Extraction of Hydroxycinnamoyltartaric Acids from Berries of Different Grape Varieties

Urška Vrhovšek[†]

HBLA&LA-Klosterneuburg, Wienerstrasse 74, A-3400 Klosterneuburg, Austria

The concentrations of the hydroxycinnamoyltartaric acids (caftaric, *cis*- and *trans*-coutaric, and fertaric acid) were determined in berries of six white grapevine cultivars and compared with their concentrations in oxidation-protected juices. The analyses were performed by direct injection in a reverse phase HPLC-DAD system. Differences among the cultivars in extraction yield appear to be independent of absolute amount. The extraction of fertaric acid was steadily the highest (average = 70.9%), followed by caftaric acid (average = 59.0%) and both *p*-coutaric isomers (average = 36.2% for *cis*- and 33.6% for *trans*-). Four different technologies of juice preparation were performed (in pilot-scale press) to verify the influence of technology on the extraction yield of hydroxycinnamoyltartaric acids and their enzymatic oxidation during the pressing. Losses during juice preparation depended strongly on pressing management. The lowest recovery of all examined compounds occurred in must obtained by whole cluster pressing. Overnight settling of the juice caused further oxidative losses only in the treatments in which the juice had been left on skins before pressing, irrespective of SO_2 addition.

Keywords: *White grape; hydroxycinnamoyltartaric acids; extraction yield; enzymatic oxidation*

INTRODUCTION

Hydroxycinnamates (HCA) are the most important group of nonflavonoid phenols in wine. The four most abundant ones are *trans*-caftaric, *cis*- and *trans*-coutaric, and *trans*-fertaric acids. In wine they are present also in the free form (*trans*-caffeic, *trans*-*p*-coumaric, and *trans*-ferulic acids). They are involved in the browning reactions of must and wine, are precursors of volatile phenols, and have antimicrobial and antioxidant activity.

After developing and validating an HPLC method for separation and quantification of each HCA (Vrhovsek et al., 1997), we determined the concentration of these compounds in Slovenian (Vrhovsek, 1998) and Austrian white wines (Vrhovsek et al., 1997). The differences among samples from different wineries were found to be important, highlighting the effect of wine-making technologies on HCA (Vrhovsek, 1998).

Not all technological factors influencing the concentration of hydroxycinnamates in white wines are known, indicating the need for relevant studies. Two such factors, grape cultivar and method of juice extraction, appear to be of special practical importance. Quantitative studies of the balance of each compound, that is, its origin, initial and final amounts, and conversion to other compounds appear necessary to understand and monitor the complex changes occurring in grapes and wine.

As far as we know, there are only a few studies that compare the balance of HCA during white wine vinification (Singleton, 1984; Somers et al., 1987; Betés-Saura et al., 1996) and no data about the extractability of each HCA from the berries during processing. Such data are necessary to link information on grape composition and results of wine-making.

In recent years several new technological options for making white wine have been proposed to oenologists. Skin contact, with or without addition of SO_2, has become a common practice in many wineries (Arnold and Noble, 1979; Test et al., 1986; Cheynier et al., 1989a) with the aim to improve the extraction of aroma components located in the skins and to increase the varietal character of the wines. Whole cluster pressing, as is the usual practice in the production of sparkling wine (Boulton et al., 1996), is a technology that is gaining increasing importance in making still white wines, especially in Germany and Austria (Schneider, 1992). It minimizes the time between berry breakage and juice separation and may thus provide juices with a much lower amount of phenols and lower levels of suspended solids (Seckler, 1996). It is interesting to verify how these different technological choices can affect the content of HCA of the must.

The experiments reported in this paper have been carried out over two years. During the 1995 vintage Pinot Blanc grape and the relevant juice produced at the laboratory under complete protection from oxidation were analyzed to estimate the yield of extraction of HCA without the interference of oxidation processes. Grapes from the same lot were processed in a pilot-scale press with four different technologies of must preparation, to produce must in which are measured the total HCA losses in real wine-making conditions. Comparison of the concentrations obtained in unoxidized juice and in musts allowed us to estimate the relative importance of losses due to incomplete extraction from grape and further oxidation.

