15th Students' Congress of SCTM

PHENOLIC PROFILE OF MERLOT WINES DETERMINED BY UPLC-ESI-IT-MS

Aleksandar Piperevski[1,2],

Violeta Ivanova-Petropulos[1], Viktor Sándor[3], Ferenc Kilár[3]

[1]Faculty of Agriculture, University "Goce Delčev" - Štip, Krste Misirkov 10A, Štip, Republic of N. Macedonia

[2]Imako Vino Winery, Mihajlo Apostolski 34/5 2000 Stip, R. North Macedonia

[3]Institut of Bioanalisis, Faculty of Medicine, University of Pécs – Hungary, 7624 Pécs, Honvéd utca 1.

INTRODUCTION

- ***Polyphenols - large family of naturally occurring, organic compounds***
- ***Important constituents of red and white wines, contributing to the taste, color, mouthfeel and overall quality.***
- ***Associated with the health-promoting properties of red wine***

INTRODUCTION

- Flavonoids and non-flavonoids

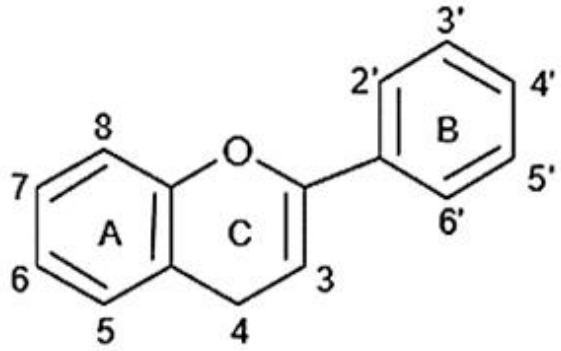
Flavonoids:

- Anthocianins, flavan-3-ols, flavonols and dihydroflavonols

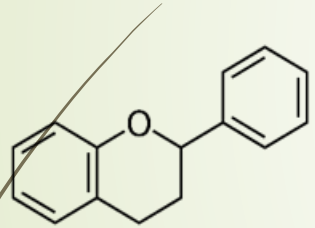
Non-flavonoids

- Phenolic acids and their derivatives and Stilbenes

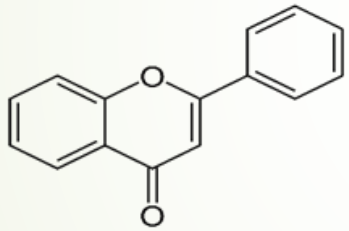
Flavonoids



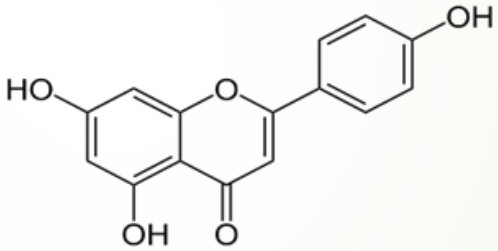
Two phenolic rings **A,B**
Pyran ring **C**



