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Inhibitory effects of various antibrowning agents on apple slices

S.M. Son, K.D. Moon, C.Y. Lee *

Department of Food Science and Technology, Cornell University, Geneva, NY 14456, USA

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Abstract

A comprehensive study to evaluate the relative antibrowning activity of 36 known compounds was conducted. Five chemical groups, including carboxylic acids, ascorbic acid derivatives, sulfur containing amino acids, phenolic acids and other miscellaneous compounds, were tested on apple slices under the same conditions. Among the compounds tested, oxalic acid, oxalacetic acid, ascorbic acid-2-phosphate, cysteine, glutathione, *N*-acetylcysteine, kojic acid and 4-hexyl resorcinol belonged to a group that showed the highest inhibitory activity on apple browning. The minimal concentrations of oxalacetic acid, oxalic acid, cysteine and kojic acid, for an effective antibrowning activity, were 0.25, 0.05, 0.05 and 0.05%, respectively. Oxalic acid showed a synergistic effect when 0.02% oxalic acid was used with 1% ascorbic acid or its derivatives.

Keywords: Antibrowning agents; Apple; Oxalic acid; Oxalacetic acid; Inhibitory activity on browning

1. Introduction

With the consumers' increased awareness of health benefits from consuming fresh fruits and vegetables, there is an increasing demand for higher quality of fresh-cut fruits and vegetables in a convenient form. In order to meet these demands, the food industry has focused on the development of new processing techniques for minimally processed fruit and vegetable products. Processing operations, such as peeling, cutting and shredding, induce enzymatic browning and enhance the ethylene synthesis, respiration, softening and microbial contamination, which all correlate with quality deterioration (Ahvenainen, 1996). Of these, enzymatic browning has been the major concern with regard to aesthetic quality and it was successfully controlled by sulfites until 1986. However, due to the government ban on the use of sulfites for fresh fruits and vegetables (Food and Drug Administration, 1987), a replacement has been urgently sought (Duxbury, 1988; Langdon, 1987). Nicolas, Richard-Forget, Goupy, Amiot, and Aubert (1994) reported that several chemical compounds inhibit enzymatic browning in apples and apple products. However, most of the reported inhibitory activities were measured individually at different conditions in different commodities. Therefore, the overall relative inhibitory activity of individual compounds is not clear. The main purpose of this study was to find the most effective antibrowning agent(s) for apple slices, by comparing the antibrowning activity of commonly known compounds under the same conditions.

2. Materials and methods

2.1. Apples

Liberty apple cultivar was chosen for this study because it is highly prone to browning (Kim, Smith, & Lee, 1993). The apples were harvested at commercial maturity in the New York State Agricultural Experiment Station orchard during the 1998 growing season and stored in the cold room (0–2°C and 90–95% RH). The moisture content of the apples was 83.6%, soluble solid content, 13.6% Brix, organic acid content, 0.69% and the pH, 3.3.

2.2. Antibrowning agents

Five major groups of chemical compounds tested (Table 1) were either certified ACS grade or analytical grade obtained from Fisher Scientific (Fair Lawn, NJ)

* Corresponding author. Tel.: +1-315-787-2271; fax: +1-315-787-2284.

E-mail address: cyl1@cornell.edu (C.Y. Lee).

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Table 1
Five major chemical groups tested for antibrowning activity

Carboxylic acids	Ascorbic acid derivatives	Sulfur-containing amino acids	Phenolic acids	Others
Acetic	Ascorbic	Cysteine	Caffeic	CaCl2
Citric	Ca-ascorbate	Histidine	Chlorogenic	NaCl
Formic	Fe-ascorbate	Glutathione	Cinnamic	4-Hexyl resorcinol
Fumaric	Mg-ascorbate	Methionine	Coumalic	Honey
Lactic	Na-ascorbate	*N*-acetylcysteine	Ferulic	
Malic	Ascorbic-2-phosphate		Gallic	
Malonic			Kojic	
Pyruvic	Erythorbic			
Oxalic	Na-erythorbate			
Oxalacetic				
Succinic				
Tartaric				

or Sigma Chem. Co. (St. Louis, MO). Concentration of each dipping solution started at 1% and then decreased to lower concentrations, except for phenolic acids and 4-hexyl resorcinol which started at 0.1% due to their limited solubility in the water.

