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Selenium organic compounds and B-vitamins in red wine

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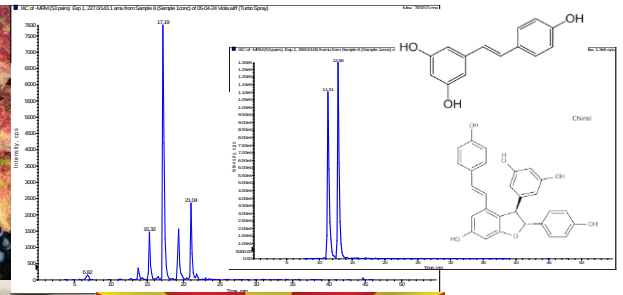
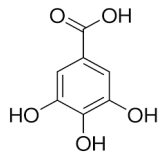
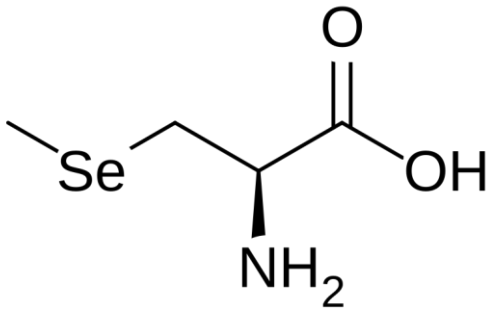


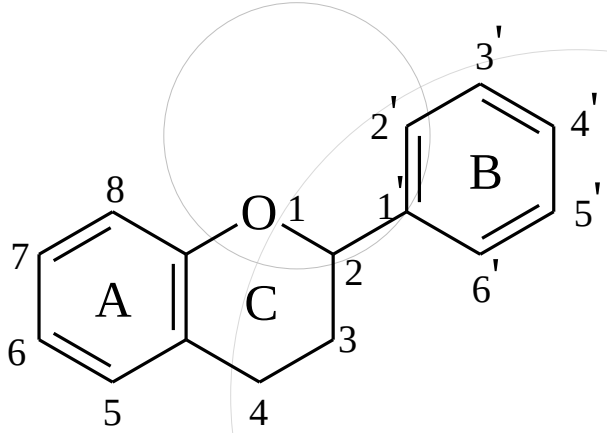


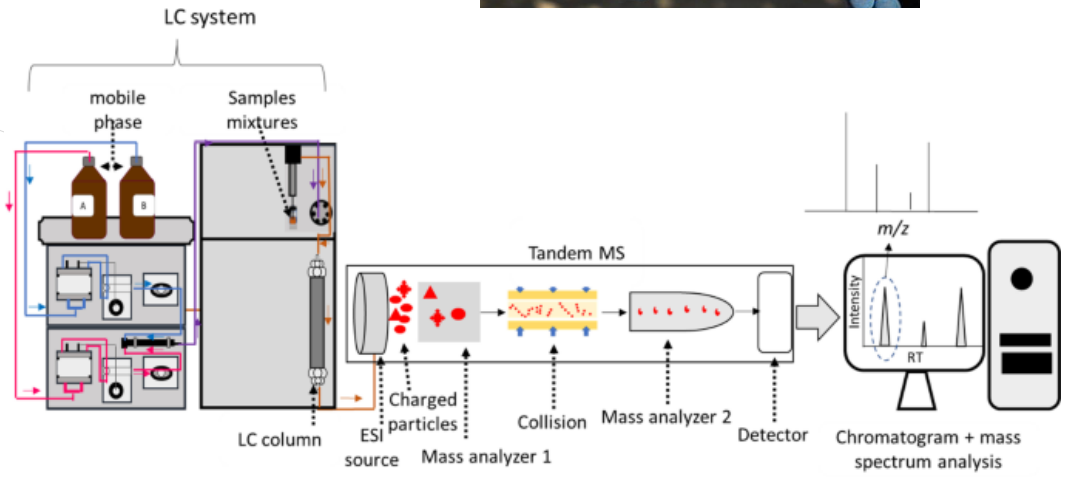


PREVIEW

- ✓ Selenium bioactive compounds
- ✓ B-group vitamins
- ✓ Kratošija – important Balkan variety







INTRODUCTION

- **Wine** – a very complex medium containing various organic and inorganic substances, influencing the wine quality.
- Macro- and microelements are important constituents of wine, possessing:
  - **nutritional value** (Ca, Cr, Co, K, Mg, Mn, Na, **Se**, and Zn),
  - **toxic effects** on the human body (As, Cd, and Pb).

