

ANALYSIS OF WINE AND GRAPE SAMPLES WITH MALDI-TOF-MS

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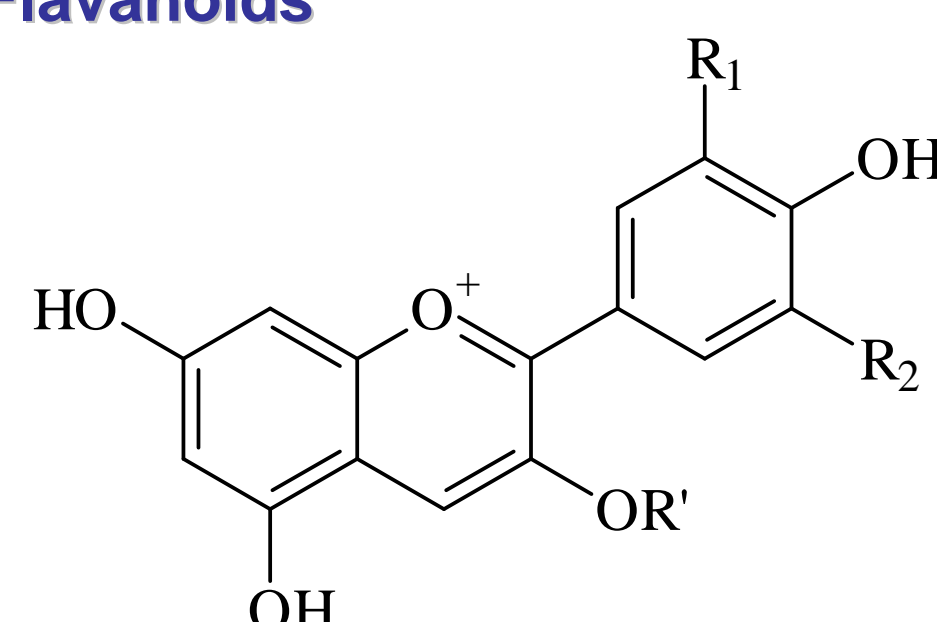
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INTRODUCTION

Polyphenols play an important role in the organoleptic characteristics of wine; in particular, tannins confer astringency and structure to the beverage by formation of complexes with the proteins of saliva. This knowledge is very important to predict the aging attitude of wine, and to attempt to resolve problems about color stability — in particular in the case of premium red wine that are destined to long aging periods. [1] Wine phenolics are divided into two groups: non-flavonoids (hydroxybenzoic and hydroxycinnamic acids and stilbens) and flavonoids (anthocyanins, flavonols and flavan-3-ols).

Flavonoids

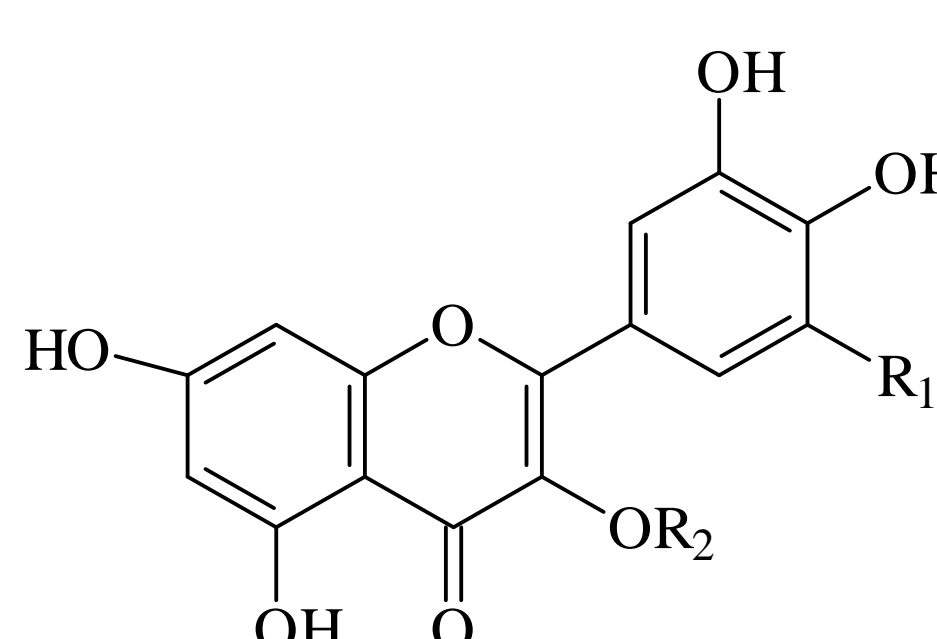


Anthocyanidins	R ₁	R ₂	M _r
Cyanidin	OH	H	287
Peonidin	OCH ₃	H	301
Delphinidin	OH	OH	303
Petunidin	OH	OCH ₃	317
Malvidin	OCH ₃	OCH ₃	331