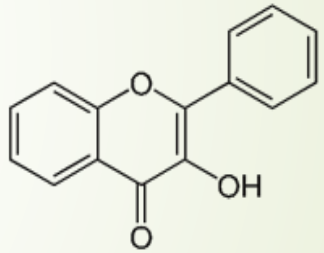
Flavan



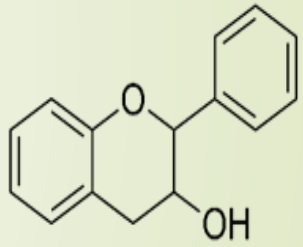
Flavanon



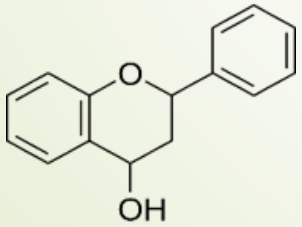
Flavon



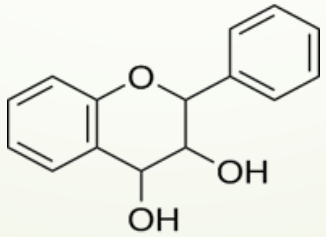
Flavonol



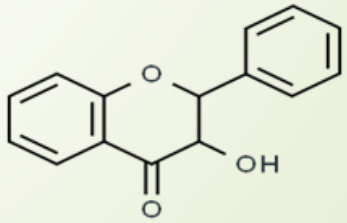
Flavan-3-ol



Flavan-4-ol

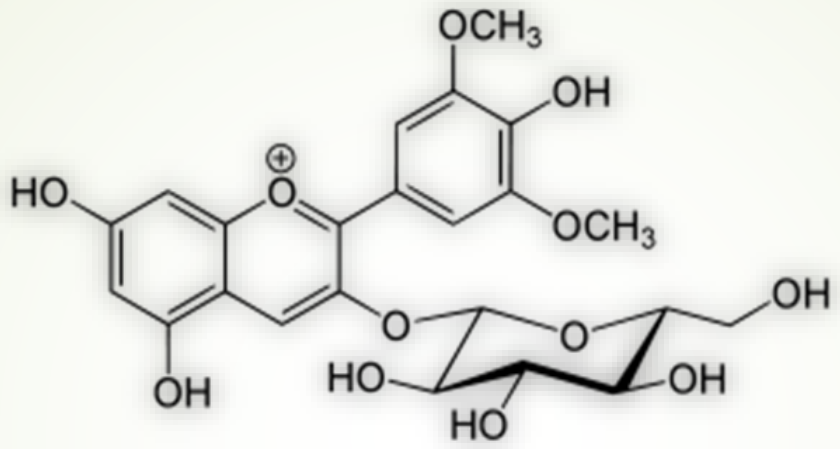


Flavan-3,4- diol



Dihidroflavonol

Anthocianins

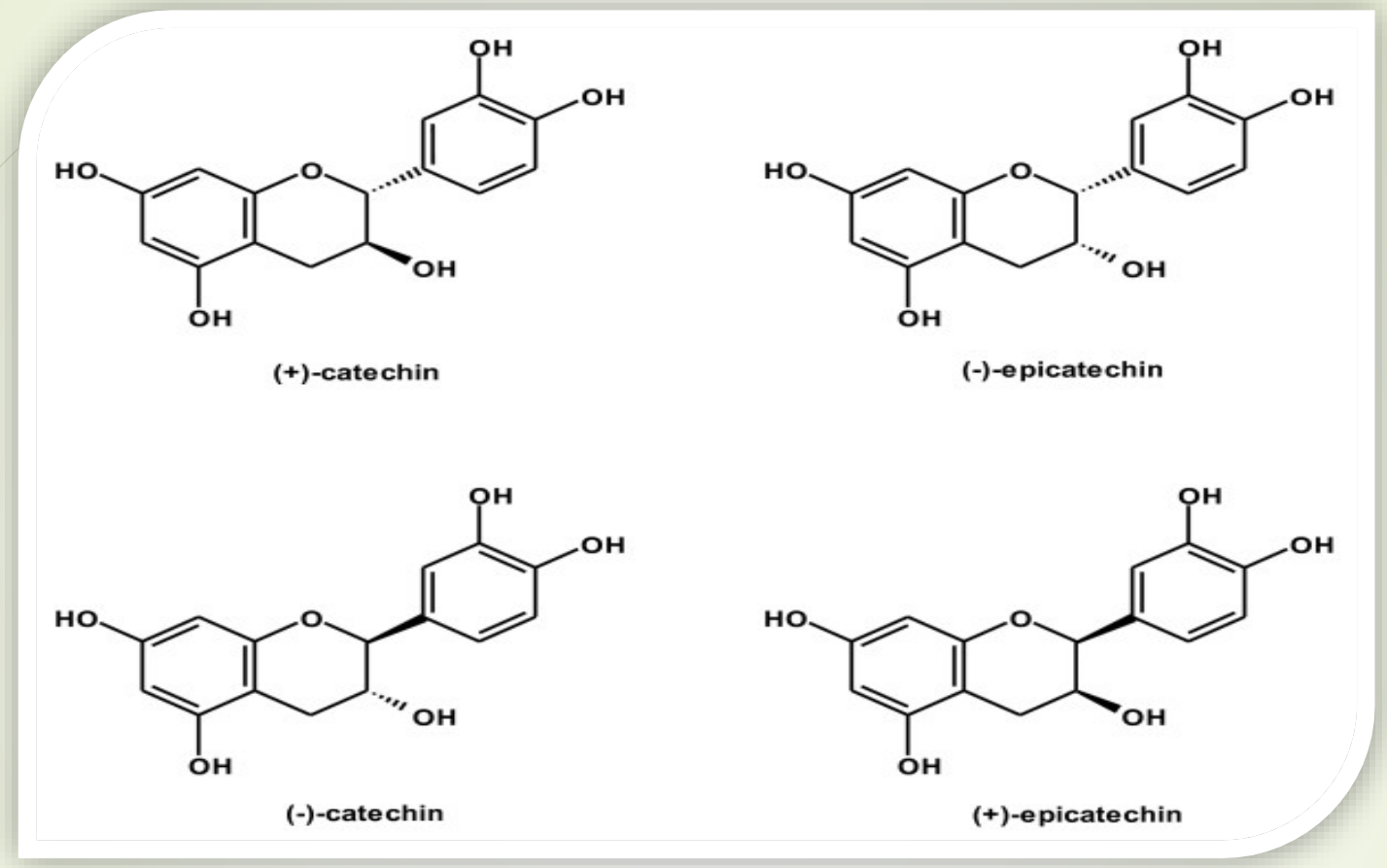


- **Malvidin, delphinidin, peonidin, cyaniding, petunidin**

As:

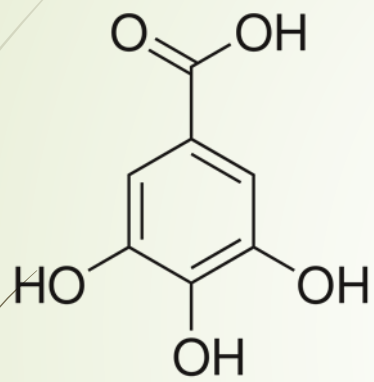
- **Monoglucosides**
- **Acetylglucozides**
- ***p-coumarylglucozides***

