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Effects of Anti-Browning Agents on Polyphenoloxidase Activity and Total Phenolics as Related to Browning of Fresh-Cut 'Fuji' Apple

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Abstract: The objective of this study was to study the influences of anti-browning agents on the correlation between phenolics, and PPO activity and browning of fresh-cut 'Fuji' apple. Fresh apples were washed with distilled water, peeled, cut in to 1.5 cm cubes, and treated with distilled water (WC), chlorinated water (CW, 0.01%, v/v), cysteine solution (CS, 0.5%, w/v) and ascorbic acid solution (AA, 0.5%, w/v). The WC treatment was considered as a control. All samples were stored in the dark at 4°C and RH 90% for 7 days. Color, browning index, total phenolics, and PPO activity of the samples were evaluated. PPO activity and browning index of all samples increased during storage. For total phenolics, WC and CW treatments did not show observed changes during storage, although CS and AA treatments showed an increase. Browning index of WC and CW treatments during storage was found to be highly correlated with PPO activity and color degradation, as indicated by changes in color parameters, but CS and AA treatments were not. Total phenolics of fresh-cut apples during storage were found to be moderately correlated with browning index and not correlated with color degradation.

Keywords: Polyphenoloxidase, phenolics, anti-browning, apple, storage

INTRODUCTION

Fresh-cut products are valued for the convenience that increased shelf-life allows, and consumers have become more critical of synthetic additives used to preserve foods or enhance characteristics such as color and flavor. Consumers have also placed a greater premium on foods which retain their natural nutritional and flavor qualities. Thus, fresh-cut fruits and vegetables have become increasingly popular due to their convenience and fresh characteristics (Schlimme *et al.*, 1995; Niyomlao *et al.*, 2002). However, discoloration of fresh-cut products has been shown to be the main factor in the loss of aesthetic, nutritional qualities and market value of fresh-cut fruits and vegetables (Kim *et al.*, 2002).

Enzymatic browning of fruit is a well-known phenomenon caused by the oxidation of phenolic compounds into quinones (Macheix *et al.*, 1991; Nicolas *et al.*, 1994). This reaction is mainly catalyzed by polyphenol oxidase (PPO, EC 1. 14. 18. 1) in the presence of oxygen (Lee and Whitaker, 1995) and gives rise to a brown pigmentation. This brown discoloration leads to organoleptic and nutritional modifications in the plant tissues, thus causing unfavorable quality changes in the food products (Carbonaro and Mattera, 2001; Kim *et al.*, 2002).

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The level of polyphenoloxidase activity at harvest and its variation during fruit storage have been considered important for the prediction of susceptibility to browning. The study of different apple varieties is important, since PPO activity is cultivar-dependent (Zocca and Ryugo, 1975). It is generally agreed that PPO is the enzyme mainly responsible for browning (Lattanzio *et al.,* 1994). An increase in PPO activity after peeling and cutting would be expected. The contribution of other enzymes to total browning may also be relevant (Rolle *et al.,* 1987).

Phenolics are secondary plant metabolites that have an important role in providing flavor and color characteristics of fruit products. There is general agreement that the concentration of phenolics is very high in young fruits and then rapidly decreases during fruit development. After harvest, the concentration of total phenols remains essentially constant or decreases slightly, and individual phenolic compounds have been shown to vary in their browning rates.

Chlorogenic acid has been shown to be the major phenolic compound in apples, decreasing rapidly during the early stage of fruit development to reach an almost steady level at maturity, while catechins have been reported to vary irregularly and to decrease during maturation. (Vamos-Vigyazo *et al.,* 1976; Burda *et al.,* 1990).

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; Coseteng and Lee, 1987; Klein, 1987). Thus it has been discovered that browning is mainly related to PPO activity, or to phenolic content. Nevertheless, the studies were carried out with many varieties with none-hurdle treatment, the results are divergent as to which of the two factors, the enzyme or the substrate, play the decisive role in the overall phenomenon of enzymatic browning (Coseteng and Lee, 1987; Burda *et al.*, 1990; Amiot *et al.,* 1992).

The aim of this work was to follow the PPO activity and phenolic content of fresh-cut apples with none- and/or anti-browning agent treatments during cold storage and to establish potential relationships with enzymatic browning.

MATERIALS AND METHODS

'Fuji' Apple Samples

Fresh apple were purchased from a local traditional market (Garagdong, Seoul, Korea) and transported to the laboratory within 1 h of purchase. Samples were used in each series of experiments. Ascorbic acid (AA) and cysteine (CS) were of reagent grade.

