Scientific Paper

Determination of Polyphenols in White Grape Berries cv. Rebula†

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Abstract

Rebula white grapes polyphenols are determined for the first time. We analyzed grapes sampled from 13 different vineyards in Goriška Brda. Total polyphenols were determined according to the Folin-Ciocalteu method. For the individual polyphenols determination we used high performance liquid chromatography (HPLC) - diode array detection (DAD) technique. The separation of polyphenols from grapes was performed using narrow bore C18 Luna column (Phenomenex, 2 x 150 mm, 3µm) and monitored at 320 nm. The majority of white grapes polyphenols was represented by four hydroxycinnamic acids (HCAs). According to their UV-Vis spectra and chromatographic retention properties they corresponded to *trans*-caftaric, *trans*-coutaric, *cis*-coutaric and *trans*-fertaric acid. To confirm the identity of separated polyphenols, they were also directed to the ion trap mass spectrophotometer (Finnigan LCQ Deca) fitted with electrospray ionization (ESI) probe. Spectra were recorded in negative mode between *m/z* 100 and *m/z* 500. The mass spectrometer was programmed to do two consecutive scans: a full mass (MS) and an MS^2 scan of the most abundant ion in the full mass spectra. The results confirmed the assumptive identification of three HCAs; *trans*-caftaric, *trans*-coutaric and *trans*-fertaric acids in white grape berries cv. Rebula based on HPLC-DAD analysis.

Key words: White grapes, HPLC-DAD, MS/MS, hydroxycinnamic acids.

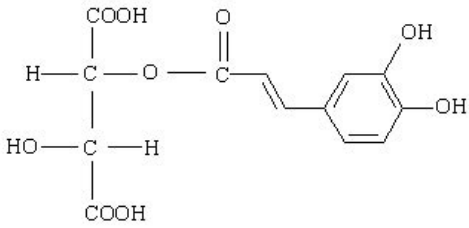
Introduction

Wine production is one of the most important agricultural activities in Slovenia. In the wine region in the west part of Slovenia (northern Primorska) are Rebula grapes (old cultivar) the most common and important white grapes, especially for the Goriška Brda region. Therefore it is not unexpected that the white wine production in this region is mainly based on this type of grapes.

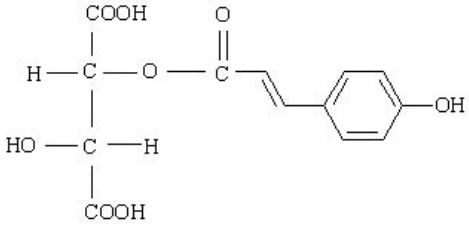
White wines are usually made from the free running juice, without grape mash, having no contact with the grape skins. This was thought to be the main reason for the relatively low polyphenol content and thus for the lower antioxidant activity of white wine in comparison to red wine.[1,2,3] The white wines usually contain on average 225 mg/L of total polyphenols (as gallic acid) on the contrary to red wines, which contain on average 1800 mg of total polyphenols (as gallic acid)/L.[4] Lately it has been shown that processing white wine by imposing a short period of grape skin contact in the presence of alcohol produces polyphenol-rich white wine with antioxidant characteristics similar to those of red wine.[2]

Tartaric esters of hydroxycinnamic acids (HCAs) represent the main non-flavonoid polyphenols in white grapes, mainly concentrated in the grapes flesh and thus in the wines made from white varieties (or produced without maceration of the solid parts). They represent 80% of all polyphenols of white grapes juice.[5,7] The four most abundant ones are *trans*-caftaric, *trans*- and *cis*-coutaric and *trans*-fertaric acids (Figure 1). They are involved in the browning reactions of must and wine, are precursors of volatile phenols and have antimicrobial and antioxidant activity.[1,8,9] Their antioxidant properties may exert a positive health effect that is attributed to moderate wine consumption.[10]

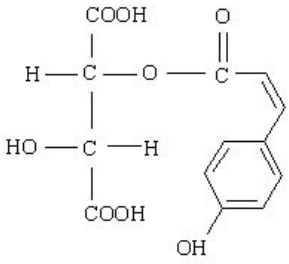
White grape juice HCAs were investigated a lot in the past.[7-9,11-14] The main HCA was in different cultivars *trans*-caftaric acid, ranging from 16-299 mg/L, followed by coutaric acids (*trans*- and *cis*-), which were found to vary between 18-44.0 mg/L. *Trans*-fertaric acid was present in white grapes in much lower amounts (1.2-15.9 mg/L).[8,11]