Scheme 1. Anthocyanins

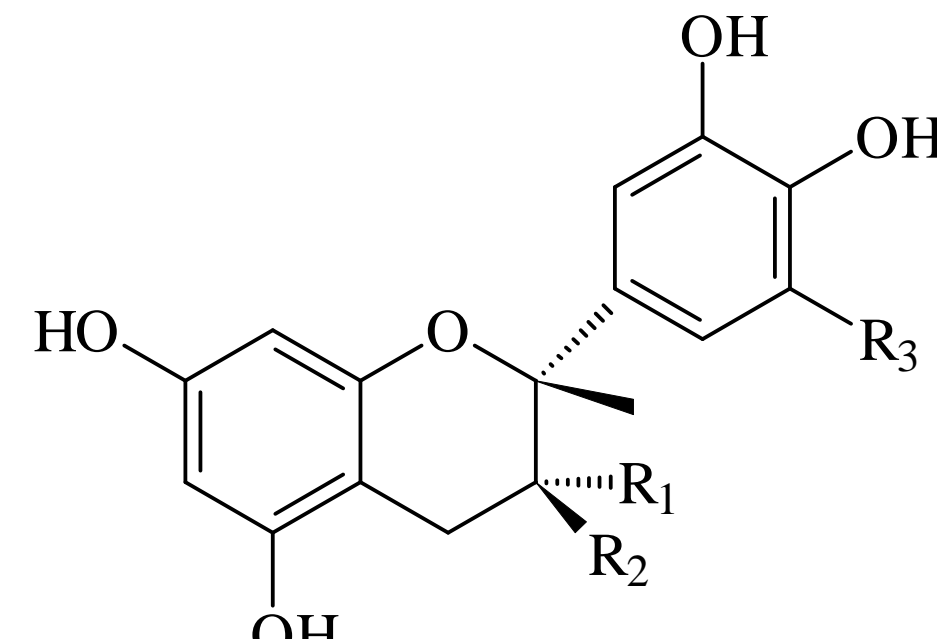
(R'=glucoside, acetylglucoside, p-coumarylglucoside)

Anthocyanins are red pigments, localized in the skin of the grapes. Those compounds are present in aglycone forms in the grapes skin and glycoside forms in the wines. The anthocyanin profile is a useful tool to characterize and to determine the origin of the products and in the identification of possible adulterations in case of the red wines.



Flavonols	R ₁	R ₂	M _r
Quercetin	H	HO	302
Myricetin	OH	H	318

Scheme 2. Flavonols



Flavan-3-ols	R ₁	R ₂	M _r
(+)-Catechin	H	OH	290
(-)-Epicatechin	OH	H	290

Scheme 3. Flavan-3-ols

[1] Mass Spectrometry Reviews, 2003, 22, 218-250.

SAMPLES and METHODS

Grapes and wines from Vranec, Merlot, Smederevka and Chardonnay varieties (2007 vintage) have been analyzed. Wines were produced with traditional techniques for wine-making in the experimental cellar of the Wine Department, Institute of Agriculture in Skopje. The grape samples (skin, seed and pulp) have been extracted with acetone/water (80/20 V/V). The wine and grape samples have been analyzed by MALDI-TOF-MS without any other preparation.

Parameters of MALDI-TOF-MS measurements:

- Bruker instrument with FlexControl 2.4 software
- Positive and reflection mode, an accelerating voltage of 20 kV was used.
- Mass range: 50–700 m/z (or 50–1400 m/z in some cases)
- Accumulating data from 1000 consecutive laser shots
- Tested matrices: α-cyano-4-hydroxycinnamic acid (CHCA), sinapinic acid (SA), 2,5-dihydroxy-benzoic acid (DHB), fullerene [C70]
- Preparation of the matrices: 10 mg dissolved in 1 mL 0.1%TFA (H₂O)/ACN (2:1) (in case of [C70] saturated solution was prepared in toluene)
- Sandwich method: the sample crystallized between two layers of fullerene

