Effect of Organic Farming Practices on the Level of Latent Polyphenol Oxidase in Grapes

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ABSTRACT: The levels of polyphenol oxidase (PPO) in organic as compared with conventional Monastrell grapes were examined in this study. The enzyme was partially purified using the Triton X-114 method, and in both cases it was obtained in a latent state, being activated by different methods (acid shocking, detergents, and proteases). PPO purified from both organic and conventional Monastrell grapes had the same isoenzymatic form, appearing as the same single band in polyacrylamide gel electrophoresis. However, diphenolase activity of activated PPO in organic grapes was 2 times higher than in conventional grapes, independent of the activation method used. Moreover, the proteolytic activation method, using trypsin, was the most effective for this latent PPO.

Keywords: organic culture, grape, polyphenol oxidase, latency, activation

Introduction

Organic food is an expanding sector of the agricultural industry in many parts of the world, and it is possible to find organically produced food in most supermarkets in Europe and North America.

The overall goal of organic farming is to use agricultural methods that have the smallest impact on the environment and provide the greatest benefit to people (Becharrell and MacFie 1991; Bourn and Prescott 2002).

One main difference between organic and conventional farming is in the use of fertilizers. The latter uses chemical fertilizers as well as manure, compost, sewage sludge, and other soil amendments, whereas most certified organic farming allows only the use of manure, compost, and natural soil additives. A second difference between the 2 systems is the use of pesticides and herbicides. Conventional farming, once again, uses any product available on the market (not forbidden by law), whereas organic farming allows only a few pesticides that are believed to leave no residue on the products, for example, copper ammonium carbonate, copper sulfate, copper oxychloride, sulfur, rotenone, pyrethrum, and soft soap (IFOAM 1998).

The application of pesticides and fertilizers has previously been reported to modulate the biosynthesis and nature of phenolics in plants (Lea and Beech 1978; Nicolas and others 1994; Daniel and others 1999; Carbonaro and Mattera 2001), indirectly affecting the enzymatic browning of fruits and vegetables (Martinez and Whitaker 1995). The degree of browning also depends on the presence of oxygen, reducing substances, metallic ions, pH, temperature, and the activity of different oxidizing enzymes, especially polyphenol oxidase (PPO) (monophenol, dihydroxyphenylalanine: oxygen oxidoreductase, EC 1.14.18.1), which is a copper-containing enzyme. It catalyzes 2 different reactions in the presence of molecular oxygen: the hydroxylation of monophenols to o-diphenols (monophenolase activity) and the oxidation of o-diphenols to o-quinones (diphenolase activity) (Sánchez-Ferrer and others 1995). In some plants, this enzyme exists in an inactive or latent state (Mayer and Harel 1979; Moore and Flurkey 1990; Sánchez-Ferrer and others 1993a; Núñez-Delicado and others 1996) and is activated by a variety of treatments or agents, including acid and alkaline shock (Kenten 1957), urea (Swain and others 1966), anionic detergents such as sodium dodecyl sulfate (SDS) (Laveda and others 2000; Núñez-Delicado and others 2003), proteases (Laveda and others 2003), and fatty acids (Golbeck and Cammarata 1981).

It is well known that grapes contain high polypohenol oxidase activity (Valero and others 1988; Sánchez-Ferrer and others 1989b) and that enzymatic browning occurs rapidly in the damaged berry or after the crushing of fresh grapes for juice and wine production (Singleton and Rossi 1965; Kidron and others 1978; Sánchez-Ferrer and others 1989b). This reaction causes a change in the color and the flavor of the juice and greatly diminishes the quality of the final product (Valero and others 1988). Thus, to produce high-quality wine, it is important to know how to control the level of this enzyme in the grapes.

The objective of this work was to study the influence of organic farming practices on the level of PPO expressed in Monastrell grapes, a winemaking variety, compared with that expressed in conventionally grown grapes. The influence of different activation processes on the latent enzyme was also studied and compared.

Materials and Methods

Sampling and raw material

Monastrell grapes at vinification maturity, organically and conventionally grown, were kindly supplied by San Isidro wineries (Jumilla, Murcia, Spain). Organic grapes were grown under controlled cultivation conditions in which only sulfur and cuprous oxychloride were added to the soil, whereas in the case of conventionally grown grapes, sulfur, zinc, manganese, malathion, fenarimol, fenitrotion, and pH regulator were added to plants.

