Inhibition of Browning by Sulfur Amino Acids. 2. Fruit Juices and Protein-Containing Foods

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Enzymatic and nonenzymatic browning reactions may adversely affect the quality, nutritional value, and safety of foods. A need therefore exists to develop methods to control such reactions in a variety of foods. Reflectance measurements were used to compare the relative effectiveness of a series of compounds in inhibiting browning in freshly prepared and commercial fruit juices including apple, grape, grapefruit, orange, and pineapple juices. The potential inhibitors tested include ascorbic acid, a commercial formulation called Sporix, sodium sulfite, *N*-acetyl-L-cysteine, L-cysteine, and reduced glutathione. For comparison, related studies were also carried out with several protein-containing foods such as casein, barley flour, soy flour, nonfat dry milk, and the commercial infant formula Isomil. The results revealed that under certain conditions SH-containing *N*-acetyl-L-cysteine and the tripeptide reduced glutathione may be as effective as sodium sulfite in preventing both enzymatic and nonenzymatic browning. The unique electronic and nucleophilic properties of sulfhydryl compounds that enable them to act as inhibitors of both enzymatic and nonenzymatic browning are discussed. These sulfur amino acids merit further study to assess their potential for preventing long-term food browning under practical storage and processing conditions.

INTRODUCTION

The following information, summarized from recent reviews (Friedman et al., 1990; Handwerk and Coleman, 1988; King and Bolin, 1989), offers a brief overview of our current knowledge of browning in citrus products. (a) Browning of citrus products has been a major problem throughout the history of the citrus processing industry. (b) Browning induces deterioration in color, flavor, and taste during processing of citrus fruit juices and concentrates and during long-term storage of dehydrated products. (c) The food ingredients responsible for browning include (1) free amino acids; (2) carbohydrates such as glucose, fructose, and sucrose; (3) vitamin C; and (4) polyhydroxybenzenes. (d) Characterized browning products include (1) furfuraldehyde derivatives; (2) pyrrole derivatives; (3) brown polymers; (4) aldehydes and ketones; and (5) cyclopentenones. (e) Maillard-type nonenzymatic browning plays a prominent and diverse role in that it produces beneficial flavors as well as objectionable color and taste changes. (f) Vitamin C browning is a special case, leading to the destruction and loss of this important vitamin and to the production of furfurals as a result of degradative transformations. Vitamin C and its degradation products also participate with amino acids in Maillard browning.

Better and more practical means need to be developed to reduce deleterious changes during browning of citrus products. Nonenzymatic browning has generally been recognized to occur in all fruit juices tested, including apple (Toribio and Lozano, 1984), grapefruit (Lee and Nagy, 1988), lemon (Robertson and Samanego, 1986), and pear (Bevridge and Harrison, 1984). Such browning damages the appearance, quality, and occasionally the safety of the juices (Stich et al., 1981), although we were unable to confirm the reported increase in the formation of heat-induced mutagens in fruit juices (Ekasari et al., 1986, 1989; Friedman et al., 1990).

Research to improve both the quality and the safety of the juices is needed both to assess the risk of consuming such browning products and to devise improved processing conditions to minimize or prevent nonenzymatic browning.

Sodium sulfite has been widely used to minimize both enzymatic and nonenzymatic (Maillard type) browning in a variety of foods. However, because of a number of reports that some humans, especially asthmatics, may be sensitive to sulfite (Brown, 1985; Fan and Book, 1987; FDA, 1986, 1987), alternatives for browning prevention are needed. A number of attempts to develop such alternatives appear to have been only partly successful (Andres, 1985; Sapers and Douglas, 1987; Sapers and Ziolkowski, 1987).

In related studies (Friedman and Molnar-Perl, 1990; Molnar-Perl and Friedman, 1990) we found that certain SH-containing sulfur amino acids are good inhibitors of browning in heated amino acid–glucose systems and at cut surfaces of apples and potatoes. This paper extends these studies to fruit juices and to protein-containing foods. Our main objective was to compare the effectiveness of the browning inhibitors ascorbic acid, Sporix, and sodium sulfite to that of three SH-containing compounds, L-cysteine, *N*-acetyl-L-cysteine, and reduced glutathione. Experimentally, we used tristimulus reflectance colorimetry to measure the effectiveness and extent of browning and browning prevention.