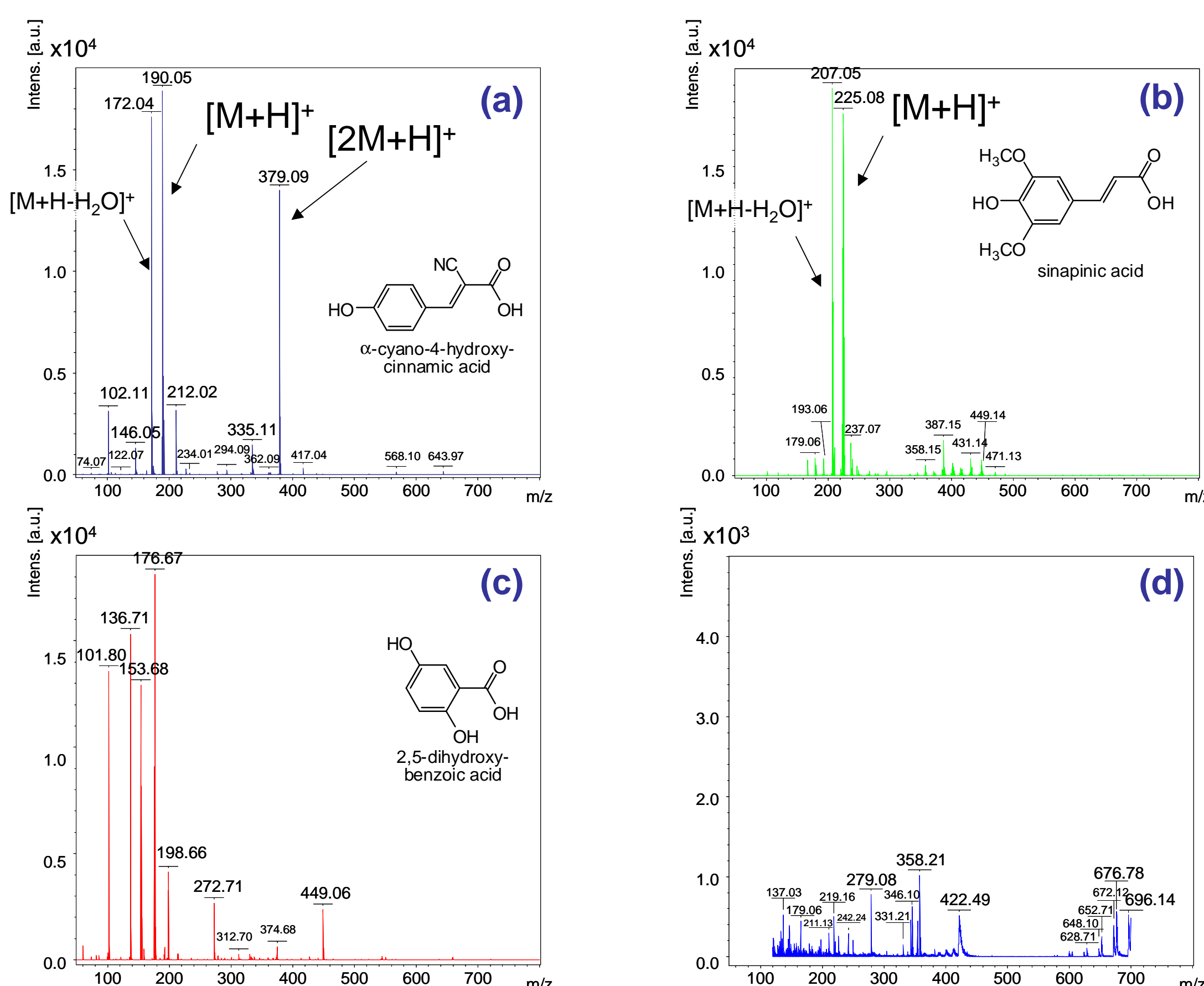


Fig. 1. MALDI-TOF-MS spectrum of the matrices tested (a) CHCA, (b) SA, (c) DHB, (d) C70



Vranec



Merlot



Smederevka



Chardonnay



RESULTS

Important factors for MALDI measurements:

- ✓ selection of an appropriate matrix
- ✓ optimal mixing and drying of matrix and sample
- ✓ adjustment of laser strength
- ✓ selection of calibration standards
- ✓ correct interpretation of the spectra

