UDC 547.96:663.1:663.221
ISSN 1330-9862 *original scientific paper*

(FTB-1150)

Identification of Phenolic Acids and Changes in their Content during Fermentation and Ageing of White Wines Pošip and Rukatac

Irena Budić-Leto[1]* *and Tomislav Lovrić*[2]

[1]Institute for Adriatic Crops and Karst Reclamation, Put Duilova 11, P.O.Box 288, HR-21000 Split, Croatia

[2]Faculty of Food Technology and Biotechnology, University of Zagreb, Pierottijeva 6, HR-10000 Zagreb, Croatia

Received: May 7, 2002
Accepted: June 18, 2002

Summary

Identification of phenolic acids was performed and changes in their content during the production of autochthonous Croatian white wines Pošip and Rukatac (*Vitis vinifera*, L.) were registered. In both varieties (Pošip, Rukatac) the following phenolic acids were identified: gallic, protocatechuic and vanillic acids as hydroxybenzoic acids; and caffeic, *p*-coumaric and ferulic acids as hydroxycinnamic acids. It was found that there is a difference between hydroxybenzoic acid group and hydroxycinnamic acid group content and between their influences on the wine colour (colour intensity and hue).

Key words: phenolic acids, Pošip, Rukatac, HPLC, autochthonous cultivars

Introduction

Phenolic acids are phenolic compounds of the nonflavonoid family, present in several parts of grapes, but mainly in grape juice. They are biosynthesised through shikimic acid pathway. Phenolic compounds have been characterised as »potential causes of wine instability«, because of their involvement in formation of sediments (*1*) and yellow or brown pigments, which is one of the most severe problems in white wine making. There are two main groups of phenolic acids: hydroxybenzoic acids (HBA) and hydroxycinnamic acids (HCA). Hydroxybenzoic acids have a general structure of C_6–C_1 type of benzoic ring derived directly from benzoic acid and they differ in accordance with hydroxylations and methoxylations of the aromatic ring. Hydroxycinnamic acids (C_6–C_3) derive from cinnamic acid. Both mentioned groups of phenolic acids are commonly present in grapes either as derivations in form of esters with tartaric acid or with anthocyanins in red grape. Extraordinary circumstances, like high temperature and too acid medium, lead to hydrolysis of bound HCA forms. Different conditions of microbiological, chemical and mechanical attacks on plant may cause enzymatic degradations of various phenols as a consequence of plant's defence. In these cases, free HCA are released from the bound ones producing much more stable compounds. It was reported that high free HCA content, which is probably associated with the original maturation of fruit, is accompanied with browning (*2*).

During fermentation and ageing of wine, various reactions take place, in which HCA and HBA acids change their forms and content. It has been shown that some of these acids are involved in wine browning.

* Corresponding author; Phone: +385 (0)21 316 578; Fax: +385 (0)21 316 584; E-mail: irena@krs.hr

Some authors have used caffeic acid as a model to study oxydative reactions related to browning that white wine undergoes in relation to temperature and pH. It has been shown that the formation of brown colour, measured at 420 nm, correlated well with oxydation of caffeic acid (*3*).

Paper chromatography and thin-layer chromatography have been used for separation and qualitative detection of phenolic acids for a long time. The determination of these compounds has greatly progressed by using high performance liquid chromatography (HPLC), which enables separation and both qualitative and quantitative analysis. Reversed-phased HPLC is now commonly used for separation of complex mixtures of phenolic compounds in wines (*4*).

Various aspects of phenolic compounds involved in different reactions during wine making have been studied predominantly in classic wine cultivars (international varieties) (*5*). Some conclusions are drawn regarding the difference in phenolic profile depending on grape cultivars, vintages, technology and particulary on components (*6*).

The objective of this study was to identify and quantify the free forms of phenolic acids and to determine the changes in their content during fermentation and ageing of autochthonous Croatian (Dalmatian) white wines Pošip and Rukatac (*Vitis vinifera*, L.) as well as their potential influence on the colour of wine.

Material and Methods

Preparation of samples

The grapes of Pošip and Rukatac cultivars (*Vitis vinifera*, L.) were harvested at technological stage of ripening in the vineyards of Korčula (coastal subregion of Dalmatia), Republic of Croatia in 1996. The experiments were carried out with 50 kg of grapes in three repetitions. The grapes were crushed and destemmed after the harvest, the juice left to settle, and then treated with bentonite (0.1 g/L) and SO_2 (100 mg/kg of grapes). Fermentation was carried out without adding selected yeast strains (spontaneously), at the temperature between 18 and 23 °C. After eight months, the wines were racked and then stored at 12–16 °C in 0.75 L-glass bottles with cork caps. The analyses were performed at the end of fermentation, after eight months, and after ten months of storage in glass bottles. Identification and quantitative determination of phenolic acid were carried out in all samples. In addition, colour changes were registered.

