Oxidation of Chlorogenic Acid, Catechins, and 4-Methylcatechol in Model Solutions by Combinations of Pear (*Pyrus communis* Cv. Williams) Polyphenol Oxidase and Peroxidase: A Possible Involvement of Peroxidase in Enzymatic Browning[†]

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To clarify the role of pear peroxidase (POD) in enzymatic browning, oxidation of 4-methylcatechol, chlorogenic acid, and (−)-epicatechin catalyzed by purified polyphenol oxidase (PPO), purified POD, or combinations of the two enzymes was followed by HPLC. It was shown that pear POD had no oxidative (oxygen dependent) activity. However, in presence of PPO, POD enhanced the phenol degradation. Moreover, when PPO was entirely inhibited by NaCl after different oxidation times, addition of POD led to a further consumption of the phenolic compound. Two mechanisms have been proposed to explain this additional consumption. First, our results have demonstrated that, whatever the substrate used, PPO oxidation generated H_2O_2, the amount of which varies with the phenolic structure. Second, quinonic forms are used by POD as peroxide substrate. These two mechanisms associated with the kinetic properties of pear PPO and POD are consistent with an effective involvement of pear POD in enzymatic browning.

Keywords: *Enzymatic browning; pear; peroxidase; polyphenol oxidase*

INTRODUCTION

Browning of damaged tissues of fruits and vegetables during postharvest handling and processing is one of the main causes of quality loss (Mathew and Parpia, 1971). The brown color development is primarily related to the oxidation of phenolic compounds. This reaction, mainly catalyzed by polyphenol oxidase (EC 1.14.18.1; PPO), results in the formation of *o*-quinones which subsequently polymerize, leading to brown pigments (Nicolas *et al.*, 1994). Peroxidases (EC 1.11.1.7; POD) may also contribute to enzymatic browning. These enzymes, the primary function of which is to oxidize hydrogen donors at the expense of peroxides, are highly specific for H_2O_2. However, they accept a wide range of hydrogen donors, including polyphenols. POD are able to oxidize hydroxycinnamic derivatives and flavans (Robinson, 1991; Nicolas *et al.*, 1994), *i.e.* the main phenolic structures implicated in enzymatic browning. They also oxidize flavonoids (Miller and Schreier, 1985; Richard and Nicolas, 1989), which are not PPO substrates but are found degraded in bruised fruits. Part of this degradation has been ascribed to co-oxidation reactions (Richard-Forget, 1992). Involvement of PODs in enzymatic browning has been assumed by numerous authors (Burnette, 1977; Williams *et al.*, 1985; Nicolas *et al.*, 1994) and has also been reported in slow processes such as pineapple internal browning (Teisson, 1972). This involvement remained however questionable for two main reasons, *i.e.* the high affinity of PPO for its natural substrate and the low H_2O_2 level in fruits. In 1993, Jiang and Miles described, in addition to the

NADH oxidation pathway, another source of H_2O_2 generation. According to these authors, autoxidation and tyrosinase-catalyzed oxidation of (+)-catechin can generate H_2O_2, probably via superoxide. This H_2O_2 could then be used as an electron acceptor by POD.

A more precise understanding of the implication of POD in enzymatic browning is an essential step for a more efficient control of these undesirable reactions, particularly in heat-processed products which frequently contained residual POD activity. Pears are particularly prone to enzymatic browning and this greatly restricts their use as processed products such as juice or purée. We therefore decided to investigate the capacity of hydroxycinnamic esters (chlorogenic acid) and flavans ((−)-epicatechin) to generate H_2O_2 during pear PPO oxidation and, at the same time, the capacity of POD to use the generated H_2O_2 to further oxidize the phenolic compound. The possible use of quinonic forms by POD as peroxide substrate was also considered.