Comparison of different matrices in detection of anthocyanin profile

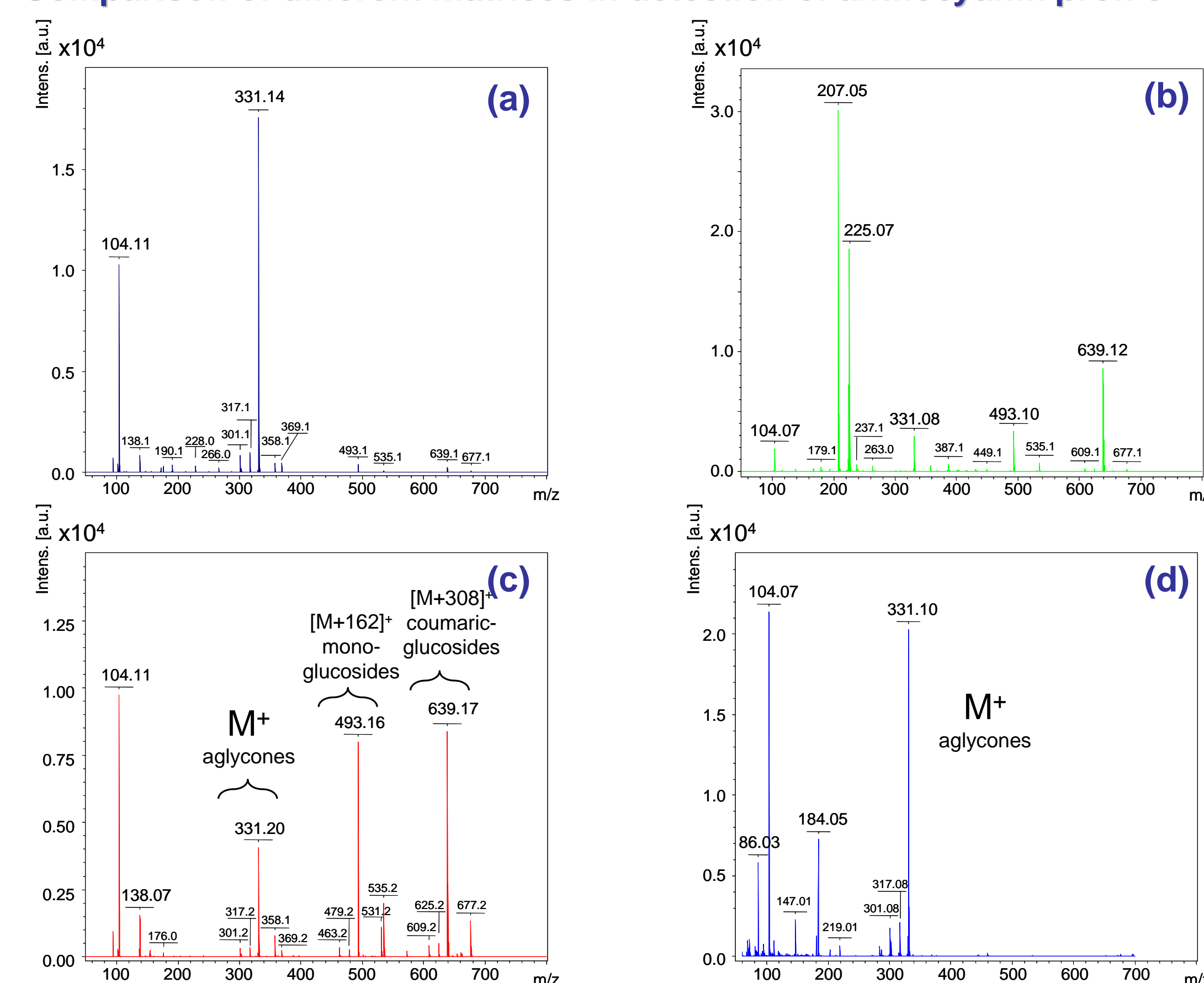


Fig. 2. MALDI-TOF-MS spectrum of the skin extract of Vranec grape matrix: (a) CHCA, (b) SA, (c) DHB, (d) C70 „sandwich”

Results from MALDI-TOF measurements:

- ✓ DHB was superior with respect to all the matrices tested
- ✓ a new „sandwich” method with [C70] matrix gives the possibility to analyse hydroscopic samples
- ✓ ionising the samples on [C70] matrix higher laser energy needed
⇒ fragmentation (anthocyanins detected only in aglycon form)
- ✓ 172 different grape and wine samples have been analysed in [C70] sandwich
- ✓ external calibration (CHCA and SA)
- ✓ MALDI „fingerprint” of samples