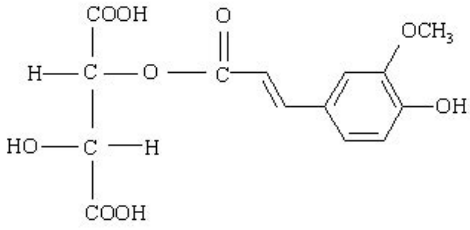
trans-caftaric acid



trans-p-coutaric acid



cis-p-coutaric acid



trans-fertaric acid

Figure 1. Major HCAs of white grapes.[1]

The analysis of phenolic compounds in grapes is of considerable commercial importance, since it is known that they contribute to the flavor of white wine.[1]

A common spectrophotometric method for total polyphenol content according to Folin-Ciocalteu has been widely used in the area of enology and viticulture.[4] Unfortunately this method is not specific, since all the polyphenols (reducing components) can contribute to the final result. More specific is reverse phase-high performance liquid chromatography (RP-HPLC). This technique represents the most popular and reliable technique for individual white grape hydroxycinnamates assay according to the literature data.[7-9,11-14] The chromatographic separation and detection of plant polyphenols have been constantly improving in the following years. Nowadays the HPLC separation of polyphenols is usually coupled with diode array detection (DAD) at different wavelengths, specific for the polyphenols.[15] Other methods of detection, like mass spectrometry (MS), have been developed and used in a number of plant polyphenol analyses, especially in the recent years. The HPLC-MS/MS equipment has become more accessible for the scientific purposes. MS together with tandem MS/MS approach is gaining popularity, since more specific identification can be provided with the molar weights of eluted peaks and their fragments. A lack of HCAs standards is a huge problem in their basic chromatographic identification. Among all the HCAs present in white grapes is only *trans*-caftaric acid commercially available. In addition these compounds have similar UV-Vis characteristics, therefore their identification, based on UV-Vis spectra is unfortunately not enough to make serious conclusions.

As it was already mentioned, Rebula is very old grape cultivar, quite spread and popular in Goriška Brda region. Rebula grape can be found only in this part of Slovenia. It represents our history and national inheritance and as such could be used for promotion of Slovenia. For that we need more data about local grapes, especially in the area of polyphenols, which are important constituents of grape. As already stated, they influence the wine sensory characteristics (flavor, coulor and taste) but they have also antimicrobial and antioxidant activity.[1,8] Polyphenol potential of Rebula grapes, as well as others' domestic cultivars (Pinela, Zelen, Glera, Verduc, etc.) was not investigated very much in the past. In fact, domestic cultivars like Rebula, already well adapted to the area of growing, could contain larger amounts of polyphenols, metabolites that are known to be stimulated by stress conditions in the environment.[15]

We employed different analytical techniques to determine the polyphenol potential and content of local Rebula grape berries. A representative grape sample was obtained from 13 different vineyards in Goriška Brda. In addition to HPLC-DAD-ESI-MS-MS/MS analysis of individual HCAs, total polyphenols assay according to spectrophotometric Folin-Ciocalteu method[4] was chosen to position the Rebula grapes to other white grape varieties. In this study we present for the first time the HCAs composition and total polyphenol content of Rebula grapes.

Results and discussion

The HPLC-DAD analysis of Rebula juice has showed the presence of one major and three minor peaks in the first part of chromatographic analysis (Figure 2), having their maximum absorbance in the 300-330 nm region of UV-Vis spectra (Figure 3). Considering that information, we classified them to the HCAs, since only that group of polyphenols absorbs in the 300-330 nm region.[15]

The recorded UV-Vis spectra of peaks 1 and 4 were quite similar, as well as those recorded for peaks 2 and 3 (Figure 3). In accordance to data about spectral and chromatographic properties of white grape HCAs' we tentatively concluded that peak 1 (λ_{max} = 250 nm and 329 nm, shoulder at 301 nm) corresponds to *trans*-caftaric acid, peak 2 (λ_{max} = 247 and 314 nm) to *cis*-coutaric acid, peak 3 to *trans*-coutaric acid (λ_{max} = 245 and 315 nm) and finally peak 4 to *trans*-fertaric acid (λ_{max} = 250 nm and 329 nm, shoulder at 301 nm).[11-15] Same chromatographic profile (HPLC-DAD) of major HCAs (peaks 1, 3 and 4) was observed in whole berries of Rebula sample (combined juice and three sequenced skin extracts). Peak 2, tentatively *cis*-coutaric acid, was not detected in the same sample. Other white grape cultivars (Chardonnay, Pinot Blanc, Sauvignon, etc.) are known to contain *cis*-coutaric acid mainly in the grape skin.[8,9] The HPLC-DAD results of total grape sample haven't confirmed its presence in Rebula skins.

