Food Chemistry **38** (1990) 27–39

Polyphenol Oxidase from Artichoke (*Cynara scolymus* L.)

Onofrio Leoni, Sandro Palmieri*

Istituto Sperimentale per le Colture Industriali, Italian Ministry of Agriculture, Via di Corticella 133, 40129 Bologna, Italy

Vincenzo Lattanzio

Università della Basilicata, Potenza, Via Nazario Sauro 85, 85100 Potenza, Italy

&

Christiaan F. Van Sumere

Laboratorium voor Plantenbiochemie, RUG, K. L. Ledenganckstraat 35, 9000-Gent, Belgium

(Received 26 April 1989; revised version received and accepted 13 October 1989)

ABSTRACT

Polyphenol oxidase (PPO) was purified from hearts of artichoke. Starting with a crude 20 mM acetate buffer pH 5·7 extract obtained from an acetone powder originating from the plant material, the enzyme was purified 65-fold (overall yield 9%) by means of hydrophobic followed by gel filtration chromatography. The purified artichoke polyphenol oxidase (APPO), which migrated as a single band during gel filtration chromatography and chromatofocusing, showed an isoelectric point of 4·5 and a molecular weight of 116,000 dalton. The best substrates for the enzyme at pH 6·0 were 5-o-caffeoylquinic acid (5-o-CQA, according to IUPAC 1976 nomenclature) (relative activity 100%) and caffeic acid (relative activity 69%). Pyrocatechol, which was also oxidized by APPO (relative activity 42%), proved to be a less suitable substrate, whereas 1,5-dicaffeoylquinic acid and catechin were only very poorly oxidized. With each of the main substrates the optimum pH was widespread between pH 5·0 and 7·0 for 5-o-CQA and pyrocatechol, while

* To whom correspondence should be addressed.

for caffeic acid the maximum activity was widely extended between pH 6·5 and 8·0. The kinetic constants of the enzyme determined at pH 7·0 and 30°C with both 5-o-CQA and caffeic acid as substrate proved to be quite similar ($Km = 4·2 \pm 0·5$ mM, $V_{max} = 135·6 \pm 14·7$ U/mg and $Km = 5·3 \pm 0·3$ mM, $V_{max} = 115·1 \pm 6·1$ U/mg, respectively). The activation energy of the enzyme with caffeic acid was $21·7 \pm 0·2$ kJ/mole at pH 6·5. Cu^{++} and Fe^{+++} proved to activate APPO-5-o-CQA oxidation. However, APPO activation by these ions does not appear to be important enough to ascribe them a role in the enzymatic browning of stored artichoke heads. Although both ascorbic and citric acids are known to considerably improve the shelf life of artichoke heads, only ascorbic acid proved to be significantly inhibitory towards APPO.

INTRODUCTION

Although it is well established that polyphenol oxidase (PPO; EC 1.14.18.1, formerly EC 1.10.3.1) catalyses *in vitro* the *o*-hydroxylation of monophenols and the oxidation of *o*-diphenols to *ortho*-quinones, the true physiological function of PPO in plants remains unknown, although several hypotheses have been proposed. Some of the most important are the synthesis of *o*-diphenols, electron transport, the catalysis of IAA oxidation (thereby affecting plant growth indirectly) and, finally, the production of free radicals, which appear to be involved in the defense of plants against pathogens and phytophagous insects (Mayer & Harel, 1979; Mayer, 1987).

Latent forms of PPO have been found in higher plants (Vaughn & Duke, 1984), and such enzymes may also be present in the thylakoid membranes of artichoke heads. In addition, such latent forms of PPO might be activated by chemical or physical stimuli and, as a result, may play an important role during the shelf life of artichoke heads and the selection of suitable storage conditions.

