

Control of Enzymatic Browning in Apple with Ascorbic Acid Derivatives, Polyphenol Oxidase Inhibitors, and Complexing Agents

ABSTRACT

Novel browning inhibitors were evaluated in raw apple juice and on the cut surface of apple plugs, using quantitative measurements of color changes during storage to assess treatment effectiveness. Ascorbic acid-2-phosphate (AAP) and -triphosphate (AATP) showed promise for cut surfaces but were ineffective in juice. Ascorbic acid-6-fatty acid esters showed anti-browning activity in juice. Cinnamate and benzoate inhibited browning in juice but induced browning when applied to cut surfaces. Combinations of β -cyclodextrin with ascorbic acid (AA), AAP or ascorbyl palmitate were effective in juice but not on cut surfaces. Combinations of AA with an acidic polyphosphate were highly effective with both juice and cut surfaces.

INTRODUCTION

THE CONTROL of enzymatic browning in raw fruits and vegetables used in salad bars and other food service applications represents a difficult problem for the food processing industry, especially with recent restrictions in the use of sulfites in such foods by the Food and Drug Administration (Anon., 1986, 1987). Alternative treatments to control enzymatic browning, mostly formulations of ascorbic acid (AA), erythorbic acid (EA) or their sodium salts with citric acid, have been developed (Anon., 1977; Labell, 1983; Andres, 1985b; Duxbury, 1986; Langdon, 1987). However, these alternatives are considered to be less effective than sulfite because of insufficient penetration into the cellular matrix (Taylor et al., 1986). Furthermore, AA is easily oxidized by endogenous enzymes (Ponting and Joslyn, 1948) or by iron- or copper-catalyzed autoxidation. When oxidized by these reactions or in the course of its intended role as a browning inhibitor, AA may fall into a concentration range where it exerts prooxidant effects (Mahoney and Graf, 1986). EA appears to be more easily oxidized than AA (Borenstein, 1965; Sapers and Ziolkowski, 1987).

Recently, Seib and Liao (1987) have described the preparation of ascorbic acid-2-phosphate (AAP) and ascorbic acid-2-triphosphate (AATP), compounds that are stable against oxygen and release ascorbic acid when hydrolyzed by phosphatase. Ascorbyl palmitate (AP), a fat soluble analog of ascorbic acid, is an effective antioxidant for vegetable oils (Cort, 1974).

A number of other anti-browning treatments, including reducing agents, acidulants, chelating agents, polyphenol oxidase (PPO) inhibitors, inorganic salts and enzymes, have been investigated but are not in commercial use (Vamos-Vigyazo, 1981; Joslyn and Ponting, 1951). Of particular interest are cinnamic and benzoic acids, which are well-characterized PPO inhibitors (Shannon and Pratt, 1967; Pifferi et al., 1974; Walker and Wilson, 1975). Promising results have been obtained with

these compounds in apple juice (Walker, 1976) and on apple and potato slices (Gajzago et al., 1981; Zent and Ashoor, 1985).

Chelating agents such as cyanide, diethyldithiocarbamate, 2-mercaptobenzothiazole, azide and EDTA inhibit PPO by interacting with its prosthetic group, copper (Mayer and Harel, 1979; Vamos-Vigyazo, 1981). Polyvinylpyrrolidone will bind to the phenolic substrates of PPO, and thereby, prevent their conversion to quinones (Loomis, 1968). An acidic polyphosphate, Sporix, which has been described as an effective chelating agent (Friedman, 1986), has been tested as a sulfite substitute in apples (Zent and Ashoor, 1985) but is not yet approved for food use in the U.S. A blend of food-grade phosphates, citric acid and dextrose is being marketed as a sulfite alternative for fruits and vegetables (Duxbury, 1986). Cyclodextrins, cyclic oligosaccharides composed of 6 or more glucose units with α -1, 4-linkages, which form inclusion complexes with various organic and inorganic compounds, have been used to debitter grapefruit juice by removing naringin, a flavanone, and limonin, a terpene (Shaw and Buslig, 1986). Szejtli (1982) has observed that the discoloration of some fruits, caused by the enzymatic oxidation of polyphenols, may be retarded by cyclodextrins.

Effective sulfite substitutes might be based on stabilized forms of AA, used alone or in unique combinations with other types of browning inhibitors. The objective in the present study was to evaluate the performance of two classes of AA derivatives, employed individually or in combination with AA, cinnamate or benzoate, cyclodextrins and Sporix as browning inhibitors for apple.

