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Application of a Novel Small-Scale Sample Cleanup Procedure Prior to MALDI-TOF-MS for Rapid Pigment Fingerprinting of Red Wines

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Abstract This study evaluates the anthocyanin and derived pigment composition of *Vitis vinifera* red wines of Vranec, Merlot, and Cabernet Sauvignon produced in 2006, 2007, and 2008 vintages from the Tikveš wine region in the Republic of Macedonia. Their profile was established using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) technique. A total of 22 anthocyanins and derived pigments have been identified in the samples including 10 anthocyanins, 1 ethyl-bridged flavanol–anthocyanin adduct, and 11 pyranoanthocyanins. MALDI-TOF-MS analysis was performed after solid-phase extraction of the wines by using, for the first time, the Zip-Tip® C18 stationary phase, introducing a novel small-scale sample cleanup procedure prior to the rapid MALDI-TOF-MS fingerprinting of wine samples. 2′,4′,6′-Trihydroxyacetophenone (dissolved in acetonitrile/water 1:1, *v*/*v*) was used as a matrix. The qualitative screening of anthocyanins and derived pigments with MALDI-TOF-MS confirmed the presence of glucoside, acetylglucoside, and *p*-coumaroylglucoside derivatives of anthocyanins in the wine samples. Furthermore, pyranoanthocyanins formed by reactions of anthocyanins with pyruvic acid and acetaldehyde, as well as flavanol–pyranoanthocyanins and ethyl-bridged flavan-3-ol-anthocyanin adduct pigments have been detected in the samples.

Keywords Wine · Anthocyanins · Pyranoanthocyanins · Ethyl-bridged pigments · 2′,4′,6′-Trihydroxyacetophenone · MALDI-TOF-MS

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Introduction

The color of wine is a value of quality and the most influential factor for choosing wine in a market. It is mainly determined by the composition and concentration of anthocyanins. The main anthocyanins in wines from *Vitis vinifera* grape varieties are 3-*O*-glucosides, 3-*O*-acetylglucosides, 3-*O*-*p*-coumaroylglucosides, and, to a lesser extent, 3-*O*-caffeoylglucosides of delphinidin, cyanidin, petunidin, peonidin and malvidin (Wulf and Nagel 1978; Ivanova et al. 2011a). During vinification, the concentration of monomeric anthocyanins, especially acylated anthocyanins, decreases as a result of the interaction of anthocyanins with

themselves (self-association) or with other phenolic compounds (copigmentation) such as flavonols, flavan-3-ols, or phenolic acids (Wrolstad et al. 2005; Guadalupe and Ayestarán 2008; He et al. 2012). These reactions occur during the maceration processes and wine aging. They are followed by an irreversible formation of more complex and stable anthocyanin-derived pigments, such as pyranoanthocyanins, ethyl-bridged flavanol–anthocyanin adducts, and other pigments influencing wine expression such as in color, mouth feel, and sensory properties of red wine (Wrolstad et al. 2005; Monagas et al. 2005; Jensen et al. 2008).

Many studies have been performed on the structure and formation mechanisms of anthocyanin derivatives, as well as on the conditions that enable their formation (Berg and Akiyoshi 1975; Bakker and Timberlake 1986; Bakker and Timberlake 1997; Fulcrand et al. 1998; Alcalde-Eon et al. 2006). Different techniques have been used for pigment analysis and determination of their molecular masses. The most commonly used technique is high-performance liquid chromatography (HPLC), for the separation of the pigments, coupled to electrospray ionization mass spectrometry, as one of the most powerful techniques for structure characterization of wine components that is especially valuable for the identification of new derived wine pigments (Mateus et al. 2003; Kelebek et al. 2007; Jemal et al. 1998; Ivanova et al. 2011a). Furthermore, nuclear magnetic resonance or atmospheric pressure chemical ionization is also used for the characterization of phenolic compounds (Mateus et al. 2002, 2004; Ferrari et al. 2011).

In addition, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) is a powerful new technique that has a great potential in food analysis, as well as on wine and grape analysis (Sugui et al. 1998, 1999; Wang et al. 1999; Wang and Sporns 1999; Reed et al. 2005; Carpentieri et al. 2007; Ivanova et al. 2011b). This technique allows successful determination of the molecular weights in complex samples directly from ion abundances in the mass spectrum without previous isolation or cleanup of the sample (Hanton 2001; Ivanova et al. 2011b). The MALDI technique, for the first time, was demonstrated by Karas et al. 1987, originally being developed for the analysis of large biomolecules, such as proteins. MALDI-MS has also been applied to carbohydrates (Mock et al. 1991) and fructooligosaccharides in plants and food samples (Metzger et al. 1994; Stahl et al. 1997; Wang et al. 1999). In addition, MALDI-TOF-MS has been used for the identification of wine and grape anthocyanins and confirmation of the dominant anthocyanin compounds such as malvidin and its derivatives, using different matrices (2,5-dihydroxybenzoic acid (2,5-DHB), α-cyano-4-hydroxycinnamic acid (CHCA), and sinapic acid (SA)), as well as C_{70} fullerene applied for the first time for this purpose, without sample preparation (Ivanova et al. 2011b). The advantages of MALDI-TOF-MS over other methodologies include the ease of use, speed of analysis, high sensitivity, wide applicability combined with a good tolerance toward contaminants, as well as the ability to analyze complex mixtures such as wine (Stefova and Ivanova 2011).

