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Exogenous glycine betaine maintains postharvest blueberry quality by modulating antioxidant capacity and energy metabolism

Yu Zhang [a], Xiaohong Kou [b], Guohe Zhang [a], Donglan Luo [a], Sen Cao [a,b,*]

[a] School of Food Science and Engineering, Guiyang University, Guizhou Province, 550005, PR China
[b] School of Chemical Engineering and Technology, Tianjin University, Tianjin, 300072, PR China

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ABSTRACT

Blueberries are susceptible to decay and spoilage due to increased respiration rates and metabolism after harvest. The aim of this study was to examine the impact of postharvest application of exogenous glycine betaine (GB) on blueberry storage quality, antioxidant system, and energy metabolism in order to enhance understanding of the regulatory mechanisms of GB on blueberry quality. Fresh blueberries were soaked for 5 min in different concentrations of GB. Preliminary pre-tests revealed that 10 mmol L^{-1} GB treatment significantly retarded the quality deterioration, decay and dent rate, while increasing the firmness and soluble solid content. Concurrently, exogenous GB treatment resulted in an increase in the content of anthocyanosides, total flavonoids, total phenols, and reduced glutathione, also exhibited higher antioxidant enzyme activities, including superoxide dismutase, catalase, ascorbate peroxidase, polyphenol oxidase, phenylalanine ammonia-lyase, glutathione reductase, dehydroascorbate reductase, and monodehydroascorbate reductase. In GB-treated blueberries, endogenous GB content was significantly higher. The hydrogen peroxide value, superoxide anion, and malondialdehyde content, and higher levels of free radical scavenging rate (DPPH), which significantly improved antioxidant capacity. Meanwhile, succinate dehydrogenase, cytochrome C oxidase, H^{+}-ATPase, and Ca^{2+}-ATPase, decreased and slowed the senescence of blueberries, the ATP content and energy charge were observed to be elevated. In summary, exogenous GB treatment maintained the quality of post-harvest blueberries by increasing their antioxidant capacity and inhibiting the decrease in the activity of energy metabolism-related enzymes. The results indicate that 10 mmol L^{-1} GB treatment may be an efficacious approach for maintaining the quality and prolonging the shelf life of blueberries, offering novel insights into the preservation of this fruit.

1. Introduction

Blueberries are popular all over the world due to their high nutritional value, which is rich in nutrients and bioactive substances, include vitamins, amino acids, anthocyanins, polyphenols and flavonoids, among others, which are widely welcomed by consumers and are one of the top five healthy fruits (Chu et al., 2018; Yang et al., 2018). The storage period of blueberries is very short, the new cell growth rate decreases after harvesting, and a decrease in enzyme activity accelerates the decay and deterioration of blueberries, this ultimately results in a decline in the quality of blueberries, thus limiting the transportation of blueberries and fresh sales, which seriously affects the commodity value of blueberries and causes great economic losses to the blueberry industry. The current preservation techniques used for blueberries include low temperature, thymol, and γ-aminobutyric acid (Chea et al., 2019; Ding et al., 2023). These techniques delay the aging of the fruits to a certain extent and maintain the quality of the fruits. However, there are also drawbacks associated with these techniques, including high cost and energy consumption, low efficiency, and the presence of chemical residues. Therefore, it is imperative that new technologies for blueberry preservation be proposed without delay to ensure the storage quality of blueberries and prolong their marketing period.

ROS are metabolites produced by organisms during normal aerobic respiration that directly affect the postharvest quality of fruits (Wang et al., 2019, 2019b). With prolonged storage, various abiotic stresses disrupt plant metabolic homeostasis and increase in the production of reactive oxygen species (ROS) (Torres, 2010), resulting in reduced fruit quality. The quality of blueberries is closely related to their antioxidant

* Corresponding author. School of Food Science and Engineering, Guiyang University, Guizhou Province, 550005, PR China.
E-mail addresses: 1478205938@qq.com (Y. Zhang), kouxiaohong@tju.edu.cn (X. Kou), 1578108852@qq.com (G. Zhang), luodonglan1991@163.com (D. Luo), cs5638myself@126.com (S. Cao).

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system. Therefore, improving the antioxidant system is an effective measure for improving the quality of blueberries. However, insufficient energy supply is an important cause of physiological disorders in plants. Energy is a fundamental requirement for the normal physiological metabolism of postharvest fruits and plays a pivotal role in the control of postharvest fruit senescence (Huang et al., 2021 a; Li et al., 2016). Adenosine triphosphate (ATP) is primarily formed through oxidative phosphorylation in mitochondria and subsequently transported by adenine nucleotide transposons (ANT), which provide energy for cellular activities (Klingenberg, 2008; Li et al., 2024). Prior research has indicated that exogenous application of melatonin maintains storage quality of jujubes by enhancing anti-oxidative ability and suppressing the activity of cell wall-degrading enzymes (Tang et al., 2020); Nitric oxide treatment maintains postharvest quality of table grapes by mitigation of oxidative damage (Zhang et al., 2019). Furthermore, retardation of postharvest softening of blueberry fruit by methyl jasmonate is correlated with altered cell wall modification and energy metabolism (Wang et al., 2021); Melatonin alleviates pericarp browning in litchi fruit by regulating membrane lipid and energy metabolisms (Wang et al., 2020). The findings indicate that the modulation of antioxidant and energy metabolism represents a pivotal strategy for the retardation of fruit senescence. Meanwhile, glycine betaine inhibits postharvest softening and quality decline of winter jujube fruit by regulating energy and antioxidant metabolism (Zhang et al., 2023); Exogenous phytosulfokine alpha (PSK alpha) application delays senescence and relieves decay in strawberry fruit during cold storage by triggering extracellular ATP signaling and improving ROS scavenging system activity, which reveal the correlation between energy metabolism, antioxidant metabolism and fruit quality (Aghdam et al., 2021).

Glycine betaine (GB, N,N,N-trimethylglycine) is a small amphoteric ionic compound, an alkaloid with a chemical structure similar to that of an amino acid, and a quaternary ammonium base with the molecular formula $C_5H_{11}NO_2$. Betaine is widely found in both plants and animals. Lycium barbarum and legumes contain betaine, and the primary source of betaine is molasses derived from sugar beets (de Zwart et al., 2003; Sakamoto & Murata, 2002). Glycine betaine, an alkaloid, is a novel, natural, effective, safe and environmentally friendly biological preservative that is widely used for the post-harvest preservation of fruits and vegetables. Chen et al. (2021) reported that the postharvest application of GB significantly reduced the incidence of CI in banana fruits during refrigeration. Habibi et al. (2022) indicated that exogenous GB treatment could maintain the bioactive compounds, antioxidant activity and quality of "Moro" blood orange fruits during the period of long-term storage, and the exogenous application of GB treatment attenuated low-temperature-induced skin browning of "Nango" pears. This was achieved by modulating antioxidant enzymes and proline metabolism (Sun et al., 2019). The positive effects of the GB treatment on the quality of other fruits and vegetables have been demonstrated, its physiological effects on blueberries are worth exploring. There is a paucity of reports on the subject of GB treatment in the postharvest preservation of blueberries. A growing body of research now corroborates the hypothesis that antioxidant systems and energy are involved in the metabolism and regulation of postharvest physiological and biochemical aspects of fruit. Therefore, this study focused on the effects of GB treatment on the antioxidant system and energy metabolism of postharvest blueberries in order to maintain their quality. The results of this study provide theoretical support for the preservation of post-harvest blueberries.

2. Materials and methods

2.1. Plant material and sample treatment

The experimental materials "Brightwell" blueberries were sourced from the Qiandongnan Miao and Dong Autonomous Region of Guizhou Province. The fruits were harvested on July 1, 2023. Blueberries with maturity of 80–90% were tested, and the color was dark blue. After harvesting, the fruits were placed in tray boxes and transported to the experimental site at a temperature of 22–25 °C and a RH of 80–90%. A total of 45 kg of blueberries were harvested for the experiment and randomly divided into six equal groups for the test. One group was subjected in distilled water for 5 min, acting as the control group, and the other five groups were soaked in GB at concentrations of 5, 10, 20, 30 and 40 mmol L^{-1} for 5 min, the temperature of the solution used for treatment is room temperature, 20–22 °C. After treatment, the fruit was left to dry naturally at room temperature until there were no more drops of water on the surface. Subsequently, the fruits were placed into polyethylene plastic bags and transported to the storage are at 4 ± 1 °C with the relative RH of 85–90% for 20 d. Fruits with diameters of 12–16 mm were selected at five-day intervals (0, 5, 10, 15, and 20 d) and the physiological indices of the fruits were determined. The fruits were subsequently subjected to a process of cutting and freezing, then rapidly frozen at −80 °C for subsequent analysis.

2.2. Measurement of firmness

Fruit firmness was determined using an XT-Plus texture analyzer (Stabie Micro System, United Kingdom, UK) according to the method described by Angeletti et al. (2010). A P/2 probe (2 mm in diameter) was used with a penetration height of 5 mm. Ten fruits were used for each treatment. The results are expressed in Newtons (N).

2.3. Measurement of decay rate, depression rate and total soluble solids (TSS)

The decay and depression rates were determined using the counting method.

After treatment, blueberries were stored at 4 ± 1 °C for 20 d. Samples were taken every 5 d. Fruits with visible mold and flowing juice were rotted, and were selected to be recorded as the decay rate.

$$\text{Decay rate (\%)} = (\textit{Number of decayed fruit / Total fruit}) \times 100\%$$

Fruits with a depression on the surface were dented, and picking out was recorded as the depression rate.

$$\text{Depression rate (\%)} = (\textit{Number of depressed fruits / Total fruit}) \times 100\%$$

TSS was determined using a handheld refractometer. Calibration was carried out with distilled water before each determination, scale readings were taken, and the resulting values were recorded. The determination was repeated three times for each sample and averaged. The decay rate, depression rate, and TSS values were expressed as mass fractions (%).