To verify the varietal differences in the extractability of each HCA, the yields of extraction from grape to juice

[†] Address correspondence to the author at LIMNOS, Podlimbarskega 31, 1000 Ljubljana, Slovenia (telephone +386 61 557 472; fax +386 61 557 386).

10.1021/jf980461s CCC: $15.00
Published on Web 09/16/1998

fully protected from oxidation were analyzed in six white cultivars of the 1996 vintage.

MATERIALS AND METHODS

Grape Samples. Grapes (cv. Pinot Blanc) were harvested in 1995 at the stage of technical maturity in the vineyard of the Research Station (Höhere Bundeslehranstalt und Bundesamt für Wein und Obstbau) Klosterneuburg. A small sample was used for laboratory determinations and the bulk (260 kg) for microvinification. The grapes had a sugar reading of 21.9 °Brix, 9.6 g/L titratable acidity, and a pH value of 3.25.

Grapes from the same vineyard were collected in 1996 from vines of cv. Pinot Blanc (two samples), Chardonnay, Grüner Veltliner, Sauvignon Blanc, Riesling, and Riesling Italico. Data for their composition are given in Table 2.

Laboratory Trials. *Pinot Blanc 1995*. The individual undamaged berries were snipped carefully without breaking the berry skin, and those not intact were discarded. Mean berry weight was determined from a 50 berry sample to which, as a protection against oxidation, were added potassium metabisulfite (1 g) to inhibit polyphenol oxidase (PPO) and ascorbic acid (1 g) to reduce any quinone formed, before the crushing and pressing, which were carried out in a vacuum (Cheynier et al., 1989b). Smaller additions of these substances were regarded as being adequate by Singleton et al. (1984), but to ensure maximum effect, we used the stated amounts because excess had no apparent deleterious effect on chromatography. The antioxidant treatment proved effective as no GRP was detected in any of the juices or extracts examined.

Each berry sample, protected from oxidation and under vacuum, was ground in a porcelain dish to homogenize skins and pulp without crushing the seeds. The volume of unoxidized juice was measured to calculate the exact dilution, and a sample of it was taken to measure its HCA concentration. The skins were then extracted three times with 30 mL portions of 6% (w/v) perchloric acid in water (Cheynier et al., 1989b). Perchloric acid was added to precipitate proteins and inhibit PPO. The unoxidized juice and the three skin extracts were combined, and the total volume was measured to refer the data (milligrams per liter) to the volume of unoxidized juice. This sample was considered representative of the total content of HCA in berries.

The sampling, extraction, and analysis of grapes were performed in duplicate.

Variety Trial 1996. In this trial, the relationship between the concentration of HCA in grape berries and in juices of the six cultivars mentioned above was examined according to the same methodology as described for Pinot Blanc 1995.

Pilot-Scale Press 1995. To test four different technologies of juice preparation, the above-described, hand-harvested Pinot Blanc grapes (~260 kg) were divided into four equal portions. Split and botrytized berries were carefully removed from the bunches before pressing. The grapes of three lots were destemmed and crushed. The grapes of the fourth lot remained intact, that is, neither destemmed nor crushed (whole cluster). All four lots were pressed in a 50-kg hydraulic basket press (pressure up to 2.5 bar). Four different white wine technologies were performed: T1, standard juice extraction (control), pressing immediately after crushing, without extended skin contact, and addition of 50 mg/L SO_2 immediately after pressing; T2 (skin contact + SO_2), crushing, immediate addition of 50 mg/kg SO_2, and skin contact for 3 h at 15 °C before pressing; T3 (skin contact − SO_2), crushing, no addition of SO_2, skin contact for 3 h at 15 °C before pressing, and addition of 50 mg/L SO_2 immediately after pressing; T4, whole cluster pressing, no crushing, intact bunches pressed, and addition of 50 mg/L SO_2 immediately after pressing.

