PINEAPPLE RESPONSES TO POSTHARVEST APPLICATIONS OF ABA, CHITOSAN, AND DECROWNING ON THE SEVERITY OF INTERNAL BROWNING AND OTHER FRUIT QUALITIES

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Highlight
Postharvest applications of ABA, chitosan, and decrowning on pineapple internal browning.

Abstract
The shelf life of pineapple is significantly influenced by storage temperature and can be prolonged by maintaining an optimal temperature range of 5-12°C. However, there is still the problem of internal browning (IB) in the long-term storing of fresh harvest at cold temperatures. Postharvest application of 380 µM ABA (Abscisic Acid) to the crown, which is a source of ABA endogenous was found to suppress IB, while the concentration of 95 µM was not effective. Therefore, this research aimed to determine the response of GP3 and MD2 clones to postharvest treatment with the application of 50 mg/L ABA, chitosan and decrowning on the IB severity and other fruit qualities. The experimental design used a Completely Randomized Design with 3 factors of clone (GP3 and MD2), decrowning (crown and crownless), and fruit coating [chitosan 1%, ABA 50 mg/L, ABA + chitosan mix, and control (H_2O)]. The fruits were kept at 7°C and observed at 0, 3, 6, 9, 16, 23, 30, and 37 days. The results showed that MD2 was significantly lower IB than GP3 and IB severity negatively correlated with ascorbic acid (AsA) content. MD2 had lower fruit weight loss (FWL) and skin dehydration (SD), higher AsA, soluble solid content (SSC), and SSC/titratable acidity (STA) ratios compared to GP3. The crown + ABA treatment decreased the IB severity of GP3, with a level of 0.75% after 37 days which was lower than crown + H_2O by 9.17% and crownless + H2O by 8.42%. ABA treatment also showed higher SD and FWL, while AsA, SSC, TA, and STA were not different from the control.

Keywords
pineapple; postharvest; internal browning; ascorbic acid.

Introduction
According to a survey conducted in 2019, Indonesia is the fourth-largest producer of pineapple globally, following Costa Rica, the Philippines, and Brazil, with a total output of 2,196,456 tons [1]. This is due to pineapple being one of the main agricultural products in the country. However, the issue of internal browning (IB) is particularly important in the global pineapple industry as it can lead to substantial losses during canning and after shipping in refrigerated ocean containers. For example, the Australian pineapple industry incurs losses of US$1.3 million annually due to IB, out of a total production value of around US$30 million [2]. GP3 pineapple clone is a type of

Smooth Cayenne pineapple, while MD2 is a hybrid product that has 50% of the same characteristics as the clone type. Some of the advantages of the MD2 pineapple clone compared to others include its attractive skin colour and ripe fruit (golden yellow), with a higher content of vitamin C, total soluble solids, and resistance to cold storage [3]. Furthermore, its cultivar is more resistant to IB occurrence compared to the Smooth Cayenne cultivar [4,5]. The resistance of Smooth Cayenne to IB during storage of export pineapples has been surpassed by that of the hybrid pineapple. MD2 cultivar helps consumers to consume fresh products in non-tropical countries which can only enjoy canned pineapple preparations from the cultivar Smooth Cayenne[3].

According to a previous report [5], IB severity on MD2 is smaller than Smooth Cayenne cultivars due to its resistance to IB induction [6]. Generally, the initiation of IB occurs through an enzymatic reaction mechanism that depends on the enzyme activity of phenolic compounds, polyphenol oxidase (PPO) and peroxidase (POD), as well as O_2. Browning occurs due to the reaction between oxidized phenolic compounds in the presence of PPO and/or POD enzymes that form highly reactive o-quinones to combine with their counterparts or other phenolic compounds [7]. Meanwhile, symptoms occur in the flesh of the fruit close to the core with the initial appearance of translucent flesh [8]. These symptoms develop with changes in colour to brown and black, which occurs internally without any external signs of the fruit [9]. In the experiment on the crowned and uncrowned pineapples, the highest content of gibberellic acid (GA) was found in the uncrowned fruit. This indicated that the crown was one of the media translocating abscisic acid (ABA) in pineapple and antagonistic to GA. It was also discovered that crowned pineapple fruit with a maturity level of 70% had a positive correlation with an increase in the incidence of IB, GA content, reactive oxygen species (ROS), malondialdehyde (MDA), phenolic content, phenylalanine ammonia-lyase (PAL), and soluble solid content/fatty acid ratios (SFA) [10]. Meanwhile, there was no relationship with PPO activity in pineapple after 9 days of storage at 20°C. Research on ABA infiltration was carried out by spraying its solution on pineapples to determine the severity of IB and GA levels. The results showed that 380 µM ABA reduced the severity of IB and GA after 9 days of storage, as well as PPO after 6 days. while 95 µM had no significant effect [11]. It was also discovered that the combined treatment of 200 mg/L ABA and storage temperature of 5°C can reduce the severity of IB and GA4 [12].

