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Received for review April 16, 1982. Revised manuscript received November 19, 1982. Accepted December 13, 1982. New Jersey Agricultural Experiment Station Publication No. D-10501-3-82, supported by State Funds and a grant-in-aid from General Foods Corporation, White Plains, NY.

The Browning Capacity of Grapes. 1. Changes in Polyphenol Oxidase Activities during Development and Maturation of the Fruit

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The investigation over a 3-year period of the development of polyphenol oxidase activities throughout the growth and maturation of five varieties of grape reveals the extreme complexity of the phenomenon from the green stage up until the full maturation of the fruit. Although they remain comparable, the crude total polyphenol oxidase activities and the solubilized polyphenol oxidase activities differ for certain years in the intensity and the speed of their development. The varietal factor appears to be important and characteristic in the case of the crude soluble polyphenol oxidase activities.

Browning during grape juice processing is a well-known phenomenon, the causes of which are essentially enzymatic in origin. In the presence of the oxygen in the air, the polyphenol oxidase of the grape (*o*-diphenol oxidase, EC 1.10.3.1) catalyses the oxidation of certain phenolic compounds occurring naturally in the fruit; the quinones thus formed lead by polymerization to the creation of brown pigments which are characteristic of the browning phenomenon.

This phenomenon causes a radical change in the color and the flavor and is thus a considerable handicap which greatly diminishes the quality of the processed products. It is therefore desirable to find ways of avoiding this, in particular by finding varieties having the lowest possible level of sensitivity to browning. This latter is in connection with many parameters—parameters which are often lacking in definition. Among those, both polyphenol oxidase and phenolic compounds levels play an important role. In that first part of work we have been interested in polyphenol oxidase. Data concerning phenolic compounds will be reported in a following paper. Quite a number of studies have been made concerning polyphenol oxidase in the grape (Mayer et al., 1965; Poux, 1966; Constantinides, 1967; Ivanov and Ivanova, 1968; Montedoro, 1969a,b; Montedoro and Cantarelli, 1969; Harel and Mayer, 1971; Lerner et al., 1972; Traverso-Rueda and Singleton, 1973; Dubernet and Ribéreau-Gayon, 1973; Harel et al., 1973; Dubernet, 1974; Dubernet and Ribéreau-Gayon, 1974; Lerner et al., 1974; Kidron et al., 1977, 1978; Wissemann and Lee, 1980a,b).

In our research, we therefore started by studying the variations in polyphenol oxidase activities throughout the development and the maturation of a number of varieties and we compared the results obtained over several consecutive years.

MATERIALS AND METHODS

Plant Material. The study was carried out from 1979 to 1981 on a number of varieties from the same vineyard. In 1979, four varieties were selected for study throughout their development: two of these were known to be sensitive to browning (Grenache, a red variety and Clairette, a white variety) and two were known to resist browning (Carignane, a red variety, and Ugni blanc, a white variety). To the results obtained in 1979 were added others obtained in 1980 and 1981 for most of the above varieties plus Maccabeo (white variety). Between July 1st and Sept 30th, four samplings were taken from each variety in 1979 and 19 samplings were taken in 1980. In 1981, 14 samplings were taken between June 20th and Sept 30th. In 1979 and 1980, the grapes sampled were freeze-dried immediately after being harvested. In 1981, they were analyzed fresh when picked. At the time of testing, the deseeded grapes are immersed in liquid nitrogen and ground immediately in a ball grinder (Dangoumau type) while still in the presence of liquid nitrogen. The powders obtained by this method are used for the preparation of enzymatic extracts.

Preparation of Enzymatic Extracts and Measurement of Polyphenol Oxidase Activity. To 5 g of the grape powder placed in suspension in 50 mL of 0.1 M phosphate buffer, pH 7.2, is added 400 mg of poly(ethylene glycol), 1.5 g of Polyclar AT (to set the phenolic compounds ocurring naturally in the fruit) and 75 μL of a solution of mercapto-2-ethanol at 10/100. After homogenization at 0 °C in an Ultra-Turax for 30 s and gentle agitation at 0 °C for 30 min, the mixture is centrifuged (40000*g*, 2 °C,

