

Inhibitory effect of sericin hydrolysate on polyphenol oxidase and browning of fresh-cut products

¹Puangphet, A., ²Tiyaboonchai, W. and ^{1*}Thongsook, T.

¹Department of Agro-Industry, Faculty of Agriculture Natural Resources and Environment, Naresuan University, Phitsanulok 65000, Thailand

²Department of Pharmacy Practice, Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok 65000, Thailand

Article history

Received: 19 September 2014

Received in revised form:

9 January 2015

Accepted: 12 January 2015

Abstract

Sericin was extracted from silk cocoon shells (white cocoon shells) by heat treatment. Molecular weight of the extracted sericin appeared between 35 and 100 kDa and serine and aspartic acid were major amino acids. Sericin hydrolysate (SH), produced by hydrolysis of sericin using Alcalase, showed ability to inhibit polyphenol oxidase (PPO) and browning of fresh-cut products. Using the response surface method (RSM), optimum conditions to obtain high degree of hydrolysis (DH) and producing SH of high inhibitory ability on PPO and browning were at an enzyme to substrate ratio of 0.52% (w/w), a temperature of 50 °C and pH of 8.8. SH lowered PPO activity from apple extract by 95%, from eggplant extract by 79% and from bean sprouts and banana flower extracts by 70%. SH delayed changes in L* value and browning index (BI) values of fresh-cut apple and eggplant pieces. Results showed that SH is a promising non-sulfite anti-browning agent from natural sources.

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Keywords

Sericin

Sericin hydrolysate

Polyphenol oxidase (PPO)

Browning

Inhibitor

Introduction

Cut-surface browning of fresh-cut products is one of the major problems affecting consumer acceptability. Most of them are caused by the action of polyphenol oxidase (PPO) (EC 1.10.3. 1) (Garcia and Barrett, 2002). After plant tissues were damaged and the cut surface was exposed to oxygen in the air, oxidation of phenolic compounds was catalyzed by PPO resulting in the production of dark-colored pigments (Constabel and Barbehenn, 2008; Whitaker 1994).

Application of antibrowning agents is a popular approach for retarding enzymatic browning in fresh-cut products (Suttirak and Manurakchinakorn, 2010). Usually sulfites and their derivatives were used to control enzymatic browning in food industries. However, there has been an effort to avoid the use of sulfites in foods due to safety, regulatory, and labeling issues. Natural inhibitors were used to replace sulfites to prevent browning in fresh-cut products (Barbagallo *et al.*, 2012). Ascorbic and citric acids are commonly used in food products, but their effectiveness is less than that of sulfites. In addition, high cost and short-term function limited their abilities to prevent enzymatic browning. Recent studies showed that sulfhydryl (or thiol) compounds

are good PPO inhibitors (Ding *et al.*, 2002; Altunkaya, 2011). Protein and peptide can be used to replace sulfites for PPO inhibition. Several research groups demonstrated that protein and peptide from natural sources were able to inhibit PPO activity (Schurink *et al.*, 2007) for example, whey protein (Altunkaya, 2011), silk protein (Kato *et al.*, 1998; Thongsook and Tiyaboonchai, 2011), wheat protein (Ortiz-Estrada *et al.*, 2012) and rice bran protein (Kubglomsong and Theerakulkait, 2014). In addition, the decrease in molecular weight of protein or peptide by enzymatic hydrolysis enhances the functions of proteins (Klompong *et al.*, 2007).

Sericin is a water-soluble silk protein derived from the silkworm *Bombyx mori* and constitutes about 20–30% of the total cocoon weight. Mostly, it was found in the degumming wastewater from silk manufacture. Sericin consists of 18 kinds of amino acids and is rich in serine (about 32%) and is affected by a high content of the hydroxyl group (Wu *et al.*, 2007). Sericin has been studied for various potential applications including antibiotic-antibacterial activity, antioxidant behaviour, anti-tyrosinase activity, anticarcinogenic effects, UV protective properties, and coagulant and moisturizing capabilities in cosmetic preparations (Aramwit *et al.*, 2012). Sericin which is a non-dietary protein has

*Corresponding author.

