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PHENOLIC, VOLATILE AND ELEMENTAL COMPOSITION OF MACEDONIAN WINES

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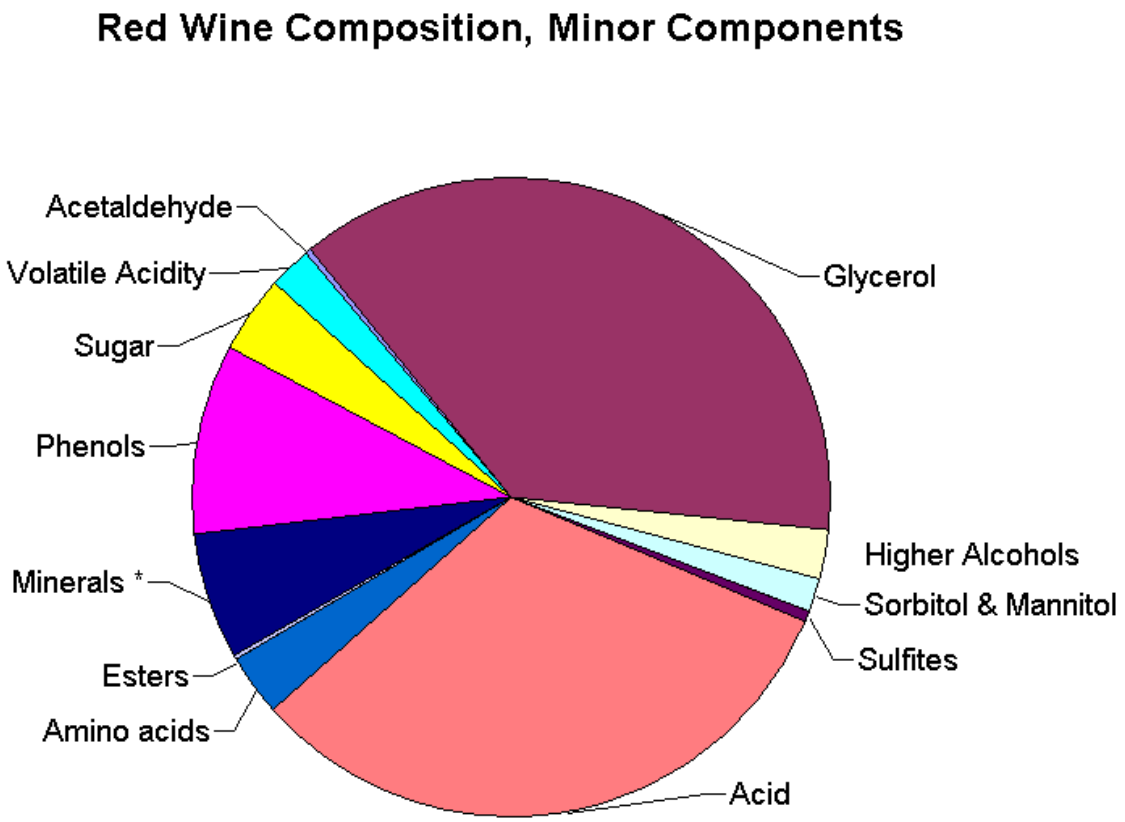
Trajće Stafilov, Marina Stefova, Ernst Lankmayr , Ferenc Kilar

Chemical composition of wine



Types of wine

By the colour

white

red

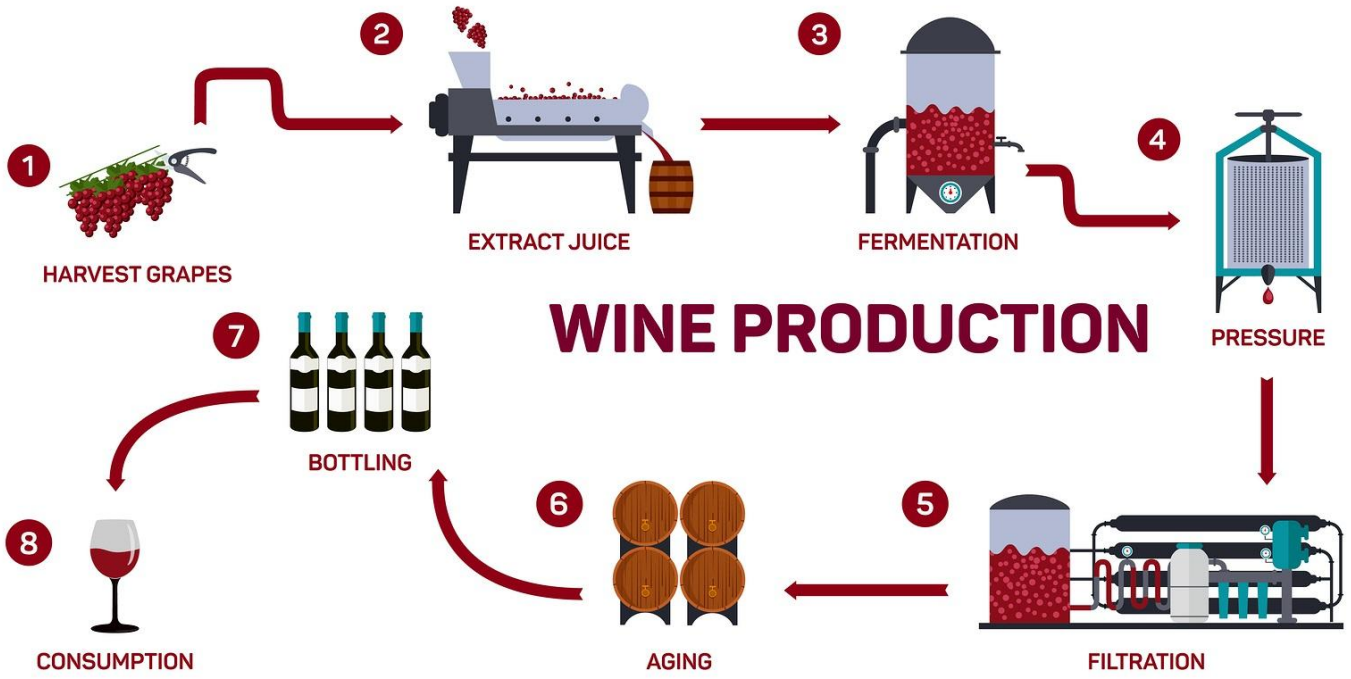
rosé

Still or sparkling

still

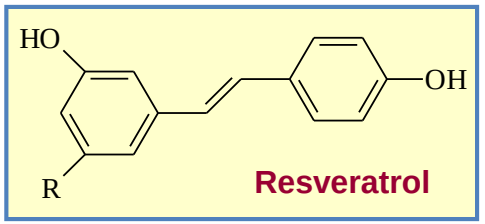
sparkling

Winemaking


1
HARVEST GRAPES
2
EXTRACT JUICE
3
FERMENTATION
4
PRESSURE
WINE PRODUCTION
5
FILTRATION
6
AGING
7
BOTTLING
8
CONSUMPTION

PHENOLIC COMPOSITION OF MACEDONIAN WINES

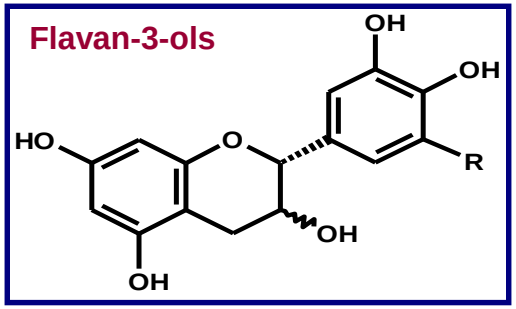
- ✓ **Determine the colour, mouthfeel, astringency and bitterness of wine.**
- ✓ **Influenece the sensorial characteristics of grape and wine**
- ✓ **Antioxidant, antimicrobal, anticancerogenic effects, prevention of cardiovascular diseases.**

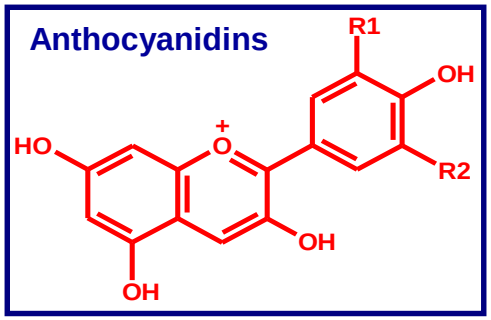


Two groups of polyphenols:

Non-flavonoids: **Hydroxybenzoic acids, Hydroxycinnamic acids and derivatives, Stilbenes and stilbene glucosides**

Flavonoids: **Flavanones, Flavonols, Flavones, Flavan-3-ols, Anthocyanidins**





Analytical techniques for analysis of phenolics

HPLC-DAD-MS
MALDI-TOF

Wine and grape samples

***Vitis vinifera* varieties, 2007 vintage**

Red grapes and wines:
Vranec and Merlot

White grapes and wines:
Smederevka and Chardonnay

HPLC-DAD-MS

- C18 column, DAD
- Binary solvent system: polar solvent (aqueous acetic acid, phosphoric acid, or formic acid) and a less polar organic solvent (methanol or acetonitrile, possibly acidified)
- HPLC-DAD is limited technique for compounds with similar UV-Vis spectra (flavanols and flavonols)

- **HPLC-MS** powerful and more sophisticated technique for characterization of the phenolics

- Confirmation of the structure of the main phenolic compounds
- Detection of a novel compounds

