Inhibition of Polyphenol Oxidase by Thiols in the Absence and Presence of Potato Tissue Suspensions†

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To contribute to the development of sulfite alternatives to prevent enzymatic browning in fruits and vegetables, the inhibition of polyphenol oxidase (PPO) by structurally different sulfhydryl compounds (thiols) and by sodium bisulfite in the absence and presence of suspensions of dehydrated potatoes was measured. Relative potencies (I_{50} values), defined as the millimolar concentration of the inhibitor needed to reduce 400 units of PPO activity by 50%, were calculated from regression equations of the concentration dependence of the inhibition at 25 °C. For solutions of pure PPO, the I_{50} values ranged as follows: *N*-(2-mercaptopropionyl)glycine, 0.058 (the most potent inhibitor); L-cysteine methyl ester, 0.099; 2-mercaptopyridine, 0.100; L-cysteine ethyl ester and reduced glutathione, 0.12; 2-mercaptoethanesulfonic acid, 0.14; sodium bisulfite, 0.21; homocysteine, 0.23; *N*-acetyl-L-cysteine, 0.27; L-cysteine, 0.35; and cysteinylglycine, 0.43. Studies on the influence of temperature with six inhibitors, each tested at the I_{50} concentration listed above, in the range 25–55 °C showed that although heat alone partly inactivated the enzyme, the presence of thiols such as cysteine, *N*-acetyl-L-cysteine, and reduced glutathione markedly increased the inactivation. However, this was not the case for cysteine ethyl and methyl esters. Inhibition studies with NDA 1725 and Russet potato suspensions at 25 °C revealed that the inhibitors were less effective in the heterogenous food milieu and that the relative effectiveness of structurally different thiols differed from those observed in PPO solutions. Mechanisms are described to explain the inhibition of PPO and the prevention of enzymatic browning by SH-containing compounds. Possible approaches to applying the results to the development of useful browning inhibitors for foods are also discussed.

Keywords: *N-Acetyl-L-cysteine; L-cysteine ethyl ester; enzymatic browning prevention; polyphenol oxidase inhibition; potatoes; reduced glutathione; sodium bisulfite; thiols*

INTRODUCTION

As part of an effort to improve handling and processing of potatoes, we would like to find new ways to inactivate enzymes that catalyze enzymatic browning and glycoalkaloid biosynthesis. In previous studies, we (Friedman, 1991; Friedman et al., 1986) and others (Dudley, 1989; Golan-Goldhirsh and Whitaker, 1984) showed that sulfhydryl (SH or thiol) compounds such as cysteine, *N*-acetyl-L-cysteine, and reduced glutathione are good inhibitors of the enzyme polyphenol oxidase (PPO) which catalyzes enzymatic browning in fruits and vegetables (Mayer, 1987; Schwimmer, 1981), including potatoes (Matheis, 1987a,b; Sanchez-Ferrer et al., 1993). In related studies (Friedman and Molnar-Perl, 1990; Friedman et al., 1992; Molnar-Perl and Friedman, 1990a,b), we showed that SH-containing amino acids and peptides are good inhibitors of both enzymatic and nonenzymatic browning in freshly prepared and commercial fruit juices, apples, fresh and dehydrated potatoes, heated amino acid–carbohydrate mixtures, and protein-rich foods. The effectiveness of these compounds approached that of sodium sulfite in some applications. Although sodium sulfite is highly effective as an antibrowning agent, its use is being discontinued because many individuals, especially asthmatics, are sensitive to it (FDA, 1990; Sapers, 1993). It has also been reported to cross-link nucleic acids and proteins (Shapiro and Gazit, 1977) and to be genotoxic in mice (Pal and Bhunya, 1992).

During the course of our studies, it became apparent that the potencies of different thiols as PPO inhibitors varied widely, depending on both the structure of the thiol itself and the environment in which the inhibition is carried out. It was therefore of interest to compare the relative effectiveness of structurally different thiols in inhibiting the oxidase in a food matrix as well as the pure enzyme solution. Our ultimate objective is to find the most potent SH-containing amino acids and peptides that are compatible with foods and that can replace sodium sulfite as antibrowning agents. To address these issues, this study evaluates the effectiveness of a series of sulfhydryl compounds in inhibiting PPO in solution and in dehydrated potato tissue suspensions.

We selected the NDA 1725 high-glycoalkaloid potato variety for most of these studies rather than another food because we hope to transform it with the aid of molecular biology techniques to a low-glycoalkaloid cultivar (Friedman, 1992; Stapleton et al., 1991, 1992, 1994). Russet potatoes were also included for comparison.