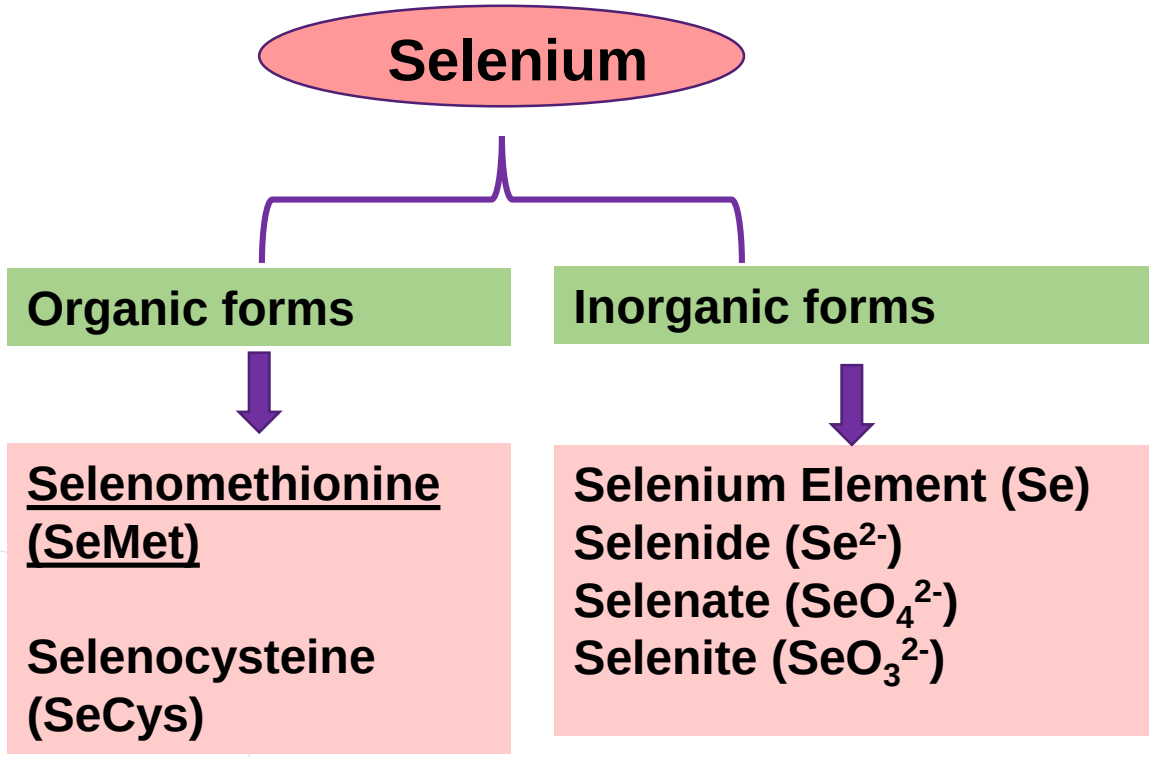
- According to the International Code of Oenological Practices established by **OIV maximum** acceptable limits for some metals.

- **Red wine is an essential component of the diet**, since ***moderate consumption*** of wine is associated with lower risk and mortality from cardiovascular disease.
- **Health benefits seems to be due to polyphenols in wine**.

Elements in wine	OIV - maximum acceptable limits (mg/L)
As	0.2
Cd	0.01
Cu	1
Pb	0.15
Na	80
Zn	5

Bioactive Selenium species

- **Selenium (Se)** is an element found in the soil, and it naturally appears in water and some foods.
- **Se** as a crucial microelement, is an nutrient for normal activity of human body.
- Many recent studies have indicated that selenium, as **selenoamino acids and selenoproteins**, plays an important role in the immune system:
- It reduces the presence of free radicals, produced from oxygen metabolism, which may contribute to the development of chronic diseases such as cancer and heart disease.
- Se deficiency remains a problem in many human populations.
- **Se** content of most of foods is **very low.**
- **Se** requirement of the body can be satisfied with dietary **supplements**, and/or with **Se-enriched foods**.
- The **organic forms of Se** are more retrained for further bioactivity (e.g protein incorporation) than that of inorganic Se forms.



Bioactive Selenium species

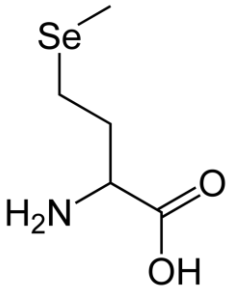
Se-containing compounds like:

- ✓ **Selenoaminoacids** such as:
  - **Selenomethionine (SeMet)**,
  - **Se-methylselenocysteine (SeMeCys),**
  - **Selenocysteine (SeCys)**, **selenocystine (SeCys2), selenohomocysteine (SeHoCys2)**, and **c-glutamyl-Se-methylselenocysteine (c-Glu-MeSeCys)**

  are chemo protective agents, reducing the incidence of different cancers in model systems.

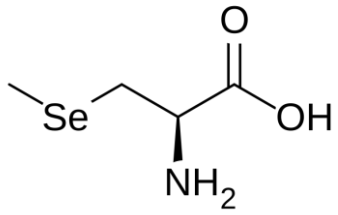
The presence of these forms depend on the type of food and the selenium content as well as on the level of Se used for the enrichment of plants.

Bioactive Selenium species



- ✓ **Selenomethionine is a bioactive form of selenium** that possesses antioxidant properties, playing a significant role in cardiovascular health, autoimmune conditions, and the prevention of certain cancers.
- ✓ **Selenomethionine, the major dietary form of Se**.

- ✓ **Selenomethionine oxide** is the oxidized form of the amino acid selenomethionine. It is formed when the sulfur atom in selenomethionine is oxidized.
- ✓ It has protective mechanism against oxidative stress. When a protein that contains selenomethionine is exposed to oxidants, the selenomethionine can be oxidized to selenomethionine oxide instead of the protein being damaged.



Se-methylselenocysteine, is a naturally occurring organoselenium compound found in certain plants, also know as methylselenocysteine, is an analog of S-methylcysteine in which the sulfur atom is replaced with a selenium atom.