2.3. Slicing and dipping

Selected apples of uniform size and color were washed by hand and sliced longitudinally into 10 equal pieces using a stainless steel hand slicer. Ten apple slices were treated in 500 ml of dipping solution for 3 min and drained. The excess liquid was removed on cheese cloth. Samples were kept on the laboratory bench at room temperature for 3 h.

2.4. Color measurement

The changes in flesh color of the treated apple slices were measured by a Hunter Colorimeter (Hunterlab D-25, USA) at 10 min intervals for 3 h. The degree of browning was expressed by "δL" value (L value at initial$-L$ value at given time). The results were expressed as a mean value from three replications of the 10 measured samples.

2.5. Statistical analysis

Statistical analysis was carried out using a PC SAS package (SAS, 1990). Duncan's multiple range tests ($P=0.05$) were performed to determine any significant difference among various treatments.

3. Results and discussion

3.1. Carboxylic acids

Since most of the carboxylic acids have shown inhibitory effects on enzymatic browning due to their metal-chelating characteristics or lowering of pH (Furia, 1964), twelve carboxylic acids, commonly found in fruits and vegetables, were tested for their antibrowning activity. Change in color of apple slices treated in individual carboxylic acid solution at 1% is shown in Fig. 1. Based on Duncan's multiple range test ($P=0.05$), the 12 carboxylic acids can be divided into three groups. Oxalic acid, oxalacetic acid, tartaric acid and malonic acid are the best inhibitor group that showed only a slight change in color. Pyruvic acid, citric acid, malic acid and lactic acid belong to a medium inhibitor group followed by the weak inhibitor group of acetic acid, succinic acid, fumaric acid and formic acid. The latter group of acids was the most ineffective, showing severe browning on apple slices during the 3 h period. Oxalic acid, oxalacetic acid, tartaric acid, and malonic acid were much better inhibitors than ascorbic acid on apples at the same concentration ($P<0.05$).

Among the carboxylic acids tested, citric acid has been widely used commercially as an antibrowning agent. Citric acid has been reported extensively for its inhibitory activity on polyphenol oxidase (Langdon, 1987; Pizzocaro, Torreggini, & Gilardi, 1993) and its antibrowning activity in minimally processed fruits and vegetables (Ahvenainen, 1996; Duxbury, 1986; Rocha, Brochado, & Morais, 1998). Since oxalic acid and oxalacetic acid showed much more potent antibrowning activity than citric acid, they can be used effectively at lower concentrations for fresh-cut apple slices. Oxalic acid occurs extensively in nature as a free acid or as potassium or calcium salts. Some vegetables, such as spinach, rhubarb and beet root are reported to contain as much as 356–780, 260–620, and 97–121 mg/100 g of fresh material, respectively (Hodgkinson, 1977). A high oxalate intake in the diet may reduce the absorption of certain minerals due to the formation of insoluble salts. The reported daily consumption of oxalic acid in western diets ranged from 70 to 150 mg (Zarembski & Hodgkinson, 1962). Since the concentration of oxalic acid (1.6–2.5 mg/100 g) in fresh-cut apple slices, treated

