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Abscisic Acid Promotes Ripening in Sapodilla (*Manilkara zapota* L.) Fruit after Refrigerated Storage

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ABSTRACT

Sapodilla is a climacteric fruit with high marketing potential but limited export viability due to its short shelf life. During transport, the fruit is refrigerated in a semi-ripe state and is expected to be ready for consumption upon reaching consumers. This study aims to determine the physical and chemical characteristics of sapodilla fruit treated with abscisic acid after refrigerated storage. Half-ripe sapodilla fruits (n=66) were selected, washed, and stored at 10°C for 10 days. The fruits were then divided into control (n=33) and treatment (n=33) groups. Samples in the treatment group were sprayed with a 0.1% ABA solution before further storage at room temperature for 16 days. During this period, weight loss (%), firmness (N/mm²), total soluble solids (°Brix), titratable acidity (%), and reducing sugar levels (%) were observed. The Wilcoxon rank-sum test ($\alpha = 0.05$) was used to analyze mean differences between groups for each parameter. Significant differences between groups emerged on the 12th day of storage at room temperature. On day 12, ABA-treated samples exhibited significantly lower firmness (0.13 ± 0.01 N/mm²) and reduced sugar levels (0.207 ± 0.074%) compared to the control (0.26 ± 0.06 N/mm² and 0.427 ± 0.040%, respectively). The titratable acidity of ABA-treated samples (3.57 ± 0.26%) was also significantly lower than that of the control (5.96 ± 0.45%). However, ABA-treated sapodilla exhibited higher total soluble solids (17.6 ± 1.53 °Brix) than the control (14.1 ± 0.58 °Brix). Similarly, vitamin C content was higher in ABA-treated samples (0.032 ± 0.002%) compared to the control (0.006 ± 0.007%). These changes suggest a synergistic relationship between ABA and ethylene. Therefore, the application of ABA can accelerate ripening in sapodilla fruit after refrigeration.

Keywords: Abscisic acid, climacteric fruit, fruit ripening, post-harvest, sapodilla, shelf-life

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INTRODUCTION

Sapodilla (*Manilkara zapota* L.) belongs to the Sapotaceae family and is known for its sweet taste and high nutritional value. It contains 69.46% moisture, 20.0 g of carbohydrates, 15.96% total sugars, and various vitamins (vitamin A, vitamin C, pantothenic acid, and niacin) and minerals (calcium, potassium, and iron) (Bangar et al., 2022; Shinwari & Rao, 2020). Sapodilla fruit also exhibits antioxidant activity and phytochemicals, such as phenols, flavonoids, and anthocyanins (Cortes et al., 2022). In Indonesia, sapodilla, commonly known as *sawo*, has gained economic significance. According to BPS-Statistics Indonesia (2020), sapodilla fruit production increased from 144,966 tons in 2019 to 186,706 tons in 2020. This rise in production indicates the potential expansion of sapodilla cultivation to meet growing market demand and even possible export opportunities.

However, as a climacteric fruit, sapodilla continues to ripen after harvest, posing challenges during transportation from orchards to consumers or distributors. During ripening, total soluble solids increase as starch is converted into soluble sugars, but these levels decline as the fruit progresses into senescence (Baidya et al., 2020). The firmness of fleshy fruits irreversibly decreases due to transpiration, respiration, and other postharvest metabolic processes. Physical weight loss (PWL) increases over time as moisture content decreases. Additionally, titratable acidity, which indicates organic acid content, declines as the fruit ripens, as these acids are either utilized in respiration or converted into sugar (Blissett et al., 2019). These physicochemical changes significantly influence the fruit's sensory properties, which are critical in shaping consumer purchasing decisions. Therefore, controlling ripening-related physicochemical properties is essential to maintaining fruit quality.

