

4TH Vranec World Day

5th of October, 2022, Skopje, Republic of N. Macedonia

Aromatic and polyphenolic profile of wines from the Vranec variety

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УНИВЕРЗИТЕТ
„ГОЦЕ ДЕЛЧЕВ“
ШТИП



УНИЛАБ



INTRODUCTION

HOW MANY GRAPE VARIETIES ARE GROWN?

Several thousand to be precise,
but a **few hundred** are actually
used for wine making.

The vine plant can produce fruit for
up to 100 years.

by starting with the highest

variety of soil types,

, Asia, Mediterranean and
land, most of North America

GRAPE VARIETIES IN R. N. MACEDONIA

➤ **Red Grape Varieties:** Vranec, Stanušina, Kratošija, Merlot, Pinot Noir, Cabernet Sauvignon, Cabernet Franc, Karadrka



Stanušina



Vranec



Merlot

➤ **White Grape Varieties:** Smederevka, Žilavka, Župjanka, Traminac, Temjanika (Riesling), Chardonnay, Semilion, Sauvignon Blanc, Muscat Ottonel, Grenache Blanc-Belan



Smederevka



Žilavka



Chardonnay

VRANEC variety

- The most important grape variety used for red wine production in R. Macedonia.
- It represents about 50 % of the total red wine production in the country.
- It is grown in all vineyards, mostly in the Tikveš wine region, where more than 80% of the Macedonian vineyards are located.
- The wine produced from this variety has an intense dark red colour, aroma of plum, sour cherry and wild berries, rich in polyphenols.

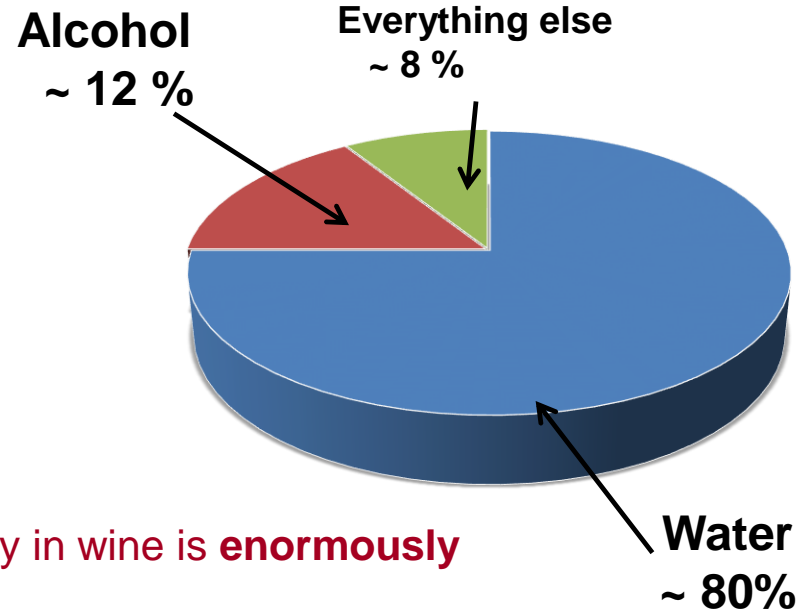
Chemical composition of wine

❖ A bottle of red wine contains over 1000 chemical compounds

❖ Quite amazing when you consider wine is **>80% water + alcohol**

Wine is a complex matrix of many components:

- ✓ Water, Alcohols, Organic acids, Carbohydrates, Aldehydes, Esters
- ✓ Minerals, Nitrogen compounds
- ✓ **Phenolic compounds**
- ✓ **Aromas**
- ✓ Vitamins



- ✓ The chemistry of flavour, colour and astringency in wine is **enormously complicated**.
- ✓ Many chemical and biochemical pathways are not well **understood**.

AROMA COMPOUNDS

- The volatile composition is a very important factor affecting the wine aromatic attributes and hence its quality.
- Some volatile compounds originate from the grapes
- Most of them are formed during the fermentation and storage of wines.
- Different parameters influence the aroma composition of the grapes: grape varietal characteristics, light intensity, temperature, soil, climate, degree of maturation, cultivation practices, etc.

AROMA COMPOUNDS

- Crushing, pressing, fermentation temperature, maceration, yeast strain, SO_2 , wine dealcoholisation and supercritical extraction, affect the extraction of grape aroma compounds in the juice.
- During wine ageing under different conditions, the volatile composition could be changed due to appearance of some volatiles that could decline the wine aroma quality.

AROMA COMPOUNDS

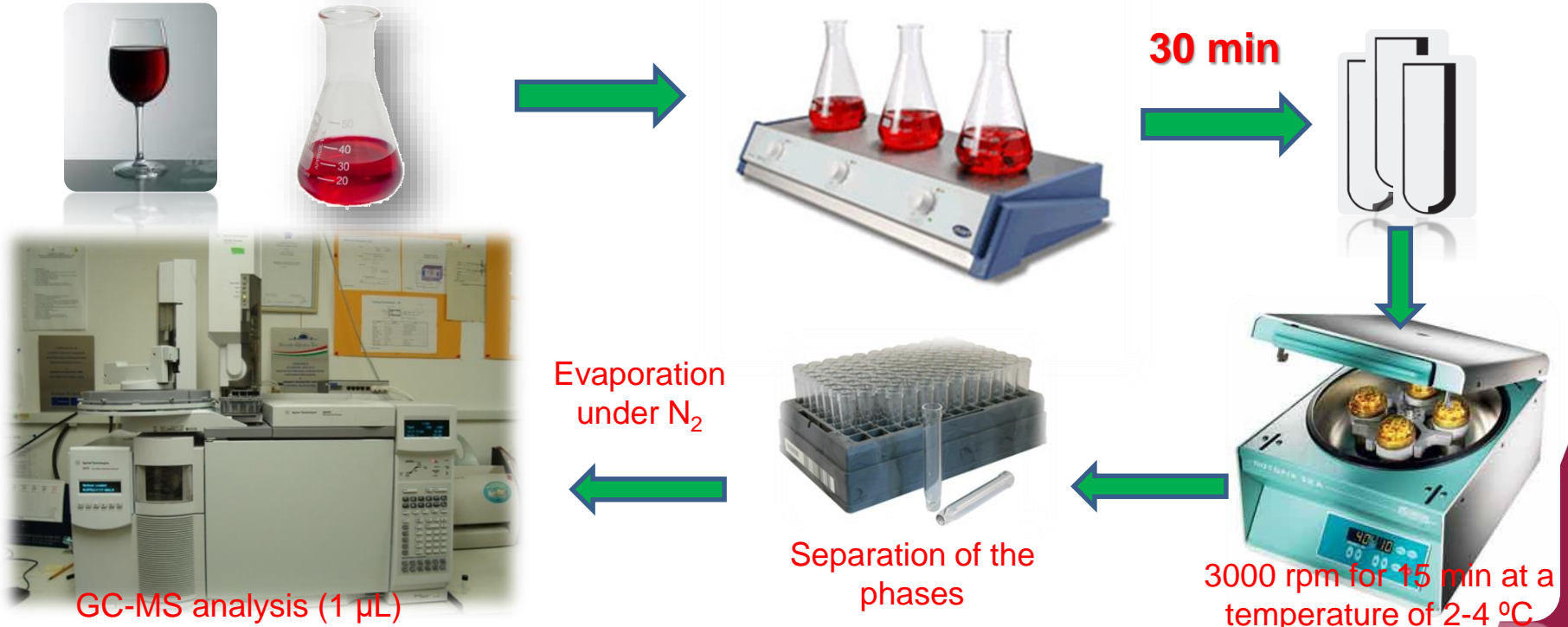
- Different groups of volatile compounds have been identified in grapes and wines:
 - ✓ higher alcohols, esters, aldehydes, lactones, terpenes, C13-norisoprenoids, volatile phenols, fatty acids, carbonyls, sulphur and nitrogen compounds.
 - ✓ More than **1000** aroma compounds with different polarities, volatilities and odour impact have been identified in wines.
 - ✓ The main aroma compounds in wine are **higher aliphatic alcohols, ethyl esters** and **acetates** (mainly formed from the yeast metabolism during the alcoholic fermentation).

