

Responses of MD2 pineapple to postharvest application of calcium in combination with hot water treatments during storage

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Abstract

The post-harvest handling of pineapple fruits is targeted at providing an ideal quality for consumers. This study aimed to determine the impact of calcium combined with hot water treatments associated with the postharvest quality of MD2 pineapple. The treatments employed were A (Control: No use of CaCl₂ and hot water treatment), B (0% CaCl₂ + hot water treatment), C (2% CaCl₂ + hot water treatment), D (4% CaCl₂ + hot water treatment), E (6% CaCl₂ + hot water treatment), implemented in two pineapple harvest stages, dark green skin color and light green skin color. The calcium content in the flesh and skin, together with the water content, firmness, total soluble solids, and total acidity level of the fruits were analyzed postharvest during storage conditions. Regarding the dark green skin color, the treatment employing CaCl₂ (6%) with hot water treatment for five mins gave the best performance, providing the most elevated calcium content in the flesh, an ideal level in the skin, and optimal values of water content, total soluble solids (TSS) and total acidity (TA) in the fruits. On the contrary, for the light green skin color stage, the treatment that provided the best response was the one that only used hot water treatment, having the most elevated calcium content in the flesh. Also, the treated pineapples had an ideal level in the skin, optimal values of water content, firmness of the flesh, and TSS and TA in the fruits examined. In conclusion, the postharvest application of calcium together with hot water treatments can affect the postharvest quality of MD2 pineapple depending on its physiological harvest stage.

1. Introduction

Pineapple is one of the main horticultural commodities grown in Indonesia. Currently, consumers' interest in fresh pineapple varieties is increasing, being MD2 the cultivar most explored and exported in the industry (Bin Thalip *et al.*, 2015; Paull and Chen, 2018). MD2 pineapple is well known for its glossy, green to golden skin color, with a uniform and attractive cylindrical shape. Besides, this cultivar has a high vitamin C, more yellow flesh color, less acidity and sweeter taste, with more aromatic properties than other varieties. Because of that, the consumer predisposition towards this pineapple variety (Bin Thalip *et al.*, 2015; Paull and Chen, 2018). Commonly in Indonesia MD2 is harvested according to its skin color characteristics, based on consumer preferences, employing two

parameters, when the skin color is still dark green or when this is light green. This property reflects two maturity stages in the fruit, which are considered ideals in order to obtain the most adequate fruit physico-chemical quality (Bin Thalip *et al.*, 2015; Cano-Reinoso, Kharisun, Wibowo *et al.*, 2022).

Pineapple is a non-climacteric fruit that will not have considerable quality changes after being harvested, more than the normal senescence (Paull and Chen, 2018). MD2 like many other fruits requires an appropriate handling in order to avoid any mechanical damage or pathogenic attacks that could cause a shorter shelf life (Paull and Chen, 2018). It is a problem that must be faced constantly. In an appropriate storage condition, MD2 pineapple can maintain an optimal quality for consumption for up to 30 days; nevertheless, some

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destinies of exportation demand a longer storage time, creating challenges for the producers (Paull and Chen, 2018; Cano-Reinoso, Kharisun, Wibowo *et al.*, 2022). Normally pineapple is stored in low temperature and high humidity conditions (around 7°C and 95% relative humidity (RH), respectively). Nevertheless, these conditions are not enough to maintain the quality of the fruit during long transit periods (Paull and Chen, 2018; Cano-Reinoso, Kharisun, Wibowo *et al.*, 2022).