MATERIALS AND METHODS

Materials. Russet Idaho potatoes were obtained from a local store. NDA 1725 high-glycoalkaloid potatoes, the same experimental variety described previously (Friedman and Dao, 1992), were provided by J. J. Pavek of the ARS Potato Breeding Program, Aberdeen, ID. The following compounds were obtained from Sigma Chemical Co., St. Louis, MO: *N*-acetyl-

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	HS-R	Name
1.	$HS\text{-}CH_2CH(NH_2)COOH$	L-cysteine
2.	$HS\text{-}CH_2CH(NH_2)COOCH_3$	L-cysteine methyl ester
3.	$HS\text{-}CH_2CH(NH_2)COOCH_2CH_3$	L-cysteine ethyl ester
4.	$HS\text{-}CH_2CH(NHCOCH_3)COOH$	N-acetyl-L-cysteine
5.	$HS\text{-}CH_2CH(NH_2)CONHCH_2COOH$	cysteinyl-glycine
6.	$HS\text{-}CHCONHCH_2COOH$ $NHCOCH_2CH_2CH(NH_2)COOH$	reduced glutathione (aspartyl-cysteinyl-glycine)
7.	$HS\text{-}CH_2CH_2CH(NH_2)COOH$	homocysteine
8.	$HS\text{-}CH_2CH_2CONHCH_2COOH$	N-mercaptopropionyl-glycine
9.	$HS\text{-}CH_2CH_2SO_3H$	mercaptoethane sulfonic acid
10.		mercaptopyridine

Figure 1. Structures of sulfhydryl compounds (HS–R) evaluated in the study.

L-cysteine, lot 40H0342; L-cysteine, lot 39F0688; L-cysteine ethyl ester, lot 103F0250; L-cysteine methyl ester, lot 100H131; cysteinylglycine, lot 32H0907; glutathione (reduced), lot 42H8070; homocysteine, lot 51H3850; 2-mercaptoethanesulfonic acid, lot 118F0279; *N*-(2-mercaptopropionyl)glycine, lot 82H0666; 2-mercaptopyridine, lot 100H3404; mushroom tyrosinase (polyphenol oxidase, EC 1.14.18), lot 112H9580, 800 units (U)/mg. Sodium bisulfite came from Mallinckrodt, Milwaukee, WI.

Polyphenol Oxidase Assay. The assay was carried out as described by Worthington (1993) with modifications as follows:

Materials included 0.5 M potassium phosphate, monobasic, buffer, pH 6.5; 1 mM L-tyrosine; PPO diluted to 400 U/mL.

Procedure. A Perkin-Elmer (Norwalk, CT) Lambda 6 UV–vis spectrophotometer was used. The cell was maintained at 25 °C with a temperature-controlled circulating pump. A mixture of 1 mL of phosphate buffer, 1 mL of 1 mM L-tyrosine, and 0.9 mL of deionized water was equilibrated at 25 °C and oxygenated by bubbling oxygen gas into the solution through a capillary tube for 5 min. The oxygen-saturated substrate was then transferred to a cuvette, and 100 μL of diluted enzyme solution (400 U/mL) was added. The absorbance at 280 nm (A_{280}) was recorded for 10 min. The change in absorbance (ΔA_{280}) was then determined from the linear part of the curve. There was usually a nonlinear lag of 2–3 min.

Activity (U/mg) = (ΔA_{280}/min) × 1000/mg of enzyme in reaction mixture, where 1 U causes a ΔA at 280 nm of 0.001/min at 25 °C, pH 6.5.

Inhibition of Pure PPO. Pure PPO (400 U/mL) was mixed with different concentrations of the various inhibitors and incubated at 25 °C for 1 h. Enzyme activity of the resulting solutions was then measured. One milliliter of phosphate buffer and 1 mL of 1 mM L-tyrosine were then oxygenated by bubbling oxygen into the solution for 5 min. One milliliter of the enzyme–inhibitor mixture was added, and the change in absorbance (ΔA_{280}) was determined from the linear portion of the curve as above.

Inhibition of PPO Added to Potato Powders. Fresh potatoes were rinsed, patted dry, cubed, and immediately frozen in liquid nitrogen. Samples were then lyophilized to dry powders. The freeze-dried potatoes were ground in a Wiley mill to pass a 1 mm screen. This was used to make a 5% suspension of potato powder in phosphate buffer. The oxidase (400 U/mL) was then incubated with 1 mL of the suspension and several concentrations of the different inhibitors, as indicated in the figures, in a ratio of inhibitor to PPO identical to that used for the pure enzyme (also 400 U/mL). The mixture was stirred for 4 h at room temperature and then centrifuged in a Beckman microfuge for 5 min. One milliliter of supernatant was used for the assay of residual PPO activity as described earlier for the pure PPO, starting with oxygenation of buffer and tyrosine.

Effect of Temperature. Enzyme solution (400 U/mL) in phosphate buffer and various inhibitor solutions were preheated in a water bath to the appropriate temperature. The

