Characterization of Polyphenol Oxidase from Photosynthetic and Vascular Lettuce Tissues (*Lactuca sativa*)

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To examine the undesired enzymatic browning in iceberg lettuce (*Lactuca sativa* L.), polyphenol oxidase (PPO) from vascular and photosynthetic tissues was characterized. PPO activity was determined by measuring dioxygen consumption with a Clark electrode. The enzyme in photosynthetic and vascular tissues had native molecular weights of ≥150 000 and ≥136 000, respectively. In addition, two PPO-active bands (40 000 and 46 000) were found in vascular tissue only. The following enzyme characteristics were found for PPO from both vascular and photosynthetic lettuce tissues: substrate specificity exclusively for *o*-dihydroxy substrates; p*I* 3.6; pH optimum 5–8; temperature optimum 25–35 °C; pH stability 5–8 for 20 h at 5 °C; temperature stability 0–70 °C for 5 min. Michaelis–Menten characteristics for a range of *o*-dihydroxy substrates and dioxygen were determined. The enzyme in vascular and photosynthetic tissues in lettuce could be classified as a 1,2-benzenediol:oxygen oxidoreductase (EC 1.10.3.1).

INTRODUCTION

Polyphenol oxidase (PPO) is widespread in nature (Vámos-Vigyázó, 1981; Mayer, 1987). Interest in PPO in fruits and vegetables is increasing, since PPO is held responsible for the undesired enzymatic browning that develops after tissue injury. When fruits and vegetables are processed without heating and additives (antioxidants), enzymatic browning, therefore, is very difficult to avoid.

PPO characteristics are thoroughly investigated from apple (Goodenough *et al.*, 1983; Janovitz-Klapp *et al.*, 1989; Trejo-Gonzalez and Soto-Valdez, 1991), grape (Nakamura *et al.*, 1983; Sánchez-Ferrer *et al.*, 1988; Valero *et al.*, 1988), potato (Chen *et al.*, 1992), and mushroom (McCord and Kilara, 1983; Chen *et al.*, 1992). PPO in leaves is sparely investigated, but Fujita *et al.* (1991) and Ganesa *et al.* (1992) have studied PPO characteristics in lettuce and leaves from broad bean, respectively.

The function of PPO in plants is unknown, but Schwimmer (1981) suggests that PPO could be involved in resistance to infection and in biosynthesis of plant constituents, while Mayer (1987) suggests that PPO might function as an oxygen scavenger in photosynthetic tissue.

In the literature, no studies could be found concerning the properties of PPO from various parts of tissues in leaves from the same plant.

The aim of this work was to characterize PPO from photosynthetic and vascular tissues in lettuce and to investigate the possibility of isoenzymes.

MATERIALS AND METHODS

Eight heads of fresh iceberg lettuce (*Lactuca sativa* L. cv. Pennlake) were purchased from a commercial grower near Copenhagen, Denmark. Vascular tissue (108 g) was cut from the midrib in the lower third of 8–12 outer leaves from each lettuce head (3 cm × 8 cm). Photosynthetic tissue (475 g) was cut from the foliar parenchyma in the upper third of the same outer leaves (3 cm × 8 cm). Samples were frozen in liquid nitrogen, freeze-dried, ground into fine powder, and then stored at −20 °C in airtight bags for less than 4 months until further use.

Extraction. Plant powder (75.0 mg) was extracted with 15.0 mL of water using magnetic stirring for 2 h in an ice bath. Samples were centrifuged at 15000*g* for 20 min at 2 °C. All extractions were performed in duplicate. After filtration, samples were stored at 5 °C for not more than 1 day.

Enzyme and Protein Assays. PPO activity was assayed by measuring initial dioxygen (O_2) consumption with a Clark electrode (Rank Brothers, Cambridge, U.K.) at 30 °C by a modification of the method of Wesche-Ebeling and Montgomery (1990). To determine PPO activity (standard assay conditions), 2.825 mL of 0.1 M air-saturated acetate buffer (pH 5.5) and 75 μL of enzyme extract (vascular sample, 0.11 mg of protein/mL; photosynthetic sample, 0.23 mg of protein/mL) were added to the reaction chamber and allowed to equilibrate before measuring. To initiate the reaction, 0.1 M chlorogenic acid (100 μL) was added. All assays were performed in duplicate. PPO activity was expressed as nanomoles of dioxygen consumed per second (nkat) under the assumption that water contains 7.4 mg of O_2/L at 30 °C with a salinity of 5.8 g/L (Montgomery *et al.*, 1964).

Protein was determined according to the method of Bradford (1976) using bovine serum albumin (Sigma) as a standard.