Email: tipawant@nu.ac.th

Tel: 66 55 96 2746; Fax: 66 55 96 2703

been reported to activate immune system (Keawkorn *et al.*, 2013). Sericin is used to prepare not only film applicable for wound healing; it is also an edible film that can be used in the food industry as a substitute for traditional plastic films (Sothornvit *et al.*, 2010).

Some studies demonstrated that sericin can inhibit tyrosinase or PPO (Kato *et al.*, 1998; Yamada *et al.*, 2000). Sericin hydrolysate (SH) prepared by enzymatic hydrolysis of sericin was more effective in inhibiting PPO than sericin (Thongsook and Tiyaboonchai, 2011). However, most studies on SH focused on its antioxidant activity (Fan *et al.*, 2010; Wu *et al.*, 2008).

Therefore, there was no literature on optimum conditions for enzymatic hydrolysis of sericin to obtain SH with high PPO inhibitory ability. Moreover, effect of SH on PPO inhibition was only studied *in vitro* especially on tyrosinase activity (Fan *et al.*, 2010; Wu *et al.*, 2008). No research has focused on testing its anti-browning ability on real food systems. Therefore, the objective of this study was to investigate optimum conditions for enzymatic hydrolysis of sericin to obtain SH with high PPO inhibitory ability. Efficiency of SH on the inhibition of PPO extracted from different plant sources and application of SH as an anti-browning agent for fresh-cut products were also examined.

Materials and Methods

Materials

Silk cocoon shells, the Thai-foreign hybrid variety (white cocoon shells), were kindly provided by Queen Sirikit Sericulture Center, Nan province, Thailand. The impurities were separated from the cocoon shells before being used. Thereafter, the sample was vacuum-packed in polyethylene plastic bags and stored at room temperature until used. Tyrosinase (commercial PPO) from mushroom (EC 1.14.18.1) was purchased from Sigma (St. Louis, MO) containing tyrosinase, PPO and catechol oxidase activity. PPO activity was 98,800 units/mg solid. Alcalase (from *Bacillus licheniformis*) were purchased from Sigma. Other chemicals in experiments were reagent-grade.

Compositions of silk cocoon shells

Silk cocoon shells were determined for their chemical compositions using standard methods according to AOAC (Association of Official Analytical Chemists) (2000).

Sericin extraction

Silk cocoon shells were cut into about 1 cm x

1 cm. Two grams of silk cocoon were weighed into a glass container after which 100 mL of distilled water were added. The mixture was autoclaved at 121°C for 45 min (Puangphet *et al.*, 2013). Sericin solution was obtained after the insoluble portion (fibroin) was separated. The extracted sericin was an odorless white color solution. The protein content in the sericin solution was analyzed by the Kjeldahl method (AOAC, 2000).

Analysis of amino acid profile of the extracted sericin

Sericin solution was prepared in the dried form by the precipitation of 95% ethanol before analysis of the amino acid profile at a ratio of sericin solution to 95% ethanol being 4 to 1. The mixture was incubated at 4°C for 4 h. After that, the solution was centrifuged at 10,000 rpm and 4°C for 30 min to collect the precipitate. The precipitate was freeze-dried to obtain sericin powder. Sericin powder was determined for the amino acid content by high performance liquid chromatography (HPLC) using the Waters Associates AccQ-Tag method (Liu *et al.*, 1995). The mobile phase consisted of two solvents including the sodium acetate buffer (pH 4.9) and 60% acetonitrile. The column used was the Hypersil Gold column C18 (Thermo Fisher Scientific, San Jose, CA) and a fluorescence detector was used as well. Running condition was at 35°C.