Flavan-3-ols

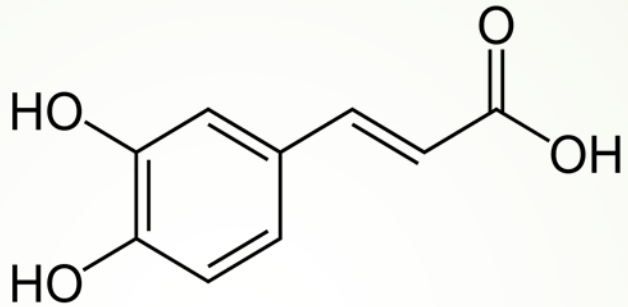


- **Monomers: Catechin, Epicatechin**
- **Dimers: (B1, B2, B3 ,B4), polypmers**

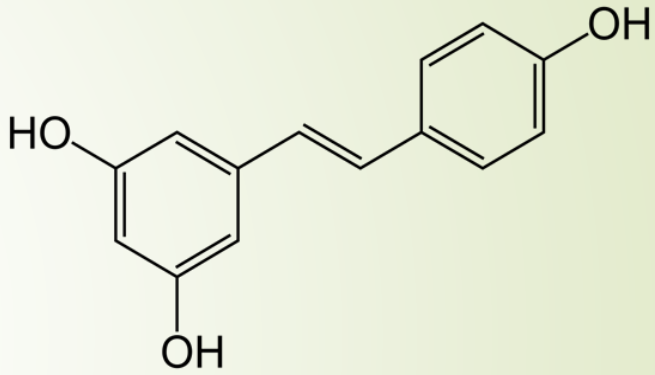
Non-flavonoids



Gallic acid



Ferulic acid



Resveratrol

- **Phenolic acids**
- **Stilbens**

Merlot variety

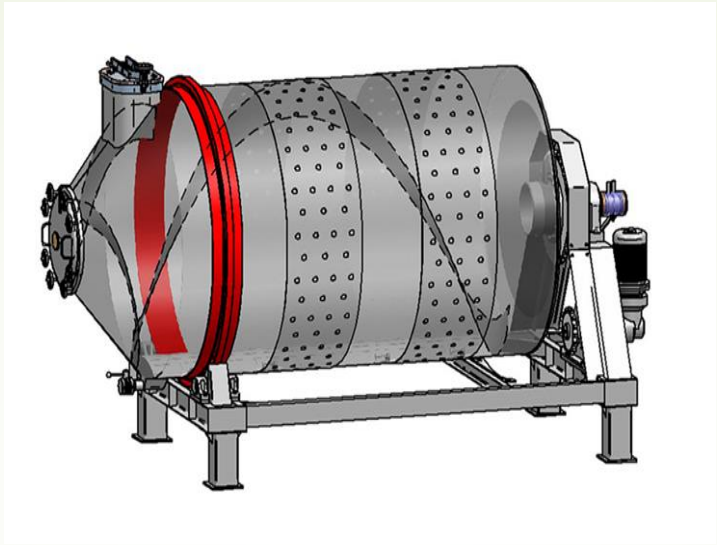
- International red variety
- In Macedonia, it can be found in Veles, Kumanovo, Ovcepole, Bitola and other wine districts. It ripens at the beginning of September. The cluster is small, cylindrical, long and compressed. The berry is small, dark blue coloured and with thin skin.
- The Macedonian Merlot is elegant and complex wine, rich in coluor and aroma.

Merlot grapes

Types of fermenters for winemaking

CLASICAL FERMENTER

- Cylindrical stainless steel vessels with a conical bottom.
- Pump that transports the fluid from the bottom to the top.
- Temperature regulation
- Fermentation lasts from 6 to 9 days.



ROTO FERMENTER

- Cylindrical vessel in horizontal position.
- Supplied with a rotation program.
- The contact area between the must, the skins and the seeds is better.
- Maceration lasts longer and is more effective.

PUNCHDOWN FERMENTER

- The punch-down mechanism breaks down the cap created during fermentation and submerge it into the must.
- The pneumatic cylinder continuously pushes the shovel-shaped punch-down agitator up and down.

EXPERIMENTAL

- **Wine samples: Merlot produced with 3 types of fermentation**

Winemaking: Grapes with optimal maturity (22 º Brix)

- **Pectinase enzymes are then added (Sodinal, Speed up Rouge 20g/100L).**
- **30 mg/L SO_2.**
- ***Saccharomyces cerevisiae* (Sodinal FERMCTIVE ROUGE, 20g /100L previously activated in hot water at 30ºC).**
- **Temperature of fermentation (24ºC).**
- **Supplement of ammonium phosphate (10g /100L)**
- **Tartaric stabilization and sedimentation (0-4ºC).**
- **Before analysis, wine samples are stored for 3 months in a dark and cool.**

EXPERIMENTAL

Wine analysis

- UPLC- Ultra Hightperformance Liquid Chromatografy (**Agilent 1290 UHPLC instrument)**
- **Agilent 6530 Accurate- Mass Q-TOF**
- CORTEX UPLC **C18** column (**2,1 x 150 mm , 1,6 µm**)
- ***Multi –step gradient***

  (*eluent A:* **1% CH_3COOH in water**, *eluent B:* 1**% CH_3COOH in methanol**)
- ***Elution*** (0-10 min 5-20% B, 10-45 min 20-50% B, 45-50 min 50-80% B, 50-60 min 80-90% B.
- ***Injection volume:*** 10µL
- ***Sample preparation:*** **filtration of wines with 0,45 µm filters**

