Int.J.Curr.Microbiol.App.Sci (2016) *5*(10): 212-228

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 5 Number 10 (2016) pp. 212-228**
Journal homepage: http://www.ijcmas.com

Original Research Article http://dx.doi.org/10.20546/ijcmas.2016.510.023

Antibrowning Activity of Bioactive Peptides from Lab-Cultured Skim Milk Hydrolysate

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Keywords

Antibrowning activity, Antioxidative activity, Skim milk hydrolysate (SMH), Bioactive peptides.

Article Info

Accepted:
12 September 2016
Available Online:
10 October 2016

A B S T R A C T

The application of antibrowning agents is one of the most effective methods for controlling the browning reaction in fruits and vegetables. Therefore, in this study skim milk hydrolysate (SMH) at different concentrations were tested for antibrowning reaction of fruits and vegetables compared with ascorbic acid and citric acid. Fresh Chinese pear and potatoes slices were treated with distilled water (as control), ascorbic acid (AA), citric acid (CA), SMH- *L. plantarum* 1 (SMH1), SMH- *Ln. mesenteroides* (SMH2) and combining SMH- *L. plantarum* 1+ SMH- *Ln. mesenteroides* (SMH1+SMH2) at different concentrations (1, 2, 3 and 4% w/v) and dipped for 5 min. Then all slices were placed in Petri dishes and exposed to air at room temperature for 0, 3, 6, 12 and 24 min. The degree of color (*L**, a*, b*), browning index (BI) and total phenolic content (TPC) of the samples were evaluated. The results indicate that the slices of Chinese pear and potato treated with combining SMH1+SMH2 inhibited browning reaction comparable to AA as shown by reduction of TPC. It can be concluded that we found new antibrowning agents from natural source to achieve greater inhibition of browning like as ascorbic acid and citric acid. That would be useful in the formulation of functional foods.

Introduction

The market for fresh-cut fruit salads and vegetables is expanding rapidly as a consequence of increasing consumer demand for healthy eating, convenience and their fresh-like character (Buckley *et al*., 2007; Gorny, 2003). The International Fresh-cut Produce Association defines fresh-cut products as fruits or vegetables that have been trimmed and/or peeled and/or cut into a 100 % usable product that is bagged or pre-packed to offer the consumer high nutrition, convenience and flavor while still maintaining its freshness (Garrett, 2002). Since minimal processing results in quality deterioration associated with water loss, softening, microbial contamination, increased respiration and ethylene, and cut-surface browning, minimally processed products become more perishable (Rolle and Chism, 1987; Lee *et al*., 2003). Slowing or preventing the enzymatic browning of sliced fruit is a continuing problem for the

processors of fresh-cut fruit. Browning is due to many factors, including cell disruption and the release of polyphenol oxidases (PPOs; Shapton and Shapton, 1998; Garcia and Barret, 2002; Martìn-Belloso *et al*., 2006).

Browning is the main physiological disorder that impairs sensory properties and discourages consumer purchase of fresh-cut fruits. Enzymatic browning reactions in fruits are primarily catalyzed by polyphenol oxidase (PPO) in the presence of oxygen (Martinez and Whitaker, 1995). Extensive research has been focused on control of browning in fresh-cut fruits and several approaches to browning inhibition have been explored. Browning in fruits increases after being cut damaged or stored. Many reports have attempted to establish a relationship between the degree of browning and the phenolic content and enzymatic activity of apples (Vamos-Vigyazo *et al*., 1976; Klein, 1987; Jeong *et al*., 2008) and banana (Apintanapong *et al*., 2007). Thus browning in cut fruits and vegetables is mainly related to polyphenol oxidase (PPO) activity which, in the presence of O_2, converts phenolic compounds in fruits and vegetables into dark colored pigments (Burda *et al*., 1990; Amiot *et al*., 1992; Suttirak and Supranee, 2010)**.**

Enzymatic browning is a significant problem in a number of fruits and vegetables such as strawberry (Chisari *et al*., 2007), grape (Munoz *et al*., 2004), potato (Lee and Park, 2007), and lettuce (Gawlik-Dziki *et al*., 2007). The discoloration in fruits and vegetables by enzymatic browning, resulting from conversion of phenolic compounds to o-quinones which subsequently polymerize to be a brown or dark pigment. The enzymes involved in these processes are PPO and peroxidase (POD) (Jiang *et al*., 2004), since PPO and POD are the main enzymes involved in the phenolic oxidation of many fruits and vegetables. Phenolic compounds are a group of chemical substances in plants, which play an important role during enzymatic browning because they are substrates for the browning enzymes. The phenolics are normally complex organic substances, which contain more than one phenolic group. Polyphenolics can be divided into many different subcategories, such as flavonoids and non-flavonoid components.

Application of antibrowning agents is a popular approach for retarding enzymatic browning in fresh-cut fruits and vegetables. Surface treatments by dipping fresh-cut products in the appropriate antibrowning agents can effectively help to delay discoloration. Nature identical antibrowning agents are a favorite group because they are generally recognized as safe (GRAS) status and are non-toxic.

