DR.SC.-10 REPORT ON DISSERTATION EVALUATION[1]			
GENERAL INFORMATION AND PERSONAL CONTACT INFORMATION OF THE DOCTORAL CANDIDATE			
First and last name, and title of the doctoral candidate:		Anica Bebek Markovinović, MSc	
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:		425/PT	
Dissertation title in:	*language of the dissertation:*	English	
	Croatian:	Primjena tehnologije preprekama i 3D ispisa u razvoju funkcionalne hrane na bazi jagode	
	English:	Application of hurdle technology and 3D printing in the development of strawberry-based functional food	
Area/field/branch (if the doctoral study is performed in a branch)**:**		Biotechnology / Food Technology / Engineering	
MENTOR(S)			
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DISSERTATION EVALUATION (evaluation must specify original scholarly/scientific contribution and new finding)			

[1] Please send the filled -out form DR.SC.-10, in electronic and written format, and signed, to the appropriate Registrar's Office

The dissertation by MSc Anica Bebek Markovinović was conducted at the Laboratory of Chemistry and Technology of Fruits and Vegetables, Faculty of Food Technology and Biotechnology, University of Zagreb, under supervision of Associate Professor PhD Danijela Bursać Kovačević.

The dissertation follows the "Scandinavian model" and is based on 10 published scientific papers. It spans 275 pages and includes 8 tables, 163 references, a basic documentation card in both Croatian and English, an extended summary in Croatian and English, and the curriculum vitae of the candidate and the supervisor. The dissertation is divided into the following chapters: Introduction, Theoretical Part, General Discussion, Conclusions and Perspectives, and Literature, with the published scientific papers attached as separate units. The papers on which the dissertation is based have been published in renowned journals such as Current Opinion in Food Science, Horticulturae, Foods, Molecules, Antioxidants and Food Chemistry, with the author holding first authorship on 9 out of the 10 papers included in the dissertation.

Introduction

The introductory section highlights the growing trend toward increased fruit and vegetables consumption, driven by their association with a healthy lifestyle and the prevention of chronic diseases. This shift has spurred significant interest in the food industry to produce functional foods, particularly fruit juices, which can effectively meet daily nutritional needs. Among these, strawberries (Fragaria x ananassa Duch.) stand out as a prime candidate for functional juice production, given their rich polyphenolic content and demonstrated physiological benefits. Traditionally, fruit juices are preserved using thermal processes to extend shelf life. However, these methods have a negative effect on the bioactive compounds and ultimately reduce the biological value of the product. Therefore, it is crucial to explore new non-thermal technologies, such as pulsed electric field, high-power ultrasound, and high hydrostatic pressure, to maintain the health benefits of the product during storage while preserving its bioactive components. A key focus is on investigating hurdle technologies, where a combination of non-thermal and/or thermal methods are applied in a specific sequence under milder processing conditions than when applied individually. This approach aims to retain the original product quality while extending shelf life. The principles of sustainable non-thermal technologies like pulsed electric field and high-power ultrasound are outlined, as well as the application of 3D printing technology in the development of functional fruit-based products. Finally, the research objectives of the dissertation are clearly formulated. They focus on optimizing the process parameters for hurdle technologies that combine pulsed electric fields and high-power ultrasound in the processing of strawberry juice. Additionally, this study explores the optimization of 3D printing parameters for producing functional 3D strawberry-based products, enhanced with Arbutus unedo L.

Theoretical part

The theoretical section of the dissertation delves into the significance of the strawberries as a valuable fruit variety for functional juice production. The technology of pulsed electric field and high-power ultrasound are discussed in detail, highlighting their potential applications with hurdle technology frameworks. Additionally, an overview is provided on the importance of 3D printing technology in food production, focusing on 3D printing processes and the necessary conditions for raw materials to be suitable for printing. To give a more detailed understanding of 3D printing in the production of fruit-based functional products, a scientific review article is included as a part of this chapter (Publication No. 1: Tomašević, I., Putnik, P., Valjak, F., Pavlić, B., Šojić, B., Bebek Markovinović, A., Bursać Kovačević, D. (2021) 3D printing as a novel tool for fruit-based functional food production. Current Opinion in Food Science, 41, 138–145. doi: 10.1016 /j.cofs.2021.03.015), in which the applicant is one of the co-authors. Considering that 3D printing of fruit-based products often requires the addition of thickening agents due to the physicochemical properties of the fruits, the next subsection describes Arbutus unedo L. (strawberry tree) fruits. This part explores the potential of strawberry tree fruits for use in functional food

