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CHANGES AND IMPORTANCE OF OLIGOMERIC PROCYANIDINS DURING MATURATION OF GRAPE SEEDS

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Key Word Index—*Vitis vinifera*; Vitaceae; grape; seeds; procyanidins; flavans; HPLC.

Abstract—Flavans and procyanidins from the seeds of different grape varieties were separated and identified using HPLC techniques. The compounds identified were (+)-catechin and (−)-epicatechin, dimeric procyanidins B1, B2, B3 and B4, trimeric procyanidin C2 and gallic acid. During maturation of the grape berries, the flavan-3-ol content fell in the seeds whereas procyanidin levels increased. This suggests an interrelationship between the compounds. There was also evidence of varietal differences in the amounts of phenolic compounds in grape seeds.

INTRODUCTION

The quality of food products from raw plant material is generally dependent on phenolic compounds, mainly, in the case of grapes and wines, tannins [1–4] and hydroxycinnamoyltartaric esters [5–7]. Their levels vary widely according to variety and physiological stage [8]. In grape berries, seeds are rich in low M_r condensed tannins [1, 9, 10] and may contribute, in so far as they are released into the medium, to organoleptic qualities. The purpose of this work, using HPLC techniques, was to separate and quantify phenolic compounds in seeds from various grape varieties and to monitor their evolution during the development and the maturation of the fruit. Analyses were carried out on two red grape varieties (Grenache and Carignane) and on two white ones (Ugni blanc and Maccabeo). These varieties were chosen for their different browning potential [8, 11]. By comparison with previous results [8], this study will enable comparison of the relative variations of phenolic compounds in the fruit and seeds during their development. Moreover, it will be possible to specify the characteristics of the plant material before technological treatments.

RESULTS

Chromatograms from HPLC separation of grape seed phenolics were fairly complex with eight peaks indentified viz. gallic acid, (+)-catechin and (−)-epicatechin, dimeric procyanidins B1, B2, B3 and B4, trimeric procyanidin C2. The order of elution was similar to that described for beer [12] and, with the exception of gallic acid whose λ_{max} is 274 nm, each compound has a maximum absorption at 280 nm, identical to that of standard compounds. The free gallic acid content was low in all cases but alkaline hydrolysis of a total phenolic extract releases large amounts of free gallic acid, ten times as much as in the initial extract. In consequence, this compound is likely to be present in the form of esters, such as epicatechin 3-*O*-gallate previously identified in this plant material [13–15] but which could not be separated under our analytical conditions.

Changes in (−)-epicatechin and (+)-catechin reached a maximum at *véraison* (Fig. 1), the varietal differences being mainly in the relative amounts. (+)-Catechin was generally the chief component (60 μmoles/g dry wt in Ugni blanc at *véraison*). The amount of dimeric procyanidins was rather low, B3 being the most abundant in Grenache and Ugni blanc (Fig. 2). Except in Maccabeo, the highest levels of dimeric procyanidins were found in the very young berries; they declined during grape development and remained steady during maturation. However, an increase in B2 occurred after *véraison* in two cases. The trimeric procyanidin C2 was only present as traces and could not be determined quantitatively. However, the largest quantities were observed in the two white varieties. Amounts of free gallic acid were always low and varied only slightly (Fig. 1).

Interesting observations can be made when the results are expressed per berry. Whereas the evolution of monomeric flavan-3-ols displayed a maximum at *véraison*, procyanidins B1, B2 and B4 increased continuously during maturation, which is the main period for the accumulation of these compounds in seeds (Fig. 3).

DISCUSSION

The varietal differences observed illustrate the high levels of flavan-3-ols and derived tannins in Grenache and Ugni blanc seeds. The hydroxycinnamoyl esters content of the berries of these two varieties are also known to be high [8], as is their potential browning [7]. This phenomenon may be accentuated by the diffusion of grape seed tannins as occurs during red wine-making with pomace contact [16].

