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Research article

Effect of sericin coating on reducing browning of fresh-cut mango cv. 'Nam Dok Mai No. 4'

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Abstract

The effects were investigated of a sericin-based edible coating on the browning inhibition of fresh-cut 'Nam Dok Mai No.4' mango during storage. Mango fruits were cut in the longitudinal direction and cross-sectional direction and then these fresh-cut mangos were dipped in sericin solution or distilled water (control) and samples were placed in a rigid-plastic box and stored at 10°C to accelerate browning. The results showed that the sericin-coated fruit samples had lower browning scores, browning index and enzymatic browning activity compared to the control set. Fresh-cut mango coated with sericin solution had higher L^* color values than the control. The hue angle and chroma values of samples treated with sericin remained constant while non-treated fruit had gradual decreases during storage. The sericin treatment also decreased the browning enzymatic activities of polyphenol oxidase, peroxidase, phenylalanine ammonialyase and the phenolic content. On the other hand, there was no significant difference in the firmness and ascorbic content between the treatment and control sets. Sericin-treated mango had a storage life of 3 d while the control set had a storage life of 1 d.

Introduction

'Nam Dok Mai' mango (*Mangifera indica* L.) belongs to the family Anacadiaceae, as an Indochinese type mango; this type is considered one of the choicest fruits of the world because of attractive color, delicious taste and excellent nutritional properties (Suwapanich, 2006). In particular, 'Nam Dok Mai No. 4' has been promoted to expand the export value and nowadays, the demand for fresh-cut goods has increased globally to support the current modern lifestyle (James and Ngarmsak, 2010). Fresh-cut mango is very perishable based on dehydration, discoloration with browning symptoms and spoilage (Baldwin et al., 1998).

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The browning incidence in fresh-cut produce is caused by polyphenoloxidase (PPO; EC.1.10.3.1), peroxidase (POD; EC 1.11.1.7) and phenylalanine ammonialyase (PAL; E.C. 4.3.1.5) that are related to discoloration (browning) and changes in the texture and flavor of fruits (Vamos-Vigyazo 1981). PPO rapidly oxidizes *o*-diphenols into *o*-quinones, resulting in the eventual formation of browning pigments via a non-enzymatic pathway (Alberio et al., 2015). Several chemical antibrowning agents have been estimated for their inhibition of PPO activity in fruit and vegetables. Sulfites are one of the most effective browning inhibitors (Chen et al., 2000); however, the use of sulfites for the inhibition of browning reaction has been prohibited by the U.S. Food and Drug Administration (FDA) due to adverse health effects (Coetzer et al., 2001). Recently, there has been increasing interest in using natural antibrowning agents such as honey, aloe vera gel, pineapple juice, whey protein, seaweed extracts and rice bran extracts (Chen et al., 2000; Valverde et al., 2005; Chaisakdanugull et al., 2007; Yi and Ding; 2014, Alberio et al., 2015; Augusto et al., 2016; Sukhonthara et al., 2016).

The FDA has now included sericin protein and its derivatives in the "Generally Recognized as Safe - GRAS" list (Food and Drug Administration, 2001). The main characteristic of this agent is its antioxidant and antibrowning function; therefore it has been proposed as a functional food. However, usage of commercial foods that contain this protein or any related products is still limited, with examples being the use of sericin as an ingredient in salad dressing (Takechi et al., 2011) and the use of sericin to decrease the specific volume and to darken the crust color of bread (Takechi and Takamura, 2014). The current research focused on the effects of sericin coating on the reduction of browning in fresh-cut mango cv. 'Nam Dok Mai No. 4'.

