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**APPLICATION OF HPLC-DAD FOR
PHENOLIC EVALUATION OF RED WINES
DURING MACERATION**

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INTRODUCTION

- Wine is a very important product in Macedonia
- It has been produced long time ago
- Three wine regions in Macedonia:
 1. Povardarie-Vardar region (the biggest)
 2. Pelagonia-Polog region
 3. Pčinja-Osogovo region



INTRODUCTION

Grapes varieties

Various grape varieties are grown in Macedonia, including:

- **Red varieties:** Vranec, Cabernet Sauvignon, Merlot, Stanušina, Kratošija, Kabernet Franc, Kadarka, Pinot Noir, etc.
- **White varieties:** Smederevka, Charodonnay, Žilavka, Župjaka, Sauvignon Blanc, Muscat Otonel, etc.





CABERNET SAUVIGNON

- This variety covers most of the land in our country.
- In Tikveš region this variety matures in September.
- Wines are rich with anthocyanins and extract, have fine specific taste, softness and intense red color.

VRANEC

- Vranec is the most important red variety in of Macedonia.
- It is represents about 70% of all red grape varieties grown in the country.
- From this grape variety are produced quality red wines with a purple red colour.





STANUŠINA

- Macedonian authentic indigenous variety that can be found only in the Tikveš region.
- Stanušina wines are red and pink.
- Popova Kula Winery is the first winery which started producing wine of this variety.

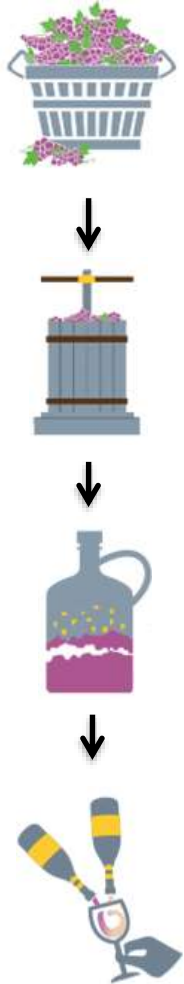
AIM OF THE STUDY

- Evaluation of the phenolic composition of **Stanušina** wines (exclusive and authentic wines of Macedonia),
- Comparison them with **Vranec** wines (the most popular in Macedonia, as well as in the Balkan region) and **Cabernet Sauvignon** wines (the most known international red variety).
- **Stanušina wine was studied for the first time, and the extraction of phenolic compounds during maceration was monitored with the time (3, 6 and 9 days).**



EXPERIMENTAL

Winemaking



Analysis of phenolic compounds

Spectrophotometric analyses:

