Inhibition of Browning by Sulfur Amino Acids. 3. Apples and Potatoes

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Sodium sulfite, widely used to inhibit enzymatic and nonenzymatic browning in fruits and vegetables, has been reported to be an irritant to some consumers. In an effort to develop sulfite alternatives, Russet Burbank potatoes, Washington Golden Delicious apples, and Washington Red Delicious apples were subjected to enzymatic browning in air and evacuated plastic pouches in the absence and presence of the following potential browning inhibitors: L-cysteine, *N*-acetyl-L-cysteine, reduced glutathione, sodium bisulfite, sodium sulfhydrate, and sodium hydrosulfite. Studies on the effects of concentration of inhibitors, storage conditions, and pH revealed that *N*-acetyl-L-cysteine and reduced glutathione were nearly as effective as sodium sulfite in preventing browning of both apples and potatoes. In contrast, a previously proposed mixed solution of salicylic and ascorbic acids and potassium sorbate was effective only for short periods. *N*-Acetyl-L-cysteine and reduced glutathione are promising alternatives to sulfite in preventing browning in fruits and vegetables.

INTRODUCTION

Enzymatic and nonenzymatic browning has generally been recognized to occur in apples and potatoes (Toribio and Lozano, 1984). Such browning damages the appearance, organoleptic properties, nutritional quality and, occasionally, safety of these commodities. The highly effective browning inhibitor sodium sulfite cannot be used in many food applications because some individuals, especially asthmatics, are sensitive to it (FDA, 1986, 1987). A need therefore exists to develop new inhibitors that could substitute for sulfite in fruits, fruit juices, vegetables, wine, etc. In this study, we compared the effectiveness of the following potential inhibitors in minimizing or preventing browning in apples and potatoes: L-cysteine, *N*-acetyl-L-cysteine (NAC), reduced glutathione (GSH), sodium bisulfite, sodium sulfhydrate, and sodium hydrosulfite. Our data suggest that NAC and GSH may be as effective as sodium sulfite in some food applications.

MATERIALS AND METHODS

Materials. The compounds were purchased from the following sources: L-cysteine, free base, U.S. Biochemical Corp., Cleveland, OH; *N*-acetyl-L-cysteine and reduced glutathione, Sigma, St. Louis, MO; sodium bisulfite, citric acid, and potassium sorbate, Mallinckrodt, St. Louis, MO; sodium hydrosulfite (sodium dithionite, $Na_2S_2O_4$, Fisher S-310) and sodium sulfhydrate ($NaSH \times H_2O$, Fisher S-423) Fisher Scientific, Chicago, IL; L-ascorbic acid, Eastman, Rochester, NY. Washington Golden and Red Delicious apples and White Russet Burbank potatoes were purchased in a local store.

Browning Procedures. Stock solutions (0.5 M) of sodium sulfite, sodium sulfide, sodium hydrosulfite, L-cysteine, NAC, GSH, and salicylic acid were adjusted to pH 7.0–7.5 before dilution to concentrations of 0.05, 0.025, 0.01, and 0.05 M and 5, 10, 25, and 50 mM. The precipitate formed by dissolving sodium sulfhydrate was filtered off. The mixed-acid solutions proposed by Langdon (1987) were prepared as follows: mixed acids solution a contained 1% citric acid, 1% ascorbic acid, and 0.2% potassium sorbate; solution b contained 0.5% citric acid, 0.3% ascorbic acid, and 0.2% potassium sorbate.

