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Physicochemical characteristic and internal browning of pineapple as affected by calcium and gibberellic acid dipping application

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Abstract: Postharvest applications of calcium and gibberellic acid (GA) have proved to maintain optimal fruit quality and control decay during cold storage. This study evaluated the effect of calcium and gibberellic acid dipping application on pineapple physicochemical characteristics and internal browning. The experiment implemented two factors. The first factor relates to two dipping times (five and ten minutes) and the second factor related to four treatments, GA, Ca, mix GA-Ca, and control (no GA or Ca applied). Total soluble solids (TSS), total acidity (TA), TSS/TA ratio, sugar, citric and ascorbic acid content, together with internal browning severity and incidence were determined. The treatment of Ca, essentially using a dipping of five minutes delivered the best performance, having the lowest severity and incidence of internal browning (4.44 and 22.22%, respectively), the highest citric acid (0.61%), ascorbic acid content (405.18 mg kg^{-1}) and the lowest TSS/TA ratio (25.53). Meanwhile, the other treatments were considered less satisfactory, due to their highest internal browning severity and incidence, without a notable impact on the citric acid and ascorbic acid content, especially with a dipping time of ten minutes. In conclusion, dipping applications of calcium in postharvest can enhance pineapple quality and reduce internal browning.

1. Introduction

Pineapple is an important crop worldwide, mainly exported as canned and fresh fruit (Hassan *et al*., 2011). Low acid hybrids are the pineapple cultivars more needed by the industry nowadays. These hybrids are attractive to consumers due to their more yellow shell colour, higher sugar content and uniformity (Chen and Paull, 2017; Paull and Chen, 2018; Cano-Reinoso *et al.*, 2021 a). Therefore, the control of the optimal

physico-chemical characteristics of these hybrids has become a primordial activity for the farmers.

To regulate the fruit decay, pineapple is commonly subjected to postharvest storage, where the deterioration of its physico-chemical properties is delayed, especially during long exportation periods (Hassan *et al.*, 2011; Paull and Chen, 2018; Cano-Reinoso *et al*., 2022 b). During cold storage, the fruit is affected by physiological disorders such as chilling injury and internal browning (De Freitas and Resender Nassur, 2017; Paull and Chen, 2018). These conditions highly endanger the shelf-life of the fruits. Because of that, several postharvest technics have been developed to extend the cold storage life of the horticultural products, mitigating the impact of any physiological disorders (De Freitas and Resender Nassur, 2017; Noichinda *et al*., 2017).

One of these technics is the dipping of the fruit employing calcium mineral sources. Calcium treatments during postharvest have been proved to maintain optimal fruit quality and enlarger the fruit cold storage life (De Freitas and Resender Nassur, 2017). In addition, calcium inhibits fruit softening by increasing the cell wall strength, which mitigates the cell breakdown, a normal phenomenon occurring during postharvest decay (Hocking *et al*., 2016; De Freitas and Resender Nassur, 2017). Besides, applications with this mineral have shown a positive impact on nutritional flavour, antioxidant capacity and reduction of internal browning (De Freitas and Resender Nassur, 2017). For example, in pineapple, postharvest calcium treatments have provided significant results in the internal browning reduction, the activity of oxidative enzymes like phenylalanine ammonia-lyase (PAL) and polyphenol oxidase (PPO), and an increase of the total phenols content (Youryon and Wongs-Aree, 2015; Youryon *et al*., 2018).

Another technique for controlling fruit decay during cold storage is by dipping applications using plant regulators like gibberellic acid (GA). This plant regulator has demonstrated outstanding results in reducing postharvest decay in fruits and vegetables due to its antagonist properties in ethylene sensing and production (Pusittigul *et al*., 2012; Mohamed *et al.*, 2016). Additionally, GA used during postharvest has delivered positive results improving the physicochemical attributes of fruits, especially those related to antioxidant accumulation and weight loss reduction during cold storage (Pusittigul *et al.*, 2012; Dong *et al.*, 2019). For example, in pineapple, GA employed during postharvest enhanced the shelf-life of the fruit up to 24 days, with an increase in its total soluble solids, total acidity, and percentage of weight loss (Pusittigul *et al*., 2012; Mandal *et al.*, 2015).

However, most of the calcium used on pineapple after harvest has been documented as calcium infiltrations or mixed with other techniques such as hot water treatments. At the same time, in the case of GA, more studies are needed to develop a correct characterization of its impact on pineapple quality. Because of that, few papers have been reported on postharvest calcium and GA employing dipping technics; moreover, they have studied their effect on low-acidic hybrids and possible synergy between them. Therefore, this study aims to evaluate the effect of calcium and GA dipping application on pineapple physicochemical characteristics and internal browning.

2. Material and Methods

Experiment design and treatments

The research was implemented in a pineapple packing house located in Lampung, Sumatra island of Indonesia, between January and February of 2020. In this experiment, the MD2 pineapple cultivar was used. This low acid hybrid is well known in the industry because of its outstanding qualities related to higher sugar and antioxidant content, more uniformity and yellowish shell colour than other acid hybrids (Bin Thalip *et al.*, 2015; Cano-Reinoso *et al*., 2021 b). The study was set using an experiment design with two factors. The first factor related to two dipping times (five and ten minutes), and the second factor concerning four treatments consisted of three solutions of, GA, Ca, mix GA-Ca, and C (Control - no use of GA and Ca). Three replications per treatment were used in this experiment. Furthermore, randomly fruit samples were picked from every treatment to be examined employing intervals of eight days.

