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# LC-ESI-MS/MS characterization of organic selenium compounds in red wine

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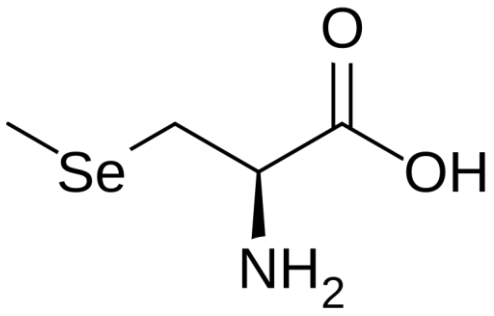
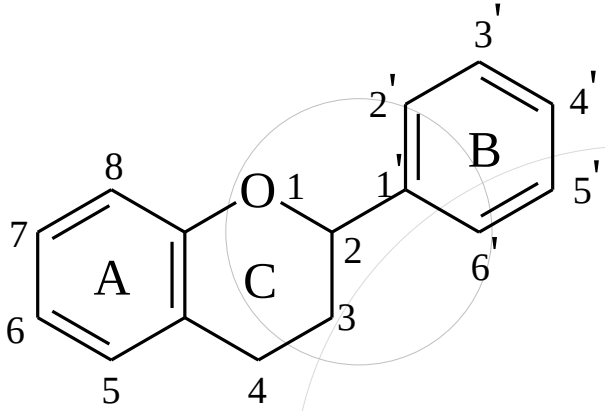
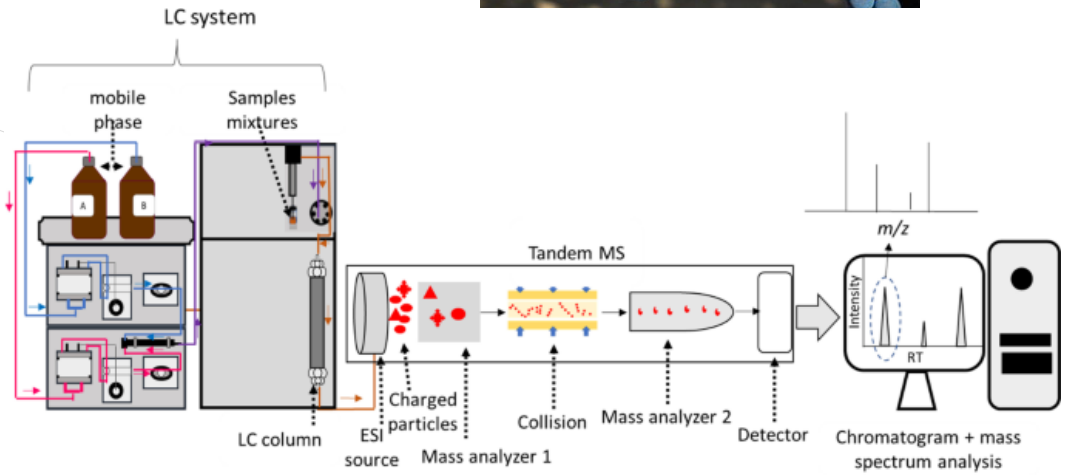
# PREVIEW

FLAVOURsome

- ✓ **Selenium bioactive compounds**
- ✓ **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).