One of the efforts that can be made to conserve the quality during these long transit periods is the dipping of the fruit using calcium mineral sources. Ca^{2+} is needed to provide appropriate cell wall stability and integrity, a process that is inhibited during the fruit senescence, creating physiological disorders like internal browning, due to the cell wall breakdown (De Freitas and Resender Nassur, 2017). Besides, Ca^{2+} can also influence other physiological processes in fruits associated with the reduction of respiration, delaying of ripening, and hormonal signaling (Hocking *et al.*, 2016; De Freitas and Resender Nassur, 2017). These findings have also been supported by Pusittigul *et al.* (2014) and Cano-Reinoso, Soesanto, Kharisun *et al.* (2022), where the use of calcium mineral sources during postharvest caused a reduction of the internal browning, increasing the ascorbic acid (AsA), and extending the pineapple shelf life up to 40 days. These previous facts demonstrated the potential of calcium treatments to enhance the physico-chemical quality of the fruit. However, in terms of fresh pineapple, not many studies have been documented about the employment of this mineral in postharvest, as most have been more focused on fresh-cut products.

Parallely, another alternative to boost the employment of minerals during postharvest is the use of hot water treatments. Dipping fruits in hot water can cause an increase in their antioxidant content, catalyzation of the activity of antioxidative enzymes, reduction of internal browning, and as a consequence, prolonging their shelf life (Fallik and Illić, 2017). This circumstance has been attributed to the physiological stress that this type of treatment can cause, creating the activation of hypertensive response, triggering several mechanisms that are beneficial for the fruit and its shelf life, in order to cope with the stress sensed (Saidi *et al.*, 2011; Fallik and Illić, 2017). For instance, a previous postharvest study conducted by (Gonçalves *et al.* (2000) in fresh pineapple employing hot water treatments at 38°C and 40°C for 10 and 20 mins, they got to prolonged the postharvest shelf life of the fruit due to a reduction in internal browning incidence, and low activity of enzyme promoting oxidative species such as polyphenol oxidase (PPO), peroxidase (POX), and phenylalanine ammonium lyase (PAL). Also, in this study, CaCl_2 was administered

at a concentration of 2%. However, no sufficient literature has been reported on the employment of hot water treatments in the postharvest of fresh pineapple, specifically in combination with calcium dipping applications. Therefore, this study aimed to determine the impact of calcium combined with hot water treatments on the postharvest quality of MD2 pineapple.

2. Materials and methods

2.1 Experiment design and treatments

This research was carried out in the packing house of a pineapple field located in Lampung Indonesia, between March and April of 2020. The MD2 pineapple cultivar was used for this experiment. The fruit was harvested and sorted employing a weight range between 1.46 kg – 1.7 kg with two parameters of skin color classification associated with two harvest stages, when this was still dark green or when it was light green (Figure 1), with the idea of elaborating two groups of study. The experimental design implemented was a randomized block design with three replications per treatment, implemented in the previous two groups of study mentioned.

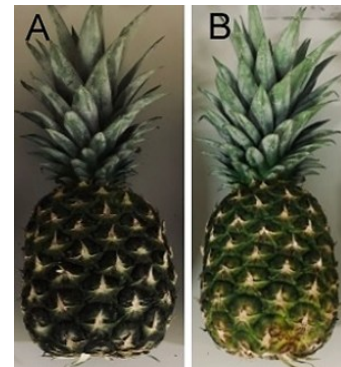


Figure 1. Skin color classification was employed for the experiment associated with two harvest stages of the fruits. A: Dark green skin color, B: Light green skin color.

The treatments applied were CaCl_2 (0, 2, 4, and 6%) mixed with hot water immersions of 50°C for 5 mins, following the method described by Gonçalves *et al.* (2000) and Cano-Reinoso, Soesanto, Kharisun *et al.* (2022), employing a heat electric resistance and a water thermometer to monitor the temperature. In addition, a control treatment without any calcium or hot water dipping application was employed. The water temperature employed in this research does not affect the moisture content of pineapple fruit, which ranges between 80-90% (Paull and Chen, 2018), as demonstrated by Gonçalves *et al.* (2000). CaCl_2 is a calcium salt-free of phosphorous that it has been widely implemented as a supplementary source for calcium uptake in fruits and plants, due to its natural origin (Martín-Diana *et al.*, 2007; De Freitas and Resender

Nassur, 2017). Because of that, this calcium source was chosen for this experiment.

