

## Measurement of Enzymatic Browning at Cut Surfaces and in Juice of Raw Apple and Pear Fruits

### ABSTRACT

Reflectance procedures were developed to measure the extent of enzymatic browning at cut surfaces and in the raw juice of apple and pear fruits. Reflectance  $L$  and  $a$  measurements, made at transversely cut surfaces of plugs bored from fruit halves, were linear or bilinear with log time and related to the extent of browning in six apple cultivars. With apple and pear juices, tristimulus values changed linearly with time in samples undergoing browning. Differences between initial and final tristimulus values were better indices of browning than the slopes of time curves. The suitability of these procedures for evaluating the effectiveness of browning inhibitors was demonstrated with  $SO_2$  and ascorbic acid treatments.

### INTRODUCTION

BROWNING of raw fruits and vegetables due to mechanical injury during postharvest handling and processing is an important cause of quality and value loss in affected commodities. This reaction results from the polyphenol oxidase (PPO)-catalyzed oxidation of phenolic compounds to *o*-quinones which subsequently polymerize to form dark-colored pigments (Joslyn and Ponting, 1951; Mayer and Harel, 1979; Vamos-Vigyazo, 1981). Enzymatic browning in unblanched, cut fruits and vegetables may be controlled by the application of sulfur dioxide (Joslyn and Braverman, 1954), ascorbic acid (Bauernfeind and Pinkert, 1970), and various other antioxidants, chelating agents, salts, enzymes and enzyme inhibitors (Vamos-Vigyazo, 1981). In recent years, however, concern over adverse health effects from sulfite, the most effective browning inhibitor, has stimulated a search for alternative antibrowning compounds (Taylor and Bush, 1983; Labell, 1983; Andres, 1985).

To evaluate the effectiveness of experimental treatments in controlling enzymatic browning and to compare them with conventional treatments, accurate measurements of the extent of browning are required. Spectrophotometric procedures, usually entailing absorbance measurements at 420 nm, have been used to measure brown pigments in clarified juices (Toribio et al., 1984) and in vegetable extracts (Hendel et al., 1955). However, such procedures are slow relative to the rate of enzymatic browning in macerated fruits and vegetables and are not applicable to the evaluation of browning at cut surfaces.

Tristimulus reflectance colorimetry (usually the measurement of  $R_d$  or Hunter  $L$  values) has been used to follow the extent of enzymatic browning in juices (Smith and Cline, 1984) and apple slices (Ponting et al., 1972). Published information on sample preparation and presentation for colorimetry, the sensitivity of different tristimulus color scales in responding to enzymatic browning and the kinetics of such changes is limited. While reflectance methods are rapid and nondestructive, preliminary studies have shown them to be limited in accuracy and precision with heterogeneous samples or samples that were subject to physical changes during the time of measurement (Sapers, 1985). The objective of this study was to develop

accurate and precise, nondestructive tristimulus reflectance procedures, that could be applied to the cut surfaces or juice of raw fruits, yielding data that could be correlated with visual and spectrophotometric assessments of browning and used to determine the effectiveness of treatments to control enzymatic browning.

### MATERIALS & METHODS

#### Browning at cut surfaces

Apple and pear samples representing common cultivars were obtained from local food stores during the fall and winter of 1985-86 and stored briefly at 4°C until needed. One hour prior to use, fruits were removed from the refrigerator and equilibrated to room temperature (ca 20°C). Each apple or pear was cut in half along the stem axis, and the halves were positioned in a Petri dish, cut side down, under an electric cork borer (Sargent-Welch, Skokie, IL) so that uniform plugs could be bored perpendicular to the cut surface, on either side of the point of greatest thickness, with a 22 mm stainless steel cutting tube. At the start of an experiment, a transverse cut was made in the plug, at least 1 cm from the skin end (to exclude the effects of bruising), exposing fresh surface. An arrow was cut at the opposite end of the plug to mark its orientation during reflectance measurements.

Colorimetry was performed with a Gardner XL-23 Tristimulus Colorimeter (Pacific Scientific, Silver Spring, MD), operated with large diameter illumination and with a 19 mm opening aperture plate. The instrument was standardized against a white tile ( $Y = 84.60$ ,  $X = 82.21$ ,  $Z = 97.64$ ) before each measurement. The transversely cut surface of a plug was centered over the aperture, oriented so that the arrow cut in the opposite end pointed away from the colorimeter operator. Values of the tristimulus coordinates in the  $L$ ,  $a$ ,  $b$  and  $Y$ ,  $X$ ,  $Z$  systems were recorded at 1, 10, 30, 60, 90, 120, 150, 180, 240, 300, 360, and 420 min. Between measurements, plugs were held in covered glass crystallizing dishes to minimize dehydration at the cut surface. The tristimulus coordinates were plotted against log time, and the slopes of linear portions of these curves were obtained by linear regression.