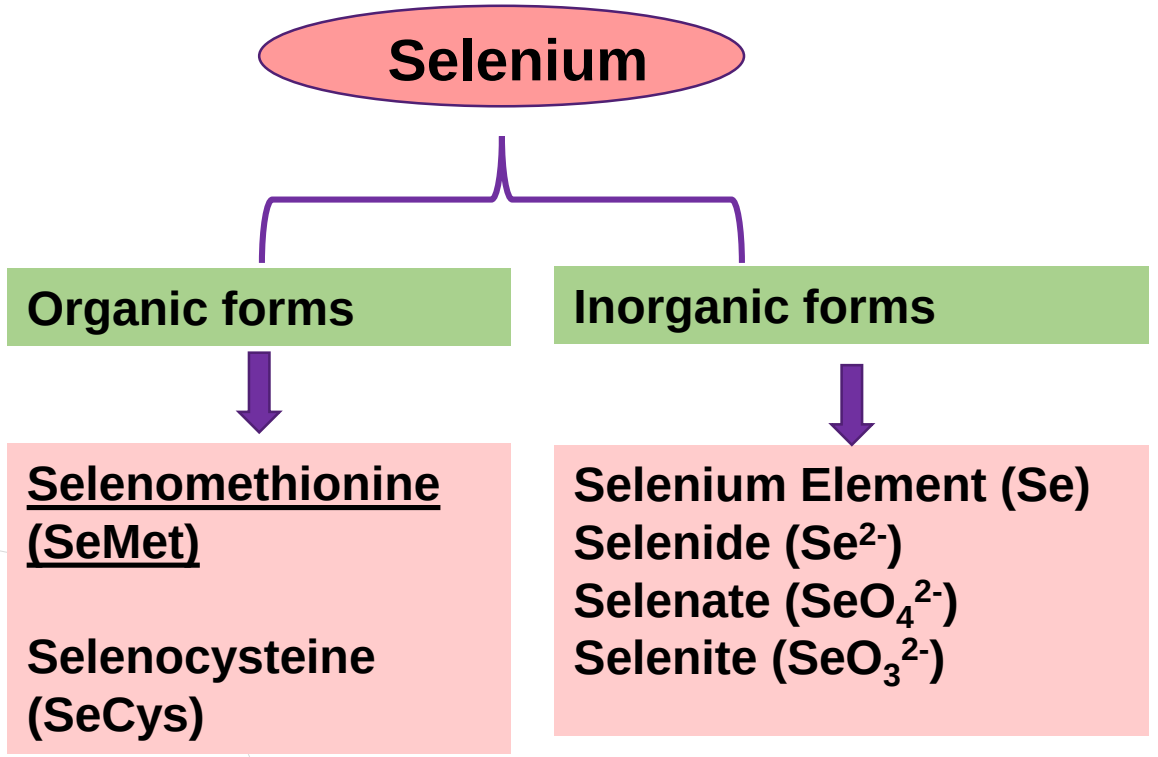
- According to the International Code of Oenological Practices established by **OIV maximum** acceptable limits for some metals.

- **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

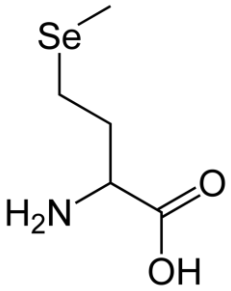
Se-containing compounds like:

- ✓ **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.

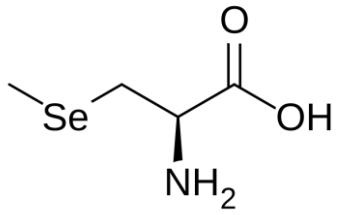
The presence of these forms depend on the type of food and the selenium content as well as on the level of Se used for the enrichment of plants.

# Bioactive Selenium species



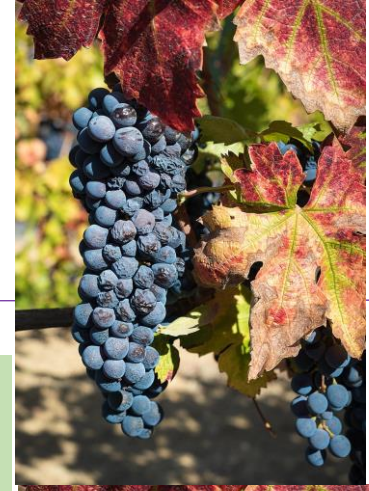

- ✓ **Selenomethionine is a bioactive form of selenium** that possesses antioxidant properties, playing a significant role in cardiovascular health, autoimmune conditions, and the prevention of certain cancers.
- ✓ **Selenomethionine, the major dietary form of Se**.

- ✓ **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.

# 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

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## 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