Every one of the treatments used in this experiment employed fungicide and wax before cold storage. The fungicide administrated was Prochloraz in concentrations of 2 cc/L, while the waxing applied was OE 6012 in solutions of 100 ml/L. The fungicide was administrated before the waxing, and both of them were applied in dipping applications of ten seconds, just after the calcium and hot water treatment mentioned above. After that, all the fruits were arranged into a box per treatment and stored in cold storage with a temperature of 7°C and relative humidity of 95%, for 40 days, as recommended by Paull and Chen (2018) and Cano-Reinoso, Renaldy Tamalea and Wibowo (2022). Observations and analyzes were carried out with a total of 40 days of storage.

2.2 Calcium content determination

The calcium content determination in the flesh and skin of the fruits was carried out by atomic absorption spectrometry (AAS 932 Plus, GBC scientific equipment, USA). A composition of three fruits and the skin per replication was analyzed in every treatment, based on the method described by Benton-Jones (2001) and Cano-Reinoso, Kharisun, Wibowo *et al.* (2022). First, 5 mL of juice extracted from the flesh/skin was taken. After that, the juice was filtrated and put in a digestion tube. Next, 5 mL of 65% nitric acid through the digestion tube walls was added and left overnight. Later, the samples were heated at 125°C for one hour and subsequently lifted and cooled. Thereafter, 3 mL of 30% hydrogen peroxide (H₂O₂) through the digestion tube walls were added, reheated for one hour, removed and cooled. Next, 5 mL of nitric acid with distilled water (1:10) was added and shaken using a Vortex Shaker. Finally, the samples were transferred to a flask 25/50 mL quantitatively and pitch with distillate water creating an extract ready for the calcium mineral analysis. Results are expressed in dry basis content.

2.3 Total soluble solids and total acidity determination

Following the method described in Cano-Reinoso, Renaldy Tamalea and Wibowo (2022), the TSS and TA were calculated in each fruit sample. TSS was measured employing a hand-held refractometer (MASTER-53 α , Atago, Japan); meanwhile, the TA was determined by titration to pH8.1 with 0.1 M NaOH using phenolphthalein as an indicator and expressed as a percentage of citric acid.

2.4 Water content and firmness in the fruit

The water content of the fruit samples was calculated according to the method described in Siti Roha *et al.*

(2013). Approximately 25 g of a composition of the flesh of three fruits per replication were oven-dried at 60°C for 24 hours to constant weight for moisture determination. On the other hand, the flesh firmness of the fruit was determined following the method of Cano-Reinoso *et al.* (2021). The firmness was calculated in the centre region of each fruit flesh, adjacent to the core. A penetrometer (CT3 texture analyzer, Brookfield Ametek, USA) with a 7 mm diameter flat probe was used, implemented the respective test parameters (regular test, trigger: 10 g, deformation: 7 mm, and speed: 1.0 m/s); the results were expressed in N.

2.5 Statistical analysis

Statistical analyses were performed using SPSS Version 22.0 software (SPSS Inc., USA). All data were analyzed by ANOVA of one-way. Mean significant differences at $P < 0.05$ were determined by Duncan's multiple range test.

3. Results and discussion

3.1 Calcium content in the fruit

The calcium content of the skin was higher than in the flesh in both types of skin color. In the case of the flesh, treatment D obtained the most superior value for the pineapples with a dark green skin color (1236.94 mg/kg), while for the ones with a light green skin color, the most superior result in the flesh was observed in treatment A (1264.38 mg/kg) (Tables 1 and 2). Intermedial doses of CaCl₂ could cause a reduction of the calcium content in the flesh of pineapples with green dark skin color, while elevated doses of this fertilizer produced the same phenomenon, but in the case of the green light skin color. In contrast, in terms of calcium in the skin, no significant differences were obtained in the dark green skin color, while in the light green, the most superior outcome was evidenced in the control (4245.82 mg/kg) (Tables 1 and 2). Superior doses of CaCl₂ together with the hot water treatment applied, could cause a reduction of the calcium content in the skin. Furthermore, pineapples with a light green skin color tend to have a lower concentration of calcium in the skin than those with dark green skin.