Winemaking

Kratošija grapes, harvest at 260 °Brix

Added:

Potassium metabisulfite, $K_2S_2O_5$

Enzyme Lafaze HE GRAND CRU

I

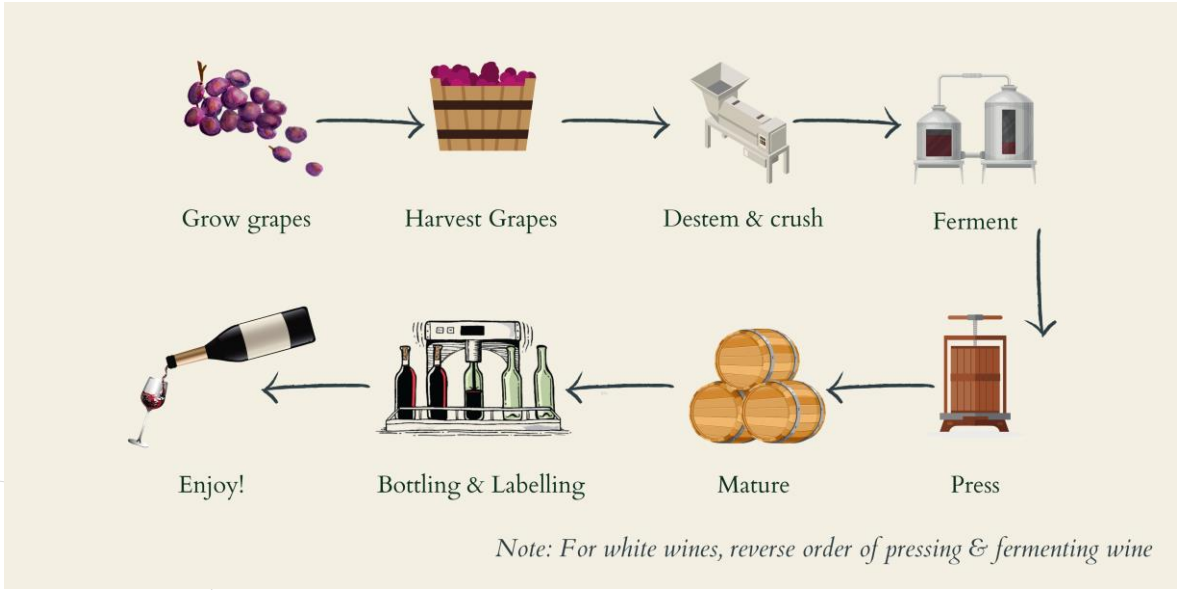
Kratošija 1 with yeast: Zymaflore™ Xpure (Laffort, Bordeaux, France)

Yeast recommended for wines with **high aromatic purity**, **freshness**, expression of **black fruit notes** and great smoothness on mouthfeel.

II

Kratošija 2 with yeast: Lalvin ICV D80 (Lallemand, Blagnac, France)

Yeast brings high foremouth volume, big mid-palate mouthfeel and intense fine grain tannin to reds. **It is one of the best strains for contributing big tannin volume** and is characterized by ripe fruit, smoke and a licorice finish.



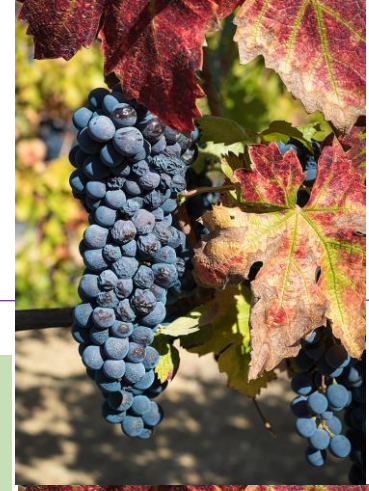
21 day alcoholic fermentation (18-20 °C)

Punching down:

Two times a day - 14 days

Once a day – 7 days

KRATOŠIJA – IMPORTANT BALKAN VARIETY



- ✓ Kratošija is considered as an **old ancient variety**, grown at the Macedonian vineyards in the period when wine was produced in amphora.
- ✓ In the period of Yugoslavia, Kratošija was replaced with Vranec variety.
- ✓ Nowadays, Kratošija is found mainly in combination with Vranec vines and at small locations alone, mainly in the Tikveš wine region.
- ✓ **Expansion of the vine areas is expecting in the coming years**.

ORIGIN of Kratošija

- ✓ In some publications, **Kratošija is considered as autochthonous Montenegrin variety** (Pajović Šćepanović et al. 2019a; 2019b; 2024)
- ✓ The results of DNA analyses obtained by Calò et al. (2008) showed that **Kratošija is a synonym of Zinfandel (USA), Primitivo (Italy) and Crljenak Kaštelanski (Croatia).**
- ✓ Moreover, previous investigations of Maletić et al. (2004) indicated that **Zinfandel has a Croatian origin** because of its microsatellite allelas typical for the Croatian gene pool.
- ✓ Consequently, more additional genetic investigations are necessary to be done.
- ✓ **Therefore, it would be the best if Kratošija is considered as a Balkan variety**.

The first published paper about Macedonian Kratošija wine

Food Analytical Methods
https://doi.org/10.1007/s12161-025-02899-4

RESEARCH

Check for updates

Targeted Characterization of Flavonoids and Nonflavonoids in Kratošija Red Wine with HPLC-ESI-MS/MS Technique

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Abstract
In this study, a rapid and sensitive HPLC-mass spectrometric method was applied for identification and semi-quantification of flavonoids and nonflavonoids in Kratošija wines. Wines were produced by inoculation of two commercial yeasts (Zymaflore™ Xpure (Laffort) and Lalvin ICV D80 (Lallemand)) in order to study the effect of the yeast on the phenolics profile of wines. The targeted analyses of phenolic compounds have been performed using HPLC with a tandem mass spectrometer in a single reaction monitoring mode (SRM) or fragmentation spectrum mode to determine each individual compound. A total of 26 phenolic compounds, including 13 nonflavonoids (10 phenolic acids and 2 stilbenes and 1 stilbenoid) and 13 flavonoids (6 flavan-3-ols, 3 flavonols, 2 flavones, 1 flavanone, and 1 flavanonol), have been determined. Flavones chrysin and luteolin, flavanone naringenin, flavanonol taxifolin, and stilbenoid *ε*-viniferin were reported for the first time in Macedonian red wine. The effect of yeast on the phenolic profile of wines was noticed, as a result of the different fermentation rates in accordance to their specifications. Flavan-3-ols were present in a higher content in the wine fermented with Lalvin ICV D80, while wine fermented with Zymaflore™ Xpure presented higher amounts of all other phenolic compounds.