EXPERIMENTAL

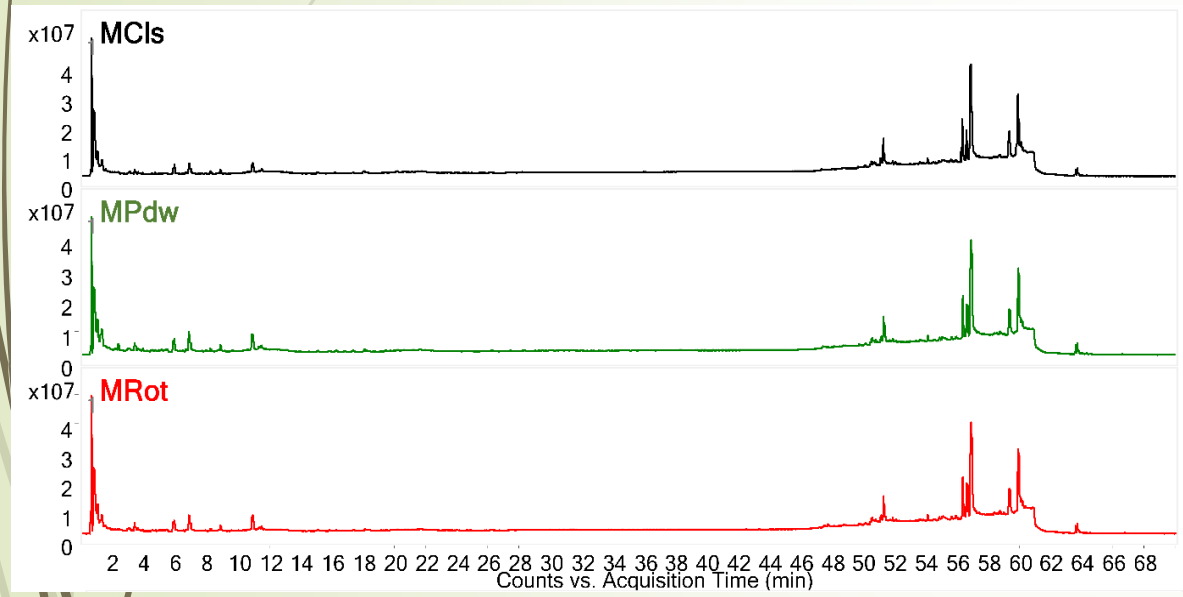
- Identification of the compounds by the MS/MS spectra
- Ion intensities were extracted at the *m/z* values of the molecular M^+ or the quasi-molecular $[M+H]^+$ and $[M-H]^-$ ions of the detected compounds

Standard solutions for quantification purpose

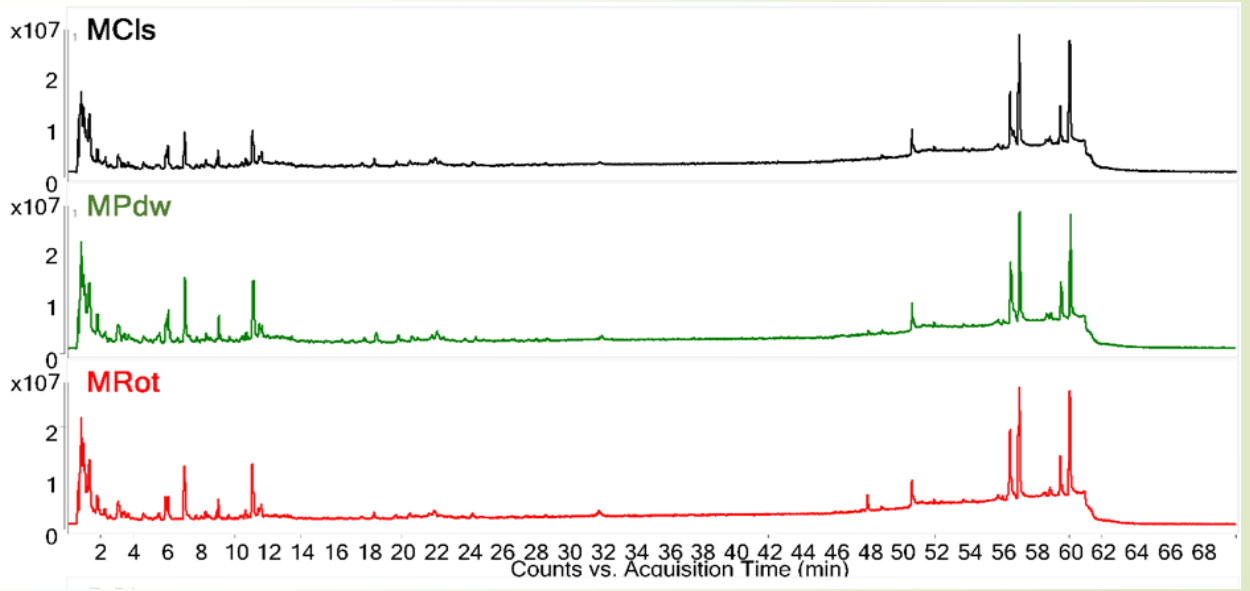
- **Calibration curves constructed (gallic acid, ferulic acid, *p* - coumaric acid, *p* - dihydroxybenzoic acid, caffeic acid, syringic acid, rutin,, quartzetin and resveratrol)**
- **Concentration of the standard is 500 µg/mL**
- **Mixture of standards with concentration 40 µg/mL**
- **Dilution in 6 points (0,625 µg/ml, 1,25 µg/ml, 2.5 µg/ml, 5 µg/ml, 10 µg/ml и 20 µg/ml**
- ***Validation* (LOQ, LOD, reputability, reproducibility, inter-day and intra- day)**

RESULTS AND DISCUSSION

***59 polyphenolic compounds identified and semi-quantifed* <u>using positive and negative ion mod</u>**



Chromatograms in *<u>positive</u>* ion mod



Chromatograms in *<u>negative</u>* ion mod

RESULTS AND DISCUSSION

Following phenolic compounds have been identified:

- ***Phenolic acids - 10***
- ***Stilbens - 4***
- ***Flavanols-7***
- ***Dyhidroflavonols - 4***
- ***Flavan-3-ols - 11***
- ***Anthocyanins - 12***

