Effect of Several Enological Practices on the Content of Catechins and Proanthocyanidins of Red Wines

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Several microvinification experiments were conducted with Vranac red grapes to understand the effect of several enological techniques on the content of (+)-catechin, (−)-epicatechin, and procyanidin B-type dimers and trimer C-1 in wines, which were quantitated by HPLC. Both the length of maceration time and the presence of a high quantity of pomace, seeds, and stems in contact with the must during fermentation led to wines with a higher content of catechins and proanthocyanidins. The addition of a reasonable quantity of supplementary seeds effects catechin and simple lower procyanidin oligomer concentrations higher than those present in wines made by classical production methods.

INTRODUCTION

The study of catechins and proanthocyanidins in grapes and wines has become a topic of interest due to their positive role in human nutrition as captors of free radicals (Uchida et al., 1987; Masquelier, 1988) and in relation to certain vascular diseases (Masquelier, 1987). Grape seeds and cluster stems are richer in catechins and proanthocyanidins than grape skins and pulps, in both red and white cultivars (Lea et al., 1979; Bourzeix et al., 1986; Lee and Jaworski, 1989; Kovac et al., 1990).

The catechins and proanthocyanidins contained in grapes are extracted into wine during the fermentation and maceration of grape clusters. The enological literature (Singleton and Esau, 1969; Peynaud, 1971; Paronetto, 1977) does not give enough information about the exact role of the different parts of grape clusters (stems, skins, pulp, and seeds) in the content of catechins and proanthocyanidins of red wines. Therefore, we have studied the influence of several winemaking techniques (time of maceration, destemming of grape clusters, and addition of a supplementary quantity of seeds to musts) on the content of catechins and proanthocyanidins in red wine to obtain a better understanding of the factors which may affect the diffusion of these substances from whole grape berries and rachis (whole clusters) to wine and also to establish the effect of such techniques on the physicochemical and organoleptic characteristics of wines.

MATERIALS AND METHODS

Winemaking. Early harvested red grapes of the cultivar Vranac, native of Montenegro, were collected in 1989 in the vineyards of Grokombinat "13. Jul", Titograd, Republic of Montenegro, Yugoslavia. Thirteen different microvinification experiments were conducted. Characteristics of these experiments are given in Table I. The experiments were carried out with 70 kg of grapes, which were crushed and, in some cases, destemmed. Then, the must was treated with SO_2 (100 mg/kg of grapes). The fermentation started after sowing with yeast (2% dry wt/wt), and the mash or cap was immersed twice a day. The fermentation temperature was 30 °C. When the maceration was completed, the must was pressed (about 8 bar), resulting in 38 L of wine. In some cases the fermentation continued after pressing. At the end of the spontaneous sedimentation, the wines were racked, treated with SO_2 (50 mg/L of wine), and then stored at 14 °C prior to analysis.

Table I. Some Characteristics of Microvinification Experiments

expt	state of clusters	time of maceration, days	seeds added[a]	total acidity,[b] g/L	reducing sugars,[c] %
V-1	whole cluster	2		6.1	19.4
V-2	whole cluster	3		6.5	19.6
V-3	whole cluster	4		6.1	18.8
V-4	whole cluster	5		6.2	19.1
V-5	whole cluster	6		5.8	19.1
V-6	whole cluster	7		6.1	18.8
V-7	whole cluster	14		5.7	19.4
V-8	berries only	7		5.8	19.6
V-9	berries only	14		5.8	19.6
V-10	berries only	7	60	5.4	19.6
V-11	berries only	14	60	6.1	20.2
V-12	berries only	7	120	5.5	19.9
V-13	berries only	14	120	6.1	19.4

[a] Wet weight/kg of grapes. [b] Expressed as tartaric acid. [c] Each must was chaptalized (except must V-11) to adjust its reducing sugars content to that of must V-11.

