

## Research article

# Sericin and Pineapple Fruit Extract as Natural Agents Inhibiting Browning and Improving Antioxidant Activity in Fresh-cut Ripe Mangoes cv. Nam Dok Mai

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## Abstract

### Keywords

browning;  
sericin;  
pineapple juice;  
antioxidants;  
fresh-cut fruit

Recently, the use of natural agents to improve quality has attracted a lot of attention on fresh-cut produce and minimally processed fruit. The aims of this work were to investigate the efficiency of sericin and fresh pineapple juice (selected natural agents) on browning inhibition and antioxidant activity of fresh-cut 'Nam Dok Mai' mangoes during refrigeration (4°C). Based on a preliminary study and our previous work, 2% sericin (S) and 50% pineapple juice (PJ) were selected. Biological parameters related to enzymatic browning incidence, antioxidants and antioxidant enzyme activity of the fresh-cut mangoes immersed in 2% S, 50% PJ and 50% PJ incorporated with 2% S (PJ+S) were investigated. Immersion in S, PJ or PJ+S inhibited enzymatic browning due to delaying of discoloration, lowering of browning index (BI) and increases in polyphenol oxidase (PPO) and peroxidase (POD) activities compared to control treatment. The lowest PPO and POD activities and total phenolic content were found in the S treated fresh-cut mangoes. PJ+S treated fresh-cut mangoes had antioxidant activities higher than other treatment. All treatments enhanced the activities of antioxidant enzymes, especially PJ immersion. Therefore, S and PJ show potential as natural agents that can inhibit browning and improve antioxidant activity in fresh-cut mangoes during refrigeration.

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## 1. Introduction

'*Nam Dok Mai*' mango is a commercially famous mango cultivar originated in Thailand. There are two strains of '*Nam Dok Mai*' mango as '*Si Thong*' and '*No.4*' but '*Nam Dok Mai Sri Thong*' strain is the most famous cultivar for export from Thailand. In the first half of the year 2021, the value of Thailand's mango exports was 2,935 million baths [1]. The flesh of '*Nam Dok Mai Sri Thong*' mango is smooth and silky, and the fruit has beautiful golden-hued skin, sweet fragrance, a very sweet taste, and a less fibrous texture. Additionally, the '*Nam Dok Mai Sri Thong*' mango can be produced almost all year-round in Thailand. It is known for its health benefits and is rich in nutrition values including high vitamins, carotenoids, and dietary fibre. Other health benefits also include improving the digestive system, balancing blood pressure and strengthening immunity [2].

Fresh-cut mangoes or minimally processed mangoes are very popular in Thailand. They are highly attractive to customers because of their high nutritious value, low price, and good flavour. However, minimally process fruit or fresh-cut product is very susceptible to moisture loss, discoloration, and microbial spoilage, which are causes of shelf-life limitation [3]. They are products that although they have been cleaned, peeled, and cut for convenience still contain living tissue that can undergo cellular respiration. Nowadays, the fresh-cut industry is growing rapidly and continues to expand because of the huge increase in demand for fresh-cut products. The major problems of minimally processed fruit or fresh-cut produce are desiccation and browning, which start when the peels of fruits such as apple, banana and mango are removed, and tissues are cut. Desiccation and browning result in reducing fruit quality, color, shelf life, safety and nutritional value during storage, factors that cause consumers to reject the product, especially due to their appearance [4]. These symptoms occur when the fruits are cut, and the plant tissues are exposed to oxygen. Phenolic compounds such as *o*-diphenols are rapidly oxidized to *o*-quinones by polyphenol oxidase (PPO), enzymes located in the plastid, leading to polymerization that turns to brown pigment of melanin [5].

At present, using natural agents to alleviate browning symptom of fresh cut produce become a trending for food safety and human health concerns. Current research focused on the use of natural anti-browning agents that can improve nutritional values and to avoid the usage of artificial chemicals because of consumer concerns about human health and the side effects of such chemicals. The use of natural anti-browning agents is the best choice and in favourable for consumers. Recent reports revealed that natural agents such as honey [6], purslane [7], lemon [8], onion [9], *Aloe vera* gel [10], sericin [11] and pineapple extract [12] could be used to control browning symptoms in fresh-cut produce. Pineapple (*Ananas comosus*) is one of the important commercial fruits in Thailand. Pineapple is a non-climacteric fruit that is popular due to its unique aroma and flavour. It contains nutrients that are good for human health [13]. Chaisakdanugull *et al.* [12] reported that pineapple juice, due to its organic acid content, may assist in the inhibition of enzymatic browning in fresh-cut produce. The dominant organic acid in pineapple juice is citric acid, which chelates copper and lowers pH, leading to the inhibition of PPO activity. Sericin is a protein created by silkworms (*bombyx mori*). Sericin is generally recognized as a safe agent that has anti-browning and antioxidant functions [14]. Sericin (S) is rich in amino acids that have polar hydroxyl, carboxyl, and amino side-groups. It inhibits activity of PPO by reducing *o*-quinones, thus preventing melanin polymerization and by chelating the copper from PPO molecule [15]. Chimvaree *et al.* [16] reported that sericin decreased browning incidence by inhibition of PPO and PAL activities, resulting in delaying color changes on the surfaces of minimally processed "*Nam Dok Mai No. 4*" mangoes.