All treatments, including the control used fungicide and wax before cold storage. The fungicide product implemented was Prochloraz in doses of 2 cc l^{-1}, while the waxing product applied was Sta-Fresh 2952 in doses of 74 g l^{-1}. Both fungicide and waxing, in that order, were used in dipping applications for ten seconds, just after the dipping of the fruits in GA or Ca. The calcium source used was Calcibor (Alba Milagro Internation, Lombardia, Italy) - (12.9% w/v CaO and 2.6% w/v B) in doses of 4 l 2000 l^{-1}; meanwhile, the GA product employed was ProGibb (40%

of GA_3 active component, Valent USA Corp., Walnut Creek, California - USA) in doses of 100 mg l^{-1}. The Calcibor doses implemented were prepared according to the producer recommendation; meanwhile, the GA doses were arranged based on the previous experiments of Mandal *et al*. (2015) and Dong *et al.* (2019). Concerning the mix GA-Ca treatment, it was employed a sequenced application (one solution of GA or Ca at time). Besides, the dipping times implemented in this experiment (five and ten minutes) were based on the previous studies of Pusittigul *et al*. (2012) and Islam *et al*. (2013).

The fruits were selected according to their weight and shell colour characteristics and arranged by their respective treatments inside cold storage for 40 days (Temperature: 7°C, relative humidity: 95%). The preference for fruit weight for the research was between 1.4 and 1.5 kg, with a shell colour where 10-20% of the area from the base already turned yellow. The MD2 pineapple is typically harvested and exported with the previously described weight and shell colour characteristics (Bin Thalip *et al*., 2015; Paull and Chen, 2018).

Determination of the total soluble solids (TSS), total acidity (TA) content

According to the procedure described in Shamsudin *et al*. (2020), the TSS and TA were measured in each fruit per replication of every treatment arranged. TSS was calculated employing a hand-held refractometer (MASTER-53 α, Atago, Japan), while the TA was measured by titration to pH 8.1 with 0.1 M NaOH using phenolphthalein as an indicator and expressed as a percentage of citric acid.

Sugar, citric acid and ascorbic acid (AsA) content

Pineapple sugar and citric acid were measured using the method described in Siti Roha *et al*. (2013), employing a High-Performance Liquid Chromatography (HPLC) - (Hitachi, USA) model L-2000 instrument with a Refractive Index detector model L-2490. A juice extracted from the fruit flesh adjacent to the core was employed for this procedure. Samples were obtained from each fruit per replication of every treatment implemented. For the sugar content, standard solutions of 500, 1000, 1500, 2000 mg l^{-1} of glucose, fructose and sucrose were prepared with the aim of developing a curve of sugar level; furthermore, in the case of the citric acid and AsA determination, the standard solutions were 1000 mg l^{-1} and, 200, 400, and 800 mg l^{-1}, respectively, having the same objective in mind. The standard solutions were dissolved in distilled water and filtered through a Millipore 0.45 µm membrane filter. Finally, the sugar, citric acid and AsA content was quantified, comparing the peak area by a chromatographic procedure.

The chromatographic condition for the sugar determination was:
Column: Purospher® STAR NH2 (250 x 4 (mm), 5 µm). Guard column: LiChocart® 4-4 / LiChrospher® 100 NH_2, 5 µm. Column temperature: Room temperature (22°C). Mobile phase: Acetonitrile: distilled water (80:20). Flow rate: 1 ml min^{-1}. Injection volume: 20 µL. Duration of analysis: 15 min.

Meanwhile, for the citric acid and AsA was:
Column: Purospher® STAR NH2 (250 x 4 (mm), 5 µm). Guard column: LiChocart® 4-4 / LiChrospher® 100 NH_2, 5 µm. Column temperature: Room temperature (22°C). Mobile phase: Pipette of 0.14 ml H_2SO_4 (0.0025 M) concentrated at 97% is introduced in a volumetric flask, then add 1000 ml of distilled water. Injection volume: 20 µl. Duration of analysis: 15 min.

Browning severity and incidence

The internal browning severity was calculated employing the following score classification and transformed into a percentage: 1 (No flesh browning, 0%), 2 (20% of browning in the flesh), 3 (40% of browning in the flesh), 4 (60% of browning in the flesh), 5 (80% of browning in the flesh) and 6 (100% of browning in the flesh). Figure 1 shows a schematic example of the previous score classification described. Furthermore, the incidence was measured by accounting the percentage of fruits affected by internal browning in each treatment during every observation.

Statistical analysis

Statistical analyses were performed using SPSS Version 22.0 software (SPSS Inc.; Chicago, IL: USA).

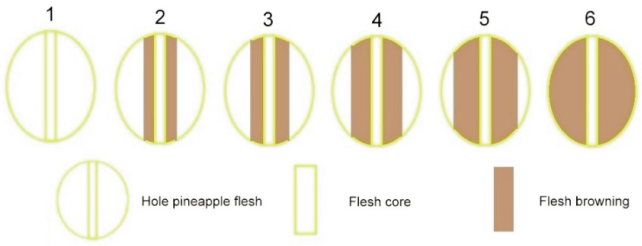

Fig. 1 - Scheme of the score classification used to determine the internal browning severity in the experiment during each observation. Score: 1 (0%), 2 (20%), 3 (40%), 4 (60%), 5 (80%), and 6 (100%).

All data were analyzed by two-ways ANOVA. Mean significant differences at $p<0.05$ were determined by Duncan's multiple range tests and Kruskal-Wallis test (in case of the internal browning data).