Conventional Analysis. Total acidity and reducing sugar content of musts were determined according to official OIV methods (Office International de la Vigne et du Vin, 1990). In young wines, the following parameters were determined by standard methods: color intensity, expressed as $E_{420}^{1cm} + E_{520}^{1cm} + E_{620}^{1cm}$ (Glories, 1984); tint, expressed as $E_{420}^{1cm}/E_{520}^{1cm}$ (Sudraud, 1958), free anthocyanins (Ribereau-Gayon and Stonestreet, 1965); Folin–Ciocalteu index (Office International de la Vigne et du Vin, 1990; based on Singleton and Rossi, 1965), and coloring matter qualitative estimation (Bourzeix and Heredia, 1985), which allows for an estimation of the relative amount of red monomers, expressed as $[(E_{538}^{0.1\%HCl} \times 100)/(E_{538}^{0.1\%HCl} + E_{525}^{50\%HCOOH} + E_{525}^{HCOOH})]$; red polymers, as $[(E_{525}^{50\%HCOOH} \times 100)/(E_{525}^{0.1\%HCl} + E_{525}^{50\%HCOOH} + E_{525}^{HCOOH})]$, and brown polymers, as $[(E_{525}^{HCOOH} \times 100)/(E_{538}^{0.1\%HCl} + E_{525}^{50\%HCOOH} + E_{525}^{HCOOH})]$, after the fractionation of wine phenolics on a Polyclar AT column with three different acidic solvents (0.1% HCl, 50% formic acid, and formic acid). The absorbance at 280 nm of wine samples diluted 1:50 was multiplied by 50 to obtain the relative ultraviolet absorption.

HPLC Separation of Catechins and Proanthocyanidins. The analysis of catechins and proanthocyanidins was carried out in the ethyl acetate fraction obtained by using the wine phenolics fractionation procedure described previously (Revilla et al., 1990).

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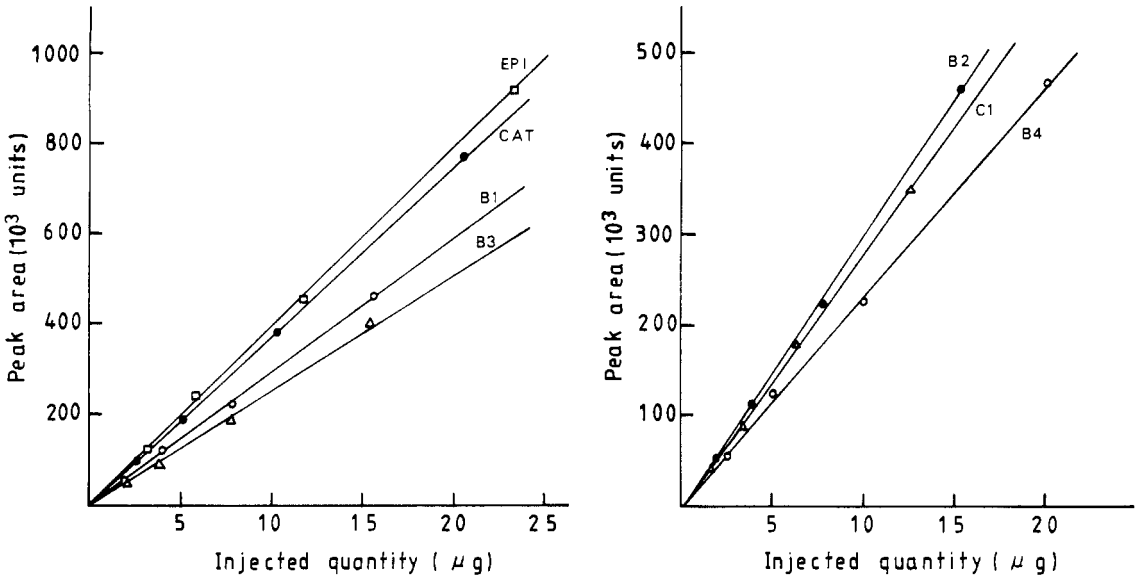

Figure 1. Calibration lines for several catechins and proanthocyanidins. CAT, catechin; EPI, epicatechin; B1, procyanidin B_1; B2, procyanidin B_2; B3, procyanidin B_3; B4, procyanidin B_4; C1, procyanidin C_1.