Molecular weight distribution of the extracted sericin

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed by a discontinuous buffer system according to Laemmli (1970). SDS-PAGE of sericin was studied by using an 8% stacking gel and a 4% separating gel. Samples were prepared in a Tris-HCl buffer (pH 6.8) containing 10% SDS, 0.5 mL β -mercapto-ethanol, 50% glycerol, 1% bromophenol blue. The gel was run in a vertical slab-gel electrophoresis apparatus at 150 V for 1 h by Dual Mini Slab Kit (Mini-PROTEAN 3 Cell: Bio-Rad, Richmond, CA). The molecular weights of the peptide band were detected by the Coomassie brilliant blue stain method. A protein standard with 13 prestained proteins covering wide range molecular weights from 3.5 to 245 kDa (GeneDirex, Las Vegas, NV) were used as standard proteins.

Production of sericin hydrolysate (SH)

The response surface method (RSM) was applied to predict optimum conditions for hydrolysis of sericin using Alcalase to produce SH with the highest degree of hydrolysis (%DH), PPO inhibition, and browning inhibition. Protein content of the sericin

solution used in this experiment was 1.38 mg/100 mL sericin solution. The hydrolysis of sericin was conducted according to Thongsook and Tiyaboonchai (2011) and Wu *et al.* (2008). The solution was centrifuged at 10,000 rpm at 4 °C for 10 min to collect SH. A set of the optimal exact three factor designs (Borkowski, 2003) was employed in this study, requiring 11 experiments. Since Alcalase has broad substrate specificities and is active between pH 6.5 and 8.5 with an optimum temperature of 60°C as reported by the manufacturer, therefore, the range of temperature and pH selected in this experiment was 50-60°C and pH 7-9, respectively. Whereas enzyme to substrate ratio (E/S) was 0.1-1% weight of protein [W/W]. Since the hydrolysis reaction occurred rapidly at the first hour of the reaction and after 3 h %DH only changed slightly, the hydrolysis time for the RSM experiment was 3h. The determination responses were DH, PPO inhibition, and browning inhibition. DH was determined by the pH-stat method and calculated according to Wu *et al.* (2008) and Adler-Nissen (1986), respectively. SH produced from each experiment was concentrated (50% increase in concentration) by the evaporation at 60°C before testing and is an odorless light brown colored solution.

Inhibitory effect of SH on commercial PPO

The inhibitory effect of SH on PPO or PPO inhibitions for SH obtained from the RSM experiment was tested on commercial PPO. PPO inhibition was conducted by the colorimetric method using catechol as a substrate and the absorbance at 420 nm was monitored by a spectrophotometer (Genesys 20: Thermo Fisher Scientific, Waltham, MA). Total reaction volume was 3 mL containing 2.45 mL of 0.1 mol/L (pH 6.5) phosphate buffer, 1 mL of 0.175 mol/L pyrocatechol, and 0.05 mL of PPO solution (commercial PPO extracted from the mushroom with an activity of 100 unit/mL prepared in 0.1 mol/L phosphate buffer [pH 6.5]). The activity of PPO was the initial slope of the plot of the absorbance values versus the reaction times. One unit of PPO activity was defined as the amount of enzyme which caused a change of absorbance of 0.001 per min mL⁻¹ at 420 nm. For the determination of PPO inhibition of SH, 1 mL of SH was substituted into the phosphate buffer. PPO inhibition was calculated as follows:

$$\text{PPO inhibition (\%)} = 1 - \frac{\text{PPO activity of the assay containing inhibitor}}{\text{PPO activity of the assay without inhibitor}} \times 100 \quad (1)$$

Inhibitory effect of SH on PPO extracts

SH was prepared by hydrolyzing 5% sericin

(W/V) under the optimized condition. Phosphate buffer pH 6.5 containing 1% polyvinylpyrrolidone (PVP) was used to extract the PPO from apple and bean sprouts (20 g: 40 mL extracting solution). A buffer containing 1% PVP and 1% Triton X-100 was used to extract PPO from eggplant and banana flower. The mixture was blended and centrifuged at 6000 rpm and 4 °C for 15 min to obtain the PPO extracts. The assay mixture was composed of 1 mL 0.175 mol/L pyrocatechol, 1.45 mL buffer, and 50 µL PPO extract. One milliliter of inhibitor was added to replace the buffer. PPO inhibition was calculated as equation (1).