2.4. Measurement of total anthocyanin content (TAC), reduced glutathione (GSH), total phenol content (TPC) and total flavonoid content (TFC)

The determination of anthocyanin content was carried out using the pH difference method as described by Huang et al. (2016), with slight modifications. Extraction was performed using a 60% ethanol solution. Two test tubes were used: one with 1 mL of extract and 9 mL of KCl buffer (pH 1.0) and the other with 1 mL of ethanol extract and 9 mL of KCl buffer as a blank control. Two other test tubes were used: one with 1 mL extract and 9 mL NaAc buffer (pH 4.5) and the other with 1 mL ethanol extract and 9 mL NaAc buffer as blank controls. The mixture was allowed to stand for 40 min, after which the absorbance of the two systems was measured at 510 and 700 nm. The units have been expressed in mg g^{-1}.

The GSH levels were quantified in accordance with the methodology delineated by Ma et al. (2021). The TCA solution was then homogenized. Two test tubes were taken and 1.0 mL of supernatant and 1.0 mL of sodium phosphate buffer were added, followed by the addition of 4 mM

DTNB solution (0.5 mL) to one test tube and sodium phosphate buffer was added to each tube. The tubes were kept warm at 25 °C for 10 min, after which the absorbance was measured at a wavelength of 412 nm. The units have been expressed in mmol g^{-1}.

The total phenolic and flavonoid contents were determined using the method described by Shi et al. (2023), with slight modifications.

Total phenolics were extracted with 70% ethanol solution, 1 mL of ethanol extract was aspirated, 3 mL of Forintol reagent was added, 6 mL of sodium carbonate solution was added, and the volume was fixed to 20 mL with distilled water Total flavonoids were extracted with 75% ethanol solution, 1 mL of ethanol extract was sucked up and added to 4 mL of 0.6% TBA solution, boiled in a water bath for 15 min, centrifuged, 1 mL of extract was added to 2 mL of $AlCl_3$ solution and 3 mL of CH_3COOH solution, and 75% ethanol was fixed to the scale. The solution was then allowed to stand for 1 h. Absorbance was quantified at 760 and 420 nm, with the results expressed in mg g^{-1}.

2.5. Measurement of peroxidase (POD), polyphenol oxidase (PPO), phenylalanine ammonia-lyase (PAL), catalase (CAT), ascorbate peroxidase (APX) and superoxide dismutase (SOD)

The POD activity was quantified using the methodology proposed by Centeno et al. (2017). The reaction system consisted of 0.5 mL of crude enzyme solution, 3.0 mL of guaiacol, and 200 μL of 5 mmol L^{-1} hydrogen peroxide. The PPO levels were determined in accordance with the methodology described by Shi et al. (2023). One gram of each sample was ground into a homogenate in 15 mL of phosphate buffer. Take 3.9 mL of phosphate buffer, add 1 mL of catechol and 3 mL of supernatant, hold at 37 °C for 10 min, remove and cool add 2 mL of trichloroacetic acid to terminate the reaction. The PAL activity was quantified using the methodology proposed by Xie et al. (2020). Approximately 3 g of the sample was ground into a homogenate with 5 mL of borate buffer. A volume of 3 mL of borate buffer and 0.5 mL of crude enzyme solution should be added, and the mixture should be held at 37 °C for a period of 60 min. Subsequently, 0.1 mL of 6 mol L^{-1} hydrochloric acid should be added in order to terminate the reaction. One unit of enzyme activity was defined as a 0.01 decrease in the absorbance of the reaction system at 490 nm, 420 nm, and 290 nm per minute. The results were expressed as U g^{-1}.

The activities of CAT, APX, and SOD were determined in accordance with the methodologies described by Wang et al. (2020 b), Sun et al. (2011), and o-phenyltriol autoxidation. For the CAT activity assay, 1 g of the sample was weighed and homogenized in 15 mL of sodium phosphate extract. A volume of 0.1 mL of the supernatant was aspirated, and 2.9 mL of 20 mol L^{-1} H_2O_2 was subsequently added. Pipette 0.1 mL of supernatant and add 2.9 mL of H_2O_2 solution. Grind and homogenise with sodium carbonate to determine APX activity. The reaction system was composed of the following elements: 4.6 mL sodium carbonate buffer, 0.1 mL ascorbic acid, 0.2 mL supernatant, and 0.1 mL 3% hydrogen peroxide. For the SOD activity assay, 15 mL of sodium phosphate buffer was used to homogenise a 3 g sample, and the reaction system was constituted of the following elements: 2.35 mL of Tris-HCl, 0.85 mL of buffer, 0.7 mL of o-toluene trisol solution, and 0.6 mL of supernatant. The absorbance was measured at 240, 290, and 325 nm. The change in absorbance of 0.01 at 240, 290, and 325 nm per minute of reaction system was taken as one unit of enzyme activity. The results were expressed as U g^{-1}.

2.6. Measurement of glutathione reductase (GR), monodehydroascorbate reductase (MDHAR) and dehydroascorbate reductase (DHAR)

Extracts were prepared according to Huang et al., (2021 b). 1 g of sample was added to 5 mL of pre-cooled 50 mM phosphate buffer (containing 2.0 mM AsA, 0.2 mM EDTA and 2.0 g L^{-1} PVPP) and centrifuged.

The GR reaction system consisted 200 μL of supernatant, 3.0 mL of 100 mM PBS (containing 1.0 mmol L^{-1} EDTA), 500 μL of 20 mmol L^{-1} H_2O_2, and 800 μL of 3.0 mmol L^{-1} nicotinamide adenine dinucleotide phosphate ($NADP^+$). The MDHAR reaction system was composed of the following elements: 0.2 mL of supernatant, 1.7 mL of 50 mmol L^{-1} PBS, 200 μL of 2.0 mmol L^{-1} AsA, and 100 μL of 4.0 mmol L^{-1} NADPH. The degree of DHAR activity was quantified by the addition of 200 μL of supernatant, 1.6 μL of 0.1 mmol L^{-1} 4-hydroxyethylpiperazine acetate-potassium hydride buffer, 100 μL of 20 mmol L^{-1} glutamic acid, and 8.0 mmol L^{-1} docosahexaenoic acid to a cuvette. Absorbance was determined via measurement at 240, 340, and 265 nm. One unit of GR, MDHAR, or DHAR activity was defined as an increase in absorbance of 0.01 per minute. The GR, MDHAR, and DHAR results have been expressed as U g^{-1}.

2.7. Measurement of H_2O_2 content, $O_2^{\cdot-}$ generation rate, free radical scavenging rate (DPPH) and malondialdehyde (MDA)

H_2O_2 content was determined using the method described by Huan et al. (2017) with minor modifications. Acetone (5 mL) was then ground into a homogenate and centrifuged. Aspirate 1 mL of the supernatant, add 0.1 ml of a 10 % solution of titanium tetrachloride in hydrochloric acid and 0.2 ml of concentrated ammonia and leave to react for 5 min, centrifuge, rinse the precipitate repeatedly with acetone at −20 °C until the pigment is removed, add 3 mL of 2 mol L^{-1} sulfuric acid to dissolve the precipitate, and measure its absorbance at 412 nm. The results were expressed in terms of μmol g^{-1}.

The method proposed by Wang et al. (2019 a) was employed to ascertain the rate of $O_2^{\cdot-}$ generation. A total of 5 mL of sodium phosphate buffer was employed to homogenise the samples, which were subsequently centrifuged. 1.0 mL of supernatant, 1.0 mL of 50 mmol L^{-1} phosphate buffer and 1.0 mL of 1 mmol L^{-1} hydroxylamine hydrochloride solution were aspirated and held at 25 °C for 1 h. 1.0 mL of 17 mmol L^{-1} p-aminobenzenesulphonic acid solution and 1.0 mL of 7 mmol L^{-1} α-naphthylamine solution were added and held at 25 °C for 20 min, and the absorbance was measured at 530 nm. The results are expressed as μmol min^{-1} g^{-1}.

The determination of DPPH activity was conducted in accordance with the methodology described by Xu and Chang (2007). Two milliliters of the appropriately diluted sample solution and 2 mL of 0.20 mM DPPH solution were mixed well and stored at 37 °C for 30 min, protected from light. Two milliliters of anhydrous ethanol mixed with 2 mL of 0.20 mmol L^{-1} DPPH solution was used as the blank group, and 2 mL of anhydrous ethanol mixed with 2 mL of the sample solution was used as the background group (adjusted to zero). The absorbance of the sample, blank, and background groups was measured at 517 nm. The absorbance of the sample, the blank, and the background groups was measured at 517 nm. The results were expressed as percentages (%). Calculations were performed using the following formula: DPPH (%) = $1 - ((A_i\text{-}A_j)/A_c)$.

The term "A_i" is used to indicate the absorbance of the sample group, "A_j" is used to indicate the absorbance of the background group, "A_c" is used to indicate the absorbance of the blank group.

The method described by Du et al. (2024) was employed to determine the MDA content with minor modifications. Grinding with 10% TCA solution, centrifugation. Two milliliters of supernatant (2 mL of TCA instead of extract was used as the control tube) and 2 mL of 0.67% thiobarbituric acid solution were added. The mixture was boiled for 20 min, centrifuged again, and the absorbance was measured at 450, 532, and 600 nm. The results were expressed in μmol g^{-1}.

2.8. Measurement of energy metabolism-related indicators

The relevant indices of energy metabolism were determined according to the method of Jin et al. (2013), with minor modifications.

