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STUDY OF THE PHENOLIC PROFILE OF VRANEC AND MERLOT WINES PRODUCED UNDER DIFFERENT VINIFICATION CONDITIONS

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INTRODUCTION

1. *Polyphenols are large family of naturally occurring, structurally diverse, organic compounds abundant in plants. Phenolic compounds such as anthocyanins, flavonols and tannins are important constituents of red wine contributing to the taste, color, mouthfeel and quality.*

2. *They are also associated with the health-promoting properties of red wine.*

INTRODUCTION

- In this study, phenolic profile of *Vitis Vinifera* red wines Vranec and Merlot (*vintage 2021*), produced in Republic of N. Macedonia, has been evaluated.

- Wines have been produced with three winemaking techniques, including classical fermentation, roto process and punchdown method, in order to study and compare the effect of vinification on the individual phenolic compounds.

- The phenolic profile was determined using an UPLC technique coupled with DAD and MS detectors.

- ESI-IT-MS method with alternating ionization polarity was used for identification of the phenolic compounds

VRANEC

- Vranec is the most important red variety in Nort Macedonia
- It s represent 70% of all red grape varieties grown in our country
- From this grape variety are produced quality red wines

MERLOT

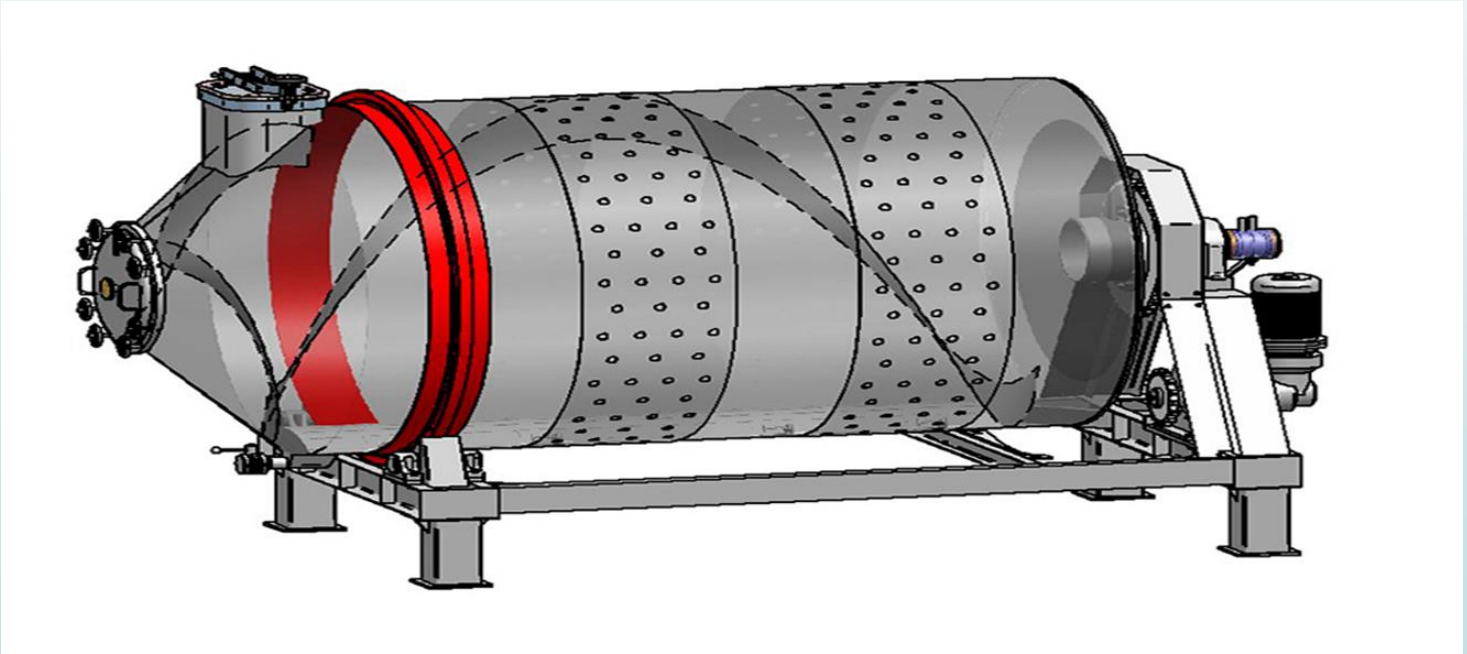
- In Macedonia, it can be found in Veles, Kumanovo, Ovcepole, Bitola and other wine districts. It ripens at the beginning of September. The cluster is small, cylindrical, long and compressed. The berry is small, dark blue coloured and with thin skin.
- The Macedonian Merlot is a wine of terrific elegance and complexity, rich yet linear pretty, but with depth and power.

CLASICAL FERMENTER

- Cylindrical stainless steel vessels with a conical bottom.
- Supplied with a pump that transports the fluid from the bottom to the top.
- Temperature regulation, cooling with circulating water.
- 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

- Punch-down fermenters are equipped with a cap submerging system, which consists of a punch-down agitator, pneumatic cylinder and a control unit.
- The punch-down mechanism makes it possible to break down the cap that was created during fermentation and submerge it into the must. The pneumatic cylinder (larger fermenters have two) continuously pushes the shovel-shaped punch-down agitator up and down, which makes the agitator gently pierce the cap and submerge it into the must.