Furthermore, the presence of phenols in artichokes is well established (Lattanzio & Van Sumere, 1987) and therefore certain diphenols such as 5-*o*-CQA and caffeic acid or other related polyphenols may well be involved in both the enzymatic and non-enzymatic browning which results from disrupted cell compartmentalization. If present in artichokes, PPO could catalyse the production of quinones, giving rise to secondary reactions with the ultimate formation of intensely coloured products (Rhodes *et al.*, 1981). On the other hand, browning reactions may also be due to the oxidation of polyphenols catalysed by transition metals, chiefly iron (Mathew & Parpia, 1971). In fact, iron seems to be the metal most commonly involved in phenol browning via metal–polyphenol complex formation. It is also well established that both ascorbic acid (a reducing compound) and citric acid (a metal chelator) decrease plant tissue discoloration (Vande Casteele *et al.*,

1981; Van Sumere, unpublished results). These substances are also inhibitors of browning reactions in artichoke heads (Lattanzio *et al.*, 1989), which themselves contain considerable amounts of both *o*-diphenols (Lattanzio & Van Sumere, 1987) and iron (Lattanzio *et al.*, 1981). The fact that the quality and shelf life of artichoke heads, whether stored at 4°C or at room temperature, has been shown to improve remarkably after treatment with 1% ascorbic acid or citric acid suggested that these compounds might affect 5-*o*-CQA-iron complex formation and may also regulate phenylalanine ammonialyase and PPO activity (Lattanzio *et al.*, 1989). Thus PPO may play an important role in the browning of artichoke heads and as such could be of great interest in the quality and shelf life technology of artichokes. For these reasons, we decided to study some molecular properties of APPO in more detail. The results are described in this paper.

MATERIALS AND METHODS

Plant material

Artichoke heads (cv. Catanese) of marketable quality were cultivated in South Italy (Policoro, Matera) and harvested in January 1986.

Acetone powder preparation

Isolated artichoke hearts were frozen in liquid nitrogen, broken in a Pulsematic 8 Osterizer and then homogenized in cold acetone (−20°C) with an Ultraturrax model TP18/2N IKA-Werk (FRG) at low speed. The slurry was then filtered through a Büchner funnel with Whatman no. 4 filter paper, and the solid portion was repeatedly washed with cold acetone. Finally, a dry powder was obtained by drying the solid under vacuum.

Enzyme (APPO) extraction

The acetone powder (stored at −20°C) was extracted for 80 min in the presence of Dowex AG1-X8 and 1-X2 and insoluble PVP, with 1:100 (w/v) degassed 20 mM acetate buffer pH 5·7 (containing 0·2M KCl, 0·2M $(NH_4)_2SO_4$ and 1 mM $CuSO_4$ under nitrogen.

Enzyme (APPO) Purification

The crude enzyme solution was loaded onto a 1 × 35 cm column of Phenyl-Sepharose CL-4B (Pharmacia Fine Chemicals) equilibrated with the above-mentioned buffer solution. The column was then thoroughly washed with

starting buffer until the absorbance reached the base line values. The enzyme was eluted with 1 mM copper sulfate and then concentrated by ultrafiltration with an Amicon cell equipped with a YM30 membrane. A second purification step consisted of the gel filtration of the concentrated enzyme fraction on a 2·5 × 100 cm column of Sephadex G-150, equilibrated with 50 mM Tris-HCl pH 7.2.

Enzyme (APPO) assay

Polarographic method
Enzyme activity was determined polarographically at 30°C by measuring the oxygen uptake with a Clark electrode connected to a Gilson K-IC Oxygraph (Medical Electronics, Middleton, Wisc., USA). The reaction mixture consisted of 1 ml of 20 mM substrate in a buffer solution of suitable molarity and pH and 2 μl of appropriately diluted APPO solution.

Spectrophotometric method
Enzyme activity was measured with a Cary model 219 recording spectrophotometer at 30°C by determining the absorbance increases at 420 nm, 475 nm and 500 nm, using caffeic acid, D, L–DOPA or 5-o-CQA as a substrate. The typical reaction mixture was placed in a quartz cell with a 1 cm path-length and contained 20 μl of appropriately diluted APPO solution and 2·48 ml of substrate solution (4 mg/ml) in 0·5M phosphate buffer pH 6·8.

Unit definition

One unit of APPO was defined as the amount of enzyme causing uptake of 1 μmole of oxygen per min at 30°C when the polarographic method was used, while, with the spectrophotometric assays, the enzymic unit was defined as the amount of enzyme causing a change in absorbance of 0·001 ABS per min.

