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Evolution of grape polyphenol oxidase activity and phenolic content during maturation and vinification

by

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L'évolution de l'activité polyphénoloxidasique de raisin et du contenu phénolique pendant la maturation et la vinification

Résumé: L'activité de la polyphénoloxydase (PPO) et la teneur en composés phénoliques ont été étudiées au cours de la maturation de deux cépages cultivés au sud-est de l'Espagne, et au cours de la vinification à des concentrations différentes d'anhydride sulfureux.

Les activités crésolasiques et catécholasiques (mesurées à pH 7,0 et à pH 4,5, respectivement) ont augmenté pendant la période étudiée, et la teneur en composés phénoliques a diminué d'une façon rapide quand elle s'est exprimée en concentration (mg acide gallique/g de raisin frais), tandis que, exprimée en quantité par baie (mg/baie), elle a été presque constante. Au cours de la vinification, l'activité de l'enzyme a été la plus élevée immédiatement après le broyage des raisins frais, et cette activité n'a pas été détectée à la fin de la fermentation. La teneur en composés phénoliques a aussi diminué au cours de la vinification jusqu'à niveau constant, celui-ci a été en rapport direct avec le niveau de SO_2 employé.

Key words: enzyme, polyphenol, phenol, sulphur, berry, maturation, must, wine, vinification, Spain.

Introduction

Browning during grape juice processing is a well-known phenomenon, the causes of which are essentially enzymatic in nature. In the presence of atmospheric oxygen, the polyphenol oxidase (PPO) (monophenol, dihydroxy-L-phenylalanine: oxygen oxidoreductase, E. C. 1.14.18.1) catalyzes the oxidation of natural phenolic substances; the quinones thus formed undergo rapid secondary reactions creating the undesirable coloured products.

These processes which cause a radical change in the colour and the flavour of the juice and greatly diminish the quality of the final product must be prevented to produce wine of good quality. One of the most widely used inhibitors of enzymatic browning of fruits and vegetables is sulphur dioxide, which appears to be the most practical oxidation inhibitor in the wine industry (IVANOV 1967).

The activity of PPO is known to be considerably influenced by several factors, primarily variety, stage of development and environmental conditions (MAYER and HAREL 1979; SAPIS *et al.* 1983). For this reason — although quite a number of studies have been made concerning grape PPO in other climates and countries (POUX 1966; DUBERNET and

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RIBÉREAU-GAYON 1973; TRAVERSO-RUEDA and SINGLETON 1973; KIDRON *et al.* 1978; WISSEMANN and LEE 1980; SAPIS *et al.* 1983) — in this paper we followed the PPO activity and phenolic content during fruit development and the vinification process of two wine grape varieties grown in an area with Mediterranean climate like south-eastern Spain.

Materials and methods

Grape material: The experiments were carried out in 1987 with two *Vitis vinifera* varieties: Airen (white) and Monastrell (red), grown at Villarrobledo (Albacete) and Jumilla (Murcia), respectively. A random sample was collected weekly from the middle of July till harvest and was analyzed the same day.

Vinification: Wine from Airen grapes was prepared in Ayuso wineries, Villarrobledo, in four 16 l batches with 0, 50, 100 and 150 ppm SO_2. Wine from Monastrell grapes (rosé) was prepared in Umbría wineries, Jumilla, in four 16 l batches with 0, 100, 200 and 300 ppm SO_2. Samples were taken at various stages of vinification and were analyzed the same day.

Preparation of crude PPO extract: PPO was extracted at 4 °C according to the method of LERNER *et al.* (1971); 250 g of fresh grapes were, together with 125 ml of 100 mM phosphate buffer pH 7.3 containing 10 mM sodium ascorbate, homogenized in a blender for 15 s, filtered through 8 layers of gauze and centrifuged at 4,000 *g* for 15 min. The precipitate was extracted for 30 min with 10 ml of 1.5 % Triton X-100 in 100 mM phosphate buffer pH 7.3 and then centrifuged at 15,000 *g* for 1 h. An ammonium sulphate fractionation was carried out and the fraction precipitating between 45 and 95 % saturation collected and redissolved. This solution, after dialysis, was used as enzyme source.