#### Browning in juice

Juice samples were prepared from individual apples or pears with an Acme Supreme Model 6001 Juicerator (Acme Juicer Manufacturing Co., Lemoyne, PA), lined with Whatman No. 1 filter paper. A 25 or 50 mL aliquot of thoroughly mixed juice was pipetted into a cylindrical clear glass optical cell (57.1 mm i.d.) to a depth of about 10 or 20 mm for colorimetry. Care was taken to exclude foam from the cell. Colorimetry was performed as described above but with the optical cell placed in a 50 mm diameter support ring in place of the 19 mm aperture plate. Tristimulus values were recorded at 1, 2, 3, 4, 6, 8, 10, 15, 20, 30, 40, 50, 60, 75 and 90 min and were plotted against time rather than log time.

To permit the direct comparison of reflectance and spectrophotometric data for browning apple juice, 10 mL aliquots of a 100-125 mL juice sample (mixed slowly with a magnetic stirrer) were taken for spectrophotometry at 15 min intervals, when reflectance measurements also were made. The aliquots were clarified by a modification of the method of Meydavi et al. (1977) entailing rapid mixing with an equal volume of 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 the filtrates was determined at 420 nm with a Perkin-Elmer Model 552 UV-visible spectrophotometer (Perkin-Elmer, Oak Brook, IL)

## Evaluation of browning inhibitors

To determine the suitability of the tristimulus reflectance procedure for evaluating browning inhibitors applied to cut surfaces, Red Delicious and Stayman Winesap apple plugs (2 per half, taken on either side of the core axis) were cut in half, yielding 4 pairs of plug halves per fruit, each pair having a common cut surface. Treatments were applied to one plug half from each pair, the other half serving as a control, so that 4 levels of a treatment and corresponding untreated controls could be compared, using only 1 apple. Treatments consisted of 90 sec dips in freshly prepared 0.01%, 0.02%, 0.04% or 0.08% NaHSO<sub>3</sub> solutions or in freshly prepared 1% citric acid monohydrate solutions containing 0.4%, 0.8%, 1.6% or 3.2% ascorbic acid. After dipping, the plugs were drained, blotted dry with absorbent tissue and then held for 7 hr at 20°C during which time tristimulus reflectance measurements were made at intervals.

The suitability of the reflectance procedure for evaluating browning inhibitors in juice was tested with Golden Delicious and Granny Smith apples. The freshly prepared juice from 2 apples was briefly stirred to assure uniformity and then divided into 5 25-mL portions, rapidly dispensed by burette into optical cells containing 1 mL H<sub>2</sub>O (the control), 1 mL 0.406% sodium bisulfite (100 ppm SO<sub>2</sub> in the juice), 0.75 mL H<sub>2</sub>O + 0.25 mL 0.1% ascorbic acid (AA) (10 ppm), 0.50 mL H<sub>2</sub>O + 0.50 mL 0.1% AA (20 ppm), and 1.0 mL 0.1% AA (40 ppm). Reflectance L- and a-values for controls and treated juices were measured at intervals during 1½ hr at 20°C. To compensate for sedimentation in the optical cells, the samples were briefly stirred prior to each reading.

## RESULTS & DISCUSSION

### Measurement of browning at cut surfaces

Preliminary observations of the cut surfaces of apple and pear plugs indicated that enzymatic browning occurred gradually over several hours with unblemished tissue. Little or no variation in the degree of browning could be seen along the length of the bore hole remaining in the fruit half after removal of a plug, indicating that the exact location of the transverse cut probably was not critical. Tristimulus reflectance measurements made at the cut surface of fruit plugs yielded values which were linear or occasionally bilinear when plotted against log time and appeared to be related to the extent of browning (Fig. 1). The logarithmic relationships were unexpected and may be a consequence of the gradual depletion of PPO substrates in the free juice adhering to disrupted cell layers at the cut surface. Initial flat regions or deviations from linearity in the reflectance curves may be indicative of the depletion of endogenous browning inhibitors such as ascorbic acid prior to the onset of browning (Ponting and Joslyn, 1948), or of changes

in the albedo of the cut surface due to physical processes such as the evaporation of free juice.