MATERIALS & METHODS

Systems for evaluation of browning inhibitors

Ripe apples were obtained from local food stores during 1986 and 1987 and stored at 4°C for no more than 5 days before being used. All procedures for sample preparation, colorimetry and data analysis were described in detail in an earlier publication (Sapers and Douglas, 1987). Briefly, two systems were used to evaluate the effectiveness of browning inhibitors: raw juice prepared from Granny Smith apples and the cut surface of plugs obtained from Red Delicious and Winesap apples. These cultivars were used since they underwent enzymatic browning at rates suitable for our study.

In the juice system, 30 mL portions of juice, obtained from several composited apples with an Acme Supreme Juicerator, were mixed with 1-4 mL of treatment solutions or H₂O (controls) in cylindrical optical glass beakers (57.1 mm i.d.) at zero time (within 1 min of juice preparation). The samples were covered to prevent evaporation and stored for as long as 24 hr at room temperature. L- and a-values were measured at frequent intervals with a Gardner XL-23 Tristimulus Colorimeter, operated in the reflectance mode and standardized against a white tile, by placing the beakers over a 32 mm diameter aperture at the sample port. The L- and a-values were plotted against time, yielding linear curves, sometimes having an initial region of zero slope indicating the absence of browning. "Lag" times corresponding to this region and the changes in L (or a) from the initial values to those at a specified time (ΔL or Δa) were obtained. An index of treatment

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Table 1—Inhibition of enzymatic browning at cut surface of Red Delicious plugs by dips containing ascorbic acid-2-phosphates and ascorbic acid in water or 1% citric acid^a

Expt.	Treatment ^b	Dip ^c solvent	Percent inhibition		Lag time (min)
			6 hr	24 hr	
A	45.4 mM AAP	H ₂ O	107 ^{de}	120 ^{de}	>360 ^d
	45.4 mM AA	H ₂ O	0 ^f	15 ^f	10 ^e
	45.4 mM AAP + 22.7 mM AA	H ₂ O	118 ^d	140 ^d	>360 ^d
	68.1 mM AA	H ₂ O	71 ^e	70 ^{ef}	225 ^d
B	45.4 mM AATP	H ₂ O	87 ^d	100 ^d	>360 ^d
	45.4 mM AA	H ₂ O	-18 ^e	18 ^e	20 ^e
	45.4 mM AATP + 22.7 mM AA	H ₂ O	90 ^d	90 ^d	>360 ^d
	68.1 mM AA	H ₂ O	-2 ^e	6 ^e	30 ^e
C	45.4 mM AAP	1% CA	77 ^e	78 ^{de}	240 ^d
	45.4 mM AA	1% CA	94 ^{de}	67 ^e	300 ^d
	45.4 mM AAP + 22.7 mM AA	1% CA	99 ^{de}	106 ^d	280 ^d
	68.1 mM AA	1% CA	106 ^d	100 ^{de}	>360 ^d
D	45.4 mM AATP	1% CA	38 ^e	58 ^d	<10 ^e
	45.4 mM AA	1% CA	52 ^{de}	4 ^e	100 ^{de}
	45.4 mM AATP + 22.7 mM AA	1% CA	42 ^e	48 ^{de}	10 ^e
	68.1 mM AA	1% CA	79 ^d	48 ^{de}	262 ^d

^a Based on measurement of L-value; means of 4–8 replicates.

^b AAP = ascorbic acid-2-phosphate; AATP = ascorbic acid-2-triphosphate; AA = ascorbic acid.

^c CA = citric acid.

^{d,f} For each experiment, means within a column, followed by different superscripts, are significant by the Bonferroni LSD test ($p < 0.05$).

effectiveness, the percent inhibition, was calculated from the ΔL (or Δa) values for treated samples and corresponding controls as follows:

$$\% \text{ Inhibition at time } t = \frac{\Delta L \text{ control} - \Delta L \text{ treatment}}{\Delta L \text{ control}} \times 100$$

where $\Delta L = L_t - L_{\text{initial}}$

A spectrophotometer (The Color Machine, Pacific Scientific Co., Silver Spring, MD) was used in the same manner as the tristimulus colorimeter to measure browning in juice in experiments carried out during the fall of 1987. With this instrument, it was possible to obtain spectral reflectance data as well as values of the tristimulus coordinates. Percent inhibition values could be calculated for the change in percent reflectance at a suitable wavelength (i.e., 410–440 nm) in the same way as for the change in L or a.

In some preliminary studies, carried out before the acquisition of the spectrophotometer, browning in juice samples was measured spectrophotometrically. Ten mL aliquots were taken at intervals from 75 mL portions of treated or control juice (gently stirred) and clarified by the addition of 10 mL 95% ethanol and 0.3g Celite Analytical Filter Aid (Fisher Scientific, Pittsburgh, PA) followed by filtration through Whatman No. 50 paper under suction. The absorbance of filtrates was determined at 420 nm. Percent inhibition values were calculated from the changes in absorbance in treated and control samples over time.

