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Procyanidin composition of Chardonnay, Mauzac and Grenache blanc grapes

by

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La composition en procyanidines des grappes de Chardonnay, Mauzac et Grenache blanc

Résumé: Procyanidines dimères galloylées et non-galloylées, et trimères sont dosées dans trois cépages blancs: Chardonnay, Mauzac et Grenache blanc. Les procyanidines sont extraites des différentes parties de la grappe (pépins, rafles, pellicules et pulpe), puis quantifiées par CLHP. La procyanidine B_1 est la plus abondante dans les rafles et pellicules, tandis que la procyanidine B_2 est la plus abondante dans les pépins.

Key words: procyanidin, polyphenol, analysis, bunch, berry skin, seed, rachis, white variety of vine.

Introduction

The procyanidins are a class of natural products which arise from flavan-3-ols and exist in a range of forms (dimers, trimers, tetramers, pentamers and polymers).

Grape and wine procyanidins are of undoubted importance and they contribute to the organoleptic properties of wine (TIMBERLAKE and BRIDLE 1976), oxidation reactions (SIMPSON 1982; OSZMIANSKI *et al.* 1985; CHEYNIER *et al.* 1988; LEE and JAWORSKI 1988; CHEYNIER and RICARDO-DA-SILVA 1991), haze formation and interactions with proteins (OH *et al.* 1985; YOKOTSUKA and SINGLETON 1987; POWERS *et al.* 1988; JOUVE *et al.* 1989; RICARDO-DA-SILVA *et al.* 1991 a), ageing behaviour of wines (HASLAM 1980) and physiological effects (LAPARRA *et al.* 1978; MASQUELIER 1988; RICARDO-DA-SILVA *et al.* 1991 b).

Different procyanidins have been isolated and identified in grapes: procyanidins B_1, B_2, B_3 and B_4 (WEINGES and PIRETTI 1971; PIRETTI *et al.* 1976; CZOCHANSKA *et al.* 1979; LEA *et al.* 1979; HASLAM 1980; BOURZEIX *et al.* 1986; ROMEYER *et al.* 1986; LEE and JAWORSKI 1987), procyanidin B_7 (BOUKHARTA 1988; RICARDO-DA-SILVA *et al.* 1991 c), procyanidin B_5, B_6 and B_8 (RICARDO-DA-SILVA *et al.* 1991 c), procyanidin trimer C_1 (LEA *et al.* 1979; BOUKHARTA 1988; OSZMIANSKI and SAPIS 1989; RICARDO-DA-SILVA *et al.* 1991 c), procyanidin trimer C_2 (ROMEYER *et al.* 1986), procyanidin trimer: epicatechin-(4β→8)-epicatechin-(4β→8)-catechin (LEA *et al.* 1979; RICARDO-DA-SILVA *et al.* 1991 c). Recently four trimeric procyanidins with a C_4-C_6 linkage between monomer units have been isolated and identified in grape seeds (RICARDO-DA-SILVA *et al.* 1991 c). Many procyanidins in natural products are esterified with gallic acid. Epicatechin 3-O-gallate (SU and SINGLETON 1969; CZOCHANSKA *et al.* 1979; BOUKHARTA *et al.* 1988; OSZMIANSKI and SAPIS 1989), procyanidin B_2 3'-O-gallate (CZOCHANSKA *et al.* 1979; BOUKHARTA *et al.* 1988; OSZMIANSKI and SAPIS 1989; RICARDO-DA-SILVA *et al.* 1991 c), (+)-catechin-gallate and catechin-catechin-gallate (LEE and JAWORSKI 1990), procyanidin B_1 3-O-gallate, B_2 3-O-gallate, B_4 3'-O-gallate, B_2 3,3'di-O-gallate and epicatechin-(4β→8)-epicatechin 3-O-gallate-(4β→8)-catechin (RICARDO-DA-SILVA *et al.* 1991 c) are also present in grapes.

Various authors have proposed analytical methods for the quantitative determination of grape and wine procyanidins (LEA 1980; SALAGOITY-AUGUSTE and BERTRAND 1984; BOURZEIX *et al.* 1986; JAWORSKI and LEE 1987; LUNTE *et al.* 1988; REVILLA *et al.* 1989; RICARDO-DA-SILVA *et al.* 1990). However, few studies concerning white grape oligomeric procyanidin composition were performed (JOSLYN and DITTMAR 1967; LEA *et al.* 1979; BOURZEIX *et al.* 1986; ROMEYER *et al.* 1986; LEE and JAWORSKI 1987, 1989; KOVAC *et al.* 1990).