The severity of IB after storing the IB-susceptible variety (Trad-See-Thong) at 10°C started on the 10th day of storage and accelerated on the 8th day after being transferred to 25°C for a day. In the IB tolerant cultivar (Pattavia), the severity started after 19 days of storage at 10°C and accelerated to 15 days after being transferred to 25°C for a day [13]. Long storage at cold temperatures has been found to initiate IB, while wax treatment can reduce the incidence by 87.5% at day 20 [14]. The incidence of IB was reported in several pineapple cultivars in Malaysia, such as Mauritius, Sarawak, Gandol, Babagon, and Maspine. The results showed that Mauritius cultivars previously stored at 8°C and 12°C for up to 4 weeks followed by a week-long storage period at 28°C can induce IB events [15]. Moreover, chitosan is a linear polysaccharide consisting of the monomers N-acetylglucosamine and D-glucosamine with the molecular formula [$(C_6H_{11}NO_4)_n$]. It is widely used in extending the shelf life of fruit by suppressing respiration rates, such as guava fruit as reported by [16], bananas [17,18], strawberries [19], and avocados [20]. Chitosan coating was also developed to maintain shelf quality (ascorbic acid (AsA), total phenol, and antioxidant activity) and extend the shelf life of strawberries and apples [21].

The content of AsA in pineapple will affect resistance to IB. This is because the higher the AsA content, the lower the physiological damage to IB. The suppressing activity is related to that of AsA compounds as antioxidants (acidification and chelating of PPO enzymes) and ROS scavengers in protecting cells from damage [7,22–25], AsA content in pineapple also has a negative correlation with IB physiological damage and PPO enzyme activity [7,10–12,26–28]. The application of postharvest treatment can decrease IB severity in fresh pineapple in a short and effective time, which makes it applicable to the large pineapple industry. According to and [10,11], spray treatment by immersing pineapple fruit in 50 mg/L ABA solution will successfully reduce IB severity. It was discovered that a recommended concentration, lower than 380 µM and an optimum temperature provided a more efficient and effective effect, without reducing fruit qualities. The effect of chitosan is also expected to reduce O_2 in preventing aerobic conditions to suppress the browning enzymatic reaction. Therefore, the combination of both postharvest application of ABA and chitosan on crowned and decrowned pineapples in this research will have a significant impact in reducing the IB severity. It is also expected that GP3 pineapple clone will balance the fruit's resistance to meet exportation requirements.

Methods

This research was conducted at Great Giant Food Co. Ltd. (GGF), East Lampung, Lampung, Indonesia, in August

September 2022. Fresh pineapple was harvested from GGF with a weight of 825-1,124 g/fruit and an export standard maturity level with a yellow skin colour of 0%. The experiment used a 2 x 2 x 4 factorial in a Completely Randomized Design. The 3 factors were clones (GP3 and MD2), Decrowning (Crown and Crownless), Coating [(chitosan [$(C_6H_{11}NO_4)_n$] 1%, Abscisic Acid (ABA) [$(C_{15}H_{20}O_4)$ Phytotechlab, Lenexa, Kansas, USA] 50 mg/L, ABA 50 mg/L + chitosan 1% mix, and control (H_2O)]. Both destructive and non-destructive observations were made 7 times on days 3, 6, 9, 16, 23, 30, and 37 with 3 replicates. The application of H_2O and chitosan treatment was carried out by soaking the fruit in H_2O and single chitosan. Subsequently, ABA and the combination of ABA + chitosan were applied by spraying their solution until the entire surface of the fruit was wet. In the ABA + chitosan treatment, the fruit was first sprayed with ABA, and dried for 30 minutes, followed by the application of chitosan. Pineapple fruit was air-dried for 30 minutes before being packaged in cardboard with a capacity of 10-11 sheets per carton that has been perforated (GGF packaging cardboard). All fruits were stored at 7°C for 37 days.