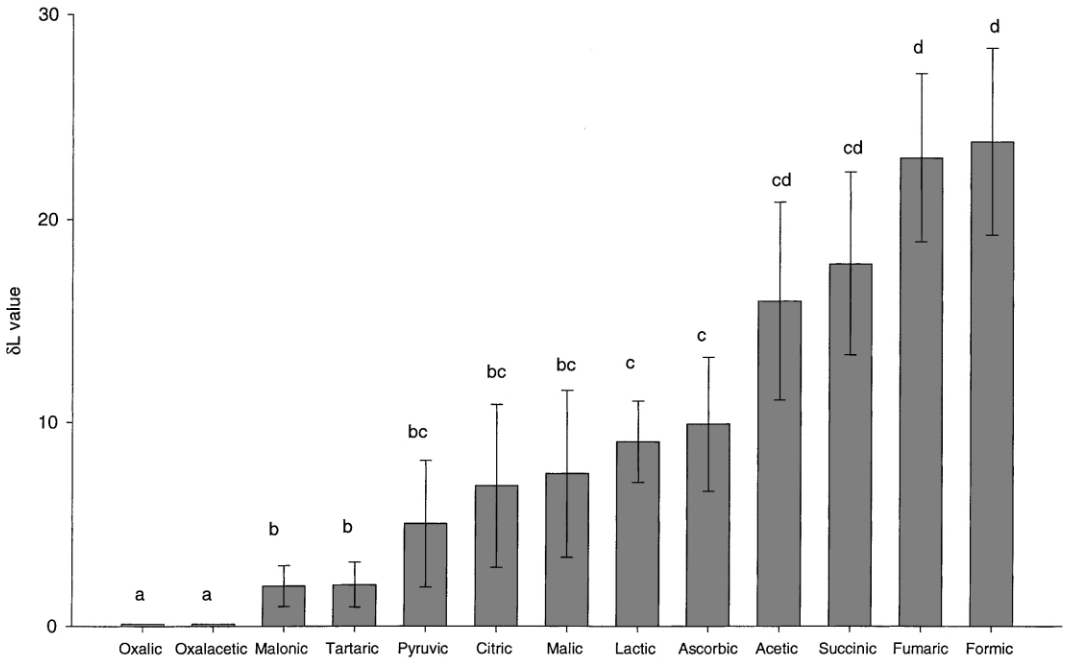

Fig. 1. Browning of apple slices after 3 h treatment with 1% carboxylic acids and 1% ascorbic acid (reference). Duncan's multiple range tests ($P=0.05$) were performed. Any treatments which were not significantly different from one another are illustrated as the same letter on the bar graph.

in 0.05% oxalic acid solution, is far below the level in spinach, rhubarb, or beet root, there is no concern about any additional amounts of oxalate in our diet.

3.2. Ascorbic acid and its derivatives

Ascorbic acid prevents enzymatic browning by reducing the quinone products to their original polyphenol compounds (Walker, 1977). Ascorbic acid has been widely used as an antibrowning agent for processing of fruits and vegetables. The antibrowning activities of ascorbic acid and its derivatives were examined on the apple slices after treatment in a 1% dipping solution (Fig. 2). Initial observation on treated apple slices revealed that 1% solutions of ascorbic acid and its derivatives appeared to effectively inhibit browning. After the reducing power of ascorbic acid was depleted, within 20 min, the brown color developed rapidly thereafter. Among ascorbic acid and its derivatives tested, ascorbic acid-2-phosphate was the most potent with a smallest δL value of 0.9, but the rest were as ineffective as the ascorbic acid ($P<0.05$). This result was similar with the previous studies of some researchers (Sapers et al. 1989; Sapers & Miller, 1992) on apple and potato.

3.3. Sulfur-containing amino acids

The sulfur-containing amino acids are well known for their antibrowning activity and they have been successfully used for apples (Goerge, Harold, David, & Chien, 1999), potato (Gurbuz & Lee, 1997), liychi fruit (Jiang & Fu, 1998) and other fruit beverages (McEvily, Iyengar, & Otwell, 1992). However, the exact inhibition mechanism of sulfur-containing compounds on PPO is not yet clear. Kahn (1985) proposed that cysteine directly reacts with the PPO and forms stable complexes with copper. However, it is also known that cysteine reacts with quinone products and forms colorless conjugated compounds (Molnar-Perl & Friedman, 1990; Richard-Forget, Goupy, & Nicolas, 1992). Five sulfur-containing compounds were tested as dipping solutions for apple slices (Fig. 3). There was no change in the L value of apple slices dipped in a 1% solution of cysteine, glutathione or *N*-acetylcysteine. Methionine and histidine showed weak antibrowning activity among sulfur-containing compounds tested but they were much more effective than ascorbic acid ($P<0.05$). Molnar-Perl and Friedman reported that N-acetylcysteine and reduced glutathione were excellent antibrowning agents in fruits and vegetables. However, sulfur-containing compounds such as cysteine and glutathione at high concentration, may produce an unpleasant odor in fruits and vegetables when used as dipping agents (Mathew & Parpia, 1971).