The aim of this study was to test the applicability of the MALDI-TOF-MS methodology for fingerprinting the pigment profile of red wines after a fast and simple sample preparation procedure. The suitability of the method was demonstrated by performing a detailed anthocyanin and pigment profile determination of Vranec wine, the most widespread and typical variety for Macedonia and the Balkans, and Cabernet Sauvignon, and Merlot wines, as worldwidely known and popular varieties, all produced in Tikveš wine region in the Republic of Macedonia. The MALDI-TOF-MS technique was employed for fast fingerprinting of anthocyanins and derived pigments in wines after solid-phase extraction with Zip-Tip pipette tips, used for the first time for fast and efficient wine sample cleanup.

Materials and Methods

Chemicals and Reagents

2′,4′,6′-Trihydroxyacetophenone was from Sigma-Aldrich (St. Louis, MO, USA). The standard of malvidin-3-glucoside was purchased from LGC Promochem GmbH, Szentendre (Hungary). Acetonitrile (HPLC-grade) was purchased from Scharlau Chemie S.A. All the other used reagents were of analytical purity grade. Zip-Tip® pipette tips filled with C18 stationary phase were from Millipore.

Wine Samples

In total, nine red wine samples from three *V. vinifera* varieties (Vranec, Merlot, and Cabernet Sauvignon) from three different vintages (2006, 2007, and 2008) were subject of the investigation. All wine samples were kindly provided by Tikveš Winery, Kavadarci, Republic of Macedonia.

Sample Cleanup by Solid-Phase Extraction Using Zip-Tip® C18 Pipette Tips

For the extraction of wine pigments, a solid-phase extraction procedure was applied using reversed-phase Zip-Tip® pipette tips. The Zip-Tip® C18 tips were washed with a 1-μL mixture of acetonitrile/0.1 % (*v*/*v*) and trifluoroacetic acid (TFA) in water (1/1, *v*/*v*) and conditioned with 1 μL of 0.1 % TFA in water. Then, the wine sample (1 μL) was aspirated and dispensed through the tip. One microliter of 0.1 % (*v*/*v*) TFA in water was used to remove the sugars and acids which were not retained by the C18 stationary phase under these conditions. Elution of pigments from the stationary phase was performed

with a 1-μL solution mixture of acetonitrile/0.1 % (*v*/*v*) TFA in water (1/1, *v*/*v*).

MALDI-TOF-MS and MS/MS Analyses

The matrix solution was prepared by dissolving 10 mg of 2′,4′,6′-trihydroxyacetophenone (THAP) in 1 mL of acetonitrile/water (1/1, *v*/*v*). After the solid-phase extraction with Zip-Tip® C18 tips, 1 μL of the eluate was mixed with 1 μL of the matrix solution on the target plate (MTP 384 massive target plate; Bruker Daltonics, Bremen, Germany). After sample crystallization, the target plate was introduced into an Autoflex II MALDI-TOF/TOF MS instrument (Bruker, Daltonics, Bremen, Germany). The samples with the matrix were ionized by nitrogen laser pulse (λ=337 nm, 50 Hz). The laser power was adjusted between 20 and 30 % of its maximum intensity. Pulsed ion extraction was applied with 80 ns of delay; ion source voltages 1 and 2 were 19 and 17.05 kV, and the ion source lens voltage was set to 8.2 kV.

Mass spectrometry data were acquired in the positive ionization mode and reflectron operation mode; the reflectron voltage was set to +20 kV. The mass spectra were recorded in the *m*/*z* ranges of 350–1,400 or 750–1,800 and were at the sum of 300 consecutive laser shots on a sample spot. The precursor isolation window for the MS/MS experiments was set to ±1 % of the mass of the precursor ion. Data processing was performed using FlexAnalysis 2.4 software package (Bruker Daltonics, Bremen, Germany).

