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CHEMICAL AND PHENOLIC PROFILE OF STANUŠINA RED WINES DETERMINED BY HPLC-DAD

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CHEMICAL COMPOSITION OF WINE

- Wine production in Macedonia takes place in 149 officially registered wineries
- The total bottling capacity is about **650,000** hl per year.
- Macedonia is mainly **export-oriented country (Germany, Croatia, Slovakia, Poland, China, UK….)**.

Regarding the export value of agricultural products, WINE is:

- In **first place** in terms of export of alcoholic beverages, and
- In second place immediately after the **tobacco**.

CHEMICAL COMPOSITION OF WINE

To determine the chemical composition of wines, the following methods are used:

- Methods published and recommended by the International organization of vine and wine (**OIV**), and
- Methods compliant with the standards recommended by the International Organization for Standardization (**ISO**).

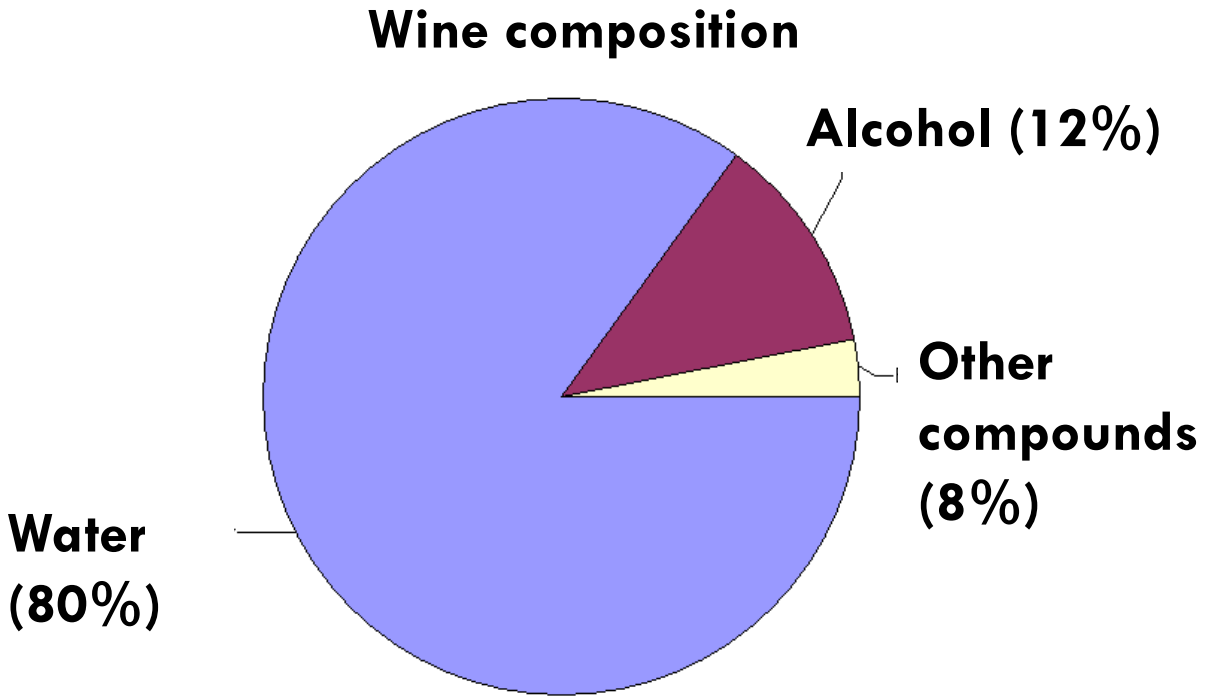
CHEMICAL COMPOSITION OF WINE

❖ **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.**

CHEMICAL COMPOSITION OF WINE

Ethanol

- ✓ Affects the quality of the wine: **the stability of the wine - sensory characteristics**

Carbohydrates (sugars) and sweetness of wine

- ✓ Reducing sugars: **Glucose** and **fructose**
- ✓ Dry wines contain small amounts of unfermented sugar

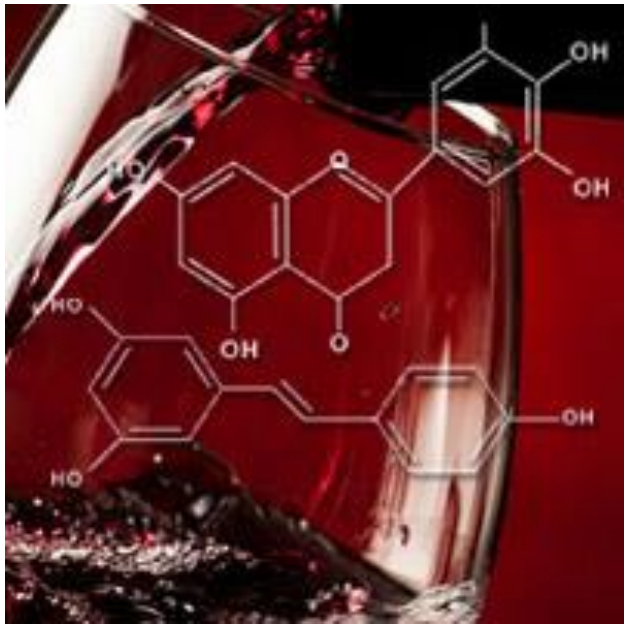
Acids and Acidity

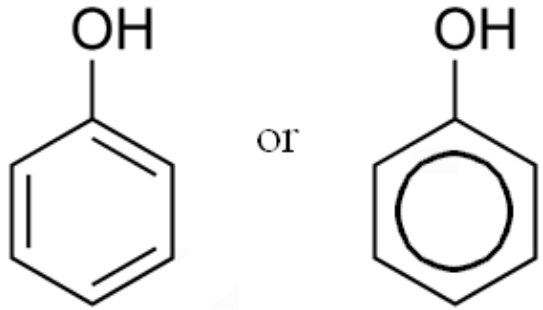
- ➢ The main acids in wine are: **tartaric**, **malic**, **lactic**, **citric.**
- ➢ These acids and other minor acids which determine the **acidity** of the wine.
- ➢ Total acidity is determined in grape juice, must and wine.
- ➢ **Acetic acid is the main volatile acid which determines the volatile acidity.**