The destructive and non-destructive characteristics were observed. The destructive variables included:

a. the IB severity, which was measured using the fruit score, by observing the surface area of the transverse fruit pieces experiencing a change in colour from transparent to blackish brown. Meanwhile, a score of 1 indicated mild category (< 5%), 2 was moderate (6-10%), 3 was moderately severe (11-20%), and 4 weight categories (> 20%), with the formula:

(1) IB severity (%) = [(Σ no. of the IB in category x category value)/(total no. of fruits x the highest category value)] x 100%

The scoring of IB severity was based on the United States Standards for Grades of Pineapples with modification [29].

b. AsA was determined using the titrimetric approach outlined in AOAC Method 967.21 [30]. This involved adding 2 ml pineapple juice or AsA (as Dye factor), with 5 ml of metaphosphoric acid (HPO3), and shaking until homogeneous. The solution was titrated using 2,6-dichlorophenol indophenol (2,6-D). The AsA value was calculated by the formula:

(2) AsA (ppm) = [(Σ actual 2,6-D volume x Dye-factor)/(sample volume)] x 1000

c. Soluble solid content (SSC) was measured by dropping fruit juice on a digital refractometer prism glass and the value obtained was expressed in the Brix value.

d. Titratable Acidity (TA) was measured by mixing 5 ml of fruit juice with 5 drops of phenolphthalein indicator, which was shaken until homogeneous. The solution was titrated using 1 N NaOH compound and the TA value was calculated by the formula:

(3) TA (%) = [(Σ NaOH volume x 0.064 x NaOH molarity)/(sample volume)] x 100%

e. and SSC/TA ratio (STA), the comparison between SSC and TA values. Non-destructive observation variables include:

- Fruit weight loss (FWL), which was determined by weighing the fresh weight on the daily observation and the initial weight with a digital scale. The FWL value was calculated by the formula:

(4) FWL (%) = [(Σ Weight on the day of observation – the initial weight)/ (weight from the initial weight)] x 100%

Furthermore,

- Dehydration of the fruit skin was determined by scoring 10 samples around the open eyeball on each of the 3 sides of the fruit. The degree of shrinkage level was indicated by score, where a score of 1 represented the mild (<25%), 2 was moderate (26-50%), and 3 was in the heavy category (>26%). The fruit was calculated for the severity of skin dehydration (SD) with the formula:

(5) SD (%) = [(Σ no. of the skin around the eye in the category x category value)/(total no. of skin eyes x the highest category value)] x 100%

The data were processed by comparing the mean with the 95% confidence interval (mean±CI) (α=0.05) for each treatment group and post hoc. test with Duncan's Multiple Distance Test (DMRT). Data were displayed on bar and line charts with CI bars and tables as well as letter notation to compare their significance. Statistical data were processed using the IBM SPSS Statistics Version 26 program.

Results and discussion

The experiment with pineapple clones gave different responses to long shelf life at 7°C for 37 days on the IB severity. The results showed that MD2 clone was more resistant, as evidenced by its 1.40% (mild symptoms) IB severity compared to the 11.63% (moderately severe) in GP3 on day 37, as presented in Figure 1.A. MD2 pineapple cultivar also had higher resistance than Smooth Cayenne cultivars [5]. It was also discovered that when the fruit was stored at 7°C for a shelf life of 23 days, the IB began to occur, but there was no incidence of IB for 16 days of storage and below.