Substrate Specificity. The substrate specificity of PPO was determined using various 0.02 M substrates: chlorogenic acid, caffeic acid, hydroquinone, resorcinol, catechol, 4-methylcatechol, gallic acid, tyrosine, DL-DOPA (DL-dihydroxyphenylalanine), dopamine (3-hydroxytyramine), (−)-epicatechin, (+)-catechin (Sigma), and *p*-coumaric acid (Fluka). Enzyme activity was determined under standard assay conditions.

Kinetic Parameters of Various Substrates. Michaelis constants were determined under standard assay conditions using various concentrations of substrates ranging from 0.67 to 3.33 mM and 50 μL of enzyme solution. PPO activity in assay solutions containing varying concentrations of the two substrates, chlorogenic acid and dioxygen, was determined. Gas mixtures (15, 10, 5% O_2) of dioxygen and dinitrogen (Hede Nielsen, Denmark) were successively bubbled through the buffer at 30 °C for a minimum of 10 min, ending with the lowest dioxygen concentration, before the enzyme and substrate were added as described by Janovitz-Klapp *et al.* (1990). The initial dioxygen concentration was determined for each gas mixture with a Clark electrode. Constants (K_m^{app} and V_{max}^{app}) for the phenolic substrates and for dioxygen were calculated from plots of initial rates versus substrate concentrations according to the methods of Lineweaver–Burk or Hanes.

K_m for dioxygen and chlorogenic acid, respectively, and V_{max}, were calculated from the replots of intercepts and slopes from Lineweaver–Burk plots versus the reciprocal of the concentration of dioxygen as described by Wold (1971).

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Effect of pH. PPO activity as a function of pH was determined under standard assay conditions using various buffers: acetate (0.1 M, pH 3.5–6.0), phosphate (0.1 M, pH 5.5–7.5), and Tris (0.1 M, pH 7.0–9.0). To determine pH stability, the enzyme was preincubated in acetate (0.1 M, pH 3.5, 4.0, and 5.0), phosphate (0.1 M, pH 6.0 and 7.0), or Tris (0.1 M, pH 8.0 and 9.0) for 20 h at 5 °C. Residual PPO activity was measured under standard assay conditions.

Effect of Temperature. PPO activity as a function of temperature was determined under standard assay conditions using temperatures from 5 to 54 °C.

Thermal stability of PPO was determined by heating the enzyme solution at various temperatures between 0 and 90 °C for 5 min. Residual PPO activity was measured under standard assay conditions.

Activation energy (E_a) was obtained using the Arrhenius equation.

Molecular Weight Estimation. Native molecular weights of PPO in unpurified extracts were estimated by SDS electrophoresis on a vertical electrophoresis unit (No. 2001, LKB) using a high molecular weight standard (Bio-Rad), according to the method of Laemmli (1970). Power (maximum 500 V) and current (20 mA) were controlled by an electrophoresis power supply (EPS 500/400, Pharmacia). Separating gel contained 12% polyacrylamide and 0.17% bis(acrylamide), whereas stacking gel contained 3% polyacrylamide and 0.04% bis(acrylamide). The gel was divided in two parts before protein and PPO detection. Nondenatured samples were specifically colored for polyphenol oxidases according to a modification of the method of Lee (1991) using 20 mM 4-methylcatechol and 0.05% *p*-phenylenediamine in 0.1 M acetate (pH 5.5). Ascorbic acid (1 mM) was used to destain and stabilize the color. Tyrosinase (Sigma) was used as reference. Denatured and nondenatured samples were silver stained by a modification of the method of Blum *et al.* (1987), which substituted ethanol for methanol and adjusted the developing solution to pH 11.5.

Molecular weights (M_w) of PPO in lettuce extracts were estimated as described by Weber and Osborn (1969) from mobilities of specifically colored bands using a standard curve.

Estimation of p*I*. p*I* was estimated by isoelectric focusing electrophoresis (IEF) using a gel made of 1% agarose (Serva), 2% ampholyte (pH 3–10, Bio-Rad), 5% sorbitol, and 10% glycerol in water. IEF was made using a Bio-Phoresis horizontal electrophoresis cell (Bio-Rad) at 10 °C, a power supply (Model 3000 X_i, Bio-Rad), and an IEF marker covering pH 2.4–5.65 (BDH). Focusing was carried out for 90 min at constant power (7 W). The maximal voltage and current were 2000 V and 50 mA, respectively. The anodic and cathodic solutions were 1 M phosphoric acid and 1 M sodium hydroxide, respectively. One half of the gel was stained with Coomassie according to the method of Bradley *et al.* (1989). The other half was specifically colored for PPO (see Molecular Weight Estimation). By comparing migration of PPO active bands with migration of IEF standards, p*I* values were found.