3.4. Phenolic acids

Phenolic compounds have shown complicated and intriguing reactions in enzymatic browning. The intensity of brown color caused by PPO and phenolics in the presence of oxygen, depends on the type of phenolic compounds involved (Lee & Jaworski, 1988; Amiot, Tacchini, Aubert, & Nicolas, 1992). Certain phenolic acids inhibit the PPO activity by binding in the active

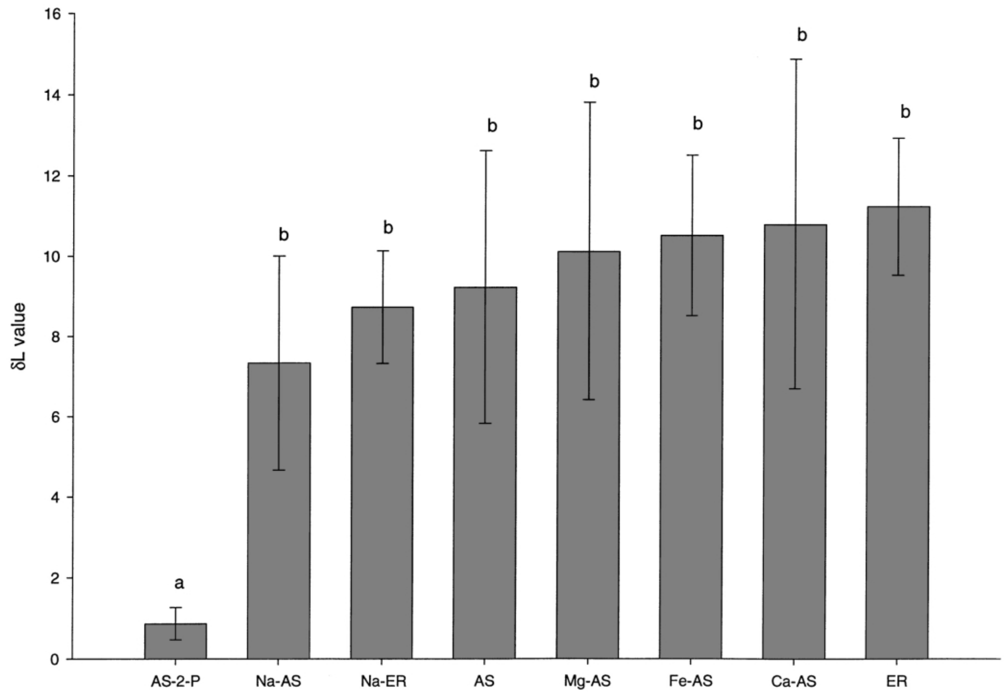

Fig. 2. Browning of apple slices after 3 h treatment with 1% ascorbic acid and its derivatives (AS, ascorbic acid; ER, erythorbic acid; and AS-2-P, ascorbic acid 2-phospate). Duncan's multiple range tests ($P = 0.05$) were performed. Any treatments which were not significantly different from one another are illustrated as the same letter on the bar graph.