Extraction of phenolic acids

Solid phase extraction (SPE) was applied for separation and extraction of phenolic acids in juice and wine samples by using C18 and SAX cartridge (500 mg, Varian) following the procedure described by Guillen *et al.* (*7*).

Determination of phenolic acids

Phenolic acids of juice and wine samples, contained in acid phenolic fraction after SPE, were identified and qualitatively determined by using the HPLC.

Analyses were performed in a Varian HPLC system with a 9010 pump, a Rheodyne injection valve furnished with a 10-μL loop, a UV-VIS detector 9050 and a Varian 4400 Integrator at λ = 280 nm.

Separation was carried out by using a LiChrosorb RP-C18 column (Chrompack), 250 mm × 3 mm I.D. and 5 μm particle size.

The mobile phase was a linear gradient of *V*(methanol):*V*(acetic acid):*V*(water) = (10:2:88) as solvent A and *V*(methanol):*V*(acetic acid):*V*(water) = (90:2:8) as solvent B. Flow rate was 1.0 mL/min.

The quantitative determinations were carried out by using the calibration curves of the corresponding acids.

The average concentration of phenolic acids was calculated for each of the samples. The results represent the average of three repetitions.

Conventional parametres such as relative density, actual alcohol, total extract, reducing sugars, pH-value, total acidity, volatile acidity, ash, free and total SO_2 were measured according to Office International de la Vigne et du Vin (O.I.V.) methods (*8*).

Total phenols were determined by the official AOAC spectrophotometric method with Folin-Ciocalteu reagent according to Singleton and Rossi (*9*).

Colour intensity and hue were estimated by measuring the absorbance at 420, 520 and 620 nm according to O.I.V. methods (*8*). Spectrophotometric measurements were made in a Varian UV-VIS DM 200 spectrophotometre in a 10 mm cell.

Reagents

Methanol (HPLC-gradient grade) was supplied from Merck (Darmstadt, Germany). HBA acids: Gallic (3,4,5-trihydroxybenzoic), protocatechuic (3,4-dihydroxybenzoic), vanillic (4-hydroxy-3-methoxybenzoic) and HCA acids: caffeic (3,4-dihydroxycinnamic), *p*-coumaric (4-hydroxycinnamic) and ferulic (4-hydroxy-3-methoxycinnamic) were supplied from Merck and Fluka. Deionized water was used to prepare all standard solutions and HPLC mobile phase.

Results and Discussion

Analytical data for eight-month-old wine Pošip and Rukatac is shown in Table 1.

In both grape juices (musts) of Pošip and Rukatac gallic, protocatechuic, vanillic, caffeic, *p*-coumaric and ferulic acid were identified and quantitatively determined as shown in Tables 2 and 3.

In the juices, the total content of hydroxybenzoic acid (HBA) group was higher than total content of hydroxycinnamic acid (HCA) group. The predominant acids were: protocatechuic (2.41 mg/L) in Rukatac; (4.22 mg/L) in Pošip, and gallic (0.85 mg/L) in Rukatac; (1.50 mg/L) in Pošip.

Table 1. Analytical data for eight-month-old wine Pošip and Rukatac

Parametre	Pošip	Rukatac
ρ (specific gravity) (20/20 °C)	0.9932	0.9907
φ (alcohol)/ vol %	12.22	11.52
γ (total extract)/ (g L^{-1})	23.63	15.5
γ (reducing sugar)/ (g L^{-1})	< 1	< 1
γ (total acidity)/ (g L^{-1})	6.73	4.22
γ (volatile acidity)/ (g L^{-1})	0.61	0.47
γ (free SO_2)/ (mg L^{-1})	7	12
γ (bound SO_2)/ (mg L^{-1})	153	97
pH	3.17	3.51
m(ash) / g	1.69	1.35
γ (total phenols) / (mg L^{-1})	273	231

ters (caftaric and coutaric acids) has been reported by some authors (*11*).

It was observed that the level of vanillic acid increased at the end of fermentation as well as after eight and ten months of wine ageing. Namely, considering that one of the ways of biosynthesis of vanillic acid is through β-oxydation of ferulic acid, the registered increased level of vanillic acid could be attributed to the transformation of ferulic acid to forming of vanillic acid. The possible conversion of ferulic to vanillic acid was suggested to occur during the fermentation and ageing of Monasterll wines by Lazaro *et al*. (*12*).