Both of sericin (S) and pineapple juice (PJ) have been shown to exhibit anti-browning function in fresh-cut fruit but so far there is no evidence or report to show the synergistic effect of sericin incorporated with pineapple juice on inhibiting browning and inducing antioxidant properties in fresh-cut or minimally processed '*Nam Dok Mai Sri Thong*' mango. So, the effects of sericin and

pineapple juice added separately and in combination on physical quality, browning incidence, and antioxidant properties of fresh-cut or minimally process ‘*Nam Dok Mai Sri Thong*’ mango are of particular interest in this study.

## 2. Materials and Methods

### 2.1. Preparation of fresh-cut mango

‘*Nam Dok Mai Sri Thong*’ mangoes were obtained from a commercial orchard at Chachoengsao province, Thailand. The mangoes were harvested at full-mature stage (approximately 115-120 days after anthesis). Mangoes of uniform size that had no diseases symptoms and physical damage were selected and then transported to the laboratory at the Division of Postharvest Technology, King Mongkut’s University of Technology Thonburi within 6 h. Mangoes were ripened by immersion in 600  $\mu\text{L L}^{-1}$  ethephon for 3 min and then left at 25°C for 2 days. After ripening, the mangoes were cleaned with 200  $\mu\text{L L}^{-1}$  NaOCl for 2 min. Then, mango peels were removed, and the fleshly tissues were half-cut lengthwise using a sharp knife. Each half-cut fruit was cut crosswise into 4 equal pieces.

### 2.2 Preparation of pineapple juice

Ripe pineapples cv. ‘Trad-see-thong’ (150 days after flower induction) were obtained from a fruit wholesale market (Talad Thai market), Bangkok, Thailand. The fruits were washed by rinsing with tap water and dipping in 200  $\mu\text{L L}^{-1}$  NaOCl for 30 min. After that, pineapple peels were removed. The whole peeled fruits were blended, and the juice was extracted. The pineapple juice (PJ) was then diluted into 50% (v/v) and 75% (v/v) pineapple juice (PJ) using sterilised water.

### 2.3 Preparation of sericin solution

Sericin (S) was obtained from Faculty of Science and Technology, Thammasat University, Thailand. In this study, 2% (w/v) of sericin was used which was in accordance with the method of Chimvaree *et al.* [16].

### 2.4 Preliminary experiment

A preliminary study was carried out with three treatments including untreated (control), 50% PJ dip and 75% PJ dip. The fresh-cut mangoes were immersed in 50% or 75% PJ for 1 min. Four equal pieces of samples were packed into a polyethylene terephthalate clamshell box and then refrigerated at 4±1°C for 6 days. There were three replications in each treatment. Sampling was done every two days to determine the color changes, which were expressed as lightness ( $L^*$ ) value, browning index (BI) and visual appearance.

### 2.5 Main experiment

The fresh-cut mangoes were immersed in 50% PJ for 1 min, 2% S for 10 s or the immersion in 50% PJ for 1 min followed by 2% S for 10 s (PJ+S). Untreated fresh-cut mangoes were used as control samples. Four pieces of the fresh-cut mangoes were placed in a polyethylene terephthalate clamshell packaging then stored at 4±1°C for 6 days. There were three replications in each treatment.

Sampling was done every two days to determine biochemical parameters which related to enzymatic browning incidence and antioxidant capacity, including antioxidant enzymes activities.

### 2.5.1 Color and browning index (BI)

The color of the cut surfaces of mango pieces was evaluated by a portable colorimeter CR-400 (Minolta, Japan). Color changes expressed as  $L^*$  (lightness),  $a^*$  (green-red) and  $b^*$  (blue-yellow) values were measured. The  $\Delta E^*$  value or color difference was calculated following formula (1). The BI value of fresh-cut mangoes was computed according to equation (2) [17].

$$\Delta E^* = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \quad (1)$$

where  $\Delta L^* = (L^*_0 - L^*_x)$ ,  $\Delta a^* = (a^*_0 - a^*_x)$ ,  $\Delta b^* = (b^*_0 - b^*_x)$ , 0 = initial day, x = date of measurement

$$BI = [100 (x - 0.31)] / 0.172 \quad (2)$$

where  $x = (a^* + 1.75L^*) / (5.645L^* + a^* - 0.3012b^*)$

### 2.5.2 Total phenolic content (TP)

Phenolic compounds of sample were extracted using 60% ethanol. Total phenolic content was determined using a modification of Slinkard and Singleton [18]. Total phenolic content was recorded as  $\mu\text{g}$  gallic acid equivalent per g fresh weight (FW) ( $\mu\text{g GE/g FW}$ ).