3. Results

TSS, TA, and TSS/TA ratio

There was no significant impact evidenced coming from the dipping time, the treatments implemented and the interaction between these two factors for the TSS, TA, and TSS/TA ratio. Concerning the TSS, the mean results exposed that the control had the highest value (16.93%), meanwhile the treatment of GA-Ca with a dipping of ten minutes provided the lowest outcome (14.47%). Regarding the TA, the mean results show that the treatment of Ca with a dipping of five minutes delivered the highest outcome, while the GA-Ca treatment with five minutes dipping had the lowest one (0.61 and 0.53%, respectively). On the other hand, the mean outcomes for the TSS/TA ratio delivered the highest value in the treatment of GA with five minutes dipping (38.17%), and a most inferior result in the treatment of Ca also with a dipping of five minutes (26.16%). The TSS, TA and TSS/TA ratio mean values corroborated the previous results described concerning the single factors influence and their interaction; there was no a clear positive or negative trend in the mean values when the dipping times were increased or reduced and combined with the treatments administrated (Table 1).

Sugar content

In terms of pineapple sugar after cold storage of 40 days, the fructose, glucose and sucrose content did not demonstrate significant differences coming from the dipping time, the treatments administrated, and their interaction. Concerning the mean values of these two factors, just the sucrose exposed significant differences. In this case, the treatment of GA with ten minutes dipping had the highest result, and the same treatment but with dipping of five minutes, the most inferior outcome (8.25 and 5.97%, respectively). Despite this particularity, for the mean results of the fructose, glucose and sucrose content, it was difficult to determine a positive or negative tendency coming from the rise or reduction of the dipping times and their combination with the treatments used, reaffirming the individual factors impact and their interaction outcomes (Table 1).

Citric acid and AsA content

The citric acid and AsA did not present a representative impact from the dipping time and the treatments studied; however, the interaction between these two factors was significant, impacting the mean outcomes among these variables after 40 days of cold storage. For the citric acid, the lowest outcomes were obtained in the treatments of GA and Ca

Table 1 - Results of the single and interaction effect from treatments and dipping times with their respective combined mean values on the TSS, TA, and sugar content, after 40 days of cold storage

	TSS (%)	TA (%)	TSS/TA	Glucose (%)	Fructose (%)	Sucrose (%)
			Studied factors (single and interaction effect)			
Treatment	-	-	-	-	-	-
Dipping time	-	-	-	-	-	-
Treatment x Dipping time	-	-	-	-	-	-
			Mean values (Treatment vs. Dipping time)			
GA 5 min	16.13 ± 0.48 ab	0.39 ± 0.06 b	43.32 ± 6.71 a	3.35 ± 0.22 a	3.41 ± 0.21 a	5.97 ± 0.56 b
Ca 5 min	14.80 ± 0.00 bc	0.62 ± 0.13 a	25.53 ± 4.48 b	2.48 ± 0.63 a	2.77 ± 0.56 a	7.72 ± 1.03 ab
GA-Ca 5 min	15.20 ± 0.70 bc	0.56 ± 0.04 ab	27.82 ± 4.02 ab	3.25 ± 1.02 a	3.49 ± 0.99 a	6.77 ± 1.50 b
GA 10 min	15.27 ± 0.27 bc	0.50 ± 0.10 ab	33.01 ± 5.88 ab	2.71 ± 0.18 a	2.99 ± 0.19 a	8.25 ± 0.45 a
Ca 10 min	14.67 ± 0.18 bc	0.59 ± 0.11 ab	26.80 ± 4.96 ab	3.01 ± 0.12 a	3.28 ± 0.11 a	7.11 ± 0.62 ab
GA-Ca 10 min	14.47 ± 0.29 c	0.50 ± 0.06 ab	29.91 ± 4.78 ab	2.06 ± 0.86 a	3.23 ± 0.43 a	6.54 ± 0.75 b
Control	16.93 ± 0.84 a	0.58 ± 0.04 ab	29.46 ± 2.57 ab	2.97 ± 0.22 a	3.27 ± 0.28 a	7.77 ± 0.09 ab

* Results marked with (+) indicate that the P-value ($p < 0.05$) for the single or interaction can be considered to draw conclusions; meanwhile, results marked with (-) indicate that the P-value ($p > 0.05$) for the single or interaction are not significant to draw conclusion in the text. Mean values ± SE in each column followed by the same lower-case letters are not statistically different by Duncan's multiple range test ($p < 0.05$). Treatments: GA, Ca, mix GA-Ca, Control - no use of GA and Ca. Dipping time: 5 and 10 minutes.

(GA: 34% - five minutes; Ca: 37% - ten minutes). Moreover, the treatment of Ca but using a dipping of five minutes had the highest outcome (0.61%). No notable differences were observed between the mean results of the treatment GA-Ca in both dipping times; however, those outcomes were higher than the control (Table 2).

Concerning the AsA, similar with the previous variable, when the Ca treatment was implemented with a dipping of five minutes the highest result was obtained (405.18 mg kg^{-1}). Besides, the most reduced outcome was determined in the same treatment but with a dipping of ten minutes (111.38 mg kg^{-1}). On top of that, a high remarkable mean result was observed in the treatment of GA with ten minutes dipping (208 mg kg^{-1}). In this case, not all the mean outcomes of the treatment GA-Ca in both dipping times provided a positive increase, as the control obtained a superior value, essentially when five minutes dipping was used (Table 2). Compressing the previous information, it was noticed that for the citric acid and AsA, Ca treatments caused a rise in their values employing a short dipping of five minutes, contrary effect detected on the GA treatments, which demonstrated positive increases with a long dipping of ten minutes. This situation was not clearly observed in the GA-Ca treatments.