Chromatofocusing

Chromatofocusing was carried out at room temperature on a 1 × 40 cm column equilibrated with degassed 25 mM Bis-Tris buffer pH 7.1. The enzyme solution, which was previously equilibrated by dialysis with the same buffer, was applied to the column and eluted with Polybuffer 74, pH 4·0. Fractions of 4 ml were collected and monitored at 280 nm.

Protein determination

Soluble protein concentration was measured by the method described by Bradford (1976) using the Bio-Rad Protein Assay.

Molecular weight determination

The molecular weight of APPO was estimated according to Determann (1967) using gel filtration chromatography with a 1·6 × 46·5 cm BioGel column. The protein molecular weight markers, obtained from Pharmacia Fine Chemicals, were: Blue dextran 2000 for determining the void volume of the column, thyroglobulin (669 000 dalton), ferritin (440 000), catalase (232 000), aldolase (158 000), bovine serum albumin (67 000), ovalbumin (43 000), chymotrypsin (25 000) and ribonuclease A (13 700). The proteins were eluted with a 50 mM Tris-HCl, pH 7·2.

Kinetic constants determination

Determination of *Km* and V_{max} were performed according to Lineweaver-Burk to obtain the double reciprocal plot ($1/V$ versus $1/S$). APPO activity was measured polarographically at 30°C using 0·2M phosphate buffer, pH 7·0, with different substrate concentrations.

Effect of temperature on enzyme activity and determination of activation energy

The effect of temperature on the rate of APPO-catalysed oxidation of caffeic acid was determined in 0·5M phosphate buffer, pH 6·5, by measuring the enzyme activity at temperatures ranging from 4 to 50°C with both the polarographic and spectrophotometric methods. The activation energy was obtained by plotting the logarithm of the reaction rate against the reciprocal of the absolute temperature.

Electrophoresis

SDS-PAGE was carried out as described by Laemmly (1970) using a Bio-Rad vertical cell Protean II, a 7% polyacrylamide gel concentration and a 0·1% staining solution of Coomassie Blue R-250. The zymogram was performed according to Davis (1964); the staining solution was prepared using catechol as substrate (Park & Luh, 1985).

Activator and inhibitor studies

These studies were carried out with 40 mM caffeic acid as substrate using the polarographic technique at 30°C. To study the inhibitory effects of ascorbic and citric acids, the assay was carried out in 0·5M phosphate buffer, pH 7·0, adding suitable volumes of inhibitor stock solutions (both 0·1M) starting from 10 μl. The effect of the activators (Cu^{++} and Fe^{+++}) was observed using 1–2 mM $CuSO_4$ and $FeCl_3$ in 20 mM acetate buffer, pH 6.5.

RESULTS AND DISCUSSION

Extracting PPO from artichoke hearts, which have remarkably high phenol concentrations, is not an easy task. Therefore, the extraction of this enzyme was thoroughly studied under varying conditions, e.g. pH and ionic strength and the presence of reductants, surfactants and resins to remove phenols. The best procedure proved to be the extraction of an acetone powder (see 'Methods') with acetate buffer, pH 5·7, containing both Dowex AG1-X8 and 1-X2 and insoluble PVP. However, it should be mentioned that this procedure gave a crude extract that was barely suitable for further purification. As stated above, the main drawback was the presence of large amounts of phenols, which tend to oxidize and bind covalently to the polypeptide molecules (Sabir *et al.*, 1974; Van Sumere *et al.*, 1975; Yamamura *et al.*, 1980; Leoni *et al.*, 1985).