The grinding process was conducted with a solution of 0.6 mmol L^{-1} perchloric acid, and the pH was adjusted to 6.5–6.8 with 1 mmol L^{-1}

potassium hydroxide. Subsequently, the solution was subjected to filtration through a 0.45 μm filter membrane and subsequently loaded into a brown liquid phase injection bottle for HPLC analysis. The results obtained were ATP, ADP, AMP, and EC levels were measured. The chromatographic column was operated at a flow rate of 1 mL min^{-1}, the column oven temperature was 40 °C, and the injection volume was 20 μL. The unit was expressed as mg kg^{-1}. EC = [*ATP*+*1/2ADP*]/[*ATP* + *ADP* + *AMP*].

Milling with Tris-HCl bufferSamples and centrifuged again. The supernatant was discarded and the precipitate was rinsed with Tris-HCl washing solution and suspended in 2 mL of washing solution to obtain the crude mitochondrial extract. The activity of the CCO was determined by mixing 0.2 mL of the extract with 0.5 mL of 0.4 g L^{-1} cytochrome C. Hold at 37 °C for 2 min, after which 0.5 mL of 4 g L^{-1} dimethylbenzene diamine was added. The system was then held under the same conditions for a further 10 min, after which the absorbance was measured at a wavelength of 510 nm. The activity of SDH was determined by sequentially adding 0.3 mL of extract, 3 mL of 0.2 mmol L^{-1} potassium phosphate buffer, 1 mL of 1 mmol L^{-1} sodium succinate, 0.1 mL of 1 mmol L^{-1} sodium dichlorophenol, and 0.1 mL of 3.3 g L^{-1} phenazine

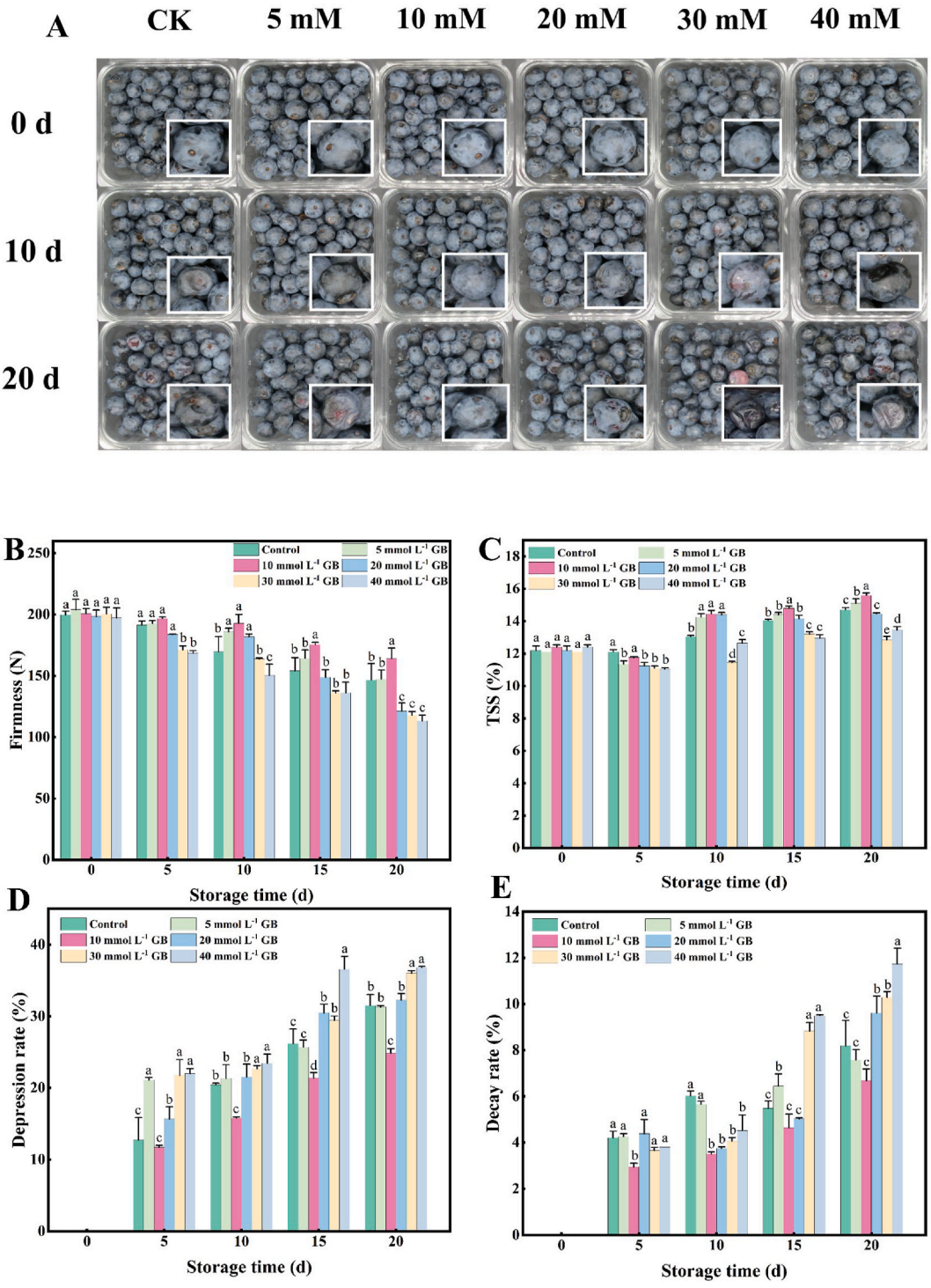

Fig. 1. Effects of different concentrations of GB treatments on the appearance of blueberries (A), firmness (B), TSS (C), depression rate (D), and decay rate (E) at 20 d of storage. *$P < 0.05$, **$P < 0.01$.

methylsulfate, and absorbance was measured at 600 nm. One unit of CCO and SDH was defined as an increase of 0.01 in the absorbance at 510 and 600 nm, respectively. Units are expressed as U g^{-1}.

H^+-ATPase and Ca^{2+}-ATPase levels were measured using appropriate ultratrace enzyme kits. ATPase activity was specified as one unit of ATPase activity expressed as U mg^{-1} per h per milligram of tissue in which the ATPase breaks down ATP to produce 1 μmol of inorganic phosphorus.

2.9. Measurement of endogenous GB content

GB content determination was conducted out with reference to Shan et al. (2016) with slight modifications. The sample was weighed and ground in 95% methanol. The solution was then shaken at 45 °C for 30 min, centrifuged, and the supernatant was collected. The precipitate was then added to 95% methanol and this process was repeated twice. The resulting filtrate was then combined and the volume was determined to be 30 mL. 4 mL of the filtrate was concentrated to near dryness by nitrogen blowing, dissolved in 2 mL of acetonitrile, filtered through a 0.22 μm membrane, and loaded into the injection bottle for HPLC analysis, and the results were expressed as mg g^{-1}. High performance liquid chromatography (HPLC) column: ODS-SP (4.6 × 150 mm, 5 μm); Column temperature: 25 °C; Flow rate: 0.7 mL min^{-1}; Injection volume: 10 μL; Mobile phase: acetonitrile: water = 80:20 isocratic elution; Detection wavelength: 195 nm.

3. Results

3.1. Changes in blueberry appearance and quality indicators

As shown in Fig. 1A, after 20 d of storage, shriveling, mildew, and rotting were significantly higher in the 30 mmol L^{-1} and 40 mmol L^{-1} GB treatment groups than in the control group, and the 5, 10, and 20 mmol L^{-1} GB treatment groups delayed to a certain extent the color change of the fruit to black, rotting, and juicing. In a comprehensive comparison, the 10 mmol L^{-1} GB treatment group showed the best appearance.

Firmness gradually decreased, depression and decay rates gradually increased, and TSS content showed an increasing trend (Fig. 1B–E). In comparison with the control group, the 30 mmol L^{-1} and 40 mmol L^{-1} GB treatment groups accelerated the rotting and softening of the fruits and reduced their quality, whereas the 5, 10 and 20 mmol L^{-1} GB treatment groups delayed the softening of the fruits to some extent. After 15 d of storage, the freshness preservation effect of the 5 mmol L^{-1} GB and 10 mmol L^{-1} GB treatments was significantly superior to that of the other treatment groups, and at the 20th day of the storage period, the depression and decay rates of the 10 mmol L^{-1} GB treatment were 1.25 ($P < 0.01$) and 1.27 ($P < 0.05$) times lower than those of the control group, respectively. The 10 mmol L^{-1} GB treatment had 11.41% ($P < 0.05$) higher firmness and 1.13 ($P < 0.01$) and 1.26 ($P < 0.05$) lower decay and depression rates, respectively, than the 5 mmol L^{-1} GB treatment after 20 d of storage. In a comprehensive comparison, the storage quality of blueberries treated with 10 mmol L^{-1} GB was found to be the most optimal.

From the above experimental results, it can be concluded that the 10 mmol L^{-1} GB treatment had the best effect on maintaining the appearance and quality of blueberries during storage and significantly reduced the decay rate of blueberries. Consequently, blueberries treated with 10 mmol L^{-1} GB were selected for subsequent studies to investigate the effects of exogenous GB treatment on the antioxidant and energy metabolism of blueberries.

3.2. Changes in total anthocyanin content, GSH, total phenol content and total flavonoid content

The anthocyanin content of both groups gradually decreased during storage, whereas the GSH content decreased during the first 5 d and then slightly increased, with an overall decreasing trend (Fig. 2A and B). The anthocyanin content in the GB treatment group was significantly higher than that in the control group on days 5, 10, and 15 of the storage period ($P < 0.05$), and the GSH content was significantly higher than that in the control group after day 5 of the storage period ($P < 0.01$). The total phenolic content of the control group exhibited a gradual decline with an increase in storage time, whereas in the GB-treated group, it increased on day 5 of the storage period and then decreased, which was significantly increased by GB treatment (Fig. 2C). Total flavonoid content showed a fluctuating declining trend in both treatment groups, decreasing slightly throughout the storage period, with higher total flavonoid content maintained at 5 and 15 d of storage (Fig. 2D).

