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Characterization of some Italian *V. vinifera* L. grape varieties on the basis of their flavonol profile

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Abstract. "Suspect screening metabolomics" is a mid-way approach between "targeted" and "untargeted" analysis. For this aim, a new database of putative grape and wine metabolites (*GrapeMetabolomics*) was expressly constructed. Currently, this database contains around 1,100 compounds. By performing UHPLC/QTOF mass spectrometry analysis in both positive and negative ionization mode, in a grape extract averaging 320–450 putative compounds are identified. Most of these compounds are important grape metabolites, including flavonols, anthocyanins, and stilbene derivatives. In the present study, this approach was focalized on the characterization of flavonols of 18 important Italian red and white grape varieties and the method provided the identification of 15 flavonols. By performing statistical analysis (Principal Component Analysis and Cluster Analysis), the effect of the variety on the flavonol composition of the grapes was studied. Both the red and white samples fell into three different groups, respectively, on the basis of their flavonol profiles. Because the samples were cultivated in the same vineyard, their profile potentially was not affected by cultural or environmental factors. Anyway, these preliminary results will have to be confirmed by the study of grape samples collected in different years and from different vineyards.

1. Introduction

Suspect screening metabolomics" is a mid-way approach between "targeted" and "untargeted" analysis. For this aim, a new database of putative grape and wine metabolites (*GrapeMetabolomics*) was expressly constructed. Currently, this database contains around 1,100 compounds. By performing Ultra-High Performance Liquid Chromatography/Quadrupole Time of Flight Mass Spectrometry (UHPLC/QTOF) analysis in both positive and negative ionization mode, averaging 320–450 putative compounds are identified in a grape extract, depending on the variety. Most of these compounds are important grape metabolites: e.g., in Raboso Piave grape extract around 80 polyphenols were identified with an identification score of 95–99%, including flavonols, anthocyanins, and stilbene derivatives [1]. This profound knowledge of the grape chemistry can be effectively applied in the study of grapevine diseases, cultural practices, and for monitoring of climate change effects.

Flavonols have antioxidant activity and are bioactive compounds occurring in dietary plants useful for human health and nutrition [2]. Quercetin blocks the aggregation of human platelets by ADP and thrombin, and proved to be an inhibitor of carcinogens and cancer cell growth in many experimental and human tumors [3]. *V. vinifera* grape flavonols have also been widely studied because are involved in the phenomenon of copigmentation with anthocyanins, which induces a bathochromic shift to purplish hues of red wines [4,5]. These compounds are also secondary metabolites widely studied as markers in grape chemotaxonomy [6–8].

In a recent work, the method was used to study flavonol profiles of several hybrid grape varieties and 24 compounds were identified, four of them were found in grape for the first time [9]. In the present study, this approach was focalized on the characterization of flavonol profiles of several important Italian grape varieties. By performing statistical analysis (Principal Component Analysis and Cluster Analysis), the effect of the variety on the grape flavonol composition was studied.

2. Samples and methods

About 100 berries of the 18 red and white grape varieties listed in Table 1 were collected at full ripening in 2013 from the CRA-VIT Vine Germplasm Collection (Susegana, Treviso, Italy). For sample preparation, twenty berries were weighed; homogenized using liquid nitrogen and the resulting powder was immediately extracted with pure methanol in ratio 2:1 v/w under stirring for 20 min. After addition of 200 μL of 4′,5,7-trihydroxy flavanone 500 mg/L solution as internal standard, the sample was centrifuged at 10°C for 20 min. The solution was filtered

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Table 1. Investigated *V. vinifera* grape varieties.

Variety	Acronym	Berry color
Corvina	Cor	Red
Enantio	Ena	Red
Lambrusco Grasparossa	LaG	Red
Montepulciano	Mnt	Red
Nebbiolo	Neb	Red
Nero d'Avola	NdA	Red
Raboso Piave	Rab	Red
Rossese	Ros	Red
Uva di Troia	UvT	Red
Falanghina	Fal	White
Fiano	Fia	White
Garganega	Gar	White
Glera	Gle	White
Moscato Bianco	MoB	White
Tocai Friulano	ToF	White
Trebbiano Toscano	TrT	White
Verdicchio	Vrd	White
Vernaccia	Vnc	White

with Acrodisc GHP 0.22 μm filter (Waters) and collected in a vial for LC/MS analysis. For each sample two replicate analyses were performed.

