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# Inhibition Studies on Apple Polyphenol Oxidase

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Inhibition studies by reductant, carboxylic acid, and halide compounds have been carried out on purified apple polyphenol oxidase (PPO). When ascorbic acid, cysteine, or bisulfite was added, a lag period was observed in the color formation whereas oxygen uptake was immediate. The most efficient compound in decreasing the remaining activity was bisulfite followed by cysteine and ascorbic acid. All tested aromatic carboxylic acids were pure competitive inhibitors. For a same substitution, inhibition decreased in the order cinnamic, benzoic, phenylpropionic, and phenylacetic. In each series, inhibition was slightly enhanced by *p*-hydroxy substitution and greatly decreased by *m*-methoxy substitution. Inhibition increased as pH was lowered from 5 to 3.6, and the results indicated that the neutral form of the carboxyl group was mainly responsible for inhibition. Sodium halides were inhibitors and the decreasing order was NaF, NaCl, NaBr, and NaI. When pH was varied between 3.5 and 5, fluoride exhibited the same behavior as the carboxylic inhibitors. The $K_i$ for HF was close to 4 μM; thus it was by far the most potent inhibitor of apple PPO. Chloride was a noncompetitive inhibitor, and an equation is given for the variation of the apparent $K_i$ of chloride with pH.

Enzymatic browning in fruits and vegetables is often an undesirable reaction, the prevention of which has always been a challenge to food scientists (Ponting, 1960). The oxidative browning of fruits is mainly due to polyphenol oxidase (EC 1.14.18.1; PPO). PPO has been the subject of recent reviews (Vamos-Vigyazo, 1981; Whitaker, 1985; Mayer, 1987). It is a copper enzyme that in the presence of oxygen catalyzes the oxidation of phenolic substrates in quinones, which then are polymerized to brown, red, or black pigments.

According to Mayer and Harel (1979), PPO inhibitors can be grouped into two classes: compounds that interact with copper in the enzyme and those that affect the active site for the phenolic substrate. Among the second type of inhibitors, aromatic carboxylic acids of the benzoic and cinnamic series have been widely studied since the first works of Kuttner and Wagreich (1953) and Krueger (1955). Although most authors found that these compounds were competitive inhibitors of PPO due to their structural similarities with phenolic substrates, some papers indicated that the type of inhibition was dependent on the substrate being used for assay and was either competitive, noncompetitive, or mixed (Soler-Martinez et al., 1965; McRae and Duggleby, 1968; Duckworth and Coleman, 1970; Rivas and Whitaker, 1973; Pifferi et al., 1974; Walker and Wilson, 1975; Walker and McCallion, 1980; Gunata et al., 1987). For the first type of inhibitors, inhibition by metal ion chelators such as azide (Healey and Strothkamp, 1981), cyanide (Duckworth and Coleman, 1970), and diethyldithiocarbamate, which are more or less specific for copper, as well as inhibition by inorganic halides has been studied for PPO from different sources (Krueger, 1955; Sharon and Mayer, 1967; Ben-Shalom et al., 1977; Penafiel et al., 1984; Martinez et al.,

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1986; Rouet-Mayer and Philippon, 1986). In the latter case, it has been shown by the same authors that inhibition is strongly pH dependent, increasing when the pH was decreased. A third class of reagents can be used to inhibit the browning reaction, namely, some reducing compounds such as bisulfite, thiol compounds, and ascorbic acid or its derivatives. The main effect of these reagents is either to reduce the *o*-quinones back to the *o*-dihydroxyphenols such as ascorbic acid (Varoquaux and Sarris, 1979; Golan-Goldhirsh et al., 1984) or to form with *o*-quinones colorless addition products such as bisulfite (Embs and Markakis, 1965; Wedzicha, 1984) and thiol compounds (Sanada et al., 1972; Cheynier et al., 1986). In another way, according to Khan (1985), amino acids, including cysteine, can inhibit PPO through the formation of stable complexes with copper. With the exception of bisulfite, which is known to cause direct and irreversible PPO inactivation (Sayavedra-Soto and Montgomery, 1986), the direct inhibition of the enzyme by ascorbic acid and thiol compounds remains to be proved (Muneta, 1981; Golan-Goldhirsh and Whitaker, 1984; Hsu et al., 1988).

