The Browning Capacity of Grapes. 3. Changes and Importance of Hydroxycinnamic Acid-Tartaric Acid Esters during Development and Maturation of the Fruit

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The fractionation and the determination of hydroxycinnamoyl esters (caffeoyl, *p*-coumaroyl, and feruloyl tartaric acid esters) were carried out by using HPLC for four varieties of grape (Grenache, Carignane, Ugni blanc, Maccabeo) during the development and maturation of the fruit. Less complete quantitative findings were also obtained for the Clairette variety. In the case of all varieties investigated, the overall hydroxycinnamoyl tartaric acid ester contents dropped considerably as development progressed, as did the contents for each of the esters determined separately. Important differences were noted, however, from one variety to the next; these concerned in particular the proportion of caffeoyl tartaric, a good polyphenol oxidase substrate, in relation to the other hydroxycinnamoyl esters, a fact which may partially explain the great differences in potential browning capacity for the different grape varieties.

In our earlier studies, we were able to show to what extent the polyphenol oxidase activities of grapes vary with the year, the variety, the stage of development and the degree of maturation of the berry (Sapis et al., 1983a). However, the browning capacities of the mature berries depend to a large extent on factors other than the polyphenol oxidase activities, and the measurements carried out under different conditions (Sapis et al., 1983b) would seem to indicate that the phenolic substrates must be taken into consideration. Among the numerous phenolic compounds present in the grape (Genevois, 1952; Ribereau-Gayon et al., 1972; Singleton and Esau, 1969), the hydroxycinnamic derivatives play an important part from the points of view of both their quantity and the capacity of some of them to get a participation in browning reactions. The presence of *p*-coumaric, caffeic and ferulic acids in grapes was discovered early (Genevois, 1952; Ribereau-Gayon, 1963), but the nature of the derivatives they form has been interpreted in a number of manners up until recently (Sondheimer, 1958; Weurman and de Rooij, 1958; Hennig and Burkhardt, 1960; Wulf and Nagel, 1976; Ong and Nagel, 1978a). Ribereau-Gayon (1965) was the first to demonstrate that the phenolic acids of the grape combine with the tartaric acid to form esters, certain of which were already known elsewhere (Scarpati and Oriente, 1958; Scarpati and d'Amico, 1960) and which were to be found in other raw material (Tadera et al., 1970; Suzuki et al., 1970). Singleton and Noble (1976) considered that the nature of the hydroxycinnamic derivatives in grapes was not yet fully identified, but recent analyses based on high-performance liquid chromatography and physical and chemical analyses have now confirmed that hydroxycinnamoyl tartaric acid esters are present in the berry of *Vitis vinifera* (Ong and Nagel, 1978a; Singleton et al., 1978; Okamura and Watanabe, 1979; Baranowski and Nagel, 1981); in particular, chlorogenic acid, which is very common in a wide range of plants (Sondheimer, 1958), is not present in the grape, but the hydroxycinnamic acids play an additional part through acylation of the various anthocyanins in the grape (Albach et al., 1965; Hrazdina and Franzese,1974; Wulf and Nagel, 1976).

The variations in the phenols or the hydroxycinnamic acids as a whole in relation to the variety and the level of maturation of the grape have been discussed in a number of papers (Singleton, 1961; Ribereau-Gayon, 1972; Dumazert, 1973; Ong and Nagel, 1978a,b; Nagel et al., 1979), but we considered it important to report in the present work on the individual variations of each of the hydroxycinnamoyl tartaric acid esters throughout the development and maturation of the fruit of several white and red varieties which are common in France and which have, as we have demonstrated elsewhere (Sapis et al., 1983b), greatly differing browning capacities.

MATERIALS AND METHODS

Plant Material. Five varieties of grape were chosen for their differing browning capacities (Sapis et al., 1983b): two red varieties (Grenache and Carignane) and three white varieties (Maccabeo, Ugni blanc, and Clairette, the last named of which was studied in less depth). The analyses were carried out in part in 1979 and more extensively in 1980.

The grapes were immediately immersed in liquid nitrogen after picking and then freeze-dried. Each sample was deseeded (with the exception of an initial sample in which the seeds were too small) and then ground in the presence of liquid nitrogen. The powders obtained—light green for the white grapes and for the red before veraison, medium to dark pink for the red grapes from the veraison to complete maturity—were, in all cases, free from browning. They were kept in a freezer at −20 °C.