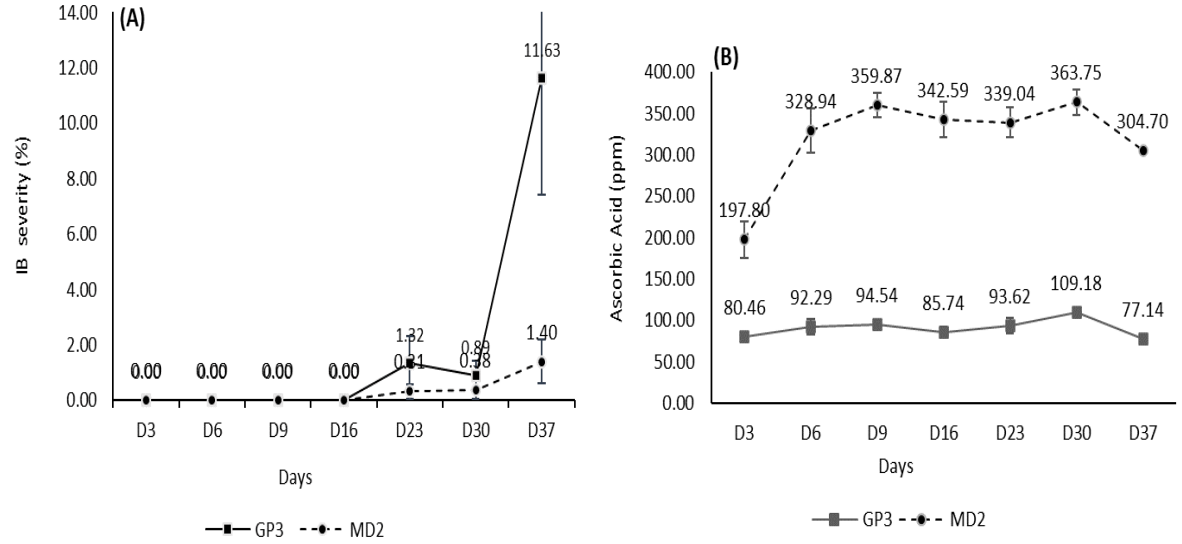

Figure 1. Comparative response between the development of GP3 and MD2 clones stored at 7°C for 37 days on the IB severity (A) and ascorbic acid (B). The values are mean±CI. *Source: Author.*

There was a significant difference in AsA content, where MD2 clone had higher values than GP3 as presented in Figure 1.B, which negatively correlated with the IB severity on day 37 in Figure 1.A. AsA has been shown to decrease the activity of the PPO enzyme, thereby reducing the IB severity [7,10–12,26–28]. According to [31], there was no correlation between AsA content and IB severity, which was also reported in GP3 pineapple clone. It was also discovered that the genetic factors of pineapples have a considerable influence on IB severity [6]. This is in line with a previous study, where the pineapple sclerenchyma cultivar MD2 observed using scanning electron microscopy had a thicker sclerenchyma fibre layer structure and was twice as large as susceptible cultivars (Trad-See-Thong). Furthermore, the tolerant (Pattavia) of IB, MD2 sclerenchyma cells formed concentric rings around the phloem and xylem. Based on this research, it was concluded that ASA can suppress IB severity when its content in pineapple fruit is above or equal to 197.80 ppm.

All treatments of MD2 clone showed no significant difference in the IB severity. However, in GP3 clone, the interaction of crown and ABA 50 mg/L was significant compared to other treatment interactions. This did not include GP3 x Crownless x Chitosan treatment, because it had an insignificant effect compared to others, as illustrated in Figure 2.A. The application of ABA treatment in GP3 pineapple clone with crown intact was included in the mild category, and the others were moderate, except for the combination of ABA and Chitosan. This treatment can be used as in reducing the IB severity at a temperature of 7°C up to a shelf life of 37 days. Generally, the crown is a source of exogenous ABA in pineapple, which contributes to donors, and is antagonistic to GA compounds. Gibrelic acid (GA) endogenous in pineapple has a positive correlation with IB severity [10–12].

There was no significant difference between the treatment of decrowning and clones on AsA levels, as illustrated in Figure 2.B. This experiment concluded that AsA was formed by the respective genetic clones (GP3 and MD2).