HPLC analyses

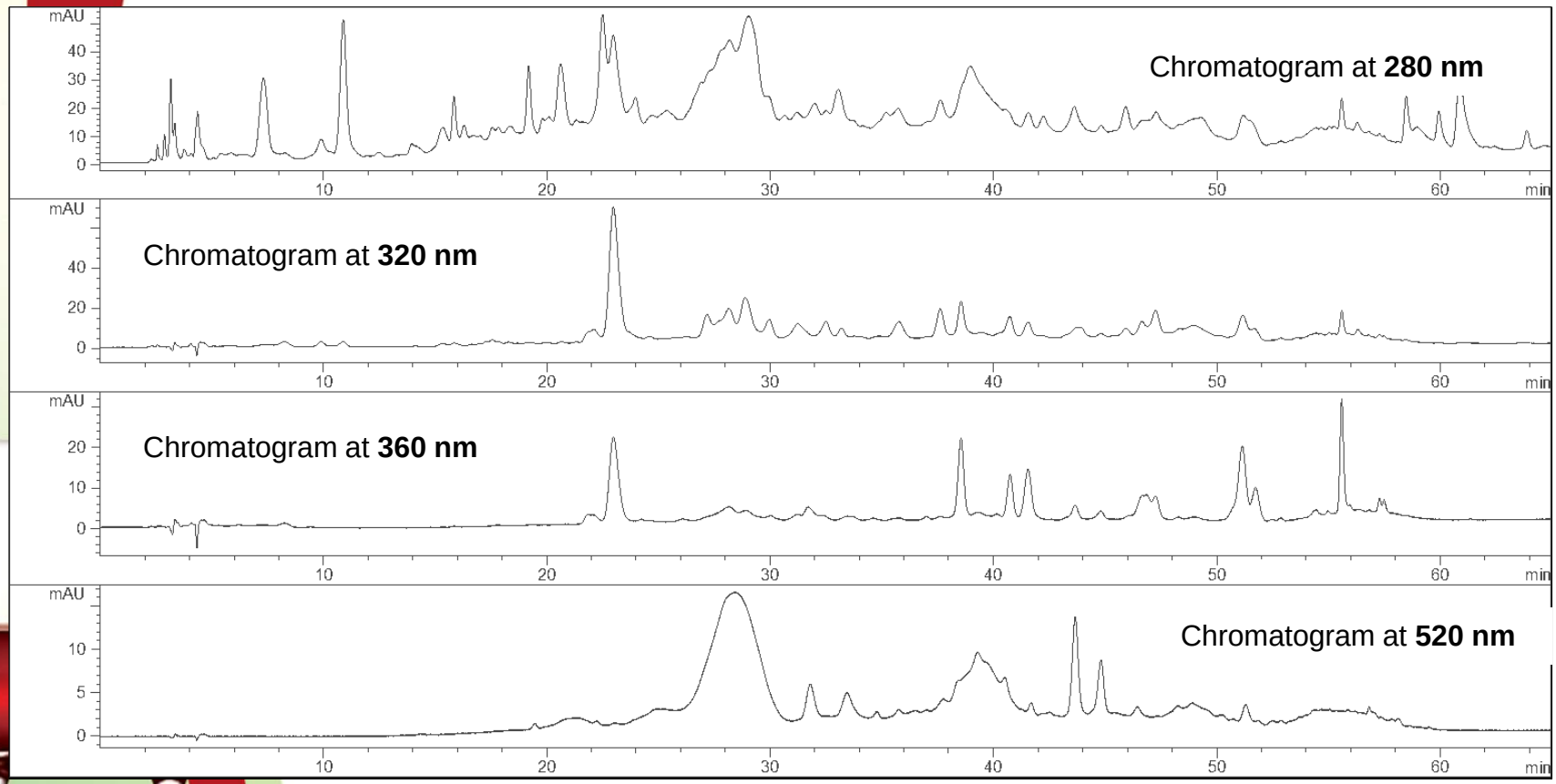
Agilent series 1100, equipped with a DAD and ion trap MS

- Column: Phenomenex C18 (60x4,6; 3µm)
- Gradien elution: **A** - 1 % acetic acid in water
- **B** - 1 % acetic acid in metanol pH 2,5-3
- Flow rate: 0,2 mL/min; room temperature,
- Injection volume: 5 µL
- Run time: 60 min

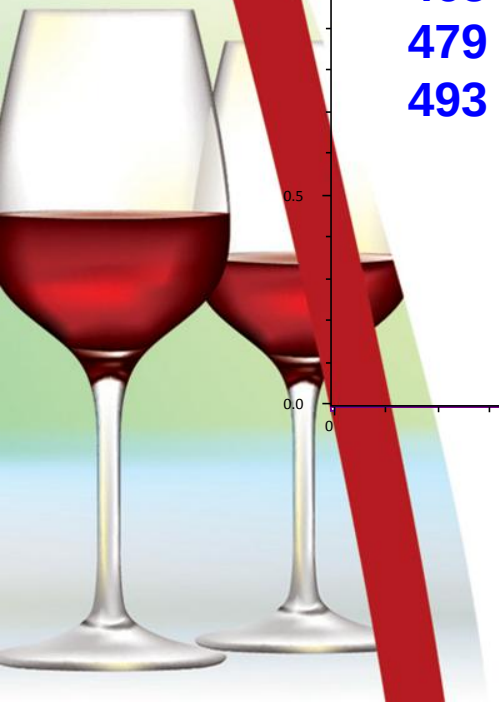
t_R/min	B/ %
0	5
10	20
45	50
50	80
60	90

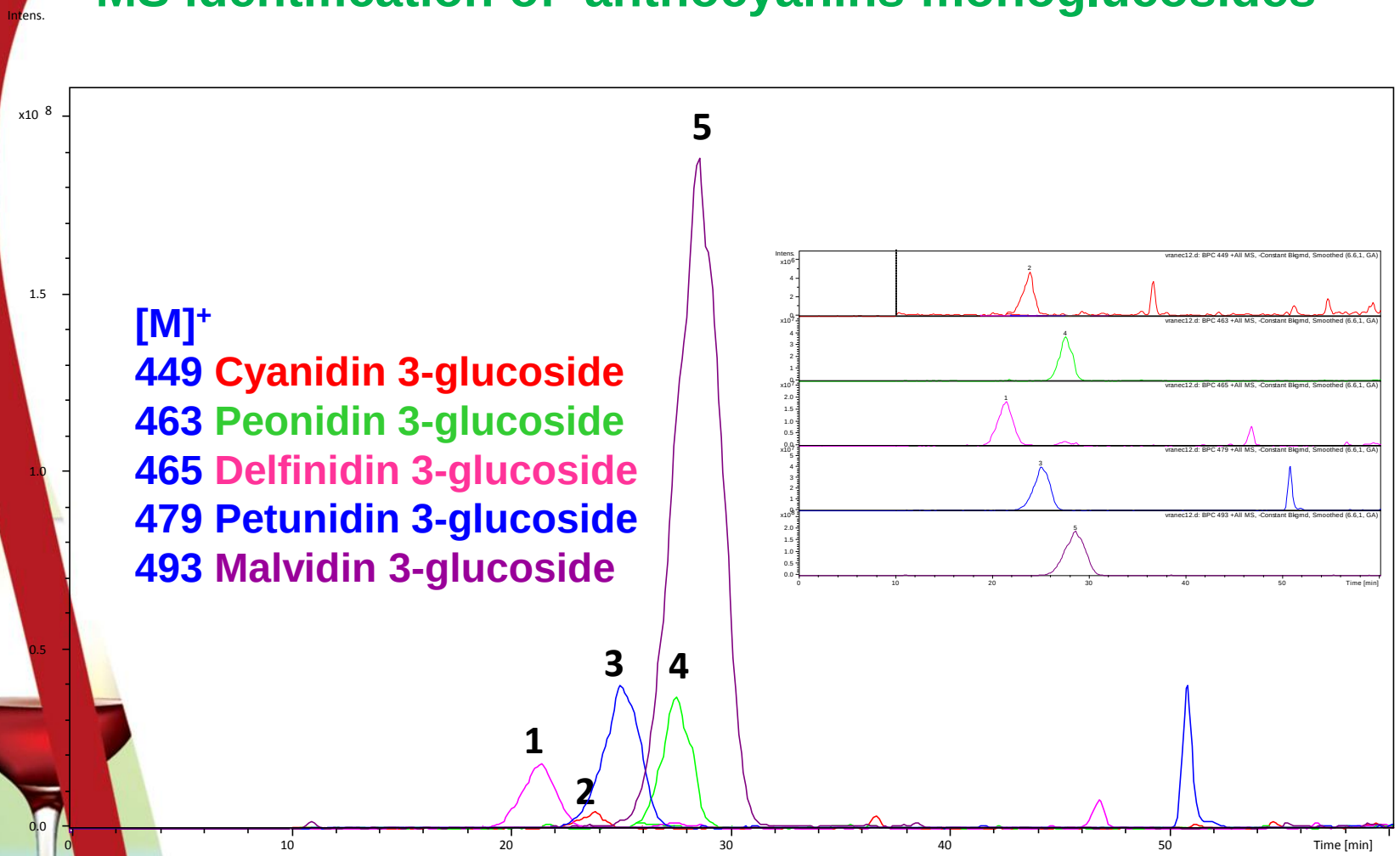
- **The ESI parametars**:
- Nebuliyer, 15 psi; Dry gas (N_2) flow 5 L/min
- Dry gas temerature, 325 ºC
- Scanning range from *m/z* 50 to *m/z* 2200 in negative and positive mode