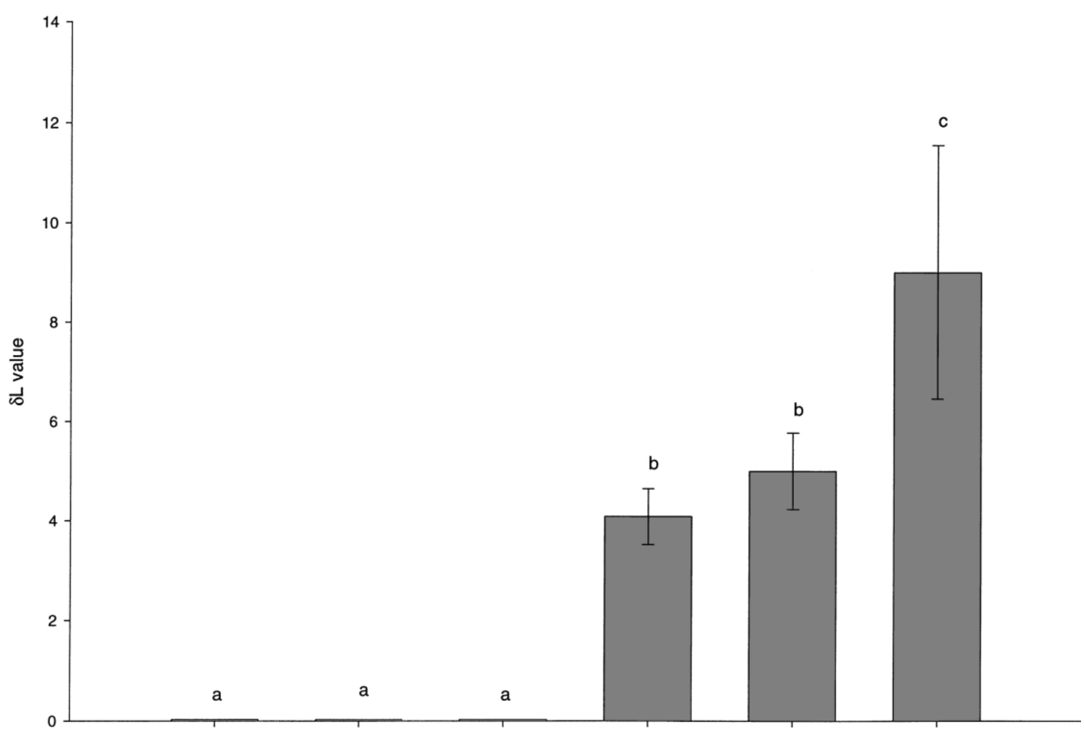

Fig. 3. Browning of apple slices after 3 h treatment with 1% sulfur-containing compounds and 1% ascorbic acid (reference). Duncan's multiple range tests ($P = 0.05$) were performed. Any treatments which were not significantly different from one another illustrated as the same letter on the bar graph.

site of the enzyme, whereas others can promote the browning reaction (Janovitz-Klapp, Richard, Goupy, & Nicolas, 1990). Among the phenolic acids tested (0.1%) in this study, kojic acid showed the highest inhibitory effect ($P<0.05$; Fig. 4). *p*-Coumalic acid, ferulic acid, cinnamic acid and gallic acid showed the similar inhibitory activity as ascorbic acid, but chlorogenic acid and caffeic acid were as much weaker ($P<0.05$). Some researchers (Chen, Wei, & Marshall, 1991; Chen, Wei, Rolle, Otwell, Balaban, & Marshall 1991; Kahn, Lindner, & Zakin, 1995) reported the inhibition mechanism of kojic acid on PPO and proposed that kojic acid acts as a reducing agent as well as an inhibitor to the enzyme per se.

3.5. Other chemicals

Many researchers have been looking for novel anti-browning agents to replace sulfites. One of them is 4-hexyl resorcinol, known as an effective antibrowning agent for some fruits and vegetables (Monsalve-Gonzales, Barbosa-Canovas, Cavalieri, McEvily, & Iyengar, 1993; Monsalve-Gonzales, Barbosa-Canovas, McEvily, & Iyengar, 1995; Saper & Miller, 1998). Among natural substances, Oszmianski and Lee (1990) reported that a peptide in natural honey solution is responsible for retarding the browning in apple and grape juice. Chloride ion was shown to be a noncompetitive inhibitor on apple PPO (Janovitz-Klapp et al., 1990). Among other chemicals tested, 4-hexyl resorcinol (0.1%) was the most effective in preventing the browning of apple slices with no change in the L value (Fig. 5). Apple slices dipped in honey solution were not significantly different in δL with ascorbic acid, sodium chloride and calcium chloride ($P<0.05$).