3.3. Changes in SOD, APX, CAT, PAL, POD and PPO

The GB-treated fruits exhibited higher SOD activity on days 5–15 of the storage period than the control group, and the trends in APX activity were similar in the control and GB-treated groups (Fig. 3A and B). The activities of CAT and PPO exhibited an upward and then downward trend in both groups, with the GB treatment maintaining higher CAT activities on days 5, 10, and 20 of the storage period and higher PPO activities on days 10 and 20 of the storage period (Fig. 3C and D). The POD activity of GB-treated fruits exhibited a gradual decline, whereas the control exhibited an increasing trend at 0–5 days and 10–15 days of storage, followed by a gradual decline at 5–10 days and 15–20 days of storage (Fig. 3E). GB treatment significantly decreased POD activity, which was 10.06% ($P < 0.05$) and 32.41% ($P < 0.01$) reduction in comparison to the control group. Fig. 3F shows that the activity of PAL was 1.25, 1.12 and 1.13 times higher ($P < 0.05$) than that of CK on days 5–15 of the storage period of GB-treated fruits. The effect of GB treatment on antioxidant enzyme activity has also been observed in glycine-treated postharvest betaine-treated bananas (Chen et al., 2021).

3.4. Changes in MDHAR, DHAR and GR

GR, MDHAR, and DHAR are produced in fruits and vegetables and protect tissues and cells from oxidative damage. Throughout the storage period, MDHAR, DHAR, and GR activities gradually decreased in both the control and GB-treated groups, with a similar decreasing trend. In particular, during the late storage period, the GB treatment significantly inhibited the decrease in the activities of the MDHAR, DHAR, and GR enzymes during storage (Fig. 4). MDHAR and DHAR were 35.34% and 21.82% ($P < 0.05$) higher than those of the control, respectively, on day 20 of the storage period, and GR activity was 1.33 times ($P < 0.01$) higher than that of the control. Throughout the storage period, the GB-treated group demonstrated elevated levels of MDHAR, DHAR, and GR activity, which further substantiates the hypothesis that GB treatment has the potential to mitigate oxidative damage in cells and enhance the antioxidant activity of blueberries.

3.5. Changes in H_2O_2, DPPH, MDA, and $O_2^{\cdot-}$

Blueberry fruit showed a gradual increase in the rate of hydrogen peroxide and $O_2^{\cdot-}$ production throughout the storage period (Fig. 5A and B). In the late storage period (days 10–20) GB treatment significantly inhibited the increase in hydrogen peroxide content, which was 23.94%, 17.54%, and 20.08% lower than that of the control on days 10, 15, and 20 of the storage period, respectively ($P < 0.01$). At days 10–15 of the storage period, GB treatment significantly suppressed the $O_2^{\cdot-}$ production rate, which was 1.08 and 1.10 times higher in the control group than in the GB-treated group, respectively ($P < 0.05$). These results suggest that post-harvest spraying with glycine betaine reduces hydrogen peroxide content and $O_2^{\cdot-}$ production during storage and maintains blueberry fruit quality. The initial fruit MDA content was relatively low, with a gradual increase observed over the storage period, remaining at a relatively high

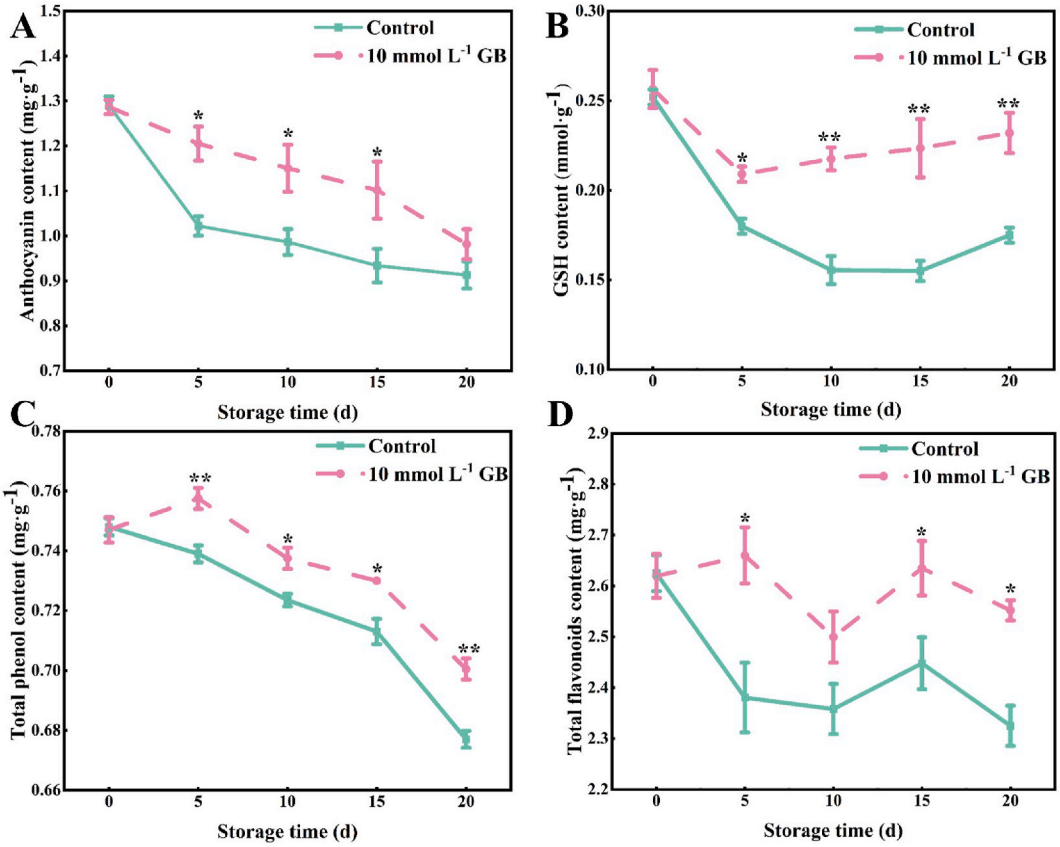

Fig. 2. Effect of GB treatment on blueberry total anthocyanin (A), GSH (B), total phenol (C), and total flavonoids (D) content. Data are shown as mean ± SD standard deviation (n = 3). *$P < 0.05$, **$P < 0.01$.

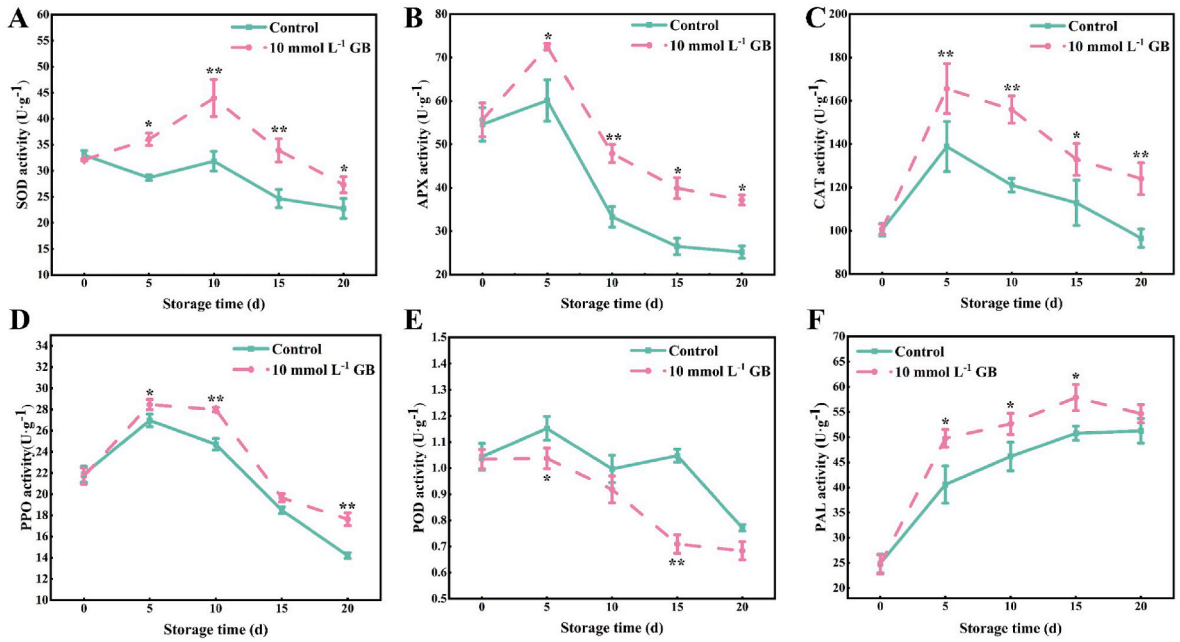

Fig. 3. Effects of GB treatment on blueberry SOD (A), APX (B), CAT (C), PPO (D), POD (E), and PAL (F) activities. Data are shown as mean ± SD standard deviation (n = 3). *$P < 0.05$, **$P < 0.01$.

level on the 20th day of the storage period (Fig. 5C), which significantly inhibited the increase in MDA content ($P < 0.01$). During the storage period, the trend in the DPPH content was opposite to that of the MDA content, showing a decreasing trend. The change in DPPH clearance rate was similar in the control and treated groups, decreasing at days 0–10 of the storage period, and then increasing and decreasing at days 15–20 of the storage period (Fig. 5D). The DPPH content of the GB-treated group was significantly different on days 15 and 20 of the storage period,