Analyses were performed in both positive and negative ionization mode using an Agilent UHPLC 1290 Infinity ultra-high performance-liquid chromatography system coupled to Agilent 1290 Infinity Autosampler (G4226A) and Agilent 6540 accurate-mass Q-TOF Mass Spectrometer (nominal resolution 40,000) with Jet Stream Ionization source (Agilent Technologies, Santa Clara, CA). Chromatography was performed using a Zorbax reverse-phase column (RRHD SB-C18 3×150 mm, 1.8μ m) and mobile phase composed of A) 0.1% v/v aqueous formic acid, B) acetonitrile containing 0.1% v/v formic acid. QTOF conditions: sheath gas nitrogen 10 L/min at 400°C; drying gas nitrogen 8 L/min at 350°C; nebulizer pressure 60 psi, nozzle voltage 1 kV, capillary voltage 3.5 kV. Signals in the m/z 100–1700 range, were recorded.

Statistical analyses were performed by using PAST 3.01 software (Paleontological statistics software package for education and data analysis; Hammer, Ø., Harper, D.A.T., Ryan, P.D. 2001).

3. Results and discussion

Suspect screening metabolomics provided the identification of 15 compounds listed in Table 2. Figure 1 shows the extracted ion chromatogram of [M-H]$^-$ flavonol signals recorded in the UHPLC/QTOF analysis of Rossese grape extract.

In general, white grapes mainly contain flavonols in which the B-ring is mono- and di-substituted (kaempferol, quercetin, isorhamnetin), red grapes also contain tri-substituted compounds (myricetin, laricitrin, syringetin) [6, 10]. Due to the lack of available standards just a semi-quantitative analysis was performed, but the method was suitable to perform a comparative study among the varieties in order to identify those characterized by peculiar flavonol contents.

Table 2. Flavonols identified in the grape varieties investigated. Numbers correspond to the peaks in Fig. 1.

Flavonol	[M-H]$^-$ ion (m/z)
1. myricetin-3-*O*-glucuronide	493.0624
2. myricetin-3-*O*-glucoside	479.0831
3. dihydroquercetin-3-*O*-hexoside	465.1038
4. rutin	609.1461
5. quercetin-3-*O*-galactoside	463.0882
6. quercetin-3-*O*-glucuronide	477.0675
7. laricitrin-3-*O*-hexoside	493.0988
8. quercetin-3-*O*-glucoside	463.0882
9. dihydroquercetin-3-*O*-rhamnoside	449.1089
10. kaempferol-3-*O*-galactoside	447.0933
11. kaempferol-3-*O*-glucoside	447.0933
12. kaempferol-3-*O*-glucuronide	461.0725
13. syringetin-3-*O*-glucoside	507.1144
14. isorhamnetin-3-*O*-hexoside	477.1038
15. dihydrokaempferol-3-*O*-rhamnoside	433.1140

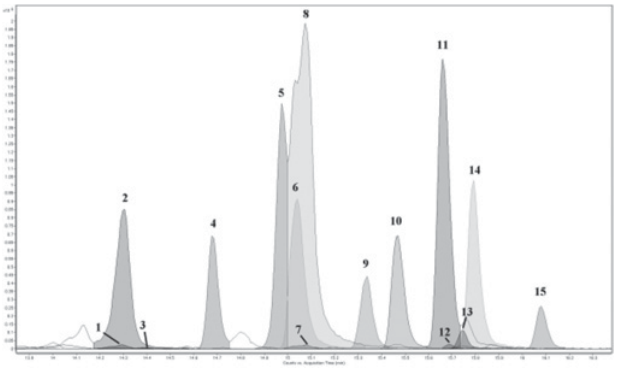

Figure 1. Extracted ion chromatogram of [M-H]- flavonol signals recorded in the UHPLC/QTOF analysis of Rossese grape extract.