The purpose of this paper is to provide further insight into some inhibitors of purified apple PPO and study the pH effect on the inhibition.

## MATERIALS AND METHODS

**Materials.** Apples from the variety Red Delicious picked at the commercial maturity were used as an enzyme source. The PPO was 120-fold purified from the cortex in three steps, extraction, fractional precipitations by ammonium sulfate, and hydrophobic chromatography with Phenyl-Sepharose CL4B (Pharmacia), according to Janovitz-Klapp et al. (1989).

**Assay Procedure.** The substrate was 20 mM in 4-methylcatechol in a McIlvaine buffer at pH 4.5. PPO activity was routinely assayed by polarography at 30 °C using air-saturated substrate solution. For inhibition studies by carboxylic acids and halide salts at pH 4.5, 4-methylcatechol was varied from 20 to 2 mM in the control and with three concentrations of inhibitor. When the pH was varied, the study was performed in the same substrate concentration range for control and for one concentration of inhibitor close to the apparent $K_i$ obtained at pH 4.5. It has been checked that changing citric acid concentrations from 50 to 70 mM, corresponding to a pH change from 5 to 3.5 with the McIlvaine buffer solutions, had no effect on the enzyme activity. All assays were performed in duplicate. Apparent $K_m$ and $V_m$ were determined by using a nonlinear regression data analysis program developed for the IBM PC by Leatherbarrow (1987). For ascorbic and erythorbic acids, cysteine, and sodium bisulfite, absorbance changes were also monitored at 395 nm by spectrophotometry under the above substrate conditions. PPO activity was expressed as nanomoles of oxygen consumed per second (nkat) when measured by polarography. It was expressed as absorbance unit change at 395 nm per second (total cuvette volume = 3 mL) when assayed by spectrophotometry. p$K$ values of carboxylic acids and fluorhydric acid were either measured or taken from the *Merck Index* (1983, Tenth Edition).

## RESULTS AND DISCUSSION

**Effects of Ascorbic Acid, Cysteine, and Bisulfite.** The effects of increasing amounts of ascorbic acid, cysteine, and bisulfite were studied on apple PPO both spectrophotometrically and polarographically (Figure 1). As usually found for PPO from other sources (Embs and Markakis, 1965; Muneta, 1981; Golan-Goldhirsh and Whitaker, 1984), in the presence of reducing compounds, a lag period was apparent before any change in absorbance whereas the oxygen uptake was immediate. When erythorbic acid was used, the results obtained were equivalent to those observed with ascorbic acid (data not shown). Therefore, the two isomers are equally effective for the inhibition of the browning reaction, in agreement with the results of Sapers and Ziolkowski (1987). For all tested compounds, the higher the reductant concentration was, the longer was the lag period and the lower was the rate following the lag period. By polarography, there was no effect on enzyme activity until 0.5 mM of either ascorbic acid or cysteine whereas at this concentration sodium bisulfite was slightly inhibiting (<10%). By spectrophotometry in the same concentration range, the effectiveness of the reductants in the inhibition of the color formation was in the decreasing order sodium bisulfite, cysteine, and ascorbic acid. By comparison with the oxygen uptake, it was apparent that a nonnegligible part of the activity loss during the lag phase was only due to the decrease in the oxygen concentration in the cuvette. Moreover, for all tested compounds the residual activity for color formation was nearly zero for 0.5 mM in reductant. This concentration is about twice that of the initial content in dissolved oxygen for air-saturated aqueous solution at 30 °C (Wise and Naylor, 1985). Thus, during the lag phase, a great part of the oxygen was consumed either for the ascorbic acid oxidation or for the formation of a conjugated product between S-containing reductants and *o*-quinones with always a stoichiometry of 1 mol of oxygen for 2 mol of reductant. However, compared to ascorbic acid, it was apparent that after the reductant consumption, the remaining activity was decreased with cysteine. Therefore, the addition product formed by cysteine with *o*-quinones of 4-methylcatechol could be a slight inhibitor of the reaction, in agreement with the earlier findings of Sanada et al. (1972). These authors indicated that conjugation products of cysteine with DOPA exhibited inhibitory effects on the melanin formation by mushroom tyrosinase. In another way, Singleton et al. (1985) claimed that the *S*-glutathionylcaftaric acid (conjugation product of caffeoyltartaric acid and glutathione) was not a strong inhibitor of the grape PPO. The remaining activity was much more decreased with bisulfite. This suggests that the inhibition of bisulfite is enhanced by its direct effect on the PPO, which according to Sayavedra-Soto and Montgomery (1986) was greatly increased by pH below 5.

