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Extending Storage Life of Fresh-Cut Apples Using Natural Products and Their Derivatives

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Prevention of browning of apples slices has been difficult to achieve because of the rapidity of the enzymatic oxidation of phenolic substrates even under reduced atmospheric pressure storage. Combinations of enzymatic inhibitors, reducing agents, and antimicrobial compounds containing calcium to extend storage life were tested to decrease the browning of Red Delicious apple slices stored at 5 and 10 °C under normal atmospheric conditions. Treatments were devised to prevent browning for up to 5 weeks at 5 °C with no apparent microbial growth using dipping solutions of compounds derived from natural products consisting of 4-hexylresorcinol, isoascorbic acid, a sulfur-containing amino acid (*N*-acetylcysteine), and calcium propionate. Analyses of organic acids and the major sugars revealed that the slices treated with the combinations of antibrowning compounds retained higher levels of malic acid and had no deterioration in sugar levels at 5 and 10 °C, indicating that higher quality was maintained during storage.

Keywords: *Cysteine; homocysteine; N-acetylcysteine; S-carbamylcysteine; isoascorbic acid; 4-hexylresorcinol*

INTRODUCTION

Fresh-cut or minimally processed refrigerated fruit has become an important area of potential growth in the rapidly expanding fresh-cut produce industry (Luo and Barbosa-Canovas, 1996). However, deterioration of the fruit after minimal processing resulting from wound-induced biochemical and physiological changes associated with water loss, respiration, and cut-surface browning has been accompanied by microbiological spoilage (Rolle and Chism, 1987). The marketing of fresh-cut fruit has been limited to 5−7 days as compared to 15−20 days for preparations from vegetative and root tissues because of the more rapid deterioration (Watada, 1997). Much of the more recent research was initiated after the use of sulfite to prevent browning of fresh-cut fruits and vegetables was banned by the Food and Drug Administration (1986). Enzymatic browning of minimally processed apples has been a major problem affecting utilization of the fruit; slices of various cultivars were prepared and stored for 12 days at 2 °C, but rapid browning of the cultivars most suitable for fresh-cut use had occurred during the first 3 days of storage (Kim et al., 1993). Various approaches to control the extent of browning have been investigated, with one being the use of different modified atmosphere (N_2/CO_2) conditions with low-temperature storage (0 °C) to prevent enzymatic browning of apple slices (Annese et al., 1997). However, this was not found to be effective because the high phenolic contents of the fruit diminished the effectiveness of elevated CO_2 atmospheres in preventing browning of fresh-cut apples and pears; it was concluded that the reduction of O_2 levels to near 0% was necessary to inhibit polyphenol oxidase (PPO) mediated browning of many fresh-cut fruit products (Gorny, 1997). Other approaches have used browning