3.6. Minimal concentration of oxalic acid, oxalacetic acid, cysteine and kojic acid for an effective antibrowning activity

In order to find the minimal concentration for an effective inhibition of apple browning, concentrations of

Table 2
The δL value of apple slices after 3 h dipped in various concentrations of oxalic acid, oxalacetic acid, cysteine, and kojic acid

Concentration (%)	Oxalic acid	Oxalacetic acid	Cysteine	Kojic acid
1	0	0	0	NT[a]
0.75	0	0	0	NT
0.5	0	0	0	NT
0.25	0	0	0	NT
0.1	0	0.93	0	0
0.075	0	2.67	0	0
0.05	0	4.5	0	0.01
0.025	2.5	6.3	1.1	3.2

[a] NT, not tested.

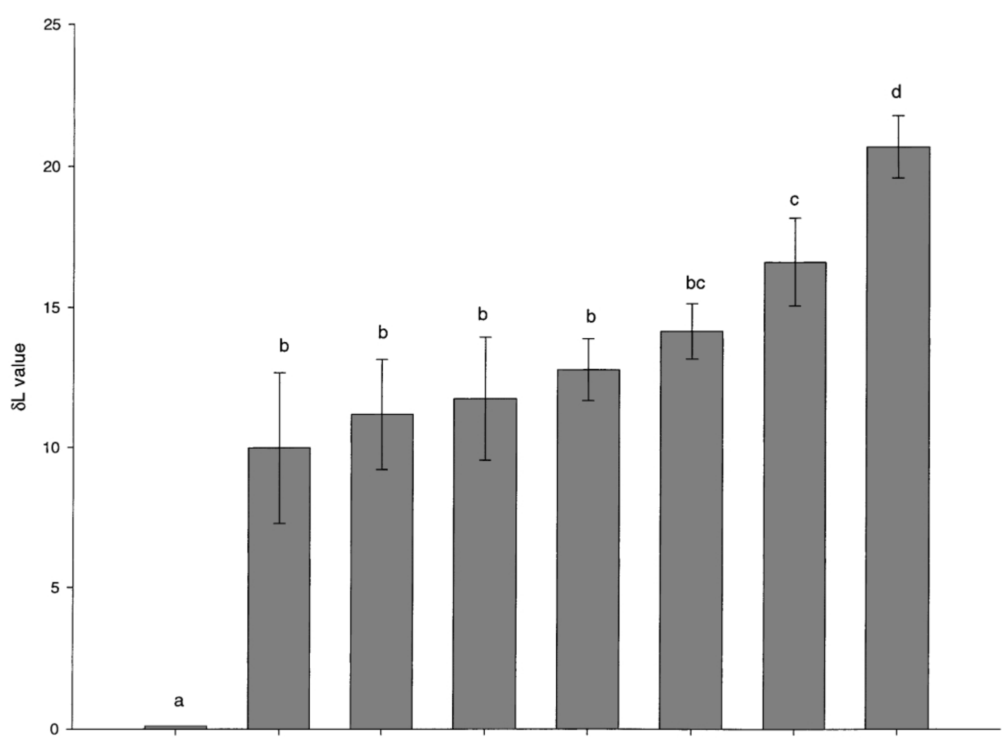

Fig. 4. Browning of apple slices after 3 h treatment with 0.1% phenolic acids and 1% ascorbic acid (reference). Duncan's multiple range tests ($P=0.05$) were performed. Any treatments which were not significantly different from one another are illustrated as the same letter on the bar graph.