UV-Vis Chromatograms

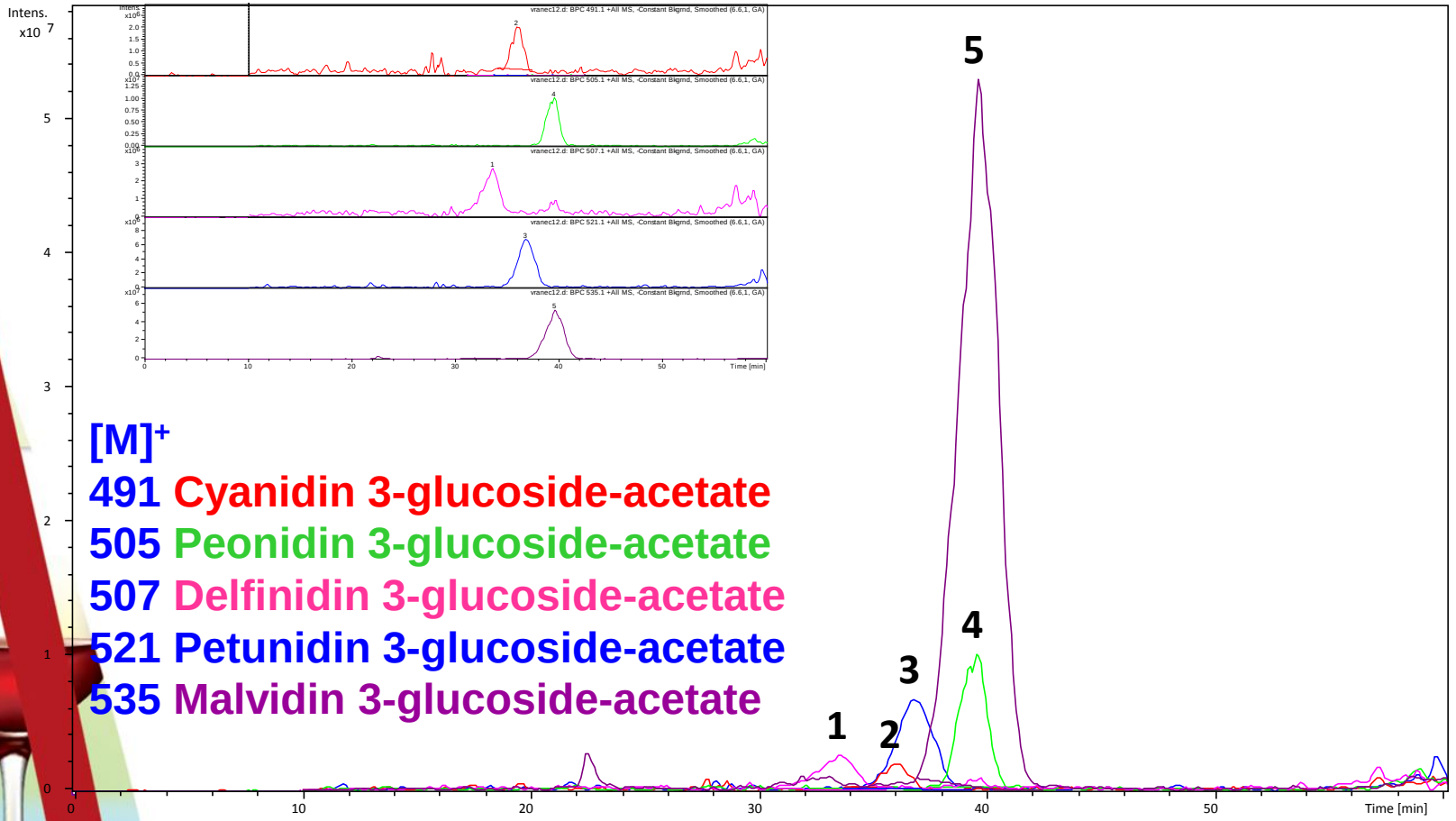


MS identification of anthocyanins monoglucosides





MS identification of anthocyanins acetylglucosides



[M]+

491 Cyanidin 3-glucoside-acetate

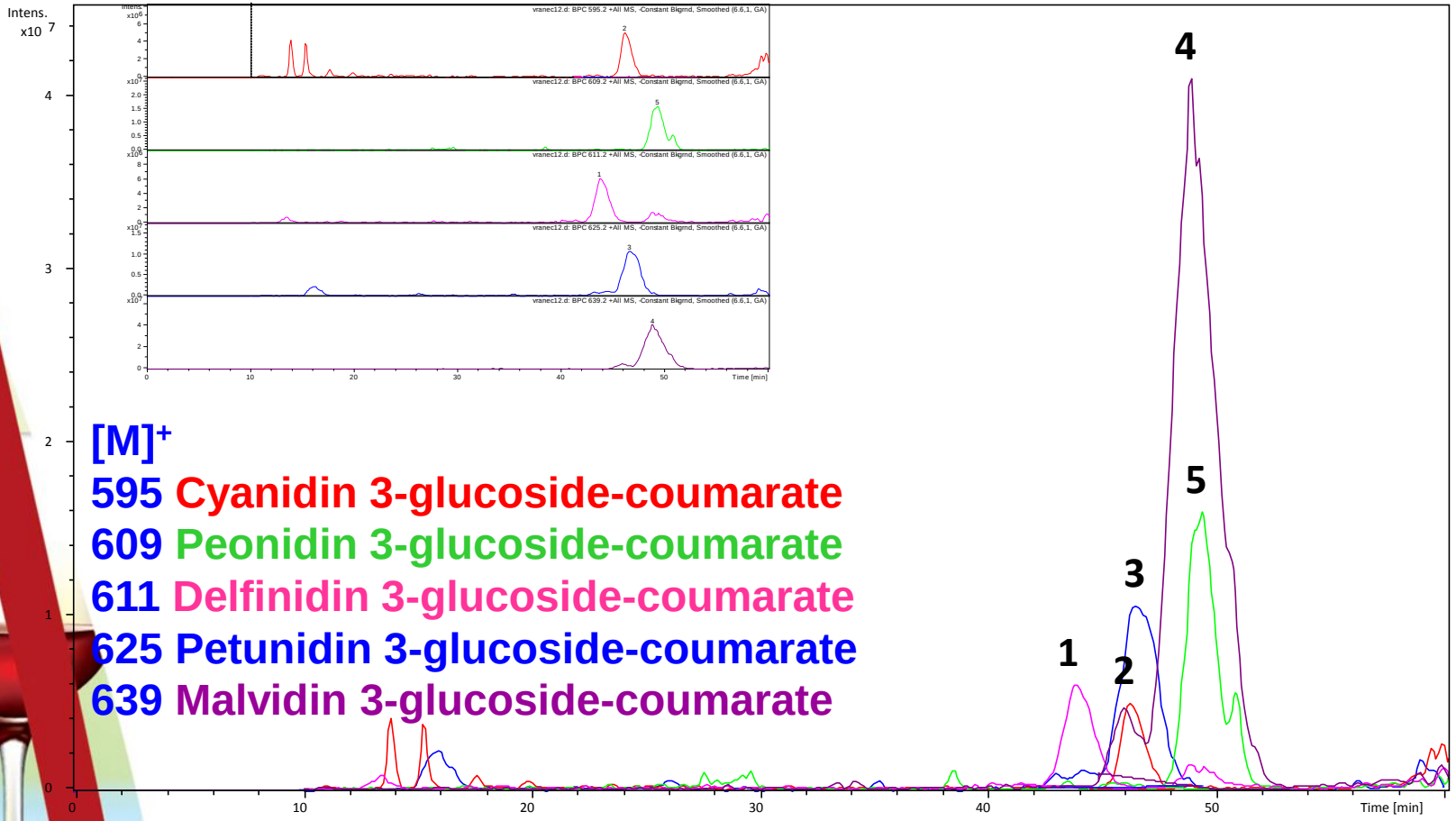
505 Peonidin 3-glucoside-acetate

507 Delfinidin 3-glucoside-acetate

521 Petunidin 3-glucoside-acetate

535 Malvidin 3-glucoside-acetate

MS identification of anthocyanins coumaroylglucosides



$[M]^+$

595 Cyanidin 3-glucoside-coumarate

609 Peonidin 3-glucoside-coumarate

611 Delfinidin 3-glucoside-coumarate

625 Petunidin 3-glucoside-coumarate

639 Malvidin 3-glucoside-coumarate

Food Chemistry 124 (2011) 316–325

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Polyphenolic content of Vranec wines produced by different vinification conditions

Violeta Ivanova [a,b], Ágnes Dörnyei [c,d], László Márk [e], Borimir Vojnoski [b], Trajće Stafilov [a], Marina Stefova [a], Ferenc Kilár [c,d,*]

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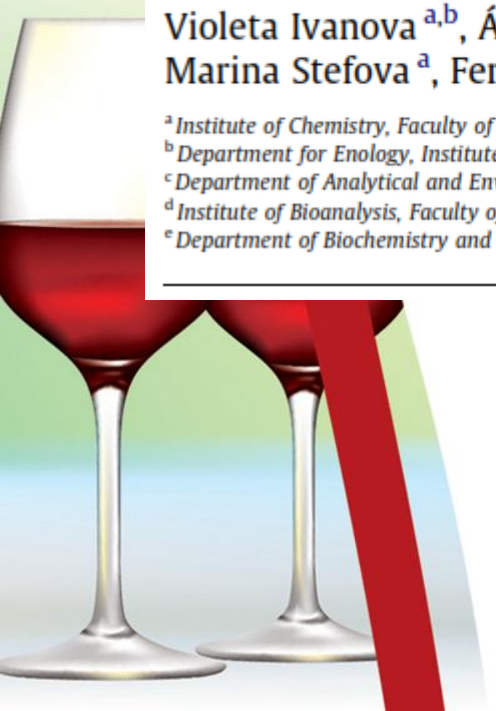


ANALYSIS OF ANTHOCYANINS IN WINE

HPLC system: Agilent 1100 Series, DAD (G1315B) and a LC/MSD Trap VL (G2445C VL) electrospray ionization mass spectrometry (ESI-MS^n) system.