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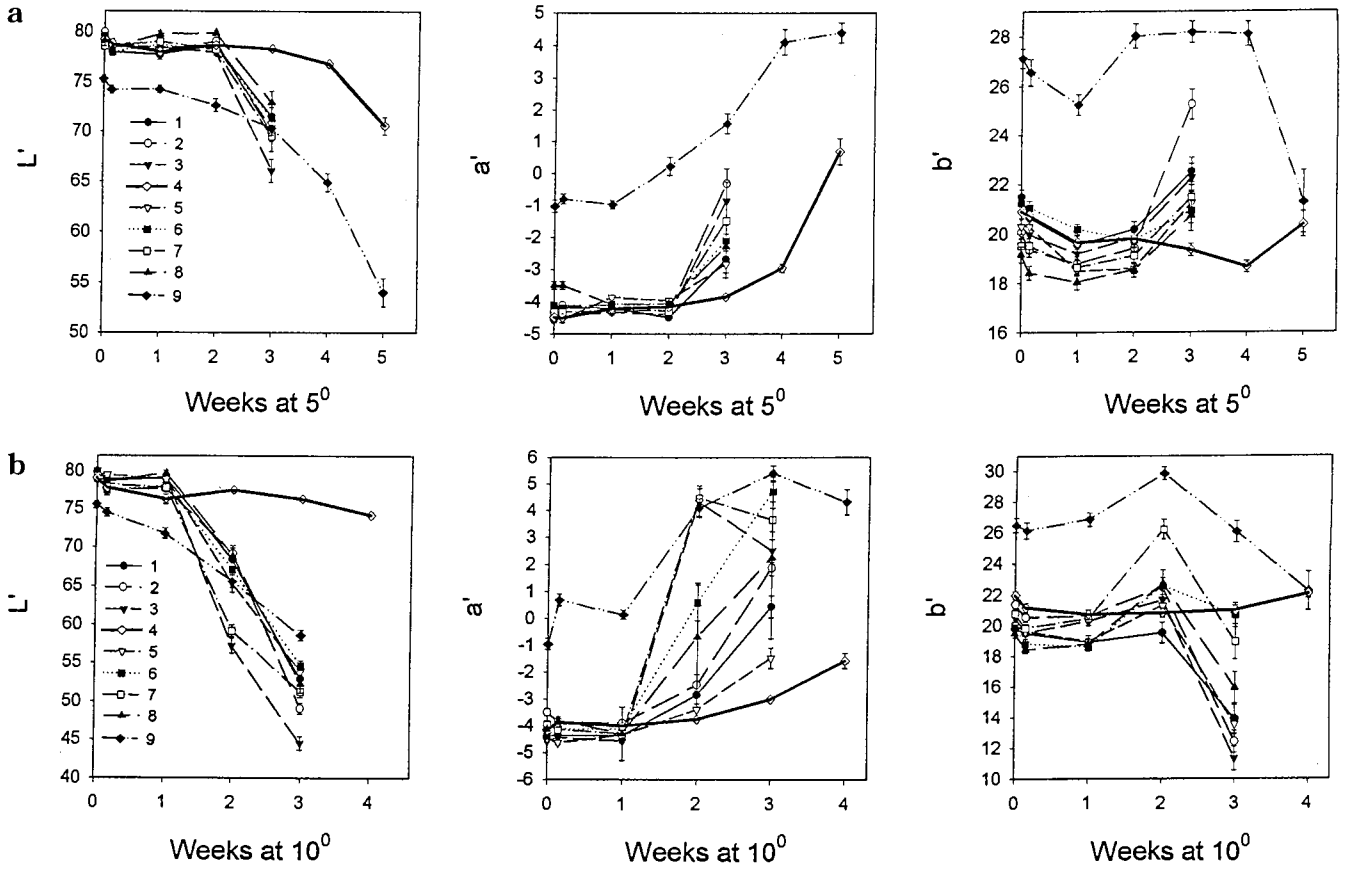

Figure 1. (a) Reflectance measurement (L', a', and b' values) of apple slices treated with the following antibrowning agents and stored at 5 °C: (1) 0.001 M 4-hexylresorcinol, 0.5 M isoascorbic acid, 0.05 M calcium chloride, and 0.025 M acetylcysteine; (2) 0.001 M 4-hexylresorcinol, 0.5 M isoascorbic acid, 0.05 M calcium tartrate, and 0.025 M acetylcysteine; (3) 0.001 M 4-hexylresorcinol, 0.5 M isoascorbic acid, 0.05 M calcium glucoheptanoate, and 0.025 M acetylcysteine; (4) 0.001 M 4-hexylresorcinol, 0.5 M isoascorbic acid, 0.05 M calcium propionate, 0.025 M acetylcysteine; (5) 0.001 M 4-hexylresorcinol, 0.5 M isoascorbic acid, 0.05 M calcium lactate, and 0.025 M acetylcysteine; (6) 0.001 M 4-hexylresorcinol, 0.5 M isoascorbic acid, and 0.025 M acetylcysteine; (7) 0.001 M 4-hexylresorcinol plus 0.5 M isoascorbic acid; (8) 0.001 M 4-hexylresorcinol, 0.5 M isoascorbic acid, and 0.05 M calcium chloride; (9) water check. Standard deviation bars show the level of significance $p = 0.05$. (b) Reflectance measurement (L', a', and b' values) of apple slices treated with antibrowning agents [see (a) for description of treatments] and stored at 10 °C. Standard deviation bars show the level of significance $p = 0.05$.