RESULTS AND DISCUSSION

PHENOLIC ACIDS
Negative ion mod $[M-H]^-$

Phenolic acids	t_R	$t_R(\pm)$	MS (*m/z*)	MS-MS (*m/z*) fragments
Gallic acid	1.8	0.02	169	125
Caffeic acid	8.1	0.01	153	109
Ferulic acid	13.8	0.04	193	193
p-Coumaric acid	11.4	0.01	163	179, 149, 134
p-Dihydrohibenzoic acid	3.3	0.11	153	315, 285
Syringic acid	9.5	0.05	197	183, 153, 138
Ellagic acid	3.2	0.06	300	284, 275, 229
Sinapinic acid	3.2	0.04	223	208, 179, 164
Vanillic acid	3.8	0.01	167	124, 123, 108
Cinnamic acid	2.3	0.01	147	104, 103

RESULTS AND DISCUSSION

FLAVONOLS
Positive ion mod $[M-H]^+$

Flavonols	t_R	$t_R(\pm)$	MS (*m/z*)	MS-MS (*m/z*) fragments
Myricetin-3-glucoside	11.8	0.06	493	319
Myricetin-3-glucorinide	22.1	0.02	495	319
Quarcetin-3-glucoside	21.9	0.01	465	303
Quarcetin-3-glucoronide	21.6	0.02	303	303
Quarcetin	31.2	0.04	285	/
Syringetin-3-glucoside	28.1	0.01	509	347
Kaempferol	3.1	0.04	285	255, 227

RESULTS AND DISCUSSION

STILBENES
Negative ion mod $[M-H]^-$

Stilbenes	t_R	$t_R(\pm)$	MS (*m/z*)	MS-MS (*m/z*) fragments
***cis*-Resveratrol-*3-O*-glucoside**	**4.2**	**0.01**	**389**	**227**
trans*-Resveratrol-*3-O-glucoside	**4.7**	**0.02**	**389**	**227**
Resveratrol	**1.4**	**0.01**	**227**	**185, 159**