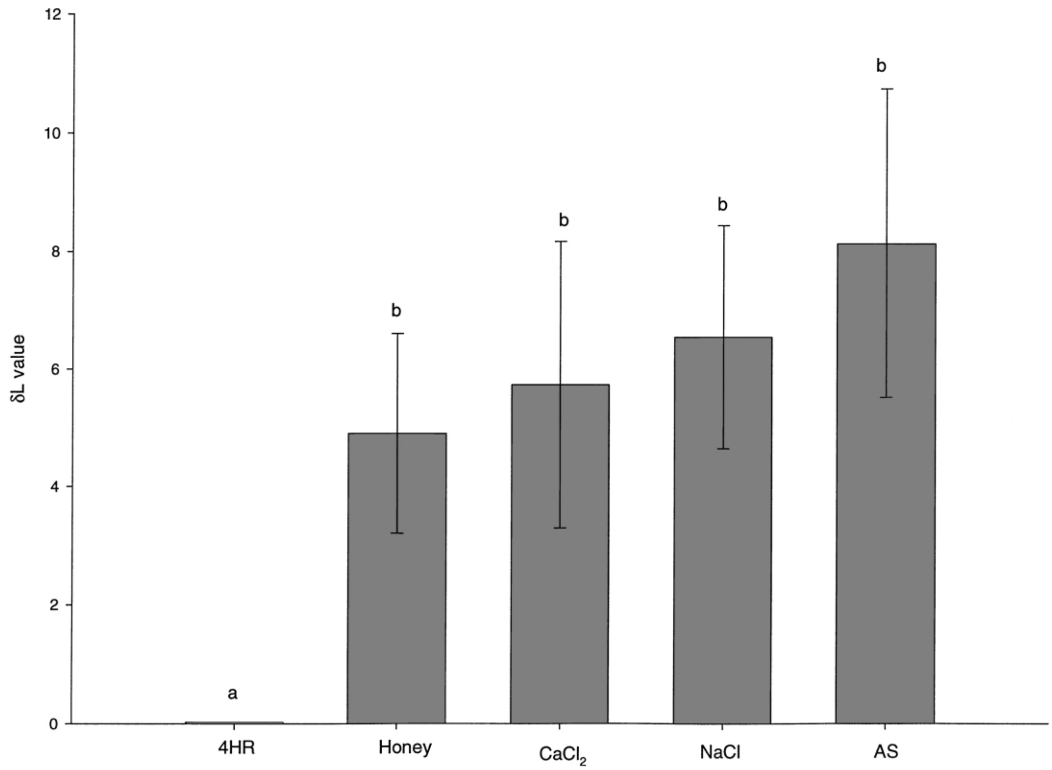

Fig. 5. Browning of apple slices after 3 h treatment with 0.1% 4-hexyl resorcinol (4HR), 1% honey, 1% NaCl, 1% $CaCl_2$ and 1% ascorbic acid (reference). Duncan's multiple range tests ($P=0.05$) were performed. Any treatments which were not significantly different from one another are illustrated as the same letter on the bar graph.

