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RESEARCH PAPER

Responses of GP3 and MD2 pineapple clones to the postharvest application of wax and chitosan-based coatings

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Abstract

The maintenance of fresh pineapple shelf life is a significant challenge in the market. In this context, coating is a good method used to maintain the quality of the fruits during storage. Since the materials required for the process are dependent on imported products, there is a need to develop alternatives from locally available resources. Therefore, this study aimed to determine the responses of GP3 and MD2 pineapple clones to different materials as post-harvest treatments, including Sta-Fresh 2952, OE6012, palm stearin, and chitosan. The treatments were stored at 7°C and observed on days 0, 7, 14, 21, 28, and 35. The results showed that GP3 clones were more susceptible to internal browning (IB) disorders, leading to an early onset on day 14 compared to MD2. Furthermore, a Brix value of 13.9% and vitamin C content of 52 ppm were reported at the end of storage, which were significantly lower compared to MD2 with 16.4% and 586 ppm, respectively. Based on these findings, the quality of GP3 clones was not suitable for consumption as fresh pineapple. The coating could not prevent IB disorders or respiration rate but had significant effects on fruit weight loss. Meanwhile, the Sta-Fresh 2952 treatment consistently reduced weight loss by 11.8% over 35 days of shelf life.

1. Introduction

Ananas comosus [(L.) Merr.] is an important tropical fruit from the Bromeliaceae family, accounting for 20% of the total production volume (Lobo and Paull, 2017). Indonesia is among the largest producers, with a total production of 2,447,243 tons in 2020, and Lampung Province contributed 27.07% to the volume (Fakhriandi and Erdi, 2021). The opening of Indonesian fresh pineapple market opportunities to foreign countries needs to be used for business expansion and development. The Great Giant Pineapple company, which currently produces canned products using GP3 clones, has a plan to penetrate the market share dominated by the MD2 variety. The MD2 cultivar is a popular pineapple clone on the market with several better qualities, including bright golden ripe fruit, sweeter taste, higher vitamin C content, lower fiber, and longer shelf life compared to other cultivars (Thalip *et al.,* 2015). Smooth Cayenne pineapple was more tolerant to *Phytophthora* sp. and *Erwinia chrysanthemi* compared with MD2 pineapple. However, the MD2 clone had higher resistance to internal browning (IB) (Bartholomew *et al*., 2002; Sanewski *et al*., 2018). IB is a common postharvest chilling injury symptom caused by a complex physiological disorder in tropical and subtropical fruits after exposure to low temperatures (0–20°C). This symptom is often characterized by the appearance of brownish spots near the fruit core (Luengwilai *et al.,* 2016). The maintenance of shelf life has proven to be a significant challenge when penetrating the fresh pineapple market. An effective method to extend the shelf life of fruits is through coating, but the required materials are dependent on imported products, such as Sta-Fresh 2952, derived from natural, synthetic and modified natural resin products (Vargas *et al.,* 2008). Based on these findings, there is a need to develop alternatives produced independently and are readily available.

The coating is a process that includes covering the surface of fruits with an emulsion to maintain quality and

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extend shelf life. This is an effective method for delaying maturity and extending the shelf life of horticultural products. The application of wax coating is often carried out to prevent excessive water loss from commodities due to evaporation and slow down wilting by covering some of the stomata (pores). Furthermore, the process regulates oxygen needed for respiration to reduce fruit damage, as well as covers small scratches on the fruit. Several studies have shown that the smaller the air cavity, the slower the respiration, oxidation, and chlorophyll degradation processes, leading to reduced color changes in the fruit. Indonesia was the largest palm oil producer in the world with a production of 42.5 million tons in 2019 (Mc. Carthy, 2020). Therefore, stearin, which was a palm oil derivative product, had a great opportunity to be used as an endogenous raw material for available alternative coating products. This study aimed to determine the responses of GP3 and MD2 pineapple clones to different coating materials as post-harvest treatments, including Sta-Fresh 2952, OE6012, palm stearin, and chitosan.