In the cut surface system, individual apples were cut in half along the stem axis, and as many as 4 plugs were bored from each half with an electric cork borer using a 22 mm diameter stainless steel cutting tube. Plugs were cut transversely at their midpoints, yielding half-plugs sharing a common cut surface. One half-plug was dipped for 90 sec in a treatment solution, while the other half (control) was dipped in water (or the treatment solvent) for 10 sec to remove adhering juice. Following treatment, the half-plugs were rolled on adsorbent tissue to remove excess treatment solution from the circumferential surface (but not the freshly cut surface). Colorimetry was performed with the XL-23 by centering the transversely cut surface of a half plug over a 19 mm diameter aperture at the sample port. L- and a-values were recorded at frequent intervals over 6 hr and also after 24 hr at room temperature. Between measurements, the half-plugs were stored in covered crystallizing dishes to minimize dehydration. The L- and a-values were plotted against log time, yielding linear or bilinear curves, sometimes preceded and followed by regions of zero slope. A lag time corresponding to the duration of the initial zero slope region was obtained graphically from the intersection of the zero slope and linear regions of each curve. The slopes of linear portions were determined by regression analysis. Percent inhibition values were calculated from the changes in L- and a (ΔL and Δa) over specified time intervals for treated half-plugs and corresponding controls, as described above.

Anti-browning treatments

Various AA derivatives, competitive inhibitors of PPO, and combinations of these compounds were screened for anti-browning activity in the juice system, and if promising, were evaluated further in the cut surface system with both Red Delicious and Winesap apples. Compounds tested included ascorbic acid (reagent grade; J.T. Baker Chem. Co., Phillipsburg, NJ); sodium ascorbate (Sigma Chemical Co., St. Louis); AAP and AATP, which were prepared by Prof. Seib (Lee et al., 1978; Seib and Liao, 1987); ascorbyl-6-palmitate (NF₄-FFC grade; Roche Chemical Div., Hoffman-La Roche, Inc., Nutley, NJ); ascorbyl-6-decanoate and ascorbyl-6-laurate, synthesized in our laboratory by the method of Cousins et al. (1977); t-cinnamic acid (Sigma); sodium benzoate (USP-FCC; Pfizer Chemicals Div., Pfizer, Inc., New York, NY); α -, β - and γ -cyclodextrins (Sigma); and Sporix (International Sourcing, Inc., South Ridgewood, NJ). All AA derivatives were compared with AA at equimolar concentrations, chosen so that AA would provide a low to moderate degree of protection against browning, thereby making it possible to detect improvements in anti-browning activity. Water-soluble browning inhibitors were added to the juice as concentrated aqueous solutions. The AA-6-fatty acid esters, which were sparingly soluble in water, were added as concentrated ethanolic solutions. Dips containing water soluble AA derivatives were prepared with distilled water or 1% citric acid solution. Only the former was used with combinations containing cinnamate or benzoate, which would have precipitated at a low pH. Additionally, these combinations were prepared with an equivalent amount of sodium ascorbate rather than AA to avoid precipitation. Dips containing ascorbyl-6-palmitate (AP) were prepared by first dissolving 2–8% of the ester, and in some trials, 1–5% of an emulsifying agent such as EC-25 or Durlac-100 (Durkee Industrial Foods, Cleveland, OH) in ethanol, and then adding 1–3 mL of the ethanolic solution to 75 mL of a hot (ca 75°C) solution containing 0.1–0.4% carboxymethylcellulose (Sigma) and/or 0.05M phosphate buffer, pH 7. Because of their limited solubility, the cyclodextrins were added in the solid state instead of as concentrates and were dissolved by stirring.

Except for preliminary studies, all treatments were compared in experiments with two or more trials (individual apples or batches of juice with duplicate plugs or juice aliquots per treatment), arranged in a randomized, complete block design where each block represented one trial. Each experiment was subjected to analysis of variance to determine the treatment effects on responses. The Bonferroni LSD test (Miller, 1981) was used to separate means.

RESULTS & DISCUSSION

Ascorbic acid-2-phosphates

Because of the reported stability of the ascorbic acid-2-phosphates to oxidation (Seib and Liao, 1987), these compounds,

Table 2—Inhibition of enzymatic browning in Granny Smith juice by ascorbic acid-6-fatty acid esters and ascorbic acid^a