Inhibitory effect of SH on browning of fresh cut products

Browning inhibitions for SH obtained from the RSM experiment were tested on apple pieces. The tests were conducted according to the method described by Zocca *et al.* (2011) with some modifications. Red delicious apples (*Malus domestica*) and green eggplants (*Solanum xanthocarpum* Schrad. & Wendl.) were purchased from a local market. The plants sample were cut into 1 cm x 1 cm pieces and soaked in 5 mL of SH or other inhibitor solutions (1% citric acid, 1% NaCl, 0.1% ascorbic acid, 1% ascorbic acid) for 15 min. Samples soaked in distilled water were used as control. Thereafter, 1 mL of 10 mmol/L catechol was added onto the sample pieces and incubated at the room temperature for 25 min. Changes in surface color (L* value) were monitored using the HunterLab colorimeter (MiniscanXE & ColorFlex, Hunterlab 11491, Reston, VA). After the optimum conditions were chosen, SH was tested for its inhibitory effect on both apple and green eggplant pieces using SH prepared from the hydrolysis of 5% sericin (W/V) under the optimized condition. L* value and browning index (BI) of the fresh-cut samples were monitored. The L* value indicated the lightness of an object scale that varied from 0 (black) to 100 (white). BI which represents the real color was calculated from L*, a* and b* value according to Bal *et al.* (2011).

$$\text{BI} = 100 \times \frac{(X - 0.31)}{0.17} \quad (2)$$

$$X = \frac{a_t^* + 1.75L_t^*}{5.645L_t^* + a_o^* - 3.012b_t^*}$$

Where:

- a_o^{*}: the initial color measurement of sample pieces after cutting
- L_t^{*}, a_t^{*} and b_t^{*}: are the color measurements of

Table1. Experimental design of enzymatic hydrolysis with three responses according to a genetic algorithm design (Equations beneath the table indicate relationships between each response and the variables)

| Run | Independent variables | | | Responses | | |
|-----|-----------------------|----------------|----------------|-------------------|-------------------|-------------------|
| | X ₁ | X ₂ | X ₃ | (Y ₁) | (Y ₂) | (Y ₃) |
| 1 | 1 | 0 | 0 | 14.79 ± 1.57 | 30.18 ± 0.50 | 52.98 ± 1.12 |
| 2 | 0 | 1 | 0 | 13.00 ± 0.40 | 30.53 ± 1.49 | 54.75 ± 0.84 |
| 3 | 0 | 0 | 1 | 19.36 ± 0.41 | 33.68 ± 1.49 | 55.39 ± 0.46 |
| 4 | -1 | -1 | -1 | 4.34 ± 0.40 | 28.6 ± 0.74 | 47.82 ± 1.39 |
| 5 | 1 | -1 | -1 | 6.94 ± 1.63 | 29.47 ± 1.49 | 45.54 ± 1.60 |
| 6 | -1 | 1 | -1 | 4.63 ± 0.40 | 26.43 ± 1.16 | 48.97 ± 0.18 |
| 7 | -1 | -1 | 1 | 17.20 ± 0.62 | 33.27 ± 0.41 | 53.41 ± 0.12 |
| 8 | 1 | 1 | -1 | 7.23 ± 1.22 | 31.29 ± 0.41 | 50.10 ± 0.47 |
| 9 | 1 | -1 | 1 | 20.67 ± 1.03 | 35.56 ± 1.16 | 53.94 ± 1.41 |
| 10 | -1 | 1 | 1 | 16.47 ± 0.41 | 31.93 ± 0.99 | 51.34 ± 1.89 |
| 11 | 1 | 1 | 1 | 19.36 ± 0.41 | 37.19 ± 0.99 | 51.21 ± 0.64 |