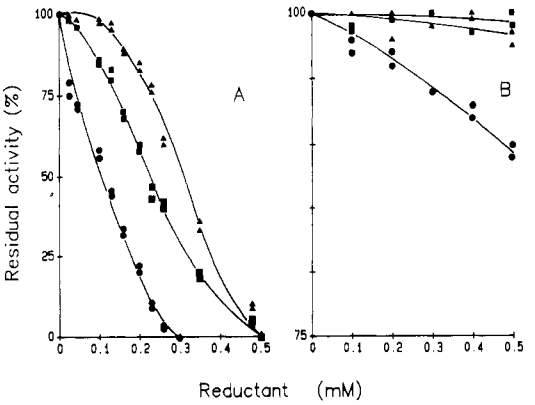


**Figure 1.** Effect of reductant concentration on purified apple PPO measured by spectrophotometry (A) and by polarography (B): (▲) ascorbic acid; (■) cysteine; (●) sodium bisulfite. Assays were performed at 30 °C and pH 4.5 with 20 mM 4-methylcatechol.

**Inhibition by Carboxylic Acids.** First, the inhibitory properties on apple PPO of the benzoic acid series and the corresponding cinnamic acid series have been compared using 4-methylcatechol as substrate. In each case, the type of inhibition was deduced from Lineweaver–Burk double-reciprocal plots. The inhibitory constants

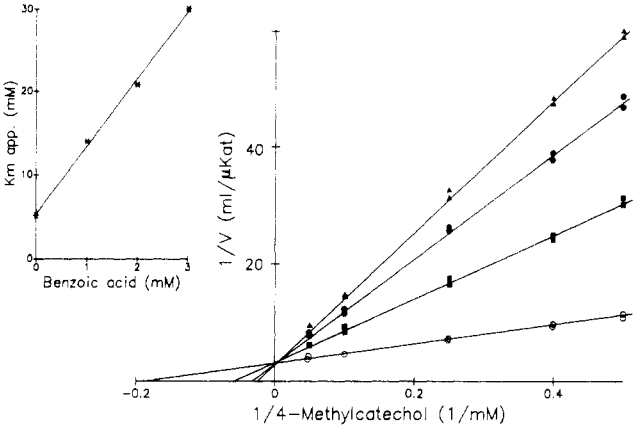


**Figure 2.** Inhibition of purified apple PPO by benzoic acid: (○) control; (■) 1 mM benzoic acid; (●) 2 mM benzoic acid; (▲) 3 mM benzoic acid. Inset: Effect of benzoic acid concentration on apparent $K_m$.

**Table I. Inhibition Effects of Carboxylic Acids on Purified Apple PPO in Relation to Their Structures[a]**

| acid | substitution p | m | m′ | app $K_i$, mM |
|---|---|---|---|---|
| **Benzoic Series** | | | | |
| benzoic | | | | 0.64 |
| *p*-hydroxybenzoic | OH | | | 0.57 |
| vanillic | OH | $OCH_3$ | | 10 |
| syringic | OH | $OCH_3$ | $OCH_3$ | 34.5 |
| **Cinnamic Series** | | | | |
| cinnamic | | | | 0.092 |
| *p*-coumaric | OH | | | 0.04 |
| ferulic | OH | $OCH_3$ | | 0.29 |
| sinapic | OH | $OCH_3$ | $OCH_3$ | 15 |
| **Phenylacetic, Phenylpropionic, and Sorbic Acids** | | | | |
| phenylacetic | | | | 13 |
| phenylpropionic | | | | 1.4 |
| *p*-hydroxyphenylpropionic | OH | | | 1.1 |
| sorbic | | | | 0.51 |

[a] Assays were performed at pH 4.5 with 4-methylcatechol, $K_m$ = 5.2 mM (Janovitz-Klapp et al., 1989).