Extensive literature on inhibitors of browning in fresh-cut fruits include using ascorbic acid (AA) (Gorny *et al*., 2002), citric acid (CA) (Jiang *et al*., 2004), or their derivatives (Moline *et al*., 1999) and some sulfur-containing amino acids have been used as substitutes for sulfite to prevent browning, alone or in combination (Dudley & Hotchkiss, 1989). Several studies have shown that Natureseal® (AS1: ascorbic acid and AS5: citric acid) products can reduce browning in fresh-cut fruit slices (Abbott *et al*., 2004; Rupasinghe *et al*., 2005; Toivonen, 2008).

The use of synthetic chemicals for controlling browning is becoming less acceptable to consumers (Lu and Toivonen, 2000). Therefore, efforts have been focused on use of natural materials (Sapers, 1993). Whey protein concentrate (WPC) edible coatings in combination with anti-browning

agents effectively extended the shelf-life of minimally processed apple slices by two weeks when stored in packed trays at cold storage (Lee *et al*., 2003).Whey protein-based coatings without incorporation of antioxidants were more effective in reducing enzymatic browning of 'Golden Delicious' apples than hydroxypropyl methylcellulose based coatings (Perez-Gago *et al*., 2005). Earlier study (Abubakr *et al*., 2012) showed that skim milk hydrolysate (SMH) from fermentation with the selected LAB has antioxidant activity, thus it may be possible that the peptide generated may also have antibrowning activity. Therefore, this study evaluated the antibrowning activity of SMH fermented by *Lactobacillus plantarum* 1 and *Leuconstoc mesenteroides* on fruits and potatoes slices. This would be useful to maintain the acceptable quality of minimally processed fruits and potatoes.

Materials and Methods

Samples

The samples used were apple, banana, Chinese pears and potatoes obtained from supermarket in Nilai. Each sample was washed by distilled water then cut by knife into slices and exposed to air at room temperature. Development of brown color on exposed side of the slices was monitored visually and the time taken for observable brown color formation was noted. Slices of Chinese pear and potatoes showed fast browning color formation than banana and apple, and thus Chinese pear and potatoes were selected for further study.

Slices preparation and treatment condition

The Chinese pears and potatoes were initially washed in water then peeled and cored. After that, each sample was cut into slices. The slices were dipped into ascorbic acid (AA), citric acid (CA), solutions of SMH-*L. plantarum* 1 (SMH1), SMH-*Ln. mesenteroides* (SMH2) and combing SMH-*L. plantarum* 1+ SMH-*Ln. mesenteroides* (SMH1+SMH2) at different concentration for all (1, 2, 3 and 4% w/v) for 5 min (Rocha *et al.,* 1998; Lu *et al*., 2007). Then all slices were placed in Petri dishes and exposed to air at room temperature for 0, 3, 6, 12 and 24 min. Chinese pear and potato slices were dipped in distilled water served as control. The experiments were done in triplicate.

Color assessment

The changes in surface color of all the treated and control Chinese pear slices and potato slices and control (without treatment) were measured by a Hunter colorimeter (Minolta CR-300, Minolta Corp., Ramsey, NJ) and measurements were made immediately after dipping the slices for 5 min as described above. The color was recorded using the CIE-L*, a*, b* scale, where L* represents lightness, a* represents chromaticity on a green (–) to red (+) axis and b* represents chromaticity on a blue (–) to yellow (+) axis. Numerical values of a* and b* were converted into hue angle ($Hue=\tan^{-1}(b^*/a^*)$) (Jeong *et al*., 2008).

Browning index (BI)

Twenty grams of all the treated Chinese pear slices, potato slices and control were homogenized in a laboratory blender for 2 min. Homogenates were centrifuged at 10000 rpm for 15 min at 4 ^{0}C, filtered through Whatman no. 2 filter paper (Whatman, Maidstone, Kent, UK) and the absorbance of clear juice was determined immediately at 420 nm and reported as the browning index (BI). Greater absorbance at 420 nm corresponds to greater browning (Jeong *et al*., 2008).

Estimation of total phenolic content (TPC)

1 g of all the treated Chinese pear slices, potato slices and control (without treatment) were extracted three times with 15 ml of 50% methanol by maceration for 2 hr, then filtered and the final volume was made with 50% methanol up to 50 ml. Gallic acid was weighed (10 mg) and dissolved in 100 ml of 50% methanol. Lower concentrations of gallic acid (1, 2, 4, 6, 8 and 10 mg/ml) were prepared by serially diluting stock solution (100 mg/ml). Samples and various concentrations of standard (1ml each) were taken in test tubes, diluted with 10 ml of distilled water, 1.5 ml Folin Ciocalteu's reagent was added and was kept at room temperature for 5 min to which 4 ml of Na_2CO_3 (20% w/v) was added, the final volume adjusted to 25 ml with distilled water, then agitated and allowed to stand for 30 min at room temperature. Absorbance was measured at 765 nm by a spectrophotometer (Varian Carry 50 Conc) for three parallel determinations. Quantification was done on the basis of a standard curve of gallic acid (0 – 30 mg/ml) (Appendix D Figure 1). Results were expressed as mg gallic acid equivalents (GAE) and percentage w/w (Hodzic *et al*., 2009).

Statistical analysis

The results were presented as mean ± standard deviations of triplicate determinations and were statistically analyzed by two-way analysis of variance (ANOVA) using (Minitab, Inc.) version 15 (Germany), $p \leq 0.05$ were considered statistically significant. Correlation analysis was conducted between color (*L**, a*, b*), total phenolic content (TPC) and browning index (BI). Attempts were made to establish the relationships between the parameters studied using "R" as the correlation factor.