EXPERIMENTAL

- **Wine samples**
- Optimal maturity (22 º Brix)
- Mechanical crushed in destemmer mechanical grinder.
- Pectinase enzymes are then added (Sodinal, Speed up Rouge 20g/100L).
- Must is sulfited to 30mg/L SO_3.
- Fermentation is started by adding yeast Saccharomyces cerevisiae (Sodinal FERMCTIVE ROUGE, 20g /100L previously activated in hot water at 30ºC).
- Controlled the temperature of fermentation (24ºC).
- Supplement of ammonium phosphate (10g /100L) was added.
- Tartar stabilization and sedimentation (0-4ºC).
- From each wine, 10 bottles of 0.75L are individually filled and closed with a cork stopper (sampling is performed under a nitrogen atmosphere).
- All 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 wather, eluent B 1% CH_3COOH in methanol)
- **Elution programme**

  (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
- **Filtered**

  0,45µm filters

EXPERIMENTAL

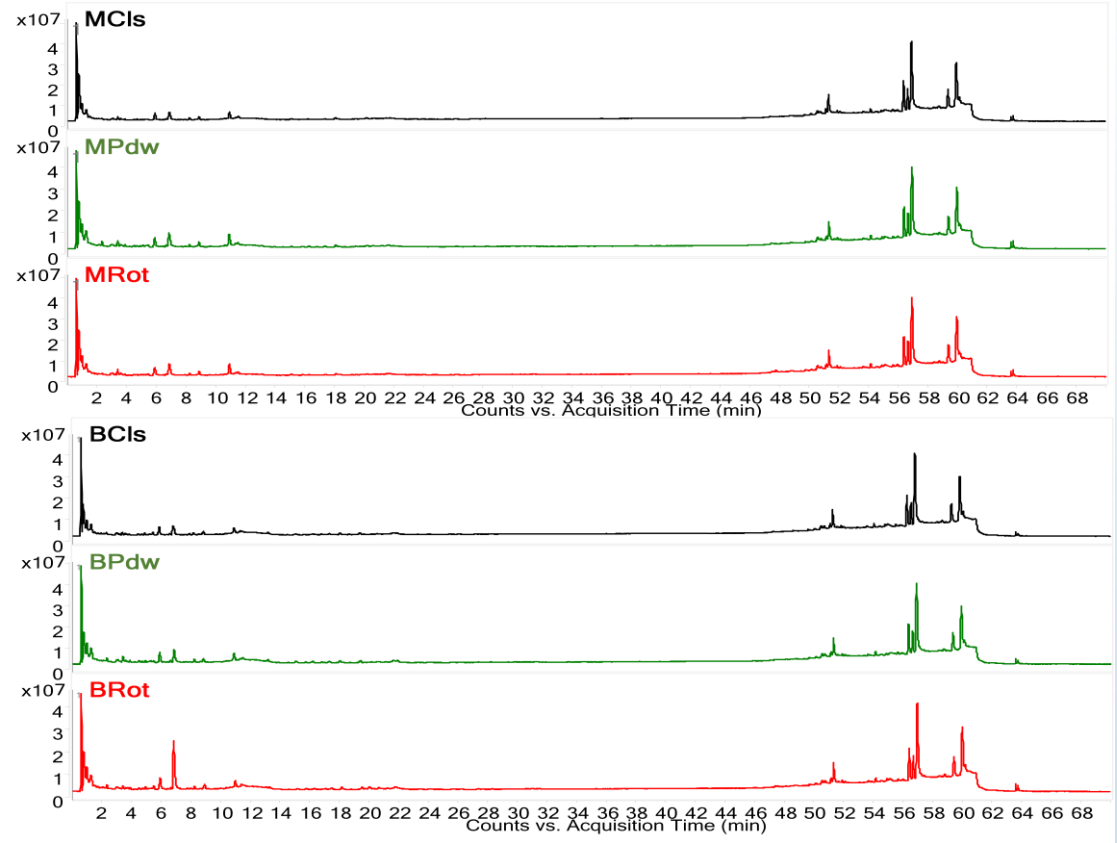
- Identification of the component was performed by the MS/MS spectra
- Ion intensities were extracted at the *m/z* values of the molecular M^+ or the quasi-molecular $[M+H]^+$ and $[M-H]^-$ jons of the detected compounds