2. Materials and methods

This study was carried out from June to August 2022 at the Postharvest Laboratory of Great Giant Pineapple Co. Ltd. Plantation Group 4, East Lampung. The pineapple was stored for 35 days in cold storage at 7°C and observations were carried out on days 0, 7, 14, 21, 28, and 35 after the application of the treatments. This study used a completely randomized design with 2 factorial (2×5), pineapple clones (GP3 and MD2) and coatings [K (control), C (chitosan 1% w/v), S (Sta-Fresh2952 10% w/v equivalent to 5.5°Bx), O (OE6012 at 10% v/v equivalent to 5.5°Bx), and P (Palm stearin at 1% w/v)]. The solid palm stearin material was melted at a temperature of 60°C before adding chitosan (1% w/v), citric acid (2% v/v), and Tween 80/Polysorbate polysorbate 80 (2% v/v) to obtain °Brix value of 5.5. Statistical analyses were performed using the MINITAB version 19 software, and the data were analyzed through one-way analysis of variance (ANOVA). The process was continued with Duncan's multiple range tests (DMRT) at a significant difference of $P<0.05$.

2.1 Materials

The pineapple fruits were selected based on fruit color and size uniformity, with a weight range of 1000-1200 g harvested at the age of 144 days after the induction of flowering.

2.2 Process of coating and the application on pineapple fruit

For palm stearin-based coating, a concentration of 1% was used and mixed with 2% Tween80 emulsifier, 1% chitosan, and 2% citric acid. The mixture was boiled and stirred evenly at 60°C until a homogenous solution was formed. Meanwhile, chitosan coating was made from pure chitosan with a concentration of 1%, which was dissolved in 2% citric acid at 60°C. The Sta-Fresh2952 and OE6012 were existing products diluted using water into a concentration of 10%. After the preparation of the treatments, pineapple fruits were washed with clean water and coated. Subsequently, the samples were air-dried, packaged, and stored in a room at 7°C and 90-95% RH.

2.3 Analysis

2.3.1 Acidity

Acidity measurement aims to determine the level of acidity of the fruit. Observations were made on the acidity and free acid content, which was measured as the level of citric acid using a titration method with 0.1 N NaOH and phenolphthalein as an indicator. The working procedure that needs to be done is take 5 mL of pineapple juice sample into an Erlenmayer tube then add 3 drops of 1% phenolphthalein solution to the sample solution. Then, the NaOH solution was dropped into the sample solution of fruit juice while being shaken until the solution changed color to light pink.

2.3.2 Total soluble solid

The measurement of total soluble solid describes the level of sweetness of the pineapple, the step that needs to be prepared is to give pineapple juice and drops to taste on the sensor glass of the Refractometer measuring instrument. The soluble solid was measured in °Brix (%) using an Atago MASTER-M.

2.3.3 Respiration rate

The respiration rate was measured based on the method proposed by Cano-Reinoso (2022). Measurement of CO_2 concentration was conducted in a sealed container with a capacity of 13 L using a detector HT-2000 Digital CO_2 meter (CO_2 range: 0-9999 mg/kg, 10-99% RH, 0-50°C; Dongguan Xintai Instrument; China). The fruit was weighed with a digital scale before the measurement and the samples were placed in a tightly closed container with a CO_2 meter. Subsequently, the level of the gas was measured in a cold storage room of 7°C and changes in CO_2 concentration were calculated for 1 hr on each fruit.

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2.3.4 Weight loss

Measurements were made by comparing the difference between the initial weight and the final weight of the observation. The sample was weighed on a digital balance and was reported as a percentage loss in weight based on the original mass (Maftoonazad and Ramaswamy, 2005).

2.3.5 Vitamin C

The vitamin C content was assessed with the 2,6-dichloroindophenol titrimetric technique of the AOAC Method 967.21(Association of the Official Analytical Collaboration (AOAC) International., 2015), while coating analysis was carried out using a scanning electron microscope (SEM). In this titration, metaphosphoric acid was added to the sample preparation to prevent other metal catalysts from oxidizing vitamin C. The method included the determination of the level in foodstuffs through the reduction reaction of 2,6-dichlorophenol indophenol in the presence of vitamin C, such as ascorbic acid. The endpoint of the titration was indicated by a color change to pink under acidic conditions (Nielsen, 2017).