The main objective of the present study was to determine the major procyanidin composition of stems, skins and seeds of three white grape varieties (Chardonnay, Mauzac and Grenache blanc). To our knowledge, this is the first time that procyanidins: B_1 3-O-gallate, B_2 3-O-gallate, B_2 3'-O-gallate, trimer C_1 and trimer 2 (epicatechin-(4β→8)-epicatechin-(4β→8)-catechin), are quantified in grapes.

Materials and methods

1. Grapes

Chardonnay and Mauzac grapes were performed from a vineyard in Limoux (South of France) and Grenache blanc grapes from Colombiers, also in the South of France. Chardonnay and Mauzac grapes were hand-harvested in 1988 and Grenache blanc grapes in 1989, all at commercial maturity (22.8, 19.0 and 23.1 °Brix, respectively).

2. Procyanidin standards

All procyanidins were obtained from a grape seed extract prepared as described by BOURZEIX *et al.* (1986) and identified following the procedure described by RICARDO-DA-SILVA *et al.* (1991 c) and RIGAUD *et al.* (1991).

Each procyanidin was isolated by means of liquid chromatography over Fractogel TSK HW-40 (s) (Merck, Darmstadt, F.R.G.) using methanol as the eluant, followed by HPLC at the semi-preparative scale on a Spherisorb ODS-2 (5 µm packing, 250 mm × 8 mm i.d.) column, protected with a guard column of the same material (Knauer, Bad Homburg, F.R.G.) and heated at 41 °C.

The HPLC elution conditions and other details were described in a previous paper (RICARDO-DA-SILVA *et al.* 1991 c).

3. Extraction

Details regarding extraction and obtention of methanol extracts of grape skins, seeds, pulps and stems were described by BOURZEIX *et al.* (1986).

4. Sample purification

The use of polyamide chromatography with three successive elutions (1st: water (pH 7.0); 2nd: acetonitrile/water (30 : 70 v/v); 3rd: acetone/water (75 : 25 v/v)) to eliminate HPLC interfering compounds such as phenolic acids and to separate catechins from procyanidins allowed us to obtain a pure procyanidin fraction. This fraction was further analysed by reversed phase HPLC (Fig.).

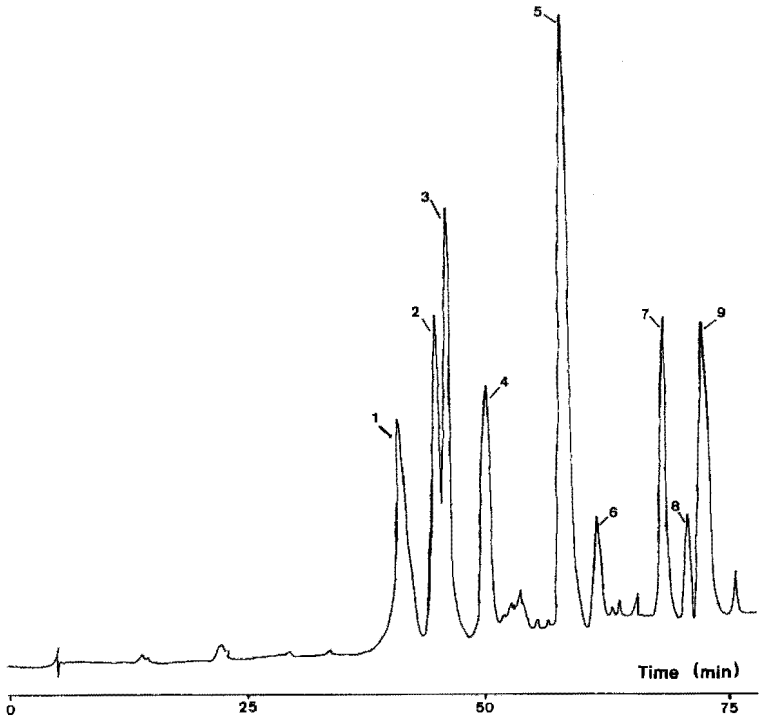
More details of the purification were given in a previous study (RICARDO-DA-SILVA *et al.* 1990).

