Histochemical Localization and Developmental Expression of Peroxidase and Polyphenol Oxidase in Strawberries

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ABSTRACT. Levels and histochemical localization of peroxidase and polyphenol oxidase, and levels of anthocyanins and (+)-catechin, were studied in fruit of two strawberry (*Fragaria ×ananassa* Duch.) cultivars ('Oso Grande' and 'Chandler'), which show different degrees of susceptibility to enzymatic browning after processing. Although the levels of anthocyanins at the processing-ripe stage may be important in determining pigment stability, and therefore market suitability, the color stability of 'Chandler' is apparently determined by the lower endogenous levels of peroxidase and polyphenol oxidase in the processing-ripe stage, which are also accompanied by a lower (+)-catechin content. Polyphenol oxidase was localized almost exclusively in the cortex and to a lesser extent in the pith, showing a complementary pattern to that shown by peroxidase, which was localized in the vascular bundles. Since peroxidase and polyphenol oxidase showed a complementary localization pattern in the fruit, these results strongly suggest a synergic role for these two oxidative enzymes in pigment decay and the associated browning reaction, which occurs in processed strawberry fruit and their derived foods.

Strawberries (*Fragaria ×ananassa*) are very susceptible to undesirable alterations in texture, flavor, and color as a consequence of injury during processing (Spayd and Morris, 1981). One of the most important causes of color deterioration in processed strawberry fruit is enzymatic browning, a phenomenon caused by the oxidation of polyphenolic compounds by both polyphenol oxidase and peroxidase, which leads to the formation of dark brown polymers of a quinoidal nature (López-Serrano and Ros Barceló, 1998). This process is accompanied by changes in the native color of the jam, which may lead to substantial economic loss due to unacceptability by consumers.

Pigment (anthocyanin) instability in canned syrup strawberries has been associated in part with oxidative phenomena caused by a basic peroxidase isoenzyme which remains active after appertization (treatment at 100 °C in a boiling water bath followed by a fast cooling) (López-Serrano and Ros Barceló, 1996). This isoenzyme is located in the concentric array of the vascular bundles and in the vascular connections with the seeds (López-Serrano and Ros Barceló, 1995a), and it is capable of efficiently oxidizing (+)-catechin at extremely low H₂O₂ concentrations (López-Serrano and Ros Barceló, 1997). This peroxidase isoenzyme, together with strawberry polyphenol oxidases (Cano et al., 1997; Civello et al., 1995; Espin et al., 1997; Spayd and Morris, 1981; Wesche-Ebeling and Montgomery, 1990), is considered to be the main factor responsible for pigment decay in processed strawberries and their derived foods (López-Serrano and Ros Barceló, 1999).

In fact, when phenolic and pigment (anthocyanin) stability in processing-ripe strawberries were studied in response to aging

under mildly oxidizing conditions, it was found that phenolic metabolism in strawberries may be either oxidative (H₂O₂ independent) or peroxidative (H₂O₂ dependent), and that treating strawberry slices with H₂O₂ stimulates oxidative phenomena that take place in the absence of H₂O₂, such as anthocyanin and (+)-catechin degradation, and brown polymer formation (Lopez-Serrano and Ros Barceló, 1999). These results suggested a synergic role for both peroxidase and polyphenol oxidase in (+)-catechin and anthocyanin degradation, and in brown polymer formation, during the aging of strawberry fruit under mildly oxidizing conditions.

The color stability of processed strawberry fruit is known to differ according to the cultivar (Lopez-Serrano and Ros Barceló, 1995b, 1996). For example, 'Oso Grande' and 'Chandler', two widely used strawberry cultivars in the food industry, both show distinctive features. The former lacks anthocyanins in its heart and is therefore considered a white heart cultivar, while 'Chandler', which shows a high heart anthocyanin level, is considered as a red heart cultivar. The color (pigment) stability of processed 'Chandler' is greater than that of 'Oso Grande' (Lopez-Serrano and Ros Barceló, 1995b), and is therefore more suitable for processing, although differences in anthocyanin content are slight. In this report, we studied the levels and histochemical localization of both peroxidase and polyphenol oxidase, the two enzymes involved mainly in enzymatic browning in processed strawberries (López-Serrano and Ros Barceló, 1999; Spayd and Morris, 1981) during fruit ripening, in order to explain differences in color stability between the two strawberry cultivars. In addition, levels of anthocyanins at the processing-ripe stage, which determine strawberry color, and of (+)-catechin, a pro-oxidant compound (Richard-Forget and Gaillard, 1997; Subramanian et al., 1999), were also studied.

