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Research article

Inhibitory effect of rice bran protein and its fractions on enzymatic browning in potato puree

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Abstract

The protein was extracted from defatted rice bran (*Oryza sativa* L.) cv. Khao Pathum Thani 1, Khao Suphan Buri 1, Khao Dawk Mali 105, Khao Chinat 1 and Khao Gokho 15 using alkali extraction at pH 9.5, followed by precipitation at pH 4.5 to obtain rice bran protein extract (RBPE). The RBPE from Khao Dawk Mali 105 (K-RBPE) inhibited browning in potato puree more than the RBPE from the other cultivars. The protein concentration of K-RBPE was varied to 0%, 0.25%, 0.5%, 0.75%, 1.0%, 1.5% and 2.0% (g protein/100 mL extract). K-RBPE at 1.0% inhibited potato puree browning to a similar level as 1.5% and 2.0% during storage for 6 hr. Khao Dawk Mali 105 rice bran proteins were fractionated using the Osborne procedure and the effect was investigated of its protein fractions on the inhibition of enzymatic browning in potato puree. The albumin rice bran protein fraction (Ab-RBPF) inhibited potato puree browning to a similar level as the glutelin fraction (Gt-RBPF) but the former had the highest potato polyphenol oxidase (PPO) inhibition among all the fractions. However, 1.0% K-RBPE inhibited potato puree browning more effectively than 1.0% Ab-RBPF. Furthermore, 1.0% K-RBPE had an inhibitory effect on browning similar to that of 10 mM ascorbic acid and 4-hexylresorcinol; and higher than for 10 mM ethylenediaminetetraacetic acid (EDTA) and citric acid; however, it was less effective than 10 mM cysteine and sodium metabisulfite. In addition, K-RBPE could inhibit potato PPO more effectively than Ab-RBPF, citric acid and EDTA, respectively.

Introduction

Enzymatic browning has been considered as the major reaction that affects the quality of fruit and vegetables, and leads to undesirable changes in the quality of products during processing and storage as it usually impairs the color properties of the products (Kubglomsong and Theerakulkait, 2014a). Polyphenol oxidase (PPO) is a key enzyme involved in the enzymatic browning reaction by catalyzing the phenolic compounds to produce colored polymers or pigments (Marshall et al., 2000; Ding et al., 2002).

There have been several studies to develop techniques for inhibiting PPO activity in foods. Sulfites have been used extensively because of their high effectiveness in controlling browning and their low cost; however, sulfites are subjected to regulatory restrictions because of the adverse effects on health and quality of products (Girelli et al., 2004). Thus, extracts from natural sources have been sought to replace chemical additives that prevent enzymatic browning reaction by retarding PPO activity, including amino acids, peptides or proteins (Kahn, 1985), rice bran extract (Theerakulkait and Boonsiripiphat, 2007; Sukhonthara and Theerakulkait, 2011; Threranukool et al., 2018) and rice bran protein extract (Kubglomsong and Theerakulkait, 2014a; Kubglomsong and Theerakulkait, 2014b; Kubglomsong et al., 2018).

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Thai farmers have cultivated rice for a long time and have improved it continuously so that now there are more than 100 cultivars of Thai rice (Rice Department, 2020). Rice bran is a by-product from the rice milling process (Juliano, 1985). Rice bran is a source of numerous nutrients such as proteins, peptides and several amino acids that have antioxidant properties, especially glutamic acid, asparagine, taurine, glutathione and glutamine (Hamada et al., 1998; Parrado et al., 2006).