RESULTS AND DISCUSSION

To keep the enzyme intact and minimize the division into subunits or conformational changes, the enzyme was given a very gentle treatment. Kinetic studies of grape catechol oxidase showed only linearity when membrane-bound unpurified chloroplasts were used (Lerner and Mayer, 1976). It was not necessary to remove low molecular weight compounds from the enzyme extract, because no background activity was found to interfere.

Separation of lettuce into vascular and photosynthetic tissues was not absolutely complete. Insignificant amounts of vascular tissue were unavoidable in the part called photosynthetic tissue. Very few chloroplasts were expected to be in the part called vascular tissue. Latex channels and supporting tissue were included in the vascular tissue.

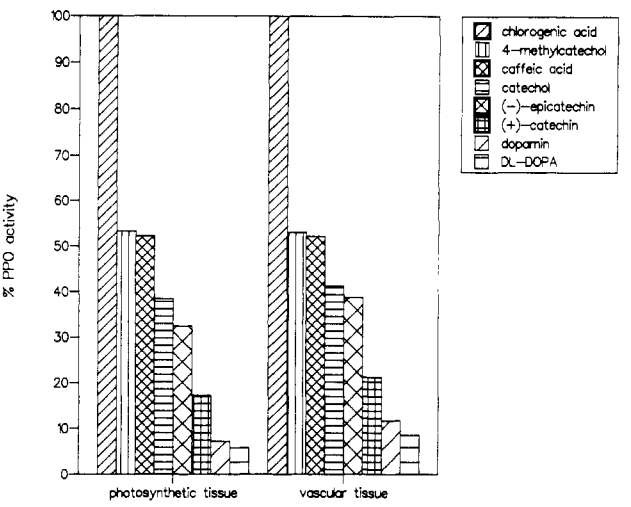

Figure 1. Substrate specificity relative to chlorogenic acid obtained by PPO in iceberg lettuce tissues (*L. sativa*). Enzyme activity was measured by dioxygen consumption.

Amost comparable PPO activities were found in vascular and photosynthetic tissues when the enzyme activity was expressed as PPO activity per gram of protein (specific activity). PPO activity per gram of fresh weight in vascular tissue was only about one-seventh that in photosynthetic tissue. To compare PPO characteristics in vascular and photosynthetic tissues from lettuce, specific activity was used.

Substrate Specificity. To examine the substrate specificity of PPO in photosynthetic and vascular tissues, respectively, activities and kinetics of the enzyme against some mono-, di-, and trihydroxy substrates were determined. Identical activities against various *o*-dihydroxy substrates in percent of PPO activity against chlorogenic acid were found for the two kinds of tissues (Figure 1). No activities were found against monohydroxy substrates (*p*-coumaric acid and tyrosine), *p*-dihydroxy substrate (hydroquinone), *m*-dihydroxy substrate (resorcinol), or trihydroxy substrate (gallic acid).

The substrate specificity found in this work is identical to the substrate specificity of purified PPO from Koshu grapes measured by a polarographic method (Nakamura *et al.*, 1983). Activity against *o*-dihydroxy substrates only, but with alternative succession, is found for purified PPO from lettuce (Fujita *et al.*, 1991) and for partially purified PPO from apples using spectrophotometric methods (Trejo-Gonzalez and Soto-Valdez, 1991).

In the present study, activity against DL-DOPA was too low to establish kinetic parameters. In a screening survey for distribution of PPO in terrestrial and aquatic plants, Sherman *et al.* (1991) used DL-DOPA as a general substrate without any comments of low or varying reactivities against PPOs.

In this study, no activity was found (data not shown) when ascorbate was added to the reaction mixture to examine the tyrosine-hydroxylating activity of PPO (EC 1.14.18.1) in lettuce. Wichers *et al.* (1984) concluded that ascorbate served as a cofactor for the tyrosine-hydroxylating activity and as an inhibitor of the catechol oxidase activity of PPO from suspension cultures of *Mucuna pruriens*.

The presence of other enzyme activities using dioxygen as substrate, e.g., ascorbate oxidase (EC 1.10.3.3), was ruled out by checking ascorbic acid as substrate.

From these results we can conclude that PPO in vascular and photosynthetic tissues can be classified as a 1,2-benzenediol:oxygen oxidoreductase (EC 1.10.3.1) with the recommended name catechol oxidase.

Table 1. Michaelis-Menten Characteristics for Some o-Dihydroxy Substrates and PPO Activity in Iceberg Lettuce Tissue (*L. sativa*)[a]

substrate	K_m^{app} (mM), photosynth tissue	K_m^{app} (mM), vascular tissue	V_{max}^{app} (nkat/g of protein), photosynth tissue	V_{max}^{app} (nkat/g of protein), vascular tissue
chlorogenic acid[b]	2.4	1.4	407	318
4-methylcatechol[b]	9.2	11.5	1098	813
caffeic acid[c]	1.0	0.9	150	145
catechol[c]	1.0	*d*	66	*d*
(-)-epicatechin[b]	0.03	0.03	61	95
dioxygen[b]	0.3	0.3	901	459

[a] Chlorogenic acid (0.1 M) was the second substrate in the dioxygen experiment. [b] Lineweaver-Burk plot. [c] Hanes plot. [d] Undetectable.

