INHIBITION OF APPLE POLYPHENOLOXIDASE (PPO) BY ASCORBIC ACID, CITRIC ACID AND SODIUM CHLORIDE

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ABSTRACT

The inhibiting effect of ascorbic acid, citric acid and sodium chloride on Polyphenoloxidase (PPO) of Golden Delicious apple cubes was studied.

Dipping in ascorbic acid (0.2–10 g/L range) and in NaCl (0.2–1 g/L range) solutions for 5 min increases the PPO activity. Citric acid solutions (0.2–10 g/L range) have little or no inhibition of PPO.

A 90–100% PPO inhibition was obtained with a 5 min dip in mixtures of ascorbic acid and citric acid (10 + 2 g/L), and of ascorbic acid and sodium chloride (10 + 0.5 g/L).

INTRODUCTION

Browning occurs during fruit processing. At least five causes of browning in processed and/or stored fruit and vegetables are known: enzymatic browning of the phenols, Maillard reaction, ascorbic acid oxidation, caramelization and formation of browned polymers by oxidized lipids.

The oxidation of the o-diphenols to o-quinones by polyphenoloxidase (E.C. 1.14.18.1:usually named PPO) is the most important cause of the change in color as the o-quinones quickly polymerize and produce brown pigments (melanin) (Mayer and Harel 1979; Vamos-Vigyazo 1981). Enzymatic browning also causes a loss in the nutritional value through oxidation of ascorbic acid.

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In the food industry, enzymatic browning can be avoided by using thermal inactivation of PPO, but heat can cause softening.

Instead of blanching, chemical additives can be used to prevent enzymatic browning: bisulfite (Ponting 1960; Walker 1977; Sayavedra-Soto and Montgomery 1986), ascorbic acid and its analogs (Bauernfeind and Pinkert 1970; Eskin *et al.* 1971; Walker 1977; Sapers and Ziolkowski 1987; Hsu *et al.* 1988), and cysteine as reducing agent (Walker and Reddish 1964; Montgomery 1983; Dudley and Hotchkiss 1989).

The chemical action of the bisulfites is to react with the o-quinones forming colorless complex compounds (Embs and Markakis 1965; Lu Valle 1952; Wedzicha 1984). Ascorbic acid reduces the o-quinones to colorless dihydroxyphenols (Varoquaux and Sarris 1979; Golan-Goldhirsh and Whitaker 1984).

Although the bisulfites are efficient, they are banned in the USA for use in raw fruit and vegetables by a ruling of the FDA (1986). The presence of bisulfites can be dangerous to human health, especially in asthmatic patients (Taylor and Bush 1986), so alternative chemical additives are needed that are without toxic effects.

Langdon (1987) showed that different combinations of ascorbic and citric acid prevent enzymatic browning of sliced potatoes. Santerre *et al.* (1988) confirmed that combinations of ascorbic acid, erythorbic acid and citric acid were efficient in preventing browning of sliced apples. According to Ponting *et al.* (1972) sliced Golden Delicious apples could be protected from browning by using a mixture of ascorbic acid (0.5%) and calcium chloride (0.05%) at pH 7. De Poix *et al.* (1980), in a study on the action of sodium and calcium chloride on preventing browning of apple puree, pointed out that the addition of chlorides delays the occurrence of browning. For a given chloride concentration, the latent period before the advent of browning is proportional to the amount of ascorbic acid added.

Sapers and Douglas (1987) showed that the treatment of cut apple surfaces with 1% citric acid monohydrate solutions containing 0.4, 0.8, 1.6 or 3.2% ascorbic acid, is effective in the inhibition of browning.

The phosphate esters of ascorbic acid (ascorbic acid-2-phosphate and ascorbic acid-2-triphosphate) were investigated as alternative sources to ascorbic acid for the inhibition of browning at the cut surfaces of raw apples (Sapers *et al.* 1989). The phosphate esters were more effective than similar concentrations of ascorbic acid in the prevention of browning in Red Delicious and Winesap apples.