GAS CHROMATOGRAPHY

- **Gas chromatography/mass spectrometry (GC/MS)** is a highly efficient separation technique for volatiles' analysis and for characterization of the wine bouquet.
- GC-MS with polar column for separation of components in low concentration, as well as, in a complex matrices, as wine is.
- Extraction methods: solid-phase extraction (SPE), solid-phase microextraction (SPME), stir bar sorptive extraction (SBSA), or **Liquid-liquid extraction** methods using organic solvents (dichloromethane), showing high repeatability and possibility of carrying out simultaneous extractions.

Liquid-liquid extraction of wine aroma compounds

50 mL wine + 25 mL dichloromethane + 200 µL internal standard of 1-octanol



GC-MS analysis of aroma compounds in VRANÆC wine

GC-MS analysis



Agilent 5975 Mass Spectrometer coupled to
an Agilent 6890N Gas Chromatograph

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

Working parameters:

Injector temperature 240 °C;

MS source 230 °C;

MS Quad 150 °C,

Transfer line 280 °C

40 °C for 3 min

180 °C at 3 °C /min.

260 °C with 20 °C /min

260 °C for 10 min

Carrier gas - He with a flow rate of 1.5 mL/min.

Splitless mode for injection

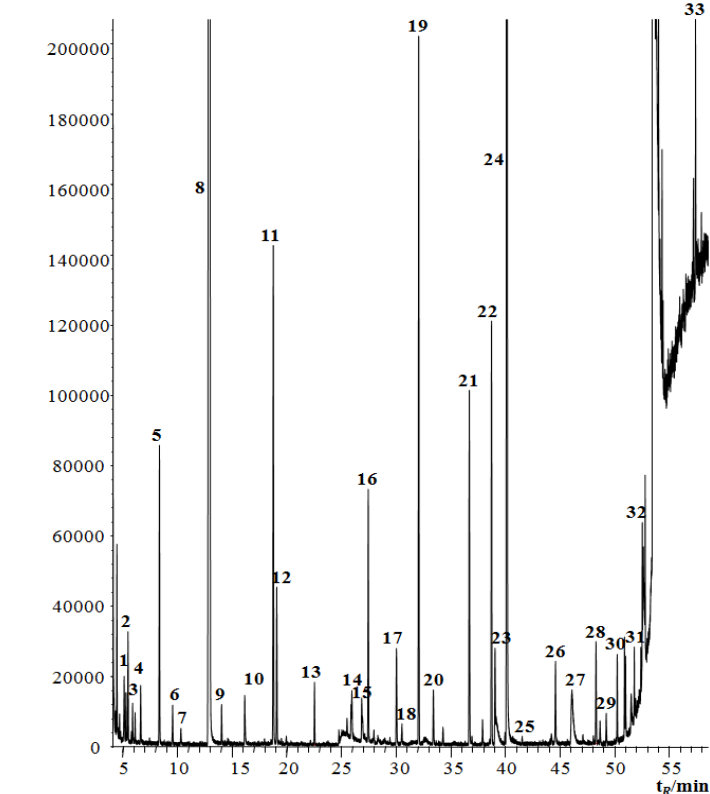
Mass range of 50–400 m/z , recorded at 1 scan/s.

Ivanova et al. *Food Analytical Methods*, 5, 1427-1434, 2012

Ivanova et al. *Food and Bioprocess Technology*, 6(6) 1609-1617, 2013

Analysis of the volatile composition of Vranec wine

GC/MS chromatogram of the volatile compounds in Vranec wine



46 volatile compounds

In Vranec wine identified and quantified

14 alcohols - secondary products mainly produced during the yeast metabolism;

19 esters - are formed by esterification of alcohols and acids followed by water molecule elimination;

2 fatty acids - products of yeast metabolism, and they could inhibit the alcoholic fermentation

1 furan

1 sulphur compound - derived from sulphur-containing amino acids during the microbial transformations, or from the elemental sulphur