PHENOLIC COMPOSITION OF WINE

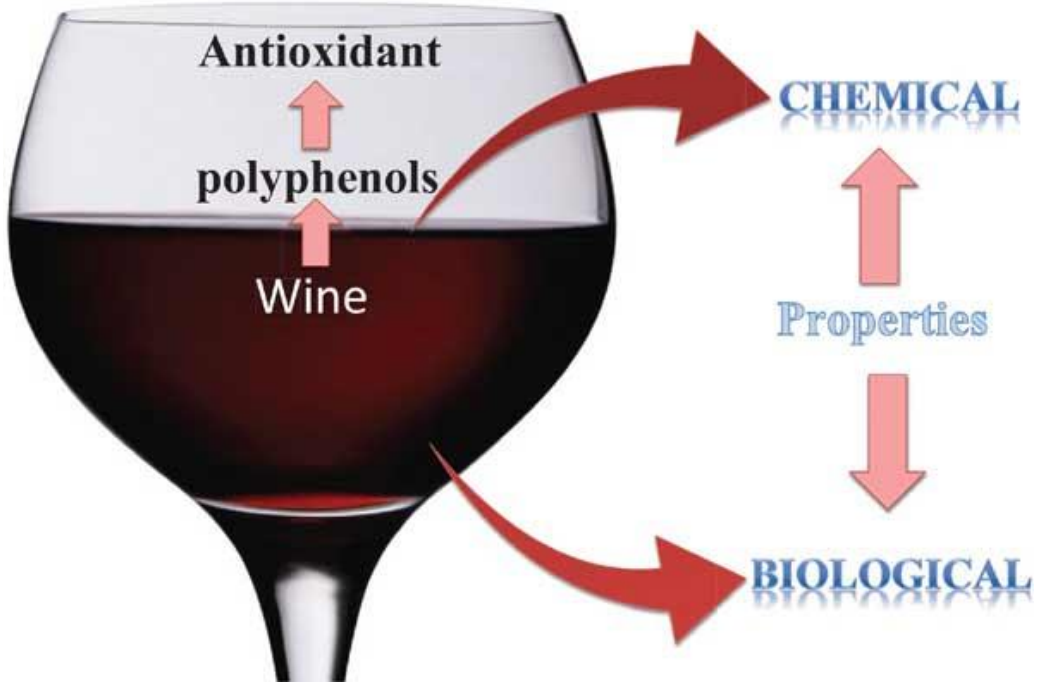
Very important components in wine and grapes responsible for the quality of wine,

- 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 COMPOSITION OF WINE

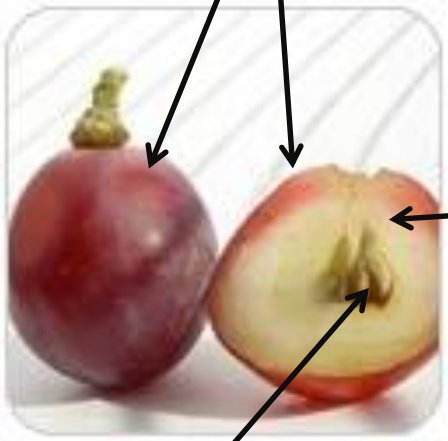
FLAVONOIDS

- Anthocyanins
- Flavan-3-ols
- Flavonols
- Flavons.
  Flavanons....

NON-FLAVONOIDS

- Phenolic acids
- Stilbens

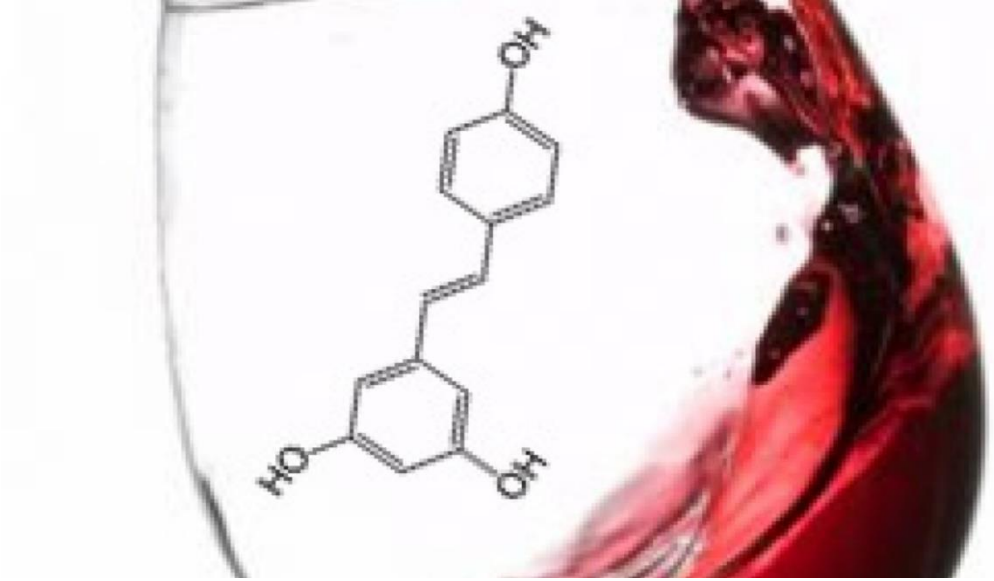
Skin: anthocyanins. Tanins, flavonols



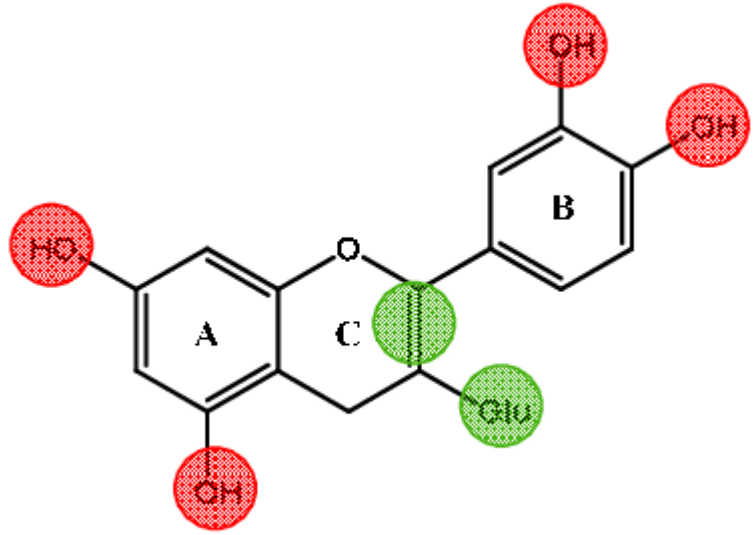
Pulp

Seeds: flavan-3-ols
MONOMERS

FLAVONOIDS

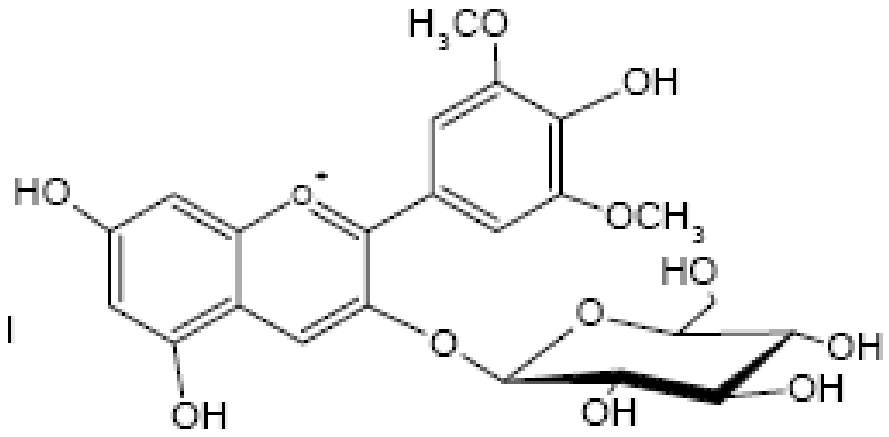


- Includes over **2000 phenolic compounds** in the plant world
- Many are brightly coloured
- In grapes, source is **seeds/skin**s and tissue
- Much of the structure and colour of wine is from this group of compounds
- Anthocyanins (red pigments), procyanidins (colour co-factors and tannin precursors)
- Flavonols (anti-oxidant compounds)
- Catechins (yellow pigments)