Internal browning

The monitoring of internal browning after 40 days of cold storage provided significant differences coming from the treatments implemented but not from the dipping times, and the interaction among these factors. In the mean outcomes it was possible to observe that samples employing the treatments of Ca obtained the lowest severity and incidence of internal browning, more remarkable when a dipping of five minutes was implemented (severity: 4.44%, incidence: 22.22%), compared with the other treatments and the control. On the contrary, samples using the treatments of GA and GA-Ca had a more superior internal browning severity and incidence, more evidenced with dipping times of ten minutes, suggesting a negative effect of the GA, concerning this variable (severity: 42%, incidence: 100%, for the GA treatment with ten minutes dipping) (Table 2).

4. Discussion and Conclusions

TSS, TA, and TSS/TA ratio in the fruit

TSS in pineapple low acid hybrids should be minimal as 12%, although some authors have recommended higher values close to 14% (Paull and Chen, 2015, 2018; Cano-Reinoso *et al.*, 2021 b). These val-

Table 2 - Results of the single and interaction effect from treatments and dipping times with their respective combined mean values on the citric acid, ascorbic acid (AsA), and internal browning, after 40 days of cold storage

	Citric acid (%)	AsA (mg kg^{-1})	Browning severity (%)	Browning incidence (%)
	Studied factors (single and interaction effect)			
Treatment	-	-	+	+
Dipping time	-	-	-	-
Treatment x Dipping time	+	+	-	-
	Mean values (Treatments vs. Dipping time)			
GA 5 min	0.34 ± 0.03 b	170.79 ± 48.88 b	37.78 ab	88.89 ab
Ca 5 min	0.61 ± 0.10 a	405.18 ± 64.31 a	4.44 c	22.22 c
GA-Ca 5 min	0.53 ± 0.14 ab	148.11 ± 63.87 b	37.78 bc	88.89 ab
GA 10 min	0.52 ± 0.04 ab	208.00 ± 22.56 b	42.22 ab	100 a
Ca 10 min	0.37 ± 0.03 ab	111.38 ± 36.12 b	11.11 bc	66.67 bc
GA-Ca 10 min	0.55 ± 0.05 ab	186.03 ± 66.56 b	28.89 bc	100 a
Control	0.44 ± 0.04 ab	157.46 ± 32.30 b	42.22 ab	77.78 ab

* Results marked with (+) indicate that the P-value ($p < 0.05$) for the single or interaction can be considered to draw conclusions; meanwhile, results marked with (-) indicate that the P-value ($p>0.05$) for the single or interaction are not significant to draw conclusion in the text. Mean values ± SE in each column followed by the same lower-case letters are not statistically different by Duncan's multiple range test, and Kruskal-Wallis test (for the internal browning data) ($p<0.05$). Treatments: GA, Ca, mix GA-Ca, Control - no use of GA and Ca. Dipping time: 5 and 10 minutes.

ues were assessed in the results obtained after 40 days of cold storage, knowing that it is common the reduction of TSS content through the postharvest life of pineapples (Lu *et al.*, 2011; Hu *et al*., 2012). Therefore, as all treatments provided an optimal performance, it is possible to infer that no harmful impact on this variable was received, even with the application of different dipping times. In addition, the lowest result observed in the treatment of Ca-GA with ten minutes dipping can be attribute to the time implemented. Typically, GA influences cell enlargement, this process generates more soluble solids as sugars, which can be assimilated in the cell (Wang and Irving, 2011; Gupta and Chakrabarty, 2013). Furthermore, this situation can cause more Ca^{2+} ions to crosstalk, creating cell wall channels, with the objective of maintaining optimal membrane stabilization (Gupta and Chakrabarty, 2013, Hocking *et al*., 2016; Cano-Reinoso *et al*., 2022 a). Nevertheless, during this process several reactive oxygen species (ROS) are released, and those can interfere and destroy organic molecules presented as soluble solids (Wang and Irving, 2011; Gupta and Chakrabarty, 2013). Because of that, a longer dipping time could be a trigger for more ROS production, decreasing the TSS available in the cell structures, and as a consequence the more reduced level observed in this treatment.

On the other hand, values of TA between 0.4 and 06% are recommended for optimal quality in MD2 pineapple; nevertheless, higher percentages have been reported in former studies (Chen and Paull, 2017; Paull and Chen, 2018; Cano-Reinoso *et al*., 2021 b). The outcomes of this research delivered values among this ideal range for almost all the treatments applied in both dipping times. Previous experiments using calcium as a postharvest treatment in pineapple had not presented any remarkable impact on TA (Pusittigul *et al*., 2014; Youryon *et al*., 2018). Nonetheless, in some fruits like apricots (Hajilou and Fakhimrezaei, 2013) and apples (Shirzadeh *et al.*, 2011), postharvest calcium applications increased the TA content. Calcium may positively affect the accumulation of organic acids through its impact on fruit metabolism, which are highly associated with the TA level. Calcium has been proved to delay fruit senescence by regulating the opening of stomata, creating a reduction of the degrading of organic acids, main source of fruit respiration (Van Meeteren and Aliniaeifard, 2016; De Freitas and Resender Nassur, 2017). This fact can explain why the treatment of Ca with five minutes dipping had the highest results. Five minutes could be the ideal time to produce the most positive impact on the fruit metabolism. However, this dipping time could cause a negative influence when the treatment of GA was administrated. GA has been detected to reduce the TA degrading in postharvest of pineapple (Mandal *et al*., 2015; Dong *et al.*, 2019). Nevertheless, as mentioned before, GA tends to ROS production, this dipping time could cause that the ROS level overcame and interfere the organic acid production to maintain a stable fruit metabolism, causing the lowest content with this treatment.