Analysis of anthocyanins and other pigments:

Sample preparation: **Dilution of wines, 1:4 with HCl 0.1N**

Solvents: **A** - water/acetonitrile/formic acid (**87:3:10**, v/v/v)

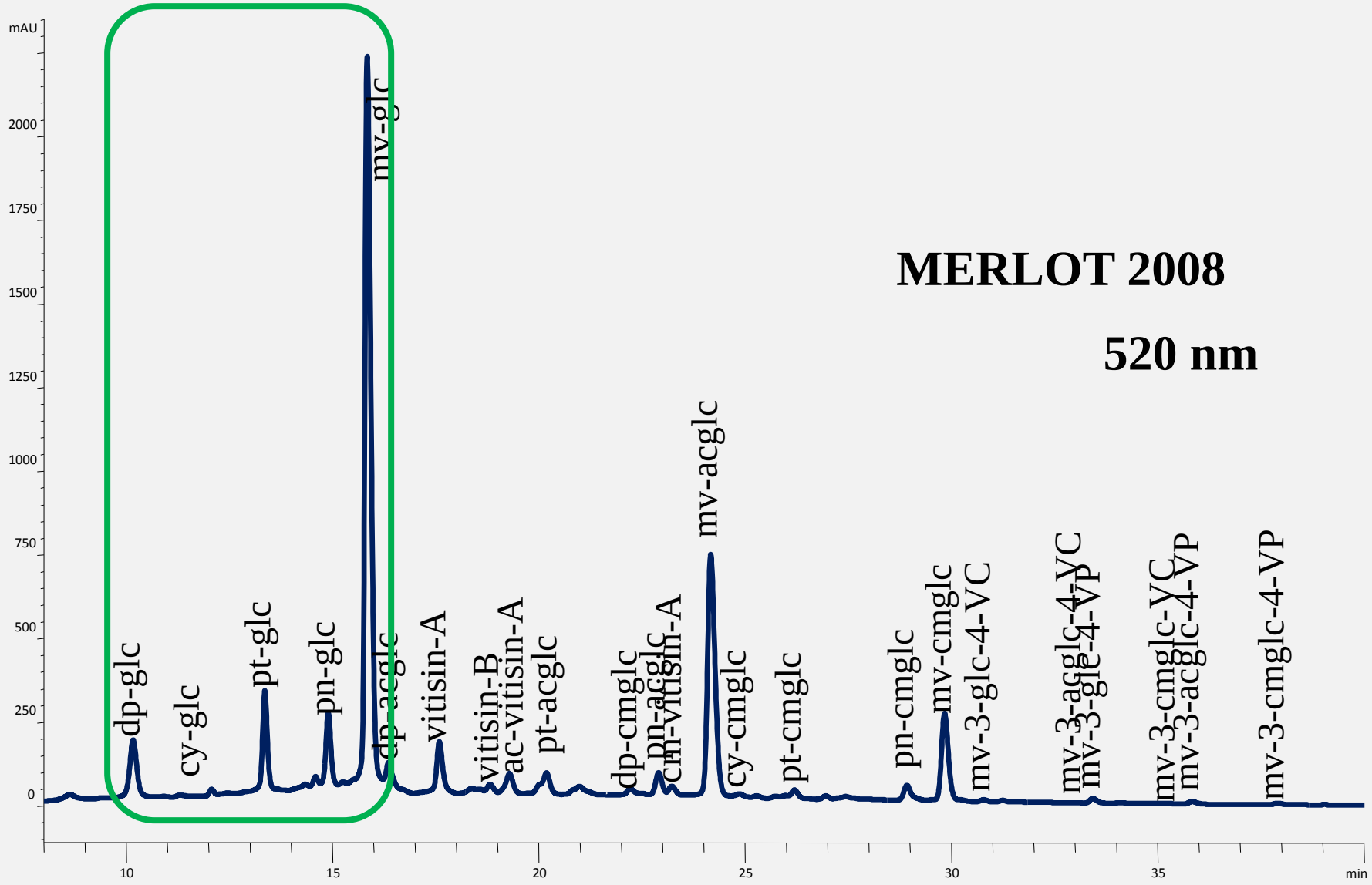
B - water/acetonitrile/formic acid (**40:50:10**, v/v/v)

Flow rate: 0.63 mL/min

DAD: 520 nm

The linear gradient for solvent B:

t/min	B (%)
0	6
15	30
30	50
35	60
38	60
46	6



Chromatographic separation of ANTHOCYANINS AND PYRANOANTHOCYANINS

Concentration of anthocyanins

ANTHOCYANIN PROFILES (molar %)

	Merlot 2006	**Merlot** 2007	**Merlot** 2008	**CabSv** 2006	**CabSv** 2007	**CabSv** 2008	**Vranec** 2006	**Vranec** 2007	**Vranec** 2008
mv-3-glc	54.51	48.21	48.92	44.91	50.81	51.70	47.58	50.15	48.73
mv-3-acglc	14.09	14.45	20.69	7.08	20.67	20.32	3.13	5.54	7.24
mv-3-cmglc	5.72	6.78	6.81	8.41	5.26	6.72	4.57	6.80	7.64
TA*	**47.6**	**159.7**	**194.2**	**351.1**	**96.1**	**193.6**	**16.1**	**53.6**	**507.6**

* mg/L, as malvidin 3-glucoside; ND, not detected; dp, delphinidin; cy, cyanidin; pt, petunidin; pn, peonidin; mv, malvidin glc, 3-glucoside; acglc, 3-(6''-acetyl)-glucoside; cmglc, 3-(6''-coumaroyl)-glucoside

Journal of Food Composition and Analysis 41 (2015) 1–14

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journal homepage: www.elsevier.com/locate/jfca

Original Research Article

Phenolic compounds and antioxidant activity of Macedonian red wines

Violeta Ivanova-Petropulos [a,*], Isidro Hermosín-Gutiérrez [b], Borbála Boros [c], Marina Stefova [d], Trajče Stafilov [d], Borimir Vojnoski [e], Ágnes Dörnyei [c,f], Ferenc Kilár [c,f]

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Food Research International

journal homepage: www.elsevier.com/locate/foodres

Identification of polyphenolic compounds in red and white grape varieties grown in R. Macedonia and changes of their content during ripening

Violeta Ivanova [a,*], Marina Stefova [b], Borimir Vojnoski [c], Ágnes Dörnyei [d,e], László Márk [f], Violeta Dimovska [a], Trajče Stafilov [b], Ferenc Kilár [d,e]

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[c] Department for Enology, Institute of Agriculture, Ss. Cyril and Methodius University, Aleksandar Makedonski bb, 1000 Skopje, Republic of Macedonia
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[e] Institute of Bioanalysis, Faculty of Medicine, University of Pécs, Szigeti út 12., H-7624 Pécs, Hungary
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MALDI-TOF-MS analyses

Important factors:

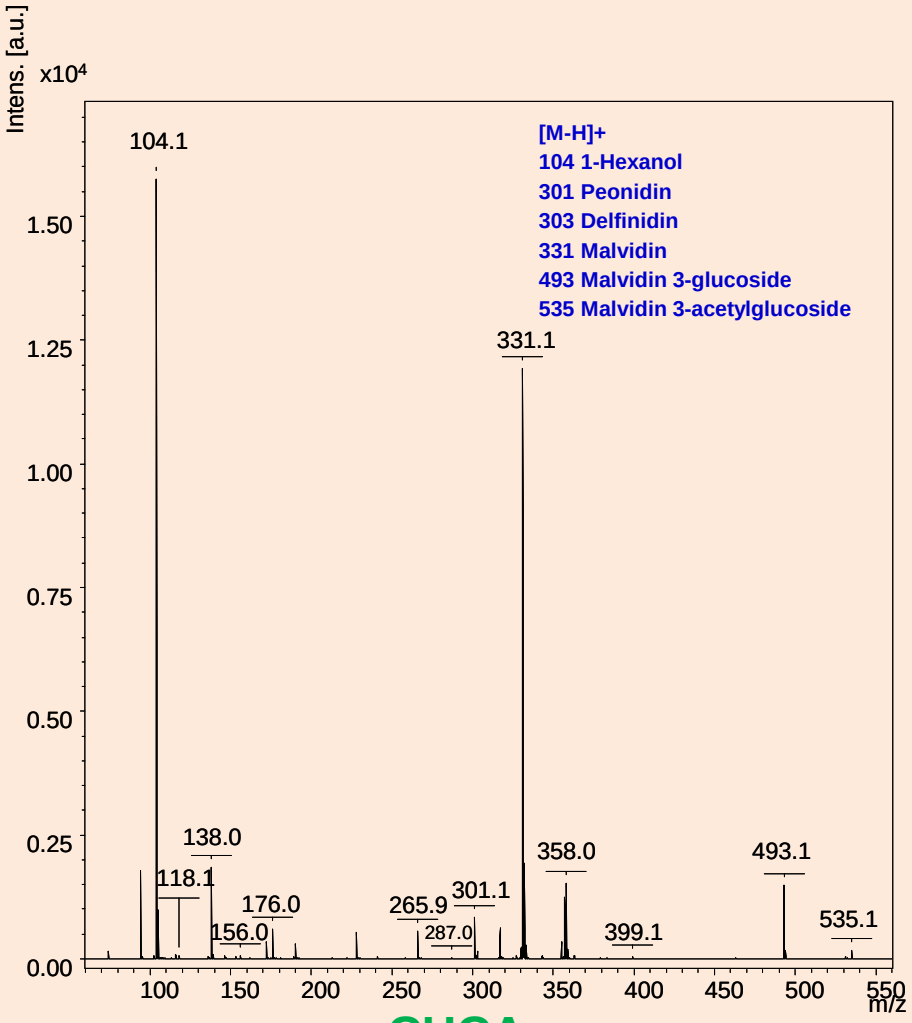
- ✓ selection of an appropriate matrix
- ✓ optimal mixing and drying of matrix and sample
- ✓ adjustment of laser strength
- ✓ selection of calibration standards
- ✓ correct interpretation of the spectra

Different MALDI matrices were tested:

1. Alpha-Cyano-4-hydroxycinnamic acid (CHCA)
2. Sinapic acid (SA)
3. 2,5-Dihydorxybenzoic acid (2,5-DHB)
4. Fullerene [C70]
5. Measurenments without matrix