Temperature control is a widely used postharvest handling technique to preserve fruit quality. Cold storage slows the general metabolism of harvested fruits by reducing respiration rates and enzyme activity, thereby delaying ripening (Brizzolara et al., 2020). This method is particularly beneficial for exported fruits, which may take weeks or months to reach their destination. Typically, fruits are harvested in a semi-ripe condition and stored at low temperatures to extend shelf life during transit. However, once the fruits reach their destination, consumers and distributors expect them to be ready for immediate sale or consumption. Thus, the ripening process must be re-induced to develop desirable sensory properties.

Abscisic acid (ABA) is a growth-regulating substance involved in fruit development, maturation, and ripening. The effect of ABA application on fruit ripening is closely associated with its synergistic relationship with ethylene, a plant hormone crucial in climacteric fruit ripening. During climacteric fruit ripening, ABA levels peak earlier than ethylene levels, highlighting its role in regulating ethylene production (Bai et al., 2021). Previous research mentioned that the core signaling pathway between ABA and ethylene is conserved in climacteric fruits, with key regulators (i.e., 9-cis epoxycarotenoid dioxygenase (NCED), abscisic acid-responsive elements (ABRE) binding proteins (AREB) or binding factors (ABFs), 1-aminocyclopropane-1-carboxylic (ACC) synthase (ACS) and ACC oxidase (ACO), and ethylene response factors (ERFs)) and ethylene-related transcription factors (i.e., Mcm1, Agamous, Deficiens and SRF or MADS-ripening inhibitor (MADS-RIN), Tomato AGAMOUS-LIKE1 (TAGL1), Colorless Non-ripening (CNR), and Non-ripening (NOR)) are highly likely mediating ABA-ethylene interactions (Bai et al., 2021). However, the precise mechanism of ABA-mediated ethylene regulation during climacteric fruit ripening remains unclear, requiring further research. This study investigates whether ABA can be applied to sapodilla fruit to induce ripening after refrigerated storage. To mimic the postharvest transportation condition, sapodilla fruits were stored at low temperatures before ABA application.

MATERIALS AND METHODS

Sample Preparation

The sapodilla fruits used in this study were handpicked by farmers in Wonosari, Gunungkidul, Indonesia (7° 58.0393' S, 110° 36.0668' E). Fruits at a half-ripe maturity stage were selected based on the physical characteristics described by (Brito & Narain, 2002). Each selected fruit weighed between 80–90 g, was fresh, free from disease, and had no physical damage. A total of sixty-six sapodilla fruits were obtained and arranged in groups of three on Styrofoam trays.

Refrigerated Storage Treatment

Before refrigeration, the fruits were washed with water containing 20 ppm chlorine and then air-dried. The cleaned fruits were subsequently stored in a refrigerator at 10 °C for ten days, with relative humidity maintained at 95%.

Application of Abscisic Acid Solution

To prepare a 0.1% abscisic acid (ABA) solution, 100 mg of ABA (HiMedia, Mumbai, India) was dissolved in 500 mL of distilled water and 500 mL of 96% ethanol. Subsequently, 0.05% (0.5 mL/L) Tween 20 was added as a surfactant. This solution was applied to the treatment group (n = 33) after ten days of refrigerated storage. Before application, the refrigerated fruits were placed on a metal drying tray and allowed to rest for ten minutes. The ABA solution was then sprayed onto the fruits to the point of run-off while slowly rotating them to ensure even coverage. The treated samples were subsequently air-dried in a dark place and stored at room temperature for 16 days.

Measured Parameters

The ripening process of ABA-treated sapodilla fruits was monitored over 16 days of storage at room temperature. Physicochemical parameters were assessed, including physical attributes such as weight loss and firmness, as well as chemical characteristics such as total soluble solids (TSS), titratable acidity (TTA), reducing sugar levels, and vitamin C content. Measurements were conducted every two days, except for weight loss, which was recorded daily. Each parameter was measured in triplicate to ensure reliability.