Results and Discussion

The primary aim of this study was to develop and utilize a novel small-scale sample cleanup procedure that will be applied prior to the MALDI-TOF-MS for rapid fingerprinting of wine samples. Previously, we had used the MALDI-TOF-MS technique for fingerprinting purposes in case of wine and grape samples, and we had tested the applicability of several matrices including 2,5-dihydroxybenzoic acid (2,5-DHB), α-cyano-4-hydroxycinnamic acid, sinapic acid (SA), as well as C_{70} fullerene. 2,5-DHB was superior for the identification only of the main anthocyanins in wine and grape (Ivanova et al. 2011b), opposite of previously published results (Carpentieri et al. 2007) where different kinds of chemical compounds were detected in wine samples using this matrix. Furthermore, C_{70} fullerene was applied for the first time for wine analysis with MALDI-TOF-MS, without any prior sample cleanup procedure (Ivanova et al. 2011b), giving good quality spectra and matrix peaks with very low intensities in range of *m*/*z* 100–700. Since a low number of pigments in wine were detected using the 2,5-DHB and C_{70} fullerene matrices in the previous study and in order to be able to detect more anthocyanins, as well as derived pigments in wine, a sample cleanup, including the extraction of these compounds from the complex matrix, is necessary to be performed. Therefore, the solid-phase extraction with Zip-Tip® C18 pipette tips has been used for the extraction of anthocyanins and derived pigments from the wine samples in this study. Zip-Tip C18 is a 10-μL pipette tip with a micro-volume (0.5 μL) bed of reversed-phase medium fixed at its end, i.e., a miniature reversed-phase column. This type of tip was chosen since it was considered that it would be appropriate for purifying and concentrating the wine samples in order to provide better data quality. Furthermore, the whole extraction procedure (described in

Table 1 Phenolic compounds found in Vranec, Cabernet Sauvignon, and Merlot wines identified by MALDI-TOF-MS analysis

Phenolics	MS (*m*/*z*) M^+	MS/MS (*m*/*z*) Fragment ions
Anthocyanins		
Non-acylated glucosides		
Dp-3-glc	465	303
Pt-3-glc	479	317
Pn-3-glc	463	301
Mv-3-glc	493	331
Acetylglucosides		
Mv-3-acetylglc	535	331
p-Coumaroylglucosides		
Dp-3-*p*-coumglc	611	303
Cy-3-*p*-coumglc	595	287
Pt-3-*p*-coumglc	625	317
Pn-3-*p*-coumglc	609	301
Mv-3-*p*-coumglc	639	331
Ethyl-bridged flavan-3-ol-anthocyanin adducts		
(Epi)cat-ethyl-Mv-3-*p*-coumglc	955	665, 357
Pyranoanthocyanins		
Vitisin A	561	399
p-Cm-vitisin A	707	399
Vitisin B	517	355
Ac-vitisin B	559	355
p-Cm-vitisin B	663	355
A-type vitisin of Pt-3-*p*-coumglc	677	369
10-DHP-pymv-3-glc (pinotin A)	625	463
10-MHP-pymv-3-cmglc	755	447
10-(Epi)Cat-pymv-3-glc	805	643, 491
10-(Procyanidin dimer)-pymv-3-glc	1,093	931, 803
10-(Procyanidin dimer)-pymv-3-acglc	1,135	931, 845

The details on the MALDI-TOF-MS are described in the "MALDI-TOF-MS and MS/MS Analyses" section

Dp delphinidin, *Cy* cyanidin, *Pt* petunidin, *Pn* peonidin, *Mv* malvidin, *Cat* catechin, *Epicat* epicatechin, *glc* 3-glucoside, *acglc* 3-(6″-acetyl)-glucoside, *cmglc* 3-(6″-coumaroyl)-glucoside, *10-MHP* 10-(4‴-monohydroxyphenyl), *10-DHP* 10-(3‴,4‴-dihydroxyphenyl), *pymv* pyranomalvidin, *vitisin A* 10-carboxy-pyrmv-3-glc, *vitisin B* 10-H-pymv-3-glc, *A-type vitisin* 10-carboxy-pyranoanthocyanins

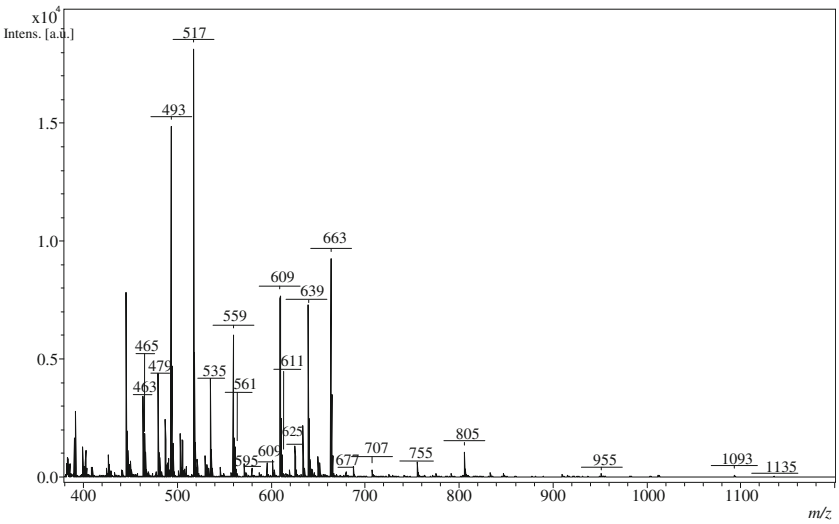

Fig. 1 Positive-ion MALDI-TOF mass spectra of Vranec wine, produced in 2008. *m/z* values of identified compounds are presented in Table 1

the "Sample Cleanup by Solid-Phase Extraction Using Zip-Tip® C18 Pipette Tips" section) required only few minutes and small volumes (1 μL) of solvents (acetonitrile and 0.1 % (*v*/*v*) TFA in water).

