DR.SC.-01 REQUEST FOR APPROVAL OF THE DISSERTATION TOPIC[1]

GENERAL INFORMATION AND PERSONAL CONTACT INFORMATION OF THE DOCTORAL CANDIDATE

First and last name, and title of the doctoral candidate:	Ana Boban, mag. chem.		
Provider of the study programme:	University of Zagreb Faculty of Food Technology and Biotechnology		
Name of the study programme:	Biotechnology and Bioprocess Engineering, Food Technology and Nutrition		
Scientist ID of the doctoral candidate:	445/PT		
Approval of topic for acquiring a PhD *(please fill in appropriate box)*:	■ within programme-based doctoral study	☐ on the basis of scientific achievement	☐ Dual doctorate (Cotutelle de these)
First and last name of mother and/or father:	Dragica Boban and Joško Boban		
Date and place of birth:	February 11, 1997, Split, Croatia		
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CURRICULUM VITAE OF THE DOCTORAL CANDIDATE

Education *(in chronological order, with most recent first)*:	1. University of Split - Faculty of Chemistry and Technology, Croatia, Graduate Study of Chemistry, Master's Degree in Chemistry (2019/2020) 2. University of Split - Faculty of Chemistry and Technology, Croatia, Undergraduate study of Chemistry, Bachelor's Degree in Chemistry (2017/2018)
Work experience *(in chronological order, with most recent first)*:	August 23, 2021 -present - Institute for Adriatic Crops and Karst Reclamation; Ph.D. student on a research project funded by the Croatian Foundation
Bibliography and active participation in conferences:	1. Boban, A., Vrhovsek, U., Anesi, A., Milanović, V., Gajdoš Kljusurić, J., Jurun, Z., & Budić-Leto, I. (2024). Modulation of Aromatic Amino Acid Metabolism by Indigenous Non-*Saccharomyces* Yeasts in Croatian Maraština Wines. *Foods, 13*, 2939. doi: 10.3390/foods13182939 2. Ivić, S., Jeromel, A., Kozina, B., Prusina, T., Budić-Leto, I., Boban, A., Vasilj, & V., Jagatić Korenika, A-M. (2024). Sequential Fermentation in Red Wine cv. Babić Production: The Influence of *Torulaspora delbrueckii* and *Lachancea thermotolerans* Yeasts on the Aromatic and Sensory Profile. *Foods, 13(13)*, 2000. doi: 10.3390/foods13132000 3. Boban, A., Milanović, V., Veršić Bratinčevića, M., Botta, C., Ferrocinoc, I., Cardinali, F., Ivić, S., Rampanti, G., Budić-Leto, I. (2024). Spontaneous fermentation of Maraština wines: the correlation between autochthonous mycobiota and phenolic compounds. *Food Research International, 180,* 114072. doi: 10.1016/j.foodres.2024.114072 4. Boban, A., Vrhovsek, U., Anesi, A., Milanović, V., Budić-Leto, I. Autochthonous non-*Saccharomyces* extra-cellular metabolism of tryptophan, tyrosine, and phenylalanine. //

[1] Please name file as: DR.SC.-01 – Last name and first name of Doctoral Candidate.doc
Please send the filled -out form DR.SC.-01, in electronic and written format, and signed, to the appropriate Registrar's Office.

Open International Conference on Grapevine Physiology and Biotechnology, Logroño, Spain, 07.07.2024-11.07.2024. (poster presentation)