Results and Discussion

Total phenolic content (tpc) and browning reaction

The TPC values varied with type of fruits and potato slices; Chinese pear slices contained higher levels of phenolic compounds with value 66.8 mg GAEs/g than apple, potato and banana slices with values 50.3, 39.2 and 20.9 mg GAEs/g, respectively. Browning of cut surfaces was observed within three minutes on Chinese pear slices compared to apple, potato and banana slices within 4, 4, and 6 minutes, respectively (Table 1). There was no significant difference ($p \geq 0.05$) between the samples for browning reaction. Based on the shortest time taken for brown color to develop, Chinese pear was selected as type of fruits and potatoes as type of vegetables for future study.

Color assessment and browning index (BI)

The effect of antibrowning agents (AA, CA, SMH1, SMH2 and SMH1+SMH2) on Chinese pear and potato slices was investigated. Browning reaction was assessed by measuring degree of color (insoluble pigments *L**, a*, b*) and browning index (soluble pigments BI) of Chinese pear and potato slices before and after treated with antibrowning agents. The degree of color and BI of Chinese pear and potato slices treated with antibrowning agents was not affected ($p \geq 0.05$) by the concentration of antibrowning agents used 1 to 4 % (w/v). All the antibrowning agents at concentration 1 % were observed able to inhibit the browning reaction in both the Chinese pear and potato slices. The *L** and dE* values of Chinese pear and potato slices treated with all antibrowning agents were found to increase slightly and remained

approximately constant thereafter within 24 min while a* and b* values decreased within 24 min compared to the control. The *L** values of Chinese pear slices treated whit AA was 70.8 %, followed by SMH1+SMH2, SMH1, CA and SMH2 with values 70.1, 69.7, 67.0 and 66.4 %, respectively after 24 min. The a* values of Chinese pear slices treated with CA was 0.14, followed by SMH1+SMH2, AA, SMH1and SMH2 with values 0.22, 0.22, 0.36 and 0.44, respectively after 24 min. The b* values of Chinese pear slices treated with CA was 16.5, followed by AA, SMH1+SMH2, SMH2 and SMH1 with values 18.5, 18.7, 20.1 and 21.26, respectively at 24 min. The dE* values of Chinese pear slices treated with CA was 73.4, followed by AA, SMH1+SMH2, SMH1 and SMH2 with values 72.6, 71.3, 69.9 and 67.3, respectively at 24 min. The BI values of Chinese pear slices were lower when treated with CA, SMH1+SMH2, AA, SMH1 and SMH2 with values 0.26, 0.28, 0.29, 0.30 and 0.34, respectively at 24 min (Tables 3, 4, 5, 6 and 7). In contrast, the control becomes darker (*L** = 46.2 %, a* = 5.85, b* = 23.7, dE* = 46.1 and BI= 0.65) (Table 2). Similar colour values were reported for potato slices when treated with all the antibrowning agents (Table 8, 9, 10, 11, 12 and 13) and no significant difference was observed between Chinese pear and potato slices treated with the five antibrowning agents evaluated. The results indicated that the Chinese pear and potato slices treated with SMH1+SMH2 was comparable to AA and CA for prevent browning reaction.

Estimation of total phenolic content (TPC)

TPC of Chinese pear and potato slices slightly decreased with increasing concentration of antibrowning agents and time of exposure to air at room temperature. There were no significant differences ($p \geq 0.05$) between antibrowning agents and TPC of Chinese pear and potato slices treated within 24 min compared to the control. The TPC values of Chinese pear slices treated with AA was 59.5 mg GAEs/g, SMH1+SMH2, SMH1, CA and SMH2 with values 59.7, 61.1, 62.5 and 63.0 mg GAEs/g, respectively after 24 min (Table 14). In contrast, the TPC value of control was higher (80.2 mg GAEs/g) after 24 min. Similarly, lower values of TPC were observed for potato slices treated with AA (30.9 mg GAEs/g), and SMH1+SMH2, CA, SMH2 and SMH1 with values 31.5, 32.3, 33.5 and 33.9 mg GAEs/g, respectively after 24 min. In contrast, the TPC value of control was increased within 24 min (48.2 mg GAEs/g) Table 15. The results indicate that the slices of Chinese pear and potato treated with combining SMH1+SMH2 inhibited browning reaction comparable to AA as shown by reduction of TPC.

Relationship between color (*L**, a*, b*, dE*), total phenolic content (TPC) and browning index (BI)

Attempts were made to establish the relationships among the parameters studied for antibrowning agent treated Chinese pear and potato slices. A negative correlation between TPC and *L**, dE* and BI but, a positive correlation between TPC and a*, b* for all treated samples was observed (Table 16 and Table 18). While, the results showed a positive correlation between BI and *L**, dE* but, a negative correlation between a* and b* for all treated samples was observed (Table 17 and Table 19). The color changes in Chinese pear and potato slices treated with AA, CA, SMH1, SMH2 and SMH1+ SMH2 determined by absorbance at 420 nm (soluble pigments) expressed as the BI and lightness (insoluble pigments) expressed as

*L** values were a negative correlated with the total phenolic content. TPC in Chinese pear and potato slices are closely related to the color changes that occur in Chinese pear and potato slices during long time.

