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Enhancing Cucumber (*Cucumis sativus* L.) Quality and Shelf Life Through Innovative Post-Harvest Application of Glycine Betaine

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Abstract

Cucumber fruits are known for their short shelf life post-harvest, primarily due to their high-water content, elevated metabolic activity, and susceptibility to microbial spoilage. These attributes underscore the necessity for effective storage methods and quality preservation strategies. This study aimed to evaluate the effects of glycine betaine at concentrations of 0 and 15 mM on various quality parameters of cucumber fruit during a 12-day storage period at 4°C. The findings revealed that glycine betaine treatments significantly mitigated weight loss and preserved essential quality indicators, including total soluble solids concentration, pH, fruit firmness, total chlorophyll content in the skin, and ascorbic acid levels. Notably, the treatment with 15 mM glycine betaine resulted in the lowest weight loss and the highest firmness on days 6 and 9 of storage. Furthermore, this treatment was associated with the highest levels of soluble solids, total chlorophyll, and vitamin C, alongside the lowest pH and sensory quality reduction on days 6 and 9, demonstrating a statistically significant improvement compared to the control group. These results suggest that glycine betaine is an effective agent for enhancing the post-harvest quality and longevity of cucumbers.

Keywords: Ascorbic acid, Cucumber, Glycine betaine, Post-harvest, Soluble solids.

1. Introduction

Cucumber fruit is one of the most important commercial vegetables grown in various regions around the world (Charles-Rodríguez et al., 2023; Malekzadeh et al., 2017). Nutritionally, cucumbers contain phenolic compounds, cucurbitacin's, vitamins, antioxidants, blood sugar reducers, anti-inflammatory agents, and anticancer compounds, along with a high-water content, fiber, and low calories. This has made cucumbers very popular among consumers. However, this product is highly perishable, and during storage, transportation, and post-harvest operations, it can spoil, leading to a rapid loss of quality characteristics (Łaźny et al., 2023). Water loss, tissue firmness reduction, drying, yellow or brown discoloration, rotting, and freezing all contribute to a shortened shelf life (Wang et al., 2023). For producers, brokers, distributors, and consumers, extending the shelf life of cucumbers is essential for reducing waste and maintaining quality. Various methods have been employed to achieve this, including chemical methods (Łaźny et al., 2023), physical methods (Wang et al., 2023), and edible coatings (Charles-Rodríguez et al., 2023). Among these methods, chemical and physical approaches are costly, non-biodegradable, and harmful to the environment and human health. In contrast, edible coatings are a natural, environmentally friendly, and cost-effective solution (Charles-Rodríguez et al., 2023). In this process, coating layers act as an

insulator on the surface of coated products, altering the ethylene gas composition around the product, which prevents water evaporation and reduces physiological weight. Therefore, they delay the aging process and help maintain the shelf life of fruits and vegetables. Currently, edible coatings for fruits and vegetables are made using various natural materials such as polysaccharides (cellulosic compounds, starch, chitin, and gums), proteins (soy protein, milk, gelatin, corn, zein, and wheat gluten), and lipids (oils, butters) and resins. Among edible coatings, CHITOSAN is the most widely used due to its abundance, biocompatibility, biodegradability, low cost, ability to form better coatings, and natural antimicrobial and antioxidant properties. The beneficial effects of CHITOSAN coatings on some cucumber species have been reported.

Glycine betaine (GB), a quaternary ammonium compound, acts as an osmotic regulator in plant cells, maintaining higher cellular solubility and providing resistance to various abiotic stresses (Mahmoudi et al., 2022; Wang et al., 2016). It has been reported that exogenous application of GB increases the cold tolerance of Arabidopsis and tomato plants (Dou et al., 2022; Malekzadeh et al., 2014; Moretti et al., 2002; Xing & Rajashekar, 2001). Recent studies have also shown that exogenous GB treatment may effectively reduce chilling injury (CI) and improve post-harvest quality in various harvested products, such as bananas (Ali et al., 2023), peaches (Shan., 2016). Exogenous glycine betaine treatment enhances chilling tolerance of peach fruit during cold storage. Postharvest Biology and Technology, 114, 104-110hawthorn (Razavi et al., 2018), and bell peppers (Wang et al., 2016a). The aim of this study is to investigate the effects of post-harvest application of different concentrations of glycine betaine coating on the preservation of quality and extension of the shelf life of cucumber fruit during storage at 4±1°C.