2.3.6 Internal browning

Assessment of internal browning (IB) was performed by identifying changes in the flesh color of the fruit, and evaluation was carried out every 7 days until the end of observation. Flesh coloration was determined using the color index on pineapple and the IB was visually graded from 0 (normal), 1 (light), 2 (medium), and 3 (severe browning).

2.3.7 Scanning electron microscopy

The microstructure of the pineapple-coated surface was analyzed using SEM (ZEISS/EVO MA 10; German). The SEM analysis was performed in the Technical Implementation Unit of the Integrated Laboratory and the Center for Technological Innovation, University of Lampung, Indonesia. Coated rectangular pineapple pieces with a size of 5 mm × 5 mm × 2 mm were obtained from the phloem tissue and cut for the observation of the film-coated surface. Meanwhile, a small piece of tissue in the middle of the flesh adjacent to the core was split with a tweezer. The slices were dehydrated with ethanol solutions and dried at a critical point of liquid CO_2 with a desiccator. The samples were mounted on aluminium specimen stubs using conductive silver glue and sputter-coated with gold.

3. Results and discussion

3.1 Acidity

For fresh fruit, low-acid hybrids are the most exported and required by the industry (Cano-Reinoso *et al.*, 2021). The results showed that there was no significant difference in the acidity levels of GP3 and MD2 clones ($p>0.05$), as shown in Figures 1 and 2. The different coating did not affect the acidity level ($p>0.05$), with an average value of 0.6%. This parameter was influenced by fruit varieties, seasons, and regions. During summer, the average value ranged from 0.3-0.4% and increased up to 0.7% during winter, which was acceptable by most consumers (Newett *et al*., 2006).

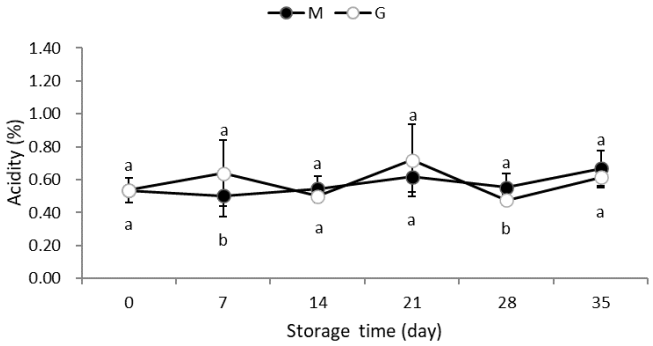

Figure 1. Effect of different clones; MD2 (M) and GP3 (G) on the acidity level of pineapple stored at 7°C for 35 days. Data are expressed as mean±SD. Data with different notations are statistically significantly different by DMRT ($p\leq0.05$).

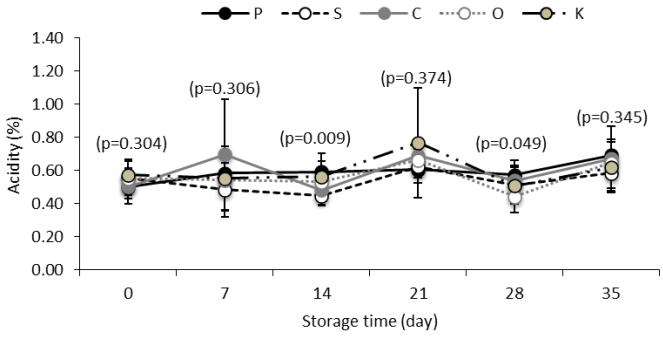

Figure 2. Effect of different coating on both pineapple clones; palm stearin (P), Sta-Fresh2952 (S), chitosan (C), OE6012 (O), and control (K) on the acidity level of pineapple stored at 7°C for 35 days. Data are expressed as mean±SD. P-values of ≤0.05 are statistically significantly different.