- Total phenols
- Antioxidant activity
- Colour, hue



HPLC analysis

- Anthocyanins
- Phenolic acids
- Flavan-3-ols



RESULTS AND DISCUSSION

General chemical composition

Stanušina

Alcohol: 13.57 %

Total acid: 5.5 g/L

Total phenols: 795 ml/L GAE

Antioxidant activity: 102 mg/L TE

Vranec

Alcohol: 11.52 %

Total acid: 6.5 g/L

Total phenols: 1014 ml/L GAE

Antioxidant activity: 108 mg/L TE

Cabernet Sauvignon

Alcohol: 12.97 %

Total acid: 5.9 g/L

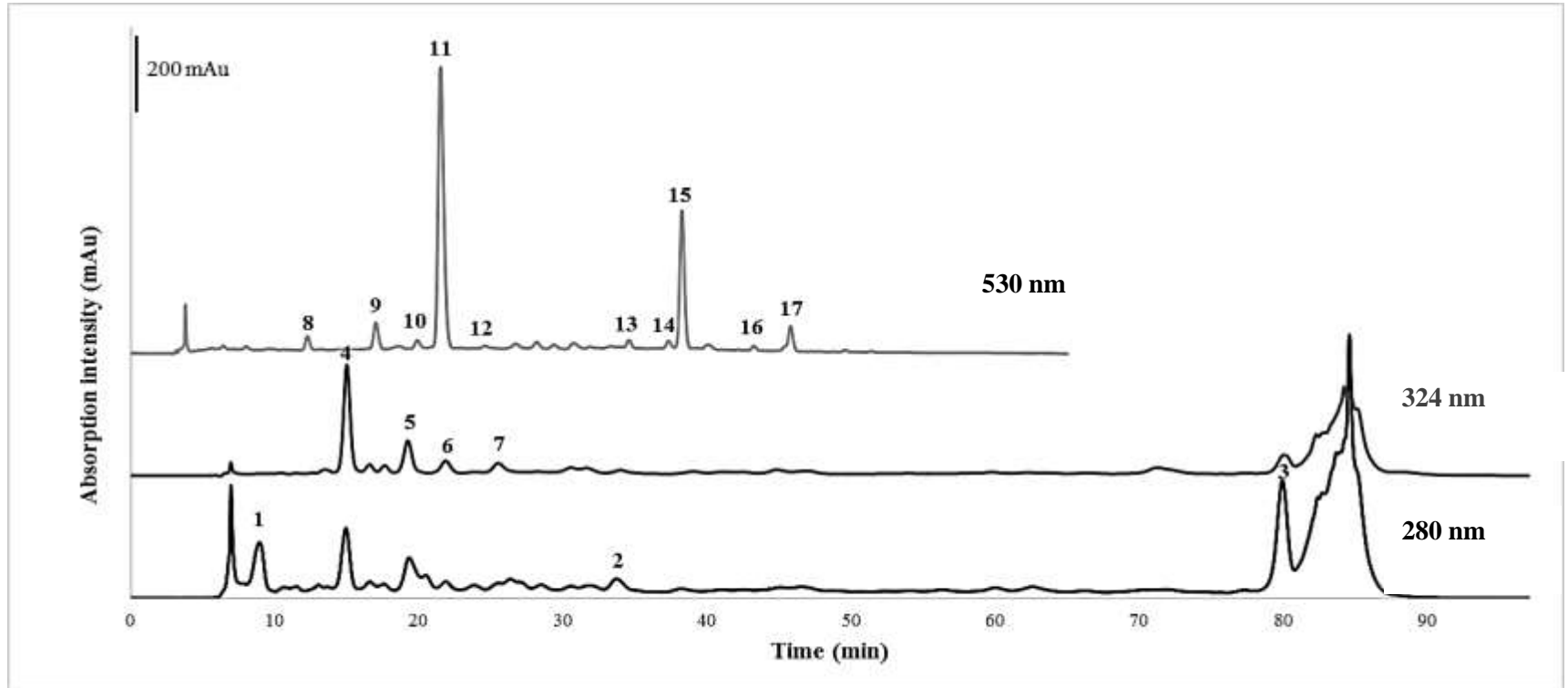
Total phenols: 1547 ml/L GAE

Antioxidant activity: 115 mg/L TE



RESULTS AND DISCUSSION

HPLC analyses



UV-Vis chromatogram of Cabernet Sauvignon wine sample recorded at:

- **530 nm** for separation and quantification of **anthocyanins (10 compounds)**.
- **324 nm** for separation and quantification of **flavan-3-ols and hydroxybenzoic acids (4 compounds)**.
- **280 nm** for separation and quantification **hydroxycinnamic acids derivatives (2 compounds)**.