Table 1 shows the procedures employed and the results for APPO purification, which amounted to a 65-fold enrichment of the enzyme. In all trials hydrophobic interaction chromatography using Phenyl-Sepharose proved to be more efficient than gel filtration on G-150 Sephadex. In fact,

TABLE 1
Purification of Artichoke Polyphenol Oxidase

Step	*Total volume (ml)*	*Protein (mg/ml)*	*Total protein (mg)*	*Total activity (μmol O_2/min)*	*Activity (U/ml)*	*Specific activity (U/mg)*	*Yield (%)*	*Purification (fold)*
Crude	1 400	0·74	1 036	19 740	14·1	19·1	100	1
Phenyl sepharose chromatography	32·5	0·17	5·5	2 860	88·0	518	14·5	27
Gel filtration G-150 Sephadex	23·5	0·06	1·4	1 776	74·0	1 233	9·0	65

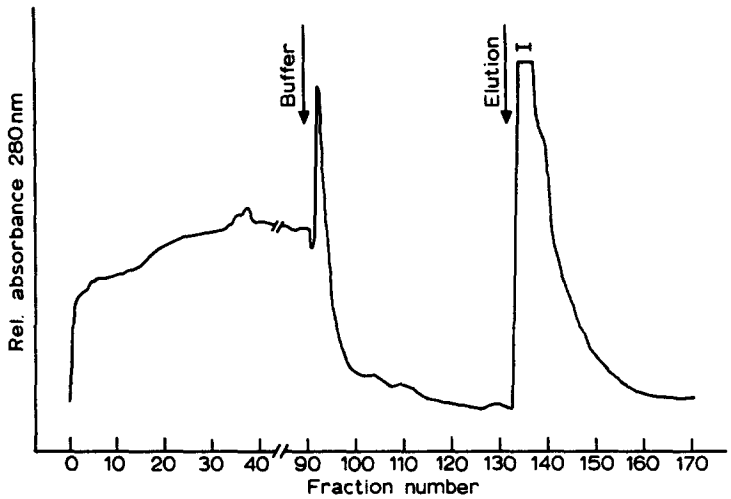

Fig. 1. Typical chromatogram of crude polyphenol oxidase on phenyl-Sepharose CL-4B column. The enzyme was pooled as indicated.

APPO binds rather strongly and steadily during chromatography to Phenyl-Sepharose. Subsequently, the column was washed thoroughly with starting buffer until the absorbance reached a zero value at 280 nm. The enzyme was eluted in a single peak, using 1 mM copper sulfate in water as reported in Fig. 1.

Using gel filtration over BioGel, the molecular weight of APPO was shown to be approximately 116 000 dalton (Fig. 2). The molecular weight of

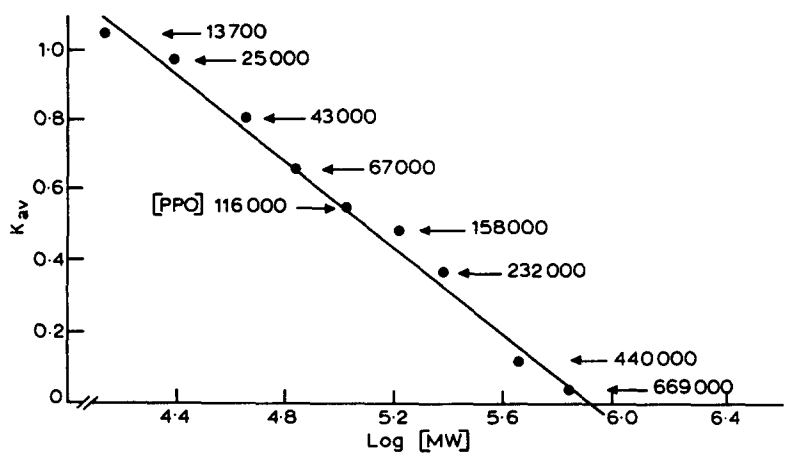

Fig. 2. Calibration curve for determining the molecular weight of APPO. The logarithmic values of the molecular weights of eight standard marker proteins and APPO were plotted against their mobilities on gel filtration chromatography in a column 1·6 × 46·5 cm packed with Bio-gel A 0·5. $K_{av} = (V_{el} - V_o)/(V_t - V_o)$; V_{el} = Elution volume; V_t = Total volume; V_o = Exclusion volume.

APPO seems thus to be similar to that of other PPOs isolated from mushroom (Bouchilloux *et al.*, 1963), potato (Anisimov *et al.*, 1978) and sugar cane (Gross & Coombs, 1975). The APPO, which appears homogeneous on the basis of molecular weight determinations, showed a pH of 4·5. However, the zymogram demonstrated the presence of three isoenzymes of very similar relative electrophoretic mobility and band intensity. In addition, SDS polyacrylamide gel electrophoresis showed an acceptable level of purity of the enzyme preparation, which appears to consist of three similar subunits. A molecular weight of *ca.* 40 000 dalton has been calculated for the monomer.