MATERIALS AND METHODS

Materials. Williams pears, picked at commercial maturity, were used as an enzyme source. Pear PPO was 120-fold purified from the cortex in four steps: extraction, ammonium sulfate precipitation, and hydrophobic (Phenyl Sepharose CL4B) and ionic exchange (DEAE Sepharose CL6B) chromatography (Gauillard and Richard-Forget, 1997). Pear POD was 40-fold purified from the cortex according to the procedure developed for apple (Richard and Nicolas, 1989). The procedure included four steps: extraction, ammonium sulfate precipitation, and hydrophobic (Phenyl Sepharose CL4B) and affinity (ConA Sepharose) chromatography. Before use, the purified POD extract was dialyzed overnight against McIlvaine buffer, pH 5.5. Phenyl Sepharose CL4B, DEAE Sepharose CL6B, and ConA Sepharose were from Pharmacia (Uppsala, Sweden). Horseradish peroxidase (HRP), catalase (bovine liver), and all other chemicals were reagent grade quality and supplied by Sigma (St. Louis, MO).

Assay Procedures. PPO activity was polarographically assayed (Gauillard and Richard-Forget, 1997). POD activity

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Involvement of Peroxidase in Enzymatic Browning

J. Agric. Food Chem., Vol. 45, No. 7, 1997 **2473**

was routinely assayed at 30 °C, using 40 mM guaiacol and 10 mM H_2O_2 in a McIlvaine buffer at pH 5.5, in a total volume of 3 mL. Assays were carried out at 470 nm with guaiacol and 400 nm with chlorogenic acid, 4-methylcatechol, and catechins, using a Uvikon 810 (Kontron) spectrophotometer. One unit of peroxidase activity was defined as the amount of enzyme that could cause a change of 1 absorbance unit per second. Activity was expressed in $mUDO \cdot s^{-1}$. For the kinetic parameter (V_m and K_m) determinations, substrate concentrations ranged from 2.38 to 64 mM for guaiacol, 0.5 to 5 mM for chlorogenic acid, 1 to 20 mM for 4-methylcatechol, 0.8 to 8 mM for catechins, and 0.1 to 10 mM for H_2O_2.

Due to their poor solubility in water, flavonols and phenolic acids were dissolved in methanol. The final concentration of methanol in the assay mixture was 2.5%, which, according to Richard and Nicolas (1989), has no effect on POD activity. Reactions were followed by the absorbance decrease at 350 nm for quercetin and its glycosides and at 280 nm for phenolic acids. For the determination of apparent K_m values (H_2O_2 concentration equal to 1 mM), the phenolic concentrations ranged from 0.0125 to 0.05 mM.

Assays were performed in duplicate and kinetic constant values were determined with a nonlinear regression analysis developed for IBM by Leatherbarrow (1987).

Oxidation Systems. *Phenol Oxidation by Combinations of Pear PPO and POD.* All of the enzymatic reactions were carried out with purified pear PPO (5 $nkat \cdot mL^{-1}$) or/and dialyzed POD (10 $mUDO \cdot mL^{-1}$), with or without addition of 0.2 $mg \cdot mL^{-1}$ catalase, in a reaction vessel at pH 5.5 and 30 °C, in the presence of 0.2 mM vanillic acid (internal standard for HPLC analysis) using air agitation. In preliminary experiments, we had checked that vanillic acid was neither a substrate of pear POD nor an inhibitor. The concentration of phenolic substrates varied from 1 to 4 mM. For each time tested, 0.5 mL of reaction mixture was withdrawn from the reaction vessel and immediately mixed with an equal amount of stopping solution containing 2 mM NaF. The residual phenols and quinones were separated and quantified by HPLC (9010 pump and 9050 UV detector driven by a 9020 workstation from Varian) on 10 μL samples using the isocratic conditions described by Richard *et al.* (1991). The variation coefficient of the method was 1.5%, with 1 mM chlorogenic acid used as reference.