3.2 Total soluble solid

Figure 3 shows that there was a significant difference ($p<0.05$) in the level of sweetness between the two pineapple clones. MD2 was consistently sweeter with a value of 16.4% compared to GP3 at 13.9%. Based on Figure 4, the different coating treatments had no significant effect ($p>0.05$) on the level of sweetness until the end of shelf life. This condition showed that MD2 clones had a sweeter taste characteristic than GP3, a Smooth Cayenne cultivar. The differences in observed

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values were due to the occurrence of a transpiration metabolic process during shelf life, leading to changes in fruit quality. Temperature and post-harvest treatments also had a significant effect on the Brix value. The difference in Brix values could only be seen from the influence of pineapple clones. This was reported by Ahmad (2014) who stated that differences in fruit varieties affected quality.

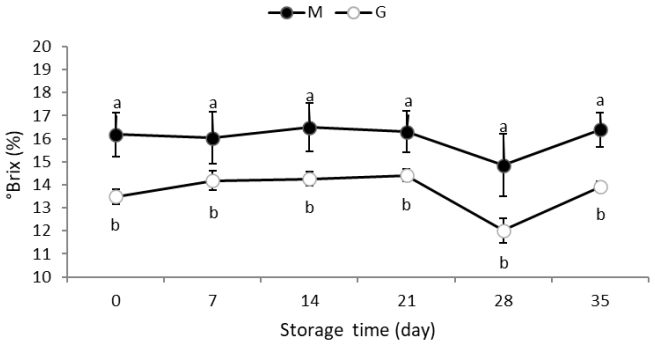

Figure 3. Effect of different clones; MD2 (M) and GP3 (G) on the °Brix level of pineapple stored at 7°C for 35 days. Data are expressed as mean±SD. Data with different notations are statistically significantly different by DMRT (p≤0.05).

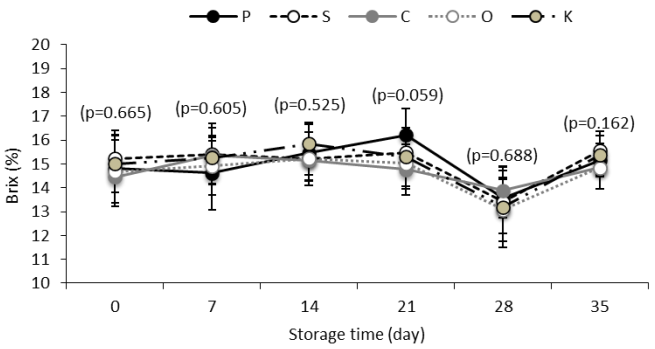

Figure 4. Effect of different coating on both pineapple clones; palm stearin (P), Sta-Fresh2952 (S), chitosan (C), OE6012 (O), and control (K) on the °Brix level of pineapple stored at 7°C for 35 days. Data are expressed as mean±SD. P-values of ≤0.05 are statistically significantly different.

3.3 Respiration rate

Respiration is the chemical process by which fruits and vegetables convert sugar and oxygen into carbon dioxide, water and heat (Khan *et al.,* 2017). Based on the observations, there was a significant difference ($p<0.05$) in the respiration between MD2 (M) and GP3 (G) clones from day 0 to 14, as shown in Figure 5. Furthermore, the condition stabilized without a significant difference ($p>0.05$) from day 21 to 35. The coating treatment had no significant effect on the respiration rate ($p>0.05$), as shown in Figure 6. This was because fruit storage at 7°C was the optimal temperature for pineapple, which suppressed the rate to a range of 1.3 to 4.4 mL CO_2/kg.h^{-1}. At a temperature of 23°C, the value reported for the parameter was 22 mL CO_2/kg.h^{-1} (Bartholomew *et al.*, 2002). Storage of fruit at low temperatures is an effective method to extend the shelf life of fresh produce and reduce respiration activity (Blongkod *et al.*, 2016).

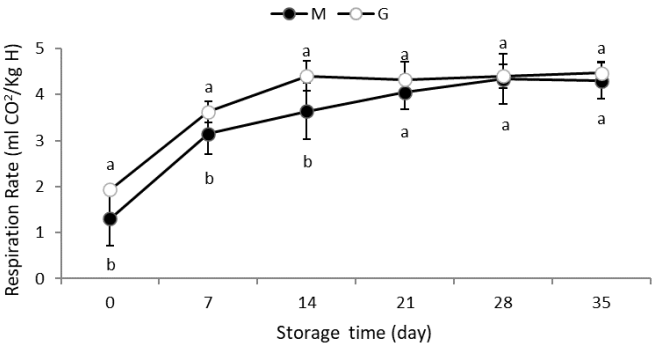

Figure 5. Effect of different clones; MD2 (M) and GP3 (G) on the respiration rate of pineapple stored at 7°C for 35 days. Data are expressed as mean±SD. Data with different notations are statistically significantly different by DMRT (p≤0.05).