Treatment Condition

The apples for each experiment were initially washed in distilled water to eliminate surface contamination. After peeling and coring, each apple was cut into approximately 1.5 cm cubes and then randomly selected for the different experiments. The apple cubes were then dipped in either distilled water (DW treatment), 0.5% ascorbic acid solution, 0.5% cysteine solution, or chlorinated water (CW treatment, 100 ppm of active chlorine) for 2min. Excess liquid was removed manually using a tissue.

The apple samples were stored in low density polyethylene (LDPE) bags without sealing in the dark at 4°C, RH 90%, for 7 days. Samples were evaluated for several physicochemical parameters after various storage periods.

Color Assessment

Color characteristics (Hunter L, a and b) were measured using a Chromameter (Model CR-200, Minolta Co., Osaka, Japan) calibrated with a white tile (L = 98.64, a = 0.43, b = 0.03). Color was measured at the same location (six sides of each cube) using 10 apple cubes for each treatment. Numerical values of a and b were converted into hue angle (H^o = tan-1 (b/a)) and chroma (chroma = $(a^2+b^2)^{1/2}$) (Ozoglu and Bayind, 2002).

Assay for PPO Activity

The assay procedure used was a modified method of Lee and Smith (1979). Enzymatic activity was assayed by measuring the rate of increase in absorbance at 420 nm and 25°C in a UV/VIS spectrophotometer (Shimadzu Corp., Tokyo, Japan). The reaction mixture contained 2.8 ml of 0.6 M catechol solution, freshly prepared in 0.05 M sodium phosphate buffer at pH 6.5, and a fixed quantity of enzyme. The reference cuvette contained only the substrate solution. The reaction was conducted at 25°C. The unit for enzymatic activity was defined as a change of 0.001 in the absorbance value under the conditions of the assay. All determinations were performed in triplicate.

Total Phenolics

The assay procedure used was a modified method of Folin and Ciocalteau (1927). Each replicate of 10 apple cubes was crushed and homogenized with water, then centrifuged at 3000 rpm for 10 min at 4°C. Total phenolic content was measured using Folin-Ciocalteau reagent. Aliquots (0.5 ml) of the clear apple juice were diluted in 9.5 ml of deionized water, and 5 ml of diluted Folin-Ciocalteau reagent (1 ml plus 9 ml of deionzed water) was added to 1 ml of the resulting solution. Then, 4 ml of sodium carbonate solution (0.075 g sodium carbonate/ml) was added. After 1 hour at 30°C, the absorbance of the solution was measured at 760 nm. Chlorogenic acid was used to obtain the standard curve, and the concentration of phenolics was calculated directly from that curve, since the standard and samples were treated identically. Total phenolics were expressed as mg% (mg/100g) fruit fresh weight.

Browning Index (BI)

Frozen apple (20 g) from each replicate of 10 fruits was homogenized with a laboratory blender for 2 min. The homogenates were centrifuged at 10000 rpm for 15 min at 4°C and filtered through Whatman No 2 filter paper, then the absorbance of the resulting clear juice was measured immediately at 420 nm to determine BI. Higher values of absorbance at 420 nm correspond to higher browning of the tissue.

Statistical Analysis

All experiments were done in triplicate. Analysis of variance (ANOVA) on data was done using SAS computer system. Means were compared using LSD method at a probability level of 0.05. Relationships among measurement variables were studied using standard correlation, R^2 being the correlation factor.

RESULTS AND DISCUSSION

Color Changes

The influence of CW, AA and CS on the Hunter L, a, b and chroma values and Hue angle of fresh-cut apple cubes are shown in Figure 1. The results revealed that CS was generally more effective than AA and CW, regardless of storage time ($P<0.05$). Furthermore, DW treatment was not as effective as the chemical treatments under all conditions. All samples changed color during the first few days of storage, as can be observed by the increase in a value and the decrease in hue angle (Figure 1). The Hunter L value decreased (decrease of lightness) and the chroma value increased, but at a later stage of storage (Figure 1). An increase in chroma value indicates an increase in overall pigmentation. Mendel *et al.* (1997) reported that enzymatic discoloration highly correlated with total phenolic ($r = 0.89$) and free tyrosine levels ($r = 0.85$) in potatoes. The values obtained are highly dependent on the method of measurement and on the state of the surface of the examined object (Kuczinsky *et al.*, 1992). Most authors use the decrease in lightness to evaluate the extent of browning. A browning study on cut surfaces of apples showed that the L and a tristimulus values were linear or occasionally bilinear with log time and related to the extent of browning (Sapers and Douglas, 1987).