Furthermore, the TSS/TA ratio for MD2 pineapple should range between 18 and 25, although current authors have suggested values close to 30 (Chen and Paull, 2017; Paull and Chen, 2018; Cano-Reinoso *et al*., 2021 b). Numbers more superior than 30 are considered not suitable for an ideal consumption (Ding and Syazwani, 2016; Paull and Chen, 2018). The most elevated and inferior TSS/TA ratio values observed in the treatments of GA and Ca were more associated with the impact of their TA content, as this displayed more remarkable differences among both treatments with five minutes dipping than the TSS.

Pineapple sugar content

Fructose, glucose, and sucrose are the major sugars present in pineapple. Sucrose is the most accumulated during fruit ripening, ranging around 7-9%, and essentially responsible for the sweet taste in low acid hybrids like MD2 (Nadzirah *et al*., 2013; Lu *et al.*, 2014; Paull and Chen, 2018). Pre-and postharvest applications with GA have shown an increase in the sugar content of pineapple (Mandal *et al*., 2015); also in fruits like mango (Islam *et al.*, 2013), and peaches (Çetinbaş and Koyuncu, 2013). Few studies have reported a dipping time ideal for GA (Pusittigul *et al*., 2012; Islam *et al*., 2013). Five minutes have been suggested as optimal to generate a positive effect on sugar content in pineapple; nevertheless, results of this experiment suggested another outcome, as this dipping time delivered the lowest result. In pineapple, sucrose phosphate synthase (SPS) and sucrose synthase (SS) are the main enzymes responsible for the sucrose accumulation, while the invertase enzyme (IE) mainly responsible for its hydrolyzation (Chen and Paull, 2017; Paull and Chen, 2018). The five minutes dipping with the treatment of GA may affect theses enzymes through the cold storage, essentially promoting a superior activity of IE, causing

more sucrose hydrolyzation and conversion into fructose and glucose, including other organic sugars. Contrary process evidenced with this treatment but employing ten minutes dipping, obtaining the highest value. This dipping time could generate a more pronounced activity of SPS and SS, having a more elevated sucrose content and reduced fructose and glucose mean outcomes. More emphasis in these enzyme activities under GA applications for future experiments is recommended.

Pineapple citric acid and AsA

In hybrids like MD2 the citric acid value should be between 0.4 and 07% for ideal consumption (Lu *et al*., 2014; Paull and Chen, 2018; Cano-Reinoso *et al*., 2022 b). Values within that range were obtained in most of the treatments implemented. Citric acid is the predominant organic acid in pineapple, impacting its taste (Chen and Paull, 2017; Paull and Chen, 2018). This acid is considered a plant antioxidant, which has been detected to improve the tolerance to disease attacks and delaying fruit senescence in pre- and postharvest studies (Patrignani *et al*., 2015; Yang *et al.*, 2019). The citric acid of this experiment are highly related to the TA results described previously. This acid accounts for most of the TA content in pineapple (Chen and Paull, 2017; Paull and Chen, 2018). As mentioned before, calcium can impact organic acids accumulation positively, like the citric acid, by regulating the opening of stomata, causing a reduction of its metabolizing process during fruit respiration, essentially with short dipping times. This information can clarify why the treatment of Ca with five minutes dipping obtained the highest result. Nevertheless, as mentioned in the TA outcomes, this dipping time could not be sufficient to provide an adequate production of ROS that does not alter the citric acid metabolization, primordially when GA is used, reason why this treatment had the lowest outcome. Furthermore, this lowest value could be attribute also to its impact on the organic acid metabolizing enzymes. A high activity of the aconitase enzyme (ACO) has been associated with the citric acid reduction in pineapple low acid hybrids (Saradhuldhat and Paull, 2007). Therefore, it could be possible to infer that a short dipping time together with the treatment of GA can produce a superior activity of ACO during cold storage. On the contrary, an opposite situation can occur when same dipping time was used but with the treatment of Ca. The more reduced picks of citric acid content observed in the figure 2 could be linked to the high ACO activity during postharvest. However, this phenomenon should be examined in detail for future studies.

On the other hand, AsA is considered a soluble vitamin and the most representative antioxidant compound in pineapple (Kongsuwan *et al*., 2009; Akram *et al*., 2017; Noichinda *et al.*, 2017). In MD2, a value higher than 300 mg kg^{-1} has been established as ade-

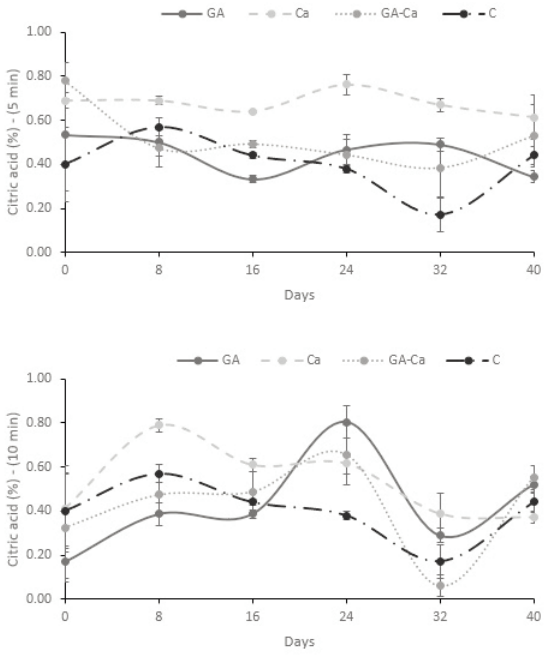

Fig. 2 - Effects of the treatments applied using both dipping times (5 and 10 min) on the citric acid content during cold storage. Treatments: GA, Ca, mix GA-Ca, C (Control-no use of GA and Ca). Values are the mean three replicates, and vertical bars represent ± SE.