Since treatments to inhibit enzymatic browning might be evaluated by comparing one or several plugs taken from opposite halves of a single fruit (each plug receiving a different treatment level), the similarity of reflectance vs time relationships for multiple plugs taken from individual Red Delicious and Stayman apples was examined (Table 1). Regression slopes for the linear portion of reflectance vs log time curves as well as differences between the final and initial L- or a-values ( $\Delta$  values) for the entire curve generally were similar for different plugs obtained from the same fruit. Since some variation in slopes or  $\Delta$  values was obtained within individual apples (see Red Delicious I B-1, for example), the procedure was modified so that a treated plug could be compared with a control representing the same plug. This was done by using half-plugs, one half receiving the treatment and the same surface on the other half serving as its control. Variation in cut apple surfaces could be related to the location of plugs and transverse cuts relative to the core, with vascular bundles showing up as streaks or spots, depending on the orientation of the surface. Such heterogeneity largely could be eliminated by boring plugs on either side of the core axis at the widest part of the apple.

To determine which of the tristimulus coordinates gave the most accurate indication of browning at cut surfaces, Hunter L, a, b and CIE Y, X, Z values were measured for plugs taken from 4 fruits for each of 6 apple cultivars (Cortland, Granny Smith, Idared, McIntosh, Red Delicious and Stayman) over a 3 hr period. This study was not extended to pear fruits since the cultivars examined in preliminary studies (Anjou, Bartlett, Bosc, Red Bartlett and Seckel) browned too slowly to be useful in evaluating browning inhibitors. Values of Y, X, Z and L for the browning apple surfaces decreased with time, while values of a and b increased. In all cases, a linear relationship was seen between the tristimulus coordinate and the logarithm of time, correlation coefficients for the regression usually exceeding 0.9. However, changes in X, Z and b appeared to be unrelated to the extent of browning. Ponting et al. (1972) reported that total reflectance values ( $R_d = Y$ ) correlated better with browning in apple slices than did a- or b-values (determined with the  $R_d$  coordinate system). Bolin et al. (1964) was able to use the L-value ( $L = 10^{1/2}$  to determine the effectiveness of SO<sub>2</sub> in

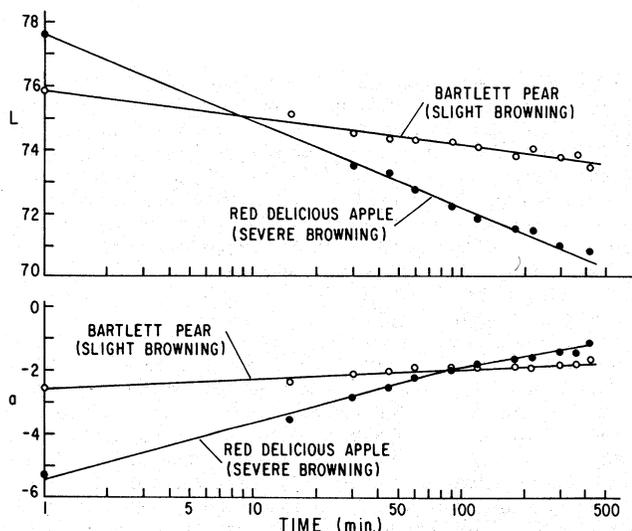


Fig. 1—Reflectance L- and a-values at cut surfaces of apple and pear plugs held at 20°C.

Table 1—Reflectance characteristics of cut surfaces of plugs from opposite sides of Red Delicious and Stayman apples undergoing browning at 20°C

