Research Article

Control of Enzymatic Browning in Apple and Potato Purees by Using Guava Extract

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Abstract

Control of polyphenol oxidase (PPO) and browning prevention in apple and potato purees by using guava extract (GE) compared with distilled water (DW) were studied at 4 concentrations, including 25%GE (0.24 g wet guava pulp/mL of the extract), 50%GE (0.48 g wet guava pulp/mL of the extract), 75%GE (0.72 g wet guava pulp/mL of the extract), and 100%GE (0.97 g wet guava pulp/mL of the extract). After storage for 5 h, the browning value of apple and potato purees blended with 100%GE was significantly lower than those treated with 50%GE, 25%GE, and DW ($p \leq 0.05$). The L^* value of apple puree treated with 100%GE was significantly higher than those treated with others ($p \leq 0.05$), while L^* value of potato puree treated with 100%GE was not significantly higher than that treated with 75%GE ($p > 0.05$), but significantly higher than those treated with others ($p \leq 0.05$) during storage. The inhibitions of both PPOs were increased by increasing in guava extracted concentration. The most effectiveness in inhibition of apple and potato PPO activity was 74.53±0.41% and 64.62±0.52%, respectively. Moreover, 100%GE exhibited a higher inhibitory effect on apple PPO than 0.02% w/v citric acid and 0.02% w/v ascorbic acid, while it exhibited a higher inhibitory effect on potato PPO than 0.02% w/v citric acid, and showed similar inhibitory effect to 0.02% w/v ascorbic acid. 100%GE showed the highest total phenolic compound and 0.02% w/v ascorbic acid content values of 880.73±2.15 µg gallic acid equivalent (GAE)/mL sample and 122.67±2.02 µg/mL, respectively. In conclusion, GE possessed effective inhibitors against enzymatic browning in apple and potato purees.

***Key Words*:** Apple; Enzymatic browning; Guava; Polyphenol oxidase; Potato

Introduction

"Fuji" apple was introduced in Japan in 1962 by crossing between red "Delicious" and "Ralls Janet". It is one of the main apple cultivar consumed all over the world, since it has a good quality and sensory characteristics. (Stebbins et al., 1991; Yoshida et al., 1995). Potato (*Solanum tuberosum* L.) is one of the world major agricultural crops, consumed daily by millions of people from diverse cultural backgrounds (Chiavaro et al., 2006). Potatoes are grown in approximately 80% of all countries and worldwide production stands in excess of 300 million tons per year, which a figure exceeded only by wheat, maize, and rice (Pedreschi et al., 2005). However, browning occurred during handing, processing, and storage after harvest is the main problem which contributes to loss in quality of apple and potato (Kaaber et al., 2002). Browning also leads to development of off-flavours and losses in nutritional quality (Severini et al., 2003). Currently, the browning is an important and challenging research topic (Komiyama et al., 1991; Murata et al., 1995; Sannomaru et al., 1998;

Toivonen, 2006). One of the main problems associated with preserving tfruits and vegetables is the enzymatic browning, catalyzed by polyphenol oxidase (PPO) (Gómez-López, 2002). PPO (EC 1.14.18.1), also called tyrosinase, is a copper-containing enzyme which catalyzes the hydroxylation of monophenols to o-diphenols (monophenolase or cresolase activity) and the oxidation of o-dihydroxyphenols to o-quinones (diphenolase or catecholase activity), utilising molecular oxygen. These quinones are highly reactive, electrophilic molecules which covalently modify one crosslink to a variety of cellular constituents. The reactions produce undesirable blackening or browning in food processing and post-harvest physiology of plant products and are the main focus of interest in PPO in food technology (Aydemir, 2004; Guerrero-Beltran, 2005). Several chemical browning inhibitors have been assessed for the inhibition of PPO activity in fruits and vegetables. Sulfites are among the most effective browning inhibitor (Chen et al., 2000). However, because of adverse health effects, the use of sulfites for this purpose has been restricted by the U.S. Food and Drug Administration (Coetzer et al., 2001). Currently, alternative natural PPO inhibitors have been investigated by several researchers (Kahn, 1985; Oszmianski and Lee, 1990; Lozano-de-Gonzales et al., 1993; Chen et al., 2000; Son et al., 2000; Jang et al., 2002; Lee et al., 2002; Girelli et al., 2004; Kim et al., 2005; Chaisakdanugull et al., 2007; Lee et al., 2007; Lee, 2007; Roldán et al., 2008; Gacche et al., 2009; Soysal, 2009; Altunkaya and GÖkmen, 2011; Zocca et al., 2011; Sukhonthara and Theerakulkait, 2012; Fante et al., 2013; Schulbach et al., 2013; Kubglomsong and Theerakulkait, 2014). The development of natural alternatives to those costly and potentially toxic inhibitors would be desirable. Since 1940s, guava has shown many interesting properties such as antimicrobial, antimalarial effects, anticancer/antitumour effects, cardiovascular, hypotensive effects, wound healing antioxidant, free radical scavenger and radioprotective activities (Gutiérrez et al., 2008).

