



Research article

## Alleviation of internal browning and fruit rot disease in ‘Pattavia’ pineapple using ethanolic shellac-modified coconut oil coating

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### Abstract

**Importance of the work:** Internal browning (IB) and fruit rot disease are the main problems of harvested pineapple that limit its quality.

**Objective:** To investigate the effect of ethanolic shellac-modified coconut oil (ES-MCO) coating against IB and fruit rot disease in ‘Pattavia’ pineapples.

**Materials & Methods:** Fruits were coated with ES-MCO (consisting of 8% shellac and 2% MCO) and stored at 13°C and 95% relative humidity for 20 d. Uncoated fruits were used as the control. The IB, physiochemical qualities and rot disease were determined.

**Results:** The ES-MCO treatment maintained fruit quality through a reduction in the respiration rate, resulting in a slower weight loss and color changes (peel and pulp). The browning index and browning scores of the coated fruits were lower than those of the control by 8.8% and 33.26%, respectively, while maintaining the total soluble solids/titratable acidity (TSS/TA) ratio and the ascorbic acid content. The ES-MCO treatment significantly reduced the polyphenol oxidase and peroxidase activity levels (browning enzymes), by 1.56–5.52 and 1.16–2.14 times, respectively, relative to the control. These results correlated with the reduction in IB and the increase in the total phenolic content. Compared to the control, the ES-MCO treatment slowed down the increase in the malondialdehyde content that is involved with the oxidative stress response, as well as suppressing rot disease.

**Main finding:** Coating pineapple with ES-MCO alleviated IB and rot disease and delayed adverse changes in the physiochemical qualities caused by a reduction in the respiration rate.

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## Introduction

Pineapple (*Ananas comosus* L. Merr.) is a tropical fruit and ranks as one of the 15 most popular fruits in the world due to its high production and consumption (FAO Statistical Database, 2022). Thailand is a major producer and exporter of pineapple (Anupunt et al., 2000). It is consumed as fresh and frozen fruit and used as the raw material for the production of processed goods, such as juice and canned pineapple. ‘Pattavia’ is a popular cultivar of pineapple because of its good taste as well as being able to be planted in many areas in Thailand (Dittakan et al., 2018), where the most productive regions are along the east and west coasts of the Gulf of Thailand (Anupunt et al., 2000). In 2022, the export volume of fresh and frozen pineapples increased 3.6-fold compared to 2020 (Office of Agricultural Economics, 2023). However, there are major obstacles to the export of fresh pineapple, especially internal browning (IB; Boonyarittongchai and Supapvanich, 2017) and fruit decay caused by various fungal pathogens and spoilage microorganisms, such as *Rhizopus*, *Geotrichum*, *Neurospora*, *Candida* and *Aspergillus* (Fulgence et al., 2021).

Low-temperature storage is a common method to preserve the quality of fruit and vegetables during transportation and storage because it helps delay cell metabolism, senescence and eventual ripening (Sevillano et al., 2009). However, low-temperature storage may induce a physiological disorder called chilling injury (CI). Pineapples are especially susceptible to CI after exposure to low temperature (0–20°C; Paull, 1990). The recommended storage temperature for pineapples in the commercial sector is 13°C; however, this temperature still causes CI, which leads to the presence of IB symptoms on the pulp tissue adjacent to the core (PAC) tissue (Pusittigul et al., 2012; Youryon et al., 2018).

The primary signal of the cool response pathway in plants is an increase in reactive oxygen species (ROS), such as the superoxide anion ( $O_2^-$ ), hydrogen peroxide ( $H_2O_2$ ) and the hydroxyl radical ( $HO\bullet$ ), which cause the loss of membrane fluidity and subsequent cell damage (Tarmizi and Dolhaji, 2022) because membrane lipids are degraded through a peroxidation reaction with  $H_2O_2$ , resulting in malondialdehyde (MDA) release (Nukuntornprakit et al., 2015). Cell membrane deterioration results in the leakage of phenolic compounds and the polyphenol oxidase (PPO) enzyme (Luengwilai et al., 2016). The mechanism of internal browning involves the reaction of the PPO enzyme with a phenolic compound in the presence of oxygen (Moon et al., 2020). PPO converts monophenols and

o-dihydroxy phenols to o-quinones; afterward, o-quinones bind with free amino acids to form the dark pigments that cause browning (Stewart et al., 2001). Peroxidase (POD) is the enzyme involved in various plant processes (Quiroga et al., 2000), with an especially important role in plant defense responses and browning reactions that are related to lignin biosynthesis (Aquino-Bolaños and Mercado-Silva, 2004).

Prevention of IB has been reported in many freshly produced fruits. In this regard, an edible coating is one of the most effective and simple technologies to suppress enzymatic browning, maintain quality and extend the shelf life of fruits (Nimitkeatkai et al., 2006). Several materials are used for producing edible coatings, such as proteins, lipids, polysaccharides and composite materials (Hassan et al., 2018). The coating has the same function as modified atmosphere packaging (MAP) (Dhall, 2013), which acts as a barrier to control the movement of  $CO_2$ ,  $O_2$ , flavor, moisture and various dissolved compounds between the plant tissue and the environment (Hassan et al., 2018). Polysaccharide-based coatings have lower gas permeability properties, whereas protein-based coatings have better mechanical properties and lipid-based coatings have the greatest potential to control moisture permeability (Bourtoom, 2008), leading to reduced transpiration and also improved brightness (Blancas-Benitez et al., 2022).

