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Effect of Rice Bran Protein Extract on Enzymatic Browning Inhibition in Vegetable and Fruit Puree

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ABSTRACT

The effect of rice bran protein extract (RBPE) on enzymatic browning inhibition in vegetable and fruit puree was investigated. Protein from defatted rice bran was prepared by alkaline extraction and isoelectric point precipitation. RBPE was prepared by dispersing rice bran protein precipitate in distilled water (DW) at 0.8% protein (weight per volume). Potato, banana or apple was blended with RBPE and their color values (L*, a* and b*) were measured. Polyphenol oxidase (PPO) inhibition was studied by measuring absorbance at 420 nm with catechol as the substrate. It was found that the browning values in potato, banana and apple puree treated with RBPE were lower than those treated with DW ($P \leq 0.05$). The L* values and hue angles in potato, banana and apple puree treated with RBPE were higher than those treated with DW ($P \leq 0.05$). RBPE showed the highest percentage browning inhibition in potato puree, followed by apple and banana puree, respectively ($P \leq 0.05$). Moreover, RBPE had a higher percentage of PPO inhibition in potato than apple and banana, respectively ($P \leq 0.05$). Therefore, RBPE could inhibit enzymatic browning in vegetable and fruit puree, especially potato puree.

Keywords: enzymatic browning, rice bran protein, potato, banana, apple

INTRODUCTION

Enzymatic browning has a major impact on the quality of many fruit and vegetables. It might also produce undesirable changes in flavor, texture and nutritional values of the product during handling, processing and storage, resulting in a shorter shelf life and reduced market value (Fu *et al*., 2005). Enzymatic browning is mainly caused by polyphenol oxidase (PPO; EC 1.14.18.1) that catalyzes the hydroxylation of monophenols to *o*-diphenols and the oxidation of *o*-diphenols to *o*-quinones in the presence of oxygen. These *o*-quinones are very reactive compounds which self-polymerize or interact with other molecules in foodstuffs, leading to high molecular weight dark pigments commonly called melanins (Cantos *et al*., 2002; Girelli *et al*., 2004). There are several methods to inhibit enzymatic browning such as applying chelators, reducing agents and acidulants (Buta and Moline, 2001; Zhu *et al*., 2007); however, there is an increasing consumer demand for substituting synthetic compounds with natural substances (Jang *et al*., 2002). Therefore, natural antibrowning agents have been widely investigated.

Rice is one of the important staple foods for the world's population with global rice production in 2012 of about 690 million t (International Rice Research Institute, 2013). This huge amount of production results in commensurately large amounts of rice byproducts

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(Sereewatthanawut *et al*., 2008). Rice bran is the major byproduct generated amounting to about 5–8% of the rice grain during the milling process (Juliano, 1985). It is a source of protein, minerals, unsaturated fat, vitamins and dietary fiber (Abdul-Hamid and Luan, 2000). Due to its high content of hypoallergenic protein (Helm and Burks, 1996), rice bran has a high potential to be used as an ingredient in food applications.

There have been several studies on the inhibition of enzymatic browning in fruit and vegetables using proteins, peptides and amino acids such as the proteins, protein hydrolysates and amino acids from milk (Kahn, 1985), peptide from honey (Oszmianski and Lee,1990; Ates, 2001), hen egg white lysozyme (Li *et al*., 2006), wheat bran protein (Campas-Ríos *et al*., 2012; Ortíz-Estrada *et al*., 2012), sericin (Kato *et al*., 1998; Thongsook and Tiyaboonchai, 2011) and peptides from onion (Gnangui *et al*., 2010). From previous study, rice bran water extract has been reported as an enzymatic browning inhibitor (Kaewka *et al*., 2009; Theerakulkait and Boonsiripiphat, 2009; Sukhonthara and Theerakulkait, 2012); however, no adequate information has been published to date on the effect of rice bran protein on enzymatic browning in fruit and vegetables. Therefore, the objective of this research was to study the effect of rice bran protein extract on enzymatic browning inhibition in vegetable and fruit puree.