Rice bran proteins are complex. They can be fractionated by their solubility properties in water, salt, alkaline and alcohol using the Osborne classification (Osborne, 1924). The soluble fractions are albumins, globulins, glutelins and prolamins which are present in rice bran proteins at 37%, 36%, 22% and 5%, respectively (Betschart et al., 1977). Chanput et al. (2009) found that rice bran proteins contained prolamins, albumins, globulins and glutelins that exhibited antioxidant properties. However, their antioxidant properties depended on the rice bran cultivar (Iqbal et al., 2005). PPO catalyzes the oxidation of phenolic compounds and results in the enzymatic browning reaction (Ali et al., 2016). Thus, proteins that exhibited antioxidant properties could possibly inhibit enzymatic browning. However, there has been no adequate published information on the inhibition of enzymatic browning in potato puree by rice bran protein extract from different cultivars. In addition, the effect of rice bran protein fractions on enzymatic browning in potato puree and their comparison with commercial anti-browning agents has not been investigated. The purposes of this study were to investigate the effect of protein extract from different cultivars of rice bran on the inhibitory efficiency of enzymatic browning in potato puree. The protein fractions of rice bran cultivar with the most effective inhibition were also studied for their enzymatic browning inhibition of potato puree and compared with commercial browning inhibitors.

Materials and Methods

Rice bran samples

Rice bran samples (RB) from five different cultivars were used in this study, namely *Oryza sativa* L. cv. Khao Dawk Mali 105, *O. sativa* L. cv. Khao Pathum Thani 1, *O. sativa* L. cv. Khao Suphan Buri 1, *O. sativa* L. cv. Khao Chinat 1 and *O. sativa* L. cv. Khao Gokho 15. All rice bran samples were defatted using hexane according to the method of Kubglomsong and Theerakulkait (2014a).

Preparation of rice bran protein extract

Rice bran protein was extracted using distilled water (DW) and the pH was adjusted to 9.5 using 1.0 N NaOH, followed by isoelectric point precipitation at pH 4.5 using 1.0 N HCl (Gnanasambandam and Hettiarachchy, 1995). The precipitate was rice bran protein concentrate. Rice bran protein extract (RBPE) was prepared by dispersing rice bran protein concentrate in DW at the protein concentrations described below. All experiments were performed at ambient temperature (25°C).

Browning inhibition of rice bran protein extract from different cultivars of rice bran

Potatoes (*Solanum tuberosum* L.) were peeled and blended for 20 s with each RBPE prepared at a protein concentration of 0.75% (g protein/100 mL extract) or DW (control) at a ratio of potatoes to samples at 2:1 (weight per volume). The color values (L*, a* and b*) of the samples were recorded using a spectrophotometer (CM-3500D, Minolta) at 0 hr, 0.5 hr and 6 hr after blending and keeping at ambient temperature. The browning values and total color differences (ΔE) were calculated according to the equations reported by Labuza et al. (1990) and Girelli et al. (2004), respectively. RBPE from the rice cultivar that showed the highest browning inhibition of potato puree was selected for further study.

Browning inhibition of rice bran protein extract at different protein concentration

RBPE prepared from selected rice cultivars from the previous step was varied in protein concentration at 0%, 0.25%, 0.5%, 0.75%, 1.0%, 1.5% and 2.0% (g protein/100 mL extract) to study the browning inhibition of potato puree according to the method described above.

Fractionation of rice bran proteins using the Osborne procedure

Rice bran protein fractions (RBPF) were fractionated using the Osborne procedure (adapted from Likitwattanasade and Hongsprabhas, 2010). First, 100 g of the defatted rice bran from the selected cultivar was extracted for 4 hr using 400 mL of DW, and then centrifuged at 3,000×g for 30 min at 25°C. The obtained supernatant was the albumin fraction. The pellet from the albumin extraction step was similarly extracted using 400 mL of NaCl at a concentration of 5 g/100 mL to recover the globulin fraction (supernatant), while the pellet from the globulin fraction extraction was again extracted using 400 mL of NaOH at a concentration of 0.1 M to yield the glutelin fraction (supernatant). The pellet after glutelin fraction extraction was further extracted using 400 mL of ethanol at a concentration of 70 mL/100 mL to obtain the prolamin fraction (supernatant). The protein fractions: albumin (Ab-RBPF), globulin (Gb-RBPF), glutelin (Gt-RBPF) and prolamin (Pl-RBPF) were adjusted using 0.1 M HCl to their isoelectric pH levels at 4.1, 4.3, 4.8 and 5.0, respectively, and then centrifuged at 3,000×g for 30 min to collect the RBPF. Each RBPF sample was washed twice with DW (adapted from Likitwattanasade and Hongsprabhas, 2010). All protein fractions were used for the browning inhibition study of potato puree according to the method described above. The potato PPO activity inhibition of each fraction was measured using the modified method of Lozano-de-Gonzalez et al. (1993).