Materials and Methods

Fresh-cut mango and coating solution preparation

'Nam Dok Mai No. 4' mango (*Mangifera indica* L.) fruits were harvested at the full-mature (after 85 d of anthesis) stage with selection based on uniform size and no physical damage or infection. Fruits were hastened in ripening by immersion in 400 µL/L ethephon and then placed in a room at 28 ± 2ºC for 3 d. Ripe mangos with a firmness of 10–12 N were selected for use. They were peeled and halved and then the fleshy sections were cut using a sharp knife one in the longitudinal direction and three times in the cross sectional direction.

Sericin powder was produced for this study by the Department of Biotechnology, Faculty of Science and Technology, Thammasat University, Pathum Thani, Thailand. A coating solution was prepared based on 2 g of sericin powder which was dissolved in distilled water (100 mL). Then, the solution was heated and dissolved in an autoclave at 105°C for 5 min.

Previously, a screening test was carried out for the selection of sericin concentrations to reduce browning in fresh-cut mango. The results showed that 2% sericin tended to reduce the browning symptoms of fresh-cut mango, so 2% sericin was used in this experiment. Each half slice of fresh-cut mango was separately dipped in the sericin solution at a concentration of 0 or 2%. The fresh-cut mangos were placed in a rigid-plastic box and then stored at 10°C and 85–90% relative humidity for 4 d to accelerate the browning.

Physical changes

Color changes in the surface of fresh-cut mango were measured using a Minolta DP-301 colorimeter (Konica Minolta; Tokyo, Japan). The CIE color system was recorded as Hue angle, Chroma, ΔE and L* value expressed as lightness.

The browning score was evaluated as the incidence of visual browning on the cutting surface area which was rated based on a hedonic sensory testing scale, where 1 = no browning (excellent quality); 3 = slight browning (1–25%); 5 = moderate browning (26–50%); 7 = severe browning (51–75%); and 9 = extreme browning (76–100%). Brown color was expressed as an average value.

The firmness of the fresh-cut mango was determined using a texture analyzer (TA-XT Plus; Stable Micro System Ltd.; Surrey, United Kingdom). The firmness was measured as the maximum penetration force (N) reached during tissue breakage and was determined using a 7 cm × 5 cm flat-knife blade as a probe. The equipment settings used were 1 cm with a stroke speed of 5 mm.

Browning enzymes assay

PPO and POD activities were determined using 5 g of mango mixed with 10 mL of 0.05 mM sodium phosphate buffer (pH 7) with 0.25 g of polyvinylpyrrolidone (PVPP) which was homogenized on ice for 1 min. The homogenate of the mango fruit was centrifuged at 12,000 revolutions per minute (rpm) for 20 min at 4°C (Jiang, 2000). Supernatants were collected and filtered using Whatman paper No.1. The supernatant was used as crude enzyme extract. PPO activity assay was determined using 0.05 mM sodium phosphate buffer pH 7 (1 mL), 10 mM of phenolic substrate (1 mL) and enzyme crude extract (1 mL) that were mixed well. The enzyme solution was added to a quartz cuvette and the enzymatic browning activity was measured using a spectrophotometer at an optical density (OD) of absorbance at 420 nm (OD_{420}). The change in absorbance at 420 nm was recorded automatically after 5 min of reaction time at 25°C. One unit of enzyme activity was determined as a change in absorbance of 0.001 per minutes per milligram protein extract.

The POD activity was determined using the method of Zhang et al. (2005), against guaiacol as substrate; 100 µL supernatant was mixed with 2.9 mL 0.5 M sodium phosphate buffer (pH 7.0), 500 µL of 20 mM H_2O_2 and 500 µL of 2 mM guaiacol. The increase in the absorbance at 470 nm due to the guaiacol oxidation was recorded for 2 min. One unit of enzyme activity was defined as the amount that caused a change of 0.01 in the absorbance per minute.