Kinetic Parameters. Michaelis-Menten kinetics for different substrates are outlined in Table 1. In lettuce we did not find significantly different K_m^{app} values for PPO in vascular tissue and photosynthetic tissue. Low K_m^{app} values are often found for biological substrates, whereas higher K_m^{app} values are common for artificial substrates.

K_m^{app} values for various substrates and PPO in photosynthetic and vascular tissues in lettuce are in accordance with values obtained by PPO in several fruits and vegetables. Using chlorogenic acid as substrate and PPO as enzyme, the following K_m^{app} values are reported: 8 mM for purified PPO from sweet potato (Lourenco *et al.*, 1992), 5 mM for partially purified PPO from apple (Janovitz-Klapp *et al.*, 1989), and 0.7 mM for PPO in lettuce (Fujita *et al.*, 1991). Using 4-methylcatechol as substrate and PPO as enzyme, the following K_m^{app} values are reported: 26 mM from sweet potato (Lourenco *et al.*, 1992), 8.1 mM from apple (Janovitz-Klapp *et al.*, 1989), and 9 mM for purified PPO from grape (Valero *et al.*, 1988; Sánchez-Ferrer *et al.*, 1988). The K_m^{app} value found in this study for PPO and (-)-epicatechin is lower than that reported by Fujita *et al.* (1991) from lettuce (0.91 mM).

In Figure 2A a Lineweaver-Burk plot of the rate of PPO from photosynthetic tissue against chlorogenic acid is shown. A Lineweaver-Burk plot using PPO from vascular tissue shows the same pattern (data not shown). From these Lineweaver-Burk plots it is clear that the mechanism for PPO in lettuce is not a Ping-Pong mechanism (Wold, 1971) because the slopes are different. From the kinetic parameters it is not possible to conclude whether the enzyme mechanism using chlorogenic acid is ordered or random, since it is difficult to establish if all of the lines in Figure 2A intercept on or under the horizontal axis.

For the substrate 4-methylcatechol and PPO from grape an interception on the *x*-axis and a random mechanism were found (Lerner and Mayer, 1976), whereas an interception under the *x*-axis and an ordered mechanism were found for apple PPO (Janovitz-Klapp *et al.*, 1990). For the substrate chlorogenic acid and PPO from apple a random mechanism was found (Janovitz-Klapp *et al.*, 1990).

Replots of intercepts and slopes versus reciprocal of dioxygen concentration are shown in Figure 2B,C. From the curves the true K_m and V_{max} values are calculated. Our estimates of true K_m values for chlorogenic acid are 1.6 and 2.6 mM for PPO from photosynthetic and vascular tissues in lettuce and 0.4 and 0.5 mM for dioxygen (photosynthetic and vascular tissues). These K_m values for dioxygen are in accordance with the values obtained from apple PPO (0.3 mM; Janovitz-Klapp *et al.*, 1990) and grape PPO (0.5 mM; Lerner and Mayer, 1976) using the same estimation method. Air-saturated buffer at 30 °C has a dioxygen concentration of 0.23 mM, and therefore the velocity of the enzyme reaction is approximately one-fourth of the maximum during routine activity assay. V_{max} was estimated as 1340 and 1110 nkat/g of protein for photosynthetic and vascular tissues, respectively. It is not possible from the true K_m and V_{max} data to distinguish between the PPOs from the two tissues.

If PPO was to act as a dioxygen scavenger in photosynthetic tissue, a high affinity for dioxygen and, therefore, a low value of K_m would have been suspected.

Additional data are required to make any conclusions about the order of binding of the two substrates to lettuce PPO and the mechanism of the enzyme.

Optimum pH. In both vascular and photosynthetic tissues a broad pH optimum of 5–8 was found for the enzyme (Figure 3).

In whole lettuce, apple, and sweet potato a lower pH optimum for PPO using chlorogenic acid as substrate (pH 4.5) is reported (Fujita *et al.*, 1991; Janovitz-Klapp *et al.*, 1989; Lourenco *et al.*, 1992). Chubey and Dorrell (1972) observed two pH optima in unpurified PPO from parsnip root: pH 5.2 and 4.4.

Optimum Temperature. In both vascular and photosynthetic tissues a temperature optimum of 25–35 °C was found for the enzyme (Figure 4).