After pressing, a sample of juice was withdrawn from each lot for analysis while the rest was allowed to settle overnight. Excess amounts of SO_2 (40 mg/50 mL of juice) and ascorbic acid (40 mg/50 mL of juice) were immediately added to every juice sample on collection. The samples were stored at −18 °C; they proved to be stable for several months at this temperature.

Table 1. Concentration of Hydroxycinnamoyltartaric Acids in Grape Berries and in the Unoxidized Juice of Pinot Blanc and Percent Yield of Extraction in the Juice (Season 1995)

	grape			juice			extraction yield (%)	
	mg/L			mg/L				
compd	mean	SD	%[a]	mean	SD	%[a]	mean	SD
CTA	178.0	2.8	77	85.5	2.1	87	48.0	0.4
cis-CoTA	11.3	1.1	5	2.7	0.1	3	24.0	1.0
trans-CoTA	40.6	7.1	17	8.9	0.4	9	22.2	2.8
trans-FTA	2.7	0.0	1	1.4	0.1	1	50.0	2.7
total HCA	232.6			98.5			36.1	
GRP	nd[b]						nd	

[a] Percent of concentration of total hydroxycinnamoyltartaric acids. [b] nd, not detectable.

Chemicals. *trans*-Caftaric acid (CTA), *trans*-coutaric acid (*trans*-CoTA), and *trans*-fertaric acid (FTA**)** were isolated from Grenache grapes (grown in the vineyard of University of California, Davis) as described by Vrhovsek et al. (1997) following the method of Singleton et al. (1978). The *cis*-isomers were obtained by UV illumination of the *trans*-standards, and 2-*S*-glutathionylcaftaric acid (GRP) was obtained by aerating a solution of CTA (1 g/L) with 2 mol equiv of reduced glutathione in the presence of crude grape PPO, in aqueous solution at room temperature. All other chemicals used were purchased from commercial sources.

HPLC Analysis. The analysis of hydroxycinnamates was performed by direct injection in a reverse phase HPLC-DAD system (Hewlett-Packard 1090 with HP 9000, series 300 workstation and diode array detector) (Vrhovsek et al., 1997). Peak identification was obtained by comparison of retention time and spectral characteristic with those of authentic compounds. Quantification was based on peak areas at 320 nm, and the amounts of all compounds were estimated using a standard calibration method. Results were expressed in milligrams per liter.

HPLC analyses were carried out on juice samples as soon as possible, even though juices, once protected by high doses of SO_2 and ascorbic acid and filtered, are slow to show further changes. Samples were membrane-filtered (0.45 μm) before injection into HPLC using a precolumn HP-ODS Hypersil RP-C18 (20 × 2.1 mm, 5 μm), followed by a sequence of two coupled columns HP-ODS Hypersil RP-C18, 5 μm (200 and 100 mm length; each 2.1 mm bore). The mobile phases were (A) formic acid in water (0.5%), pH 2.3, and (B) methanol; injection volume = 10 μL; flow rate = 0.2 mL/min; oven temperature = 40 °C; analytical wavelength = 320 nm. The elution program was a linear gradient from 8 to 16% of B during the first 25 min, increased to 60% B at 55 min, isocratic at 60% B for 10 min, and back to the initial conditions of 8% B in 2 min.

Statistical Analysis. The analysis of variance (two-way ANOVA) and the least significant differences of means (lsd) for the percent yield of extraction in the juices (season 1996) were computed by the statistical package Genstat 5, release 3.2, Lawes Agricultural Trust, Rothamsted Experimental Station.