Effect of rice bran protein extract and its protein fractions compared with commercial browning inhibitors on browning and polyphenol oxidase activity inhibition

The RBPE and its protein fractions selected from previous study were used and compared with 10 mM citric acid, ascorbic acid, 4-hexylresorcinol, ethylenediaminetetraacetic acid (EDTA), cysteine and sodium metabisulfite for potato puree browning inhibition and potato PPO inhibition as mentioned above.

Statistical analysis

All experiments were performed in three replicates. One-way analysis of variance and Duncan's multiple range tests were applied to identify significant differences between treatments at the $p \leq 0.05$ test level.

Results and Discussion

Browning inhibition of protein extract from different cultivars of rice bran in potato puree

Fig. 1 shows the browning inhibition of potato puree treated with rice bran protein extract prepared from five different rice cultivars: Khao Suphan Buri 1 (S-RBPE), Khao Pathum Thani 1 (P-RBPE), Khao Dawk Mali 105 (K-RBPE), Khao Chinat 1 (C-RBPE), and Khao Gokho 15 (R-RBPE). The potato puree treated with K-RBPE had the highest L* values, and the DW-treated sample had the lowest L* values at 6 hr storage ($p \leq 0.05$). The browning values of the DW-treated sample were higher than for each RBPE-treated sample during 0.5–6 hr storage, and the K-RBPE-treated sample had the lowest browning values at 6 hr storage ($p \leq 0.05$); this result was similar for the ΔE* values (Fig. 1).

The results indicated that RBPE had browning inhibitory efficiency with potato puree. This might have been due to the protein in the extracts inhibiting the enzymatic browning in potato puree. Kahn (1985) reported that protein could inhibit enzymatic browning through a reaction with the quinone to form colorless complexes, or through direct inhibition of the enzyme and form stable complexes with Cu^{2+} at the active site of PPO. Cysteine and histidine that showed high chelating activity and had been found in rice bran protein might be involved in enzymatic browning inhibition of potato puree (Juliano, 1985; Luh et al., 1991; Marshall et al., 2000; Parrado et al., 2006). In addition, rice bran protein contains high amounts of sulfur amino acids (Parrado et al., 2006), glutamic acid, aspartic acid, glutathione and glutamine that showed antioxidative properties (Hamada et al., 1998; Sereewatthanawut et al., 2008). This might be related to the inhibition of PPO which catalyzes the oxidation reaction (Ali et al., 2016). RBPE from the different rice cultivars showed different browning inhibitory efficiency. This might have been due to the different types and amounts of amino acids in the different cultivars of rice bran. Houston et al. (1969) found that the amounts of methionine, histidine, glutamic acid and arginine in six rice brans were significantly different. In addition, several researchers also found that the amino acid compositions in RBPE from different rice cultivars were different (Juliano, 1985; Prakash and Ramanatham, 1995; Prakash, 1996). The current results showed that K-RBPE had the highest browning inhibitory efficiency; therefore, K-RBPE was selected for further study.