Moreover, guava contains high phenolic contents which were significantly correlated with antioxidant capacity (Alothman et al., 2009; Jiménez-Escrig et al., 2001; Zabidah et al., 2011). Alothman et al. (2009) found Thai seedless guava had high polyphenol contents (129-191 gallic acid equivalents/100 g or GAE/100g) and FRAP (30.1-30.8 μmol FeII/g fresh weight) and DPPH$^{\bullet}$ (84.9-94.6% inhibition) values in comparison with honey pineapple and banana. Jiménez-Escrig et al. (2001) reported pulp and peel fractions of guava showed high contents of polyphenols (2.62-7.79%) and a remarkable antioxidant capacity (FRAP = 238-462 μmol Trolox/ g of dry matter, DPPH$^{\bullet}$ = 1.92-3.70 EC50 g of dry matter/g DPPH$^{\bullet}$). Zabidah et al. (2011) demonstrated guava juice had the highest total phenolic content (24.64 mg GAE/100 mL) compared to other juices (bambangan and cocoa pulp juice), which HPLC results showed that higher catechin, vanillic acid and ferulic acid contents may provide the antioxidant activity in guava juice. Nowadays, the effect of guava extract on browning of apple and potato has not been previously investigated. Therefore, the main objective of this research was to investigate the effect of guava extract on browning prevention in apple and potato purees.

Materials and Methods

Mature green fruit of guavas were obtained from local market in Phetchaburi. The ascorbic acid, citric acid, 2,6-dichloroindophenol sodium salt, gallic acid, and sodium metabisulfite were purchased from Ajax Finechem Pty Ltd (Australia). Folin-Ciocalteu reagent, polyvinylpyrrolidone, pyrocatechol, and Triton X-100 were purchased from Sigma-Aldrich (USA).

Preparation of guava extract

Guava extract (GE) was prepared by homogenizing 150 g of guava pulp (peel and deseed) with 150 mL of distilled water (DW) in a blender, and then filtrated though a cheese cloth to obtain 155 mL of filtrate (100%GE, 0.97 g wet guava pulp/ mL of the extract). 75%GE, 50%GE, and 25%GE were prepared by dilution of 100%GE.

Apple and potato purees preparation and browning prevention

Apple and potato were purchased from local

market in Phetchaburi. Apple and potato purees were prepared by blending 150 g of apple or potato pulp for 20 s with 150 mL of DW, 25%GE (0.24 g wet guava pulp/mL of the extract), 50%GE (0.48 g wet guava pulp/mL of the extract), 75%GE (0.72 g wet guava pulp/mL of the extract), and 100%GE (0.97 g wet guava pulp/mL of the extract). DW was mixed in purees as the control sample. The puree samples were poured into the liquid tester of ColorimetricSpectrophotometer (Hunter Lab, ColorFlex) for measure of (L^*, a^*, and b^*) at 1, 3, and 5 h of storage time at room temperater (25° C). The browning values (($\Delta L^*/L_0^*$) x 100) were calculated; when ΔL^* was equal to $L_0^* - L^*$; L^* was the L^* value at any time and L_0^* was the initial L_0^* measurement (Labuza et al., 1990).