Expt.	Treatment ^b	Percent inhibition			Lag time (min)
		2 hr	4 hr	6 hr	
1	1.14 mM AP	82 ^a	72 ^d	52 ^c	20 ^e
	1.14 mM AA	100 ^c	68 ^d	23 ^d	180 ^d
	1.14 mM AP + 0.28 mM AA	85 ^d	74 ^d	58 ^c	20 ^e
	1.42 mM AA	98 ^c	96 ^c	52 ^c	240 ^c
2	1.14 mM AL	54 ^d	56 ^c	29 ^c	35 ^e
	1.14 mM AA	96 ^c	16 ^d	-26 ^d	150 ^d
	1.14 mM AL + 0.28 mM AA	66 ^d	62 ^c	58 ^c	35 ^e
	1.42 mM AA	94 ^c	78 ^c	-17 ^d	180 ^c
3	1.14 mM AD	102 ^c	86 ^d	72 ^d	150 ^f
	1.14 mM AA	96 ^c	68 ^e	18 ^f	180 ^e
	1.14 mM AD + 0.28 mM AA	102 ^c	100 ^c	99 ^c	>360 ^e
	1.42 mM AA	96 ^c	90 ^{cd}	41 ^e	240 ^d

^a Based on measurement of percent reflectance at 440 nm; means of duplicates.

^b AP = ascorbyl-6-palmitate; AL = ascorbic acid-6-laurate; AD = ascorbic acid-6-decanoate; AA = ascorbic acid.

^{c-f} For each experiment, means within a column, followed by different superscripts, are significantly different by the Bonferroni LSD test ($P < 0.05$).

Table 3—Inhibition of enzymatic browning in Granny Smith juice by cinnamate, benzoate, or their combinations with ascorbic acid or ascorbic acid derivatives

Expt.	Treatment ^a	Percent inhibition ^b			Lag time (min)
		2 hr	4 hr	24 hr	
4	0.67 mM CINN	98	84	—	> 120
	1.33 mM CINN	105	98	—	> 120
	2.67 mM CINN	105	103	—	> 120
	1.14 mM AA	30	22	—	30
5	0.67 mM CINN	88	79	43	60
	1.14 mM AA	5	10	24	30
	0.67 mM CINN + 1.14 mM AA	95	99	52	> 390
6	0.67 mM CINN + 0.57 mM AAP	101 ^c	—	79 ^{cd}	> 360
	0.67 mM CINN + 0.57 mM AP	101 ^c	—	89 ^c	> 360
	0.67 mM CINN + 0.57 mM AA	101 ^c	—	76 ^d	> 360
	0.57 mM AA	16 ^d	—	11 ^e	30
7	3.1 mM BENZ	66	42	—	60
	6.3 mM BENZ	102	63	—	80
	12.6 mM BENZ	110	99	—	240
	0.57 mM AA	48	30	—	70
8	6.9 mM BENZ	74 ^d	42 ^e	16 ^d	65 ^a
	1.14 mM NaA	97 ^c	62 ^d	1 ^d	170 ^d
	6.9 mM BENZ + 1.14 mM NaA	100 ^c	102 ^c	84 ^c	> 360 ^c
9	6.9 mM BENZ	68 ^d	40 ^d	0 ^d	50 ^{cd}
	1.14 mM AAP	24 ^e	24 ^d	29 ^d	10 ^d
	6.9 mM BENZ + 1.14 mM AAP	94 ^c	84 ^c	68 ^c	210 ^c
	1.14 mM AA	98 ^c	82 ^c	1 ^d	200 ^c

^a CINN = cinnamate; AA = ascorbic acid; AAP = ascorbic acid-2-phosphate; AP = ascorbyl palmitate; BENZ = sodium benzoate; NaA = sodium ascorbate.

^b For Expts 4-6, based on change in absorbance at 420 nm; for Expts 7-9, based on change in L-value.

^{c-e} Means of 4-6 replicates; for each experiment, means within a column, followed by different superscripts, are significantly different by the Bonferroni LSD test ($p < 0.05$). Preliminary experiments 4, 5 and 7 were not replicated.

Table 4—Inhibition of enzymatic browning at cut surface of apple plugs by dips containing cinnamate and ascorbic acid or ascorbic acid-2-phosphate^a

Expt.	Cultivar	Treatment ^b	Percent inhibition			Lag time (min)
			2 hr	6 hr	24 hr	
E	Winesap	10.0 mM CINN	74 ^c	5 ^d	-156 ^e	70 ^d
		45.4 mM NaA	-28 ^d	-30 ^d	-11 ^d	10 ^d
		10.0 mM CINN + 45.4 mM NaA	98 ^c	82 ^c	68 ^c	292 ^c
F	Winesap	10.0 mM CINN	72 ^c	11 ^d	-180 ^e	80 ^d
		45.4 mM AAP	96 ^c	113 ^c	128 ^c	> 360 ^c
		10.0 mM CINN + 45.4 mM AAP	103 ^c	97 ^c	83 ^{cd}	345 ^c
		45.4 mM AA	15 ^d	-14 ^d	10 ^d	39 ^d

^a Based on measurement of L-value; means of 4 replicates.