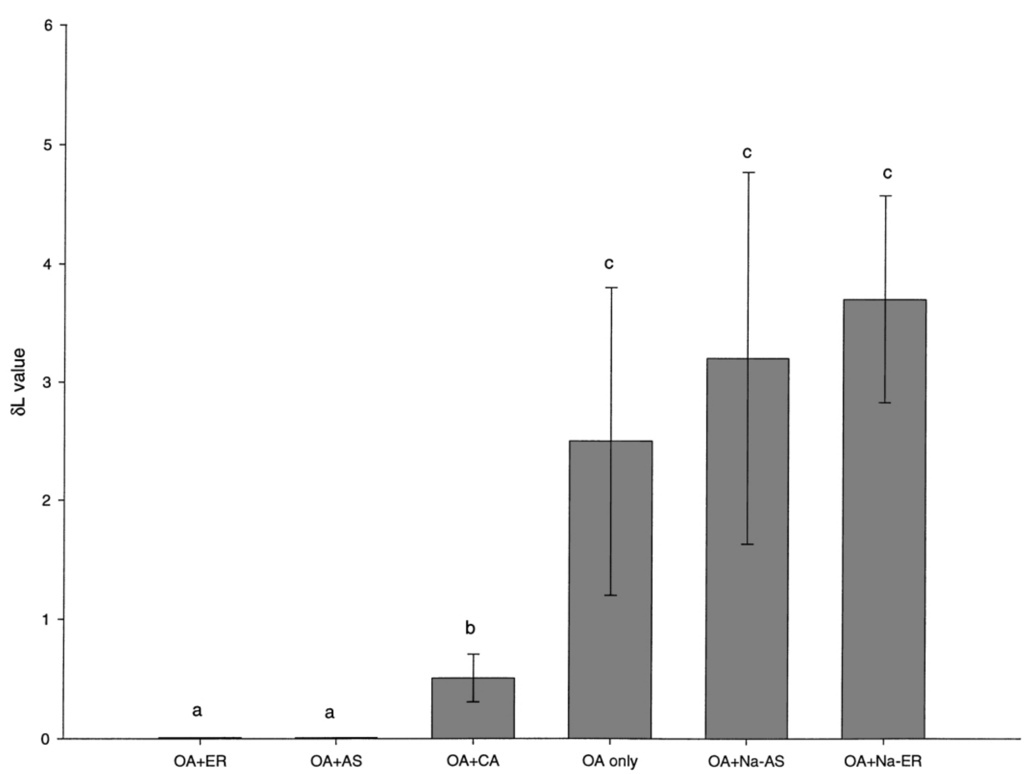

Fig. 6. Browning of apple slices after 3 h dipped in 0.02% oxalic acid mixed with 1% of ascorbic acid, citric acid, erythorbic acid or their derivatives. Duncan's multiple range tests ($P=0.05$) were performed. Any treatments which were not significantly different from one another are illustrated as the same letter on the bar graph.

oxalic acid, oxalacetic acid, cysteine and kojic acid in dipping solution were gradually decreased from 1 to 0.025% and changes in color were measured as shown in Table 2. At the concentration of 0.1% level, only oxalacetic acid showed the change in L value (0.93), whereas other compounds showed no color change except as low as 0.05%. Eventually, at 0.025% level, oxalic acid showed a δL value of 2.5; cysteine, 1.1; kojic acid, 3.2. Antibrowning activity of oxalic acid was similar to that of cysteine and kojic acid in apple slices with a minimal concentration of 0.05%.

3.7. Synergistic effect of mixed antibrowning agents

Mixtures of ascorbic acid, pyrophospate, calcium chloride and citric acid are commonly used, commercially, on fruits and vegetables (Ahvenainen, 1996; Duxbury, 1988; Langdon, 1987). The synergistic effect on antibrowning was examined by mixing 0.02% oxalic acid with 1% of each, ascorbic acid, sodium ascorbic acid, erythorbic acid, sodium erythorate and citric acid. As discussed above (Fig. 1), oxalic acid was the most potent browning inhibitor among the dozen carboxylic acids tested. It showed efficacy as low as 0.05%. When 0.02% oxalic acid was combined with 1% erythorbic acid or 1% ascorbic acid as a dipping solution, there was a strong synergistic effect, showing no change in color of the apple slices (Fig. 6). Citric acid also showed a synergistic effect with oxalic acid, although it was less effective than erythorbic acid or ascorbic acid. Based on this result, we believe that apple browning can be effectively prevented by combining 1% ascorbic acid or citric acid with less than 0.02% oxalic acid as a dipping solution.

4. Conclusion

In order to select the most effective antibrowning agent(s) on apple slices, five chemical groups were tested for their inhibitory activity under the same conditions. Among 12 carboxylic acids, oxalic acid and oxalacetic acid were the most potent browning inhibitors. Ascorbic acid 2-phosphate was the best among seven ascorbic acid derivatives, while cysteine, glutathione, and N-acetylcysteine were effective inhibitors among sulfur-containing compounds. Kojic acid showed the highest potency among phenolic acids tested and 4-hexyl resorcinol was also very effective in preventing apple browning. Each compound has its own strength and weakness in terms of variable potency, availability, safety, and cost. However, it appears that oxalic acid has a strong potential for practical applications, if the concentration is properly controlled, especially, considering the synergistic effect with ascorbic acid or erythorbic acid.

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