*** X₁ = enzyme:substrate (%), X₂ = temperature (°C), X₃ = pH, Y₁ = %DH, Y₂ = PPO Inhibition, Y₃ = L*

$$Y_1 = 13.7349 + 1.4451X_1 - 0.1806X_2 + 6.3222X_3 - 0.3907X_1^2 - 0.0723X_1X_2 - 0.5486X_2^2 + 0.1445X_1X_3 - 0.3251X_2X_3 - 0.6931X_3^2 \quad (3)$$

$$Y_2 = 29.1228 + 1.6594X_1 - 0.0073X_2 + 2.7705X_3 - 0.6067X_1^2 + 0.8699X_1X_2 + 1.4108X_2^2 + 0.2266X_1X_3 + 0.0804X_2X_3 + 1.7909X_3^2 \quad (4)$$

$$Y_3 = 55.3130 - 0.0943X_1 + 0.1111X_2 + 2.1845X_3 - 2.2404X_1^2 + 0.3439X_1X_2 - 0.6767X_2^2 + 0.1943X_1X_3 - 1.3149X_2X_3 - 2.104X_3^2 \quad (5)$$

sample pieces after treatments

Effect of SH on odor of fresh-cut apple

A triangle test was used to determine the effect of SH on odor of fresh-cut apple by using 10 untrained panelists. Red delicious apple slices were cut into 3 cm x 3 cm pieces and dipped in either SH or distilled water for 5 min at 25°C. After that the excess solution was removed from the pieces by leaving a stainless steel sieve for 1 min before placing in plastic cups sealed with aluminum foil.

Statistical analysis

All the data except RSM were treated for significance by the one-way analysis of variance (One-way ANOVA) at $p \leq 0.05$ with the aid of spss 17.0 for windows software and Duncan's new multiple range test applied for comparison tests at 95% confidence level. Moreover, ANOVA was used for RSM at 0.05 of significant level. The optimum levels of different independent variables were analyzed by "Response optimizer" of the Minitab software.

Results and Discussion

Properties of white cocoon shells and the extracted sericin

Thai-foreign hybrid variety cocoon shells contained high fiber and protein content, 27.93% and 23.43%, respectively. Moisture, ash and fat content were found to be slightly (7.56 %, 1.13%, and 0.45%, respectively). The protein content of sericin solution extracted from sericin by heat treatment at 121 °C for 45 min was 0.51 mg/100 mL sericin solution.

Major amino acids were serine and aspartic acid (33.73 and 20.82 mg/100 mg, respectively). Glycine and threonine were 9.53 and 8.57%, respectively. Arginine, glutamic acid, tyrosine were 5.22, 4.98 and 4.60, respectively. Valine, lysine, alanine and histidine ranged 1.11 to 2.77% whereas histidine was 1.11%. Results according to Wu *et al.* (2008) reported that the majority of amino acids in hot water-soluble sericin are serine, aspartic acid and glycine.

The molecular weight of protein in sericin was studied by SDS-PAGE. Sericin extracted from white cocoon shells by heat treatment showed continuous band of protein ranging from 35-100 kDa on SDS-

Table2. Effect of SH and citric acid on the activity of PPO extracted from different sources

| Source of PPO extracts | PPO activity (unit) ^{**} ; (mean ± SD) | | | PPO inhibition (%); (mean ± SD) | |
|------------------------|-------------------------------------------------|------------------------------|------------------------------|---------------------------------|----------------|
| | Untreated* | SH | 1% Citric acid | SH | 1% Citric acid |
| Apple | 0.1920 ± 0.0018 ^a | 0.0087 ± 0.0004 ^c | 0.1094 ± 0.0074 ^b | 95.50 ± 0.26 | 43.05 ± 3.87 |
| Eggplant | 0.4766 ± 0.0247 ^a | 0.0978 ± 0.0159 ^b | 0.1033 ± 0.0062 ^b | 79.49 ± 3.34 | 78.33 ± 1.31 |
| Bean sprouts | 0.1348 ± 0.0042 ^a | 0.0405 ± 0.0007 ^b | 0.0185 ± 0.0014 ^c | 69.99 ± 0.58 | 86.31 ± 1.10 |
| Banana flower | 1.2240 ± 0.0113 ^a | 0.3880 ± 0.0147 ^c | 0.4808 ± 0.0192 ^c | 68.30 ± 1.20 | 60.72 ± 1.57 |