RESULTS AND DISCUSSION

Laboratory Pinot Blanc Experiment 1995. The average amount of juice obtained was 65.0% (w/w). The concentrations of HCA in the berries and juice of Pinot Blanc grapes are shown in Table 1. The complete absence of GRP in the extracted tissues and juice of the berries indicated proper protection from oxidation. The reduction in the concentration of HCA from that in the grape to that in the juice was due to incomplete extraction. The amount of grape CTA extracted into the

Table 2. Concentration of Hydroxycinnamoyltartaric Acids in Grape Berries and in the Oxidation-Protected Juice of Several White Grape Cultivars and Percent Yield of Extraction (Season 1996)

	cultivar[a]						
	PB1	PB2	Chardonnay	GV	Sauvign	Riesling	Riesl Ital
compd (mg/L) in grape							
caftaric acid	341.0	202.9	207.0	257.7	177.5	369.5	270.2
SD	0.0	31.0	29.7	36.3	9.8	43.1	15.8
cis-coutaric acid	16.7	16.0	18.7	11.8	21.0	16.0	19.6
SD	1.3	0.6	1.6	0.6	0.8	1.5	1.5
trans-coutaric acid	93.0	55.4	61.9	55.3	68.9	64.6	61.2
SD	5.7	5.4	8.3	7.4	2.8	5.6	7.0
trans-fertaric acid	3.2	3.3	3.7	3.5	1.7	16.8	4.0
SD	0.4	0.6	0.4	0.4	0.1	1.7	0.4
total HCA[b]	454	278	291	328	269	467	355
compd (mg/L) in juice							
caftaric acid	234.5	116.3	110.7	140.9	113.4	191.5	169.9
SD	4.9	15.2	16.5	22.8	4.6	31.4	6.9
cis-coutaric acid	7.3	5.0	5.7	4.6	7.0	5.6	8.1
SD	1.1	0.6	0.7	1.1	0.1	1.0	1.3
trans-coutaric acid	36.7	14.9	15.9	20.8	20.9	22.4	24.9
SD	4.3	1.3	2.1	5.8	1.1	4.9	3.7
trans-fertaric acid	2.7	2.1	2.3	2.5	1.2	12.4	2.9
SD	0.4	0.4	0.2	0.2	0.1	1.8	0.0
total HCA[b]	281	138	135	169	142	232	206
extracted (% of grape in juice)							
caftaric acid	68.8	57.4	53.4	54.6	63.9	51.7	63.1
SD	1.5	1.3	0.3	1.1	0.9	2.5	6.2
cis-coutaric acid	43.4	30.9	30.5	38.9	33.1	35.0	41.6
SD	2.9	2.9	1.1	7.5	1.0	2.9	10.0
trans-coutaric acid	39.3	27.2	25.7	37.3	30.3	34.4	41.3
SD	2.2	5.0	0.0	5.5	0.4	4.6	10.8
trans-fertaric acid	82.8	63.5	61.7	71.1	70.0	73.6	73.7
SD	0.1	2.0	0.2	1.1	2.8	3.5	6.6
berry composition							
sugar content (Brix)	20.6	22.5	23.9	20.1	21.2	20.3	19.5
titratable acidity (g/L)	13.7	11.8	12.5	11.6	12.7	13.2	11.0
pH	3.4	3.5	3.5	3.3	3.1	3.3	3.4
av amount of juice (% w/w)	64	64	64	75	69	60	68

[a] Cultivar identification: PB1, PB2 = Pinot Blanc; GV = Gruner Veltliner; Sauvign = Sauvignon Blanc; Riesl Ital = Riesling italico (Welschriesling). [b] HCA, hydroxycinnamoyltartaric acid.

juice fully protected from oxidation was 48%. The extraction yield of *cis*-CoTA from grapes was much lower than for CTA. This is entirely reasonable, given the preferential localization of CoTA in the berry skin. Thus, in the juice fully protected from oxidation only 24.0% of the original amounts in the grapes was extracted. The extraction yield of *trans*-CoTA from the grape was 22.2%. In the Pinot Blanc sample FTA was found to have the highest extraction yield from grape into unoxidized juice, similar to the extraction yield obtained for CTA and much higher than that of both isomers of CoTA.