5. HPLC analysis

Samples were filtered through 0.45 µm membrane filters prior to injection (injection volume: 50 µl) onto the HPLC column. The HPLC apparatus was a Millipore-Waters (Milford, CT, USA) system, equipped with 6000A and EM 45 pumps, UV-V detector with four simultaneous detection channels (model 490) and connected to a data module (model 730) integrator. The detection wavelength was 280 nm. A second channel set at 313 nm was used to control the presence of phenolic acids. The column was reversed-phase Nucleosil C-18 from SFCC (Neuilly-Plaisance, France) (5 µm packing, 250 mm × 4.6 mm i.d.), protected with a guard column of the same material. The elution conditions were as follows: solvent A, acetic acid/bidistilled water (10 : 90 v/v) and solvent B, bidistilled water. A linear gradient was run from 10 vol. A + 90 vol. B to 82 vol. A + 18 vol. B during 47 min and then to pure A during 8 min. The flow rate was 0.8 ml · min^{-1} and column temperature was 32 °C.

Finally the column was washed isocratically with a mixture of solvents (methanol/bidistilled water/acetic acid, 50 : 35 : 15 v/v) at the same flow rate as before, followed by reconditioning of the column.

The response factors were determined for each procyanidin by injection of known dilutions.



Example of the procyanidin fraction (from Chardonnay grape seed extract). 1. procyanidin B_3; 2. procyanidin B_1; 3. procyanidin trimer 2; 4. procyanidin B_4; 5. procyanidin B_2; 6. procyanidin B_2 3-O-gallate; 7. procyanidin B_2 3-O-gallate; 8. procyanidin B_1 3-O-gallate; 9. procyanidin C_1.

Exemple d'une fraction procyanidolique (extrait des pépins de la variété Chardonnay).

Results and discussion

The procyanidin composition of Chardonnay, Mauzac and Grenache blanc grapes is presented in Tables 1, 2 and 3. For all grape varieties studied, procyanidin B_2 was the major component in seeds, and procyanidin B_1 in stems and skins.

Procyanidins B_2 and B_4 were practically present only in grape seeds.

Significant differences were observed in procyanidin profiles of grape seeds, stems or skins for all white grape varieties studied.

Pulp of all grape varieties was devoid of procyanidins.

In all different parts of the grape cluster, procyanidin gallates were present in much lower concentrations than procyanidins non galloylated.

Trimeric procyanidins with C_4-C_8 linkages (trimers C_1 and 2) between monomer units were also present in important amounts compared to dimeric procyanidins. In Chardonnay, Mauzac and Grenache blanc grapes, procyanidin trimer 2 was the second major important procyanidin quantified in stems and skins.

Mauzac grapes presented the highest contents of procyanidins in the entire grape cluster, followed by Chardonnay. Grenache blanc exhibited a very low procyanidin concentration compared to Mauzac and Chardonnay. For example, procyanidin B_2 content in Mauzac grape seeds was almost 6-fold higher than in Grenache blanc.

However, Grenache blanc skins contained almost the same procyanidin concentrations as Chardonnay and Mauzac skins, meaning that, if pomace contact occurs during wine making, the release of procyanidins from the skins, which seems to be the major source of flavans in wines (CHEYNIER *et al.* 1989), may induce the same result in wines, made separately from all three white grapes.

Table 1

Procyanidin composition (on a fresh wt base) of Chardonnay grapes

Composition en procyanidines (référée au matériel frais) de la grappe de Chardonnay

Procyanidins	Seeds			Stems			Skins			Total grape cluster
	(mg/kg clusters)		(mg/g seeds)	(mg/kg clusters)		(mg/g stems)	(mg/kg clusters)		(mg/g skins)	(mg/kg)
Procyanidin B_1	75.6	33[1])	1.63	112.5	48[1])	2.73	44.5	19[1])	0.27	232.6
Procyanidin B_2	141.4	92	3.05	6.6	4	0.16	6.5	4	0.04	154.5
Procyanidin B_3	65.9	66	1.42	29.0	29	0.70	5.2	5	0.03	100.1
Procyanidin B_4	73.1	96	1.58	1.1	2	0.03	1.7	2	0.01	75.9
Procyanidin B_1 3-O-gallate	6.5	57	0.14	3.7	33	0.09	1.2	10	0.01	11.4
Procyanidin B_2 3-O-gallate	8.2	55	0.18	5.2	35	0.05	1.4	10	0.01	14.8
Procyanidin B_2 3'-O-gallate	13.6	72	0.29	4.2	22	0.10	1.1	6	0.01	18.9
Procyanidin C_1	85.9	74	1.85	24.5	21	0.60	6.1	5	0.04	116.5
Procyanidin Trimer 2	91.6	51	1.98	63.6	35	1.54	25.6	14	0.16	180.8

[1]) Distribution within grape clusters in %.