Materials and Methods

PLANT MATERIAL. 'Oso Grande' and 'Chandler' strawberries were field-grown at Huelva, Spain, during the 1998 and 1999 growing season, and harvested at different developmental stages from flowering to the processing-ripe state. Fruit were sorted

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visually into five maturity stages according to Spayd and Morris (1981): I = immature green (small green fruit), II = mature green (pale green to whitish colored fruit), III = inception (first appearance of red color to 50% or less of full color), IV = firm ripe (well-developed red color, firm), and V = processing-ripe (dark red color, slightly soft). The strawberries were used directly in their fresh state for histochemical studies or frozen at -20°C for 1 month until analysis.

CHEMICALS. (+)-Catechin, tyramine, 3-methyl-benzotiazolinone hydrazone (MBTH), tropolone and 3,5,3',5'-tetramethylbenzidine-HCl were obtained from Sigma Chemical Co. (Madrid, Spain). 4-Methoxy- α -naphthol was from Aldrich Chemie (Madrid, Spain). The other chemicals and solvents used in this study were of the maximum purity available.

PHENOLIC EXTRACTION. Frozen processing-ripe fruit (50 g) were extracted with 100 mL cold acetone, and the mixture homogenized with a mechanical blade (Sorvall Omnimixer 230; Kendro Lab. Prod., Newtown, Conn.). The homogenate was filtered through filter paper and acetone was removed in vacuo. The aqueous phase obtained was acidified to pH 1.0 with HCl and extracted twice with 50 mL ethyl acetate. The acidified aqueous phase was used directly to measure the anthocyanin content. The ethyl acetate phase was evaporated in vacuo and the phenolic residue dissolved in 1.0 mL methanol. The methanolic samples were diluted with 5 mL 2.5% (v/v) acetic acid and prepurified by absorption on Sep-Pack C18 cartridges (Millipore Corp., Waters Chromatography, Milford, Mass.). Elution was first with 2.5% acetic acid and then with methanol. The methanolic fraction was recovered and dried under a N_2 stream before being dissolved in 1.0 mL methanol, and analyzed directly by high-performance liquid chromatography (HPLC).

DETERMINATION OF (+)-CATECHIN. (+)-Catechin was determined by HPLC (López-Serrano and Ros Barceló, 1999) in a 4.6 mm ID $\times$ 25 cm Waters Spherisorb S5-ODS2 column (Millipore Corp.) using a Waters system, comprised of a model 600 controller, model 600 pump, Rheodyne 7725i manual injector, and a Waters 996 Photodiode Array detector. The data were processed with Waters Millennium 2010 LC version 2.10 software.

Elution was performed at a flow rate of $1\text{ mL}\cdot\text{min}^{-1}$ at 25°C , using as solvent A, 2.5% acetic acid in water, and as solvent B, acetonitrile, by means of a linear gradient from 0% to 10% B (for 5 min), from 10% to 30% B (for 20 min) and from 30% to 50% B (for 20 min). (+)-Catechin was quantified from a HPLC-calibration curve at 280 nm.

DETERMINATION OF ANTHOCYANINS AND TOTAL PHENOLIC COMPOUNDS. Total anthocyanins (mainly composed by pelargonidin-3-glucoside) were estimated by the pH differential method (Wrolstad, 1976), and expressed as pelargonidin-3-glucoside equivalents. Total phenolic compounds were determined as described by García-Florenciano et al. (1990) based on a tungsten-molybdate assay.

HISTOCHEMICAL STAINING FOR PEROXIDASE AND POLYPHENOL OXIDASE. For histochemical localization of peroxidase, thin sections (100 to 500 μm) obtained from fruit at the mature green stage II, were incubated directly (Ros Barceló, 1999a) in a reaction medium containing 50 μM 3,5,3',5'-tetramethylbenzidine-HCl in 0.1 M Tris-acetate buffer, pH 5.0, in the absence and in the presence of 0.33 mM H_2O_2 , and incubated at 25°C for 15 to 30 min.

Histochemical localization of polyphenol oxidase was performed by incubating thin strawberry sections in a reaction medium containing 5 mM tyramine and 2 mM 3-methyl-benzotiazolinone hydrazone (MBTH) in 50 mM K-phosphate buffer, pH 6.8, in the absence and in the presence of 5 mM tropolone, and incubated at 25°C for 15 to 30 min.