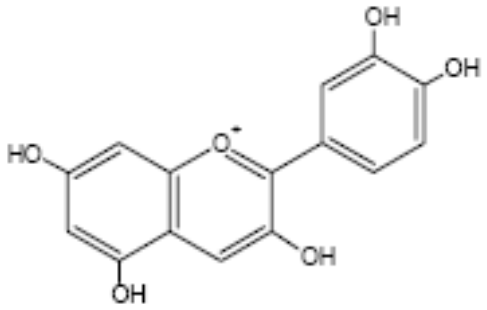


ANTHOCYANINS – RED WINE PIGMENTS

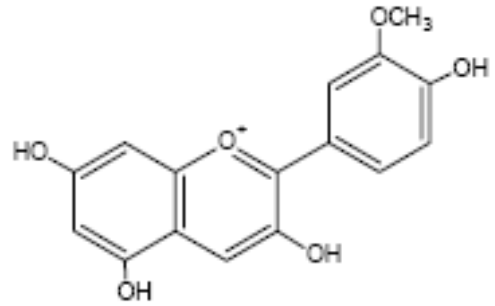
- ✓ Dominant anthocyanin in all varieties is **malvidin.**
- ✓ Total anthocyanins in young reds typically 500 mg/L.
- ✓ Highly reactive compounds
- ✓ Colour, hue and density of young red wines result of a complex series of delicately balanced equilibria
- ✓ Particularly sensitive to pH and SO_2 levels



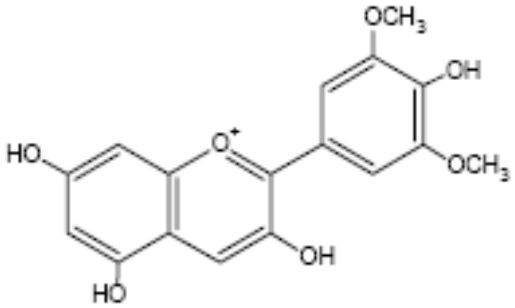
Malvidin 3-glucoside



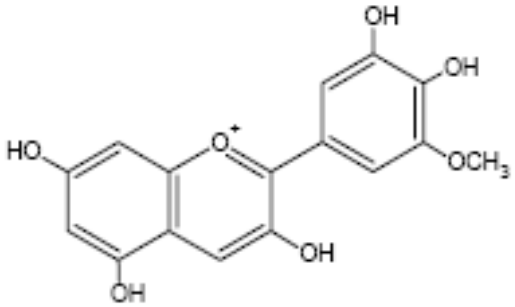
Cyanidin



Peonidin



Malvidin

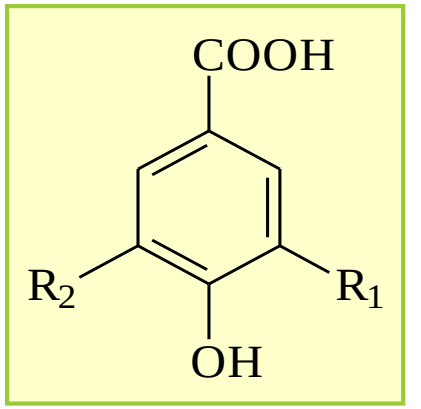


Petunidin

NON-FLAVONOIDS – BITTERNESS COMPOUNDS

Benzoic and cinnamic acid derivatives

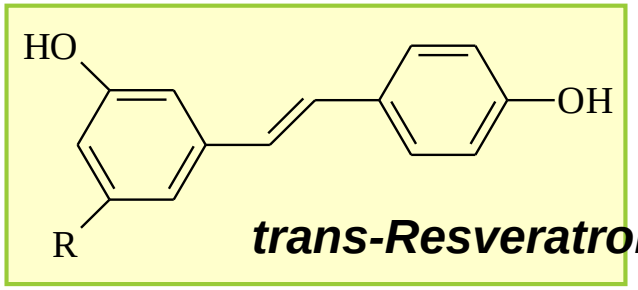
- ✓ Compounds that oxidise to cause browning in wines (oxidised form is yellow/brown)
- ✓ Generally odourless (but can be precursors to volatile phenol fault compounds)
- ✓ Have a bitter flavour
- ✓ Also Coumarins – from oak,
- ✓ Can exist as the glycoside (bitter) or aglycone (acidic)



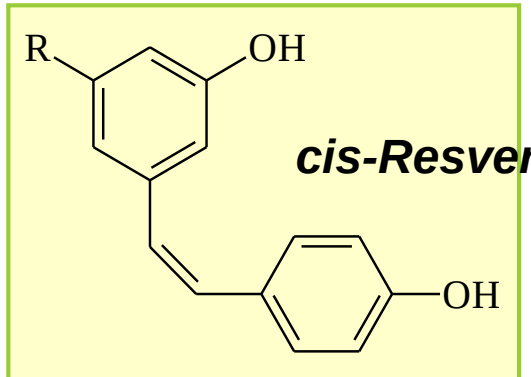
Gallic acid

Stilbenes

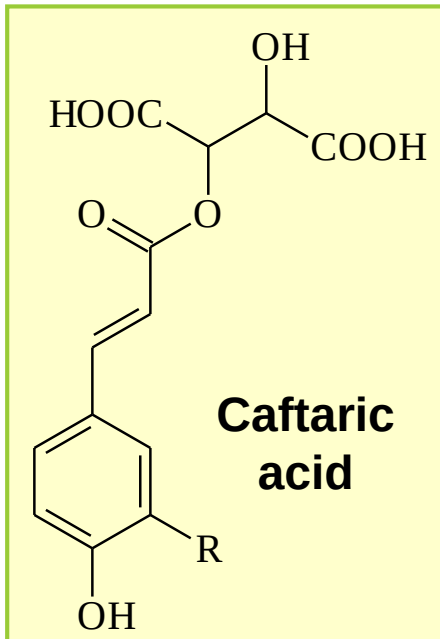
- ✓ They are synthesized in the vine
- ✓ Defense system against fungal infection, most commonly *Botrytis cinerea*, and for protection against UV radiation.



trans-Resveratrol



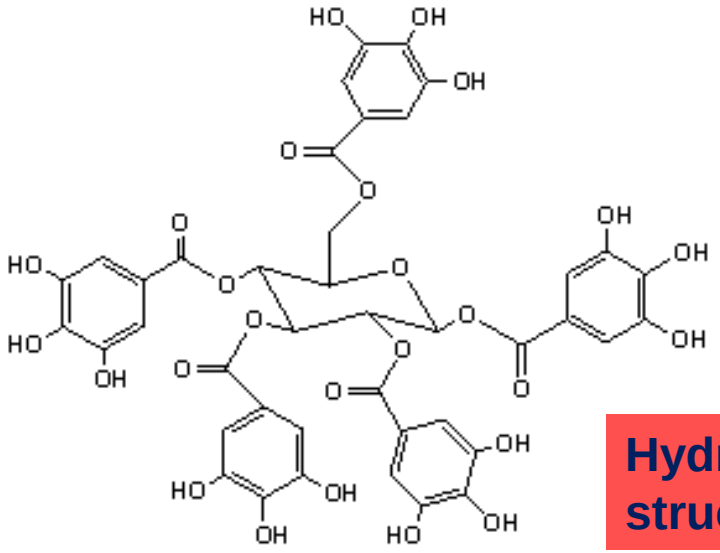
cis-Resveratrol



Caftaric acid

WHAT ARE TANNINS?