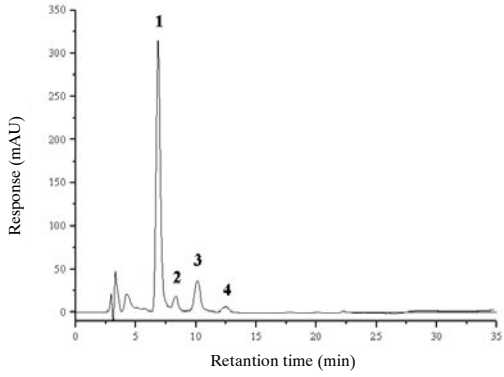


Figure 2. HPLC-DAD separation of Rebula white grape juice at full ripeness, monitored at 320 nm. Numbers denote the following tentative components (identified based on UV-Vis and chromatographic properties): 1: *trans*-caftaric acid; 2: *cis*-coutaric acid 3: *trans*-coutaric acid; 4: *trans*-fertaric acid.

Our assumptions about some Rebula HCAs identifications were tentatively confirmed by the ES-MS and MS/MS analysis of these HCAs (peaks 1, 3 and 4 on figure 2). They produced molecular (and fragment) ions with *m*/*z* ratios of 310.9 (179, 149) (Figure 4), 295 (163) and 324.9 (193.1) that tentatively corresponded to molecular weights of caftaric acid, coutaric acid and fertaric acid, respectively. The white grape HCAs in the nature mainly exist in the *trans*-form.[15] Therefore we concluded that HCAs' peaks 1, 3 and 4 were most likely *trans*-versions of HCAs. According to the UV-Vis (Figure 3), chromatographic retention properties (Figure 2) and literature[5,7,8,13,16,17] we would expect peak 2 to have the same molecular (and fragmentation)

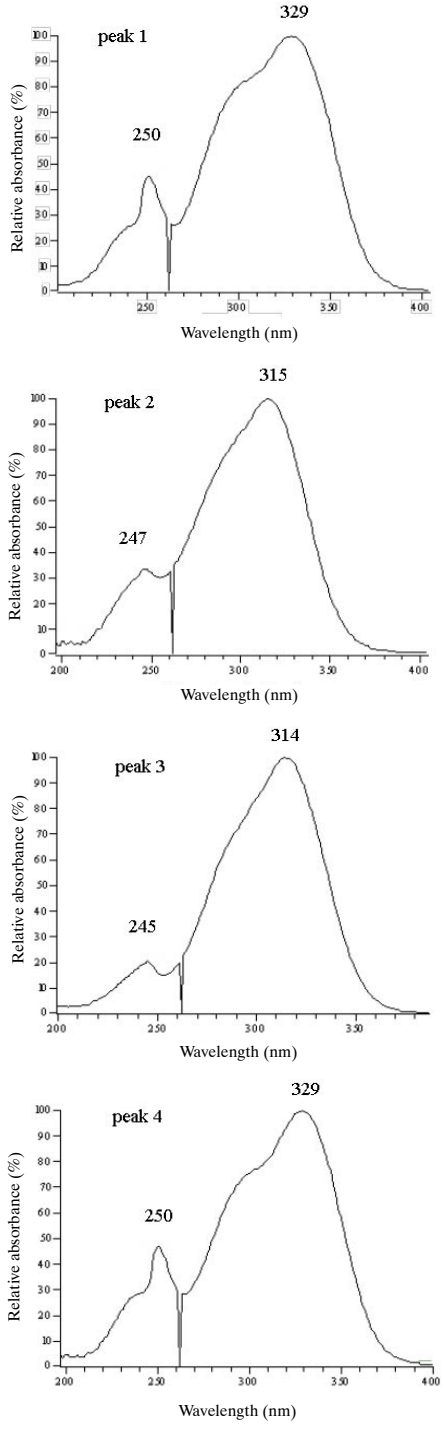


Figure 3. UV-Vis spectra of monitored HCAs in the Rebula grapes juice. The numbers correspond to the peaks of figure 2.