Vacuum and pressure infiltration of ascorbic and erythorbic acid into the cut surfaces of raw apples improved the efficiency of inhibitors (2.25% sodium ascorbate or erythorbate and 0.2% calcium chloride) (Sapers *et al.* 1990).

The influence of ascorbic acid on PPO activity is still controversial. Varoquaux and Sarris (1979) reported that ascorbic acid neither prevented nor activated PPO. Activation of PPO by ascorbic acid has been reported (Krueger 1950). Golan-

Goldhirsh and Whitaker (1984) showed there was rapid inactivation of the mushroom PPO incubated with ascorbic acid without the phenolic substrate. Recently, Hsu *et al.* (1988) reported an inhibiting effect of ascorbic acid on the PPO of mushrooms.

In the present work the inhibiting effect of ascorbic acid, citric acid and sodium chloride, used alone or in mixtures, on the apple PPO activity was studied. As a comparison the PPO inhibition obtained using potassium metabisulfite was also analyzed.

MATERIALS AND METHODS

Raw Materials

Fresh apples (*Malus domestica* Borkh., cv. Golden Delicious) were obtained from Valtellina (Italy), picked at commercial maturity and stored at 2–4C.

After washing in running water the apples were peeled, cored and mechanically diced (14 mm).

PPO Inhibition

Inhibition of PPO in apple cubes was obtained by:

(1) Dipping in aqueous solutions of: (a) L (+) ascorbic acid (AA), (b) citric acid (CA); (c) sodium chloride (NaCl); (d) potassium metabisulfite (PBS). The reagents were of analytical grade (Merck, Darmstadt, Germany). The concentrations used were: (a) 0.2–1.0–2.0–10.0 g/L of ascorbic acid; (b) 0.2–1.0–2.0–10.0 g/L of citric acid; (c) 0.2–0.5–1.0 g/L of sodium chloride; (d) 0.1–0.3–0.5–1.0 g/L of potassium metabisulfite.

(2) Dipping in aqueous solutions of the following mixtures: (a) L (+) ascorbic acid and citric acid (AA + CA); (b) potassium metabisulfite and citric acid (PBS + CA); (c) L (+) ascorbic acid and sodium chloride (AA + NaCl).

The concentrations of the mixtures were: (a) AA 1 g/L and CA 1 g/L; AA 1 g/L and CA 2 g/L; AA 10 g/L and CA 1g/L; AA 10 g/L and CA 2 g/L; (b) PBS 0.1 g/L and CA 1 g/L; PBS 0.1 g/L and CA 2 g/L; PBS 0.3 g/L and CA 1 g/L; PBS 0.3 g/L and CA 2 g/L; (c) AA 10 g/L and NaCl 0.2 g/L; AA 10 g/L and NaCl 0.5 g/L; AA 10 g/L and NaCl 1 g/L.

The solid/liquid ratio was 1:5 and deionized water at 16–18C was used.

The dipping time was 5 min and the apple cubes were stirred every 30 s with a plastic spatula. Lengthening the dipping time to 15 min did not significantly influence the PPO inhibition (data not reported). After dipping, the apple cubes were left to drip for 2–3 min and kept at 2C prior to analysis. Apple cubes dipped in deionized water for 5 min, were used as control.

TABLE 1.
ASCORBIC ACID AND POTASSIUM METABISULFITE INHIBITION OF APPLE PPO ACTIVITY

Ascorbic acid				Potassium metabisulfite			
Conc. (g/L)	activity ($0.001\Delta A_{420} min^{-1} ml^{-1}$) X	s.d.	Inhibition %	Conc. (g/L)	activity ($0.001\Delta A_{420} min^{-1} ml^{-1}$) X	s.d.	Inhibition %
0	594	30	0	0	605	38	0
0.2	880	41	-48*	0.1	516	32	15
1.0	670	31	-13	0.3	120	8	80
2.0	780	33	-31	0.5	27	0.9	96
10.0	890	36	-50	1.0	0	-	100

X = means of 5 replicates
s.d. = standard deviation
(*) = negative values indicate PPO activation

PPO Activity

The apple cubes were immersed in liquid nitrogen and ground immediately in a stainless steel blender.

