DR.10 DOCTORAL THESIS EVALUATION REPORT[1]			
DOCTORAL CANDIDATE			
First and last name:		Melissa Prelac	
Registration number:		443/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:		University Doctoral Study 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*	The adsorption of phytochemicals from onion peel and olive leaf aqueous extracts onto biochar from grapevine pruning residues	
	In Croatian	Potencijal biougljena od ostataka rezidbe vinove loze u adsorpciji fitokemikalija iz vodenih ekstrakata ljuske luka i lista masline	
	In English		
MENTOR(S)			
		Full name, title:	**Institution, country:**
Mentor:		Igor Palčić, Ph.D.	Institute of Agriculture and Tourism, Poreč, Croatia
Mentor:		Maja Repajić, Ph.D., Associate Professor	University of Zagreb Faculty of Food Technology and Biotechnology
THESIS EVALUATION COMMITTEE			
Appointed Thesis Evaluation Committee[2]:		**Full name, title:**	**Institution, country:**
		1. Verica Dragović-Uzelac, Ph.D., Full Professor	University of Zagreb Faculty of Food Technology and Biotechnology
		2. Josip Ćurko, Ph.D., Associate Professor	University of Zagreb Faculty of Food Technology and Biotechnology

[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. Nikola Major, Ph.D.	Institute of Agriculture and Tourism, Croatia
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	7.	
Session of the competent body and agenda item within which the Committee was appointed:		

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 dissertation by Melissa Prelac, MSc, was conducted at the Institute of Agriculture and Tourism in Poreč, Croatia, under the supervision of Ph.D. Igor Palčić and Associate Professor, Ph.D. Maja Repajić.

Doctoral candidate Melissa Prelac has prepared and submitted for evaluation a doctoral dissertation titled "The adsorption of phytochemicals from onion peel and olive leaf aqueous extracts onto biochar from grapevine pruning residues". The dissertation follows the Scandinavian model and consists of 117 pages, 5 figures, 236 references, and 3 supplementary materials, which include published scientific papers. The work is structured into several core chapters: general introduction, theoretical background, general discussion, conclusions and prospects, references, supplementary materials, and the candidate's curriculum vitae.

The introductory part of the dissertation is logically structured and thematically cohesive, encompassing a broad theoretical framework and a relevant contextualization of the research problem. It highlights the issue of global population growth, which leads to increased pressure on food systems and the environment, along with a rise in agro-industrial waste. Residues such as grapevine prunings, onion peels, and olive leaves often remain unutilized, despite containing valuable bioactive compounds, particularly polyphenols with beneficial biological effects. To extract these compounds in a sustainable way, green extraction methods are increasingly being applied, such as maceration, ultrasound-assisted extraction, and microwave-assisted extraction, with water being used as an environmentally friendly solvent. At the same time, biochar obtained through the pyrolysis of plant biomass, is being increasingly investigated as an adsorbent due to its porosity and large surface area.

The theoretical part of the dissertation is based on a multidisciplinary approach to the valorization of agri-food waste in the context of climate change and sustainable resource management. The work first examines the growing impact of climate change and the need

for more efficient use of by-products from the agri-food industry, contributing to waste reduction and increased added value within the circular bioeconomy. Special emphasis is placed on biochar produced by the pyrolysis of grapevine pruning residues, which is being investigated as a functional material due to its favorable adsorption properties. Such biochar has potential for the adsorption and stabilization of organic compounds, especially polyphenols, making it suitable for further application in environmental and food technologies.

The dissertation also provides a detailed analysis and description of the potential of onion peels and olive leaves as sources of high-value bioactive compounds, primarily polyphenols, which exhibit significant antioxidant, anti-inflammatory, and anti-microbial properties. Since these materials are often overlooked and discarded, their valorization represents a tangible contribution to sustainable biomass management. In addition, the work emphasizes the use of green, water-based extraction methods (maceration, ultrasound-assisted extraction, and microwave-assisted extraction) as alternatives to conventional solvents, thereby reducing the environmental footprint of the process.

The theoretical section is concluded with the formulation of the hypothesis, research objectives, and expected scientific contribution. Based on a detailed review of previous studies, the research hypotheses were established as follows: that biochar will exhibit favorable physicochemical properties as an adsorbent, and in the Langmuir and Freundlich isotherm models the coefficient of determination (R^2) will exceed 0.90; that water will be an effective solvent for the extraction of polyphenols from onion peels and olive leaves; that modern extraction methods, higher temperatures, and lower solid-to-liquid ratios will result in a higher polyphenol content and antioxidant activity; and that biochar will achieve greater adsorption of less polar polyphenols present in the extracts.

Based on these hypotheses, the following research objectives were defined:

(i) to analyze and characterize biochar derived from grapevine pruning residues as an adsorptive material through the development of Langmuir and Freundlich adsorption models;

(ii) to optimize the process of polyphenol extraction from onion peels and olive leaves using water as a solvent, taking into account the extraction method, temperature, and solid-to-liquid ratio;

(iii) to determine the potential of biochar from grapevine pruning residues in the adsorption of polyphenols from onion peel and olive leaf extracts.