5. Boban, A., Vrhovsek, U., Carlin, S., Milanović, V., Gajdoš Kljusurić, J., Budić-Leto, I. Impact of autochthonous non-Saccharomyces yeasts on wine aroma complexity // 5th European Horticulture Congress, Bucharest, Romania, 12.05.2024-17.05.2024. (oral presentation)
6. Milanović, V., Cardinali, F., Boban, A., Gajdoš Kljusurić, J., Osimani, A., Aquilanti, L., Garofalo, C., Budić-Leto, I. (2023). White grape variety Maraština as a promising source of non-*Saccharomyce*s yeasts intended as starter cultures. *Food Bioscience,* 55, 103033. doi: 10.1016/j.fbio.2023.103033
7. Budić-Leto, I., Boban, A., Smokrović, K., Maltar-Strmečki, N. Antioxidant activity and metal micronutrient content of Croatian wines determined by electron spin resonance spectroscopy // Book of Abstracts -1st Joint International Conference of Biochemistry of Trace Elements & 21st International Conference of Heavy Metals - ICOBTE&ICHMET 2023 / - (ur.). Wuppertal, Germany: COST European Cooperation in Science and Technology, 2023. str. 511-511. (poster presentation)
8. Boban, A., Milanović, V., Jurun, Z., Mucalo, A., Budić-Leto, I. Fermentation potential of indigenous non-Saccharomyces yeasts isolated from Maraština grapes of Croatian vineyards // Book of Abstracts - ŒNOMacrowine 2023 - 12th International Symposium of Œnology, Bordeaux- 9th edition In Macrowine / Oeno Macrowine (ur.). Bordeaux: Université de Bordeaux, 2023. str. 189-189. (poster presentation)
9. Boban, A., Milanović, V., Vrhovsek, U., Masuero, D., Budić-Leto, I. Profiling of lipids in wines from monoculture fermentation with indigenous Metschnikowia yeast species // Book of Abstracts - ŒNOMacrowine 2023 - 12th International Symposium of Œnology, Bordeaux- 9th edition In Macrowine / Oeno Macrowine (ur.). Bordeaux: Université de Bordeaux, 2023. str. 197-197. (poster presentation)
10. Milanović, V., Cardinali, F., Ferrocino, I., Boban, A., Franciosa, I., Gajdoš Kljusurić, J., Mucalo, A., Osimani, A., Aquilanti, L., Garofalo, C., Budić-Leto, I. (2022). Croatian white grape variety Maraština: First taste of its indigenous mycrobiota. *Food Research International, 162*, 111917. doi:10.1016/j.foodres.2022.111917
11. Gajdoš Kljusurić, J., Boban, A., Mucalo, A., Budić-Leto, I. (2022). Novel application of NIR spectroscopy for non-destructive determination of 'Maraština' wine parameters. *Foods, 11(8),* 1172. doi:10.3390/foods11081172
12. Gajdoš Kljusurić, J., Boban A., Mucalo, A., Budić-Leto, I. Near infrared spectroscopy as authentication tool of protect design of origin for Dalmatian wine produced from grape Maraština. // Book of Abstract, 1st SensorFint International Conference, COST Action 19145 „European Network for assuring food integrity using non-destructive spectral sensors"/ Michael Burnard, Andreja Kutnar (ur.). Izola, 2022. pp. 67-67 (poster, presentation)
13. Boban, A., Vrhovsek, U., Carlin, S., Mucalo, A., Budić-Leto, I. (2022). A Targeted and an Untargeted Metabolomics Approach to the Volatile Aroma Profile of Young 'Maraština' Wines. *Metabolites, 12*, 1295. doi:10.3390/metabo12121295
14. Boban, A., Vrhovsek, U., Carlin, S., Mucalo, A, Budić-Leto, I. A targeted and untargeted approach to volatile metabolite characterization of "Maraština" wines produced by spontaneous fermentation. // 10th International Congress of Food Technologists, Biotechnologists and Nutritionists - book of abstracts, Zagreb: Croatian Society of Food Technologists, Biotechnologists and Nutritionists, 2022. pp. 59-60 (poster presentation)
15. Milanović, V., Cardinali, F., Boban, A., Gajdoš Kljusurić, J., Mucalo, A., Osimani, A., Aquilanti, L., Garofalo, C., Budić- Leto, I. Oenological characterization of non-*Saccharomyces* yeasts isolated from Croatian white grape variety Maraština. // 10th International Congress of Food Technologists, Biotechnologists and Nutritionists - book of abstracts / Komes, Draženka (ur.). Zagreb: Croatian Society of Food Technologists, Biotechnologists and Nutritionists, 2022. pp. 27-28 (poster presentation)
16. Boban, A., Masuero, D., Vrhovsek, U., Budić-Leto, I. (2023). Comprehensive Lipid Profile of 'Maraština' Grape Skins from Dalmatia. *Applied Science*, *13,* 6446.

