the storage period of strawberries, treatment of 5 mmol L^{-1} showed the highest amount of ABTS scavenging capacity (87.22 %) (P < 0.05). According to these results, we suggest that the higher phenolic and flavonoid accumulations in strawberry fruit treated by GABA are most likely together with an increase in DPPH radicals and ABTS scavenging capacities (Figure 2 and 3).

3.4. Effect of exogenous GABA treatment on total anthocyanin and Ascorbic Acid Content

The anthocyanin composition is an important quality parameter of strawberries because it contributes significantly to their visual appeal, flavor, and nutritional value. Based on the data presented in Figure 4A, there was no significant difference observed between the control and GABA-treated groups during the initial and third days of storage. However, from the 6th to the 12th day of storage, both the control and treated groups exhibited an increasing trend in total anthocyanin content. Figure 4A clearly illustrates that strawberry fruit treated with GABA displayed a significantly higher accumulation of total anthocyanins throughout the cold storage period (P < 0.01), whereas the control group showed a relatively mild increasing trend. The results indicate that on the 12th day of storage, the GABA-treated group exhibited the highest anthocyanin content at a concentration of 10 mM GABA.

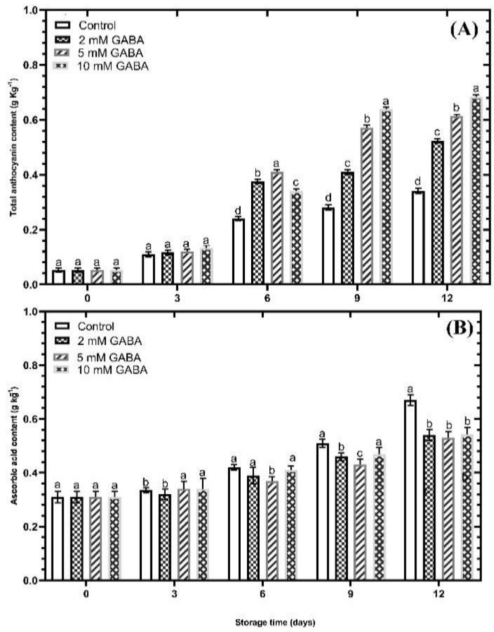

Figure 4. The impact of GABA treatment on the total anthocyanin (A) and Ascorbic acid contents (B) in strawberry fruit during storage. Values are presented as means ± SD (standard error). Different letters in the same storage period indicate significant differences (P < 0.05).

Based on the information depicted in Figure 4B, no significant difference was found in the ascorbic acid content between the control and GABA-treated groups (2 mM) during the initial, third, and sixth days of storage. Nonetheless, starting from the 9th *and 12th day of storage, both the control and* treatment groups displayed a gradual increase in total ascorbic acid content. However, a negligible increase in the amount of ascorbic acid was recorded upon increase in GABA concentration.

3.5. Effect of exogenous GABA Treatment on endogenous GABA content

As Figure 5 illustrates, strawberry fruit treated with GABA remarkably showed higher endogenous GABA accumulation during cold storage ($P < 0.05$), but the control fruit exhibited minor fluctuations of endogenous GABA. In contrast, exogenous GABA treatments (2, 5, and 10 mM) generally show an increase in endogenous GABA content, although the magnitude of this increase varies depending on the GABA concentration. At harvest, there is no significant difference observed between the treatments.

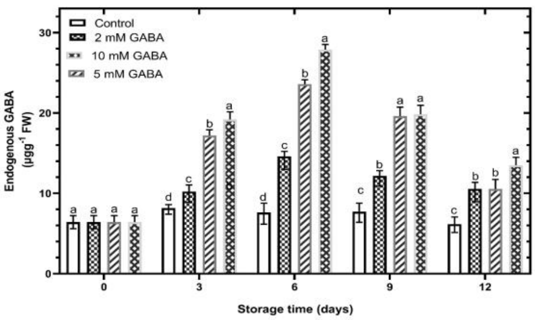

Figure 5. The impact of GABA treatment on endogenous GABA accumulation in strawberry fruit during storage. Values are presented as means ± SD (standard error). Different letters in the same storage period indicate significant differences ($P < 0.05$).

However, from day 3 onwards, the 5- and 10-mM treatments clearly distinguish themselves from the control and the 2 mM treatment, exhibiting a greater increase in endogenous GABA. On 6 d of storage, a slight increase in GABA content was observed across all treatments, followed by a decline by 12 d. Overall, it appears that treatment with exogenous GABA can lead to an increase in endogenous GABA content over time. This effect is particularly evident in the higher concentration treatments (5 and 10 mM).

4. Discussion

The application of exogenous GABA can have an impact on various physical features of fruits, including their color, firmness, and levels of bioactive compounds such as phenolics and other antioxidants (Badiche, Valverde, Martínez-Romero, Castillo, Serrano, & Valero, 2023; X. Lee et al., 2022; Wu et al., 2023). Research has indicated that GABA can stimulate the ripening of strawberries by promoting the accumulation of anthocyanin or the production of ethylene (C. Lee et al., 2022; X. Lee et al., 2022; Yu, Ma, Sheng, & Shen, 2023). Our prior investigation has also validated that the application of exogenous GABA can improve the postharvest ripening process of cucumbers (Malekzadeh et al., 2017).