Keywords Flavonoids · Nonflavonoids · Yeast fermentation · Red wine · Kratošija · HPLC-ESI-MS/MS

HPLC-ESI-TQMS analysis of selenium species

LC 20 Shimadzu, ESI-TQMS 8030

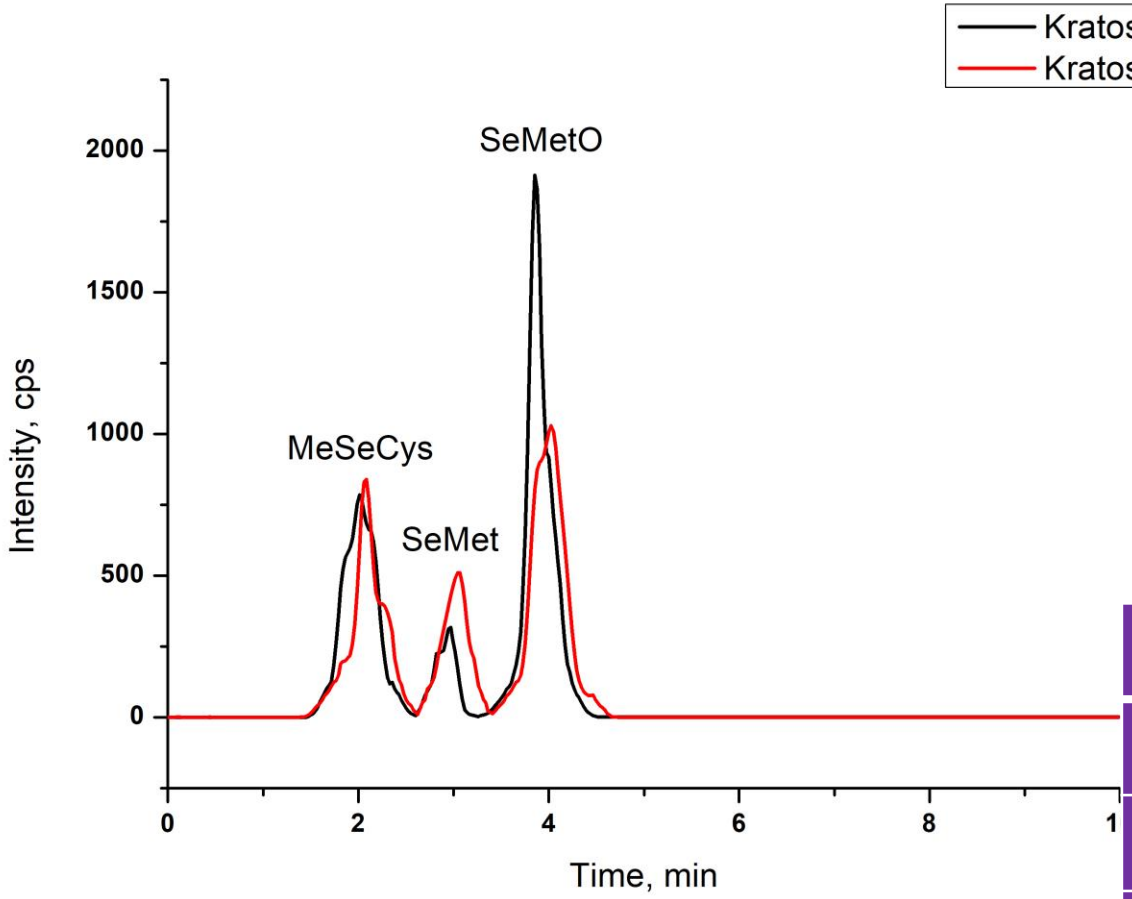
- ✓ Positive mode, ESI-TQMS, Sample volume: 5 mL injection
- ✓ Column: ZIC-HILIC column (100 x 2.1 mm, 3.5 µm)
- ✓ **Eluent A: MeOH/eluent B: H_2O (75/15, *v/v*) isocratic mode**

