DR.10 DOCTORAL THESIS EVALUATION REPORT[1]			
DOCTORAL CANDIDATE			
First and last name:		Ana Boban, M.Sc.	
Registration number:		445/PT	
GENERAL INFORMATION ABOUT THE STUDY PROGRAMME			
Institution responsible for organizing the studies:		University of Zagreb Faculty of Food Technology and Biotechnology	
Name of study programme:		Biotechnology and Bioprocess Engineering, Food Technology and Nutrition	
Name of partner institution *(in case of dual doctorate)***:**			
Name of doctoral study programme at partner institution *(in case of dual doctorate)***:**			
Area / field / branch *(if the doctoral study programme is performed within a branch)***:**		Biotechnical sciences / Food Technology	
INFORMATION ON THE DOCTORAL THESIS			
Thesis language		English	
Thesis title:	*In the language of the thesis*	Impact of indigenous non-*Saccharomyces* yeasts on the aroma profile of young wine from 'Maraština' cv. (*Vitis vinifera* L.)	
	In Croatian	Utjecaj autohtonih kvasaca koji ne pripadaju rodu *Saccharomyces* na profil arome mladoga vina sorte 'Maraština' (*Vitis vinifera* L.)	
	In English	Impact of indigenous non-*Saccharomyces* yeasts on the aroma profile of young wine from 'Maraština' cv. (*Vitis vinifera* L.)	
MENTOR(S)			
		Full name, title:	**Institution, country:**
Mentor:		Irena Budić-Leto, PhD, scientific advisor with tenure	Institute for Adriatic Cops and Karst Reclamation
Mentor:			
THESIS EVALUATION COMMITTEE			
Appointed Thesis Evaluation Committee[2]:		**Full name, title:**	**Institution, country:**
		1. Karin Kovačević Ganić, Ph.D, Full professor	University of Zagreb Faculty of Food Technology and Biotechnology, Croatia
		2. Jasenka Gajdoš Kljusurić, Ph.D, Full professor	University of Zagreb Faculty of Food Technology and Biotechnology, Croatia

[1] Forward the completed and signed DR.10 form to the appropriate Student Office in digital and print format.
[2]Committee members 6 and 7 only in case of a dual doctorate

	3. Vesna Milanović, Ph.D, Associate professor	Department of Agricultural, Food and Environmental Sciences, Polytechnic University of Marche, Italy
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Session of the competent body and agenda item within which the Committee was appointed:	8th regular session of the Faculty Council of the Faculty of Food Technology and Biotechnology in Zagreb in the academic year 2024/2025 held on May 21st, 2025 (ad 19)	

DOCTORAL THESIS EVALUATION

Thesis form:	☐ monograph ☒ Scandinavian model ☐ artistic work	
Thesis is evaluated:	☒ for the first time	☐ after revision
Thesis should be:	☒ accepted ☐ revised ☐ rejected	

Explanation for the above opinion *(specifying original scientific or artistic contribution and new discovery)*:

The doctoral dissertation by Ana Boban, M.Sc. was prepared at the Institute for Adriatic Crops and Karst Reclamation in Split, under the supervisor of Irena Budić-Leto, Ph.D scientific advisor with tenure.

The dissertation, known as The Scandinavian model, is based on four scientific papers published in first-quartile (Q1) journals.

The dissertation consists of a total of 140 pages and includes 3 figures, 2 tables, 195 literature references, 2 appendices, the CVs of the candidate and mentor, a basic documentation card, and extended summary in English and Croatian. The dissertation is structured into the following chapters: General Introduction, Theoretical Background, General Discussion, Conclusions, References, and two Appendices that include the four scientific articles and three additional tables.