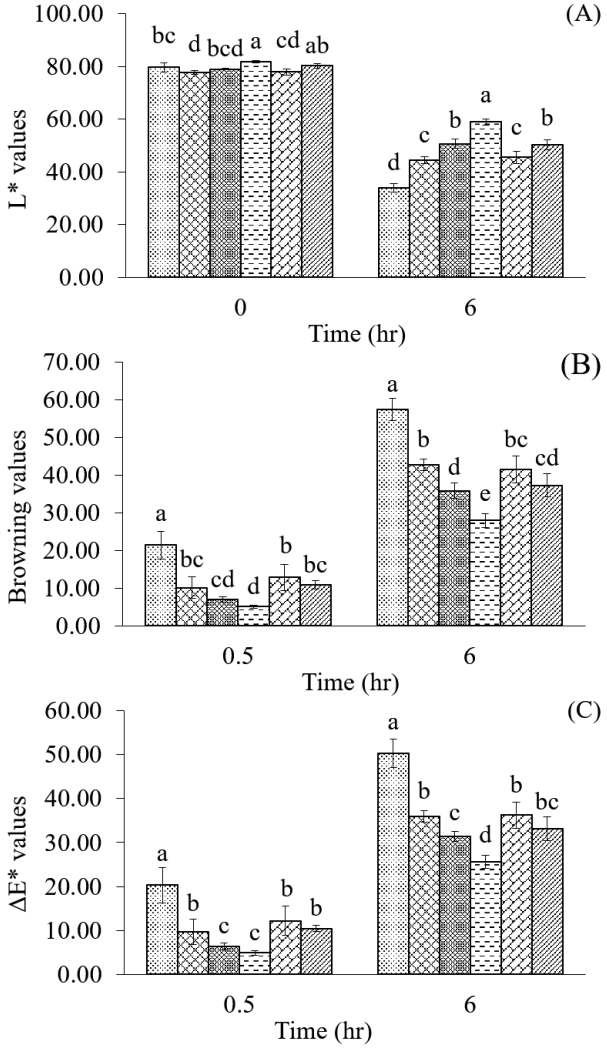

Fig. 1 Values for L* (A), browning (B) and ΔE* (C) of potato puree blended with DW (▨), S-RBPE(▨), P-RBPE (▨), K-RBPE (▨), C-RBPE (▨) and R-RBPE (▨) at the same protein concentration and stored at room temperature for 6 hr, where DW = distilled water; S-RBPE = rice bran protein extract from Khao Suphan Buri 1; P-RBPE = rice bran protein extract from Khao Pathum Thani 1; K-RBPE = rice bran protein extract from Khao Dawk Mali 105; C-RBPE = rice bran protein extract from Khao Chinat 1; R-RBPE = rice bran protein extract from Khao Gokho 15 and different letters indicate significant ($p \leq 0.05$) differences within each storage period

Effect of protein concentration of rice bran protein extract on browning inhibitory efficiency with potato puree

The effects of different protein concentrations of K-RBPE on the browning inhibitory efficiency with potato puree are shown in Fig. 2, At 6 hr storage, the potato puree treated with 1%, 1.5% and 2% of K-RBPE had L* values significantly higher than those treated with 0%, 0.25%, 0.5% and 0.75% of K-RBPE. The browning values and ΔE* values of 0%, 0.25%, 0.5% and 0.75% of K-RBPE-treated puree were significantly higher than those of 1%, 1.5% and 2% treated puree at 6 hr storage. Based on these results, the browning inhibitory efficiency with potato puree treated with 1% K-RBPE was similar to that of the samples treated with 1.5% and 2%. Moreover, K-RBPE had a light brown color and a slight rice bran odor, so using a high protein concentration of the extract might result in unacceptable color and odor. Thus, the protein concentration at 1% was selected for further study.

Inhibitory effect of rice bran protein fractions on potato puree enzymatic browning and potato polyphenol oxidase activity

The rice bran protein fractions were fractionated from K-RBPE using the Osborne procedure and then RBPF (Ab-RBPF, Gb-RBPF, Gt-RBPF and Pl-RBPF) was dispersed in DW to an equal protein concentration. The browning inhibition of RBPF in potato puree is shown in Fig. 3. At 6 hr storage, the puree treated with Ab-RBPF had the highest L* values among all fractions ($p \leq 0.05$). The L* values of the puree treated with Gb-RBPF and Gt-RBPF were not significantly different whereas, the Pl-RBPF treatment had the lowest L* values ($p \leq 0.05$). The browning values and ΔE* values during 6 hr storage of the puree treated with Ab-RBPF were the lowest, followed by those of Gt-RBPF, Gb-RBPF and Pl-RBPF, respectively ($p \leq 0.05$). Based on the L* values, browning values and ΔE* values, Ab-RBPF had the strongest potato puree browning inhibition, with Gt-RBPF and Gb-RBPF being the second highest and Pl-RBPF the lowest browning inhibitory effect. These results agreed with the potato PPO activity inhibition by these fractions as shown in Table 1 where the Ab-RBPF treatment had the highest PPO activity inhibition, followed by Gt-RBPF, Gb-RBPF and Pl-RBPF, respectively ($p \leq 0.05$).