Protein transfer to nitrocellulose membranes (0.45 μm , Bio-Rad, Hercules, Calif.) from thin strawberry sections and staining for peroxidase activity using 4-methoxy- α -naphthol was performed exactly as described previously (Calderón et al., 1993). Staining for polyphenol oxidase activity of the protein blotted onto nitrocellulose membranes was performed as in the histochemical localization.

EXTRACTION OF THE PROTEIN FRACTION. Fruit, sampled at different developmental stages, were homogenized (1:1, w/v) in a mortar with a pestle in 100% (w/v) acetone at -20°C and the residue filtered in vacuo through filter paper. The precipitate was washed with acetone at -20°C until all the pigments were removed and then dried under a N_2 stream. The acetone powder was resuspended in 50 mM Tris-HCl, pH 7.5, containing 1.0 M KCl and 100 mM CaCl_2 , and stirred gently for 4 h at 4°C . After centrifugation at 27,000 g_n for 20 min, the supernatants were dialyzed overnight against 50 mM Tris-HCl, pH 7.5, and concentrated, initially, with Aquacide I (Calbiochem, Calbiochem-Novabiochem Corp., San Diego, Calif.), and then using the Centriprep-10 (Amicon, Amicon Inc., Beverly, Mass.) system. The protein fraction was stored at 0°C for 24 h and used subsequently for further studies.

DETERMINATION OF ENZYMATIC ACTIVITIES. Peroxidase activity was determined in reaction media containing 1.0 mM 4-methoxy- α -naphthol and 0.33 mM H_2O_2 , by monitoring the increases in absorbance at 593 nm ($\epsilon_{593} = 21,000\text{ molar}^{-1}\cdot\text{cm}^{-1}$), as described by Ferrer et al. (1990). Polyphenol oxidase activity was determined using 1 mM (+)-catechin by monitoring the increases in absorbance at 390 nm ($\epsilon_{390} = 4,680\text{ molar}^{-1}\cdot\text{cm}^{-1}$) as described by López-Serrano and Ros Barceló (1997). Activities were expressed in nkat (i.e., nmoles of substrate oxidized/s). The spectrophotometric determinations of enzymatic activities were carried out at 30°C in 0.1 M Tris-acetate, pH 5.0, in a Uvikon 940 spectrophotometer (Kontron Instruments, Madrid, Spain).

ISOELECTRIC FOCUSING AND ZYMOGRAPHIC STAINING. Isoelectric focusing of peroxidase and polyphenol oxidase on polyacrylamide gels in 3.5 to 10 pH gradients, and staining for peroxidase activity with 4-methoxy- α -naphthol, and for polyphenoloxidase

Table 1. Endogenous anthocyanins, total phenols, peroxidase, polyphenol oxidase, and (+)-catechin concentrations in 'Oso Grande' and 'Chandler' strawberries at the processing-ripe stage (n = 3).

Chemical	Cultivar		LSD, 0.05
	Oso Grande	Chandler	
Anthocyanins (Plg-3-Glu, $\mu\text{g}\cdot\text{g}^{-1}$ FW)	267	343	12
Total phenolics (chlorogenic acid, $\text{mg}\cdot\text{g}^{-1}$ FW)	9.0	9.8	0.3
Peroxidase (nkat $\cdot\text{g}^{-1}$ FW)	1.6	1.0	0.2
Polyphenoloxidase (nkat $\cdot\text{g}^{-1}$ FW)	12.7	6.5	0.2
(+)-Catechin ($\mu\text{g}\cdot\text{g}^{-1}$ FW)	811	608	20

Fig. 1. Changes in peroxidase activity during development of strawberry fruit of 'Oso Grande' (●) and 'Chandler' (▼). Fruit were sorted visually into five maturity stages: I = immature green (small green fruit), II = mature green (pale green to whitish colored fruit), III = inception (first appearance of red color to 50% or less of full color), IV = firm ripe (well-developed red color, firm), and V = processing-ripe (dark red color, slightly soft) fruit. Symbols are means $\pm$ SE (n = 3).

Fig. 2. Changes in the peroxidase isoenzyme pattern resolved by isoelectric focusing in 3.5 to 10 pH gradients during development of strawberry fruit of 'Oso Grande' for three developmental stages: I = immature green (small green fruit), II = mature green (pale green to whitish colored fruit), and V = processing-ripe (dark red color, slightly soft) fruit. Peroxidase isoenzymes were revealed with 4-methoxy- α -naphthol in the presence of H_2O_2 .