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Grapes were picked from 3 single plantations of organic culture and 3 single plantations of conventional culture. At each plantation, 10 vines, situated at different places, were selected, and 0.5 kg of grapes was harvested (at vinification maturity) from each of them. Two mixtures were made, the 1st with grapes from each organic plantation and the 2nd with grapes from each conventional plantation. These 2 mixtures were frozen at –80 °C until used as PPO source. Samples of frozen grapes (50 g each) both from conventional and organic culture types were picked at random to extract PPO. All PPO extracts were made in triplicate.

Reagents

Biochemicals were purchased from Sigma (Madrid, Spain) and used without further purification. Triton X-114 (TX-114) was obtained from Fluka (Madrid, Spain) and condensed 3 times as described by Bordier (1981), but using 100 m*M* sodium phosphate buffer, pH 7.3. The detergent-rich phase of the 3rd condensation had a concentration of 25% TX-114 (w/v). The TX-114 concentration was estimated from 278 nm absorption of dilute solution (A_{278} = 1.25 for 0.05%; w/v) (Werck-Reichart and others 1991). The phenolic substrates 4-*tert*-butylcatechol (TBC), 4-methylcatechol (4MC), and chlorogenic acid (CGA) were obtained from Fluka (Madrid, Spain).

Enzyme purification of grape PPO

Grape PPO was extracted using a modification of the method described earlier by our group (Sánchez-Ferrer and others 1989a). A sample of 50 g of frozen grapes was thawed at room temperature and homogenized for 1 min with 50 mL cold buffered 100-m*M* sodium phosphate (pH 7.3) containing 25 m*M* ascorbic acid and serine protease inhibitors (1-m*M* phenylmethanesulfonyl fluoride [PMSF] and 1-m*M* benzamidine hydrochloride), both of which were added immediately before use. The homogenate was filtered through 8 layers of cheesecloth and centrifuged at 4000 × *g* for 10 min at 4 °C. The supernatant was discarded, and the precipitate was resuspended with 20 mL of 4% (w/v) TX-114 in 100 m*M* sodium phosphate buffer (pH 7.3) for 15 min at 4 °C. This solution was subjected to temperature-induced phase partitioning, and the mixture was warmed at 37 °C for 15 min. During this time, the solution became spontaneously turbid because of the formation, aggregation, and precipitation of large mixed micelles of detergent, which contained hydrophobic proteins, chlorophylls, and phenolic compounds. This solution was centrifuged at 10000 × *g* for 15 min at 25 °C. The detergent-rich phase was discarded, and the clean detergent-poor supernatant, containing soluble grape PPO, was used as the enzyme source. This enzyme solution was stored at –20 °C.

Enzyme activity

Enzymatic activity was determined spectrophotometrically (Varian, Cari 50-Bio, Victoria, Australia) at 400 nm with TBC (ϵ_{400} = 1150/*M*/cm) (Sánchez-Ferrer and others 1993a). One unit of enzyme was defined as the amount of enzyme that produced 1 μmol of the *tert*-butyl-quinone/min.

Unless otherwise stated, the standard reaction medium at 25 °C contained 25 μg of protein, 10m*M* sodium phosphate buffer, pH 6.0, and 2.5m*M* TBC in a final volume of 1 mL. Each sample was assayed in triplicate.

Optimum pH

pH studies were carried out using 10 m*M* sodium acetate buffer (pH 3.0 to 5.5) and 10 m*M* sodium phosphate buffer (pH 6.0 to 7.5) in the presence or absence of 3.5 m*M* SDS.

Optimal activation by SDS

Activation studies were carried out in the standard reaction medium (1 mL) adding different detergent concentrations.

Trypsin activation

Activation studies were carried out by incubating 25 μg/mL grapes PPO and 0.5 mg/mL trypsin for different times. After that, the activated PPO activity was measured in the standard reaction medium conditions in a final volume of 1 mL.

Electophoresis

Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE) was carried out as described by Angleton and Flurkey (1984). Samples were mixed with glycerol, SDS, and bromophenol blue before being applied to 10% polyacrylamide gels. Electrophoresis was carried out for 1 h at 25 °C in a Mini Protein Cell (Bio-Rad). The gels were stained for PPO activity in 100 mL of 10 m*M* sodium acetate buffer (pH 5.0) containing 5m*M* L-3,4-dihydroxyphenylalanine (L-dopa) and 3m*M* 3-methyl-2-benzo-thiazolinone hydrazone (MBTH) hydrochloride hydrate.

Protein determination

The protein content was determined according to Bradford's dye binding method, using bovine serum albumin (BSA) as a standard (Bradford 1976).