$K_i$ were calculated from secondary plots of apparent $K_m$ against inhibitor concentration. Good straight lines were obtained, and a typical example is shown in Figure 2 for benzoic acid. All aromatic carboxylic acids tested were competitive inhibitors, and their $K_i$ are reported in Table I. It appeared that cinnamic acids were more potent inhibitors than their benzoic homologues since the $K_i$ values were between 2 and 30 times lower than in the benzoic series. Moreover, in both series, *p*-hydroxy substitution slightly enhanced the inhibitory properties, whereas adding one or two methoxy groups in the meta position greatly decreased the inhibitor affinity for the enzyme. In another way, when the carboxyl group was separated from the benzene cycle by a methylene group as in phenylacetic acid, the inhibition was greatly reduced. However, it was partially restored by an additional methylene group as in phenylpropionic acid and again enhanced by a *p*-hydroxy substitution (*p*-hydroxyphenylpropionic acid). Sorbic acid was almost equally efficient as a competitive inhibitor as benzoic acid. Therefore, it appears that the presence of the benzene nucleus is not an absolute structural requirement for the inhibitory effect since it can be replaced by conjugated double bonds, in agreement with the results of Soler-Martinez et al. (1965).

The effect of pH on the inhibition by three carboxylic acids, namely, sorbic, cinnamic, and benzoic acids, has been studied. The results are illustrated in Figure 3. As

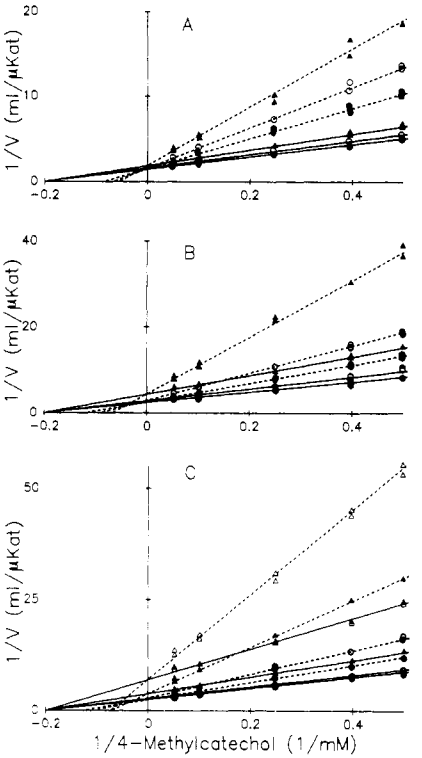


**Figure 3.** Effect of pH on inhibition of purified apple PPO by 1 mM sorbic acid (A), 0.1 mM cinnamic acid (B), and 0.5 mM benzoic acid (C): (●) pH 5; (○) pH 4.5; (▲) pH 4; (△) pH 3.6. The full lines are for the control experiments, and the dashed lines are for the experiments with the added carboxylic acid.