As for the choice of matrix, THAP has been used as convenient matrix for the analysis of oligosaccharides and polyphenols in food samples (Wang et al. 1999), giving small homogeneous crystals and better resolution of the glucosides. THAP was also successfully used as a matrix for peptide and oligonucleotide analysis (Kussmann et al. 1997). Applying this matrix for wine analysis, homogeneous sample preparation and high ionization efficiency were achieved, confirming its suitability for wine phenolic compound identification. Thus, the proposed MALDI-TOF-MS analysis combined with solid-phase extraction using Zip-Tip®C18 pipettes and THAP matrix provided a rapid method for qualitative screening of wine pigments, taking only few minutes in total and greatly reducing the analysis time compared to traditional HPLC-DAD-MS methods.

The solid-phase extraction method with Zip-Tip® C18 pipettes was applied for wine cleanup, and in total, 22 compounds have been tentatively identified by MALDI-TOF-MS technique. Table 1 and Fig. 1 present the identified compounds, belonging to five groups: anthocyanins, vitisin-type pyranoanthocyanins, hydroxyphenyl-pyranoanthocyanins, flavanyl-pyranoanthocyanins, and acetaldehyde-mediated flavanol–anthocyanin adducts. The identification was performed on the basis of the monoisotopic molecular mass of the flavylium cations (M^+ signals) or the protonated quasimolecular ions ($[M+H]^+$ signals) under positive ionization mode and confirmed by MS/MS fragmentation. The identification was additionally confirmed using the standard of malvidin-3-glucoside analyzed under the same experimental conditions and/or with comparison of the results in already published

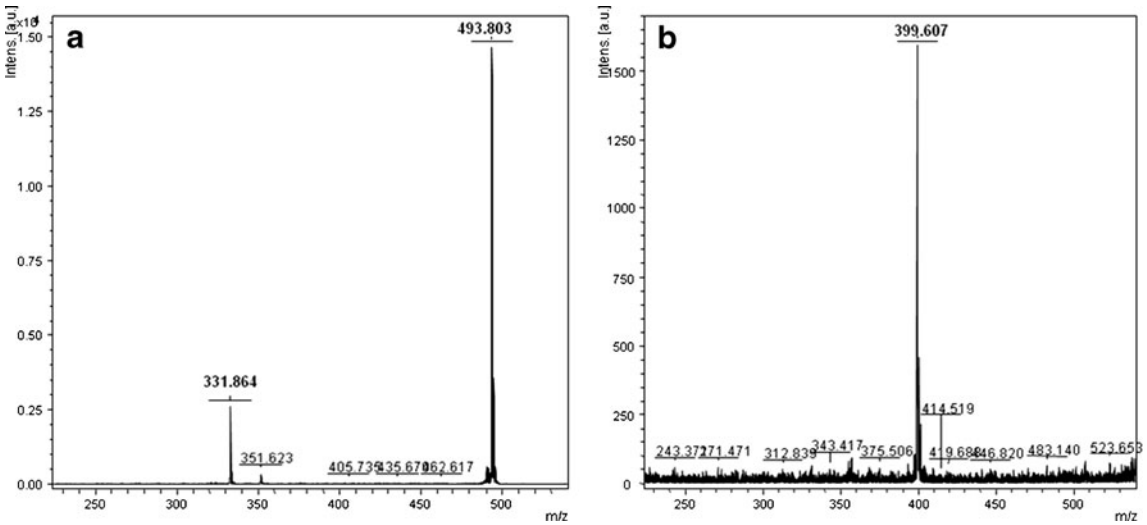

Fig. 2 MALDI-MS/MS fragmentation of the ions at *m/z* 493 (**a**) and *m/z* 707 (**b**) under positive mode