inhibitors having several different types of biochemical functions in combination with modified (reduced oxygen) atmospheres and low-temperature storage (Friedman, 1996; Nicoli et al., 1994). Reducing agents such as ascorbic acid or sodium erythorbate (isoascorbate), often with the addition of calcium chloride, have been widely employed to prevent the browning of apple slices as when used in conjunction with vacuum infiltration, by which inhibition of browning was achieved for 7 days in 4 °C storage (Sapers et al., 1990). Another approach to prevention of apple slice browning utilized sulfhydryl (SH)-containing amino acids and peptides with storage in partially evacuated containers to inhibit the action of PPOs, by which browning inhibition was maintained for 24 days at 4 °C (Molnar-Perl and Friedman, 1990). A recent advance has been the use of compounds such as 4-hexylresorcinol, which are competitive inhibitors of the PPOs considered to be the major initiators of the browning reactions (Monsalve-Gonzalez et al., 1995). Combinations of 4-hexylresorcinol, ascorbic acid, and calcium chloride have been effective in the prevention of browning of apple slices stored for up to 8 weeks at 0.2 °C with vacuum packaging; however, during storage the ethanol content of the slices increased, which was attributed to undesirable anaerobic respiration (Luo and Barbosa-Canovas, 1996). In this study, we have attempted to lengthen the time in cold storage of minimally processed apple slices kept in containers under normal atmospheric conditions by investigating combinations of the reducing agents, enzymatic inhibitors, and calcium-containing compounds, all derived from natural products, that are effective in preventing browning and inhibiting microbial spoilage. Effects on maintenance of quality as indicated by changes in sugar and organic acid levels were also evaluated.

MATERIALS AND METHODS

Plant Material. Apples (*Malus domestica* Borkh.) cv. Delicious (colloquially known as Red Delicious) were harvested at Rice Brothers Orchards, Gettysburg, PA, and placed in cold storage at 0 °C. The Delicious cultivar was chosen for experimentation because of its wide popularity as a food and the rapid browning of slices after preparation. Fruit were washed in a 10% Clorox solution preceding treatment. Apples were manually sliced into wedges, and all core tissue was removed.

Bioassays. Preliminary treatments to determine the relative efficacy of the various enzymatic inhibitors, reducing agents, and calcium salts in preventing browning were done by dipping the apple slices for 30 s into test solutions and then placing them in 15 × 100 mm plastic Petri dishes. Control samples were dipped in distilled water. Samples were stored at both 5 and 10 °C, and visual observations of the extent of browning were made. The two storage temperatures were

Table 1. Visual Observations of Apple Slices during Storage at 5 (a) and 10 °C (b) after Treatment with Antibrowning Solutions Containing Various Calcium Salts (Treatments Are Shown in Figure 1)

treatment		week 1	week 2	week 3	week 4	week 5
1	a	2[a]	3	5+	−	−
	b	3	5	5+	−	−
2	a	2	4	5+	−	−
	b	2	4	5+	−	−
3	a	1	3	5+	−	−
	b	3	4	5+	−	−
4	a	0	0	1	1	2
	b	0	1	2	2	−
5	a	1	2	5+	−	−
	b	2	4	5+	−	−
6	a	1	2	5+	−	−
	b	3	4	5+	−	−
7	a	1	3	5+	−	−
	b	3	4	5+	−	−
8	a	1	3	5+	−	−
	b	3	4	5+	−	−
9	a	3	4	5+	5+	5+
	b	5	5+	5+	5+	5+

[a] Numbers refer to browning index: 0 = none, 1 = trace, 2 = slight, 3 = moderate, 4 = severe, 5 = extremely severe; (+) = presence of decay, (−) = missing data.