Standard solutions

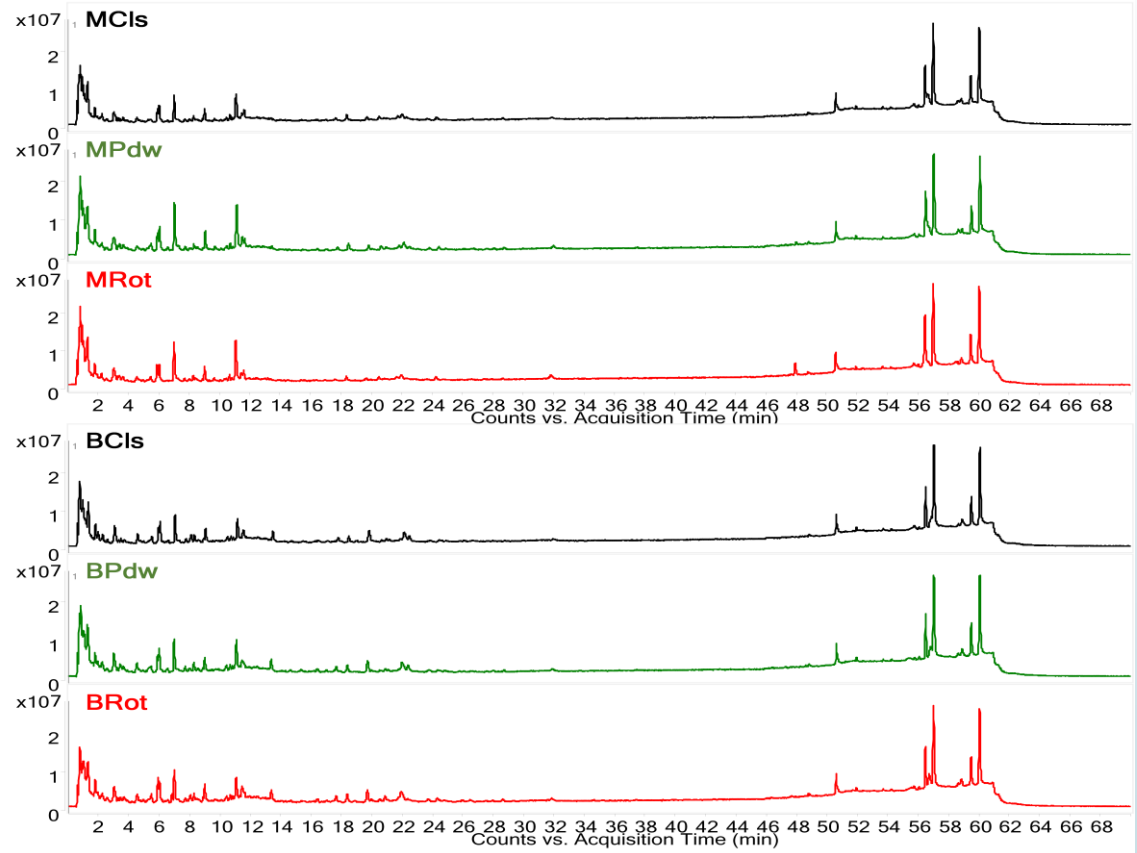
- 9 standard solutions was made (gallic acid, ferulic acid, *p* - coumaric acid, *p* - dihydroxybenzoic acid, caffeic acid, syringic acid, rutin,, quartzetin and resveratrol)
- Concentration of the standard is 500 µg/mL
- Mixture of standards with concentration 40 µg/mL
- Dilution in 6 points (0,625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

RESULTS AND DISCUSSION

- ***59 polyphenolic compounds were identified***



Positive mod



Negative mod

RESULTS AND DISCUSSION

- ***Phenolic acids***
- ***Stilbens***
- ***Flavanols***
- ***Dyhidroflavonols***
- ***Flavan-3-ols***
- ***Anthocyanins***

RESULTS AND DISCUSSION

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

Phenolic acids	t_R	$t_R(\pm)$	MS (m/z)	MS-MS (m/z)
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

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

Flavonols	t_R	$t_R(\pm)$	MS (m/z)	MS-MS (m/z)
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

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

Dihydroflavonols	t_R	$t_R(\pm)$	MS (m/z)	MS-MS (m/z)
Dihydromyricetin-*3*-*O*-fhamnoside	13,4	0,05	465	339, 301
Quarcetin-*3*-*O*-galactozide	3,5	0,01	463	301, 300
Astibin	1,7	0,08	449	303, 285
Myricetin-*3*-*O*-hexozide	2,8	0,02	479	317, 316

RESULTS AND DISCUSSION

Flavan-3-ols in negative ion mod $[M-H]^-$

Flavan-3-ols	t_R	$t_R(\pm)$	MS (m/z)	MS-MS (m/z)
(+)-Catechin	7,1	0,01	289	245, 205, 179
(-)-Epicatechin	11,0	0,02	289	245, 205, 179
(-)-Epicatechin-*3-O*-gallat	15,1	0,02	441	289, 169
Procyanidin B2	5,4	0,03	577	451, 425, 407
Procyanidin B3	6,1	0.01	577	451, 425, 407
Provyanidin B4	7,2	0,02	577	451, 425, 407
Procyanidin B1	9,1	0,02	577	451, 425, 407
Gallocatechin	2,4	0,01	305	261, 221, 219
(*epi)*-Catechin-*3-O*-vanillat	1,6	0,02	439	271, 261, 167
(epi)-Catechin-*3-O-cumarat*	0,6	0,01	435	341, 289, 269
Catechin-*3-O*-gallat	1,4	0,03	441	331, 271, 227

RESULTS AND DISCUSSION

Stilbens in negative ion mod $[M-H]^-$

Stilbens	t_R	$t_R(\pm)$	MS (m/z)	MS-MS (m/z)
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