Physical Parameters Measurement

Weight loss was determined using an analytical scale (OHAUS Scout Pro, New Jersey, USA) by subtracting the weight at each measurement point from the initial weight and expressing the result as a percentage. Firmness was assessed using a fruit penetrometer (MRC Lab Equipment, Essex, United Kingdom) with a force specification of 13 kg and a penetration depth of 100 mm. Measurements were taken at three points on each fruit: tip, middle, and base.

Chemical Characteristics Measurement

Total soluble solids (TSS) were measured using an ATAGO Pal-3 hand refractometer (ATAGO, Tokyo, Japan). Sapodilla fruits were cut into sections and blended (Miyako, Jakarta, Indonesia) to extract juice. Three drops of the juice were placed on the refractometer prism, and the TSS value was recorded in °Brix. Titratable acidity (TTA) was determined following the method described by (Sadler & Murphy, 2010) and expressed as a percentage. Reducing sugar levels was quantified using the modified Nelson-Somogyi method (Romadhoni et al., 2017), while vitamin C content was analyzed according to (Satpathy et al., 2021).

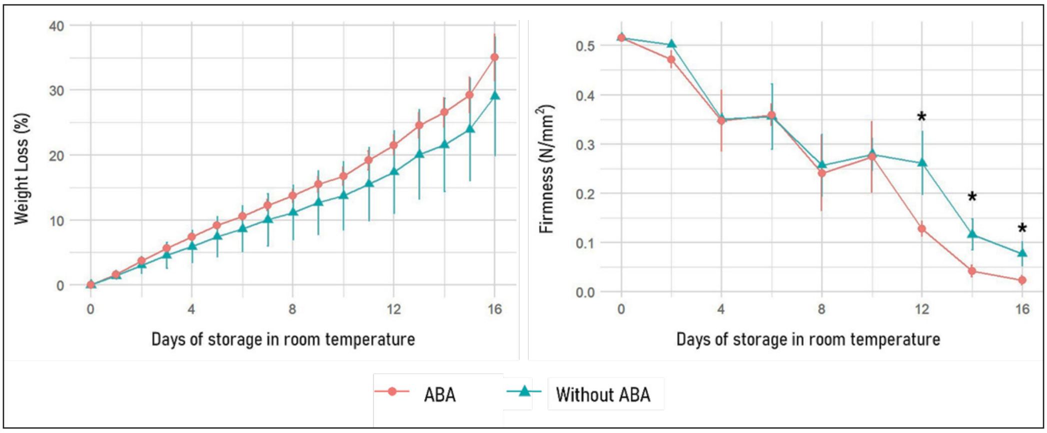
Statistical Analysis

All statistical computations and graphical visualizations were performed using R in RStudio (version 2023.06.0). The observed parameter values between treatments on the same day were analyzed for significant differences using the non-parametric Wilcoxon rank-sum test with a significance level of 0.05. Line plots were generated using the ggplot2 package.

RESULTS

ABA Application and Weight Loss

Figure 1 illustrates the changes in weight loss and firmness of sapodilla fruit under different treatments following ten days of refrigerated storage. Both ABA-treated and untreated fruits exhibited increasing weight loss after being transferred to room temperature (Figure 1A). No significant differences were observed between the treatments across all measured days.



(A) (B)

Figure 1. Changes in physical observation of (A) weight loss and (B) firmness in 16 days of room temperature storage between sapodilla fruit treated with and without abscisic acid (ABA) after refrigerated storage (10 days at 10°C). Asterisks (*) represent significant differences between two treatments within the same day of storage ($p = 0.05$)

ABA Application Reduces Firmness After Refrigerated Storage

The firmness measurements are presented in Figure 1B. Regardless of treatment, the firmness of sapodilla fruit continuously declined throughout the observation period. However, fruits treated with ABA exhibited significantly lower firmness than the control group on days 12 ($P = 0.023$), 14 ($P = 0.020$), and 16 ($P = 0.013$) of room temperature storage. On day 12, ABA-treated fruits had a firmness of 0.13 ± 0.01 N/mm², compared to 0.26 ± 0.06 N/mm² in the control samples. By the final observation day, firmness values dropped to 0.02 ± 0.01 N/mm² and 0.08 ± 0.02 N/mm² for ABA-treated and untreated fruits, respectively.