PAL assay was modified from Jiang and Joyce (2003); a sample of 5 g of mango flesh was homogenized in 10 mL of 0.1 M sodium borate buffer (pH 8.0) containing 0.25 g of PVPP, 5 mM β-mercaptoethanol and 2 mM ethylenediaminetetraacetic acid at 4°C. The homogenate was centrifuged at 12,000 rpm for 20 min at 4°C and then the

supernatant was collected for enzyme activity measurement. The PAL activity was determined by incubating a mixture of 0.1 mL of enzyme extract and 2.90 mL of 0.1 M sodium borate buffer (pH 8.0) containing 3 mM L-phenylalanine for 1 hr at 37°C. The reaction was stopped by adding 0.5 mL of 5 N HCl.

The protein contents in the extracts were determined following the method of Bradford (1976).

Biochemical characteristics

The browning index of fresh-cut mangoes was measured according to Supapvanich et al. (2011). Each sample of 2 g of the fresh-cut mango was homogenized with 65% ethanol (volume per volume) then stirred at room temperature for 1 hr. The extract was filtered using Whatman No. 1 filter paper. The absorbance at 420 nm was measured using a spectrophotometer (model UV 1800; Shimadzu; Kyoto, Japan). The browning index was expressed as OD_{420} per 100 g fresh weight.

The total phenol content was measured using methanol-extracted analysis as described by Swain and Hillis (1959), with some modification. Each sample of 5 g of mango flesh was homogenized with 25 mL methanol in a homogenizer (Heidolph Silent Crusher M; Schwabach, Germany). The homogenate of mango was kept at 4°C for 12 hr and then centrifuged at 15,000 rpm for 20 min using a Sorvall RC 5C Plus centrifuge (Kendro Laboratory Products; Connecticut, United States). The supernatants were stored at -20°C until measurement. The total phenolic content was measured using the Folin-Ciocalteu method. Briefly, 150 µL of supernatant was mixed with 2,400 µL of distilled water before adding 150 µL of 0.25 N Folin-Ciocalteu phenol reagent and vortex mixing in a test tube. The mixture was placed at room temperature to react for 3 min and then 300 µL of 1 N Na_2CO_3 solution was added. The solutions were mixed well and incubated at room temperature (25°C) for 2 hr. The absorbance at 725 nm was measured using a spectrophotometer (model UV 1800; Shimadzu; Kyoto, Japan) and the result converted into gallic acid equivalents (GAE; measured as milligrams per 100 g fresh weight) using a gallic acid (0–0.1 mg/mL) standard curve. Methanol was used as the blank. Data were reported as grams per 100 g fresh weight.

Ascorbic acid was measured according to Schaffert and Kingsley (1955) modified method. Fruit extract was obtained by homogenizing 5 g of mango with 5% metaphosphoric acid (on ice). After filtration using Whatman No.1 paper, the extract was determined by the spectrophotometric method at OD_{540} nm, using an ascorbic acid (0–50 µg/mL) standard curve, with 5% metaphosphoric acid as a blank. The ascorbic acid content was expressed as grams per 100 g fresh weight.

Statistical analysis

Statistical analysis was carried out using the ANOVA procedure in the general linear model procedure of the SPSS software (version 15.0; IBM Corp.; White Plains, NY, USA) for completely randomized design experiments. Each treatment contained three replicates consisting of four pieces of fresh-cut mango. Significance was tested at $p \leq 0.05$ using Duncan's multiple range test.

Results and Discussion

Effect of sericin on surface color changes of fresh-cut mango

Fig.1 shows the superficial color of fresh-cut mango 'Nam Dok Mai No. 4' coated with sericin solution during storage under and accelerated temperature regime (10°C). On the initial day of storage, the L^* value (lightness) of the fresh-cut mango treated with sericin was significantly lower than that of control fruit. Afterward, the L^* value of the sericin-coated samples was significantly higher than for the control fruit until the end of storage ($p \leq 0.01$). It was noticed that there was a lower L^* value ($p \leq 0.01$) at the beginning (initial day) for the sericin-treated fresh-cut mango compared to the control; however the L^* value of the sericin-treated samples remained higher than for the control for the remainder of storage time (Fig. 1A). The low L^* value on the initial day of the coating treatment was expressed as slight changes in the color of the sericin coated fresh-cut mango. Similarly, Thongsook and Tiyaboonchai (2011) reported that the L^* values of apple slices coated with sericin were significantly lower than for uncoated fruit at day 0 and that afterward, the L^* values of these slices was higher than that of uncoated apple during 3 days of storage ($p \leq 0.01$).

