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Changes in Physicochemical Characteristics, Peel Color, and Juice Attributes of 'Moro' Blood Orange Fruit Treated with Glycine Betaine and Methyl Salicylate during Cold Quarantine Storage

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Abstract: Cold quarantine storage is the practice of subjecting citrus fruit to low temperatures after harvesting to comply with stringent international phytosanitary standards for export, but fruit quality can be affected during storage. Therefore, this study investigated the effects of glycine betaine (GB) and/or methyl salicylate (MeSA) on physicochemical changes, chemical attributes of juice, and peel color of 'Moro' blood orange at cold quarantine storage (2 °C) for 60 days. Fruit were treated with GB (15 and 30 mM) by vacuum infiltration at 30 kPa for 8 min and vapor treatment of MeSA (100 µM) for 18 h as well as the combination of both GB concentrations with MeSA. The key findings of this research revealed that the combined treatment of 30 mM GB and 100 µM MeSA significantly mitigated weight and firmness losses in 'Moro' blood orange fruit during the cold quarantine period. Furthermore, there was a decrease in titratable acidity (TA) across all treatments, with the highest TA recorded for the 30 mM GB + 100 µM MeSA combination. Conversely, total soluble solids (TSS), TSS/TA ratio, and juice pH increased in all treatments, with the control treatment displaying the highest values. Regarding peel color parameters, which encompass L^* (lightness), b^*, hue angle ($h°$), chroma (C^*), and a^*, as well as the citrus color index (*CCI*), these exhibited characteristic changes during cold quarantine storage. However, the application of GB and MeSA, especially at the 30 mM GB + 100 µM MeSA level, noticeably delayed these peel color variations. Overall, GB and MeSA treatments offer significant advantages in preserving the physicochemical characteristics and chemical attributes of 'Moro' blood oranges during cold quarantine storage. These findings underscore the potential of GB and MeSA treatments for maintaining the quality of 'Moro' blood oranges during cold quarantine storage, with a noteworthy synergistic effect between MeSA and GB in preserving fruit quality.

Keywords: chroma; firmness; juice content; titratable acidity; weight loss

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1. Introduction

Blood oranges (*Citrus sinensis* L. Osbeck) are a desirable citrus crop due to their high content of bioactive compounds, particularly anthocyanin pigments, known for their health-related properties. This makes blood oranges popular among consumers who value their nutritional benefits. The quality attributes of blood oranges, including anthocyanin, color, flavor, texture, aroma, juiciness, sugar content, seedlessness, size, freshness, and nutritional value, all play a crucial role in promoting consumer acceptance and satisfaction with blood oranges [1].

Postharvest life extension and maintenance of citrus fruit quality can be achieved by storing them at temperatures ranging from 1 to 4 °C. For the disinfestation of citrus

fruit, cold quarantine storage at temperatures between 0.5 and 2 °C is widely used and accepted by regulatory agencies of most importing countries. This method mainly controls the Mediterranean fruit fly and can be implemented on a commercial scale. Chemical treatments for the disinfestation of fruit flies are not permitted, and physical treatments such as controlled atmosphere, dielectric heating, heat treatments, irradiation, and ultrasound treatments can be expensive and difficult to implement due to the fruit's threshold tolerance to treatment. However, it is essential to note that while cold quarantine storage is widely accepted, this method may cause cold damage. Therefore, protecting citrus fruit from such damage needs to be clearly defined and addressed [2].

Elicitors are substances that can induce or enhance the production of bioactive compounds and nutritional quality in fruit and vegetables. In recent times, elicitor compounds have gained popularity as a means of preserving fruit quality during cold storage. One such treatment involves the use of methyl salicylate (MeSA), a natural volatile compound, which has been found to effectively maintain the quality of fruits like pomegranate [3] and sweet cherry [4] during cold storage. In addition, the application of postharvest glycine betaine (GB) treatment, which is a quaternary ammonium compound, has become prevalent in the treatment of vegetables during storage including sweet pepper [5] and zucchini [6], climacteric fruits including peach [7–10], loquat [11], hawthorn [12], pear [13–15], plum [16], banana [17], and jujube [18], as well as non-climacteric fruits including pomegranate [19] and strawberry [20].