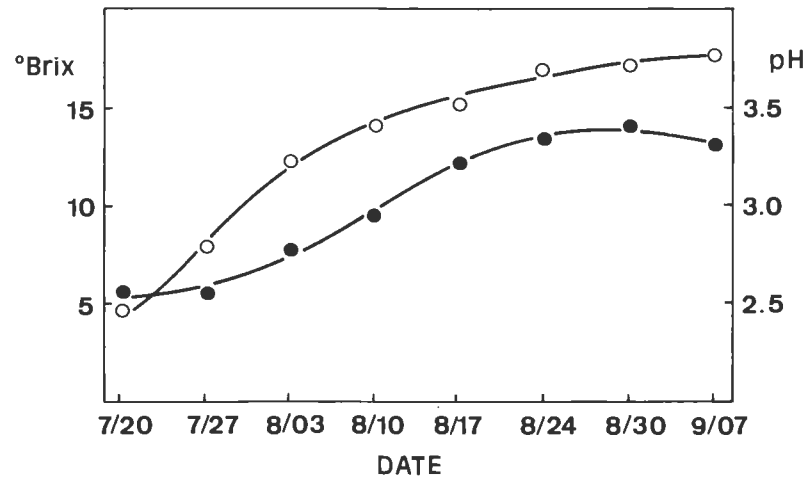

Fig. 1: Changes in the sugar content (○) expressed in °Brix and pH (●) of the Airen grape berries during maturation.

Changements du contenu sucré (○) exprimé comme °Brix et pH (●) des baies du cépage Airen pendant maturation.

Grape juice and wine samples (100 ml) were centrifuged at 20,000 *g* for 30 min. The precipitate was extracted for 30 min with 8 ml of 1.5 % Triton X-100 in 100 mM phosphate buffer pH 7.3. The suspension was centrifuged at 20,000 *g* for 1 h and the supernatant used immediately as source of the enzyme.

PPO assay: Both cresolase and catecholase activities were measured spectrophotometrically by the appearance of 4-methyl-*o*-benzoquinone at 400 nm (ε = 1350 M^{-1} cm^{-1}) (MAYER *et al.* 1966) at 30 °C, since recently has been established that the