Statistical analysis

The significance of the difference between means of PPO activity in conventional and organic grapes was estimated by Student *t*-test. These analyses were carried out using SPSS version 12 (SPSS Inc., Chicago, Ill., U.S.A.).

Results and Discussion

Enzymatic browning of musts and wines is mainly dependent on the oxidation of endogenous phenols by PPO (Sapis and others 1983). Mayer (1987) has reviewed the importance of this enzyme in winemaking, which has attracted the attention of many investigators since the 1960s for many reasons: its kinetic parameters (Lerner and Mayer 1976; Sánchez-Ferrer and others 1988), its latency (Sánchez-Ferrer and others 1989a), its location (Ivanov 1967), its changes during maturation (Sánchez-Ferrer and others 1989b; Valero and others 1989), and the best moment for harvesting grapes to produce a high-quality wine without, or at least with reduced, enzymatic browning (Valero and others 1989).

In this work, PPO samples from organic and conventional Monastrell grapes, a Spanish winemaking variety, were partially purified using a modification of the method described by Sánchez-Ferrer and others (1989a), which consists of phase partitioning in TX-114 (for details, see "Materials and Methods" section). The enzyme thus obtained was in a latent state and could be activated by different methods (acid shocking, detergents, or proteases), which is in agreement with data previously described (Sánchez-Ferrer and others 1989b).

The partially purified conventional and organic Monastrell grape PPO appeared as a single band in PAGE (Figure 1) when L-dopa and MBTH were used as a substrate, in both cases. This result was similar to that obtained for soluble potato PPO (Sánchez-Ferrer and others 1993a), iceberg lettuce PPO (Chazarra and others 1996), banana pulp PPO (Sojo and others 1998), and persimmon fruit (Núñez-Delicado and others 2003). Moreover, Figure 1 shows the same band in both cases, indicating the presence of the same PPO isoenzyme in Monastrell grapes grown under conventional and organic farming conditions.

When the PPO activity was studied with TBC at different pH values in the absence of SDS, both conventional and organic grapes showed an optimum pH at 4.0 (Figure 2a, filled symbols). These data were in accordance with the optimum pH described for conventional grape PPO (Sánchez-Ferrer and others 1989b). The low pH optimum is a result of the enzyme being induced by acid shocking. This low pH was displaced to pH 5.0 when the assays were conducted in the presence of 0.1% SDS, in both cases (Figure 2a, open symbols). This effect of pH displacement in the presence of SDS has also been described with persimmon fruit latent PPO (Núñez-Delicado and others 2003).

Figure 2a also shows that PPO activity in organic Monastrell grapes (circles) was double ($P < 0.05$) that observed in conventional Monastrell grape (squares), both in the presence or in the absence of SDS. The highest value in the activation process was obtained at pH 6.0 in both cases (Figure 2b), at which value the activation was 8.6-fold and 7-fold for organic and conventional Monastrell grapes, respectively.

To further characterize the differences between the 2 types of farming, the effect of SDS concentration was studied at pH 6.0. Figure 3 shows that the activation process was saturable, reaching its maximum activation at 3.5 m*M* SDS in both organic and conventional Monastrell grapes. Once again, PPO activity in the presence of SDS was approximately 2 times higher in organic than in conventional Monastrell grapes ($P < 0.001$).

This SDS activation was also dependent on the substrate used. Three substrates, TBC, 4MC, and CGA, were tested (Table 1), a clear dependence between the degree of hydrophobicity of the