Laboratory Variety Trial 1996. Despite the fact that absolute concentrations of each HCA varied a lot among the samples (Table 2), the extraction yields of single HCA were found to be very similar for all varieties. A two-way ANOVA was computed for the yields (percent) by taking into consideration the two factors compounds and grape sample. The difference among compounds was highly significant ($\alpha < 0.001$), explaining 85.3% of the variance. The extraction of FTA was steadily the highest (average = 70.9%) and the extraction yield of both CoTA isomers the lowest (average = 36.2 and 33.6%, respectively, for *cis*- and *trans*-). The average extraction yield of CTA was 59.0%. Given that the lsd of means was 3.3%, only the difference between the two CoTA isomers was not significant.

From the technological point of view, these results should mean that during the pressing FTA should be extracted easily and therefore sooner than CTA and the slowest extraction should be expected for both CoTA-isomers.

The relative amount of CTA in each cultivar seems to be quite stable in relation to the total amount of HCA, whereas the relative amounts of CoTA and FTA varied very much. Different absolute amounts of each HCA do not seem to play an important role in their extraction yield. The different sample accounted only for a residual 9.0% of the variance of the extraction yields. The variability between the two different samples of Pinot Blanc was at least comparable to the variability between the samples belonging to different varieties (Table 2). The interaction compound−sample was not significant ($\alpha = 0.283$).

Our results on extraction yield of CTA and CoTA are in good agreement with the data on localization of CTA and CoTA in the berry, because in the flesh the concentration of CTA is much higher than that of CoTA (Boursiquot et al., 1986; Di Stefano and Maggiorotto, 1995). On the other hand, higher concentrations of CoTA were found in the skins; therefore, it could be supposed that CoTA needs more time to be extracted. Our results obtained for FTA showed the highest extraction yield of all HCA, and this suggests that FTA, the concentration of which has been reported to be from 3.0 to 14.2 times higher in the skin that in the pulp of other *Vitis vinifera* varieties (Boursiquot, 1987), is present in quite higher total amounts in the vacuoles of grape flesh cells of the varieties considered in this study.

Table 3. Concentration of HCA (Milligrams per Liter) after Pressing and Overnight Settling in Musts of Pinot Blanc 1995

		T1: control	T2: maceration with SO_2	T3: maceration without SO_2	T4: whole cluster pressing
CTA	must	51.0	47.0	30.0	16.0
	must after overnight settling	52.0	34.0	22.8	17.9
cis-CoTA	must	1.7	2.0	2.3	0.9
	must after overnight settling	1.5	1.8	1.9	1.0
trans-CoTA	must	4.5	5.8	4.5	1.9
	must after overnight settling	4.3	3.8	3.1	1.9
GRP	must	45.0	42.0	46.0	45.0
	must after overnight settling	45.0	42.0	45.5	45.0

p-Coumaric and fertaric acids are the precursors of volatile phenols in wine. The red wine vinification allows—with respect to the white wine vinification—a longer skin contact time and therefore a better extraction of HCA from the skin. A quicker extraction of FTA in relation to CoTA is in agreement with and could partially explain the higher ratio ferulic/coumaric volatile derivatives, that is, 4-vinylguaiacol + 4-ethylguaiacol/4-vinylphenol + 4-ethylphenol, which was observed in white wines compared to red and rosé wines (Rapp and Versini, 1996).

Because only a few data about FTA localization are available so far, this strongly recommends further investigation in this field.

Microvinification Trial Pinot Blanc 1995. *Caftaric Acid*. Changes in the amount of CTA during pressing are mainly due to two processes: the incomplete extraction of CTA from grape into juice and the enzymatic oxidation of CTA. Comparison of the CTA concentrations in the fresh musts (Table 3) of the variants T1, T2, T3, and T4 with that of the nonoxidized juice obtained in the laboratory from the same grape (Table 1) indicates that the high amount of CTA originally present in grapes was additionally oxidized during crushing and pressing. In agreement with the results of Cheynier et al. (1993), the usual technological levels of SO_2 were not high enough to completely prevent oxidation.