This might have been due to the highest reported antioxidative activity of Ab-RBPF followed by the glutelin, globulin and prolamin fractions (Adebiyi et al., 2009). This activity might have affected the enzymatic browning reaction which is an oxidation reaction (Marshall et al., 2000). Padhye and Salunkhe (1979) reported that rice albumin contained higher amounts of polar uncharged amino acids than the other fractions, whereas the prolamin fraction contained the lowest amounts. Schurink et al. (2007) explained that peptides containing polar uncharged amino acids such as cysteine and serine had high PPO inhibition. This might be related to the PPO inhibition of Ab-RBPF. Moreover, Wang et al. (2014) reported that Gt-RBPF contained high amounts of sulfur-containing amino acids such as cysteine that have been reported to inhibit PPO activity. This might have resulted in different enzymatic browning inhibitory efficiencies with potato puree. Thus, the Ab-RBPF was selected for further study.

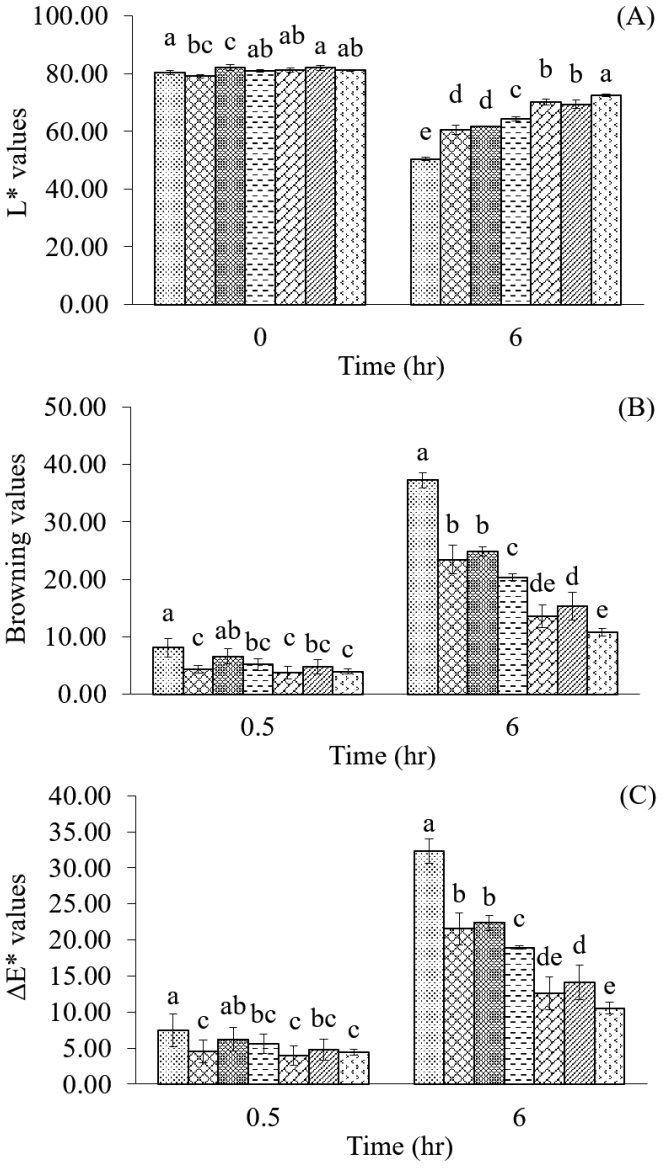

Fig. 2 Values for L* (A), browning (B) and ΔE* (C) of potato puree blended with K-RBPE at 0% (▨), 0.25% (▩), 0.50% (▩), 0.75% (▬), 1.00% (▨), 1.50% (▨) and 2.00% (g protein/100 mL extract) (▬), stored at room temperature for 6 hr, where K-RBPE = rice bran protein extract from Khao Dawk Mali 105 and different letters indicate significant ($p \leq 0.05$) differences within each storage period