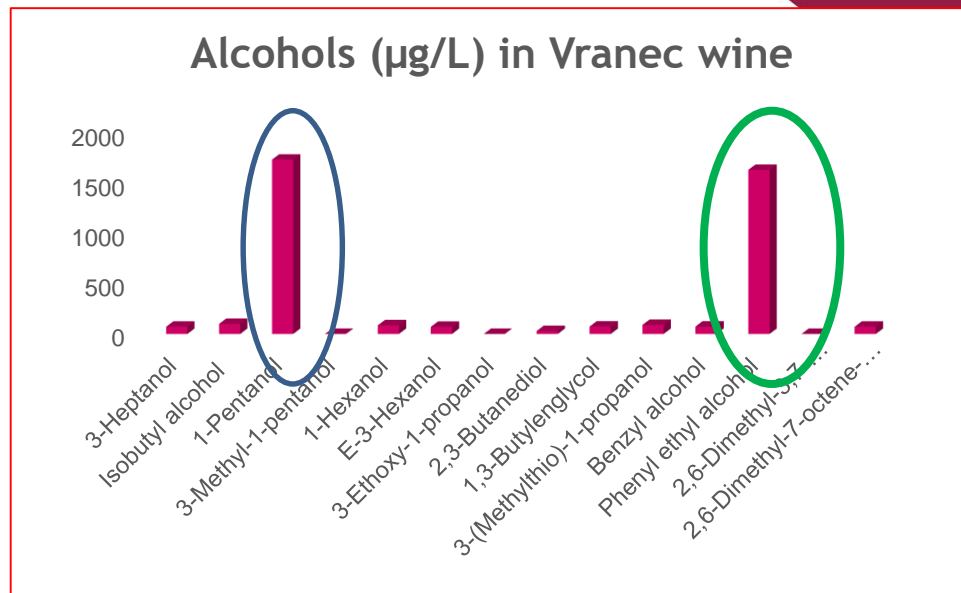
2 phenols

1 lactone

3 other compounds

Higher alcohols in Vranec wine

Alcohols(µg/L)	Vranec
3-Heptanol	72.2
Isobutyl alcohol	100
1-Pentanol	1736
3-Methyl-1-pentanol	0
1-Hexanol	86.1
E-3-Hexanol	70.8
3-Ethoxy-1-propanol	0
2,3-Butanediol	29.8
1,3-Butylenglycol	73.9
3-(Methylthio)-1-propanol	90.4
Benzyl alcohol	73.6
Phenyl ethyl alcohol	1634
2,6-Dimethyl-3,7-octadiene-2,6-diol	0
2,6-Dimethyl-7-octene-2,6-diol	71.82
Total alcohols (µg/L)	4040

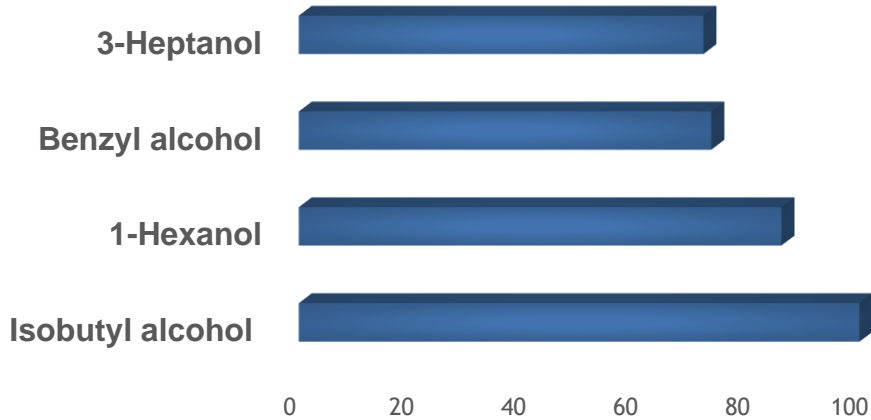


- ✓ 1- pentanol (unpleasant aroma, its esters: pentyl butyrate, smells like apricot, amyl acetate (pentyl acetate), smells like banana)
- ✓ 2-phenyl ethanol (Saccharomyces cerevisiae metabolite, pleasant floral odor, rose-like taste)
- ✓ formed by the yeast, from the sugars or from the amino acids.

Higher alcohols in Vranec wine

- ✓ Are major portion of the secondary products of yeast metabolism

Alcohols ($\mu\text{g/L}$) in Vranec wine



3-Heptanol: strong herbaceous odor and a pungent, slightly bitter taste

Benzyl alcohol: floral type odor and an fruity type flavor

1-Hexanol: winey fatty fruity wine-like-notes, coconut, berry, fruit-flavor

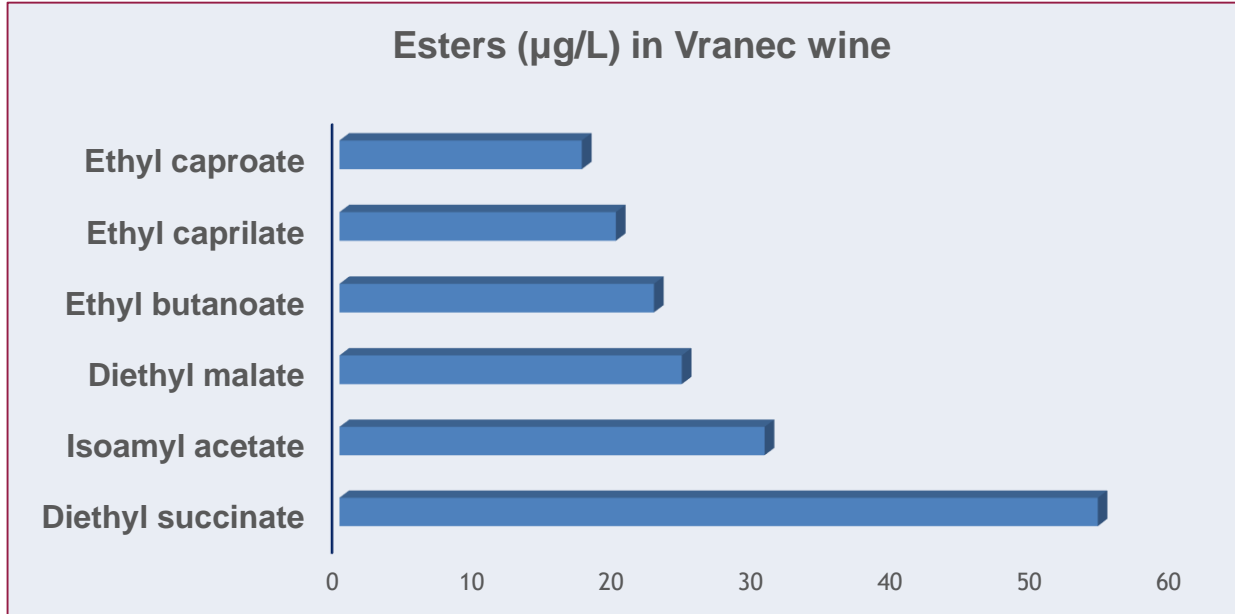
Isobutyl alcohol - sweet whiskey taste

Yeast can produce: isobutanol from valine, isoamyl alcohol from leucine, n-propanol from threonine.