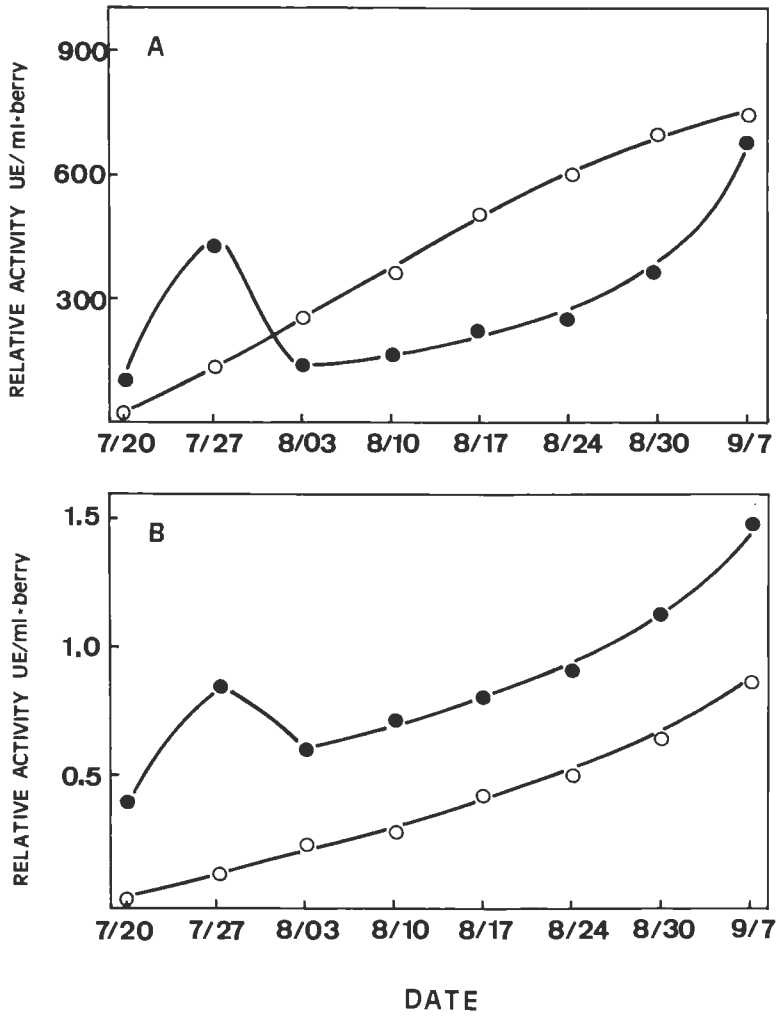

Fig. 2: (A) Trends in catecholase activity expressed per grape berry during grape maturation. (B) Trends in cresolase activity expressed per grape berry during grape maturation. (○) Airen grape, (●) Monastrell grape.

(A) Tendances de l'activité catécholasique exprimée par baie de raisin pendant maturation. (B) Tendances de l'activité crésolasique exprimée par baie de raisin pendant maturation. (○) Cépage Airen, (●) cépage Monastrell.

o-quinone has a half life of 1400 s (VALERO *et al.* 1988). Catecholase activity was determined using 4-methylcatechol (4MC) (30 mM), in 10 mM sodium acetate buffer pH 4.5, as substrate; the substrate for measuring cresolase activity was 4-methylphenol (*p*-cresol) (0.5 mM) in 10 mM phosphate buffer pH 7.0 (SANCHEZ-FERRER *et al.* 1988).

Phenolic compounds: The phenolic compounds were extracted from the grapes according to the method of KIDRON *et al.* (1978). The juices and wines were brought to 80 % ethanol and insoluble material was removed by filtration. Total phenols were determined by the colorimetric method of SINGLETON and ROSSI (1965) and the *o*-diphenols by the molybdate method (MAPSON *et al.* 1963) from the same previous phenolic extract.