Table II. Some Analytical Data of Wines Made with Entire Grape Clusters and with Different Lengths of Maceration[a]

	exptl						
	V-1	V-2	V-3	V-4	V-5	V-6	V-7
maceration time, days	2	3	4	5	6	7	14
color intensity, $E^{1cm}_{420}+E^{1cm}_{520}+E^{1cm}_{620}$	2.034	2.269	2.348	2.299	2.623	2.427	2.636
tint, $E^{1cm}_{420}/E^{1cm}_{520}$	0.54	0.55	0.54	0.56	0.52	0.54	0.55
Folin–Ciocalteu index	26.0	34.4	35.9	36.3	37.8	44.2	43.2
relative UV absorption (280 nm)	57.5	70.5	72.0	72.5	76.0	81.2	81.5
free anthocyanins, mg/L	530	680	560	400	340	600	270
red monomers,[b] %	43.0	50.6	47.1	33.0	25.7	48.3	20.6
red polymers,[c] %	40.7	34.7	37.0	43.2	46.3	36.2	48.0
brown polymers,[d] %	16.3	13.7	15.9	23.8	28.0	15.5	31.4
total catechins and proanthocyanidins (HPLC), mg/L	196	254	275	313	352	458	532
(+)-catechin, mg/L	55	78	80	84	88	130	137
(−)-epicatechin, mg/L	25	36	38	46	56	65	76
procyanidin B_1, mg/L	73	73	83	85	97	115	137
procyanidin B_2, mg/L	18	31	33	45	54	70	78
procyanidin B_3, mg/L	10	14	16	16	17	27	33
procyanidin B_4, mg/L	10	12	14	20	22	29	43
procyanidin C_1, mg/L	5	10	11	17	18	22	28

[a] For further details, see Table I. [b] $(E^{0.1\%HCl}_{538} \times 100)/(E^{0.1\%HCl}_{538} + E^{50\%HCOOH}_{525} + E^{HCOOH}_{525})$. [c] $(E^{50\%HCOOH}_{525} \times 100)/(E^{0.1\%HCl}_{538} + E^{50\%HCOOH}_{525} + E^{HCOOH}_{525})$. [d] $(E^{HCOOH}_{525} \times 100)/(E^{0.1\%HCl}_{538} + E^{50\%HCOOH}_{525} + E^{HCOOH}_{525})$.

This involves the use of Sep-Pak C_{18} cartridges (Waters Associates, catalog no. 51910). This fraction, dried under vacuum at 30 °C and then dissolved with 500 μL of 50% methanol, was assayed by HPLC, using a Waters Associates chromatograph equipped with M6000A and M45 pumps, a U6K universal injector, a 490 programmable multiwavelength visible–ultraviolet detector, a 730 data module, and a 760 system controller. The HPLC separation was carried out by injecting 50 μL on a 250 mm × 4.6 mm Nucleosil 5-μm C_{18} column (SFCC, France) at 32 °C, using a linear gradient with a flow rate of 0.8 mL/min. Mobile phase A was 10% acetic acid, and mobile phase B was deionized, distilled water. The linear gradient was started with 10% A to 82% A in 47 min and continued with 82% A to 100% A in 8 min. It was then run with 100% A for 10 min and then back to initial conditions after the column was washed with methanol/acetic acid/water (50:15:35) for 45 min. The effluent was monitored at 280 and 313 nm, with a sensitivity of 0.1 AUFS. The HPLC quantitation of catechins and proanthocyanidins was achieved by an external standard procedure, using multiple-point calibration (see Figure 1).