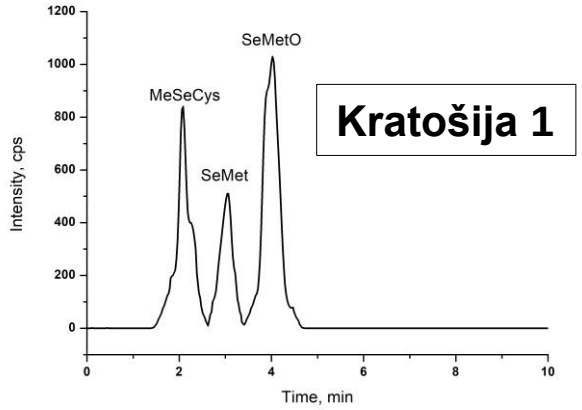
Calibration parameters

Linerity range: 0.01- 25 ppm

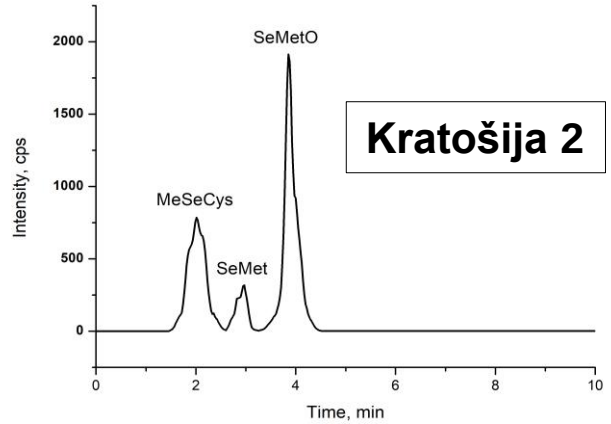
Selenium compounds	t_r(min)	LOD [ug/L]	LOQ [ug/L]	Slope	R^2
Se-methylselenocysteine (SeMeSeCys)	2.1	0.100	0.200	444890	0.999
Selenomethionine (SeMet)	3.4	0.100	0.300	903460	0.999
Selenomethionine oxide (SeMetO)	4.2	0.100	0.200	853321	0.999

EIC of Selenium species





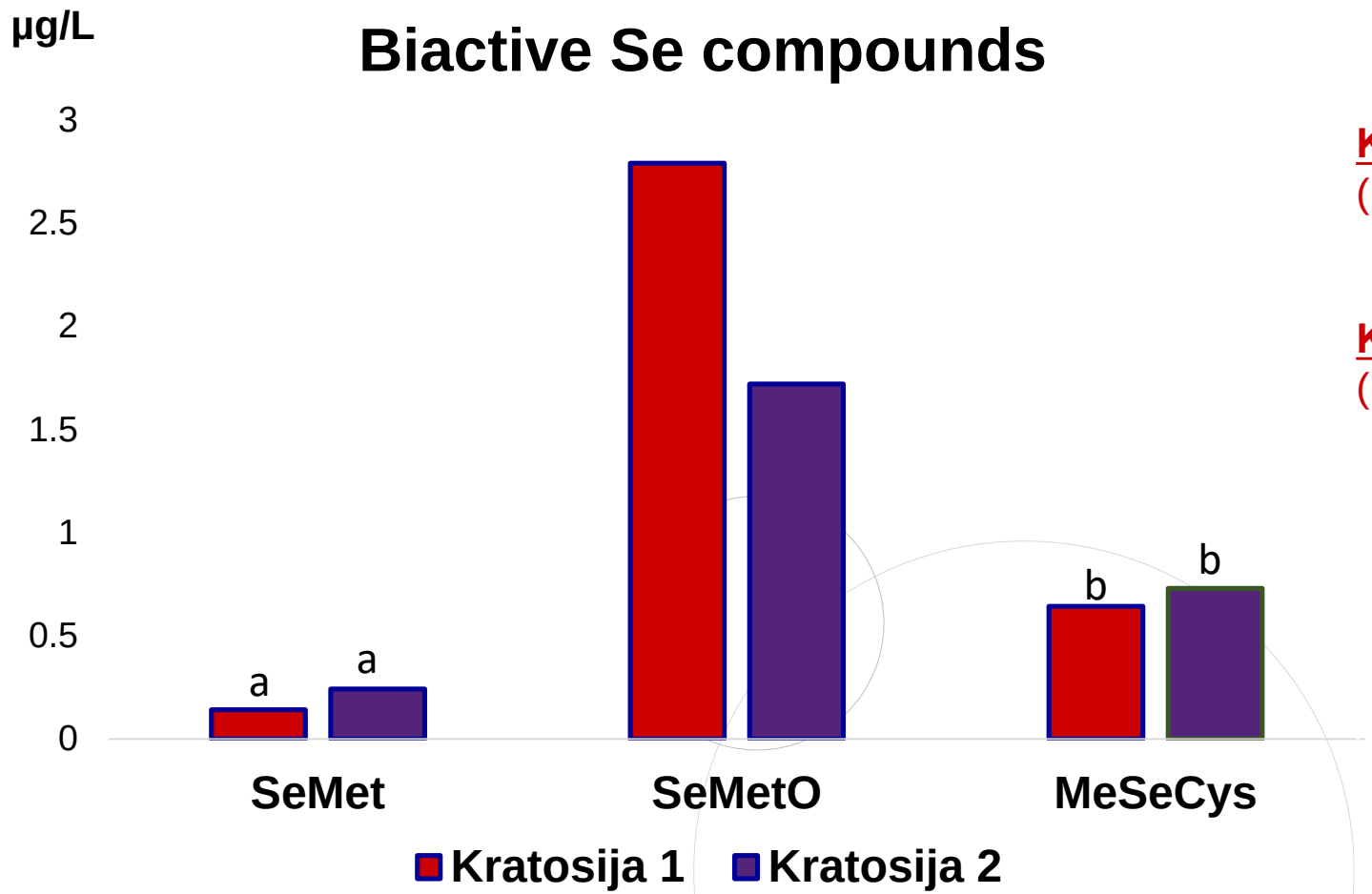
Kratošija 1



Kratošija 2

Selenium compound	t_r(min)	MS (m/z) M^+	MS/MS (m/z) Fragment ion
Se-methylselenoselenocysteine (MeSeCys)	2.1	184	167.00; 94.95; 55.05
Selenomethionine (SeMet)	3.4	198	181.00; 109.05; 56.00
Selenomethionine oxide (SeMetO)	4.2	212.90	148.95; 116.95; 85.05