Addition of Pear POD to Oxidized Phenol, after Inhibition of PPO. Preliminary experiments were carried out to find a specific PPO inhibitor with no action on POD activity. This was obtained with NaCl (2 M) in McIlvaine buffer, pH 5.5. PPO-catalyzed oxidation of phenolic compound was performed as previously described. For each time tested, 0.5 mL was withdrawn and mixed with 0.5 mL of NaCl (4 M) supplemented or not with 50 mUDO POD. Contents of the solutions (immediately after mixing and 10 min later) were analyzed by HPLC. The HPLC method was the same as above.

H_2O_2 Detection and Quantification. The procedure was adapted from Jiang and Miles (1993); 5 $nkat \cdot mL^{-1}$ of purified pear PPO was added to 1 mM phenolic compound dissolved in McIlvaine buffer, pH 5.5, in a total volume of 3.5 mL. After different oxidation times, the reaction mixture was shaken for 2 min with activated charcoal and centrifuged (5 min, 10000g). Five hundred microliters of 50 mM guaiacol (dissolved in McIlvaine buffer, pH 5.5) and 0.6 mg of HRP were added to 1.5 mL of supernatant. The absorbance was immediately followed at 470 nm. The same experiment was performed with addition of 0.2 $mg \cdot mL^{-1}$ of catalase in the reaction mixture. As blank, phenolic compound without addition of PPO was similarly reacted with guaiacol and HRP. Quantification of H_2O_2 was done with known H_2O_2 solutions (in the range 0−100 μM).

RESULTS AND DISCUSSION

Kinetic Properties of Pear POD. When H_2O_2 was omitted from our reaction mixtures, pear POD was not able to oxidize 4-methylcatechol, chlorogenic acid, nor catechins. This suggested that pear POD had only a

Table 1. Kinetic Parameters for the Oxidation of Guaiacol, 4-Methylcatechol, Chlorogenic Acid, and Catechins by Pear POD at pH 5.5[a]

substrate	K_m (phenol) (mM)		V_m (%/guaiacol)	V_m/K_m
	phenol	H_2O_2		
guaiacol	45.0	1.5	100	2.2
4-methylcatechol	12.9	11.5	14	1.1
chlorogenic acid	3.5	5.5	60	17.1
(+)-catechin	1.5	0.4	50	33.3
(−)-epicatechin	1.5	0.15	90	60

[a] POD activity was spectrophotometrically assayed, at 470 nm with guaiacol and 400 nm with 4-methylcatechol, chlorogenic acid, and catechins.

very weak oxidative (oxygen dependent) activity if any, in contrast to that of many plant POD (Whitaker, 1985). Similar results have been reported by Richard and Nicolas (1989), concerning apple POD. 4-Methylcatechol, chlorogenic acid, and catechins were however oxidized by pear POD in the presence of H_2O_2, following a classical mechanism for POD, *i.e.* a Ping-Pong bireactant mecanism. The Michaelis constants are reported together with the V_m values in Table 1. Thus, significative differences among Michaelis constants appear either for phenolic compounds or for H_2O_2. Although the comparison is only approximative since the absorption coefficients of the different oxidation products are unknown, such differences are also noticed for the V_m values. In term of efficiency (V_m/K_m), (−)-epicatechin appeared to be the best substrate, followed by chlorogenic acid and (+)-catechin. Our results also confirmed the high affinity of POD for H_2O_2 with catechins as substrate. This affinity was however weaker when chlorogenic acid was used as phenolic substrate. Additional experiments have also demonstrated that, in the presence of H_2O_2, pear POD was able to oxidize quercetin and its glycosides. The determined apparent K_m values (H_2O_2 concentration equal to 1 mM) were roughly constant, close to 0.1 mM, whatever the flavonol (results not presented). It was however noticed that the presence of a glycosyl residue greatly reduced the degradation velocity, in agreement with the reports of Richard and Nicolas (1989). Cinnamic acids (*p*-coumaric, caffeic, and ferulic acids) were also oxidized by pear POD, with apparent K_m values (H_2O_2 concentration equal to 1 mM) close to 0.2 mM (data not shown).