Table 2. Visual Observations of Apple Slices during Storage at 5 (a) and 10 °C (b) after Treatment with Antibrowning Solutions Containing Various Sulfur Amino Acids (Treatments Are Shown in Figure 2)

treatment		week 1	week 2	week 3	week 4	week 5	week 6
1	a	2[a]	3	5	5+		
	b	3	4	5+	−	−	−
2	a	3	4	5	5+	−	−
	b	3	5	5	−	−	−
3	a	3	4	5	5	5+	5+
	b	3	5	5+	5+	5+	−
4	a	2	3	3	4	5	5+
	b	2	3	5	5	5+	−
5	a	2	3	3	4	5	5+
	b	1	3	4	5	5+	−
6	a	0	1	2	3	4	4
	b	1	3	4	4	5	−
7	a	0	0	0	1	2	2
	b	0	0	1	1	2	−
8	a	1	2	3	4	4	5
	b	2	3	4	5	5	5
9	a	3	4	5	5	5+	5+
	b	3	4	5	5	5+	5+

[a] Numbers refer to browning index: 0 = none, 1 = trace, 2 = slight, 3 = moderate, 4 = severe, 5 = extremely severe; (+) = presence of decay, (−) = missing data.

chosen because 5 °C would be correct for commercial storage and 10 °C represents an improper storage temperature condition.

Solutions tested included reducing agents [ascorbic acid or isoascorbic (erythorbic) acid], from 0.1 to 0.5 M; SH compounds (cysteine hydrochloride, homocysteine, *N*-acetylcysteine, and *S*-carbamylcysteine], from 0.01 to 0.1 M; a competitive inhibitor of PPO, 4-hexylresorcinol, from 0.0005 to 0.005 M; readily available calcium salts (chloride, glucoheptanoate, lactate, propionate, and tartrate), from 0.025 to 0.1 M; and sodium benzoate as an antimicrobial compound, from 0.005 to 0.05 M.

Combinations of the most effective compounds were then tested on large samples of apple slices. Apples were sliced and cored after being washed in 10% Clorox. The slices were dipped for 30 s in test solutions (pH range 2.1−3.15) and drained, and then 25 slices were placed in a zip-lock plastic bag. Two replicates of each treatment were prepared for each experiment, and a minimum of two experiments were performed. The bags of apple slices were stored at both 5 and 10 °C for up to 6 weeks.

Tristimulus reflectance colorimetry was used to assess the extent of browning in sliced produce (Sapers and Douglas, 1987). The color of the sliced apples was determined (as L', a', and b' values) through the bag surface without opening within 2 h of treatment using a Minolta CR-300 Chroma meter. A decrease in L' value indicated a loss of whiteness (brightness), and a more positive a' value indicated browning had occurred, whereas a more positive b' indicated yellowing. One measurement was made on each of 20 slices per replicate. Additional measurements were made after 24 h and at weekly intervals for the duration of the experiments along with visual assessments of microbial growth. Samples were removed from the bags at weekly intervals for further chemical analyses.

Analysis of Sugars and Organic Acids. Two grams of flesh tissue was homogenized with a Polytron homogenizer (Brinkmann Instruments, Westbury, NY) in imidazole buffer (20 mM, pH 7.0). The extracts were centrifuged, and the supernatants were dried in vacuo in derivatizing vials. Derivatization of sugars was carried out according to procedures described by Li and Schuhmann (1980). One microliter of the derivatized samples was injected for gas chromatographic separation and quantification. A Hewlett-Packard 5890 gas chromatograph equipped with a flame ionization detector was used. A known amount of β-phenyl-D-glucopyranoside was included in all samples as an internal standard. A 25 m cross-linked methyl silicon gum capillary column (0.2 mm i.d., 0.33 μm film thickness) was used. Chromatograph temperatures were as follows: injector, 250 °C; detector, 275 °C; and column, 100−250 °C programmed at 10 °C/min. Organic acids were analyzed after extraction with imidazole buffer and purification with a Baker-10 solid phase extraction system. Supernatants from the extracts were passed through quaternary amine columns that had been previously conditioned with hexane and methanol. The samples were then eluted from the columns with 0.1 N HCl. The eluates were concentrated to dryness in vacuo in derivatized vials. Procedures of derivatization and chromatography for organic acids were the same as those for sugars except that column temperatures were programmed from 180 to 250 °C at 10 °C/min. Separated sugars and organic acids were compared with derivatized standards for qualitative and quantitative determinations. A Hewlett-Packard ChemStation was used to calibrate the peaks, record the data, and calculate the results.