Quantification of Bioactive Selenium compounds in red wine



Kratošija 1 with yeast: Zymaflore™ Xpure (Laffort, Bordeaux, France)

Kratošija 2 with yeast: Lalvin ICV D80 (Lallemand, Blagnac, France)

Selenomethionine (SeMet)
Selenomethionine oxide (SeMetO)
Se-methylselenocysteine (SeMeSeCys)

B-vitamins

- ✓ **Vitamins, are essential compounds in wine, particularly B vitamins.**

- ✓ These vitamins can function as important cofactors for yeast. They play an important role in the enzymatic reactions that **enable yeast to grow and improve fermentation efficiency**.

- ✓ **A lack of vitamins can result in slow fermentation and undesirable aromatic profiles.**

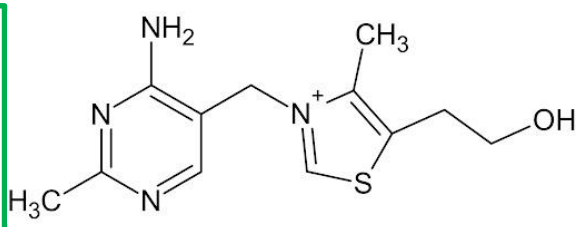
- ✓ Vitamins in oenology have remained mostly uninvestigated in the late decades. The current knowledge regarding the topic remains primarily based on investigations led between the 1950s and 1970s.

- ✓ **While HPLC, GC, HPTLC, or electrophoresis-based methods have been developed in other matrixes, there is, no published method to perform such a simultaneous vitamin analysis in neither grape musts nor wines yet.**

B-group vitamins

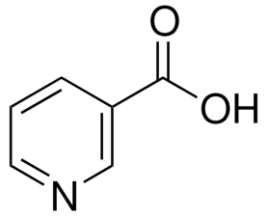
- B-group vitamins are water soluble compounds: **thiamine (B1)**, riboflavin (B2), **niacin (B3)**, **pantothenic acid (B5)**, pyridoxine (B6), biotin (B7), folic acid (B9), and cobalamin (B12) are generally grouped under the “B-complex” or “B-group” denomination.
- B-group vitamins have been shown to display a catalytic function, and act as coenzymes in diverse metabolisms.

- ✓ **Thiamine (B1)** consists of a pyrimidine ring and a thiazole ring, both connected by a carbon link.
- ✓ **Thiamine contents** in grape musts have been reported to range between **80 µg/L and 1.2 mg/L.**
- ✓ B1 is characterized by the capacity of being accumulated in high amounts by yeasts.
- ✓ Thiamine contents almost **entirely disappear during alcoholic fermentation**, as wine is estimated to possess only 3% to 5% of the initial concentration in fresh juices.



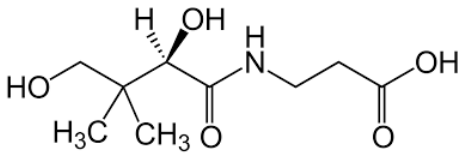
Thiamine

- ✓ **Nicotinic acid**, also known as **niacin** or **vitamin B3.**
- ✓ Nicotinamide content in grape musts ranges between **0.86 and 2.56 mg/L**, which drops to values between **0.11 and 0.42 mg/L** in wines as a result of high consumptions exerted by yeasts.
- ✓ Nicotinic acid increase malic acid fermentation speeds when added to the fermenting medium.



Nicotinic acid

- ➢ Grapes contain an average of **6.8 and 8.5 mg/L pantothenic acid (B5)** in red and white cultivars, respectively.
- ➢ **Pantothenic acid** is extracted in high proportions from the grapes to the musts, because 66% to 93% of the initial content can be found in the musts, leading to must concentrations ranging between 0.16 µg/L and **10.50 mg/L**.



Pantothenic acid

HPLC-ESI-TQMS analysis of B-group vitamins

❖ All the experiments to determine the vitamin contents in grape musts and wines have been carried out over previous decades with analytical methods with low sensitivity.