Extraction of the Phenolic Compounds. The phenolic compounds in freeze-dried powder were extracted by using 80% ethanol at 0 °C (Macheix, 1974a); three successive extractions were used to remove all the soluble phenolic compounds in the raw material.

The extract obtained was evaporated in a vacuum and reduced to 10 mL of aqueous medium and then extracted by using petroleum ether (v/v) to eliminate chlorophylls and carotenoids. Next, the hydroxycinnamoyl tartaric acid esters and most of the other phenolic compounds were extracted by using ethyl acetate and experimental conditions defined previously (Fleuriet and Macheix, 1972). The extract was dry-evaporated and mixed with 5 mL of methanol to give the final extract used for the chromatographic analyses.

Paper Chromatography. This was used to isolate and purify the reference compounds which were used for the

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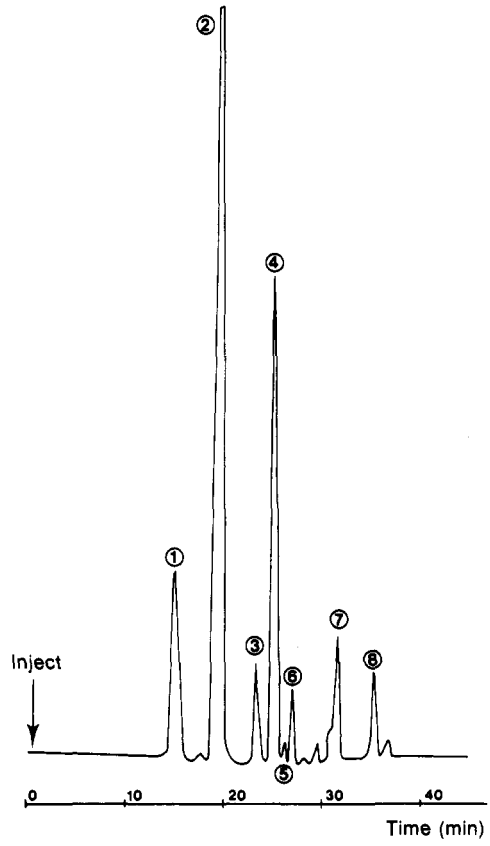

Figure 1. Fractionating the hydroxycinnamic derivatives and the isoquercitrin by HPLC from the Grenache variety sampled on July 7, 1980. Peak identification: 1, *cis*-caffeoyl tartaric acid ester; 2, *trans*-caffeoyl tartaric acid ester; 3, *cis*-*p*-coumaroyl tartaric acid ester; 4, *trans*-*p*-coumaroyl tartaric acid ester; 5, *cis*-feruloyl tartaric acid ester; 6, *trans*-feruloyl tartaric acid ester; 7, unknown peak; 8, isoquercitrin.

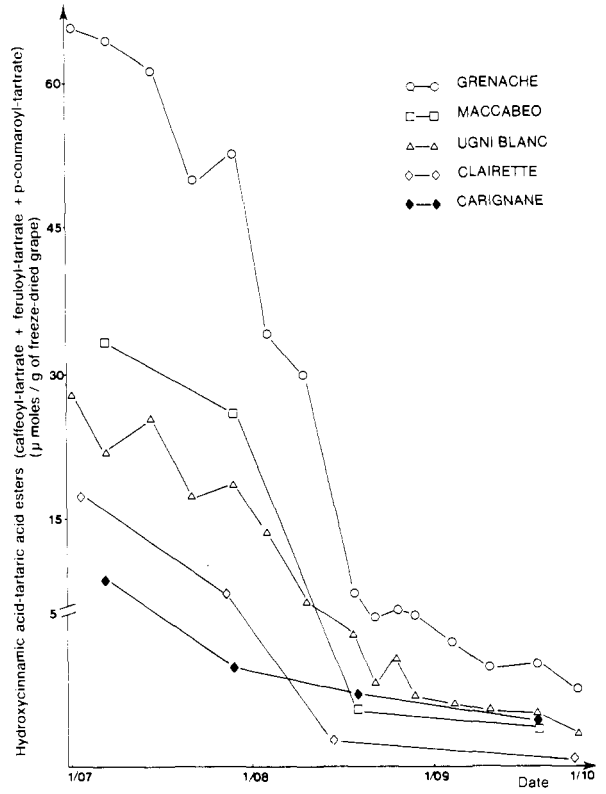

Figure 2. Evolution of the overall hydroxycinnamic esters content of the five grape varieties during development and maturation.