The amount of CTA remained the highest in the case of the control variant, whereas whole cluster pressing resulted in the lowest amount of CTA among all vinifications performed, which was expected because it was possible to produce the must by means of lower pressures.

Comparing the T2 and T3 musts—which differ in the amount of CTA by ~17 mg/L (Table 3)—it can be concluded that additional losses of CTA occurred during the 3 h of skin contact and during the pressing when the crush was not protected from oxidation. This could be due to the protective effect of sulfite ions, which can reduce the concentration of quinones (Cheynier and Moutounet, 1992) and therefore the losses due to addition of free quinones on CTA or on catechins or procyanidins (Cheynier and Ricardo da Silva, 1991). Another factor that could possibly explain the higher levels of CTA of T2 compared to T3 is a higher yield of extraction of phenolic compounds because of the SO_2 addition.

From a comparison of the CTA concentrations in the musts T1 and T2 it appears that—at least for the kind remaining on skins tested—no additional CTA comes from contact with solid parts.

The sum of the losses due to the incomplete extraction of CTA and its enzymatic oxidation during pressing and settling was 70−90% of the amount of CTA originally present in grape.

cis-Coutaric Acid. The values of *cis*-CoTA in musts (Table 3) were always lower with respect to the concentration in the relevant unoxidized juice (Table 1), showing the occurrence of oxidative losses during crushing and pressing (Table 3). As for the CTA, the concentration was again the lowest with whole cluster pressing. The concentrations of *cis*-CoTA were slightly higher in the juices with 3 h of skin contact with respect to the control vinification. These results are consistent with the localization of this compound in the skin, from which *cis*-CoTA needs more time to be extracted.

trans-Coutaric Acid. The balance for *trans*-CoTA losses followed almost the same pattern as for *cis*-CoTA. As for the *cis*-isomer, the localization in the skin could be an explanation for the lowest concentration in the whole cluster pressing must.

When the concentrations in musts (Table 3) were compared to the concentration in the fully protected juice (Table 1), it was observed that the percentages of *trans*-CoTA and CTA losses during the pressing—and for T2 and T3 also during settling—were comparable. These results could be explained because CoTA, unlike CTA, cannot be regenerated once oxidized. Therefore, the reported stronger affinity of PPO to CTA than to CoTA (Gunata and Moutounet, 1988) can only reduce the initial oxidation rate of CoTA but cannot prevent its oxidation during wine-making. Such results could also be due to the possible presence of *Botrytis* laccase, having lower substrate specificity (Gunata and Moutounet, 1988).

Fertaric Acid. As a consequence of the low amount of FTA and of the high concentration of GRP in musts T1−T4, it was not possible to obtain a precise quantification of the former, which elutes immediately after GRP. These data have been therefore omitted in Table 3.

2-S-Glutathionylcaftaric Acid (GRP). All of the musts (Table 3) contained large quantities of GRP. This confirms that oxidation of CTA and CoTA by grape PPO to the corresponding *o*-quinone and the subsequent reaction of the latter with the available glutathione to form GRP took place during crushing and pressing. Oxidation of CTA and CoTA to GRP was evident in juices processed both with and without SO_2, which confirms that the amounts of SO_2 added immediately after pressing were not able to inhibit PPO activity.

It must be pointed out that in media containing quite different amounts of substrate (CTA) due to different

vinification techniques, very similar amounts of GRP were formed. This could mean that the limiting factor for GRP formation could be PPO activity or the amount of glutathione, but not the amount of CTA. Pinot Blanc has been reported to have a medium content of glutathione in comparison to other varieties. The molar ratio HCA/glutathione was reported to be 1.8 in the must and 2.5 in grape (Cheynier et al., 1989b). In light of our data and data from the literature, the amount of glutathione could be the limiting factor for GRP formation. Cheynier et al. (1989b) reported that the maximum level of GRP formed during must oxidation was highly correlated with and sometimes equal to the measured amount of glutathione.