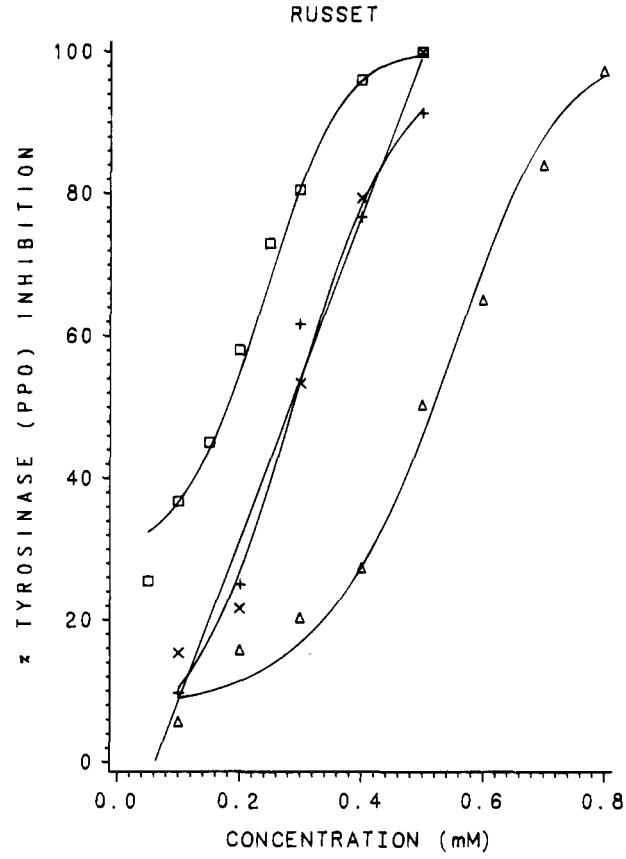

Figure 2. Concentration dependence of the inhibition at 25 °C of endogenous PPO (~1.5 U/g) in Russet potato powder suspensions by sodium bisulfite (□), *N*-acetyl-L-cysteine (×), L-cysteine (+), and glutathione (△).

solutions (100 μL) were then mixed and allowed to incubate at 35, 45, or 55 °C for 10 min in a water bath. The solutions were cooled to 25 °C and added to an oxygenated (5 min) mixture of 1 mL of phosphate buffer, 1 mL of tyrosine solution, and 0.8 mL of water. Enzyme activity was determined as above.

Measurement and Inhibition of Endogenous PPO in Potato Powders. One gram of freeze-dried powder (NDA 1725 or commercial Russet) was rehydrated in 5 mL of cold 0.1 M potassium phosphate buffer, pH 6.0, and centrifuged (15000*g*) at 4 °C for 30 min. One milliliter of the supernatant was incubated with several concentrations of the inhibitor for 1 h. One milliliter of phosphate buffer (0.5 M, pH 6.5) and 1 mL of L-tyrosine solution (1.0 mM) were oxygenated by bubbling O_2 into the mixture for 5 min. One milliliter of the supernatant or supernatant–inhibitor mixture was added. The mixture was then transferred into a cuvette, and the change in absorbance (ΔA_{475}) was recorded. The extent of reaction was calculated from the linear portion of the curve. Reproducible results were obtained for the low levels of endogenous PPO when activity was measured spectrophotometrically at 475 nm at 25 °C (Stark et al., 1985). The 475 nm absorbance was used for this experiment because we could not obtain a good reading at 280 nm with the low levels of endogenous PPO present in potatoes.

Statistics. The data were analyzed using SAS PROC GLM (SAS, 1987) programs, fitting the observed variations in the extent of inhibition of PPO as a function of inhibitor concentration. Regression equations were used to calculate standard errors (SE) and the concentration of inhibitor needed to achieve 50% inhibition (I_{50} values) from duplicate and triplicate separate determinations. The corresponding 95% confidence intervals (CI) were constructed on I_{50} values using Fieller's theorem (Zerbe, 1978).

RESULTS AND DISCUSSION

This section discusses effectiveness of the sulfhydryl compounds in inhibiting the activity of PPO (a) in

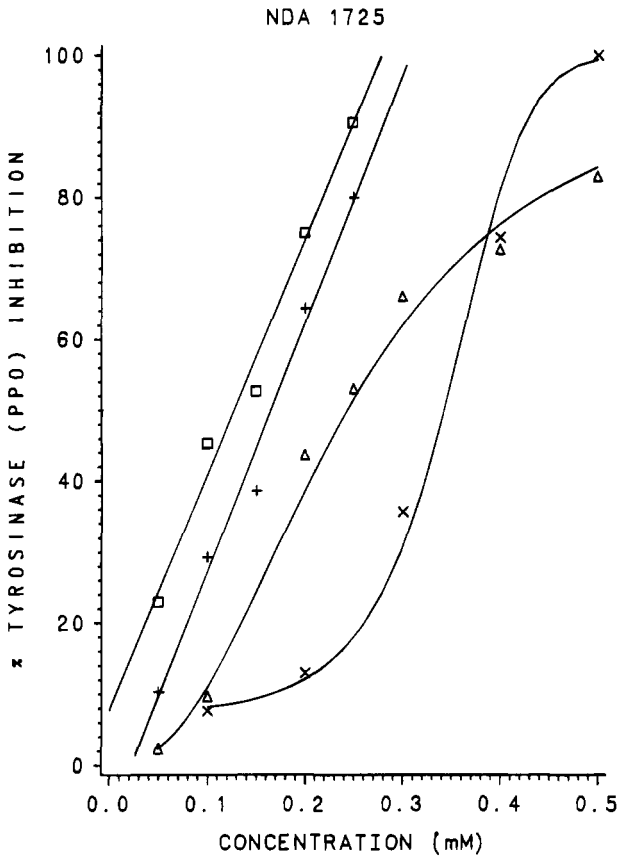

Figure 3. Concentration dependence of the inhibition at 25 °C of endogenous PPO (~1.5 U/g) in NDA 1725 potatoes by sodium bisulfite (□), *N*-acetyl-L-cysteine (+), L-cysteine (×), and glutathione (△).