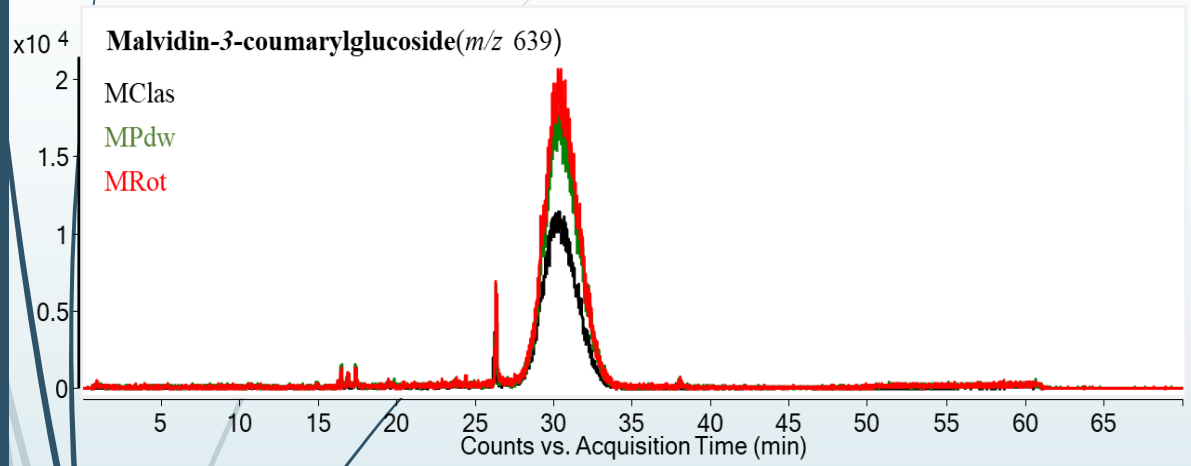
RESULTS AND DISCUSSION

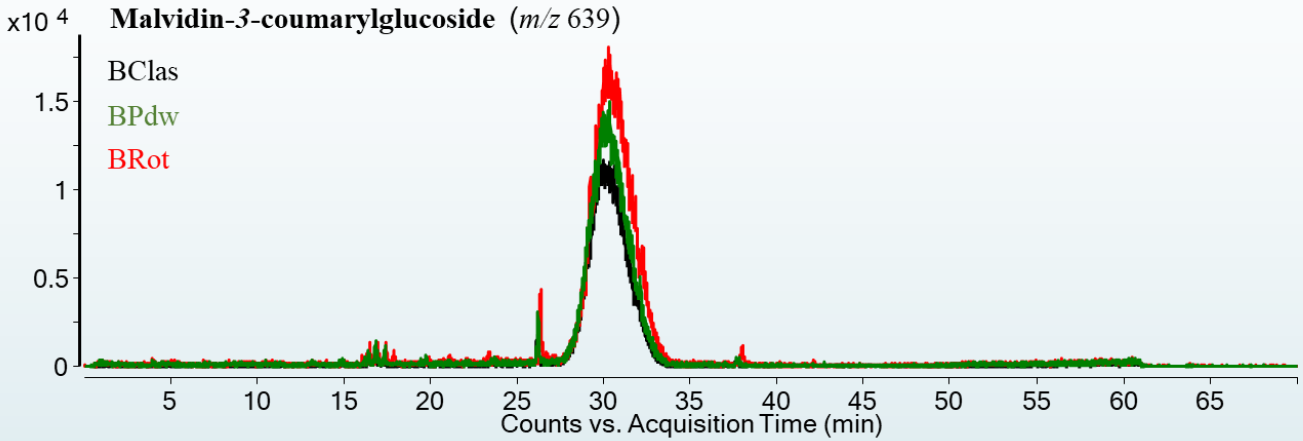
Anthocyanins in molecular ion mod M^+

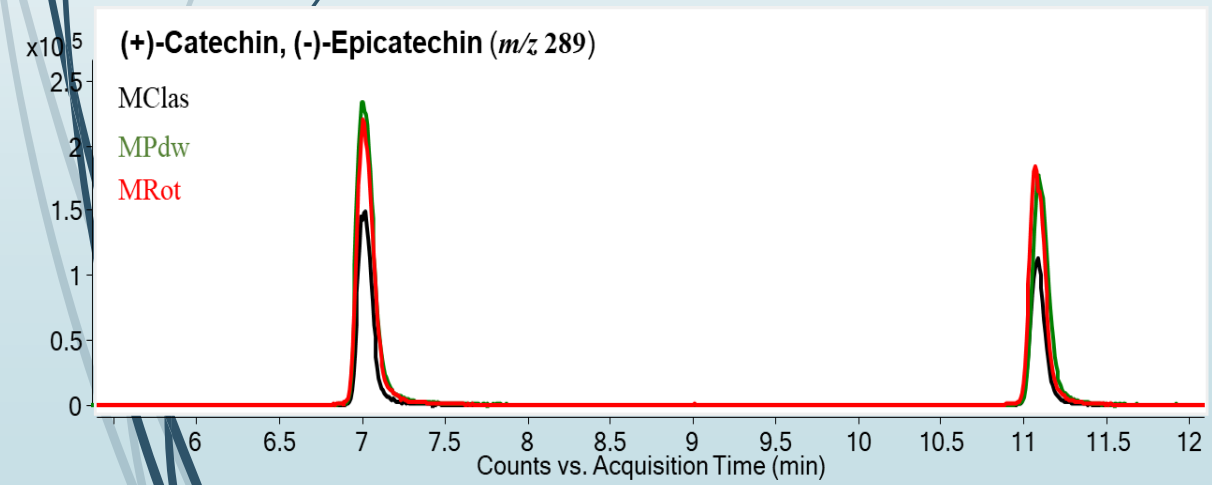
Anthocyanins	t_R	$t_R(\pm)$	MS (m/z)	MS-MS (m/z)
Delphinidin-3-glucoside	22,8	0,02	465	303
Cyanidin-3-glucoside	24,4	0,01	449	287
Petunidin-3-glucoside	21,4	0,04	479	317
Peonidin-3-glucoside	11,0	0,01	463	301
Malvidin-3-glucoside	11,8	0,07	493	331
Delphinidin-3-acetylglucoside	20,1	0,04	507	303
Cyanidin-3-acetylglucoside	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