Conclusions. In T2 and T3 a decrease of CTA, *cis*-CoTA, and *trans*-CoTA during the overnight settling was observed, whereas in T1 and T4 the concentration remained stable. This seems to indicate higher PPO activity in must obtained with skin contact and possibly increased consumption due to enhanced formation of condensation products (Cheynier et al., 1989c; Cheynier and Ricardo da Silva, 1991) of the oxidized HCA in the quinone form with catechins and proanthocyanidins extracted from skins.

From the analysis of the grape, the unoxidized juice, and four different musts of Pinot Blanc, it was observed that the sum of average HCA losses varied with the vinifications examined. In light of the results from the literature and the results of this study, we can conclude that there are three main sources of losses during pressing: the yield of extraction of each HCA from grape into the juice, the enzymatic oxidations due to PPO activity, and the condensation reactions. Our study allowed us to evaluate the former factor and also to obtain some data about the overall losses connected with the two latter processes and about the main product of enzymatic oxidation (GRP). The extraction yield was clearly the most important in determining both the final amount and pattern of HCA in the must. In our experiments about the extractability of single HCA from grape, FTA was found to have the highest extraction yield from grape into the juice fully protected from oxidation. The extraction yield of CTA was lower than that of FTA and higher than the extraction yield of both *cis*- and *trans*-CoTA. Therefore, the losses (percent) due to incomplete extraction from the berry are lower for CTA than for CoTA. CTA is then oxidized in higher absolute amount than CoTA.

ABBREVIATIONS USED

HCA, hydroxycinnamic acids and their tartaric esters (also called hydroxycinnamates); CTA, *trans*-caffeoyltartaric acid; *cis*-CoTA, *cis*-*p*-coumaroyltartaric acid; *trans*-CoTA, *trans*-*p*-coumaroyltartaric acid; FTA, *trans*-feruloyltartaric acid; GRP, 2-*S*-glutathionylcaffeoyltartaric acid; PPO, polyphenol oxidase.

ACKNOWLEDGMENT

I gratefully acknowledge Josef Weiss for his supervision, Silvia Wendelin, Manfred Pastler, and Herbert Frohner for technical assistance, and Reinhard Eder for his help and suggestions.

LITERATURE CITED

Arnold, R. A.; Noble, A. C. Effect of pomace contact on the flavour of Chardonnay wine. *Am. J. Enol. Vitic.* **1979**, *30*, 179−181.

Betés-Saura, C.; Andrés-Lacueva, C.; Lamuela-Raventos, R. M. Phenolics in white free run juices and wines from Penedes by high-performance liquid chromatography: Changes during vinification. *J. Agric. Food Chem*. **1996**, *44*, 3040−3046.

Boulton, R. B.; Singleton, V. L.; Bisson, L. F.; Kunkee, R. E. Preparation of musts and juice. In *Principles and Practices of Winemaking*; Chapman and Hall Press: New York, 1996.

Boursiquot, J. M. Contribution à l'étude des esters hydroxycinnamoyltartriques chez le genre Vitis. Recherche d'application taxonomique. Thèse Ecole Nationale Supérieure Agronomique de Montpellier, 1987.

Boursiquot, J. M.; Sapis, J. C.; Macheix, J. J. Etude de la distribution des esters hydroxycinnamoyltartriques dans la pulpe et la pellicule des baies de raisin mur. Relation avec la couleur des varietes. *Bull. Liaison Groupe Polyphenols* **1986**, *13*, 431−433.

Cheynier, V.; Moutounet, M. Oxidative reactions of caffeic acid in model systems containing polyphenol oxidase. *J. Agric. Food Chem*. **1992**, *40*, 2038−2044.