### 2.5.3 Polyphenol oxidase activity (PPO) and peroxidase activity (POD) assays

The cut surface tissues of the fresh-cut mangoes were homogenized with cold (4°C) 0.05 M sodium phosphate buffer (pH 7) mixed with 0.25 g of polyvinylpyrrolidone (PVPP). The enzyme extract was collected, and this process was done under cold conditions. PPO activity was expressed as units/100g FW and was measured with a spectrophotometer at OD 410 nm. Data was calculated from the increased absorbance at 410 nm wavelength per min [19]. POD activity was measured using a spectrophotometer at OD 470 nm. Data was calculated from the increased absorbance at 470 nm per min., then recorded after guaiacol oxidation for 2 min. The data was expressed as units/100 g FW [20].

### 2.5.4 Antioxidant activity assays

Ferric reducing antioxidant potential (FRAP) [21] and DPPH free radical scavenging activity [22] were used for antioxidant assays. Sample extracts was obtained by the method described in Section 2.5.2. The ferric reducing ability or FRAP was recorded in terms of  $\mu\text{mole Trolox equivalents per g fresh weight}$  ( $\mu\text{mol TE/g FW}$ ). The free radical scavenging ability (DPPH) was evaluated by incubating the reaction for 30 min in the dark. The percentage of DPPH free radical scavenging capacity of sample extracts was calculated by equation (3).

$$\text{DPPH free radical scavenging activity (\%)} = \frac{(A_0 - A_{30})}{(A_0)} \times 100 \quad (3)$$

where:  $A_0$  = the absorbance of the reaction mixture measured at the initial time (0 min);  $A_{30}$  = the absorbance of the reaction mixture measured at the endpoint (30 min).

### 2.5.5 Antioxidant enzymes (catalase (CAT), superoxide dismutase (SOD), and ascorbic acid peroxidase (APX)) assays

The pulp of fresh-cut mangoes was extracted using the method described in Section 2.5.3. The SOD activities of the samples were determined using a spectrophotometric assay that had been modified from the protocol of Ukeda *et al.* [23]. SOD activity was expressed as units per g fresh weight (unit/100 g FW). Data was obtained at the absorbance at 470 nm after 20 min of incubation time. CAT activity was evaluated using a slight modifying method of Aebi [24]. CAT activity was expressed as units per 100g fresh weight (unit/100 g FW). APX activity was determined following the protocol of Cuvi *et al.* [25]. APX activity was expressed as units per 100 g fresh weight (unit/100 g FW).