Exogenous application of elicitor compounds on blood oranges can have beneficial effects on reducing chilling injury (CI), increasing bioactive compounds, and antioxidant activity during cold storage. On the other hand, postharvest elicitor treatments are a promising approach to enhance the postharvest life of blood oranges by mitigating physiological disorders, preserving quality attributes, and boosting healthy phytochemicals [1]. The mechanisms through which postharvest treatments of GB and MeSA can reduce postharvest losses and control CI in blood oranges involve several processes. These include enhancing antioxidant enzyme activities such as catalase, ascorbate peroxidase, and superoxide dismutase, promoting proline accumulation, reducing electrolyte leakage, malondialdehyde, and hydrogen peroxide content, increasing phenylalanine ammonia-lyase activity, and decreasing polyphenol oxidase activity. Additionally, these mechanisms suppress polyphenol oxidase and peroxidase activities. Together, these processes inhibit lipid peroxidation and maintain cell membrane integrity, ultimately preventing fruit losses and chilling symptoms [21].

The cold temperatures can prolong the storage life of blood oranges by reducing fruit metabolism and other processes. Previous research has indicated that MeSA or GB can effectively mitigate CI and preserve the quality of blood oranges stored at 3 °C [21,22]. However, there is currently no scientific evidence on the combined effects of organic osmolyte (GB) and plant growth regulators (MeSA) on blood oranges during cold quarantine storage, and their potential roles have not been explored. Therefore, this study aimed to assess the impact of GB and/or MeSA treatments on the physicochemical attributes of 'Moro' blood oranges during cold quarantine storage.

2. Materials and Methods

2.1. Fruit Treatments and Storage Conditions

Blood oranges (cv. Moro) were harvested at commercial maturity based on the ratio of total soluble solids (TSS) to titratable acidity (TA) (TSS/TA ≅ 10). They were then transported to the postharvest laboratory, where they were checked for uniform size and the absence of any defects or rind injuries. The fruit were subjected to a vacuum infiltration treatment with an aqueous solution of 15 and 30 mM GB at 30 kPa for 8 min. Vapor treatment of 100 µM MeSA was applied to the fruit by placing them in 20 L plastic containers for 18 h. The combination of GB with MeSA (15 mM GB + 100 µM MeSA, 30 mM GB + 100 µM MeSA) was also performed under the same conditions. The optimal concentrations of GB and MeSA were determined based on previous study [23]. The control

fruit received no treatment. Each treatment was conducted on three replicates of five fruit, which were then placed in polyethylene bags with 16 holes and stored for 60 days at 2 °C with 90% relative humidity (RH). The following parameters were measured after 1, 30, and 60 days of cold storage plus shelf-life conditions (20 °C) for two days.

2.2. Weight Loss

The fruit weight was measured individually before storage (*W*1) and at each sampling time (*W*2) using a digital balance (HL-i, A&D, Tokyo, Japan) with an accuracy of 0.001, to determine the fruit weight loss (*W*L). The percentage of weight loss was calculated using the following formula [24]:

$$Weigt\ loss\ (\%) = \frac{W1-W2}{W1} \times 100 \quad (1)$$

2.3. Firmness

To determine the firmness of each treatment, a texture analyzer (TA-XT2, Surrey, UK) was used with a 3.5 mm diameter probe to compress the equatorial area of the fruit at a rate of 10%. The measured data were reported in newton [21].

2.4. Chemical Attributes of Juice

The TSS were measured using a portable refractometer (RBX0032A, Petro Centre, Singapore) and recorded as a percentage (%). TA was measured by titration with NaOH 0.1 N to pH 8.2 as an endpoint with a pH meter (Jenway 351, London, UK). The maturity index was calculated by dividing the TSS value by the TA value. The pH of the juice was measured using a pH meter [24].

2.5. Juice Content

The juice content of the fruit was determined by first weighing the fruit using a digital balance (HL-i, A&D, Tokyo, Japan). Next, the juice from each treatment was manually squeezed from hand-peeled fruit and weighed. The fruit juice content was then calculated using the following formula [24]:

$$Juice\ content\ (\%) = \frac{Weigt\ of\ juice}{Weigt\ of\ fruit} \times 100 \quad (2)$$

2.6. Peel Color

The color of the peel of each fruit was measured using a colorimeter (CR400/4P, Minolta Camera Co., Osaka, Japan) on two opposite sides of the equatorial area of 15 fruit. The L^* value represented lightness (0 = black to 100 = white), while a^* and b^* values represented green (−) to red (+) and blue (−) to yellow (+), respectively. Hue angle (h°), chroma (C^*), and citrus color index (*CCI*) were then calculated using the following formula [24]:

$$Chroma = \sqrt{a^{*2} + b^{*2}} \quad (3)$$

$$h^\circ = Arctan\frac{b^*}{a^*} \quad (4)$$

$$CCT = 1000 \times \frac{a^*}{L^* \times b^*} \quad (5)$$

2.7. Statistical Analysis

The experiment was carried out using a completely randomized design (CRD) with three replications, and the collected data were subjected to a two-factor analysis of variance (ANOVA) to analyze the effects of both treatments and storage times. The SAS software (version 9.4) for Windows was used to perform the data analysis. Mean comparisons were

carried out using the least significant difference (LSD) test, and the standard errors (SE) of means were considered with a significance level of $p < 0.05$.