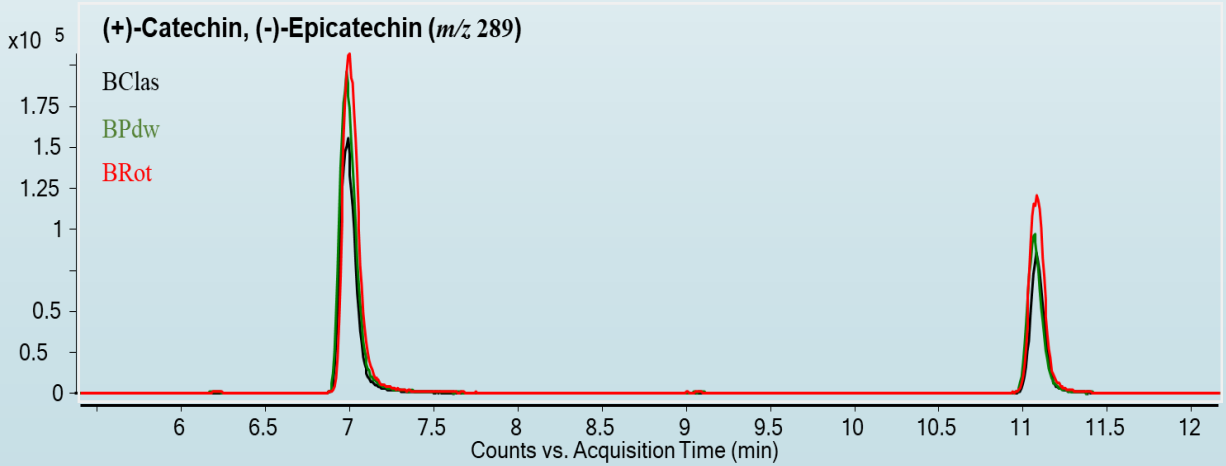
RESULTS AND DISCUSSION

Relative quantification in % (average peaks area and intensity)