ions like peak 3 which was not the case. Peak 3 had the molecular ion of 298 *m*/*z* and fragmentation ion of 245 *m*/*z*, which corresponded to epicatechin. Therefore we cannot confirm the tentative identification of peak 2 as *cis*-coutaric acid as previously proposed. To test whether the absence of *cis*-coutaric acid in Rebula grapes is genetic, we analyzed a Chardonnay white grape juice in the same manner (HPLC-ESI-MS-MS/MS) as Rebula juice. The MS-MS/MS identification confirmed the presence of *cis*-coutaric acid in this grape cultivar (data not shown), which has been already described.[8,13] Again it has been proved that identification of plant polyphenols relying only on chromatographic and UV-Vis characteristics can sometimes be misleading.

To check the linearity of the response of detector, a linear regression analysis of absolute areas versus concentration of the *trans*-caftaric acid was used. The linearity was determined by the square correlation coefficients of the calibration curve generated by three repeated injections of standard solutions at 7 concentrations levels, with concentration range expected in real samples ($R^2 = 0.9994$).

The limit of detection (LOD) was calculated from the relation: LOD = 3(SD/S), where S stands for the slope of the calibration curve (5-100 mg *trans*-caftaric acid/L juice) and SD is the standard deviation of y-intercept of the corresponding regression line. For *trans*-caftaric acid in the grape juice matrix the LOD was calculated to be 3 mg/L. These results suggest that the proposed HPLC method is sufficiently sensitive for the determination of HCAs in grapes.

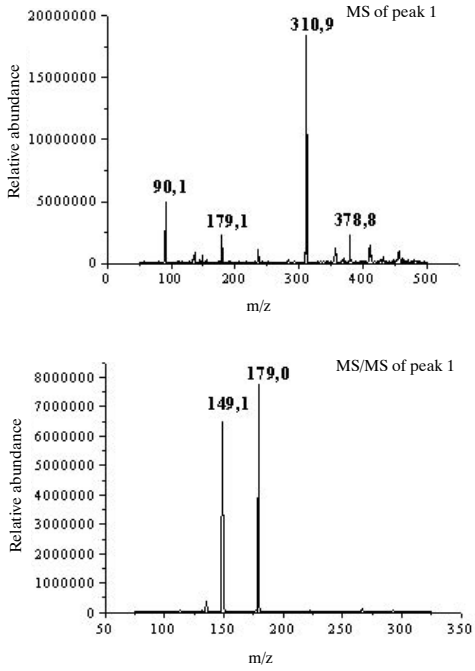


Figure 4. MS and MS/MS spectra of chromatographic peak 1 from figure 2.

The matrix effect on grape juice HCAs HPLC determination was studied with the use of standard addition method and evaluated based on the recovery of *trans*-caftaric acid. Known amounts of *trans*-caftaric acid were added to a grape juice at three different concentration levels (5, 50 and 100 mg/L). The recovery measurements were done only for *trans*-caftaric acid, the only commercially available grape HCA standard and also representing more than 80% of all the HCAs in grape juice.[7] Calculated recovery of 102 ± 2% has shown only minor effect of matrix on the HPLC determination of *trans*-caftaric acid in grape juice. Precision was studied in a real sample in two ways: peak areas and retention times. The repeatability of peak areas and retention times were calculated by the RSD (%) of six injections carried out on the same day. The RSD of all peaks through all day was 1.3% for the peak areas and their retention times as well.

White wines are usually made from the free running juice, without grape mash, having no contact with the grape skins.[1,2,3] In the recent years some vineries include the short term maceration in the white wine making process.[2] That is why we present the quantity and quality results of major polyphenols in the Rebula juice as well as whole berries (without seeds) (Table 1).