PPO activity inhibition study

Apple and potato PPO activities were determined by measuring the increase in absorbance of reaction mixture at 420 nm with a spectrophotometer (UV-VIS Spectrophotometer, UV-mini-1240, Shimadzu corporation analytical instruments division, Japan) at 25 °C. The apple PPO (or potato PPO) was obtained by homogenizing 50 grams of apple (or potato) with 50 mL of a cold 0.1 M sodium phosphate buffer (pH 6.8, 4 °C) containing 10.0 g/L polyvinylpyrrolidone (PVP) and 5.0 g/L Triton X-100, then centrifuged for 10 mins (4 °C) at 8,000 x g (Hettich Universal 32 R, Hettich Zentrifugen, Germany). The supernatant was collected and stored at -20 °C for use as crude enzyme until analysis. The reaction mixture was consisted of 0.1 mL of the enzyme, 0.9 mL of 0.05 M sodium phosphate buffer pH 6.8, 1.0 mL of 0.2 M catechol substrate solution in 0.05 M sodium phosphate buffer pH 6.8 and 1.0 mL of GE (inhibitor) or DW (control). The change in absorbance at 420 nm of reaction mixture was recorded for 1 min. One unit of enzyme activity was defined as the amount of enzyme responsible for a change of 1 absorbance unit at 420 nm/min at 25 °C, pH 6.8. The percent of PPO inhibition was calculated as follows (Chaisakdanugull et al., 2007):

PPO inhibition (%) = [(activity of control - activity of the treatment)/activity of control] × 100

Comparison of GE to antibrowning agents of enzymatic browning

Commercial antibrowning agents (citric acid, ascorbic acid, and sodium metabisulfite) were tested at final concentration of 0.02% w/v or 200 mg/mL. The reaction mixture consisted of 0.1 mL of the enzyme, 0.9 mL of 0.05 M sodium phosphate buffer pH 6.8, 1.0 mL of 0.2 M catechol substrate solution in 0.05 M sodium phosphate buffer pH 6.8, and 1.0 mL of 100%GE, DW or each antibrowning agent.

Total phenolic content of GE

The total phenolic contents of GE were estimated with Folin–Ciocalteu reagent by a modified method of Cai et al. (2004) and expressed the result as µg gallic acid equivalent (GAE/mL sample). The 50 µL of GE was mixed with 250 µL of freshly prepared Folin–Ciocalteu reagent, and 3 mL of pure water. The mixture was allowed to react for 10 min at ambient temperature. Then, 0.75 mL of 20% sodium carbonate was added. After incubation in a water bath at 40 0C for 20 mins, the sample was then cooled before the absorbance of resulting blue color at 765 nm was measured. The total phenolic content of GE was calculated on the basis of the standard curve of gallic acid.

Ascorbic acid content of GE

Ascorbic acid content of GE was determined by the titrimetry using 2,6-dichloroindophenol. A 1 mL sample of GE was mixed with 5 mL for 10 s and titrated against the dye solution (2,6-dichloroindophenol solution) until the end-point (change from blue to a permanent pink colour) for 10 s. The titrations were repeated in triplicates, and blank (DW) and standard (ascorbic acid solution, 1 mg/mL) were also carried out following the above procedure.

Statistical analysis

Three replications of each experiment were performed. All data were analyzed and tested by one-way analysis of variance. Significant difference

($p \leq 0.05$) among various treatments was detected by using Duncan's multiple range tests.