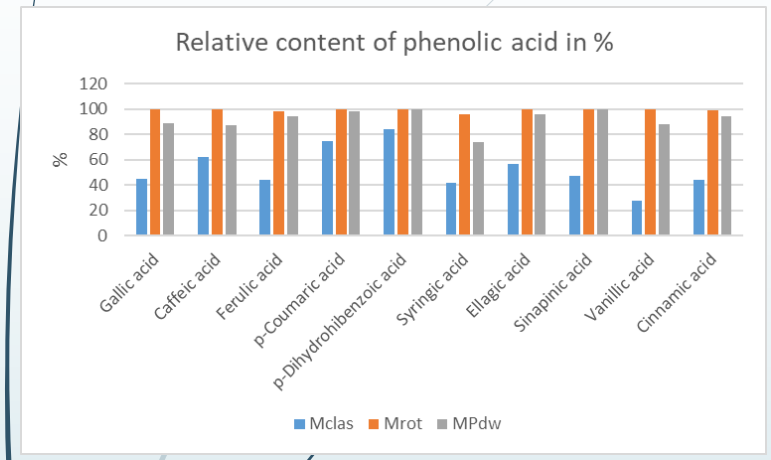


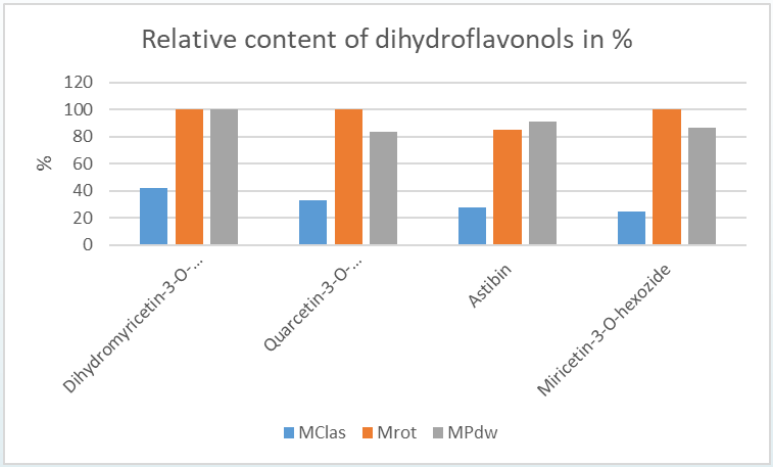


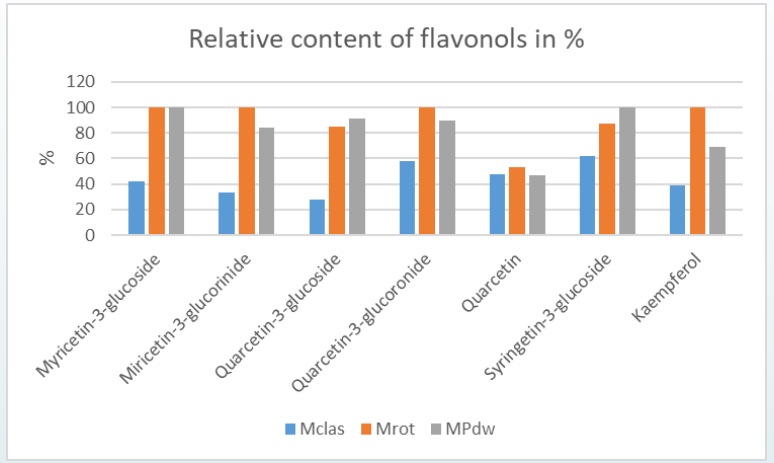


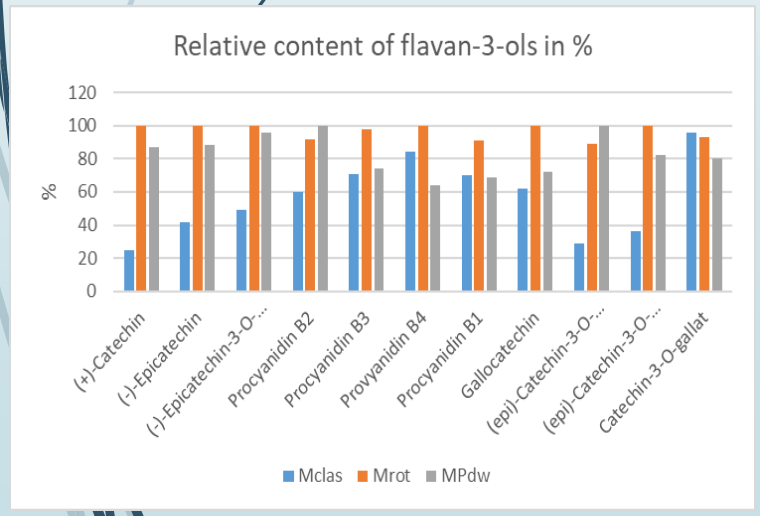
RESULTS AND DISCUSSION

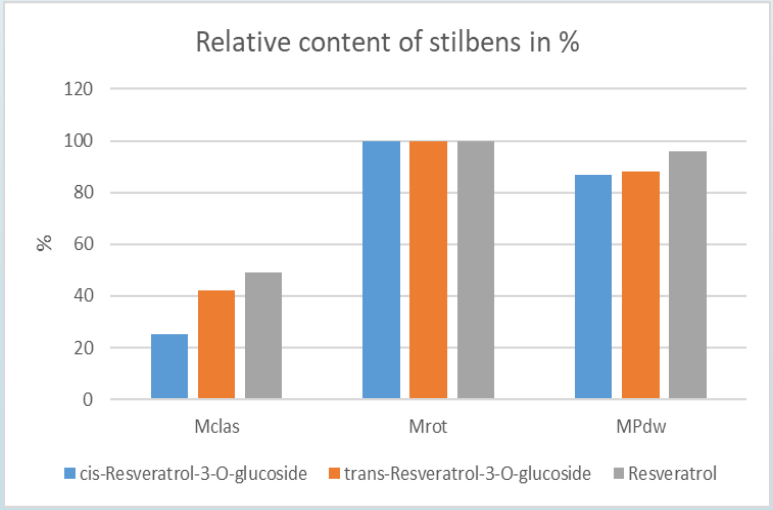
Relative content of phenolic compounds in Merlo Wine