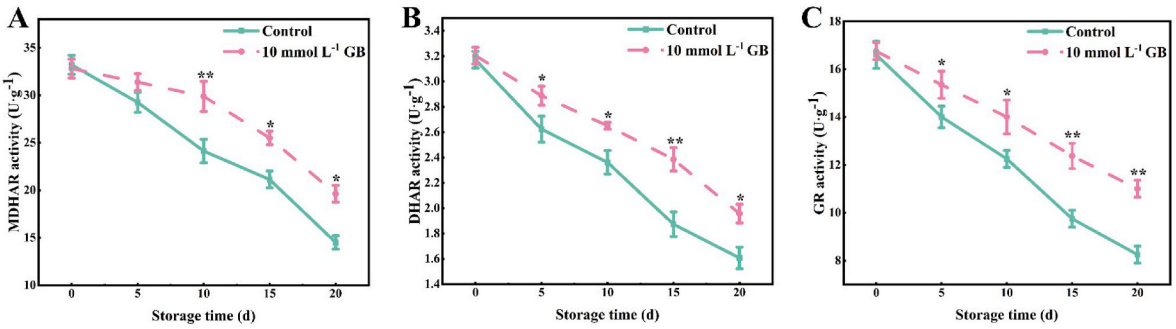

Fig. 4. Effects of GB treatment on blueberry MDHAR (A), DHAR (B), and GR (C) activities. Data are shown as mean ± SD standard deviation (n = 3). *$P < 0.05$, **$P < 0.01$.

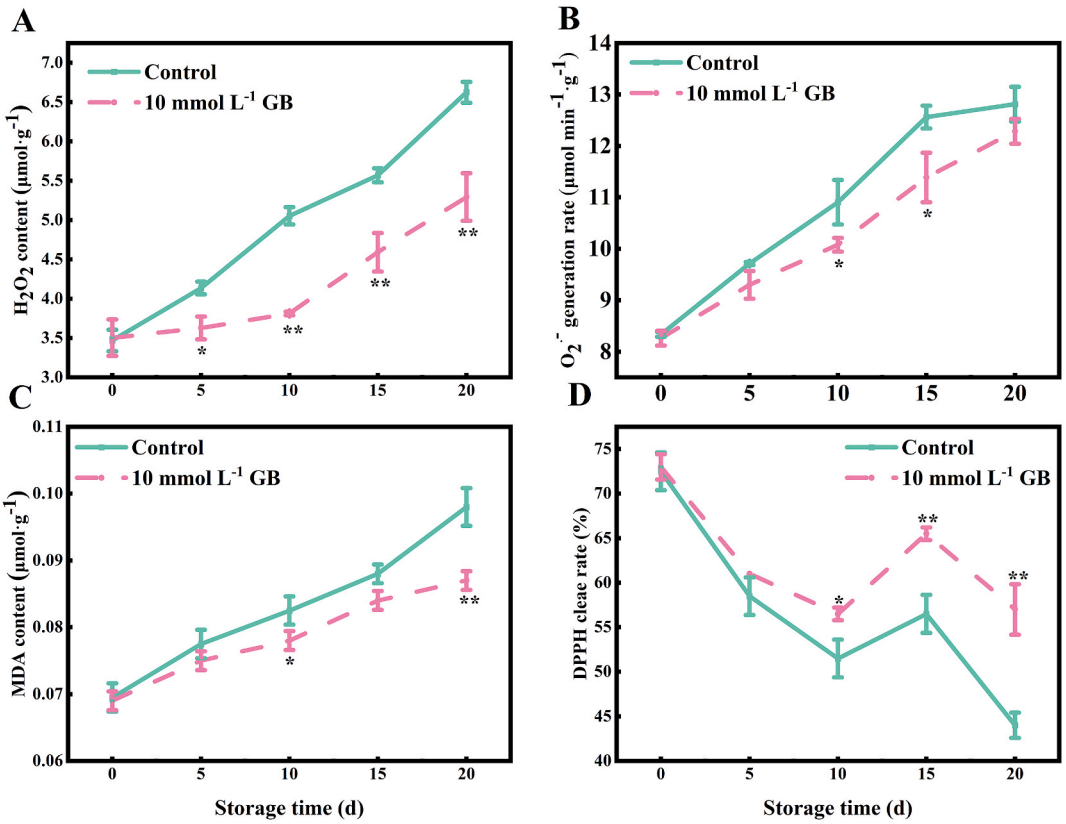

Fig. 5. Effect of GB treatment on blueberry H_2O_2 (A), $O_2^{\cdot-}$ (B), MDA (C), and DPPH (D) content. Data are shown as mean ± SD standard deviation (n = 3). *$P < 0.05$, **$P < 0.01$.

decreasing by 10.27% and 21.92% from the initial value, respectively ($P < 0.01$). The DPPH scavenging rate reached its maximum value on the first day in two groups, and the DPPH scavenging rate in the GB-treated group was 1.30 times higher than that in the treated group on the 20th day of the storage period ($P < 0.01$). The GB-treated fruits exhibited reduced levels of MDA and elevated values of DPPH, which may have been attributed to an increase in both non-enzymatic and enzymatic antioxidants.

3.6. Changes in ATP, ADP, AMP, EC, H^+-ATPase, Ca^{2+}-ATPase, SDH, and CCO

The ATP and ADP content exhibited a gradual decline, while the AMP content demonstrated a gradual increase(Fig. 6A–C). Compared to the control group, GB treatment suppressed the decrease in energy charge by increasing the content of ATP and ADP and decreasing the level of AMP. As a result of the changes in ATP, ADP, and AMP content, the energy charge tended to decrease throughout the storage process (Fig. 6D). GB significantly inhibited the decrease in energy charge on days 10, 15, and 20 of the storage period ($P < 0.01$), and the energy charge of the GB-treated fruits was 2.69% higher than that of the control group at the 20th d of the storage period ($P < 0.01$). The H^+-ATPase activity and Ca^{2+}-ATPase activity of the GB group and the control group showed a gradual decreasing trend. The GB treatment maintained a higher H^+-ATPase activity during storage, and the Ca^{2+}-ATPase activity of GB-treated blueberry fruits was observed to be higher that of the control during storage (Fig. 6E and F). As shown in Fig. 6 G, GB-treated blueberry fruits exhibited higher SDH activity on day 5 of the storage period in comparison to the control group ($P < 0.05$), and CCO activity exhibited a gradual decline during storage in both the GB and control groups. However, the GB treatment resulted in a significant increase in CCO activity on days 10 and 15 ($P < 0.01$), with a 1.09-fold difference on

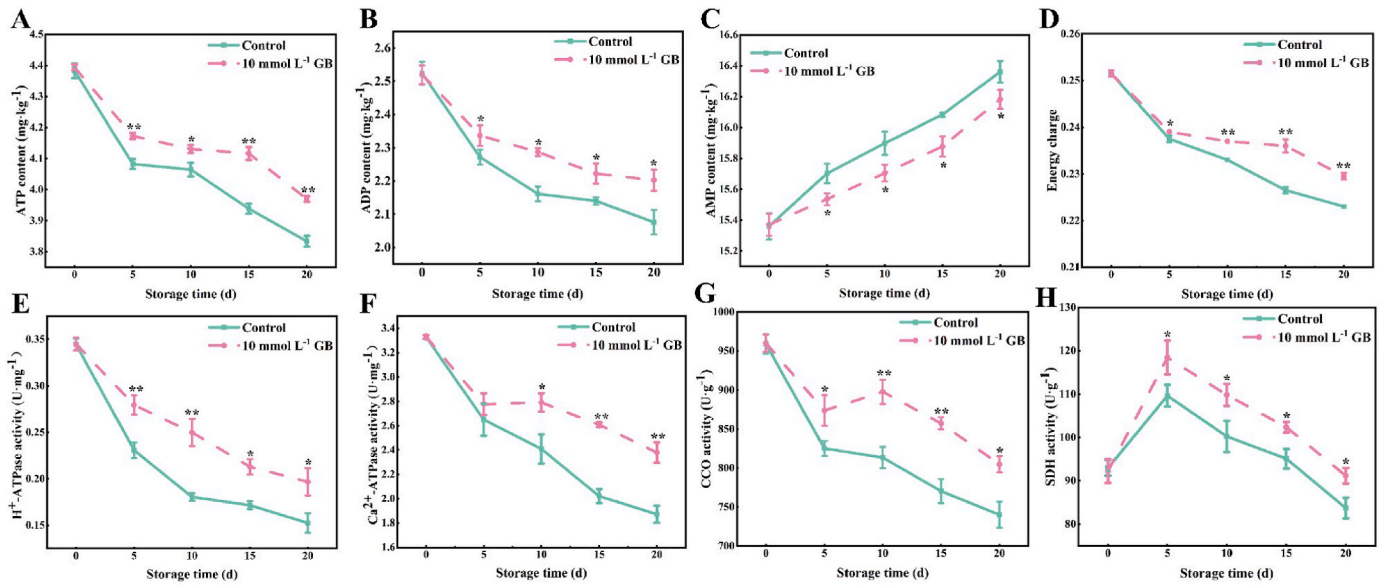

Fig. 6. Effects of GB treatment on blueberry ATP (A), ADP (B), AMP (C), EC (D), H^+-ATPase (E), Ca^{2+}-ATPase (F), CCO (G), and SDH (H) activities. Data are shown as mean ± SD standard deviation (n = 3). $^*P < 0.05$, $^{**}P < 0.01$.

day 20 of the storage period ($P < 0.05$)) (Fig. 6 H). Consistent with the results reported in previous studies, loquats improved fruit quality by increasing the activities of energy-metabolizing enzymes (Song et al., 2017).