Shellac is naturally produced from the lac insect (*Kerria lacca*) and has lipid film-forming properties (Ma et al., 2021). Reports have shown that a shellac coating could maintain the quality, extend the shelf life and prevent browning by reducing the respiration rate, weight loss, fruit decay, plant metabolisms and enzymatic browning activity levels in various fruits, including jujube (Promyou and Supapvanich, 2011), pear (Zhou et al., 2011), mangosteen (Thuong et al., 2015), rambutan (Jitareerat et al., 2017) and pineapple (Hu et al., 2012). In addition, a shellac coating mixed with hydrochloric acid reduced PPO, POD and phenylalanine ammonia lyase (PAL) activity levels on the litchi pericarp that suppressed browning (Nanglia et al., 2022). Furthermore, a shellac coating has many advantages as a food preservative; however, it has limited antimicrobial properties (Kouassi et al., 2012; Ma et al., 2021). Therefore, a shellac coating with increased antimicrobial properties has been developed by adding antimicrobial substances, such as essential oils and organic acids. For example, Jo et al. (2014) reported that carnauba-shellac wax containing lemongrass oil could maintain the quality and reduce the microbial population of apples. On the other hand, Ma et al. (2021) found that shellac

mixed with tannic acid improved overall quality, enhanced the antifungal effect and prolonged the shelf life of mango.

Sripong et al. (2015) reported on producing modified coconut oil (MCO) using the glycerolysis technique, with the MCO containing monoglycerides (lauric acid, monolaurin, dilaurin), which had antifungal properties against *Colletotrichum gloeosporioides* Penz. growth, a cause of anthracnose disease in mango. Antimicrobial activity of monoglycerides has been reported in *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas* spp., *Streptococcus* spp., *Candida albicans* and *Aspergillus* spp. (Effiong et al., 2019). Jitareerat et al. (2017) reported that shellac mixed with MCO (ES-MCO) could prevent fruit rot disease and water loss in gamma-irradiated rambutan fruit. In addition, Thuong et al. (2015) demonstrated that the treatment of ES-MCO combined with 1-methylcyclopropene (1-MCP) significantly retarded fruit rot disease in mangosteen, as did a fungicide treatment.

The literature review above has shown that a shellac coating has the potential to delay IB and maintain the quality of various fruits, while MCO has antimicrobial properties. Therefore, the present study aimed to investigate the effect of shellac combined with modified coconut oil (ES-MCO) on the alleviation of IB and fruit rot disease in ‘Pattavia’ pineapple.

## Material and Methods

### ES-MCO preparation

The solution of ES-MCO was prepared by dissolving 8% shellac (Bleached Shellac Dewaxed EXL.3-CIRCLES; Creasia Group; Thailand) in 95% ethanol to obtain ethanolic shellac (ES) and then mixing it with 2% MCO. The MCO was prepared by converting the fatty acids of virgin coconut oil to monoglycerides using the glycerolysis technique (Sripong et al., 2015).

### Pineapple preparation and treatment

Pineapple fruits cv. ‘Pattavia’ (smooth cayenne) were harvested at the commercial stage from an orchard in Chonburi province, Thailand. The pineapple fruits collected showed no signs of physical damage or insects or disease symptoms, with an average weight of a fruit in the range 1.5–1.8 kg; the fruits were transported to the packhouse of V.S. Freshco Co. Ltd. located in Nakhon Pathum province within 3 hr. The fruits were washed with tap water and disinfected in 200 parts per million

sodium hypochlorite (NaOCl) for 5 min. The top of the crown was removed, leaving one-quarter of the crown on the fruit. Then, the fruits were dipped into the ES-MCO solution for 20 s and air-dried, whereas uncoated fruits served as the control. All samples were kept at 13°C and 95% relative humidity (RH) for 20 d. Fruit samples were randomly collected to determine the physiochemical qualities and fruit decay initially and at 5 d intervals. Each treatment had four replications with two fruits per replication.

### Measurement of color, browning index and browning score

The color was assessed of the fruit peel and pulp (excluding the browning area) at three points (top, middle, and bottom of longitudinal regions) using a colorimeter (Minolta 400; Japan). Values of lightness ( $L^*$ ), red-green ( $a^*$ ) and yellow-blue ( $b^*$ ) were recorded. The browning index of the pulp was used as an indicator of browning intensity, and this value was calculated according to Palou et al. (1999) using the formula: browning index =  $[100(x - 0.31)] / 0.172$ , where  $x = (a^* + 1.75L^*) / (5.645L^* + a^* - 3.012b^*)$ . The browning score was evaluated as described by Boonyaritthongchai and Supapvanich (2017), where 0 = 0% (no browning), 1 = 1–20% (internal browning), 2 = 21–40% (internal browning), 3 = 41–60% (internal browning) and 4 = > 60% (internal browning).

### Polyphenol oxidase and peroxidase analysis

The PPO activity was determined according to the method described by Benjamin and Montgomery (1973). A sample (1 g) of pulp adjacent to the core (PAC) tissue was homogenized with 5 mL of 0.05 M sodium phosphate buffer (SPB), pH 7, containing 0.1 g of polyvinylpyrrolidone (PVP) and 100  $\mu$ L of triton-X 100, and then centrifuged at 15,000 $\times$ g for 25 min at 4°C. Next, 50  $\mu$ L of the supernatant was reacted with a mixture of 1.95 mL SPB and 1 mL of 0.5 M catechol. The increase in absorbance at the 420 nm wavelength of the reaction was recorded at 25°C for 60 s. The unit of PPO activity was calculated as the increased absorbance per minute. The data were expressed as units per milligram of protein.

The POD activity was determined as described by Wang et al. (2005). A sample (3 g) of PAC tissue was homogenized with 10 mL of 0.05 M SPB, pH 7, containing 0.1 g of PVP, and then centrifuged at 15,000 $\times$ g for 25 min at 4°C. Next, 1 mL of the supernatant was reacted with a mixture of 0.5 mL of SPB, 0.5 mL of 0.5% guaiacol and 1 mL of 1% hydrogen

peroxide. The increase in absorbance at 470 nm of the reaction was recorded at 25°C for 150 s. The unit of POD activity was calculated as the increased absorbance per minute. The data were expressed as units per milligram of protein.