Statistical Analysis. Data from the Minolta Chroma meter were transferred to a laptop computer and stored as a DOS file. The data were then imported to a Lotus 123 spreadsheet, which after minimal manipulation was imported into a SigmaStat 5 program. One-way nonparametric Kruskal−Walis ANOVA ($p \leq 0.05$) was run, and mean comparisons were performed using Dunnet's test.

RESULTS AND DISCUSSION

Reflectance measurements confirmed the preliminary observations that certain combinations of compounds derived from natural products when applied to apple slices could inhibit browning and lengthen storage life at low temperature to ~1 month without use of modified atmospheric conditions. The combinations were composed of 4-hexylresorcinol, isoascorbic acid, cysteine, and derivatives as well as calcium chloride and readily available calcium salts of organic acids. Several series of experiments using larger quantities of apples were then performed to attempt to maximize the activity as browning inhibitors of mixtures of several different types of components on the basis of these preliminary findings. First, the use of different calcium salts was investigated in an attempt to determine whether another salt might be more effective than the commonly used calcium chloride, which had been used in combination with hexylresorcinol and ascorbic acid to inhibit the browning of vacuum packaged apple slices at 0.5 °C (Luo and Barbosa-Canovas, 1996). Several other calcium

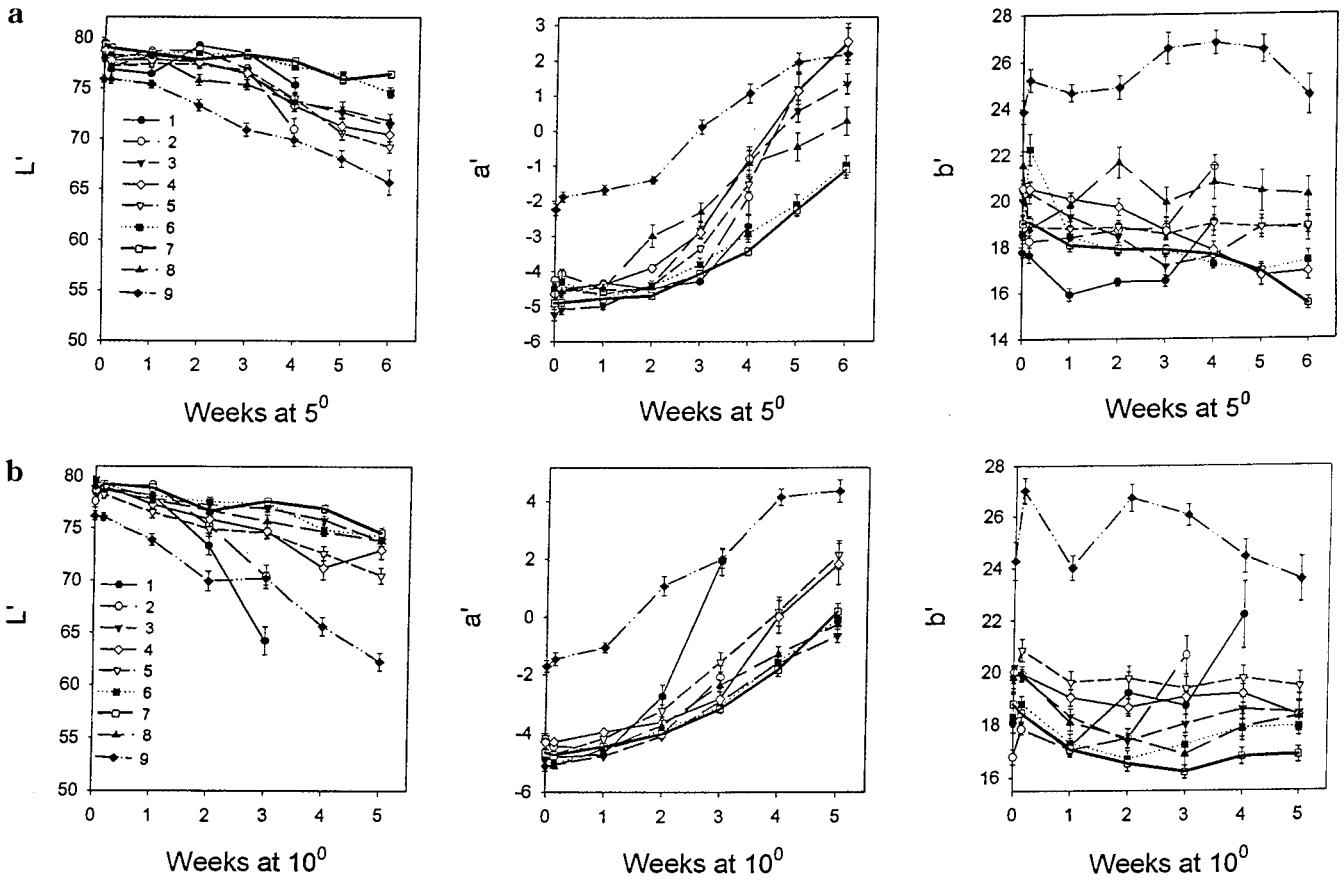

Figure 2. (a) Reflectance measurement (*L′*, *a′*, and b′ values) of apple slices treated with the following antibrowning agents and stored at 5 °C: (1) 0.001 M 4-hexylresorcinol plus 0.5 M isoascorbic acid; (2) 0.001 M 4-hexylresorcinol, 0.5 M isoascorbic acid, and 0.05 M calcium chloride; (3) 0.001 M 4-hexylresorcinol, 0.5 M isoascorbic acid, and 0.05 M calcium propionate; (4) 0.001 M 4-hexylresorcinol, 0.5 M isoascorbic acid, 0.05 M calcium propionate, and 0.025 M cysteine hydrochloride; (5) 0.001 M 4-hexylresorcinol, 0.5 M isoascorbic acid, 0.05 M calcium propionate, and 0.025 M carbamylcysteine; (6) 0.001 M 4-hexylresorcinol, 0.5 M isoascorbic acid, 0.05 M calcium propionate, and 0.025 M homocysteine; (7) 0.001 M 4-hexylresorcinol, 0.5 M isoascorbic acid, 0.05 M calcium propionate, and 0.025 M acetylcysteine; (8) 0.001 M 4-hexylresorcinol, 0.5 M isoascorbic acid, 0.05 M calcium propionate, and 0.025 M sodium benzoate; (9) water check. Standard deviation bars show the level of significance $p = 0.05$. (b) Reflectance measurement (*L′*, *a′*, and *b′* values) of apple slices treated with antibrowning agents [see (a) for description of treatments] and stored at 10 °C. Standard deviation bars show the level of significance $p = 0.05$.

