DR.10 DOCTORAL THESIS EVALUATION REPORT[1]			
DOCTORAL CANDIDATE			
First and last name:		Josipa Dukić, M.Sc.	
Registration number:		449/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*	Utilization of by-products and waste from sugar beet (*Beta vulgaris* L.) processing by non-thermal extraction techniques	
	In Croatian	Iskorištavanje nusproizvoda i otpada iz prerade šećerne repe *(Beta vulgaris* L.) primjenom netoplinskih tehnika ekstrakcije	
	In English	Utilization of by-products and waste from sugar beet (*Beta vulgaris* L.) processing by non-thermal extraction techniques	
MENTOR(S)			
		Full name, title:	**Institution, country:**
Mentor:		Anet Režek Jambrak, Full Professor, PhD	University of Zagreb Faculty of Food Technology and Biotechnology, Croatia
Mentor:			
THESIS EVALUATION COMMITTEE			
Appointed Thesis Evaluation Committee[2]:		**Full name, title:**	**Institution, country:**
		1. Verica Dragović-Uzelac, Full Professor, Ph.D	University of Zagreb Faculty of Food Technology and Biotechnology, Croatia
		2. Tomislav Bosiljkov, Full Professor, Ph.D	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. Ana Butorac, Ph.D	Selvita d.o.o., Zagreb, Croatia
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	6.	
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Session of the competent body and agenda item within which the Committee was appointed:	6th regular session of the Faculty Council of the Faculty of Food Technology and Biotechnology in Zagreb in the academic year 2024/2025 held on March 26th, 2025 (ad 15)	

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 of Josipa Dukić, M.Sc. was prepared in the Laboratory for sustainable development at the University of Zagreb Faculty of Food Technology and Biotechnology under the supervision of prof. Anet Režek Jambrak, Ph.D.

The doctoral thesis, known as The Scandinavian model, is based on 4 published scientific papers of the PhD candidate. The dissertation consists of a total of 163 pages, 11 figures, 3 tables, 193 literature references, the applicant's and mentor's CV, a basic documentation card and an extended summary in English and Croatian. It is divided into the following chapters: General introduction, Theoretical background, Scientific papers, General discussion, Conclusions and References. The papers on which the PhD thesis is based were published in the journals Foods, Applied Sciences, Ultrasonics Sonochemistry and Molecules where the applicant is the first author of three papers.

General introduction:

The introductory section outlines the global problem of population growth and the increasing problem of climate change, which requires the development of sustainable food processing methods that have less impact on the environment. New technologies, such as non-thermal extraction techniques, offer an alternative to conventional methods by reducing energy and processing time while aiming to maintain food quality and reduce waste. The focus of interest is the extraction of proteins and other bioactive compounds from sugar beet processing waste and by-products that have a positive impact on human health. Non-thermal extraction techniques, high-power ultrasound and high-voltage electrical discharge are proposed as new and promising techniques for the extraction of said compounds to achieve sustainable development goals, the concept of "zero waste" and the circular economy.

Theoretical background:

The theoretical section provides basic information on sustainability in food technology, briefly describes the history of sustainable development and explains the concepts of the circular economy and zero waste. In addition, the plant material sugar beet and the by-products and waste products generated during processing are described in detail. Waste and by-products from sugar beet processing are presented as a good source of protein, with a focus on the RuBisCO enzyme and other bioactive compounds such as the flavonoid vitexin. The health benefits of the compounds mentioned are listed and the non-thermal extraction techniques (high-power ultrasound and high-voltage electrical discharge) are presented in detail. The table provides a very clear and systematic overview of the research to date (over the last 10 years) which includes the extraction of valuable compounds from sugar beet and its by-products and waste products using ultrasound and high-voltage electrical discharge. The following hypotheses were formulated on the basis of a detailed review of the research results available to date:

- non-thermal extraction techniques enable more efficient extraction of proteins and other bioactive compounds from sugar beet processing by-products and waste compared to conventional thermal extraction technique;
- in addition, the antioxidant activity of bioactive compounds varies depending on the used non-thermal extraction technique and its operating parameters

Based on the hypotheses put forward, the following research objectives were defined:

- to evaluate the effects of high-power ultrasound and high-voltage electrical discharge on the efficiency of extraction of proteins and other bioactive compounds from sugar beet processing by-products and waste;
- to determine antioxidant activity of extracts from sugar beet processing by-products and waste using high-power ultrasound and high-voltage electrical discharge.

In addition to the objectives pursued, the chemical composition of the plant raw materials (sugar beet processing by-products and waste) was investigated and the influence of the chemical composition on the thermal and kinetic properties was determined.