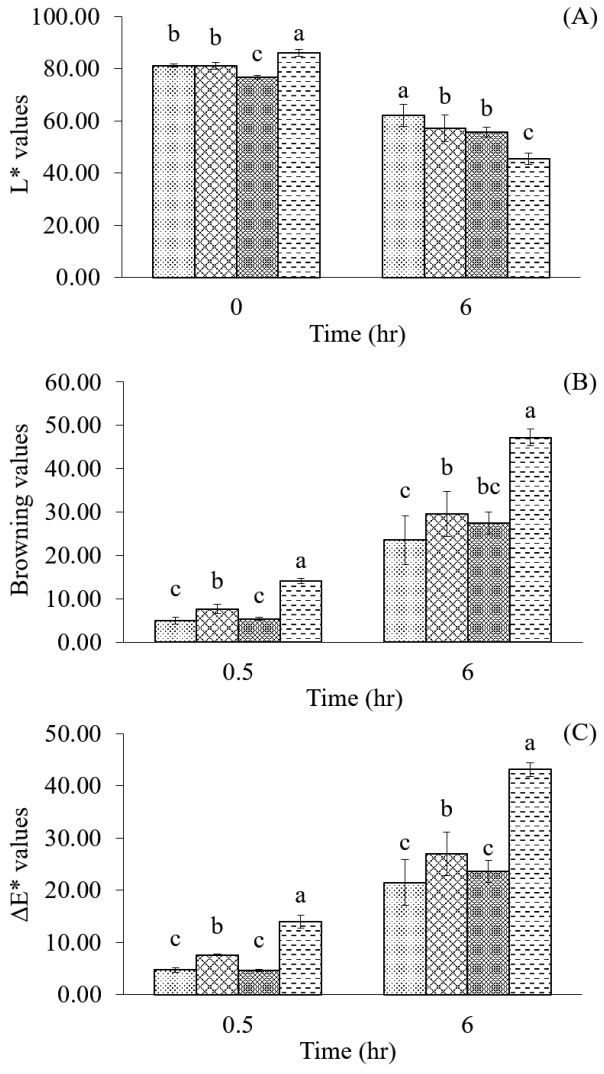

Fig. 3 Values for L* (A), browning (B) and ΔE* (C) of potato puree treated with K-RBPF at the same protein concentration: Ab-RBPF (▨), Gb-RBPF (▩), Gt-RBPF (▩) and Pl-RBPF (▭), stored at room temperature for 6 hr, where Ab-RBPF = albumin rice bran protein fraction; Gb-RBPF = globulin rice bran protein fraction; Gt-RBPF = glutelin rice bran protein fraction; Pl-RBPF = prolamin rice bran protein fraction and different letters indicate significant ($p \leq 0.05$) differences within each storage period

Table 1 Potato polyphenol oxidase inhibition (%) of Ab-RBPF, Gb-RBPF, Gt-RBPF and Pl-RBPF

Inhibitor	Potato PPO inhibition (%)
Ab-RBPF	20.52±1.25[a]
Gb-RBPF	10.19±3.50[c]
Gt-RBPF	14.94±3.09[b]
Pl-RBPF	4.21±1.64[d]

PPO = polyphenol oxidase; Ab-RBPF = albumin rice bran protein fraction; Gb-RBPF = globulin rice bran protein fraction; Gt-RBPF = glutelin rice bran protein fraction; Pl-RBPF = prolamin rice bran protein fraction.
means ± SD in a column with different letters indicate significant ($p \leq 0.05$) differences.

Effect of K-RBPE and Ab-RBPF compared with commercial browning inhibitors on potato puree browning and potato polyphenol oxidase activity

Fig 4. shows the potato enzymatic browning inhibitory efficiency of K-RBPE and Ab-RBPF at 1.0% and of 10 mM 4-hexylresorcinol (4-HR), ascorbic acid (ASA), citric acid (CT), cysteine (CYS), EDTA and sodium metabisulfite (SM). These results showed that at 6 hr storage, the potato puree treated with K-RBPE had higher L* values than Ab-RBPF and were also higher than those of CT and EDTA ($p \leq 0.05$); however, it was similar to the ASA treatment ($p > 0.05$). At 6 hr storage, the browning values of the EDTA-treated puree were higher than those treated with CT, Ab-RBPF, ASA, K-RBPE, 4-HR, CYS and SM, respectively ($p \leq 0.05$). The ΔE* values of the K-RBPE-treated puree were significantly less than those treated with Ab-RBPF, ASA, CT and EDTA at 6 hr storage.