In order to determine the impact of free phenolic acids on the colour of wine, absorbances at 420, 520 and 620 nm were measured in all samples during the ageing of wine.

Table 2. Changes in phenolic acids content during fermentation and ageing of wine Pošip

	γ (phenolic acid) / (mg L^{-1})					
	Gallic	Protocatechuic	Vanillic	Caffeic	*p*-coumaric	Ferulic
Must	1.50	4.22	0.21	0.13	0.12	0.47
Wine after fermentation	0.26	4.21	0.38	2.51	2.36	2.13
Eight-month-old wine	0.12	1.71	0.58	1.78	2.50	1.43
Ten-month-old wine in glass bottles	0.48	2.31	1.18	5.03	5.94	1.72

Table 3. Changes in phenolic acid content during fermentation and ageing of wine Rukatac

	γ (phenolic acid) / (mg L^{-1})					
	Gallic	Protocatechuic	Vanillic	Caffeic	*p*-coumaric	Ferulic
Must	0.85	2.41	0.80	1.01	0.47	0.32
Wine after fermentation	0.05	1.98	0.90	1.48	1.82	2.46
Eight-month-old wine	0.14	1.18	1.09	2.12	2.56	3.20
Ten–month-old wine in glass bottles	0.46	1.38	1.52	2.41	2.79	3.87

Caffeic, *p*-coumaric and ferulic acids were found (immediately after pressing) in lower concentration in both Pošip and in Rukatac.

It is known that HCA are present in fruits in esters form, and only a few natural circumstances or technological processing operations can cause them to accumulate in the free form (*10*). According to the obtained results (Tables 2 and 3) it could be presumed that sulfiting of must, which was applied before fermentation, diminished polyphenoloxidase activity, initially present in the juices, and potential enzymatic degradation of combined forms of HCA, that could be responsible for the low concentration of caffeic, *p*-coumaric and ferulic acids determined in the juices (musts).

During the fermentation, the noticeable trend of the decrease of gallic and protocatechuic acids and the increase of vanillic, caffeic, *p*-coumaric and ferulic acids was registered. The decrease of protocatechuic acid content after fermentation was almost negligible Pošip (from 4.22 ± 1.02 mg/L to 4.21 mg/L ± 0.96). The increase of the content of caffeic, *p*-coumaric and ferulic acids at the end of fermentation could be due to possible hydrolysis of HCA esters (caftaric, coutaric, fertaric) during fermentation. Complete hydrolysis of hydroxycinnamic es-

Table 4. Optical density at 420, 520 and 620 nm during the ageing of wine Pošip

	Pošip 240 day	Pošip 540 day
A (420 nm)	0.110	0.176
A (520 nm)	0.040	0.059
A (620 nm)	0.035	0.042
A (colour intensity)	0.185	0.276
A (hue)	2.750	2.983

Table 5. Optical density at 420, 520 and 620 nm during the aging of wine Rukatac

	Rukatac 240 day	Rukatac 540 day
A (420 nm)	0.190	0.142
A (520 nm)	0.050	0.068
A (620 nm)	0.003	0.005
A (colour intensity)	0.243	0.215
A (hue)	3.800	2.088

*Colour intensity = $A_{420} + A_{520} + A_{620}$
*hue = A_{420} / A_{520}

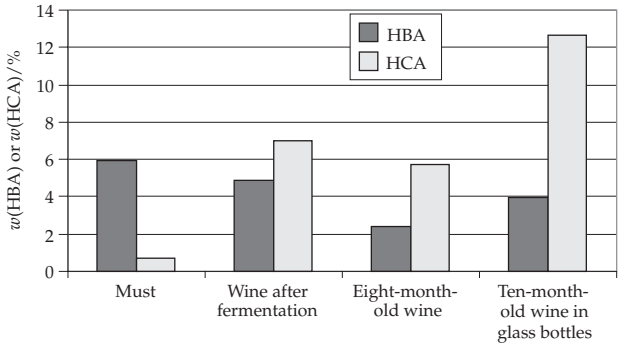

Fig. 1. Mass fraction of HBA and HCA in must and wine of Pošip at different stages of production

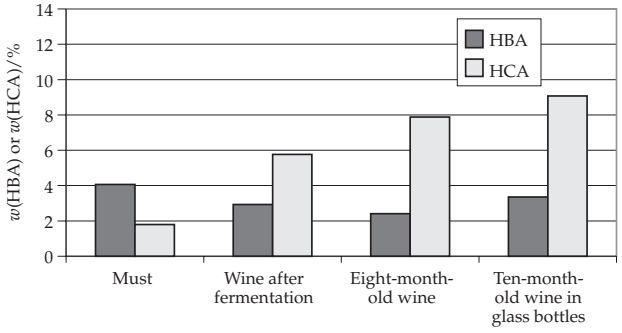

Fig. 2. Mass fraction of HBA and HCA in must and wine of Rukatac at different stages of production

It was already suggested that absorbance values at 420 nm correlate directly to the susceptibility of the browning of wine. According to the results obtained in this research, which are shown in Tables 2 and 4 for wine Pošip and in Tables 3 and 5 for wine Rukatac, a positive linear correlation between colour intensity and gallic, protocatechuic, vanillic and ferulic acid content was found.