Comparison of Vranec grape and wine samples

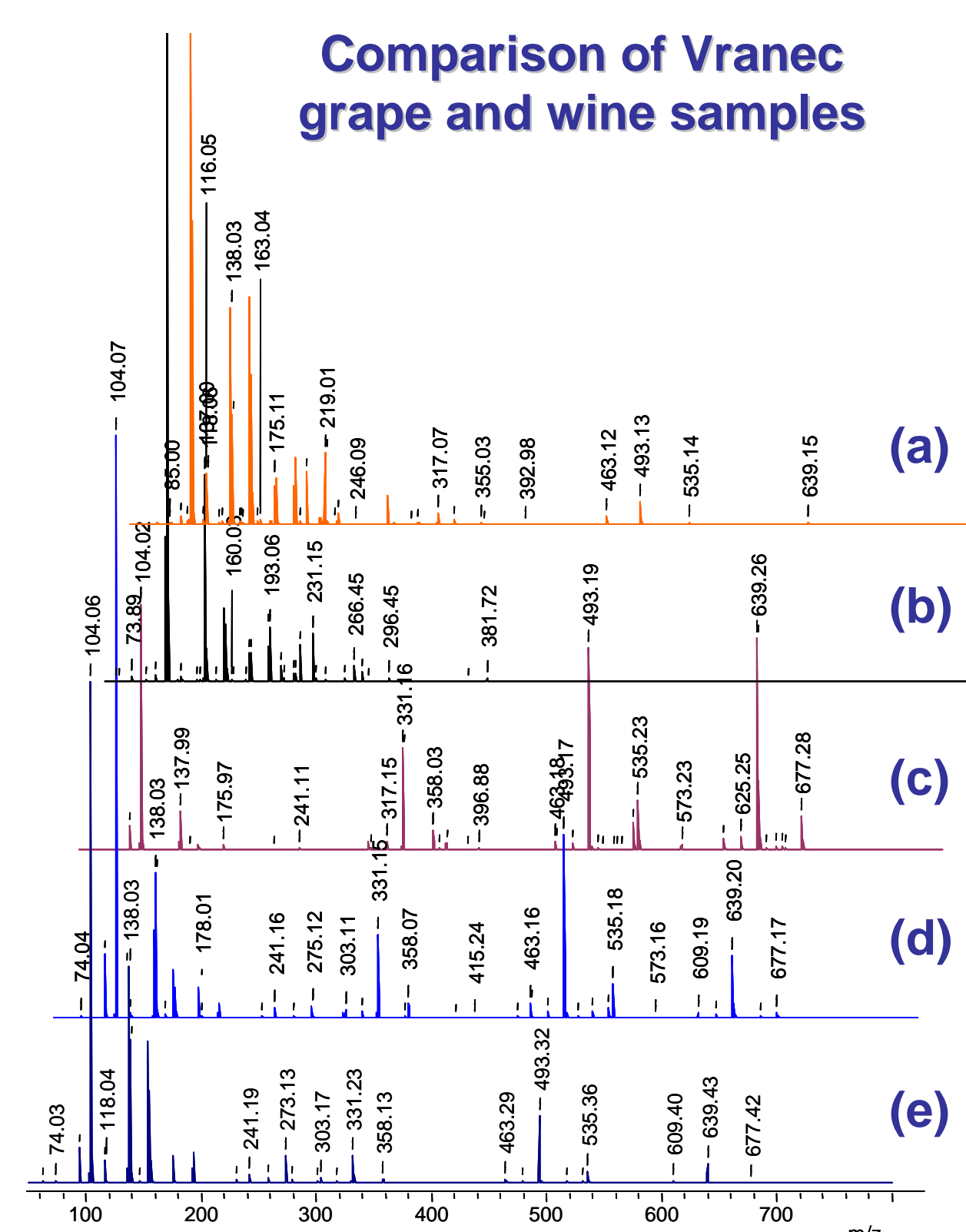


Fig. 3. MALDI-TOF-MS spectra of Vranec grape and wine samples (DHB matrix): (a) pulp, (b) seed, (c) skin, (d) wine (6 days of maceration), (e) wine (10 days of maceration)