The results of total polyphenol content (as mg gallic acid/L juice) and total HCAs content (as mg *trans*-caftaric acid/L juice) presented in the table 1 show that Rebula juice is not a very rich source of polyphenols, which is usual fact for white grape juices.[2,4] There are no data available describing the total polyphenol content (according to Folin-Ciocalteu method) in white grapes juice, therefore we compared our results to the nearest approximate, the white wine total polyphenols. As stated in the literature white wines contain total polyphenols between 40-1200 mg gallic acid/L and Rebula wine was found to contain around 100 mg of total polyphenols/L (as gallic acid).[4,18] Our results of Rebula juice total polyphenols (118.6 mg gallic acid/L) correspond to these data. It is normal that wine contains lower amounts of polyphenols, since degradation might occur during fermentation process due to oxidation.[19] For comparison, red wines can contain 190-3800 mg of total polyphenols/L (as gallic acid).[4]

In 21 different white grape cultivars juice was the main HCA *trans*-caftaric acid, ranging from 16-295 mg/L, followed by coutaric acids (*trans*- and *cis*-), which were found to vary between 18-44.0 mg/L. *Trans*-fertaric acid was present in white grapes juice in much lower amounts (1.2-15.9 mg/L).[11,13] The same HCAs and in similar amounts (expressed as mg *trans*-caftaric acid/L) were also found in Rebula grapes. On average was their majority in the juice represented by *trans*-caftaric acid (83.8 mg/L), followed by *trans*-coutaric acid with 12.6 mg/L and finally there were 2.4 mg/L of *trans*-fertaric

acid. In another study there were 135 to 281 mg of total HCAs/L juice quantified in 7 different grape cultivars (Pinot Blanc, Chardonnay, Sauvignon Blanc, etc.).[8] The Rebula juice contained 99.0 mg of total HCAs/L (as *trans*-caftaric acid) and as such was not exceptional to other white grape cultivars in quality and quantity of polyphenols described in the literature.[8,11,13]

The comparison of levels of caftaric, coutaric and fertaric acid isomers in the juice is essential since polyphenol oxidase does not exhibit the same affinity to all substrates, as do *o*-diphenols which are involved in the browning wine phenomena.[17,20,21]

The ratio of *trans*-caftaric acid (to all measured HCAs) from 13 different vineyards was on average 84.6 ± 1.2%, which is according to literature a good background of grape to have high browning capacity latter in wine.[17] The grapes with higher proportions of *trans*-caftaric acid among all HCAs (80-90%) in the juice are known to posses higher browning capacity.[17] This ratio was quite stable in the Rebula juice sampled from 13 selected vineyards (1.6% RSD), showing that this characteristic does not depend on the place of Rebula grape growing and could be cultivar dependent. This has already been confirmed for other white grape cultivars.[13]

Table 1. The results of quantification of polyphenols in Rebula juice and grape berries from Goriška Brda (mean values and standard deviations, n = 13).

	juice (mg/L)	grape (mg/L)
Total polyphenols [α]	118.6 ± 22	537.7 ± 114.8
Total HCAs [β]	99.0 ± 15.1	175.3 ± 26.4
% *trans*-caftaric acid [γ]	84.6 ± 1.2	80.2 ± 1.2
% *trans*-coutaric acid [γ]	12.7 ± 1.1	18.1 ± 1.2
% *trans*-fertaric acid [γ]	2.4 ± 0.3	1.7 ± 0.3

[α] As gallic acid equivalents.
[β] As *trans*-caftaric acid equivalents.
[γ] Percent of concentration of total HCAs measured.

In the table 1 we compare the concentration of total HCAs in Rebula grape berries (without seeds) (175.3 mg *trans*-caftaric acid/L) to those determined in Rebula juice. Total amount of HCAs in Rebula grapes was in 43.5% represented by those located in the berry skin, as already reported for other white grape cultivars like Pinot Blanc, Chardonnay, Sauvignon Blanc and Riesling.[8] In the Rebula grapes higher ratio of *trans*-coutaric acid to total HCAs amount (18.1%) compared to grape juice (12.7%) was determined (Table 1). These differences are quite clear if we take in account known facts about *trans*-coutaric acid being preferentially localized in grape skin and having lower ability to extract from grape skin to grape juice compared to *trans*-caftaric and *trans*-fertaric acids.[8,9] According to the literature, *trans*-fertaric acid is 70.9% extracted from grape to juice, followed by *trans*-caftaric acid with 59.0% yield and both coutaric isomers (36.2% yield for *trans*- and 33.6% for *cis*- respectively).[8] Rebula juice *trans*-cafaric acid represented 59.5% of its original amounts in the grapes, *trans*-fertaric acid 79.7% and *trans*-coutaric only 39.8%. Based on these results we concluded that Rebula grape berries as a whole are not rich source of HCAs (175.3 mg *trans*-caftaric acid/L). These HCAs have similar extraction properties (from grape to juice) compared to other white grape cultivars like Pinot Blanc, Chardonnay, Sauvignon Blanc and Riesling.[8]