Cultivar	Fruit Side Plug	Slope <sup>a</sup>	L		a	
			Slope <sup>a</sup>	$\Delta L^b$	Slope <sup>a</sup>	$\Delta a^b$
Red Delicious	I	A 1	-2.7	-5.2	1.7	3.2
		A 2	-2.9	-5.6	1.8	3.5
	B	B 1	-2.1	-4.2	1.3	2.6
		B 2	-2.9	-5.9	1.7	3.4
	Mean $\pm$ S.D. <sup>c</sup>		-2.6 $\pm$ 0.4	-5.2 $\pm$ 0.7	1.6 $\pm$ 0.2	3.2 $\pm$ 0.4
	II	A 1	-2.1	-4.3	1.4	2.8
		A 2	-1.5	-2.8	1.0	1.8
	B	B 1	-2.0	-4.0	1.3	2.8
		B 2	-2.4	-4.7	1.6	3.3
	Mean $\pm$ S.D.		-2.0 $\pm$ 0.4	-4.0 $\pm$ 0.8	1.3 $\pm$ 0.2	2.7 $\pm$ 0.6
Stayman	I	A 1	-3.9	-7.7	2.4	4.9
		A 2	-3.2	-6.5	1.9	4.3
	B	B 1	-4.0	-8.1	2.4	5.0
		B 2	-3.6	-7.2	2.2	4.8
	Mean $\pm$ S.D.		-3.7 $\pm$ 0.4	-7.4 $\pm$ 0.7	2.2 $\pm$ 0.2	4.8 $\pm$ 0.3
	II	A 1	-2.8	-5.5	1.6	3.2
		A 2	-3.7	-7.4	2.3	4.5
	B	B 1	-3.3	-6.4	2.0	4.0
		B 2	-3.8	-7.8	2.3	4.6
	Mean $\pm$ S.D.		-3.4 $\pm$ 0.4	-6.8 $\pm$ 1.0	2.0 $\pm$ 0.3	4.1 $\pm$ 0.6

<sup>a</sup> Linear portion of L or a vs log time curve (at least 5 data points); correlation coefficients for regression > 0.98.

<sup>b</sup> Difference between 180 min and 1 min values.

<sup>c</sup> Standard deviation.

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inhibiting browning in apple wedges. Since the most characteristic manifestation of enzymatic browning is sample darkening (changes in hue being commodity-dependent), a negative correlation between browning and the Y- (or L-) value, which is defined as the luminosity or lightness function (Clydesdale, 1978), would be expected.

The absolute values of the reflectance measurements could not be used to compare different samples undergoing browning because of apple-to-apple variability in natural pigmentation. Therefore, the slopes of the reflectance curves (change in L- or a-value per log cycle time) as well as the differences between final and initial values of L or a ( $\Delta L$  or  $\Delta a$ ) were compared, the latter approach being applicable to curves with bilinear or nonlinear regions for which a single slope value would be meaningless (Table 2). The apples compared in this study varied greatly in degree of browning, both between and within cultivars. Such variability probably results from differences in PPO activity, polyphenol content and/or ascorbic acid content (Vámos-Vigyazo, 1981). The extent of apple-to-apple variability within samples would mitigate against the use of more than one apple to carry out comparisons of multilevel treatments to control browning. Among the cultivars compared, Idared and Granny Smith tended to brown less while Stayman browned more; Red Delicious, Cortland and McIntosh were intermediate in browning. Slopes and  $\Delta$  values for L and a both appeared to be related to the extent of browning, changes in L being larger and consequently more sensitive than changes in a. Because of occasional inconsistencies between L and a, perhaps due to changes in sample albedo, both  $\Delta L$  and  $\Delta a$  values should be used to determine the extent of browning in apple plugs. Measurements should be made at times appropriate to the samples and treatments.

### Evaluation of browning inhibitors applied to cut surfaces

To demonstrate the applicability of the reflectance procedure to cut apple surfaces treated with browning inhibitors, un-

treated cut surfaces of Red Delicious and Stayman plug halves, which undergo severe browning, were compared with surfaces of the corresponding plug halves that had been dipped in solutions containing different concentrations of sulfite or ascorbic acid. The extent to which these treatments inhibited browning was expressed on a percent basis, i.e., the percent difference between the control and treatment  $\Delta L$  or  $\Delta a$  values after a specified storage time t:

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

where  $\Delta L$  (or  $\Delta a$ ) is the difference between the L- (or a-value) at time t and the value at 1 min. Positive values of the percent inhibition between 0 and 100 would indicate that the treatment is effective as a browning inhibitor to the extent calculated. Values greater than 100%, if significant, would indicate sample bleaching by the treatment, while negative values would indicate that the treatment promoted rather than inhibited browning. The inhibition data clearly showed that the reflectance procedure could detect the differing degrees of browning inhibition obtained in plugs treated with different levels of sulfite or ascorbic acid and then stored for different periods of time (Table 3). Inhibition data for Red Delicious apples (not shown) were similar to those obtained with Stayman apples.