#### *Total phenolic analysis*

The total phenolic (TP) content was determined according to the method of Singleton et al. (1999). A sample (2 g) of PAC tissue was homogenized with 10 mL of 80% ethanol and passed through Whatman No. 1 filter paper. Then, 40 µL of the filtered sample was mixed with 1.58 mL of distilled water, 0.1 mL of concentrated Folin-Ciocalteu phenol reagent and 0.3 mL of 20% sodium carbonate, followed by incubation at 40°C for 30 min. The mixed sample was measured using an ultraviolet (UV)-1800 spectrophotometer (Shimadzu; Japan) at 765 nm. The TP content was calculated as milligrams of gallic acid equivalent (GAE) using a gallic acid calibration curve. The result was expressed as milligrams of GAE per 100 g fresh weight (FW).

#### *Malondialdehyde analysis*

The MDA content was determined according to Zhang et al. (2011). A sample (2 g) of PAC tissue was homogenized with 5 mL of 0.1% trichloroacetic acid (TCA). The supernatant was collected using centrifugation at 15,000×g for 25 min at 4°C. A sample (2 mL) of the supernatant was reacted with 2 mL of 15% TCA containing 0.5% thiobarbituric acid. The mixture was heated at 95°C for 20 min and then cooled immediately in an ice bath. The mixture was measured using the UV-1800 spectrophotometer at 532 and 600 nm wavelengths. The data were calculated based on the formula:  $MDA = [(Abs\ 532\ nm - Abs\ 600\ nm) \times 10^6 \times dilution] / 155,000$ , where the MDA was in units of nanomoles per gram FW.

#### *Determination of total soluble solids and titratable acidity*

Pineapple juice was collected from the pineapple pulp and used to determine the TSS and TA using an auto-refractometer (Pocket Refractometer PAL-1, Japan) and the titratable method (meq. citric acid = 0.064) described by Association of Official Analytical Chemists (1990), respectively. The result was reported as a TSS/TA ratio.

#### *Total ascorbic acid analysis*

The total ascorbic acid (AsA) was determined as described by Roe et al. (1948), with some modifications. A sample (2 g) of PAC tissue was homogenized with 10 mL of ice-cold 5% metaphosphoric acid. The extract was passed through Whatman No. 1 filter paper. Then, 0.4 mL of the filtered sample was mixed with 0.2 mL of 0.02% 2,6-dichlorophenol-indophenol, 0.4 mL of 2% thiourea and 0.2 mL of 2% dinitrophenol hydrazine and incubated at 37°C for 3 hr. An amount (1 mL) of 85% sulfuric acid was added to the mixture solution and incubated at room temperature for 30 min. The sample was measured using the UV-1800 spectrophotometer at 540 nm. The AsA content was calculated as milligrams of AsA equivalents using an AsA calibration curve. The result was expressed as milligrams of AsA per 100 g FW.

#### *Determination of weight loss*

Each fruit sample was weighed on a digital balance to determine the initial weight and this was compared with the final weight. The percentage of weight loss was calculated according to the formula:  $(N_0 - N_1) / N_0 \times 100$ , where  $N_0$  and  $N_1$  are the initial and final weights, respectively.

#### *Determination of respiration rate*

The respiration rate was determined as described by Gemma et al. (1994). The fruit was kept in a plastic airtight container (5,000 mL volume) at 13°C for 2 hr. Then, a gas sample (1 mL) was withdrawn from the container using a hypodermic syringe. The respiration rate was detected based on gas chromatography (GC-2014; Shimadzu; Japan). The respiration rate was expressed as milligrams of CO<sub>2</sub> per kilogram per hour.

#### *Incidence and severity of fruit rot disease*

The incidence of fruit rot during storage was evaluated based on the visible fungal growth on the peduncle, crown or peel surface of the fruit. Any fruit with visible fungal growth on the surface affected was considered to have fruit rot in this study. The percentage of disease incidence was calculated according to the formula:  $(F_0 / F_1) \times 100$ , where  $F_0$  and  $F_1$  are the number of fruit incidences and the total number of fruits, respectively.

Disease severity was determined according to the method described by Bartholomew et al. (2003) using the scores: 0 = no appearance of fungal growth; 1 = fungi appear on the peduncle and crown but not on the fruit peel; 2 = fungi appear on the peduncle, crown and on 1–5% of the fruit peel; 3 = fungi appear on the peduncle, crown and on 6–10% of the fruit peel; 4 = fungi appear on the peduncle, crown and 11–20% of the fruit peel; and 5 = fungi appear on the peduncle, crown and > 21% of the fruit peel.

### Statistical analysis

All data in this experiment were presented as mean  $\pm$  SD values of four replicates, where each replicate had two fruits. Comparison of the means of the control and ES-MCO-treated pineapple fruits was performed using independent samples t test analysis in the SAS version 9.0 software (SAS Institute; USA). All analyses were considered significant at  $p < 0.05$  on the same day.