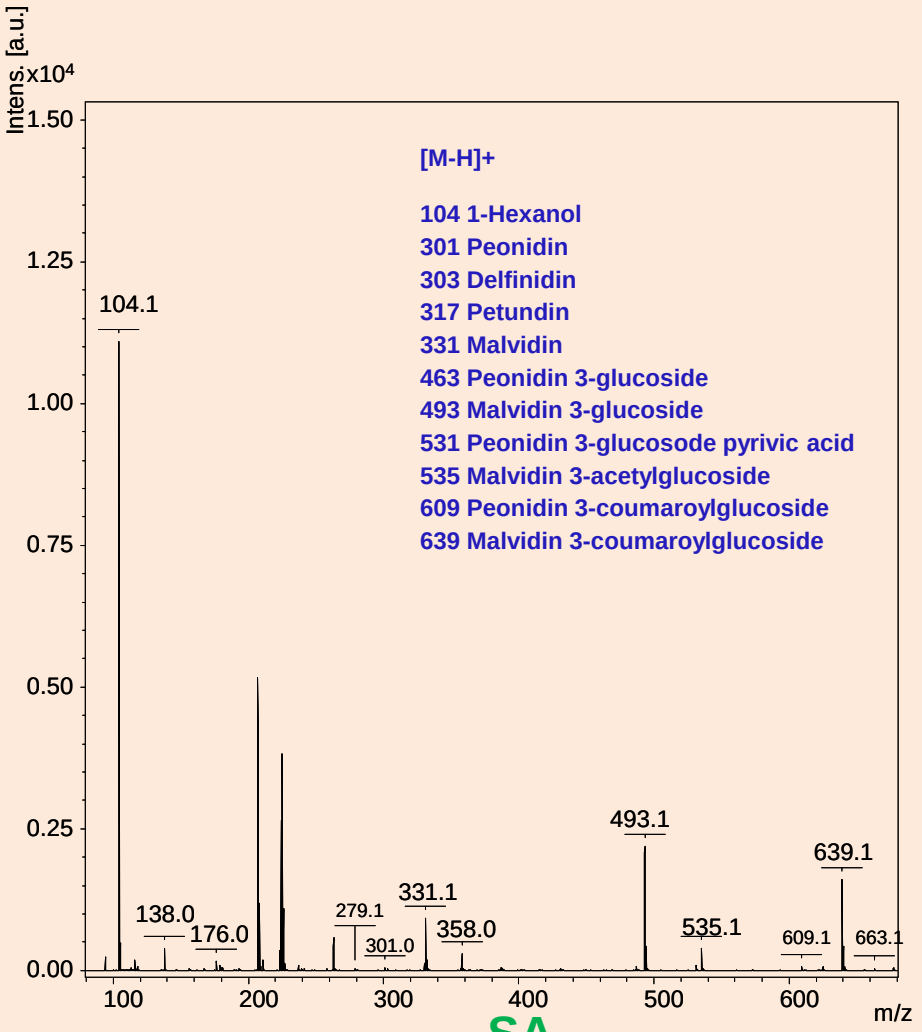
Sendwich method was applied for all sample measurements

MALDI-TOF-MS spectra of wine with different matrices



CHCA

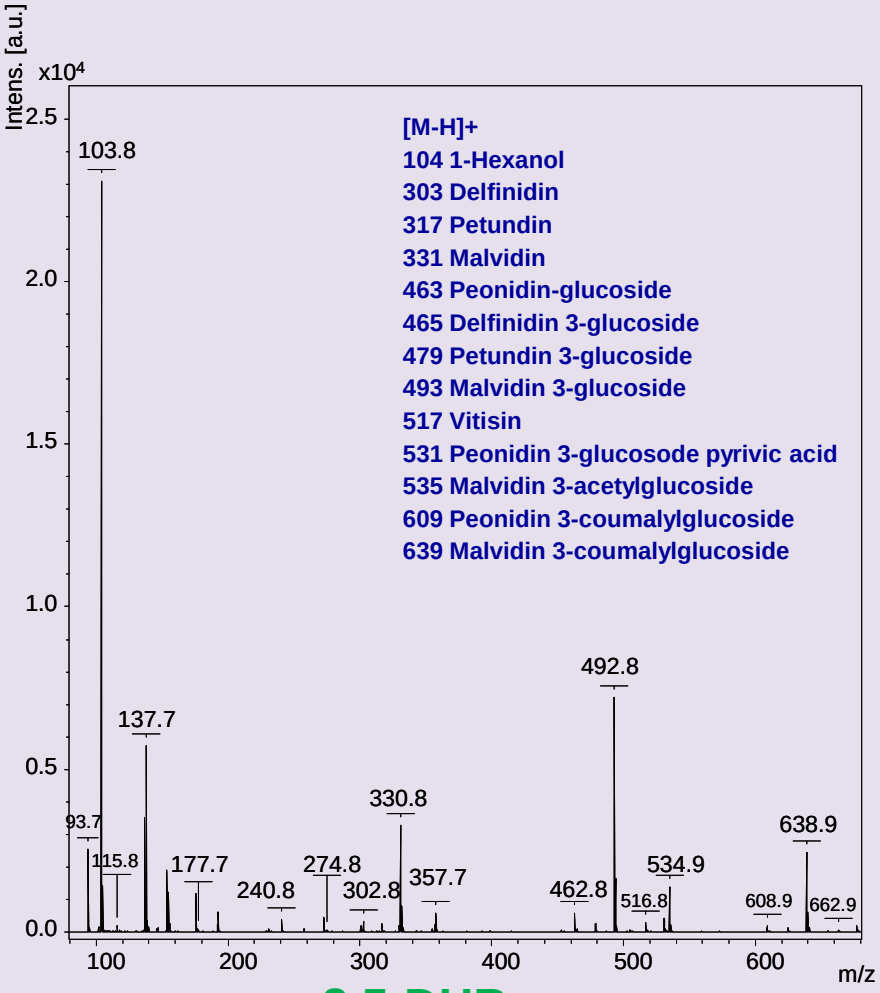
(Alpha-Cyano-4-hydroxycinnamic acid)



SA

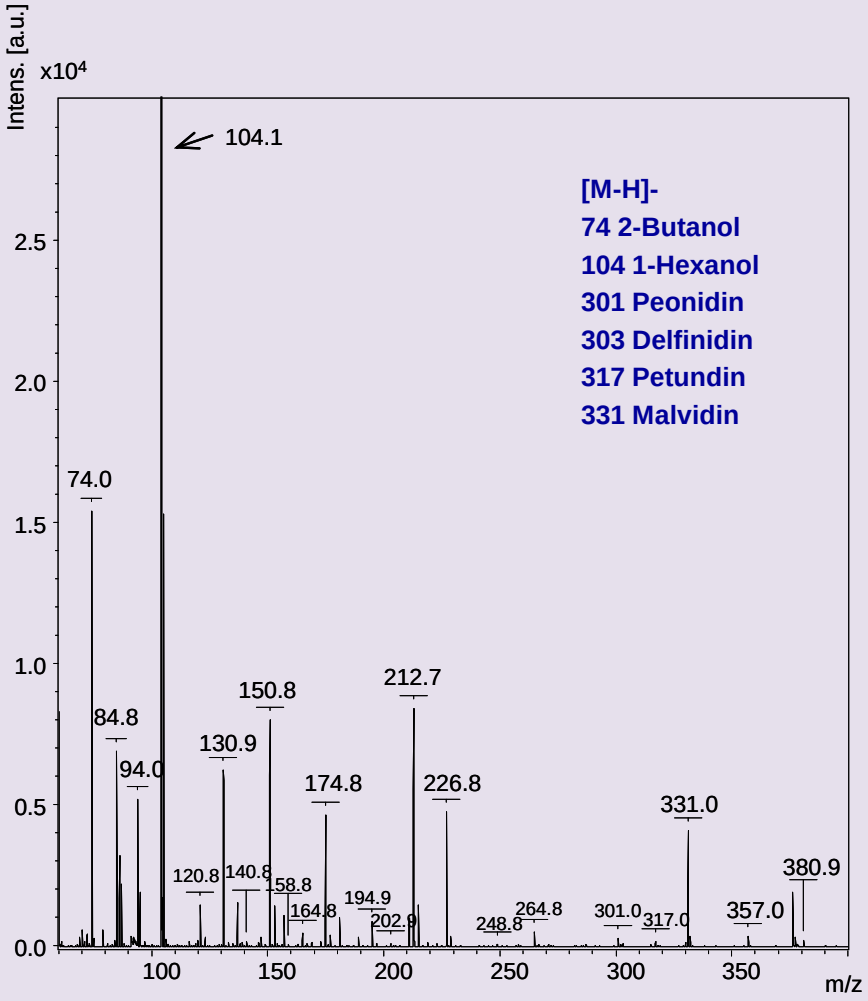
(Sinapic acid)

MALDI-TOF-MS spectra of wine with different matrices



2,5-DHB

(2,5-Dihydorxybenzoic acid)



C70

(Fullerene)

Food Anal. Methods (2011) 4:108–115
DOI 10.1007/s12161-010-9143-7

Rapid MALDI-TOF-MS Detection of Anthocyanins in Wine and Grape Using Different Matrices

Violeta Ivanova · Ágnes Dörnyei · Marina Stefova · Trajće Stafilov · Borimir Vojnoski · Ferenc Kilár · László Márk

Food Anal. Methods (2014) 7:820–827
DOI 10.1007/s12161-013-9687-4

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

Violeta Ivanova Petropulos · Ágnes Dörnyei · Marina Stefova · Trajče Stafilov · Borimir Vojnoski · László Márk · Isidro Hermosín-Gutiérrez · Ferenc Kilár

VOLATILE ELEMENTAL COMPOSITION of wines

✓Different groups of volatile compounds, have been identified in wines in a wide concentration range, affecting the wine aroma even present in a low concentration:

Alcohols
Esters
Aldehydes

Lactones
Terpenes
Volatile phenols

✓Among the volatiles, **alcohols** and **esters** (fruity nuances) are the main compounds present in a highest content in the wines.

Sample preparation and GC-MS analysis

Before the GC-MS analysis, volatile compounds are usually extracted by:

- solid-phase extraction (SPE)
- solid-phase microextraction (SPME)
- stir bar sorptive extraction (SBSA)
- **liquid-liquid extraction methods using organic solvents (dichloromethane)**