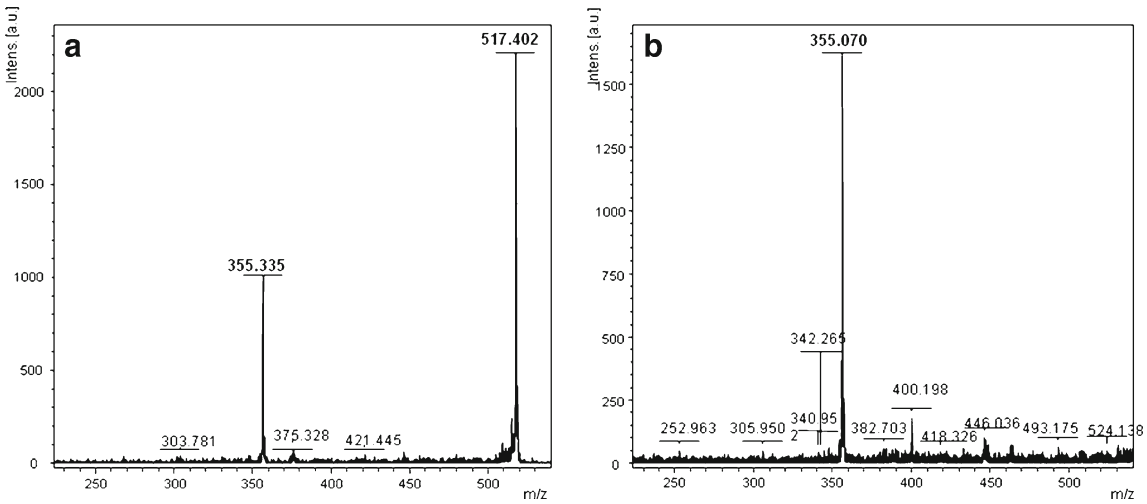

Fig. 3 MALDI-MS/MS fragmentation of the ions at *m/z* 517 (**a**) and *m/z* 559 (**b**) under positive mode

data. Thus, the cation of malvidin-3-glucoside (M^+) (*m/z* 493) was detected in all samples. The molecular ion peaks that appeared in the mass spectrum, at *m/z* 535 and 639, corresponded to the malvidin-3-acetylglucoside and malvidin-3-*p*-coumaroylglucoside, respectively. The fragmentation of these three ions (*m/z* 493, 535, and 639) produced fragment ion at *m/z* 331 as a result of the elimination of glucose, acetylglucoside, and *p*-coumaroylglucoside moieties, respectively (Ivanova et al. 2011a). Molecular peaks at *m/z* 465, 479, and 463 in the MALDI-TOF-MS spectrum of the wine extract were identified as 3-glucosides of delphinidin, petunidin, and peonidin, respectively. 10-Carboxy-pyranoanthocyanins or A-type vitisins (vitisin A and *p*-coumaroyl-vitisin A) were detected on the basis of their M^+ signals at *m/z* 561 and 707, respectively, giving fragment at *m/z* 399, corresponding to the loss of glucose (162 u) and *p*-coumaroylglucoside (308 u) moieties, respectively. The molecular ion peak at *m/z* 677 and a fragment ion at *m/z* 369 were identified as A-type vitisin of petunidin-3-*p*-coumaroylglucoside (Blanco-Vega et al. 2011). The MALDI-MS/MS fragmentation of malvidin-3-glucoside (*m/z* 493) and *p*-coumaroyl-vitisin A (*m/z* 707) is presented in Fig. 2.

Another group of wine-derived pigments has been identified belonging to the group of 10-H-pyranoanthocyanins (B-type vitisins, or simply pyranoanthocyanins). Molecular ion peaks at *m/z* 517, 559, and 663 were identified as pyranomalvidin-3-glucoside (vitisin B), pyranomalvidin-3-acetylglucoside (acetyl-vitisin B), and pyranomalvidin-3-*p*-coumaroylglucoside (*p*-coumaroyl-vitisin B), all producing a fragment ion at *m/z* 355 as a result of the elimination of glucoside (162 u), acetylglucoside (204 u) and *p*-coumaroylglucoside (308 u) groups, respectively (Ivanova et al. 2011a; Blanco-Vega et al. 2011). The MALDI-MS/MS fragmentation of vitisin B (*m/z* 517) and acetyl-vitisin B (*m/z* 559) is presented in Fig. 3.

In addition, three 10-flavanyl-pyranoanthocyanin pigments have been detected in the wines, showing signals at *m/z* 805 (fragment ions *m/z* 643, 491), *m/z* 1,093 (fragment ions *m/z* 931, 803), and *m/z* 1,135 (fragment ions *m/z* 931, 845). These pigments referred to 10-(epi)catechin-pyranomalvidin-3-glucoside, 10-(procyanidin dimer)-pyranomalvidin-3-glucoside,