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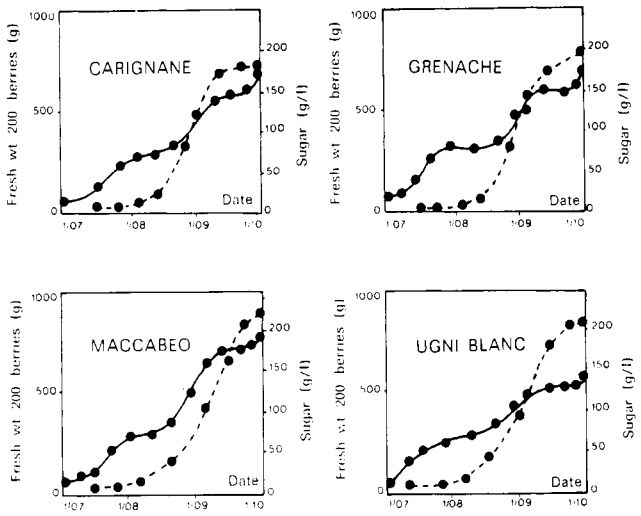

Figure 1. Changes in *fresh weight* of 200 berries (—) and *sugar content* of juice (---) during development and maturation of four grape varieties in 1980.

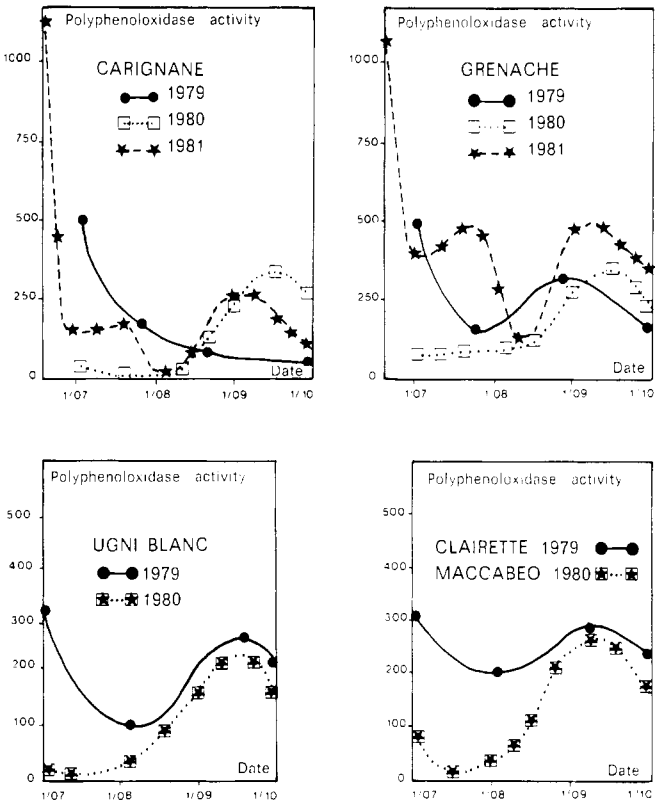

Figure 2. Changes in *solubilized* polyphenol oxidase activities during development and maturation of some grape varieties in 1979, 1980, and 1981 (enzyme activity is defined as micromoles of oxygen consumed per minute per gram of fresh weight).

20 min). The supernatant is passed through a column of Sephadex G-25 gel which is kept at 4 °C and which has previously been equilibrated by using the same 0.1 M phosphate buffer, pH 7.2. The polyphenol oxidase activity is measured for 200 μL of percolate by using a polarographic method (Mayer et al., 1965; Dubernet, 1974; Kidron et al., 1978) by measuring the consumption of oxygen. The reaction takes place at pH 4.8, the pH of the maximum activity of the enzyme (Harel and Mayer, 1971) at 30 °C in a thermostated cell (Oxygraph, Gilson Medical Electronics) and in the presence of a substrate surplus (1500 μL of a 0.1 M solution of methyl-4-pyrocatechol). The enzymatic activity measured in this manner is called the *solubilized polyphenol oxidase activity*.