3. Results

3.1. Weight Loss

All treatments resulted in an increase in weight loss during cold quarantine storage, with control samples displaying the highest weight loss (Figure 1). However, treatments led to a significant reduction in weight loss at all sampling times. The combined treatment of GB and MeSA, especially at 30 mM GB + 100 µM MeSA, had the most positive effect on weight loss reduction.

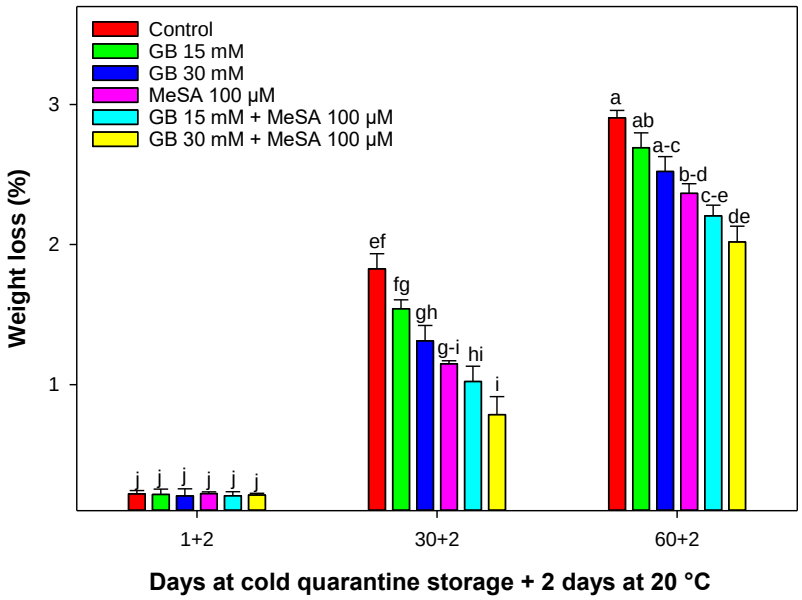

Figure 1. Changes in weight loss in control and treated fruit during cold quarantine storage. Vertical bars represent ± standard errors (SE) of means. Different letters above the bars on columns indicate significant difference at $p < 0.05$ level of probability based on LSD test.

3.2. Firmness

All treatments showed a decrease in firmness during cold quarantine storage, with the control samples exhibiting the most significant loss of firmness (Figure 2). All treatments were effective in reducing fruit firmness loss at all sampling times, but GB treatments displayed a synergistic effect in combination with MeSA displaying fruit firmness values of 27% and 34% higher when 15 mM GB and 30 mM GB, respectively, were applied as a combined treatment with MeSA respectively as compared to control fruit.

3.3. Chemical Attributes of Juice

Chemical attributes of juice (TSS, TA, TSS/TA, and juice pH) changed during cold quarantine storage in all treatments (Figure 3). TSS, TSS/TA, and juice pH increased in all treatments and the highest and lowest values were observed in control and 30 mM GB + 100 µM MeSA treatment, respectively. TA significantly decreased in all treatments and treated fruit had the highest TA, especially for 30 mM GB + 100 µM MeSA during cold quarantine while the lowest TA was observed in control samples. On the other hand, a dose-dependent effect related to GB concentrations applied was observed to delay the evolution of these parameters, applied alone or combined with MeSA treatments.