Table III. Some Analytical Data of Wines Related to Destemming Experiments[a]

	expt			
	V-6	V-7	V-8	V-9
maceration time, days	7	14	7	14
stems	yes	yes	no	no
color intensity, $E^{1cm}_{420}+E^{1cm}_{520}+E^{1cm}_{620}$	2.427	2.636	2.448	2.481
tint, $E^{1cm}_{420}/E^{1cm}_{520}$	0.54	0.55	0.55	0.56
Folin–Ciocalteu index	44.2	43.2	33.2	35.3
relative UV absorption (280 nm)	81.2	81.5	71.5	72.8
free anthocyanins, mg/L	600	270	520	460
red monomers,[b] %	48.3	20.6	44.5	37.8
red polymers,[c] %	36.2	48.0	40.3	45.0
brown polymers,[d] %	15.5	31.4	15.2	17.2
total catechins and procyanidins (HPLC), mg/L	458	532	357	393
(+)-catechin, mg/L	130	137	99	102
(−)-epicatechin, mg/L	65	76	63	74
procyanidin B_1, mg/L	115	137	59	60
procyanidin B_2, mg/L	70	78	68	73
procyanidin B_3, mg/L	27	33	19	24
procyanidin B_4, mg/L	29	43	29	36
procyanidin C_1, mg/L	22	28	20	24

[a] Wines V-6 and V-7 were made with entire cluters and wines V-8 and V-9 with destemmed grapes. Maceration lasted 7 days for wines V-6 and V-8 and 14 days for wines V-7 and V-9. [b] $(E^{0.1\%HCl}_{538} \times 100)/(E^{0.1\%HCl}_{538} + E^{50\%HCOOH}_{525} + E^{HCOOH}_{525})$. [c] $(E^{50\%HCOOH}_{525} \times 100)/(E^{0.1\%HCl}_{538} + E^{50\%HCOOH}_{525} + E^{HCOOH}_{525})$. [d] $(E^{HCOOH}_{525} \times 100)/(E^{0.1\%HCl}_{538} + E^{50\%HCOOH}_{525} + E^{HCOOH}_{525})$.

Standards. Catechin and epicatechin were purchased from Fluka AG (Switzerland). Standards of procyanidins B_1, B_2, B_3, B_4, and C_1 were kindly supplied by Dr. M. Moutounet, Institut des Produits de la Vigne, Montpellier, France.

RESULTS AND DISCUSSION

Effect of the Time of Maceration. Results for maceration of whole grape clusters after 2, 3, 4, 5, 6, 7, and 14 days, respectively, are summarized in Table II. There was a progressive increase in the content of catechins and proanthocyanidins with the length of maceration time. The sum of catechins and procyanidins increased 336 mg/L or 230% from 2 to 7 days of maceration. There are significant differences for individual catechins and procyanidins (Figure 2). Concentrations of (−)-epicatechin