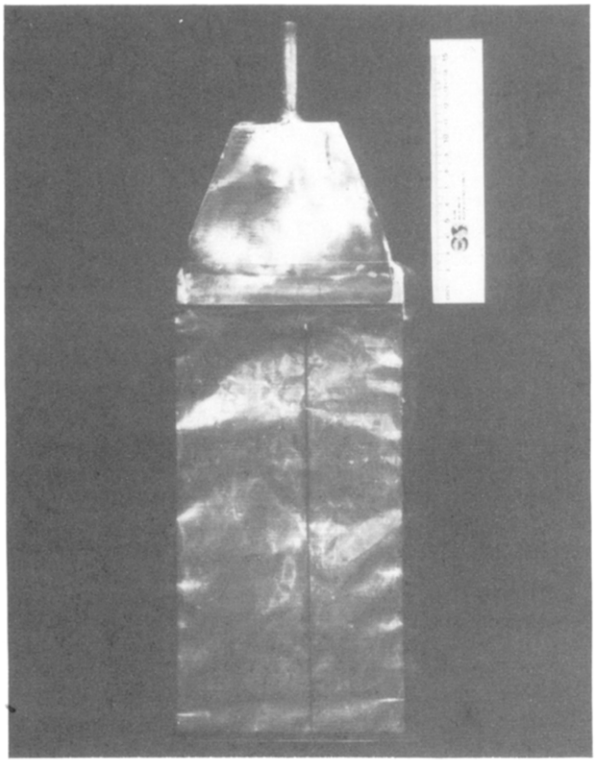

Figure 1. Illustration of connection between vacuum source and polyethylene pouch.

Apples and potatoes were peeled and then sliced into 4–5 mm thick slices. These were immediately immersed for 1–2 min in a treatment bath containing one of the inhibitors listed above. The excess solution was then blotted off, and the slices were placed on culture dishes (A values) or in plastic bags (B values). The bags were evacuated with the aid of a Cryovac apparatus (Cryovac, Simpsonville, SC). The plastic pouches (6 × 11 in.) were resealed twice in this way and a third time without vacuum. Deaeration of the bags was done with the aid of a PAC apparatus (Packaging Aids Corp., San Francisco, CA).

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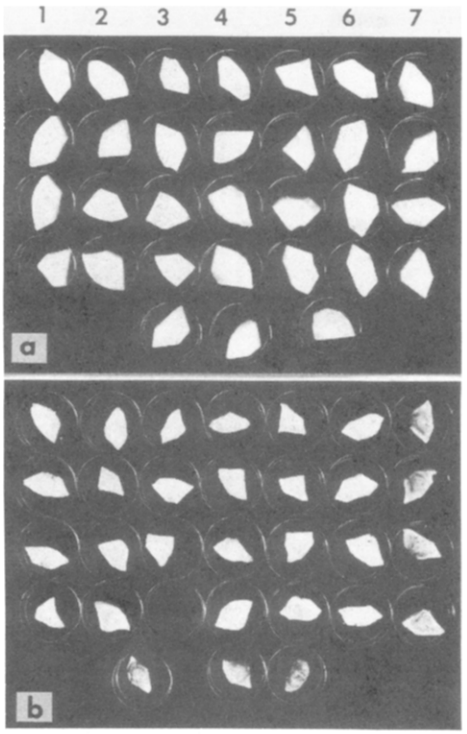

Figure 2. Photograph of slices of Washington Golden Delicious apples after 24 h (a) and after 1 week (b) stored in air. Samples from left to right (1–7) were treated with the following solutions: (1) sodium bisulfite; (2) sodium sulfhydrate; (3) sodium hydrosulfite; (4) L-cysteine; (5) *N*-acetyl-L-cysteine; (6) reduced glutathione; (7) sodium salicylate. The horizontal rows from top to bottom were treated with the following decreasing concentrations of the same bath: 50, 25, 20, and 5 mM. The fifth horizontal row shows the samples treated with mixed baths a and b, respectively (Langdon, 1987).