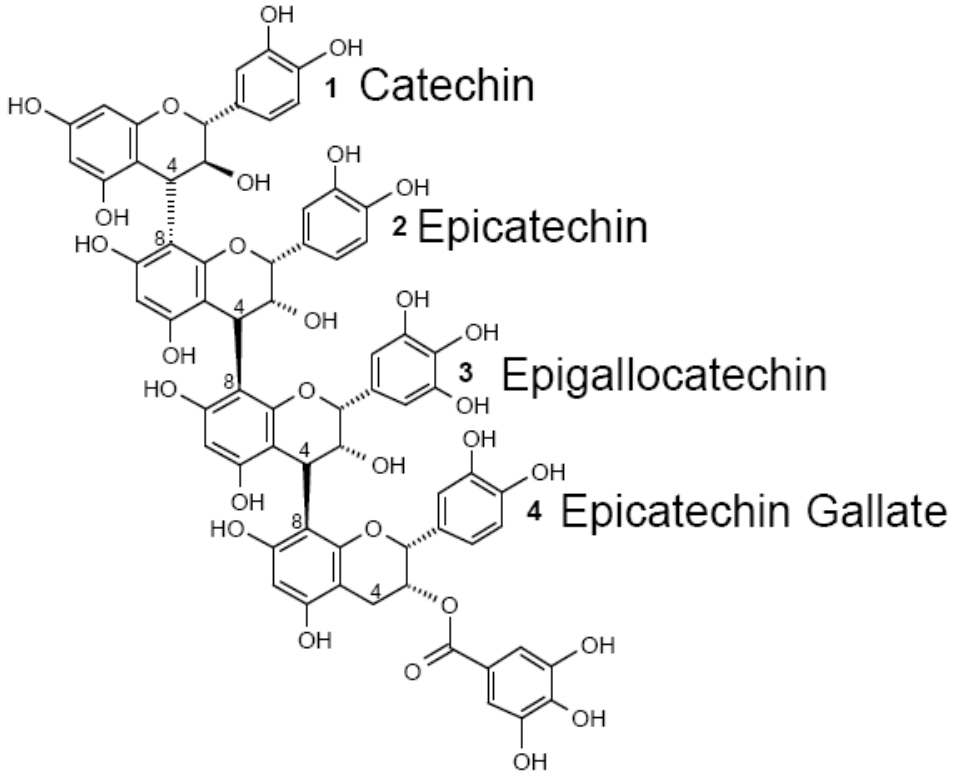
- ✓ Large molecular weight compounds made up of smaller phenolic units.
- ✓ Characterised by their precipitation reaction with proteins.
- ✓ Tannins react with saliva proteins.
- ✓ Molecular weights range from 500 to over 3000.
- ✓ Act as ‘oxygen’ soaks – assist in preventing oxidation of red wines



Hydrolysable tannin structure

- ❑ **Condensed tannins from grape** (skins/seeds)
- ❑ **Hydrolysable tannins** less from grapes but extracted from **oak barrels**

Condensed Tannin Structure



STANUŠINA GRAPE VARIETY
МК. СТАНУШИНА

- Red grape variety **indigenous** to the Republic of Macedonia and is found nowhere else in the world.
- Little known outside of the native country.
- It is capable of producing very **high quality wines** and is mainly grown in the Tikveš region.
- The introduction of international grape varieties have caused a sharp decline of Stanušina from the country's vineyards in recent times.
- Local wineries have begun initiatives to reintroduce this uniquely old Macedonian domestic variety back into the region.
- The grapevine is **highly resistant** to drought and pests and can be cultivated without irrigation.
- The fruit ripens very late but gives good yields. Producing on average 15-20 t/ha.
- The juice obtained contains approx 18-20% sugar and 6-9 g/l acids.
- During a good year it can provide a base for wine of **high quality and medium strength with 11-12% of alcohol**.

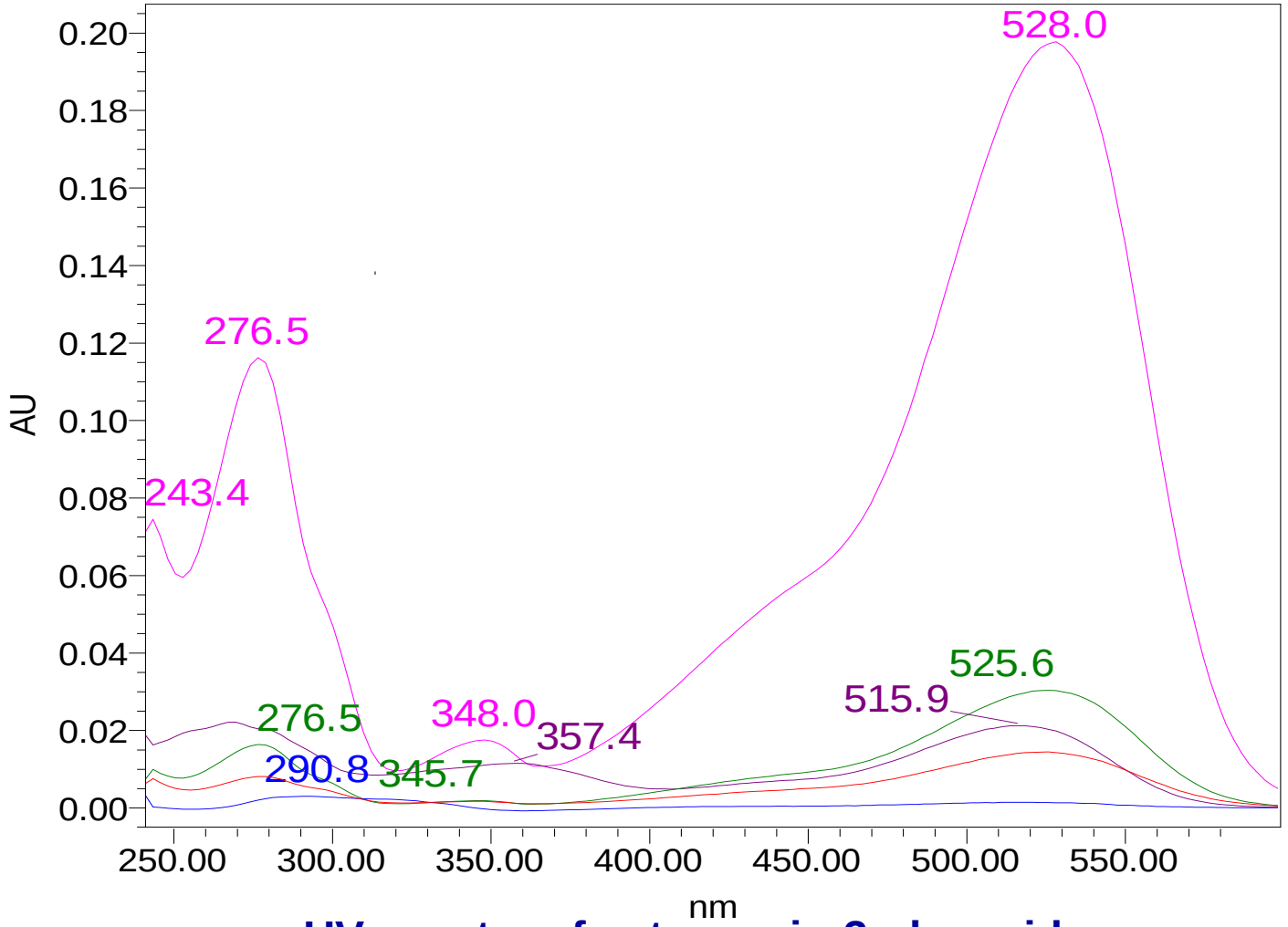
ANALYTICAL TECHNOLOGIES FOR ANALYSIS OF POLYPHENOLS

