IDENTIFICATION OF ANTHOCYANINS AND ANTHOCYANIN-DERIVATIVES IN VRANEC WINES DURING AGING

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INTRODUCTION

Color of wine is associated with the presence of anthocyanins, derived from the red grape berries. The main anthocyanins in wines are 3-*O*-glucosides, 3-*O*-acetylglucosides, 3-*O*-*p*-coumroylglucosides of delphinidin, cyanidin, petunidin, peonidin and malvidin. During aging anthocyanins react with other wine moleculs, by means of cycloaddition to the O-5 and C-4 positions of the anthocyanins and new anthocyanin-derived stable pigments, namely pyranoanthocyanins are formed.

The aim of the work was identification and quantification of individual anthocyanins and anthocyanin-derivatives, in Vranec wines, applying HPLC-DAD-ESI-MS (Ion Trap).

MATERIALS AND METHODS

Wine samples: Red wine samples from Vranec *V. vinifera* variety (vintages: 2006, 2007 and 2008) were kindly provided by Tikveš Winery, Kavadarci.

HPLC-DAD-ESI-MS^n analysis: Column Zorbax Eclipse XDB-C18 (250 x 4.6mm; 5 µm particle size).

Mobile phase: water/acetonitrile/formic acid (87:3:10, *V/V/V*, solvent A) and water/acetonitrile/formic acid (40:50:10, *V/V/V*, solvent B) at flow rate of 0.63 mL/min. Proportions of solvent B: 0 min, 6%; 15 min, 30%; 30 min, 50%; 35 min, 60%; 38 min, 60%; 46 min, 6%. **ESI** was operated in positive ionization mode.

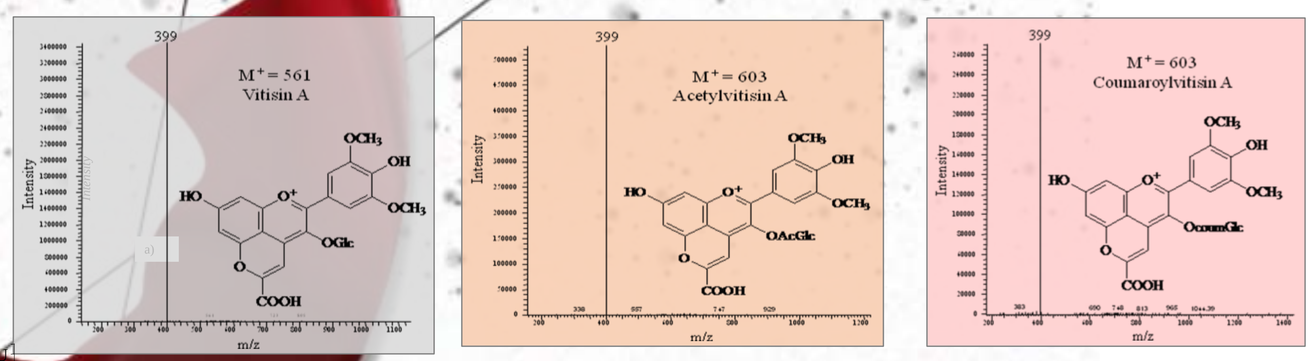
Statistical analysis: means, standard deviation and relative standard deviation, ANOVA.