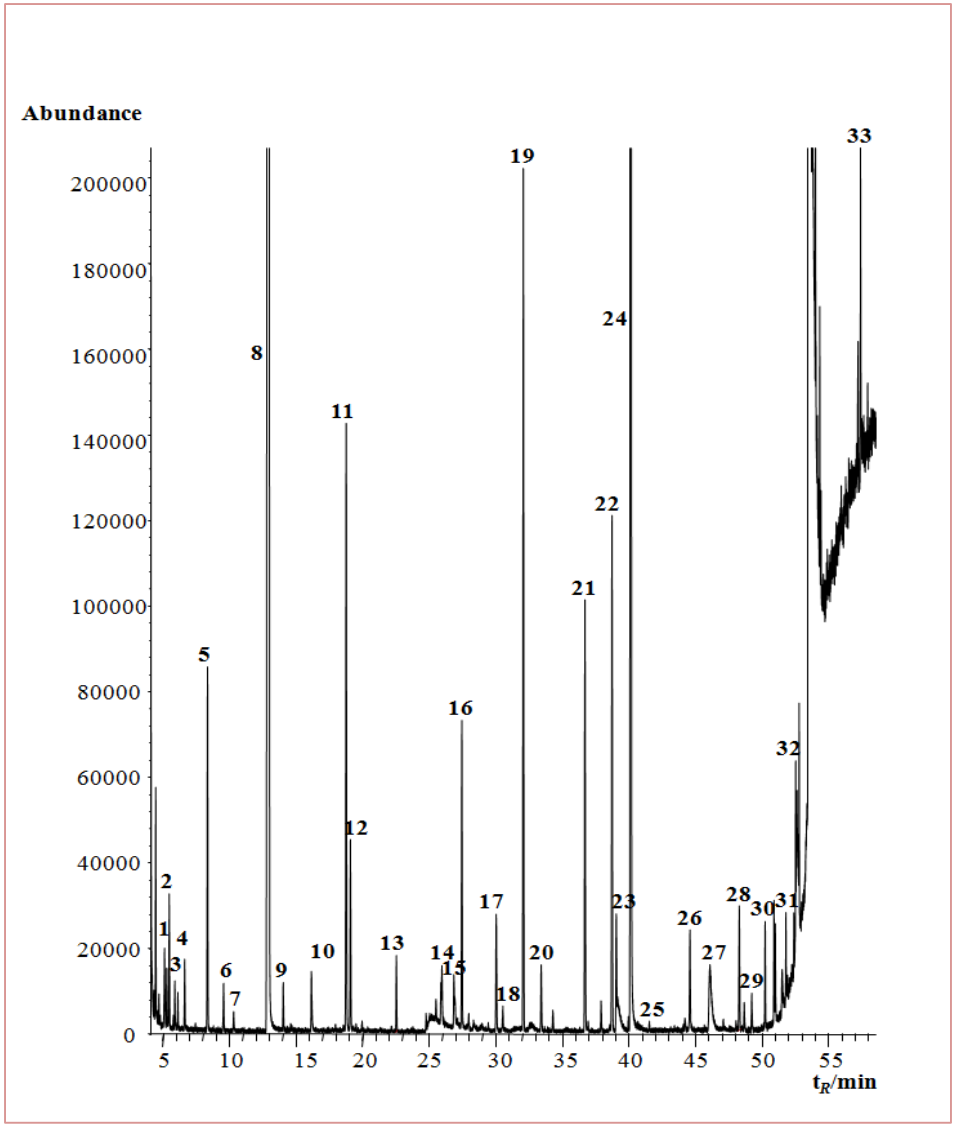
Separation - *p*olar capillary column, Carbowax type from Agilent, (30 m × 0.25 mm ID and 0.25 μm film thickness)

Working parameters:
Injector temperature 240 ºC; MS source 230 ºC; MS Quad 150 ºC,
Transfer line 280 ºC.

40 ºC for 3 min
180 ºC at 3 ºC /min.
260 ºC with 20 ºC /min
260 ºC for 10 min

METHOD APPLICATION

- Analysis of the volatile composition of five Kékfrankos wines
- 33 volatile compounds were identified and quantified



GC/MS chromatogram of the volatile compounds in Kékfrankos wine

12 alcohols quantified - secondary products mainly produced during the yeast metabolism

Volatile compounds in Kekfrankos wines samples	Wine 1/µg/L	Wine 2/µg/L	Wine 3/µg /L	Wine 4/µg/L	Wine 5/µg/L
Alcohols					
1-Hexanol	743±0.25	1578±5.63	676.70±1.72	29.15±4.27	43.84±5.52
1-Pentanol	**11600±9.50**	**80232±5.53**	**39895.3±4**	**161.77±0.24**	**1125.38±1.81**
1,3-Butylen glycol	598±0.49	2503±6.49	1176.10±2.20	4.34±0.38	32.39±1.97
2,3-Butanediol	280±8.48	147±29.88	/	/	9.85±20.52
3-Heptanol	575±0.42	346±27.1	329.23±3.16	6.01±1.77	5.12±1.42
3-(Methylthio)-1-propanol	713±0.49	723±3.93	452.34±3.47	15.76±2.01	16.00±2.23
Benzyl alcohol	738±0.41	1079±4.14	8019.63±165	85.11±8.69	900.98±99.21
E-3-Hexanol	567±0.07	240±5.12	197.84±8.28	3.90±1.80	7.35±3.82
Isobutyl alcohol	840±1.94	2728±4.62	1245.27±2.97	25.88±1.36	22.99±1.59
Phenyl ethanol	**14170±5.26**	**50983±7.62**	**15412.94±85.4**	**69.84±0.20**	**1826.87±4.11**
Tyrosol	1480±3.43	3248±9.21	1794.19±6.14	180.11±8.28	310.51±9.34
Vinyl guiacol	103±3.58	209±16.84	323.98±143.4	14.46±6.75	33.30±12.47
Total alcohols (µg/L)	**32407±30.3**	**144016±126**	**69522±425**	**596±35.7**	**4335±164**

✓ **2-phenyl ethanol, the most important phenol-derived higher alcohol (present from 11.7 to 43.7% of the total alcohols) and 1-pentanol (26 - 57% of the total alcohols) - major components in the overall volatile content of the wines.**

✓ **Kékfrankos wines presented similar content of alcohols compared to other varieties from the world.**

Food Anal. Methods (2012) 5:1427–1434
DOI 10.1007/s12161-012-9401-y

Validation of a Method for Analysis of Aroma Compounds in Red Wine using Liquid–Liquid Extraction and GC–MS

Violeta Ivanova · Marina Stefova · Trajče Stafilov · Borimir Vojnoski · Ildiko Bíró · Anita Bufa · Ferenc Kilár

Food Bioprocess Technol (2013) 6:1609–1617
DOI 10.1007/s11947-011-0760-y

COMMUNICATION

Volatile Composition of Macedonian and Hungarian Wines Assessed by GC/MS

Violeta Ivanova · Marina Stefova · Borimir Vojnoski · Trajče Stafilov · Ildiko Bíró · Anita Bufa · Attila Felinger · Ferenc Kilár

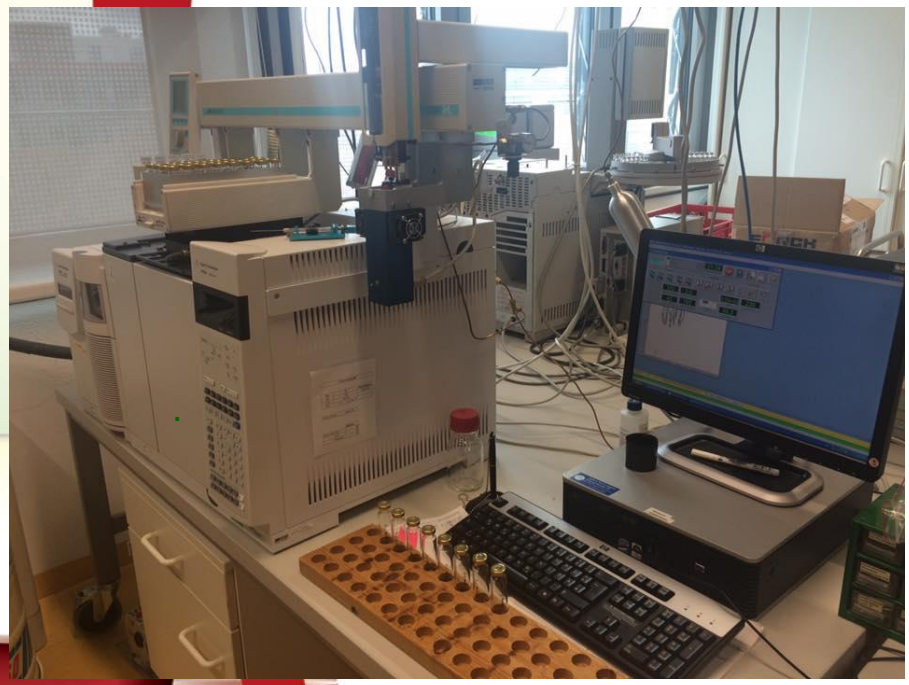
HS-SPME-GC-MS analyses

- An automated HS-SPME combined with GC-MS is **highly efficient separation technique** for extraction and separation of wine aroma compounds.

HS-SPME conditions

- 500 µL of wine transferred into a headspace vial.
- the headspace flushed with nitrogen
- SPME fiber : DVB/Carboxen/PDMS 50/30, 2 cm stable flex (Supelco, Bellfonte, USA).
- Prior to the extraction, samples were equilibrated at 40°C for 5 minutes.
- SPME fiber exposed into the headspace for 20 min at 40°C and transferred to the GC-injector at 270°C.

HS-SPME-GC-MS analyses of volatile compounds



Agilent system (GC 7890, MS 5975c VL MSD)