Results

Effect of GE on browning in apple and potato puree

Browning values and the changes of L^*, a^*, and b^* values of apple and potato purees blended with 25%GE, 50%GE, 75%GE, 100%GE, and DW which were stored at 25°C for 5 h were shown in Fig. 1 and Fig. 2, respectively. The browning value was calculated from $(\Delta L^*/L_0^*) \times 100$. The lower browning values indicated the greater effectiveness of browning inhibition. After storage for 1, 3, and 5 h, 100%GE was not significantly lower than treated with 75%GE ($p>0.05$) but significantly lower than those treated with 50%GE, 25%GE, and DW in apple puree ($p\leq0.05$). Their browning values of apple after 5 h of 100%GE, 75%GE, DW, 50%GE and 25%GE were 26.00±0.43, 28.43±0.80, 42.66±1.25, 44.92±0.94, and 49.49±2.92, respectively. L^* is the luminosity, a^* is the position on the green (-) to red (+) axis, and b^* is the position on the blue (-) to yellow (+) axis (Girelli et al., 2004). The decrease of L^* value means darker color, and the increase of a^* value means higher red color. Thus, the decrease of L^* value and the increase of a^* value indicate with a high browning color (Moline et al., 1999). L^* value of apple puree treated with 100%GE was significantly higher than those treated with others ($p\leq0.05$) during storage for 1 and 5 h. The a* values of apple puree treated with 100%GE was lower than those treated with others ($p\leq0.05$) during storage for 5 h, while its b^* value was similar to that treated with 75%GE ($p>0.05$) after stored for 5 h.

Browning value of potato puree treated with 100%GE was similar to those treated with 75%GE and 50%GE ($p>0.05$), but significantly lower than those treated with 25%GE and DW ($p\leq0.05$) after stored for 1 h. After storage for 3 and 5 h, 100%GE was not significantly lower than that treated with 75%GE ($p>0.05$) but significantly lower than those treated with 50%GE, 25%GE and DW ($p\leq0.05$). Their browning values after 5 h of 100%GE, 75%GE, 50%GE, 25%GE, and DW were 11.99±0.21, 12.91±0.54, 18.88±0.89, 23.19±1.38, and 38.67±0.83, respectively. L^* value of potato puree treated with 100%GE was not significantly higher than that treated with 75%GE ($p>0.05$) but significantly higher than those treated with others ($p\leq0.05$) during storage. The a^* values of potato puree treated with 100%GE were not significantly lower than those treated with 75%GE ($p>0.05$) but significantly lower than those treated with others ($p\leq0.05$) during storage for 1-5 h, while its b^* value was not significantly higher than those treated with 75%GE and 50%GE ($p>0.05$) but significantly higher than those treated with 25%GE and DW ($p\leq0.05$) after stored for 5 h.

Effect of GE on apple and potato PPOs

The effect of various concentration of guava extract on apple and potato PPOs is shown in Table 1, which % inhibition of PPO activity ranged from 8.59±0.38% to 74.53±0.41%. Inhibition of PPO activity increased when the concentration of GE increased. 100%GE showed the highest % inhibition of apple and potato PPO activities compared with those treated with 25%GE, 50%GE and 75%GE ($p<0.05$). This means that phenolic compounds and ascorbic acid in GE may affect to the inhibition of both PPO activities.

Table 1 % Inhibition of apple and potato PPO activity by GE

GE	% PPO inhibition	
	apple	potato
25%	17.76±0.98^{d}	8.59±0.38^{d}
50%	31.78±1.94^{c}	21.50±1.06^{c}
75%	61.62±0.84^{b}	56.51±1.31^{b}
100%	74.53±0.41^{a}	64.62±0.52^{a}

$^{a, b, c, d}$ Mean values with different letters in the same column are significantly different ($p\leq0.05$).

Comparison of GE and various anti-browning agents on apple and potato PPOs

The effect of 100%GE and various antibrowning agents (final conc. 0.02%w/v) on apple and potato PPOs inhibition are shown in Table 2. 100%GE

exhibited significantly higher inhibitory effect on apple PPO than ascorbic acid and citric acid ($p \leq 0.05$), and exhibited significantly higher inhibitory effect on potato PPO than citric acid ($p \leq 0.05$). 100%GE exhibited slightly higher inhibitory effect on potato PPO than that of ascorbic acid ($p > 0.05$). Sodium metabisulfite was the most effective inhibitor on apple and potato PPOs.

Table 2 Comparison of GE and various antibrowning agents on the activity of apple and patato PPO. The reaction contained 100%GE or antibrowning agents at final concentration of 0.02%w/v.