The presence of procyanidins has already been confirmed in grapes [10, 17] but we report here for the first

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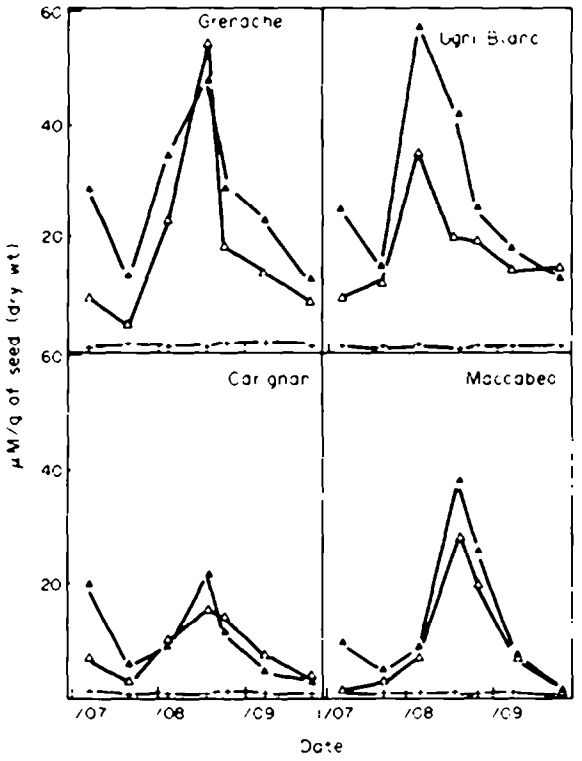

Fig. 1. Evolution of (+)-catechin (▲), (−)-epicatechin (△) and gallic acid (+) in four grape varieties during development and maturation (µmoles/g).

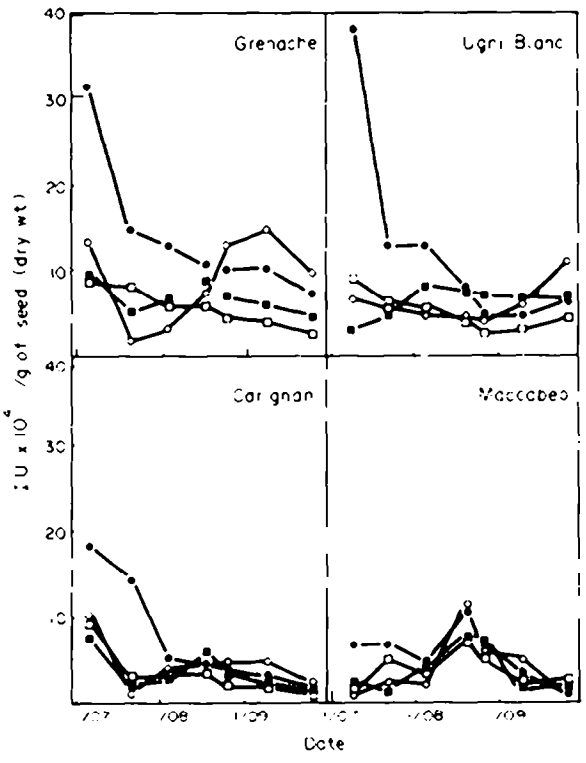

Fig. 2. Evolution of procyanidins B_1 (■), B_2 (○), B_3 (●) and B_4 (□) in four grape varieties during development and maturation (IU: integrator unit).