According to a previous report [9], the decrowning was not correlated with AsA content. The IB severity was not correlated with AsA, while the IB-tolerant Pattavia cultivar had lower AsA content than the Trad-See-Thong cultivar [31].

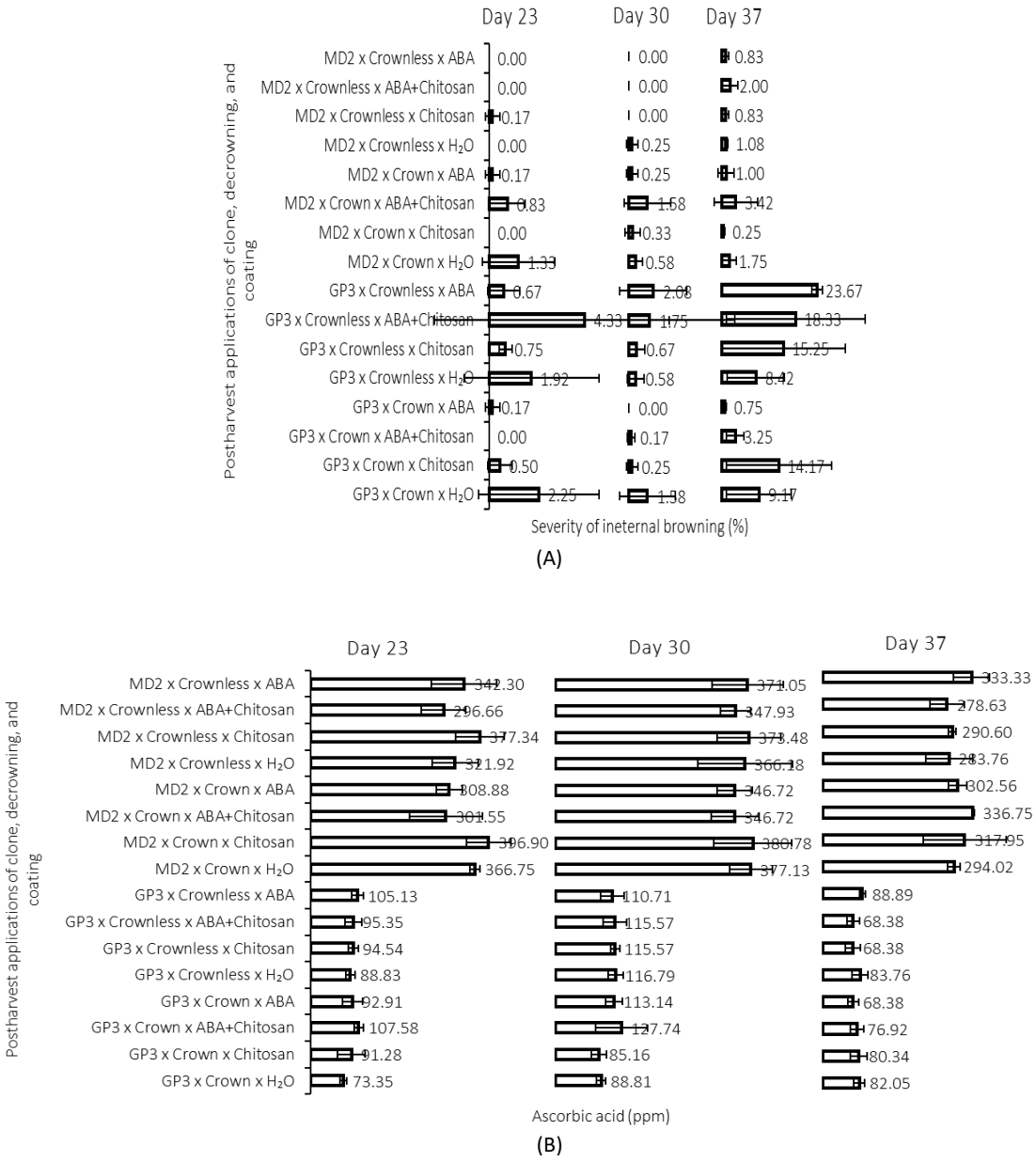

Figure 2. Response of GP3 and MD2 pineapple clones to postharvest applications of ABA, chitosan, and decrowning on the development of IB severity (A) and ascorbic acid (B) stored at 7°C for 37 days. The values are mean±CI. *Source: Author.*