An important issue in fresh-cut fruits and vegetables processing is the control of discoloration (pinking, reddening or blackening) or browning at cut surfaces. Oxidative browning is usually caused by the enzyme polyphenol oxidase (PPO) which, in the presence of O_2 converts phenolic compounds in fruits and vegetables into dark colored pigments (Beaulieu and Baldwin, 2002). Various antibrowning agents as alternatives of sulfite have been were investigated. The CA and AA have been reported extensively for their antibrowning activity in minimally processed fruits and vegetables (Son *et al*., 2001; Lu *et al*., 2007). CA is a chelating agent and acidulates, reducing pH and chelating copper in the active site of polyphenol oxidase and, therefore, inactivating the enzyme polyphenol oxidase (Gurbuz & Lee, 1997; Lee *et al*., 2003; Lu *et al*., 2007).

Many previous studies have proved that when the color became darker, producing a decrease in *L** values and more yellow, producing an increase in a* and b* values in fruits and vegetables (Lee, 1999; Apintanapong *et al*., 2007; Lu *et al*., 2007). In this research, the effect of five antibrowning agents on Chines pear and potato slices browning was investigated using tristimulus colorimeter that can be applied directly to cut surfaces. The results showed good browning inhibitory activity through increase *L** values and decrease a* and b* values with increasing the time of exposure to air at room temperature for all treatment compare with control (without treated). However, when the samples slices were treated with combining antibrowning agents SMH1+SMH2 showed inhibited browning reaction comparable to AA beater than used each one alone. No reports have been found regarding the effect of protein with antioxidant activity on the activity of browning in Chinese pear and potato slices. However, effects of AA and CA have been reported in other fresh cut fruits. Therefore, this study was first report using SMH protein as antibrowning agents (new natural source) to prevent or inhibit browning in fruits and vegetables. Suttirak and Supranee, (2010) reported that the use of mixtures of various antibrowning agents conducive to increased antibrowning efficiency is presumably due to the collaborative inhibitory mechanism of the constituents these results were similar to the results obtained from mixing of SMH1 and SMH2 beater than used each one speared to inhibit browning reaction in this study. The effects in color of apple slices have been observed previously with mixed antibrowning agents (Son *et al*., 2001). The effectiveness on browning prevention is mainly dependent on the produce types and cultivars, and concentrations of antibrowning agents. On the other hand, efficiency of respective AA, CA and OA on delaying browning can be enhanced by addition of an antibrowning agent possessing a different inhibitory mechanism and/or superior stability (Suttirak and Supranee, 2010).

Enzymatic browning is a significant problem in a number of fruits and vegetables such as strawberry (Chisari *et al*., 2007), grape (Munoz *et al*., 2004), potato (Lee and Park, 2007), and lettuce (GawlikDziki *et al*., 2007). The discoloration in fruits and vegetables by enzymatic browning, resulting from conversion of phenolic compounds to o-quinones which subsequently polymerize to be a brown or dark pigment. Thus it has been discovered that browning is mainly related to PPO activity, or to phenolic

content. Our study demonstrated that the antibrowning agents affected the total phenolic content as compared to control. For all samples the TPC decreased with increasing concentration of antibrowning agents and longer exposure time. A decrease in degree of browning correlates with decrease in phenolic content. Jeong *et al*., (2008) reported that influenced the antibrowning agents treatments. Some authors who worked with apples and peaches noticed an increase in degree of browning with an increase in phenolic content and suggest that the degree of browning is determined by the amount of phenolic compounds present in the fruits (Coseteng and Lee, 1987; Lee *et al*., 1990). Aminot *et al*., (1992) found that the degree of browning measured by absorbance at 420 nm (soluble pigments) and lightness *L* (insoluble pigments) was closely related to amount of phenols degraded. Prohens *et al*. (2005) indicated that the selection for a reduced degree of browning in commercial varieties has resulted probably in the indirect selection of materials with lower concentrations of phenolic compounds.

The browning index (BI) of Chinese pear and potato slice measured by absorbance at 420 nm (soluble pigments) had small absorbance with increasing concentration of antibrowning agents and exposure time compared to control which had higher BI.

Table.1 Total phenolic content and time for observable browning color of fruits and potato slices

Samples	TPC (mg GAEs/g)	Time (min)
Chinese pear	66.8 ± 0.089	3 ± 0.12
Apple	50.3 ± 0.322	4 ± 0.35
Banana	20.9 ± 0.268	6 ± 0.26
Potato	39.2 ± 0.322	4 ± 0.11

[a] Results were mean values of triplicate determinations ± sd.

Table.2 Effect of distilled water on degree of color and browning index (BI) of Chinese pear slices as control.

Time (min)	Degree of color [a]				Browning index (BI)[b]
	*L**	a*	b*	dE*	
0	55.1 ± 0.01	2.42 ± 0.07	16.9 ± 0.19	60.2 ± 0.01	0.0 ± 0.00
3	54.9 ± 0.54	3.15 ± 0.05	17.4 ± 0.02	52.8 ± 0.23	0.55 ± 0.11
6	53.4 ± 0.16	3.70 ± 0.76	19.8 ± 0.90	50.1 ± 0.12	0.59 ± 0.09
12	48.5 ± 0.31	4.32 ± 0.12	22.6 ± 0.11	47.6 ± 0.09	0.62 ± 0.01
24	46.2 ± 0.11	5.85 ± 0.01	23.7 ± 0.13	46.1 ± 0.45	0.65 ± 0.14

[a] is insoluble pigments.
[b] is soluble pigments.