Esters in Vranec wine

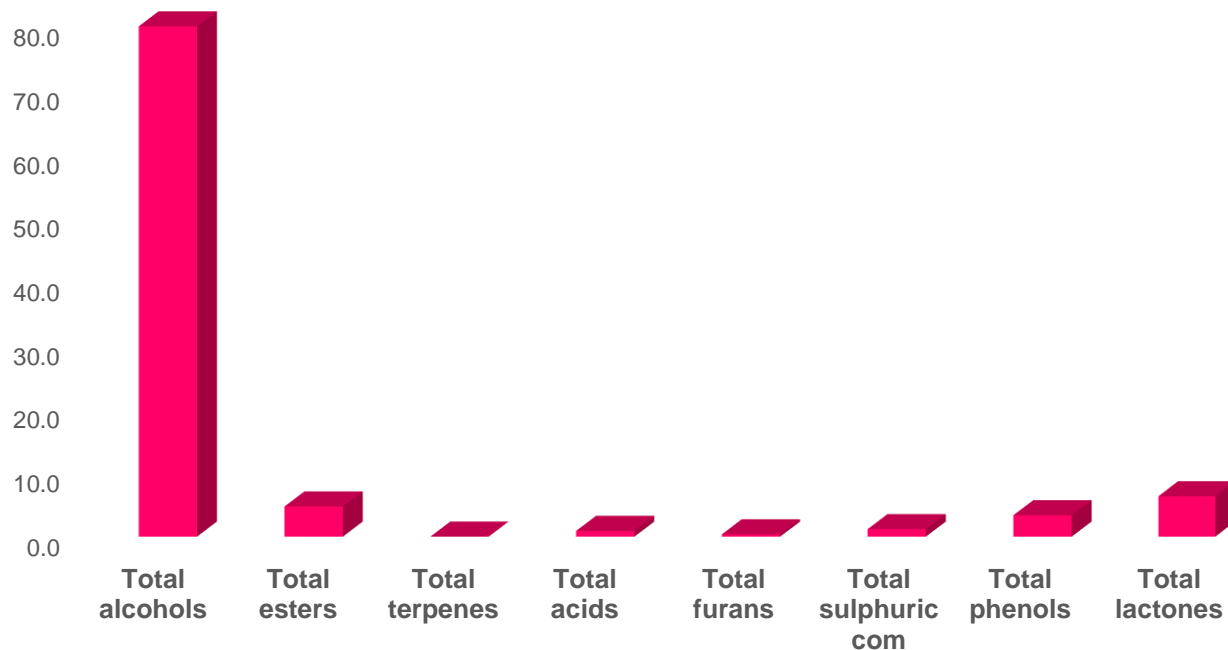
Esters ($\mu\text{g/L}$)	Vrane wine
Ethyl propanoate	11.42
Ethyliso butyrate	9.85
Ethyl butanoate	22.4
Isoamyl acetate	30.3
Butyl formate	12.5
Ethyl caproate	17.3
Hexyl acetate	0
Ethyl caprilate	19.7
Ethyl-3-hydroxybutanoate	17.1
Ethyl caprylate	10.4
Diethyl succinate	54.2
Methyl-4-hydroxybutanoate	0
Phenyl ethyl acetate	12.6
Diethyl malate	24.4
Acetyl glycineethyl ester	12.5
Ethyl palmitate	n.d.
E-11-Hexadecanoic acid ethylester	n.d.
Succinic acid, 2-hydroxy-3-methyl-diethylester	23.3
Total esters ($\mu\text{g/L}$)	328

Esters in Vranec wine



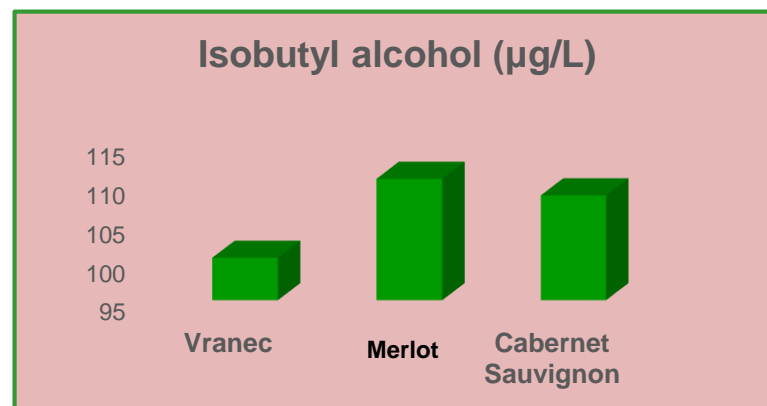
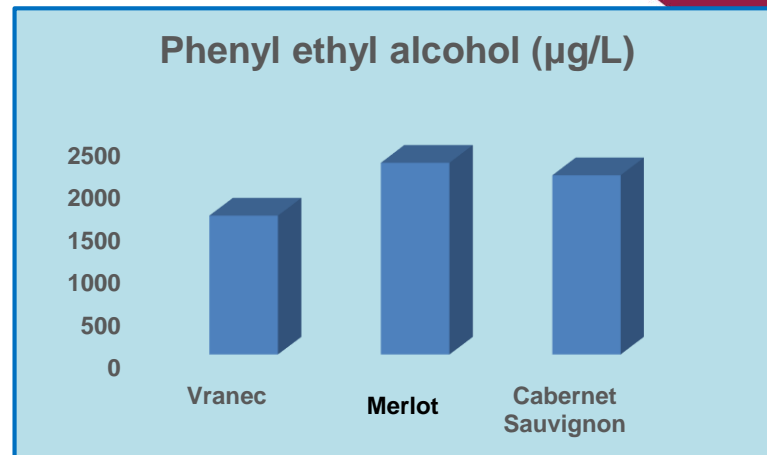
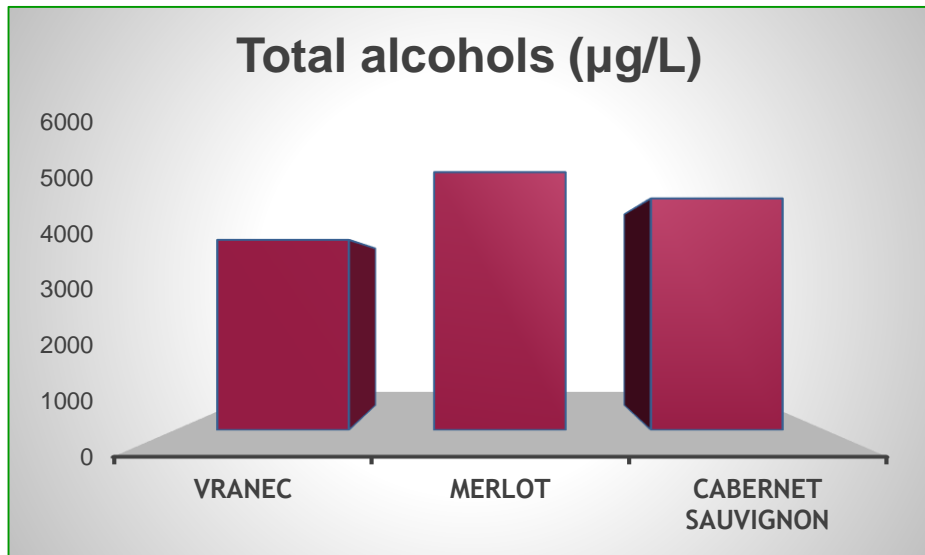
- ✓ Ethyl caproate (ethyl hexanoate) - green apple aroma
- ✓ Ethyl caprilate (ethyl octanoate) - strong caramel and fruity odor (apricot)
- ✓ Diethyl malate - floral and fruity aroma, over-ripe, peach and prune
- ✓ Isoamyl acetate - pleasant fruity notes (banana and strawberry aroma)
- ✓ Diethyl succinate - characteristic volatile compounds of the malolactic fermentation in young wines, its concentration increases during wine storage and aging, floral and fruity aroma.