Figure 3 shows the pH optimum for APPO with four different substrates: caffeic acid, 5-*o*-CQA, pyrocatechol and 1,5-dicaffeoylquinic acid. All of these substrates showed a broad range of activity, viz. pH 6·5–8·0 for caffeic acid and pH 5·0–7·0 for the other substrates. Moreover, with 1,5 dicaffeoylquinic acid, the activity proved to be very low, while these optimal pH values were very similar to other plant PPOs.

In addition, substrate specificity was determined both polarographically and spectrophotometrically at pH 6·0. Although the latter technique is not considered very accurate (Mayer & Harel, 1979), the results with both techniques were similar. Those obtained by polarography are listed in

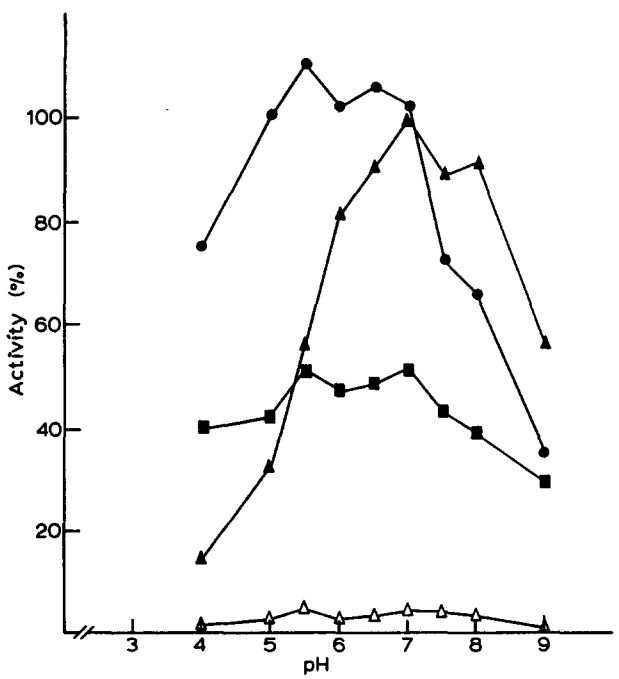

Fig. 3. Effect of pH on artichoke polyphenol oxidase activity: ●, 5-*o*-CQA; ▲, caffeic acid; ■, pyrocatechol; △, 1,5-dicaffeoylquinic.

TABLE 2
Substrate Specificity of Polyphenol Oxidase from Artichoke Head

Phenolic substrate	*Relative activity* (%)
5-*o*-CQA	100
Caffeic acid	69
Pyrocatechol	42
Pyrogallol	8
1,5-dicaffeoylquinic acid	6
Catechin	6
L-DOPA	5
Tyrosine	0
p-Coumaric	0
Ferulic acid	0
Cinnamic acid	0
Fisetin	0
Kaempferol	0
Quercetin	0
Luteolin	0
Myricetin	0

Table 2, in which it appears clear that APPO does not catalyse the oxidation of monophenols such as tyrosine or *p*-coumaric acid. This demonstrates that the artichoke enzyme does not show any cresolase activity. In fact, APPO was active only towards *o*-diphenols, mainly 5-*o*-CQA (100%), caffeic acid (69%) and pyrocatechol (42%). Lower activities were also detected for other polyphenol substrates such as pyrogallol, catechin, L-DOPA and 1,5-dicaffeoylquinic acid. An analogous behaviour has also been observed for other PPOs isolated from fruits and plants (Gregory & Bendall, 1966; Wong *et al.*, 1971; Hasegawa & Maier, 1980).