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

UV-VIS SPECTRA OF PHENOLICS

Phenolic compounds show characteristic absorbances in the UV/Vis region:

- Anthocyanins: 520 nm,
- Flavonols: 360 nm,
- Hydroxycinnamic acids: 320 nm.
- Flavan-3-ols: at 280 nm



UV spectra of antocyanin-3-glucosides

THE AIM OF THE WORK

1. **To study the phenolic composition of wines of the Stanušina variety using UV-Vis spectroscopy and HPLC-DAD**

2. **To study the influence of maceration time on phenolic composition**

EXPERIMENTAL

Winemaking

- **Yeast** for fermentation, Excellence SP Saccharomyces cerevisiae (LamotheAbiet, Canejan/Bordeaux, France)
- 80 mg/L **SO_2**
- **Nutrients**: 20 g/hL Oenostim activator, Lamothe-Abiet, France)

Basic chemical parameters

Alcohol (OIVMA-AS312-01 A), **dry extract** (OIV-MA-AS2-03B), **specific density** (OIV-MA-AS2-01), **total acidity** (OIV-MAAS313–01), **volatile acidity** (OIV-MA-AS313–02), **free and total SO_2** (Ivanova-Petropulos and Mitrev 2014)

Spectrophotometric analyses

- **Total phenols, 280 nm**
- **Antioxidant activity, DPPH method, 515 nm**
- **Colour and hue:** 420 nm (browning degree), 520 nm и 620 nm (anthocyanins)

HPLC analyses

HPLC-DAD system: Dionex DX500

- **Anthocyanins and related pigments:** column Gemini RP-C18 (250 × 4,6 mm; 5 μm)
- **Hydroxycinnamic acids**
- **Flavan-3-ols**

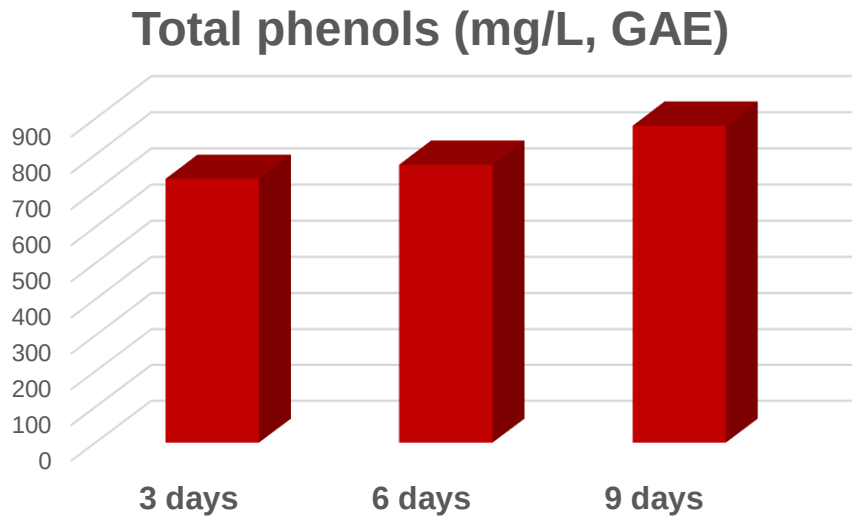
column Aquapore ODS-300 RP-C18, (250 × 4,6 mm; 7 μm)

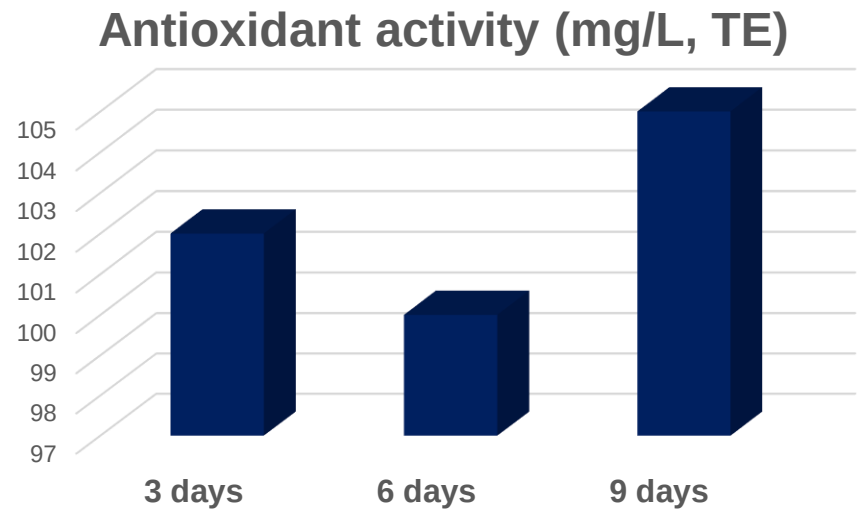
RESULTS AND DISSECTION
BASIC CHEMICAL COMPOSITION