**Table II. Inhibition of Purified Apple PPO by Sorbic, Cinnamic, and Benzoic Acids at Different pH[a]**

| | pH 3.6 | 4 | 4.5 | 5 |
|---|---|---|---|---|
| **Sorbic Acid (1 mM), p$K$ = 4.76** | | | | |
| app $K_m$ | | 19 | 15.4 | 12.5 |
| app $K_i$[b] | | 0.4 | 0.51 | 0.98 |
| $\beta$ = [AH]/([A⁻] + [AH])[c] | | 0.85 | 0.65 | 0.37 |
| $K_{i,AH}$[d] | | 0.34 | 0.33 | 0.36 |
| **Cinnamic Acid (0.1 mM), p$K$ = 4.46** | | | | |
| app $K_m$ | | 13.9 | 10.7 | 7.81 |
| app $K_i$[b] | | 0.06 | 0.094 | 0.20 |
| $\beta$ = [AH]/([A⁻] + [AH])[c] | | 0.74 | 0.48 | 0.22 |
| $K_{i,AH}$[d] | | 0.044 | 0.045 | 0.044 |
| **Benzoic Acid (0.5 mM), p$K$ = 4.20** | | | | |
| app $K_m$ | 13 | 11.8 | 9.26 | 7.35 |
| app $K_i$[b] | 0.33 | 0.39 | 0.64 | 1.21 |
| $\beta$ = [AH]/([A⁻] + [AH])[c] | 0.80 | 0.61 | 0.33 | 0.14 |
| $K_{i,AH}$[d] | 0.27 | 0.24 | 0.21 | 0.17 |

[a] Michaelis and inhibition constants are expressed in mM. [b] $K_m$ value of 5.2 mM was taken for 4-methylcatechol (Janovitz-Klapp et al., 1989). [c] $A^-$ and AH are the anion and the undissociated forms of the acid, respectively. [d] The undissociated form AH was postulated to be the only species responsible for inhibition.

already stated (Kuttner and Wagreich, 1953; Krueger, 1955), the extent of inhibition is pH dependent. Between pH 3.6 and 5 and without inhibitor, the $K_m$ value of apple PPO for 4-methylcatechol was independent of pH. This confirms our previous results that hydrogen ion can be considered as a noncompetitive inhibitor for apple PPO for pH lower than pH 5 (Janovitz-Klapp et al., 1989). When the carboxylic acid was added at a constant concentration, the $V_m$ value, which decreased when the pH decreased, was independent of the presence of the inhibitor. In contrast, the apparent $K_m$ increased as the pH

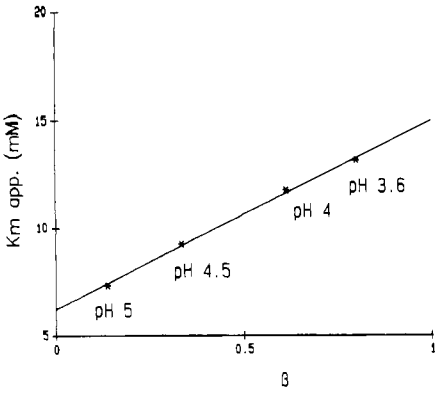


**Figure 4.** Effect of pH on apparent $K_m$ of purified apple PPO in the presence of benzoic acid. $\beta$ was calculated by using a p$K$ value of 4.20 for benzoic acid.

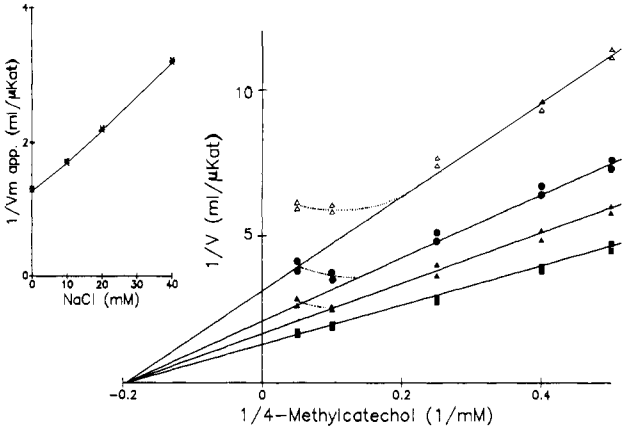


**Figure 5.** Inhibition of purified apple PPO by sodium chloride: (■) control; (▲) 10 mM NaCl; (●) 20 mM NaCl; (△) 40 mM NaCl. The dashed lines indicated the deviation from pure noncompetitive inhibition. Inset: Effect of NaCl concentration on apparent $V_m$.

was lowered (Table II). Therefore, when the pH was decreased, all tested carboxylic acids remained pure competitive inhibitors but their effectiveness increased.