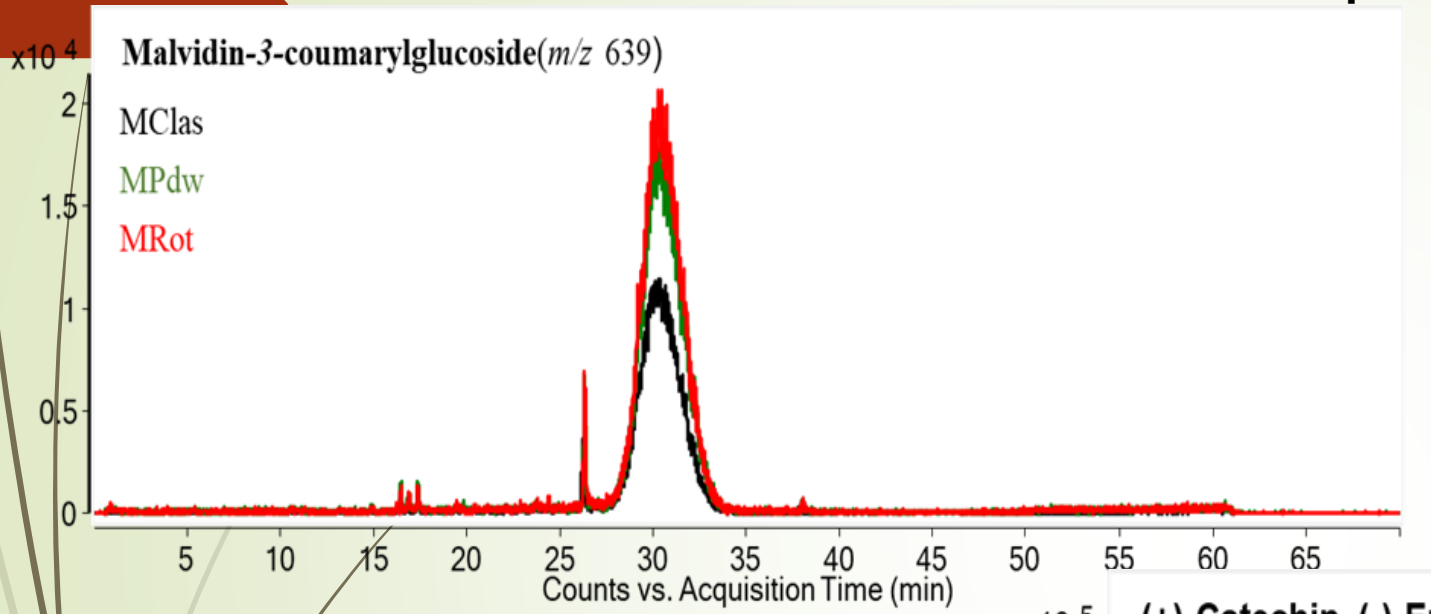
RESULTS AND DISCUSSION

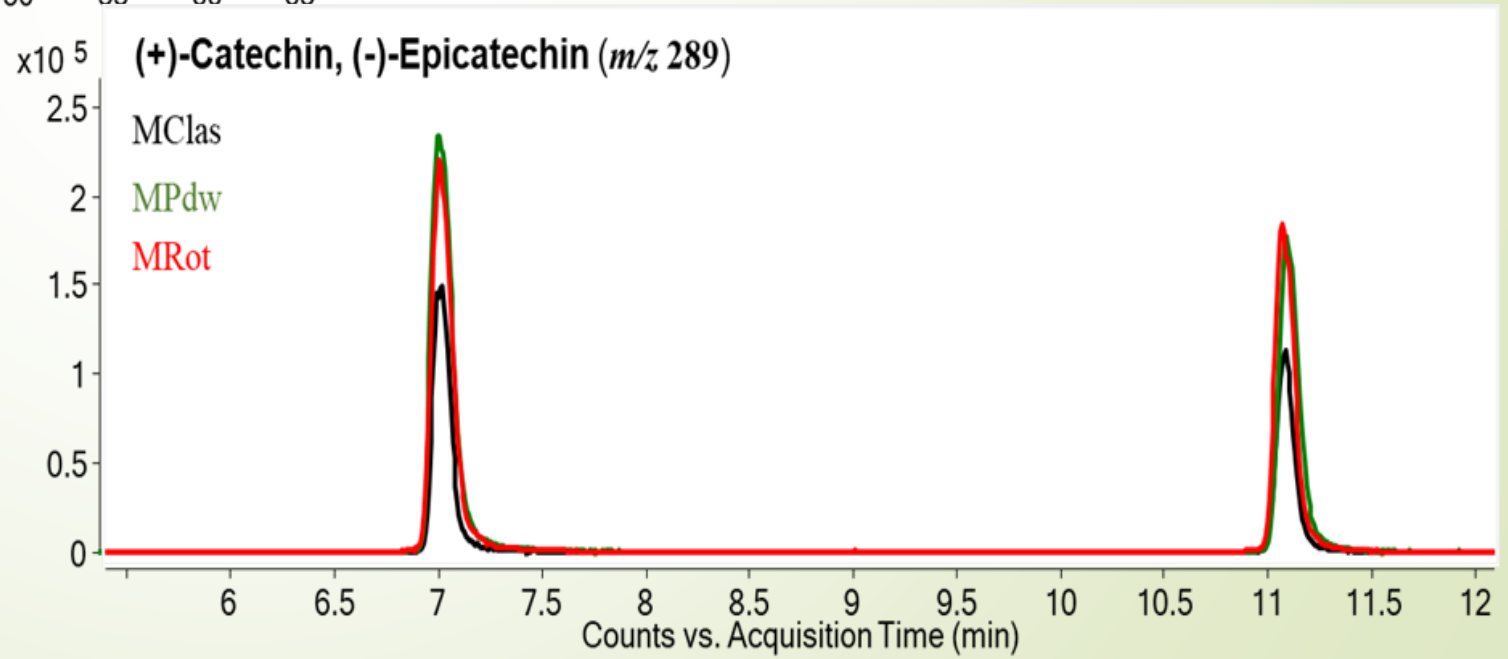
ANTHOCYANINS
Positive ion mod $[M-H]^+$

Anthocyanins	t_R	$t_R(\pm)$	MS (m/z) M^+	MS-MS (*m/z*)
Delphinidin-*3*-glucoside	22.8	0.02	465	303
Cyanidin-*3*-glucoside	24.4	0.01	449	287
Petunidin-*3*-glucoside	21.4	0.04	479	317
Peonidin-*3*-glucoside	11.0	0.01	463	301
Malvidin-*3*-glucoside	11.8	0.07	493	331
Delphinidin-*3*-acetylglucoside	20.1	0.04	507	303
Cyanidin-*3*-acetylglucoside	18.4	0.01	491	287
Petunidin-*3*-acetylglucoside	18.4	0.02	521	317
Peonidin-*3*-acetylglucoside	20.9	0.02	505	301
Malvidin-*3*-acetylglucosid	21.1	0.02	535	331
Delphinidin-*3*-coumarylglucoside	22.1	0.04	611	303
Malvidin-*3*-coumarylglucoside	25.1	0.01	639	331

RESULTS AND DISCUSSION

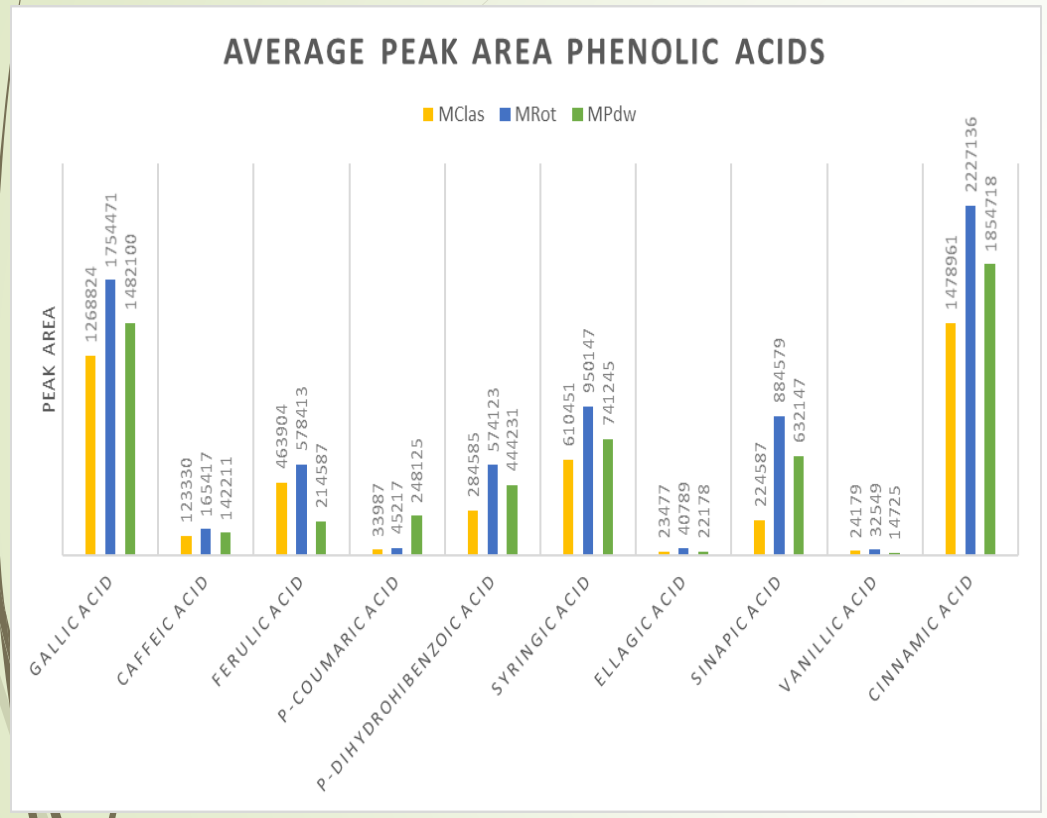
Relative quantification (average peaks area and intensity)