The difference between the total polyphenols (as mg gallic acid/L) and total HCAs (as mg *trans*-caftaric acid/L) in the juice and berries is due to the non-selectivity of the Folin-Ciocalteu method. This analytical method comprises all the polyphenols in the juice and grapes, not only the HCAs.[4,22] The HCAs are according to the literature predominant polyphenols in the white grape flesh (more than 80% of all polyphenols).[5,7] The majority of white grape polyphenols is located in the skin (flavonols, flavan-3-ol, oligomeric procianidins).[2,7,23,24] The profile of polyphenols in the Rebula skins is most certainly in favor of other polyphenols in addition to HCAs (Table 1). If we compare HPLC and spetrophotometric results we can see 3.5 times higher amount of total polyphenols in Rebula skins than in juice and less than once higher amount of total HCAs. The deviations of spectrophotometric results to those obtained by chromatography could be a consequence of different individual polyphenols present in the samples possessing variable reducing properties. The precise HPLC profile of polyphenols in the skin was not measured, only the HCAs profile, therefore we cannot conclude which polyphenols contributed to total amount of polyphenols present in the Rebula grapes skin.

Experimental

Rebula grapes were sampled from 13 different vineyards in Goriška Brda region in the beginning of October 2004. A vineyard sample was represented with 10 clusters of grapes. Ten undamaged berries were carefully sniped from each cluster of a vineyard sample without breaking the berry skin forming the final 100-berry sample. This sample was put in a porcelain dish and 2 g of potassium metabisulfite was added to inhibit the polyphenol oxidase. Skins and pulp were crushed gently and than the juice was collected, its volume measured and than a sample (20 mL) was taken for the HPLC and spectrophotometric assay. The skins were than extracted three times for 10 minutes with 60 mL portions of 6% (v/v) perchloric acid in water. Previously collected juice and three sequenced skin extracts were

combined and the total volume was measured to refer the data (mg/L) to the volume of the juice of the berry sample. This sample was considered as representative of the total content (100%) of HCAs in grape berries.[8] The juice and the combined sample (juice and skin extracts) were than subjected to spectrophotometric Folin-Ciocalteu method for total polyphenol assay[4] and HPLC-DAD analysis for the individual HCAs. Separated HCAs from Rebula juice were tentatively identified by HPLC-ESI-MS/MS technique.

Total polyphenol determination according to Folin-Ciocalteu[4] is based on reduction of yellow Folin-Ciocalteu reagent in basic environment to blue pigments by polyphenols. The reducing polar impurities (acids, sugars, SO_2, proteins) present in the juice can interfere the results.[22] Therefore, we had to remove them from the juice with the solid phase extraction (SPE) (Phenomenex Strata X 200 mg).

The SPE columns were preconditioned with 5 mL of methanol, followed with 5 mL of deionised (DI) water and than 3 mL of free run juice (and combined sample) was slowly added. The impurities were removed with 1 mL of DI water and finally the polyphenols were eluted in 100 mL calibrated flask with 5 mL of methanol. The methanol extract was diluted with 60 mL of DI water, followed by 5 mL of Folin-Ciocalteu reagent, well stirred and after 0.5 ~ 8 minutes 15 mL of 20% Na_2CO_3 was added. The solution was brought to 100 mL with DI water, stirred and the absorbance of a solution was read at 765 nm after 120 min. The calibration was performed with gallic acid and the total polyphenols were presented as mg gallic acid/L grape juice.[4]

For the individual HCAs determination we used high performance liquid chromatography (HPLC) - diode array detection (DAD) technique. The juice was filtered through 0.45 μm PTFE syringe filters (Chromafil) prior to analysis. The equipment we used was an HP 1100 liquid chromatograph, coupled with a DAD. The separation of polyphenols from direct inject of 5 μL grape juice was performed using narrow bore C18 Luna column (Phenomenex, 2 x 150 mm, 3 μm), protected by a guard column packed with the same material. The column was kept at 40 °C. Eluent (A) was 2.3% formic acid (pH 2,1) and (B) acetonitrile (HPLC grade), flow rate 0,13 mL/min. The gradient elution was as follows: 0 minutes 12% B; 15 minutes, 22% B; 20 minutes, 55% B; 25 minutes 12% B; 50 minutes 12% B. Since no significant effects from matrix were observed, the HCAs were quantified by preparing calibration curves with *trans*-caftaric acid (0.8 -180 mg/L) in mobile phase.