high-performance liquid chromatography. The phenolic compounds of the grape were separated by successive monodimensional chromatographies on No. 3. Whatman paper by using solvents of the type normally used for fruit extracts (Macheix, 1974a): butyl acetate/acetic acid/water (4/1/5 v/v), butanol/acetic acid/water (4/1/5 v/v), and methyl isobutyl ketone/formic acid/water (3/1/2 v/v). Each compound separated was developed in ultraviolet light and then eluted; some quantitative data were obtained by spectrophotometric determination of the eluates, particularly in the case of the Clairette variety.

High-Performance Liquid Chromatography (HPLC). The equipment used is a Spectra Physics SP 8000 with microprocessor. The phenolic compounds are separated by using an RP C 18 column with a granulometry of 5 μm (reversed-phase polarity), a length of 25 cm, and an internal diameter of 4.6 mm; it has a concave slope gradient of 6% up to 40% of acetonitrile in water adjusted to pH 2.6 with phosphoric acid. Detection is by means of spectrophotometry with a fixed wavelength of 312.5 or 254 nm.

HPLC determinations of each of the three hydroxycinnamoyl esters are obtained by peak integration or by planimetry, by bringing the surface of each peak to a standard concentration calculated to give maximum absorption and having the corresponding molar extinction coefficient. It is possible in this manner to determine the cis and trans forms of a given hydroxycinnamic derivative. The molar extinction coefficients given by Kahnt (1966) are those of the free-state acids, whereas 5 μm for the cinnamic esters remained unknown. Variations in determination are corrected by internal standardization. The final results are expressed in micromoles of each of the esters per gram of freeze-dried grape or per berry.

RESULTS AND DISCUSSION

Each of the red and white varieties investigated revealed the presence of tartaric acid esters of caffeic acid (CT), *p*-coumaric acid (pCT), and ferulic acid (FT); Figure 1 illustrates the distribution of various derivatives in a selected sample of Grenache. Isoquercitrin (quercetin 3-glucoside) is also present in considerable quantity together with flavanes [(+)-catechin, (−)-epicatechin] which are revealed at 254 nm. Other nonidentified peaks are present but have very low absorption at 312.4 nm; these could be benzoic acids (Ribereau-Gayon, 1964).

Each hydroxycinnamoyl derivative was fractionated into its two cis and trans isomers. Several experimental propositions (Romeyer, 1981) demonstrated that under our experimental conditions, the cis is eluted first, thus confirming a number of published findings (Hartley and Buchan, 1979; Baranowski and Nagel, 1981).

The evaluation of phenol content using HPLC for the two varieties Grenache and Ugni blanc was based on 15 samplings taken from the beginning of July up until maturation and for the varieties Maccabeo and Carignane on 4 samplings. The reproducibility of the extraction is satisfactory in all cases with results obtained being ±13.6%.

Evolution of the Overall Hydroxycinnamic Ester Content during Development and Maturation. The overall content is obtained by adding the CT, pCT, and FT contents for each variety (Figure 2): during development and maturation, the hydroxycinnamic esters content decreases sharply between the beginning of July and the end of September for all the varieties, with this decrease becoming very much slower at the end of development and the onset of maturity. The results obtained are similar to

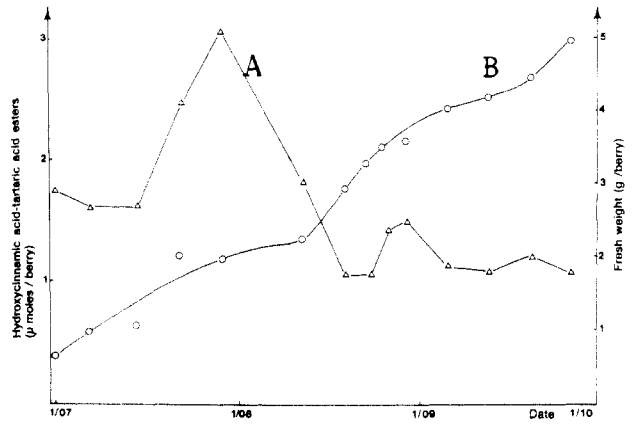

Figure 3. (A) Evolution of the hydroxycinnamic esters content per grape berry during development and maturation. (B) Development curve of the fruit.

those found for other grape varieties (Dumazert et al., 1973; Ong and Nagel, 1978b) as well as to those found more generally for numerous types of fruits (Macheix, 1974b; Fleuriet, 1977).