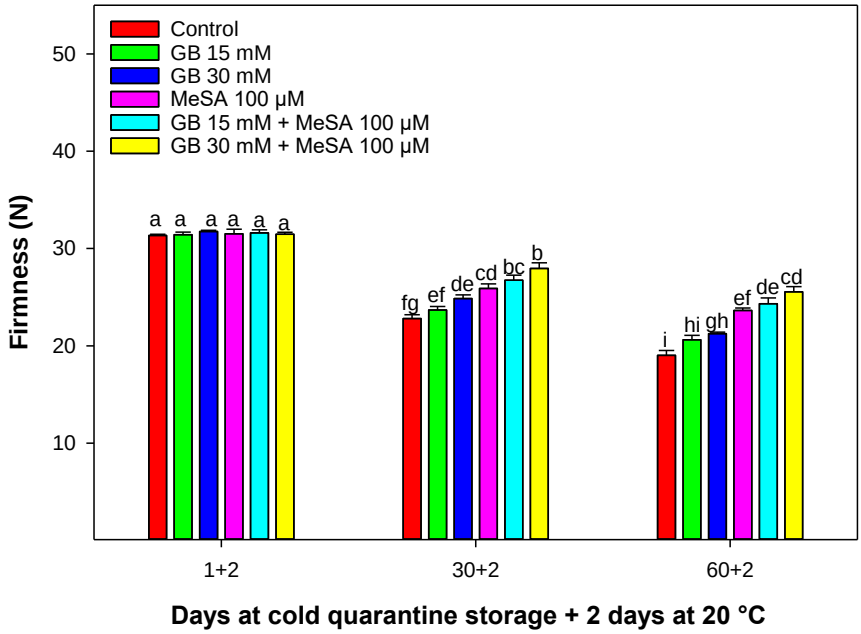

Figure 2. Changes in firmness in control and treated fruit during cold quarantine storage. Vertical bars represent ± standard errors (SE) of means. Different letters above the bars on columns indicate significant difference at $p < 0.05$ level of probability based on LSD test.

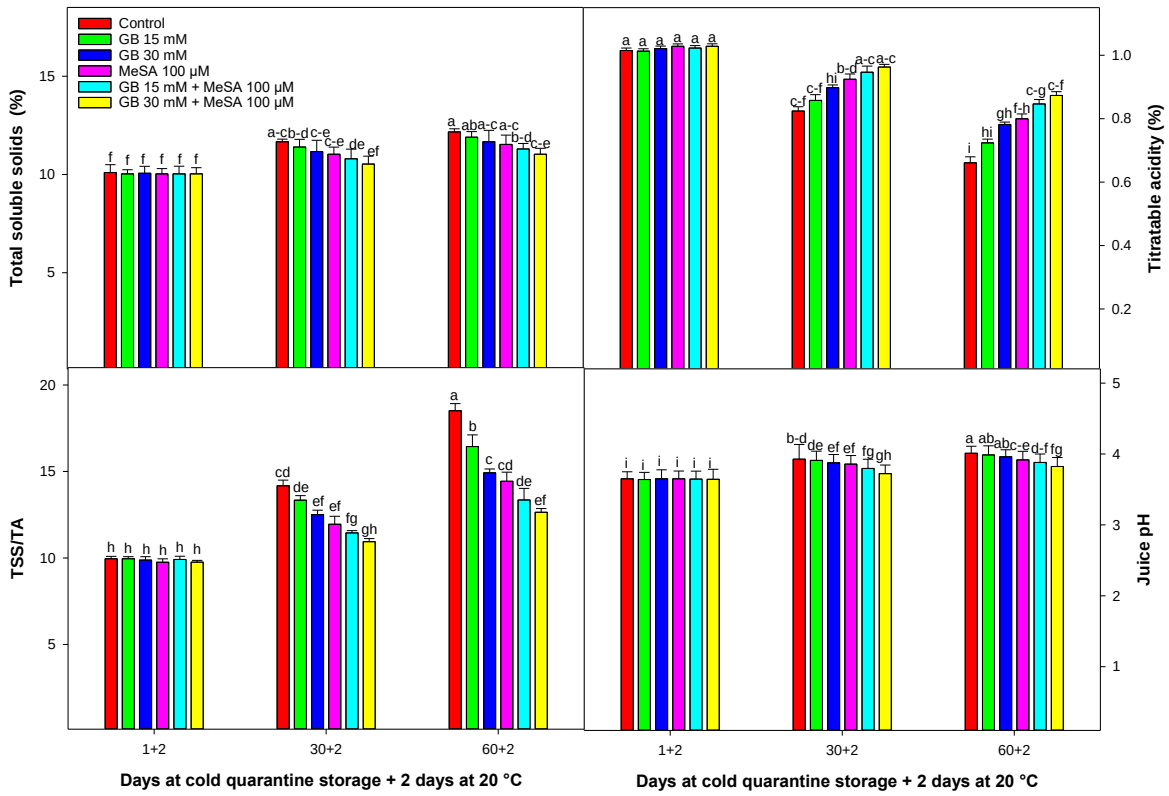

Figure 3. Changes in total soluble solids (TSS), titratable acidity (TA), TSS/TA, juice pH in control and treated fruit during cold quarantine storage. Vertical bars represent ± standard errors (SE) of means. Different letters above the bars on columns indicate significant difference at $p < 0.05$ level of probability based on LSD test.