ABA Application Enhances Total Soluble Solids and Reduces Sugar Levels

As shown in Figure 2A, the total soluble solids (TSS) content in untreated sapodilla fruit gradually declined after six days of room temperature storage, with a pronounced drop between days 10 and 12. In contrast, ABA-treated fruits exhibited an increase in TSS levels after day 10, continuing to rise throughout the remaining observation period. A significant difference ($P = 0.008$) was observed on day 12, where the TSS content in ABA-treated fruits (17.6 ± 1.53 °Brix) was notably higher than in the control group (14.1 ± 0.58 °Brix). By day 16, TSS levels reached 23.1 ± 1.53 °Brix in ABA-treated samples, whereas control samples measured 15.3 ± 0.58 °Brix.

A similar trend was observed in reducing sugar content (Figure 2B). Both treatments initially showed a decline after four days of room temperature storage, with a divergence occurring between days 10 and 12. On day 12, ABA-treated fruits exhibited a continuous increase in reducing sugar content (0.427 ± 0.040%), while untreated fruits continued to decline (0.207 ± 0.074%). A statistically significant difference emerged in the final three days of observation, with ABA-treated fruits reaching 0.475 ± 0.034% and untreated fruits at 0.261 ± 0.007% on day 16 (P = 0.048).

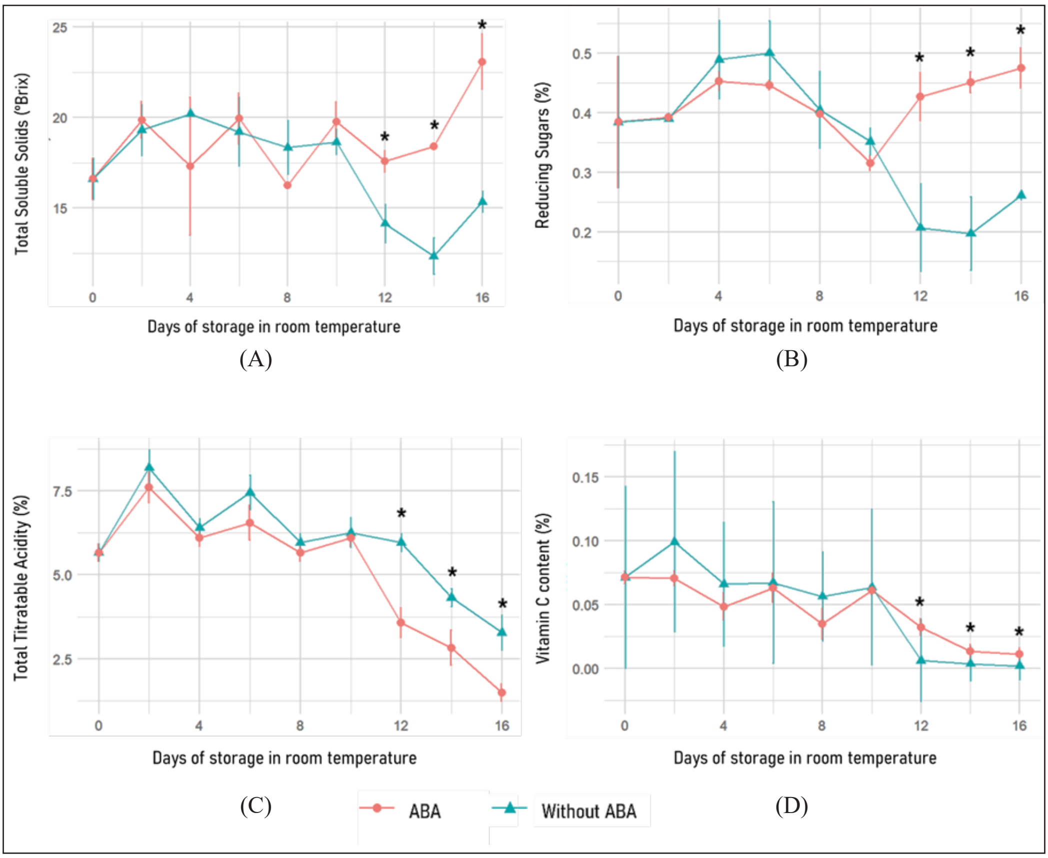


Figure 2. Changes in chemical observation of (A) total soluble solids, (B) reducing sugar level, (C) total titratable acidity, and (D) vitamin C level in 16 days of room temperature storage between sapodilla fruit treated with and without abscisic acid (ABA) after refrigerated storage (10 days at 10°C). Asterisks (*) represent significant differences between the two treatments within the same day of storage (p = 0.05)

ABA Treatment Reduces Total Titratable Acidity

Both ABA-treated and untreated fruits exhibited a progressive decline in total titratable acidity (TTA) throughout the 16-day room temperature storage period after ten days of refrigerated storage at 10 °C (Figure 2C). Similar to the TSS trends, significant differences in

TTA were observed on days 12, 14, and 16, with untreated fruits maintaining higher acidity than ABA-treated samples. On day 12, TTA values were 5.96 ± 0.45% for control samples and 3.57 ± 0.26% for ABA-treated fruits. The decline persisted until day 16, resulting in TTA values of 3.28 ± 0.52% (control) and 1.49 ± 0.26% (ABA-treated) ($P = 0.003$).

ABA Application Increases Vitamin C Content