Aromatic profile of Vranec



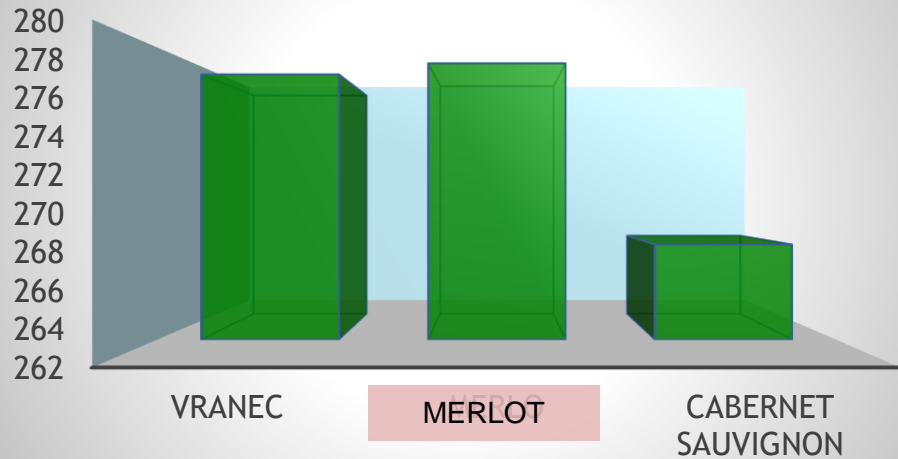
In general: complex aroma profile of Vranec wines

Comparison of aromatic profile of Vranec, Merlot and Cabernet Sauvignon

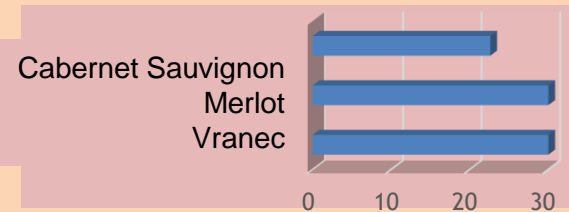


Comparison of individual aromatic compounds in Vranec, Merlot and Cabernet Sauvignon

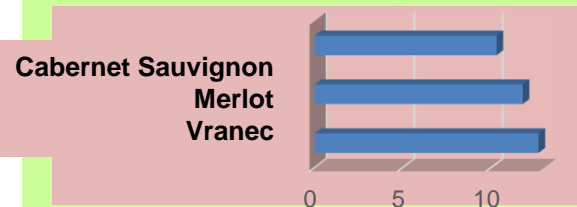
Total esters ($\mu\text{g/L}$)



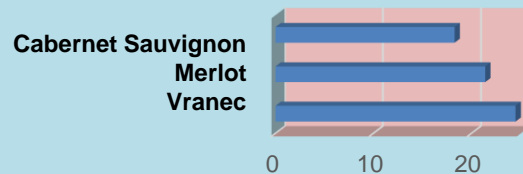
Isoamyl acetate ($\mu\text{g/L}$) (banana)



Phenyl ethyl acetate (rose)



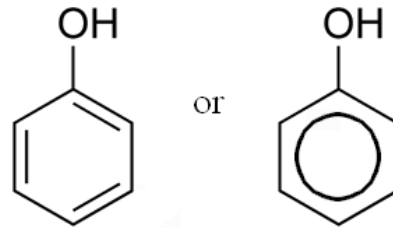
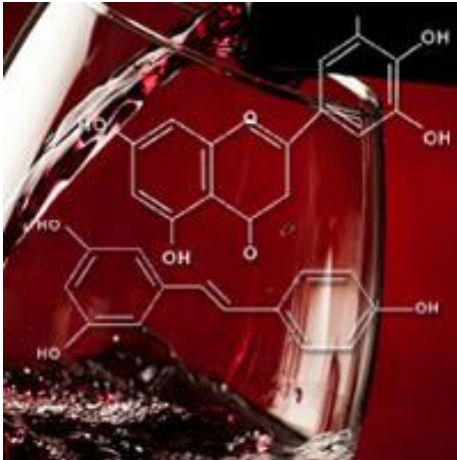
Diethyl malate ($\mu\text{g/L}$) (over-ripe, peach and prune)



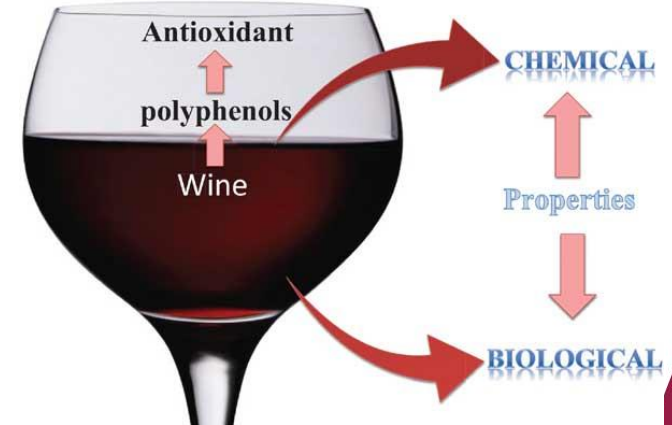
Phenolic composition of wine

Very important components in wine and grapes responsible for the quality of wine,

- Beneficial effects on health: antioxidant, antimicrobial, anticancer ...
- Determine the color, taste, astringency and bitterness of the wine.
- They are responsible for the differences between red and white wines, especially the color and taste of reds.
- These substances are present in different parts of the grapes and are extracted during vinification.