Antibrowning agent	% PPO inhibition	
	apple	potato
100% GE	74.53±0.41[b]	64.62±0.52[b]
Citric acid	7.74±0.70[d]	0.88±0.30[c]
Ascorbic acid	45.64±1.76[c]	63.99±1.28[b]
Sodium metabisulfite	96.59±1.39[a]	98.81±1.11[a]

[a, b, c] Mean values with different letters in the same column are significantly different ($p \leq 0.05$).

Total phenolic compounds and ascorbic acid content of GE

Total phenolic compounds and ascorbic acid of various concentration of guava extract are shown in Table 3, which total phenolic compounds ranged from 265.40±3.46 to 880.73±2.15 µg GAE/mL sample and ascorbic acid ranged from 41.56±0.81 to 122.67±2.02 mg/mL. Total phenolic compounds and ascorbic acid increased when the concentration of GE increased. 100%GE showed the highest total phenolic compounds and ascorbic acid compared with those treated with 25%GE, 50%GE, and 75%GE ($p \leq 0.05$).

In Table 2 and 3, 100%GE is the most potent enzymatic browning inhibitor against PPO in apple and potato. At ascorbic acid content of 100%GE equal to 122.67±2.02 mg/mL, %PPO inhibition of 100%GE was significant higher than 0.02% w/v or 200 mg/mL ascorbic acid. This showed the important role on %PPO inhibition of phenolic compounds in guava extract. Moreover, phenolic compounds and ascorbic acid amounts increased when concentration of GE increased. This result indicated that %PPO inhibition in both fruits depends on concentration of GE.

Table 3 Total phenolic compounds and ascorbic content of GE

GE	Total phenolic compounds (µg GAE/mL)	Ascorbic acid (µg/ml)
25%	265.40±3.46[d]	41.56±0.81[d]
50%	471.40±4.00[c]	65.89±1.14[c]
75%	682.07±3.06[b]	94.94±1.68[b]
100%	880.73±2.15[a]	122.67±2.02[a]

[a, b, c, d] Mean values with different letters in the same column are significantly different ($p \leq 0.05$).

Discussion and Conclusion

The browning, L^*, a^*, and b^* values showed significant difference in browning color between GE and DW. Because of these values, it suggested that GE was a good browning inhibitor against browning in apple and potato. However, these values did not show the difference of browning prevention between 100%GE and 75%GE. 100%GE also showed the highest value of total phenolic compounds and ascorbic acid. From previous works, some researchers reported that guava fruit contained major phytochemicals such as ferulic acid, cinnamic acid, vanillic acid, gallic acid, protocatechuic acid, and ascorbic acid providing antibrowning properties (Gutiérrez et al., 2008; Zabidah et al., 2011). Some phenolic phytochemicals such as cinnamic acid and ferulic acid were effective in inhibition of enzymatic browning in potato (Macrae and Duggleby, 1968) and apple (Walker, 1976; Walker and Wilson, 1975; Özoglu and Bayindirli, 2002). Gallic acid and protocatechuic acid reported to be effective inhibitors of tyrosinase activity (Kubo et al., 2003; Momtaz et al., 2008; Miyazawa et al., 2003). Ascorbic acid was effectively browning inhibitor in clouding apple juice (Özoglu and Bayindirli, 2002; Li et al., 2007) and potato PPO (Lee et al., 2002;

Duangmal et al., 1999). The mechanism of phenolic in browning prevention might influence PPO activity directly by acting as, for example, competitive or non-competitive inhibitor, while the action of ascorbic acid inhibition consist of chemical reduction of quinones to diphenols, leading to the formation of uncolored compounds . Therefore, it can be assumed that the phenolic compounds and ascorbic acid in GE play an important role in enzymatic browning inhibition ability of GE.

GE showed ability to reduce the browning values of apple and potato puree and control of PPOs in apple and potato. It also demonstrated concentration-dependent inhibition of browning. The results suggested that GE has potential to be used as a natural antibrowning agent for apple and potato. However, a study of the effective compounds in GE will require in terms of PPO inhibitor in apple and potato in the future.