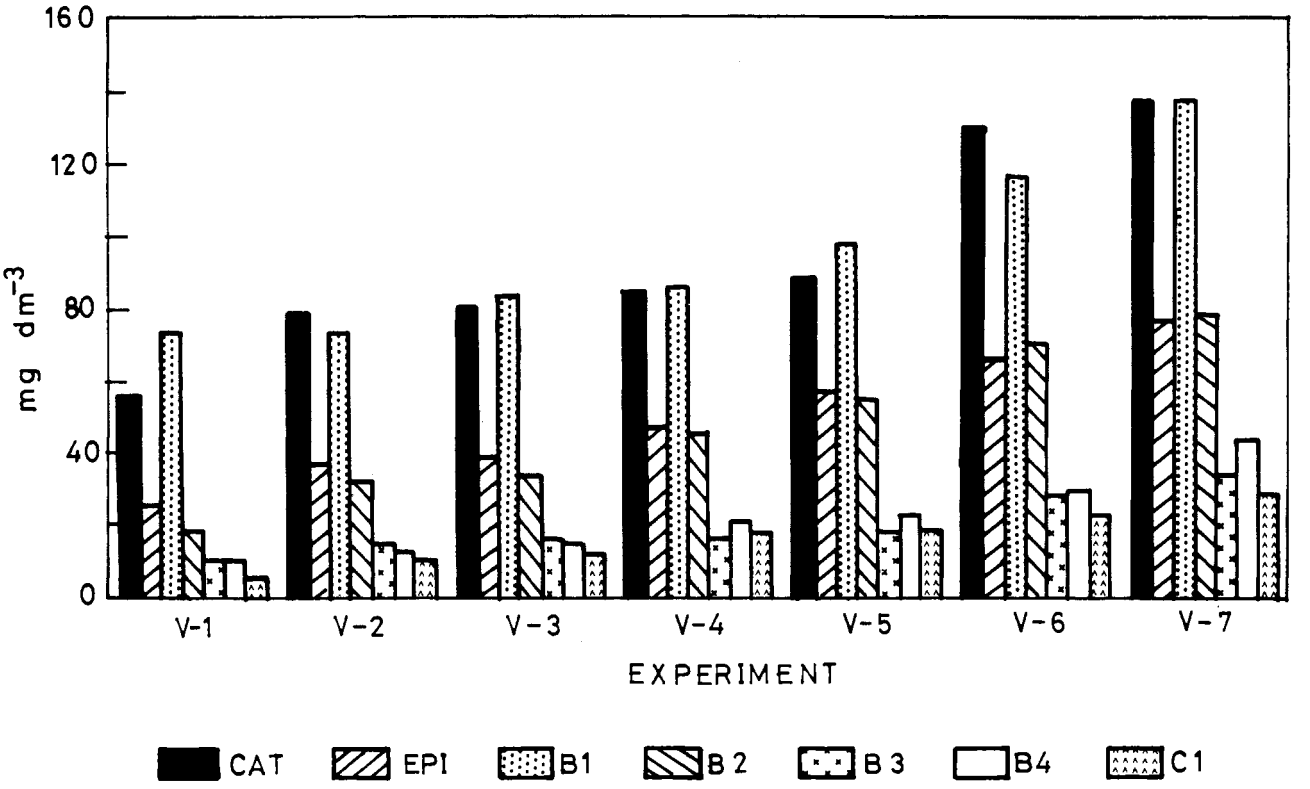

Figure 2. Content of several catechins and proanthocyanidins (mg dm^{-3}), as determined by HPLC, in the wines obtained with different lengths of maceration time, using entire grape clusters (wines V-1–7). For key to substances, see Figure 1.

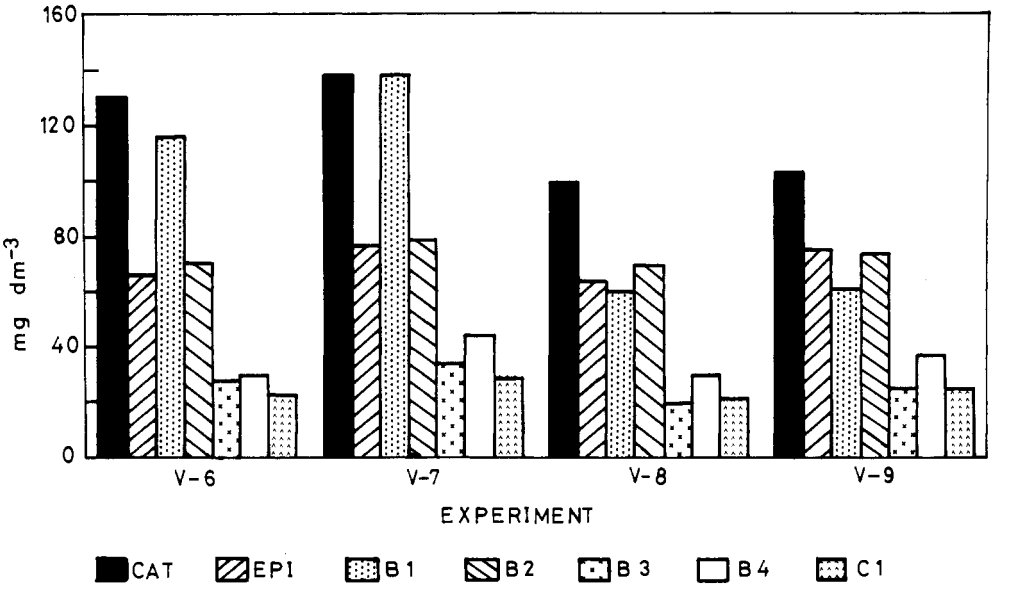

Figure 3. Content of several catechins and proanthocyanidins (mg dm^{-3}), as determined by HPLC, in the wines related to destemming experiments. Wines V-6 and V-7 were made with entire clusters and wines V-8 and V-9 with destemmed clusters. Maceration lasted 7 days for wines V-6 and V-8 and 14 days for the others. For key to substances, see Figure 1.