The effect of temperature on the rate of APPO-catalysed caffeic acid oxidation was determined both polarographically and spectrophotometrically by measuring the reaction rate at several temperatures between 4 and 50°C. Also in this case, similar results were obtained. Figure 4 shows the spectrophotometric findings, and demonstrates that, with increasing temperature, APPO increased its activity up a maximum of 25°C. The same optimum was also observed for PPO isolated from apricot (Mihalyi *et al.*, 1978) and apple cv. Starking (Vamos-Vigyazo, 1981). It is also interesting that at 50°C APPO activity was *ca.* 10% of the original. In addition, the activation energy of APPO with caffeic acid substrate in 0·5M phosphate buffer, pH 6·5, was $21{\cdot}7 \pm 0{\cdot}2$ kJ/mole, a value similar to the 18·6 kJ/mole determined for PPO purified from banana (Palmer, 1963).

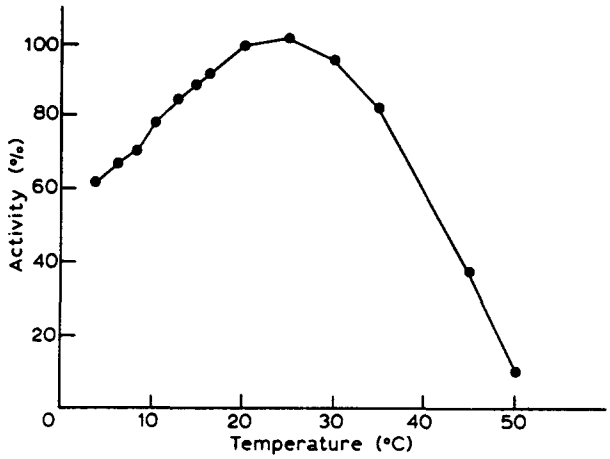

Fig. 4. Effect of temperature on artichoke polyphenol oxidase activity. Caffeic acid was used as substrate in buffered solution at pH 6·5. This curve was obtained using the spectrophotometric data.

Furthermore, the Lineweaver-Burk plots for APPO with 5-*o*-CQA and caffeic acid as substrates are shown in Fig. 5, while Table 3 shows the corresponding extrapolated *Km* values and the maximum velocities of the reactions. Although these substrates appear to be very similar for APPO, differing little in enzyme affinity, 5-*o*-CQA seems to be the substrate of choice. These results are comparable to those found for other PPOs purified from potato tubers, Japanese pear and eggplant (Alberghina, 1964; Tono *et al.*, 1986; Fujita & Tono, 1988). These authors call their PPOs chlorogenic acid oxidases. In our case, given that 5-*o*-CQA is the most

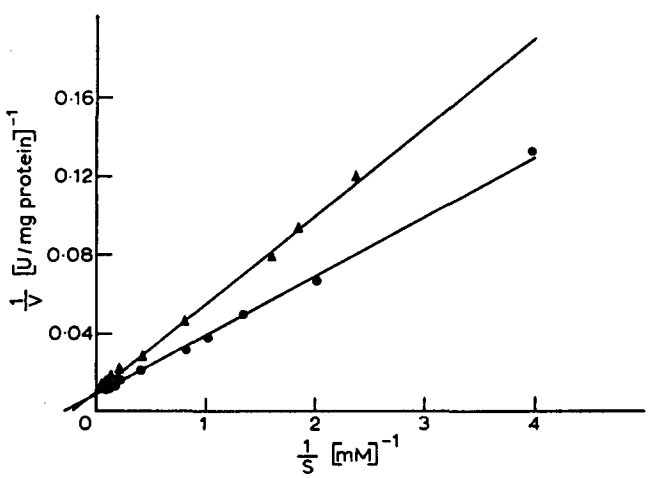

Fig. 5. Lineweaver–Burk plot with chlorogenic acid (●) and caffeic acid (▲) as substrate.

TABLE 3
Michaelis Constants of Artichoke Polyphenol Oxidase for the Two Best Substrates (5-*o*-CQA and caffeic acid) at 30°C, 0·2M phosphate buffer pH 7·0

Substrate	*Km* (*mM*)	V_{max} (*U/mg protein*)
5-*o*-CQA	4·2 ± 0·5	135·6 ± 14·7
Caffeic acid	5·3 ± 0·3	115·1 ± 6·1

TABLE 4
Effect of Cupric and Ferric Ions on Artichoke Polyphenol Oxidase

Ion	*mM*	*Relative activity* (%)
None	—	100
Cu^{++}	1	108
Cu^{++}	2	120
Fe^{+++}	1	110

abundant phenol in artichoke head, it appears reasonable and convenient to call APPO chlorogenic acid oxidase also.