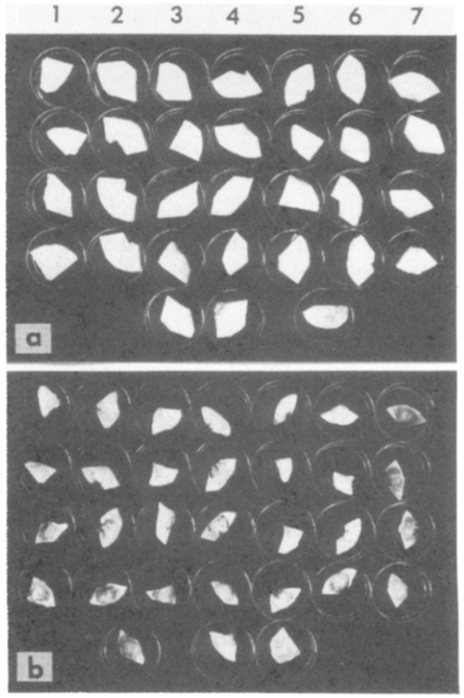

Figure 3. Photographs of slices of Washington Delicious apples stored in air for 24 h (a) and for 1 week (b). Conditions are as in Figure 2.

The extent of browning in the absence and presence of inhibitors was estimated with a Minolta Chroma Meter, CR 100, which measures tristimulus reflectance L values as described by Sapers and Douglas (1987) and Sapers and Ziolkowski (1987). The standard error in preliminary experiments was about ±5%.

Table I. ΔL Values of Control Slices of Potato and Apples after Specified Times

species	treatment[a]	time, h	ΔL values of control samples
White Russet Burbank potato	A	4	20.2
		7	26.1
	B	2	14.2
		5	16.6
Washington Red Delicious apple	A	6	12.6
		24	12.6
	B	2	6.4
		5	6.9
Washington Golden Delicious apple	A	6	6.2
		24	6.9
	B	2	3.5
		5	3.5

[a] A: Immersed in inhibitor solution, placed on dish, then measured after 4 and 7 h. Since there was no browning, the time chosen was arbitrary. B: After opening of the polyethylene pouches following storage at 4 °C for 24 days, slices were placed on dishes and readings taken at different time periods.

Table II. Prevention of Enzymatic Browning of Cut Surfaces of White Russet Burbank Potato by Treatment with Various Inhibitors

inhibitor	treatment	time, h	inhibition,[a] %, with treatment baths of (mM) 5	10	25	50
sodium bisulfite	A	4	93	105	102	101
		7	97	102	101	99
	B	2	94	89	103	98
		5	73	85	96	103
sodium sulfhydrate	A	4	97	98	99	100
		7	77	99	95	98
	B	2	97	99	98	98
		5	100	99	95	98
sodium hydrosulfite	A	4	96	97	102	93
		7	100	104	103	102
	B	2	100	99	98	96
		5	97	99	101	95
L-cysteine	A	4	96	97	102	96
		7	93	95	96	98
	B	2	83	82	92	96
		5	79	80	95	100
N-acetyl-L-cysteine	A	4	95	97	102	101
		7	88	98	96	106
	B	2	80	92	99	99
		5	78	96	99	99
glutathione (reduced)	A	4	100	93	97	100
		7	95	85	95	101
	B	2	83	87	96	96
		5	70	90	95	98
sodium salicylate	A	4	70	76	71	76
		7	72	84	92	89
	B	2	33	39	39	25
		5	30	44	33	−27
mixed acids	A	4	no data available			
		7	no data available			
	B	2	61 (b)		21 (a)	
		5	−4 (b)		−22 (a)	

[a] Inhibition % = (ΔL control − ΔL treatment) × 100/ΔL control. ΔL values are differences in L values between 2 min and specified time. The values measured after slicing form the basis of comparison for all conditions tested. Mixed acids indicate the treatment baths (a or b) proposed by Langdon (1987).