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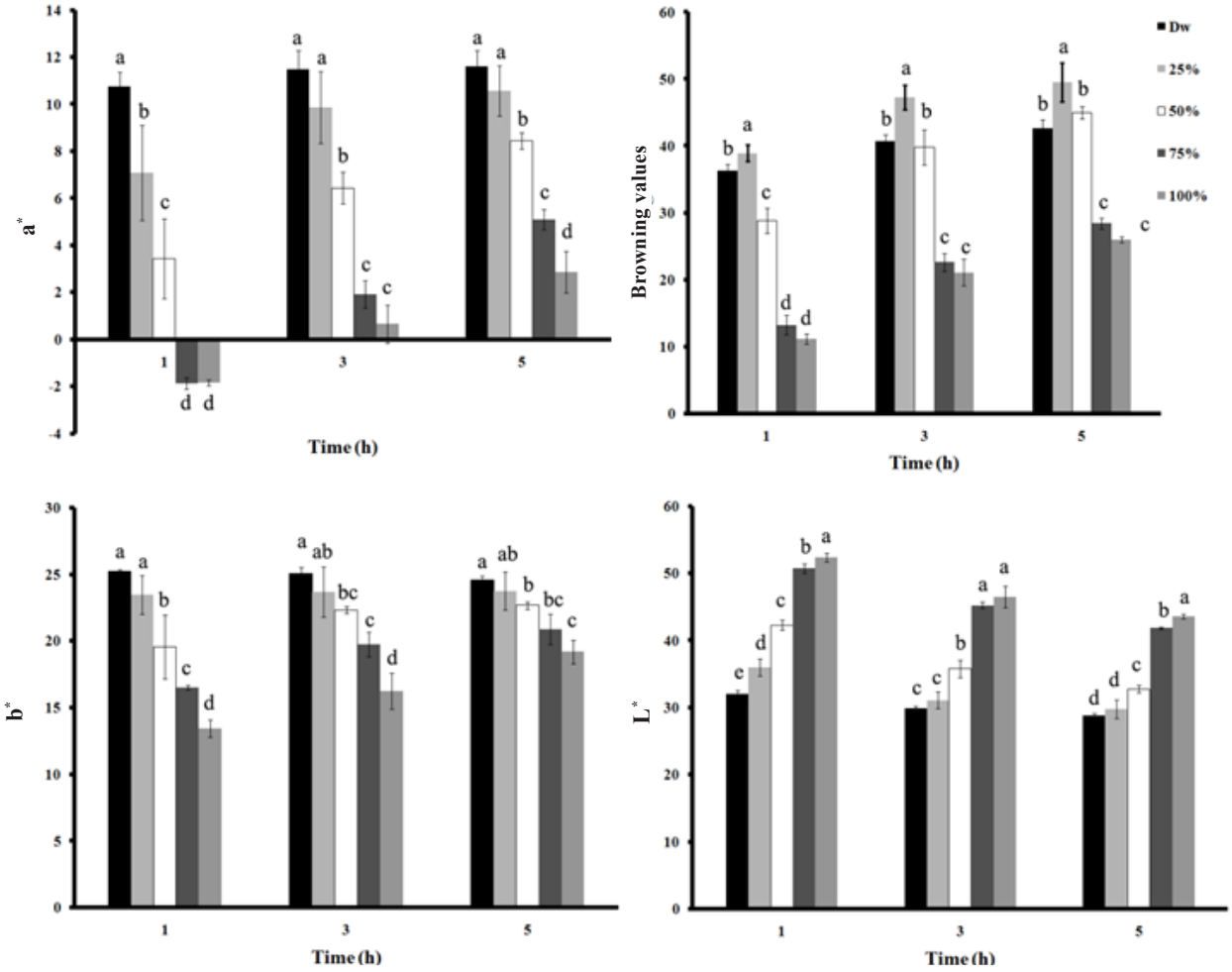

Figure 1 L*, a*, b*, and browning values ((ΔL*/L*0) x100) of apple puree blended in 25%GE, 50%GE, 75%GE, 100%GE and DW and stored at room temperature (25° C) for 5 h.
[a, b, c, d, e] Mean values with different letters in the same storage time are significantly different ($p \leq 0.05$). Error bars indicate ± SD.