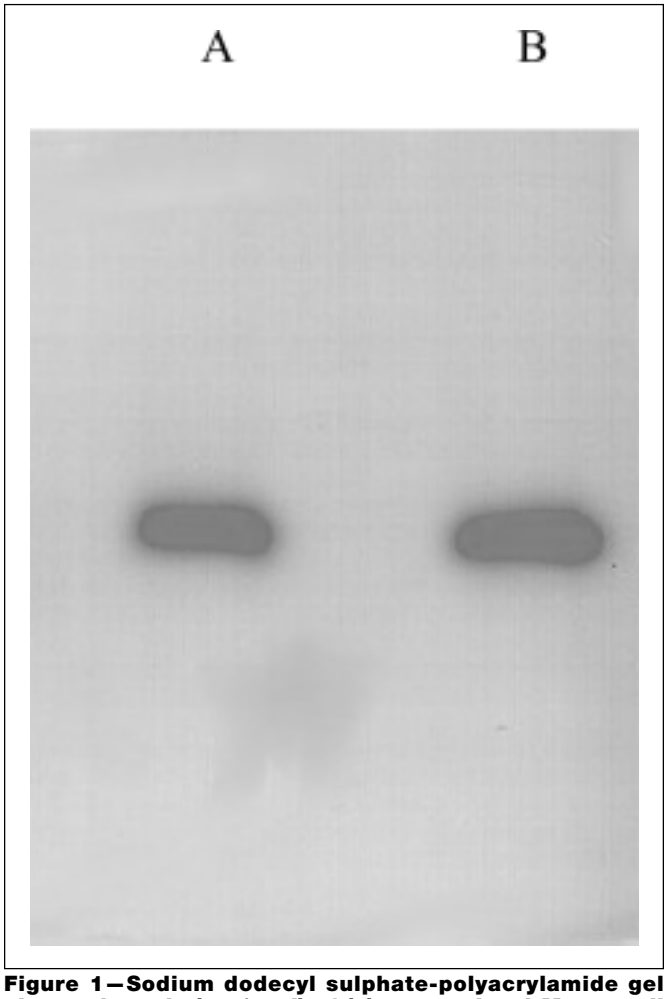

Figure 1—Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (10% gel) of (a) conventional Monastrell grape polyphenol oxidase and (b) organic Monastrell grape polyphenol oxidase, stained with 5m*M* L-3,4-dihydroxyphenylalanine (L-dopa) and 2m*M* 3-methyl-2-benzo-thiazolinone hydrazone hydrochloride hydrate in sodium acetate buffer (pH 4.0).

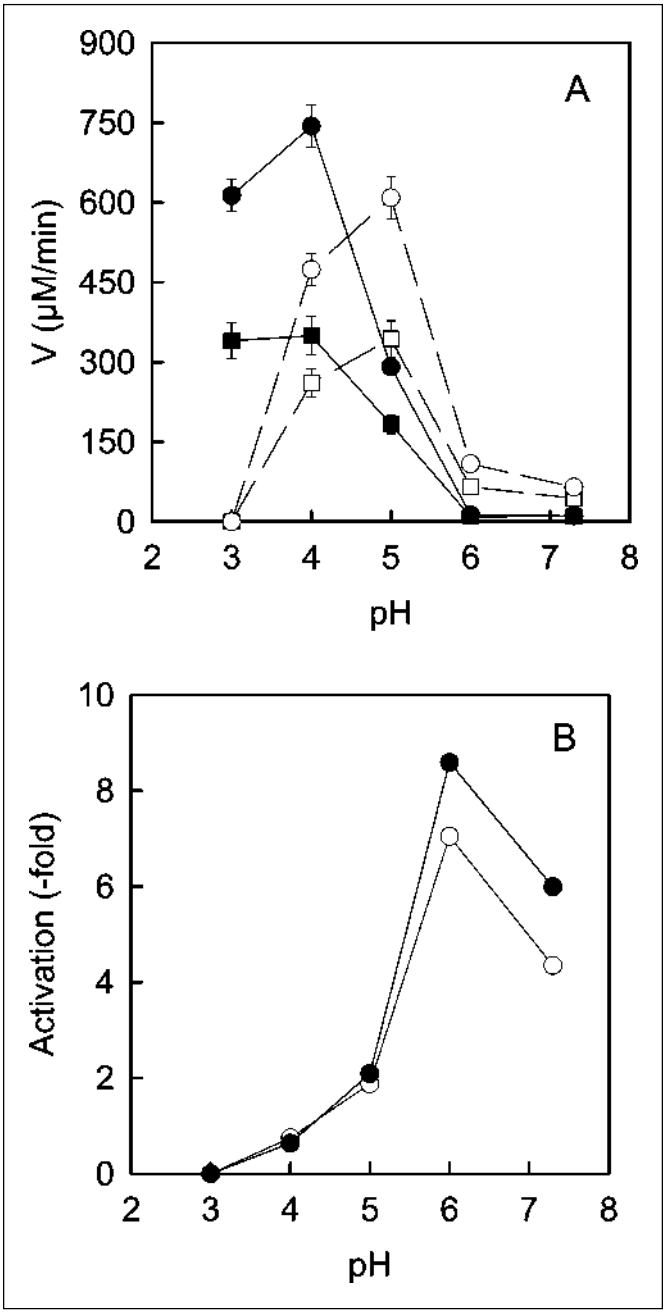

Figure 2—(a) Effect of pH on Monastrell grape polyphenol oxidase activity in 10 m*M* sodium acetate (pH 3.0 to 5.0), 10-m*M* sodium phosphate (pH 6.0 to 7.5). The reaction medium at 25 °C contained 2.5 m*M* 4-tert-butylcatechol and 25 μg/mL of partially purified polyphenol oxidase (PPO). Organic Monastrell grape PPO in the presence (○) or absence (●) of 3.5 m*M* SDS. Conventional Monastrell grape PPO in the presence (□) or absence (■) of 3.5 m*M* sodium dodecyl sulfate (SDS). (b) Effect of pH on SDS activation of organic (●) and conventional (■) Monastrell grapes.