2. Material and methods

2.1. Plant Materials

Cucumber fruits (Cucumis sativus L.) of the Emerald variety were harvested in October 2023 from a greenhouse located in Qom and were quickly transferred to the post-harvest physiology laboratory. Fresh fruits were manually selected for uniformity in size and shape and to ensure there was no visible damage. Initially, the fruits were washed with a 0.05% hypochlorite solution and then rinsed with distilled water before being air-dried. After air-drying, their initial physical and chemical characteristics were measured (day 0). The fruits were then pre-treated with glycine betaine (0, 15 mM per liter). Before treatment, glycine betaine (Sigma-Aldrich) solutions were prepared in 1% acetic acid and distilled water, respectively. After treatment, the weight of the samples was measured, and they were stored in polyethylene containers at 4°C and 85-90% relative humidity for a duration of 12 days. Measurements included weight loss, sensory quality, tissue firmness, total soluble solids concentration, pH content, ascorbic acid, and chlorophyll in the fruit skin. All measurements were conducted on days 0, 3, 6, 9, and 12 after the start of the experiment, with three replications for all measured parameters.

2.2. Percentage of Weight Loss

The measurement of weight loss in the samples during the storage period was expressed as a percentage of the initial weight using a digital scale with an accuracy of 0.001 grams, calculated using the following formula:

Percentage of Weight Loss=100× (Initial Weight−Final WeightInitial Weight) Percentage of Weight Loss=100× (Initial WeightInitial Weight−Final Weight)

2.3. Measurement of Physicochemical Characteristics

To determine the total soluble solids concentration of the cucumber samples, a digital refractometer was used at a temperature of 20°C. The pH of homogenized cucumber samples (5 grams with 50 milliliters of distilled water) was measured using a pH meter.

2.4. Tissue Firmness

The firmness of the cucumbers was measured using a fruit firmness tester (model FT011) with an 8 mm diameter probe, and the firmness of the cucumber fruit was reported in kilograms per square centimeter.

2.5. Measurement of Chlorophyll in Fruit Skin

Chlorophyll from cucumber skin samples was extracted using 80% acetone, and the optical density of the extracts was read at two wavelengths, 645 and 663 nanometers, and calculated (Malekzadeh et al., 2023).

2.5. Statistical Analysis

The experiment was conducted as a factorial design in a completely randomized design with three replications. Each replication included four fruits for each measurement stage. Overall, the experimental treatments included different concentrations of glycine betaine (0, 15 mM) storage times (0, 3, 6, 9, and 12 days). During the measurement period, four fruits from each replication in each treatment were removed, and the desired indices were measured. Statistical analysis of the data from this study was performed using SPSS software version 26, and the least significant difference (LSD) test was used to compare the means.

3. Results and Discussion

The results of the variance analysis of the data from this study indicated that the main effects of glycine betaine, and storage time, as well as the interaction effects of glycine betaine × storage time, were significant at the 1% probability level for all studied characteristics.

3.1. Physiological Weight Loss Percentage

Figure1, illustrates the effect of glycine betaine (GB) treatment on the physiological weight loss (PWL) of cucumber fruits during storage. The results demonstrate that increasing the concentration of glycine betaine led to a reduction in weight loss over time compared to the control.

At the initial 0 days of storage, the PWL was similar between the control and 15 mM GB treatments. However, as the storage time increased, the PWL increased for both treatments, but the rate of increase was lower for the 15 mM GB treatment. After 12 days of storage, the PWL was 8.57% for the control, while it was only 7.30% for the 15 mM GB treatment, a statistically significant difference.

The interactive effects of glycine betaine and storage time revealed that higher concentrations of GB (e.g., 15 mM) were more effective in reducing weight loss compared to lower concentrations.

These findings suggest that the application of glycine betaine can help maintain the quality and extend the shelf life of cucumber fruits by reducing physiological weight loss during storage. The stabilizing effect of GB on cell membranes and its ability to enhance the integrity of the fruit's cell wall are likely the underlying mechanisms responsible for the observed reduction in weight loss.