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17. Boban, A., Jurun, Z., Mucalo, A., Budić-Leto, I. Impact of native *Pichia kluyveri* and *Metschnikowia pulcherrima* on Maraština wines. // 1st European GREEN Conference Osijek, 2023. 2022, 1 (poster presentation)
18. Škugor Rončević, I., Vladislavić, N., Boban, A., Bosnić, Lj., Buzuk, M., Buljac, M. Primjena modificirane ugljikove elektrode za voltametrijsko određivanje teških metala u medu.// 26. hrvatski skup kemičara i kemijskih inženjera s međunarodnim sudjelovanjem, 4. Simpozij "Vladimir Prelog", Book of abstract / Galić, Nives ; Rogošić, Marko (ur.). Zagreb: Hrvatsko društvo kemijskih inženjera i tehnologa, 2019. str. 90-90 (poster presentation)

TITLE OF THE PROPOSED TOPIC

Croatian:	Utjecaj autohtonih kvasaca koji ne pripadaju rodu *Saccharomyces* na profil arome mladoga vina sorte 'Maraština' (*Vitis vinifera* L.)
English:	Impact of indigenous non-*Saccharomyces* yeasts on the aroma profile of young wine from 'Maraština' cv. (*Vitis vinifera* L.)
Title in the language of the dissertation (if it is not Croatian or English)	-
Area/field/branch (if the doctoral study is performed in a branch)**:**	Biotechnology Sciences/Food Technology

PROPOSED OR POTENTIAL MENTOR(S)

(name the second mentor in case of interdisciplinary research or if there is another reason for more than one mentor)

	First name and last name, and title:	**Institution, country:**	**E-mail:**
First mentor:	Ph.D. Irena Budić-Leto, senior scientist in permanent position	Institute for Adriatic Crops and Karst Reclamation	irena.budic-leto@krs.hr
Second mentor:	-	-	-

MENTOR'S COMPETENCES – list of up to five relevant works published in the last five years

First mentor:	1. Boban, A., Vrhovsek, U., Anesi, A., Milanović, V., Gajdoš Kljusurić, J., Jurun, Z., & **Budić-Leto, I.** (2024). Modulation of Aromatic Amino Acid Metabolism by Indigenous Non-*Saccharomyces* Yeasts in Croatian Maraština Wines. *Foods, 13*, 2939. (Q1, IF=4.7) 2. Ivić S., Jeromel A., Kozina B., Prusina T., **Budić-Leto I**., Boban A., Vasilj V., Korenika A-MJ. (2024) Sequential Fermentation in Red Wine cv. Babić Production: The Influence of Torulaspora delbrueckii and Lachancea thermotolerans Yeasts on the Aromatic and Sensory Profile. Foods. *13(13),* 2000. (Q1, IF=4.7) 3. Milanović, V., Cardinali, F., Boban, A., Gajdoš Kljusurić, J., Osimani, A., Aquilanti, L., Garofalo, C., **Budić-Leto, I.** (2023). White grape variety Maraština as a promising source of non-*Saccharomyces* yeasts intended as starter cultures. *Food Bioscience,* 55, 103033. (Q1, IF=4.8) 4. Milanović, V., Cardinali, F., Ferrocino, I., Boban, A., Franciosa, I., Gajdoš Kljusurić, J., Mucalo, A., Osimani, A., Aquilanti, L., Garofalo, C., **Budić-Leto, I.** (2022). Croatian white grape variety Maraština: first taste of its indigenous mycobiota. *Food Research International, 162,* 111917. (Q1, IF=7.1) 5. Boban, A., Masuero, D., Vrhovsek, U., Budić-Leto, I. (2023) Comprehensive Lipid Profile of 'Maraština' Grape Skins from Dalmatia. *Applied Sciences, 13,* 6446. (Q2, IF=2.7).
Second mentor:	-