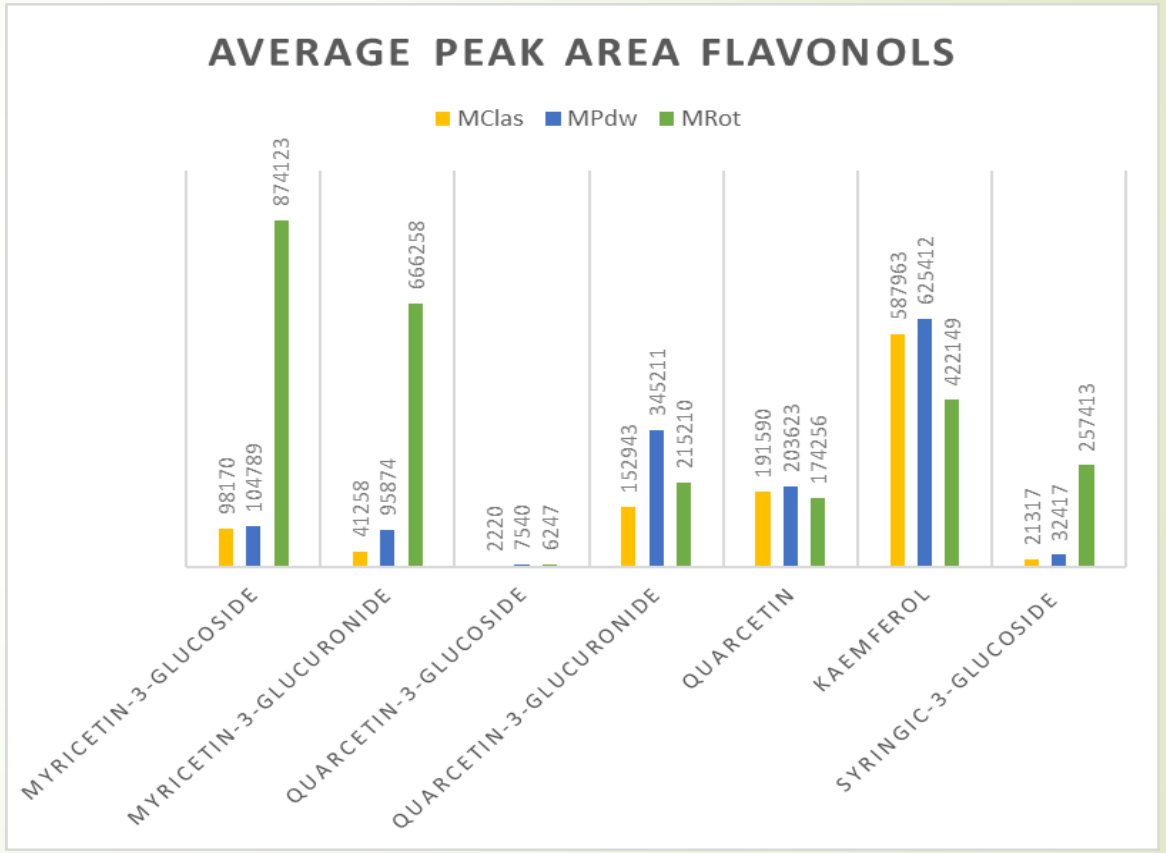




RESULTS AND DISCUSSION

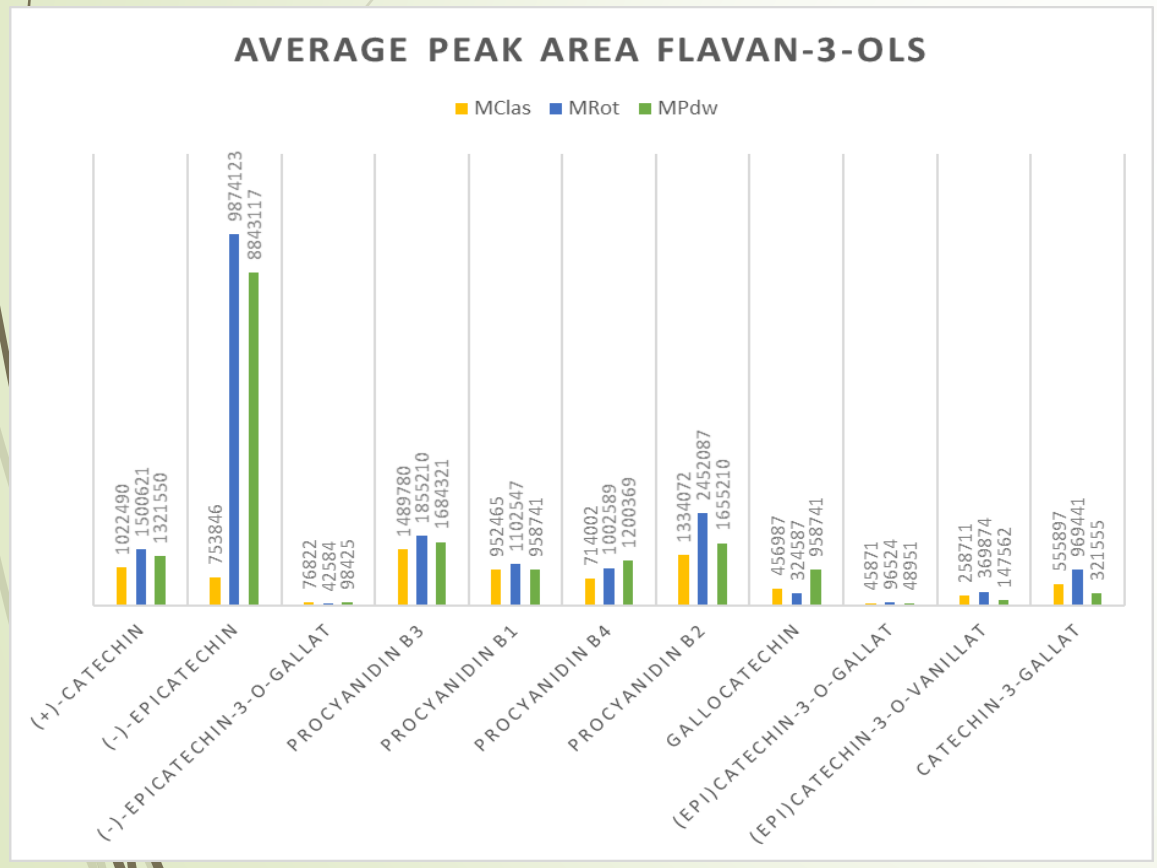
Relative amounts of polyphenolic compounds expressed as average peak area (n=3)