# Winemaking

## Kratošija grapes, harvest at 260 °Brix

***Added:***

**Potassium metabisulfite, $K_2S_2O_5$**

***Enzyme Lafaze HE GRAND CRU***

I

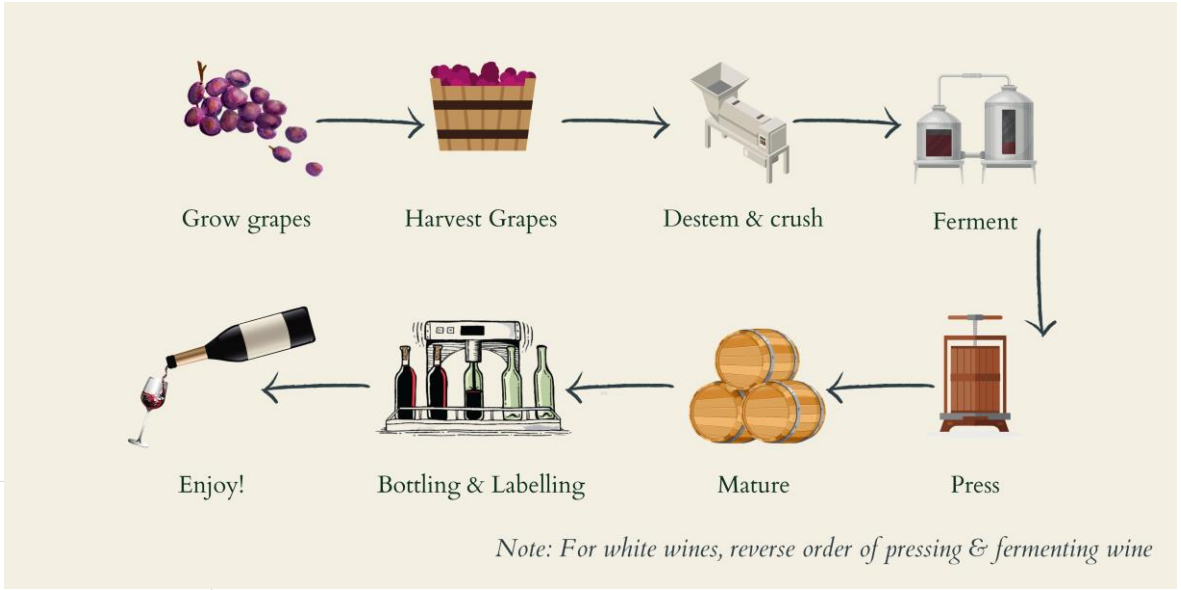
**Kratošija 1 with yeast: Zymaflore™ Xpure (Laffort, Bordeaux, France)**

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***

# HPLC-ESI-TQMS analysis of selenium cmpounds

**LC 20 Shimadzu, ESI-TQMS 8030**

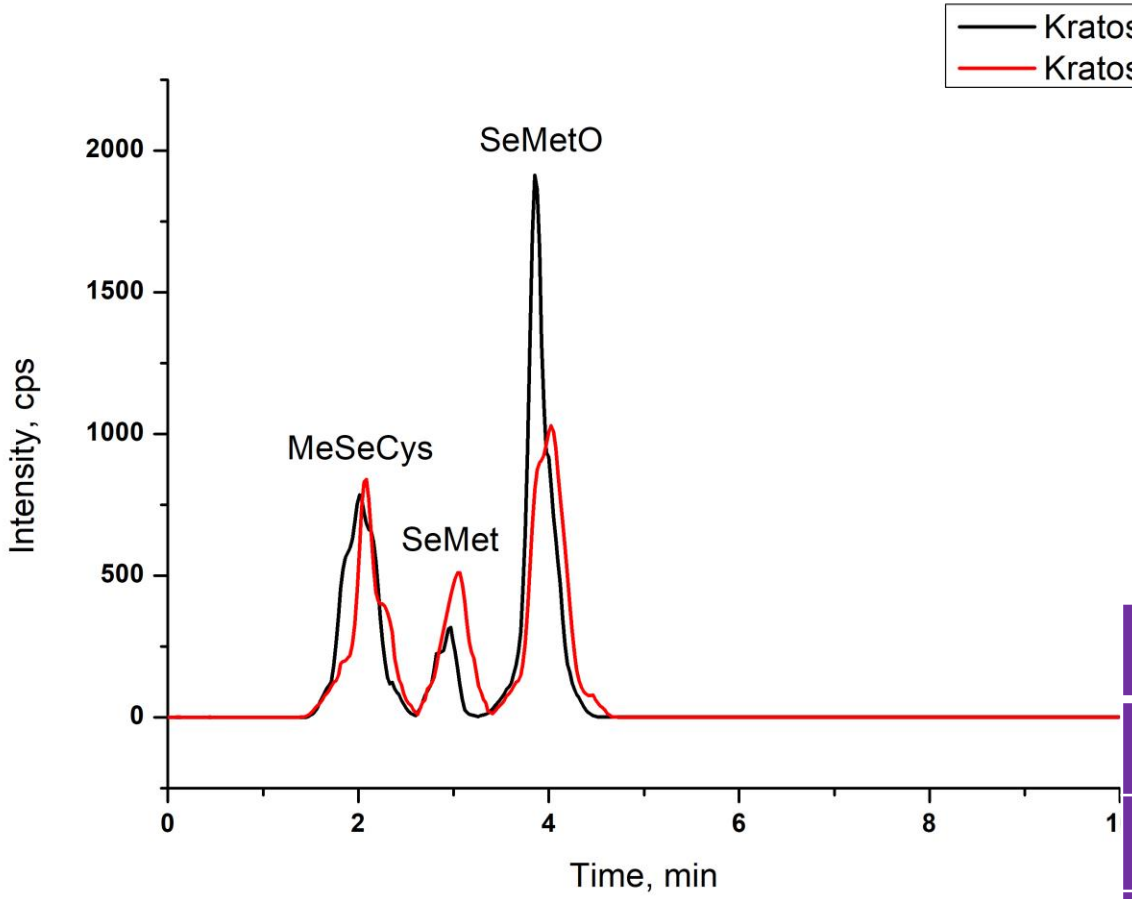
- ✓ Positive mode, ESI-TQMS, Sample volume: 5 mL injection
- ✓ Column: ZIC-HILIC column (100 x 2.1 mm, 3.5 µm)
- ✓ **Eluent A: MeOH/eluent B: $H_2O$ (75/15, *v/v*) isocratic mode**