quate to have a longer shelf-life, especially during cold storage (Lu *et al.*, 2014; Paull and Chen, 2018; Cano-Reinoso *et al.*, 2022 b). AsA increase in fruits has been associated with a higher activity of the enzyme ascorbic peroxidase (APX) (Akram *et al.*, 2017). Furthermore, more superior Ca^{2+} ion assimilation in fruits' primary cell wall matrix has been linked also to a higher production of AsA (Sadak *et al.*, 2010; Farouk, 2011). These facts can explain why treatment of Ca obtained the highest AsA content, due to the synergy between Ca^{2+} ion influences on the cell wall and the AsA generation. Besides, the five minutes dipping could be the ideal time to have an adequate ROS level that does not interfere with the AsA metabolization, similar with the situation described for the TA. However, an opposite phenomenon could have occurred in the same treatment but with ten minutes dipping; the ROS production could have overcome and reached inadequate levels, causing a

disturbance in the AsA production and Ca^{2+} ion assimilation, reason why this treatment obtained the lowest outcome. Figure 3 shows how at 24 days of cold storage there is a break point in the trend of both dipping times. At this moment, the decay symptoms and senescence process of the fruit could have been more intense, and this situation could have provoked a hypertensive response (HR). HR are plant physiology mechanisms activated under stress conditions characterized by antioxidants production to cope with stressfully circumstance (Goñi *et al*., 2017). Moreover, a more pronounce activity of APX could have been encouraged during that period. In the case of the GA, despite its positive effects reported on AsA and Ca assimilation (Mandal *et al*., 2015; Dong *et al.*, 2019), the dipping times employed together with its mix with Ca could have mitigate these characteristics, and as a consequence, no any superior AsA level observed with GA treatments.

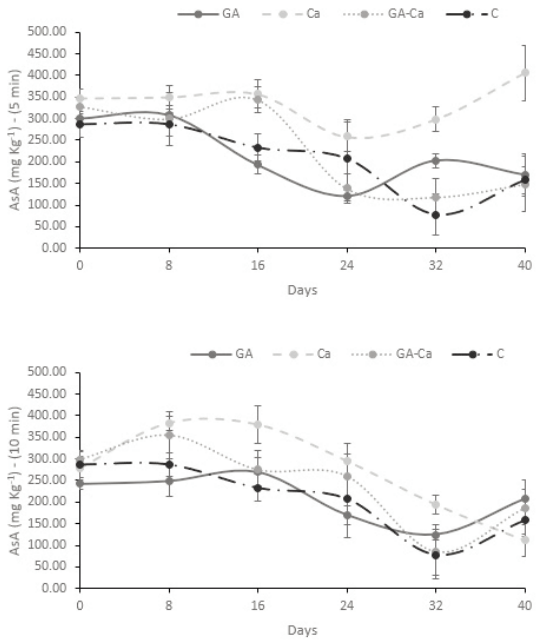

Fig. 3 - Effects of the treatments applied using both dipping times (5 and 10 min), on the AsA content during cold storage. Treatments: GA, Ca, mix GA-Ca, C (Control- no use of GA and Ca). Values are the mean three replicates, and vertical bars represent ± SE.

Internal browning

Internal browning in pineapple has been linked to an increase in the activity of the phenylalanine ammonia-lyase (PAL) and polyphenol oxidase (PPO) enzymes during cold storage (Youryon *et al.*, 2018; Paull and Chen, 2019). Postharvest calcium treatments have shown a reduction of PAL and PPO associated with a more elevated activity of their antagonisms enzyme, catalase (CAT), peroxidase (POD), and superoxidase dismutase (SOD); besides, the already mentioned superior antioxidant capacity (Pusittigul *et al*., 2012; Youryon *et al*., 2018; Cano-Reinoso *et al.*, 2022 b). These antagonism enzymes can interfere in the production of ROS like hydrogen peroxide H_2O_2, and the superoxide radical O_2^-. This ROS inhibition creates a reduction of the membrane lipid peroxidation, giving more consistency to the cell wall matrix, which cause a longer shelf-life. Furthermore, Youryon *et al.* (2018) reported that Ca^{2+} ions could bind to the negative charge molecules associated with PAL and PPO, suppressing their metabolic disturbances. This previous information can help clarify why the treatments of Ca caused a low severity and incidence of internal browning, and why this circumstance was also linked to the highest AsA and citric acid results, especially with five minutes dipping. This dipping time can be ideal to maintain a low ROS, PAL, and PPO activity during postharvest.

On top of that, results show that there may be a negative impact in all treatments employing GA, due to their high severity and incidence of internal browning, primordially with ten minutes dipping. Pusittigul *et al*. (2012) demonstrated that an elevated endogenous concentration of GA in pineapple fruit can enhance the activity of PAL and PPO. Therefore, it is important to control the gibberellins level in the fruit, especially under the use of exogenous GA applications. This information suggests that the treatments with GA, essentially with long dipping times, like ten minutes, could not only facilitate these enzyme activities, also encourage superior ROS concentration. However, more studies are recommended for the future, especially to clarify GA interaction with Ca impacting the occurrence of internal browning.