By performing statistical analysis (Principal Component Analysis and Cluster Analysis) using the normalized signals of the compounds as variables, the effect of variety on the flavonol composition of grapes was studied. Figure 2 shows the PCA diagram of the red grape varieties. The first principal component (PC1) accounted for 50.2% and PC2 for 29.9% of the total variance, with cumulative variance of 80.1%. Cluster analysis divided the varieties into three groups: **A**) Montepulciano, Nebbiolo, Rossese, and Uva di Troia; **B**) Corvina, Nero d'Avola, Lambrusco Grasparossa, and Raboso Piave; **C**) Enantio (Fig. 2). In general, samples of group **A** had higher contents of quercetin-3-*O*-glucoside and kaempferol-3-*O*-glucoside, and lower myricetin-3-*O*-glucoside; group **B** was more heterogeneous and in general the samples had lower contents of flavonols; Enantio was characterized by high content of both myricetin-3-*O*-glucoside and quercetin-3-*O*-glucoside, and high total flavonol content in agreement with previous findings [6].

Figure 3 shows the PCA diagram of the nine white samples. In this case PC1 accounted for 67.5% and PC2 for 24% of the total variance, with cumulative variance of

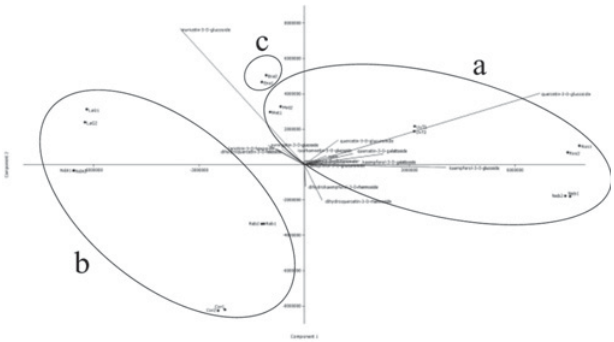

Figure 2. PCA plot of red grape varieties. PC1 accounted for 50.2% and PC2 for 29.9% of the total variance.

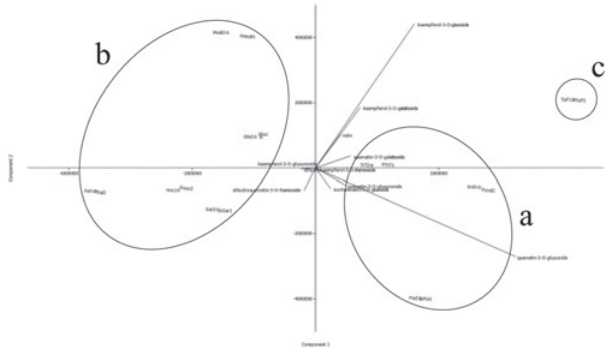

Figure 3. PCA plot of white grape varieties. PC1 accounted for 67.5% and PC2 for 24% of the total variance.

91.5%. Cluster analysis yet divided the varieties into three groups: **A**) Fiano, Trebbiano Toscano, and Verdicchio; **B**) Falanghina, Garganega, Glera, Moscato Bianco, and Vernaccia; **C**) Tocai Friulano. In general, samples of group **A** were characterized by higher quercetin-3-*O*-glucoside and group **B** by lower content of flavonols. Tocai Friulano grape showed the highest contents of flavonols, in particular rutin, quercetin-3-*O*-glucoside and glucuronide, kaempferol-3-*O*-glucoside.

4. Conclusions

Flavonols are an ubiquitous class of secondary metabolites used in grape chemotaxonomy because their profile is mostly influenced by genetic factors [11]. Both red and white samples investigated fell into three different groups, respectively, on the basis of their flavonol profile, in particular the myricetin-3-*O*-glucoside, quercetin-3-*O*-glucoside, and kaempferol-3-*O*-glucoside contents. All samples were cultivated in the same vineyard therefore potentially were not affected by cultural or environmental factors. Anyway, these preliminary results will have to be confirmed by the study of grape samples collected in different harvests and from different vineyards.

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