In PPO from parsnip root an optimum temperature of 30 °C is reported using chlorogenic acid as substrate in the enzyme assay (Chubey and Dorrell, 1972). Temperature optimum for PPO in apple and grape is in the area 25–45 °C using 4-methylcatechol as substrate (Trejo-Gonzalez and Soto-Valdez, 1991; Valero *et al.*, 1988).

E_a values for PPO from photosynthetic and vascular tissues in lettuce are estimated as 25 and 28 kJ/mol, respectively.

Activation energies for enzyme-catalyzed systems are generally 20–50 kJ/mol, which compares to the E_a values found in this study. For purified PPO from kiwi fruit (17 kJ/mol), partly purified PPO from wild rice (20 kJ/mol), and purified PPO from artichoke (22 kJ/mol) slightly lower E_a values for PPO are reported (Park and Luh, 1985; Owusa-Ansah, 1989; Leoni *et al.*, 1990).

Stability of Enzyme. PPO in vascular tissue of lettuce was stable for 20 h at 5 °C in buffers ranging from pH 4 to 8 (Figure 5). In photosynthetic tissue the PPO was relatively stable between pH 5 and 8. At higher and lower pH values, the activity decreased slowly.

Only a few examples of pH stability of PPO are reported, but the pH stability of PPO in vascular and photosynthetic tissues in lettuce found in this work is similar to that reported by Fujita *et al.* (1991) for PPO in whole lettuce, using the same method.

PPO in both photosynthetic and vascular tissues was stable for 5 min in temperatures ranging from 0 to 70 °C. At higher temperatures the activity decreased rapidly. At 90 °C, no activity remained after 5 min (Figure 6).

A temperature stability covering 0–70 °C for 5 min is in contrast to a decreasing temperature stability using 30–90 °C for 5 min stated by Fujita *et al.* (1991), even though the same method and vegetable were used. Miller *et al.* (1990) reports that PPO from cucumber is relatively

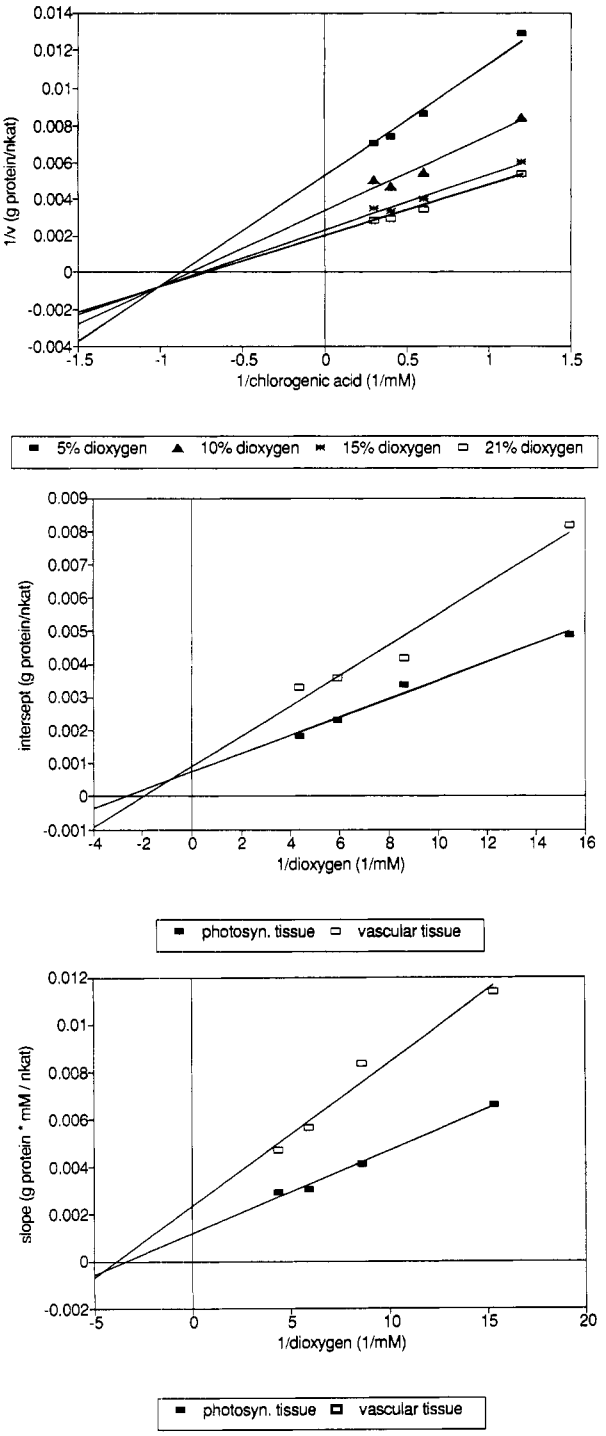

Figure 2. (A, top) Effect of dioxygen concentration on the initial specific activity of lettuce PPO from photosynthetic tissue at pH 5.5 and 30 °C using chlorogenic acid as the other substrate. (B, middle) Replots of intercepts versus the reciprocal dioxygen concentration using chlorogenic acid as the other substrate for PPO from photosynthetic and vascular tissues. (C, bottom) Replot of slopes vs the reciprocal dioxygen concentration using chlorogenic acid as the other substrate for PPO from photosynthetic and vascular tissues.

stable for 10 min at 0–70 °C, which is comparable with the results from the present study.

