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Actual Browning and Peroxidase Level Are Not Correlated in Red and White Berries from Grapevine *(Vitis vinifera)* Cultivars[1]

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Abstract

The measurement of the peroxidase activity (EC 1.11.1.7) in ten grapevine cultivars (Airen, Clairette, Riesling, Servant, Carignan, Cinsaut Noir, Gamay Rouge, Grenache, Monastrell and Mourvedre) with *o*-phenylenediamine as substrate reveals that peroxidase activity is not correlated with actual browning in either red or white cultivars, even under conditions in which the catecholase activity of grape polyphenoloxidase is minimal. This is probably due to the fact that for peroxidase to act on flavonol and anthocyanin substrates the previous action of α-and β-glycosidases is necessary to remove sugar moieties from the glycosides in order to liberate the phenolic aglycones that are the true substrates of the enzyme.

Introduction

Must browning is the result of the oxidation of certain phenolic compounds occurring naturally in the berries, which leads to a modification of color and an alteration in the sensory qualities of the wines (6, 7). Grape browning is initiated by the enzymatic oxidation of phenolic compounds by polyphenoloxidases and/or peroxidases (6, 7). Thus, among factors which may affect browning are the levels of endogenous phenols and of the enzymes themselves.

Correlations between polyphenoloxidase and the phenolic content are frequently sought to explain the browning intensity of grape cultivars (7, 9). However, the levels of polyphenoloxidase and polyphenols are not correlated with the browning of grape cultivars, except in some red varieties with a high polyphenolic content (7, 11).

The search for correlation between must browning and grape peroxidase started with the report of Bolcato et al. (1). To date, it is well known that grape peroxidase is capable of oxidizing the benzoic acid (10, 14), the anthocyanidin (2) and the flavonol (8) constituents of the grape berry, so that a participation of peroxidase in the enzymatic browning of grapes cannot be discarded. The aim of the present work was to study correlations between enzymatic browning and peroxidase levels in ten grapevine cultivars of white and red varieties.

Materials and Methods

Plant material

The seeded *Vitis vinifera* cultivars Airen, Clairette, Riesling, Servant, Carignan, Cinsaut Noir, Gamay Rouge, Grenache, Monastrell and Mourvedre were grown in field at the "Hacienda Nueva" Viticultural Experimental Station of the C.R.I.A (Murcia, Spain) and sampled in August at veraison. Clusters were transported to the laboratory and frozen immediately at -30 °C for later analysis.

Tissue homogenization and peroxidase fractions

After removal of the seeds, the grapes were homogenized in a mortar with pestle at 4 °C in the presence of 50 mM ammonium tartrate, 6 mM ascorbic acid, 1.0 M LiCl, 250 mM Tris [tris(hydroxymethyl)aminomethane]-

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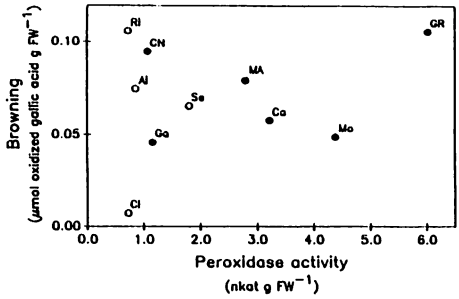

Figure 1. Relationship between browning and peroxidase activity in grapevine cultivars for white (○ and red (●) berries. Ai, Airen; Ca, Chardonnay; Cl, Clairette; CN, Cinsaut Noir; Ga, Grenache; GR, Gamay Rouge; MA, Monastrell; No, Mourvedre; Ri, Riesling; Se, Servant. Values are means (n = 3). Standard errors were within 10%.

HCl buffer, pH 7.5, containing polyvinylpolypyrrolidone in a ratio of 0.1 g/1.0 g fresh tissue (3). The homogenate was centrifuged at 20,000 g for 15 min and the supernatant dialyzed overnight against 50 mM Tris-HCl buffer, pH 7.5. The dialyzed extracts constituted the soluble protein fraction used in further studies.