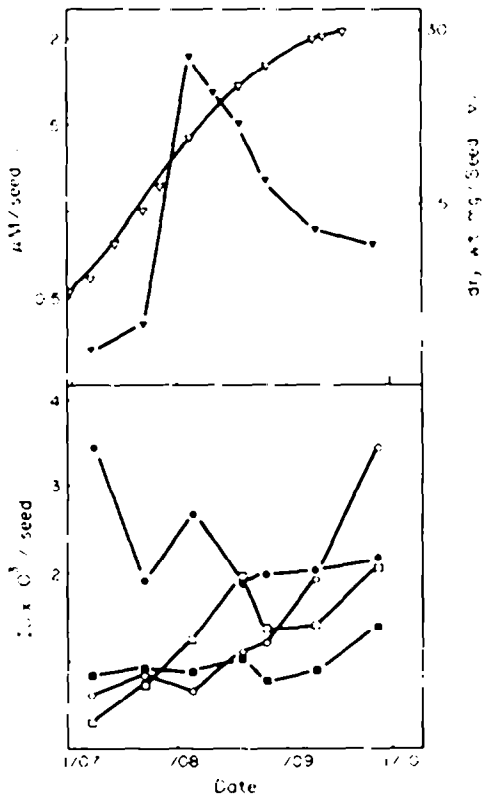

Fig. 3. Evolution of flavan-3-ols and procyanidins per grape seed during development and maturation of the Ugni Blanc variety. (+)-Catechin and (−)-epicatechin (▼), B_1 (■), B_2 (○), B_3 (●) and B_4 (□). Evolution of seed dry wt (▽).

time their separation and quantitative determination by HPLC in grape seeds. The trimeric procyanidin C2 had not been identified as yet in grapes although its presence was likely when dimers occur [18]. As a result of missing standards several compounds which were previously reported in grapes were not identified in our analyses, in particular gallocatechin, epigallocatechin and epicatechin 3-*O*-gallate [13, 19–22]. The latter compound is probably responsible for the large quantity of gallic acid found after alkaline hydrolysis. The importance of free (+)-catechin and the occurrence of B3 and C2 emphasizes the fact that the chief grape procyanidin unit is (+)-catechin. This relative abundance vs (−)-epicatechin proves that grape seeds display a genetically homogeneous behaviour [23].

The marked decrease in flavan-3-ols after veraison cannot be explained by diffusion towards the pulp of the fruit since these compounds do not occur there [8]. On the other hand, the accumulation of dimeric procyanidins during maturation suggests that they are synthesized from monomeric forms as suggested by several authors [24–26] with [27] or without [28] enzymatic control. In consequence, seed maturation is characterized by a very clear relative enrichment in dimeric compared to monomeric forms. Similar conclusions cannot be extended to higher polymerized forms as they are less abundant (C2) and difficult to separate.

Thus, the model system constituted by the grape berry and its seeds is particularly interesting for the study of

both the physiological aspects of oligomer procyanidins and the technological consequences which may be induced.

EXPERIMENTAL

Plant material. After picking, grapes were immediately frozen in liquid N_2 then stored at $-20°$. Seeds were separated just before extraction.

Extraction. Phenolic compounds were extracted three times with 80% EtOH at 0°. The extract was then evapd under vacuum to 10 ml of aq. medium. Carotenoids were extracted with petrol, then flavonoids and other phenols were separated by EtOAc according to ref. [29]. Just before injection into the HPLC column, 1 ml of the EtOAc extract was evapd to dryness under N_2 and then dil with an equal vol. of H_2O [8]. The extract was finally filtered through a 0.45 μm Millipore filter.

HPLC. Gradient separations were carried out on a reversed-phase column (RP, C18, 250 × 4.6 mm packed with Lichrosorb, particle size 5 μm). The column was fitted with a guard column before the injector and protected with a C_{18} silica gel precolumn placed after the injector. The column was equilibrated with a mobile phase of 10% MeCN (90% H_2O) adjusted to pH 2.6 with H_3PO_4. The solvent gradient profile was: 0–10 min: 10–15% MeCN; 10–20 min: 15% MeCN; 20–25 min: 15–20% MeCN; 25–45 min: 20% MeCN; 45–75 min: 20–40% MeCN. Flow rate 1 ml/min. The eluate was monitored at 280 nm, 0.2 AUFS, using a scanning spectrophotometer with autocontrol and variable wavelength.

Identification. Phenolic compounds were determined by co-chromatography and by scanning reference compounds [(+)-catechin, (−)-epicatechin, gallic acid from Fluka] and natural compounds previously extracted from plant material (procyanidins) in other laboratories.

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