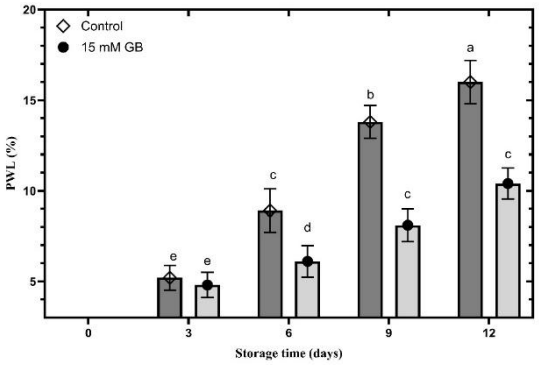

Figure 1. Effect of Glycine Betaine on PLW (%) in Cucumbers during Storage at 4º C.

The results showed that the application of glycine betaine in cucumber products can effectively prevent physiological weight loss during the storage period. Increasing the concentration of glycine betaine led to a reduction in the rate of weight loss over the storage time compared to the control sample (Alaei & Mahna, 2023; Badawy et al., 2024). This protective effect is likely due to the ability of glycine betaine to stabilize cell membranes and increase the integrity of the fruit's cell wall, which prevents moisture evaporation and weight reduction of the product (Chen et al., 2021). Additionally, the results showed that higher concentrations of glycine betaine (such as 15 mM) were more effective in reducing weight loss than lower concentrations. These findings demonstrate the potential of using glycine betaine to maintain quality and increase the shelf life of cucumbers during storage.

3.2. Total Soluble Solids (TSS)

The Fig 2 demonstrates the changes in Total Soluble Solids (TSS) percentage over a 12-day storage period for a control sample and a sample

containing 15 mM Glucose Betaine (GB). The control sample, represented by a diamond symbol, exhibits a gradual decrease in TSS over the storage time. In contrast, the 15 mM GB sample, denoted by a black circle, displays a different trend. It initially decreases, then increases, and ultimately reaches a higher TSS level compared to the control at the end of the 12-day storage period. This data suggests that the addition of 15 mM GB may have a beneficial effect on maintaining or even enhancing the TSS of the sample over an extended storage time, in comparison to the control. Further research would be necessary to elucidate the underlying mechanisms and potential applications of this observation. At the time of harvest (day 0), the TSS in the GB-treated sample is approximately 15% higher than the control sample. Throughout the storage period, the TSS in the GB-treated sample remains consistently higher than the control. By day 12 of storage, the TSS in the GB-treated sample is about 25% higher than the control sample. This increase in TSS in the GB-treated samples may be due to the reduced water evaporation and the preservation of the fruit's sugar content during storage. The higher TSS in the GB-treated fruit can potentially lead to improved quality and better taste of the fruit during storage. These results suggest that the use of glycerol betaine compounds can be an effective method for maintaining quality and increasing the shelf-life of fruits after harvest.

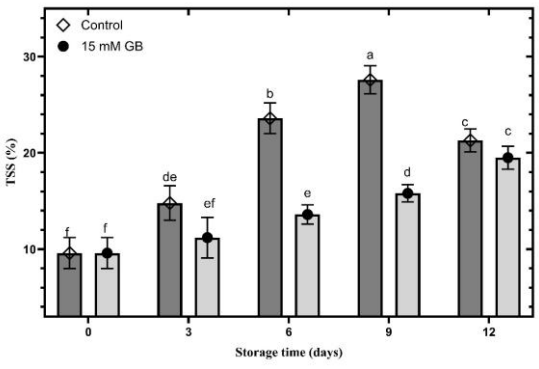

Figure 2. Effect of Glycine Betaine on TSS (%) in Cucumbers during Storage at 4º C.

The Total Soluble Solids (TSS) content, which represents the total dissolved solids in the fruit, plays a crucial role in the post-harvest quality and shelf-life of cucumbers (Habibi et al., 2023; Huang et al., 2022). Higher TSS levels are generally associated with improved fruit quality, as they indicate a higher concentration of sugars, acids, and other dissolved solids that contribute to the fruit's taste and nutritional value. By maintaining elevated TSS levels during storage, the post-harvest quality and consumer acceptability of cucumbers can be enhanced, leading to extended shelf-life and reduced waste (Ahmad & Ali, 2019; Ahmed et al., 2017; Zhang et al., 2023).

3.3. Titratable Acidity (TA)

The graph depicts the changes in titratable acidity (TA) of cucumbers over a 12-day storage period, comparing a control sample and a sample treated with 15 mM glycerol betaine (GB). At the initial time of harvest (day 0), the TA of the GB-treated sample (point a) is slightly higher than the control sample. However, during the storage period, the TA of the control sample decreases more rapidly, as shown by the steeper slope (points b to i). In contrast, the TA of the GB-treated sample declines at a slower rate, maintaining a higher TA level throughout the 12-day storage period. This suggests that the application of 15 mM GB was effective in retarding the loss of titratable acidity in the cucumbers, potentially contributing to improved quality and shelf-life.