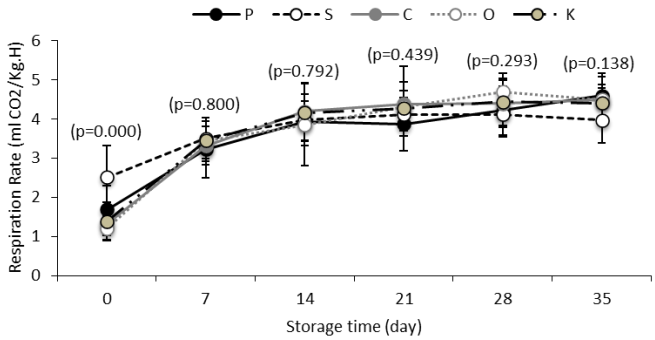

Figure 6. Effect of different coating on both pineapple clones; palm stearin (P), Sta-Fresh2952 (S), chitosan (C), OE6012 (O), and control (K) on the respiration rate of pineapple stored at 7°C for 35 days. Data are expressed as mean±SD. P-values of ≤0.05 are statistically significantly different.

3.4 Weight loss

Figure 7 shows that MD2 (M) had a weight loss of 13.9% after storage, and this value was 2% lower ($p<0.05$) compared to GP3 (G) with 15.9%. The use of Sta-Fresh 2952 (S) coating was more stable in suppressing the rate of weight loss starting from 3 weeks post-treatment until the end of shelf life. Meanwhile, the application of chitosan and palm stearin coatings maintained quality and shelf life during storage by reducing weight loss until the 14th day. The treatment also had a greater weight loss value, ranging from 14.9% to 17.4%, which was significantly different from existing products Sta-Fresh 2952 (S) ($p<0.05$) as shown in Figure 8. Hanani *et al.* (2012) reported that the application of chitosan-stearin edible coatings improved the quality and shelf-life of star fruits during storage time at room temperature. This was achieved by reducing weight loss, maintaining appearance, and slowing down respiratory CO_2 and ethylene production while preventing the decline of oxygen concentration.

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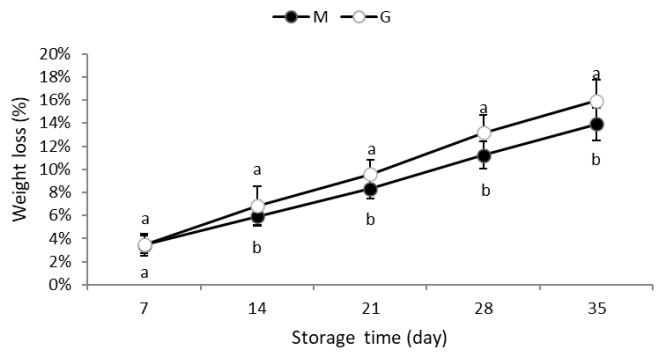

Figure 5. Effect of different clones; MD2 (M) and GP3 (G) on the respiration rate of pineapple stored at 7°C for 35 days. Data are expressed as mean±SD. Data with different notations are statistically significantly different by DMRT (p≤0.05).

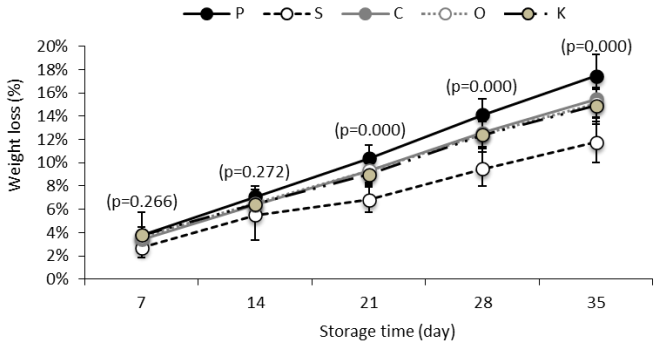

Figure 6. Effect of different coating on both pineapple clones: palm stearin (P), Sta-Fresh2952 (S), chitosan (C), OE6012 (O), and control (K) on the respiration rate of pineapple stored at 7°C for 35 days. Data are expressed as mean±SD. P-values of ≤0.05 are statistically significantly different.