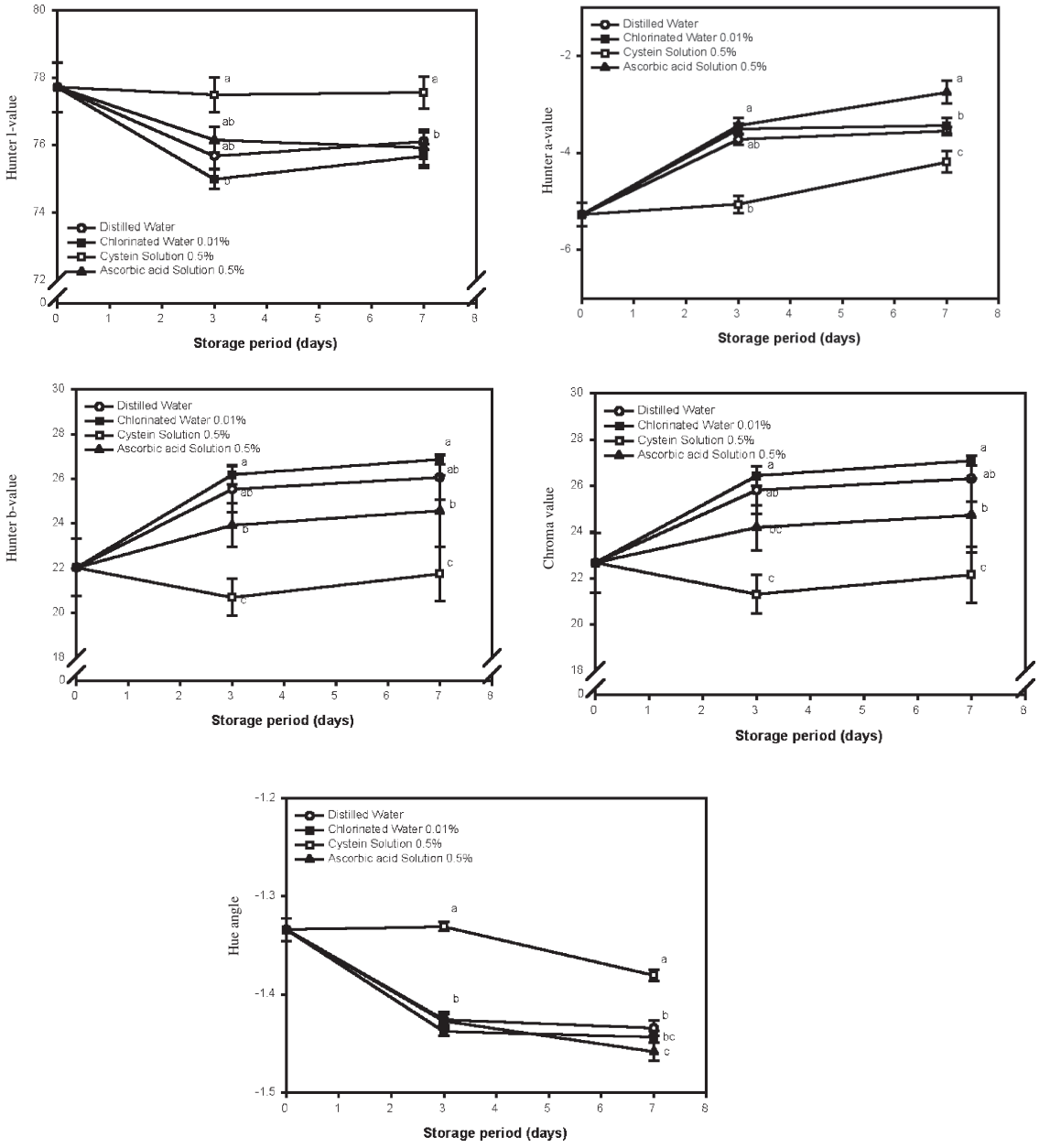

Figure 1: Change of hunter Color parameters in Fresh-cut 'Fuji' apple during storage at 5°C. [1]Data represent mean ± standard deviation of three replications. [2]All value presents the mean ± SD of triplicate determinations and spots within different letters are significantly different at $p<0.05$ by Duncan's multiple range test