Figure 2 shows the trend of the calcium content in the flesh through the postharvest life of the pineapples. It can be observed that in both types of skin color, the calcium content decreases proportionally with the increase of the storage days of the fruit; also, in both cases, there is a slight increase during the late postharvest days. Moreover, Figure 2 shows that the mean values of the fruits with dark green skin color tend to be a little bit superior to those with light green skin, as the shape of the baseline peak shape is less biased toward

Table 1. Effect of the treatments employed on the calcium and physicochemical characteristics of the fruits with dark green skin color after 40 days of cold storage.

Treatment	Dark green skin color					
	Ca flesh (mg/kg)	Ca Skin (mg/kg)	Water content (%)	Firmness (g.f)	TSS (%)	TA (%)
A	1089.30±60.86 ^{ab}	4019.30±190.41 ^a	92.69±0.15 ^a	929.75±82.86 ^a	14.53±0.031 ^a	0.90±0.13 ^a
B	908.97±2.17 ^b	4351.54±500.50 ^a	92.76±0.40 ^a	777.17±58.84 ^a	14.53±0.49 ^a	0.89±0.04 ^a
C	997.06±45.89 ^{ab}	4273.53±453.72 ^a	92.40±0.11 ^a	719.25±51.72 ^a	14.69±0.32 ^a	0.94±0.06 ^a
D	995.07±148.79 ^{ab}	4352.47±370.38 ^a	92.89±0.25 ^a	903.08±77.82 ^a	14.24±0.49 ^a	0.79±0.02 ^a
E	1236.94±26.48 ^a	3961.41±142.32 ^a	93.33±0.37 ^a	1001.75±159.88 ^a	14.11±0.42 ^a	0.85±0.03 ^a

Values are presented as mean ± standard error. Values with different superscripts in the same column are statistically significantly different by Duncan's multiple range test ($p < 0.05$).

Table 2. Effect of the treatments employed on the calcium and physicochemical characteristics of the fruits with light green skin color after 40 days of cold storage.

Treatment	Light green skin color					
	Ca flesh (mg/kg)	Ca Skin (mg/kg)	Water content (%)	Firmness (g.f)	TSS (%)	TA (%)
A	1072.49±34.81 ^{ab}	4245.82±175.72 ^a	92.74±0.02 ^a	569.83±0.87 ^a	12.68±0.54 ^a	0.93±0.07 ^{ab}
B	1264.38±102.81 ^a	3513.17±263.14 ^b	93.45±0.35 ^a	571.67±0.78 ^a	13.82±0.24 ^a	0.87±0.04 ^{ab}
C	1002.25±69.50 ^b	3015.18±46.30 ^b	93.39±0.18 ^a	697.00±1.12 ^a	14.09±0.26 ^a	0.80±0.03 ^b
D	881.79±75.63 ^b	3339.89±96.29 ^b	92.96±0.27 ^a	803.67±0.39 ^a	13.71±0.41 ^a	0.88±0.03 ^{ab}
E	944.29±18.27 ^b	3507.22±369.57 ^b	92.84±0.17 ^a	624.08±0.41 ^a	13.38±0.57 ^a	0.96±0.15 ^a

Values are presented as mean ± standard error. Values with different superscripts in the same column are statistically significantly different by Duncan's multiple range test ($p < 0.05$).