MATERIALS AND METHODS

Materials. Sodium bisulfite was obtained from Mallinckrodt, St. Louis, MO; L-ascorbic acid from Eastman, Rochester, NY; Sporix (phosphoric acid derivatives) from International

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Table I. Prevention of Browning in Fresh Apple Juice with Various Inhibitors

inhibitor	treatment time	inhibition, %, with inhibitor at (mM) 0.62	1.55	3.10	4.65	6.20	12.40
ascorbic acid	1–2 min	24	82	94	100	96	105
	1 h	0	57	77	92	94	105
	2 h	0	26	38	44	71	98
	6 h	−2	20	13	23	40	69
	24 h	−8	20	8	21	24	32

inhibitor	treatment time	inhibition, %, with inhibitor at (10^{-3} %) 4.55	11.36	22.72	34.13	45.50	91.00
Sporix	1–2 min	26	29	19	35	21	24
	1 h	11	9	22	34	14	13
	2 h	10	8	19	25	13	11
	6 h	11	6	12	22	14	13
	24 h	9	9	−1	1	1	1

inhibitor	treatment time	inhibition, %, with inhibitor at (mM) 0.227	0.568	1.136	1.704	2.27	4.54
sodium bisulfite	1–2 min	66	63	106	106	92	103
	1 h	30	38	103	107	94	96
	2 h	21	25	105	106	96	95
	6 h	20	16	101	109	104	106
	24 h	28	6	104	102	105	93

inhibitor	treatment time	inhibition, %, with inhibitor at (mM) 0.568	1.136	1.704	2.27	4.54	9.08
L-cysteine	1–2 min	103	101	101	78	99	114
	1 h	96	96	98	88	103	108
	2 h	100	99	97	95	107	119
	6 h	94	101	97	104	106	121
	24 h	83	89	86	97	97	106
glutathione (reduced)	1–2 min	103	98	98	107	96	99
	1 h	94	108	93	114	89	91
	2 h	94	109	96	110	92	102
	6 h	94	116	99	114	95	103
	24 h	74	102	96	110	99	93
N-acetyl-L-cysteine	1–2 min	92	101	100	96	114	97
	1 h	87	98	92	99	112	102
	2 h	83	102	95	98	108	108
	6 h	65	97	100	90	107	99
	24 h	43	100	93	96	107	103

Sourcing, Chicago, IL; and L-cysteine, free base, from U.S. Biochemical Corp., Cleveland, OH. *N*-Acetyl-L-cysteine and reduced glutathione were obtained from Sigma, St. Louis, MO. Fruit juices and other materials were purchased in local stores. Fresh juices were prepared from Washington Golden Delicious apples and d'Anjou pears.

Instruments. The extent of browning was measured by recording the reflectance or *L* values with a Minolta Chroma Meter, Type CR 100, as described by Sapers and colleagues (Sapers and Douglas, 1987; Sapers and Ziolkowski, 1987). The values of the tristimulus coordinates *L*, *a*, and *b* were recorded and used to calculate the extent of browning in the absence and presence of inhibitors. A Radiometer pHM 26 meter and a Beckman 39030 thin-probe combination electrode were used to record pH values.

Procedures. (*1*) *Fresh Juices.* Fruits were peeled, ground with a hand-operated fruit grinder, and pressed through three layers of gauze. Samples (2 mL) of the fresh juice were then immediately pipetted into matched vials with screw caps (Kimble No. 62910-1) which contained previously prepared solutions of different amounts of the inhibitors dissolved in 3 mL of phosphate buffer, pH 7.2. The final volume of all solutions was uniformly 5 mL. Tristimulus values were recorded 1–2 min and 1, 2, 6, and 24 h after pressing.

(*2*) *Commercial Juices.* The following commercial juices were evaluated: grape juice (Welch's, Concord, MA), apple cider (S. Martinelli, Watsonville, CA), apple juice (Tree Top, Selah, WA), pineapple (Castle & Cooke, San Francisco, CA), grapefruit (Ocean Spray Cranberries, Plymouth, MA), and orange (L.K.S. Products, Dublin, CA).

Table II. Prevention of Browning in Fresh Pear Juice with Various Inhibitors

inhibitor	treatment time	inhibition, %, with inhibitor at (mM) 0.62	1.55	3.10	4.65	6.20	12.40
ascorbic acid	1–2 min	78	91	82	79	84	73
	1 h	24	60	78	81	87	81
	2 h	5	36	32	54	81	80
	4 h	0	35	49	32	38	61
	24 h	40	40	28	24	29	48

inhibitor	treatment time	inhibition, %, with inhibitor at (10^{-3} %) 4.55	11.36	22.72	34.13	45.50	91.00
Sporix	1–2 min	50	45	71	54	68	65
	1 h	37	23	59	31	43	52
	2 h	10	9	36	15	25	20
	4 h	10	10	36	8	24	18
	24 h	17	19	46	24	32	45