and procyanidins B_2, B_3, B_4, and C_1 increased, but concentrations of (+)-catechin and procyanidin B_1 remained relatively stable from the second to the sixth day of maceration. Concentrations of catechins and procyanidins increased 20% from 7 to 14 days of maceration (Table III). The extraction of catechins and proanthocyanidins during the maceration of pomace seems to be quite different from that of anthocyanins, whose content increases followed by a decrease after several days of maceration (Ribéreau-Gayon, 1982). Other colorimetric data did not change significantly. Color intensity ($E_{420}^{1cm} + E_{520}^{1cm} + E_{620}^{1cm}$) and tint ($E_{420}^{1cm}/E_{520}^{1cm}$) were unchanged. The percentage of red monomers appears to decrease during maceration; on the other hand, the percentages of red polymers and brown polymers increased. Total anthocyanins showed an erratic behavior. The concentration of total phenols estimated by the Folin–Ciocalteu index and the UV absorbance increased between 2 and 7 days of maceration. A preliminary sensory analysis has shown better quality ratings for wines which were in contact with pomace for 6 and 7 days.

Influence of Destemming. Experiments V-8 and V-9 were carried out to show that stems affect the phenolic composition of wines. Whole grape clusters (skins, pulps, and seeds) were together in the must. Extraction after 7 and 14 days was compared with wines made with and

Table IV. Some Analytical Data of Wines Made with Destemmed Clusters[a]

	expt					
	V-8	V-10	V-12	V-9	V-11	V-13
maceration time, days	7	7	7	14	14	14
stems	no	no	no	no	no	no
seeds/kg of grapes, g	0	60	120	0	60	120
color intensity, $E^{1cm}_{420}+E^{1cm}_{520}+E^{1cm}_{620}$	2.448	2.799	2.836	2.481	2.868	2.724
tint, $E^{1cm}_{420}/E^{1cm}_{520}$	0.55	0.58	0.60	0.56	0.60	0.64
Folin–Ciocalteu index	33.2	57.7	70.5	35.3	62.2	77.5
relative UV absorption (280 nm)	71.5	93.3	97.8	72.8	94.8	99.8
free anthocyanins, mg/L	520	550	550	460	520	520
red monomers,[b] %	44.5	37.9	32.8	37.8	32.5	34.8
red polymers,[c] %	40.3	40.1	40.9	45.0	41.5	37.5
brown polymers,[d] %	15.2	22.0	26.3	17.2	26.0	27.7
total catechins and procyanidins (HPLC), mg/L	357	941	1386	393	1142	1786
(+)-catechin, mg/L	99	235	334	102	275	423
(−)-epicatechin, mg/L	63	155	212	74	186	255
procyanidin B_1, mg/L	59	175	248	60	207	270
procyanidin B_2, mg/L	68	166	274	73	227	386
procyanidin B_3, mg/L	19	64	79	24	68	124
procyanidin B_4, mg/L	29	78	123	36	89	171
procyanidin C_1, mg/L	20	68	118	24	90	157

[a] Wines V-8 and V-9 were made without addition of seeds. The other wines are made with addition of seeds (60 g/kg of grapes for V-10 and V-11; 120 g/kg of grapes for V-12 and V-13). Maceration lasted 7 days for wines V-8, V-10, and V-12 and 14 days for the others. [b] $(E^{0.1\%HCl}_{538} \times 100)/(E^{0.1\%HCl}_{538} + E^{50\%HCOOH}_{525} + E^{HCOOH}_{525})$. [c] $(E^{50\%HCl}_{525} \times 100)/(E^{0.1\%HCl}_{538} + E^{50\%HCOOH}_{525} + E^{HCOOH}_{525})$. [d] $(E^{HCOOH}_{525} \times 100)/(E^{0.1\%HCl}_{538} + E^{50\%HCOOH}_{525} + E^{HCOOH}_{525})$.