### Measurement of browning in apple and pear juice

Raw juice might represent a more useful system than the cut surface of plugs for the comparison of multilevel treatments to inhibit browning since it would be homogeneous and more easily manipulated. However, preliminary experiments with a number of apple cultivars (Jonathan, McIntosh, Red Delicious, Rome, Stayman and Winesap) and pear cultivars (Bartlett, Red Bartlett and Seckel) indicated that browning in the freshly pre-

Table 2—Measurement of browning at cut surface of apple plugs held at 20°C by tristimulus reflectance colorimetry

Cultivar	Apple	Extent of browning (180 min) <sup>a</sup>	L - value		a - value	
			Slope (r) <sup>b</sup>	$\Delta L^c$	Slope (r) <sup>b</sup>	$\Delta a^c$
Idared	1	V. sl.	-0.7 (-0.85)	-1.5	0.8 (0.95)	0.8
	4	V. sl.	-2.8 (-0.99)	-3.4	1.4 (0.99)	1.9
	2	Sl.	-2.8 (-0.99)	-3.0	2.8 (0.99)	2.5
	3	Sl.	-3.4 (-0.99)	-3.9	2.0 (0.99)	2.4
	Mean $\pm$ S.D. <sup>d</sup>		-2.4 $\pm$ 1.2	-3.0 $\pm$ 1.0	1.8 $\pm$ 0.8	1.9 $\pm$ 0.8
Granny Smith	1	None	-1.9 (-0.93)	-0.6	1.2 (0.98)	1.1
	3	V. sl.	-1.8 (-0.99)	-2.4	1.1 (0.99)	1.4
	4	Sl.	-2.7 (-0.96)	-2.9	1.9 (0.99)	1.6
	2	Mod.	-3.9 (-0.97)	-5.9	2.7 (0.99)	4.0
	Mean $\pm$ S.D.		-2.6 $\pm$ 1.0	-3.0 $\pm$ 2.2	1.7 $\pm$ 0.7	2.0 $\pm$ 1.3
Red Delicious	2	Mod.	-2.0 (-0.98)	-4.3	1.4 (0.98)	2.8
	4	Mod.	-2.1 (-0.98)	-4.9	1.8 (0.99)	3.4
	1	Sev.	-2.6 (-0.99)	-6.0	1.5 (0.99)	3.6
	3	Sev.	-3.2 (-0.99)	-5.9	2.0 (0.99)	3.8
	Mean $\pm$ S.D.		-2.5 $\pm$ 0.6	-5.3 $\pm$ 0.8	1.7 $\pm$ 0.3	3.4 $\pm$ 0.4
Cortland	2	Sl.	-3.7 (-0.99)	-5.6	1.0 (0.99)	1.4
	3	Mod.	-5.1 (-0.99)	-7.7	2.1 (0.99)	3.0
	1	Sev.	-4.2 (-0.97)	-8.8	1.3 (0.99)	2.8
	4	Sev.	-6.2 (-0.99)	-9.1	2.7 (0.99)	4.3
	Mean $\pm$ S.D.		-4.8 $\pm$ 1.1	-7.8 $\pm$ 1.6	1.8 $\pm$ 0.8	2.9 $\pm$ 1.2
McIntosh	2	Mod.	-3.2 (-0.97)	-6.4	2.2 (0.99)	3.4
	3	Mod.-Sev.	-3.2 (-0.99)	-7.0	2.6 (0.99)	3.7
	1	Sev.	-4.6 (-0.99)	-6.4	1.9 (0.99)	4.1
	4	Sev.	-4.5 (-0.99)	-7.5	2.1 (0.99)	3.9
	Mean $\pm$ S.D.		-3.9 $\pm$ 0.8	-6.8 $\pm$ 0.5	2.2 $\pm$ 0.3	3.8 $\pm$ 0.3
Stayman	3	Mod.-Sev.	-2.5 (-0.99)	-5.2	2.2 (0.99)	4.0
	1	Sev.	-4.4 (-0.99)	-8.1	2.8 (0.99)	5.9
	2	Sev.	-4.6 (-0.99)	-8.0	3.1 (0.99)	5.6
	4	Sev.	-5.1 (-0.99)	-9.6	3.4 (0.99)	6.1
	Mean $\pm$ S.D.		-4.2 $\pm$ 1.1	-7.7 $\pm$ 1.8	2.9 $\pm$ 0.5	5.4 $\pm$ 1.0

<sup>a</sup> V. sl. = very slight; Sl. = slight; Mod. = moderate; Sev. = severe.