Structure of phenol



PHENOLIC COMPONENTS

FLAVONOIDS

Anthocyanins

Flavan-3-ols

Flavonols

Flavons.
Flavanons....

NON-FLAVONOIDS

Phenolic acids

Stilbens

Skin: anthocyanins.
Tanins, flavonols



Seeds: flavan-3-ols
MONOMERS

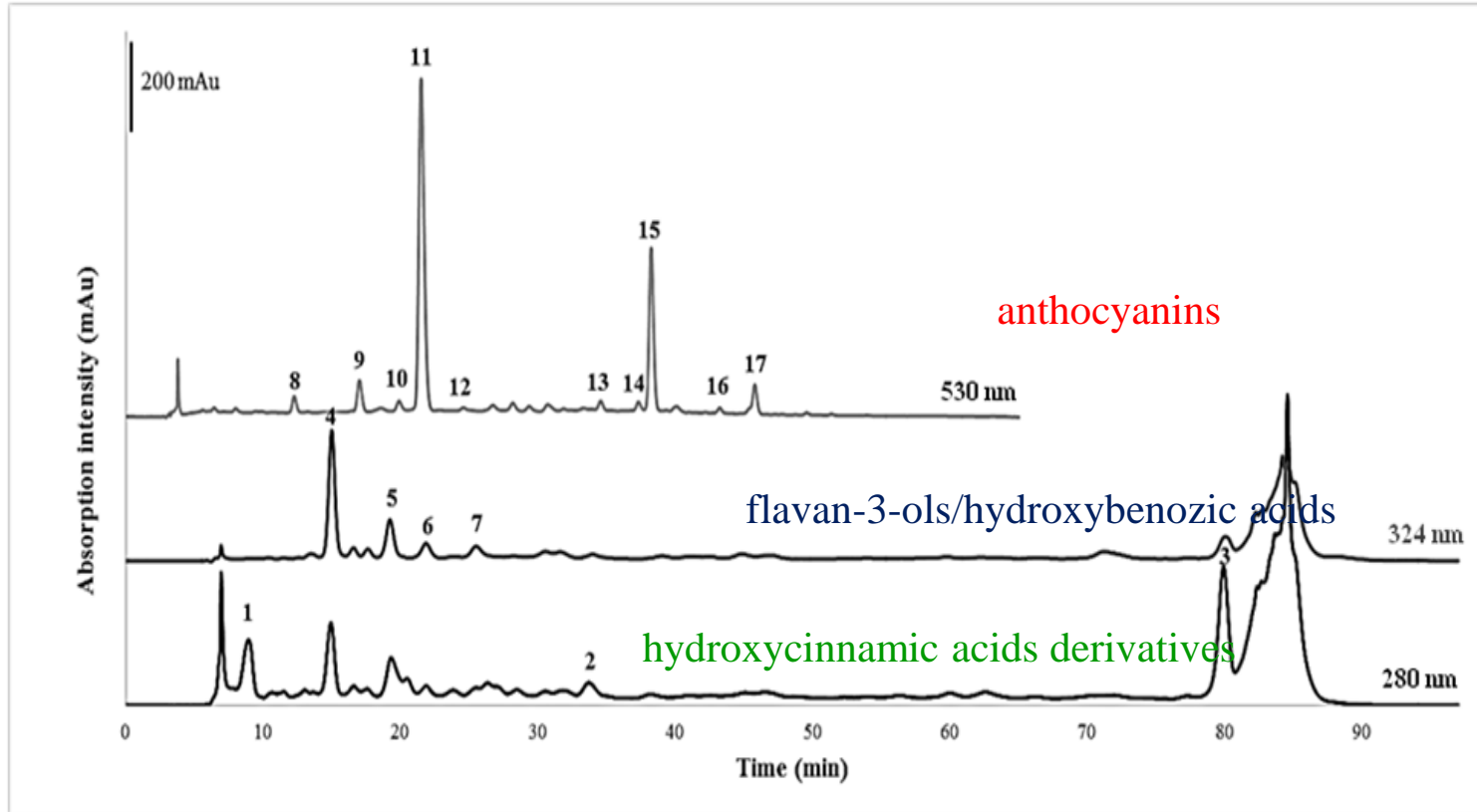
Analytical technologies for analysis of polyphenols

HPLC-DAD

HPLC-DAD-MS (high-performance liquid chromatography coupled with diode-array detector, mass detector)

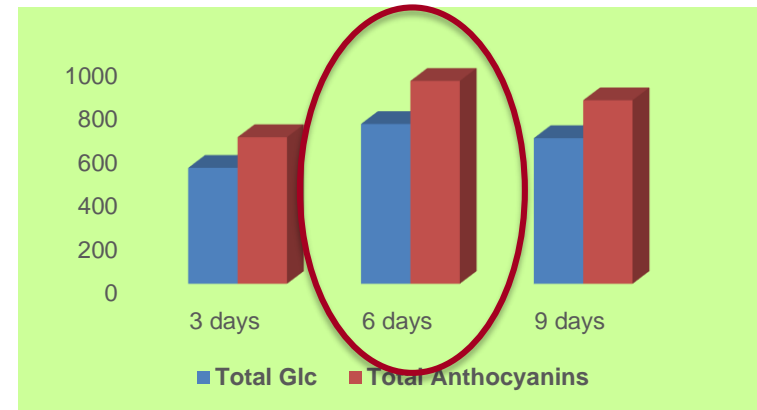
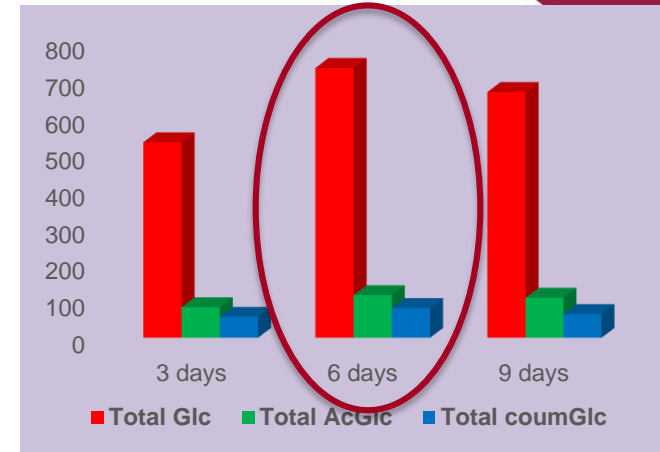