LC 20 Shimadzu, ESI-TQMS 8030

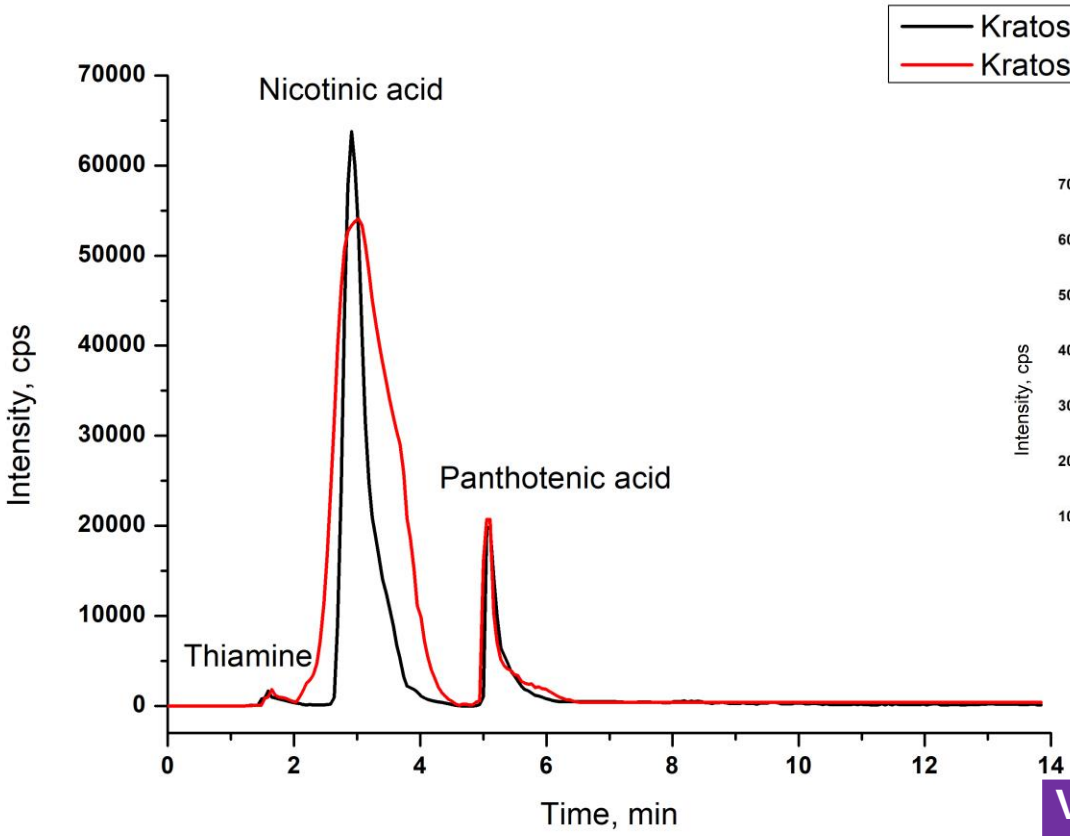
- ✓ Positive mode, ESI-TQMS, Sample volume: 5 mL injection
- ✓ Column: ZIC-HILIC column (100 x 2.1 mm, 3.5 µm)
- ✓ Mobile phase: MeOH/ 8 mM HCOOH (pH 2.8) in gradient elution, as follows: 0-3 min 80%B, 4-7 min 50% B, 9-13 min 70% B and 13-20min 80%B.

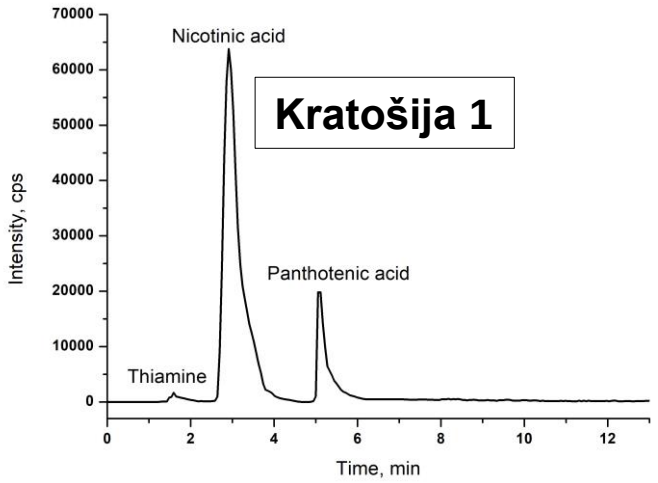
Calibration parameters

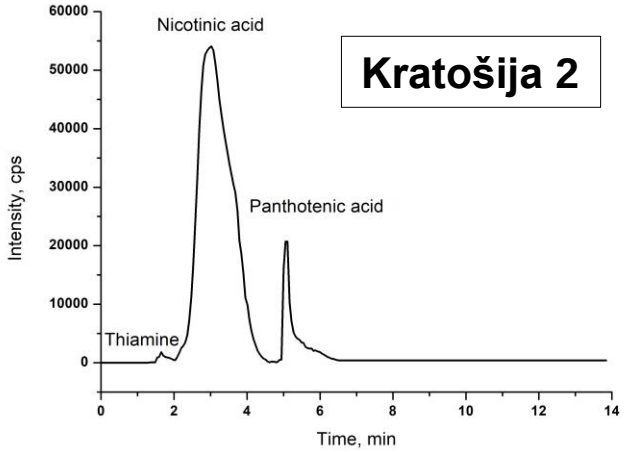
Linerity range: 0.01- 25 ppm

B-group Vitamins	t_r(min)	LOD [µg/L]	LOQ [µg/L]	Slope	R^2
Thiamine (B1)	1.67	0.02	0.05	31061	0.998
Nicotinic acid (B3)	3.3	0.03	0.09	3245	0.999
Panthotenic acid (B5)	4.85	0.02	0.06	42370	0.999