MATERIALS AND METHODS

Materials and chemicals

Potato (*Solanum tuberosum* L.), banana (*Musa* (AAA Group) 'Gros Michel') and apple (*Malus pumila* cv. Fuji) were purchased from a local market in Bangkok, Thailand. Full-fatted rice bran from the aromatic rice (*Oryza sativa* L. cv. Khao Dawk Mali 105) was obtained from Patum Rice Mill and Granary Public Co. Ltd., Pathum Thani, Thailand. Polyvinylpyrrolidone and catechol were purchased from Sigma Chemical (St Louis, MO, USA). Triton X-100 was purchased from Fluka Chemika (Buchs, Switzerland). The other chemicals were analytical grade.

Preparation of defatted rice bran

Full-fatted rice bran was sieved through a 50-mesh screen and extracted twice with hexane at a ratio of rice bran to hexane of 1:3 (weight per volume; w/v) for 30 min and centrifuged at 4,000×*g* at 25 ºC for 30 min. The defatted rice bran was air-dried overnight under a fume hood, ground and sieved through a 50-mesh screen and stored in aluminum foil bags at -20 ºC until used (a modified method of Wang *et al*., 1999).

Preparation of rice bran protein extract

Rice bran protein was extracted by dispersing defatted rice bran in distilled water (DW) at a ratio of rice bran to DW of 1:4 (w/v). The pH of the slurry was adjusted and maintained at 9.5 with 1.0 N NaOH during stirring with an overhead stirrer at 500 rpm at room temperature for 45 min, then centrifuged at 15,200×*g* at 25 ºC for 30 min. The supernatant was adjusted to pH 4.5 with 1.0 N HCl and centrifuged using the same conditions. The rice bran protein precipitate (RBPP) was obtained (a modified method of Gnanasambandam and Hettiarachchy, 1995; Theerakulkait *et al.*, 2006). The protein content of RBPP was determined by the Kjeldahl method (Association of Official Analytical Chemists, 1995); the value of 5.95 was used as a protein conversion factor (Juliano, 1985). Rice bran protein extract (RBPE) was prepared by dispersing RBPP in DW at 0.8% protein (w/v). DW was used as a control. Browning inhibition in potato, banana and apple puree and PPO inhibition were studied.

Effect of rice bran protein extract on browning inhibition in vegetable and fruit puree

Samples of potato, banana or apple were blended with RBPE at a ratio of potato, banana or apple to RBPE of 2:1 (w/v) for 20 s. DW was used as a control. The color values (L*, a* and b*) of the

samples were measured by a spectrophotometer (CM-3500D; Minolta; Tokyo, Japan) at 0, 1, 2, 3, 4, 5 and 6 hr of storage at room temperature. The browning value was calculated as $(\Delta L^*/L_0^*) \times 100$; where ΔL^* is the change of L^* at storage time and L_0^* is the initial L^* measurement (Labuza *et al*., 1990). The percentage browning inhibition was calculated as (Browning $value_{control}$ - Browning $value_{inhibitor}$) × 100 / Browning $value_{control}$. The hue angle was calculated using $\tan^{-1}b^*/a^*$ for the first quadrant (+a*, +b*), $180 + \tan^{-1}b^*/a^*$ for the second quadrant (–a*, +b*) and the third quadrant (–a*, –b*) and $360 + \tan^{-1}b^*/a^*$ for the fourth quadrant (+a*, –b*), respectively (McGuire, 1992; McLellan *et al*., 1995).

Effect of rice bran protein extract on vegetable and fruit polyphenol oxidase inhibition

Potato, banana or apple (200 g) was blended for 20 s with 200 mL of cold 0.1 M sodium phosphate buffer (pH 6.6) containing 1% polyvinylpyrrolidone and 0.5% Triton X-100. The homogenate was centrifuged at 25,560×*g* at 4 ºC for 30 min (a modified method of Galeazzi *et al*., 1981). The supernatant was collected and stored at -20 ºC to be used as crude enzyme. One unit of enzyme activity was defined as the amount of enzyme causing a change in absorbance of 0.01 at 420 nm in 1 min at 25 ºC and pH 6.6. The PPO activity in crude enzyme of each plant was adjusted to 490 unit.mL^{-1} by 0.1 M sodium phosphate buffer (pH 6.6).