The separated Rebula juice HCAs were also subjected to the ion trap mass spectrophotometer (Finnigan LCQ Deca) fitted with ESI probe. For the separation, Thermo Separation Products HPLC system was employed, involving a gradient pump (P4000), an autosampler (AS 3000) with 5 μL injection volume. The column was the same as for HPLC-DAD analysis, the gradient solvent system as well. The column was kept at ambient temperature, and there was no pre-column installed (to keep the backpressure within the limit frame 3500 psi). The sample of grape juice was 3-times pre-concentrated with SPE like previously described in the sample preparation for Folin-Ciocalteu method. The methanol SPE extract was evaporated at 30 °C in the vacuum, and the dried polyphenols were diluted back in 1 mL of DI water. This sample was than subjected to the HPLC separation and consequent MS and tandem MS/MS analysis.

Spectra were registered in the negative mode over range *m*/*z* 100-500. Nitrogen gas was used as sheath and auxiliary gas. The capillary temperature was 225 °C, the source voltage 5 kV, source current 80 μA, the capillary voltage –15 V, tube lens offset –30.00 V. The mass spectrometer was programmed to do a series of two scans: MS and MS/MS scan of the most abundant ion in the MS scan. The collision gas was helium and the energy of collision was 28%.

Conclusions

Spectro(photo)metric techniques were applied for the determination of Rebula white grape berries polyphenols for the first time. A DAD in addition to MS/MS analysis of separated peaks was convincing enough to see the tentative identification of major Rebula grape berries polyphenols, the HCAs, which was also in accordance to the literature. Results have shown that polyphenols in Rebula juice and berries do not differ to other white grapes cultivars, in quantity as well as quality of polyphenols.[4-9,11-14,17,18] Compared to other white grape cultivars is the Rebula grape HCAs content not very high. The total polyphenol results have shown 3.5 times higher amount of total polyphenols in Rebula skins compared to the grape flesh. The exact nature of these polyphenols remains to be studied in the future. Comparison of chromatographic and spectro(photo)metric results indicated the profile of polyphenols in Rebula skins in favor of other polyphenols in addition to HCAs. More polyphenols in the grape berry skin compared to grape flesh have been already shown in the literature.[2,7,23,24] The physiological explanation for this is the protection of the grape inside. The initial hypothesis in this study has not been confirmed; the Rebula grape polyphenols are not exceptional to other white grape cultivars. As already told, the white wine production in Goriška Brda region is mainly based on this local sort of grapes and polyphenols (HCAs) are important constituents of grapes, very much involved in the formation of white wine sensory

characteristics (flavor, colour, taste). Our results are the first report about these important compounds in Rebula grapes and as such could contribute to Rebula wine making process when necessary.