*Different letters of each parameter in the same row (PPO activity) indicated significant differences (P<0.05)
 ** One unit of PPO activity was defined as the amount of enzyme which caused a change of 0.001 in absorbance/ min. The assay mixture composed of 1 mL 0.175 M catechol, 1.45 mL buffer, 50 µl PPO extract. One mL of inhibitor was added to replace buffer

PAGE (data not shown). A broad diffuse protein band observed on SDS-PAGE was commonly found for proteins extracted using heat treatment (Aramwit *et al.*, 2010; Zhang *et al.*, 2004). Similarly to our study, Aramwit *et al.* (2010) observed a broad diffuse protein band of molecular weight ranging from 25-150 kDa for the silk sericin extracted by high temperature and pressure.

Optimization of enzymatic hydrolysis of sericin by Alcalase

Table 1 shows experimental design and response values including degree of hydrolysis (%DH), PPO inhibition and L* values indicating the browning of the tested apple pieces. The quadratic models (equations (3), (4) and (5)) showed relationships between %DH (Y₁), PPO inhibition (Y₂) and L* (Y₃) and the independent variables studied. Analysis of variance (ANOVA) for DH quadratic model indicated that the predicted models did not show lack of fit. R² values of the response value Y₁, Y₂ and Y₃ were 0.97, 0.89 and 0.85 respectively. Optimum hydrolysis conditions were obtained using superimposing contour plots of the responses. The optimum conditions were based on high level of %DH, PPO inhibition and L* value. In the overlaying plots (Figure 1), regions with a shaded area do not fit the optimization criteria, while the non-shaded area meets the optimization criteria. When the E/S ratio was set at the highest point (X₁=1), the optimum temperature and pH were chosen using the following criteria: DH > 4.34%, PPO inhibition >26.43% and L* >45.54 (Figure 1A). Due to an economic reason, 50°C was chosen as the optimum temperature. When temperature was set at 50°C (X₂=-1), the E/S ratio and pH were chosen using the following criteria: DH > 4.34% PPO inhibition >26.43% and L* >45.54 (Figure 1B). The optimum levels of different independent variables were analyzed by “Response optimizer” of the Minitab

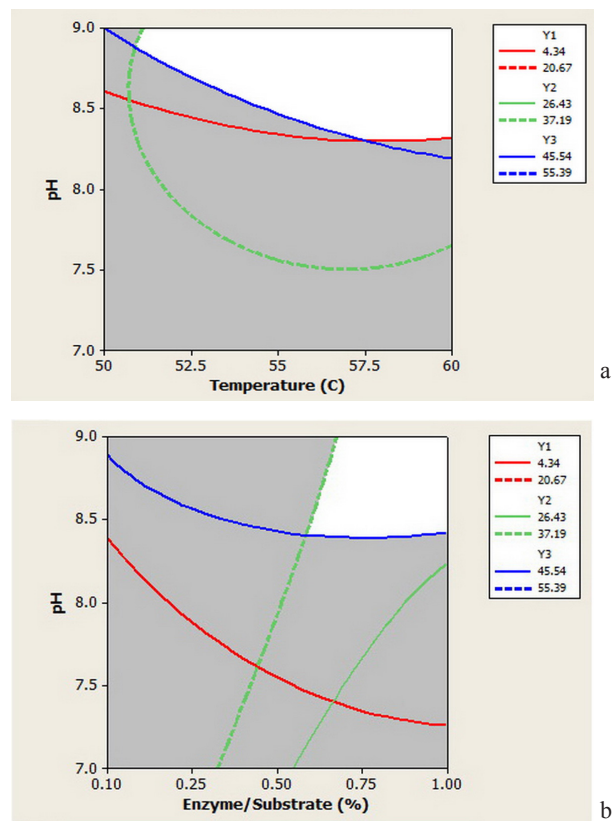


Figure1. Superimposed contour plots showing the regions of best combinations of hydrolysis conditions to generate maximum DH, PPO inhibition and L*