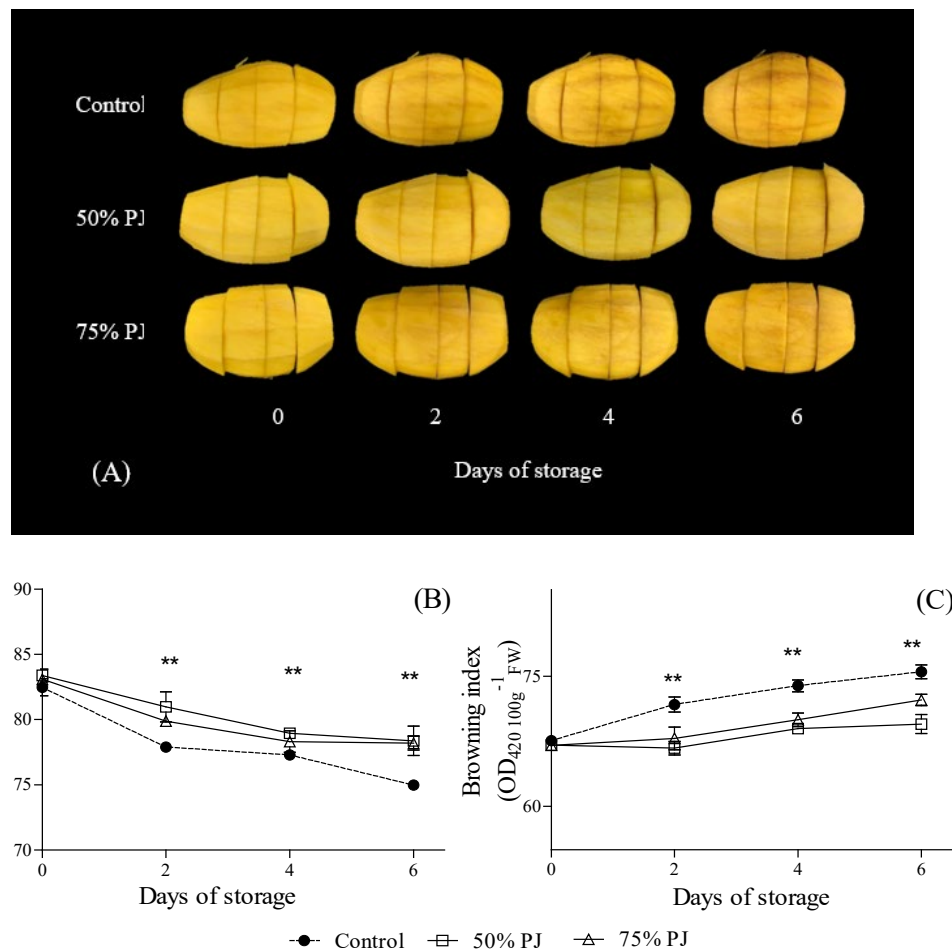
### 2.6 Statistical analysis

The averaged data was collected from 4 replications. The experiments were conducted using a completely randomized design. Analysis of variance at 95% and 99% confidence levels was performed using the version 18 SPSS software.

## 3. Results and Discussion

### 3.1 Preliminary study

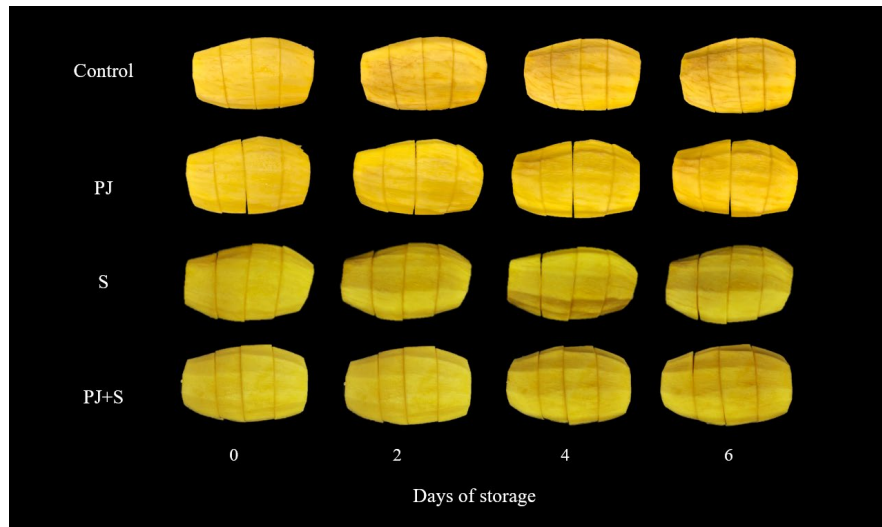
The results, shown in Figure 1, revealed that PJ immersion inhibited browning on the mango piece surface under the cold storage. Untreated samples had developed clear browning by day 2 of storage and the browning continuously increased throughout storage time. On the other hand, no browning was observed on the surfaces of both PJ treated mango pieces over the storage time. However, the darkness of mango surfaces gradually increased for both 50%PJ and 75%PJ treated samples during storage. Both PJ treatments showed significant delay in decrease of  $L^*$  values when compared with the untreated or control samples ( $P < 0.05$ ). There was no significant difference in  $L^*$  values between the 50% and 75% PJ treatments. The BI value of untreated mango pieces was significantly higher than those of 50% and 75% PJ treated mango ( $P < 0.05$ ), and it evidently increased under cold storage. There was no significant difference between the two PJ treatments under cold storage for 4 days. Moreover, the BI value of 50% PJ treated mango pieces was obviously lower than the value of 75% PJ treated and control samples on day 6 of the cold storage. The  $L^*$  value decrease with increase in browning index of mango pieces were associated with browning enhancement under cold storage. This result was in agreement with Boonyariththongchai *et al.* [26], who suggested that pineapple extract prevented discoloration of minimally processed mangoes under storage. Supapvanich *et al.* [27] also revealed that the core of pineapple fruit extract potentially reduced browning symptoms in fresh-cut wax apples because of the reduction of enzymatic browning. Chaisakdanugull *et al.* [12] showed that pineapple juice could prevent browning of banana flesh because the organic acids present in the juice inhibited enzymatic browning. Regarding the visual appearance and superficial color values of mangoes treated with PJ, 50% PJ immersion was selected for further study. Mango pieces dipped into 75% PJ displayed more browning and surface drying when compared to 50% PJ, an effect that was probably due to the 75% PJ containing higher amounts of sugar and organic acids than 50% PJ that caused more osmotic dehydration on the mango cut surface. These results led to the appearance of dried surface and browning on the 75% PJ treated mango pieces. A similar phenomenon was reported by Rux *et al.* [28], who observed that fresh-cut apples dipped into a 30% sugar solution showed higher browning index than those dipped in 10 and 20% sugar solution, while both these concentrations produced fewer browning symptoms than the control set.