EIC of Vitamins B

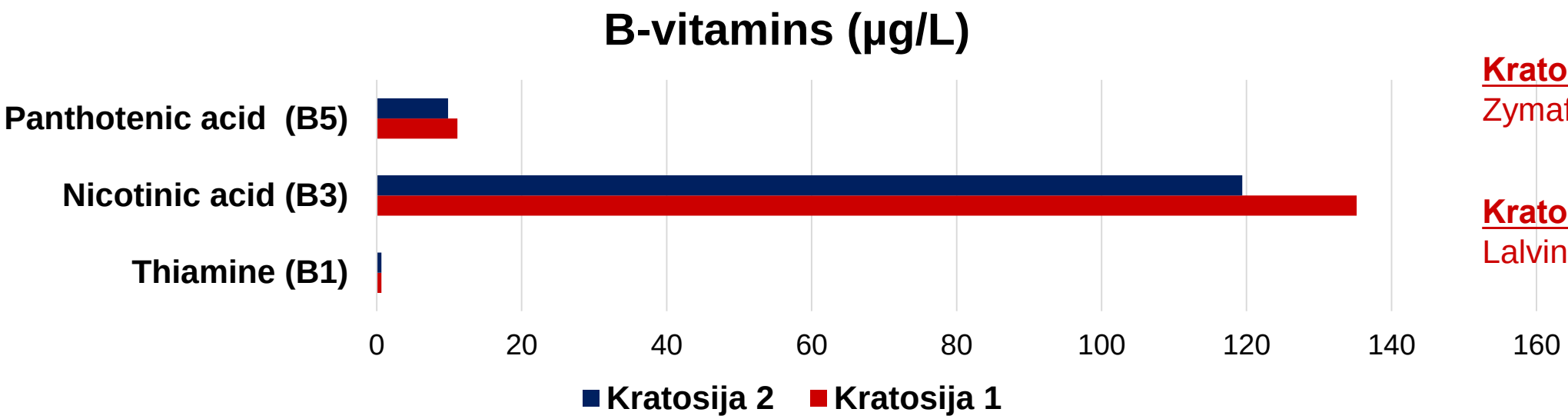






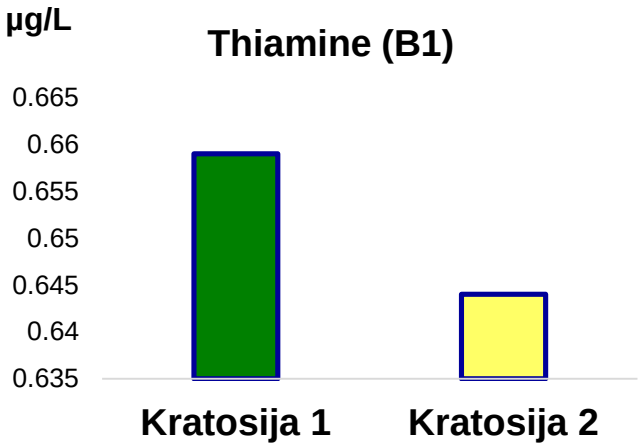
Vitamin B	t_r(min)	MS (m/z) M^+	MS/MS (m/z) Fragment ion
Thiamine (B1)	1.67	264.5	122.10
Nicotinic acid (B3)	3.3	123.50	96.05; 80.05; 53.10
Panthotenic acid (B5)	4.85	219.5	202.10; 90.05; 30.05

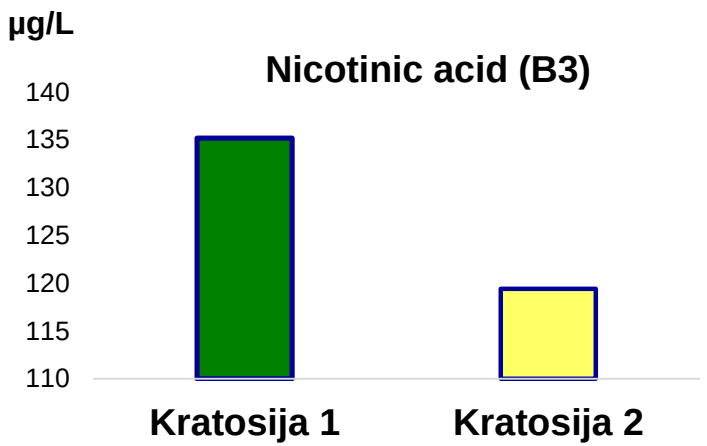
Quantification of B-group vitamins in red wine

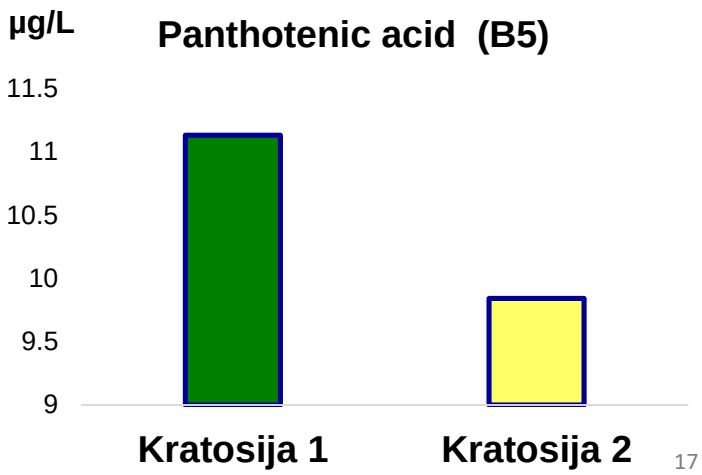


Kratošija 1 with yeast: Zymaflore™ **Xpure**

Kratošija 2 with yeast: Lalvin ICV **D80**







CONCLUSIONS

- HPLC coupled to ESI-TQMS in positive mode was applied for determination of **Se organic bioactive compounds** and **B-vitamins** in **Kratošija red wine**.
- **Se** compounds and **B** vitamins **determined for the first time in Macedonian wine.**
- Wine presented relatively **high content** of Se-compounds as well as nicotinic acid, which means that the analyzed red wine have a potential to be considered as a functional beverage.
- Influence of yeast was noticed.
- **Further analyses have to be performed focusing on the influence of yeast fermentation on Se-compounds and B-vitamins as well as other white and red varieties.**

CEEPUS Network
TEACHING AND LEARNING BIOANALYSIS

Tikveš lake, with Tikveš vineyards