In conclusion, calcium and GA dipping application affected the pineapple physicochemical characteristics and internal browning. The treatment of Ca with dipping time of five minutes delivered the best results. This treatment caused the lowest severity and incidence of internal browning, together with the highest AsA, citric acid content, and lowest TSS/TA ratio, after 40 days of cold storage. Also, this treatment had the closest values to an ideal fruit quality. On the other hand, the control, the treatments using GA, and its mix with calcium, in both dipping times, did not provide satisfactory results, primordially because those delivered a high severity and

incidence of internal browning and did not cause a remarkable enhancement in the fruit antioxidant content. Finally, the results described here can be consider preliminary, since the majority of the current investigations concerning the employment of Ca and GA as postharvest treatments on pineapple have not yet clarified what the optimal doses and dipping time to be implemented are; therefore, further studies are recommended.

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References

AKRAM N.A., SHAFIQ F., ASHRAF M., 2017 - *Ascorbic acid-a potential oxidant scavenger and its role in plant development and abiotic stress tolerance.* - Front. Plant Sci., 8: 1-5.

BIN THALIP A.A., TONG P.S., CASEY N.G., 2015 - *The MD2 "Super Sweet" pineapple (*Ananas comosus*).* - Utar Agric. Sci. J., 1: 14-17.

CANO-REINOSO D.M., KHARISUN K., SOESANTO L., WIBOWO C., 2022 a - *Effect of calcium and silicon fertilization after flowering on pineapple mineral status and flesh translucency*. - Plant Physiol. Rep., 27: 96-108.

CANO-REINOSO D.M., SOESANTO L., KHARISUN WIBOWO C., 2021 a - *Review: Fruit collapse and heart rot disease in pineapple: Pathogen characterization, ultrastructure infections of plant and cell mechanism resistance*. - Biodiversitas, 22: 2477-2488.

CANO-REINOSO D.M., SOESANTO L., KHARISUN WIBOWO C., 2021 b - *Effect of pre-harvest fruit covers and calcium fertilization on pineapple thermotolerance and flesh translucency.* - Emir. J. Food. Agric., 33: 834-845.

CANO-REINOSO D.M., SOESANTO L., KHARISUN WIBOWO C., 2022 b - *Effect of pre-and postharvest treatments with salicylic acid on Physicochemical Properties of Pineapple cv. MD2*. - CMUJ Nat. Sci., 21: e2022039.

ÇETİNBAŞ M., KOYUNCU F., 2013 - *The ripening and fruit quality of 'Monroe' peaches in response to pre-harvest application gibberellic acid*. - Akdeniz Üniversitesi Ziraat Fakültesi Derg., 26: 73-80.

CHEN N.J., PAULL R.E., 2017 - *Production and postharvest handling of low acid hybrid pineapple.* - Acta Horticulturae, 1166: 25-34.

DE FREITAS S.T., RESENDER NASSUR R.C.M., 2017 - *Calcium treatments*, pp. 52-68.- In: PAREEK S. (ed.) *Novel postharvest treatments of fresh produce*. CRC Press, Boca Raton, USA, pp. 731.

DING P., SYAZWANI S., 2016 - *Physicochemical quality, antioxidant compounds and activity of MD-2 pineapple fruit at five ripening stages.* - Int. Food Res. J., 23: 549-555.

DONG Y., ZHI H., WANG Y., 2019 - *Cooperative effects of pre-harvest calcium and gibberellic acid on tissue calcium content, quality attributes, and in relation to postharvest disorders of late-maturing sweet cherry.* - Sci. Hortic. (Amsterdam), 246: 123-128.

FAROUK S., 2011 - *Ascorbic acid and α-tocopherol minimize salt-induced wheat leaf senescence.* - J. Stress Physiol. Biochem., 7: 58-79.

GOÑI M.G., QUIRÓS-SAUCEDA A.E., VELDERRAIN-RODRÍGUEZ G.R., OVANDO-MARTÍNEZ M., ROURA S.I., GONZÁLEZ-AGUILAR G.A., PAREEK S., 2017 - *Salicylic acid treatments*, pp. 119-148. - In: PAREEK, S. (ed.) *Novel postharvest treatments of fresh produce*. CRC Press, Boca Raton, USA, pp. 731.

GUPTA R., CHAKRABARTY S.K., 2013 - *Gibberellic acid in plant: still a mystery unresolved.* - Plant Signal. Behav., 8: e25504.

HAJILOU J., FAKHIMREZAEI S., 2013 - *Effects of post-harvest calcium chloride or salicylic acid treatments on the shelf-life and quality of apricot fruit*. - J. Hortic. Sci. Biotechnol., 88: 600-604.

HASSAN A., OTHMAN Z., SIRIPHANICH J., 2011 - *Pineapple (*Ananas comosus *L. Merr.),* pp. 194-218. - In: YAHIA E. (ed.) *Postharvest biology and technology of tropical and subtropical fruits: Mangosteen to white sapote*. Woodhead Publishing, Mexico City, Mexico, pp. 485.

HOCKING B., TYERMAN S.D., BURTON R.A., GILLIHAM M., 2016 - *Fruit calcium: Transport and physiology*. - Front. Plant Sci., 7: 1-17.

HU H., LI X., DONG C., CHEN W., 2012 - *Effects of wax treatment on the physiology and cellular structure of harvested pineapple during cold storage*. - J. Agric. Food Chem., 60: 6613-6619.

ISLAM M.K., KHAN M.Z.H., SARKAR M.A.R., YEASMIN S., ALI M.K., UDDIN M.H., 2013 - *Postharvest quality of mango (*Mangifera indica *L.) fruit affected by different levels of gibberellic acid during storage*. - Malaysian J. Anal. Sci., 17: 499-509.

KONGSUWAN A., SUTHILUK P., THEPPAKORN T., SRILAONG V., SETHA S., 2009 - *Bioactive compounds and antioxidant capacities of phulae and nanglae pineapple*. - Asian J. Food Agro-Industry., special isuse: 44-50.