HPLC-DAD analysis of Vranec wine



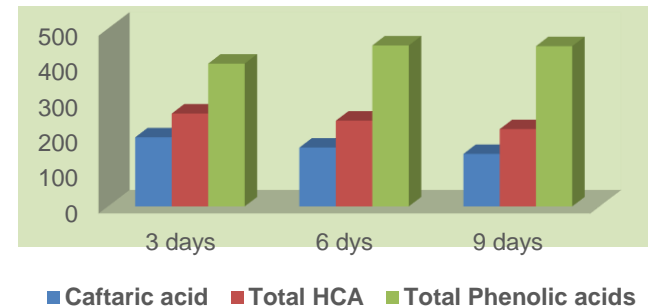
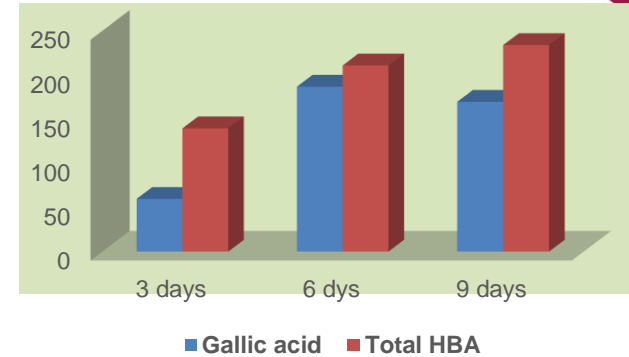
Quantification of anthocyanins in Vranec wines during maturation

Anthocyanins (mg/L)	Vranec wine		
Time of maceration	3 days	6 days	9 days
Dp-Glc	5.88	12.2	10.3
Pt-Glc	30.4	48.6	43.2
Pn-Glc	9.13	21.1	17.8
Mv-Glc	485	649	595
Total Glc	531	732	667
Pt-AcGlc	3.65	5.89	5.63
Pn-AcGlc	6.30	8.53	8.48
Mv-AcGlc	73.3	102	94.8
Total AcGlc	83.3	116	109
Pn-coumGlc	3.56	6.92	5.31
Mv-coumGlc	53.5	74.1	59.2
Total coumGlc	57.1	81.1	64.5
Total Anthocyanins	671	929	840



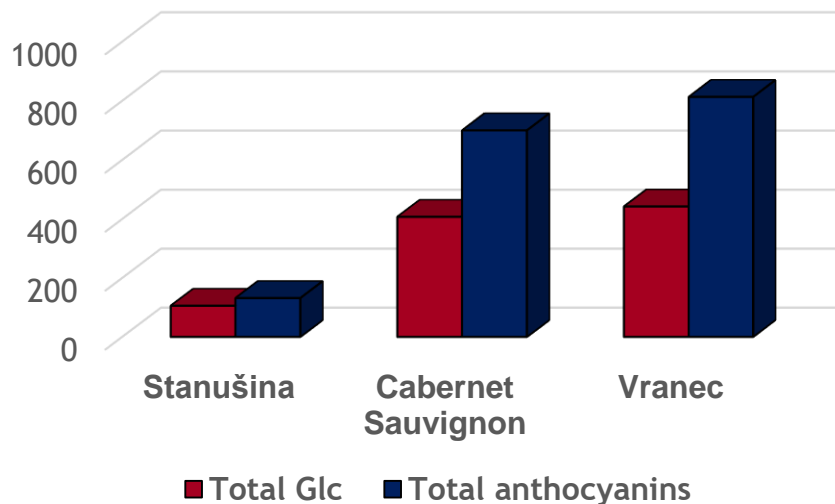
Quantification of phenolic acids in Vranec wines during macuration

Phenolic acids	Vranec wines		
	3 days	6 dys	9 days
Time of maceration	3 days	6 dys	9 days
Protocatecuic acid	47.6	n.d.	26.7
Gallic acid	59.9	187	170
Syringic acid	32.5	24.4	37.1
Total HBA	140	211	234
<i>p</i> -Coumaric acid	14.1	12.8	8.40
Caftaric acid	195	166	148
Coutaric acid	23.3	29.4	25.5
Caffeic acid	10.9	10.9	16.2
Fertaric acid	18.2	22.7	19.5
Total HCA	262	242	218
Total Phenolic acids	402	453	451
(+)-Catechin	20.5	220	251