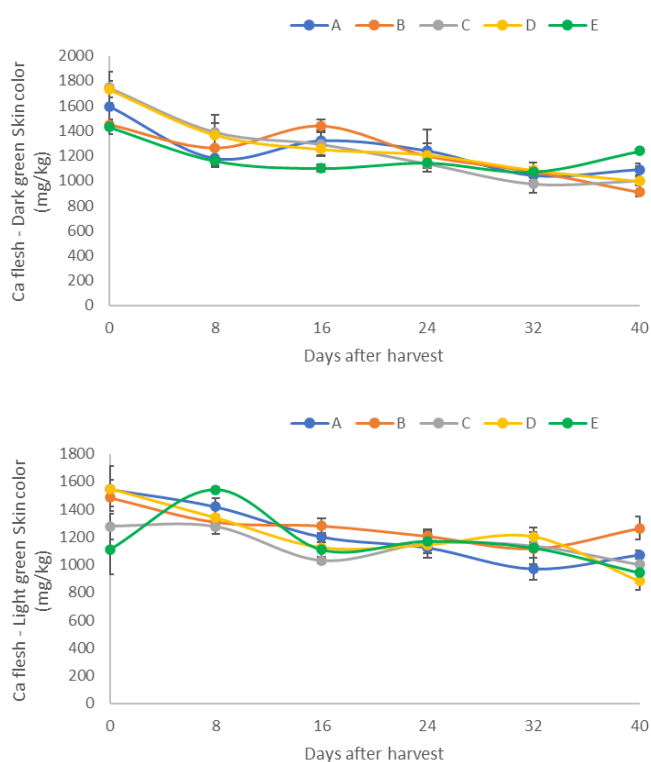


Figure 2. Effect of the treatments employed on the trend of calcium content in the pineapple flesh during 40 days of storage for the fruits with dark and light green skin color. The data presented are the mean of triplicates. Error bars represent standard error.

low calcium content, especially between the middle days of the pineapple's storage.

Calcium is a mineral that has been associated with

the proper stability of cell walls in plants, providing more tolerance to physiological disorders and diseases, delaying fruit senescence, and as a result enhancing its quality (De Freitas and Resender Nassur, 2017; Gulbagca *et al.*, 2020). Regarding the flesh of the fruits, the use of high doses of CaCl_2 mixed with hot water treatments leads to a more elevated accumulation of Ca^{2+} ions after 40 days of cold storage in the dark green skin color, an opposite situation occurring in the light green skin color, where the absence of CaCl_2 with the use of hot water treatments provided the most superior outcome. It is considered that pineapples in the dark green color stage are harvested younger than those of light green skin color, counting the days after flower induction (Paull and Chen, 2018; Vásquez-Jiménez and Bartholomew, 2018). Besides, it has been proven the younger the fruit, the activities of cell wall degrading enzymes like pectin methyl esterase (PEM) or polygalacturonases (PG) are reduced, affecting the postharvest shelflife (De Freitas and Resender Nassur, 2017; Gulbagca *et al.*, 2020). Therefore, in relation to the dark green skin color, the application of hot water treatments could have provoked a more elevated activity of PEM and PG than the usual one, together with the production of reactive oxygen species (ROS), those that can cause a loss of integrity in the cell wall, especially with the progress of the storage days. Because of that, high doses of calcium chloride were necessary not only to deal with this circumstance but also to enhance Ca^{2+} ions assimilation into the cell wall matrix.