RBPE diluted at 1:10 (v/v) or DW (1.0 mL), 0.9 mL of 0.05 M sodium phosphate buffer pH 6.6 and 1.0 mL of 0.2 M catechol in 0.05 M sodium phosphate buffer pH 6.6 were mixed and incubated for 30 s. Then, 0.1 mL of crude enzyme was added and mixed. The absorbance at 420 nm was immediately measured for 1 min using the spectrophotometer (a modified method of Lee *et al*., 2002). The percentage PPO inhibition was calculated as $(\Delta A_{420,\ control} - \Delta A_{420,\ inhibitor}) \times 100 / \Delta A_{420,\ control}$; where ΔA_{420} is the change in A_{420} between time *t* and the initial time t_0 (Özoğlu and Bayındırlı, 2002).

Statistical analysis

All experiments were performed with three replications. Statistically significant difference was assessed by one-way analysis of variance. Significant differences ($P \leq 0.05$) among treatments were detected using Duncan's multiple range test.

RESULTS AND DISCUSSION

Effect of rice bran protein extract on vegetable and fruit puree browning and polyphenol oxidase inhibition

In previous study, the concentration of RBPE at 0.8% protein (w/v) could inhibit browning in potato, banana and apple puree and it was found that 0.8% protein (w/v) RBPE inhibited browning in potato puree slightly less than 1% protein (w/v) RBPE; however, when the protein concentration increased from 1 to 1.5 and 2% protein (w/v), the inhibitory effect was not significantly increased (data not shown). Moreover, the RBPE itself was a light brown color and had a rice bran odor. This might lead the products to be unacceptable if RBPE were applied at a high concentration. Therefore, RBPE at 0.8% protein (w/v) was used in the current study. Kubglomsong and Theerakulkait (2014) also found that RBPE at 0.8% protein (w/v) effectively inhibited enzymatic browning in potato.

The browning values of potato, banana and apple puree treated with RBPE compared to DW are shown in Figure 1. The browning values of potato puree treated with RBPE were lower than those treated with DW for all storage times ($P \leq 0.05$) with values at 6 hr storage of 31.97 and 58.21, respectively (Figure 1a). The browning values of banana puree treated with RBPE were lower than those treated with DW at 2 to 6 hr storage ($P \leq 0.05$) with values at 6 hr storage of 18.04 and 20.84, respectively (Figure 1b). Moreover, the browning values of apple

puree treated with RBPE were lower than those treated with DW for all storage times ($P \leq 0.05$) with values at 6 hr storage of 31.66 and 38.20, respectively (Figure 1c).

The L* value provides a measure of lightness. A decrease in the L* value indicates a darker color that is related with a higher browning formation (Sapers and Douglas, 1987; İyidoğan and Bayındırlı, 2004). The L* values of potato, banana and apple puree treated with RBPE compared to DW are shown in Figure 2. It was found that the L* values of potato puree treated with RBPE were higher than those treated with DW for 1 to 6 hr ($P \leq 0.05$; Figure 2a), the L* values of banana puree treated with RBPE were higher than those treated with DW for 2 to 6 hr ($P \leq 0.05$; Figure 2b) and the L* values of apple puree treated with RBPE were higher than those treated with DW for all storage times ($P \leq 0.05$; Figure 2c). The L* values of potato, banana and apple puree treated with RBPE at 6 hr storage were 52.79, 52.68 and 37.19, respectively; whereas, those treated with DW had the L* values of 33.65, 50.93 and 29.49, respectively.