3.7. Changes in endogenous GB content

There was a gradual increase in GB content in blueberry fruit throughout the storage process (Fig. 7). Exogenous GB treatment enhanced endogenous GB throughout storage time, fruits treated with 10 mmoL L^{-1} of GB showed higher levels of GB accumulation during storage. At 5th, 10th and 15th day of storage, there was a highly significant difference ($P < 0.01$) between the treated group and the control group, with the GB content of the treated fruits being 1.25, 1.30 and 1.99 times higher than that of the control group, respectively.

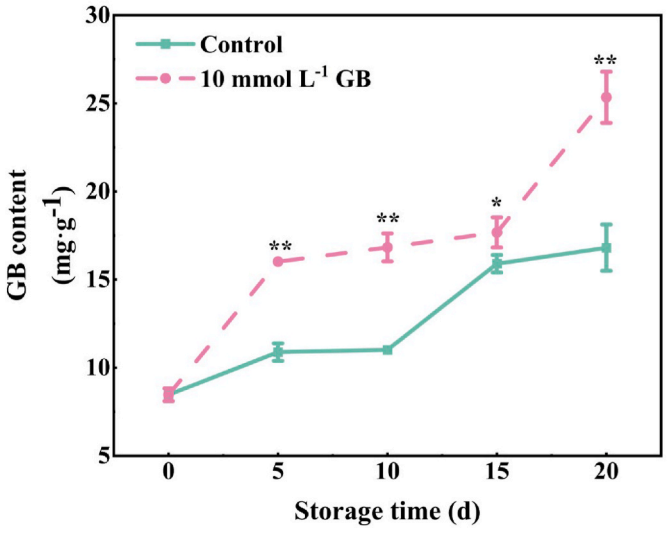

Fig. 7. Effects of GB treatment on blueberry endogenous GB content. Data are shown as mean ± SD standard deviation (n = 3). $^*P < 0.05$, $^{**}P < 0.01$.

4. Discussion

The postharvest quality of blueberry fruits is a critical factor in transportation and fresh marketing. The findings indicate that exogenous GB can maintain the quality of post-harvest blueberries, which is associated with changes in antioxidant energy and energy metabolism. The exogenous GB maintained a high level of antioxidants over time (Fig. 2). SOD catalyzes the $O_2^{\cdot-}$ catabolism reaction to hydrogen peroxide and O_2, and CAT and APX convert hydrogen peroxide to O_2 and H_2O, GR, MDHAR, and DHAR are produced in fruits and vegetables and protect tissues and cells from oxidative damage (Wang et al., 2020, 2020b). Razavi et al. (2018) stated that GB treatment enhanced antioxidant enzyme activity thereby reducing reactive oxygen species (ROS) accumulation and attenuated cold damage in hawthorn fruits. The data from the study demonstrated that GB significantly enhanced the activities of antioxidant enzymes, including SOD, APX, and CAT. Additionally, the fruit quality attributes exhibited marked improvement, exceeding those of the control, and demonstrated enhanced ROS scavenging capabilities (Figs. 3–4). ROS accelerate fruit softening and antioxidant enzymes play a crucial role in fruit ripening (Mittler, 2002). It can be proposed that increased antioxidant enzyme activity may be one of the mechanisms that are partially responsible for maintaining blueberry fruit quality. Consistent with our findings, GB treatment was also found to increase antioxidant enzyme activity in bell peppers (Wang et al., 2016) and zucchini fruits (Yao et al., 2018). H_2O_2 and superoxide anions are important physiological indicators of fruit and vegetable senescence. $O_2^{\cdot-}$ and hydrogen peroxide are the main products of ROS, which attack membrane lipids and accelerate cell death, and various biotic and abiotic stresses disrupt the balance of ROS metabolism, promote the accumulation of H_2O_2 and superoxide anions, and exacerbate fruit and vegetable senescence (Huang et al., 2024; Xia et al., 2015). MDA is a product of lipid peroxidation when plants are subjected to oxidative stress and is typically used as an indicator of the extent of membrane damage caused by lipid peroxidation (Nie et al., 2020). Reducing $O_2^{\cdot-}$ and hydrogen peroxide levels can alleviate lipid peroxidation, which may be responsible for the reduction of MDA. The DPPH free radical scavenging capacity has also been employed as an indicator of the potency of the plant antioxidant system (Fig. 5 Wang et al., 2013). The present study demonstrated that GB treatment enhanced the antioxidant system's capacity to defend against oxidative stres.

On the other hand, energy supply is essential for cell wall synthesis

and physiological processes in plant cells, and it is also a key factor in controlling postharvest fruit senescence (Aghdam et al., 2018; Verbančič et al., 2018). The energy state of the cell depends on the levels of ATP, ADP and AMP. Insufficient ATP levels may lead to lipid peroxidation, resulting in the accumulation of ROS, which can disrupt the cell membrane (Hu et al., 2021). With the extension of storage time, ATP levels decrease, and AMP levels increase in fruits (Cao et al., 2014; Ge et al., 2019). The GB treatment maintained high ATP levels, inhibited EC decline and effectively maintained blueberry quality. It has been demonstrated that MeJA treatment can effectively maintain higher levels of ATP in loquat fruits (Cao et al., 2014), that the TSP has been demonstrated to be capable of maintaining the quality of apple fruits by regulating respiration and mitochondrial energy metabolism (Ge et al., 2019), and furthermore, MeJA has been demonstrated to maintain high levels of ATP by stimulating the activity and transcript levels of enzymes involved in energy metabolism (Wang et al., 2021). Consistent with the results of previous studies, the levels of ATP and energy charge in both groups of fruits exhibited a gradual decline with increasing storage time, and the AMP content was higher. (Fig. 6A–D). Both H^+-ATPase and Ca^{2+}-ATPase are capable of catalysing the breakdown of ATP into ADP, they play a pivotal role in the synthesis and supply of energy, catalysing the conversion of ATP to ADP and thus contributing to the synthesis and supply of energy (Ge et al., 2019). SDH catalyzes the oxidation of succinate and the reduction of ubiquitin in the TCA cycle and ETC pathway, whereas CCO serves as a terminal enzyme of ETC and generates ATP through oxidative phosphorylation in the mitochondria (Huang et al., 2021 a). The results indicated that GB treatment maintained higher energy levels and ATPase activities, thus maintaining blueberry quality, and that GB had a vigorous effect on the activities of H^+-ATPase, Ca^{2+}-ATPase, CCO, and SDH (Fig. 6E–H).

Endogenous GB content was stressed by the environment during low temperature storage, and exogenous GB treatment stimulated endogenous GB accumulation, improved the antioxidant defence system, exhibited higher accumulation of total phenols, flavonoids and anthocyanosides, as well as delayed the decline of enzymes related to energy metabolism, and prolonged the post-harvest shelf-life (Fig. 7). It has also been reported in the literature that glycine betaine is effective in reducing cold injury in fruit. Pan et al. (2019) investigated the effect of glycine betaine on energy metabolism cold damage in papaya fruits during cold storage and showed that the enhanced cold tolerance of GB-treated papaya fruits during cold storage could be attributed to improved energy status and increased activity of enzymes related to energy metabolism. Meanwhile, the CTS-GB NPs effectively mitigated the chilling injury and maintained the nutritional quality of plums (Roghayeh et al., 2022). These results suggest that GB treatment can be used as an effective technique to control fruit cold damage and maintain quality, laying the foundation for post-harvest storage of fruits and vegetables. Correlation analysis showed that hardness was highly significantly positively correlated with APX, MDHAR, DHAR, GR, DPPH, anthocyanin, ATP, ADP, EC, and H^+-ATPase ($P < 0.01$), and highly significantly negatively correlated with decay rate, denaturation rate, hydrogen peroxide, MDA, superoxide anion, and AMP ($P < 0.01$). Consequently, exogenous GB treatment increased the endogenous GB content of the fruit, activated the antioxidant defence system and the energy metabolism of the blueberry fruit, and provided the energy necessary for the necessary biochemical changes occurring in the fruit, reducing low temperature stress and maintaining blueberry quality (Fig. 8).

5. Conclusions

The results demonstrated that GB significantly maintained postharvest blueberry fruit quality, retards the decline of ATP, ADP, H^+-ATPase, Ca^{2+}-ATPase, SDH, and CCO, while maintaining a higher electrical charge (EC). Meanwhile, GB treatment resulted in an increase in the accumulation of endogenous GB, which led to an enhancement in antioxidant enzyme activities. This effectively inhibiting the deterioration of blueberry fruits, and providing technical support for the postharvest preservation of blueberries. Furthermore, this study offers a theoretical foundation for the development and utilisation of other alkaloids as postharvest preservation strategies for fruits and vegetables.

CRediT authorship contribution statement

Yu Zhang: Writing – original draft, Data curation. **Xiaohong Kou:** Methodology, Conceptualization. **Guohe Zhang:** Software. **Donglan Luo:** Visualization, Investigation. **Sen Cao:** Validation, Supervision, Software.