RESULTS AND DISCUSSION

The degree of browning was monitored by reflectance measurements of the apples and potatoes immediately after slicing (A values in Tables I–IV) and after storage of the slices in deaerated polyethylene pouches for 24 days at 4 °C (B values in Tables I–IV). To compare the effectiveness of the various potential inhibitors, changes in reflectance of L values were calculated according to the method of Sa-

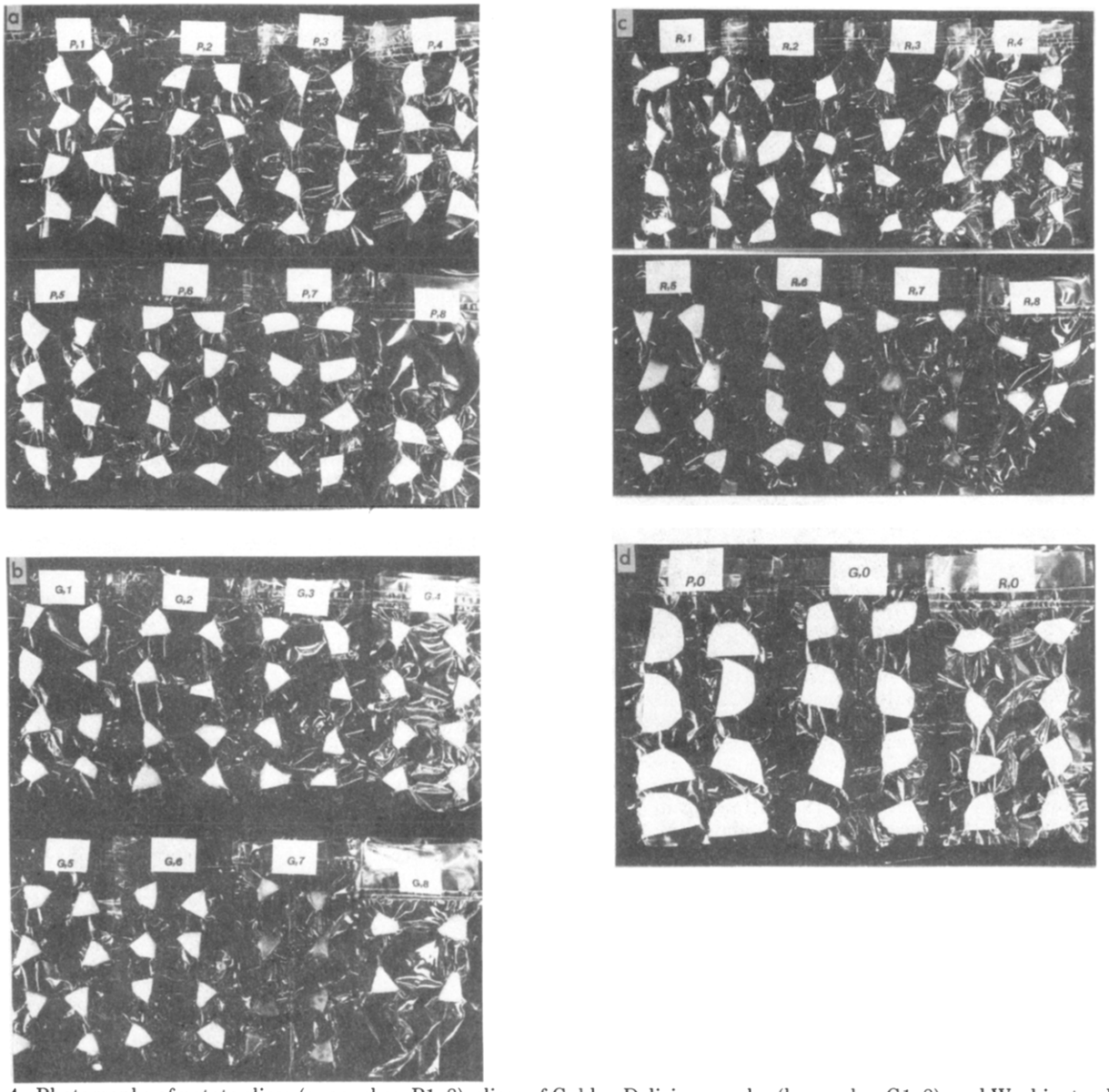

Figure 4. Photographs of potato slices (a, pouches, P1–8), slices of Golden Delicious apples (b, pouches G1–8), and Washington Red Delicious apples (c) (pouches R1–8). All were stored for 24 days at 4 °C after deaeration of the pouches. The numbers 1–7 associated with letters P, G, and R describe the treatment solutions listed in Figure 2. P8, G8, and R8 represent slices treated with Langdon's (1987) mixed bath b (top) and a (bottom), respectively. The four rows associated with pouches P1–7, G1–7, and R1–7 from top to bottom represent samples treated with the following increasing concentrations: 5, 10, 25, and 50 mM. Part d shows untreated control samples for potatoes (P0), Washington Golden Delicious apples (G0), and Washington Red Delicious apples (R0), respectively.

pers and Ziolkowski (1987). The relative potency of a given inhibitor was determined as the difference between the control value and the values observed in the presence of the inhibitor after the specified time period. Thus, both A and B values are compared to the same "initial" time and reflectance value, obtained immediately after slicing. The percent inhibition was calculated as