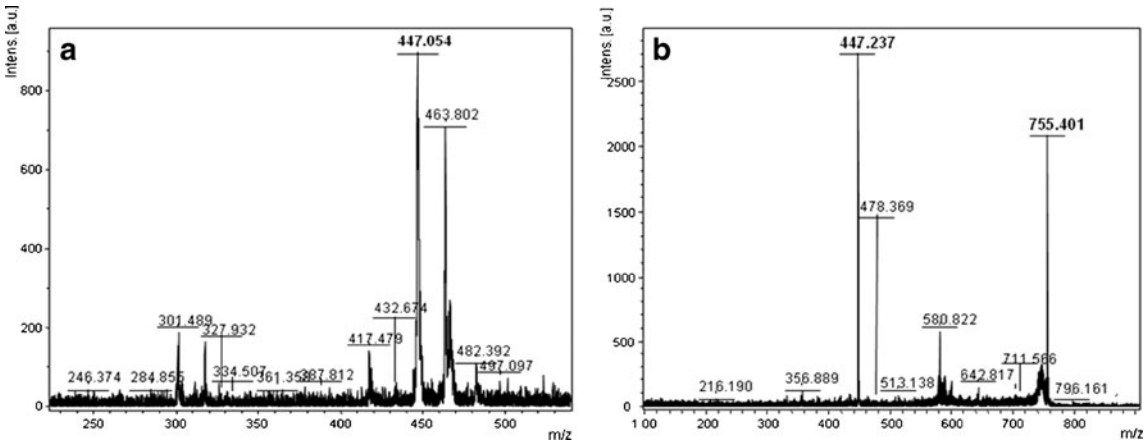

Fig. 4 MALDI-MS/MS fragmentation of the ions at *m/z* 625 (**a**) and *m/z* 755 (**b**) under positive mode

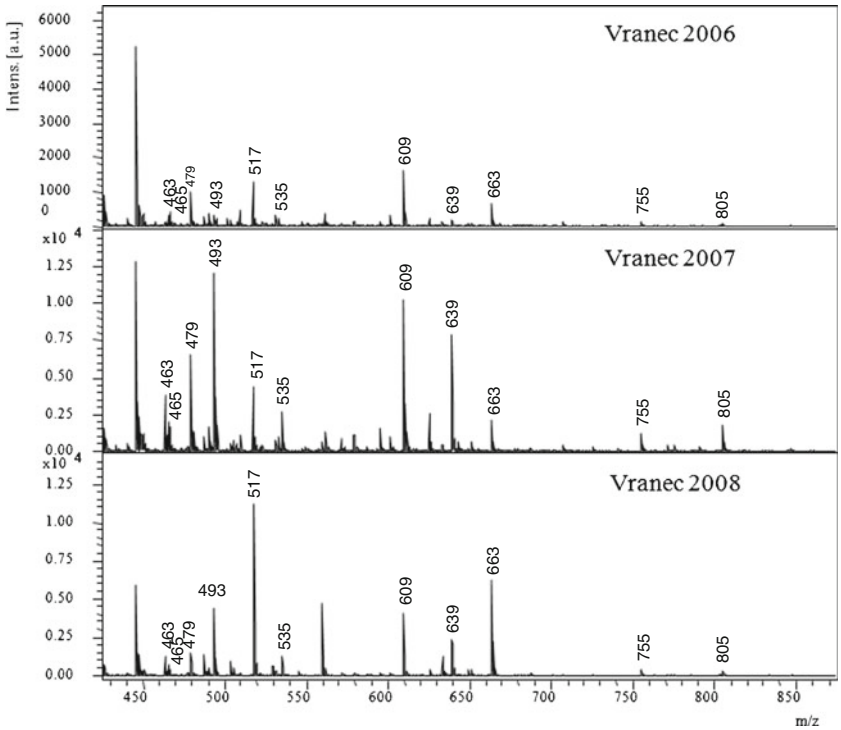

Fig. 5 Positive-ion MALDI-TOF-MS mass "fingerprint" spectra of Vranec wines (*2006*, *2007*, and *2008* years of production) obtained after SPE with Zip-Tip C18 pipette tips. *m/z* values of the most abundant identified compounds are presented in Table 1

and 10-(procyanidin dimer)-pyranomalvidin-3-*p*-acetylglucoside, respectively. Fragmentation of the first molecular ion (*m/z* 805) yielded aglycone cation at *m/z* 643 as a result of loss of glucoside, and the molecular ions at *m/z* 1,093 and 1,135 yielded cations at *m/z* 931 as a result of the elimination of acetylglucoside groups. The fragment ion at *m/z* 491 corresponded to a retro-Diels–Alder fission of the (+)-catechin moiety (152 U). The fragments at *m/z* 803 and 845, for both components, respectively, were formed by the cleavage of interflavonoid bond of procyanidin dimers producing loss of flavan-3-ol monomer ion followed by