Cheynier, V.; Ricardo da Silva, J. M. Oxidation of grape procyanidins in model solutions containing *trans*-caffeoyltartaric acid and polyphenol oxidase. *J. Agric. Food Chem*. **1991**, *39*, 1047−1049.

Cheynier, V.; Rigaud, J.; Souquet, J. M.; Barillère, J. M.; Moutounet, M. Effect of pomace contact and hyperoxidation on the phenolic composition and quality of Grenache and Chardonnay wines. *Am. J. Enol. Vitic.* **1989a**, *40*, 36−42.

Cheynier, V.; Souquet, J. M.; Moutounet, M. Glutathione content and glutathione to hydroxycinnamic acid ratio in *Vitis vinifera* grapes and musts. *Am. J. Enol. Vitic.* **1989b**, *40*, 320−324.

Cheynier, V.; Basire, N.; Rigaud, J. Mechanism of *trans*-caffeoyltartaric acid and catechin oxidation in model solutions containing grape polyphenoloxidase. *J. Agric. Food Chem*. **1989c**, 37, 1069−1071.

Cheynier, V.; Masson G.; Rigaud, J.; Moutounet, M. Estimation of must oxidation during pressing in Champagne. *Am. J. Enol. Vitic.* **1993**, *44*, (4), 393−399.

Di Stefano, R.; Maggiorotto, G. Antociani, acidi idrossicinnamici e flavonoli del frutto, delle foglie, dei raspi e dei tralci della vite (Anthocyanins, hydroxycinnamic acids and flavonols in berries, leaves, stems and shoots of vine). *Riv. Vitic. Enol.* **1995**, *2*, 51−65.

Gunata, Y. Z.; Moutounet, M. Activites de la polyphenoloxidase du raisin et de la laccase de *Botrytis cinera* sur les derives hydroxycinnamiques comparees a d'autres substrats phenoliques. *Rev. Fr. Oenol.* **1988**, 42−47.

Rapp, A.; Versini, G. Fluchtige phenolische Verbindungen in Wein. *Dtsch. Lebensm.-Rundsch.* **1996**, *92*, 42−48.

Schneider, V. Entrappen oder nicht entrappen? *Weinwirtsch. Tech.* **1992**, 63−65.

Seckler, J. Steigerung der Weinqualität durch schonende Traubenverarbeitung. *Rebe Wein* **1996**, 281−285.

Singleton, V. L.; Timberlake, C. F.; Lea, A. G. H. The phenolic cinnamates of white grapes and wine. *J. Sci. Food Agric.* **1978**, *29*, 403−410.

Singleton, V. L.; Zaya, J.; Trousdale, E.; Salgues, M. Caftaric acid in grapes and conversion to a reaction product during processing. *Vitis* **1984**, *23*, 113−120.

Somers, T. C.; Vérette, E.; Pocock, K. F. Hydroxycinnamate esters of *Vitis vinifera*: changes during white vinification and effects of exogenous enzymic hydrolysis. *J. Sci. Food Agric.* **1987**, *40*, 67−78.

Test, S. L.; Noble, A. C.; Schmidt, J. O. Effect of pomace contact on Chardonnay musts and wines. *Am. J. Enol. Vitic.* **1986** *37*, 133−136.

Vrhovsek, U. Hydroxycinnamic acids in Slovenian white wines varieties Pinot blanc and Chardonnay. *Sodobno Kmetijstvo* **1998**, *5*, 226−232.

Vrhovsek, U.; Wendelin, S.; Eder, R. Quantitative Bestimmung von Hydroxyzimtsäuren und Hydroxyzimtsäurederivaten (Hydroxycinnamaten) in Weissweinen mittels HPLC [Quantitative determination of hydroxycinnamic acids and their derivatives (hydroxycinnamates) in white wine by means of HPLC]. *Mitt. Klosterneuburg* **1997**, *47*, 164−172.

Received for review May 7, 1998. Revised manuscript received August 3, 1998. Accepted August 5, 1998. The work was supported by Austrian and Slovenian Ministry for Research and Science.

JF980461S