LU X., SUN D., LI Y., SHI W., SUN G., 2011 - *Pre- and postharvest salicylic acid treatments alleviate internal browning and maintain quality of winter pineapple fruit*. - Sci. Hortic. (Amsterdam), 130: 97-101.

LU X.H., SUN D.Q., WU Q.S., LIU S.H., SUN G.M., 2014 - *Physico-chemical properties, antioxidant activity and mineral contents of pineapple genotypes grown in China*. - Molecules, 19: 8518-8532.

MANDAL D., LALREMRUATA HAZARIKA T.K., NAUTIYAL B.P., 2015 - *Effect of post-harvest treatments on quality and shelf life of Pineapple (*Ananas comosus *[L.] Merr. 'Giant Kew') fruits at ambient storage condition*. - Int. J. Bio-Resource Stress Manag., 6: 490.

MOHAMED M.A.A., EL-KHALEK A.F., ABDELAZIZ A.M.R.A., 2016 - *Increasing storability of Mango cv. "Zibda" fruits by 1-methylcyclopropene and gibberellic acid postharvestaApplications.* - Egypt. J. Hortic., 43: 277-298.

NADZIRAH K.Z., ZAINAL S., NORIHAM A., SITI ROHA A.M., NADYA H., 2013 - *Physico-chemical properties of pineapple variety N36 harvested and stored at different maturity stages.* - Int. Food Res. J., 20: 225-231.

NOICHINDA S., BODHIPADMA K., WONGS-AREE C., 2017 - *Antioxidant potential and their changes during postharvest handling of tropical fruits*, pp. 633-662. - In: PAREEK S. (ed.) *Novel postharvest treatments of fresh produce*. CRC Press, Boca Raton, USA, pp. 731.

PATRIGNANI F., SIROLI L., SERRAZANETTI D.I., GARDINI F., LANCIOTTI R., 2015 - *Innovative strategies based on the use of essential oils and their components to improve safety, shelf-life and quality of minimally processed fruits and vegetables*. - Trends Food Sci. Technol., 46: 311-319.

PAULL R.E., CHEN C.C., 2018 - *Postharvest physiology, handling and storage of pineapple*, pp. 295-323. - In: SANEWSKI G.M., D.P. BARTHOLOMEW, and R.E. PAULL (eds.) *The pineapple: Botany, production and uses*. CABI Publishing, London, UK, pp. 355.

PAULL R.E., CHEN N.J., 2015 - *Pineapple translucency and chilling injury in new low-acid hybrids.* - Acta Horticulturae, 1088: 61-66.

PAULL R.E., CHEN N.J., 2019 - *Tropical and sup-tropical fruits*, pp. 513-527. - In: DE FREITAS S.T., and S. PAREEK (eds.) *Postharvest physiological disorders in fruits and vegetables*. CRC Press, Boca Raton, USA, pp. 853.

PUSITTIGUL I., KONDO S., SIRIPHANICH J., 2012 - *Internal browning of pineapple (*Ananas comosus *L.) fruit and endogenous concentrations of abscisic acid and gibberellins during low temperature storage*. - Sci. Hortic. (Amsterdam), 146: 45-51.

PUSITTIGUL I., SIRIPHANICH J., JUNTEE C., 2014 - *Role of calcium on internal browning of pineapples.* - Acta Horticulturae, 1024: 329-338.

SADAK M.S., BADR N.M., GABALLAH M.S., RADY M.M., 2010 - *Increasing sunflower salt tolerance using Nicotinamid and α -tocopherol*. - Int. J. Acad. Res., 2: 263-270.

SARADHULDHAT P., PAULL R.E., 2007 - *Pineapple organic acid metabolism and accumulation during fruit development.* - Sci. Hortic. (Amsterdam), 112: 297-303.

SHAMSUDIN R., ZULFIKLI N.A., KAMARUL ZAMAN A.A., 2020 - *Quality attributes of fresh pineapple-mango juice blend during storage*.- Int. Food Res. J., 27:141-149.

SHIRZADEH E., RABIEI V., SHARAFI Y., 2011 - *Effect of calcium chloride ($CaCl_2$) on postharvest quality of apple fruits.* - African J. Agric. Res., 6: 5139-5143.

SITI ROHA A.M., ZAINAL S., NORIHAM A., NADZIRAH K.Z., 2013 - *Determination of sugar content in pineapple waste variety N36*. - Int. Food Res. J., 20: 1941-1943.

VAN MEETEREN U., ALINIAEIFARD S., 2016 - *Stomata and postharvest physiology*, pp. 157-191.- In: PAREEK S. (ed.) *Postharvest ripening physiology of crops*. CRC Press, Boca Raton, USA, pp. 620.

WANG Y.H., IRVING H.R., 2011 - *Developing a model of plant hormone interactions.* - Plant Signal. Behav., 6: 494-500.

YANG C., CHEN T., SHEN B., SUN S., SONG H., CHEN D., XI W., 2019 - *Citric acid treatment reduces decay and maintains the postharvest quality of peach (*Prunus persica *L.) fruit*. - Food Sci. Nutr., 7: 3635-3643.

YOURYON P., SUPAPVANICH S., KONGTRAKOOL P., WONGS-AREE C., 2018 - *Calcium chloride and calcium gluconate peduncle infiltrations alleviate the internal browning of Queen pineapple in refrigerated storage*. - Hortic. Environ. Biotechnol., 59: 205-213.

YOURYON P., WONGS-AREE C., 2015 - *Postharvest application of calcium chloride affects internal browning reduction during low temperature storage of "sawi" pineapple*. - Acta Horticulturae, 1088: 197-200.