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

Photographs document the inhibition as shown in Figures 1–5. Table I shows the ΔL values of the control slices in the absence of inhibitors after specified time periods.

The data in the tables and figures reveal the following.

(1) The ΔL value of the control samples ranged widely, from 3.5 for Washington Golden Delicious apples to 26.1 for the White Russet Burbank potatoes. This variation depended also on the mode of browning (A and B conditions in Table I).

(2) The reproducibilities of the ΔL values also varied widely. Additional studies showed that L values of fresh apples and potatoes ranged between 70 and 80. The reproducibility in the readings of the fresh samples was ± 0.5 L units. This corresponds to $\pm 2\%$ for the samples with ΔL of 26.1 and $\pm 14\%$ for those with a value of 3.5.

(3) *N*-Acetyl-L-cysteine and reduced glutathione, applied at 25 or 50 mM concentrations, appear to be as effective as sodium sulfite in preventing browning of apples and potatoes [see Tables II–IV; Figures 2, 3, and 5, vertical columns (*N*-α-acetyl-L-cysteine), Figure 6 (reduced glutathione), and Figure 4, pouches P5, P6, R5, R6, G5, and G6].

(4) Sodium salicylate actually promoted enzymatic browning, especially during storage. Thus, the listed inhibition percentages of −328 and −414 (Table IV) indicate that browning in sodium salicylate treated samples were 3.28 and 4.14 times greater than in untreated samples (see Tables II–IV; Figures 2, 3, and 5, seventh vertical column, and Figure 4, pouches P7, R7 and G7).