^b CINN = cinnamate; NaA = sodium ascorbate; AA = ascorbic acid; AAP = ascorbic acid-2-phosphate.

^{c-f} For each experiment, means within a column, followed by different superscripts, are significantly different by the Bonferroni LSD test ($p < 0.05$).

which were not reducing agents *per se*, were first tested in juice and then on plugs. In the juice system, AAP and AATP proved to be less effective than AA at concentration as high as 1.14 mM (data not shown). We hypothesize that the failure of the AA-2-phosphates in apple juice resulted from insufficient endogenous acid phosphatase activity due to enzyme inactivation during juice preparation and/or to the low juice pH (3.3) which is substantially less than the optimal pH for acid

phosphatase obtained from plant tissues (Ninomiya et al., 1977; Sugawara et al., 1981; Paul and Williamson, 1987).

In contrast to the juice results, both AAP and AATP showed considerable activity as browning inhibitors when applied as dips at concentrations of 45.4 mM (0.8% AA) to the cut surfaces of Red Delicious plugs. It is evident from percent inhibition values calculated for changes in L (and a; data not shown) that the AA derivatives were more effective than equivalent

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Table 5—Inhibition of enzymatic browning in Granny Smith juice by cyclodextrins (CD) and combinations of CD's with ascorbic acid (AA) and AA derivatives

Expt.	Treatment ^a	Percent inhibition ^b			
		30 min	60 min	120 min	24 hr
10	2.9 mM β-CD	56	48	—	—
	5.9 mM β-CD	73	60	—	—
	11.9 mM β-CD	92	90	—	—
11	5.9 mM β-CD	86	70	57	—
	5.9 mM β-CD + 1.14 mM AA	100	100	65	—
	1.14 mM AA	98	22	-1	—
12	5.9 mM β-CD + 0.57 mM AAP	98 ^d	90 ^c	83 ^c	—
	5.9 mM β-CD + 0.57 mM AP	104 ^c	95 ^c	85 ^c	—
	5.9 mM β-CD + 0.57 mM AA	98 ^d	80 ^d	57 ^d	—
13	5.9 mM β-CD + 0.57 mM AAP	98 ^c	97 ^d	89 ^d	81 ^c
	0.67 mM CINN + 0.57 mM AAP	104 ^c	104 ^c	103 ^c	63 ^d
	0.57 mM AA	78 ^d	30 ^e	16 ^e	12 ^e

^a AAP = ascorbic acid-2-phosphate; AP = ascorbyl palmitate, CINN = sodium cinnamate.

^b Based on change in absorbance at 420 nm.

^{c-e} Means of 4-6 replicates; for each experiment, means within a column, followed by different superscripts, are significantly different by the Bonferroni LSD test ($P < 0.05$). Preliminary experiments, 10 and 11 were not replicated.

Table 6—Inhibition of enzymatic browning in Granny Smith juice by Sporix and combinations of Sporix with ascorbic acid (AA)

Expt.	Treatment	Percent inhibition ^a			Lag time (min)
		1 hr	2 hr	6 hr	
14	0.29% Sporix	73	73	74	10
	0.58% Sporix	107	111	119	>360
	0.88% Sporix	101	110	119	>360
	0.57 mM AA	68	4	-22	40
15	0.29% Sporix + 0.57 mM AA	109 ^c	113 ^c	114 ^c	>360 ^c
	0.29% Sporix	68 ^{c-d}	73 ^d	78 ^d	10 ^e
	0.57 mM AA	58 ^d	14 ^e	-5 ^e	25 ^d
16	0.29% Sporix + 0.57 mM AA (pH 3.1)	102	104	110	>360
	0.29% Sporix + 0.57 mM AA (pH 3.3) ^b	68	38	33	30
	0.57 mM AA (pH 3.3)	43	14	6	20

^a Based on change in L-value.

^b Adjusted to pH of control (3.3) with 10% NaOH.

^{c-e} Means of 4 trials; means within a column, followed by different superscripts, are significantly different by the Bonferroni LSD test ($p < 0.05$). Preliminary experiments 14 and 16 were not replicated.

Table 7—Inhibition of enzymatic browning at cut surface of Winesap plugs by combinations of Sporix and ascorbic acid (AA)

Expt.	Treatment	Percent inhibition ^a			Lag time-L (min)	Slope-L (min ⁻¹)
		2 hr	6 hr	24 hr		
G	22.7 mM AA + 0.24% Sporix	88	69	58	40	-2.0
	22.7 mM AA + 0.48% Sporix	102	97	81	>360	0
	0.24% Sporix	55	43	43	10	-2.5
	0.48% Sporix	64	52	30	10	-1.3
	22.7 mM AA	6	-5	28	20	-5.6
H	22.7 mM AA + 0.24% Sporix	100	107	111	>360	0
	0.24% Sporix	57	51	-34	10	-2.1
	22.7 mM AA	49	58	71	20	-4.5
I	56.8 mM AA + 0.24% Sporix	102 ± 5 ^b	109 ± 10	109 ± 25	>360	0

^a Based on change in L-value.