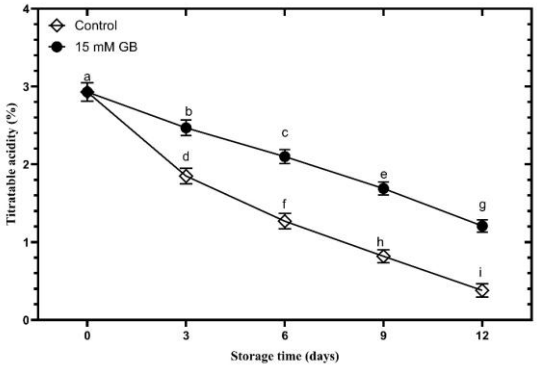

Figure 3. Effect of Glycine Betaine on TA (%) in Cucumbers during Storage at 4º C.

3.4. pH

Exogenous application of glycine betaine led to a decrease in the pH of cucumber fruits (Fig4). The results showed that glycine betaine treatments caused a decrease in pH and reduced the observable increase

in pH during storage. During the storage of fruits, the pH of fruit extracts increases, and acidity decreases. Throughout the storage period, the pH of fruits increases, and researchers have indicated that in most fruits, the increase in pH is related to the reduction of organic acids during storage (Amiri et al., 2022; Asgarian et al., 2022). These results are consistent with findings reported by other researchers, indicating that the decrease in acidity during storage signifies fruit aging. The increase in pH may be attributed to the consumption of organic acids in respiration processes during storage (Abdalla et al., 2022; Almas et al., 2021). The reduction in pH changes may be related to the effects of treatments on the biochemical status of the fruit and the associated reductions in respiration rates and metabolic activity (Chen et al., 2008).

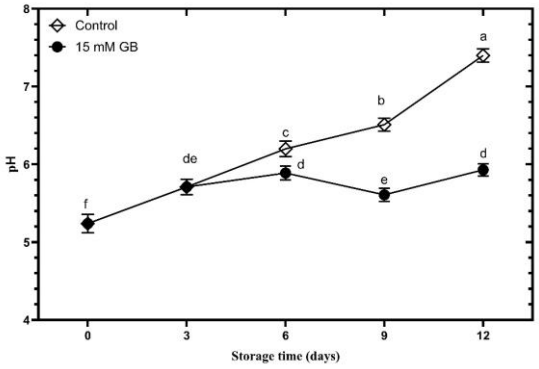

Figure 4. Effect of Glycine Betaine on pH in Cucumbers during Storage at 4º C.

3.5. Fruit Firmness

The results obtained in Fig 5, indicate that increasing concentrations of glycine betaine led to increased firmness and a reduction in the storage time of cucumber firmness. The comparison of the interactive effects of glycine betaine × storage time showed that increasing concentrations of glycine betaine at similar times led to a reduction in the process of firmness loss in fruits (Fig 5). Tissue firmness is one of the important quality characteristics in the acceptance of fresh fruits and vegetables by consumers (Malekzadeh et al., 2017; Nomberg et al., 2022). The firmness of fruits is a primary attribute that determines post-harvest life and fruit quality. The main factor in the reduction of firmness in fruits and vegetables is the degradation of pectin polymers. Cucumber is a fruit that quickly loses its firmness after harvest, leading to a shortened shelf life and increased susceptibility to fungal contamination (Hou et al., 2023). Researchers have reported that coating cucumbers reduces respiration, which may be responsible for maintaining fruit firmness during storage (Fahmy et al., 2015). The results showed that using glycine betaine treatment can reduce the loss of fruit firmness. These results align with findings from other researchers regarding cucumbers (Fahmy et al., 2015; Hou et al., 2023; Malekzadeh et al., 2017). Therefore, utilizing the postharvest properties of glycine betaine can delay the aging and spoilage of fruits.

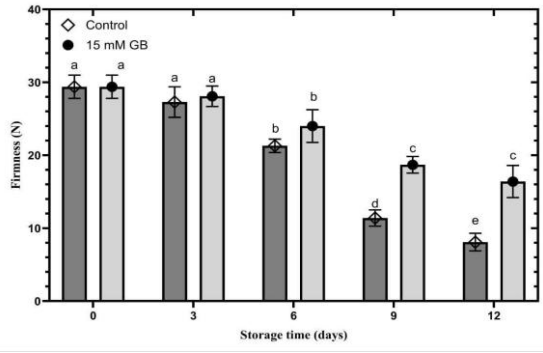

Figure 5. Effect of Glycine Betaine on Firmness Content in Cucumbers during Storage at 4º C.