Separation - HP5MS column, 30m*0,25mm*1µm, Agilent Technologies

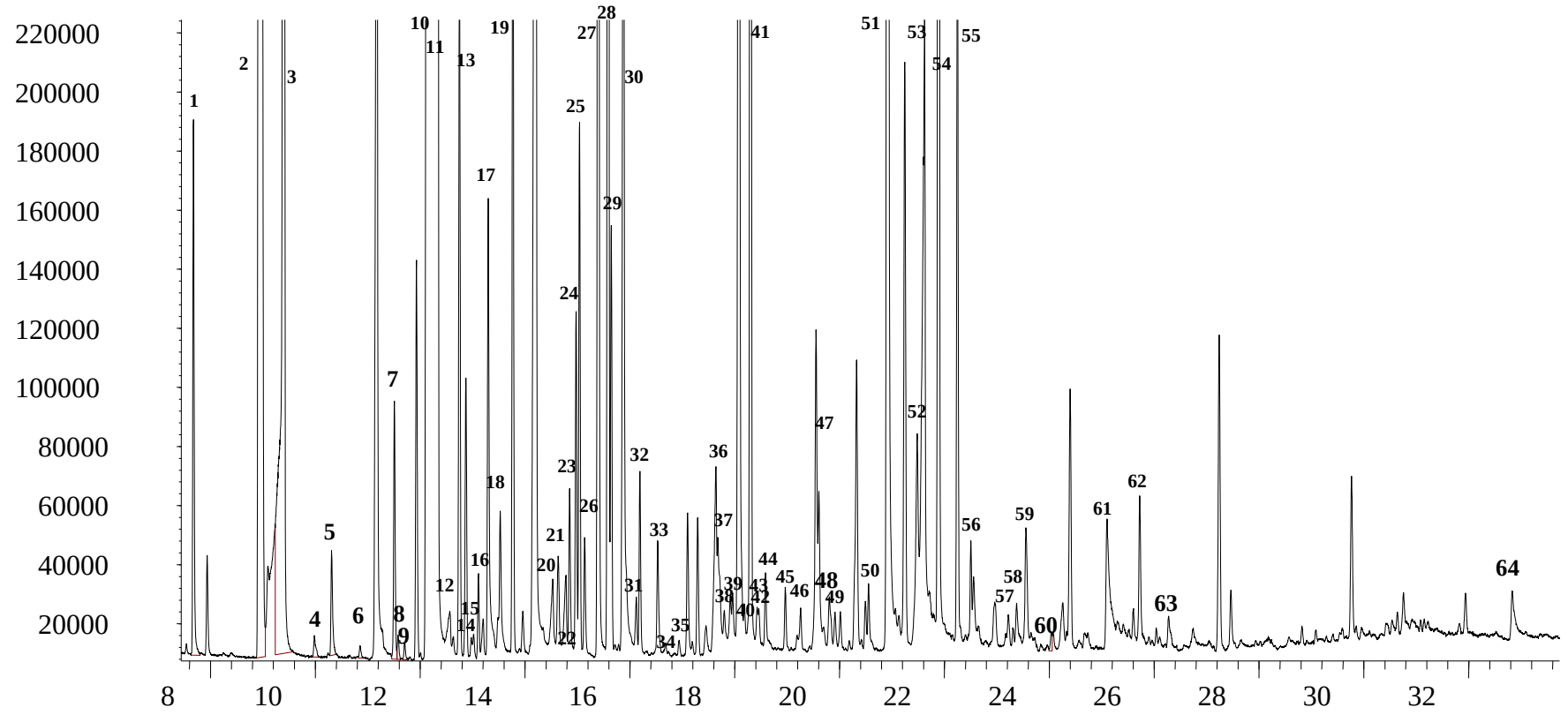
Working parameters:
Injector temperature 270 ºC; MS source 230 ºC; MS Quad 150 ºC,
Transfer line 280 ºC.

-10 ºC for 1 min
270 ºC at 8 ºC /min

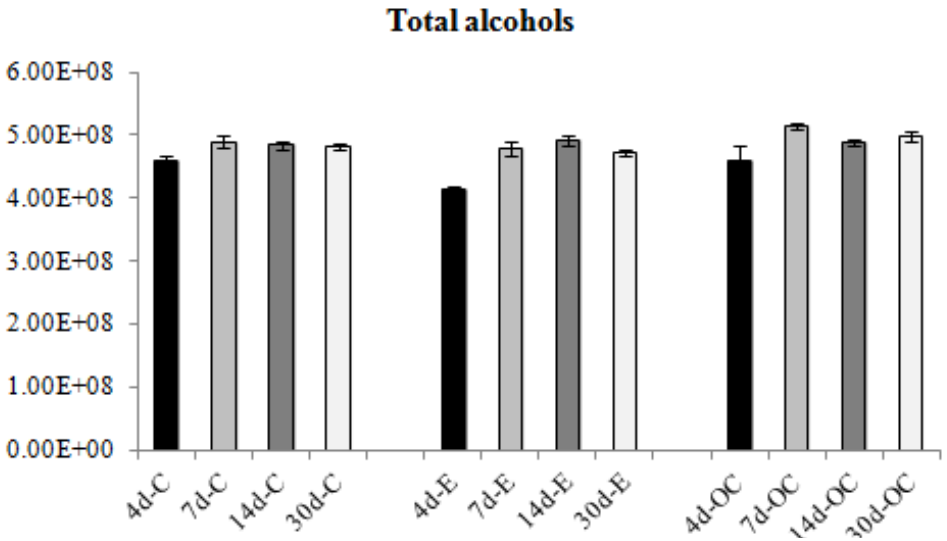
Wine samples: **Vranec**

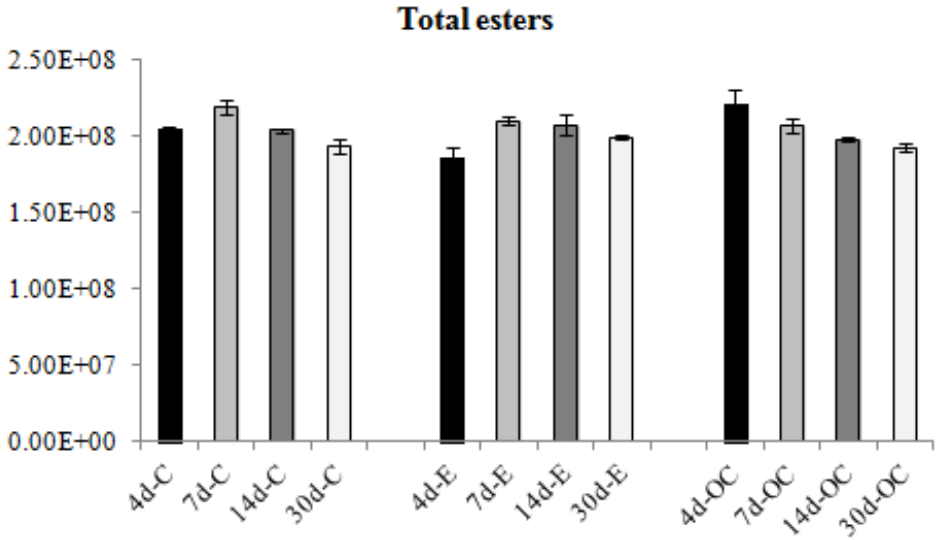
1) **Control wine**
2) **Wine with addition of enzyme**
3) **Wine with addition of oak chips**
4) **Maceration time: 4, 7, 14, 30 days**

GC-MS chromatogram of wine after HS-SPME



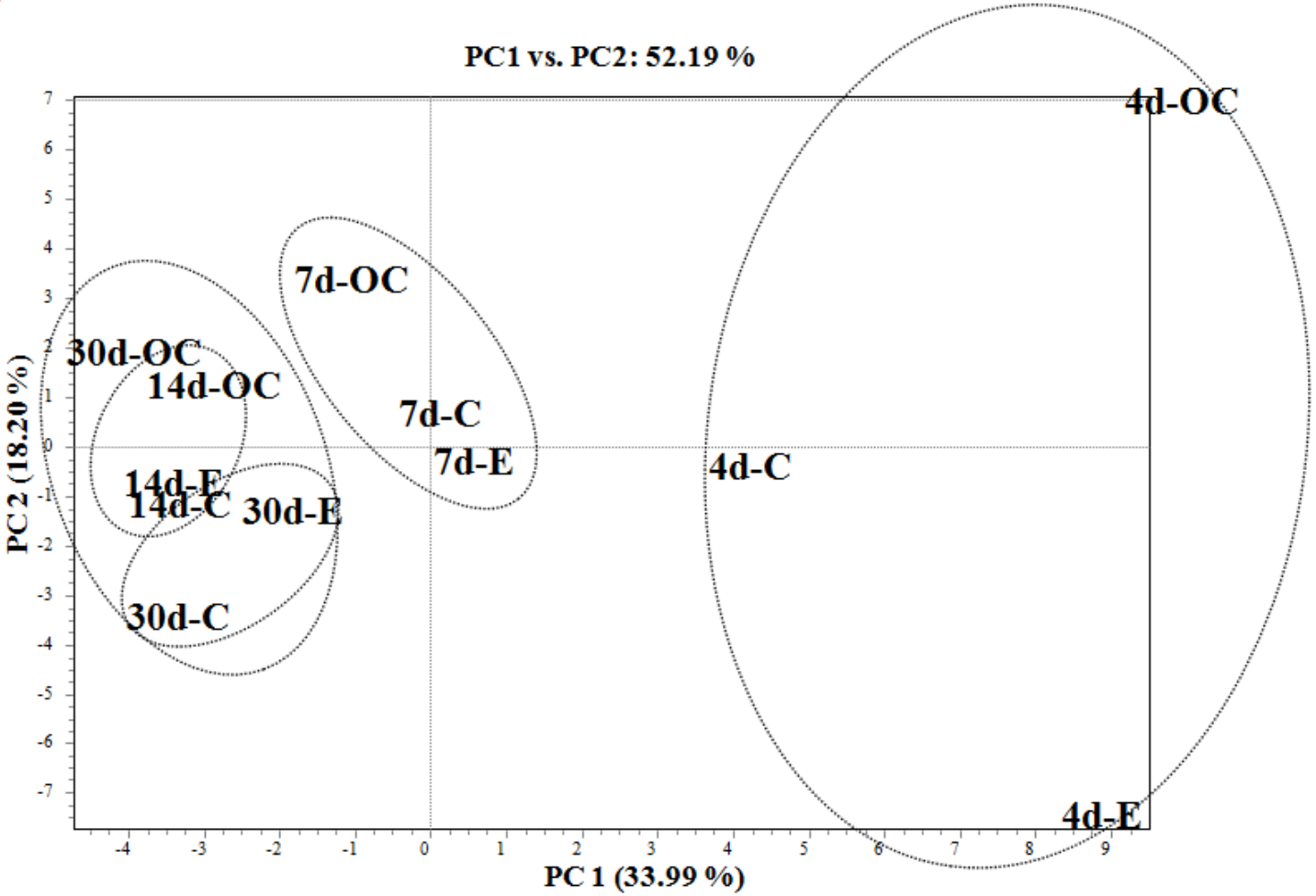
Quantitative analysis





PCA analysis

- Separation according to the **maceration time**



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journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Study of the influence of maceration time and oenological practices on the aroma profile of Vranec wines

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ELEMENTAL COMPOSITION of wine determined by ICP-OES and ICP-MS

ICP-OES and ICP-MS operating conditions

Parameter	ICP-OES	ICP-MS
RF Power	1350 W	1350 W
Cooling gas flow	12.5 L min^{-1}	14 L min^{-1}
Auxiliary gas flow	0.6 L min^{-1}	1.3 L min^{-1}
Nebulizer gas flow	0.83 L min^{-1}	0.91 L min^{-1}
Nebulizer	Cross flow	Meinhard Type A
Spray chamber	Scott type	Cyclonic
Integration time	24 s	1000 ms for each m/z, 50 ms dwell time, peak hopping
Replicates	5	4