salts in addition to $CaCl_2$ had been infiltrated into whole apples under pressure, and no improvement in long-term storage over use of $CaCl_2$ was found (Scott and Wills, 1979; Beavers et al., 1994). However, treatment of apple slices with a mixture of components, 4-hexylresorcinol, isoascorbic acid, and *N*-acetylcysteine, where the calcium salts were the variables, did result in a significant increase in storage time only with the use of calcium propionate. This treatment (4) maintained the apple slices in essentially unchanged appearance for 4 weeks at 5 °C as confirmed by reflectance measurements of the *L′* (whiteness), *a′* (browning), and *b′*-(yellowing) values (Figure 1a). Tissue breakdown resulting from physiological deterioration or microbial spoilage led to termination of measurements of the other treatments after 3 weeks (Table 1). Storage of the slices for 3 weeks at 10 °C after treatment with the same propionate mixture was possible as shown by similar measurements (Figure 1b; Table 1). Incorporation of calcium propionate in treatment mixtures significantly increased the length of time in cold storage at both temperatures for the apple slices in comparison to the similar mixture containing calcium chloride. The increase in storage life can be attributed to a decrease in physiological breakdown as was found by the incorporation of calcium salts into treatments of whole apples (Scott and Wills, 1979) and the enhanced effectiveness of propionate salts in decreasing microbial growth (Freese et al., 1973).

The other component of the browning inhibition treatment mixtures that was examined was the use of different compounds containing the SH− moiety present in cysteine and other sulfur amino acids that had been investigated previously as browning inhibitors in apples and potatoes stored in deaerated containers (Molnar-Perl and Friedman, 1990). The SH− compounds were considered to be effective inhibitors of PPO (Friedman et al., 1986). Further action of the SH− compounds has been described as reaction with quinones formed by PPO activity, resulting in the formation of colorless adducts instead of the brown pigments (McEvily et al., 1992a). The compounds investigated here were cysteine and related compounds for which the reactivity of the SH− moiety was affected by alteration of the cysteine molecule by derivatization or use of a homologue. The treatment mixtures that contained SH− compounds (0.025 M) were significantly more effective in prolonging storage life and preventing decay (Table 2), although these concentrations were much less than the concentrations (0.5 M) of isoascorbic acid, the other reducing agent component of the treatments; this suggested that the mode of action of the SH− compounds might be

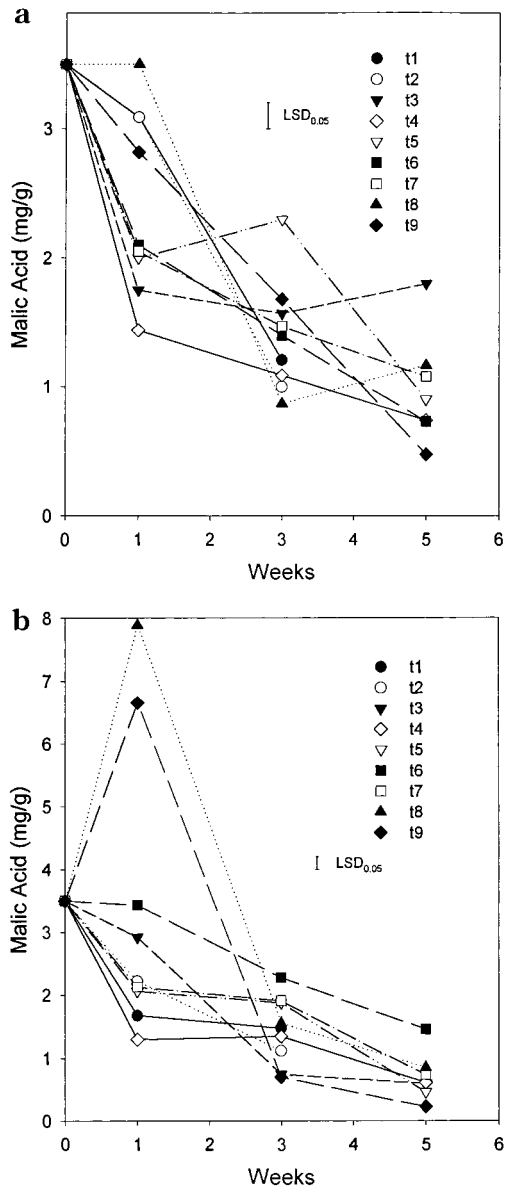

Figure 3. Changes in malic acid in apple slices during 5 weeks of storage at (a) 5 and (b) 10 °C (treatments identified in Figure 2). Vertical bars represent LSD at $p = 0.05$.