These first results, associated with the low affinity of pear PPO for its natural substrates (Gauillard and Richard-Forget, 1997), are in agreement with an effective involvement of pear POD in enzymatic browning. Thus, in the presence of an oxidizing substrate, pear POD will be able to degrade not only the main pear endogenous substrates of enzymatic browning but also some phenolic compounds which are bad substrates or even inhibitors of PPO.

Oxidation of Phenolic Compounds by Combination of PPO and POD. Oxidation of 1 mM 4-methylcatechol, 1 mM chlorogenic acid, and (−)-epicatechin by purified PPO, purified POD, and a mixture of purified PPO and POD, in the presence or not of catalase, has been followed by HPLC (Figure 1A−C). Preliminary experiments have shown that catalase did not modify the PPO-catalyzed oxidation rate. In accordance with the kinetic data, no degradation of 4-methylcatechol, chlorogenic acid, and (−)-epicatechin was noticed with the POD extract. For the three compounds tested, the rate of phenol consumption by PPO was significantly enhanced by the addition of POD. Supplementation with POD led to an additional con-
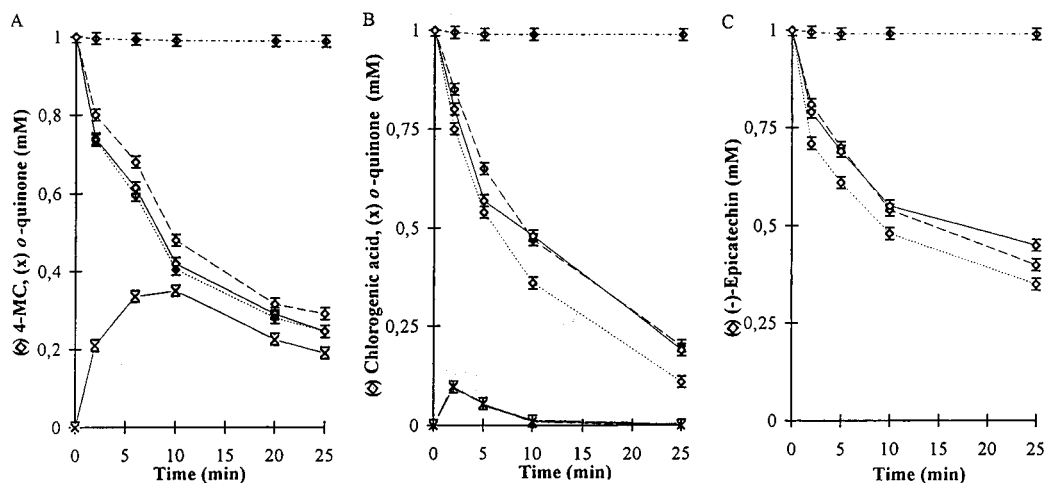
Figure 1. Oxidation of 4-methylcatechol (A), chlorogenic acid (B) and (−)-epicatechin (C) by 10 mUDO·mL^{-1} POD (- -), 5 nkat·mL^{-1} PPO (− −), and a combination of PPO (5 nkat·mL^{-1}) and POD (10 mUDO·mL^{-1}) with (−) or without (···) catalase (0.2 mg·mL^{-1}). Quinones amounts (×) are reported for 4-methylcatechol and chlorogenic acid. Reactions were performed at pH 5.5.