3.6. Chlorophyll

The fig 6 depicting the chlorophyll content (mg/gFW) of a sample over a 12-day storage period. The graph shows the control group, which represents the chlorophyll content without any treatment, and the 15 mM GB treatment group, which demonstrates the chlorophyll content of the sample treated with 15 mM of an unspecified compound (likely glycine betaine or a similar compound). The data indicates that the chlorophyll content of the control group remains relatively stable throughout the 12-day period, while the 15 mM GB treatment group initially exhibits a higher chlorophyll content but then experiences a decrease over time. By the end of the 12-day storage period, the chlorophyll content of the 15 mM GB treatment group is lower than the control group.

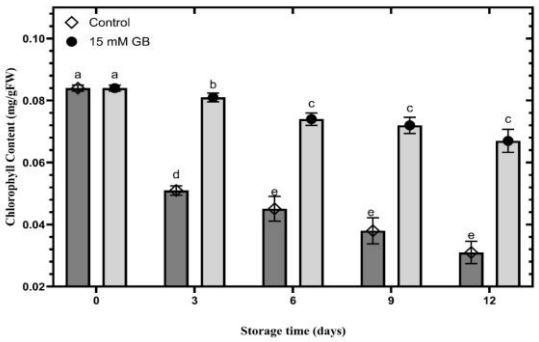

Figure 6. Effect of Glycine Betaine on Chlorophyll Content in Cucumbers during Storage at 4º C.

3. Conclusion:

In previous studies, the effect of glycine betaine (GB) on fruit quality has been clearly demonstrated. for example, research on peach fruit showed that GB can reduce chilling injury (CI) by suppressing the expression of specific genes and enzyme activities while also helping to maintain cell membrane integrity. This study indicated that GB was able to enhance the levels of unsaturated fatty acids and increase antioxidant activities, ultimately contributing to the preservation of peach quality (Shan., 2016). Additionally, in the case of jujube, results indicated that GB could preserve the content of cell wall components and enhance the activity of antioxidant enzymes and energy metabolism. These effects delayed the softening of jujubes after harvest (Chen et al., 2021). Furthermore, another study highlighted that glycine betaine significantly reduces chilling injury in banana fruit during cold storage. This was evidenced by a decrease in the CI index, electrolyte leakage, and malondialdehyde (MDA) content, along with an increase in lightness, chlorophyll, and soluble sugars. GB not only enhanced antioxidant compounds such as total phenols, glutathione, and ascorbic acid (AsA), but it also increased the activity and gene expression of antioxidant enzymes, including ascorbate peroxidase (APX), catalase (CAT), superoxide dismutase (SOD), and peroxidase (POD). Overall, these findings suggest that the reduction of CI in banana fruit during cold storage due to GB may be attributed, at least in part, to the enhancement of the antioxidant system, including antioxidant compounds and the activities and gene expression of antioxidant enzymes.

The findings of this study demonstrate the potential of using glycine betaine (GB) to maintain the quality and extend the shelf-life of cucumber fruits during storage. The application of GB was effective in reducing physiological weight loss, preserving total soluble solids (TSS), and retarding the loss of titratable acidity (TA) and firmness in cucumber samples compared to the control. The results showed that increasing the concentration of GB, particularly at 15 mM, was more effective in mitigating the deterioration of these quality attributes over the 12-day storage period. The stabilizing effect of GB on cell membranes and its ability to enhance the integrity of the fruit's cell wall are likely the underlying mechanisms responsible for the observed improvements in weight loss, TSS, TA, and firmness. While the chlorophyll content of the GB-treated samples initially exhibited higher levels, it declined more rapidly compared to the control by the end of the storage period, suggesting that the protective effects of GB may be more pronounced for other quality parameters than for chlorophyll retention. Overall, the findings of this study indicate that the application of glycine betaine has the potential to maintain the quality and extend the shelf-life of cucumber fruits during storage, with important implications for the postharvest management and marketing of cucumber products, as they demonstrate a practical approach to reducing waste and improving the availability of high-quality cucumbers for consumers (Łaźny et al., 2023).

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