RESULTS AND DISCUSSION

Quantification of anthocyanins

| Wine | Vranec | | | Cabernet Sauvignon | | | Stanušina | | |
|--|------------|------------|------------|--------------------|------------|------------|-------------|-------------|-------------|
| | Days | 3 | 6 | 9 | 3 | 6 | 9 | 3 | 6 |
| Dp-Glc | 5.88 | 12.2 | 10.3 | 2.04 | 5.58 | 3.19 | n.d. | n.d. | n.d. |
| Pt-Glc | 30.4 | 48.6 | 43.2 | 11.7 | 20.5 | 15.5 | 4.34 | 5.13 | 2.62 |
| Pn-Glc | 9.13 | 21.1 | 17.8 | 0.35 | 5.70 | 4.31 | n.d. | n.d. | n.d. |
| Mv-Glc | 485 | 649 | 595 | 251 | 466 | 436 | 101 | 115 | 87.8 |
| Total Glc | 531 | 732 | 667 | 265 | 497 | 459 | 105 | 121 | 90.5 |
| Pt-AcGlc | 3.65 | 5.89 | 5.63 | 2.59 | 12.7 | 10.9 | n.d. | n.d. | n.d. |
| Pn-AcGlc | 6.30 | 8.53 | 8.48 | 12.9 | 16.4 | 12.9 | n.d. | n.d. | n.d. |
| Mv-AcGlc | 73.3 | 102 | 94.8 | 157 | 284 | 255 | 9.92 | 10.4 | 7.88 |
| Total AcGlc | 83.3 | 116 | 109 | 173 | 313 | 279 | 9.92 | 10.4 | 7.88 |
| Pn-coumGlc | 3.56 | 6.92 | 5.31 | 0.23 | 0.19 | 0.17 | 0.00 | 1.16 | 0.15 |
| coumGlc | 53.5 | 74.1 | 59.2 | 23.9 | 47.2 | 42.0 | 13.7 | 21.6 | 14.5 |
| Total coumGlc | 57.1 | 81.1 | 64.5 | 24.1 | 47.4 | 42.2 | 13.7 | 22.7 | 14.6 |
| Total anthocyanins | 140 | 197 | 173 | 197 | 360 | 321 | 23.7 | 33.1 | 22.5 |
| ΣGlc/ΣacG | 6.38 | 6.31 | 6.12 | 1.53 | 1.59 | 1.65 | 10.6 | 11.6 | 11.5 |
| ΣGlc/ΣcoumGlc | 9.31 | 9.03 | 10.3 | 10.9 | 10.5 | 10.8 | 7.68 | 5.30 | 6.19 |
| ΣAcGlc/ΣcoumGlc | 1.46 | 1.43 | 1.69 | 7.15 | 6.60 | 6.60 | 0.72 | 0.46 | 0.54 |