TOPIC OUTLINE

Summary in Croatian *(no more than 1000 characters with spaces):*	Aroma vina je jedan od najvažnijih atributa kvalitete vina i rezultat je interakcije hlapljivih i nehlapljivih metabolita prisutnih u vinu. Pojedini kvasci koji nisu roda *Saccharomyces* (ne *Saccharomyces* kvasci) izolirani iz ekosustava vinograda posjeduju specifičnu enzimsku aktivnost zbog čega mogu doprinijeti poboljšanju specifične arome i sortnih karakteristika vina. Cilj ovog rada je ispitati utjecaj odabranih autohtonih ne-*Saccharomyces* kvasaca na profil arome mladoga vina Maraština primjenom ciljanog (UPLC/QqQ-MS/MS) i neciljanog (SPME-GC×GC-TOF-MS) metabolomskog pristupa. Razjašnjavanje utjecaja specifičnih autohtonih kvasaca na kemijski sastav mladoga vina Maraština od velike je važnosti jer različiti sojevi istog roda kvasaca mogu proizvesti različite produkte i značajno utjecati na aromu vina. Također, stečena znanja će dati uvid u potencijalne nove starter ne-*Saccharomyces* kulture, koje će se karakterizirati s obzirom na njihove produkte i potencijalni učinak na profil arome vina.
Summary in English *(no more than 1000 characters with spaces):*	Wine aroma is one of the most important attributes of wine quality and is the result of the interaction of volatile and non-volatile metabolites present in wine. Some of non-*Saccharomyces* yeasts isolated from the vineyard ecosystem possess specific enzymatic activity and production of metabolites, which positively contribute to the aroma. The aim of this work is to determine the aroma profile of young Maraština wine with the aim of better understanding the interaction between yeast and chemical compounds of grapes by applying a UPLC/QqQ-MS/MS and SPME-GC×GC-TOF-MS. Understanding the influence of yeasts on the chemical composition of wine is of great importance because different strains of the same yeast genus can produce different products and affect the aroma of the wine. Also, the acquired knowledge will provide potential new starter non-*Saccharomyces* cultures, which will be characterized with regard to their products and the potential effect on the aroma profile of 'Maraština' wine.

Introduction and overview of research conducted hitherto *(suggested length: 7000 characters with spaces)*

Maraština is one of the most important Croatian indigenous white grape varieties, grown in the Dalmatia wine-growing region. According to the Wine Law (NN 32/19), it is used to produce quality and premium wines with a protected designation of origin - ZOI Dalmatia.

The aroma of wine is a crucial factor in defining its quality, involving compounds derived from the grapes, such as thiols, terpenes, and C_{13}-norisoprenoids, as well as higher alcohols, esters, sulfur compounds, carbonyl compounds, fatty acids, and others synthesized by yeasts. Although the aroma of wine is influenced by numerous factors, including grape variety, applied vineyard practices, climatic conditions, and vinification, yeasts and their enzymatic activity significantly affect the final aroma profile of the wine.

The use of indigenous yeasts in winemaking highlights varietal characteristics and consequently creates a regional signature in wines due to their biological diversity linked with phenotypic variations (Nisiotou et al., 2018). The microbiota of grapes is characterized by high biological diversity influenced by grape variety, sanitary status, berry ripeness, vineyard practices, climatic conditions, and soil type (Milanović et al., 2013; Guillamón & Barrio, 2017). A study by Milanović et al. (2022) identified 25 different yeast genera on Maraština grapes isolated from 11 different vineyards across the Dalmatia wine-growing region (North, Central, and South Dalmatia and Dalmatian hinterland). The study by Budić-Leto et al. (2020) showed that Maraština grapes, in addition to free forms of terpenes and norisoprenoids, contain higher concentrations of these compounds in bound forms, such as *β*-damascenone, linalool, and linalool oxide, which can contribute to the final aroma of Maraština wine, depending on the use and activity of yeasts during alcoholic fermentation.

Non-*Saccharomyces* yeasts are naturally present on grapes. They have often been referred to as "wild yeasts" due to their low fermentation activity, high production of acetic acid, acetaldehyde, and the creation of undesirable flavors (Padilla et al., 2016). Recent research shows that non-*Saccharomyces* yeasts have higher *β*-glucosidase activity compared to *Saccharomyces* yeasts, contributing to the hydrolysis of various types of glycosidic bonds such as 1,4-β and 1,6-α bonds, and promoting the formation of free terpenes, phenylpropenes, and specific esters during alcoholic fermentation (Liang et al., 2020; Zhang et al., 2021). The indigenous strain *Lachancea thermotolerans* in Pinot Grigio wine production showed increased production of lactic acid and had the highest potential to reduce ethanol content; the presence of indigenous *Starmerella bacillaris* yeast increased glycerol levels and reduced acetaldehyde and total SO_2 concentrations (Binati et al., 2020). Yeasts belonging to the *Metschnikowia* genus improved the aroma and flavor of wine due to the presence of arabinofuranosidase, which affects the increase in the concentration of varietal volatile compounds such as volatile thiols and terpenes (Capozzi et al., 2015). The study by Morreira et al. (2008) showed that *Hanseniaspora* strains produce high levels of desirable volatile compounds and higher extracellular enzyme levels, including glucosidases and proteases, compared to *Saccharomyces* strains, leading to increased concentrations of acetate esters that contribute to fruity aromas. *Pichia kluyveri* improved wine quality parameters through the production of thiols, fruity esters, and terpenes (Escribano et al., 2017).