Comparison of selected wine samples

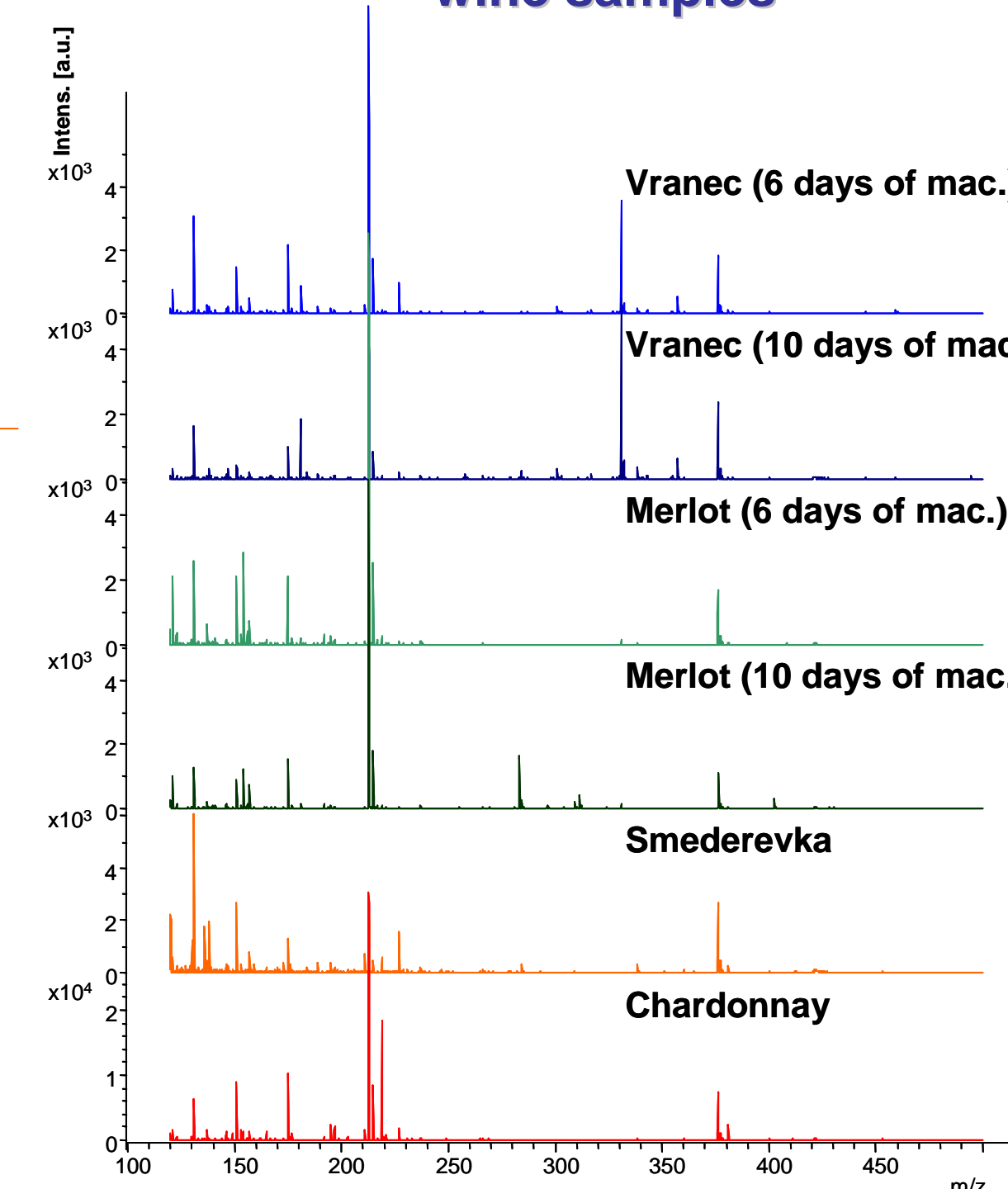


Fig. 4. MALDI-TOF-MS „fingerprints” of selected wine samples ([C70] matrix)

CONCLUSION

A simple and fast MALDI-TOF-MS method introducing a new matrix, fullerene [C70], has been developed for fingerprinting the polyphenolic (e.g. anthocyanin) content of wine and grape samples. Sandwich method was suitable for analysing all samples.

MALDI-TOF-MS could be a simple method in optimization of processes in enology and viticulture, such as grape maturation and wine ageing.

To the best of our knowledge this is the first paper of MALDI-TOF-MS analysis of typical Macedonian (e.g. Vranec or Smederevka) grape and wine.

ACKNOWLEDGEMENTS

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