Table 2. H_2O_2 Production during Oxidation of 4-Methylcatechol, Chlorogenic acid, and (−)-Epicatechin by Pear PPO (5 nkat·mL^{-1})

oxidation time	H_2O_2 Production (μM)		
	4-methylcatechol	chlorogenic acid	(−)-epicatechin
1		25	
3			70
4	3	30	
6			70
10	5	40	
12			60
20	5	40	
25			55
30	5	45	

sumption close to 100 μM with chlorogenic acid, 80 μM with 4-methylcatechol, and 70 μM with (−)-epicatechin after 10 min oxidation. This additional consumption did not vary with the level of added POD (between 10 and 20 mUDO·mL^{-1}) but increased with the initial phenolic amount (data not shown). For instance, it reached 250 μM after 10 min oxidation of 3.5 mM chlorogenic acid. These first results are in agreement with the production of H_2O_2 by PPO-catalyzed oxidation and the use of generated H_2O_2 by POD to further oxidize the phenolic compound, as suggested by Jiang and Miles (1993). Generated H_2O_2 was detected and quantified according to the protocol described by the previous authors. Results are summarized in Table 2. It clearly appeared that PPO oxidation of (−)-epicatechin generated the highest amounts of H_2O_2, with levels close to 60−70 μM. Lower amounts, between 25 and 45 μM, were obtained with chlorogenic acid, while PPO oxidation of 4-methylcatechol led to negligible quantities. These differences certainly resulted from different abilities of phenolic semiquinone radical to reduce molecular oxygen, which then can generate H_2O_2. Semiquinone radicals have been effectively described as intermediate entities in the PPO-catalyzed oxidation reaction (Pierpoint, 1969). These results are in agreement with those of Jiang and Miles (1993), who also reported a considerable production of H_2O_2 during tyrosinase oxidation of (+)-catechin and almost nil during oxidation of 4-methylcatechol. However, according to Parry *et al.* (1996), oxidation of (+)-catechin during tea fermentation did not generate sufficient amounts of H_2O_2 to be detected. The use of catalase has confirmed the different capacities of phenolic to generate H_2O_2: catalase did not modify significantly the consumption rate of 4-methylcatechol during

its oxidation by the PPO/POD combination (Figure 1A) but totally abolished the additional consumption of (−)-epicatechin resulting from the addition of POD to PPO (Figure 1C). With chlorogenic acid (Figure 1B), catalase totally inhibited the additional phenolic consumption for longer than 10 min oxidation times, but a residual increase in chlorogenic acid degradation was observed for shorter than 10 min oxidation times. This was concomitant with the presence of chlorogenic acid *o*-quinones in the reaction mixture. 4-Methylcatechol *o*-quinones, more stable than chlorogenic acid *o*-quinones (Richard-Forget *et al.*, 1992), were present in the reaction mixture during the 30 min experiment. (−)-Epicatechin *o*-quinones were never detected, due to their high instability (Richard-Forget *et al.,* 1992). Previous reports (Richard-Forget, 1992) have shown that, at pH values higher than 4.5, (−)-epicatechin *o*-quinones were involved in polymerization reaction as soon as they were generated.

According to the former results, the enhancement of (−)-epicatechin oxidation rate, resulting from the addition of POD to PPO, can be entirely ascribed to H_2O_2 generation during PPO oxidation of (−)-epicatechin. However, the H_2O_2 generation is not totally explained for chlorogenic acid and not explained at all for 4-methylcatechol. Our results suggested a possible use of quinonic form by POD as peroxide substrate.