The general introduction presents the development of our understanding of alcoholic fermentation, from spontaneous processes to the discovery of the role of microorganisms as defined by Louis Pasteur. Today, it is known that yeasts, especially *Saccharomyces cerevisiae*, play a key role in winemaking due to their resilience and ability to produce desirable aromatic compounds. However, the frequent use of this species often leads to wine uniformity, prompting interest in the application of non-*Saccharomyces* yeasts. These indigenous yeasts show greater diversity in the production of aromatic components and contribute to the expression of microbial terroir and varietal identity. Recent studies have shown their potential to enhance the sensory profile of wine and address technological challenges. Wine aroma is shaped by the interaction of volatile and non-volatile compounds, in which yeasts participate through their activation, conversion, and synthesis. The aim of the dissertation is to explore the impact of indigenous non-*Saccharomyces* yeasts on the aromatic profile of the Maraština grape variety, with an emphasis on their role in lipid and aromatic amino acid metabolism.

The theoretical section provides an overview of relevant scientific literature and fundamental concepts related to the research. It begins with a presentation of the indigenous grape variety Maraština (*Vitis vinifera* L.), including its origin, distribution, and oenological potential. This is followed by a detailed description of non-S*accharomyces* yeasts, their roles and diversity in the fermentation process, with an emphasis on enzymatic activities that can contribute to the release and synthesis of aromatic compounds from grapes. Special focus is given to the influence of non-*Saccharomyces* yeasts on the volatile profile of wine, as well as their interaction with commercial *S. cerevisiae* strains. Separate chapters explore the significance of lipids and the metabolism of aromatic amino acids during alcoholic fermentation, as important precursors of aromatic compounds in wine. Previous research on the fermentation potential and aromatic contribution of different yeasts is also discussed. Based on available data, hypotheses, objectives, and the expected scientific contributions are presented.

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

i. contain different exogenous enzymes that affect the release of aroma compounds from the grapes during alcoholic fermentation,

ii. produce metabolites during fermentation that contribute to the enhancement of wine aroma.

To test these hypotheses, the following research objectives were defined:

i. to investigate the enzymatic activity of indigenous non-*Saccharomyces* yeasts isolated from the Maraština variety,

ii. to examine the volatile and non-volatile metabolites of indigenous non-*Saccharomyces* yeasts in monoculture and in sequential fermentation of the Maraština variety.

In the general discussion, the results presented in the published scientific papers (Appendix 1) are analyzed, along with extended results statistically processed and shown in Figure 3 and three additional tables in Appendix 2. Figure 3 in the discussion presents the enzymatic characterization results that contribute to a better understanding of the potential of indigenous non-*Saccharomyces* isolates. The discussion is organized into three thematic units: (i) enzymatic characterisation of non-S*accharomyces* isolates, (ii) effect of indigenous non-*Saccharomyces* yeasts on the wine volatilome, and (iii) modulation of lipid profile and aromatic amino acid metabolism. Each section provides a clear and evidence-based analysis of results, with comparisons to relevant scientific publications.

The conclusion summarizes the main findings on indigenous non-*Saccharomyces* yeasts in Maraština wine. It was found that selected strains exhibit significant enzymatic activity that affects aroma formation and lipid profile modulation. The application of these yeasts enhances the production of key volatile compounds such as terpenes and esters, while sequential fermentations lead to changes in fatty acid and aromatic amino acid metabolism. The results confirm the importance of indigenous non-*Saccharomyces* yeasts for improving the complexity and quality of wine.

The literature used in this work includes relevant and recent scientific knowledge in the field of research.

Appendix 1 includes four scientific articles that are thematically and substantively interconnected, forming a comprehensive scientific foundation for the dissertation. The papers are arranged in logical order, and their results are analyzed and discussed in the same sequence in the Discussion chapter. Each paper provides a detailed description of the conducted research, including the experimental approach, presentation of results, and their scientific interpretation in the context of current knowledge.

Scientific paper no. 1

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.