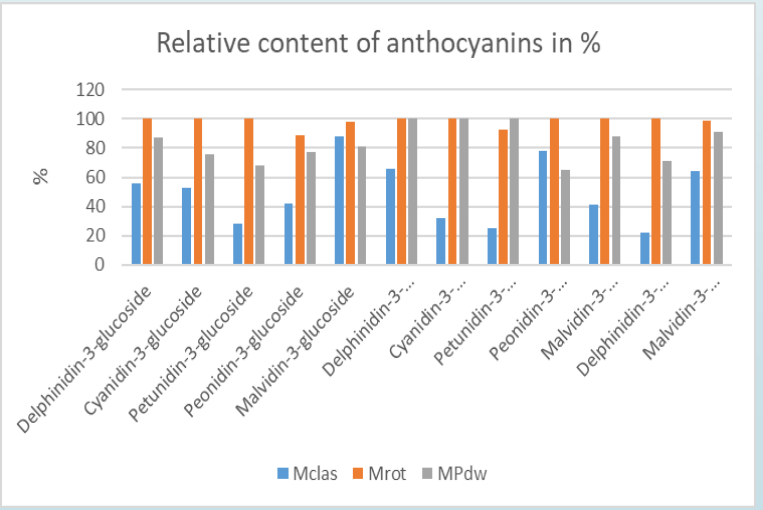






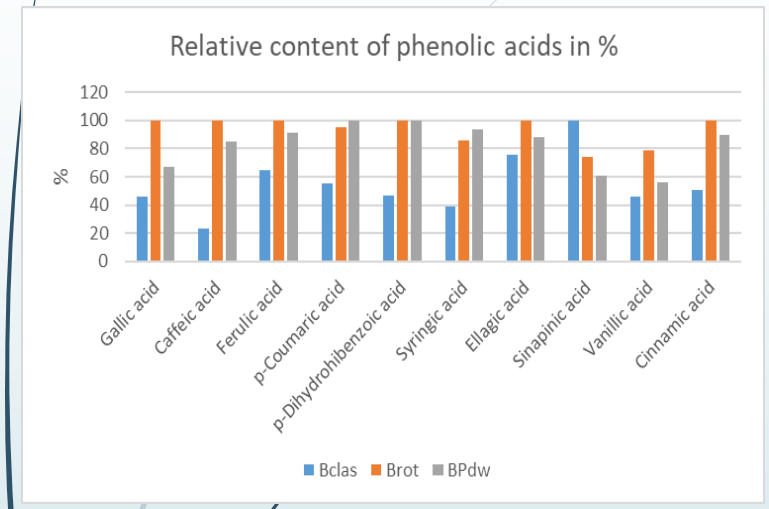


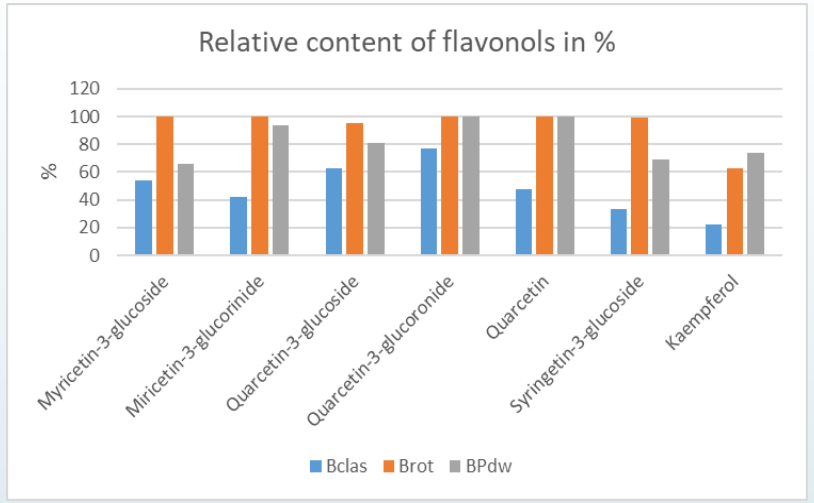


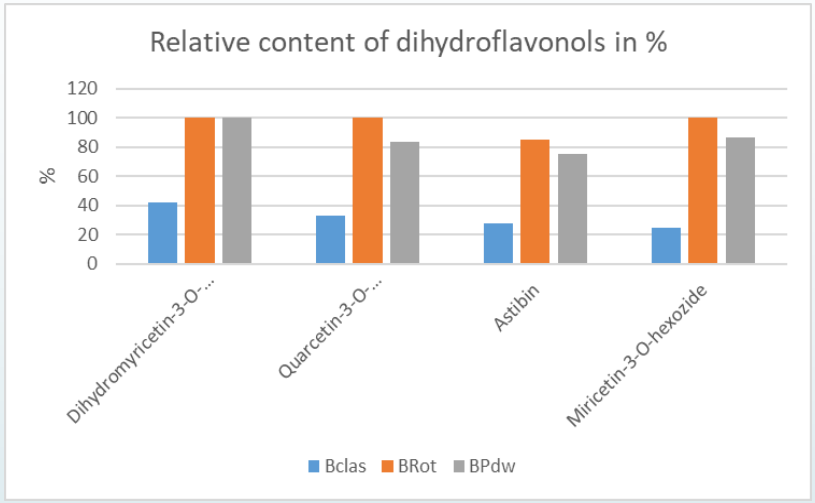


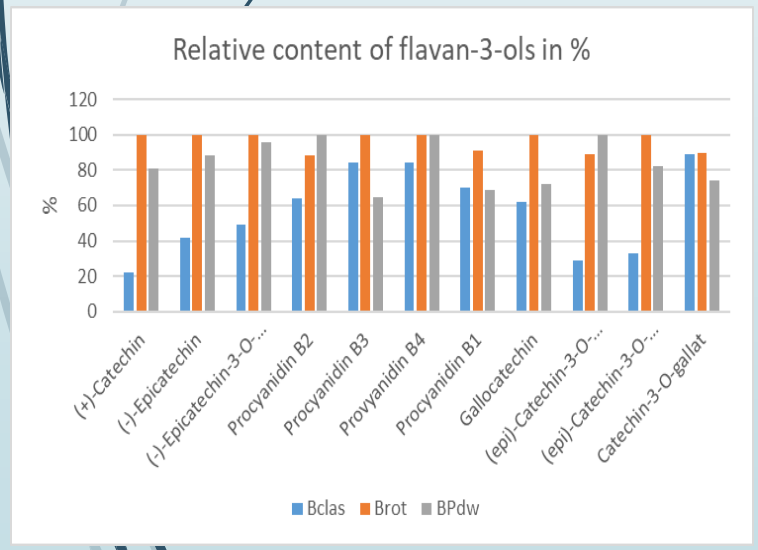
RESULTS AND DISCUSSION

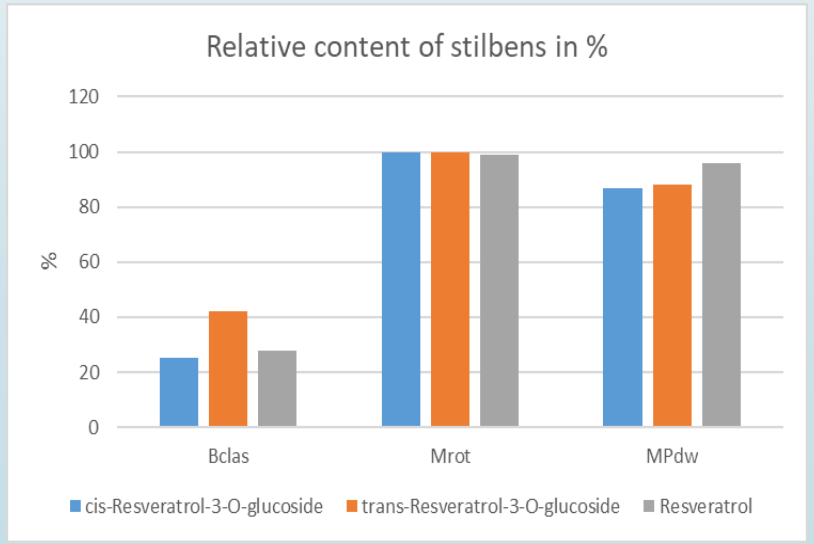
Relative content of phenolic compounds in Vranec Wine

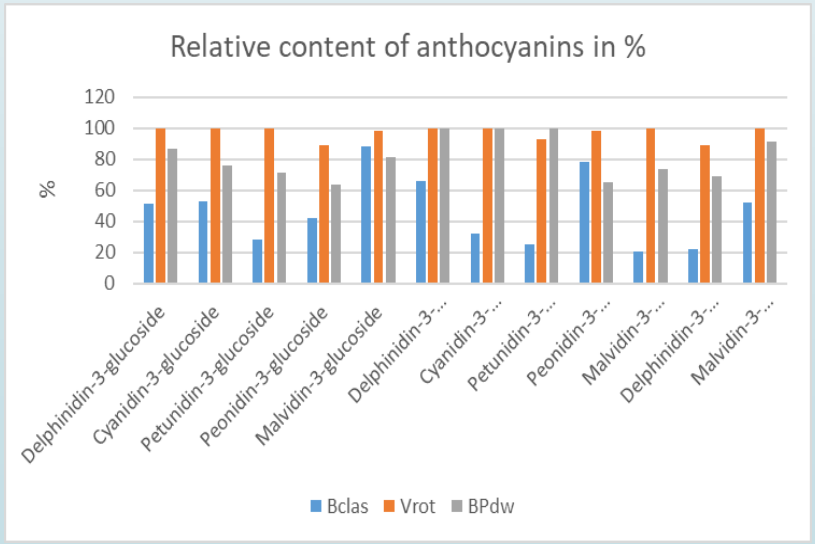












RESULTS AND DISCUSSION

Results for calibration curve

Phenolic component mg/L	BClas	BRot	BPdw	MClas	MRot	MPdw
Gallic acid	48,12	100,89	94.65	52.12	100.22	87.54
Ferullic acid	1,32	6,75	4.22	1.19	6.77	4.41
p-Coumaric *acid*	2,45	0,51	0.41	0.24	0.95	74.16
p-Dihydrohibenzoic *acid*	0,11	9,34	2.62	4.14	9.31	6.65
Caffeic *acid*	1,52	13,84	2.22	2.23	13.89	4.21
Syringic acid	118,82	278,47	261.11	120.14	278.12	110.25
Rutin	0,92	7,17	4.21	0.46	7.15	5.32
Resveratrol	1,19	5,17	2.96	2.36	5.89	3.26
Quarcetin	2,58	9,14	6.63	0.98	7.85	4.58

RESULTS AND DISCUSSION

Results of spectrophotometric measurements

- Total phenols (TP)
- Total anthocyanins (TA)
- Color intensity (CI)
- Hue (H)

Wine	Total Phenols (mg/L)	Total Antocyanins (mg/L)	Colour Intensity	Hue
BClas	1386	159	18,7	4,2
BRot	1980	236	28,9	6,0
BPdw	1657	211	25,4	4,7
MClas	1180	99	18,5	4,1
MRot	1725	151	27,3	5,8
MPdw	1499	121	23,9	4,9

Conclusion

- **Roto Method best for extraction of polyphenolic components**
- **Punchdown method – 10% lower extraction than the Roto method of fermentation**
- **The Classical method of fermentation- 30% lower extraction than the Roto method**

Conclusion

- Asistance to the wine industry in selecting the most suitable method for the extraction of polyphenols in wine

- Establishment of a validated UPLC method for determination of the polyphenolic profile of the wine

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THANK YOU FOR YOUR ATENTION