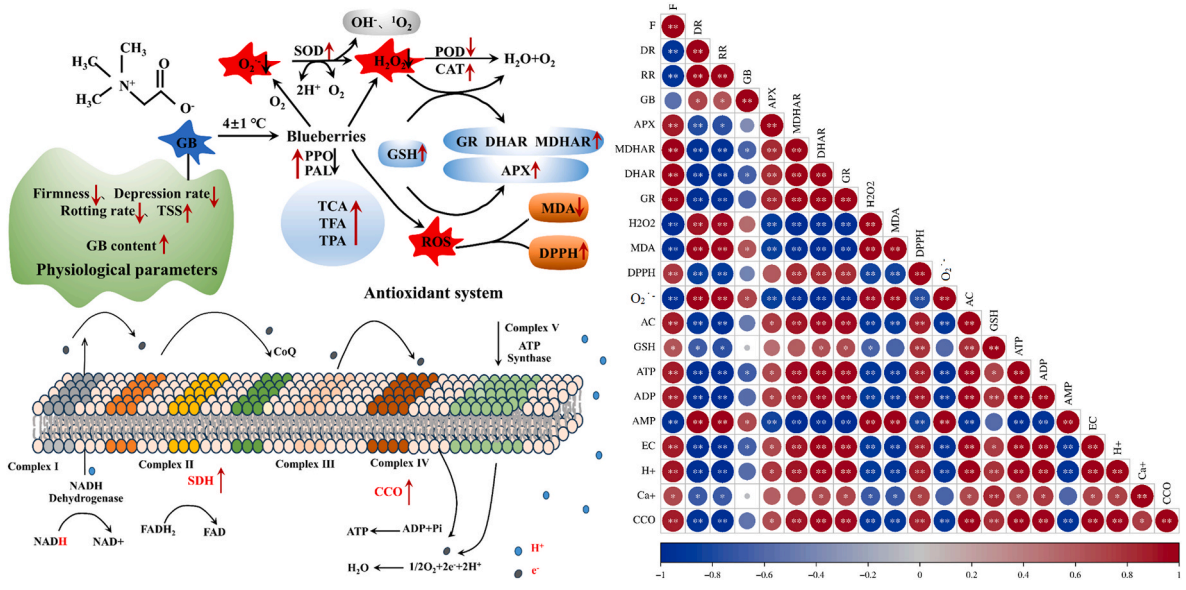

Fig. 8. Mechanistic map of GB response to the blueberry antioxidant system and energy metabolism. And correlation matrix between F (Firmness), DR (depression rate), RR (decay rate), GB, APX, MDHAR, DHAR, GR, H_2O_2, MDA, DPPH, $O_2^{\cdot-}$, AC (anthocyanin), GSH, ATP, ADP, AMP, EC, H^+ (H^+-ATPase), Ca^{2+} (Ca^{2+}-ATPase), and CCO in a correlation matrix between them. The significance levels are indicated by asterisks: $^*P < 0.05$ and $^{**}P < 0.01$.

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.

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Data availability

Data will be made available on request.

References

Aghdam, M. S., Flores, F. B., & Sedaghati, B. (2021). Exogenous phytosulfokine α (PSKα) application delays senescence and relieves decay in strawberry fruit during cold storage by triggering extracellular ATP signaling and improving ROS scavenging system activity. *Scientia Horticulturae, 279*. https://doi.org/10.1016/J.SCIENTA.2021.109906

Aghdam, M. S., Jannatizadeh, A., Luo, Z., & Paliyath, G. (2018). Ensuring sufficient intracellular ATP supplying and friendly extracellular ATP signaling attenuates stresses, delays senescence and maintains quality in horticultural crops during postharvest life. *Trends in Food Science & Technology, 76*, 67–81. https://doi.org/10.1016/j.tifs.2018.04.003

Angeletti, P., Castagnasso, H., Miceli, E., Terminiello, L., Concellón, A., Chaves, A., & Vicente, A. R. (2010). Effect of preharvest calcium applications on postharvest quality, softening and cell wall degradation of two blueberry (Vaccinium corymbosum) varieties. *Postharvest Biology and Technology, 58*, 98–103. https://doi.org/10.1016/j.postharvbio.2010.05.015

Cao, S., Cai, Y., Yang, Z., Joyce, D. C., & Zheng, Y. (2014). Effect of MeJA treatment on polyamine, energy status and anthracnose rot of loquat fruit. *Food Chemistry, 145*, 86–89. https://doi.org/10.1016/j.foodchem.2013.08.019

Centeno, D. A., Solano, X. H., & Castillo, J. J. (2017). A new peroxidase from leaves of Guinea grass (Panicum maximum): A potential biocatalyst to build amperometric biosensors. *Bioelectrochemistry, 116*, 33–38. https://doi.org/10.1016/j.bioelechem.2017.03.005

Chea, S., Yu, D. J., Park, J., Oh, H. D., Chung, S. W., & Lee, H. J. (2019). Preharvest β-aminobutyric acid treatment alleviates postharvest deterioration of 'Bluecrop' highbush blueberry fruit during refrigerated storage. *Scientia Horticulturae, 246*, 95–103. https://doi.org/10.1016/j.scienta.2018.10.036

Chen, L. L., Shan, W., Cai, D. L., Chen, J. Y., Lu, W. J., Su, X. G., & Kuang, J. F. (2021). Postharvest application of glycine betaine ameliorates chilling injury in cold-stored banana fruit by enhancing antioxidant system. *Scientia Horticulturae, 287*. https://doi.org/10.1016/j.scienta.2021.110264. Article 110264.

Chu, W., Gao, H., Chen, H., Fang, X., & Zheng, Y. (2018). Effects of cuticular wax on the postharvest quality of blueberry fruit. *Food Chemistry, 239*, 68–74. https://doi.org/10.1016/j.foodchem.2017.06.024

De Zwart, F. J., Slow, S., Payne, R. J., Lever, M., George, P. M., Gerrard, J. A., & Chambers, S. T. (2003). Glycine betaine and glycine betaine analogues in common foods. *Food Chemistry, 83*, 197–204. https://doi.org/10.1016/s0308-8146(03)00063-3

Ding, J., Liu, C., Huang, P., Zhang, Y., Hu, X., Li, H., … Qi, W. (2023). Effects of thymol concentration on postharvest diseases and quality of blueberry fruit. *Food Chemistry, 402*, 134227. https://doi.org/10.1016/J.FOODCHEM.2022.134227, 134227.

Du, M., Lian, L., Zhang, Y., Lin, H., & Wang, J. (2024). Roles of ROS metabolism and phenylpropanoid pathway in quality maintenance of postharvest Pleurotus eryngii under hyperoxia stress. *Postharvest Biology and Technology, 207*, Article 112617. https://doi.org/10.1016/j.postharvbio.2023.112617

Ge, Y., Chen, Y., Li, C., Wei, M., Li, X., Li, S., Lu, S., & Li, J. (2019). Effect of trisodium phosphate dipping treatment on the quality and energy metabolism of apples. *Food Chemistry, 274*, 324–329. https://doi.org/10.1016/j.foodchem.2018.08.142

Habibi, F., Valero, D., Serrano, M., & Guillén, F. (2022). Exogenous application of Glycine betaine maintains bioactive compounds, antioxidant activity, and physicochemical attributes of blood orange fruit during prolonged cold storage. *Frontiers in Nutrition, 9*, 873915. https://doi.org/10.3389/fnut.2022.873915, 873915.

Hu, W., Zhang, X., Godana, E. A., Gu, X., Zhao, L., & Zhang, H. (2021). Yarrowia lipolytica reduces the disease incidence of asparagus infected by Fusarium proliferatum by affecting respiratory metabolism and energy status. *Biological Control, 159*, Article 104625. https://doi.org/10.1016/j.biocontrol.2021.104625

Huan, C., Han, S., Jiang, L., An, X., Yu, M., Xu, Y., Ma, R., & Yu, Z. (2017). Postharvest hot air and hot water treatments affect the antioxidant system in peach fruit during refrigerated storage. *Postharvest Biology and Technology, 126*, 1–14. https://doi.org/10.1016/j.postharvbio.2016.11.018

Huang, R., Cheng, Y., Li, C., Guo, M., Zhu, J., Ge, Y., & Sun, T. (2021b). Postharvest application of acibenzolar- S-methyl delays the senescence of pears by mediating the ascorbate-glutathione cycle. *Scientia Horticulturae, 293*, Article 110741. https://doi.org/10.1016/j.scienta.2021.110741

Huang, T., Kou, X., Qiao, L., Li, J., Luo, D., Wang, X., & Cao, S. (2024). Maintaining quality of postharvest green pepper fruit using melatonin by regulating membrane lipid metabolism and enhancing antioxidant capacity. *Food Chemistry, 460*, Article 112619. https://doi.org/10.1016/j.foodchem.2024.140671

Huang, H., Wang, D., Belwal, T., Dong, L., Lu, L., Zou, Y., … Luo, Z. (2021a). A novel W/O/W double emulsion co-delivering brassinolide and cinnamon essential oil delayed the senescence of broccoli via regulating chlorophyll degradation and energy metabolism. *Food Chemistry, 356*, Article 129704. https://doi.org/10.1016/j.foodchem.2021.129704

Huang, L., Zhou, Y., Meng, L., Wu, D., & He, Y. (2016). Comparison of different CCD detectors and chemometrics for predicting total anthocyanin content and antioxidant activity of mulberry fruit using visible and near infrared hyperspectral imaging technique. *Food Chemistry, 224*, 1–10. https://doi.org/10.1016/j.foodchem.2016.12.037

Jin, P., Zhu, H., Wang, J., Chen, J., Wang, X., & Zheng, Y. (2013). Effect of methyl jasmonate on energy metabolism in peach fruit during chilling stress. *Journal of the Science of Food and Agriculture, 93*, 1827–1832. https://doi.org/10.1002/jsfa.5973

Klingenberg, M. (2008). The ADP and ATP transport in mitochondria and its carrier. *Biochimica et Biophysica Acta, 1778*, 1978–2021. https://doi.org/10.1016/j.bbamem.2008.04.011

Li, D., Limwachiranon, J., Li, L., Du, R., & Luo, Z. (2016). Involvement of energy metabolism to chilling tolerance induced by hydrogen sulfide in cold-stored banana fruit. *Food Chemistry, 208*, 272–278. https://doi.org/10.1016/j.foodchem.2016.03.113

Li, M., Lin, H., Chen, Y., Chen, Y., Lin, Y., Fan, Z., & Wang, H. (2024). The role of energy transport system in pericarp browning of harvested longan fruit. *Postharvest Biology and Technology, 207*. https://doi.org/10.1016/j.postharvbio.2023.112619. Article 112619.