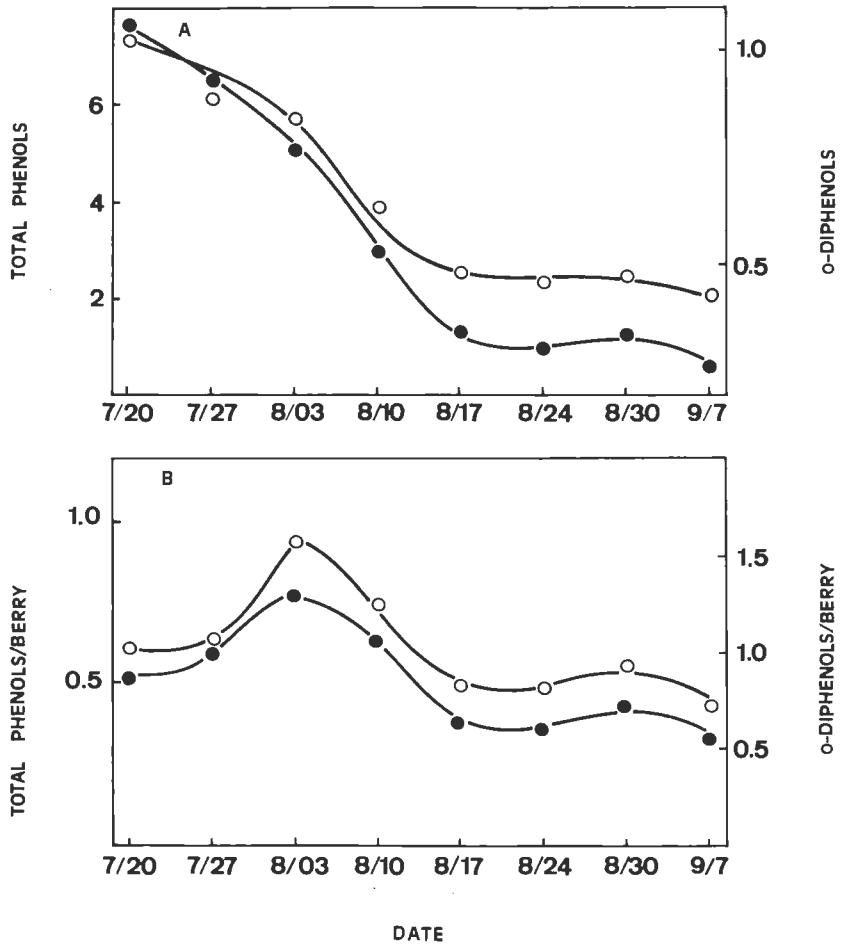

Fig. 3: (A) Changes in the concentration of total phenols (○) (mg gallic acid/g total fresh weight) and *o*-diphenols (●) (mg chlorogenic acid/g total fresh weight) for the Airen grape. (B) The same changes expressed per grape berry.

Changements de la concentration des phénols totaux (○) (mg acide gallique/g poids total de fruit frais) et des *o*-diphénols (●) (mg acide chlorogénique/g poids total de fruit frais) du cépage Airen. (B) Les mêmes changements exprimés par baie.

Colour determination: The degree of colour of the samples was determined by centrifuging 20 ml at 20,000 *g* for 10 min at 4 °C. The absorbance of the supernatant was then read against a water blank at 420 nm.

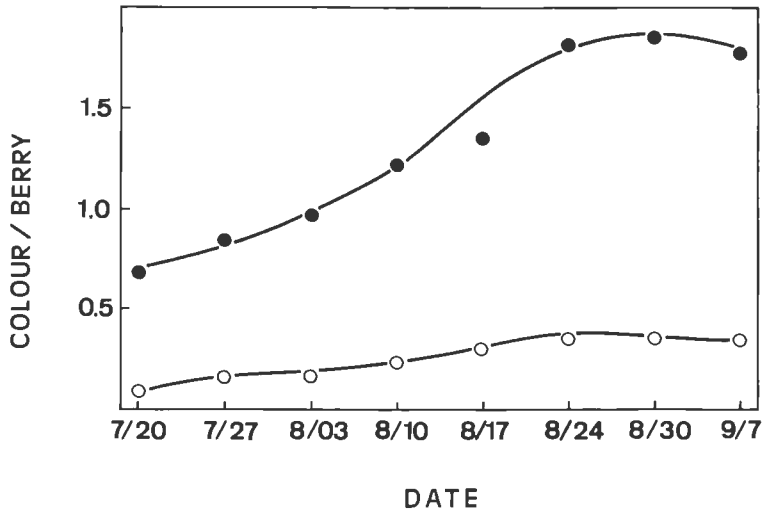

Fig. 4: Changes in colour per grape berry (absorbance at 420 nm) during grape maturation. (○) Airen grape, (●) Monastrell grape.

Changements de la couleur par baie (absorption à 420 nm) pendant la maturation du raisin. (○) Cépage Airen, (●) cépage Monastrell.

Results and discussion

Maturation studies

Grape development: In Fig. 1, the changes in sugar content (°Brix) and pH during grape maturation are shown for the Airen grape. Before veraison (between the end of July and the beginning of August), there were no appreciable changes in these parameters and afterwards they changed as expected (Sapis *et al.* 1983; Hrazdina *et al.* 1984). Monastrell grape followed a similar evolution.

Changes in PPO activity: The PPO activity for any one cultivar varied throughout the ripening period. Fig. 2 A shows the variation found in catecholase activity of grapes during maturation. The activity was very low during the first week, just before veraison, increasing afterwards in the two grape varieties. These results are in agreement with those of Wissemann and Lee (1980), who obtained a similar trend for Ravat grape, a variety with ripening rate similar to that of the grapes used in this study, and also for Dutchess and Pinot blanc varieties. Cash *et al.* (1976) also reported a rise in catecholase activity until ripeness, followed by a decline. However, Kidron *et al.* (1978) noted a rapid and intense decrease during the early stages of development, and a significant increase shortly before harvest. Fig. 2 B shows the evolution of cresolase activity during maturation. The trend is similar to that of catecholase activity, although the level is much lower. This result cannot be compared with any published data,

because changes in cresolase activity during grape development have not been reported for other grape varieties. Some authors (CASH *et al.* 1976; WISSEMANN and LEE 1981; NAKAMURA *et al.* 1983; INTERESSE *et al.* 1984) say that this activity is lost during extraction and purification.