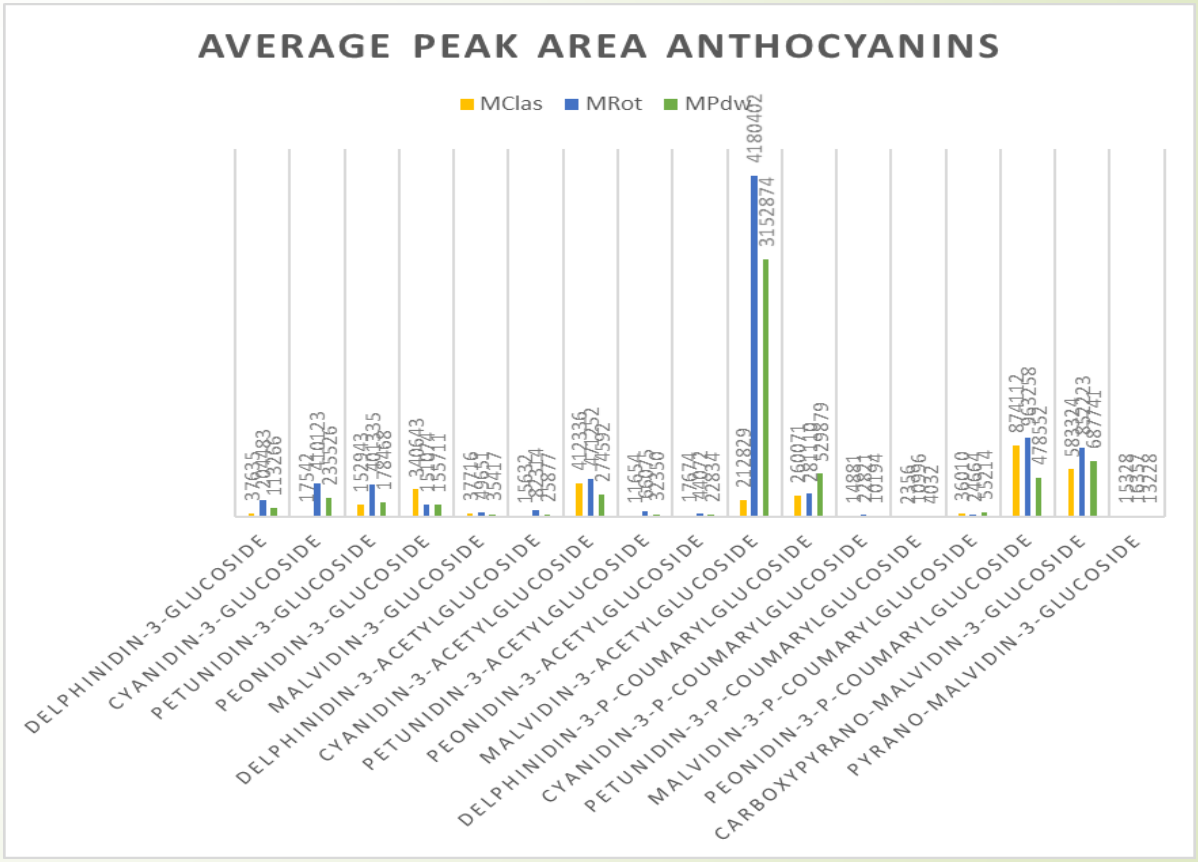




RESULTS AND DISCUSSION

Relative amounts of polyphenolic compounds expressed as average peak area (n=3)





CONCLUSION

- MS is a powerful technique for identification of various compounds without chromatographic separation of the analytes.
- Various groups of phenolic compounds have been identified: phenolic acids, flavonols, flavan-3-ols and anthocyanins.

- Roto Method – allowed extraction of highest content of polyphenolic compounds, compated to classical Method – 30% lower extraction than the roto method
- Results have high value for the wine industry in Macedonia in selecting the most suitable method for the extraction of polyphenols in wine

Acknowledgement:

CEEPUS MOBILITY GRANT CIII-RO-0010-16-2122-M-154132

THANK YOU FOR YOUR ATENTION