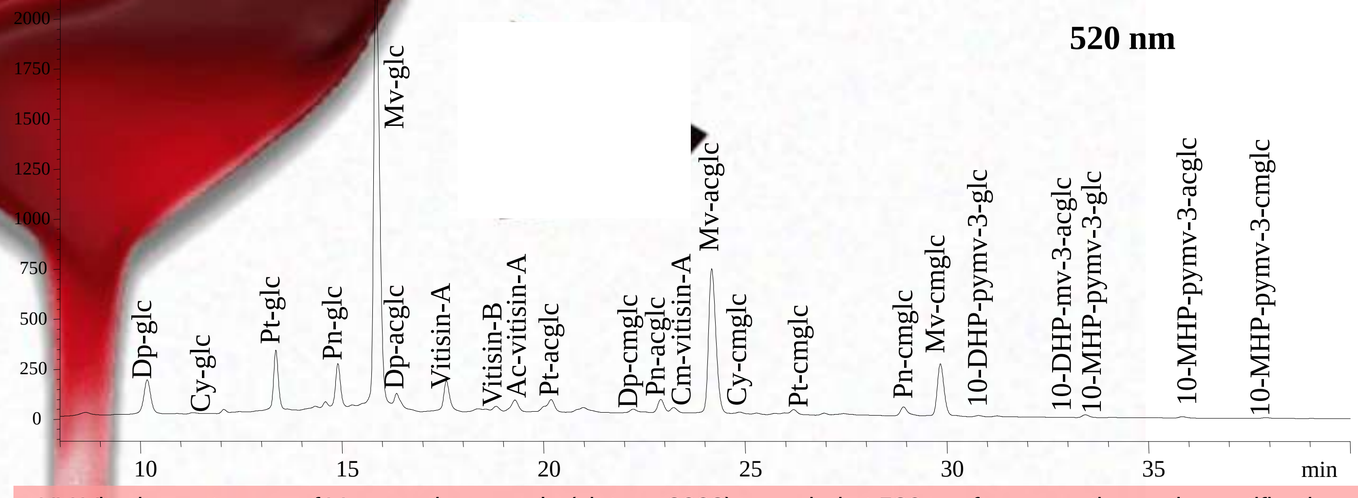
RESULTS AND DISSCUSION

MS identification of pigments in Vranec wines

Pigments	MS (m/z)	MS/MS (m/z)
Anthocyanins	**M$^+$**	**Fragment ion**
Non-acylated glucosides		
Dp-3-glc	465	303
Cy-3-glc	449	287
Pt-3-glc	479	317
Pn-3-glc	463	301
Mv-3-glc	493	331
Acetylglucosides		
Dp-3-acetylglc	507	303
Pt-3-acetylglc	521	317
Pn-3-acetylglc	505	301
Mv-3-acetylglc	535	331
p-Coumaroylglucosides		
Dp-3-p-coumglc	611	303
Cy-3- p-coumglc	595	287
Pt-3- p-coumglc	625	317
Pn-3- p-coumglc	609	301
Mv-3- p-coumglc	639	331
Pyranoanthocyanins		
Vitisins		
Vitisin-A	561	399
Ac-vitisin-A	603	399
p-Cm-vitisin-A	707	399
Vitisin-B	517	355
Ac-vitisin-B	559	355
Hydroxyphenyl-pyranoanthocyanins		
10-DHP-pymv-3-glc (pinotin A)	625	463
10-DHP-pymv-3-acglc	667	463
10-DHP-pymv-3-cmglc	771	463
10-MHP-pymv-3-glc	609	447
10-MHP-pymv-3-acglc	651	447
10-MHP-pymv-3-cmglc	755	447

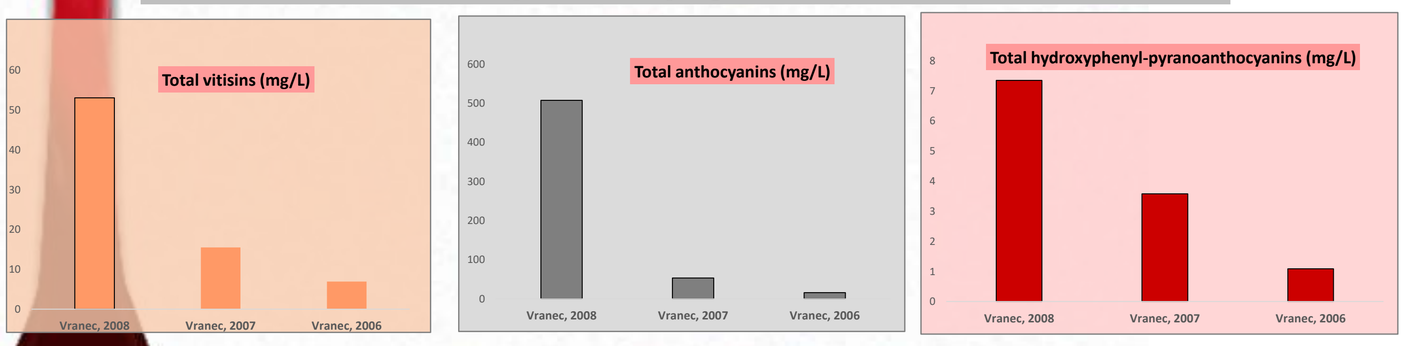
MS/MS fragmentation of A-type vitisins





UV-Vis chromatogram of Vranec wine sample (vintage 2008) recorded at 520 nm for separation and quantification of anthocyanins, pyranoanthocyanins and hydroxyphenyl-pyranoanthocyanins

Quantitative analysis of pigments in Vranec wines



Conclusions

- ✓ Malvidin-3-glucoside and its 3-acetylglucoside and 3-p-coumaroylglucoside derivatives were the major compounds.
- ✓ Vitisin A, pinotin A (10-DHP-pyranomalvidin-3-glucoside) and 10-MHP-pyranomalvidin-3-glucoside were the dominant pyranoanthocyanins and hydroxyphenyl-pyranoanthocyanins.
- ✓ Anthocyanins were the dominant pigments in all wines, regardless the aging and year of production, followed by pyranoanthocyanins and hydroxyphenyl-pyranoanthocyanins.

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