^b Mean and standard deviation for 8 trials. Preliminary experiments G and H were not replicated.

concentrations of AA as browning inhibitors (Table 1, Expt. A and B). Similar results were obtained with Winesap plugs (data not show). Browning inhibition was not improved significantly by the addition of 22.7 mM AA (.04%) in combination with AAP or AATP. Samples treated with AAP or AATP showed little or no browning after 24 hr at room temperature. When AAP and AA were compared in dips containing 1% citric acid, the two compounds were similar in browning activity (Expt. C). However, citric acid decreased the effectiveness of AATP as a browning inhibitor (Expt. D). The results suggested that treatments for apples based on the use of the ascorbic acid-2-phosphates as browning inhibitors might represent a significant advance over treatments based on AA.

The success of the AA-2-phosphates in inhibiting browning of apple plugs was due primarily to their stability, as seen by the longer lag times obtained with these derivatives, compared

to equivalent concentrations of AA. AA, applied to the cut surface of apple, may be consumed by reaction with quinones resulting from polyphenol oxidation (Ponting and Joslyn, 1948) or by autoxidation (Mahoney and Graf, 1986). Seib and Liao (1987) demonstrated that the AA-2-phosphates were much more stable to oxidation by H₂O₂ than was AA. Presumably, sufficient acid phosphatase was present at the cut surface of apple fruit to permit hydrolysis of the AA-2-phosphates at a rate sufficient to prevent browning but not great enough to generate a large excess of AA that would be subject to autoxidation. The poor performance of AATP in combination with citric acid probably resulted from acid inhibition of acid phosphatase as in juice; under favorable conditions, AATP is hydrolyzed more slowly than AAP (Seib and Liao, 1987). The suitability of the AA-2-phosphates as browning inhibitors for commodities other than apple will depend on their acidity and endogenous acid phosphatase activity.

Ascorbic acid-6-fatty acid esters

Experiments carried out with ascorbyl palmitate (AP), laurate (AL) and decanoate (AD) added to Granny Smith juice at concentrations as high as 1.14 mM (equivalent to 200 ppm AA), demonstrated that these esters were less effective than or similar to AA initially but surpassed AA as browning inhibitors after longer periods of storage (Table 2). The addition of 0.28 mM AA with AP or AL had little or no effect on the lag time before the onset of browning or the percent inhibition after storage. However, the combination of AA with AD was significantly more effective than AD alone, the former providing protection against browning for at least 24 hr.

Percent reflectance at 440 nm was used to monitor browning in these trials rather than measurements of tristimulus values since the latter parameters changed erratically prior to and during the onset of browning, probably because of light scattering by suspended particles of the fatty acid esters which were sparingly soluble in juice. Prior comparisons of tristimulus and percent reflectance data for browning Granny Smith juice indicated a high correlation between the percent reflectance at 440 nm and the L-value ($r = 0.98$) or a-value ($r = -0.99$). The spectral reflectance values for juice samples containing fatty acid esters were constant prior to the onset of browning.

Mixed results were obtained when aqueous dispersions of the fatty acid esters of AA were applied to apple plugs as dips (data not shown). AP dispersions in pH 7 phosphate buffer, stabilized with lipophilic emulsifying agents such as Durkee's EC-25 or Durlac 100, usually were more effective in controlling browning than equivalent concentrations of AA. However, the degree of inhibition was not consistent, probably because of AP precipitation on the cut surface during storage. These treatments were not as effective as the AA-2-phosphates. More stable dispersions could be prepared by substituting AL or AD for AP. However, treatment of apple plugs with the former esters tended to induce browning; similar results occurred when AP dispersions were prepared with less lipophilic emulsifying agents such as Tween 60 or Tween 80 (Sigma). We suspect that these effects were due to the disruption of membranes in cells near the cut surface by the emulsifying agents or esters, causing leakage of PPO and its substrates and thereby increasing the extent of browning.

Cinnamate and benzoate

Preliminary (unreplicated) trials indicated that cinnamic acid inhibited enzymatic browning in Granny Smith juice when added as sodium cinnamate (CINN) at concentrations between 0.67 and 2.67 mM (114–454 ppm) (Table 3, Expt. 4). The combination of CINN with AA appeared to be only slightly more effective than CINN applied alone (Expt. 5). In replicated trials, combinations of CINN with AAP, AP or AA were similar in effectiveness, greatly surpassing AA as a browning inhibitor (Expt. 6). CINN has been shown to inhibit PPO, either competitively or noncompetitively, depending on the substrate (Walker and Wilson, 1975). Walker (1976) reported that CINN, added to Granny Smith juice at concentrations greater than 0.5 mM, prevented browning for over 7 hr.