Table.3 Effect of AA (1 % w/v) on degree of color and browning index (BI) of Chinese pear slices

Time (min)	Degree of color [a]				Browning index (BI)[b]
	*L**	a*	b*	dE*	
0	64.9 ± 0.14	2.37 ± 0.23	22.6 ± 0.61	63.1 ± 0.12	0.0 ± 0.00
3	66.4 ± 0.12	1.45 ± 0.02	19.6 ± 0.41	66.0 ± 0.25	0.34 ± 0.05
6	68.0 ± 0.74	0.55 ± 0.31	19.5 ± 0.12	68.9 ± 0.47	0.31 ± 0.13
12	69.5 ± 0.19	0.43 ± 0.02	19.0 ± 0.16	70.8 ± 0.07	0.30 ± 0.24
24	70.8 ± 0.02	0.22 ± 0.27	18.5 ± 0.04	72.6 ± 0.31	0.29 ± 0.02

[a] is insoluble pigments; [b] is soluble pigments.

Table.4 Effect of CA (1 % w/v) on degree of color and browning index (BI) of Chinese pear slices

Time (min)	Degree of color [a]				Browning index (BI)[b]
	*L**	a*	b*	dE*	
0	62.8 ± 0.03	2.09 ± 0.20	19.4 ± 0.22	64.5 ± 0.11	0.0 ± 0.00
3	63.7 ± 0.46	1.59 ± 0.15	18.8 ± 0.47	67.0 ± 0.01	0.32 ± 0.23
6	65.9 ± 0.80	1.47 ± 0.21	17.5 ± 0.01	68.2 ± 0.22	0.29 ± 0.15
12	66.4 ± 0.37	0.44 ± 0.06	16.6 ± 0.32	71.4 ± 0.05	0.28 ± 0.07
24	67.0 ± 0.15	0.14 ± 0.29	16.5 ± 0.65	73.4 ± 0.33	0.26 ± 0.21

[a] is insoluble pigments; [b] is soluble pigments.

Table.5 Effect of SMH1 (1 % w/v) on degree of color and browning index (BI) of Chinese pear slices

Time (min)	Degree of color [a]				Browning index (BI)[b]
	*L**	a*	b*	dE*	
0	63.1 ± 0.21	2.40 ± 0.35	24.1 ± 0.26	60.9 ± 0.03	0.0 ± 0.00
3	64.3 ± 0.27	1.90 ± 0.13	22.9 ± 0.15	63.1 ± 0.47	0.36 ± 0.21
6	66.5 ± 0.04	0.85 ± 0.03	22.4 ± 0.78	64.8 ± 0.09	0.34 ± 0.06
12	68.1 ± 0.65	0.61 ± 0.19	22.1 ± 0.03	67.7 ± 0.12	0.33 ± 0.13
24	69.7 ± 0.19	0.36 ± 0.85	21.2 ± 0.36	69.9 ± 0.26	0.30 ± 0.03

[a] is insoluble pigments; [b] is soluble pigments.

Table.6 Effect of SMH2 (1 % w/v) on degree of color and browning index (BI) of Chinese pear slices

Time (min)	Degree of color [a]				Browning index (BI)[b]
	*L**	a*	b*	dE*	
0	61.7 ± 0.14	2.38 ± 0.35	25.6 ± 0.26	59.8 ± 0.21	0.0 ± 0.00
3	62.9 ± 0.12	1.99 ± 0.13	23.7 ± 0.15	60.4 ± 0.14	0.38 ± 0.06
6	63.7 ± 0.78	1.00 ± 0.03	22.9 ± 0.78	62.5 ± 0.12	0.37 ± 0.01
12	64.3 ± 0.03	0.75 ± 0.19	21.3 ± 0.03	65.1 ± 0.06	0.35 ± 0.28
24	66.4 ± 0.37	0.44 ± 0.85	20.1 ± 0.36	67.3 ± 0.42	0.34 ± 0.13

[a] is insoluble pigments; [b] is soluble pigments.

Table.7 Effect of SMH1+SMH2 (1 % w/v) on degree of color and browning index (BI) of Chinese pear slices

Time (min)	Degree of color a				Browning index (BI)b
	*L**	a*	b*	dE*	
0	63.5 ± 0.14	2.39 ± 0.23	22.1 ± 0.61	62.2 ± 0.12	0.0 ± 0.00
3	65.1 ± 0.12	1.76 ± 0.02	20.3 ± 0.41	65.1 ± 0.02	0.33 ± 0.14
6	66.5 ± 0.74	0.73 ± 0.31	19.7 ± 0.12	66.8 ± 0.15	0.31 ± 0.31
12	68.2 ± 0.19	0.57 ± 0.02	19.0 ± 0.16	68.7 ± 0.82	0.30 ± 0.06
24	70.1 ± 0.02	0.22 ± 0.27	18.7 ± 0.04	71.3 ± 0.18	0.28 ± 0.12

[a] is insoluble pigments; [b] is soluble pigments.