**Figure 1.** Visual appearance (A),  $L^*$  value (B) and browning index (C) of fresh-cut mangoes cv. Nam Dok Mai Sri Thong dipped and undipped (control) with pineapple juice at various concentrations under cold storage at  $4\pm 1^\circ\text{C}$  for 6 days. The shown data were means ( $n = 4$ ), and the vertical bars represent the standard deviation (SD). The asterisks show the difference between treatments at each storage period, \* represents significant difference at  $P < 0.05$ , and \*\* represents significant difference at  $P < 0.01$ .

### 3.2 Visual appearance

Figure 2 shows the change in visual appearance of mango pieces dipped with PJ, S and PJ+S solutions when compared with control or undipped fresh-cut mangoes during the refrigeration period. It clearly showed that PJ, S and PJ+S immersions inhibited browning incidence on the cut surfaces of the mango pieces. Browning symptoms occurred evidently on the surfaces of control samples and continuously increased with storage period. A slight darkness on the cut surfaces of PJ treated samples was observed after storage for 4 days compared to S and PJ+S treated samples. This

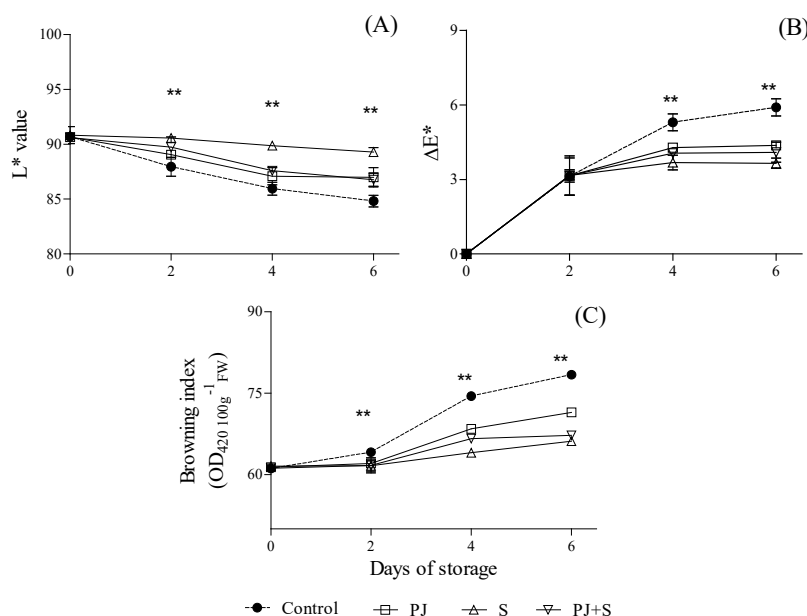


**Figure 2.** Visual appearance of mango pieces dipped and undipped (control) with 50% pineapple juice (PJ), 2% sericin (S), the combination of PJ and S (PJ+S) under cold storage at  $4\pm 1^\circ\text{C}$  for 6 days.

indicated that PJ and S had the potential to maintain visual appearance and prevent discoloration of fresh-cut mangoes throughout cold storage time. Previous studies also revealed that sericin or pineapple juice maintained visual appearance of fresh-cut fruits during storage [11, 16, 27]. We also found that S treatments (S and PJ+S) could maintain the visual appearance of fresh-cut mangoes rather than PJ treatment alone. This indicated that sericin might have greater potential to control discoloration in fresh-cut mangoes than pineapple fruit extract.

### 3.3 Color changes

The color changes in the cut surface of mangoes treated with PJ, S and PJ+S, expressed as  $L^*$ ,  $\Delta E^*$  and BI values, are shown in Figure 3. A rapid reduction in  $L^*$  value was found in the control sample, and it was clearly lower than that of all treated mango pieces over storage ( $P < 0.05$ ). Sample dipped with sericin maintained their  $L^*$  values, which were significantly higher than those samples immersed in PJ and PJ+S ( $P < 0.05$ ). The  $\Delta E^*$  value continuously increased with storage period. There were no significant differences of  $\Delta E^*$  values among treatments during storage for 2 days. Afterward, a significant increase in  $\Delta E^*$  value was observed in the untreated (control) samples which was remarkably higher than other treatments ( $P < 0.05$ ). The increased  $\Delta E^*$  values of S treated sample tended to be lower than those of PJ and PJ+S treated mango pieces; however, no significant difference in  $\Delta E^*$  value was observed among PJ, S and PJ + S treatments. In the similar vein, the increase in BI value was found in all treatments under the cold storage period and the highest increased BI was found in the untreated samples which was notably higher than other treatments throughout of the storage ( $P < 0.05$ ). The increased BI values were delayed by S, PJ+S and PJ immersion, respectively. These revealed that the decrease in  $L^*$  value was closely related to the increase in BI and  $\Delta E^*$  values of fresh-cut mangoes. This was in agreement with the report of García and Searle [29]. The application of sericin in the control of discoloration of fresh-cut produce was