Use of Quinonic Form by POD as Peroxide Substrate. The hypothesis of a possible use of quinonic form by POD was supported by the following experiments. An aliquot of 1 mM chlorogenic acid or 4-methylcatechol was oxidized by pear PPO in presence or not of catalase. After different oxidation times, the reaction was stopped by a NaCl solution supplemented or not with POD. Contents of the solutions were analyzed by HPLC over a 10 min period. Results obtained with chlorogenic acid for a 3 min oxidation time are illustrated in Figure 2. After the enzymatic reaction was inhibited by the NaCl addition (dotted lines), the oxygen uptake was immediately stopped (data not shown), a slight degradation of chlorogenic acid was apparently occurring concomitantly with a decrease in quinones content. Similar data have been reported with a NaF stopping solution (Richard-Forget *et al.*, 1992). The chlorogenic acid and *o*-quinones degradation were ascribed to nonenzymatic reactions involving *o*-quinones and their originating phenols to generate some dimers
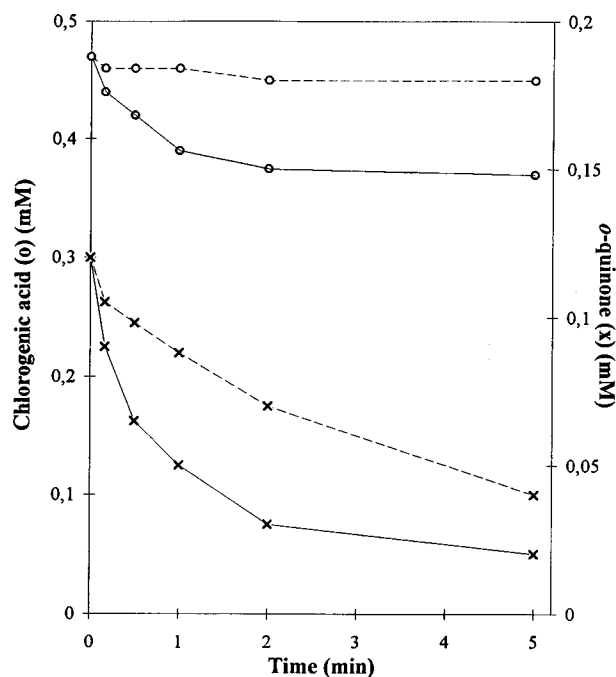
Involvement of Peroxidase in Enzymatic Browning

J. Agric. Food Chem., Vol. 45, No. 7, 1997 **2475**

Figure 2. Evolution of chlorogenic acid (○) and chlorogenic acid *o*-quinones (×) after the PPO oxidation (3 min), in the absence of catalase, was stopped by NaCl in the presence (full lines) or not (dotted lines) of POD (25 mUDO·mL^{-1}).

Table 3. Further Consumption of Chlorogenic Acid (in the presence or not of POD) after Stopping the PPO Reaction (in the presence or not of catalase) by NaCl

| | | | chlorogenic acid consumption^c (μM) | | |
| | | | NaCl stopping solution | NaCl/POD stopping solution | |
oxidation time (min)	*o*-quinone^b (μM)	H$_2$O$_2$^{b} (μM)	− catalase	− catalase	+ catalase
2	100	ND^a	20	90	60
3	120	20	25	100	70
4	70	30	15	55	35
7	40	ND	10	50	25
10	10	40	0	45	5
15	0	ND	0	45	0
20	0	40	0	40	0
30	0	45	0	30	0

^{a} ND: not determined. ^{b} *o*-Quinone and H$_2$O$_2$ values correspond to the amounts formed during the chlorogenic acid PPO oxidation. ^{c} Consumption of chlorogenic acid was estimated during the 10 min following the PPO inhibition.

(Cheynier *et al.*, 1988). These nonenzymatic reactions are favored for pH values higher than 4.5 (Richard-Forget *et al.*, 1992). With a stopping solution containing pear POD (full lines), the additional chlorogenic acid consumption was significantly enhanced, as was the decrease in *o*-quinones content. Thus, the amounts of consumed chlorogenic acid and *o*-quinones, 2 min after mixing, were equal to 95 and 90 μM, respectively; these values were close to 20 μM for chlorogenic acid and 50 μM for the *o*-quinones with the NaCl stopping solution. Similar data were obtained with 4-methylcatechol. Therefore, our results implied the existence of some reactions involving POD, phenols, and their corresponding *o*-quinones. The same experiment, as previously described, was carried out for different PPO oxidation times. The amounts of consumed chlorogenic acid (during the 10 min following the PPO inhibition by the NaCl and the NaCl/POD stopping solutions) are reported in Table 3 for reaction mixtures supplemented or not with catalase. For each PPO oxidation time, we also reported in Table 3 the amounts of *o*-quinones and