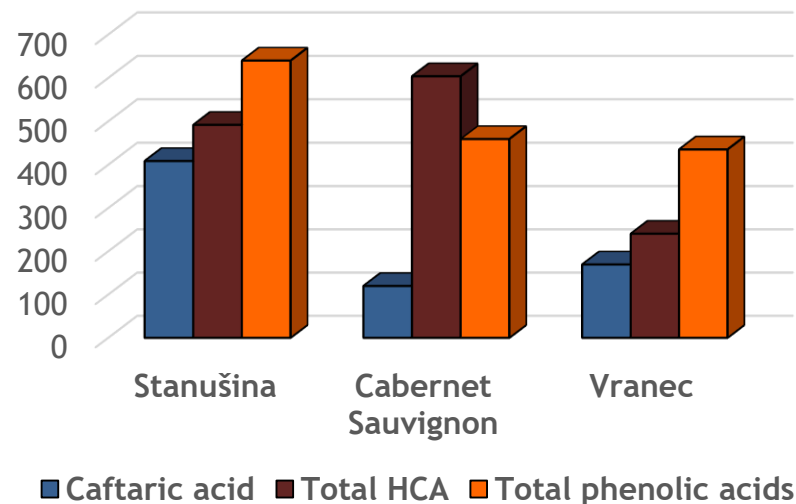


Comparison of Vranec with Stanušina and Cabernet Sauvignon

Anthocyanins

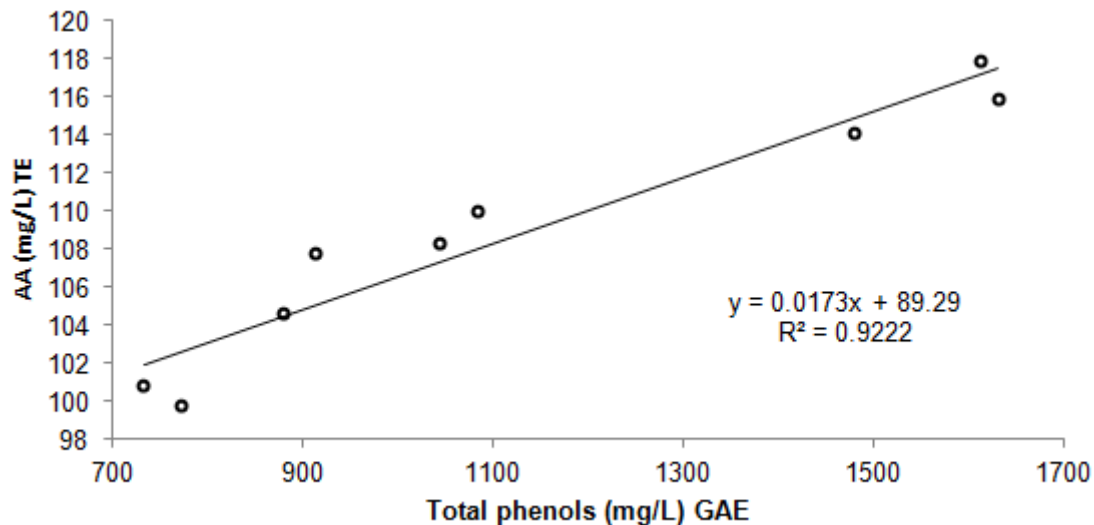


Hydroxycinnamic acids

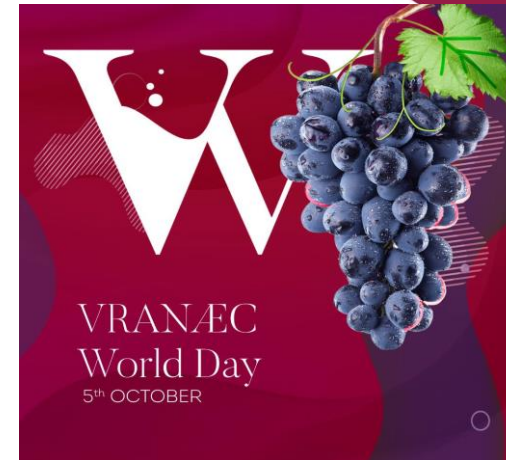
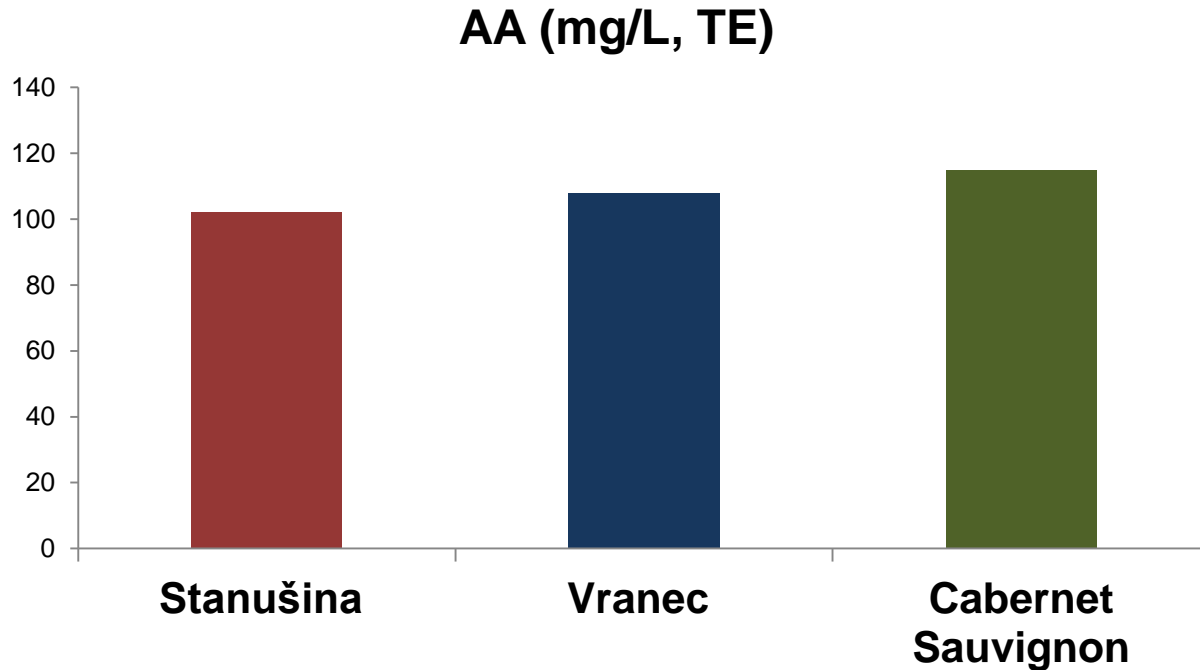


	Vranec wines		
Time of maceration	3 days	6 days	9 days
TP (mg/L, GAE)	913	1045	1084
CI	5.81	4.24	5.51
H	0.44	0.53	0.51
AA (mg/L, TE)	107	109	109

Correlation between total phenols and antioxidant activity of Vranec wines



Comparison of antioxidant activity of Vranec wines with Stanušina and Cabernet Sauvignon



CONCLUSION

- **Complex aroma profile of Vranec wines determined by GC-MS**
- **Complex polyphenols profile of Vranec wines determined by HPLC**
- **High antioxidant potential**
- **Further and continuous research on aromatic and polyphenolic profile of Vranec wine.**



ACKNOWLEDGEMENT

Organizers of the 4th Vranec world day
Wines of Macedonia



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ШТИП



УНИЛАБ

**We have a brand
Macedonia has a brand
VRANEC is our brand and pride**



Thank you for your attention!