without stems (Table III). Results show that stems contribute to higher concentration of catechins and proanthocyanidins in wines. The increases are quite similar in both pairs of experiments. Figure 3 shows concentrations of each catechin and procyanidin in wines V-6–9. As shown, some catechins and procyanidins [procyanidin B_1, procyanidin B_3, and (+)-catechin] increase in concentration when the maceration is carried out with whole clusters. This demonstrates that these three compounds are abundant in grape stems, as previously reported for seeds (Bourzeix et al., 1986). Wines made in the presence of stems have relatively similar colorimetric analyses, e.g., color intensity, tint, red monomers, red polymers, and brown polymers. The colorimetric results change dramatically in the case of wines macerated for 14 days. A similar situation may be observed for total anthocyanins. Finally, the presence of stems during maceration leads to higher values of total phenols estimated by the Folin–Ciocalteu assay and to a higher UV absorption in both cases, with similar increases. HPLC data clearly point out that the destemming of grape clusters prior to the fermentation of must produces wines with lower contents of catechins and procyanidins that those made with entire grape clusters.

Influence of the Addition of Seeds. Seeds are about 5–7% of wet weight in Vranac grapes, according to the data obtained in previous vintages. One kilogram of grapes contains approximately 60 g of seeds. Experiments to evaluate the effect of adding seeds were carried out by adding 0, 60, and 120 g of seeds (V-8, V-10, and V-12, respectively) for each kilogram of grapes and 7 days of pomace contact. Experiments show that doubling and tripling seed content per kilogram of grapes results in an average 606 mg/L increase in catechins and procyanidins per 60 g/kg addition of seeds (Table IV). Similarly, adding 0, 60, and 120 g of seeds/kg of grapes (V-9, V-11, and V-13, respectively) and 14 days of pomace contact gave similar results. Analytical data are summarized in Table IV. The presence of additional seeds increases the total content of catechins and proanthocyanidins in wines from 544 to 749 mg/L. The increases, as a percentage in relation to concentration of catechins and proanthocyanidins in wines made without addition of supplementary seeds, are quite similar for 7 and 14 days of maceration. The enrichment, as a percentage, is higher in the minor components (procyanidin B_3, procyanidin B_4, and procyanidin C_1) than in the others. This effect may be due to the different contents of several catechins and procyanidins in seeds in relation to other parts of the rachis (Bourzeix et al., 1986). This fact can be easily observed in Figure 4, which displays the content of catechins and procyanidins in wines