The first explanation is that a protonated form of apple PPO was sensitive to the carboxylic acid inhibitors. If this were so, the dissociation constants ($K_i$) of the enzyme–inhibitor complexes would be independent of pH. In fact, the apparent $K_i$'s decreased as the pH was lowered (Table II). Hence, the alternative hypothesis is that the pH affected the inhibitor and thus that the undissociated acid is responsible for inhibition by formation of a complex with copper at the active center (Robb et al., 1966). Using the p$K$ values for the three carboxylic acids, namely, 4.76, 4.46, and 4.20 for sorbic, cinnamic, and benzoic acids, respectively, we calculated the proportion $\beta$ of the neutral form for each acid at each pH (Table II). The inhibitor constants were then corrected, assuming that only the undissociated form had inhibitory properties (Table II). It can be seen that the corrected $K_i$'s of sorbic and cinnamic acids were independent of pH whereas for benzoic acid, the $K_i$ values slightly increased as the pH was lowered. In the latter case, inhibition by anion cannot be ruled out. If both forms were assumed to be competitive inhibitors, then

$$K_{m,app} = K_m\left(1 + \frac{[B^-]}{K_{i,B^-}} + \frac{[BH]}{K_{i,BH}}\right) \quad (1)$$

where $B^-$ and BH are the dissociated and neutral forms of benzoic acid and $K_{i,B^-}$ and $K_{i,BH}$ are their respective inhibitor constants. The latter values can be calculated by plotting $K_{m,app}$ versus $\beta$, the ratio of [BH] to ([BH] + [$B^-$]). A line was obtained, the extrapolation of which at $\beta = 0$ and $\beta = 1$ allowed the calculation of $K_{i,B^-}$ and $K_{i,BH}$, respectively (Figure 4). Values of 2.5 and 0.25 mM were obtained, indicating that the enzyme exhibited a much greater affinity for the neutral form than for the anion. This result can also explain why according to Pifferi et al. (1974) in the benzoic series, the esterification of the carboxyl group greatly decreased but did not suppress the inhibitory property, i.e., the affinity of the molecule for the enzyme.

One can speculate that the binding site of apple PPO recognized conjugated double bonds, which are contained either in the benzene nucleus or in an unsaturated alkyl chain. When a carboxyl group was present either directly bound to the benzene cycle (benzoic series) or to the conjugated double bonds (cinnamic series or sorbic acid), it could form a complex with the copper at the active center. In this case, when such a structure is present in the same molecule together with an *o*-diphenolic function, the interaction of copper with the *o*-diphenolic part would be greatly reduced. We have observed such an effect with caffeic and protocatechuic acids. At pH 4.5, we found $K_m$ values of 0.13 and 1.4 mM, respectively, whereas the $V_m$ values were very low compared to those obtained with 4-methylcatechol and chlorogenic acid (<5%). We have obtained 5.2 and 4.2 mM respectively for the $K_m$ of the two latter substrates (Janovitz-Klapp et al., 1989). One can think that between caffeic and chlorogenic acids, the esterification of the carboxyl group by quinic acid greatly reduced the affinity of the caffeoyl moiety for apple PPO as illustrated by the increase of $K_m$. In another way, it prevented the formation of the complex between copper and the vicinal undissociated carboxylic acid, leaving the metal free for the catalysis of the *o*-diphenol oxidation as illustrated by the large increase of $V_m$. The inhibition of substrate molecules such as protocatechuic and caffeic acids is only effective for pH lower than 5; therefore the PPO whose optimum pH is close to 7 did not exhibit such an effect.