The hue angle value of fresh-cut mango 'Nam Dok Mai No. 4' (Fig. 1B) was higher in the sericin-treated fruit than the untreated fruit throughout storage. This result clearly showed the efficacy of browning suppression on the cut surface of mango as a result of the coating used. An increase in the difference of total superficial color (ΔE) value and a decrease in the chroma of the sericin-treated mango were delayed throughout storage compared to the control (Fig. 1C–D). These results were related to the lightness (L^* values) as shown in Fig. 1A.

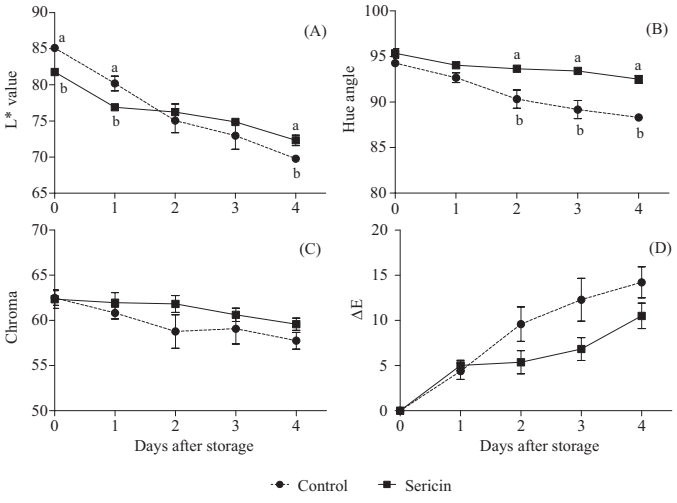

Fig. 1 L^* (lightness) value (A); hue angle (B); chroma (C); the difference of total superficial color (ΔE) (D) of fresh-cut mango cv. 'Nam Dok Mai No. 4' coated with filtered water (control set) and sericin. Coated mangoes were kept at 10°C for 4 d. Data shown are mean ($n = 3$) and error bars represent ± SD Bars in the same subfigure with the same lowercase letter are not significantly different using Duncan's multiple range test at $p \leq 0.05$.

When the mangos were peeled and cut, the cell membrane leakage increased the decompartmentalization of enzymes involved on the browning as PPO and POD and their substrates in the tissue led to discoloration. The delay in the browning development in the fresh-cut product through using a coating agent was due to the coating acting as an oxygen barrier, removing a necessary element for browning reactions to occur (Thongsook and Tiyaboonchai, 2011). Moreover, the coating with proteins, peptides or dipeptides and amino acids was more effective in preventing color changes. Edible coating can directly inhibit the enzymatic browning catalyzed reaction. The current results showed the efficacy of an edible coating based on sericin which could delay color changes in fresh-cut mango cv. 'Nam Dok Mai No. 4' throughout storage.

Effect of sericin on browning incidence and browning enzyme activity

The browning incidence and browning enzyme activity of fresh-cut mango 'Nam Dok Mai No. 4' are shown in Fig. 2. The browning index of the control set significantly increased throughout storage (at 10°C) for 4 d. Sericin application could delay browning during storage compared to the control (Fig. 2A). A similar result was reported by Puangphet et al. (2015) who reported that sericin hydrolysate delayed color changes based on the L^* value and browning index (BI) of fresh-cut apple and eggplant pieces. As shown in Fig. 2B, surface browning of fresh-cut mango appeared on the initial day of storage based on the browning score. The lowest browning score was recorded for the sericin-treated fruit ($p \leq 0.01$) and the sericin-treated fruit had a significantly higher acceptance score than untreated fruit.