Figure 2D presents the vitamin C content of sapodilla fruits under both treatments after ten days of refrigerated storage at 10 °C. While both groups showed a gradual decline over the 16-day room temperature storage, ABA-treated fruits consistently retained higher vitamin C levels than untreated fruits in the final three days. By day 16, vitamin C content reached its lowest values, with ABA-treated fruits maintaining 0.011 ± 0.002%, compared to just 0.002 ± 0.005% in untreated fruits ($P = 0.037$).

DISCUSSION

The production of sapodilla fruit in Indonesia is increasing annually, indicating its potential for global market distribution. However, extending shelf life by delaying ripening during transport while ensuring optimal ripening upon arrival is crucial for maintaining fruit quality. Low-temperature storage is a commonly used postharvest technique to slow the ripening process. In this study, abscisic acid (ABA) was investigated as a potential trigger for promoting sapodilla fruit ripening after refrigerated storage, mimicking the transportation process after harvest. While several studies (Kou et al., 2021; Wu et al., 2023; Zhang et al., 2021) have reported the ability of ABA to accelerate ripening, none have examined its application after refrigerated storage to enhance ripening and achieve the desired fruit quality for consumers.

The present study found that applying ABA after refrigerated storage significantly promoted the ripening of sapodilla fruit. Fruits in the treatment group exhibited significantly higher total soluble solids, reducing sugar content, and vitamin C levels (Figure 2), while titratable acidity (Figure 2) and firmness (Figure 1) were lower than those of the control samples. The differences in ripening parameters between treatment groups highlight the role of ABA as a ripening promoter in sapodilla fruit. However, physical weight loss showed no significant difference between treatments (Figure 1).

During storage, fruits inevitably undergo weight loss, which affects their quality. This weight loss results from water loss due to transpiration and respiration. In fresh produce such as fruits and vegetables, excessive water loss can reduce quality and cause damage. As shown in Figure 1A, although not statistically significant, the trendline of weight loss in sapodilla fruits treated with ABA was slightly higher than in untreated fruits. Similar results were observed in ABA-treated tomato and capsicum (Prajapati et al., 2019). Exogenous ABA treatment can activate enzymatic activities, including cell wall-degrading enzymes such as polygalacturonase, through the upregulation of genes responsible for pectin degradation (*PcPG1*, *PcPG2*, *PcPL*, *PcPME2*, *PcPME3*, and *PcGAL1*) and ABA biosynthesis (*PcNCED1* and *PcNCED2*) (Xu et al., 2024).