## Results

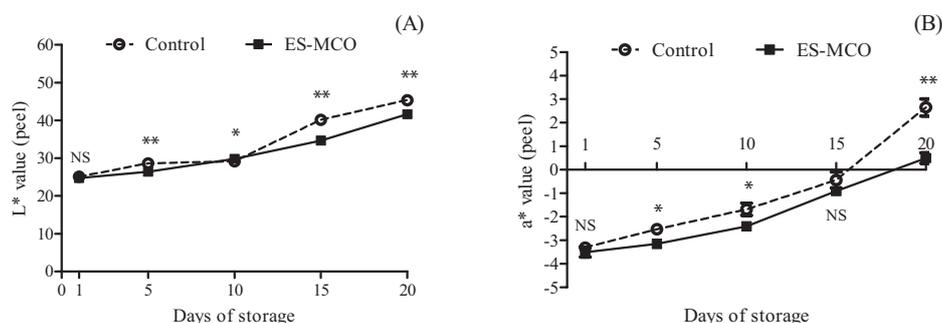
### Effect of ES-MCO treatment on peel color

The color change in the peel of the pineapple during storage at 13°C is presented as lightness ( $L^*$ ) and red-green ( $a^*$ ) values in Fig. 1. The increases in the  $L^*$  and  $a^*$  values in the peel were correlated with the ripening of the fruit throughout storage. The results showed that the  $L^*$  value of pineapple peel gradually increased from day 5 to day 20 for both the ES-MCO-treated fruit and the control fruit. The  $L^*$  value of the ES-MCO-treated fruit was significantly lower than that of the control. On the last day (day 20), the value of the

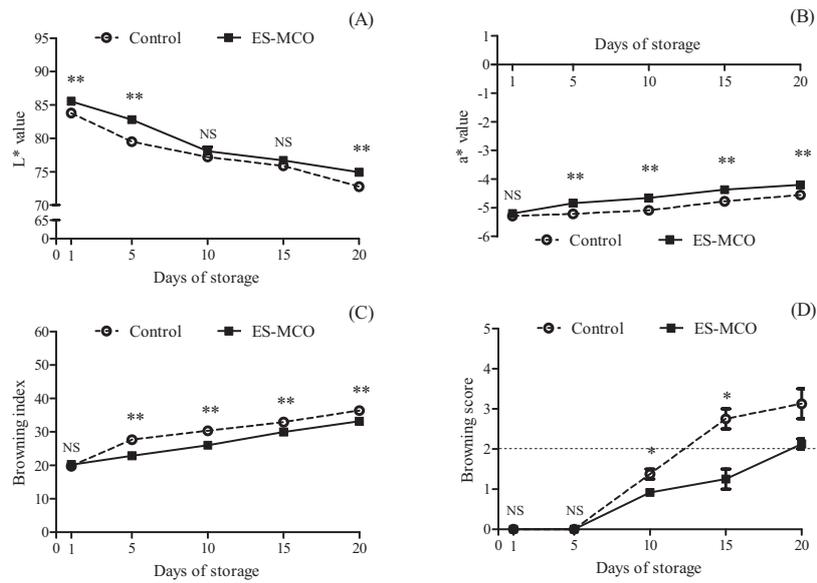
ES-MCO-treated fruit was 41.66, whereas that of the control was 45.36 (Fig. 1A). The ES-MCO-treated fruit could suppress the increase in  $a^*$  value compared to the control (Fig. 1B). The results of the  $L^*$  and  $a^*$  values were correlated with the change in peel color of the ES-MCO-treated pineapple fruit, which was still greener than the control fruit (Fig. 3). This result implied that the ES-MCO treatment could delay the color development of pineapple peel.

### Effect of ES-MCO treatment on pulp color

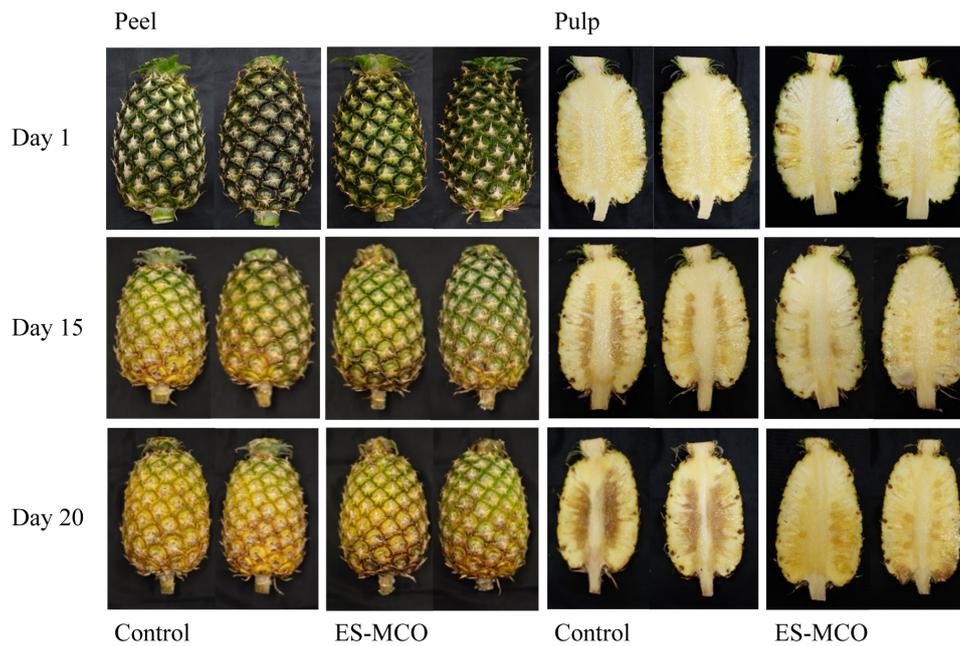
IB in pineapple fruit is commonly present after the fruit is stored at low temperatures. The present study found that the  $L^*$  value of the pulp tissue in both treatments tended to decrease with storage time. The ES-MCO treatment significantly delayed the decrease in the  $L^*$  value compared to control from day 5 to day 20 (Fig. 2A). The  $a^*$  value indicate a red-green color, where negative and positive  $a^*$  values indicate green and red colors, respectively. The  $a^*$  value of the pineapple pulp in the ES-MCO treated fruit was very close to that of the control on day 1, after which it increased slightly until the last day of storage. The  $a^*$  value of the ES-MCO-treated fruit was significantly higher than that of the control throughout storage (Fig. 2B). Slight internal browning was found in the ES-MCO-treated fruits, while the control fruit showed moderate browning on day 15 and day 20 (Fig. 3). The  $L^*$  value results in the pulp were consistent with the browning index and browning score. During storage, the ES-MCO-treated pineapple had a lower browning index and browning score compared to the control (Figs. 2C and 2D). On day 20, the browning index and browning score of the ES-MCO-treated fruit were lower than those of the control by 8.8% and 33.26%, respectively. These data suggested that the ES-MCO treatment reduced the IB symptoms in pineapple during storage at 13°C.