Molecular Weights. A molecular weight standard curve with a correlation coefficient of 0.96 was established. Only one PPO band was found in the photosynthetic tissue, whereas three were seen in vascular tissue (Table 2). Bands representing the large M_w in photosynthetic and vascular tissues are rather broad, resulting in mobilities ranging from 0 to 0.07 and from 0 to 0.09, respectively.

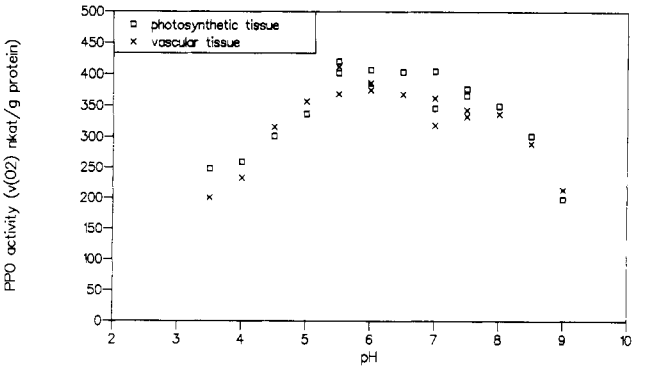

Figure 3. PPO activity in iceberg lettuce tissues (*L. sativa*) at different pH values. Enzyme activity was measured by dioxygen consumption using chlorogenic acid as substrate. $LSD_{0.95}$ values for PPO in photosynthetic tissue and vascular tissue were 11 and 9 nkat/g of protein, respectively.

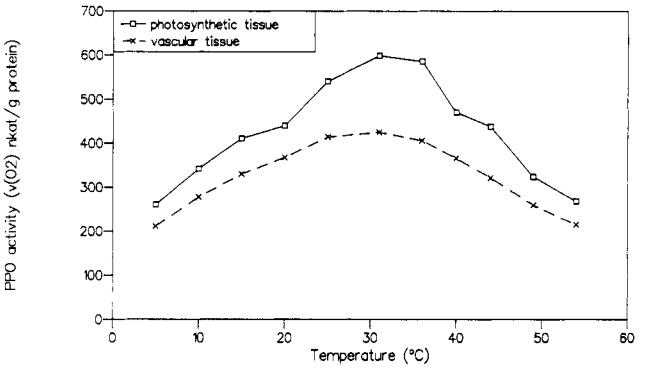

Figure 4. PPO activity in iceberg lettuce tissues (*L. sativa*) at different temperatures. Enzyme activity was measured by dioxygen consumption using chlorogenic acid as substrate. $LSD_{0.95}$ values for PPO in photosynthetic tissue and vascular tissue were 14 and 13 nkat/g of protein, respectively.

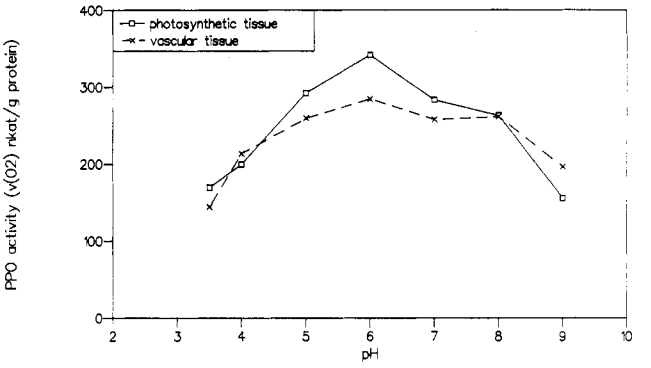

Figure 5. Remaining PPO activity in iceberg lettuce tissues (*L. sativa*) after incubation at different pH values for 20 h at 5 °C. Enzyme activity was measured by dioxygen consumption using chlorogenic acid as substrate. $LSD_{0.95}$ values for PPO in photosynthetic tissue and vascular tissue were 13 and 9 nkat/g of protein, respectively.

The low molecular weight bands of PPO (40 000 and 46 000) found in nondenatured extracts of vascular tissue probably represent active subunits of the enzyme. Lanker *et al.* (1988) found in denatured samples, when using SDS–PAGE and polyclonal anti-PPO for detection, that unpurified lettuce PPO had a M_w of 44 000, which is in accordance with our low molecular weight results. Fujita *et al.* (1991), using gel filtration, found that lettuce PPO had a molecular weight of 56 000.