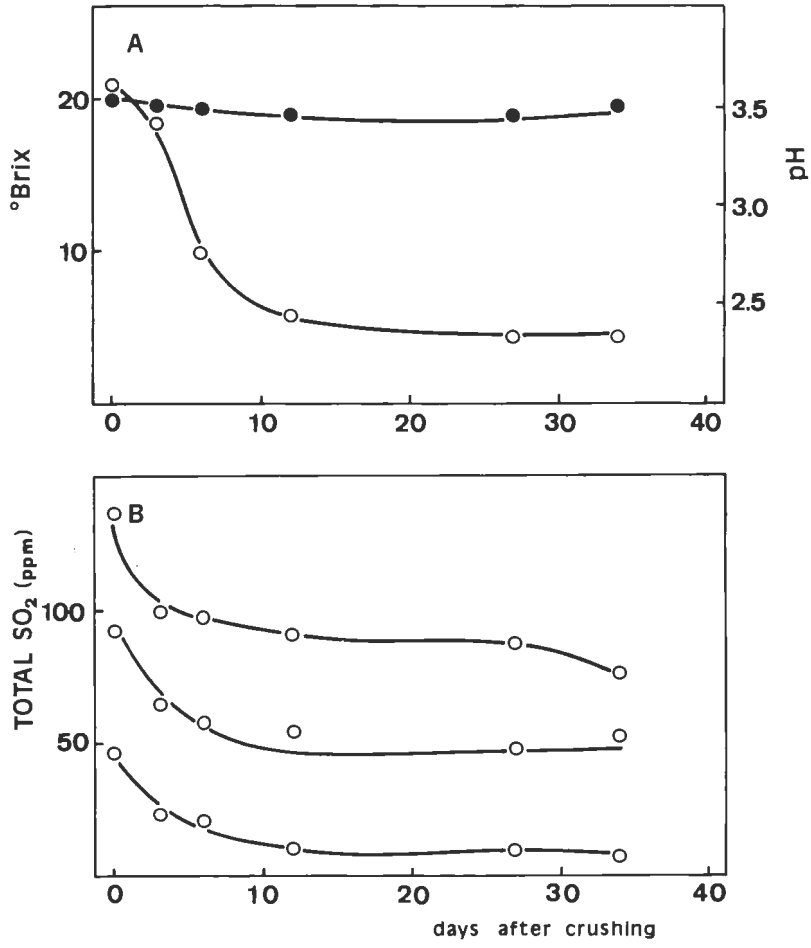

Fig. 5: (A) Changes in sugar content (○) expressed in °Brix and pH (●) of the Airen wine with 100 ppm SO_2 during vinification. (B) Changes in the total SO_2 levels (ppm) of the Airen wines.

Changements du contenu sucré (○) exprimé comme °Brix et pH (●) du vin Airen avec 100 ppm SO_2 pendant la vinification. (B) Changements des niveaux de SO_2 total (ppm) des vins Airen.

Changes in phenolic content: The evolution in total phenolic and *o*-phenolic compounds given per gram fresh weight of the berries for the Airen grape is shown in Fig. 3 A. There was a decrease until the middle of August, and finally during the completion of maturation it continued unchanged until the time of harvest. The trend was similar for the Monastrell grape. This result is qualitatively similar to those found for other grape varieties (SINGLETON 1966; KIDRON *et al.* 1978; WISSEMANN and LEE 1980).