Pineapple decrowning did not significantly affect the occurrence of IB in MD2 and GP3 clones. However, [10] stated that decrowning can exacerbate damage due to IB in Queen types of pineapple. Based on data on the level of ripeness used, the 0% maturity level used in this research was different from [10] which applied 70% after treatment. This showed that the response to IB severity will be varied due to different in maturity levels and cultivar types of pineapple. The 50 mg/L ABA can suppress IB in GP3 pineapples with intact crowns (Smooth Cayenne type). This is in line with [10,11], where postharvest treatment of 380 uM ABA suppressed the occurrence of IB in pineapple cultivars Pattavia (Smooth Cayenne type) and Trad-See-Thong (Queen type). The 50 mg/L ABA is almost the same as the 190 uM, therefore, it has half effect of the 380 uM concentration.

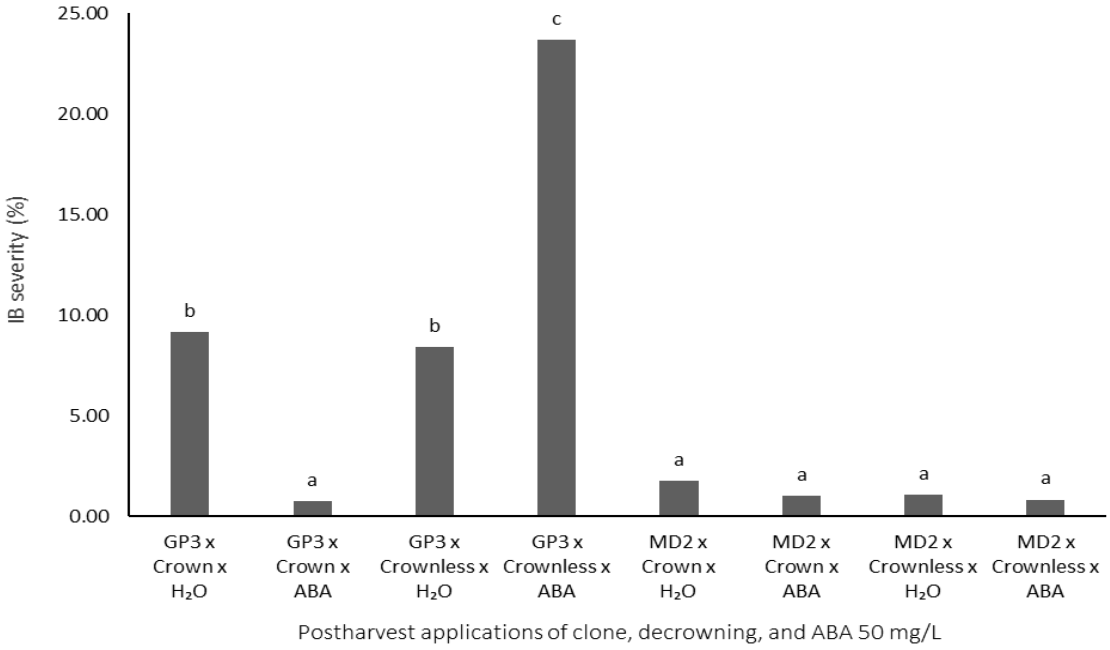

Figure 3. Effect of pineapple clones, decrowning, and 50 mg/L ABA application on IB severity stored at 7°C for 37 days. Lowercase letters indicate statistically significant differences by DMRT (p≤0.05). *Source: Author.*

The high levels of AsA value in MD2 clone showed a positive correlation in decreasing the IB severity. This can be a reference for the AsA compound infiltration experiment in IB severity reduction in GP3 or others. The increase in endogenous AsA can be obtained from the combination treatment of the application of AsA or isoascorbic acid (IAA) and plastic bagging on pineapple slices in decreasing the IB severity [32]. The application of methyl jasmonate (MJ) can prevent a reduction in AsA content in pineapple storage on PPO activity, incidence, and IB severity [28].