inhibitor	treatment time	inhibition, %, with inhibitor at (mM) 0.227	0.568	1.136	1.704	2.27	4.54
sodium bisulfite	1–2 min	62	66	74	104	100	99
	1 h	35	55	71	106	101	97
	2 h	15	55	76	105	98	100
	4 h	7	73	84	115	100	105
	24 h	45	58	86	120	106	116

inhibitor	treatment time	inhibition, %, with inhibitor at (mM) 0.568	1.136	1.704	2.27	4.54	9.08
L-cysteine	1–2 min	107	100	100	93	99	99
	1 h	96	102	101	90	106	112
	2 h	84	98	103	95	106	113
	4 h	68	94	97	94	103	107
	24 h	56	70	82	99	117	116
glutathione reduced	1–2 min	117	101	106	98	96	97
	1 h	119	101	106	98	97	96
	2 h	122	115	112	105	100	101
	4 h	124	106	113	104	102	102
	24 h	124	123	122	110	111	106
N-acetyl-L-cysteine	1–2 min	103	105	106	106	107	111
	1 h	48	105	106	103	104	111
	2 h	28	103	105	102	107	111
	4 h	22	105	108	103	110	115
	24 h	39	106	117	110	112	117

Since the inhibition was erratic at lower pH values, all juices were adjusted to neutral pH before the addition of inhibitors. To 2 mL of a particular fruit juice previously neutralized to pH 7 were added 1 mL of phosphate buffer, pH 7.2, containing inhibitors and distilled water to a final volume of 5 mL. Next, the vials were placed into a boiling water bath and kept at 100 °C for 120 min. The vial rack was then placed into a cold water bath, and the *L* values were then measured directly in the capped vials at the same intervals as for fresh juices.

(*3*) *Protein-Containing Foods.* The following protein-containing materials were used: casein (International Casein Corp., New York), barley flour (Co-Op Health Food, Berkeley, CA), soy flour (Soya Fluff 200 W, Central Soya, Ft. Wayne, IN), nonfat dry milk (Carnation Co., Los Angeles), and Isomil infant formula powder (Ross Laboratories, Columbus, OH).

In screw-cap vials, we prepared 5-mL solutions or suspensions, pH 7, containing different amounts of inhibitors. We then added one of the following to each vial with continuous stirring by a magnetic stirrer: 0.1 g of casein, barley flour, nonfat dry milk, or Isomil powder. The vials were then heated for 120 min at 100 °C and analyzed for the extent of browning as described above under Fruit Juices.

Blank control experiments were performed with all materials. The standard error in preliminary experiments was about ±5%.

RESULTS AND DISCUSSION

Prevention of Browning. The following equation was used to calculate the percent inhibition of browning under

Table III. Prevention of Browning in Commercial Fruit Juices by Sodium Bisulfite and *N*-Acetyl-L-cysteine

	inhibition, %										
	sodium bisulfite, mM					*N*-acetyl-L-cysteine, mM					
juice	1.0	2.0	4.0	8.0	16.0	2.5	10	25	50	100	200
grape	10	25	69	72	100	6	18	35	79	100	99
apple (cider)	42	50	59	93	100	1	36	52	87	100	89
apple (juice)	10	40	49	100	100	5	40	55	92	93	100
pineapple	11	39	68	102	105	10	35	54	76	100	102
grapefruit	18	25	39	63	100	32	61	75	87	93	96
orange	13	49	69	92	108	3	69	66	72	107	100

Table IV. Prevention of Browning in Protein-Containing Foods by Sodium Bisulfite and *N*-Acetyl-L-cysteine

	inhibition, %									
	sodium bisulfite, mM					*N*-acetyl-L-cysteine, mM				
protein source	2.5	25	50	100	200	25	62.5	125	250	500
casein	4	12	44	82	100	0	25	42	101	101
barley flour	3	43	61	98	95	36	42	79	96	104
soy flour	10	27	80	98	102	19	38	84	99	101
nonfat dry milk	3	23	44	94	104	19	43	78	98	101
Isomil	7	29	72	88	100	7	43	65	93	109

a variety of conditions

$$\% \text{ inhibition of browning} = \Delta L \text{ control sample} - \Delta L \text{ treated sample} \times 100/\Delta L \text{ control sample}$$

where ΔL is the difference between the measured L value at time t and the corresponding value at zero time. Zero time was defined as (a) about 1–2 min after the pressing of the fresh juices, held at room temperature, (b) after purchase of commercial juices in a local store, and (c) after preparation of the protein foods at room temperature.