**Fig. 1** Values of  $L^*$  (lightness) (A);  $a^*$  (red-green) (B) of peel of pineapple fruit after coating with ethanolic shellac - modified coconut oil (ES-MCO) compared with uncoated fruit (control) during storage at 13°C and 95% relative humidity for 20 d, where results are means  $\pm$  SD ( $n = 8$ ); \*, \*\* and NS indicate significant ( $p < 0.05$ ), highly significant ( $p < 0.01$ ) and non-significant differences between control and treatment within each day, respectively.



**Fig. 2** Values of L\* (lightness) (A); a\* (red-green) (B); browning index (C); browning score (D) of pulp of pineapple fruits after coating with ethanolic shellac - modified coconut oil (ES-MCO) compared with uncoated fruit (control) during storage at 13°C and 95% relative humidity for 20 d, where dashed line in Fig. 2D is standard of acceptable level (score  $\leq 2$ ) in terms of browning appearance, results are means  $\pm$  SD; \*, \*\* and NS indicate significant ( $p < 0.05$ ), highly significant ( $p < 0.01$ ) and non-significant differences between control and treatment within each day, respectively.



**Fig. 3** Appearance of peel and pulp of pineapple fruits after coating with ethanolic shellac - modified coconut oil (ES-MCO) compared with uncoated fruit (control) during storage at 13°C and 95% relative humidity for 1 d, 15 d and 20 d

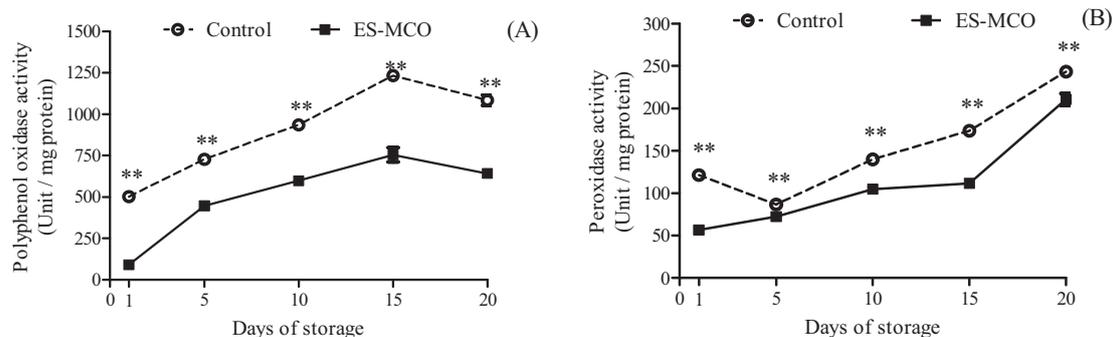
### Effect of ES-MCO treatment on polyphenol oxidase and peroxidase activity levels

Fig. 4 shows that the activity of PPO substantially increased in both treatments during the storage period. The PPO activity of the ES-MCO-treated fruit was significantly lower than that of the control. On the last day of storage, the PPO activity of the treated fruit was 642.33 units/mg protein, while that of the control fruit was 1,084.07 units/mg protein (Fig. 4A). Similar results were presented for the POD activity. On day 1, the ES-MCO and control treatments had 56.66 units/mg protein and 121.30 units/mg protein, respectively; afterward, they increased to 210.59 units/mg protein and 243.44 units/mg protein, respectively, on the last day of storage. These results showed that the PPO and POD activity levels of the ES-MCO-treated pineapples were

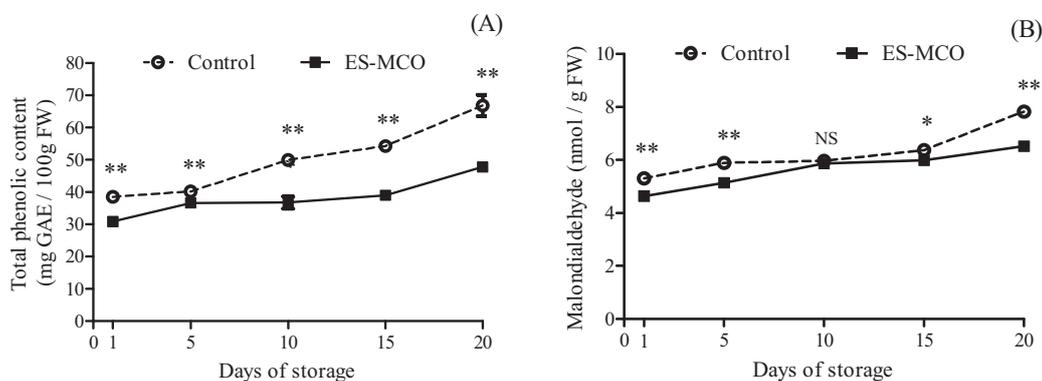
significantly lower than those of the control by 1.56–5.52 and 1.16–2.14 folds, respectively, indicating that the ES-MCO treatment suppressed the activity levels of the browning enzymes.

### Effect of ES-MCO treatment on total phenolic and malondialdehyde content

Phenolic compounds are contained in the substrate of enzymatic browning reactions (Leng et al., 2009). In the present study, the TP content of the pineapple fruits tended to increase throughout storage in both treatments. The TP content of the ES-MCO-treated pineapple was significantly lower than that of the control, with only 47.80 mg/100 g FW of TP content in the ES-MCO-treated fruit, which was lower than that in the control by 1.4 folds after storage on day 20 (Fig. 5A).