The research plan is thoroughly elaborated and divided into three sections. The first part focuses on the production and characterization of biochar, while the second and third parts simultaneously investigate the extraction and adsorption of polyphenols from different plant sources, onion peels and olive leaves. The use of green extraction methods and water as a solvent further emphasizes the environmental aspect of the research. The overall research approach is methodologically consistent and scientifically relevant, with a clear aim of valorizing agri-food waste through the development of sustainable solutions. This part of the work successfully connects theoretical assumptions with experimental design, providing a solid foundation for evaluating the scientific contribution.

The general discussion successfully integrates the results of the three studies, clearly linking the properties of biochar, the optimization of green extraction methods, and the effectiveness of biochar in polyphenol adsorption. This research has demonstrated that the investigated biochar possesses favorable physicochemical properties for use as an adsorbent. Water was confirmed as an effective solvent for the extraction of polyphenols from onion peels and olive leaves, especially when using modern methods and optimized extraction parameters. The adsorption of polyphenols onto biochar aligned well with both Langmuir and Freundlich models, showing high coefficients of determination and confirming the selective adsorption potential of the biochar. The discussion is clear, coherent, and well aligned with the research objectives.

In conclusion, this research presents an integrated approach to the valorization of agro-industrial waste through the combination of green extraction methods and the application of biochar obtained by pyrolysis of grapevine pruning residues. The focus of the study was on onion peels and olive leaves as plant materials rich in polyphenols, yet often overlooked in the context of valorization. By using water as an environmentally friendly solvent and applying maceration and ultrasound-assisted extraction at elevated temperatures, a high yield of polyphenolic compounds was achieved while preserving antioxidant activity. Ultrasound-assisted extraction at 90 °C with a solid-to-liquid ratio of 1:100 g/mL proved to be the most effective method.

The biochar produced from grapevine pruning residues exhibited favorable physicochemical characteristics as an adsorbent: high porosity, alkaline pH, significant carbon, potassium, and silicon content, and the presence of surface functional groups that support interactions with bioactive molecules. Adsorption experiments, conducted using reference standards and extracts of onion peel and olive leaf, confirmed the biochar's efficiency in binding polyphenols, with results from the Langmuir and Freundlich models showing good agreement with experimental data ($R^2 > 0.90$). Among the compounds tested, oleuropein and hydroxytyrosol from olive leaves, and quercetin-3,4'-diglucoside from onion peels, showed the highest adsorption capacity.

Furthermore, the impact of the amount of added biochar on adsorption capacity was examined. Optimal adsorption conditions included the use of 0.5 g of biochar per 3 g/L of extract, while for certain compounds from olive leaves, such as oleuropein, a higher amount of biochar (2.5 g) resulted in improved binding. These findings confirm the selective effectiveness of biochar in the adsorption of specific polyphenols, opening up possibilities for its application not only in the extraction process of valuable compounds but also in environmental technologies (e.g., wastewater treatment) and in the formulation of functional foods, cosmetics, and pharmaceuticals.

The doctoral dissertation is founded on research findings presented in three scientific publications:

Publication 1. Prelac, M., Palčić, I., Cvitan, D., Anđelini, D., Repajić, M., Ćurko, J., Kovačević, T.K., Goreta Ban, S., Užila, Z., Ban, D., Major, N. (2023) Biochar from Grapevine Pruning Residues as an Efficient Adsorbent of Polyphenolic Compounds. *Materials*, **16**, 4716. doi: 10.3390/ma16134716

The aim of the first publication was the production and characterization of biochar obtained through pyrolysis of grapevine pruning residues, to be used as an adsorbent for polyphenolic compounds. In this study, caffeic acid, gallic acid, and oleuropein were used as standard compounds. The data were modeled using Langmuir and Freundlich isotherms, with the Langmuir model providing the best values for oleuropein: KL (0.39) and R^2 (0.9934). Adsorption dynamics were examined using a customized HPLC column filled with biochar. The recovery of compounds through online HPLC extraction was 41.5% for caffeic acid, 61.8% for gallic acid, and 91.4% for oleuropein. The biochar showed a higher affinity for adsorbing less polar compounds and greater efficiency in releasing more polar ones, indicating its potential as an effective adsorbent for polyphenolic compounds. This first publication confirmed the hypothesis that the biochar would possess favorable physicochemical properties as an adsorbent and that the coefficient of determination (R^2) in the Langmuir and Freundlich isotherm models would exceed 0.90.