Fig. 3. Changes in polyphenol oxidase activity during development of strawberry fruit of 'Oso Grande' (●) and 'Chandler' (▼). Fruit were sorted visually into five maturity stages: I = immature green (small green fruit), II = mature green (pale green to whitish colored fruit), III = inception (first appearance of red color to 50% or less of full color), IV = firm ripe (well-developed red color, firm), and V = processing-ripe (dark red color, slightly soft) fruit. Symbols are means $\pm$ SE (n = 3).

activity with (+)-catechin, was performed as described by López-Serrano and Ros Barceló (1997).

Results and Discussion

Strawberry fruit of 'Oso Grande' and 'Chandler' differ in their color stability during processing (López-Serrano and Ros Barceló, 1995b). 'Oso Grande' is a strawberry cultivar that lacks anthocyanins in its heart, whereas 'Chandler' shows a moderately high heart anthocyanin level. This difference is also seen in the total anthocyanin content, which was 28% greater in 'Chandler' than in 'Oso Grande' (Table 1). The color (pigment) stability of 'Chandler' is greater than that of 'Oso Grande' after processing (López-Serrano and Ros Barceló, 1995b), and is therefore more suitable for industrial purposes, although neither the differences in anthocyanin content or total phenolic content (Table 1) are sufficient to explain these differences (López-Serrano and Ros Barceló, 1995b). In fact, 'Chandler' has a higher total phenolic content, and therefore could be considered as the more likely of the two to suffer enzymatic browning, although the opposite is true. For this reason, other factors involved in enzymatic browning, such as the peroxidase and polyphenol oxidase content, were also studied.

CHANGES IN PEROXIDASE DURING FRUIT DEVELOPMENT. Changes in peroxidase activity during fruit development in 'Oso Grande' and 'Chandler' are illustrated in Fig. 1. The results showed that, similar to other strawberry cultivars (Civello et al., 1995; Spayd and Morris, 1981), the endogenous peroxidase activity decreased asymptotically from the immature green stage I to the processing-ripe stage V, when activity reached its lowest level. Although the decline in endogenous peroxidase activity followed an identical developmental pattern in both cultivars, endogenous peroxidase levels at the processing-ripe stage were about 50% greater for 'Oso Grande' (Table 1).

The decrease in the peroxidase activity during fruit development and ripening was associated in 'Oso Grande' and 'Chandler' with the decrease of a vast array of peroxidase isoenzymes of both acidic and basic isoelectric points. Since identical results were found for 'Chandler', Fig. 2 only shows the case for 'Oso Grande'. The only peroxidase isoenzyme which remained active at the processing-ripe stage was a strongly basic peroxidase

Fig. 4. Changes in the polyphenol oxidase isoenzyme pattern resolved by isoelectric focusing in 3.5 to 7.5 pH gradients during development of strawberry fruit of 'Oso Grande' for three developmental stages: I = immature green (small green fruit), II = mature green (pale green to whitish colored fruit), and V = processing-ripe (dark red color, slightly soft) fruit. Polyphenol oxidase isoenzymes were revealed with (+)-catechin.

isoenzyme with a pI of approximately 10.5 (Fig. 2, arrow). This peroxidase isoenzyme has been purified and characterized previously (López-Serrano and Ros Barceló, 1997). The presence of this strongly basic peroxidase isoenzyme in all the monitored maturation states suggests that the decrease of the peroxidase isoenzymes of both acidic and basic isoelectric points during fruit development was not due to a dilution effect caused by cell growth, but rather to a modulation of the peroxidase isoenzyme pattern with fruit maturation.

CHANGES IN POLYPHENOL OXIDASE DURING FRUIT DEVELOPMENT. From the above results, it became apparent that endogenous

peroxidase activity may be one of the key factors in determining pigment stability in strawberries. Another enzyme that has been associated with pigment stability in strawberries is polyphenol oxidase, since processing-ripe strawberry fruit contain several phenolic compounds susceptible to oxidation by polyphenol oxidase after processing, such as (+)-catechin and anthocyanins (López-Serrano and Ros Barceló, 1999; Wesche-Ebeling and Montgomery, 1990). Furthermore, (+)-catechin oxidation by polyphenol oxidase produces H_2O_2 (Jiang and Miles, 1993), which may be used further by peroxidase, to act synergistically with polyphenol oxidase in both pigment decay and enzymatic browning (Richard-Forget and Gauillard, 1997; Subramanian et al., 1999). For this reason, changes in polyphenol oxidase during fruit development were also analyzed. In the case of polyphenol oxidase, (+)-catechin was chosen as the substrate to determine enzymatic activities, since oxidation of this endogenous strawberry compound by polyphenol oxidase may accelerate the decay of anthocyanin pigments (Wesche-Ebeling and Montgomery, 1990). In this way, (+)-catechin may be regarded as a pro-oxidant compound.