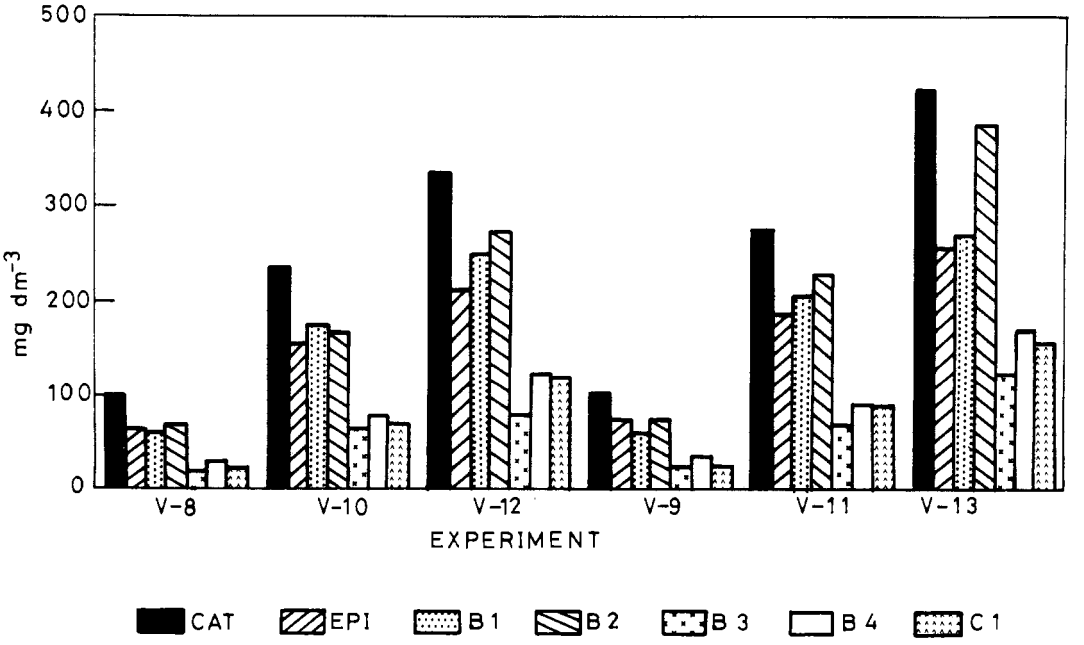

Figure 4. Content of several catechins and proanthocyanidins (mg dm^{-3}), as determined by HPLC, in the wines related to experiments with addition of seeds. Wines V-8 and V-9 were made without addition of seeds. The others were made with supplementary seeds (60 g/kg of grapes for V-10 and V-11; 120 g/kg of grapes for V-12 and V-13). Maceration lasted 7 days for V-8, V-10, and V-12 and 14 days for the others. For key to substances, see Figure 1.

"enriched" by the addition of seeds to the must (V-10, V-12, V-11, and V-13) and in control wines made under the same conditions but without addition of seeds (V-8 and V-9). The addition of seeds also gives higher color intensity. The percentage of brown polymers increases with the addition of seeds; on the other hand, the percentages of red monomers and red polymers decrease. The concentration of total anthocyanins is higher in "seed-enriched" wines, and this shows that the increase in the content of catechins and procyanidins caused by the addition of seeds may play an important role in the stabilization of copigmentation of anthocyanins (Goto, 1987). This effect was reported in a preliminary work (Kovac et al., 1991). The concentration of total phenols estimated by the Folin–Ciocalteu index and the relative UV absorption increase with the addition of seeds. Finally, it must be pointed out that a preliminary sensory evaluation of all of these young wines, prior to any stabilization or ageing process, has shown that a reasonable addition of seeds (about 60 g/kg of grapes) may be adequate to obtain wines with more pronounced characteristics and with more intense flavor and aroma than those of wines made without the addition of supplementary seeds.

Conclusions. The object of this research was to obtain red wines with a high content of catechins and proanthocyanidins, whose sensory properties may be similar to those of wines made by classic French-type vinification. The experiments using entire clusters of Vranac grapes have shown that there is a progressive increase in the content of catechins and proanthocyanidins with the length of maceration time, and wines that were in contact with pomace for 6 or 7 days show better sensory ratings than the others. Similarly, the presence of stems during fermentation gives wines with a higher content of catechins and proanthocyanidins. Several young wines made by adding a quantity of grape seeds, doubling or tripling the normal content of seeds in pomace, have shown higher contents of catechins and proanthocyanidins than those made under the same conditions but without the addition of seeds; a reasonable addition of seeds (about 60 g/kg of grapes) may give wines with more pronounced characteristics and with more intense flavor and aroma. In case, a number of new experiments must be carried out to evaluate the influence of stabilization and ageing processes in the sensory properties of Vranac wines made according to these procedures.

ACKNOWLEDGMENT

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Registry No. (+)-Catechin, 154-23-4; (−)-epicatechin, 490-46-0; procyanidin B_1, 20315-25-7; procyanidin B_2, 29106-49-8; procyanidin B_3, 23567-23-9; procyanidin B_4, 29106-51-2; procyanidin C_1, 37064-30-5.