substrate and the degree of activation being evident (TBC > 4MC > CGA). The conformational changes produced by the surfactant in the activation of latent PPO favor the access of the hydrophobic substrates to the active center, as has been previously described for latent potato leaf PPO (Sánchez-Ferrer and others 1993b).

Finally, proteolytic activation was also studied in the presence of 0.5 mg/mL trypsin (Figure 4). The activation process was time-dependent, reaching maximal activation when the incubation time was 20 min. In the case of organic grapes, the latent enzyme was activated 29-fold, which is almost double that reached in conventional grapes (17-fold). The activity of trypsin-activated organic and conventional latent PPOs was independent of pH from 3 to 7.5 (Figure 5a). However, the maximal proteolytic activation of both latent PPOs was found at basic pH values (Figure 5b). In both organic and conventional Monastrell grapes, the degree of activation obtained using trypsin as activation agent was higher (2-fold to 3-fold) than that obtained by pH or SDS.

In summary, this study revealed that organic and conventional Monastrell grape PPO is in the latent state and can be activated by different methods, such as with acid-shocking, SDS, and trypsin, the last activation method being the most effective (29-fold or 17-fold for organic and conventional grapes, respectively). In addition, the high PPO activity present in organic compared with conventional Monastrell grapes could be the result of changes in phenolic metabolism in plants grown in the absence of synthetic chemical pesticides and most of the readily soluble mineral fertilizers, especially copper sulfate. This increase in PPO activity may contribute effectively to disease resistance in organic farming via rapid oxidation of phenols to quinines, thus inhibiting the polygalacturonase of pathogens (Ohazurike and Arinze 1996).

Table 1—Sodium dodecyl sulfate (SDS) activation (-fold) of polyphenol oxidase from organic and conventional Monastrell grapes, using different substrates

	4-*tert*-Butyl catechol[a]	4-Methyl catechol[b]	Chlorogenic acid[c]
PPO[d] from organic Monastrell grapes	8.6	5.5	3
PPO from conventional Monastrell grapes	7	3.6	1.5

[a]Assayed in the standard reaction medium, in the absence or in the presence of 3.5 m*M* SDS.
[b]Assayed in the standard reaction medium but using as substrate 2.5 m*M* 4-methylcatechol, in the absence or in the presence of 3.5 m*M* SDS.
[c]Assayed in the standard reaction medium but with 2.5 m*M* chlorogenic acid, in the absence or in the presence of 3.5 m*M* SDS.
[d]PPO = polyphenol oxidase.

Conclusions

In conclusion, the results presented in this article suggest that PPO may be considered a specific endogenous marker for the differentiation of organic from conventional grapes, as has been previously described for organic peaches and pears (Carbonaro and Mattera 2001). These data indicate that wines obtained from organic grapes could be more oxidated than those obtained from conventional grapes because of the highest PPO activity observed in organic grapes.

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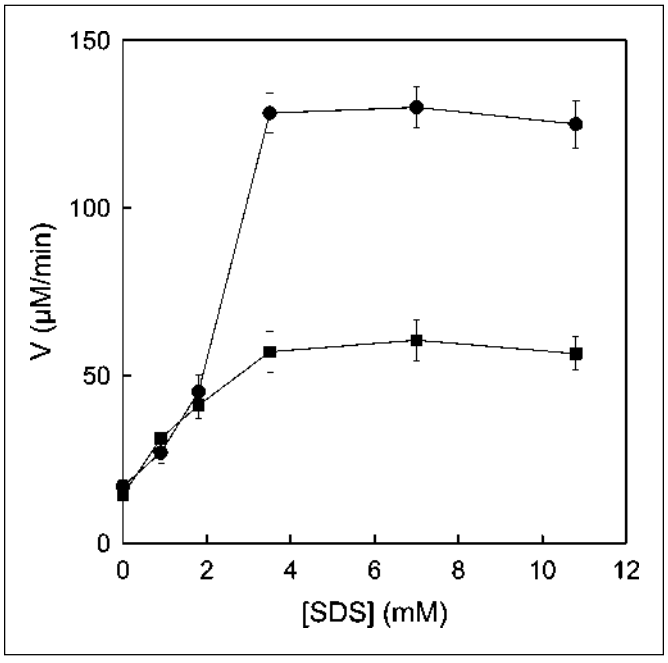