**Fig. 4** Activities of polyphenol oxidase (A); and peroxidase (B) in pulp adjacent to core tissue of pineapple fruit after coating with ethanolic shellac - modified coconut oil (ES-MCO) compared with uncoated fruit (control) during storage at 13°C and 95% relative humidity for 20 d, where results are means  $\pm$  SD ( $n = 8$ ); \*\* indicate highly significant ( $p < 0.01$ ) difference between control and treatment within each day.



**Fig. 5** Total phenolic content (A); and malondialdehyde (B) in pulp adjacent to core tissue of pineapple fruit after coating with ethanolic shellac - modified coconut oil (ES-MCO) compared with uncoated fruit (control) during storage at 13°C and 95% relative humidity for 20 d, where results are means  $\pm$  SD ( $n = 8$ ); \*, \*\* and NS indicate significant ( $p < 0.05$ ), highly significant ( $p < 0.01$ ), and non-significant differences between control and treatment within each day, respectively; FW = fresh weight.

Browning is not only known to have a positive correlation with TP content but is also involved with MDA (Leng et al., 2009). The ES-MCO-treated fruit had significantly lower levels of MDA content compared to the control, except on day 10. At the end of storage, the MDA content of the ES-MCO-treated fruit was 6.52 nmol/g FW, whereas that of the control was 7.82 nmol/g FW (Fig. 5B). This result suggested that ES-MCO reduced the accumulation of TP and the MDA content.

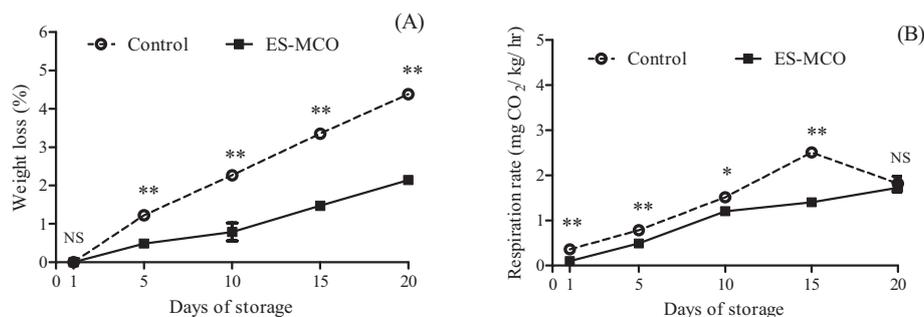
#### Effect of ES-MCO treatment on weight loss and respiration rate

The weight loss of the control fruit rapidly increased during the storage period while that of the ES-MCO treated fruit slowly increased (Fig. 6A). ES-MCO-treated pineapple had significantly lower weight loss than the control by 2.05–2.87 folds from day 5 to 20. At the end of storage, the weight loss of ES-MCO-treated fruit was 2.14%, whereas that of the control was 4.38%. Weight loss in fruit is directly correlated with respiration rate. It was found that the respiration rate of the control fruit rapidly increased and peaked on day 15 of storage before declining on day 20. ES-MCO treatment

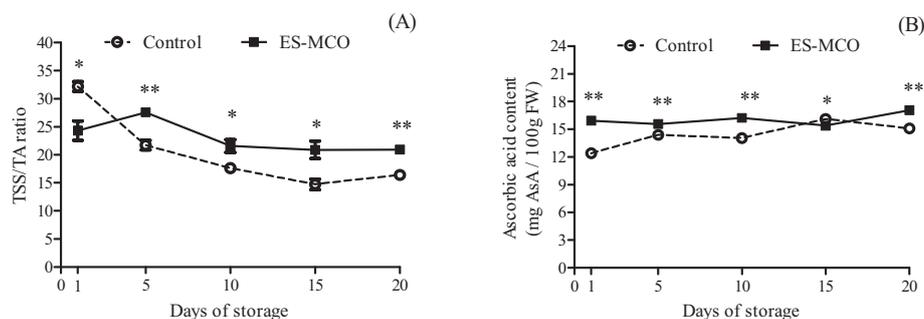
could significantly slow down the increase in respiration. Its respiration peak did not appear, even at the end of storage. On day 20, the respiration rates of the two treatments were not significantly different (Fig. 6B). ES-MCO treatment could reduce the respiration rate of pineapple by 1.04–3.27 folds compared to the control fruit. This means that ES-MCO treatment can decrease the respiration of pineapple.

#### Effect of ES-MCO coating on TSS/TA ratio and total ascorbic acid content

Commonly, TSS and TA are used as primary indices for fruit quality, particularly the taste. TSS and TA can indicate levels of sweetness and sourness, respectively. The present results showed that the TSS/TA ratio of the control fruits continually decreased during storage while that of the ES-MCO-treated fruits was relatively stable. During storage from day 5 to day 20, the TSS/TA ratio in the ES-MCO-treated fruit was in the range 20.88–27.59, whereas in the control it was 14.73–21.71. This ratio in the ES-MCO-treated fruits was 1.04–1.28 times higher than for the control (Fig. 7A).



**Fig. 6** Weight loss (A); and respiration rate (B) of pineapple fruit after coating ethanolic shellac - modified coconut oil (ES-MCO) compared with uncoated fruit (control) during storage at 13°C and 95% relative humidity for 20 d, where results are means  $\pm$  SD ( $n = 8$ ); \*, \*\* and NS indicate significant ( $p < 0.05$ ), highly significant ( $p < 0.01$ ), and non-significant differences between control and treatment within each day, respectively.