Scientific papers:

This chapter is consisted of 4 scientific papers that are systematically linked to each other. All the materials and methods used, the results obtained and the discussion on the research results presented are clearly described in each paper.

Paper no. 1:

Ostojić, S., Micić, D., **Dukić, J**., Sabljak, I., Akyüz, A., Ersus, S., Režek Jambrak, A. (2025) Thermal Characteristics and Kinetics of the Thermal Degradation of Sugar Beet Waste Leaves and Pulp in Relation to Chemical Composition. *Foods*. **14**, 307, doi: 10.3390/foods14020307.

The aim of this paper was the thermal characterization of dried sugar beet pulp, pellets, leaves and two fractions of sugar beet leaves as well as the determination of the kinetic parameters of their thermal degradation in order to gain a deeper insight into the thermal degradation process. The thermal properties of the mentioned materials were analysed by differential scanning calorimetry and thermogravimetry, and the kinetics of thermal degradation was investigated by thermogravimetric analysis. Four degradation phases in a temperature range from 25 to 700 °C were observed in the aforementioned investigations. The kinetic parameters of degradation, which were determined by the non-isothermal isoconversion method of Ortega and Friedman, did not differ significantly between the models. The highest constant degradation rate was determined for sugar beet leaf samples. The results obtained are of exceptional importance for the potential future industrial processing (scale-up) of sugar beet leaf waste. In relation to the dissertation topic proposal,

this paper extended the research objectives that provided a better insight into the chemical properties and their influence on the thermal kinetic properties of by-products and waste remaining after sugar beet processing.

Paper no. 2:

Dukić, J., Hunić, M., Nutrizio, M., Režek Jambrak, A. (2022) Influence of High-Power Ultrasound on Yield of Proteins and Specialized Plant Metabolites from Sugar Beet Leaves (*Beta vulgaris* subsp. *vulgaris* var. *altissima*). *Appl. Sci.* **12**, 8949. doi: 10.3390/app12188949.

The aim of this paper was to investigate and define the influence of high-power ultrasound on the physicochemical parameters of sugar beet leaf extract. The results obtained with ultrasound were compared with the conventional thermal extraction technique. The comparison of the two extraction techniques was based on the yield of proteins and phenolic compounds, energy consumption, power consumption, CO_2 emissions and changes in pH and electrical conductivity. Compared to the thermal extraction technique, ultrasound-assisted extraction led to a higher yield of proteins and specialized plant metabolites with significantly lower energy consumption and CO_2 emissions. The obtained results are in line with sustainable development goals. In addition, this paper confirmed the 1st hypothesis "Non-thermal extraction techniques enable more efficient extraction of proteins and other bioactive compounds from sugar beet processing by-products and waste compared to conventional thermal extraction technique".

Paper no. 3:

Dukić, J., Košpić, K., Kelava, V., Mavrić, R., Nutrizio, M., Balen, B., Butorac, A., Halil Öztop, M., Režek Jambrak, A. (2023) Alternative methods for RuBisCO extraction from sugar beet waste: A comparative approach of ultrasound and high voltage electrical discharge. *Ultrason. Sonochem*., **99**, 106535. doi: 10.1016/J.ULTSONCH.2023.106535.

The aim of this paper was to achieve a high yield of RuBisCO enzyme, which can then be used as a source of bioactive peptides in the production of functional foods. The focus was on sustainability, the use of industrial waste as a protein source, combined with non-thermal extraction techniques with low environmental impact (high-power ultrasound and high-voltage electrical discharge). Compared to high-voltage electrical discharge, high-power ultrasound proved to be a better alternative for the extraction of total soluble proteins (with a focus on the RuBisCO enzyme). By optimizing the parameters of the aforementioned extraction techniques, it is possible to influence the solubility and improve the isolation of the RuBisCO enzyme, which could then be purified and implemented in new or existing functional products. This paper also confirmed the 1st hypothesis "Non-thermal extraction techniques enable more efficient extraction of proteins and other bioactive compounds from sugar beet processing by-products and waste compared to conventional thermal extraction techniques".

Paper no. 4:

Dukić, J., Režek Jambrak, A., Jurec, J., Merunka, D., Valić, S., Radičić, R., Krstulović, N., Nutrizio, M., Dubrović, I. (2025) High-Power Ultrasound and High-Voltage Electrical Discharge-Assisted Extractions of Bioactive Compounds from Sugar Beet (*Beta vulgaris* L.) Waste: Electron Spin Resonance and Optical Emission Spectroscopy Analysis. *Molecules*, **30**(4), 796, doi:10.3390/molecules30040796.