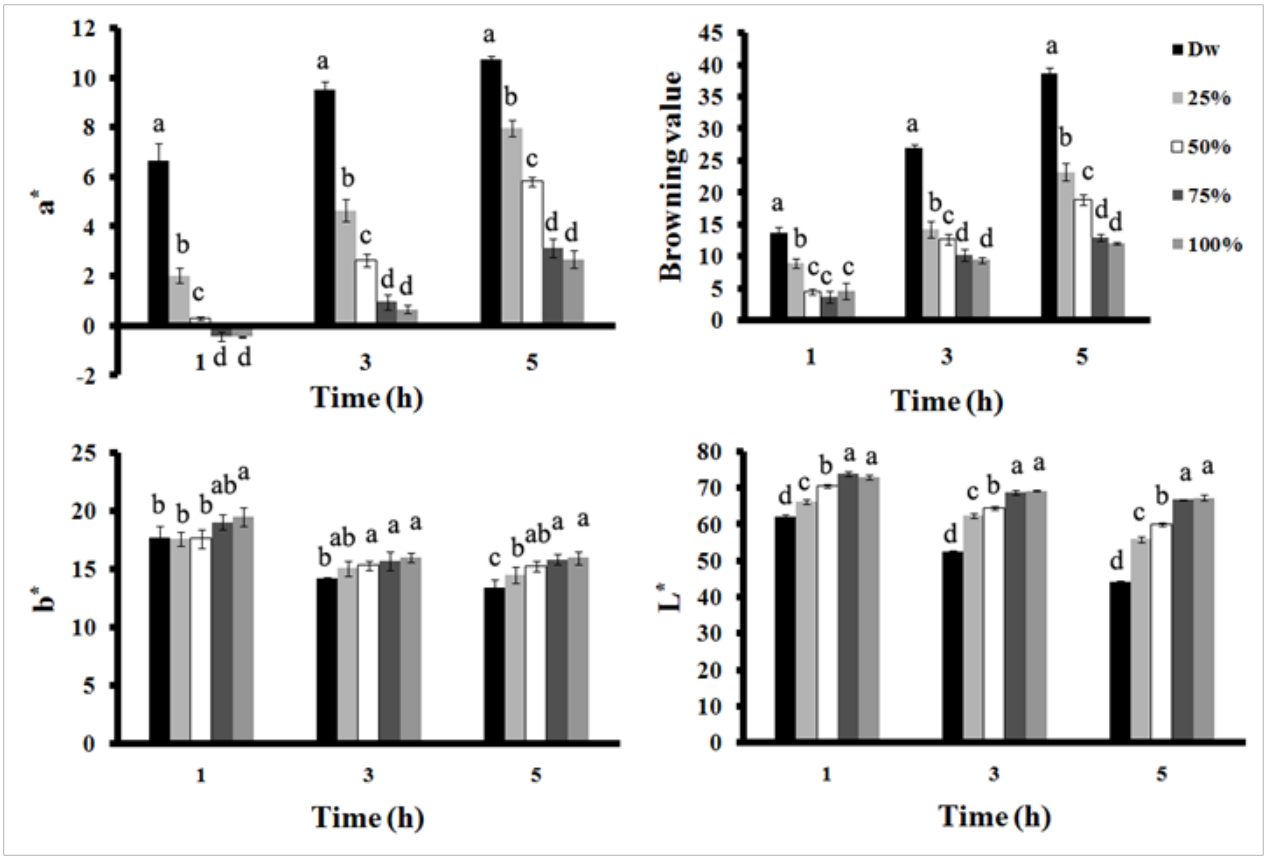

Figure 2 L*, a*, b*, and browning values ((ΔL*/L*0) x100) of potato puree blended in 25%GE, 50%GE, 75%GE, 100%GE and DW and stored at room temperature (25° C) for 5 h.
[a, b, c, d] Mean values with different letters in the same column are significantly different ($p \leq 0.05$). Error bars indicate ± SD.

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