Figure 3—Effect of sodium dodecyl sulphate (SDS) concentration on Monastrell grape polyphenol oxidase activity. The reaction medium at 25 °C contained 10 m*M* sodium phosphate buffer, pH 6.0, 2.5 m*M* 4-*tert*-butylcatechol, increasing concentrations of SDS (0 to 10 m*M*) and 25 μg/mL of partially purified polyphenol oxidase from organic grapes (●) 25 μg/mL of partially purified PPO from conventional grapes (■).

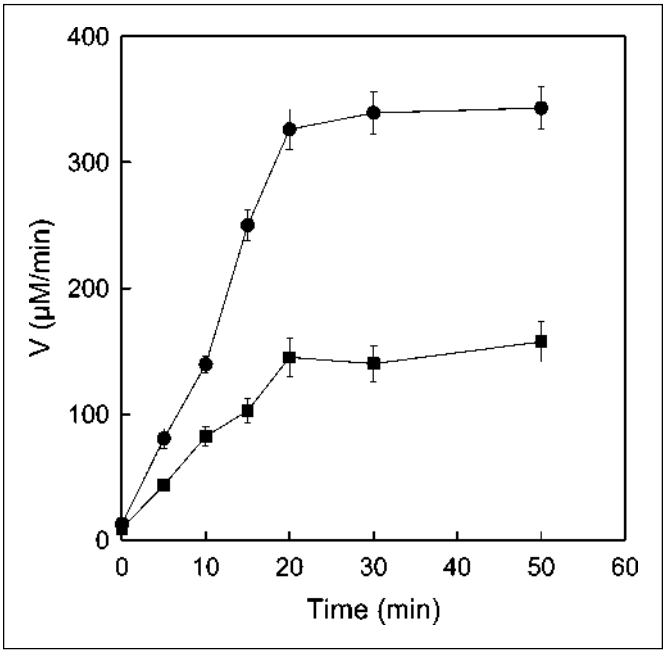

Figure 4—Effect of incubation time on the trypsin-mediated activation of Monastrell grape polyphenol oxidase (PPO). The reaction medium at 25 °C contained 10 m*M* sodium phosphate buffer, pH 6.0, 2.5 m*M* 4-*tert*-butylcatechol, and 25 μg/mL of organic Monastrell grape PPO incubated with trypsin 0.5 mg/mL for different times (●) or 25 μg/mL of conventional Monastrell grape PPO incubated with trypsin at 0.5 mg/mL for different times (■).

References

Angleton EA, Flurkey WH. 1984. Activation and alteration of plant and fungal polyphenol oxidase isoenzymes in sodium dodecyl sulphate electrophoresis. Phytochemistry 23:2723–5.

Becharrell B, MacFie JH. 1991. Consumer attitudes to organic foods. Br Food J 93:25–30.

Bordier C. 1981. Phase separation of integral membrane proteins in Triton X-114 solution. J Biol Chem 256:1604–7.

Bourn D, Prescott J. 2002. A comparison of the nutritional value, sensory qualities, and food safety of organically and conventionally produced foods. Crit Rev Food Sci Nutr 42:1–34.

Bradford MM. 1976. A rapid and sensitive method for the quantification of microgram quantities of proteins utilizing the principle of protein-dyes binding. Anal Biochem 72:248–54.

Carbonaro M, Mattera M. 2001. Polyphenoloxidase activity and polyphenol levels in organically and conventionally grown peach (*Prunus persica* L., cv. Regina bianca) and pear (*Pyrus communis* L., cv. Williams). Food Chem 72:419–24.

Chazarra S, Cabanes J, Escribano J, García-Carmona F. 1996. Partial purification and characterization of latent polyphenol oxidase in iceberg lettuce (*Lactuca sativa* L.). J Agric Food Chem 44:984–8.

Daniel O, Meier MS, Schlatter J, Frischknecht P. 1999. Selected phenolic compounds in cultivated plants: ecologic functions, heath implications, and modulation by pesticides. Environ Health Perspect 107:109–14.

Golbeck JH, Cammarata K. 1981. Spinach thylakoid polyphenol oxidase. Isolation, activation and properties of the native chloroplast enzyme. Plant Physiol 67:977–84.