The potato PPO activity inhibition by different inhibitors is shown in Table 2. The potato PPO activity inhibition by 1.0% K-RBPE was significantly higher than for 1.0% Ab-RBPF, 10 mM CT and EDTA, respectively. These results agreed with the browning inhibition in potato puree. However, the potato PPO activity inhibition by 1.0% K-RBPE was significantly less than for 10 mM 4-HR, ASA, CYS and SM, indicating that 1.0% K-RBPE was more effective at inhibiting enzymatic browning in potato puree than 1.0% Ab-fraction, 10 mM CT and EDTA. However, 1.0% K-RBPE inhibited to a similar extent as 10 mM ASA and 4-HR. K-RBPE as a crude protein might contain potential amino acids more than fractionated rice bran protein (Ab-RBPF), resulting in greater enzymatic browning inhibition in potato puree. Protein could inhibit enzymatic browning by reacting with the quinone to form colorless complexes, or through inactivation of the enzyme by forming stable complexes with Cu^{2+} at the active site of PPO (Kahn, 1985; Casado-Vela et al., 2006). Duangmal and Apenten (1999) reported that EDTA could reduce PPO activity by forming a stable complex with Cu^{2+} at the active site of the enzyme. Citric acid also showed chelating activity and acted as an acidulant; thus it could retard PPO activity (Rodrigues et al., 2014). Ascorbic acid has been used as a reducing agent to control enzymatic browning; however, its inhibition is temporary because it is oxidized irreversibly to dehydroascorbic acid by the reaction with *o*-quinones (McEvily et al., 1992; Rodrigues et al., 2014). 4-Hexylresorcinol could inhibit PPO by directly interacting with PPO instead of an enzyme substrate, resulting in inhibition of enzymatic browning (Lambrecht, 1995; Arias et al., 2007). Sodium metabisulfite and a sulfur-containing amino acid (cysteine) have been reported to effectively inhibit PPO by reacting with *o*-quinones to form a stable, colorless product (Duangmal and Apenten, 1999; Rodrigues et al., 2014), in addition, sodium metabisulfite could react with disulfide bonds on the enzyme structure leading to inactivation of the enzyme by changing its tertiary structure (Duangmal and Apenten, 1999; Rodrigues et al., 2014).