software and setting the goal of individual of response value at the maximum level. The optimum condition obtained were E/S ratio of 0.52% (w/w; mg protein enzyme/mg protein substrate), temperature of 50°C and pH of 8.8. Accordingly, the maximum DH, PPO inhibition and *L estimated were 18.22%, 33.87% and 55.99, respectively. The selected optimum conditions were used to validate the suitability of the predicted model equations, and triplicate runs were carried out at the optimum conditions. Under this condition, DH, PPO inhibition and L* was found to be 19.48±0.551%, 36.39±0.764% and 51.84±2.786

Table 3. L* values and browning index (BI) of fresh-cut apple and fresh-cut eggplant after being treated with different anti-browning agents and incubated for 25 minutes

| | Fresh-cut apple | | Fresh-cut eggplant | |
|---------------------------|-----------------|------------|--------------------|------------|
| | L* | BI | L* | BI |
| Control | 46.78±0.41 | 54.64±0.85 | 54.92±1.94 | 52.37±0.40 |
| SH | 55.44±0.43 | 40.24±0.92 | 77.44±0.88 | 8.90±0.70 |
| 1% citric acid | 42.23±0.25 | 97.89±1.24 | 77.21±0.46 | 6.48±0.61 |
| 1% NaCl | 41.68±0.59 | 89.25±0.88 | 63.41±1.30 | 31.98±0.25 |
| 0.1% ascorbic acid | 39.84±0.24 | 91.59±1.22 | 46.62±0.79 | 59.47±0.93 |
| 1% ascorbic acid | 63.47±0.35 | 6.01±0.20 | 62.95±0.43 | 10.42±0.34 |

respectively. The predicted values were found to be similar to the experimental values.

Inhibitory effect of SH on PPO extracts

In general, most of the fruits and vegetables in this study have been widely studied because they turn brown easily. The activity of PPO from apple, eggplant, bean sprouts and banana flower, after treated with SH and 1% citric acid were shown in Table 2. The results showed that, in the presence of SH, PPO activity decreased for all of samples. The degree of inhibition varied depending on sources of PPO. SH showed ability to decrease PPO activity more than citric acid on PPO from apple and banana flower whereas an opposite result was observed for bean sprout. There was no significant difference in the degree of inhibition on PPO from eggplant between SH and citric acid. Plant PPOs have broad substrates specificities and are able to oxidize a variety of mono, di or polyphenols (Queiroz *et al.*, 2008). Mishra *et al.* (2013) studied PPO activity of eight cultivars of eggplant and found that the specific activity and browning of eggplants were dependent on phenolic content and specificity of PPO. Therefore, the different characteristics of PPO from different origins were the most likely reasons of the variation in the inhibitory effect of SH on PPO extracted from different plant sources.

Inhibitory effect of SH on fresh cut products

L* value indicates the lightness of an object scale that varies from 0 (black) to 100 (white) and it was used to determined cut surface browning intensity (Gorny *et al.*, 1999; Weller *et al.*, 1995). When cut surface turned brown, lightness (L* value) decreased and BI value which indicated the purity of brown color increased. Therefore, in this study, L* and BI value were monitored and represented the browning reaction in fresh-cut products. Results in Table 3 and Figure 2 indicated that the most effective anti-