Cupric and ferric ions activated the oxidation of APPO—5-*o*-CQA (Table 4). Similar PPO activation by Cu^{++}, but not Fe^{+++}, was recorded both by Simpson *et al.* (1987) and Mathew & Parpia (1971). The activation of APPO by Fe^{+++} is of particular interest for artichoke heads, in which the quantity of iron is far from negligible (10 mg/100 g fresh weight) (Lattanzio *et al.*, 1989). However, APPO activation by these ions does not seem to be important enough to suggest that cupric and ferric ions have an important role in enzymic browning of artichoke heads during storage.

The possible APPO inhibitory effect of four non-toxic compounds, viz. ascorbic acid, citric acid, cinnammic acid and NaCl was studied. Particular attention was given to ascorbic and citric acids, which in a previous study, notably improved the shelf life of artichoke heads (Lattanzio *et al.*, 1989). Of these, only ascorbic acid (in concentrations of 5 mM upwards) proved to be significantly inhibitory towards APPO.

CONCLUSION

This study emphasizes, once more, the typical difficulties which are frequently encountered during plant PPO purification. The purification of

this enzyme is usually quite difficult and shows a scarcely reproducible degree of purity, yield and isoenzyme composition. The main difficulty during the APPO isolation and purification described in this paper was the rather poor enzyme recovery during the initial extraction of the APPO. This difficulty, which is mainly due to the high phenol content of artichokes, should be given further attention. Nevertheless, we were able to determine some important molecular properties of APPO, which are summarized in the abstract of this paper.

ACKNOWLEDGEMENT

Research work supported by CNR, Italy. Special grant IPRA Sub-Project 3.2.1, Paper No. 5325. C.F.V.S. is grateful to the Belgian IWONL and CBM for financial support.

REFERENCES

Alberghina, F. A. M. (1964). Chlorogenic acid oxidase from potato tuber slices: Partial purification and properties. *Phytochemistry*, **3**, 65.

Anisimov, V. D., Kastal'eva, T. B. & Loginova, L. N. (1978). Isolation and some properties of potato *o*-diphenol oxidase. *Biokhimiya*, **43**, 1616.

Bouchilloux, M., McMahill, P. & Mason, H. S. (1963). The multiple forms of mushroom tyrosinase. Purification and molecular properties. *J. Biol. Chem.*, **238**, 1699.

Bradford M. M., (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye-binding. *Anal. Biochem.*, **72**, 248.

Davis, B. J. (1964). Disk electrophoresis. 2 Method and application to human serum proteins. *Annals NY Acad. Sci.*, **121**, 404.

Determann, H. (1967). Gelchromatographie. Springer, New York, pp. 67–73.

Fujita, S. & Tono, T. (1988). Purification and some properties of polyphenoloxidase in eggplant (*Solanum melanogena*), *J. Sci. Food Agric.*, **46**, 115.

Gregory, R. P. F., & Bendall, D. S. (1966). The purification and some properties of polyphenol oxidase from tea (*Camelia sinensis* L.) *Biochem. J.*, **101**, 569.

Gross, D. & Coombs, J. (1975). Enzymatic colour formation in beet and cane juice. Paper presented at the 15th General Assembly of CITS, Vienna.

Hasegawa, S. & Maier, V. P. (1980). Polyphenol oxidase of Dates. *J. Agric. Food Chem.*, **28**, 891.

Laemmly, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, **227**, 680.

Lattanzio, V. & Van Sumere, C. F. (1987). Changes in phenolic compounds during the development and cold storage of artichoke (*Cynara scolymus* L.) heads. *Food Chem.*, **24**, 37.

Lattanzio, V., Lafiandra, D., Morone-Fortunato, I. (1981). Composizione chimica e valore nutritivo del carciofo (*Cynara scolymus* L.). In *Studi sul Carciofo*, ed. V. Marzi & V. Lattanzio. Industria Grafica Laterza, Bari, Italy.