Several mechanisms, including the loss of turgor pressure, pectin degradation, and damage to the fruit's cell walls, can cause texture changes such as softening in most fruits. After day 10, sapodilla fruit treated with ABA exhibited significantly lower firmness values (Figure 1). Similar firmness loss and softening effects have been reported in various fruit commodities treated with ABA. In non-climacteric strawberry fruits, both the control and ABA-treated samples showed no firmness loss after 48 hours; however, thermogravimetric analysis (TGA) revealed cell wall alterations (Castro et al., 2021). The breakdown of the xyloglucan-cellulose network and pectin solubilization were associated with cell wall deconstruction, a process mediated by ABA. Total degradation and a decrease in hemicellulose content were observed in ABA-treated samples due to ABA-induced breakdown of cell wall polymers, leading to their decomposition into low-molecular-weight compounds, primarily carbohydrates such as xylose and glucose, which are related to hemicellulose degradation (Castro et al., 2021; Castro & Morales-Quintana, 2019).

As shown in Figure 1, ABA-treated sapodilla fruit exhibited decreased firmness and slight weight loss. The relationship between ripening-related physical changes, ABA, and ethylene has also been explored in previous studies. Long-term kiwifruit storage at 0°C under varying humidity conditions showed that storage beyond eight weeks led to a rapid loss of fruit firmness (Huang et al., 2021). The fastest weight loss was observed in low-humidity conditions due to environmental factors and respiratory metabolism changes, which accelerated softening. Water loss also promoted ethylene biosynthesis and fruit softening during storage. However, these changes were not directly linked to an increase in ABA content. Instead, the strongest correlation was found between ABA concentration in fruit tissues and firmness reduction, regardless of weight loss. A higher ABA concentration was associated with decreased fruit firmness, but no correlation was observed between ABA content and weight loss. This suggests that while water loss may induce ethylene production and fruit softening, ABA concentration in tissues is more closely related to firmness than weight loss. The rapid softening of 'Hayward' kiwifruit due to dehydration may be mediated by ethylene, but ABA's specific role, whether direct or indirect, remains unclear (Huang et al., 2021).

The sapodilla samples treated with ABA have higher total soluble solids (Figure 2A) and reducing sugar content (Figure 2B) compared to control samples. Higher total soluble solids, indicative of sugar accumulation, were observed in this study and have also been reported in other fruits, including date, peach, papaya, cucumber, and tomato (Elbar et al., 2022; Utama et al., 2023). ABA plays a crucial role in various metabolic pathways, including sugar signaling. Sugar accumulation during fruit ripening can be attributed to the interaction between ABA and sugar signaling pathways, as well as the release of sugars from storage carbohydrates or the import of sugars from distal organs via phloem transport (Gupta et al., 2022).

By stimulating enzymes involved in cell wall degradation, such as pectin methylesterase (PME) and polygalacturonase (PG), ABA promotes the conversion of complex insoluble sugars into simple soluble sugars. The respiration rate in ABA-treated fruits decreased, contributing to higher sugar accumulation (Prajapati et al., 2019). Increased sugar accumulation reflects a rise in sugar content, including reducing sugars such as fructose and glucose. Previous research indicated that ABA promotes soluble sugar accumulation in different climacteric fruits. Application of 600 μM and 800 μM ABA in blueberry conferred higher soluble sugar than the control samples after 14 and 21 days of treatment. The study explained that the increase of soluble solids in the initial period of storage was likely due to a higher rate of acid and sugar biosynthesis compared to degradation at the time of harvest when the fruit was not fully ripe. However, these levels subsequently declined in the later storage period, presumably due to respiratory metabolism (Qiao et al., 2024). A recent study found the relation between key ABA signal transducers (MdbZIP23 and MdbZIP46) with the expression of the MdSWEET9b gene transporter, which affected sugar accumulation in apple fruits (Zhang et al., 2023). The study further elucidates the regulatory impacts between the ABA-signalling network and fruit sugar accumulation.