Table.8 Effect of distilled water on degree of color and browning index (BI) of potato slices as control

Time (min)	Degree of color [a]				Browning index (BI)[b]
	*L**	a*	b*	dE*	
0	60.7 ± 0.17	3.90 ± 0.07	20.1 ± 0.06	63.8 ± 0.23	0.0 ± 0.00
3	59.9 ± 0.28	3.98 ± 0.05	21.5 ± 0.26	62.3 ± 0.23	0.20 ± 0.02
6	57.0 ± 0.11	4.12 ± 0.76	22.0 ± 0.17	60.7 ± 0.35	0.21 ± 0.02
12	56.4 ± 0.09	4.67± 0.12	22.9± 0.03	58.0 ± 0.17	0.24 ± 0.03
24	54.9 ± 0.76	5.94 ± 0.01	24.8 ± 0.28	55.4 ± 0.51	0.28 ± 0.05

[a] is insoluble pigments; [b] is soluble pigments.

Table.9 Effect of AA (1 % w/v) on degree of color and browning index (BI) of potato slices

Time (min)	Degree of color [a]				Browning index (BI)[b]
	*L**	a*	b*	dE*	
0	66.2 ± 0.03	3.24 ± 0.23	24.1 ± 0.14	65.4 ± 0.02	0.0 ± 0.00
3	67.6 ± 0.67	2.09 ± 0.02	21.3 ± 0.81	67.3 ± 0.04	0.15 ± 0.02
6	68.0 ± 0.42	1.32 ± 0.31	20.2 ± 0.01	69.9 ± 0.05	0.15 ± 0.01
12	69.7 ± 0.16	0.87 ± 0.02	19.6 ± 0.11	70.9 ± 0.03	0.13 ± 0.01
24	71.4 ± 0.13	0.52 ± 0.27	19.0 ± 0.46	72.9 ± 0.03	0.13 ± 0.01

[a] is insoluble pigments; [b] is soluble pigments.

Table.10 Effect of CA (1 % w/v) on degree of color and browning index (BI) of potato slices

Time (min)	Degree of color [a]				Browning index (BI)[b]
	*L**	a*	b*	dE*	
0	64.1 ± 0.13	2.32 ± 0.20	19.8 ± 0.07	68.7 ± 0.03	0.0 ± 0.00
3	65.9 ± 0.93	1.78 ± 0.15	17.7 ± 0.11	70.3 ± 0.17	0.15± 0.01
6	66.5 ± 0.06	1.65 ± 0.21	17.2 ± 0.82	70.7 ± 0.24	0.14± 0.02
12	67.1 ± 0.29	0.95 ± 0.06	16.9 ± 0.46	72.3 ± 0.15	0.13 ± 0.01
24	67.8 ± 0.10	0.27 ± 0.29	16.0 ± 0.13	73.8 ± 0.10	0.12 ± 0.00

[a] is insoluble pigments; [b] is soluble pigments.

Table.11 Effect of SMH1 (1 % w/v) on degree of color and browning index (BI) of potato slices

Time (min)	Degree of color [a]				Browning index (BI)[b]
	*L**	a*	b*	dE*	
0	61.4 ± 0.12	3.30 ± 0.12	26.3 ± 0.14	68.4 ± 0.18	0.0 ± 0.00
3	62.1 ± 0.81	2.27 ± 0.45	24.2 ± 0.81	69.1 ± 0.96	0.17 ± 0.06
6	65.3 ± 0.40	1.54 ± 0.09	21.9 ± 0.01	69.8 ± 0.29	0.15 ± 0.03
12	66.7 ± 0.42	1.16 ± 0.26	20.3 ± 0.11	70.5 ± 0.27	0.13 ± 0.07
24	69.1 ± 0.17	0.97 ± 0.33	19.7 ± 0.46	71.0 ± 0.50	0.12 ± 0.04

[a] is insoluble pigments; [b] is soluble pigments.

Table.12 Effect of SMH2 (1 % w/v) on degree of color and browning index (BI) of potato slices

Time (min)	Degree of color [a]				Browning index (BI)[b]
	*L**	a*	b*	dE*	
0	60.1 ± 0.22	3.43 ± 0.23	26.7 ± 0.54	66.8 ± 0.10	0.0 ± 0.00
3	60.9 ± 0.19	2.76 ± 0.62	25.5 ± 0.11	67.7 ± 0.22	0.19 ± 0.01
6	62.8 ± 0.18	1.64 ± 0.13	23.9 ± 0.04	68.3 ± 0.33	0.17 ± 0.04
12	65.1 ± 0.06	1.20 ± 0.07	21.5 ± 0.44	69.3 ± 0.27	0.16 ± 0.02
24	67.9 ± 0.76	1.13 ± 0.14	20.1 ± 0.17	70.0 ± 0.63	0.13 ± 0.01

[a] is insoluble pigments; [b] is soluble pigments.

Table.13 Effect of SMH1+SMH2 (1 % w/v) on degree of color and browning index (BI) of potato slices

Time (min)	Degree of color [a]				Browning index (BI)[b]
	*L**	a*	b*	dE*	
0	64.5 ± 0.24	2.39 ± 0.20	20.0 ± 0.13	65.3 ± 0.03	0.0 ± 0.00
3	64.9 ± 0.87	1.87 ± 0.15	18.1 ± 0.12	66.8 ± 0.66	0.16 ± 0.02
6	65.7 ± 0.12	1.72 ± 0.21	17.8 ± 0.76	69.5 ± 0.02	0.15 ± 0.01
12	66.5 ± 0.34	1.10 ± 0.06	17.1 ± 0.03	70.6 ± 0.32	0.13 ± 0.03
24	69.1 ± 0.06	0.68 ± 0.29	16.4 ± 0.35	71.2 ± 0.26	0.12 ± 0.02

[a] is insoluble pigments; [b] is soluble pigments.