Furthermore, research has also shown that non-*Saccharomyces* yeasts cannot complete the alcoholic fermentation of sugars, so sequential (stepwise) or simultaneous inoculation with S. cerevisiae is required. In recent years, several research groups have studied the effect of sequential inoculation with commercial non-*Saccharomyces* and *Saccharomyces* yeasts on the composition of volatile compounds in Sauvignon Blanc (Whitener et al., 2015), Shiraz (Whitener et al., 2017), Tempranillo (Escribano-Viana et al., 2019), Riesling (Dutraive et al., 2019), and red wine varieties Trnjak, Babić, Blatina, and Frankovka (Korenika et al., 2022). Commercial non-*Saccharomyces* yeasts *Lachancea thermotolerans*, *Pichia kluyveri, Metschnikowia pulcherrima, Candida zemplinina* (synonym *Starmerella bacillaris*), and *Kazachstania aerobia*, in sequential fermentation with *S. cerevisiae*, produce higher levels of terpenes such as geraniol, trans-*β*-ocimene, cis-*α*-ocimene, linalool, *α*-terpinene, and farnesol, which significantly contribute to the creation of pleasant wine aromas compared to *S. cerevisiae* yeast fermenting in monoculture (Whitener et al., 2017; Ruiz et al., 2018).

Additionally, recent research has revealed diversity in lipid composition depending on genotype and their composition in grape skins or seeds (Perez-Navarro et al., 2019). The lipid composition of grapes, which commonly contains free fatty acids, glycerolipids, glycerophospholipids, and triterpenoids, influences fermentation development by promoting yeast growth and affecting their metabolism (Santos et al., 2011). Fatty acids are important for wine aroma because yeasts use them during fermentation to produce volatile thiols, compounds responsible for tropical notes in wines (Tumanov et al., 2018). Amino acids, such as tryptophan, phenylalanine, and tyrosine, play a significant role in yeast metabolism as nitrogen sources and produce aromatic higher alcohols like tryptophol, phenylethanol, and tyrosol, which are products of the Ehrlich mechanism (Engin et al., 2015). Amino acids also influence yeast biomass and fermentation rate through the biotransformation of grape compounds into biologically active compounds and other substances that affect the aroma and taste of wine (Álvarez-Fernández et al., 2019). Therefore, it is necessary to clarify the impact of yeast on the chemical composition of wine for each grape variety, as different strains of the same yeast species can differently affect the aroma and fundamental physical-chemical parameters in the fermentation of must from the same grape variety.

Given these insights, the objectives of the proposed topic are related to Maraština and indigenous non-*Saccharomyces* yeasts isolated from Maraština grapes in the Dalmatia wine-growing region.

Objective and hypotheses of research[2] *(suggested length: 700 characters with spaces)*

The research hypothesis is that indigenous non-*Saccharomyces* yeasts isolated from the Maraština variety:

1. contain different exogenous enzymes that affect the release of aroma compounds from the grapes during alcoholic fermentation
2. produce metabolites during fermentation that contribute to the enhancement of wine aroma

The objectives of the research are:

1. to investigate the enzymatic activity of indigenous non-*Saccharomyces* yeasts isolated from the Maraština variety.
2. to examine the volatile and non-volatile metabolites of indigenous non-*Saccharomyces* yeasts in monoculture and sequential fermentation of the Maraština variety.

Material, participants, methodology and plan of research *(suggested length: 6500 characters with spaces)*

The research will be conducted at the Institute for Adriatic Crops and Karst Reclamation in Split. The experiment will be carried out on white Maraština (*Vitis vinifera* L.) grapes from the Plastovo locality in the Skradin wine region. The Maraština grapes (150 kg) will be harvested at technological maturity, when the glucose-to-fructose ratio is one-to-one.