H$_2$O$_2$ present in reaction mixtures. It appeared that, for oxidation times shorter than 10 min, chlorogenic acid consumption (with the NaCl/POD stopping solution) was largely greater than the H$_2$O$_2$ content and partially reduced in the presence of catalase. The amounts of degraded chlorogenic acid in the presence of catalase remained however higher than those noticed with the NaCl stopping solution. For longer than 10 min oxidation times, the amounts of degraded chlorogenic acid and H$_2$O$_2$ were almost similar, close to 40 μM. These data confirmed the occurrence of another chlorogenic acid consumption pathway than that associated with the H$_2$O$_2$ production. The evolution of *o*-quinones and degraded chlorogenic acid appeared strongly correlated. For the two evolutions, the highest amounts (120 μM *o*-quinones, 70 μM consumed chlorogenic acid in the presence of catalase) were obtained for a 3 min PPO oxidation time. Moreover, when no more *o*-quinones were present in reaction mixtures, no more chlorogenic acid was degraded for reaction mixtures containing catalase. These results are another argument in favor of the use of quinones by POD to further oxidize the phenolic compound. If we assumed that 1 mol of quinone could be used by POD to oxidize 1 mol of phenol, comparison between the amounts of quinones and consumed chlorogenic acid in the presence of catalase suggested than 50 to 60% of the quinones present in reaction mixtures are used by POD, the remaining 40−50% being involved in secondary nonenzymatic reactions. Following the same assumption, almost 65% of the further chlorogenic acid consumption (for oxidation times shorter than 10 min) seemed to result from the quinone/POD pathway and 35% from the H$_2$O$_2$/POD pathway, 100% of the further chlorogenic acid consumption can be ascribed to the H$_2$O$_2$/POD pathway for the highest oxidation times. Similar data were obtained for 4-methylcatechol, with the exceptions that no significant H$_2$O$_2$ production was visualized for this phenol and that *o*-quinones were present in reaction mixtures during the 30 min experiment. All of the further 4-methylcatechol consumption was therefore ascribed to the quinone/POD pathway.

CONCLUSION

The kinetic properties of pear PPO (Gauillard and Richard-Forget, 1997) and POD (detailed in this report) are consistent with an implication of pear POD in enzymatic browning:

(1) The affinity of pear PPO for its natural substrates is lower than that usually determined for PPO from other origins.

(2) The specificity of pear POD for its hydrogen donor substrates is large; most of the phenolic compounds present in pear are oxidized by pear POD in presence of H$_2$O$_2$.

Two mechanisms implying an involvement of POD in enzymatic browning have also been proposed. First, our results have demonstrated the generation of H$_2$O$_2$ during oxidation of some phenolic compounds and the use of this generated H$_2$O$_2$ to further oxidize the phenol. On the other hand, the previously reported data are in agreement with the use of quinonic forms by POD as oxidizing substrate. The relative significance of these two pathways appeared as strongly affected by the nature of the oxidized phenol and therefore by the stability of the corresponding *o*-quinones. Thus, with (−)-epicatechin, characterized by very unstable *o*-quinones, the further consumption resulting from the addi-

2476 *J. Agric. Food Chem.,* Vol. 45, No. 7, 1997

Richard-Forget and Gauillard

tion of POD to PPO can be entirely explained by the H_2O_2 generation. With 4-methylcatechol, for which *o*-quinones are particularly stable, the use of quinonic forms by POD can explain the whole additional consumption. Chlorogenic acid appeared as an intermediate example, for which the two pathways occurred simultaneously as long as quinones were present in reaction mixtures.

Thus, our results are in agreement with a role played by POD in enzymatic browning. However, according to these results, the involvement of POD needs the presence of PPO activity to be effective. In further experiments, other phenolic compounds, nonsubstrates or inhibitors of PPO but POD substrates, such as flavonols, cinnamics acids, or thiols will be introduced in our reaction mixtures.