3.5 Vitamin C

The *v*itamin C content of GP3 and MD2 clones was significantly different ($p<0.05$) with values of 52 ppm and 586 ppm, as shown in Figures 9 and 10. MD2 had higher values compared to Smooth Cayenne and often possessed more vitamin C than regular varieties. Furthermore, high levels of ascorbic acid often affect the incidence of IB (Lobo and Paull, 2017). A vitamin C content of >500 ppm has been reported to have the ability to lower the incidence of IB. The level of ascorbic acid can change during shelf life. In this study, the levels increased slightly or remained relatively constant for several days after harvesting. Subsequently, the level

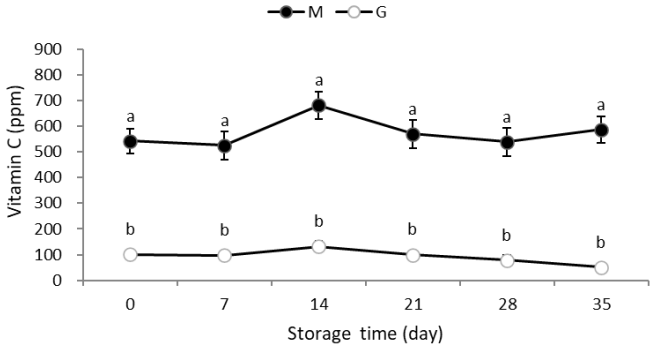

Figure 9. Effect of different clones; MD2 (M) and GP3 (G) on the vitamin C level of pineapple stored at 7°C for 35 days. Data are expressed as mean±SD. Data with different notations are statistically significantly different by DMRT (p≤0.05).

decreased after long-term storage at low and high temperatures (Rohrbach and Paull, 1982). The ascorbic acid content depended on several factors, such as variety, sunlight, air temperature, geographical conditions, and acidity. Huge differences of up to 150% in the content have been reported between different fruits of the same variety (Montero-Calderón *et al.,* 2010). Figure 11 shows that different coating treatments affected the level of vitamin C in the fruit. The highest content was obtained in the uncoated fruits (K) and was significantly different ($p<0.05$) compared to the coated samples.

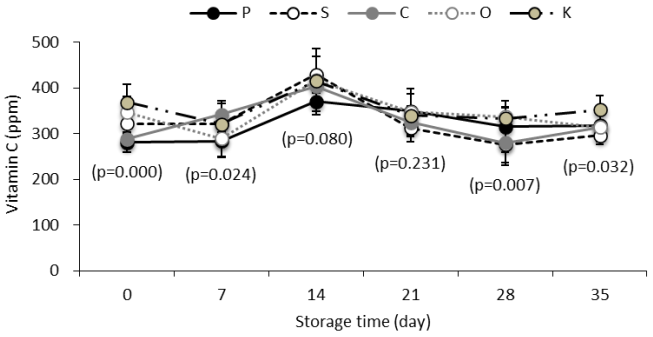

Figure 11. Effect of different coating on both pineapple clones; palm stearin (P), Sta-Fresh2952 (S), chitosan (C), OE6012 (O), and control (K) on the vitamin C level of pineapple stored at 7°C for 35 days. Data are expressed as mean±SD. P-values of ≤0.05 are statistically significantly different.