Changes in polyphenol oxidase during the various stages of fruit development of 'Oso Grande' and 'Chandler' are shown in

Fig. 5. Histochemical localization of (A–D) polyphenol oxidase and (E) peroxidase activity in (A, C, E) transverse and (B and D) longitudinal sections from strawberry fruit of 'Oso Grande' at the mature green (pale green to whitish colored) stage. The histochemical localization of polyphenol oxidase was performed by incubating thin strawberry sections in a reaction medium containing 5 mM tyramine and 2 mM MBTH in 50 mM K-phosphate buffer, pH 6.8, in the (A and B) absence and in the (C and D) presence of 5 mM tropolone, and incubating at 25 °C for 15 to 30 min. For the histochemical localization of (E) peroxidase, thin sections were incubated directly in a reaction medium containing 50 μ M 3,5,3',5'-tetramethylbenzidine-HCl in 0.1 M Tris-acetate buffer, pH 5.0, and incubated at 25 °C for 15 to 30 min.

Fig. 6. Histochemical localization of (A and B) peroxidase and (C and D) polyphenol oxidase activity in (A and D) transverse and (B,C) longitudinal sections from strawberry fruit of 'Oso Grande' at the mature green (pale green to whitish colored) stage after protein transfer to 0.45 μ m nitrocellulose membranes. The histochemical localization of peroxidase was performed by directly incubating blotted nitrocellulose membranes at 25 °C for 15 to 30 min in a reaction medium containing 1.0 mM 4-methoxy- α -naphthol and 0.33 mM H_2O_2 in 0.1 M Tris-acetate buffer, pH 5.0. Staining for polyphenol oxidase was performed by incubating blotted nitrocellulose membranes at 25 °C for 15 to 30 min in a reaction medium containing 5 mM tyramine and 2 mM MBTH in 50 mM K-phosphate buffer, pH 6.8.

Fig. 3. Polyphenol oxidase activity in strawberry fruit increases from the immature green stage I to the mature green stage II, when it decreases asymptotically until minimal activity is reached in the processing-ripe stage V (Fig. 3). Although during the two first developmental stages, the activity of endogenous polyphenol oxidase was greater in 'Chandler', the opposite was true during the last three developmental stages. In fact, at the processing-ripe stage V, the polyphenol oxidase activity of 'Chandler' was about one half that shown by 'Oso Grande', and was accompanied by a lower (+)-catechin content (Table 1). In other words, the high pigment stability of 'Chandler' could be explained by a lower peroxidase, polyphenol oxidase and (+)-catechin content.

At this point, it is necessary to call attention to the fact that when we studied (López-Serrano and Ros Barceló, 1995b) the levels of total phenols and anthocyanins in 'Oso Grande' and 'Chandler' strawberries during fruit development, it was found that total phenolics decreased with maturation, while the levels of monomeric anthocyanins increased continuously from stage III onwards. The anthocyanin degradation index, color density, polymeric color and the percentage of contribution of tannins, were also measured (López-Serrano and Ros Barceló, 1995b) as indices of desirable (or undesirable) color modifications (Wrolstad, 1976). The anthocyanin degradation index, polymeric color and tannins showed an exponential decay from immature green stage I to processing-ripe strawberries (López-Serrano and Ros Barceló, 1995b), which correlated well with the levels of peroxidase activity measured during fruit development (Fig. 1). Color density was more related to the levels of polyphenol oxidase (Fig. 3), and a strong inverse correlation was found. There seems to be, then, a strong relationship between polyphenol oxidase and peroxidase levels with both desirable (color density) and undesirable (anthocyanin degradation index, polymeric color and tannins) color modifications, respectively, during strawberry fruit maturation.

Isoenzyme patterns of polyphenol oxidase developed with (+)-catechin were identical for 'Oso Grande' and 'Chandler', and showed only minor changes during fruit maturation. Such patterns for 'Oso grande' are illustrated in Fig. 4. The increase in polyphenol oxidase levels during the transition from the immature green stage I to the mature green stage II (Fig. 3) was associated with an increase in the level of two neutral isoelectric point isoenzymes (Fig. 4, arrows), whose level was maintained until the processing-ripe stage V, despite the decrease in endogenous total polyphenol oxidase content.