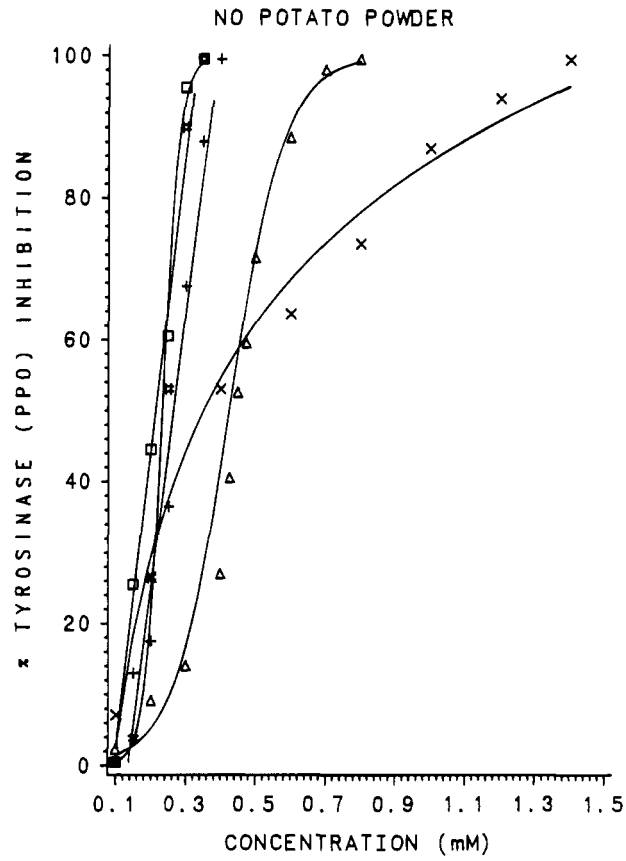

Figure 4. Concentration dependence of the inhibition at 25 °C of endogenous plus added PPO (~400 U/g) by sodium bisulfite (□), homocysteine (#), *N*-acetyl-L-cysteine (+), L-cysteinylglycine (△), and L-cysteine (×).

solution at 25 °C, (b) at several higher temperatures, (c) in potato tissue suspensions (endogenous), and (d) added to suspensions of dehydrated potatoes. The relative potencies will be compared to that of L-cysteine, the simplest SH-containing amino acid. We will also offer mechanistic rationalizations of the possible molecular basis of the thiol-induced inhibition reactions and discuss additional research needed to develop practical ways to prevent browning in foods.

Inhibition of Pure PPO. Figure 1 shows the structures of the PPO inhibitors evaluated in this study. Figures 2–8 illustrate the concentration and temperature dependence of the inhibition of PPO by sodium bisulfite and 10 thiols. The data in these figures were used to calculate I_{50} values, defined as the concentration that reduces enzyme activity of 400 U by 50%. Table 1 summarizes the I_{50} values and relative potencies.

The data for pure PPO at 25 °C show that (a) relative potencies for the 11 inhibitors evaluated varied up to 7-fold; (b) *N*-(2-mercaptopropionyl)glycine was the most potent inhibitor, followed by 2-mercaptopyridine and the two cysteine esters; (c) although L-cysteine was about 60% less effective than sodium bisulfite, reduced glutathione and cysteine methyl and ethyl esters were about 1.7–3 times more effective than bisulfite; (d) the tripeptide aspartylcysteinylglycine (reduced glutathione) was 3 times more effective as an inhibitor than was the dipeptide cysteinylglycine (the dipeptide was in fact the least effective of all compounds tested, being 20% less effective than the amino acid L-cysteine); and (e) homocysteine and *N*-acetylcysteine were respectively 50 and 30% more effective than L-cysteine.

The influence of temperature in the range 25–55 °C on the inhibition of PPO at 10 °C intervals was investigated by comparing the potencies of the inhibitors at the concentration found to produce 50% inhibition at 25 °C, *i.e.* the I_{50} values listed in Table 1. Note the actual concentration used differed for each inhibitor. Figure 6 shows that the effect of temperature was not uniform. Although the temperature dependence of the inhibition of PPO was nearly linear for sodium bisulfite, L-cysteine, *N*-acetyl-L-cysteine, and reduced glutathione, curves for the two cysteine esters are nearly flat. One possibility is that the highly reactive esters undergo significant side reactions at the higher temperatures, limiting their effectiveness as PPO inhibitors. Temperature effects on the inhibition of PPO in the presence of potato powders were not studied.

Inhibition of PPO in Dehydrated Potato Powder Suspensions. Our initial objective was to measure the effect of potential inhibitors on endogenous PPO in dehydrated potatoes. However, we found the PPO content of the potatoes to be low and difficult to extract and measure reproducibly. Although such difficulties are also documented in the literature (Matheis, 1987a,b; Muneta, 1981), we succeeded in measuring the PPO content in both Russet and NDA 1725 potatoes. Table 2 and Figures 2 and 3 show that the relative effectiveness of three thiols and sodium bisulfite in inhibiting endogenous PPO differed both within and between the two cultivars.