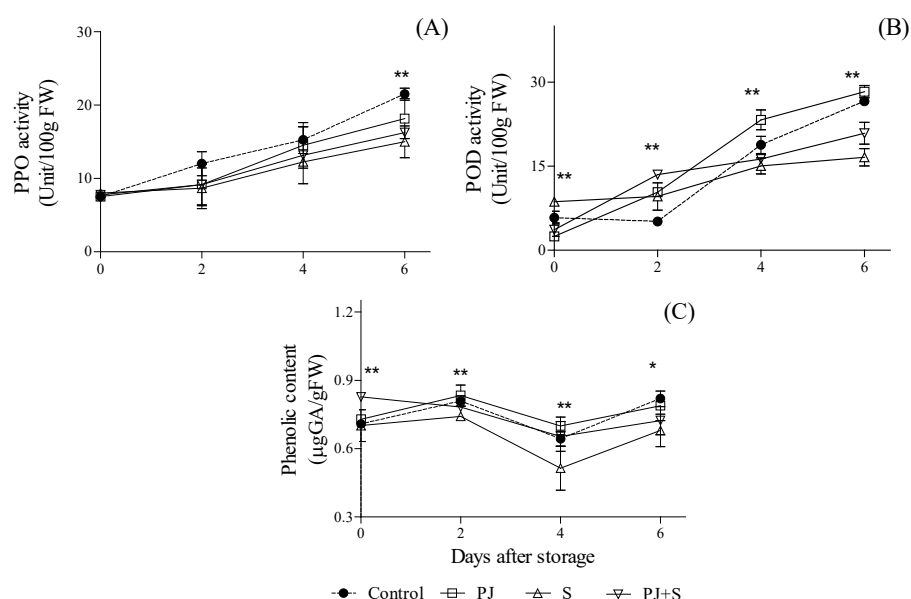


**Figure 3.**  $L^*$  value (A),  $\Delta E^*$  value (B) and browning index (C) of fresh-cut mangoes dipped and undipped (control) with 50% pineapple juice (PJ), 2% sericin (S), the combination of PJ and S (PJ+S) under cold storage at  $4 \pm 1^\circ\text{C}$  for 6 days. The data shown are means ( $n = 4$ ), and the vertical bars represent the standard deviation (SD). The asterisks show the differences between treatments of each storage period, \* represents significant difference at  $P < 0.05$ , and \*\* represents significant difference at  $P < 0.01$ .

reported for minimally processed eggplants [30], apple slices [31], and fresh-cut ‘Nam Dok Mai’ No. 4 mangoes [11, 16]. Immersion in pineapple fruit extract inhibited browning and maintained surface color in banana pulp [12], fresh-cut wax apples [27], fresh-cut mangoes [26] and fresh-cut apples [32]. These previous studies suggested that sericin and pineapple fruit extract had the ability to reduce the increased enzymatic browning reaction. Moreover, we found that the capacity of PJ+S treatment to maintain surface color was not obviously different from S or PJ treatment alone. This revealed that there was no synergistic effect on browning inhibition in the combined S and PJ treatments.

### 3.4 PPO, POD activities and total phenolic content

Figure 4 shows the PPO, POD activities and total phenolic content of the mango pieces during 6 days under cold storage at  $4^\circ\text{C}$ . Browning reaction caused by the oxidation of enzymatic browning of total phenolic content was catalysed by both PPO and POD enzymes [33-35]. The PPO and POD activities of all treatments increased with storage period. Increase in PPO activity was delayed by PJ, S and PJ+S immersion. The increased PPO activity of the undipped (control) samples was significantly higher than other treatments on day 6 under cold storage ( $P < 0.05$ ). Interestingly, the PPO activity of sericin treated sample tended to be lower than other treatments. The results revealed that a constant increase of PPO activity was associated with elevated browning incidence, whereas an increased POD activity was not observed. On day 2, POD activity of all treatments was



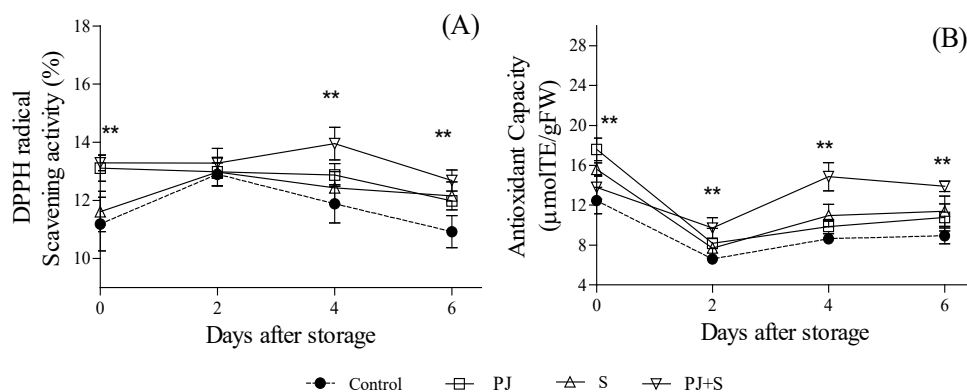
**Figure 4.** PPO activity (A), POD activity (B) and total phenolic content (C) of treated and untreated (control) fresh-cut mangoes with 50% pineapple juice (PJ), 2% sericin (S), the combination of PJ and S (PJ+S) under cold storage at  $4\pm1^{\circ}\text{C}$  for 6 days. The data shown are means ( $n = 4$ ), and the vertical bars represent the standard deviation (SD). The asterisks show the differences between treatments of each storage period, \* represents significant difference at  $P < 0.05$ , and \*\* represents significant difference at  $P < 0.01$ .