In the present study, the accumulation of H_2O_2 and MDA in strawberry fruit decreases considerably with GABA treatment in comparison to non-treated fruits. This confirms that GABA-treatment can increase the capacity of strawberry fruit against oxidative associated with post-harvest senescence. The same result was obtained by Malekzadeh et al., at which they found a significant decrease of H_2O_2 and MDA accumulation in cucumber fruit treated with exogenous GABA in comparison to non-treated fruits (Malekzadeh et al., 2017).

Phenolics and flavonoids are essential antioxidants found in plants that can help alleviate oxidative stress and protect cellular structures from damage (Peng, Wang, Wang, Wang, & Bian, 2022; Sharma, Kumar, & Chandran, 2023; Yue et al., 2023). Phenolic compounds play a crucial role in plant development and stress defense by acting as ROS scavengers and contributing to disease resistance (Peng et al., 2022; Sheng et al., 2017). These compounds can be affected by external stresses and elicitors, and their various functions are vital for the survival of plants under challenging conditions (Sheng et al., 2017; X. Wang, Gu, Chen, Huang, & Xing, 2017). The current study showed that the application of GABA increased the levels of total phenolics and total flavonoids, which is consistent with earlier findings in lemon (Badiche et al., 2023), pear (Z. Wang et al., 2021), banana (Ali et al., 2023), apple (Cheng et al., 2023), and bell pepper (Nazzal et al., 2025). Furthermore, during the post-harvest ripening period, GABA was found to enhance the accumulation of several phenolic compounds in strawberries, which is consistent with previous findings in apples (Cheng et al., 2023), grapes (Asgarian et al., 2022), peach (Wu et al., 2023), and cucumber (Malekzadeh et al., 2017). These results suggest that GABA plays a significant role in regulating the accumulation of phenolic compounds in various fruits.

Researcher found that increased anthocyanin levels have been linked to elevated endogenous H_2O_2 content, which may be triggered by suppressing glycolysis. This suppression is associated with the down regulation of genes encoding pyruvate kinase and glyceraldehyde-3-phosphate dehydrogenase (M. Luo et al., 2020). Increased accumulation of phenols, flavonoids, and anthocyanins in strawberry fruit is attributed to elevated expression levels of chalcone synthase (CHS), 4-coumarate-CoA ligase (4CL), flavanone 3-hydroxylase (F3H), and UDP-glucose:flavonoid 3-O-glucosyltransferase (UFGT). Furthermore, higher expression of polygalacturonase (PG), pectin methylesterase (PME), and expansin (EXP) genes contributes to fruit softening (Mansouri, Sarikhani, Sayyari, & Aghdam, 2021). The effects of GABA are evident through an increased activity of the phenylpropanoid pathway, leading to higher accumulation of anthocyanins and phenols (Nazzal et al., 2025).

In this study, we employed two methods (DPPH and ABTS assays) to examine the impact of GABA treatment on the antioxidant capacity of strawberry fruit throughout the cold storage period (Figure 3). The results revealed an increasing trend in the DPPH scavenging capacity of the GABA-treated fruit during the entire storage duration. Furthermore, at the conclusion of the storage period, GABA treatments exhibited a significant difference in DPPH% scavenging capacity compared to the control group ($P < 0.05$). Similar to the DPPH scavenging capacity, the ABTS scavenging capacity also displayed an increasing trend in the GABA-treated fruit throughout the storage period. In a previous study performed by Malekzadeh, it was observed that GABA treatment significantly enhances the DPPH scavenging capacity of cucumber fruit during storage at 4 °C (Malekzadeh et al., 2017). These findings were found to be correlated with the total phenolics content. Additionally, previous research reported a positive correlation between total phenolics content and antioxidant capacity (Y. Luo et al., 2020; P. Zhang et al., 2022). Building upon these results, it is proposed that the higher levels of total phenolics and flavonoids in GABA-treated strawberry fruit likely contribute to the increased DPPH and ABTS scavenging capacity.

The beneficial effects of endogenous GABA accumulation for alleviating the effects of the chilling injury have been demonstrated by (Badiche et al., 2023; D. Wang et al., 2021). Delaying post-harvest quality of cucumber fruit in response to exogenous GABA treatment was described as higher endogenous glutamate decarboxylase (GAD) enzyme activity (X. Lee et al., 2022; SHI et al., 2010). The findings were that chilling stress caused damage to fruit cells, and these cells increased endogenous GABA accumulation to decrease chilling injury.

5. Conclusion:

From this study, it can be concluded that treatment of 5 or 10 mmol L^{-1} of GABA has affected strawberry fruit by delaying the senescence and reducing the accumulation of H_2O_2 and MDA. Moreover, treatment with 5 or 10 mmol L^{-1} of GABA improves the accumulation of phenol and flavonoid, raising the antioxidant capacity. Exogenous GABA application also positively influences endogenous GABA levels. Briefly, GABA treatment in moderate concentration has a positive effect on post-harvest life and the quality of fresh strawberry fruit.

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