On the contrary, in the case of the light green skin color, the only use of hot water treatments without the application of CaCl_2 was enough to cause a positive effect on the calcium content in the flesh until the end of the storage period. Under adequate application, induction of heat in plants and fruits, in this case by dipping in hot water, can produce the activation of heat shock proteins (HSP). These proteins can encourage the creation of calcium channels in the plasma membrane, causing more elevated assimilation of Ca^{2+} ions, and as a consequence, improving the integrity of the cell wall (Song *et al.*, 2012; Cano-Reinoso *et al.*, 2021). On top of that, the ions Cl^- have been detected to promote the regulation of the osmotic potential in cells, permitting the assimilation into the cytosol of organic compounds; nevertheless, under high concentrations they could generate an imbalance in the photosynthetic net, affecting the membrane osmotic potential of the membrane, and as a result, reducing the integrity of the cell wall (Geilfus, 2018). This phenomenon could explain why higher doses of CaCl_2 did not create a positive effect on the calcium accumulation for this pineapple stage after 40 days of cold storage, contrary to what happened in the dark green skin color. The activities of PEM and PG could have been promoted at high doses of CaCl_2 due to the influences of the Cl^- ion. On top of that, Figure 2 exposed how the trend of the calcium content in the flesh in both of the skin color stages decreased as longer was the storage life for each of the treatments implemented. De Freitas and Resender Nassur (2017) and Gulbagca *et al.* (2020) explained that it is a normal physiological process in fruits to lose calcium during their postharvest life, due not only to the increase of PEM and PG activity but also because fruit senescent comes together with a more elevated production of ROS, which disrupt the creation of enzymes like calcium protein kinases, which are necessary to keep the calcium bonds into cell wall and decrease its lipid polysaccharide oxidation. Furthermore, this study did not focus on the effect of the treatments administrated on cell wall-related enzyme activities. Hence, a study addressing these pineapple fruit enzyme activities together with the physiological stage of the fruit under calcium and hot water treatment applications during storage conditions is recommended for future studies. On top of that, it is worth indicating that CaCl_2 is a natural calcium salt source free of phosphorus and helps to maintain the ratio of phosphorus-calcium in the human body. It is also widely employed in fresh fruits and fresh cut industry as food grade and harmless to human health, even though some residuals are detected (Martín-Diana *et al.*, 2007; De Freitas and Resender Nassur, 2017).

3.2 Water content and firmness of the flesh

In the case of the water content, no significant differences were observed in both types of skin color; and for both of them, the mean value obtained was on average higher than 90% (Tables 1 and 2). On the other hand, despite not exposing significant differences, the mean values of firmness in the flesh were much higher in the dark green skin color than in the light green one (Tables 1 and 2). On top of that, there was no visible relation between the treatments with calcium with hot water treatment employed and a more elevated or reduced firmness in the flesh, suggesting that the outcomes obtained could be associated with the physiological stage of the fruits collected at harvest.

The water content of the fruits is an indicator of their quality and readiness for consumption (Paull and Chen, 2019). Also, it has been demonstrated that fruits can reduce their moisture content through transpiration during postharvest (Paull and Chen, 2019). As mentioned previously, Pineapple is a fruit high in water content, which could be more than 80% (Paull and Chen, 2018). Despite that the results of this experiment did not expose significant differences, the mean values obtained for both skin color stages were superior to the minimum recommended range for an ideal consumption, demonstrating that the treatments administered did not cause a negative impact on this variable after 40 days of storage. On the other hand, the more elevated values observed in the firmness of the flesh for the fruits of the dark green skin color stage could have been associated with the younger stage in which these usually are harvested. Hu *et al.* (2012) and Cano-Reinoso, Renaldy Tamalea and Wibowo (2022) explained that during postharvest it is usual that the firmness of the flesh in fruits decreases concomitantly with their shelflife, promoted by cell wall degrading enzymes. As mentioned before, the PEM and PG enzymes do not have high activity in fruits harvested during the dark green skin color stage. This physiological condition could have encouraged higher firmness after 40 days of storage. Moreover, it has been suggested values between 400 and 700 g.f for an ideal consumption of MD2 pineapple (Paull and Chen, 2018; Cano-Reinoso *et al.*, 2022). Up to these values, it should depend on consumer preferences. On the basis of that, all the fruits of the treatments employed in the light green skin color were within that recommended range; however, some of the treatments in the dark green skin color did not provide this characteristic.

3.3 Total soluble solids and total acidity of the fruits

The TSS of the fruits did not expose significant differences in each of the skin color analyzed. However,

the TSS content of the fruits with dark green skin color was slightly more superior than those with light green skin (Tables 1 and 2). Similar to the firmness, there was no strong relation between the treatments used and the values of TSS observed in both skin color, indicating also in this case an influence of the physiological condition of the fruits at the moment of harvest for the experiment. On the other hand, the TA content in the dark green skin color did not show significant differences, while in the light green skin, those were observed. The treatment with a higher dose of CaCl_2 (treatment D), provoked a more elevated TA content in the light green skin color (0.96%) (Tables 1 and 2). Besides, in general, there were no representative differences exposed between the mean values of TA between both types of skin color.