remarkably higher than control samples ( $P < 0.05$ ). After that, POD activity of PJ treated mango was notably higher than other treatments. The POD activities of control, S and PJ+S treatments were similar on day 6 of storage. These contrasted with the browning incidence and BI value as shown in Figure 2 and 3C. Zhang and Shao [36] and Chisari *et al.* [37] suggested that as an oxidative enzyme, POD was not the primary enzyme related to the browning symptom in fruits. Interestingly, both S treatments could delay increased POD activity. The total phenolic content of all treatments seemed to remain constant during storage. Total phenolic content of S treated fresh-cut mangoes was lower than other treatments and it was significantly different when compared to the PJ treated samples. Although total phenols content is recognised as the substrate of PPO [38], the change in total phenolic content of minimally processed mango or fresh-cut mangoes was not consistent with increased browning incidence under cold storage. This indicated that PPO acts as a key enzyme that plays a major role in the development of browning symptom in fresh-cut mangoes under storage period. Moreover, we also found that the lowest discoloration of S-treated fresh-cut mangoes (shown in Figure 3) was associated with the lower total phenolic content and activities of PPO and POD when compared to other treatments. Puangphet *et al.* [30] revealed that sericin hydrolysate delayed browning in fresh-cut fruits owing to inhibitory PPO activity. Chimvaree *et al.* [16] also reported that the increased PPO activity of minimally processed ‘Nam Dok Mai No. 4’ mangoes was lowered by dipping with sericin. The efficiency of sericin in reducing PPO activity might be due to copper ion chelation [31]. Moreover, sericin contains a lot of proteins, peptides, dipeptides, and amino acids and in particular, serine which contains a high content of hydroxyl group: thus, sericin was

more functional in preventing the changing color of fresh-cut or minimally processed fruits [39]. The effect of PJ on browning inhibition of fresh-cut fruits was found to be due to organic acids content, and especially ascorbic acid and citric acid [12], and bromelain [27]. Moreover, we also found that the combination treatment of S and PJ did not have synergistic effects or associative effects on the inhibition of PPO and POD activities during storage.

### 3.5 Antioxidant activities

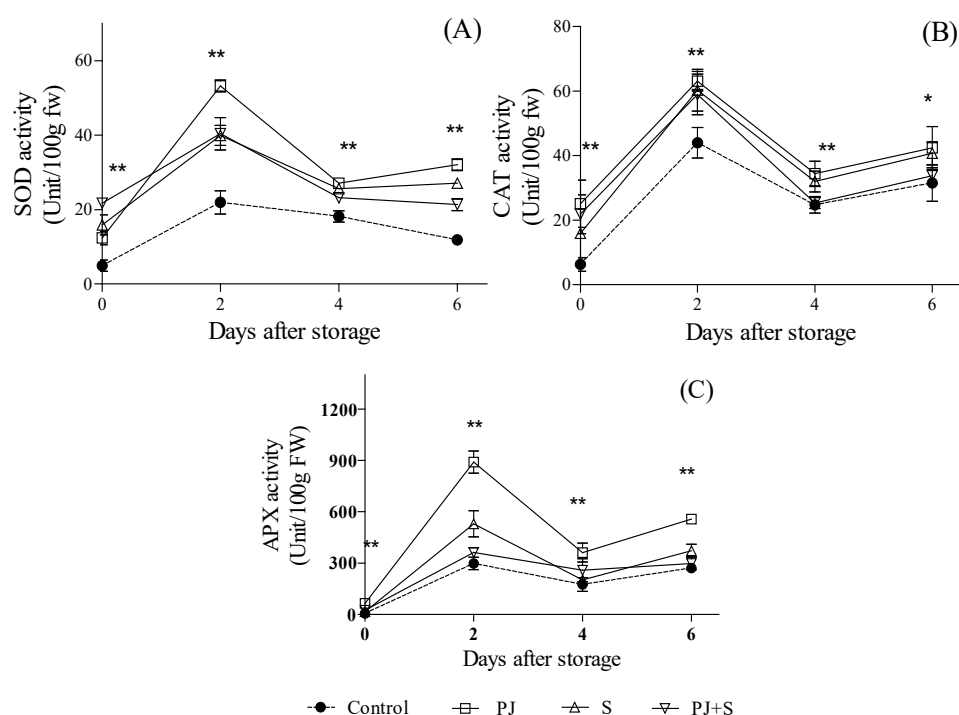
It has been established that enzymatic browning is an oxidation reaction [40]. Antioxidants can alleviate the initiation of brown pigment by acting with oxygen and reacting with the intermediate products of browning, breaking the chain reaction and inhibiting melanin formation [41]. Antioxidant ability was evaluated by determining DPPH free radical scavenging activity and FRAP ferric reducing antioxidant power, the results of which are shown in Figure 5. We found that both antioxidant activities of PJ+S treated fresh-cut mangoes were remarkably higher than other treatments during cold storage ( $P < 0.05$ ). Untreated sample revealed the lowest DPPH free radical scavenging activity and ferric reducing antioxidant power. Both of the antioxidant abilities in PJ treated fresh-cut mangoes were also higher than those of non-treated fresh cut mango. Changes in both antioxidant abilities of PJ and S treated fresh-cut mangoes were indistinguishable during cold storage. This indicated that the reduction of increased brown pigment in the fresh-cut mangoes correlated with high antioxidant activities in all treated samples when compared to the control samples. Micheal and Subramanyam [42] suggested that sericin protein contains hydroxyl groups including serine, asparagine, and threonine, which possess the function of chelators and antioxidant ability. In addition, pineapple contains bromelain, that is, a protease which can act as an antioxidant and anti-browning. The effectiveness of pineapple juice on reducing browning in fresh-cut produce relates to its organic acids and phenolic compound content [12, 43]. Our results revealed that the combination treatment of PJ and S could induce antioxidant activities in fresh-cut mangoes which may improve the nutritional value of the fresh-cut fruits.