[IFOAM] Intl. Federation of Organic Agricultural Movements. 1998. Basic standards for organic production and processing. IFOAM General Assembly; Buenos Aires, Argentina; 9-21 Nov 1998. Tholey-Theley, Germany: IFOAM.

Ivanov T. 1967. Sur l'oxydation du mout de raisin I. Activité de la polyphénoxydase du raisin des céspages "Muscat Rouge," "Dimiat," Riesling" et "Aligote." Ann Technol Agric 16:35–9.

Kenten RH. 1957. Latent phenolase in extracts of broad bean (*Vicia faba* L.). 1. Activation by acid and alkali. Biochem J 67:300–7.

Kidron M, Harel E, Mayer AM. 1978. Catechol oxidase activity in grapes and wine. Am J Enol Vitic 29:30–5.

Laveda F, Núñez-Delicado E, García-Carmona F, Sánchez-Ferrer A. 2000. Reversible sodium dodecyl sulphate activation of latent peach polyphenol oxidase by cyclodextrins. Arch Biochem Biophys 379:1–6.

Laveda F, Núñez-Delicado E, García-Carmona F, Sánchez-Ferrer A. 2003. Proteolytic activation of latent paraguaya peach PPO. Characterization of monophenolase activity. J Agric Food Chem 49:1003–8.

Lea AG, Beech FW. 1978. The phenolic of ciders: effect of culture conditions. J Sci Food Agric 29:493–6.

Lerner HR, Mayer AM. 1976. Reaction mechanism of grape catechol oxidase. A kinetic study. Phytochemistry 15:57–60.

Martinez VM, Whitaker JR. 1995. The biochemistry and control of enzymatic browning. Trends Food Sci Technol 6:195–200.

Mayer AM. 1987. Polyphenol oxidases in plants. Recent progress. Phytochemistry 26:11–20.

Mayer AM, Harel E. 1979. Review: polyphenol oxidase in plants. Phytochemistry 18:193–225.

Moore BM, Flurkey WH. 1990. Sodium dodecyl sulphate activation of a plant polyphenol oxidase. J Biol Chem 265:4982–8.

Nicolas JJ, Richard-Forget FC, Goupy PM, Amiot MJ, Aubert S. 1994. Enzymatic browning reactions in apple and apple products. Crit Rev Food Sci Nutr 34:109–57.

Núñez-Delicado E, Bru R, Sánchez-Ferrer A, García-Carmona F. 1996. Triton X-114-aided purification of latent tyrosinase. J Chromat B 680:105–12.

Núñez-Delicado E, García-Carmona F, Sánchez-Ferrer A. 2003. Partial purification of latent persimmon fruit polyphenol oxidase. J Agric Food Chem 51:2058–63.

Ohazurike NC, Arinze AE. 1996. Changes in polyphenol oxidase and peroxidase levels in cococyan tubers of different postharvest ages infected by *Sclerotiumrolfsii sacc.* Nahrung 40:25–7.

Sánchez-Ferrer A, Bru R, Cabanes J, García-Carmona F. 1988. Characterization of catecholase and cresolase activities of Monastrell grape polyphenol oxidase. Phytochemistry 27:319–21.

Sánchez-Ferrer A, Bru R, García-Carmona F. 1989a. Novel procedure for extraction of a latent grape polyphenoloxidase using temperature-induced phase separation in Triton X-114. Plant Physiol 91:1481–7.

Sánchez-Ferrer A, Bru R, Valero E, García-Carmona F. 1989b. Changes in pH-dependent grape polyphenol oxidase activity during maturation. J Agric Food Chem 37:1242–5.

Sánchez-Ferrer A, Laveda F, García-Carmona F. 1993a. Partial purification of soluble potato polyphenol oxidase by partitioning in an aqueous two-phase system. J Agric Food Chem 41:1583–6.

Sánchez-Ferrer A, Laveda F, García-Carmona F. 1993b. Substrate-dependent activation of latent potato leaf polyphenol oxidase by anionic surfactants. J Agric Food Chem 41:1583–6.

Sánchez-Ferrer A, Rodríguez-López JN, García-Cánovas F, García-Carmona F. 1995. Tyrosinase: a comprehensive review of its mechanism. Biochim Biophys Acta 1247:1–11.

Sapis JC, Macheix JJ, Cordonnier RE. 1983. The browning capacity of grapes. 1. Changes in polyphenol oxidase activities during development and maturation of the fruit. J Agric Food Chem 31:342–5.

Singleton VL, Rossi JA. 1965. Colorimetry of total phenols with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic 14:144–58.