**Inhibition by Halides.** Inorganic halides show an inhibitory effect on the PPO activity isolated from the apple. When the sodium chloride concentration was varied at pH 4.5, the inhibition was of the noncompetitive type as determined by the Lineweaver–Burk plot (Figure 5). However, for concentrations higher than 10 mM in substrate, a deviation from pure noncompetitive inhibition was observed for high chloride concentrations. The inhibition constant calculated from the secondary plot (insert) was close to 20 mM. A noncompetitive pattern of partially purified apple PPO was also obtained by Sharon and Mayer (1967). When other sodium halides were tested at the same pH, they all appeared as competitive inhibitors (Figure 6). By far, the fluoride salt was the most potent since its apparent $K_i$ was found at 0.07 mM whereas those of bromide and iodide were very close and much higher, namely, 106 and 117 mM, respectively. Again a slight deviation was observed only for bromide and iodide at 20 mM in 4-methylcatechol. For frog epidermis tyrosinase, Penafiel et al. (1984) have obtained inhibition by halides. However, they indicated that chloride ion was a competitive inhibitor of the DOPA oxidation. Unfortunately, they did not specify the type of inhibition they found for other halide salts. For apple PPO, Sharon and Mayer (1967) found also that fluoride was the most potent inhibitor followed by chloride and bromide whereas iodide was not tested. In a comparative study of tyrosinases from different sources, Martinez et al. (1986) have shown that the inhibition order

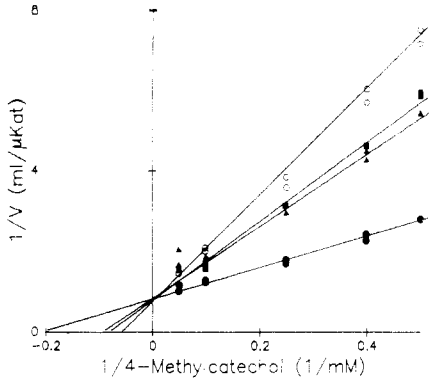


**Figure 6.** Inhibition of purified apple PPO by sodium halides: (●) control; (○) 0.15 mM NaF; (■) 0.15 M NaBr; (▲) 0.15 M NaI.

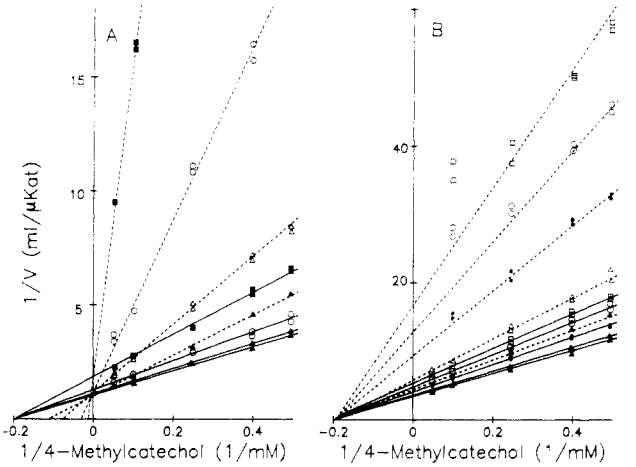


**Figure 7.** Effect of pH on inhibition of purified apple PPO by 0.15 mM sodium fluoride (A) and 20 mM sodium chloride (B): (▲) pH 5; (△) pH 4.5; (●) pH 4.2; (○) pH 4; (□) pH 3.8; (■) pH 3.5. The full lines are for the control experiments, and the dashed lines are for the experiments with the added sodium halide.

was dependent on the enzyme source. They postulated that it was the result of the balance between the stability of the copper–halide complex responsible for the inhibition and the accessibility of the active site to the halide.

The effect of pH on inhibition of apple PPO has been compared for sodium chloride and sodium fluoride between pH 3.5 and 5. The results are illustrated as Lineweaver–Burk plots in Figure 7. The fluoride salt appeared as a pure competitive inhibitor for each pH tested. Moreover, its effectiveness was greatly increased as the pH was lowered from 5 to 3.5 since the apparent $K_i$ varied from 0.98 to 0.014 mM in this pH range (Table III). In agreement with Robb et al. (1966), the same arguments used for carboxylic acids can be applied to fluoride since its ionization is pH dependent. With a p$K$ value of 3.19 for hydrofluoric acid, the corrected $K_i$ is then independent of the pH (Table III). The obtained value slightly higher than 4 $\mu$M shows that hydrofluoric acid was by far the most potent inhibitor of apple PPO that we have tested. The explanation of pH dependence for carboxylic acids and fluoride cannot be extrapolated to chloride salt because it was fully dissociated in the studied pH range. Ben-Shalom et al. (1977) and Penafiel et al. (1984) proposed that chloride and copper formed a complex after displacement of a protonated histidine residue from copper. The latter authors postulated a mechanism where chloride binds to the enzyme in competition with the substrate only when the enzyme molecule is in the protonated form. Since chloride was found to be a noncompetitive inhibitor for apple PPO, it can also interact with