<sup>b</sup> Slope and correlation coefficient for linear portion of L or a vs log time curve (at least 5 data points).

<sup>c</sup> Difference between 180 min and 1 min values.

<sup>d</sup> Standard deviation.

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Table 4—Correlation between reflectance and spectrophotometric measurements of browning in apple juice

Cultivar	Extent of browning at 30 min					L vs A <sub>420</sub> <sup>c</sup>			a vs A <sub>420</sub> <sup>c</sup>		
	Trial	Visual <sup>a</sup>	× L <sup>b</sup>	× a <sup>b</sup>	A <sub>420</sub>	Slope	Intercept	Correlation coeff.	Slope	Intercept	Correlation coeff.
Cortland	1	Mod	-6.8	2.6	0.168	-37.9	34.8	-0.96	18.5	0.5	0.99
	2	Mod-sev	-7.2	1.1	0.252	-26.2	33.2	-0.97	8.2	4.0	0.94
	3	Mod-sev	-6.9	4.9	0.238	-36.0	36.4	-0.97	15.4	1.7	0.95
	4	Mod-sev	-6.9	3.1	0.287	-21.2	34.1	-0.99	14.1	1.8	0.99
	C.V. <sup>d</sup>			2.5	53.6	21.1	26.2	4.0	-	30.7	72.1
Granny Smith	1	Sl	-3.7	1.9	0.109	-33.2	28.2	-0.98	23.4	-3.4	0.99
	2	None	-1.0	0.2	0.028	-33.9	24.8	-0.96	16.1	-1.6	0.97
	3	Mod-sev	-8.2	7.8	0.197	-33.8	33.2	-0.94	18.2	-1.7	0.93
	4	Sl	-0.3	0.3	0.036	-39.9	26.9	-0.99	20.0	-2.7	0.99
	C.V.			108.5	140.6	85.0	8.9	12.7	-	15.9	37.8
Idared	1	Mod	-6.3	5.3	0.175	-44.3	37.1	-0.98	34.8	2.9	0.97
	2	Mod-sev	-3.6	-1.2	0.184	-28.9	32.4	-0.99	3.4	5.2	0.99
	3	Mod	-4.3	2.7	0.168	-29.1	29.8	-0.99	17.7	0.1	0.98
	4	Mod	-3.4	-0.3	0.151	-28.0	32.5	-0.98	2.3	7.5	0.96
	C.V. <sup>d</sup>			30.1	182.4	8.2	24.0	9.2	-	104.6	189.0

<sup>a</sup> Sl = slight, Mod = moderate, Sev = severe.

<sup>b</sup> Difference between 30 min and 1 min values of L or a.

<sup>c</sup> Slopes, intercepts and correlation coefficients determined by linear regression based on 5 data points per trial.

<sup>d</sup> Coefficient of variation (%).

Table 5—Use of tristimulus colorimetry to evaluate the effectiveness of browning inhibitors in raw apple juice held at 20°C

Cultivar	Treatment <sup>a</sup>	% Inhibition <sup>b</sup>					
		Calculated from ΔL			Calculated from Δa		
		30 min	60 min	90 min	30 min	60 min	90 min
Golden Delicious	10 ppm AA	6	5	6	14	10	8
	20 ppm AA	23	18	15	19	15	11
	40 ppm AA	36	23	20	35	23	19
	100 ppm SO <sub>2</sub>	90	100	104	105	105	105
Granny Smith	10 ppm AA	63	38	35	48	26	30
	20 ppm AA	85	57	43	76	57	44
	40 ppm AA	96	73	50	76	60	45
	100 ppm SO <sub>2</sub>	67	92	96	70	90	91

<sup>a</sup> AA = ascorbic acid.

<sup>b</sup> (Δ control - Δ treatment) × 100 ÷ Δ control; Δ values are difference between 30, 60 or 90 min values and initial values (1.5 min for Golden Delicious and 5 min for Granny Smith).

(which took longer to give stable reflectance values) rather than at 1 min, as with the cut surface procedure. No browning was observed in these samples when the initial tristimulus measurements were made. Under the conditions of this experiment, both Granny Smith and Golden Delicious juices underwent severe browning over the course of 30-60 min at 20°C. Color changes were paralleled by decreasing L-values and increasing a-values. Browning in juices of both varieties was almost completely inhibited by the addition of 100 ppm SO<sub>2</sub>, resulting in little or no change in L or a. Ascorbic acid at concentrations of 10-40 ppm was less effective in inhibiting browning with Golden Delicious juice than with Granny Smith juice, the percent inhibition increasing with increasing ascorbic acid concentration and decreasing with time.