To this measurement are added two further measurements with a technological interest concerning the browning of grapes and juices in the early stages of grape juice processing: (1) The first is the measurement of oxygen consumption for a suspension containing only the grape powder (5 g) and 50 mL of 0.1 M phosphate buffer, pH 7.2. This measurement, carried out under the aforesaid conditions on 200 μL of homogenate makes it possible to evaluate the proportion of polyphenoloxidase activity which is technologically available in the fruit in the presence of the natural effectors: this is the *crude total polyphenol oxidase activity*. The second is (2) The measurement of the oxygen consumption on the supernatant obtained by centrifuging (40000*g*, 2 °C, 20 min) after stirring gently (0 °C, 30 min) the latter type of homogenate (5 g of grape powder in 50 mL of 0.1 M phosphate buffer, pH 7.2). This measurement makes possible to evaluate the proportion of crude activity capable of dissolving naturally, following a procedure which bears some resemblance to technological conditions, in the presence of natural activators and inhibitors present in the fruit, particularly phenolic compounds. It is called the *crude soluble polyphenol oxidase activity*.

Protein determination is carried out, after percolation on Sephadex G-25, according to the method of Lowry et al. (1951). The reducing sugars contents of the juices are obtained by using the polarimetric method (Ribéreau-Gayon et al., 1972).

RESULTS AND DISCUSSION

Grape Development and Changes in Sugar Content. As shown by the curves in Figure 1, the development of the fresh weight of 200 berries and that of the sugar content of the juice in 1980 were in accordance with the development normally found in the grape throughout its growth.

Solubilized Polyphenol Oxidase Activities. These activities were studied for Carignane and Grenache in 1979, 1980, and 1981, for Ugni blanc in 1979 and 1980, for Clairette in 1979, and for Maccabeo in 1980. The curves, given together in Figure 2, show similar overall developments for all varieties of solubilized polyphenol oxidase activities throughout development and maturation. First, a decrease is noted during the green stages of the fruit. This decrease is partially concealed in 1980 by an insufficient number of early samples; this is proved by the results obtained in 1981 which clearly show the speed and extent of this decrease during which, in 10 days, the solubilized polyphenol oxidase activities of Carignane and Grenache fall by 95 and 88 per 100, respectively, of their original levels whereas the average diameter of the berries increases by only 2 mm. Later, starting from the beginning of August, we noted an increase of activity which continues until around the end of the veraison (beginning of September). Lastly, during the completion of maturation, activity decreases regularly.

The Carignane development curve for 1979 is different, probably because an insufficient number of samplings were taken at the correct time. This explanation is borne out by the curves obtained in 1981.

When compared with the corresponding protein contents, the development of the solubilized polyphenol oxidase activities (Figure 3) shows for 1980 results which differ little from those of these same activities compared with the weight of the fresh grapes; however, the decrease in activity during the early stages is more clearly evident, particularly in the cases of Carignane, Grenache, and Maccabeo.

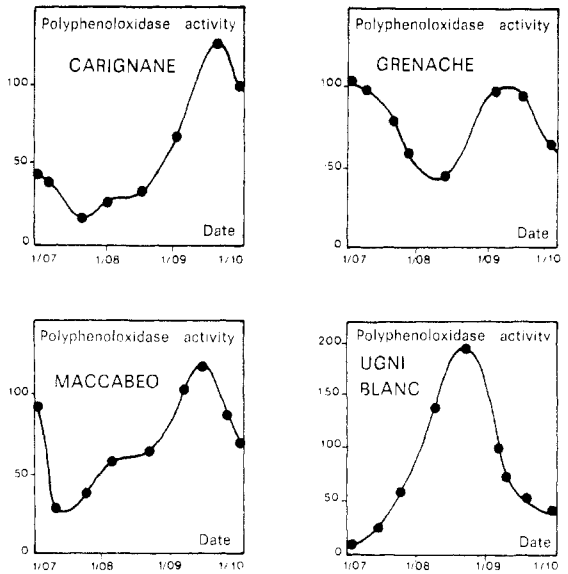

Figure 3. Changes in *solubilized* polyphenol oxidase activities during development and maturation of four grape varieties in 1980 (enzyme activity is defined as micromoles of oxygen consumed per minute per milligram of proteins).