Ma, Q., Lin, X., Wei, Q., Yang, X., Zhang, Y., & Chen, J. (2021). Melatonin treatment delays postharvest senescence and maintains the organoleptic quality of 'Newhall' navel orange (Citrus sinensis (L.) Osbeck) by inhibiting respiration and enhancing antioxidant capacity. *Scientia Horticulturae, 286*. https://doi.org/10.1016/j.scienta.2021.110236. Article 110236.

Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. *Trends in Plant Science, 7*(9), 405–410. https://doi.org/10.1016/S1360-1385(02)02312-9

Nie, Z., Huang, Q., Chen, C., Wan, C., & Chen, J. (2020). Chitosan coating alleviates postharvest juice sac granulation by mitigating ROS accumulation in harvested pummelo (Citrus grandis L. Osbeck) during room temperature storage. *Postharvest Biology and Technology, 169*, Article 111309. https://doi.org/10.1016/j.postharvbio.2020.111309

Pan, Y., Zhang, S., Yuan, M., Song, H., Wang, T., Zhang, W., & Zhang, Z. (2019). Effect of glycine betaine on chilling injury in relation to energy metabolism in papaya fruit during cold storage. *Food Science and Nutrition, 3*, 1123–1130. https://doi.org/10.1002/fsn3.957

Razavi, F., Mahmoudi, R., Rabiei, V., Aghdam, M. S., & Soleimani, A. (2018). Glycine betaine treatment attenuates chilling injury and maintains nutritional quality of hawthorn fruit during storage at low temperature. *Scientia Horticulturae, 233*, 188–194. https://doi.org/10.1016/j.scienta.2018.01.053

Roghayeh, M., Farhang, R., Vali, R., Gholamreza, G., & Lluís, P. (2022). Application of Glycine betaine coated chitosan nanoparticles alleviate chilling injury and maintain quality of plum (Prunus domestica L.) fruit. *International Journal of Biological Macromolecules, 207*, 965–977. https://doi.org/10.1016/J.IJBIOMAC.2022.03.167

Sakamoto, A., & Murata, N. (2002). The role of glycine betaine in the protection of plants from stress: Clues from transgenic plants. *Plant, Cell and Environment, 25*, 163–171. https://doi.org/10.1046/j.0016-8025.2001.00790.x

Shan, T., Jin, P., Zhang, Y., Huang, Y., Wang, X., & Zheng, Y. (2016). Exogenous glycine betaine treatment enhances chilling tolerance of peach fruit during cold storage. *Postharvest Biology and Technology, 114*, 104–110. https://doi.org/10.1016/j.postharvbio.2015.12.005

Shi, J., Xiao, Y., Jia, C., Zhang, H., Gan, Z., Li, X., … Wang, Q. (2023). Physiological and biochemical changes during fruit maturation and ripening in highbush blueberry (Vaccinium corymbosum L.). *Food Chemistry, 410*, Article 135299. https://doi.org/10.1016/j.foodchem.2022.135299

Song, H., Wang, X., Hu, W., Yang, X., Diao, E., Shen, T., & Qiang, Q. (2017). A cold-induced phytosulfokine peptide is related to the improvement of loquat fruit chilling tolerance. *Food Chemistry, 232*, 434–442. https://doi.org/10.1016/j.foodchem.2017.04.045

Sun, H., Luo, M., Zhou, X., Zhou, Q., Sun, Y., Ge, W., … Ji, S. (2019). Exogenous glycine betaine treatment alleviates low temperature-induced pericarp browning of 'Nanguo' pears by regulating antioxidant enzymes and proline metabolism. *Food Chemistry, 306*. https://doi.org/10.1016/j.foodchem.2019.125626. Article 125626.

Sun, J., You, X., Li, L., Peng, H., Su, W., Li, C., He, Q., & Liao, F. (2011). Effects of a phospholipase D inhibitor on postharvest enzymatic browning and oxidative stress of litchi fruit. *Postharvest Biology and Technology, 62*(3), 288–294. https://doi.org/10.1016/j.postharvbio.2011.07.001

Tang, Q., Li, C., Ge, Y., Li, X., Chen, Y., Hou, J., & Li, J. (2020). Exogenous application of melatonin maintains storage quality of jujubes by enhancing anti-oxidative ability

and suppressing the activity of cell wall-degrading enzymes. *LWT-Food Science and Technology, 127*, Article 109431. https://doi.org/10.1016/j.lwt.2020.109431

Torres, M. A. (2010). ROS in biotic interactions. *Physiologia Plantarum, 138*, 414–429. https://doi.org/10.1111/j.1399-3054.2009.01326.x

Verbančič, J., Lunn, J. E., Stitt, M., & Persson, S. (2018). Carbon supply and the regulation of cell wall synthesis. *Molecular Plant, 11*, 75–94. https://doi.org/10.1016/j.molp.2017.10.004

Wang, H., Cheng, X., Wu, C., Fan, J., Li, T., & Dong, C. (2021). Retardation of postharvest softening of blueberry fruit by methyl jasmonate is correlated with altered cell wall modification and energy metabolism. *Scientia Horticulturae, 276*. https://doi.org/10.1016/j.scienta.2020.109752. Article 109752.

Wang, Q., Ding, T., Zuo, J., Gao, L., & Fan, L. (2016). Amelioration of postharvest chilling injury in sweet pepper by glycine betaine. *Postharvest Biology and Technology, 112*, 114–120. https://doi.org/10.1016/j.postharvbio.2015.07.008

Wang, T., Hu, M., Yuan, D., Yun, Z., Gao, Z., Su, Z., & Zhang, Z. (2020). Melatonin alleviates pericarp browning in litchi fruit by regulating membrane lipid and energy metabolisms. *Postharvest Biology and Technology, 160*, 111066. https://doi.org/10.1016/j.postharvbio.2019.111066, 111066.

Wang, H., Wu, Y., Yu, R. P., Wu, C., Fan, G., & Li, T. (2019). Effffects of postharvest application of methyl jasmonate on physicochemical characteristics and antioxidant system of the blueberry fruit. *Scientia Horticulturae, 258*. https://doi.org/10.1016/j.scienta.2019.108785. Article 108785.

Wang, X., Xu, F., Wang, J., Jin, P., & Zheng, Y. (2013). Bacillus cereus AR156 induces resistance against Rhizopus rot through priming of defense responses in peach fruit. *Food Chemistry, 136*, 400–406. https://doi.org/10.1016/j.foodchem.2012.09.032

Wang, F., Yang, Q., Zhao, Q., & Zhang, X. (2020). Roles of antioxidant capacity and energy metabolism in the maturity-dependent chilling tolerance of postharvest kiwifruit. *Postharvest Biology and Technology, 168*. https://doi.org/10.1016/j.postharvbio.2020.111281. Article 111281.

Wang, F., Zhang, X., Yang, Q., & Zhao, Q. (2019). Exogenous melatonin delays postharvest fruit senescence and maintains the quality of sweet cherries. *Food Chemistry, 301*, Article 125311. https://doi.org/10.1016/j.foodchem.2019.125311

Xia, X. J., Zhou, Y. H., Shi, K., Zhou, J., Foyer, C. H., & Yu, J. Q. (2015). Interplay between reactive oxygen species and hormones in the control of plant development and stress tolerance. *Journal of Experimental Botany, 66*(10), 2839–2856. https://doi.org/10.1093/jxb/erv089

Xie, G., Yang, C., Fei, Y., & Ma, L. (2020). Physiological and proteomic analyses of 1-MCP treatment on lignification in fresh common bean (Phaseolus vulgaris L) during storage. *Postharvest Biology and Technology, 160*. https://doi.org/10.1016/j.postharvbio.2019.111041. Article 111041.

Xu, B. J., & Chang, S. K. C. (2007). A comparative study onphenolic profiles and antioxidant activities of legumes as affected by extraction solvents. *Journal of foodscience, 72*(2), 159. https://doi.org/10.1111/j.1750-3841.2006.00260.x

Yang, J., Li, B., Shi, W., Gong, Z., Chen, L., & Hou, Z. (2018). Transcriptional activation of anthocyanin biosynthesis in developing fruit of blueberries (Vaccinium corymbosum L.) by preharvest and postharvest UV irradiation. *Journal of Agricultural and Food Chemistry, 66*(42), 10931–10942. https://doi.org/10.1021/acs.jafc.8b03081

Yao, W., Xu, T., Farooq, S. U., Jin, P., & Zheng, Y. (2018). Glycine betaine treatment alleviates chilling injury in zucchini fruit (Cucurbita pepo L.) by modulating antioxidant enzymes and membrane fatty acid metabolism. *Postharvest Biology and Technology, 144*, 20–28. https://doi.org/10.1016/j.postharvbio.2018.05.007

Zhang, J., Wang, C., Chen, C., Zhang, S., Zhao, X., Wu, C., Kou, X., & Xue, Z. (2023). Glycine betaine inhibits postharvest softening and quality decline of winter jujube fruit by regulating energy and antioxidant metabolism. *Food Chemistry, 410*, 135445. https://doi.org/10.1016/J.FOODCHEM.2023.135445, 135445.

Zhang, Z., Xu, J., Chen, Y., Wei, J., & Wu, B. (2019). Nitric oxide treatment maintains postharvest quality of table grapes by mitigation of oxidative damage. *Postharvest Biology and Technology, 152*, 9–18. https://doi.org/10.1016/j.postharvbio.2019.01.015