The concentrations of inhibitors listed in Tables I–IV are, with one exception, in millimoles/liter. A percent value (grams/100 mL) was used for the commercial preparation Sporix because it contains phosphoric acid derivatives of unknown molecular weight. The extent of inhibition listed in the tables exceeds 100% in some cases. This is a well-recognized phenomenon in such studies (Sapers and Douglas, 1987) and may result from a combination of standard errors associated with reactions of the browning precursors with each other and with added inhibitors. The reflectance measurements of both freshly prepared and commercial fruit juices showed an inverse relationship between browning and the L values. The following initial control L values were obtained for the various juices tested: fresh apple, 6.55; fresh pear, 6.05; commercial apple cider, 3.9; commercial apple juice, 2.5; pineapple, 1.2; grapefruit, 0.7; orange, 2.11.

Measuring browning was more difficult with the protein-containing foods than with fruit juices. This is due to two factors: (a) inhomogeneity of the solution or suspensions and (b) changes in composition and conformation during the heating used to elicit browning. Since browning is reflected in decreasing L values, whereas heat denaturation of proteins is reflected by increasing L values, the two events appear competitive. The following net ΔL values were obtained with these samples after 1–2 min (zero time) and at 90 min (time t): casein, 7.09; barley flour, 6.40; soy flour, 5.30; nonfat dry milk powder, 18.8; and Isomil infant formula, 9.50.

Our findings on the effect of several parameters on browning inhibition presented in Tables I–IV can be summarized as follows.

(1) At 100 °C, maximum browning was obtained after 90 min. After that, browning leveled off. This was also true for the protein-containing samples.

(2) Ascorbic acid and Sporix in the concentrations tested did not effectively lessen browning in the freshly prepared juices. The effectiveness of ascorbic acid as a browning inhibitor for the freshly prepared juices lasted only for about 2 h (Table I). Ascorbic acid did not completely prevent browning at any of the concentrations and times listed in Table II.

(3) For the freshly pressed juice, *N*-acetyl-L-cysteine was of the same order of effectiveness as sodium bisulfite; reduced glutathione may be a better inhibitor than bisulfite with respect to duration and minimum concentration needed for complete inhibition (Tables I and II).

(4) Although L-cysteine appears to be an effective inhibitor at 1.7 mM concentrations, such high concentrations may produce undesirable odors. *N*-Acetyl-L-cysteine and reduced glutathione thus appear superior to cysteine both in relative effectiveness at the same molar concentrations in and odor formation.

(5) The extent of browning and of protection by the various inhibitors was less for the commercial juices than for the fresh ones. Inhibitors may be added to some commercial juices during production, or more likely, polyphenol oxidase enzymes that initiate enzymatic browning have been partly or completely inactivated by heat.

(6) Tasting by a few people (results not shown) suggests that, unlike cysteine, *N*-acetylcysteine and reduced glutathione produced minimal or no off-flavors at concentrations that inhibited browning. More such tests are needed to confirm this effect.

On the basis of the cited observations, we investigated more extensively the effectiveness of *N*-acetyl-L-cysteine and sodium bisulfite to inhibit browning of both commercial juices and protein-containing foods. Table III shows that for commercial juices about 5 times the molar concentration of *N*-acetyl-L-cysteine is needed to equal the inhibition produced by sodium bisulfite. This may be due to the overlapping action of inhibitors added during the preparation of the commercial juices.

For the protein-containing foods, *N*-acetyl-L-cysteine proved to be an excellent inhibitor of browning, at least as effective as sodium bisulfite (Table IV). In this connection it is worth noting that the pK value of the SH group of *N*-acetylcysteine (9.5) is much higher than the corresponding value for cysteine (8.2) (Friedman, 1973). This difference implies that the acetylated derivative may be more stable to oxidation and generally more reactive than cysteine. The greater nucleophilic reactivity may explain its greater effectiveness in some cases as an inhibitor of browning. In addition, our studies have

revealed that *N*-acetyl-L-cysteine is fully utilized by mice as a nutritional source of cysteine (Friedman and Gumbmann, 1984), without any apparent adverse manifestations.

Our results show that *N*-acetyl-L-cysteine and reduced glutathione are excellent inhibitors of browning reactions in freshly prepared fruit juices, commercial fruit juices, and protein-containing foods.

In conclusion, the described usefulness of the SH-containing amino acids in minimizing browning will vary depending on juice varieties, maturity of the fruits used to make the juices, storage times, the content of polyphenol oxidases, free amino acids, and reducing sugars. Thus, optimum conditions to inhibit browning may need to be defined for each fruit juice of interest.

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Registry No. Sporix, 10361-03-2; L-cysteine, 52-90-4; *N*-acetyl-L-cysteine, 616-91-1; glutathione, 70-18-8; ascorbic acid, 50-81-7; sodium bisulfite, 7631-90-5.