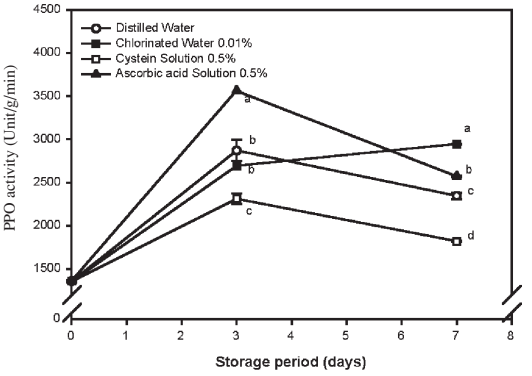

Figure 2: Change of PPO activity (unit/g/min) in fresh-cut 'Fuji' apple during storage at 5°C. [1]Data represent mean ± standard deviation of three replications. [2]All value presents the mean ± SD of triplicate determinations and spots within different letters are significantly different at $p<0.05$ by Duncan's multiple range test

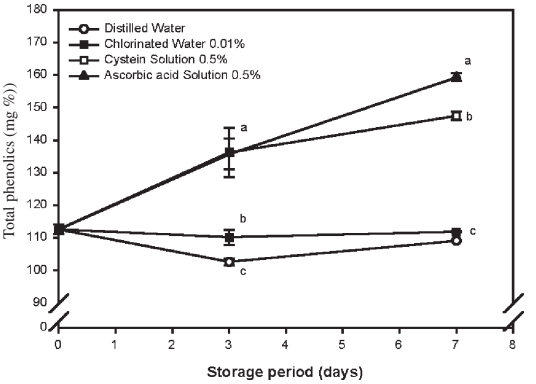

Figure 3: Change of total phenolics (mg%) in fresh-cut 'Fuji' apple during storage at 5°C . [1]Data represent mean ± standard deviation of three replications. [2]All value presents the mean ± SD of triplicate determinations and spots within different letters are significantly different at $p<0.05$ by Duncan's multiple range test

Polyphenoloxidase Activity

Figure 2 shows the changes in PPO activity in the samples as influenced by the chemical treatments during dark storage at 4°C. The activity continued to increase throughout the storage period regardless of the treatments. The rapid increase in the activity might be due to increased respiration rates following peeling and cutting. The activity of apple cubes showed a significant increase between 0 day and 3 days. This contrasts with the color data (Hunter L value) which showed a large decrease between 0 day and 3 days. The PPO activity of fresh-cut apple cubes was most effectively inhibited by 0.5% cysteine during storage at 4°C for 7 days. Apple polyphenoloxidase was at least twice as active with chlorogenic acid compared to catechins. Both classes of phenols are important for browning (Janovitz-Klapp *et al.*, 1989).

Total Phenolics

Figure 3 presents the changes in total phenolics in the samples as influenced by the chemical treatments during storage at 4°C in the dark. The total phenolics continued to significantly increase throughout the storage period in the CW and WC treatments. Ke and Saltveit (1989) reported that the wounding of iceberg lettuce caused an increase in total soluble phenolic content. Total phenolics of all samples increased, but the changes between samples treated with cysteine and ascorbic acid were unremarkable, with less than 1% total phenolics increase during the entire storage period. A similar conclusion was obtained with seven U.S. cultivars, although the authors indicated that four cultivars showed a good correlation between degree of browning and enzyme activity (Coseteng and Lee, 1987).

Browning Index (BI)

The browning index of 'Fuji' apple samples, exclusive of CS treatment, increased significantly between days 0 and 3 of storage and remained approximately constant thereafter (Figure 4). The browning index indicates the proportion of oxidized phenols during apple storage. Couture *et al.* (1993) studied the shelf-life of minimally processed lettuce and reported an increase in browning intensity (A_{340}) during 5 days of storage at 2.5°C. Amoit *et al.* (1992) reported that a strong correlation ($r = 0.986$) was obtained between A_{400} and the initial chlorogenic acid content. This suggested that this compound