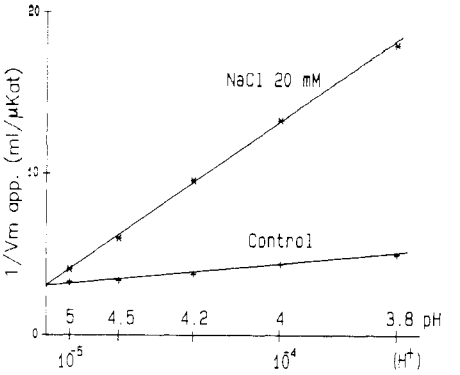


**Figure 8.** Effect of pH on apparent $V_m$ of purified apple PPO with and without sodium chloride: (■) control; (□) 20 mM NaCl.

**Table III. Inhibition of Apple PPO by Sodium Fluoride (0.15 mM; p$K$ = 3.19) at Different pH Values[a]**

| | pH | | | |
|---|---|---|---|---|
| | 3.5 | 4 | 4.5 | 5 |
| app $K_m$ | 60 | 30 | 13.6 | 7.8 |
| app $K_i$[b] | 0.014 | 0.032 | 0.093 | 0.98 |
| $\beta = [FH]/([F^-] + FH])$ | 0.33 | 0.13 | 0.047 | 0.015 |
| $K_{i,FH}$[c] | 0.0047 | 0.0042 | 0.0044 | 0.0045 |

[a] Michaelis and inhibition constants are expressed in mM. [b] A $K_m$ value of 5.2 mM was taken for 4-methylcatechol (Janovitz-Klapp et al., 1989). [c] The undissociated form FH was postulated to be the only species responsible for inhibition.

the enzyme–substrate complex in the protonated form. Therefore, the velocity equation for apple PPO catalysis in the presence of chloride can be written in the form

$$v = \frac{V_m [S]}{\left(1 + \frac{[H^+]}{K_a}\left(1 + \frac{[Cl^-]}{K_i}\right)\right)([S] + K_m)} \quad (2)$$

where $K_a$ and $K_i$ are the dissociation constants for the protonatable group of apple PPO and for the enzyme–chloride complex, respectively. The plot of the reciprocal of the apparent maximal velocity ($1/V_{m,app}$) versus the proton concentration without and with inhibitor leads to straight lines (Figure 8). The two lines intersected on the ordinate axis at the theoretical $V_m$ value. From the intercepts of the lines without and with chloride on the $[H^+]$ axis, $K_a$ and $K_i$ can be respectively calculated, yielding p$K_a$ = 3.65 and $K_i$ = 2.4 mM. The p$K_a$ value is in agreement with that found by Janovitz-Klapp et al. (1989). For a given pH, the apparent $K_i$ for chloride is

$$K_{i,app} = K_i\left(\frac{K_a}{[H^+]} + 1\right) \quad (3)$$

Thus, for pH below p$K_a$, the limiting value for $K_{i,app}$ is 2.4 mM whereas for pH greater than p$K_a$, an increase of 1 pH unit corresponds roughly to a tenfold increase of the apparent $K_i$ for chloride.

In conclusion, these inhibition studies have stressed the major importance of pH for the control of enzymatic browning. From a technological point of view, the use of sodium bisulfite is discussed more and more because of potential hazards (Taylor and Bush, 1986). Alternatives to this additive such as sodium chloride and ascorbic, citric, and sorbic acids have been proposed in order to inhibit enzymatic browning in apple and potato (DePoix et al., 1980; Langdon, 1987; Santerre et al., 1988). It is clear that a formulation using these additives must take care of the final pH for successful control of enzymatic browning.

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