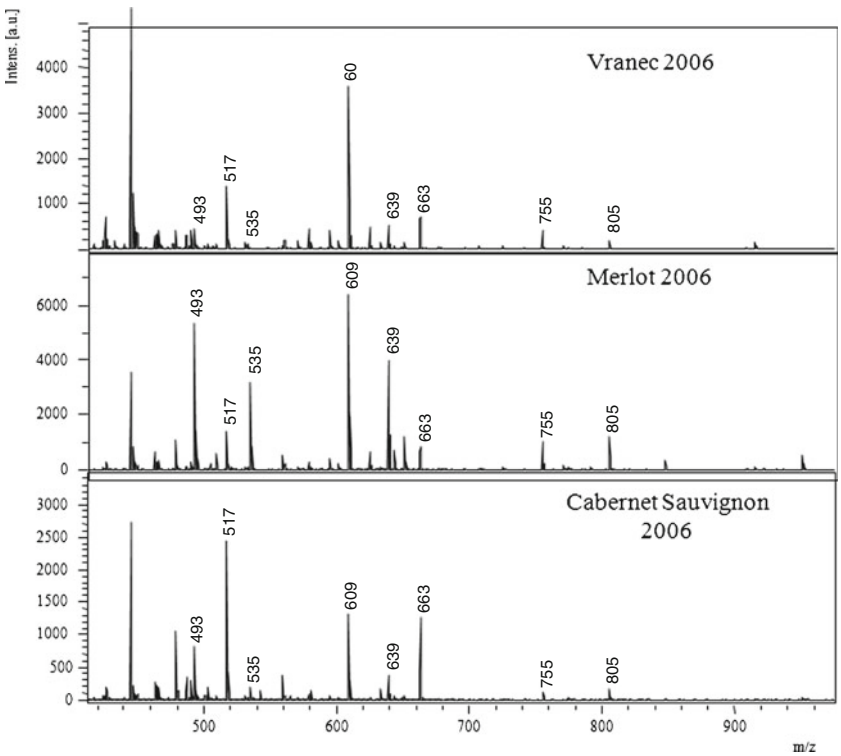

Fig. 6 Positive-ion MALDI-TOF-MS mass "fingerprint" spectra of *Vranec*, *Merlot*, and *Cabernet Sauvignon* wines, vintage 2006, obtained after SPE with Zip-Tip C18 pipette tips. *m/z* values of the most abundant identified compounds are presented in Table 1

formation of flavanyl-carboxy-pyranomalvidin-3-glucoside and flavanyl-carboxy-pyranomalvidin-3-acetylglucoside ions, respectively. One acetaldehyde-mediated flavanol–anthocyanin condensed product has been also detected in the wines. This compound was identified as (epi)catechin-ethyl-malvidin-3-*p*-coumaroylglucoside, presenting a molecular ion at *m*/*z* 955 in the MALDI-TOF-MS spectrum. Fragmentation of the molecular ion produced fragment ions at *m*/*z* 665 and 357. The first fragment (*m*/*z* 665) corresponds to the elimination of an (epi)catechin molecule (*m*/*z* 290), and the second fragment is a result of loss of *p*-coumaroylglucoside group.

Two compounds belonging to the group of hydroxyphenyl-pyranoanthocyanins were detected in wines: the compound with molecular ion at *m*/*z* 625 and fragment ion at *m*/*z* 463 was identified as 10-(3‴,4‴-dihydroxyphenyl)-pyranomalvidin-3-glucoside, also known as pinotin A (Rentzsch et al. 2010) and the compound with molecular ion at *m*/*z* 755 (fragment ions at *m*/*z* 447) was identified as 10-(4‴-monohydroxyphenyl)-pyranomalvidin-3-*p*-coumaroylglucoside. Figure 4 shows the MALDI-MS/MS fragmentation of compounds with *m*/*z* 625 and 755.

Figure 5 presents the positive-ion MALDI-TOF-MS mass "fingerprint" spectra of Vranec wines from different vintages. The relative abundance of the mass signals of the main anthocyanin, malvidin-3-glucoside, at *m*/*z* 493 is clearly different in all three samples, the highest abundance being observed in the wine produced in 2007. Similarly, the relative abundance of the mass signals at *m*/*z* 535, *m*/*z* 609, and *m*/*z* 639, attributed to malvidin-3-acetylglucoside, peonidin-3-*p*-coumaroylglucoside, and malvidin-3-*p*-coumaroylglucoside, respectively, were highest in Vranec wine from 2007, which means that not only aging conditions but also oenological practices applied for winemaking influence the content of anthocyanins in wine, as well as the edaphoclimatic conditions where the vineyards are located, and the cultural practices applied to the vine plants. Figure 6 shows the MALDI mass spectrum of Vranec, Merlot, and Cabernet Sauvignon wines from same vintage (2006). It could be clearly noticed that anthocyanins and other pigments were present with highest abundance in Merlot wine.

As a conclusion, solid-phase extraction with Zip-Tip® pipette tips is a very fast method (requires at about 1 min of preparation) with very low solvent consumption (only few microliters) that simplifies sample and spectra, followed by rapid and simple MALDI-TOF-MS identification of a big number of wine pigments. This confirms the ability of MALDI-TOF-MS as a valuable technique for fast screening of wine samples.

Conclusion

Identification of anthocyanins and derived pigments was performed with the MALDI-TOF-MS technique. A new small-scale sample cleanup procedure using solid-phase extraction prior to the rapid MALDI-TOF-MS fingerprinting of wine samples was introduced, using 2′,4′,6′-trihydroxyacetophenone as a matrix. Twenty-two color compounds were detected in the wines, developing a rapid and simple MALDI method for wine analysis and confirming the ability of this technique for fast wine screening for the detection of glucoside, acetylglucoside, and *p*-coumaroylglucoside derivatives of anthocyanins as well as pyranoanthocyanins, flavanol–pyranoanthocyanins, and ethyl-bridged flavan-3-ol-anthocyanin adduct pigments. Using this analytical methodology, hydroxyphenyl-pyranoanthocyanins were, for the first time, identified in Macedonian wines.