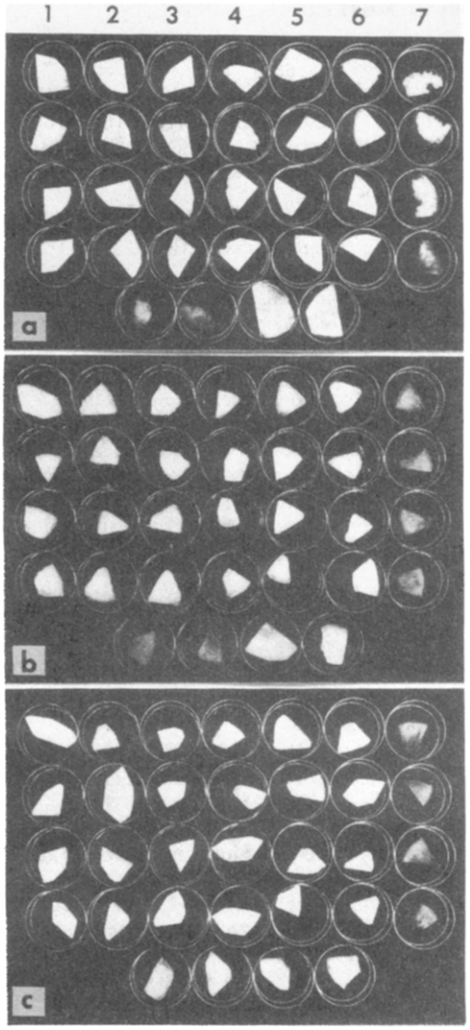

Figure 5. Photographs of slices of potatoes (a), Washington Golden Delicious apples (b), and Washington Red Delicious apples (c) after storage for 24 days in deaerated bags at 4 °C followed by storage in air for 5 h. From left to right, samples 1–7 were treated with the seven inhibitor solutions listed in Figure 2. The four horizontal rows from top to bottom illustrate samples treated with the following increasing concentrations of the same baths: 5, 10, 25, and 50 mM. The fifth horizontal row illustrates samples treated with Langdon's (1987) baths b and a and two parallel controls.

(5) The mixed-bath acids (citric plus ascorbic acids plus potassium sorbate) proposed by Langdon (1987) as sulfite alternatives appear to be of limited value. They were acceptable when measured immediately after slicing (see Tables III and IV, A values for samples treated with mixed acids; Figures 2a and 3a, fifth horizontal row, samples 1 and 2). However, during storage of the food the mixed-bath treatments seem to lose their effectiveness [see Figures 2b and 3b, fifth horizontal rows, samples 2 and 3 for storage in air; Figure 4, pouches P8, R8, and G8; Tables II–IV, B values of mixed acid treated samples (a and b data); Figure 5, fifth horizontal rows, samples 1 and 2].

(6) Previous studies disagree about optimum pH of treatments to prevent enzymatic browning. Thus, according to Langdon (1987), polyphenol oxidase enzymes have an optimum pH of about 6–7, and to be effective, the product being protected must be in a medium that maintains at least a pH of 3. However, Ponting (1971) states that an alkaline sulfite treatement was more effective than an acidic one. Our data (Table V) show that the efficiency of sulfites as inhibitors is independent of the pH of the treatement baths. Thus, Table V shows that sulfites were effective in pH ranges 2.99–3.72 and 6.26–

Table III. Prevention of Enzymatic Browning of Cut Surfaces of Washington Red Delicious Apple by Treatment with Various Inhibitors[a]

inhibitor	treatment	time, h	inhibition, %, with treatment baths of (mM) 5	10	25	50
sodium bisulfite	A	6	106	101	102	102
		24	99	98	102	102
	B	2	93	72	100	98
		5	91	66	110	98
sodium sulfhydrate	A	6	101	100	94	100
		24	99	102	101	100
	B	2	83	78	75	77
		5	75	74	72	70
sodium hydrosulfite	A	6	101	103	109	102
		24	100	102	106	102
	B	2	86	88	94	92
		5	90	81	86	72
L-cysteine	A	6	102	103	95	103
		24	98	98	95	99
	B	2	86	90	86	70
		5	71	96	81	74
N-acetyl-L-cysteine	A	6	85	102	102	103
		24	82	103	97	100
	B	2	97	97	95	100
		5	105	93	100	102
glutathione (reduced)	A	6	102	98	98	100
		24	101	98	98	100
	B	2	96	104	95	92
		5	84	100	93	93
sodium salicylate	A	6	89	87	93	92
		24	89	87	85	92
	B	2	−81	5	−31	8
		5	−90	4	−46	0
mixed acids	A	6	106 (b)		96 (a)	
		24	96 (b)		96 (a)	
	B	2	0 (b)		29 (a)	
		5	3 (b)		−7 (a)	

[a] See footnotes in Table II.