A 10 g aliquot of the ground apple was mixed with 10 ml of citric-phosphate buffer (McIlvaine) pH 6.5 using a Ultra-Turrax T25 (Janke & Kunkel) homogenizer (20500 rpm) for 60 s. The homogenate was centrifuged (45000 × g) at 4C for 30 min (Beckman J2-21 centrifuge).

The supernatant was filtered on saturated paper (Schleicher & Schull GmbH, 589) and analyzed for PPO activity at 420 nm and 25C. One milliliter catechol solution (0.175 M) and 2 ml of citric-phosphate buffer (McIlvaine) pH 6.5 were added to 0.5 ml of PPO extract (Pifferi and Cultrera 1972). The enzyme activity was calculated on the basis of the slope of the linear portion of the curve plotted with ΔA_{420} against time (up to 3 and 6 min in untreated and treated samples, respectively). One unit of enzyme activity was defined as $0.001\ \Delta A_{420}\ min^{-1}$ (ml of extract)$^{-1}$.

TABLE 2.
CITRIC ACID INHIBITION OF APPLE PPO ACTIVITY

Conc. (g/L)	activity ($0.001\,\Delta A_{420} min^{-1} ml^{-1}$) X	s.d.	Inhibition %
0	597	33	0
0.2	624	32	-4.5"
1.0	666	34	-11.5
2.0	574	27	3.8
10.0	492	20	17.6

X = means of 5 replicates
s.d. = standard deviation
" = negative values indicate PPO activation

TABLE 3.
SODIUM CHLORIDE INHIBITION OF APPLE PPO ACTIVITY

Conc. (g/L)	activity ($0.001\,\Delta A_{420} min^{-1} ml^{-1}$) X	d.s.	Inhibition %
0	784	31	0
0.2	1027	46	-31"
0.5	1480	50	-88
1.0	1483	50	-89

X = means of 5 replicates
s.d. = standard deviation
" = negative values indicate PPO activation

Residual PPO activity was expressed as ratio of treated sample versus its control. pH was determined according to the AOAC Methods (1980).

RESULTS AND DISCUSSION

PPO Inhibition Using Ascorbic Acid, Citric Acid and Sodium Chloride

The inhibition of PPO activity in apple cubes after dipping in ascorbic acid, potassium metabisulfite, citric acid and sodium chloride solutions is reported in Table 1, 2 and 3, respectively. When the concentrations of ascorbic acid, citric acid and sodium chloride in the solutions were increased, the pH of the solutions and the pH of apple cubes after dipping did not significantly change (data not reported). Concentrations between 0.2 and 10 g/L of ascorbic acid did not inhibit, but activated PPO. The increase of PPO activity could be due to an insufficient concentration of the ascorbic acid, which at low concentrations might act as a prooxidant (Kanner *et al.* 1977; Vamos-Vigyazo 1981).

The inhibition of the PPO was correlated to the amount of potassium metabisulfite; 1g/L completely inhibited PPO activity (Table 1).

Citric acid in concentrations between 0.2 and 10g/L had a low inhibiting effect and only at the maximum concentration (Table 2). Citric acid is not an antioxidant agent but its inhibiting effect could be related to the phenolase Cu-chelating power.

Sodium chloride in concentrations between 0.2 and 1 g/l activated PPO (Table 3): 1g/L increased PPO activity of about 90%. Concentrations between 0.5 and 1% of sodium chloride had an inhibiting effect on the enzymatic browning of whole apples or apple pieces (Taufel and Voigt 1964) but only concentrations of about 20% inactivated PPO isolated from the apple (Ponting and Joslyn 1948). The inhibitory effect of sodium chloride is attributed to the anion chloride: the action is of the noncompetitive type, as shown for purified PPO from apples (Sharon and Mayer 1967; Janovitz-Klapp *et al.* 1990). The increase of PPO activity observed in this study could be related to conformational changes of the enzyme or protein association or dissociation due to the modification of the ionic strength.