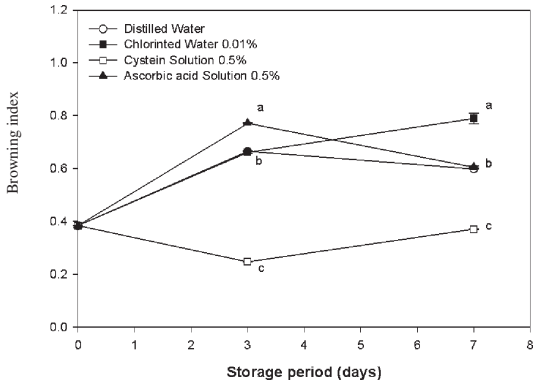

Figure 4: Change of browning index in fresh-cut 'Fuji' apple during storage at 5°C. [1]Data represent mean ± standard deviation of three replications. [2]All value presents the mean ± SD of triplicate determinations and spots within different letters are significantly different at $p<0.05$ by Duncan's multiple range test

treatments having higher PPO activity showed a higher rate of browning. Conversely, apple treatments lower in PPO activity showed a lower rate of browning. Very weak correlations ($R^2 < 0.56$) were found when total phenolics were plotted against a, b, Hue angle and chroma values (Table 2). Moderate to high correlations were obtained when total phenolics was plotted against L value and BI. Cantos *et al.* (2000) did not find any simple correlation between browning susceptibility and any of the parameters studied (PPO activation, total PPO activity, etc) in six minimally processed lettuce cultivars. Lee *et al.* (1990) reported that the degree of browning of peach cultivars correlated with their phenolic content ($R^2 = 0.67$). High correlations were obtained between BI and a, b, hue and chroma values in the CW and WC

Table 1
Correlations (R^2) between PPO activity (unit/g/min) and several parameters of fresh-cut 'Fuji' apple during storage

Samples	Distilled Water	Chlorinated Water 0.01%	Cysteine Solution 0.5%	Ascorbic acid Solution 0.5%
Color				
L value	0.98	0.91	0.78	0.74
a value	0.82	0.99	0.01	0.58
b value	0.80	0.99	0.03	0.65
Hue angle	0.83	0.99	0.01	0.61
Chroma value	0.79	1.00	0.07	0.64
Browning index	0.98	0.97	0.84	0.99
Total phenolics	0.88	0.44	0.90	0.99

R^2 at level P = 0.05.

played a prominent role in the browning of apples.

Relationships Among Color Parameters, BI, Total Phenolics and PPO Activity

High correlations ($R^2 > 0.84$) were obtained when PPO activities were plotted against BI in all the treatments (Table 1). Moderate to high correlations ($R^2 > 0.74$) were obtained when PPO activity was plotted against L value. In other words, the degree of browning in apple treatments, but weak correlations were obtained in the CS and AA treatments (Table 3).

CONCLUSIONS

In recent years, valuable progress has been made in understanding polyphenoloxidase and phenolics of apple and apple products. Many studies have been restricted to model systems with enzyme acting on a single

Table 2
Correlations (R^2) between total phenolics (mg%) and several parameters of fresh-cut 'Fuji' apple during storage

Samples	Distilled Water	Chlorinated Water 0.01%	Cysteine Solution 0.5%	Ascorbic acid Solution 0.5%
Color				
L value	0.77	0.74	0.97	0.65
a value	0.50	0.56	0.18	0.48
b value	0.47	0.43	0.02	0.55
Hue angle	0.51	0.53	0.16	0.52
Chroma value	0.46	0.42	0.00	0.55
Browning index	0.79	0.79	0.54	0.99
PPO activity	0.88	0.44	0.90	0.99

R^2 at level P = 0.05.

Table 3
Correlations (R^2) between browning index (BI) and several parameters of fresh-cut 'Fuji' apple during storage

Samples	Distilled Water	Chlorinated Water 0.01%	Cysteine Solution 0.5%	Ascorbic acid Solution 0.5%
Color				
L value	0.99	0.79	0.37	0.76
a value	0.90	0.92	-[a]	0.60
b value	0.89	0.98	0.32	0.67
Hue angle	0.91	0.93	-[a]	0.64
Chroma value	0.88	0.98	0.40	0.67
PPO activity	0.98	0.97	0.84	0.99
Total phenolics	0.79	0.79	0.54	0.99

[a] No correlation was found.

R^2 at level P = 0.05.

phenolic substrate. However, these studies would provide a better understanding of the relationship between the extent of browning and the phenolic composition of fruit.

Fresh-cut apples cause an increase in PPO activity during storage for 7 days at 4°C. This increase has been found to be highly correlated with L value and the browning index. Changes in color parameters of apple cubes during storage were found to correlate quite well with PPO activity (R^2 between 0.79 and 1.00), except for CS and AA treatments. The browning index of apple cubes during storage was found to correlate well with PPO activity ($R^2 > 0.84$) in all treatments. No correlation was found between phenolics and color parameters (L, a, b, Hue angle, and chroma value). The predominance of PPO activity in enzymatic browning seems to be highly related to the phenolics.

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