Lattanzio, V., Linsalata, V., Palmieri, S. & Van Sumere, C. F. (1989). The beneficial effect of citric and ascorbic acid on the phenolic browning reaction in stored artichoke (*Cynara scolymus* L.) heads. *Food Chem.*, **2**, 93.

Leoni, O., Iori, R. & Palmieri, S. (1985). Purification and properties of lipoxygenase in germinating sunflower seeds. *J. Food Sci.*, **50**, 88.

Mathew, A. G. & Parpia, H. A. E. (1971). Food browning as a polyphenol reaction. In *Advances in Food Research*, Vol. 19, ed. C. O. Chichester, E. M. Mrak & G. E. Steward. Advances in Food Research, Vol. 19. Academic Press, London, pp. 75–145.

Mayer, A. M. (1987). Polyphenoloxidase in plants. Recent progress. *Phytochemistry*, **26**, 11.

Mayer, A. M. & Harel, E. (1979). Polyphenol oxidases in plants. *Phytochemistry*, **18**, 193.

Mihalyi, K., Vamos-Vigyazo, L., Kiss-Kutz, N. & Babos-Szebenyi, E. (1978). The activities of polyphenol oxidase and peroxidase in fruits and vegetables as related to pH and temperature, *Acta Aliment. Acad. Sci. Hung.*, **7**, 57.

Palmer, J. K. (1963). Banana polyphenoloxidase. Purification and properties. *Plant Physiol.*, **38**, 508.

Park, E. Y. & Luh, B. S. (1985). Polyphenol oxidase of kiwifruit. *J. Food Sci.*, **50**, 678.

Rhodes, M. J. C., Wooltorton, L. S. C. & Hill, A. C. (1981). Changes in phenolic metabolism in fruit and vegetable tissue under stress. In *Recent Advances in the Biochemistry of Fruits and Vegetables*, ed. J. Friend & M. J. C. Rhodes. Academic Press, London, pp. 191–220.

Sabir, M. A., Sosulski, F. W. & Finlayson, A. J. (1974). Chlorogenic acid–protein interactions in sunflower. *J. Agric. Food Chem.*, **22**, 575.

Simpson, B. K., Marshall, M. R. & Otwell, S. (1987). Phenoloxidase from Shrimp (*Penaeus setiferus*): Purification and some properties. *J. Agric. Food Chem.*, **35**, 918.

Tono, T., Fujita, S., Kawasaki, H. & Li, Z. (1986). Purification and high L-epicatechinoloxidase activity in polyphenoloxidase of Japanese pears. *Nippon Nogeikagaku Kaishi*, **60**, 705.

Vamos-Vigyazo, L. (1981). Polyphenol oxidase and peroxidase in fruits and vegetables. *CRC Critical Reviews in Food Science and Nutrition*, **15**, 49.

Vande Casteele, K. L., Dauw-Van Keymeulen, M. I., Deberg, P.C., Maene, L. J., Flameé, M. C. & Van Sumere, C. F. (1981). The phenolics and hydrolysable tannin polyphenoloxidase of *Medinilla magnifica*. *Phytochemistry* **20**, 1105.

Van Sumere, C. F., Albrecht, J. Dedonder, A., De Pooter, H. & Pe', I. (1975). Plant proteins and phenolics. Ch. 8. In *The Chemistry and Biochemistry of Plant Proteins*, ed. J. B. Harbourne & C. F. Van Sumere. Academic Press, New York, p. 211.

Vaughn, K. C. & Duke, S. O. (1984). Function of polyphenoloxidase in higher plants. *Physiol. Plant.*, **60**, 106.

Wong, T. C., Luh, B. S. & Whitaker, J. R. (1971). Isolation and characterization of polyphenol oxidase isozymes of Clingstone peach. *Plant Physiol.*, **48**, 19.

Yamamura, I., Yonekura, M., Katsura, Y., Ishiguro, M. & Funatsu, M. (1980). Purification and some physico-chemical properties of phenoloxidase from the larvae of housefly. *Agric. Biol. Chem.*, **44**, 55.