The aim of this study was to isolate and characterize indigenous non-*Saccharomyces* yeasts from the Maraština variety and assess their oenological potential for use as starter cultures in alcoholic fermentation. Special attention was given to the evaluation of enzymatic characterization relevant for aroma development. Particularly active strains identified were *Hyp. pseudoburtonii* N-11, *M. sinensis/shanxiensis* P-7, and *H. pseudoguilliermondii* V-13 due to elevated esterase activity, while species of the genus *Hanseniaspora* showed pronounced proteolytic and *β*-glucosidase activity, key for aromatic amino acid metabolism. The analysis

revealed that enzymatic profiles depend more on the genus than the species, with clear separation between *Metschnikowia* and *Hanseniaspora.* In conclusion, the Maraština variety represents a valuable source of potentially useful non-*Saccharomyces* yeasts for the development of terroir-specific starter cultures. This paper confirmed the first hypothesis that indigenous non-*Saccharomyces* yeasts from Maraština contain various exogenous enzymes that influence the release of aroma compounds during alcoholic fermentation.

The second hypothesis — that indigenous non-*Saccharomyces* yeasts isolated from the 'Maraština' grape variety produce metabolites during fermentation that contribute to enhancing wine aroma - was confirmed through the following three scientific papers:

Scientific paper no. 2

Boban, A., Vrhovsek, U., Carlin, S., Milanović, V., Gajdoš Kljusurić, J., Jurun, Z., Budić-Leto, I. (2024) The effect of indigenous non-*Saccharomyces* yeasts on the volatile profile of Maraština wine: Monoculture versus sequential fermentation. *Applied Food Research* **4**, 100554.

This study aimed to examine how different indigenous non-*Saccharomyces* yeasts affect the volatile profile of Maraština wine when used alone or in sequential fermentation with *S. cerevisiae*. Targeted GC-MS analysis was used to assess differences in terpenes, norisoprenoids, ester, higher alcohols, volatile acids, volatile phenols, aldehydes, ketones, and lactones concentrations. The results showed that strains such as *Metschnikowia pulcherrima* K-6 and *Hanseniaspora guilliermondii* N-29 promoted increased production of key aroma compounds, significantly influencing the wine's aromatic characteristics. *Pichia kluyveri* Z-3 and *Starmerella apicola* VP-8 dominated ester production, while *M. chrysoperlae* K-11 and *M. sinensis/shanxiensis* P-7 reduced volatile phenols associated with undesirable “Brett” aromas. Sequential fermentation with *P. kluyveri* Z-3 resulted in elevated ester concentrations, and *H. guilliermondii* N-29 and *P. kluyveri* Z-3 contributed to higher levels of 2-phenylethanol and cis-3-hexenol. All tested yeasts maintained volatile acidity within acceptable limits, with increased lactic acid production compared to *S. cerevisiae* EC 1118.

Scientific paper no. 3

Boban, A., Vrhovsek, U., Masuero, D., Milanović, V., Budić-Leto, I. (2025) Effect of indigenous non-*Saccharomyces* yeasts on lipid compositions of Maraština Wine. *Foods* **14**, 269.

This study aimed to evaluate the impact of indigenous non-*Saccharomyces* yeasts on the lipid composition of Maraština wine, relevant for fermentation and aromatic profiles. Strains such as *Lachancea thermotolerans* P-25, *Hanseniaspora* spp., and *Pichia kluyveri* Z-3 significantly reduced certain saturated and unsaturated fatty acids, improving membrane fluidity and stress resistance during fermentation. Lipid changes were associated with increased ester production, important for wine quality. Different strains showed unique lipid utilization patterns and responses to ethanol stress, influencing fermentation kinetics and the volatile profile. These results highlight the role of indigenous yeasts in aroma modulation and wine stability.

Scientific paper no. 4

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.