A different pattern is seen when these results were expressed as their amount per berry (Fig. 3 B). The level of total phenolic and *o*-diphenolic compounds per berry was found to be nearly constant during fruit development: these differences in accumulation trends suggest that the decrease in phenolic concentration is only due to the increase of berry weight. This dilution effect has recently been suggested by other authors (CRIPPEN and MORRISON 1986; OZAWA *et al.* 1987), thus confirming our results. Furthermore, PIRETTI *et al.* (1980) correlated high rainfall with a decrease in phenolic concentration, due to an increase in berry size.

Fig. 4 illustrates the degree of colour of the two varieties. We found an increase in colour in both cases, although in the red variety this increase was greater, because of the biosynthesis of anthocyanins at veraison.

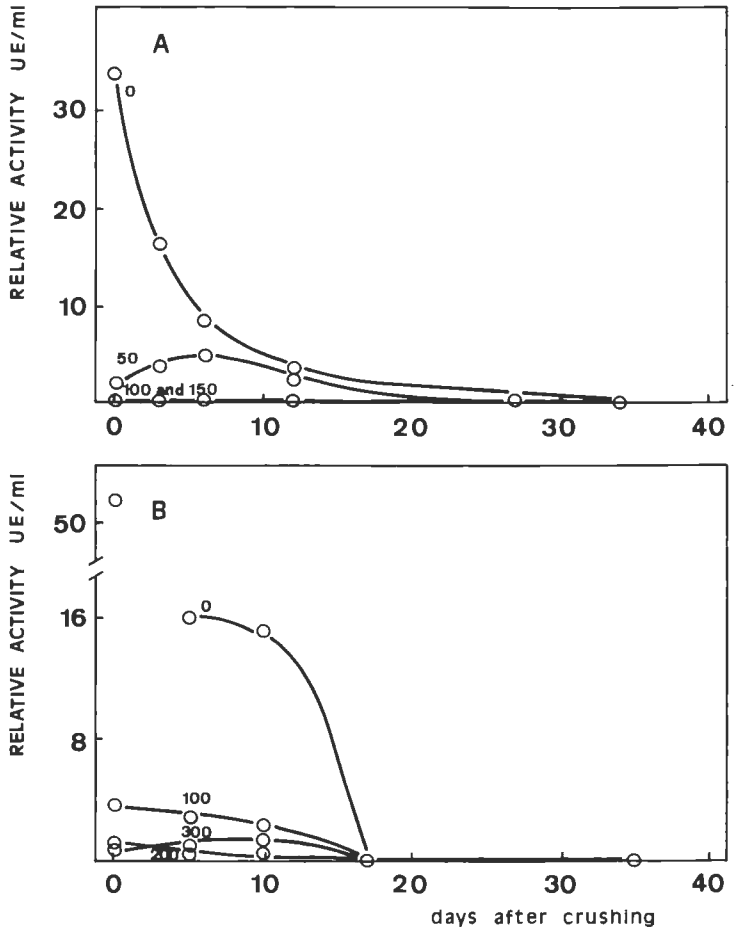

Fig. 6: Changes in catecholase activity during the vinification process at different SO_2 levels. (A) Airen wine, (B) Monastrell wine.

Changements de l'activité catécholasique pendant la vinification à différents niveaux de SO_2. (A) Vin Airen, (B) vin Monastrell.

Vinification studies

Vinification process: Since the addition of SO_2 is an important step in vinification and there is a general trend to reduce its quantity, wines were prepared with various levels of SO_2, in the same range as that used in the production of these wines.

Sugar content (° Brix), pH and SO_2 were measured and used as an index of evolution of the vinification process. Fig. 5 shows these results for the Airen wine, which were in the range normally encountered in grape musts (GORINSTEIN *et al.* 1984). Monastrell wine followed a similar evolution.

