POSTHARVEST TREATMENTS INFLUENCED THE INCIDENCE OF INTERNAL BROWNING, PHENOL, ABA, AND GA_3 CONTENTS OF TWO PINEAPPLE CLONES

David Chandra
Agricultural Science Doctoral Program, Faculty of Agriculture, University of Lampung
Indonesia 35145**,** davidchandra.unila@gmail.com
https://orcid.org/0000-0002-7614-1892

Soesiladi Esti Widodo*
Department of Agronomy and Horticulture, Faculty of Agriculture, University of Lampung
Indonesia 35145, *corresponding author: sestiwidodo@gmail.com
https://orcid.org/0000-0003-4932-2759

Muhammad Kamal
Department of Agronomy and Horticulture, Faculty of Agriculture, University of Lampung
Indonesia 35145, mkamal1961@yahoo.com
https://orcid.org/0000-0002-2935-5038

Sri Waluyo
Department of Agricultural Engineering, Faculty of Agriculture, University of Lampung
Indonesia 35145, sri.waluyo@fp.unila.ac.id
https://orcid.org/0000-0003-4334-3022

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Highlight
Pineapple postharvest treatments on IB incidence and hormones.

Abstract
Phenol is an internal browning (IB) enzymatic reaction substrate and endogenous abscisic acid (ABA) used to suppress IB incidence in the Comte de Paris cultivar (Queen type). There is no information on the correlation between pineapple IB to endogenous total phenol content (TPC), ABA, and gibberellic acid 3 (GA_3) after postharvest applications of decrowning. Therefore, this research aimed to analyze the relationship of IB incidence to total TPC, ABA, and GA_3 after postharvest treatments of decrowning and coating in GP3 and MD2 pineapple clones. The structure was based on a completely randomized design with 3 factors, namely clone (GP3 and MD2), decrowning (crown and crownless), and coating [50 mg L^{-1} ABA, 1% chitosan, ABA+Chitosan mixture, and control (H_2O)]. The results showed that the MD2 had a lower IB incidence and higher TPC than the GP3 stored at 7°C for 37 days. The increased TPC was positively correlated with IB incidence. TPC was also negatively correlated with ABA but positively with endogenous GA_3 2 weeks earlier. Coating with 50 mg L^{-1} ABA and 1% chitosan on MD2 decreased IB incidence. Pineapple crown pruning decreased ABA and increased TPC, GA_3, and IB incidence.

Keywords
Crown pruning; chitosan; coating; cold storage; fruit; physiological disorder.

Introduction
Decreased fruit quality due to internal browning (IB) is a post-harvest problem for pineapple. Cold storage at 7–12°C extends the shelf life of pineapple fruit [1–3], but long-term storage induces fruit IB before the rots (senescence). Meanwhile, IB is an enzyme reaction that uses phenol as a substrate. In the Comte de Paris cultivar (Queen type), endogenous ABA inhibited IB incidence. The relationship between endogenous total phenol content (TPC), ABA, gibberellic acid 3 (GA_3) contents as well as IB of pineapple after postharvest applications of ABA coating and decrowning in the hybrid and Smooth Cayenne groups is unknown.

Coatings with chitosan extended the shelf life of guava [4], banana [5,6], tomato [7], strawberry [8], and avocado [9] by decreasing respiration rate. According to [10], the combined application of 200 mg L^{-1} exogenous ABA with

a storage temperature of 5°C was able to suppress the incidence of IB and GA. Spraying Queen pineapple fruit with 380 µM ABA solution was positively correlated with suppressing the incidence of IB and decreasing GA content after 9 days of storage and PPO enzyme activity after 6 days [11].

Crown pruning of queen-type pineapple was reported to increase the incidence of IB, total phenolics, and endogenous GA, but decreased endogenous ABA and ascorbic acid (AsA) [12]. This process did not affect the AsA content but had a different response to the severity of IB in pineapple clone [13]. Pineapple crown pruning in the GP3 clone increased the severity of IB but did not affect the MD2 clone.