ABBREVIATIONS USED

POD, peroxidase; PPO, polyphenol oxidase.

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LITERATURE CITED

Burnette, F. Peroxidase and its relationships to food flavor and quality: a review. *J. Food Sci.* **1977**, *42*, 1−5.

Cheynier, V.; Osse, C.; Rigaud, J. Oxidation of grape juice phenolic compounds in model solutions. *J. Food Sci.* **1988**, *53*, 1729−1732.

Gauillard, F.; Richard-Forget, F. Polyphenoloxidases from Williams Pear (*Pyrus Communis* L., C. V. Williams): Activation, purification and some properties. *J. Sci. Food Agric.* **1997**, *74* (1), 49−56.

Jiang, Y.; Miles, P. W. Generation of H_2O_2 during enzymic oxidation of catechin. *Phytochemistry* **1993**, *33* (1), 29−34.

Leatherbarrow, R. J. *Enzfitter, a non-linear regression data analysis program for the IBM PC*; Elsevier: Amsterdam, 1987.

Mathew, A. G.; Parpia, H. A. B. Food browning as a polyphenol reaction. *Adv. Food Res.* **1971**, *19*, 75−145.

Miller, E.; Schreier, P. Studies on flavonol degradation by peroxidase (Donor: H_2O_2-oxidoreductase, E. C. 1.11.1.7.). Part 1: Kaempferol. *Food Chem.* **1985**, *17*, 143−154.

Nicolas, J. J.; Richard-Forget, F.; Goupy, P.; Amiot, M. J.; Aubert, S. Enzymatic browning reactions in apple and apple products. *C.R.C., Crit. Rev. Food Sci. Nut.* **1994**, *34* (2), 109−157.

Parry, A. D.; Goodsall, C. W.; Safford, D. S. The involvement of polyphenol oxidase and peroxidase in the oxidation of polyphenols during the manufacture of black tea. JIEP Groupe Polyphenols Bordeaux 1996. *Bull. Liaison* **1996**, *18*, 499−500.

Pierpoint, W. S. O-quinones formed in plant extracts. Their reactions with amino acids and peptides. *Biochem. J.* **1969**, *98*, 567−580.

Richard, F.; Nicolas, J. Purification of apple peel peroxidase. Studies of some properties and specificity in relation to phenolic compounds. *Sci. Aliment.* **1989**, *9*, 335−350.

Richard, F.; Goupy, P.; Nicolas, J.; Lacombe, J.; Pavia, A. Cysteine as an inhibitor of enzymatic browning. I. Isolation and characterization of addition compounds formed during oxidation of phenolics by apple polyphenol oxidase. *J. Agric. Food Chem.* **1991**, *39*, 841−847.

Richard-Forget, F. Ph.D. thesis, University of Paris, 1992.

Richard-Forget, F. C.; Rouet-Mayer, M. A.; Goupy, P. M.; Philipon, J.; Nicolas, J. J. Oxidation of chlorogenic acid, catechins and 4-methylcatechol in model solutions by apple polyphenol oxidase. *J. Agric. Food Chem.* **1992**, *40*, 2114−2122.

Robinson D. S. Peroxidases and catalases in foods. In *Oxidative Enzymes in Foods*; Robinson, D. S., Eskin, N. A. M., Eds., Elsevier: London, 1991; p 1.

Teisson, C. Internal bruising of pineapple. *Fruits* **1972**, *27*, 603−607.

Whitaker, J. R. Mechanisms of oxidoreductases important in food component modification. In *Chemical changes in food during processing*; Richardson, T., Finley, J. W., Eds.; AVI Publishing: Westport, CT, 1985; Vol. 8, pp 121−176.

Williams, D. C.; Lim, M. H.; Chen, O. A.; Pangborn, R. M.; Whitaker, J. R. Blanching of vegetables for freezing. Which indicator to choose? *Food Technol.* **1985**, *40*, 130−140.