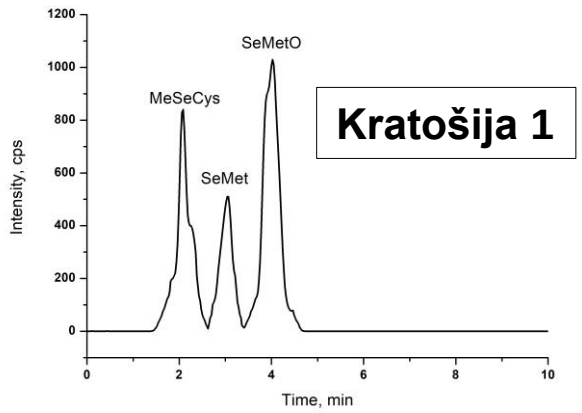
**Calibration parameters**

**Linerity range**: 0.01- 25 ppm

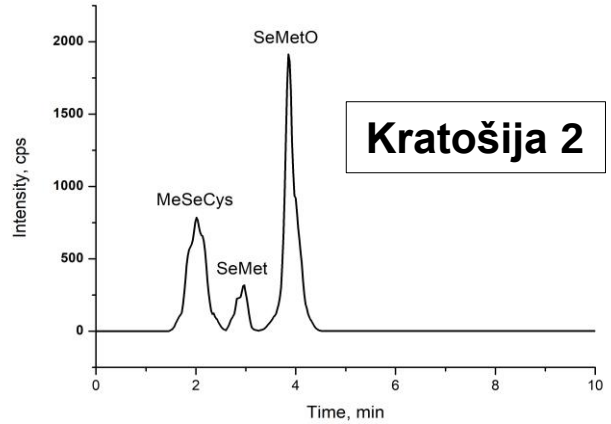
| Selenium compounds | $t_r$(min) | LOD [ug/L] | LOQ [ug/L] | Slope | $R^2$ |
|---|---|---|---|---|---|
| Se-methylselenocysteine (SeMeSeCys) | 2.1 | 0.100 | 0.200 | 444890 | 0.999 |
| Selenomethionine (SeMet) | 3.4 | 0.100 | 0.300 | 903460 | 0.999 |
| Selenomethionine oxide (SeMetO) | 4.2 | 0.100 |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