The GP3 clone pineapple is a Smooth Cayenne type with slightly thorny leaves and a relatively sweet taste. Meanwhile, MD2 is a hybrid type, which has 50% of the same characteristics as the Smooth Cayenne cultivar. The MD2 clone pineapple has an attractive skin color and ripe fruit (golden yellow), higher vitamin C and soluble solid content, and is resistant to cold storage compared to other cultivars [14]. Therefore, it was necessary to conduct additional observations on the incidence of IB, phenol, ABA, and GA_3 content in GP3 and MD2 pineapple clones. This should be achieved after postharvest application of decrowning and coating with 50 mg L^{-1} ABA as well as 1% chitosan to determine the correlation. The severity of IB as stated by [13] was different from the incidence in this research. The severity of IB is the intensity of the brown surface area of fruit, while the incidence is the number of pineapples induced by IB without looking at the intensity. This research was expected to answer the phenomenon of the relationship between variables in GP3 and MD2 pineapple clones regarding damage due to IB.

Methods

This research used pineapples harvested from Great Giant Food Co. Ltd. with a typical export maturity level of 0% and a weight of 825 - 1124 g. The experimental design included three treatment factors, namely clone (GP3 and MD2); decrowning (crown and crownless); coating [1% chitosan; 50 mg L^{-1} abscisic acid (ABA; Phytotechlab, Kansas, USA); ABA+chitosan mixture; and control (H_2O)]. Fruit coating treatments with chitosan and H_2O were carried out by quickly dipping fruit. Coating treatments with ABA and ABA+chitosan was carried out by spraying fruit. All treated pineapples were dried for 30 minutes before being packaged in perforated GGF cardboard boxes with a capacity of 10–11 per box, and fruit was stored at 7°C for 37 days. Observations on the incidence of IB, TPC, ABA, and GA_3 were carried out 7 times (days 3, 6, 9, 16, 23, 30, and 37) for three fruits each and were repeated for a total of 336.

The incidence of IB

The incidence of IB was observed and calculated by dividing fruit into two parts from the base to the tip. This was calculated based on the percentage of fruit that triggered IB from the total fruit sample.

TPC

- Sample preparation and extraction**:** Sample preparation and extraction were carried out according to [15] with a slight extraction modification. Approximately 1 ml of pineapple juice was dissolved in 19 ml of 80% methanol using a magnetic hot stirrer at 35°C for 90 minutes.

- TPC Testing: Gallic acid solution with a concentration of 100; 150; 200; 250; and 300 ppm dissolved in methanol/water solution (1/1). About 0.1 ml each of standard gallic acid and pineapple juice extract was dissolved in 7.9 ml of distilled water. Subsequently, 0.5 ml of Folin-Ciocalteu was added to the extract solution and was allowed to stand for 8 minutes after homogenizing by shaking. The extract solution was mixed with 1.5 ml of 20% Na_2CO_3, homogenized, and left in a dark place for 120 minutes. The total amount of phenolic compounds was measured using a UV-vis spectrophotometer calibrated at 766 nm and the absorption value was calculated as mg GAE/100 ml juice.

ABA and GA_3

- Sample preparation and extraction: Frozen pineapple juice samples surrounded by ice gel were packaged and sent to the Plant Physiology Laboratory, Gadjah Mada University, Yogyakarta, Indonesia within 18 hours. Pineapple juice of 10 ml was dissolved in methanol for 24 hours, while the solution was filtered and diluted by adding distilled water to the 10 ml solution. HCl was added to the solution until the pH was 2.5 and was partitioned with 60 ml of ethyl acetate. Furthermore, the ethyl acetate phase was partitioned with 60 ml of 5% $NaHCO_3$. The phase was collected in a beaker and evaporated to dryness. The results of the evaporation were dissolved in 2 ml of pure methanol and filtered

using a microfilter [16].

- ABA compound testing: ABA compounds were detected with a Shimadzu CBM 20 A HPLC system controller, LC 20AT solvent delivery unit, CTO 10 ASVP column oven, and Shimadzu SPD 20-A UV-Vis Detector. The column (Shim-pack VP ODS 5 µm 150 x 4.6 mm) was operated at a temperature of 25°C. Additionally, the mobile phase adopted was acetonitrile / H_3PO_4 0.1% (45/55; v/v) using the isocratic method and flowed at 0.6 ml/minute. ABA standard solution of 50 µl contained 0; 0.1; 0.5; 1.0; 2.5; and 5.0 ng/ml and 0.1 ml samples were injected. The eluting compound was detected at 260 nm with a retention time of 21.015 minutes (standard R2 ABA = 0.99860).