Additional experiments designed to determine the susceptibility of pure PPO to inactivation in the exact amounts present in the potatoes were not successful. With these low concentrations of PPO, the inhibition appeared to proceed too rapidly to measure accurately. To circumvent these problems, we added the same amount of PPO to the potato powder suspensions as we

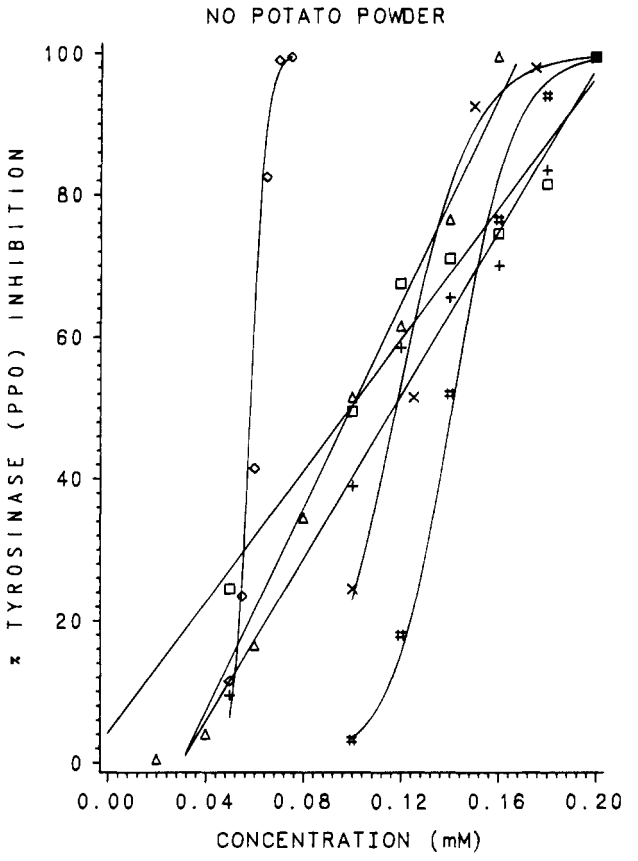

Figure 5. Concentration dependence of the inhibition at 25 °C of endogenous plus added PPO (~400 U/g) by *N*-(2-mercaptopropionyl)glycine (◇), 2-mercaptopyridine (△), reduced glutathione (×), L-cysteine methyl ester (□), L-cysteine ethyl ester (+), and 2-mercaptoethanesulfonic acid (#).

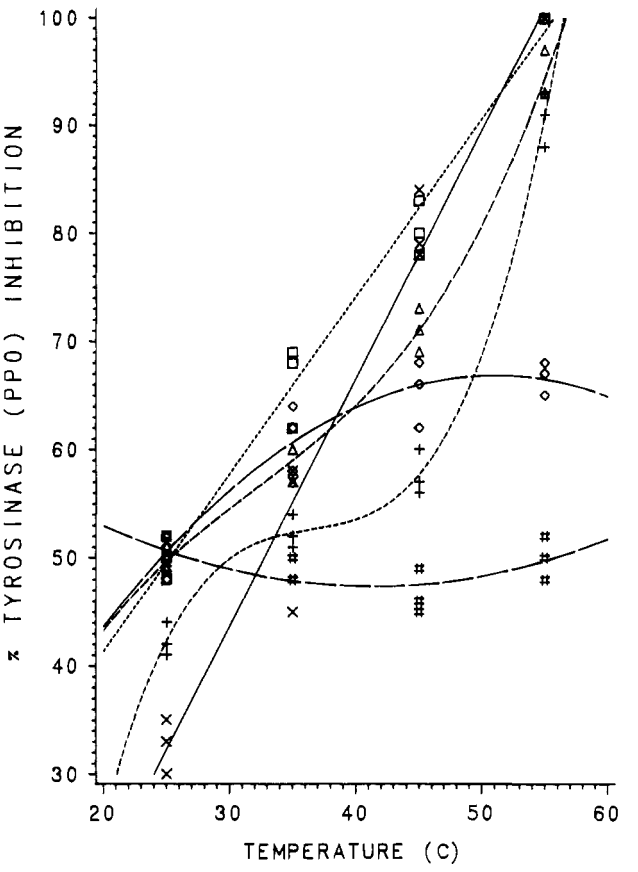

Figure 6. Effects of temperature on the inhibition of endogenous plus added PPO (~400 U/g) by sodium bisulfite (×), *N*-acetyl-L-cysteine (□), reduced glutathione (+), L-cysteine (△), L-cysteine methyl ester (#), and L-cysteine ethyl ester (◇).

used in the inhibition studies with pure PPO solutions. This experimental design permits comparison of the relative effectiveness of the inhibitors in the absence and presence of a potato matrix. The results were unexpected.

Table 1 and Figures 4, 5, 7, and 8 compare inhibitory potencies of eight thiols and sodium bisulfite in the absence and presence of potato tissue suspensions. The results for 2-mercaptoethanesulfonic acid and *N*-(mercaptopropionyl)glycine, two potent inhibitors of pure PPO, are not included because we found them to be experimentally nonreproducible. The data show that (a) the concentration of inhibitors required to inhibit PPO in the 5% potato powder suspensions is much greater than that for pure PPO; (b) for sodium bisulfite, cysteine, *N*-acetylcysteine, homocysteine, reduced glutathione, and mercaptopyridine, the I_{50} values differ between 200 and 400% and for the two cysteine esters by about 1500%; (c) compared to cysteine, the potency of sodium bisulfite is 200% greater, those for *N*-acetylcysteine and homocysteine are similar, those for glutathione, cysteine methyl ester, and cysteinylglycine are lower by 50–90%; and (d) the potency of mercaptopyridine equals that of sodium bisulfite, but mercaptopyridine cannot be used in foods.