Publication 2. Prelac, M., Palčić, I., Cvitan, D., Anđelini, D., Repajić, M., Ćurko, J., Kovačević, T.K., Goreta Ban, S., Užila, Z., Ban, D., Major, N. (2023) From Waste to Green: Water-Based Extraction of Polyphenols from Onion Peel and Their Adsorption on Biochar from Grapevine Pruning Residues. *Antioxidants*, **12**, 1697. doi: 10.3390/antiox12091697

This study examined the potential of biochar in adsorbing targeted polyphenols from onion peel extracts. The extraction was performed according to the principles of green chemistry, using deionized water and three extraction methods: maceration, ultrasound-assisted extraction, and microwave-assisted extraction. The efficiency of extraction was tested at different temperatures and solid-to-liquid ratios. The highest polyphenol yield was achieved using ultrasound-assisted extraction at 90 °C and a ratio of

1:100 g/mL. The adsorption capacity of individual polyphenols was modeled using Langmuir and Freundlich isotherms, with quercetin-3,4'-diglucoside showing the best results.

Publication 3. Prelac, M., Major, N., Cvitan, D., Anđelini, D., Repajić, M., Ćurko, J., Kovačević, T.K., Goreta Ban, S., Užila, Z., Ban, D., Palčić, I. (2024) Valorization of Olive Leaf Polyphenols by Green Extraction and Selective Adsorption on Biochar Derived from Grapevine Pruning Residues. *Antioxidants*, **13**, 1. doi: 10.3390/ antiox13010001

This study investigated the potential of biochar obtained from grapevine pruning residues for the adsorption of specific polyphenols from olive leaf extracts. Water was used as a green solvent, and extraction was carried out using maceration, ultrasound-assisted extraction, and microwave-assisted extraction, with variations in temperature and solid-to-liquid ratios. The adsorption capacity of the biochar was analyzed using the Langmuir and Freundlich isotherm models, with the Freundlich model providing a better fit to the experimental data. The most abundant compound in the extracts was oleuropein, which showed the highest correlation coefficient (R^2 = 0.9715) and Freundlich constant (Kf = 20.4). The optimal amount of biochar in the dosing experiments was 0.5 g per 3 g/L of extract, except for oleuropein and hydroxytyrosol, for which better results were achieved with 2.5 g of biochar.

Publications 2 and 3 confirm the hypotheses that water is an effective solvent for extracting polyphenols from onion peel and olive leaves, and that modern extraction methods, higher temperatures, and lower solid-to-liquid ratios result in increased polyphenol yield and antioxidant activity. Additionally, biochar achieves greater adsorption of less polar polyphenols present in the extracts.

The doctoral dissertation makes a significant scientific contribution to the sustainable valorization of agro-food waste through an integrated approach that includes optimizing green extraction methods and applying biochar as a selective adsorbent. A novel finding of the work is the demonstrated effectiveness of biochar derived from grapevine pruning residues for the selective adsorption of specific polyphenols from aqueous extracts of onion peels and olive leaves, with high conformity to the Langmuir and Freundlich models. For the first time, the study quantitatively confirms the selective adsorption capacity of biochar depending on compound polarity, the chemical composition of the adsorbent, and dosing conditions. Furthermore, the optimal conditions for aqueous extraction enable high yields and preservation of the bioactivity of polyphenols, confirming the efficiency of the applied green technologies.

This research developed a sustainable model for dual waste valorization, utilizing one type of agricultural waste as a source of valuable phytochemicals and another as a functional material for their separation and potential recovery. Thus, the work contributes to the development of sustainable technologies within the framework of the circular bioeconomy, with potential applications in water purification as well as in the food, pharmaceutical, and cosmetic industries.

Opinion and proposal:

Candidate Melissa Prelac, MSc, has prepared her doctoral dissertation according to the approved topic and in accordance with the set research objectives and hypotheses. The doctoral dissertation represents a high-quality and methodologically precise study in the field of sustainable valorization of agro-food waste. Through a coherent experimental approach, from the production and characterization of biochar to the optimization of aqueous extraction and the investigation of polyphenol adsorption, the work provides new scientific insights with potential applications in the circular bioeconomy.

The significant contribution is demonstrated in the proven selective efficiency of biochar for adsorbing specific polyphenols, as well as in optimizing green extraction parameters. The application of Langmuir and Freundlich models further confirms the scientific validity of the results. The dissertation is clearly structured and supported by relevant publications.

Based on all of the above, we propose to the Faculty Council of the University of Zagreb Faculty of Food Technology and Biotechnology that the doctoral dissertation of Melissa Prelac, MSc, entitled "The adsorption of phytochemicals from onion peel and olive leaf aqueous extracts onto biochar from grapevine pruning residues" be accepted and forwarded for further procedures for obtaining the academic degree of Doctor of Science in the scientific area of Biotechnical Sciences, field 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. Verica Dragović-Uzelac, Ph.D., Full Professor, University of Zagreb Faculty of Food Technology and Biotechnology, Croatia *(Chair of the Committee)*	
	2. Josip Ćurko, Ph.D., Associate Professor, University of Zagreb Faculty of Food Technology and Biotechnology, Croatia	
	3. Nikola Major, Ph.D., Institute of Agriculture and Tourism, Croatia	
	4.	
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	6.	
	7.	

In Zagreb, on 1st July 2025

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