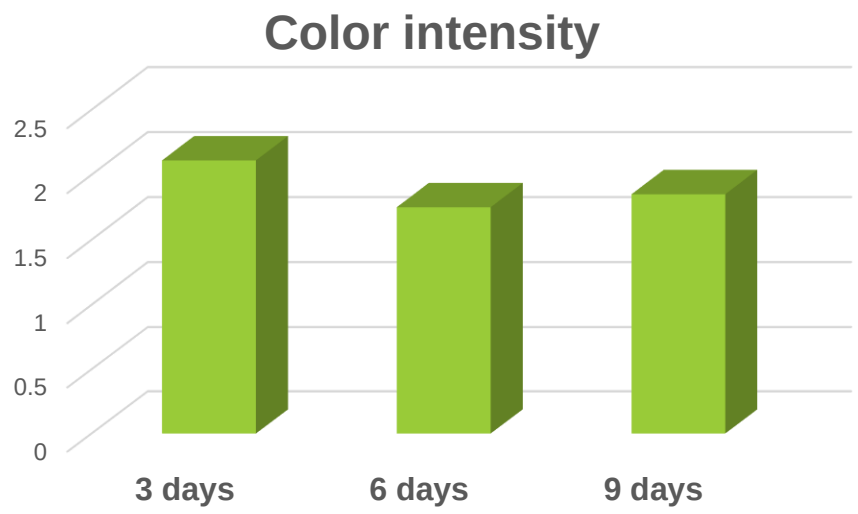
Parameters/Wines	Stanušina		
Days of maceration	3	6	9
Alcohol (%,v/v)	13.58	13.51	13.64
Total dry extract (g/L)	27.3	26.06	26.96
Specific density	0.9928	0.9924	0.9926
Volatile acidity (g/L)	0.52	0.63	0.68
Total acidity (g/L)	5.8	5.5	6.0
Free SO_2 (mg/L)	15	23	42
Total SO_2 (mg/L)	59	62	68

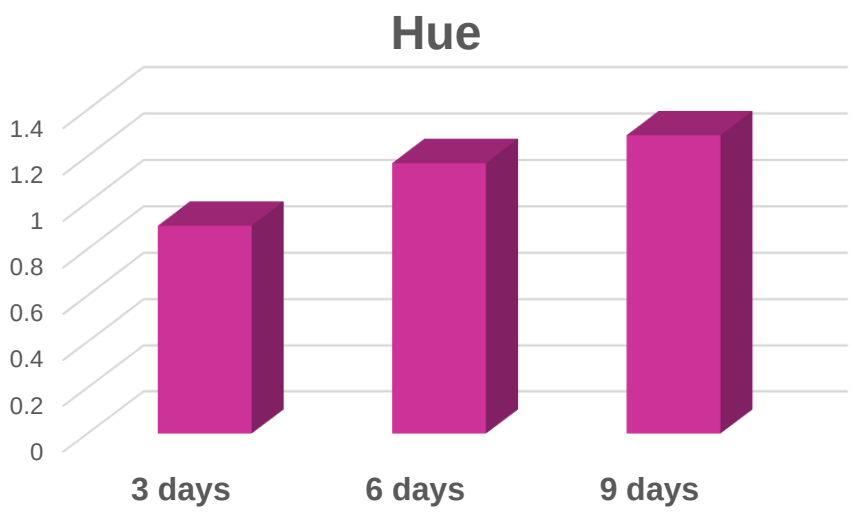
RESULTS AND DISSECTION

SPECTROPHOTOMETRIC PARAMETERS







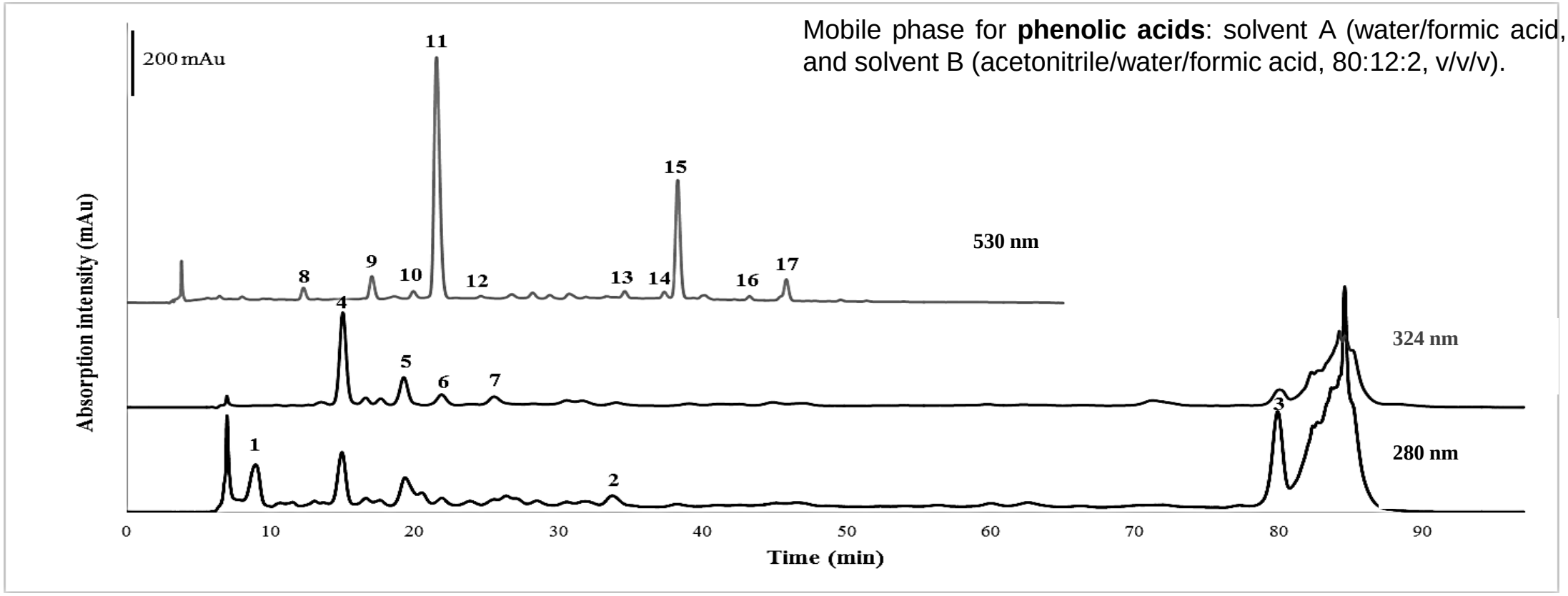


RESULTS AND DISSECTION

HPLC PARAMETERS

Mobile phase for **anthocyanins**: water/methanol (70/30, v/v) containing 6 mL/L of 70 % perchloric acid (solvent A) and water/ methanol (25/75, v/v) containing 6 mL/L of 70 % perchloric acid (solvent B).

Mobile phase for **phenolic acids**: solvent A (water/formic acid, 98/2, v/v) and solvent B (acetonitrile/water/formic acid, 80:12:2, v/v/v).