Figure 3 shows the trend of the TA content through the postharvest life of the pineapples. In Figure 3, it is possible to observe that in both types of skin color the TA have a continuing increase and decrease in its content, which could be associated with the impact of the treatments administrated, and the physiological changes suffered by the fruits during postharvest. Moreover, it can be detected that during the last days of storage, there is an increase, more pronounced in the light green skin color, especially in treatment D, compared to the other treatments (Figure 3).

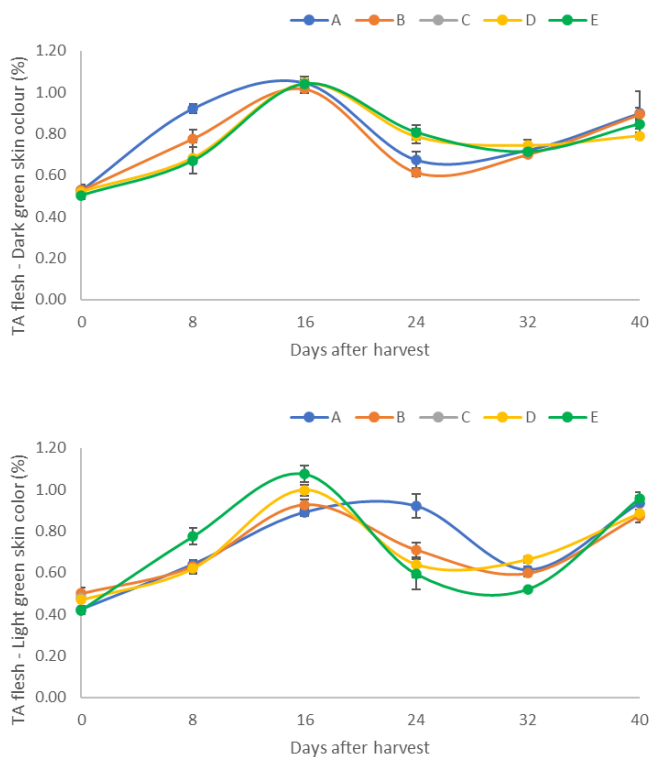


Figure 3. Effect of the treatments employed on the trend of the TA content in the pineapple during 40 days of storage for the fruits with dark and light green skin color. The data presented are the mean of triplicates. Error bars represent standard error.

The TSS content of MD2 pineapple is recommended to be as minimum as 12% for ideal consumption; nevertheless, some consumers prefer higher values in the flesh (Paull and Chen, 2018; Cano-Reinoso *et al.*, 2022). After 40 days of cold storage, the TSS outcomes in both skin color stages did not present significant differences, and the results obtained were superior to the minimum suggested value. However, it is important to mention that the mean values of the dark skin color stage were slightly higher than those of the light green skin color. TSS is primarily accounted for by the sucrose content of pineapple (Paull and Chen, 2018; Cano-Reinoso *et al.*, 2022). Therefore, it is possible to infer that the treatments implemented for the light green skin color stage caused a slight reduction of the sucrose content compared to the dark green stage. Enzymes like sucrose synthase (SC), and cell wall invertase (CWI) have been associated with this sucrose accumulation in the flesh; because of that, this reduction of the sucrose content in most of the treatments of the light green skin color stage could be linked to the lower activities of these enzymes during the postharvest shelflife of the fruits. However, more studies are needed in the future to corroborate this statement.