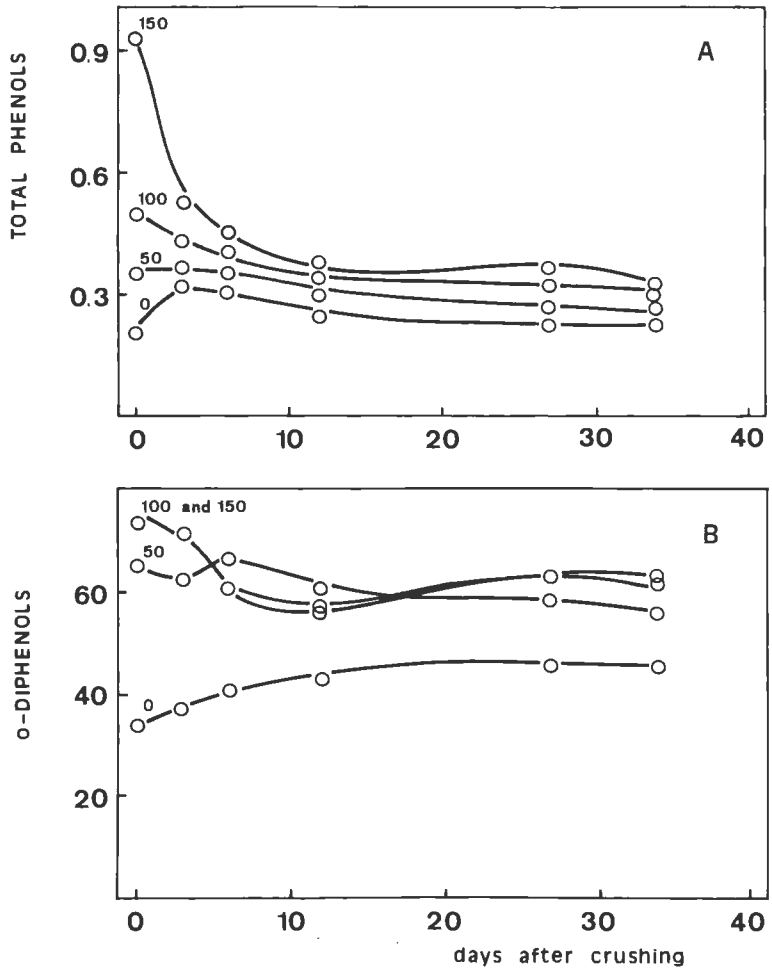

Fig. 7: (A) Total phenols (mg gallic acid/ml) during the vinification of Airen grapes at different SO_2 levels. (B) *o*-Diphenols (µg chlorogenic acid/ml) during the vinification of Airen grapes at different SO_2 levels.

(A) Le total des phénols (mg acide gallique/ml) pendant la vinification des raisins Airen, à différents niveaux de SO_2. (B) *o*-Diphénols (µg acide chlorogénique/ml) pendant la vinification des raisins Airen à différents niveaux de SO_2.

Changes in PPO activity: Fig. 6 illustrates the evolution of catecholase activity during the fermentation process for the two wines. The highest level of enzyme activity in the samples without SO_2 was found in both cases after crushing, while the samples with high levels of SO_2 (Monastrell juices with 200 and 300 ppm and Airen juices with 100 and 150 ppm) exhibited only a trace of activity. The enzyme activity, in general, decreased throughout the fermentation process and at the end of fermentation, the wine was devoid of activity. However, in the first days of fermentation, we found a slight increase in activity, in Monastrell juice with 300 ppm SO_2 and in Airen juice with 50 ppm SO_2. This small increase in activity can be explained because it coincides with a decrease in the SO_2 levels, and because the enzyme is still in the early stages of fermentation, and has not yet precipitated in significant amounts. Other authors who have followed the evolution of PPO activity during vinification (KIDRON *et*