It is of interest, in addition, to express these same results by calculating the overall hydroxycinnamoyl tartaric derivatives content of a berry throughout its development (Figure 3); the content is found to increase up until the end of July, followed by a very definite decrease preceding the veraison; this may be due to migration in an other part of the vine plant or, more probably, to the reutilization in situ of the compounds. Developments which are partly similar have already been reported for the Riesling variety (Ong and Nagel, 1978b) and for other types of fruit (Macheix, 1974b; Fleuriet, 1977), thus suggesting that, in a more general manner, the hydroxycinnamoyl derivatives in fruits are not merely derivatives of accumulation but that they are capable of being metabolized as has been observed in the case of many other parts of plants (Barz, 1977; Barz and Hoessel, 1979).

Global Varietal Differences. Varietal differences are particularly significant at the first stages of development; for example, at the beginning of July the level of esters of Grenache is 8 times higher than that of Carignane. These differences become less significant at the end of maturation, though Grenache remains constantly the richest variety in esters whereas other varieties often exhibit relative changes during maturity (Figure 2). A more detailed study of the latter stage is being extended to other varieties.

There exist annual variations both in the amount of hydroxycinnamoyl tartaric acid esters (Nagel et al., 1979) and in the polyphenol oxidase activity (Sapis et al., 1983a). These variations are possibly due to climatic factors, such as water, whose uptake may affect the level of phenolic compounds (Duteau et al., 1981). Furthermore, the varietal differences observed do not seem to be due to geographical variations (Nagel et al., 1979), since all varieties studied were grown in the same vineyard.

It may be considered, therefore, that the differences noted in the levels of hydroxycinnamic acids derivatives in the five varieties studied seem to constitute an important factor in the browning of grapes. It is essential to compare levels of *p*-coumaric, ferulic, and caffeic acids derivatives since polyphenol oxidase does not exhibit the same affinity to all substrates, as does the *o*-diphenols which are involved in the browning phenomenon (De Villiers, 1961; Arnold et al., 1980; Okamura and Watanabe, 1981).

Relative Importance of the Hydroxycinnamoyl Derivatives. The individual variations of the hydroxy-

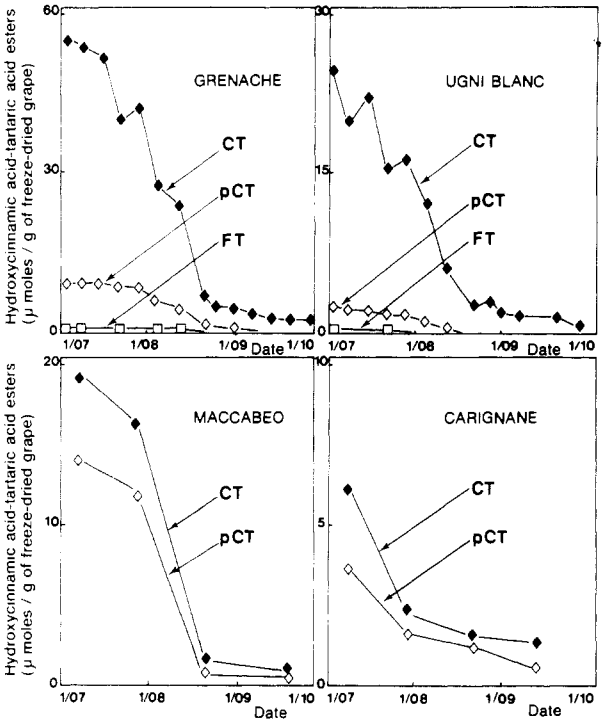

Figure 4. Evolution of the content of each of the identified hydroxycinnamic esters in four varieties of grape. (CT = caffeoyl tartaric acid ester; pCT = *p*-coumaroyl tartaric acid ester; FT = feruloyl tartaric acid ester).