Table.14 Effect of antibrowning agents (1 % w/v) on total phenols content (mg GAEs/g) of Chinese pear slices

Time (min)	Control[a]	Antibrowning agents				
		AA[b]	CA[c]	SMH1[d]	SMH2[e]	SMH1+SMH2[f]
0	66.8 ± 0.26	65.5 ± 0.32	65.3 ± 0.23	66.1 ± 0.12	66.5 ± 0.09	64.1 ± 0.32
3	72.5 ± 0.23	64.2 ± 0.17	64.3 ± 0.15	65.9 ± 0.71	66.0 ± 0.23	62.9 ± 0.26
6	75.6 ± 0.23	63.5 ± 0.35	63.4 ± 0.44	63.9 ± 0.09	64.6 ± 0.43	61.3 ± 0.40
12	76.1 ± 0.08	62.9 ± 0.26	62.5 ± 0.32	63.0 ± 0.76	63.9 ± 0.13	60.0 ± 0.32
24	80.2 ± 0.08	59.5 ± 0.32	62.5 ± 0.93	61.1 ± 0.19	63.0 ± 0.14	59.7 ± 0.32

[a] Chinese pear slices without antibrowning agents; [b] AA: Ascorbic acid; [c] CA: Citric acid; [d] SMH1: SMH- *L. plantarum* 1; [e] SMH2: SMH- *Ln. mesenteroides*,; [f] SMH1+SMH2: SMH- *L. plantarum* 1+ SMH- *Ln. mesenteroides*.

Table.15 Effect of antibrowning agents (1 % w/v) on total phenols content (mg GAEs/g) of potato slices

Time (min)	Control[a]	Antibrowning agents				
		AA[b]	CA[c]	SMH1[d]	SMH2[e]	SMH1+SMH2[f]
0	39.2 ± 0.06	38.1 ± 0.32	37.9 ± 0.12	38.6 ± 0.02	38.9 ± 0.32	37.5 ± 0.23
3	41.1 ± 0.12	36.7 ± 0.09	35.4 ± 0.21	37.1 ± 0.65	38.2 ± 0.21	36.1 ± 0.40
6	43.8 ± 0.31	35.0 ± 0.11	34.1 ± 0.67	35.6 ± 0.31	36.7 ± 0.07	35.3 ± 0.09
12	45.6 ± 0.21	33.6 ± 0.20	32.7 ± 0.14	34.7 ± 0.25	35.1 ± 0.39	33.5 ± 0.22
24	48.2 ± 0.43	30.9 ± 0.32	32.3 ± 0.32	33.9 ± 0.32	33.5 ± 0.71	31.5 ± 0.19

[a] Potato slices without antibrowning agents.
[b] AA: Ascorbic acid
[c] CA: Citric acid
[d] SMH1: SMH- *L. plantarum* 1
[e] SMH2: SMH- *Ln. mesenteroides*,
[f] SMH1+SMH2: SMH- *L. plantarum* 1+ SMH- *Ln. mesenteroides*.

Table.16 Correlations (R2) between total phenolic content (TPC mg GAEs/g) and several parameters of Chinese pear slices before and after treated by antibrowning agents a.

Quality parameters	Control [b]	AA[c]	CA[d]	SMH1[e]	SMH2[f]	SMH1+ SMH2[g]
*L**	- 0.84	- 0.97	- 0.95	- 0.98	- 0.96	- 0.98
a*	0.94	0.88	0.95	0.98	0.99	0.98
b*	0.90	0.85	0.97	0.91	0.97	0.96
dE*	- 0.98	- 0.95	- 0.99	- 0.96	- 0.98	- 0.98
BI	0.90	- 0.59	- 0.56	- 0.52	- 0.58	- 0.64

[a] Correlation is significant at the 0.05 level.
[b] Chinese pear and potato slices without antibrowning agents.
[c] Ascorbic acid, [d] Citric acid.
[e] SMH1 is SMH- *L. plantarum* 1.
[f] SMH2 is SMH- *Ln. mesenteroides*.
[g] SMH1+SMH2 is SMH- *L. plantarum* 1+ SMH- *Ln. mesenteroides*.

Table.17 Correlations (R2) between browning index (BI) and several parameters of Chinese pear slices before and after treated by antibrowning agents a.

Quality parameters	Control [b]	AA[c]	CA[d]	SMH1[e]	SMH2[f]	SMH1+ SMH2[g]
*L**	- 0.59	0.62	0.60	0.57	0.60	0.59
a*	0.50	- 0.77	- 0.52	- 0.66	- 0.65	- 0.69
b*	0.69	- 0.92	- 0.58	- 0.73	- 0.69	- 0.82
dE*	- 0.80	0.67	0.57	0.58	0.48	0.65

[a] Correlation is significant at the 0.05 level.
[b] Chinese pear and potato slices without antibrowning agents.
[c] Ascorbic acid, [d] Citric acid.
[e] SMH1 is SMH- *L. plantarum* 1.
[f] SMH2 is SMH- *Ln. mesenteroides*.
[g] SMH1+SMH2 is SMH- *L. plantarum* 1+ SMH- *Ln. mesenteroides*.