- GA_3 compound testing: GA_3 compound was obtained with a Shimadzu CBM 20 A HPLC system controller, LC 20AT solvent delivery unit, CTO 10 ASVP Shimadzu SPD M20-A Photo Diode Array Detector column oven. The column (Shim-pack VP ODS 5 µm 150 x 4.6 mm) was operated at a temperature of 30°C. The mobile phase adopted was 25% acetonitrile using the isocratic method and flowed at 0.8 ml/minute. Furthermore, 50 µl GA_3 standard solution was reported to contain 0.1; 1.0; 5.0; and 25.0 ng/ml, and 0.1 ml samples were injected. The eluting compound was detected at 206 nm with a retention time of 12.316 minutes (standard R2 GA_3 = 0.98000).

Statistical analysis

Research data were analyzed through the 95% mean ± confidence interval (CI) method (α = 0.05) with analysis of variance (ANOVA) using IBM-SPSS version 26 program.

Results and discussion

Responses of pineapple clone

IB was not detected after a shelf life of 16 days and only appeared on the 23rd day (Figure 1.A). This was supported by previous research that the incidence of IB in the cultivars Pattavia, GP3 (Cayenne type), and MD2 was not detected after a shelf life of 14 days in cold storage [17]. According to [18], IB incidence did not appear on the 21st day in the Pattvia pineapple cultivar (Smooth Cayenne type) which was stored at 25°C.

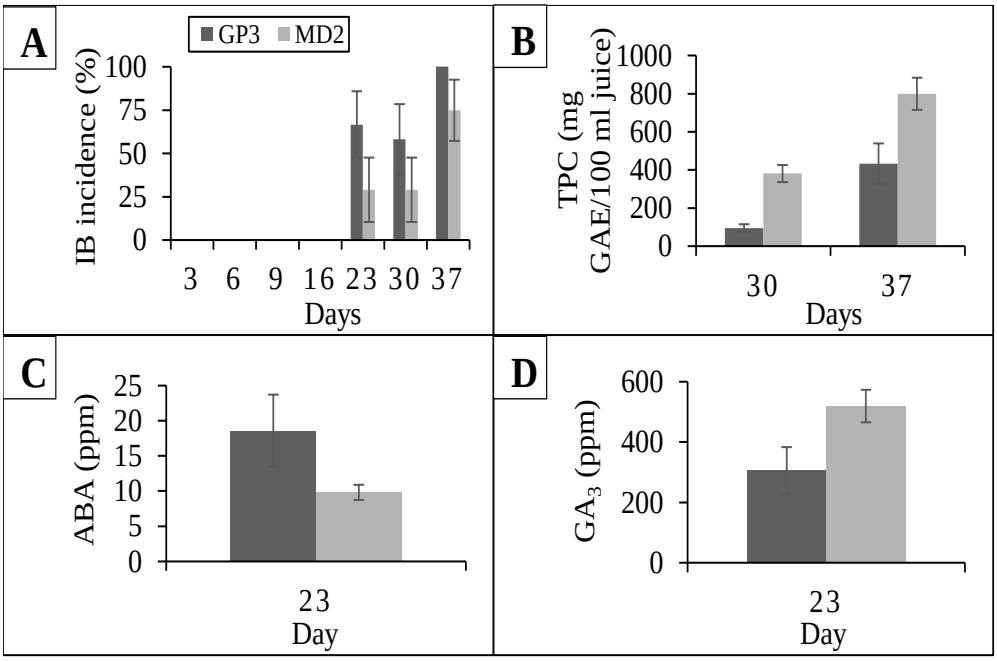

Figure 1. Responses of pineapple clone to IB incidence, TPC, ABA, and GA_3 after being stored at 7°C for 37 days.
Source: Author.