3.4. Juice Content

Juice content decreased during cold quarantine storage and control samples revealed the most significant loss of juice content (Figure 4). All treatments were effective in reducing juice content loss at all sampling times, but the most significant juice content loss reduction was observed with the combined treatment of GB and MeSA, specifically with 30 mM GB + 100 µM MeSA.

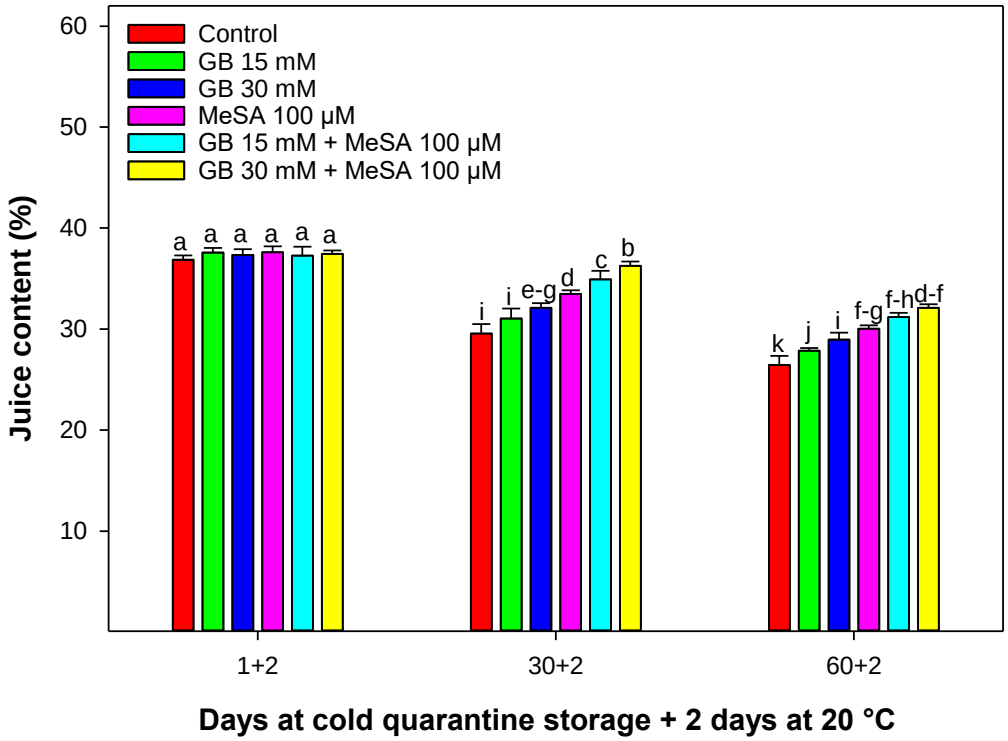

Figure 4. Changes in juice content in control and treated fruit during cold quarantine storage. Vertical bars represent ± standard errors (SE) of means. Different letters above the bars on columns indicate significant difference at $p < 0.05$ level of probability based on LSD test.

3.5. Peel Color

Color parameters, L^*, a^*, b^*, h°, C^*, and *CCI* changed during cold quarantine storage (Figure 5). In this study, L^*, b^*, h°, and C^* decreased in all treatments during cold quarantine storage, while a^* and CCI increased. The L^* value decreased with the same trends for all treatments along storage and the reduction of L^* in control samples was higher than in treated fruit. The b^* and h° values decreased in all treatments and this reduction was more pronounced in control samples. *CCI* increased during cold storage in all treatments during cold quarantine storage. Overall, the combined treatment of GB and MeSA, especially at 30 mM GB + 100 µM MeSA, had the most positive effect on color parameter changes.