The use of sericin-based edible coatings on fresh-cut mango cv. 'Nam Dok Mai No. 4' had a positive response on reducing the browning enzyme activity based on PPO, POD and PAL during storage, as shown in Fig. 2C, 2D and 2E, respectively. The increases in the PPO, POD and PAL activities were indicative of browning in the fresh-cut mango. The PPO activity of the sericin-treated fruit was significantly lower than that of the control fruit during the 4 d of storage from the initial day, which suggested that the PPO activity was correlated with the browning index, browning score and color changes. The control fruit had more enzymatic browning than from the sericin treatment (Fig. 1A–D and Fig. 2A–B). Sericin is considered as an inhibitor of PPO (Kato et al., 1998). It has been reported that proteins, peptides and amino acids are able to inhibit PPO activity as coatings with these could affect the PPO activity in at least two ways: by reacting with *o*-quinones and by chelating the essential copper at the active site of PPO (Kahn, 1985; Goetghebeur and Kermasha, 1996).

POD is one of the primary enzymes involved with the antioxidative defense mechanism expressed as an antioxidant enzyme. An antioxidant enzyme is one of the most important components in the active oxygen removal system. POD decomposed H_2O_2 by oxidation of co-substrates, such as phenolic compounds or antioxidants or both (Mittler, 2002). The POD activity in fresh-cut mangos continuously increased during storage. On the second day of storage, the POD activity in the sericin-treated fruit was significantly lower than in the control fruit. However, there was no significant difference in POD activity between the sericin treatment and the control fruit at the end of storage.

PAL is a key enzyme involved in the biosynthesis of phenolic compounds in plants (Dixon and Paiva, 1995). PAL activity could be observed in both treatments which tended to increase during refrigerated storage. The PAL activity of fresh-cut mango treated with sericin was significantly lower than that of untreated fruit during the 4 d of storage. The results showed the beneficial effect of the sericin coating, which inhibited PPO activity resulting in browning inhibition of fresh-cut mango cv. 'Nam Dok Mai No. 4' during storage. The total phenolic contents significantly affected the surface color of the fresh-cut mango cv. 'Nam Dok Mai No.4' (Fig. 2F). The phenolic content of the control sample was higher than for the sericin-treated mango during storage, which was related to the increase in the PPO and PAL activities.