Validation

One wine sample spiked with 10 µg/L multi-element solution consisting of Ag, Au, Be, Bi, Cd, Ce, Co, Cu, Dy, Er, Eu, Ga, Gd, Ge, Ho, La, Lu, Mo, Nd, Pb, Pr, Sm, Tb, Tl, Tm, U, V, Yb, Zr, for the ICP-MS analysis

Recoveries: 93 and 109 %

The procedure was evaluated by analyzing a CRM (trace elements in water, NIST SRM 1643e)

ELEMENTAL COMPOSITION of wine determined by ICP-OES and ICP-MS

25 wine samples (10 white wines, 14 red wines and 1 rose wine) from vintage 2011

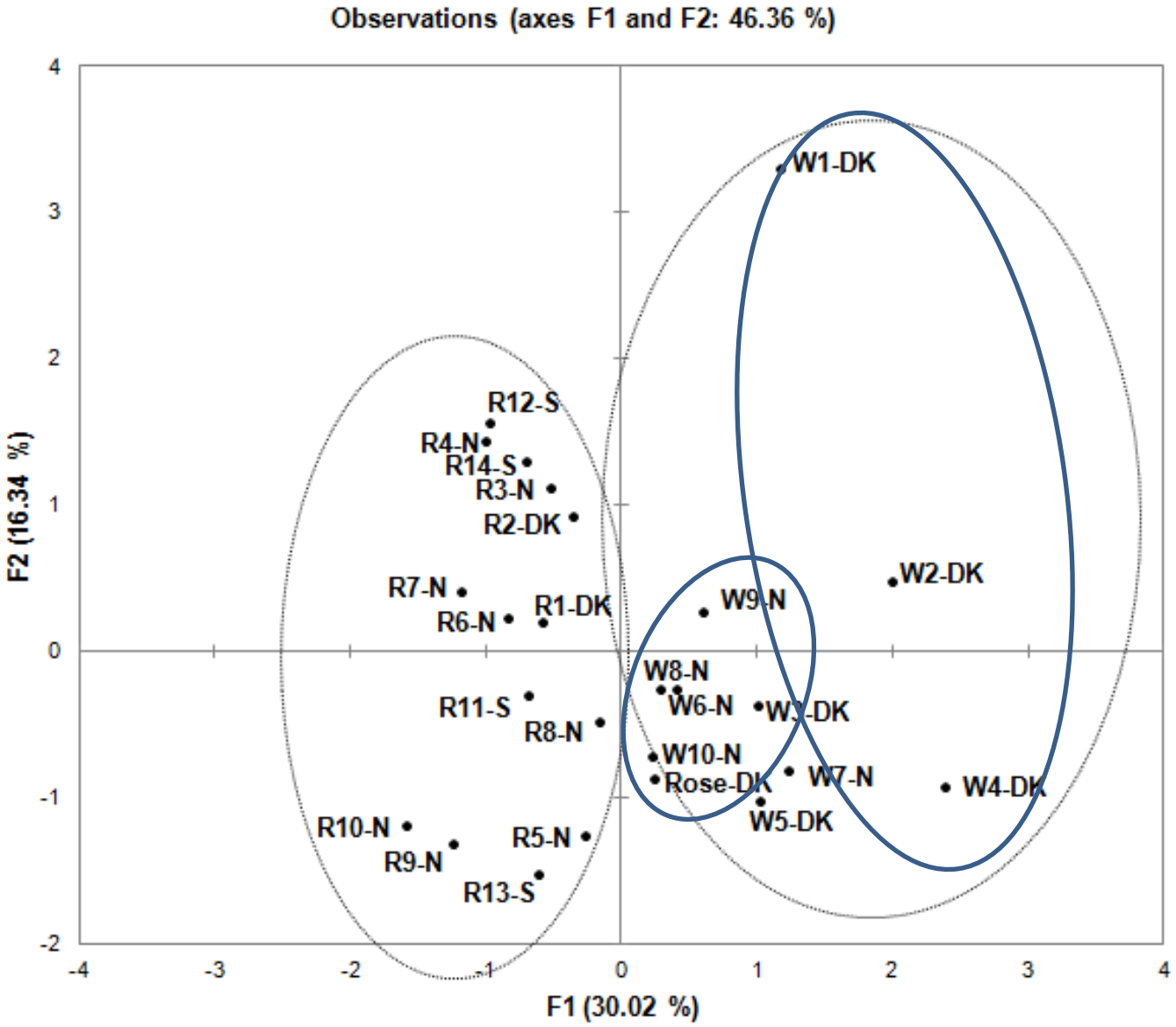
- 42 elements quantified in red, rose and white wine

Ag, Al, Au, B, Ba, Be, Bi, Ca, Cd, Ce, Co, Cu, Dy, Er, Eu, Fe, Ga, Gd, Ge, Ho, La, Lu, Mg, Mn, Mo, Na, Nd, Ni, P, Pb, Pr, S, Sm, Tb, Ti, Tl, Tm, U, V, Yb, Zn, Zr.

Ba, S, P, Ca and **Mg** were the most abundant elements in the studied wines, followed by **Cu**, **V**, **Pb** and **Na**.

Elements Ag, Au, Bi, Dy, Er, Eu, Ge, Ho, Lu, Ni, Pr, Sm, Tb, Ti, Tm, Yb were detected in a concentration lower than the LOQ.

PCA analysis

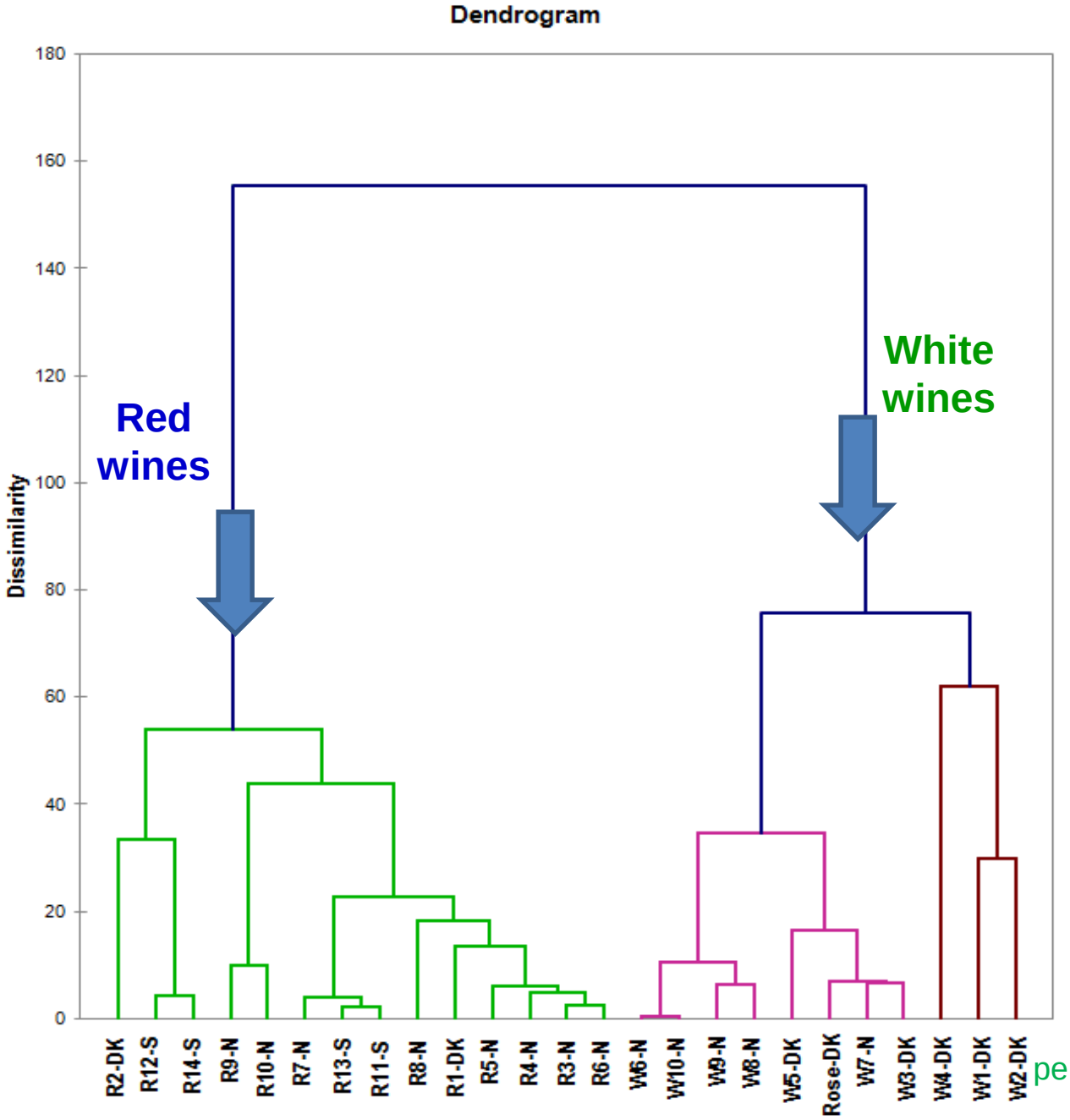


- clear separation according to the **wine type** (**white vs. red**).

- grouping according to the **region**.
Negotino region
Demir Kapija region.

Observations with F1 and F2 of the variables based on elements concentration in wines and grouping of the wines according to wine type

Cluster analysis



Wine grouping according to the wine type (red vs. white wines)

Dendogram obtained after the agglomerative Cluster Analysis performed on all elements quantified in wine samples

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MULTIELEMENT ANALYSIS OF MACEDONIAN WINES BY INDUCTIVELY COUPLED PLASMA–MASS SPECTROMETRY (ICP-MS) AND INDUCTIVELY COUPLED PLASMA–OPTICAL EMISSION SPECTROMETRY (ICP-OES) FOR THEIR CLASSIFICATION

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CONCLUSION

Macedonian wines presented:

- Complex chemical composition
- Rich in phenolics and volatiles
- Contain low concentration of heavy metals

…… other analyses are carrying out.

CONCLUSION

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