A decrease in the hue angle indicates a change of color from yellow to red which is indicative of enzymatic browning (Nicoli *et al*., 1994). The hue angles of potato, banana and apple puree treated with RBPE compared to DW are shown in Figure 3. The hue angles of potato puree treated with RBPE were higher than those treated with DW for 1 to 6 hr ($P \leq 0.05$) with values after 6 hr storage of 67.76 and 53.24, respectively (Figure 3a) The hue angles of banana puree treated with RBPE were higher than those treated with DW for a storage time of 1, 2, 3, 5 and 6 hr ($P \leq 0.05$) with values at 6 hr storage of 63.86 and 62.91, respectively (Figure 3b). The hue angles of apple puree treated with RBPE were also higher than those treated with DW for 1 to 5 hr ($P \leq 0.05$) with values at 5 hr storage of 61.86 and 61.07, respectively (Figure 3c).

The results of the L* values and hue angles indicate that potato, banana and apple puree treated with RBPE had lower browning color than those treated with DW. These were in accordance with the results for the browning value. The browning inhibition might have been

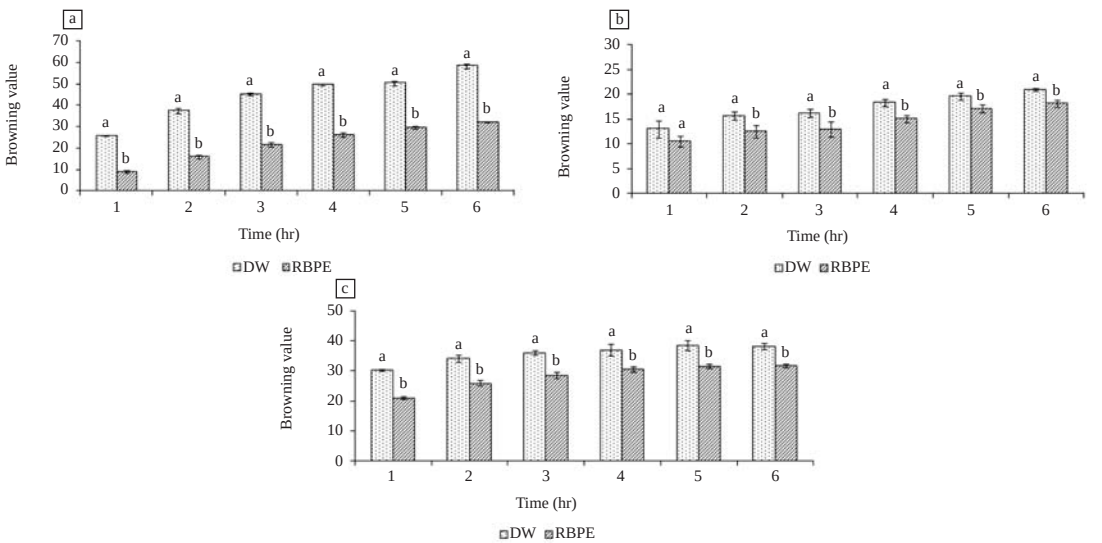

Figure 1 Browning values of potato (a), banana (b) and apple (c) puree treated with distilled water (DW) and rice bran protein extract (RBPE). All samples were stored at room temperature for up to 6 hr.

[a, b] Means with different letters are significantly different ($P \leq 0.05$) for the same storage time. Error bars indicate ± SD.

due to the proteins and peptides in RBPE. Proteins and peptides can affect the PPO activity in at least two ways: by reacting with the *o*-quinone and by chelating on copper at the active site of PPO (Kahn, 1985). Moreover, several researchers have reported that rice bran contains antioxidative peptides and sulfur amino acids (Parrado *et al*., 2006; Sereewatthanawut *et al*., 2008; Adebiyi *et al*., 2009); thus, these peptides might be the cause of browning inhibition in potato, banana and