The aim of this paper was to investigate and evaluate the effectiveness of high-power ultrasound and high-voltage electrical discharge on the extraction of bioactive components with antioxidant capacity from sugar beet leaves by varying the extraction solvent, applied amplitude and treatment time for ultrasound-assisted extraction and the extraction solvent, applied gas, applied voltage and treatment time for high-voltage electrical discharge-assisted extraction. The focus of the paper was on sustainability and use of industrial waste as a source of bioactive compounds in combination with non-thermal extraction techniques with low environmental impact. Ultrasound-assisted extraction proved to be an excellent method for the extraction of the total phenolic compounds, and high-voltage electrical

discharge-assisted extraction proved to be an excellent method for the extraction of the flavonoid compound - Vitexin. In addition, the results of the antioxidant capacity measurements were shown to correlate with each other, and the highest values were found in the samples treated with ultrasound. The characterization of the plasma by optical emission spectroscopy confirmed that neither the solvent nor the sample influenced the plasma spectra, but only the gas used (nitrogen/argon). All the results obtained provide an excellent basis for future research into the utilization of food waste and by-products. This paper confirms both hypotheses.

General discussion:

In the general discussion, the published papers are combined into a meaningful whole. The discussion is divided into three parts: (i) chemical composition of samples and their influence on thermal and kinetic properties, (ii) Optimization of protein yield from sugar beet processing by-products and waste, and (iii) optimization of Vitexin yield and antioxidant activity from sugar beet processing by-products and waste. Through the mentioned chapters, the results of the conducted research are clearly and argumentatively interpreted and compared with relevant scientific papers. In addition, the results from the attached scientific papers were further expanded and compared with new and unpublished results. Also, the scientific contribution of this dissertation was highlighted through the discussion.

Conclusions:

This chapter summarizes the most important conclusions and findings of the conducted research. It was concluded that non-thermal extraction techniques, high-power ultrasound and high-voltage electrical discharge, were successfully applied and optimized for the isolation of proteins and other bioactive compounds from sugar beet processing by-products and waste. It was also concluded that the aforementioned non-thermal techniques enable higher yields of target compounds and ensure higher antioxidant activity compared to conventional thermal extraction techniques.

References:

The literature cited in the doctoral thesis is from the research area of the doctoral thesis and includes the latest knowledge and modern methodology used during the preparation of this thesis.

In conclusion, the doctoral thesis confirms the set hypotheses, and clearly and argumentatively justifies and substantiates the objectives of the research. It is important to emphasize that this thesis represents a significant scientific contribution to the development and optimization of non-thermal extraction techniques, specifically high-power ultrasound and high-voltage electrical discharge, which in combination with green solvents contribute to higher extraction yields compared to conventional extraction techniques.

Opinion and proposal:

Based on the above, the commission considers that the dissertation of applicant Josipa Dukić, M.Sc. is made according to the set hypotheses, goals and explanation of the applied topic. The applicant presented the results systematically and clearly, and based on the discussion, reached logical conclusions. The conducted research provides new insights into the possibilities of extraction using high-power ultrasound and high-voltage electrical discharge and brings new knowledge about the sustainability of the aforementioned techniques. The research has provided a better understanding of extraction parameters for the yield, composition and antioxidant activity of compounds isolated from sugar beet processing by-products and waste. Statistical analysis of the data clearly revealed the optimal conditions for each extraction technique. The research carried out clearly demonstrates how the linear concept of the economy can be transformed into a circular form through the valorization and reuse of waste and by-products from the food industry.

Based on all of the above, we propose to the Faculty Council of the University of Zagreb, Faculty of Food and Biotechnology, that the doctoral thesis of Josipa Dukić, M.Sc. under the title " Utilization of by-products and waste from sugar beet (*Beta vulgaris* L.) processing

by non-thermal extraction techniques" ("Iskorištavanje nusproizvoda i otpada iz prerade šećerne repe (*Beta vulgaris* L.) primjenom netoplinskih tehnika ekstrakcije") to be accepted and referred to the further procedure for acquisition academic degree of Doctor of Science from the scientific field of Biotechnical Sciences, scientific field of Food Technology.

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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, Full Professor, PhD, University of Zagreb Faculty of Food Technology and Biotechnology, Croatia *(Chair of the Committee)*	
	2. Tomislav Bosiljkov, Full Professor, PhD, University of Zagreb Faculty of Food Technology and Biotechnology, Croatia	
	3. Ana Butorac, PhD, Selvita d.o.o., Zagreb, Croatia	
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In Zagreb, on April 4th,2025
(place and date)

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