Acknowledgements

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References

1. U. Vrhovšek, *Research Reports* **1999**, *73*, 39–47.
2. B. Fuhrman, N. Volkova, A. Suraski, M. Aviram, *J. Agric. Food Chem*. **2001**, *49*, 3164–3168.
3. J. A. Vinson, B. A. Hontz, *J. Agric. Food Chem*. **1995**, *43*, 401–403.
4. M. A. Amerine, C. S. Ough, Methods for analysis musts and wines, John Wiley & Sons, New York, **1988**, pp. 204–206**.**
5. C. Y. Lee, A. Jaworski, *Am. J. Enol. Vitic*. **1987**, *38*, 277–281.
6. J. L. Robichaud, A. C. Noble, *J. Agric. Food Chem*. **1990**, *53*, 343–353.
7. C. Betes-Saura, C. Andres-Lacueva, R. M. Lamuela-Raventos, *J. Agric. Food Chem*. **1996**, *44*, 3040–3046.
8. U. Vrhovšek, *J. Agric. Food Chem*. **1998**, *46*, 4203–4208.
9. R. Di Stefano, G. Maggiorotto, *Riv. Vitic. Enol*. **1995**, *2*, 51–56.
10. E. N. Frankel, A. L. Waterhouse, P. L. Teissedre, *J. Agric. Food Chem.* **1995***, 43,* 890–894.
11. B. Y. Ong, C. W. Nagel, *Am. J. Enol. Vitic.* **1978**, *29*, 277–281.
12. C. W. Nagel, J. D. Baranowski, L. W. Wulf, J. R. Powers, *Am. J. Enol. Vitic*. **1979**, *30*, 198–201.
13. V. L. Singleton, J. Zaya, E. K. Trousdale, *Phytochemistry* **1986**, *25*, 2127–2133.
14. T. Ch. Somers, E. Verette, K. F. Pocock, *J. Sci. Food Agric*. **1987**, *40*, 67–78.
15. J. J. Macheix, A. Fleuriet, J. Billot, Fruit Phenolics, CRC Press, Boca Raton, Florida, **1990**, pp. 1–34.
16. J. D. Baranowski, C. W. Nagel. *Am. J. Enol. Vitic*. **1981**, *3*, 5–13.
17. F. M. Romeyer, J. J. Macheix, J. P. Goiffon, C. C. Reminiac, J. C. Sapis, *J. Agric. Food Chem*. **1983**, *31*, 346–349.
18. T. Košmerl, V. Francetič, *Primerjava dveh metod določanja skupnih polifenolov v vinu*, in: P. Glavič, D. Brodnjak-Vončina (Eds.), Slovenski kemijski dnevi 2001, Maribor, 20. in 21. september 2001, Zbornik referatov s posvetovanja, Slovensko kemijsko društvo, Maribor, **2001**, pp. 790–795.
19. U. Vrhovšek, *Sodobno kmetijstvo* **1998**, *31, 5*, 226-231.
20. R. A. Arnold, A. C. Noble, V. L. Singleton, *J. Agric. Food. Chem*. **1980**, *28*, 675–678.
21. S. Okamura, M. Watanabe, *Agric. Biol. Chem*. **1981**, *45*, 2063–2070.
22. A. Rigo, F. Vianello, G. Clementi, M. Rosseto, M. Scarpa, U. Vrhovšek, F. Mattivi, *J. Agric. Food Chem*. **2000,** *48*, 1996–2002.
23. V. Cheynier, J. Rigaud, *Am. J. Enol. Vitic*. **1986**, *37*, 248–252.
24. A. G. H. Lea, P. Bridle, C. F. Timberlake, V. L. Singleton, *Am. J. Enol. Vitic.* **1979**, *30*, 289–300.

Povzetek

Opisana je sestava in količina polifenolov grozdja Rebula. Analizirali smo grozdje, ki smo ga vzorčili v Goriških Brdih v 13 vinogradih. Skupne polifenole smo določali po metodi Folin-Ciocalteu. K določanju identifikacije posameznih polifenolov smo pristopili s pomočjo tekočinske kromatografije visoke ločljivosti (HPLC) v kombinaciji z detektorjem s serijo diod (DAD). Polifenole jagod grozdja smo ločili na koloni C18 Luna (Phenomenex, 2 x 150 mm, 3μm) in njihovo kromatografsko ločbo spremljali pri 320 nm. Glavnino polifenolov so predstavljale štiri hidroksicimetne kisline (HCK). Po ujemanju njihovih UV-Vis spektrov in elucijskega zaporedja HPLC separacije na C18 koloni smo jih prepoznali kot *trans*-kaftarno, *trans*-kutarno, *cis*-kutarno in *trans*-fertarno kislino. Za dodatno potrditev domnevne identifikacije HCK smo ločene polifenole vodili v masni spektrometer z ionsko pastjo (Finnigan LCQ Deca) z ionizacijo v elektro spreju (ESI). Spektre smo snemali pri negativni napetosti v območju med 100 *m*/*z* in 500 *m*/*z*. Masni spektrometer je izvedel dve zaporedni seriji meritev spektra: MS in tandem MS analizo (MS^2) najbolj intenzivnega iona v MS spektru. Rezulati so delno potrdili domnevno sestavo HCK belega grozdja Rebule določeno na podlagi HPLC-DAD analiz. V vzorcu grozdja Rebula smo na podlagi mase ionov in njihovih fragmentov določili *trans*-kaftarno, *trans*-kutarno in *trans*-fertarno kislino.