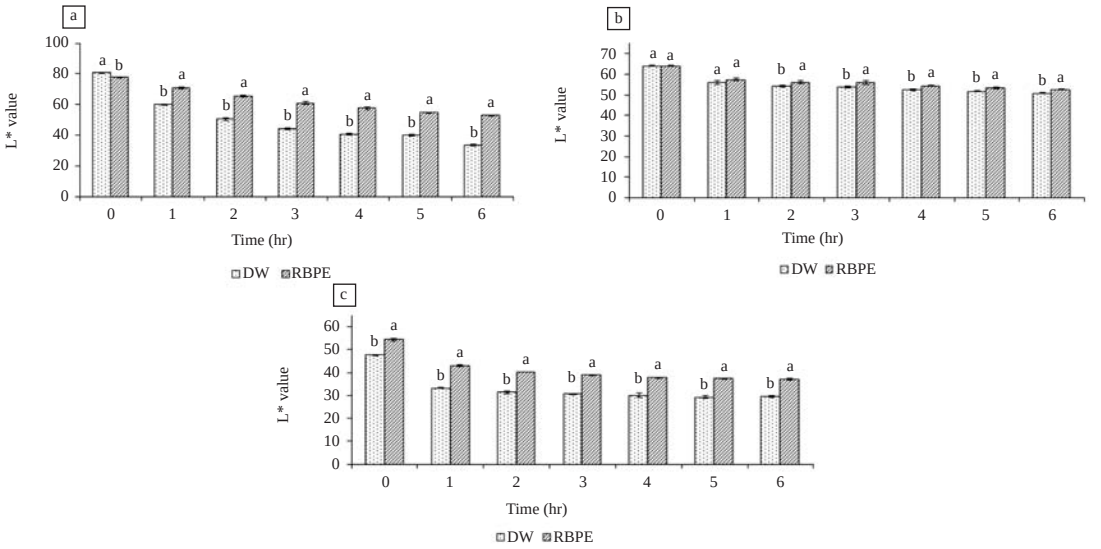

Figure 2 L* values of potato (a), banana (b) and apple (c) puree treated with distilled water (DW) and rice bran protein extract (RBPE). All samples were stored at room temperature for up to 6 hr. [a, b] Means with different letters are significantly different ($P \leq 0.05$) for the same storage time. Error bars indicate ± SD.

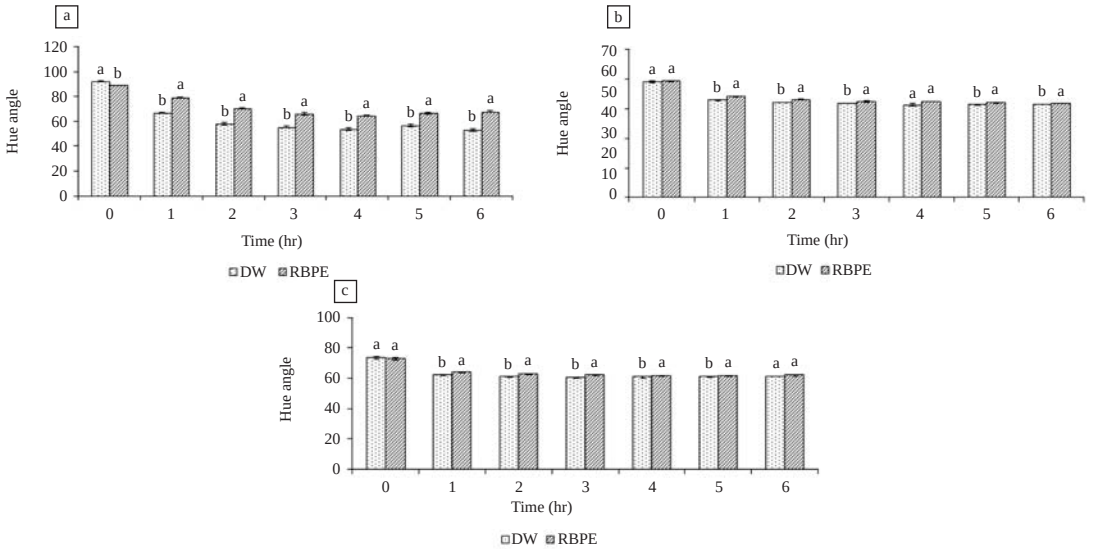

Figure 3 Hue angles of potato (a), banana (b) and apple (c) puree treated with distilled water (DW) and rice bran protein extract (RBPE). All samples were stored at room temperature for up to 6 hr. [a, b] Means with different letters are significantly different ($P \leq 0.05$) for the same storage time. Error bars indicate ± SD.

apple puree. Browning of potato and apple was also inhibited by whey protein (Tien *et al*., 2001), synthetic dipeptide (Girelli *et al*., 2004) and honey (Oszmianski and Lee, 1990).