Furthermore, TA values between 0.4 and 0.7% are suggested for pineapple at harvest (Saradhulhat and Paull, 2007; Paull and Chen, 2018). It is possible to observe that in both skin color stages, the TA was superior to the recommended range, including the control. TA is highly associated with the citric acid content of the fruit. On top of that, citric acid has been linked to antioxidant activities, scavenging ROS species, maintaining fruit cell wall integrity and the quality of fruits (Patrignani *et al.*, 2015; Yang *et al.*, 2019). Therefore, the fruits regardless of the treatments implemented in both skin color stages after 40 days of storage, could have sensed the progress of their senescence; because of that, to cope with this situation physiologically, the fruits could have increased the production of this organic acid to provide more tolerance to this condition. This circumstance could be evidenced in the trend of the TA content exposed in Figure 3. The constant physiological change of the fruits and the progress of their senescent, as mentioned previously, can cause the fluctuating trend linked to the metabolizing of organic acid, primordially in the final postharvest days. Moreover, citric acid could work also as a subtraction for the respiration of fruits (Yang *et al.*, 2019). As a consequence, also the fruits that tolerate the physiological stress that high respiration can carry out, could have elevated their organic acid content, essentially close to the end of the storage days. Additionally, aconitase enzyme (ACO) has been referred to as the enzyme responsible for the low accumulation of

citric acid in pineapple (Saradhulhat and Paull, 2007; Paull and Chen, 2018). Therefore, more experiments are recommended to study in detail the impact of the treatment used on this enzyme during postharvest associated with the physiological stage of the fruit at harvested.

Finally, it is important to mention that the scope of this study did not cover the impact of the treatment implemented on the organoleptic and microbiologic properties of the fruits. Nevertheless, regarding postharvest calcium applications, it has been proved that those can reduce the incidence of diseases and pathogens affecting fruits due to their positive effect on cell wall strength, decreasing the impact of pectolytic enzymes causing lipid peroxidation, and affecting cell wall integrity (Hocking *et al.*, 2016; De Freitas and Resender Nassur, 2017). On the other hand, some experiments have reported that calcium salt sources like CaCl_2 can produce fruits with some type of sour or salty taste; therefore, researchers have been exploring other calcium natural sources like lactate and gluconate, in order to avoid this effect on fruit taste (De Freitas and Resender Nassur, 2017). This phenomenon should be explored further in pineapple. Concerning hot water treatments, as explained before, due to their positive impact on Ca^{2+} assimilation into the cell wall, these types of treatments could help to reduce fruit decay, providing more strength to any pathogen attack, at a cellular level (Fallik and Illić, 2017). Besides, some pathogens that can surround fruit after harvest also could be treated by high temperatures. For example, in melon dipping administrations of hot water at 45°C caused inhibition of *Fusarium* rot during postharvest (Fallik and Illić, 2017); likewise, in papaya, hot water treatments at 54°C reduced the development of *Colletotrichum gloeosporioides* decay in the peel, and reduce the incidence of anthracnose (Fallik and Illić, 2017). Because of that, there is a potential to study the impact of the treatments implemented in this research on the organoleptic and microbiological properties of MD2 pineapple fruit.

In conclusion, the postharvest application of calcium together with hot water treatment affected the quality of the MD2 pineapple after the end of the storage period. Regarding the two physiological stages in which the fruits were harvested, for the dark green skin color, it is considered that the treatment employing CaCl_2 (6%) with hot water treatment for 5 mins gave the best performance, providing the most elevated calcium content in the flesh, and the ideal level in the skin, and the optimal values of water content, TSS and TA in the fruits, although the firmness of the flesh was more elevated than the recommended for consumption. On the

contrary, in relation to fruits of the light green skin color stage, the treatment providing the best response was the one only using the hot water treatment, having the most elevated calcium content in the flesh, also an ideal level in the skin, and optimal values of water content, firmness of the flesh, TSS and TA in the fruits examined. Finally, more studies related to the enzyme activities associated with the cell wall status and the metabolization of organic compounds in pineapple fruit are suggested under the treatments and physiological stages applied in this experiment.

Conflict of interest

The authors declare no conflict of interest.

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