# EIC of Selenium species



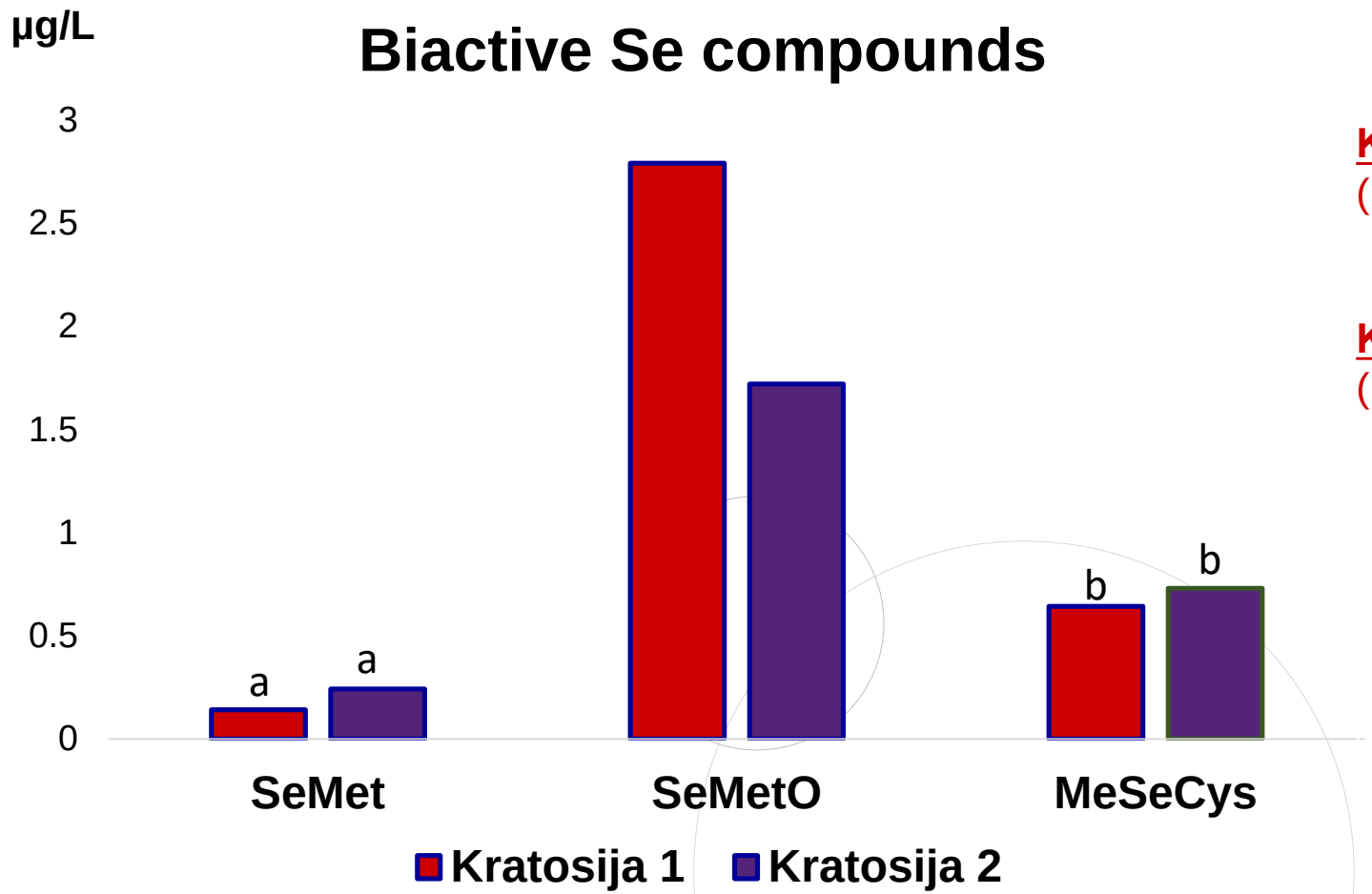

**Kratošija 1**




**Kratošija 2**

| Selenium compound | $t_r$(min) | MS (m/z) $M^+$ | MS/MS (m/z) Fragment ion |
|---|---|---|---|
| Se-methylselenocysteine (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

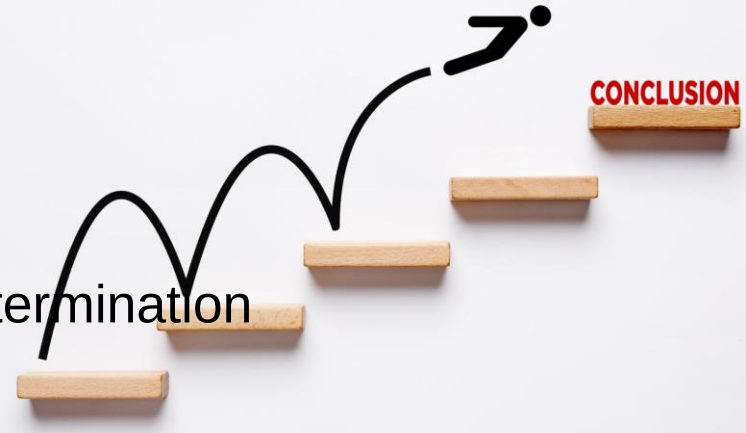



**Kratošija 1** with yeast: Zymaflore™ Xpure (Laffort, Bordeaux, France)

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

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

# CONCLUSIONS




- HPLC coupled to ESI-TQMS in positive mode was applied for determination of **Se organic bioactive compounds** in **Kratošija red wine**.
- **Se** compounds **determined for the first time in Macedonian wine.**
- Wines presented relatively **high content** of Se-compounds 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 as well as to other white and red varieties.**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