HISTOCHEMICAL LOCALIZATION OF POLYPHENOL OXIDASE AND PEROXIDASE IN STRAWBERRY FRUIT. Development of the browning reaction in processed strawberry fruit is a generalized phenomenon, in which all the tissues are involved simultaneously. This contrasts with observations in other fruit and vegetables, in which browning reactions occur as highly localized brown areas, mainly at the level of minor veins or certain subepidermal tissues (Gómez-Tena et al., 1994). The reason for this difference is uncertain but one explanation could be a wide distribution of polyphenol oxidase and peroxidase in strawberry fruit. To help clarify this possibility, the histochemical localization of polyphenol oxidase and peroxidase in the strawberry fruit was studied.

Procedures for the histochemical localization of polyphenol oxidase are based generally on the oxidation of colorless catechols to colored *ortho*-quinones. However, these reactions yield high background staining due to auto-oxidation of catechols in air, and appear to be unsuitable for use in the histochemical localization of polyphenol oxidase, especially in thin sections

obtained from the highly pigmented strawberry fruit. However, strawberry polyphenol oxidase also has cresolase (*ortho*-hydroxylating) activity (Espín et al., 1997), and this unequivocal catalytic property of polyphenol oxidase was used for the histochemical localization of polyphenol oxidase in the strawberry fruit using the tyramine/MBTH reagent (Rodríguez-López et al., 1994).

Identical results were found for 'Oso Grande' and 'Chandler' when polyphenol oxidase and peroxidase were localized histochemically in mature green stage II strawberries. The results obtained for 'Oso Grande' are illustrated in Fig. 5. Polyphenol oxidase staining was mainly in the cortex, as can be seen from tangential (Fig. 5A) and longitudinal (Fig. 5B) sections. Staining could also be seen in the central vascular bundles and the vascular connections with the seeds (Fig. 5A and B). However, in this last case, the staining was insensitive to the polyphenol oxidase inhibitor, tropolone (Kahn, 1985), when used at 5 mM (Fig. 5C and D), from which it can be deduced that the staining in the vascular bundles was due to peroxidase. This last observation was confirmed by use of 3,5,3',5'-tetramethylbenzidine, a specific peroxidase substrate (Ros Barceló 1999a), which revealed the presence of peroxidase in the vascular bundles (Fig. 5E), leading to a similar staining pattern to that observed with the tyramine/MBTH reagent in the presence of tropolone (Fig. 5C).

Staining of strawberry vascular bundles with 3,5,3',5'-tetramethylbenzidine (Fig. 5E) was observed both in the presence and in the absence of H₂O₂. The staining observed in the absence of exogenous H₂O₂ is due to the capacity of vascular tissues themselves to produce H₂O₂ (Ros Barceló, 1998a, 1998b, 1999a, 1999b), suggesting an active function for strawberry peroxidase. Confirmation of the vascular localization of peroxidase in the strawberry fruit was confirmed by blotting strawberry sections onto nitrocellulose membranes, followed by *in situ* staining with 4-methoxy- α -naphthol, which showed the exclusive localization of peroxidase in the vascular tissues, both in transverse (Fig. 6A) and longitudinal (Fig. 6B) sections. In this last case, the staining reaction was totally dependent on the presence of exogenous H₂O₂.

Since the transfer of proteins to nitrocellulose membranes is not accompanied by the transfer of low molecular weight solutes, such as endogenous H₂O₂, staining of blotted nitrocellulose membranes for polyphenol oxidase is a logical way to avoid the interference of peroxidase during the staining for polyphenol oxidase of tissues capable for producing H₂O₂. When this was done, it was found that polyphenol oxidase was localized preferentially in the cortex of the strawberry fruit and more weakly in the pith (Fig. 6C and D), showing a complementary pattern to that shown by peroxidase, which was localized preferentially in the vascular bundles (Fig. 6A and B).

Conclusions

Collectively, results herein support the view that, although the levels of anthocyanins in 'Chandler' (28% higher than in 'Oso Grande') at the processing-ripe state may be important in determining market suitability of these strawberries, color stability of 'Chandler' is apparently determined by lower endogenous levels of peroxidase (34% lower) and polyphenol oxidase (49% lower), which are also accompanied by a lower (25%) (+)-catechin content. Furthermore, since peroxidase and polyphenol oxidase showed a complementary localization pattern in the strawberry fruit, these results fit well with a synergic role for these two

oxidative enzymes in pigment decay and the associated enzymatic browning reaction which occurs in processed strawberry fruit and their derived foods.

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