The incidence of IB in the GP3 pineapple clone (Smooth Cayenne type) was higher than in the MD2 (hybrid type), as shown in Figure 1.A. These results were also supported by several other research, where the MD2 cultivar had quite high resistance to IB [17,19,20]. According to [17], the sclerenchyma observed using scanning electron

microscopy (SEM), had a thicker fiber layer structure and was larger than the susceptible cultivars Trad-see-thong and Pattavia (tolerant). In addition, MD2 sclerenchyma cells formed concentric rings around the phloem and xylem. In line with [13], the AsA content in the MD2 clone was higher than in the GP3 clone. The high content increased the reaction with ROS to prevent cell degradation, and AsA inactivated the enzyme polyphenol oxidase (PPO).

TPC of the MD2 pineapple clone which was higher than GP3 clone (Figure 1.B) did not correlate with the incidence of IB (Figure 1.A), where TPC is directly proportional to the incidence of IB. The relationship between AsA and TPC in increasing the incidence of IB was also explained by [21]. The high content of AsA suppressed the incidence of IB in pineapple, even though TPC was relatively high. The genotype influences the antioxidant pathway and when the product shows significant resistance, increased activation occurs in response to stress [22]. According to [2], pineapple genetics influences fruit resistance to IB, and three types of pineapple were stored at 4 ± 2 °C for 28 days. The incidence of IB was correlated with AsA. The highest AsA content was possessed by MD2 followed by Moris and Josapin on day 28. TPC of the three types of pineapple was similar. The AsA content and IB incidence were inversely related, with MD2 having the lowest result. According to [12], an increase in the incidence of IB was correlated with TPC in Queen pineapple. IB incidence is a complex reaction that does not only consider TPC but is related to the amount or activity of the PPO enzyme. In line with [13], the MD2 clone pineapple had an AsA content than the GP3 clone. This suppresses the incidence of IB through the antioxidant activity of AsA, and high TPC did not affect the incidence of IB.

The increase in IB incidence in pineapple clone GP3 and MD2 from day 30 to 37 was positively correlated with high TPC. According to [13], the AsA content of pineapple stored at 7°C on day 30 was not significant compared to day 37. This caused TPC to play an important role in increasing the incidence of IB. Phenolic compounds were substrates for enzymatic browning reactions catalyzed by PPO in the synthesis of o-quinone. Furthermore, the o- uinone polymerizes or reacts with other phenolic compounds to form melanin which causes browning [10,23,24]. The decrease in the incidence of IB was positively correlated with the activity of phenolic enzymes and phenylalanine amino-lyase (PAL) from days 6 to 12 in Queen pineapple. In a study conducted by [17], pineapple flesh close to fruit core (F/C) contains higher levels of phenolic compounds, followed by PPO and peroxidase (POD) enzyme activity compared to healthy fruit. An increase in TPC occurs with the shelf life of pineapple fruit (Figure 1.B). These results are also supported by [25], where phenolic levels had a greater influence on maturity than the incidence of IB. Therefore, the coated fruit also experienced an increase in phenolic compounds and was lower than the control. This means that a greater amount of IB substrate creates a high possibility of enzymatic browning reactions.

The GP3 pineapple clone on day 23 had a higher ABA content (Figure 1.C) and lower GA_3 (Figure 1.D) compared to MD2. Based on the results of [18], there was no correlation between the endogenous ABA content and the incidence of IB in the Trad-see-thong and Pattavia pineapple cultivars. The development of the endogenous ABA content tended to fluctuate from day 0 to 21. According to [10], ABA and GA content had a negative and positive correlation with the incidence of IB. In a study conducted by [13], the resistance of the MD2 pineapple clone to the severity of IB was positively correlated with the AsA content. Based on the results, the endogenous ABA and GA_3 values in initiating IB incidence depended on the AsA content values of pineapple cultivars.