It is important when using the juice system to employ cultivars that undergo sufficient browning to reveal differences between treatment levels but not so much that all treatments are ineffective. Among the cultivars compared in this study, Granny Smith and Golden Delicious not only meet these criteria but are also widely available for most of the year. Experiments designed to evaluate browning inhibitors should include both an untreated control and a sample treated with sufficient SO<sub>2</sub> to completely inhibit browning. Inclusion of the former will provide a basis for determining the extent to which an experimental treatment inhibits browning, i.e., the percent inhibition. Inclusion of the latter will permit the correction of sample reflectance values for changes unrelated to browning, i.e., the dissipation of air bubbles and development of turbidity during the first few minutes after juice preparation.

## CONCLUSIONS

ENZYMATIC BROWNING at cut surfaces of plugs from apple and pear fruits can be monitored by measuring changes in reflectance L and a values. This technique may be used with fruits that are subject to severe browning such as Stayman or Red Delicious apples to evaluate the effectiveness of new browning inhibitors. Because of fruit-to-fruit variability in the extent of enzymatic browning, multilevel treatments with browning inhibitors should be compared using several plugs from the same fruit, half of each plug serving as a control for the treatment applied to the other half. Browning in raw apple juice can be monitored by measuring reflectance L values. If the juice system is used to evaluate the effectiveness of browning inhibitors, a fruit that browns slowly such as Granny Smith apple should be employed.

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Table 3—Evaluation of treatments to inhibit browning of cut surfaces of Stayman apple plugs by reflectance tristimulus colorimetry

Browning inhibitor	Treatment level (%)	Percent inhibition <sup>c</sup>					
		Calculated from $\Delta L$			Calculated from $\Delta a$		
		2 hr	4 hr	6 hr	2 hr	4 hr	6 hr
NaHSO <sub>3</sub> <sup>a</sup>	0.01	75	69	69	69	54	48
	0.02	97	94	92	91	84	81
	0.04	96	96	80	97	89	74
	0.08	101	99	94	101	95	89
Ascorbic acid <sup>a,b</sup>	0.4	52	24	12	46	15	9
	0.8	102	91	79	100	87	75
	1.6	104	102	100	100	97	93
	3.2	102	102	101	103	100	97

<sup>a</sup> 90 sec dip.

<sup>b</sup> All solutions contain 1% citric acid.

<sup>c</sup>  $(\Delta \text{control} - \Delta \text{treatment}) \times 100 \div \Delta \text{control}$ ;  $\Delta$  values are differences between L or a values at indicated storage time and values at 1 min.

pared juice occurred too rapidly to permit sample treatment and evaluation. Juices prepared from Cortland, Golden Delicious, Granny Smith and Idared apples or from Anjou and Bosc pears browned more gradually.

Reflectance measurements, made with these slower browning cultivars, indicated an inverse relationship between browning and Z- and L- (or Y) values; a-values increased in the browning juices (Fig. 2). Plots of the tristimulus coordinates vs time were linear or bilinear over 1 hr with some nonlinearity during the first 5 or 10 min, possibly due in part to the development of turbidity or dissipation of air bubbles. Reflectance measurements usually were not extended beyond 1-2 hr because of interference due to sedimentation.

The occurrence of initial nonlinearity or bilinearity precluded the use of slopes of tristimulus coordinate vs time curves as indices of browning. No clear advantage could be seen in choosing one tristimulus coordinate over another to monitor juices undergoing browning. Johnson et al. (1976) have noted that the Z coordinate will respond to browning since brown pigments absorb at 400 nm, near the maximum for the z-function of the CIE standard observer. While the Z-value for pear juice did decrease in browning samples, it also decreased by a similar amount in some freshly prepared juices that did not yet develop brown color. Apparently, Z responds to physical changes such as the development of turbidity or the dissipation of air bubbles as well as to browning.