**Figure 5.** DPPH free radical scavenging activity (A) and FRAP ferric reducing antioxidant power (B) of fresh-cut mangoes treated and untreated with 50% pineapple juice (PJ), 2% sericin (S), the combination of PJ and S (PJ+S) under cold storage at  $4\pm 1^\circ\text{C}$  for 6 days. The data shown are means ( $n = 4$ ), and the vertical bars represent the standard deviation (SD). The asterisks show the difference between treatments of each storage period, \* represents significant difference at  $P < 0.05$ , and \*\* represents significant difference at  $P < 0.01$ .

### 3.6 Antioxidant enzymes activities

The antioxidant enzymes (SOD, CAT and APX) of the samples were measured and the data is presented in Figure 6. All S and PJ treatments could enhance antioxidant enzymes activities during storage. The lowest activities of SOD, CAT and APX were found in control samples throughout cold storage. We found that the SOD and APX activities of PJ treated fresh-cut mangoes were significantly higher than those of other samples. Sample dipped in S solution also induced all antioxidant enzymes activities. However, no synergistic and associative effects of the combination of S and PJ enhancing SOD, CAT and APX activities in fresh-cut mangoes were observed. The rise in SOD, CAT and APX activities of PJ treated mangoes might be associated with the absorption of pineapple juice into mango pulp. It is widely recognised that SOD, CAT and APX are antioxidant enzymes which can reduce oxidation reactions by scavenging free radicals [7, 23, 24, 44]. However, the mechanisms of pineapple fruit extract and sericin inducing antioxidant enzymes activities in fresh-cut fruits have not yet been elucidated.



**Figure 6.** Superoxide dismutase, SOD (A), catalase, CAT (B) and ascorbate peroxidase, APX (C) activities of fresh-cut mangoes treated and untreated (control) with 50% pineapple juice (PJ), 2% sericin (S), the combination of PJ and S (PJ+S) under cold storage at  $4\pm1^{\circ}\text{C}$  for 6 days. The data shown are means ( $n = 4$ ), and the vertical bars represent the standard deviation (SD). The asterisks show the difference between treatments of each storage period, \* represents significant difference at  $P < 0.05$ , and \*\* represents significant difference at  $P < 0.01$ .

## 4. Conclusions

The applications of S, PJ and PJ+S obviously maintained visual appearance and inhibited browning symptom of fresh-cut ‘Nam Dok Mai Sri Thong’ mangoes during refrigeration when compared to the untreated fresh-cut mangoes. The S immersion delayed the colour changes based on  $L^*$   $\Delta E^*$  value and BI in fresh cut mangoes when compared to PJ+S and PJ treatments. Both sericin and pineapple fruit extract displayed the ability to inhibit enzymatic browning due to the reduction of increased PPO activity. PJ+S immersion maintained the antioxidant activities of the fresh-cut mangoes in a superior way to S or PJ treatment alone. PJ immersion alone obviously enhanced antioxidant enzymes activities as SOD, CAT and APX when compared to S and PJ+S treatments. These results indicated that sericin and pineapple fruit extract are natural agents that have great potential to be used for controlling browning incidence as well as improving antioxidant activity in fresh-cut fruits during cold storage.

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