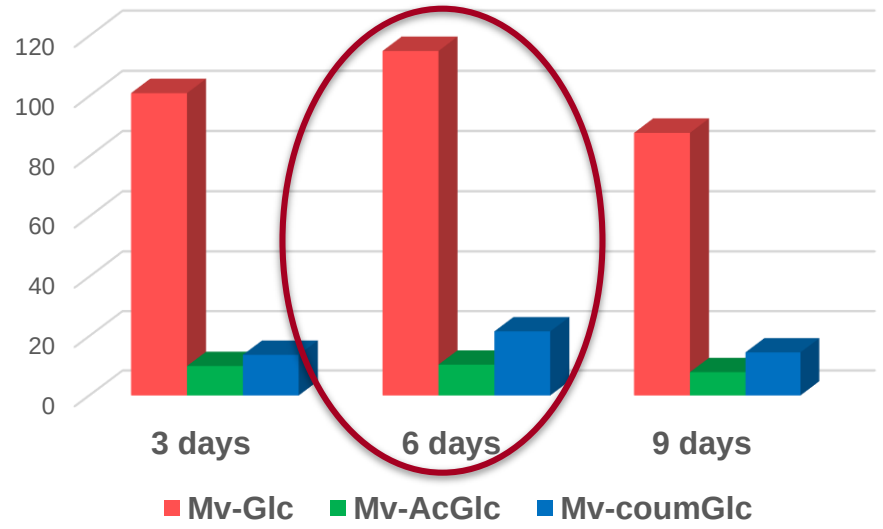
UV-Vis chromatogram of Stanušina wine

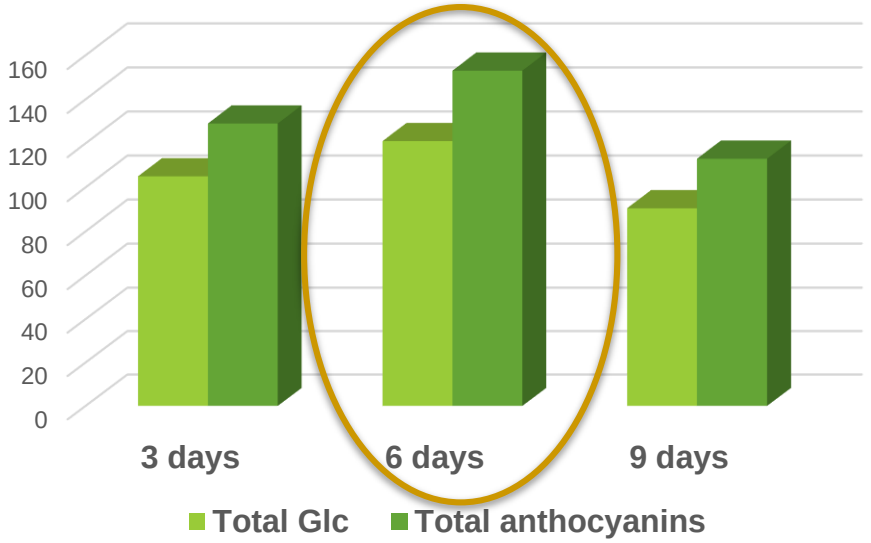
- **530** nm – for separation and quantification of anthocyanins **(10 compounds)**
- **324** nm **– for separation and quantification of flavan-3-ols and hydroxybenzoic acids (4 compounds)**
- **280** nm – for separation and quantification of **hydroxybcinnamic acids (2 compounds).**

RESULTS AND DISSECTION

QUANTIFICATION OF ANTHOCYANINS

Parameters (mg/L)	Stanušina		
Time of maceration	3 days	6 days	9 days
Dp-Glc	n.d.	n.d.	n.d.
Pt-Glc	4.34	5.13	2.62
Pn-Glc	0.00	0.00	0.00
Mv-Glc	**101**	**115**	**87.8**
Total Glc	**105**	**121**	**90.5**
Pt-AcGlc	n.d.	n.d.	n.d.
Pn-AcGlc	n.d.	n.d.	n.d.
Mv-AcGlc	**9.92**	**10.4**	**7.88**
Total AcGlc	**9.92**	**10.4**	**7.88**
Pn--coumGlc	0.00	1.16	0.15
Mv-coumGlc	**13.7**	**21.6**	**14.5**
Total coumGlc	**13.7**	**22.7**	**14.6**
Total anthocyanins	**129**	**153**	**113**
ΣGlc/ΣAcGlc	10.6	11.6	11.5
ΣGlc/ΣcoumGlc	7.68	5.30	6.19
ΣAcGlc/ΣcoumGlc	0.72	0.46	0.54

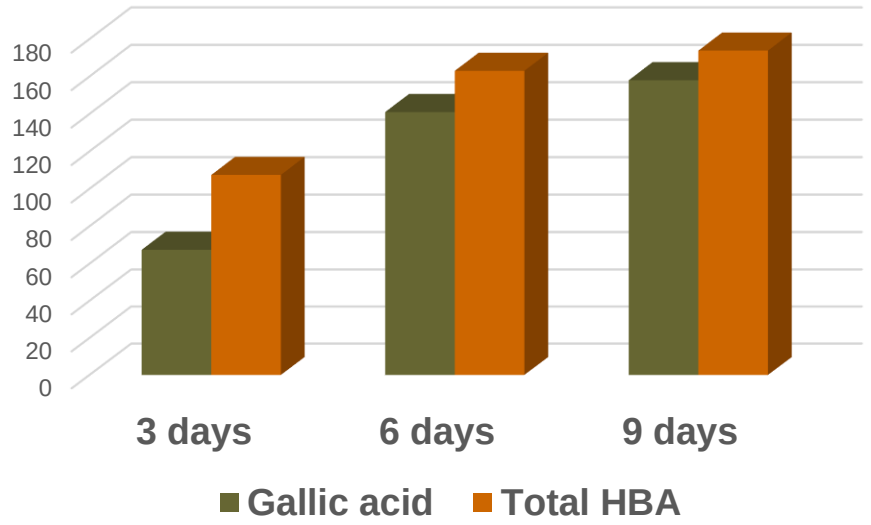


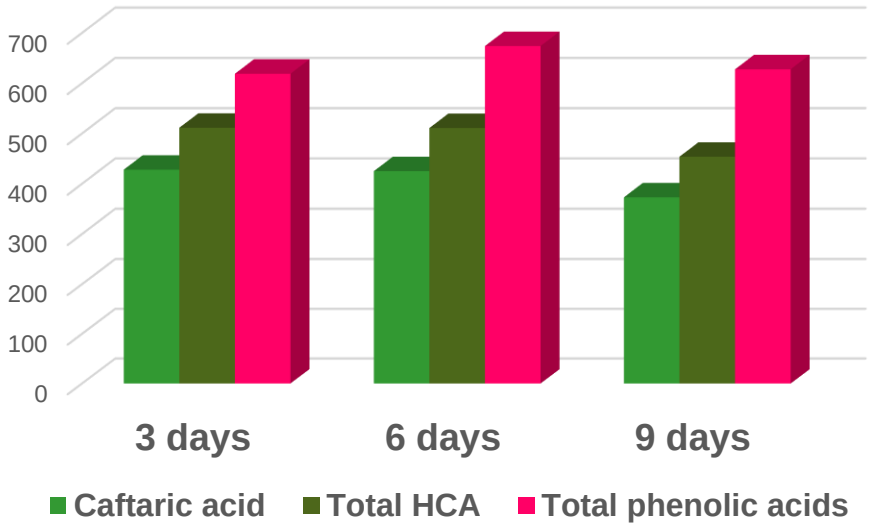


RESULTS AND DISSECTION

QUANTIFICATION OF PHENOLIC ACIDS AND FLAVAN-3-OLS

Parameters (mg/L)	Stanušina		
Time of maceration	3 days	6 days	9 days
Protocatechuic acid	18.2	n.d.	15.8
Gallic acid	**67.4**	**141**	**158**
Syringic acid	21.8	22	n.d.
Total HBA	**107**	**163**	**174**
p-Coumaric acid	2.24	3.45	4.51
Caftaric acid	**428**	**425**	**373**
Coutaric acid	18.5	26.2	24.4
Caffeic acid	**47.7**	**35.7**	**30.1**
Fertraric acid	14.9	20.5	21.8
Total HCA	**511**	**511**	**454**
Total phenolic acids	**619**	**674**	**628**
(+)-Catechin	21.8	22	n.d.