These results strikingly demonstrate that both the absolute and relative potencies of the inhibitor are strongly influenced by the structure of the inhibitor and the microenvironment in which the inhibition process takes place. Although the reasons for these differences are not immediately apparent, possibilities include (a) slow diffusion of the inhibitors to PPO and substrates in the heterogeneous dehydrated potato suspension, (b) binding of PPO to potato starch granules, (c) the action of PPO on substrates other than tyrosine such as chlorogenic acid (Dao and Friedman, 1992) or quercetin (Friedman and Smith, 1984), and (d) the reaction of the thiols with dehydroascorbic acid and carbohydrates. The latter reactions may also be beneficial since they may prevent nonenzymatic browning (Ziderman et al., 1989).

These considerations suggest that no general conclusions can be made about the PPO inhibition by thiols in specific foods. Each food category has to be evaluated separately with each sulfhydryl compound to find optimum conditions to prevent both enzymatic and nonenzymatic browning under specific conditions of storage, transport, and processing.

Mechanistic Possibilities for the Inhibition of PPO by Thiols. PPO catalyzes two reactions: hydroxylation of tyrosine to *o*-dihydroxyphenylalanine (DOPA) and oxidation of DOPA and other *o*-phenols such as chlorogenic acid to *o*-quinones (Deshpande et al., 1984; Golan-Goldhirsh et al., 1984; Wong, 1989). The quinones then undergo further oxidation to brown melanin pigments (Figure 9). Any event that can disrupt these transformations should reduce browning. Inhibition may occur directly or indirectly. Direct inhibition changes the enzyme, preventing the PPO-catalyzed formation of the *o*-quinones. Indirect inhibition affects the intermediates, preventing the further transformation of the quinone to brown pigments.

Possible inhibition mechanisms can be divided into three types: (a) copper–nitrogen bond cleavage; (b) oxidation–reduction reactions; and (c) nucleophilic addition reactions. The first type involves PPO directly. It postulates that because SH groups have a strong affinity for copper, they displace histidine residues liganded to the copper of the active site of PPO and/or

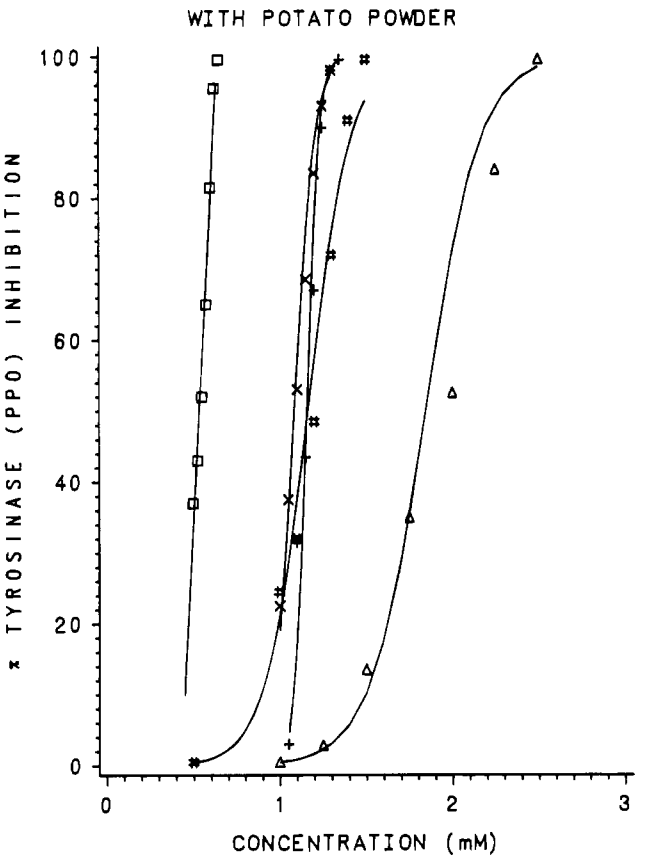

Figure 7. Concentration dependence of the inhibition at 25 °C of endogenous plus added PPO (~400 U/g) in NDA 1725 potato powder suspensions by sodium bisulfite (□), L-cysteine (×), homocysteine (#), *N*-acetyl-L-cysteine (+), and cysteinylglycine (△).