Indigenous non-*Saccharomyces* yeasts from 11 Maraština vineyards have been previously molecularly identified, isolated, and enologically characterized in the project IP-2020-02-1872, and stored in a collection established at the Institute for Adriatic Crops and Karst Reclamation. Based on enological characterization, ten different non-*Saccharomyces* yeast species have been selected for this research: *Hanseniaspora uvarum, Hanseniaspora guilliermondii, Hanseniaspora pseudoguilliermondii, Hyphopichia pseudoburtonii, Pichia kluyveri, Metschnikowia pulcherrima, Metschnikowia sinensis, Metschnikowia chrysoperlae, Lachancea thermotolerans,* and *Starmerella apicola.*

The indigenous non-*Saccharomyces* yeasts will be tested for enzyme activities related to the aroma, such as: esterase, lipase, *β*-glucosidase, *β*-galactosidase, *β*-glucuronidase, *α*-galactosidase, *α*-glucosidase, *α*-fucosidase, *α*-mannosidase, acrylamidase,

[2] The sequence of listing the objective and hypotheses depends on the area of research.

phosphatase, trypsin, and α-chymotrypsin, using the API-ZYM testing system (BioMérieux, France).

Commercial yeasts *Saccharomyces cerevisiae* EC 1118 (Lallemand), Lachancea thermotolerans OCTAVE (Eaton), and *Metschnikowia pulcherrima* FLAVIA (Lallemand) will be used for control fermentations in monoculture and sequential fermentation.

At the Sladić Winery (Plastovo, Skradin), the primary phase of must production will be carried out: crushing and maceration of grapes, pressing of the pomace, and cooling, settling, and pre-filtration of the must using plate filters. Sulfur dioxide (50 mg/L) in the form of 5% sulfurous acid will be added to the must to protect it from oxidation. The cooled must will be immediately transported to the Institute for Adriatic Crops and Karst Reclamation after pre-filtration. In the laboratory at the Institute for Adriatic Crops and Karst Reclamation, the must will be filtered using sterile filters (0.45 μm) with a vacuum filtration unit (Pall Gelman). The following experiments will then be conducted on the sterile Maraština must:

a) fermentations with monocultures of 10 indigenous non-*Saccharomyces* yeasts

b) sequential fermentations with 5 indigenous non-*Saccharomyces* yeasts combined with the commercial *Saccharomyces cerevisiae* EC 1118 yeast

c) control fermentations with commercial yeast in monoculture:

- *Lachancea thermotolerans* OCTAVE (Eaton)
- *Metschnikowia pulcherrima* FLAVIA (Lallemand)
- *Saccharomyces cerevisiae* yeast EC 1118 (Lallemand)

d) Control fermentations with commercial yeast in sequential fermentation:

- *Metschnikowia pulcherrima* FLAVIA (Lallemand) / *Saccharomyces cerevisiae* EC 1118 (Lallemand)
- *Lachancea thermotolerans* OCTAVE (Eaton) / *Saccharomyces cerevisiae* EC 1118 (Lallemand)

Established monoculture, sequential, and control fermentations with 500 mL of sterile must will be carried out in sterile Erlenmeyer flasks (500 mL) sealed with cellulose plugs that allow CO_2 to escape from the system at a controlled temperature (22 °C).

After the completion of monoculture fermentation, sequential fermentation will be established with the five indigenous yeasts that showed the best fermentation characteristics in monoculture. When ethanol concentration reaches between 3% and 5% *v/v*, the must will be inoculated with the commercial *Saccharomyces cerevisiae* EC 1118 (Lallemand) strain at a concentration of 10^6 cells/mL.

Sample collection for chemical, microbiological, and metabolomic analyses will be conducted under sterile conditions.

The dynamics of fermentation of indigenous non-*Saccharomyces* yeasts will be monitored during alcoholic fermentation in all established experiments through:

a) chemical analyses determining glucose and fructose, ethanol (vol%), volatile acids, total acids, pH, malic acid, lactic acid, tartaric acid, and yeast assimilable nitrogen (YAN) using Fourier-transform infrared spectroscopy (FTIR) (Lyza 5000, Anton Paar, Austria).

b) microbiological analyses; counting viable yeast cells (Milanović et al., 2013).

Volatile and non-volatile aroma metabolites will be identified and quantified (i) in the must at the start of fermentation – initial characteristics of the must (three samples), (ii) at the end of monoculture fermentation with non-*Saccharomyces* isolates (30 samples), (iii) during sequential fermentation before inoculation with commercial S. cerevisiae EC 1118 (15 samples), (iv) at the end of sequential fermentations (15 samples), and (v) at the end of the control fermentation experiment (15 samples).

Solid-phase microextraction (SPME) will be used to extract volatile compounds from each sample before gas chromatography (GC) analysis. For profiling wine metabolites, two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (SPME-GC×GC-TOF-MS) will be used (Whitener et al., 2017).