Pineapple crown pruning effect

Crown pruning of MD2 pineapple insignificantly increased the incidence of IB (Figure 2.A) and TPC on day 37 (Figure 2.B). This process also decreased and increased endogenous ABA and GA_3 in MD2 pineapple clone on day 23 (Figures 2. C and D). The increase in TPC in declining pineapple was supported by [12], where crown pruning of queen-type pineapple was stored at 20°C after 9 days. Pineapple crown pruning also results in wounds, leading to elevated levels of O_2^-, H_2O_2, and malondialdehyde (MDA). This induces cell membrane damage by amplifying the accumulation of reactive oxygen species (ROS) and upregulating the expression and activity of PPO as well as PAL enzyme to initiate an escalation in the incidence of IB. According to [13], crown pruning of GP3 increases the severity of IB due to the high AsA in MD2 as an antioxidant in capturing free radicals.

The response of crown pruning in GP3 and MD2 pineapples to IB incidence was not similar to Queen pineapple in increasing IB incidence [12], even though there are similarities in higher phenolic compounds and GA_3. This was also supported by [18] stating that Queen type pineapple has lower resistance to IB damage than the Smooth Cayenne type with lower resistance to IB than the MD2 cultivar [20]. According to [13], pineapple pruning only

affected the severity of IB in the GP3 clone. This was associated with the high AsA content in the MD2 clone, decreasing the incidence and severity of IB, while the GP3 clone had a lower content than MD2. However, crown pruning decreased endogenous ABA and increased total phenolic compounds and endogenous GA_3. This phenomenon strengthens the information that fruit crown was a source of endogenous ABA in pineapple, but IB damage depended on other variables.

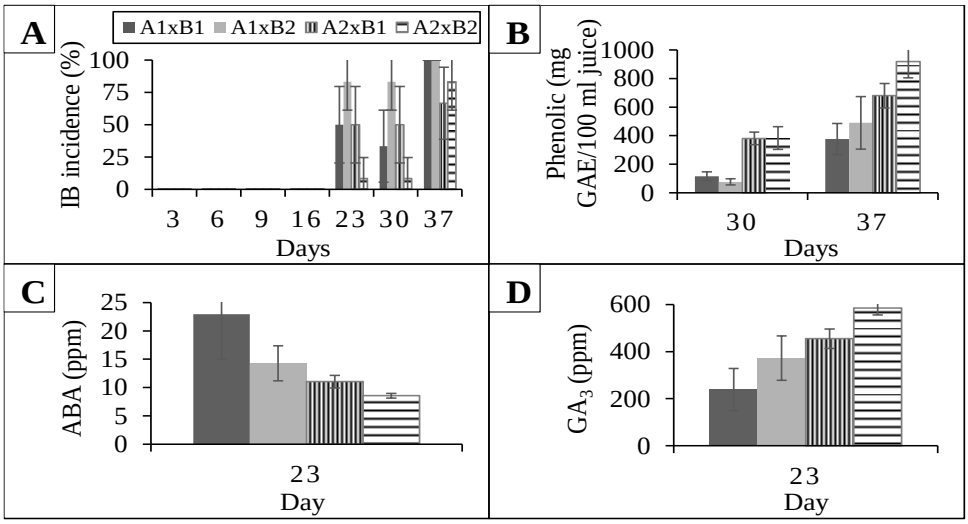

Figure 2. Effect of clone [GP3 (A1) and MD2 (A2)] and decrowning [crown (B1) and crownless (B2)] interactions on the incidence of IB, TPC, ABA, and GA_3 after being stored at 7°C for 37 days. *Source: Author.*