On the contrary, a negative linear correlation between colour intensity and caffeic and *p*-coumaric acid content was determined. It means that the increase of gallic, protocatechuic, vanillic and ferulic acid content leads to the increase of colour intensity, and increase of caffeic and *p*-coumaric ones results in the decrease of colour intensity.

However, the correlation between the hue of wine and phenolic acid content was quite different. Namely, the value of hue was more intense in the cases when the content of caffeic and *p*-coumaric acid was higher, and it was less intense in the case of increased gallic, protocatechuic, vanillic and ferulic acid content.

(Notice: The same results concerning phenolic acids were obtained from these authors in an experiment performed during the production and ageing of the wines of the above mentioned varieties at the same vintage in vinery »Jedinstvo« at Smokvica, the island of Korčula, Dalmatia).

Conclusions

By using high-performance liquid chromatography the following phenolic acids were identified in autochthonous Croatian white wines, cultivars Pošip and Rukatac: gallic, protocatechuic, vanillic, caffeic, *p*-coumaric and ferulic. In addition, changes in their content during wine making were registered. It was found that there is a difference between HBA group and HCA group content as well as in their influence on wine colour (colour intensity and hue). In the juice the total content of HBA was higher than total content of HCA. During ageing of wine in glass bottles there was increase of HCA content and decrease of HBA content. Positive linear correlation between colour intensity and gallic, protocatechuic, vanillic and ferulic acid content was registered in both Pošip and Rukatac wines.

Acknowledgments

This work was supported by the Ministry of Science and Technology of the Republic of Croatia, projects no. 009101 and 058505.

References

1. D. A. Heatherbell, *Confructa, 3* (1984) 192.
2. J. J. Macheix, A. Fleuriet, J. Billiot: *Fruit Phenolics*, CRS Press, Boca Raton, FL (1990) pp.17–23.
3. J. J. L. Cilliers, V. L. Singleton, *Am. J. Enol. Vitic. 41* (1990) 84–86.
4. R. M. Lamuela-Reventos, A. L. Waterhouse, *Am. J. Enol. Vitic. 45* (1994) 1–5.
5. V. L. Singleton, E. Trousdale, *Am. J. Enol. Vitic. 34* (1983) 27–34.
6. G. J. Soleas, J. Dam, M. Carcey, D. M. Goldberg, *J. Agric. Food Chem. 45* (1997) 3871–3880.
7. D. A. Guillen, F. Merello, C. G. Barroso, J. A. Perez-Bustamante, *J. Agric. Food Chem. 45* (1997) 403–406.
8. *Official Journal of European Communities, No 2676/90*; Ed. Commission regulation (EEZ) (1990) document 390 R 2676.
9. V. L. Singleton, J. A. Rossi, *Am. J. Enol. Vitic. 16* (1965) 144.
10. T. C. Somers, E. Verette, K. F. Pocock, *J. Sci. Food Agric. 40* (1987) 67–78.
11. V. F. Cheynier, E. K. Trousalde, V. L. Singleton, M. J. Salgues, R. Wylde, *J. Agric. Food Chem. 34* (1986) 217–221.
12. L. Lazaro, S. Almela, S. Javaloy, J. M. Lopez Roca, *Sci. Aliment. 10* (1990) 89–98.

Identifikacija fenolnih kiselina i promjene njihovih udjela tijekom fermentacije i dozrijevanja bijelih vina Pošip i Rukatac

Sažetak

Provedena je identifikacija fenolnih kiselina i praćene su promjene njihovih udjela tijekom proizvodnje vina iz hrvatskih autohtonih sorata Pošip i Rukatac (*Vitis vinifera*, L.). U obje sorte (Pošip, Rukatac) identificirane su sljedeće fenolne kiseline: galna, protokatehinska i vanilinska kao hidroksibenzojeve kiseline odnosno kafeinska, p-kumarinska i ferulična kao hidroksicimetne kiseline. Nađene su razlike u sastavu između hidroksibenzojeve i hidroksicimetne skupine kiselina te između njihova utjecaja na boju vina (intenzitet boje i nijansa).