The results of the percentage browning inhibition in potato, banana and apple puree after storage at room temperature for up to 6 hr compared to DW are shown in Table 1. It was found that RBPE could inhibit browning in potato puree to a greater level than apple and banana puree for all storage times ($P \leq 0.05$). The percentage browning inhibition in potato, apple and banana puree at 6 hr storage were 45.07, 17.11 and 13.47, respectively. Moreover, the effects of RBPE on potato, banana and apple PPO inhibition (Figure 4) indicated that RBPE produced higher percentage PPO inhibition in potato than in apple and banana ($P \leq 0.05$) with values of 15.94, 9.09 and 5.85, respectively. This agreed with the percentage browning inhibition in potato, apple and banana puree.

The results for the percentage browning inhibition and percentage PPO inhibition indicated that RBPE could inhibit enzymatic browning in potato more effectively than in apple and banana. This might have been due to the specific inhibition of RBPE on potato PPO isozymes being greater than the PPO from the other sources. The PPO isozymes in plants have been reported to be different (Flurkey, 1986). Theerakulkait and Boonsiripiphat (2009) reported that rice bran extract inhibited potato PPO more effectively than banana and apple PPO. Sukhonthara and Theerakulkait (2012) also reported that rice bran extract produced a greater level of potato PPO inhibition than banana PPO.

In addition, the effectiveness of RBPE compared to commercial antibrowning agents on enzymatic browning in potato puree was analyzed. It was found that RBPE inhibited browning in potato puree more than 5, 10 and 20 mM ascorbic acid; and 5 and 10 mM citric acid (Kubglomsong and Theerakulkait, 2014).

CONCLUSION

RBPE could inhibit enzymatic browning in potato, banana and apple puree. RBPE produced a greater level of percentage browning inhibition and percentage PPO inhibition in potato than in apple and banana. Therefore, RBPE has potential to be used as an enzymatic browning inhibitor in vegetable and fruit puree, especially potato puree.

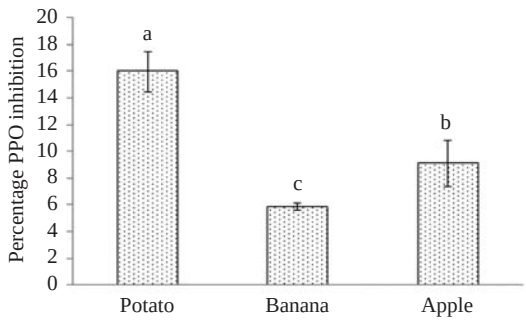

Figure 4 Percentage polyphenol oxidase (PPO) inhibition of potato, banana and apple in model system treated with rice bran protein extract.

[a, b, c] Means with different letters are significantly different ($P \leq 0.05$). Error bars indicate ± SD.

Table 1 Percentage browning inhibition in potato, banana and apple puree treated with rice bran protein extract. All samples were stored at room temperature for up to 6 hr.

Source	Percentage browning inhibition (at storage time; hr)					
	1	2	3	4	5	6
Potato	66.00 ± 2.27[a]	57.82 ± 2.95[a]	52.28 ± 2.50[a]	47.51 ± 1.56[a]	41.36 ± 1.42[a]	45.07 ± 0.96[a]
Banana	19.17 ± 4.81[c]	20.33 ± 4.53[b]	20.64 ± 5.49[b]	17.87 ± 4.80[b]	12.80 ± 1.62[c]	13.47 ± 2.77[c]
Apple	30.49 ± 2.02[b]	24.17 ± 3.23[b]	20.65 ± 1.42[b]	17.26 ± 5.38[b]	18.33 ± 1.85[b]	17.11 ± 0.80[b]

[a, b, c] Mean values (± SD) with different letters are significantly different ($P \leq 0.05$) for the same storage time.

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