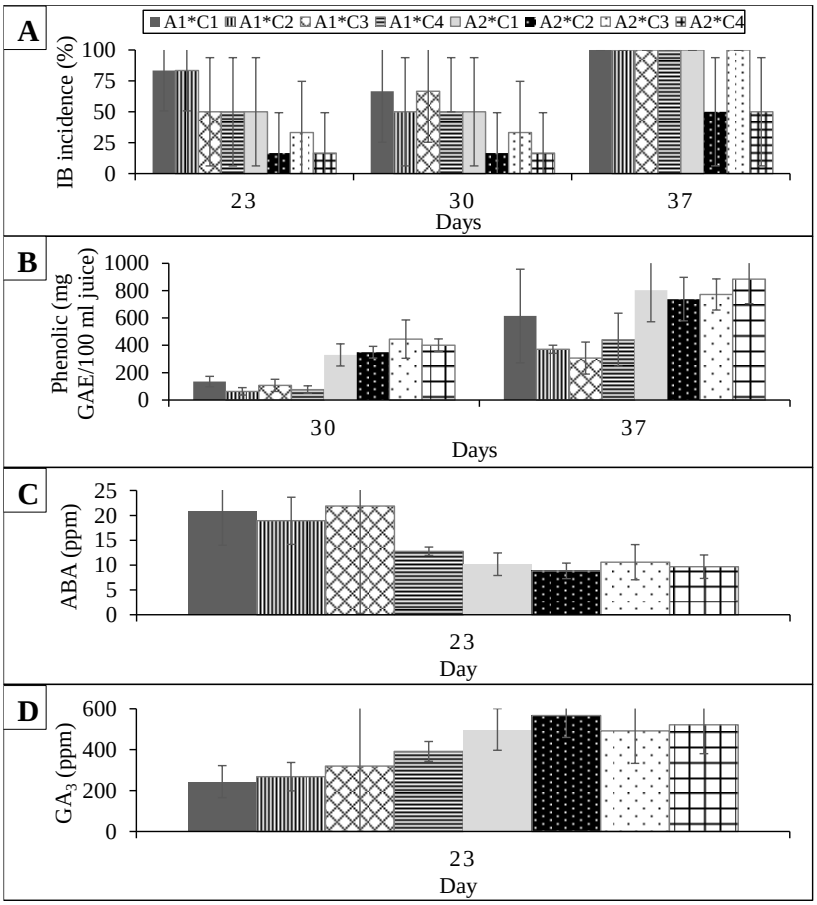

Figure 3. Effect of clone [GP3 (A1) and MD2 (A2)] and coating [H_2O (C1); 1% chitosan (C2); ABA+chitosan mix (C3); and 50 mg L^{-1} ABA (C4)] interactions on the incidence of IB, TPC, ABA and GA_3 after being stored at 7°C for 37 days. *Source: Author.*

Pineapple coating application effect

Coating with 1% and 50 mg chitosanL - 1 ABA in the MD2 pineapple clone suppressed IB incidence on the 37th day after being stored at 7°C. Therefore, the MD2 pineapple clone coated with 1% chitosan or 50 mg L^{-1} ABA suppressed the incidence of IB. Coating treatments did not affect total phenol, ABA, and GA_3 contents. Suppression of the incidence of IB in coating treatments with chitosan suppressed the entry of oxygen, hence the environment became less aerobic for phenolic oxidation. According to [26], coating fruit with chitosan suppressed browning, and PPO activity, and decreased the weight of longan fruit. The respiration rate in guava, banana, tomato, strawberry, and avocado was also suppressed [4–9]. Meanwhile, coating pineapple with ABA did not understand the inhibitory mechanism against IB. A similar result was stated by [12] that the mechanism for inhibiting the incidence of IB by ABA was unknown and required further research.

Impact

The fresh pineapple fruit industry prefers a product stored for a long time to maintain stock and delivery which requires a long time. Decrowning fruit can save packaging and storage space, but pruning increases TPC as a substrate for the browning reaction. Therefore, fruit requiring storage of up to 16-30 days must maintain pineapple crown intact. For the storage requirements of 37 days, additional post-harvest applications of 50 mg L^{-1} ABA or 1% chitosan are required.

Conclusions

In conclusion, GP3 and MD2 pineapple clones were reported to have different tolerances to endogenous TPC, ABA, and GA_3 contents which influenced the incidence of IB. The GP3 pineapple clone had a higher incidence of IB than MD2 on day 37. This was related to the large ascorbic acid content in the MD2 pineapple clone. An increase in TPC from a shelf life of 30 to 37 was positively correlated with an increase in the incidence of IB. Furthermore, postharvest coating application with 50 mg L^{-1} ABA and 1% chitosan reduced the incidence of IB. Crown pruning decreased ABA and increased TPC and GA_3 endogenous, as well as the incidence of IB.

Conflict of interest

There are no conflicts to declare.

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