This study investigated the influence of various indigenous non-*Saccharomyces* yeasts on the metabolism of aromatic amino acids (phenylalanine, tyrosine, and tryptophan) during Maraština wine fermentation. Targeted metabolomic analyses were used to detect compounds related to the degradation of these amino acids, which contribute to aroma complexity and potential bioactivity. *H. guilliermondii* N-29 and *M. pulcherrima* K-6 modulated production of tryptophol and kynurenic acid, associated with fruity notes and possible health effects. Monocultures metabolized tryptophan more efficiently, and some strains reduced phenylalanine and tyrosine levels, increasing aroma precursor concentrations. *M. pulcherrima* K-6 notably enhanced tryptophol production, while H*yp. pseudoburtonii* N-11 increased ester concentrations. The results demonstrate the significant potential of these yeasts in producing high-quality wines.

Appendix 2 contains three additional tables that present extended results and aid in interpretation. The first table provides a semi-quantitative assessment of enzymatic activity in ten selected indigenous non-*Saccharomyces* isolates. The second presents the lipid composition of Maraština grapes. The third table includes data on amino acid concentrations (tryptophan, phenylalanine, and tyrosine) and their intermediates in monoculture fermentations with all ten yeasts. These results are further discussed in the General Discussion section.

In conclusion, this doctoral dissertation confirms the stated hypotheses and successfully meets the research objectives, providing significant scientific contributions to the understanding of the enological potential of indigenous non-S*accharomyces* yeasts isolated for the first time from the Maraština variety. It clarifies their impact on the varietal and fermentative profile of young Maraština wine.

Opinion and proposal:

Based on the conducted evaluation, the Thesis Evaluation Committee considers that the doctoral thesis of the applicant Ana Boban, M.Sc. was prepared in accordance with the stated hypotheses, objectives, and explanation of the approved topic. The findings of this doctoral research constitute a significant scientific contribution to the field of enology.

The applicant presented the results clearly and systematically, and the analyses and discussion led to logical and well-supported conclusions. The conducted research provides new insights into the enzymatic activities of indigenous non-*Saccharomyces* yeasts and their impact on the production of volatile and non-volatile compounds during alcoholic fermentation. The results further contribute to the understanding of how these isolates can influence wine properties, confirming their potential in improving the quality of young wine. Statistical analysis and detailed data interpretation clearly define the specific contributions of individual yeast strains. This research significantly enriches the scientific literature on the biotechnological valorisation of indigenous yeasts and provides insight into potential new starter cultures of non-*Saccharomyces* yeasts.

Based on the above, it is proposed to the Faculty Council of the University of Zagreb, Faculty of Food Technology and Biotechnology, that the doctoral thesis by Ana Boban entitled „Impact of indigenous non-*Saccharomyces* yeasts on the aroma profile of young wine from 'Maraština' cv. (*Vitis vinifera* L.)“ (Utjecaj autohtonih kvasaca koji ne pripadaju rodu *Saccharomyce*s na profil arome mladoga vina sorte ‘Maraština’ (*Vitis vinifera* L.)) be accepted and submitted for further procedure toward the awarding of the academic degree of Doctor of Science in the scientific field of Biotechnical Sciences, scientific area of Food Technology

Separate opinion *(only if a member of the Thesis Evaluation Committee has a separate opinion)***:**

Signature

(full name of Committee member)

Additional comments *(optional)*

	Full name, title, institution, country	Signature
Signatures of Thesis Evaluation Committee members[3]	1. Karin Kovačević Ganić, Ph.D, Full professor, University of Zagreb Faculty of Food Technology and Biotechnology, Croatia *(Chair of the Committee)*	
	2. Jasenka Gajdoš Kljusurić, Ph.D, Full professor, University of Zagreb Faculty of Food Technology and Biotechnology, Croatia	
	3. Vesna Milanović, Ph.D, Associate professor, Department of Agricultural, Food and Environmental Sciences, Polytechnic University of Marche, Italy	
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	7.	

In Zagreb, on 29th May 2025

[3] Committee members 6 and 7 only in case of a dual doctorate.