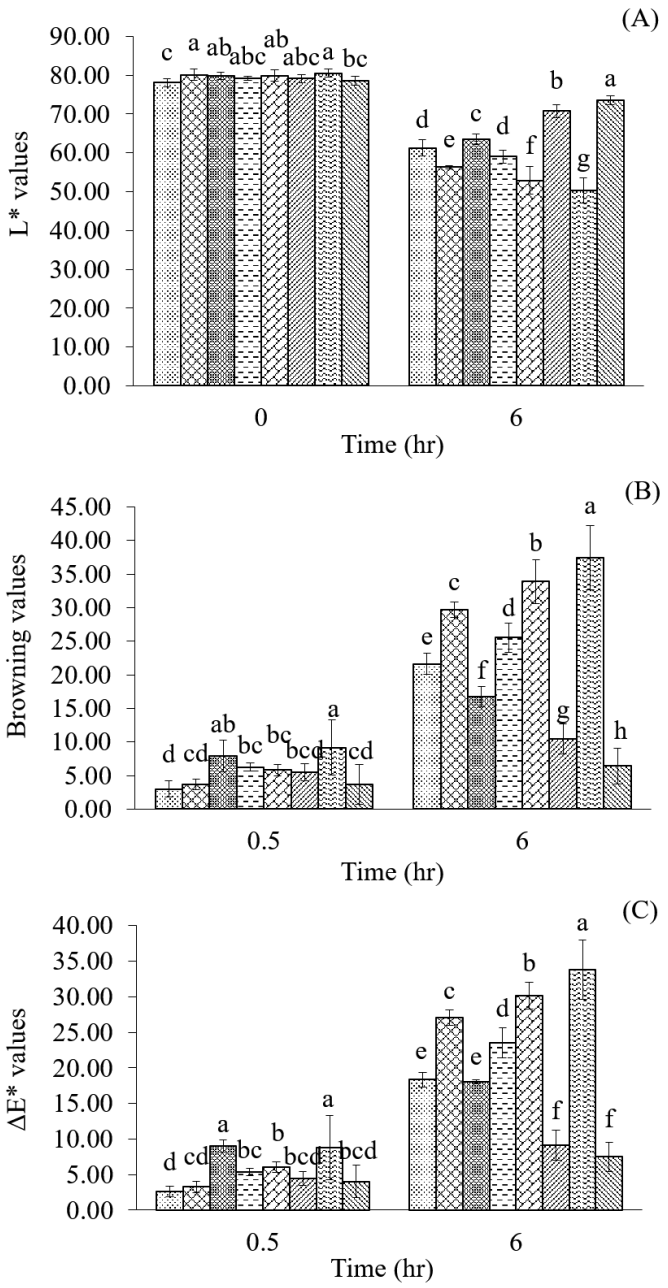

Fig. 4 Values for L* (A), browning (B) and ΔE* (C) of potato puree treated with K-RBPE (▩), Ab-RBPF (▩), 4-HR (▩), ASA (▩), CT (▩), CYS (▩), EDTA (▩) and SM (▩), stored at room temperature for 6 hr, where K-RBPE = rice bran protein extract from Khao Dawk Mali 105; Ab-RBPF = albumin rice bran protein fraction; 4-HR = 4-hexylresorcinol; ASA = ascorbic acid; CT = citric acid; CYS = cysteine; EDTA = ethylenediaminetetraacetic acid; SM = sodium metabisulfite and different letters indicate significant ($p \leq 0.05$) differences with each storage period

Table 2 Potato polyphenol oxidase inhibition of 1.0% K-RBPE, 1.0% Ab-RBPF and commercial anti-browning inhibitors at 10 mM

Inhibitor	Potato PPO inhibition (%)
K-RBPE	26.24±2.52^{c}
Ab-RBPF	23.61±0.85^{d}
4-HR	28.98±5.13^{b}
ASA	100.00±0.00^{a}
CT	13.40±3.55^{e}
CYS	99.86±0.30^{a}
EDTA	3.81±1.47^{f}
SM	100.00±0.00^{a}

PPO = polyphenol oxidase; K-RBPE = rice bran protein extract from Khao Dawk Mali 105; Ab-RBPF = albumin rice bran protein fraction; 4-HR = 4-hexylresorcinol; ASA = ascorbic acid; CT = citric acid; CYS = cysteine; EDTA = ethylenediaminetetraacetic acid; SM = sodium metabisulfite.
means ± SD in a column with different letters indicate significant ($p \leq 0.05$) differences.

In conclusion, RBPE from different cultivars could inhibit browning in potato puree to different extents. RBPE from Khao Dawk Mali 105 (K-RBPE) could inhibit potato puree browning more than RBPE from other cultivars. K-RBPE at 1.0% inhibited browning in potato puree to a similar level as the 1.5% and 2.0% treatments. Ab-RBPF had the highest browning inhibitory efficiency in potato puree and potato PPO activity inhibition compared to the other RBPF types studied. However, 1.0% K-RBPE controlled potato puree browning more effectively than 1.0% Ab-RBPF, which was similar to 10 mM ascorbic acid and 4-hexylresorcinol, and with higher efficiency than 10 mM citric acid and EDTA. However, 1.0% K-RBPE had lower inhibition than 10 mM cysteine and sodium metabisulfite. Furthermore, 1.0% K-RBPE could inhibit potato PPO activity more effectively than 1.0% Ab-RBPF, citric acid and EDTA, but less effectively than 10 mM 4-hexylresorcinol, ascorbic acid, cysteine and sodium metabisulfite. Rice bran protein extract, especially K-RBPE, has potential to be used as a natural anti-browning agent in the potato industry.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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