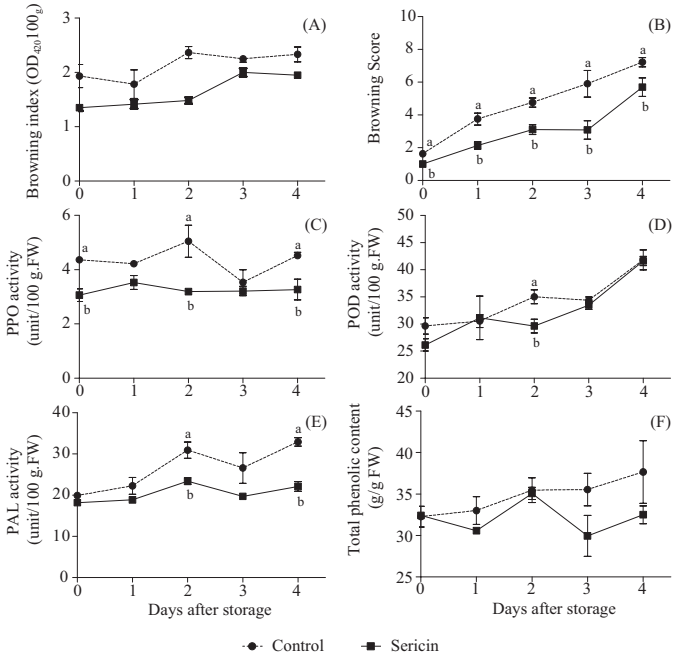

Fig. 2 Browning index based on optical density of absorbance at 420 nm (OD_{420}) (A); browning score (B); polyphenoloxidase (PPO) activity (C); peroxidase (POD) activity (D); phenylalanine ammonia-lyase (PAL) activity (E); total phenolic content (F) of fresh-cut mango cv. 'Nam Dok Mai No. 4' coated with filtered water (control set) and sericin. Coated mangoes were kept at 10°C for 4 d. Data shown are mean (n = 3) and error bars represent ± SD Bars in the same subfigure with the same lowercase letter are not significantly different using Duncan's multiple range test at $p \leq 0.05$ and FW = fresh weight

Effect of sericin on quality attributes

The changes in the firmness of samples are shown in Fig 3A which declined gradually over time for both the untreated and treated fresh-cut mango. On the initial day of storage, the firmness of the sericin-treated fruit was significantly higher than that of the control fruit; however, there was no significant difference between the firmness of fruit subjected to the sericin treatment and the control fruit for the remainder of storage. Fresh-cut mango treated with sericin maintained the greater firmness than the untreated fruit during

refrigerated storage. A change in the firmness is also associated with water loss and transpiration (Voon et al., 2006; Holcroft, 2015). In the current research, the sericin treatment could inhibit the water loss of the fresh-cut mango throughout storage. Moreover, sericin is especially rich in serine (30–33%), glycine (19%) and aspartic acid (17.8%) (Kato et al., 1998). The high content of the hydroxyl (–OH) group in the sericin-based edible coating could absorb water leading to a reduction in water loss and texture loss in the fresh-cut mango. Changes in the ascorbic acid content for the fresh-cut mango in both treatments is shown in Fig. 3B. It seemed that the ascorbic acid content of the sericin-treated fruit slightly decreased during storage on the first two days and on the last day of storage, although there was no significant difference in the ascorbic acid content between the sericin treatment and the control set of fruit throughout storage. Sericin could maintain the ascorbic acid content in fresh-cut mango resulting and acted as an antioxidant in the sericin solution. Kato et al. (1998) provided the first evidence of an antioxidant action of the silk protein sericin by showing the suppression of *in vitro* lipid peroxidation by sericin.

These results suggested that the use of edible coating prepared from sericin significantly delayed the color changes on the cut surface of fruit resulting in decreased browning incidence and the inhibition of PPO and PAL activities in fresh-cut mango cv. 'Nam Dok Mai No. 4'. Sericin is generally recognized as a safe agent which has an antibrowning property and it has been used as a material for producing edible coatings or incorporated with other ingredients. It also could be used for reducing browning symptoms and for improving the quality of other fruits and vegetables.

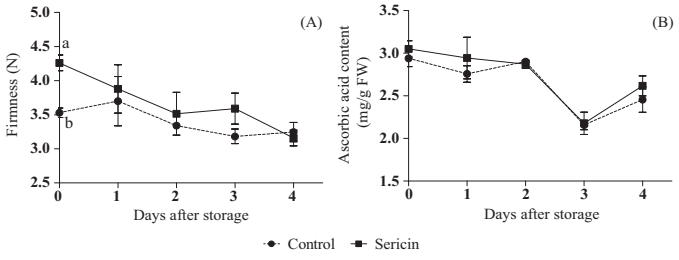

Fig. 3 Firmness (A); ascorbic acid content (B); of fresh-cut mango cv. 'Nam Dok Mai No. 4' coated with filtered water (control set) and sericin. Coated mangoes were kept at 10°C for 4 d. Data shown are mean (n = 3) and error bars represent ± SD Bars in the same subfigure with the same lowercase letter are not significantly different using Duncan's multiple range test at $p \leq 0.05$ and FW = fresh weight

Conflict of Interest

The authors declare that there are no conflicts of interest.

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