Sojo MM, Núñez-Delicado E, García-Carmona F, Sánchez-Ferrer A. 1998. Partial purification of a banana polyphenol oxidase using Triton X-114 and PEG 8000 for removal of polyphenols. J Agric Food Chem 46:4924–30.

Swain I, Mapson LW, Robb DA. 1966. Activation of *Vicia faba* tyrosinase as effected by denaturating agents. Phytochemistry 5:469–82.

Valero E, Sánchez-Ferrer A, Varón R, García-Carmona F. 1989. Evolution of grape polyphenol oxidase activity and phenolic content during maturation and vinification. Vitis 28:58–95.

Valero E, Varón R, García-Carmona F. 1988. Characterization of polyphenol oxidase from Airen grapes. J Food Sci 53:1482–5.

Werck-Reichart D, Benveniste I, Teutsch H, Gabriac B. 1991. Glycerol allows low-temperature phase separation of membrane proteins solubilized in Triton X-114; application to the purification of plant cytochromes P_{450} and b_5. Anal Biochem 197:125–31.

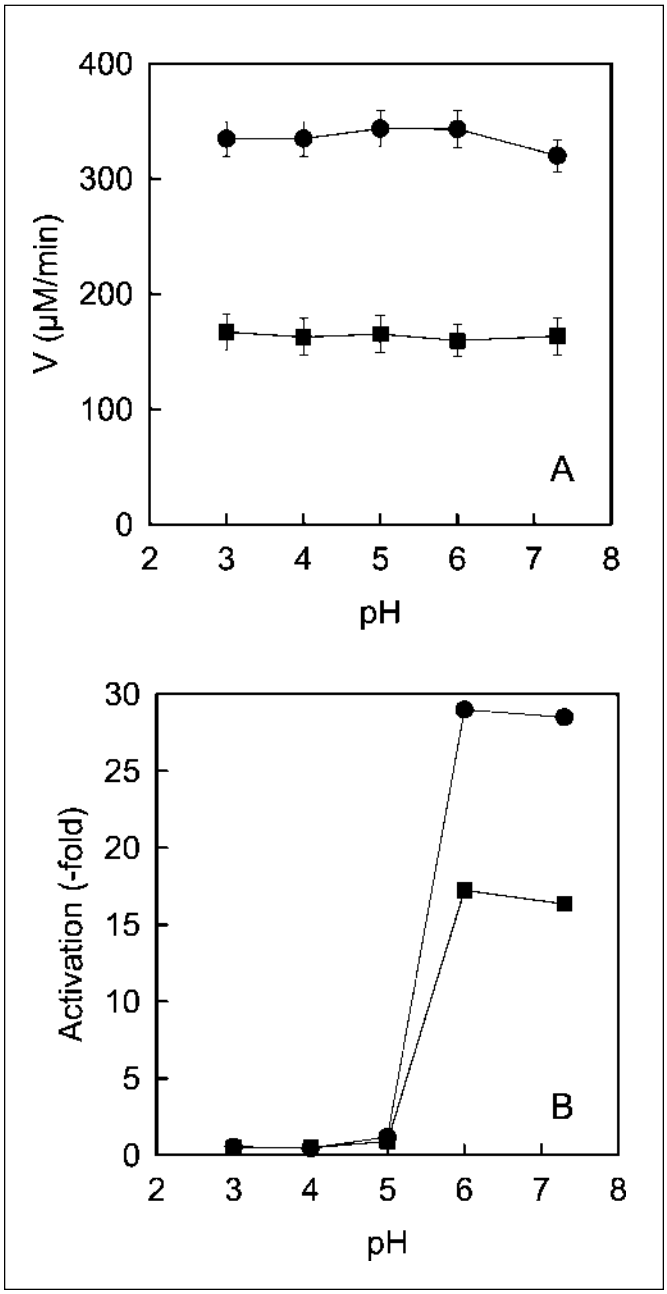

Figure 5—(a) Effect of pH on trypsin-activated Monastrell grape polyphenol oxidase (PPO), in 10 m*M* sodium acetate (pH 3.0 to 5.0) and 10 m*M* sodium phosphate (pH 6.0 to 7.5). The reaction medium at 25 °C contained 2.5 m*M* 4-*tert*-butylcatechol and 25 µg/mL trypsin-activated organic grape PPO (●) or 25 µg/mL trypsin-activated conventional grape PPO (■). (b) Effect of pH on trypsin activation of organic (●) and conventional (■) Monastrell grape.