Determination of peroxidase activity

The assay of peroxidase activity with o-phenylenediamine was carried out at 25 °C in a reaction medium containing 1.0 mM *o*-phenylenediamine, 1.0 mM H_2O_2 in 0.1 Tris-acetate buffer, pH 5.0 (15). The increase in absorbance at 435 nm was followed for one minute periods. Controls were carried out in the absence of H_2O_2. Enzyme activity was expressed in nkat using a ϵ_{435} = 7.73 x 10^3 M^{-1} cm^{-1} for the dye product and was calculated from a calibration curve of *o*-phenylenediamine oxidized in the presence of an excess (molar ratio 1:5) of H_5IO_6 (15).

Grape browning

For the determination of actual browning, grapes were homogenized in a mortar with pestle at 4 °C in the presence of 50 mM ammonium tartrate, 1.0 M LiCl, 250 mM Tris [tris(hydroxymethyl)aminomethane]-HCl buffer, pH 7.5. The homogenate was centrifuged at 20,000 g for 15 min and the supernatant incubated in the presence and in the absence of 10 mM ascorbic acid for 16 h at 25 °C. The absorbance of the extracts at 390 nm was recorded. Browning was calculated from the difference in absorbances between homogenates incubated in the absence and in the presence of ascorbic acid, and expressed in equivalents of oxidized gallic acid for which a ϵ_{390} = 9.30 x 10^3 M^{-1} cm^{-1} was used. This was calculated from a calibration curve of gallic acid oxidized in the presence of an excess (molar ratio 1:5) of H_5IO_6.

Results and Discussion

o-Phenylenediamine was selected for the measurement of the level of peroxidase activity in white and red grapes at the veraison stage (harvested 16 August) since this compound is the best substrate for grapevine peroxidase (15). *o*-Phenylenediamine is also a substrate for polyphenoloxidase (13). However, the oxidation of *o*-phenylenediamine by grape homogenates was strictly dependent on hydrogen peroxide in all varieties, and was therefore considered to be due to peroxidase activities.

A pH of 7.5 was selected for the measurement of actual browning. At this pH, the catecholase activity of grape polyphenoloxidase is minimal (4, 5, 12) while the peroxidase-mediated flavonol-oxidizing activity is maximal (8).

Under these conditions, in which the catecholase activity of grape polyphenoloxidase is minimal, no lineal correlation exists at P = 0.05 between the endogenous levels of peroxidase and browning for white (r = 0.0583, Fig. 1) or red (r = 0.2958, Fig. 1) grapevine cultivars. This absence of correlation could not have been due to a differential peroxidase isoenzyme pattern in each variety that would condition different catalytic properties of individual peroxidase isoenzymes,

since analysis of the peroxidase isoenzyme profiles of the ten cultivars revealed the exclusive presence of the vacuolar basic peroxidase isoenzyme B_5 (15).

In conclusion, these results suggest that the level of peroxidase activity in both red and white grapes from ten grapevine (*Vitis vinifera*) cultivars is not correlated with real browning. This is probably due to the fact that for peroxidase to act on flavonol and anthocyanin substrates the previous action of α- and β-glycosidases is necessary for the removal of sugar moieties from the glycosides in order to liberate the phenolic aglycones, which are considered the true substrates of the enzyme (2, 8).

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Scald Control in 'Granny Smith' Without DPA

Fruit were stored under regular storage (RA), ultra low oxygen (ULO) and controlled atmosphere storage (CA). Other samples were exposed to initial low oxygen (ILOS) conditions before ULO CA. The ILOS + ULO CA treatment resulted in lower levels of scald than ULO CA storage alone. Relative to RA, storage under ULO CA conditions led to markedly lower scald levels in a year with severe scald. Storage under the ILOS + ULO CA regime conferred low levels of scald on pre-mature, mature and post-mature fruit after both 5 and 6 months of storage. From Truter et al. (1994) J. Hort. Sci. 69:581-587.