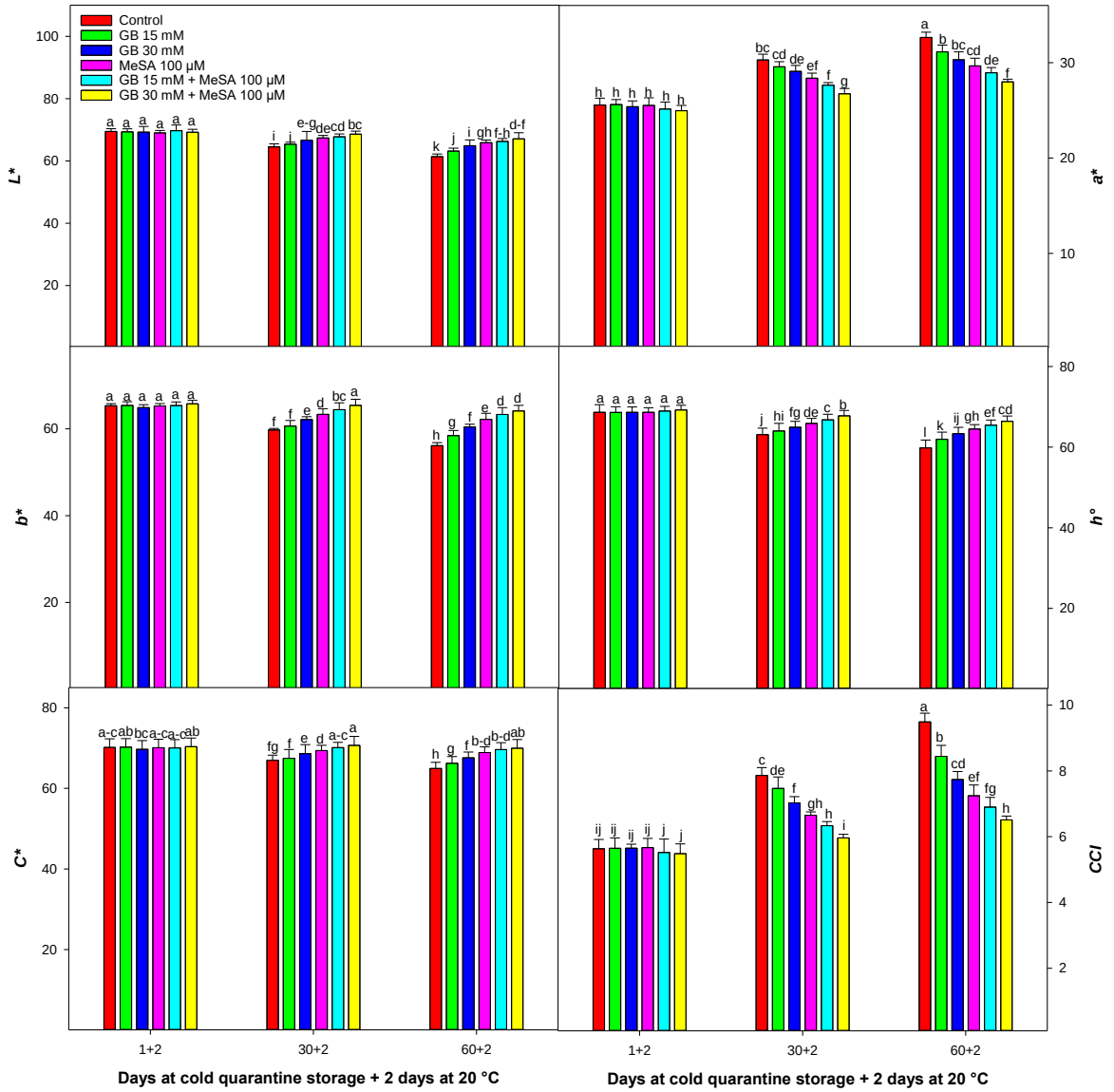

Figure 5. Changes in *L**, *a**, *b**, hue angle (*h*°), chroma (*C**), and citrus color index (*CCI*) in control and treated fruit during cold quarantine storage. Vertical bars represent ± standard errors (SE) of means. Different letters above the bars on columns indicate significant difference at $p < 0.05$ level of probability based on LSD test.

4. Discussion

Cold quarantine storage involves maintaining citrus fruit at low temperatures postharvest to meet strict phytosanitary requirements for international export [2,25]. While cold quarantine storage can have some benefits for the exportation of citrus fruit, prolonged storage at low temperatures can affect the texture, juice content, and peel color changes [24]. Therefore, postharvest elicitor treatments can help reduce these negative effects and ensure the highest possible quality of citrus fruit. In this study, the effects of postharvest treatment with GB and MeSA on 'Moro' blood orange were evaluated to determine their efficiency in mitigating weight loss, preserving firmness, maintaining the chemical attributes of juice, retaining juice content, and preserving peel color during cold quarantine storage.

Blood oranges can lose weight during cold storage due to a combination of factors, including respiration, water loss, chemical changes, transpiration, and cell-wall degradation [21]. Respiration consumes stored energy, leading to the breakdown of cells, consumption of stored energy, and water, resulting in weight loss. This can lead to weight loss and affect the fruit's quality and texture [26]. Although the respiration rate slows down during

cold storage, it still continues, leading to a continuous loss of weight, as well as a decrease in size and volume of the fruit. Proper cold storage conditions can help minimize weight loss and maintain fruit quality. In this study, the applied treatments resulted in a noteworthy reduction in weight loss. Particularly, the combined application of GB and MeSA exhibited the most pronounced positive impact on curtailing weight loss. This observation suggests that GB and MeSA played a significant role in regulating water loss, transpiration, and the rate of respiration in the treated fruit. Consequently, the treated fruit displayed lower weight loss compared to the control group, as observed in this study.