Table IV. Prevention of Enzymatic Browning on Cut Surfaces of Washington Golden Delicious Apples by Treatment with Various Inhibitors[a]

inhibitor	treatment	time, h	inhibition, %, with treatment baths of (mM) 5	10	25	50
sodium bisulfite	A	6	100	95	116	116
		24	95	95	93	104
	B	2	97	83	97	105
		5	109	80	91	100
sodium sulfhydrate	A	6	85	89	105	105
		24	92	92	123	103
	B	2	120	94	91	91
		5	109	106	97	91
sodium hydrosulfite	A	6	103	103	103	108
		24	100	97	99	93
	B	2	100	106	94	94
		5	97	106	103	100
L-cysteine	A	6	94	95	94	94
		24	100	95	99	94
	B	2	57	91	97	117
		5	0	83	100	80
N-acetyl-L-cysteine	A	6	98	111	102	111
		24	99	103	105	120
	B	2	14	41	86	100
		5	0	43	100	106
glutathione (reduced)	A	6	111	100	106	98
		24	107	93	107	96
	B	2	82	91	100	89
		5	94	93	96	103
sodium salicylate	A	6	34	50	85	55
		24	29	33	64	52
	B	2	−345	−342	−360	−377
		5	−328	−414	−397	−385
mixed acids	A	6	108 (b)		103 (a)	
		24	80 (b)		79 (a)	
	B	2	−188 (b)		−157 (a)	
		5	−271 (b)		−234 (a)	

[a] See footnotes in Table II.

Table V. pH Values of the Inhibitor Solutions at Various Concentrations

	pH values with treatment baths of (mM)			
inhibitor	5	10	25	50
sodium bisulfite	3.72	3.51	3.18	2.99
sodium sulfhydrate	8.72	8.90	9.05	9.27
sodium hydrosulfite	6.45	6.35	6.30	6.26
L-cysteine	7.20	7.25	7.30	7.34
N-acetyl-L-cysteine	7.24	7.30	7.45	7.55
glutathione (reduced)	7.34	7.32	7.35	7.33
sodium salicylate	6.37	6.34	6.35	6.37
mixed acids	3.05	2.65		

6.45. The optimum pH for the sulfur amino acids was in the range 7.20–7.55 (Table V).

(7) Preliminary taste experiments (results not shown) suggest that *N*-acetylcysteine and reduced glutathione have a lower flavor threshold than cysteine. This aspect merits further study.

In summary, *N*-acetyl-L-cysteine and reduced glutathione were excellent inhibitors of browning of apples and potatoes. These two SH-containing compounds were more efficient inhibitors than L-cysteine and approached the effectiveness of sodium sulfite. The mixed organic acids were effective only for short periods. These considerations suggest that *N*-acetyl-L-cysteine and reduced glutathione merit extensive evaluation as potentially useful inhibitors for food products.

Finally, it is likely that the mechanisms suggested in the accompanying papers for the prevention of browning by SH-containing amino acids and peptides may also apply to our findings with apples and potatoes (Friedman and Molnar-Perl, 1990; Molnar-Perl and Friedman, 1990). This study deals mainly with enzymatic browning since none of the materials were heated. In this connection, it is noteworthy that cysteine, *N*-acetyl-L-cysteine, and reduced glutathione are effective inhibitors of polyphenol oxidase (Friedman et al., 1986), the enzyme responsible for the initiation of enzymatic browning in plant foods (Schwimmer, 1981).

ACKNOWLEDGMENT

We thank H. R. Bolin and S. Schwimmer for helpful comments.

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Received for review June 12, 1989. Revised manuscript received March 21, 1990. Accepted April 3, 1990.

Registry No. NAC, 616-91-1; GSH, 70-18-8; L-cysteine, 52-90-4; sodium bisulfite, 7631-90-5; citric acid, 77-92-9; potassium sorbate, 24634-61-5; sodium hydrosulfite, 7775-14-6; sodium sulfhydrate, 16721-80-5; ascorbic acid, 50-81-7; sodium salicylate, 54-21-7.