The application of postharvest ABA in GP3 pineapple with crown intact was not significant compared to the ABA treatment and the control in MD2 clone on the IB severity. However, it had a significant effect on other GP3 treatments during 37 days of storage as shown in Figure 3. This treatment had the same resistance as MD2 clone with a fairly high AsA content. According to [10], the crown and ABA treatment as a source of endogenous ABA in decreasing GA and phenolic biosynthesis can decrease the IB of pineapple. It was also discovered that crown and ABA treatment in pineapple storage decreased GA biosynthesis [8], phenolics compound, PAL activity [11], and PPO activity [12] compared to crownless treatment.

Table. 1. Effect of pineapple clone, decrowning, and ABA 50 mg/L application on fruit qualities stored at 7°C for 37 days. *Source: Author.*

Treatment	**SSC (%)**	**TA (%)**	**STA**	**FWL (%)**	**SD (%)**
GP3 x Crown x H_2O	12.03abc	0.49ab	24.82a	13.40e	28.87a
GP3 x Crown x ABA	10.77a	0.37ab	29.75abc	14.17e	34.85bc
GP3 x Crownless x H_2O	11.33ab	0.55b	20.92a	10.27cd	28.52a
GP3 x Crownless x ABA	12.30abc	0.47ab	26.27ab	10.77d	37.11c
MD2 x Crown x H_2O	13.03abcd	0.34a	39.71c	9.52bc	31.16ab
MD2 x Crown x ABA	14.90cd	0.51ab	29.69abc	10.10cd	32.86b
MD2 x Crownless x H_2O	16.40d	0.45ab	37.66bc	7.14a	27.82a
MD2 x Crownless x ABA	14.40bcd	0.50ab	29.49abc	8.44b	34.48bc

[1]Lowercase letters indicate statistically significant differences by DMRT (p≤0.05).

GP3 x Crown x ABA treatment at a shelf life of 37 days at 7°C had no significance in SSC compared to others. However, it was lower than all MD2 treatments except for the Crown x H_2O treatment. TA and Sweetness levels

(STA) in GP3 x Crown x ABA treatment were not significantly different in all treatments, but weight loss was higher except for GP3 x Crown x H_2O treatment. Skin dehydration (SD) was higher than the control in GP3 and MD2 clones, except for MD2 x Crownless x H_2O treatment as presented in Table 1. According to a previous report [11], the ABA 380 µM exogenous treatment was not significant compared to the H_2O treatment on SSC.

Impact

MD2 pineapple had a high AsA content, therefore, it can reduce the IB severity at a storage temperature of 7°C for 37 days. This showed that MD2 clone did not need additional postharvest applications other than fruit storage at 7°C. Moreover, decrowning in pineapple storage efficiency can be carried out on MD2 clone to reduce packing costs, increase storage and transportation capacity. In pineapples with low AsA content such as GP3 clone which only ranged from 73.35 – 127.74 ppm, IB severity for 37 days at 7°C can be reduced by applying a postharvest treatment of 50 mg ABA coating /L with the crown intact. Through this method, GP3 clone that only met the demand for processed canned pineapple can be increased due to the demand for fresh pineapple to replace MD2. By applying specific recommendations for pineapple clones, yield losses during long-term storage, namely 37 days at a temperature of 7°C against IB damage, can be reduced effectively and efficiently.

Conclusions

The results showed that the MD2 clone had higher resistance compared to GP3 because the AsA content positively correlated to decreased IB severity. ABA treatment with the intact crown on GP3 clone had a significant effect on IB severity compared to control (H_2O) on crowned or decrowned pineapples. ABA treatment on fruit with intact crowns also maintained fruit quality, such as AsA, soluble solids content (SSC), titratable acidity (TA), and SSC/TA ratios (STA) but had higher fruit weight loss and skin dehydration. Therefore, by maintaining the integrity of the crown on GP3 clone stored at 7°C for 37 days, ABA treatment can be used as a reference in reducing the IB severity.

Conflict of interest

There are no conflicts to declare.

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