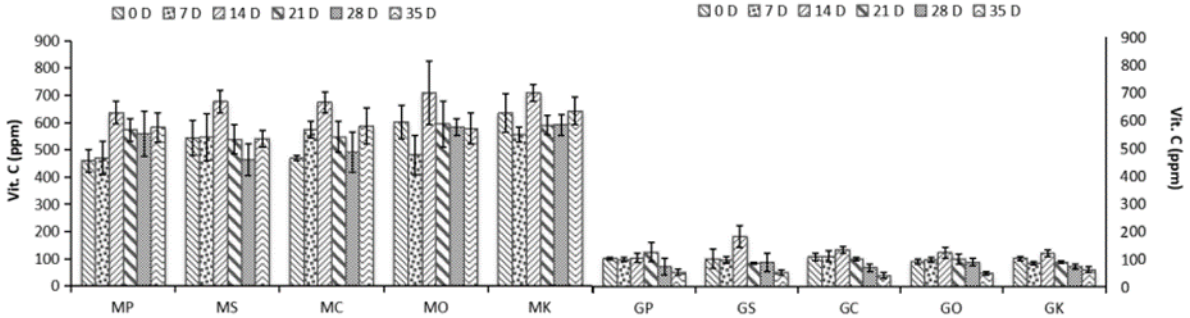

Figure 10. Vitamin C during shelf life; pineapple clones; MD2 (M) and GP3 (G) combine with different coating materials; palm stearin (P), Sta-Fresh2952 (S), chitosan (C), OE6012 (O) and control (K).

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3.6 Internal browning

The development of IB was initiated from the base of the fruit, with small translucent brown spots. The number of small brown spots increases and expands with deepened color, and damage to the cell structure (Li *et al.,* 2018). The results showed that GP3 clones were more prone to IB symptoms and the incidence rate was significantly different ($p<0.05$), as shown in Figures 12-14. IB appeared after 14 days of storage in GP3 but occurred on day 28 in MD2 with a very minimal incidence rate. IB susceptible pineapple was characterized by low ascorbic acid content in the fruit (Lobo and Paull, 2017), which acted as antioxidants by preventing the initiation of browning through reaction with oxygen. The symptoms were mostly confined to the flesh, forming translucent and isolated patches (Weerahewa and Adikaram, 2005). These findings reported that a high content of ascorbic acid reduced the occurrence of IB. The various types of coating were not significantly different ($p>0.05$) in terms of preventing the appearance of browning, as shown in Figure 14.

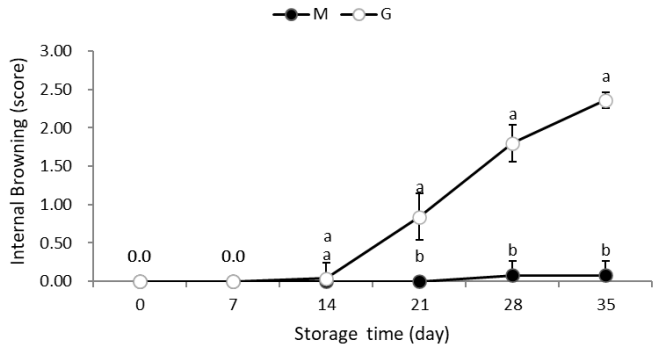

Figure 12. Effect of different clones; MD2 (M) and GP3 (G) on the IB level of pineapple stored at 7°C temperature for 35 days. Data are expressed as mean±SD. Data with different notations are statistically significantly different by DMRT ($p\leq0.05$).

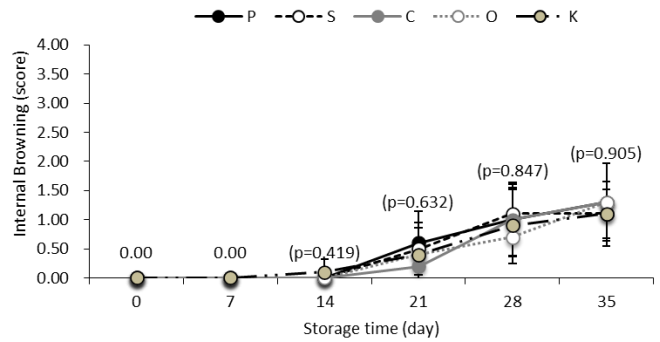

Figure 13. Effect of different coating on both pineapple clones; palm stearin (P), Sta-Fresh2952 (S), chitosan (C), OE6012 (O), and control (K) on the IB level of pineapple stored at 7°C for 35 days. Data are expressed as mean±SD. P-values of ≤0.05 are statistically significantly different.