cinnamoyl tartaric esters (Figure 4) are similar to those reported above for these compounds as a whole (Figure 2) and already reported for the maturation phase of the Riesling variety (Ong and Nagel, 1978b). However, the proportion of CT varies greatly from one variety to another. In 1980, the percentage of CT is important (80–90%) for mature Grenache and Ugni blanc which are known to possess a high browning capacity; conversely, the percentage of CT is lower (65%) for mature Carignane and Maccabeo, varieties which have in 1980 a low browning capacity (Sapis et al., 1983b). For all these varieties, the percentage of CT in relation to the others esters was constant throughout development and maturation. Very pronounced variations, however, were obtained in 1979 with Clairette for which the CT fell from 70 parts/100 in July to only 14 parts/100 in September while the FT content went from 2 to 54 parts/100 over the same period.

Thus, throughout the life of the grape but more particularly at maturity, which is an important period from the point of view of industrial processing, the differences in the composition of the hydroxycinnamic esters of the different varieties concern two points: (1) the total hydroxycinnamic ester content; (2) the proportion of *o*-diphenols esters (CT) in relation to the other compounds (pCT and FT). As a result, the browning capacities differ greatly. It must, however, be borne in mind that browning also depends on the presence of other phenolic substrates and on polyphenol oxidase activity as well as on a number of other variables (Macheix, 1971). Nevertheless, in all cases observed (Sapis et al., 1983b), the phenolic substrates of the grape constantly act as a very limiting factor in relation to browning. A study of these compounds in a much wider range of varieties with samplings taken at maturity only would lead to a clearer definition of the factors which determine browning capacities.

Registry No. *cis*-Caffeoyl tartaric acid ester, 84519-50-6; *trans*-caffeoyl tartaric acid ester, 67879-58-7; *cis*-*p*-coumaroyl tartaric acid ester, 67920-37-0; *trans*-*p*-coumaroyl tartaric acid

ester, 27174-07-8; *cis*-feruloyl tartaric acid ester, 84518-78-5; *trans*-feruloyl tartaric acid ester, 74282-22-7; isoquercitrin, 482-35-9; catechin, 154-23-4; epicatechin, 490-46-0; caffeoyl tartaric acid ester, 1234-09-9; *p*-coumaroyl tartaric acid ester, 69222-59-9; feruloyl tartaric acid ester, 1044-65-1.

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Received for review June 9, 1982. Accepted December 8, 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.

Influence of Processing on the Pigmentation of Wild Rice Grain

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Chlorophylls, pheophytins, and related derivatives in wild rice grain were analyzed by high-performance liquid chromatography (HPLC) on a reverse-phase column. High-temperature drying (>80 °C) or parching converted chlorophylls to pheophytins, pyropheophytins, and other derivatives which along with melanins contribute to the black color of this grain. HPLC was shown to be a useful technique for monitoring chlorophyll pigment alterations during the processing of wild rice.

Wild rice (*Zizania aquatica*) is an aquatic grass growing in the Upper Great Lakes region of the United States and Canada and is becoming increasingly used as a cereal grain component of many foods (Moore, 1977). This grain is unique with respect to the degree of surface pigmentation of the kernels, and the black appearance of mature seeds is a significant contributor to product identity. Withycombe (1974) studied the pigment systems of wild rice and found evidence of anthocyanin, carotenoid, tannin, and chlorophyll pigments in mature grain. Gutek et al. (1981) studied the chemistry and inheritance of the red pigments in the leaf sheath and staminate florets and found that the production of two anthocyanins was controlled by a dominant gene. However, only polyphenol and chlorophyll pigments systems appear influential in wild rice color (Withycombe, 1974), and Khoo and Wolf (1982) have recently described the location of these pigments in the structure of wild rice kernels.

Processing procedures for wild rice usually include a storage period during which a fermentation occurs, parching or drying from a moisture content of 35–50% to 7–12%, hulling, scarification of kernels, and cleaning (Lindsay et al., 1975). The heat encountered during parching should be sufficient to alter chlorophyll pigments and affect the appearance of kernels. Recently, Schwartz et al. (1981) developed a high-performance liquid chromatography method to monitor chlorophylls and their derivatives in processed foods. In this report, we present results of investigations on the application of this HPLC method to study wild rice chlorophyll pigments and relate this information to the pigment systems that characterize the appearance of wild rice grain.

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0021-8561/83/1431-0349$01.50/0