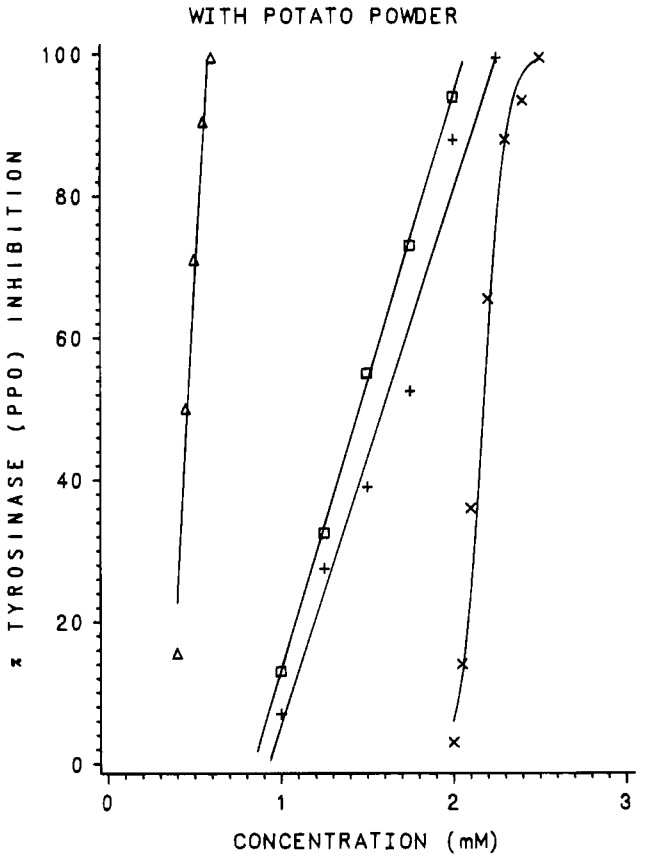

Figure 8. Concentration dependence of the inhibition at 25 °C of endogenous plus added PPO (~400 U/g) in NDA 1725 potato powder suspensions by 2-mercaptopyridine (△), L-cysteine methyl ester (□), L-cysteine ethyl ester (+), and reduced glutathione (×).

completely remove the copper from the enzyme (Lerch, 1987; Martinez et al., 1986). The resulting structural modifications of the active site, schematically illustrated in Figure 9, inactivate the enzyme.

Two oxidation–reduction reactions can, in principle, contribute to the inhibition of PPO, one direct and one indirect. In the presence of oxygen, the thiolate anion can react with cupric ion to form cuprous ions and sulfur radicals. The latter can dimerize to a disulfide bond. The net effect is cupric ion-mediated oxidation of a thiolate anion to a disulfide bond via a thiyl radical (Takagi and Isemura, 1964).

A second redox reaction involves thiol-mediated reduction of *o*-quinone intermediates back to hydroxyphenols. Such reductions may be largely responsible for the antibrowning effects of ascorbic acid (Golan-Goldhirsh et al., 1984; Sapers and Miller, 1992).

A third mechanism for inactivating PPO may involve nucleophilic addition of the thiolate anions to *o*-quinone intermediates, trapping them as thiol adducts. The addition reaction prevents formation of melanin pigments and depletes PPO substrates. Such reactions have been extensively studied (Friedman, 1973, 1994; Hurrell and Finot, 1984; Matheis and Whitaker, 1984).

The formation of the transition state and product in the addition reaction to form a thiol adduct is energetically favored because as the pair of electrons of the sulfur anion approaches the double bond, the empty d-orbitals of the sulfur atom can stabilize the high-energy electrons of the double bond polarized to the

Table 1. Millimolar Concentration of Inhibitors Required To Reduce Pure PPO and Endogenous plus Added PPO Activity in NDA 1725 Potato Suspensions by 50% (I_{50}) at 25 °C and Potencies Relative to Cysteine

	pure PPO		potato suspensions		potency relative to L-cysteine	
compound	I_{50}[a]	95% CI	I_{50}	95% CI	pure PPO	potato suspensions
L-cysteine	0.35	0.31, 0.39	1.09	1.07, 1.09	1.00	1.00
L-cysteinylglycine	0.43	0.40, 0.45	1.85	1.73, 1.98	0.81	0.59
N-acetyl-L-cysteine	0.27	0.24, 0.29	1.16	1.14, 1.18	1.30	0.94
homocysteine	0.23	0.22, 0.25	1.17	1.12, 1.22	1.52	0.93
sodium bisulfite	0.21	0.20, 0.23	0.54	0.52, 0.55	1.67	2.40
2-mercaptoethanesulfonic acid	0.14	0.14, 0.15	*b*	2.50	*b*	
L-cysteine ethyl ester	0.12	0.11, 0.12	1.60	1.49, 1.69	2.92	0.68
reduced glutathione	0.12	0.11, 0.13	2.18	2.14, 2.21	2.92	0.50
2-mercaptopyridine	0.10	0.094, 0.106	0.46	0.46, 0.49	3.50	2.37
L-cysteine methyl ester	0.099	0.083, 0.111	1.46	1.46, 1.48	3.53	0.75
N-(2-mercaptopropionyl)glycine	0.058	0.055, 0.061	*b*	6.03	*b*	

[a] The lower the I_{50} value, the more potent the inhibitor. [b] Results were not reproducible.