PPO in lettuce probably consists of four subunits, but the enzyme in vascular tissue and that in photosynthetic tissue have dissimilar abilities to separate into subunits.

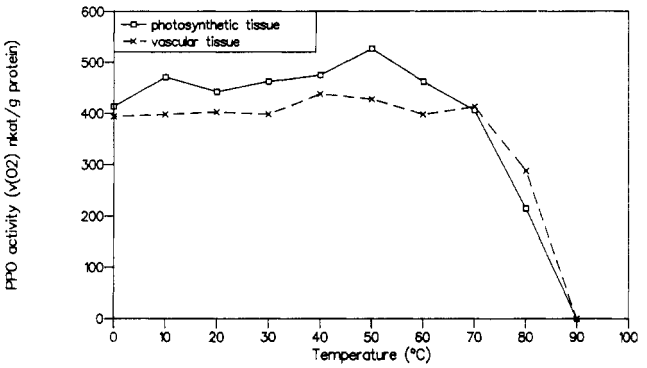

Figure 6. Remaining PPO activity in iceberg lettuce tissues (*L. sativa*) after incubation at different temperatures for 5 min. Enzyme activity was measured by dioxygen consumption using chlorogenic acid as substrate. $LSD_{0.95}$ values for PPO in photosynthetic tissue and vascular tissue were 10 and 17 nkat/g of protein, respectively.

Table 2. Molecular Weights and Isoelectric Points for Polyphenol Oxidase (PPO) in Unpurified Enzyme Preparations from Iceberg Tissue (*L. sativa*)[a]

	photosynth tissue	vascular tissue
M_w	≥150 000	≥136 000 46 000 40 000
p*I*	3.6	3.6

[a] Samples are not denatured before electrophoresis. PPO is detected by a specific color assay.

Estimation of p*I*. Only one isoelectric point (p*I* = 3.6) was found for PPO in both photosynthetic and vascular tissues in iceberg lettuce (Table 2).

p*I* for PPO in lettuce has never been reported before. The p*I* value found in this work is relatively low compared to p*I* values (4.5 and 4.8) found in apple PPO (Janovitz-Klapp *et al.*, 1989) and artichoke PPO (4.5) (Leoni *et al.*, 1990).

CONCLUSION

This examination of the characteristics of PPO in extracts from lettuce indicates that photosynthetic and vascular tissues of lettuce contain two PPO enzymes with almost identical properties, except for their molecular weights and their ability to separate into subunits. Further investigations are needed to examine the properties of PPO in specific cell components.

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LITERATURE CITED

Blum, H.; Beier, H.; Gross, H. J. Improved Silver Staining of Plant Proteins, RNA, and DNA in Polyacrylamide Gels. *Electrophoresis* **1987**, *8*, 93–99.

Bradford, M. M. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-dye Binding. *Anal. Biochem.* **1976**, *72*, 248–254.

Bradley, E. Z.; Savin, T. J.; Hall, R. E. A One-Step, Low Background Coomassie Staining Procedure for Polyacrylamide Gels. *Anal. Biochem.* **1989**, *182*, 157–159.

Chen, J. S.; Preston, J. F.; Wei, C.-I.; Hooshar, P.; Gleeson, R. A.; Marshall, M. R. Structural Comparison of Crustacean, Potato, and Mushroom Polyphenol Oxidases. *J. Agric. Food Chem.* **1992**, *40*, 1325–1330.

Chubey, B. B.; Dorrell, D. G. Enzymatic Browning of Stored Parsnip Roots. *J. Am. Soc. Hortic. Sci.* **1972**, *97*, (1), 107–109.

Fujita, S.; Tono, T.; Kawahara, H. Purification and Properties of Polyphenol Oxidase in Head Lettuce (*Lactuca sativa*). *J. Sci. Food Agric.* **1991**, *55*, 643–651.

Ganesa, C.; Fox, M. T.; Flurkey, W. H. Microheterogeneity in Purified Broad Bean Polyphenol Oxidase. *Plant Physiol.* **1992**, *98*, 472–479.

Goodenough, P. W.; Kessel, S.; Lea, A. G. H.; Loeffler, T. Mono- and Diphenolase Activity from Fruit of *Malus pumila*. *Phytochemistry* **1983**, *22* (2), 359–363.

Janovitz-Klapp, A.; Richard, F.; Nicolas, J. Polyphenoloxidase from Apple, Partial Purification and some Properties. *Phytochemistry* **1989**, *28* (11), 2903–2907.

Janovitz-Klapp, A. H.; Richard, F. C.; Goupy, P. M.; Nicolas, J. J. Kinetic Studies on Apple Polyphenol Oxidase. *J. Agric. Food Chem.* **1990**, *38* (7), 1437–1441.

Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **1970**, *227*, 680–685.

Lanker, T.; Flurkey, W. H.; Hughes, J. P. Cross-Reactivity of Polyclonal and Monoclonal Antibodies to Polyphenoloxidase in Higher Plants. *Phytochemistry* **1988**, *27* (12), 3731–3734.

Lee, P. M. Biochemical Studies of Cocoa Bean Polyphenol Oxidase. *J. Sci. Food Agric.* **1991**, *55*, 251–260.

Leoni, O.; Palmieri, S.; Lattanzio, V.; Van Sumere, C. F. Polyphenol Oxidase from Artichoke (*Cyanara Scolymus* L.). *Food Chem.* **1990**, *38*, 27–39.

Lerner, H. R.; Mayer, A. M. Reaction Mechanism of Grape Catechol Oxidase—a Kinetic Study. *Phytochemistry* **1976**, *15*, 57–60.

Lourenco, E. J.; Neves, V. A.; Da Silva, M. A. Polyphenol Oxidase from Sweet Potato: Purification and Properties. *J. Agric. Food Chem.* **1992**, *40*, 2369–2373.

Mayer, A. M. Polyphenol Oxidases in Plants—Recent Progress. *Phytochemistry* **1987**, *26* (1), 11–20.

McCord, J. D.; Kilara, A. Control of Enzymatic Browning in Processed Mushrooms (*Agaricus bisporus*). *J. Food Sci.* **1983**, *48*, 1479–1483.

Miller, A. R.; Kelley, T. J.; Mujer, C. V. Anodic Peroxidase Isoenzymes and polyphenol Oxidase Activity from Cucumber Fruit: Tissue and Substrate Specificity. *Phytochemistry* **1990**, *29* (3), 705–709.

Montgomery, H. A. G.; Thom, N. S.; Cockburn, A. Determination of dissolved oxygen by the Winkler method and the solubility of oxygen in pure water and sea water. *J. Appl. Chem.* **1964**, *14*, 280–295.

Nakamura, K.; Amano, Y.; Kagami, M. Purification and Some Properties of a Polyphenol Oxidase from Koshu Grapes. *Am. J. Enol. Vitic.* **1983**, *34* (2), 122–127.

Owusu-Ansah, Y. J. Polyphenoloxidase in Wild Rice (*Zizania palustris*). *J. Agric. Food Chem.* **1989**, *37* (4), 901–904.

Park, E. Y.; Luh, B. S. Polyphenol Oxidase of Kiwifruit. *J. Food Sci.* **1985**, *50*, 678–684.

Sánchez-Ferrer, A.; Bru, R.; Cabanes, J.; García-Carmona, F. Characterization of Catecholase and Cresolase Activities of Monastrell Grape Polyphenol Oxidase. *Phytochemistry* **1988**, *27* (2), 319–321.

Schwimmer, S. Food Color, the Phenolases and undesirable enzymatic browning. In *Source Book of Food Enzymology*; Schwimmer, S., Ed.; AVI Publishing: Westport, CT, 1981.

Sherman, T. D.; Vaughn, K. C.; Duke, S. O. A Limited Survey of the Phylogenetic Distribution of Polyphenol Oxidase. *Phytochemistry* **1991**, *30* (8), 2499–2506.

Trejo-Gonzalez, A.; Soto-Valdez, H. Partial Characterization of Polyphenoloxidase Extracted from Anna Apple. *J. Am. Soc. Hortic. Sci.* **1991**, *116* (4), 672–675.

Valero, E.; Varón, R.; García-Carmona, F. Characterization of Polyphenol Oxidase from Airen Grapes. *J. Food Sci.* **1988**, *53* (5), 1482–1485.

Vámos-Vigyázó, L. Polyphenol Oxidase and Peroxidase in Fruits and Vegetables. *CRC Crit. Rev. Food Sci. Nutr.* **1981**, *15* (1), 49–127.

Weber, K.; Osborn, M. The Reliability of Molecular Weight Determinations by Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis. *J. Biol. Chem.* **1969**, *244* (16), 4406–4412.

Wesche-Ebeling, P.; Montgomery, M. W. Strawberry Polyphenoloxidase: Its Role in Anthocyanin Degradation. *J. Food Sci.* **1990**, *55* (3), 731–734.

Wichers, H. J.; Peetsma, G. J.; Malingré, T. M.; Huizing, H. J. Purification and Properties of a Phenol Oxidase Derived from Suspension Cultures of *Mucuna pruriens*. *Planta* **1984**, *162*, 334–341.

Wold, F. *Macromolecules: Structure and Function*; Prentice-Hall: Englewood Cliffs, NJ, 1971.

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