As shown in Figure 2C, ABA-treated sapodilla fruit exhibited lower TTA levels than the control. A similar trend was observed in ABA-treated detached capsicum. Since various organic acids are catabolized in the later stages of fruit development and storage, titratable acidity naturally declines during ripening (Prajapati et al., 2019). The higher TTA content in ABA-treated fruits could be attributed to a reduced respiration rate, as organic acids serve as respiratory substrates. A lower respiration rate would slow the breakdown of organic acids, resulting in lower TTA levels.

A study investigating the effects of exogenous ABA on ripening-related genes in citrus fruit explored the potential relationship between ABA, sugar, and organic acid pathways (Wang et al., 2016). The study found that ABA significantly decreased organic acid content and regulated the expression of genes associated with sugar and acid metabolism in citrus fruits. ABA and sucrose signaling pathways played a key role in citrus fruit ripening, with transcript levels of *CsACO1* and *CsNADP-IDH* in ABA-treated fruits being higher than those in the control. When the ABA inhibitor nordihydroguaiaretic acid (NDGA) was applied, the opposite trend was observed, further supporting the role of ABA in promoting organic acid degradation. The study reported that ABA and NDGA influenced sugar accumulation and acid degradation during ripening. Sugar-related genes were upregulated, whereas acid-related genes either decreased or remained unchanged. These findings suggest that ABA and NDGA play a significant role in the degradation of organic acids such as malic acid, citric acid, and quinic acid.

The level of vitamin C was higher in fruits treated with ABA than in the control samples (Figure 2D). Similar results have been observed in other commodities. Abscisic acid potentially enhances ascorbic acid accumulation in drought-stressed tomatoes by inducing the expression of SIMAPK8 (Xu et al., 2022). Additionally, abscisic acid mitigates the inhibitory effect of auxin on ascorbic acid biosynthesis. In strawberries, the accumulation of ascorbic acid is driven by the upregulation of *FaGLDH*, *FaAPX*, and *FaMR* genes, suggesting that ABA-mediated regulatory mechanisms play a crucial role in ascorbic acid biosynthesis during fruit ripening (Zhang et al., 2023). Another study further explained that in salt-stress-induced strawberries, ABA exogenous application significantly enhances the accumulation of ascorbic acid compared to the samples in salt stress without ABA treatment (Crizel et al., 2020). Thus, the protective or acclimatory effect of ABA under stress conditions favors the ascorbic acid production.

The findings of this study suggest that ABA can be used to promote fruit ripening after refrigerated storage, presenting new research opportunities for both academia and industry. The results indicate that exogenous ABA application in sapodilla fruit after refrigeration storage can extend its shelf life by approximately two additional days compared to untreated samples. This extension could be commercially beneficial, particularly for long-distance transportation and export markets. However, it is important to note that different fruits may respond differently to refrigerated storage and ABA treatment. Future research could explore various aspects, such as optimizing ABA concentrations, determining the most effective application methods, and refining refrigerated storage processes in combination with ABA treatment.

CONCLUSION

Abscisic acid (ABA) treatment after refrigerated storage enhances the ripening qualities of sapodilla (*Manilkara zapota* L.) fruit. The application of ABA resulted in higher total soluble solids, reducing sugar content, and vitamin C levels compared to control samples. Additionally, ABA-treated sapodilla fruit exhibited lower firmness and total titratable acidity than untreated fruit. These findings indicate that ABA can effectively promote ripening in sapodilla fruit following low-temperature storage. This study highlights the potential of ABA in post-storage fruit ripening, offering insights for further research and industrial applications. Future studies could investigate the responses of different fruit types, optimal ABA concentrations, application methods, and the integration of ABA treatment with refrigerated storage optimization.

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