**Fig. 7** Total soluble solids-titratable acidity (TSS-to-TA) ratio (A); ascorbic acid content (B) of pineapple fruit after coating with ethanolic shellac - modified coconut oil (ES-MCO) compared with uncoated fruit (control) during storage at 13°C and 95% relative humidity for 20 d, where results are means  $\pm$  SD ( $n = 8$ ); \* and \*\* indicate significant ( $p < 0.05$ ), highly significant ( $p < 0.01$ ) differences between control and treatment within each day, respectively; FW = fresh weight

The results for the AsA content were similar to those for the TSS/TA ratio, with the AsA content of the ES-MCO-treated fruit being significantly higher than for the control. The AsA contents in the ES-MCO-treated fruit did not change much during storage, with values in the range 15.40–17.08 mg/100 g FW, while the values in the control were in the range 12.41–16.11 mg/100 g FW (Fig. 7B). The data showed that the ES-MCO treatment could maintain the TSS/TA ratio and the AsA content of pineapple during storage for 20 d.

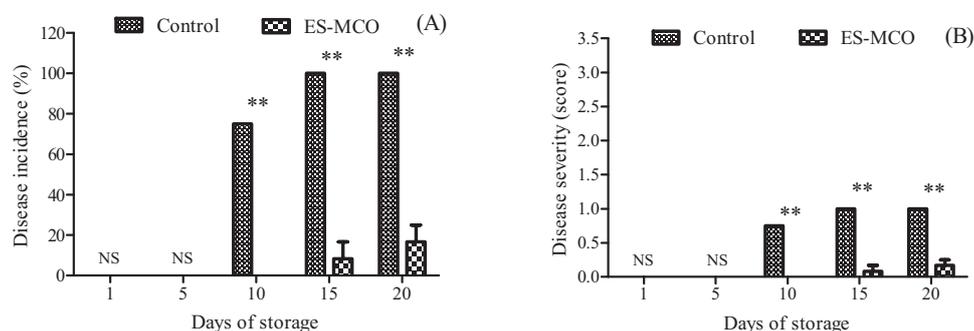
### Effect of TSS/TA treatment on disease incidence and severity

Rot symptoms first appeared in the control fruits on day 10 of storage compared to on day 15 for the ES-MCO-treated fruit. The disease incidence increased during the storage period. On the last day (day 20), the incidence for the control was 100%, whereas, in the ES-MCO-treated fruit, it was only 16.67% (Fig. 8A). The disease severity is shown in Fig. 8B. The ES-MCO-treated fruits had significantly lower severity scores than the control fruits. On day 20, the disease severity scores for the ES-MCO-treated fruits and the control fruits were 0.17 and 1.00, respectively. This result suggested that the ES-MCO treatment suppressed rot disease in pineapple fruit.

## Discussion

Nowadays, coating technology is widely used to extend the shelf life of horticultural products, with the coating playing

a role in micro-controlled atmosphere technology and affecting the respiration pathway, improving the quality, reducing CI and delaying the ripening of various fresh produce (Zhang et al., 2021). The ripening of pineapple fruit can be observed in the development of the fruit eye color. The eyes are dark green in the immature stage and change to light green and yellow when the fruit is fully ripe (Shamsudin et al., 2007). In the present study, the change in pineapple color was determined using the colorimeter and reported as lightness ( $L^*$ ), red-green ( $a^*$ ) and yellow-blue ( $b^*$ ) values. During storage, the  $L^*$  and  $a^*$  values of the pineapple peel increased, which corresponded with ripening of the pineapple. At the same time, the  $L^*$  values of the pineapple pulp decreased, and the  $a^*$  values of the pineapple pulp increased. This study found that coating pineapples with ES-MCO delayed the increase in the  $L^*$  and  $a^*$  values for the peel and decreased the  $L^*$  values for the pulp. Likewise, the  $b^*$  value for the pulp of the coated pineapples was lower than for the control (data not shown). Surprisingly,  $a^*$  values in the pulp of the ES-MCO-treated pineapple increased significantly compared to those of the control, even though those values were only slightly greater than for the control set. The ES-MCO slowed down the color change in the peel and pulp because the coating had a film-forming property that obstructed the flow of oxygen into the fruit. Consequently, respiration was reduced due to the limited amount of oxygen, while the  $CO_2$  was the by product of respiration increased inside the fruit, becoming a crucial factor in preventing chlorophyll degradation (Maftoonazad et al., 2007). Thus, the peel color of the ES-MCO-treated pineapple was greener than for the control.



**Fig. 8** Disease incidence (A); and severity (B) of fruit rot in pineapple after coating with ethanolic shellac - modified coconut oil (ES-MCO) compared with uncoated fruit (control) during storage at 13°C and 95% relative humidity for 20 d, where results are means  $\pm$  SD ( $n = 8$ ); \*\* and NS indicate highly significant ( $p < 0.01$ ) and non-significant differences between control and treatment within each day, respectively.

The ES-MCO treatment effectively reduced the respiration rate and weight loss of the pineapples. It has been reported that respiration is essential in many metabolic processes, such as the ripening process, physiological disorders, weight loss and pathological decay. Metabolic activity is associated with enzymatic oxidation caused by using oxygen to develop the activity (Wu, 2010), where a reduced respiration rate results in a decrease in various metabolic processes in plants. Some researchers discovered that this kind of lipid-based coating can slow the increase in respiration and ethylene production in citrus and jujube fruits throughout storage (Chen et al., 2019; Nasrin et al., 2020). The coating on the fruit created a thin, layer-like barrier between the fruit surface and the ambient storage atmosphere, which limited the movement of oxygen, carbon dioxide and moisture, thereby reducing the respiration rate, ethylene production and water loss (Nasrin et al., 2020). The layer of film that covered the fruit surface had the same function as modified atmosphere packaging, which can modify the internal atmospheric gases of the fruit by reducing endogenous oxygen that is a substrate for the respiration (Du et al., 1998). This impact led to a low respiration rate of the fruit. In a similar study by Sripong et al. (2015), ES-MCO treatment reduced the respiration rate, weight loss and ripening process in mangoes. Jitareerat et al. (2017) demonstrated that ES-MCO treatment reduced weight loss in gamma-irradiated rambutan by stabilizing the respiration rate.