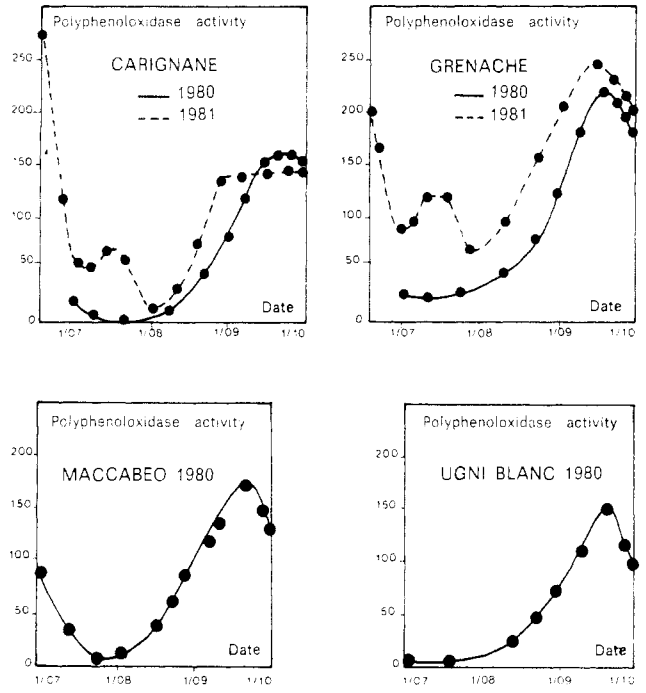

Figure 4. Changes in *crude total* polyphenol oxidase activities during development and maturation of some grape varieties in 1980 and 1981 (enzyme activity is defined as micromoles of oxygen consumed per minute per gram of fresh weight).

Crude Total Polyphenol Oxidase Activities. These activities were studied in 1980 and 1981 for Carignane and Grenache and in 1980 for Maccabeo and Ugni blanc. These activities are always lower than the solubilized polyphenol oxidase activities but their development is similar (Figure 4): a more-or-less marked decrease during the initial stages of growth of the grape (partially concealed in 1980 for the reasons mentioned earlier), a progressive increase which reaches its maximum level toward the end of the veraison (between the end of August and the middle of September), and, finally, during the completion of maturation, a slight decrease with the exception of Carignane, for which activity continues unchanged until time of harvest. It is to be noted that the ratio between the crude total activity and the solubilized polyphenol oxidase

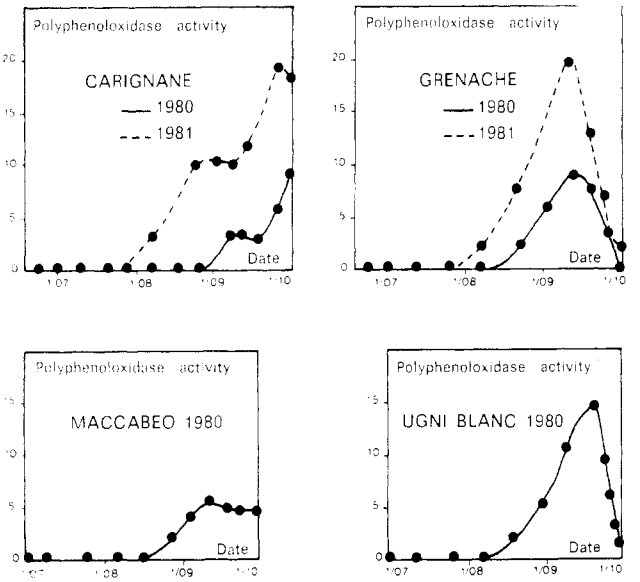

Figure 5. Changes in *crude soluble* polyphenol oxidase activities during development and maturation of some grape varieties in 1980 and 1981 (enzyme activity is defined as micromoles of oxygen consumed per minute per gram of fresh weight).

activity is greater at the end of maturation than during the early stages of development of the grape: + 30/100 on average in 1980; + 70/100 in 1981.

Crude Soluble Polyphenol Oxidase Activities. The study of the development of this type of activity during the growth and maturation of the grape is of great technological importance and especially as regards the development during the period which precedes the complete ripening of the fruit as this is the polyphenol oxidase activity which is likely to be present in the grape juices during the prefermentation oxidation processes.

In a general manner, it is noted that the crude soluble polyphenol oxidase activities are low and represent a very small part only of the total crude activities and an even smaller part of the solubilized activities. During the green stages of the development of the grape this crude soluble activity is nonexistent. It first appears at the veraison and its onset coincides with the minimum level of crude total activities and of solubilized activities recorded for that period.