Table.18 Correlations (R2) between total phenolic content (TPC mg GAEs/g) and several parameters of potato slices before and after treated by antibrowning agents a.

Quality parameters	Control [b]	AA[c]	CA[d]	SMH1[e]	SMH2[f]	SMH1+ SMH2[g]
*L**	- 0.99	- 0.97	- 0.99	- 0.98	- 0.99	- 0.98
a*	0.90	0.98	0.97	0.97	0.95	0.99
b*	0.98	0.94	0.97	0.99	0.99	0.94
dE*	- 0.99	- 0.97	- 0.96	- 0.99	- 0.99	- 0.96
BI	0.56	- 0.60	- 0.65	- 0.53	- 0.82	- 0.45

[a] Correlation is significant at the 0.05 level.
[b] Chinese pear and potato slices without antibrowning agents.
[c] Ascorbic acid, [d] Citric acid.
[e] SMH1 is SMH- *L. plantarum* 1.
[f] SMH2 is SMH- *Ln. mesenteroides*.
[g] SMH1+SMH2 is SMH- *L. plantarum* 1+ SMH- *Ln. mesenteroides*.

Table.19 Correlations (R2) between browning index (BI) and several parameters of potato slices before and after treated by antibrowning agents a.

Quality parameters	Control [b]	AA[c]	CA[d]	SMH1[e]	SMH2[f]	SMH1+ SMH2[g]
*L**	- 0.83	0.55	0.76	0.78	0.98	0.44
a*	0.65	- 0.76	- 0.51	- 0.67	- 0.88	- 0.50
b*	0.87	- 0.84	- 0.81	- 0.56	- 0.82	- 0.73
dE*	- 0.82	0.70	0.63	0.53	0.87	0.59

[a] Correlation is significant at the 0.05 level.
[b] Chinese pear and potato slices without antibrowning agents.
[c] Ascorbic acid, [d] Citric acid.
[e] SMH1 is SMH- *L. plantarum* 1.
[f] SMH2 is SMH- *Ln. mesenteroides*.
[g] SMH1+SMH2 is SMH- *L. plantarum* 1+ SMH- *Ln. mesenteroides*.

Same results were reported by Wrolstad, (1976), the lowest BI corresponds to the least browning of the apple tissues. Our study indicates that both negative and positive relationships among the parameters studied for antibrowning agent treated Chinese pear and potato slices; a negative correlation was observed between TPC and *L**, Hue dE* and BI also between BI and a*, b* while a positive correlation was observed between TPC and a*, b* also between BI and *L** for both samples treated with SMH1, SMH2 and commonly used antibrowning agents. Similarity, Dykes *et al*., (2005) reported that the negative correlation between the *L** value and total phenols (-0.69, $p < 0.01$). Also Jeong *et al*., 2008 demonstrated that the positive correlations ($R^2 < 0.56$) were found when total phenolic were plotted against a* and b*. This further confirms that the browning reaction is related with total phenol content. The mechanism for enzymatic browning involves the interaction of phenolic compounds with PPO in the presence of oxygen (Kavrayan and Aydemir, 2001; Suttirak and Supranee, 2010).

Oxidative damage in fruits and vegetables during handling, processing and Storage, due to enzymatic oxidation of endogenous phenolic compounds catalysed PPO inherent in biological tissues (Nicolas *et al.*, 1994) which leads to browning of tissue. The brown pigment formed in food and food stuffs, looks unpleasant appearance that generally associated with loss of nutritional and market values (Chen *et al*., 1991; Loganathan *et al.*, 2011). The prevention of PPO-catalysed browning in food was usually accomplished by the addition of synthetic antioxidant compounds like ascorbic acid, citric acid, benzoic acid, cysteine and glutathione were an antioxidant

compounds. Hence, there was an increase in need for substituting synthetic compounds with natural substances as food ingredients, and these types of studies could be an important topic from the standpoint of food science and technology (Murata *et al*., 1995; Loganathan *et al*., 2011). Therefore, the compounds inherent in natural origins are widely accepted by consumers in the market. Based on this notion, there have been a lot of reports on the PPO inhibitors occurring in natural resources using synthetic antioxidant compounds. However, no reports have been found on the scavenging effect of free radicals from the SMH that could prevent the oxidative damage that increase the shelf life of food stuffs. Loganathan *et al*., (2011) observed that the *Agaricus heterocystis* hot water soluble contained certain antioxidant substance or compounds which inhibited the oxidation of endogenous compounds present in the apple. We suggested that the SMH are believed to be a free radical scavenger and to prevent browning owing to its reducing power and chelating copper in the active site of polyphenol oxidase. Utilization of this SMH will be promised as a natural food additive for the prevention of color browning caused by PPO.

In conclusion, it can be concluded that SMH1 and SMH2 have both antibrowning activity comparable to AA and CA. Additionally, the LAB strains used in this study were able to generate bioactive peptides which have the antioxidant and antibrowning activities, a dual function of bioactive peptides that would be useful in the formulation of functional foods.

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How to cite this article:

Maryam A.S. Abubakr. 2016. Antibrowning Activity of Bioactive Peptides from Lab-Cultured Skim Milk Hydrolysate. *Int.J.Curr.Microbiol.App.Sci.* 5(10): 212-228.
doi: http://dx.doi.org/10.20546/ijcmas.2016.510.023