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Conflict of Interest Violeta Ivanova Petropulos declares that she has no conflict of interest. Ágnes Dörnyei declares that she has no conflict of interest. Marina Stefova declares that she has no conflict of interest. Trajče Stafilov declares that he has no conflict of interest. Borimir Vojnoski declares that he has no conflict of interest. László Márk declares that he has no conflict of interest. Isidro Hermosín-Gutiérrez declares that he has no conflict of interest. Ferenc Kilár declares that he has no conflict of interest. This article does not contain any studies with human or animal subjects.

References

Alcalde-Eon C, Escribano-Bailón MT, Santos-Buelga C, Rivas-Gonzalo JC (2006) Anal Chim Acta 563:238
Bakker J, Timberlake CF (1986) Am J Enol Viticult 37:288
Bakker J, Timberlake CF (1997) J Agric Food Chem 45:35
Berg HW, Akiyoshi MA (1975) Am J Enol Vitucult 26(3):134
Blanco-Vega D, López-Bellido FJ, Alía-Robledo JM, Hermosín-Gutiérrez I (2011) J Agric Food Chem 59:9523
Carpentieri A, Marino G, Amoresano A (2007) Anal Bioanal Chem 389:969
Ferrari E, Foca G, Vignali M, Tassi L, Ulrici A (2011) Anal Chim Acta 701:139
Fulcrand H, Benabdeljalil C, Rigaud J, Cheynier V, Mountounet M (1998) Phytochem 47:1401
Guadalupe Z, Ayestarán B (2008) Eur Food Res Technol 228:29
Hanton SD (2001) Chem Rev 101:521
He F, Liang NN, Mu L, Pan QH, Wang J, Reeves MJ, Duan CQ (2012) Molecules 17:1483
Ivanova V, Dörnyei Á, Márk L, Vojnoski B, Stafilov T, Stefova M, Kilár F (2011a) Food Chem 124:316
Ivanova V, Dörnyei Á, Stefova M, Stafilov T, Vojnoski B, Kilár F, Márk L (2011b) Food Anal Method 4:108
Jemal M, Ouyang Z, Teitz DS (1998) Rapid Commun Mass Spectrom 12:429
Jensen JS, Demiray S, Egebo M, Meyer AS (2008) J Agric Food Chem 56:1105
Karas M, Bachmann D, Bahr U, Hillenkamp F (1987) Int J Mass Spectrom Ion Processes 78:53

Kelebek H, Canbas A, Selli S (2007) Chromatographia 66:207
Kussmann M, Nordhoff E, Rahbek-Nielsen H, Haebel S, Rossel-Larsen M, Jakobsen L, Gobom J, Mirgorodskaya E, Kroll-Kristensen A, Palm L, Roepstorff P (1997) J Mass Spectrom 32:593
Mateus N, Carvalho E, Carvalho ARF, Melo A, Gonzlez-Params AM, Santos-Buelga C, Silva AMS, de Freitas V (2003) J Agric Food Chem 51:277
Mateus N, Oliveira J, Santos-Buelga C, Silva AM, de Freitas V (2004) Tetrahedron Lett 45:3455
Mateus N, Silva AM, Santos-Buelga C, Rivas-Gonzalo JC, de Freitas V (2002) J Agric Food Chem 50:2110
Metzger JO, Woisch R, Tuszynski W, Angermann R (1994) Fresen J Anal Chem 349:473
Mock KK, Daevy M, Cottrell JS (1991) Biochem Bioph Res Com 177:644
Monagas M, Bartolomé B, Gómez-Cordovés C (2005) Eur Food Res Technol 220:331
Reed JD, Krueger CG, Vestling MM (2005) Phytochemistry 66:2248
Rentzsch M, Schwarz M, Winterhalter P, Blanco-Vega D, Hermosín-Gutiérrez I (2010) Food Chem 119:1426
Stahl B, Lions A, Karas M, Hillenkamp F, Steup M (1997) Anal Chem 246:1195–1204
Stefova M, Ivanova V (2011c) Analytical methodology for characterization of grape and wine phenolic bioactives, chapter 20. In: Fruits and cereal bioactives: sources, chemistry, and applications. CRC, Boca Raton, p409
Sugui JA, Bonham C, Lo SC, Wood KV, Nicholson RL (1998) Phytochemistry 8:1063
Sugui JA, Wood KV, Yang ZY, Bonham CC, Nicholson RL (1999) Am J Enol Viticult 50:199
Wang J, Sporns P, Low NH (1999) J Agric Food Chem 47:1549
Wang J, Sporns P (1999) J Agric Food Chem 47:2009
Wrolstad RE, Durst RW, Lee J (2005) Trends Food Sci Tech 16:423
Wulf LW, Nagel CW (1978) Am J Enol Viticult 29:42