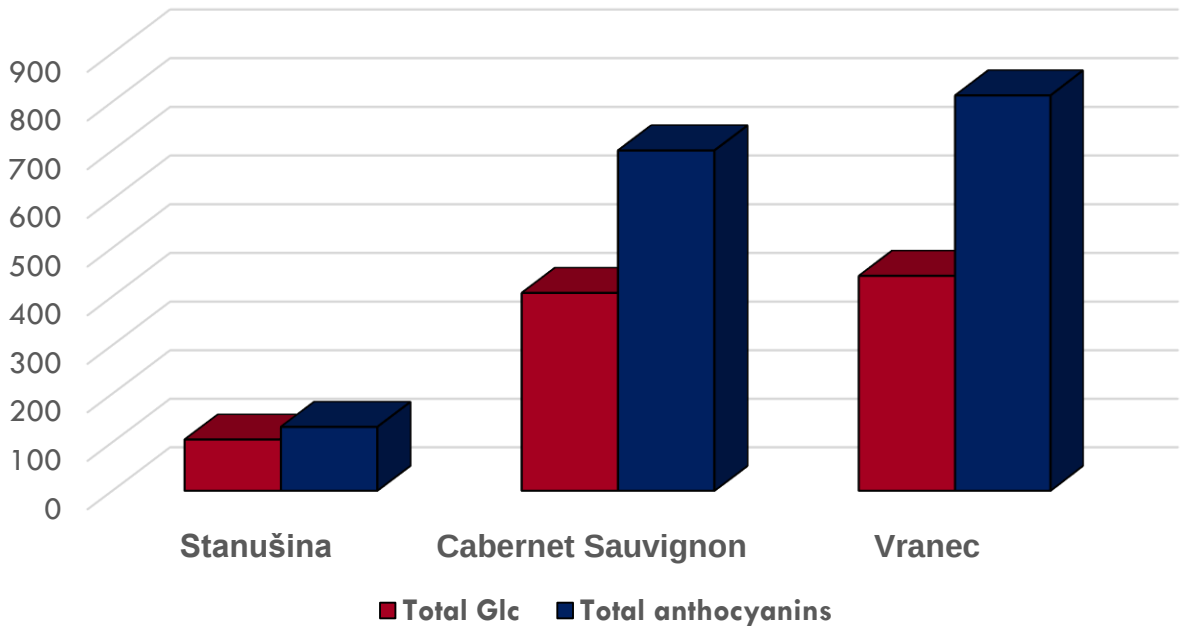




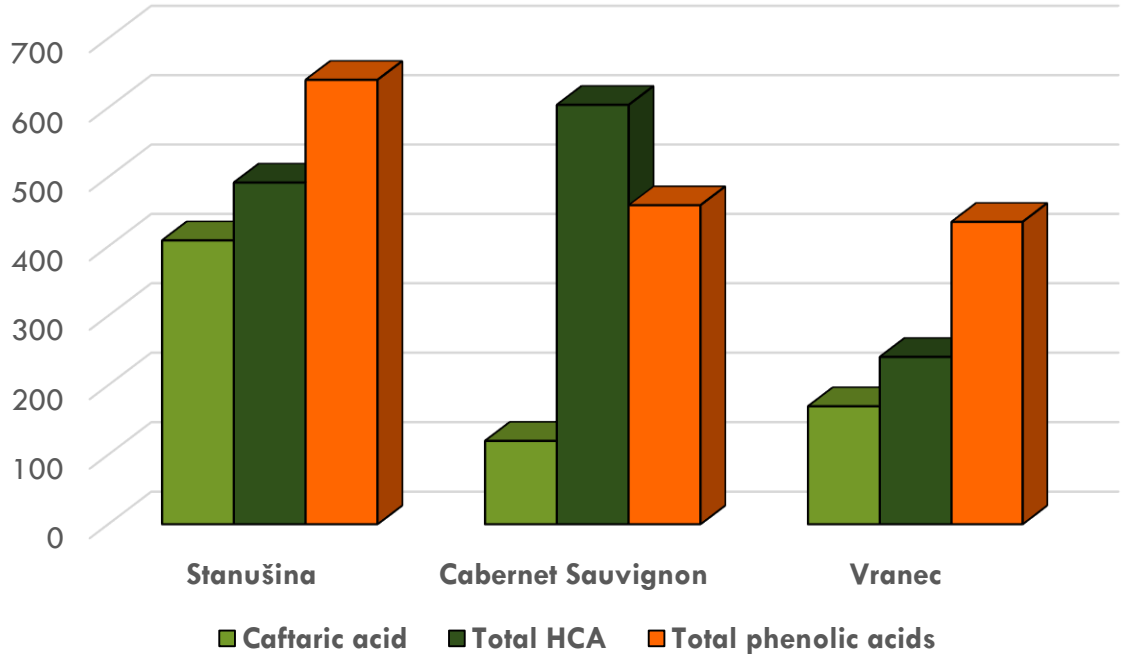
RESULTS AND DISSECTION

COMPARISON OF STANUŠINA WITH CABERNET SAUVIGNON AND VRANEC

Anthocyanins



Hydorxycinnamic acids



CONCLUSIONS

- **HPLC-DAD is valuable technique for monitoring of winemaking**
- A **complex** extraction pattern was observed that vary with maceration time.
- Hydroxycinnamic acids and anthocyanins were observed to be present in the highest content after **3 and 6 days** of maceration, respectively, followed by a slight decrease with time.
- Hydroxybenzoic acids and (+)-catechin content was highest at **9 days** of maceration.
- **Stanušina** wines showed low level of anthocyanins, but relatively **high content of hydroxycinnamic acids**, such as caftaric and caffeic acids, and **antioxidant activity** as well.

ACKNOWLEDGMENT

CEEPUS Network: Teaching and learning bioanalysis

www.ugd.edu.mk

Bilateral project between Macedonia and China: **"Dynamic monitoring of ethyl carbamate and its precursors synthesis during wine production and developing a control strategy"**

With wine and hope anything is possible

Thank you for your attention!