more than as antioxidants. Several of the compounds, *N*-acetylcysteine, *S*-carbamylcysteine, and homocysteine, incorporated in treatments that also contained 4-hexylresorcinol, isoascorbic acid, and calcium propionate were effective in maintaining the apple slices with little change in appearance for at least 5 weeks at 5 °C in three of the four experiments when reflectance measurements of L', a', and b' values (Figure 2a) were made. An experiment composed of apples from long-term controlled atmosphere was terminated after 4 weeks of storage at 5 °C because of tissue breakdown (data not shown). There was no significant difference in the changes in L', a', and b' values of the apple slices treated with the mixtures containing any of the three above-mentioned SH− compounds for 4 weeks of storage at 5 °C in the four experiments. Similar results were obtained with mixtures containing these three compounds for 4 weeks of storage at 10 °C with slices produced from the earlier group of stored apples (Figure 2b), whereas slices produced from the later group of apples from controlled atmosphere storage deteriorated after 3 weeks at 10 °C (data not shown). The treatments containing these derivatized sulfhydryl compounds where the SH− group was less available for reaction with quinones have retained inhibition of browning activity in cold storage at both temperatures longer compared to the cysteine-containing solutions. No differences in browning inhibition or prevention of microbial spoilage could be determined for the mixtures that contained the three cysteine-derived SH− compounds (Table 2).

There are other factors, such as the known biological activity in mammalian systems, that should be considered before further investigation of these compounds as browning inhibitors. *N*-Acetylcysteine, as well as cysteine, has been used in treatments to increase the nutritive value of foods such as soybean flour (Friedman et al., 1984). Also, there are several medicinal uses for *N*-acetylcysteine as a mucolytic agent and as an antidote to acetaminophen poisoning (McKinney and Sisson, 1979), so further studies of this compound should be possible. Similar possibilities may exist for *S*-carbamylcysteine, which was found to be an antineoplastic agent (Heinemann and Howard, 1964). Elevated human plasma levels of homocysteine have been associated recently with increased risk of thrombotic and atherosclerotic disease, so dietary use of the compound may not be advisable (Lentz, 1997).

No investigations of analogues of the phenolic competitive inhibitor of PPO, 4-hexylresorcinol, were done because it appeared to be the most effective of a group of synthetic analogues of natural browning inhibitors derived from 2,4-dihydroxydihydrocinnamic acid (McEvily et al., 1992b). Also, no comparison of the browning inhibitory activity of ascorbic acid and its isomer, isoascorbic acid, was incorporated in this study because an earlier experiment had indicated only a slight increase in low-temperature storage life with use of the iso form (data not shown) instead of ascorbic acid. This equivalence in activity of the isomers had been noted earlier (Sapers and Ziolkowski, 1987). The relatively high concentration of isoascorbic acid (0.5 M) was necessary as a component of the treatments because lower concentrations evaluated in the preliminary experiments were less successful in inhibiting browning when using the rapid dipping procedures that were adopted to diminish the problems such as tissue damage, waterlogging, or chemical buildup encountered when longer periods of immersion or high pressure were used during treatment (Sapers et al., 1990).

Malic acid was the major organic acid detected in apple slices (Figure 3). Levels of malic acid declined during storage at both 5 and 10 °C. Higher malic acid levels were maintained in treatments containing anti-browning compounds than in the controls after 5 weeks of storage. However, the treatments without SH− compounds retained the most malic acid at 5 °C but were not significantly different at 10 °C. Smaller quantities of citric acid (0.02−0.05 mg/g) and quinic acid (0.03−0.12 mg/g) were also present, but their levels did not fluctuate during storage. No significant changes were found in sugar levels including fructose, glucose, sorbitol, and sucrose during 5 weeks of storage at 5 and 10 °C (data not shown). These data suggest that in addition to prolonging storage life by inhibiting browning and microbial growth without imparting objectionable flavor, these treatments incorporating the anti-browning compounds also maintained higher organic

acid levels indicative of better maintenance of quality during storage of the apple slices.

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