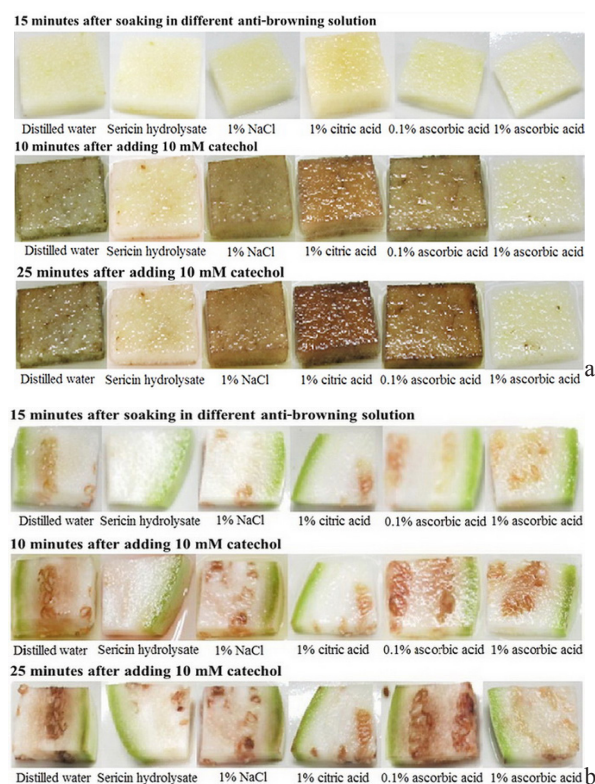


Figure 2. Changes in color of fresh-cut apple pieces (A) and fresh-cut eggplant pieces (B)

browning agent for fresh-cut apple was 1% ascorbic acid followed by SH. Citric acid and SH were comparable as anti-browning agents for fresh-cut eggplant.

SH is considered as a peptide PPO inhibitor. It has been previously reported that proteins, peptides and amino acids are able to inhibit PPO activity. It is possible that they chelated the essential copper at the active site of PPO and reacted with the o-quinones (Girelli *et al.*, 2004). Moreover, some amino acids such as sulfur-containing amino acids (methionine, cysteine) are able to inhibit PPO activity (Brosnan and Brosnan, 2006). Sericin contains high content of serine, or high content of hydroxyl group which are related to anti-PPO activity and as anti-oxidants.

Wu *et al.* (2008) reported that the effectiveness of SH on inhibiting PPO activity is related to metal ion-chelating in ferrous ion system. Their result suggested that SH might bind copper atom on the active site of PPO. However, more research on the mechanism of action of SH as anti-browning or anti-PPO action need to be conducted before one can comment on its anti-browning efficiency.

Although our result showed that SH delayed browning reaction on fresh-cut apple and eggplant, in case of apple, its effectiveness was still less than ascorbic acid. Yet, it is possible to combine SH with other anti-browning agents to enhance anti-browning efficiency. Incorporating SH with other anti-browning agents can reduce production cost in some cases when anti-browning agents with high cost were needed at high concentration.

Effect of SH on odor of fresh-cut apple

A triangle test was conducted to investigate the difference in odor between fresh-cut apple pieces treated with distilled water and SH. The treated fresh-cut apple pieces showed light apple odor and eight out of 10 panelists could not separate the difference in odor of the two samples. This indicated that SH had no influence on the odor of the fresh-cut samples.

Conclusion

In this study, SH was prepared by hydrolysis of sericin extracted from silk cocoon shells by using Alcalase. The enzyme to substrate ratio of 0.52%, temperature of 50°C and pH of 8.8 were the optimum condition for the production of SH with high anti-PPO and anti-browning efficiency. SH decreased PPO activity of PPO extracts prepared from several sources significantly. Besides, this study clearly showed that SH delayed browning of the fresh-cut products with no effect on odor of the products. Its efficiency was comparable to other kinds of anti-browning agents commonly used in food products. This new anti-browning agent from SH could be used to prevent enzymatic browning of food products which is beneficial to food industries.

Acknowledgments

The authors are grateful to the National Research Council of Thailand and the Faculty of Agriculture Natural Resources and Environment, Naresuan University for the financial support to this research, Queen Sirikit Siriculture center, Nan province, Thailand, for providing silk cocoon shells to this research and Asst. Prof. Dr. Sirilux Chaijamrus at

the Department of Biology, Naresuan University, for using spectrophotometer.

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