PPO Inhibition Using Binary Mixtures

(1) Mixtures of L (+)-Ascorbic Acid and Citric Acid (AA + CA). In Table 4 the inhibitory effect of mixtures of ascorbic acid and citric acid on apple PPO is reported. As a comparison the PPO inhibition obtained using mixtures of potassium metabisulfite and citric acid (PBS + CA) is also reported.

Ascorbic acid and citric acid inhibited PPO activity, and citric acid increased the inhibiting effect of ascorbic acid. When 2 g/L of citric acid instead of 1 g/L

TABLE 4.
ASCORBIC ACID AND CITRIC ACID, AND POTASSIUM METABISULFITE AND CITRIC ACID INHIBITION OF APPLE PPO ACTIVITY

Ascorbic acid + Citric acid				Potassium metabisulfite + Citric acid			
Conc. (g/L)	activity ($0.001\Delta A_{420}min^{-1}ml^{-1}$) X	s.d.	Inhibition %	Conc. (g/L)	activity ($0.001\Delta A_{420}min^{-1}ml^{-1}$) X	s.d.	Inhibition %
0	899	35	0	0	899	35	0
1+1	471	22	47.6	0.1+1	310	18	65.5
1+2	577	25	35.8	0.1+2	304	17	66.2
10+1	573	24	36.3	0.3+1	53	2	94.1
10+2	116	8	87.1	0.3+2	34	2	96.2

X = means of 5 replicates
s.d. = standard deviation

were added to 10 g/L of ascorbic acid the PPO inhibition increased from 36.3% to 87.1%. Citric acid also increased the inhibiting effect of metabisulfite but independelty from the concentration.

(2) Mixture of L (+)-Ascorbic Acid and Sodium Chloride (AA + NaCl). The inhibition of PPO using mixtures of ascorbic acid and sodium chloride is reported in Table 5. Ten grams/L of ascorbic acid with 0.5 g/L of sodium chloride completely inhibited the PPO activity. Sodium chloride is more efficient than citric acid, as by adding 0.5 g/L of NaCl instead of 2 g/L of citric acid to 10 g/L of ascorbic acid, the PPO inhibition was 100% compared to 87%.

After the treatments using the most efficient mixtures, i.e., ascorbic acid and citric acid (10 + 2 g/L) and ascorbic acid and sodium chloride (10 + 0.5 g/L), the pH values of the apple cubes were only slightly lower than the values of the untreated apples (data not reported). Therefore, in this case, the pH could be excluded from playing a part in the PPO inhibition.

The explanation of De Poix *et al.* (1980) of the synergic phenomena between ascorbic acid and sodium chloride is that the ascorbic acid reduces the quinones enzymatically formed and delays browning without altering the enzymatic activity, whereas the anion chloride directly inhibits the PPO.

TABLE 5.
ASCORBIC ACID AND SODIUM CHLORIDE INHIBITION OF APPLE PPO ACTIVITY

Conc. (g/L)	activity ($0.001\Delta A_{420} min^{-1} ml^{-1}$) X	s.d.	Inhibition %
0	1057	43	0
10+0.2	563	23	46.8
10+0.5	0	--	100.0
10+1	0	--	100.0

X = means of 5 replicates
s.d. = standard deviation

In this study PPO activity was increased by ascorbic acid alone (0.2–10 g/L) or by sodium chloride alone (0.2–1 g/L), on the contrary PPO activity was inhibited by the mixture of ascorbic acid and sodium chloride and the inhibition was directly related to the concentration of sodium chloride. This suggests that a more complex mechanism could be involved in the enhancement of the inhibitory action of ascorbic acid due to sodium chloride.

CONCLUSIONS

A 90–100% inhibition of PPO in apple cubes is obtained by using mixtures of ascorbic acid and citric acid (10 + 2 g/L) and ascorbic acid and sodium chloride (10 + 0.5 g/L). A similar inhibition is obtainable with 0.5% potassium metabisulfite or potassium metabisulfite and citric acid (0.3 + 2 g/L) mixture.

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