Fruit acceptability can be determined by fruit firmness, which is influenced by temperature. Lower temperatures can reduce metabolic activity and the activity of cell-wall degrading enzymes, resulting in the softening of the fruit. The softening process occurs due to the breakdown of pectin, a key component of the cell wall, through the activity of several enzymes, including polygalacturonase, pectin lyase, pectin methylesterase, and cellulose. When these enzymes are active, pectin depolymerizes, leading to softening [26]. These changes in the cell-wall composition are responsible for the decrease in citrus fruit firmness during cold storage. In this study, the treated fruit exhibited a decrease in firmness loss compared to the control fruit, with the most pronounced effect observed in the combination treatment of GB and MeSA during cold quarantine storage. This combination appeared to synergistically mitigate cell-wall degradation, resulting in the preservation of turgor pressure and the avoidance of fruit tissue collapse. Consequently, this mechanism contributed to the maintenance of fruit firmness [21].

During cold storage, citrus fruit undergo glycolysis and the Krebs cycle to produce cellular energy. However, these processes can also alter the levels of sugars and organic acids in the citrus fruit through biosynthesis and catabolism [24]. The TSS of citrus fruit juice is a reliable measure of its sugar content, which makes up around 80% of the TSS, mainly from sucrose, glucose, and fructose, while 10% consists of organic acids, such as citric, malic, and ascorbic acids, as well as other components like vitamins, proteins, free amino acids, and glucosides. The conversion of organic acids to sugars by glycolytic enzymes can lead to an increase in TSS [21]. However, prolonged cold storage can also cause a decrease in TSS due to the breakdown of sugars and other soluble constituents [24].

The TA in citrus fruit juice is closely related to the amount of organic acids present. Organic acids are essential components of citrus fruit juice, and blood oranges are known to have high levels of citric and malic acids. During cold storage, the organic acids in the fruit can be consumed for the synthesis of sugars and ATP production, which can result in a reduction of TA [23]. In this study, the treated fruit, particularly in the case of 30 mM GB + 100 µM MeSA treatment, exhibited elevated TA, which could be attributed to the potential prevention of alcoholic fermentation during cold quarantine storage [24]. The applications of GB and MeSA may have played a role in preserving organic acids, thereby delaying their breakdown and conversion into sugars. The correlation between TA and TSS was negative. Consequently, these treatments contributed to the preservation of higher TA levels in the juice of treated fruit as compared to control samples.

The TSS/TA ratio exhibited differences among the treatments in this study, and it was observed to increase during cold storage. The ratio of sugars to organic acids is not only an indicator of the harvest maturity of citrus fruit, but it also plays a crucial role in determining the taste and flavor of the fruit during cold storage [24]. Citric and malic acids are primarily responsible for the acidity of citrus fruit, and changes in their levels due to the conversion of organic acids during fruit respiration can significantly alter the TSS/TA ratio, resulting in varying qualities among different cultivars of citrus fruit [21]. As a result, the varying changes in the levels of sugars and organic acids can be associated with the differences in the TSS/TA ratio among the treatments, which exhibit a different range of values. These values were affected by lower TSS and higher TA in fruit treated with MeSA and GB alone or in combination, as compared to control fruit, displaying a delayed metabolism, as observed in different vegetal species treated with these elicitors [5,6]. The treatments involving MeSA and GB, particularly when combined, appear to exert a positive

influence on maintaining the delicate balance between sugars and organic acids throughout the cold quarantine storage period. This effect contributes substantially to enhancing the overall quality and flavor profile of the blood orange fruit, as evidenced by the MeSA and GB combination.

The results of this study indicated that the pH of the citrus fruit juice increased during storage, which can be attributed to the biochemical activity occurring within the fruit. This activity leads to the conversion of organic acids into sugar products, ultimately resulting in a shift towards a higher pH [24]. As the concentration of glucose and other sugars in the fruit increases, it can reduce the rate of respiration, which can also contribute to the observed increase in pH. Therefore, the increase in the pH of the fruit juice can serve as an indicator of the consumption of organic acids during cold storage. Furthermore, the reduction in TA can also be associated with an increase in juice pH, which is consistent with the observed results of this study [21].