With plugs, 10 mM CINN inhibited browning for several hours but then induced severe browning over extended storage times (Table 4). The combination of CINN with AA in dips was more effective in inhibiting browning than AA alone, significantly extending lag times. However, the combination of CINN with AAP showed no advantage over AAP alone. The tendency of CINN to induce browning indicated a potential problem with the use of this compound. Such behavior suggests that exogenous CINN may undergo gradual conversion at the cut surface to a PPO substrate by cinnamate-hydroxylase and other enzymes involved in the biosynthesis of polyphenols (Robinson, 1983).

Sodium benzoate (BENZ) exhibited anti-browning activity

in preliminary experiments (unreplicated) with the juice system, the effect appearing to be concentration dependent (Table 3, Expt. 7). A concentration of 6.9 mM corresponds to 0.1% BENZ, the maximum concentration permitted in foods as a preservative in the U.S. (Andres, 1985a). Combinations of BENZ with AA (added as sodium ascorbate to avoid BENZ precipitation) or AAP inhibited browning to a greater extent than either treatment alone, the effect appearing to be synergistic rather than additive in samples stored 24 hr (Expt 8 and 9). The primary effect of the combination treatments was to increase the lag time before the onset of browning. BENZ is reported to be a non-competitive inhibitor of PPO (Pifferi et al., 1974) and has been evaluated previously as an anti-browning agent in apple (Zent and Ashoor, 1985).

Dips containing BENZ, alone or in combination with AA, provided short-term protection against browning in Red Delicious and Winesap plugs but induced browning in samples stored 6 or more hours (data not shown). As with CINN, induced browning by BENZ may be an indication of its gradual conversion to a PPO substrate or stimulation of substrate synthesis by enzymes at the cut surface. Benzoic acid in plants is derived from phenylalanine via t-cinnamic acid (Alibert et al., 1972; Loffelhardt and Kindl, 1975) which is also a precursor of caffeic acid and other PPO substrates (Robinson, 1983). Benzoate formation in higher plants occurs on the thylakoid membrane; this process is apparently not reversible (Loffelhardt and Kindl, 1975). However, Zenk (1966) demonstrated that the addition of a large excess of benzoic acid to *Catalpa hybrida* leaves stimulated the hydroxylation of cinnamic acid to p-coumaric acid, a PPO inhibitor which might be hydroxylated further to caffeic acid, a substrate. Because of the possibility that benzoic acid and cinnamic acid may induce browning under some conditions, we do not recommend the use of either PPO inhibitor as a component of anti-browning formulations.

Cyclodextrins

Preliminary experiments with cyclodextrins dissolved in Granny Smith juice (Table 5, Expt. 10) indicated that β -cyclodextrin (β -CD) (cycloheptaamylose) inhibited enzymatic browning, the degree of inhibition increasing with β -CD concentration. Substantially higher concentration could not be used because of the limited solubility of this compound, 15.8 mM for a saturated solution. α -CD (cyclohexaamylose) and γ -CD (cyclooctaamylose) showed little or no anti-browning activity at concentrations as high as 27.2 mM and 10.2 mM, respectively.

The inhibitory effect of β -CD on browning in juice appeared to be enhanced slightly by adding this compound in combination with AA (Expt. 11). The combination of β -CD with AAP or AP was significantly more effective as a browning inhibitor than the combination of β -CD with an equimolar concentration of AA (Expt. 12). The combination of β -CD with AAP was similar in effectiveness to that of sodium cinnamate with AAP, both treatments being greatly superior to AA alone (Expt. 13).

The ability of β -CD to inhibit enzymatic browning in the juice system probably resulted from the ability of this compound to form inclusion complexes with PPO substrates, thereby preventing their oxidation to quinones and subsequent polymerization to brown pigments. Presumably, the PPO substrates in apple were too large to fit completely in the cavity of α -CD and too small to be retained strongly by γ -CD. Shaw and Buslig (1986) reported that β -CD polymers were more effective than α - or γ -CD polymers in removing naringin and limonin from solution. The effectiveness of β -CD as a browning inhibitor will depend on the equilibrium between free and complexed PPO substrates and the rate of complex formation. The gradual browning of apple juice at all β -CD concentrations tested indicated that complex formation did not go to completion. Browning by the uncomplexed PPO substrates might be

controlled by the addition of AA or AA derivatives, as was done in the combination treatments.