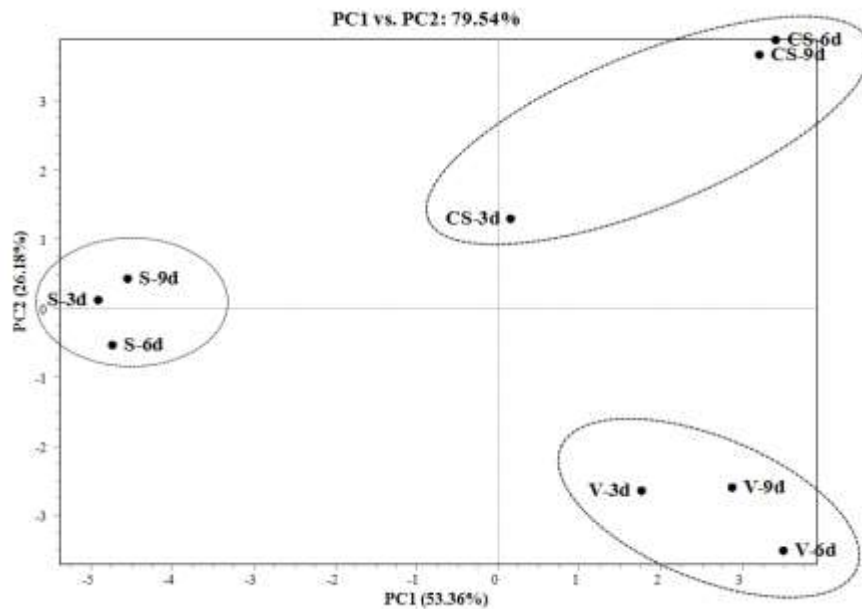
RESULTS AND DISCUSSION

Quantification of non-anthocyanins

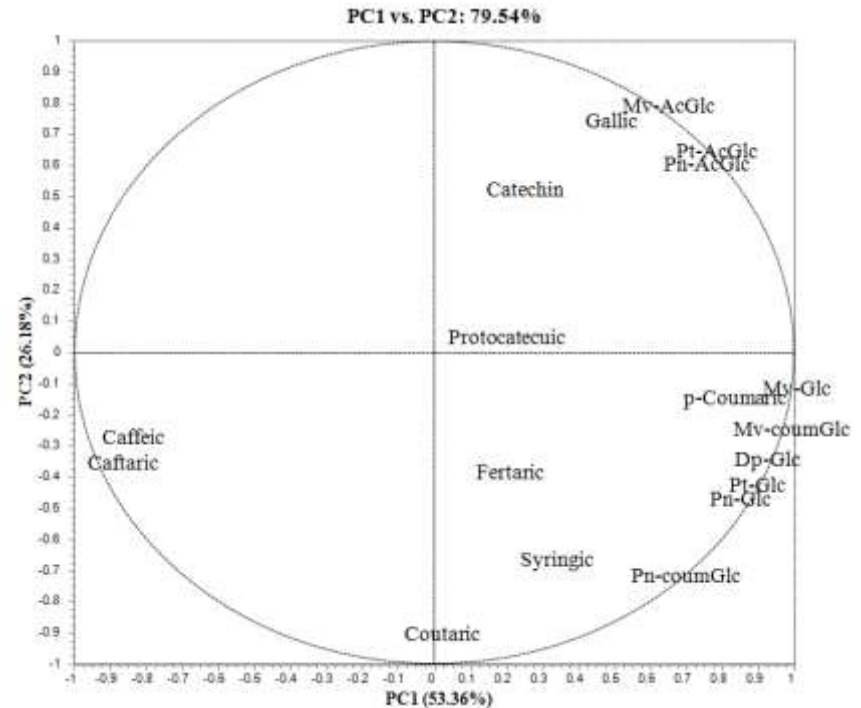
| Wine | Vranec | | | Cabernet Sauvignon | | | Stanušina | | |
|----------------------------------|------------|------------|------------|--------------------|------------|------------|------------|------------|------------|
| | 3 | 6 | 9 | 3 | 6 | 9 | 3 | 6 | 9 |
| Time of maceration (days) | 3 | 6 | 9 | 3 | 6 | 9 | 3 | 6 | 9 |
| Protocatecuic acid | 47.6 | n.d. | 26.7 | 24.7 | 18.6 | 18.6 | 18.2 | n.d. | 15.8 |
| Galic acid | 59.9 | 187 | 170 | 113.1 | 328 | 348.4 | 67.4 | 141 | 158 |
| Syringic acid | 32.5 | 24.4 | 37.1 | n.d. | n.d. | 15.5 | 21.8 | 22.0 | n.d. |
| Total HBA | 140 | 211 | 234 | 138 | 347 | 383 | 107 | 163 | 174 |
| <i>p</i>-Coumaric acid | 14.1 | 12.8 | 8.40 | 6.31 | 5.69 | 12.3 | 2.24 | 3.45 | 4.51 |
| Caftaric acid | 195 | 166 | 148 | 166 | 99.5 | 94.1 | 428 | 425 | 373 |
| Coutaric acid | 23.3 | 29.4 | 25.5 | 19.8 | 13.9 | 11.6 | 18.5 | 26.2 | 24.4 |
| Caffeic acid | 10.9 | 10.9 | 16.2 | 11.4 | 10.5 | 4.43 | 47.7 | 35.7 | 30.1 |
| Fertaric acid | 18.2 | 22.7 | 19.5 | 20.6 | 17.1 | 18.1 | 14.9 | 20.5 | 21.8 |
| Total HCA | 262 | 242 | 218 | 224 | 147 | 141 | 511 | 511 | 454 |
| Total phenolic acids | 402 | 453 | 451 | 362 | 493 | 523 | 619 | 674 | 628 |
| Catechin | 20.5 | 220 | 251 | 87.4 | 292 | 375 | 139 | 214 | 262 |

RESULTS AND DISCUSSION

Principal component analysis (PCA)



a)



b)

Principal Component score plot (a) and correlation scatterplots (b) of the variables with PC1 and PC2 based on anthocyanins, phenolic acids and catechin for the Stanušina, Vranec and Cabernet Sauvignon wines produced with 3, 6 and 9 days of maceration

CONCLUSION

- ✓ Anthocyanins reached highest content at 6th day of maceration.
- ✓ Hydroxybenzoic acids increased during winemaking and reached to the highest concentration in the wines after 9 days of maceration.
- ✓ Hydroxycinnamates reached to maximum concentration after 3 days of maceration.
- ✓ All wines showed high values of antioxidant activity.



**THANK YOU FOR YOUR
ATTENTION**