To establish the validity of the juice system without recourse to visual observations of browning, which are limited in accuracy and dependent on fruit-to-fruit variability in browning rates, reflectance L and a data for browning apple juice samples were compared with spectrophotometric measurements, i.e., the absorbance of clarified juice at 420 nm ( $A_{420}$ ) (Table 4). The  $A_{420}$  values increased linearly with time (sometimes after an initial lag) while the reflectance a-values also increased and the L-values decreased. The  $A_{420}$  and reflectance data were highly correlated. Slopes and intercepts were considerably more variable for the a vs  $A_{420}$  relationship than for the L vs  $A_{420}$  relationship, indicating that the a-value was influenced by some characteristic of juice besides the extent of browning. Consequently, the use of L (or Y) rather than the a-value to monitor browning in the juice system is recommended. In a recent study of browning in apple juice, Smith and Cline (1984) obtained a good correlation between the Hunter L-value and visual rankings of the samples. Correlations between visual rankings and values of a, b, a/L, a/b and  $\cot^{-1} a/b$  were not significant.

It is apparent from the data in Table 4 that juices from individual apples of the same cultivar varied greatly in the extent of browning. Therefore, comparisons of multilevel treatments to inhibit browning should be carried out with the juice from one fruit (or the pooled juice from several fruits), apportioned among the treatments and control.

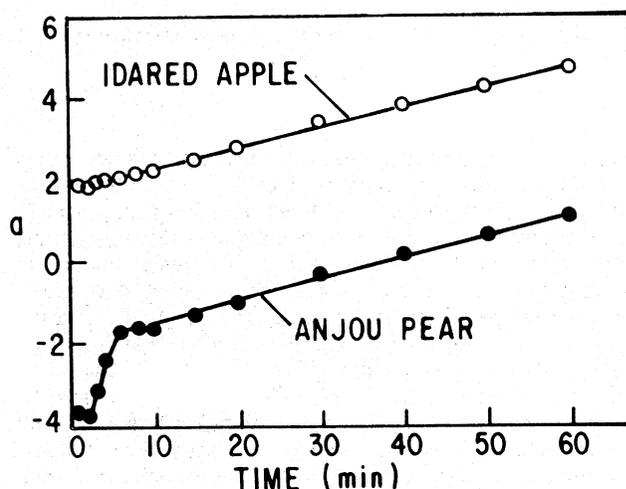
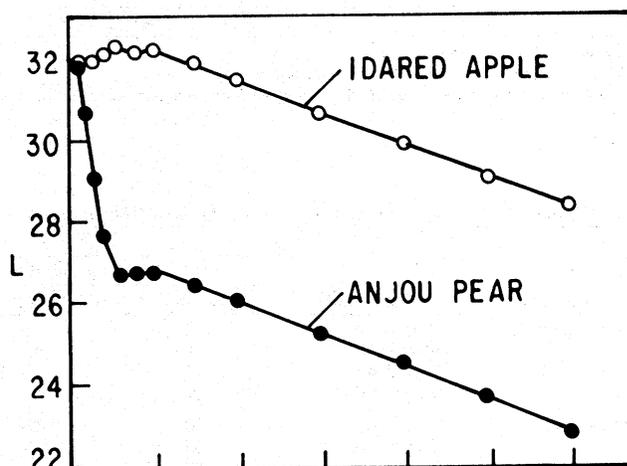
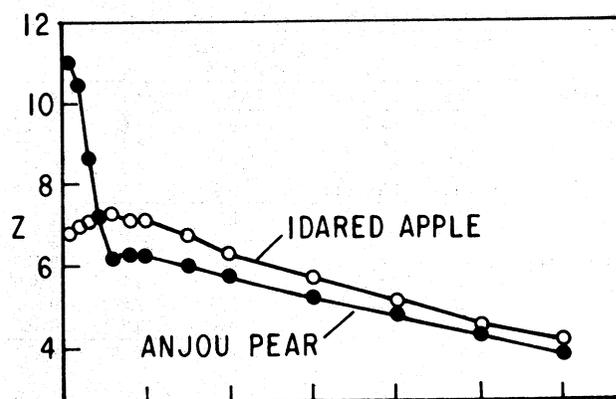


Fig. 2—Reflectance Z-, L- and a-values for apple and pear juice held at 20°C.

#### Evaluation of browning inhibitors in the juice system

Comparisons of tristimulus reflectance data for untreated apple juice samples with the same juice containing SO<sub>2</sub> or ascorbic acid illustrate the use of the juice system to evaluate browning inhibitors (Table 5). These results were expressed as percent inhibition values, calculated in the same way as for cut surface browning. However, because more time was required to prepare and stabilize samples in the juice system, the initial L- and a-values used as the basis for  $\Delta$  values were read at 1.5 min for Golden Delicious and at 5 min for Granny Smith