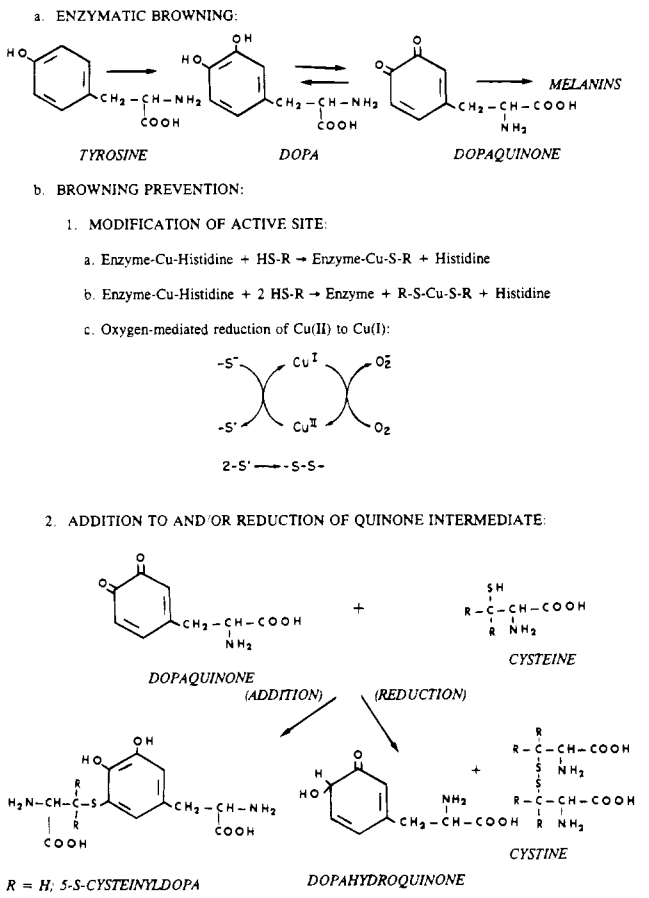

Figure 9. Possible mechanisms for the action of PPO and its inhibition by thiols.

Table 2. Millimolar Concentration of Inhibitors Required To Reduce Endogenous PPO Activity in Russet and NDA 1725 Potato Suspensions by 50% (I_{50}) at 25 °C

	Russet		NDA 1725	
compound[a]	I_{50}[b]	95% CI[c]	I_{50}	95% CI
L-cysteine	0.28	0.23, 0.33	0.34	0.32, 0.36
N-acetyl-L-cysteine	0.29	0.26, 0.32	0.17	0.15, 0.18
glutathione (reduced)	0.52	0.49, 0.55	0.24	0.23, 0.26
sodium bisulfite	0.18	0.16, 0.20	0.13	0.11, 0.14

[a] Inhibition with cysteine ethyl and methyl esters occurred too rapidly to permit accurate measurement. The estimated I_{50} values for these two compounds are below 0.05 mM. [b] mM concentration needed to inactivate 50% of PPO. The lower the number, the more potent the inhibitor. [c] 95% confidence intervals based on two to four separate determinations.

center carbon atom. Formation of the transition state is also favored by dissipation of the negative charge of the sulfur anion. The d-orbital bonding and charge dissipation are therefore responsible for the strong affinity of thiols for conjugated compounds such as *o*-quinones (Friedman et al., 1965).

To what extent each of the cited mechanistic pathways operates with different thiols is not known.

PRACTICAL APPLICATIONS

This and earlier studies show that SH-containing compounds are effective inhibitors of polyphenol oxidase. They are therefore potential sulfite substitutes for browning prevention. Since the effectiveness of a specific sulfhydryl compound is influenced by its structure, additional studies are needed to optimize the antibrowning action of a variety of structurally different SH-containing amino acids and peptides with different physicochemical properties. Factors associated with the R group in structurally different R–SH type amino acids and peptides are expected to influence inhibitory activities. Such parameters include the hydrophobic or hydrophilic nature of the R group and the presence of various functional groups such as the ϵ-NH_2 groups of lysine, the guanidino group of arginine, the imidazole group of histidine, or the carboxyl groups of aspartic or glutamic acid. These parameters are also expected to influence stabilities and solubilities of new amino acid and peptide antibrowning compounds.

Studies are also needed to define the products formed in foods during the antibrowning action of thiols. Such studies are now beginning to appear (Naim et al., 1993; Richard-Forget et al., 1992; Edwards and Wedzicha, 1992).

Whether any of the compounds evaluated in this study are usable in foods also awaits further study. Two of the most potent compounds, 2-mercaptopyridine and *N*-(2-mercaptopropionyl)glycine, are not naturally occurring amino acids of peptides and are therefore not usable in foods. The two cysteine esters also appear to be strong inhibitors. It remains to be shown whether they can serve as nutritional sources of cysteine, as does *N*-acetyl-L-cysteine (Friedman and Gumbmann, 1984).

Finally, to apply our findings to practical aspects of preventing long-term browning of food and reduction of glycoalkaloid content, answers to questions such as the following need to be explored: (1) Which SH-containing amino acid or peptide solutions could be used as a dip for apples, pears, bananas, lettuce, mushrooms, and potatoes to prevent formation of brown and black spots? (2) Will combinations of SH-containing compounds such as reduced glutathione mixed with other browning inhibitors such as ascorbic and citric acids (Langdon, 1987) or phosphoric acid (Sperber, 1993) be more effective than the individual compounds alone? (3) How safe are the various inhibitors and their reaction products for human consumption? (4) Will the use of SH-containing amino acids and peptides to inhibit browning also enhance the nutritional quality of sulfur-poor potato proteins? (5) Will the inactivation of PPO in potatoes be accompanied by simultaneous inactivation of enzymes involved in the biosynthesis of glycoalkaloids? (6) Will reduction in tyrosine content through plant breeding (Corsini et al., 1992) or chemical modification (Friedman et al., 1982) reduce the susceptibility of foods to enzymatic browning? (7) Will thiols prevent mutagen formation during nonenzymatic browning, as does sodium sulfite (Friedman et al., 1990; Krone et al., 1986)?

We are challenged to respond to these needs to improve the quality and safety of foods.

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