Ultra-high-performance liquid chromatography (UHPLC) coupled with triple quadrupole (QqQ) mass spectrometry (MS) will be used to detect lipid compounds and aromatic amino acids. Analysis of lipids and aromatic amino acids (tryptophan, phenylalanine, and tyrosine) will be conducted according to published literature (Perez-Navarro et al., 2019; Álvarez-Fernández et al., 2019).

A total of 78 samples will be analyzed using each of the metabolomic methods via SPME-GC×GC-TOF-MS and UPLC/QqQ-MS/MS at the Metabolomics Department at Fondazione Edmund Mach (FEM) in Italy.

All experimental variants will be set up in triplicate, and the analyzed data will be expressed as mean values. Statistical significance will be determined using analysis of variance (ANOVA) with post-hoc Tukey HSD test. To determine the relationships among all observed variables, multivariate analysis tools such as Cluster Analysis (CA), Factor Analysis (FA), and Principal Component Analysis (PCA) will be used. ANOVA and multivariate data analyses will be performed using STATISTICA, v. 10 (StatSoft, Inc, OK, USA) and IBM® SPSS® v. 23.0 (SPSS Inc., Chicago, Illinois, USA).

Expected scientific contribution of proposed research *(suggested length: 500 characters with spaces)*

The research will contribute to a better understanding of the enological potential of indigenous non-*Saccharomyces* yeasts that have been isolated from the Maraština variety for the first time. It will clarify their impact on the varietal and fermentation profile of young Maraština wine using targeted and untargeted metabolomics approaches, as well as FTIR analysis. The acquired knowledge will provide insights into potential new starter non-*Saccharomyces* cultures, which will be characterized based on their products and their potential effect on the wine aroma profile.

List of literature cited *(no more than 30 references)*

1. Álvarez-Fernández, M.A., Fernández-Cruz, E., Garcia-Parrilla, M.C., Troncoso, A.M., Mattivi, F., Vrhovsek, U., Arapitsas, P. (2019) *Saccharomyces cerevisiae* and *Torulaspora delbrueckii* intra- and extra-cellular aromatic amino acids metabolism. *J. Agric. Food Chem.* **67**, 7942-7953.
2. Beckner Whitener, M. E., Carlin, S., Jacobson, D., Weighill, D., Divol, B., Conterno, L., Du Toit, M., Vrhovsek, U. (2015) Early fermentation volatile metabolite profile of non-*Saccharomyces* yeasts in red and white grape must: A targeted approach. *LWT* **64**, 412-422.
3. Beckner Whitener, M. E., Stanstrup, J., Carlin, S., Divol, B., Du Toit, M., Vrhovsek, U. (2017) Effect of non-*Saccharomyces* yeasts on the volatile chemical profile of Shiraz wine. *Aust. J. Grape Wine Res.* **23**, 179-192.
4. Binati, R.L., Lemos, W.J.F., Luzzini, G., Slaghenaufi, D., Ugliano, M., Torriani, S. (2020) Contribution of non-*Saccharomyces* yeasts to wine volatile and sensory diversity: A study on *Lachancea thermotolerans, Metschnikowia* spp. and *Starmerella bacillaris* strains isolated in Italy. *Int J Food Microbiol.* **318**, 108470.
5. Budić-Leto, I., Humar, I., Gajdoš Kljusurić, J., Zdunić, G., Zlatić, E. (2020) Free and bound volatile aroma compounds of ´Maraština´ grapes as influenced by dehydration techniques. *Appl. Sci.* **10**, 8928.
6. Bisson, F.L. (1999) Stuck and Sluggish Fermentations. *AJEV* **50**, 107-119.
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International funding			
Other types of projects			
Self funding			
Session of the Ethics Committee at which consent was given to			

the research proposal[3]	

Agreement of the mentor and the doctoral candidate to request for topic approval

I declare under responsibility that I agree with the topic whose approval is requested.

Signature

Ph.D. Irena Budić-Leto

Signature

Ana Boban, mag. chem.

STATEMENT

I declare under responsibility that I have not submitted a request for approval of an identical dissertation topic at any other university[4].

Zagreb, September 26, 2024

Signature

Ana Boban, mag. chem.

Official stamp here

[3] Fill out only if needed
[4] Not required in case of dual doctorate (*Cotutelle de these*)