The curves given in Figure 5 reveal differences in development in which the year, the physiological stage, and, above all, the variety appear to play important parts. The differences between varieties are particularly clear; thus, in the case of Carignane and Maccabeo, the crude soluble polyphenol oxidase activity increases in a more-or-less regular manner from the beginning of the veraison up until complete maturation with a leveling off in mid-September (Carignane) or at the end of September (Maccabeo). In the case of Grenache and Ugni blanc, the developments of the crude soluble activities are very different and give a bell-shaped curve with a maximum situated at the end of the veraison (beginning or middle of September, depending on the year). The maximum is related to those mentioned earlier for the crude total activities and the solubilized activities (Figure 2). During the second half of September the crude soluble activities then diminish rapidly and disappear at full maturation. It is also noted that these activities vary considerably within the same variety. Carignane, for example, gave maximum levels for activities which doubled between 1980 and 1981.

Our results reveal a complex process of development of the polyphenol oxidase activities during the growth and

maturation of the grape. By permitting a clear comparison between the various types of activity, they appear to provide a synthesis of the results obtained earlier by different authors under a variety of conditions. As regards the solubilized polyphenol oxidase activity (obtained after the addition of detergent to the grapes), Kidron et al. (1978) noted, as we did, a rapid and intense decrease during the early stages of development. Afterward, however, the shape of the curves presented differs: for the Carignane, Clairette, and Semillon studied by these authors, activities decrease regularly (the speed at which this decrease occurs drops as the development of the grape advances) with a slight increase at the end of maturation. However, the results we obtained are in agreement with those of Wissemann and Lee (1980a), who obtained curves for solubilized polyphenol oxidase activity development which are similar to ours but for two only of the four varieties studied by these authors (Pinot Blanc and Dutchess).

The increase in crude total polyphenol oxidase activities which we noted throughout the growing period of the grape and stretching from the end of July up until the beginning of September confirms the results obtained by Dubernet (1974) with Malbec. However, the increase in "particular tyrosinase activity" noted by this author at the start of the development of the grape is very slight in comparison with those measured by us, furthermore, the considerable and rapid drop in activity during the green stages of the grape is not reported. This is also the case for Ivanov and Ivanova (1968) and for Montedoro (1969a), who, in various varieties of red and white grapes, report only a drop in polyphenol oxidase activity lasting from the end of the veraison up until complete maturation.

Our research was spread over a number of years and showed that the development characteristics of the polyphenol oxidase activities are common to all the varieties studied (with the exception of Carignane in 1979 and of certain samplings in 1980 which have been reported and commented upon earlier). The levels of activity measured vary, nevertheless, from one variety to another for the same year and the same variety from one year to another. It is possible that this last observation is linked to changing meteorological conditions from one year to the next which can induce variations in the physiological development of the grape.

CONCLUSIONS

The investigations carried out from 1979 to 1981 on changes in the polyphenol oxidase activities throughout the growth and maturation of the grape led to several interesting results: (1) the different approaches as regards the measurement of these activities lead to the revelation of complex changes from the green stages up to the maturation of the fruit; (2) when the results obtained for the five varieties investigated are compared, it is seen that the crude total polyphenol oxidase activities and the solubilized activities show similar developments throughout the three years of testing; nevertheless, the intensity and the speed of the physiological development—linked, probably, to the climatic conditions of each year—are likely to affect the levels of activity measured; (3) in the case of the crude soluble polyphenol oxidase activities, the variety is a factor which considerably adds to the variations due to the year and appears to be important and characteristic.

The foregoing observations, although important, should nevertheless be completed by a more detailed investigation of the polyphenol oxidases of the grape. Such an investigation should in particular be aimed at both the intracellular localization of these enzymes and the search for potentially different forms such as those which have already been identified in other plant material (Constantinides, 1967; Harel et al., 1973) during the development and maturation.

Registry No. Polyphenol oxidase, 9002-10-2.

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Received for review April 6, 1982. Revised manuscript received September 13, 1982. Accepted October 26, 1982. This work was performed as a part of a Programmed Thematic Action "Processing, storage and utilization of concentrated grape juices" conducted by the Institut National de la Recherche Agronomique.