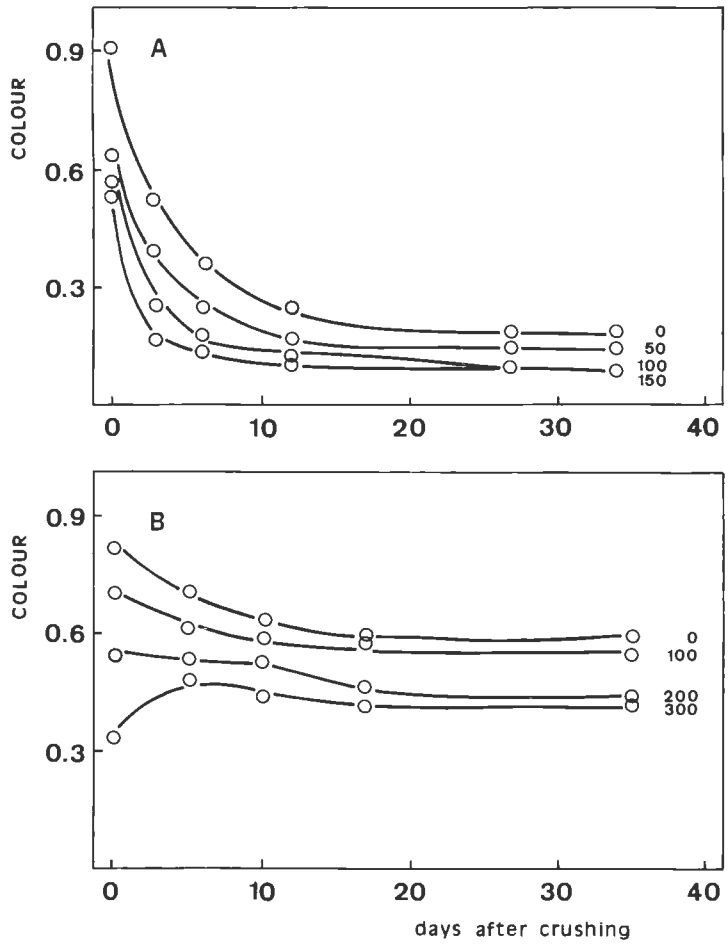

Fig. 8: Changes in colour (absorbance at 420 nm) during the vinification at different SO_2 levels. (A) Airen wine, (B) Monastrell wine.

Changements de couleur (absorption à 420 nm) pendant la vinification à différents niveaux de SO_2. (A) Vin Airen, (B) vin Monastrell.

al. 1978; WISSEMANN and LEE 1980) have not detected this fact, probably due to an insufficient number of samples in the early stages of fermentation. However, it is important to follow enzyme activity throughout the complete process in order to know the real amount of PPO in each stage.

Cresolase activity was not measured in these samples because its level is lower than that of catecholase activity, and in these conditions, the lag period characteristic of this activity can last various hours (GARCIA-CARMONA *et al.* 1987).

Changes in phenolic compounds: Figs. 7 A and B show the changes in total phenols and *o*-diphenolic compounds, respectively, for the Airen wine. Results for the Monastrell wine were similar. Total phenols, while at a maximum initially, decreased rapidly during fermentation, and afterwards remained at a constant level depending on the level of SO_2 used. A similar evolution was observed by other authors (BOURZEIX 1976; WISSEMANN and LEE 1980). The trend in *o*-diphenolics was similar, although in the juices without SO_2 we found a slight increase, possibly due to an oxidation of natural monophenols by the air oxygen and by the cresolase activity of PPO.

The degree of browning in the two wines, determined by measuring their colour at 420 nm, is shown by Fig. 8. Initially, there was a maximum that is higher when no SO_2 is present, which afterwards decreased to a constant level, in accordance with the SO_2 level added. However, in both wines, the final colour for the samples with highest SO_2 level is very similar.

These results illustrate the importance of SO_2 in the wine industry in order to prevent the browning process. However, it is important to ascertain the correct amount, as an insufficiency leads to undesirable oxidations while an excess has no additional beneficial effect.

Summary

The activity of polyphenol oxidase (PPO) and content of phenolic compounds were followed during the maturation in two varieties of wine grapes grown in south-eastern Spain and during vinification at different sulphur dioxide concentrations.

Both cresolase and catecholase activities (measured at pH 7.0 and 4.5, respectively) increased throughout the studied period and the content of phenolic compounds decreased rapidly when expressed as concentration (mg gallic acid/g total fresh weight), while when expressed as total amount per berry (mg/berry) it remained constant. During wine production, the enzyme activity was highest immediately after crushing of the fresh grapes and was not detected at the end of the fermentation process. The phenolic content also decreased during vinification to a constant level depending on the SO_2 level used.

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