The juice content of citrus fruit is an important factor in determining the overall quality of citrus fruit. The results of this study showed that the juice content decreased across all treatments during cold quarantine storage. The variation in the extent of juice content reduction among treatments could be attributed to the ability of the treatment to maintain cell membrane integrity [24]. The maintenance of membrane integrity is essential in preventing the drying of juice vesicles by protecting the sac-juice membranes from damage under low-temperature conditions [27]. Therefore, fruits that exhibit better cell membrane integrity are expected to have a lower reduction in juice content during cold storage, which is in consonance with the weight losses observed in this study.

Peel color is a crucial aspect of citrus fruit quality that greatly influences consumer preference. The color of the peel can be evaluated primarily based on three major types of pigments: chlorophylls, carotenoids, and anthocyanins. However, for a commercial perspective, it may also be important to consider factors such as visual quality and market acceptability. While chlorophylls are responsible for the green color of citrus fruit before ripening, carotenoids and anthocyanins play a significant role in determining the final color of the peel. Anthocyanins are particularly responsible for the red color of the flesh in blood oranges, but some cultivars also have them present in the peel [28]. When citrus fruits are exposed to cold temperatures, the color parameters of their peel may be affected. Specifically, the color may become darker, browner, and less orange. This is because the low temperature can cause the breakdown of chlorophyll in the peel, leading to changes in color [29]. Even though citrus fruits are not climacteric and have low ethylene production and respiration rates during maturity and ripening, color change of their peel can still occur after harvesting and during cold storage. In the present study, changes in peel color were observed in all treatments during cold quarantine storage of fruits. The lightness (L^*) value, which represents the amount of light reflected by an object on a scale from black (0) to white (100), decreased in all treatments, with control samples having the lowest L^* value. More specifically, a decrease in the L^* value indicates that the color is becoming darker. The a^* value indicates a shift towards a reddish hue. The a^* value, which ranges from green (−) to red (+), had a positive value in blood orange cultivars and increased in all treatments during cold quarantine storage. The b^* value indicates that the color is becoming less yellow and browner. The b^* value, which ranges from blue (−) to yellow (+), decreased in all treatments, possibly due to the increase in the a^* value or fruit senescence after prolonged storage [30]. Therefore, it seems that the effect of delaying color parameters was related to a delay in senescence, as it has been observed in other species treated with MeSA [3,4] and GB [7,11,18].

During cold storage in this study, the h° value, which represents the actual perceived color of orange or green and is a primary variable in changes in orange color, decreased in all treatments. The h° value ranges from 0 or 360° for red, 90° for yellow, 180° for green, and 270° for blue colors [24]. The h° values in this study were in the range of 59−67°, which corresponds to orange-yellow to yellow colors in all samples. Control samples had the lowest h° value during cold quarantine storage. Chroma (C^*) or saturation index, which

quantifies the intensity or saturation of color, decreased during cold storage [30]. In this study, CCI increased in all treatments. A comparative study on blood orange cultivars stored at 2 and 5 °C showed that changes in peel color parameters at 5 °C were higher than at 2 °C. The reason for this may be that lower temperatures reduced the biosynthesis or degradation of blood orange peel pigments [24]. The results indicate that the application of GB and MeSA treatments can influence these color changes, with the combined treatment showing the most promising results. These findings provide valuable insights into the potential strategies for maintaining fruit color quality during extended storage periods. Specifically, the combined treatment of GB and MeSA, particularly at the concentration of 30 mM GB + 100 µM MeSA, exhibited the most positive effect on color parameter changes. This finding suggests that the interaction between these two treatments could synergistically impact color preservation during cold quarantine storage.

5. Conclusions

In conclusion, the application of GB and MeSA treatments on 'Moro' blood oranges during cold quarantine storage showed significant preservation in physicochemical changes and chemical attributes of the fruit juice. The combination of 30 mM GB and 100 µM MeSA proved to be the most effective treatment, reducing weight and firmness losses while also delaying changes in peel color parameters such as L^*, b^*, h°, and C^*. Furthermore, the treated fruit exhibited higher TA, TSS, and TSS/TA compared to the control samples. These findings highlight the potential of GB and MeSA treatments in maintaining the physicochemical attributes of 'Moro' blood oranges during cold quarantine storage, with MeSA showing a synergistic effect when combined with GB in preserving the fruit's quality. Further research is needed to better understand how GB and MeSA interact with fruit physiologically. This should involve studying effects of cold quarantine storage through sensory, nutritional, and molecular analyses.

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