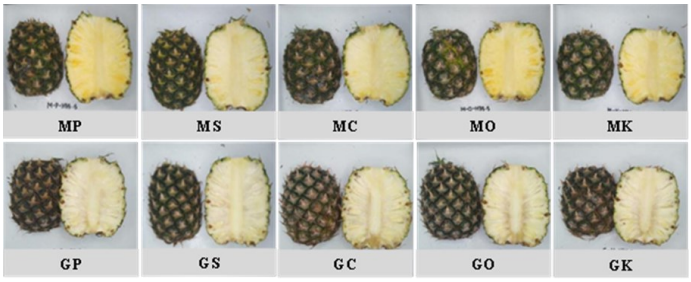

Figure 14. Pineapple flesh condition after being stored for 35 days: pineapple clones; MD2 (M) and GP3 (G) with different coating materials; palm stearin (P), Sta-Fresh2952 (S), chitosan (C), OE6012 (O) and control (K), All GP3 clones show IB with browning flesh color at the core.

3.7 Scanning electron microscopy

Microscopic studies were carried out to study the homogeneity of the coating layer on the surface of pineapple skin, as shown in Figure 15. The MK and GK images presented the condition of GP3 and MD2 without any coating layer. These images showed that uncoated pineapple had smoother surfaces compared to others. Other images reported the condition of pineapple skin with several coatings consisting of palm stearin (P), Sta-Fresh2952 (S), chitosan (C), and OE6012 (O), as well as the uncoated control (K). The images also showed the density of coating particles on the surface of the fruit skin, covering the pores. In the marked image, some parts were not completely covered by the coating, which made the surface visible. This was because the pineapple had a rough skin texture and was not covered completely with wax. According to Pereira *et al*. (2022), fresh pineapple skin had an irregular morphology with different particle sizes, with massive wrinkled and depressed blocks. Therefore, each coating material possessed different characteristics of the appearance of the layer on the surface of the pineapple skin. This

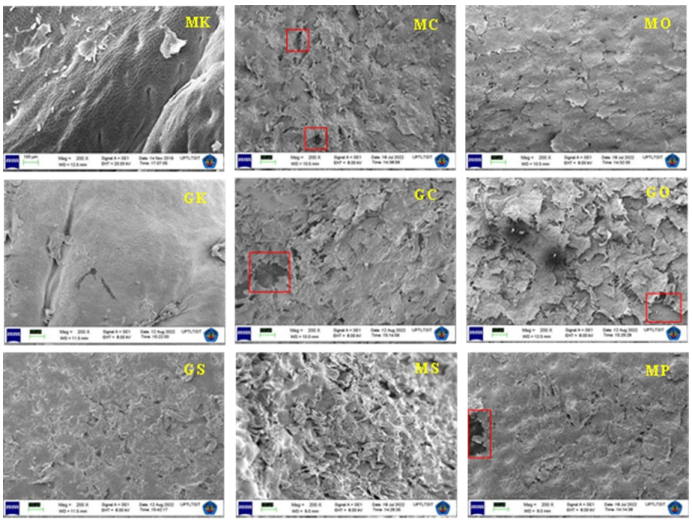

Figure 15. Display the results of various coating materials; pineapple clones; MD2 (M) and GP3 (G) with different coating materials; palm stearin (P), Sta-Fresh2952 (S), chitosan (C), OE6012 (O) and control (K) at 200× display magnification using SEM.

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difference affected the level of water loss in the fruit, as reported by a reduction in weight loss. The presence of a coating layer acted as a barrier to the transpiration process, which reduced the high potential for evaporation.

4. Conclusion

In conclusion, GP3 clones were more susceptible to IB, which started early on the 14th day after storage. In MD2 clones, IB only appeared on the 28th day with very slight severity. The use of various types of coating to maintain the quality of pineapple during its shelf life could not prevent the appearance of IB. However, the occurrence of weight loss was minimized, especially Sta-Fresh 2952, which reduced weight loss by up to 11.8% over a 35-day shelf life. These results showed that chitosan and palm stearin coating could be used as alternatives for preserving pineapple fruit up to the 14th day of shelf life without compromising quality.

Conflict of interest

The authors declare no conflict of interest.

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