Ethylene is the main hormone involved in the ripening process, including color change, nutrition, tissue softening, flavor and taste (Barry and Giovannoni, 2007). However, the current observations indicated that ‘Pattavia’ pineapple produced very low ethylene levels in the range 0–0.003 mL/kg/hr in the ES-MCO-treated and control fruit (data not shown). In addition, Boonyaritthongchai and Supapvanich (2017) reported that low ethylene production was detected in ‘Pattavia’ pineapple fruit during storage at 13°C.

IB caused by membrane structure deterioration permits the normally plastid PPO access to phenolic compounds in the presence of oxygen (Moon et al., 2020). The phenylpropanoid pathway synthesizes the phenolic compound, whereas lignin (one of the phenolic compounds) is synthesized in the last state of lignification by POD (Aquino-Bolaños and Mercado-Silva, 2004; Kumar et al., 2020). PPO and POD are crucial parts of enzymatic browning, participating in the oxidation and accumulation of phenolic compounds and resulting in tissue browning (Ma et al., 2021). The present study showed that the development of IB in pineapple fruit was accompanied

by increased PPO and POD activity levels, including the total phenolic and MDA contents, during storage at low temperature. However, ES-MCO treatment could alleviate IB by suppressing those browning enzyme activity levels (PPO and POD) and retarding the accumulation of phenolic compounds and MDA content. The coating acted as a gas barrier, modifying the internal atmosphere and resulting in lower internal O<sub>2</sub> and higher internal CO<sub>2</sub> concentrations. In conditions with low internal O<sub>2</sub>, the oxidation reaction of PPO will decrease (Worrell et al., 2002). In addition, coatings can maintain membrane integrity and reduce the PPO and POD activity levels through membrane separation between enzymes and phenolics, leading to reduced phenolic oxidation and consequently low browning and a low phenolic content (Saba and Sogvar, 2016). The results of the present study correlated with the study of Pasquariello et al. (2015), where chitosan coating inhibited the PPO and POD activity levels while sustaining membrane integrity by delaying increased MDA production. Inhibition of PPO and POD by a chitosan coating lowered flesh browning and extended the shelf life of sweet cherries and longans (Shi et al., 2013). Furthermore, Ali et al. (2019) found that aloe vera gel coating reduced the activity levels of PPO and POD, inhibited MDA accumulation and maintained the change in phenolic content of litchi fruit. Promyou and Supapvanich (2011) reported that shellac wax reduced weight loss and alleviated CI. Shellac-wax-coated jujube fruit had a high total phenolic content and antioxidant activity, while the activity levels of PPO and lipoxygenase and the content of MDA were low.

The quality attributes of fresh fruit, such as texture, flavor, and nutritive value, are important factors in consumer buying decisions. The TSS/TA ratio is the primary index indicating taste. This ratio in the uncoated pineapple decreased during storage at 13°C for 20 d because the TSS content generally decreases during storage while organic acid production increases (Dutta et al., 2016). This is related to the product from sugar metabolism (monosaccharides) in the TCA cycle, which is citric acid (Ladaniya, 2008). In addition, AsA is synthesized from monosaccharide (D-glucose) (Loewus, 1999). AsA is an important antioxidant in plants; it has the function of fighting ROS directly or indirectly (Noctor and Foyer, 1998). The present work found that ES-MCO-treated pineapple had a significantly higher TSS/TA ratio and AsA content than the control. The application of the ES-MCO coating may delay the degradation of carbohydrates (Huang et al., 2021) such as TSS; therefore,

the TSS/TA ratio of coated pineapple was higher than for the control. Basumatary et al. (2021) found that chitosan coatings containing ZnO nanoparticles maintained the TSS/TA ratio in pineapple fruit. Additionally, the TSS, TA and AsA contents in pears and mandarin were maintained by applying an edible coating (Lin et al., 2008; Ali et al., 2021). Such a coating has a barrier potential to moisture and oxygen movement in the fruit and in exchange with the local environment, which reduces transpiration, the respiration rate and the oxidative reaction of pineapple fruit (Ma et al., 2021). The coating probably reduced oxygen diffusion, inhibiting the oxidation-based deteriorative reaction of AsA in the fruit (Khodaei and Hamidi-Esfahani, 2019).

Pineapple fruit rot disease was reduced by the ES-MCO coating. MCO contains several monoglycerides, such as lauric acid, monolaurin and dilaurin, which have antifungal properties (Effiong et al., 2019; Sripong et al., 2023). This information was confirmed in harvested rambutan and mangosteen after coating with ES-MCO, which showed low disease incidence and severity (Thuong et al., 2015; Jitareerat et al., 2017). Monoglycerides are derivatives of fatty acids, with their molecules being small enough to penetrate and dissolve in the lipid phase of microbial cell membranes, resulting in the malfunction of cells. Furthermore, they inhibit the enzymes involved in energy production and nutrient transfer, leading to the death of cells (Luo et al., 2014).

In conclusion, the ES-MCO (consisting of 8% shellac and 2% modified coconut oil) treatment could retard the internal browning of 'Pattavia' pineapple stored at 13°C by suppressing the activity levels of PPO and POD and the accumulation of phenolic compounds that are directly involved with the browning reaction. Furthermore, the ES-MCO treatment delayed the respiration rate, weight loss and color changes in peel and pulp, while maintaining the AsA content and the TSS-to-TA ratio and suppressing cell membrane deterioration, as indicated by the low MDA content. Furthermore, the ES-MCO treatment controlled fruit rot disease in pineapple during storage at 13°C.

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### Conflict of Interest

The authors declare that there are no conflicts of interest.

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