Table 2

Procyanidin composition (on a fresh wt base) of Mauzac grapes

Composition en procyanidines (référée au matériel frais) de la grappe de Mauzac

Procyanidins	Seeds			Stems			Skins			Total grape cluster
	(mg/kg clusters)		(mg/g seeds)	(mg/kg clusters)		(mg/g stems)	(mg/kg clusters)		(mg/g skins)	(mg/kg)
Procyanidin B_1	103.7	39[1])	1.73	102.2	39[1])	2.09	56.7	22[1])	0.35	262.6
Procyanidin B_2	174.1	94	2.91	7.4	4	0.15	3.7	2	0.02	185.2
Procyanidin B_3	136.4	75	2.28	32.3	18	0.66	12.2	7	0.08	180.9
Procyanidin B_4	118.2	95	1.97	4.4	4	0.09	1.6	1	0.01	124.2
Procyanidin B_1 3-O-gallate	8.3	61	0.14	3.2	24	0.07	2.0	15	0.01	13.5
Procyanidin B_2 3-O-gallate	20.4	88	0.34	2.2	9	0.04	0.6	3	0.004	23.2
Procyanidin B_2 3′-O-gallate	25.8	95	0.43	1.0	4	0.02	0.4	1	0.003	27.2
Procyanidin C_1	116.7	77	1.95	26.6	17	0.55	8.9	6	0.06	152.2
Procyanidin Trimer 2	97.1	54	1.62	44.1	24	1.90	39.0	22	0.24	180.2

[1]) Distribution within grape clusters in %.

Table 3

Procyanidin composition (on a fresh wt base) of Grenache blanc grapes

Composition en procyanidines (référée au matériel frais) de la grappe de Grenache blanc

Procyanidins	Seeds			Stems			Skins			Total grape cluster
	(mg/kg clusters)		(mg/g seeds)	(mg/kg clusters)		(mg/g stems)	(mg/kg clusters)		(mg/g skins)	(mg/kg)
Procyanidin B_1	10.9	13[1])	0.19	39.9	45[1])	1.14	37.1	42[1])	0.18	87.9
Procyanidin B_2	31.7	79	0.55	2.7	6	0.08	5.9	15	0.03	40.3
Procyanidin B_3	12.0	35	0.21	11.5	34	0.33	10.8	31	0.05	34.3
Procyanidin B_4	16.6	80	0.29	1.2	6	0.03	2.9	14	0.01	20.7
Procyanidin B_1 3-O-gallate	0.8	24	0.01	1.2	36	0.03	1.3	40	0.006	3.3
Procyanidin B_2 3-O-gallate	2.5	63	0.05	0.6	15	0.01	0.9	22	0.004	4.0
Procyanidin B_2 3′-O-gallate	1.5	36	0.03	1.4	33	0.04	1.3	31	0.006	4.2
Procyanidin C_1	16.8	41	0.29	12.6	31	0.36	11.1	28	0.05	40.5
Procyanidin Trimer 2	11.3	22	0.20	17.7	34	0.51	23.6	44	0.07	52.6

[1]) Distribution within grape clusters in %.

Note that Chardonnay, grown in Limoux, France (88 vintage) presented much higher contents of procyanidins in all parts of the grape cluster than Chardonnay grown in Novi Sad (87 vintage) studied by KOVAC *et al.* (1990).

In contrast, Grenache blanc grapes studied by BOURZEIX *et al.* (1986) presented almost the same procyanidin concentration and profile as our Grenache blanc, grown in the same region (Pech Rouge-Narbonne) and harvested in 1989.

LEE and JAWORSKI (1987, 1989) found in 21 grape cultivars grown in New York that catechin-gallate and catechin-catechin-gallate were the major flavanols in berry skins and flesh. However, we have not been able to detect these compounds in any of the white grapes studied, nor as minor grape seed procyanidins (RICARDO-DA-SILVA *et al.* 1991 c).

From an industrial point of view, both seeds and stems seem to be very interesting sources for the extraction of procyanidins for practical uses.

Summary

Dimer procyanidins, galloylated or not, and trimers were analysed in three white grape varieties: Chardonnay, Mauzac and Grenache blanc. Procyanidins were extracted from the different components of the grape cluster (seeds, stems, skins and pulps) and then quantified by HPLC. Procyanidin B_1 is the major component in stems and skins while procyanidin B_2 is the major component in seeds.

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