Attempts to translate these favorable results to a β -CD dipping treatment for apple plugs were not successful. Solutions containing 8.8 mM (1%) β -CD, applied to Winesap and Red Delicious plugs by dipping for 90 sec, were ineffective in controlling browning. Similarly, dips containing 8.8 mM β -CD in combination with 22.7 – 90.8 mM AA were no more effective than the AA solutions alone in controlling browning in Winesap and Red Delicious plugs (data not shown).

The inability of β -CD to inhibit enzymatic browning in apple plugs can be understood in terms of the fundamental difference between the juice and cut surface systems. In the former, PPO substrates, PPO, O_2 , AA and browning inhibitors are all in solution so that the rate of browning is determined by their concentrations, the temperature, stirring conditions and perhaps, the surface to volume ratio (which would affect the dissolved O_2 concentration). With the cut surface system, juice released from disrupted cell layers at the freshly cut apple surface, which contains PPO, PPO substrates and other reactants, would be removed by the dipping treatment. Browning would not occur until these species diffused from the interior of the disrupted cell layers towards the surface or reacted in situ, given sufficient dissolved oxygen. An effective β -CD dipping treatment would have to complex PPO substrates before they diffused to the surface or reacted within the disrupted cells. Apparently, the rate of diffusion of β -CD from the cut surface to the interior of the disrupted cell layers was too slow to allow the complexing agent to compete with PPO for substrates.

Sporix

Sporix, an acidic polyphosphate described as having a three dimensional network structure, has been reported to inhibit enzymatic browning in fruits and vegetables (Zent and Ashoor, 1985; Friedman, 1986). In preliminary experiments the addition of about 0.6% Sporix to Granny Smith juice effectively controlled browning during 24 hr at 20°C while 0.57 mM AA (100 ppm) failed after 1 hr (Table 6, Expt. 14). If added in combination with 0.57 mM AA, a lower concentration of Sporix could be used to inhibit browning (Expt. 15). The exceptional effectiveness of the combination (seen even after 24 hr in some trials—data not shown) was due primarily to the lag time extension which appeared to be a synergistic effect rather than additive. The ability of Sporix to control browning in juice was pH-dependent. Percent inhibition and lag time values for Sporix-AA combination were decreased, although not reduced to values obtained with AA alone, when the Sporix was partially neutralized by addition of 1 meq NaOH per 100 mL juice, increasing the pH of the treated juice from 3.1 to 3.3, the pH of untreated juice (Expt. 16).

Dips containing combinations of Sporix and AA were highly effective in inhibiting enzymatic browning on the cut surface of apple plugs (Table 7). Sporix and AA alone were only partially effective under these conditions. Winesap apple plugs dipped in 56.8 mM (1%) AA in combination with 0.24% Sporix showed little or no evidence of browning after 24 hr at 20°C while untreated controls discolored within several hours (Expt. 1). Similar results were obtained with Red Delicious plugs (data not shown).

Browning inhibition by Sporix combinations can be attributed to two effects: a greatly extended lag time compared to that obtained with the individual inhibitors, as seen in juice, and a reduced rate of browning once the lag time has been exceeded. The lag time effect probably results from the inhibition of copper-containing oxidases and other copper-catalyzed oxidative processes in apple by Sporix, which is a powerful chelating agent (Friedman, 1986). These oxidative reactions normally would bring about the rapid loss of AA and permit browning to occur once the added AA was depleted (Ponting

and Joslyn, 1948). Sporix also would inhibit PPO directly by chelation of its copper (Mayer and Harel, 1979), thereby decreasing the rate of polyphenol oxidation and subsequent browning. The ability of Sporix to exert its effect on enzymatic browning by these two independent mechanisms probably accounted for the apparent synergism obtained with Sporix-AA combinations.

CONCLUSIONS

ASCORBIC ACID-2-phosphate and -triphosphate showed considerable promise as inhibitors of enzymatic browning at cut surfaces of raw apple but were ineffective in apple juice. Ascorbic acid-6-fatty acid esters showed anti-browning activity in apple juice but were of limited value when applied to cut surfaces. Cinnamate and benzoate enhanced the effectiveness of ascorbic acid or ascorbic acid derivatives as browning inhibitors in juice but tended to induce browning at cut surfaces. β -Cyclodextrin (β -CD) and β -CD combinations with ascorbic acid (AA) or AA derivatives showed considerable promise as browning inhibitors in apple juice but were ineffective at cut surfaces. The combination of Sporix with AA represented a highly effective antibrowning treatment for the juice of Granny Smith apples and the cut surface of Red Delicious and Winesap apples. Further studies should be carried out to optimize the most promising treatments, using conditions more applicable to commercial practice, and to extend these treatments to other important commodities.

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