production. To offer concise insights, another scientific paper is attached (Publication No. 2.: Bebek Markovinović, A., Brčić Karačonji, I., Jurica, K., Lasić, D., Skendrović Babojelić, M., Duralija, B., Šic Žlabur, J., Putnik, P., Bursać Kovačević, D. (2022) Strawberry Tree Fruits and Leaves (Arbutus unedo L.) as Raw Material for Sustainable Functional Food Processing: A Review. Horticulturae, 8, 881. doi: 10.3390/horticulturae8100881), where the applicant is the first author. The theoretical section concludes by clearly outlining the dissertation's hypotheses, objectives, research questions, anticipated results, and scientific contributions. Each of the remaining works on which the dissertation is based is included as a separate chapter, systematically linked. All materials and methods, results, discussions, and conclusions are clearly presented in each publication.

Publication No. 3:

Bebek Markovinović, A., Putnik, P., Duralija, B., Krivohlavek, A., Ivešić, M., Mandić Andačić, I., Palac Bešlić, I., Pavlić, B., Manuel Lorenzo, J., Bursać Kovačević, D. (2022) Chemometric Valorization of Strawberry (Fragaria x ananassa Duch.) cv. 'Albion' for the Production of Functional Juice: The Impact of Physicochemical, Toxicological, Sensory, and Bioactive Value. Foods, 11 (5), 640. doi: 10.3390/foods11050640.

The aim of this study was to investigate the potential of strawberries (Fragaria x ananassa Duch. cv. 'Albion'), harvested at two different ripening stages (75% and 100% ripeness), for juice production. Additionally, the study examined the physicochemical, sensory, toxicological and biological properties of the raw materials, the produced juices, and by-products left after juice production. The findings revealed that both ripeness level and storage conditions (4 °C/3 days) significantly influenced the quality of strawberries intended for processing. Careful control of these factors is necessary to ensure the production of high-quality, functional strawberry juice. Furthermore, the study confirmed that strawberries and their juices are safe for consumption. Interestingly, sensory evaluations indicated that strawberries, regardless of ripeness, and their corresponding juices were rated similarly in all sensory attributes, suggesting that the juicing process does not significantly affect sensory quality. Moreover, compared to fresh strawberries and juices, the by-products exhibited the highest biological potential. A chemometric analysis of all quality parameters confirmed that strawberries at 75% ripeness are also suitable for processing into functional juice, offering flexibility in harvest timing.

Publication No. 4:

Bebek Markovinović, A., Putnik, P., Stulić, V., Batur, L., Duralija, B., Pavlić, B., Vukušić Pavičić, T., Herceg, Z., Bursać Kovačević, D. (2022) The Application and Optimization of HIPEF Technology in the Processing of Juice from Strawberries Harvested at Two Stages of Ripeness. Foods, 11, 1997. doi: 10.3390/foods11141997.

The aim of this work was to investigate the effects of high-intensity pulsed electric field technology (HIPEF) on the stability of total phenols, anthocyanins, hydroxycinnamic acids, flavonols and condensed tannins in strawberry juices (Fragaria x ananassa Duch. cv. 'Albion') made from fruits harvested at different ripeness levels (75% and 100%) and stored at 4 °C for 7 days. HIPEF parameters included electric field strengths of 40 and 50 kV cm^{-1}, frequencies of 100 and 200 Hz, and treatment durations of 3 and 6 minutes. The results demonstrated that both the strength and frequency of the electric field had statistically significant effects on the content of all phenolic compounds. Conversely, treatment duration showed no significant effect on phenolic compounds except for flavonols and condensed tannins. Interestingly, storage had a favorable impact on the stability of most phenolic compounds, with the exception of flavonols. The optimization of HIPEF parameters showed that strawberry samples of both ripening stages are suitable for HIPEF treatment to produce functional fruit juices with a high content of polyphenols.

Publication No. 5:

Bebek Markovinović, A., Putnik, P., Bičanić, P., Brdar, D., Duralija, B., Pavlić, B., Milošević, S., Rochetti, G., Lucini, L., Bursać

Kovačević, D (2022) A Chemometric Investigation on the Functional Potential in High Power Ultrasound (HPU) Processed Strawberry Juice Made from Fruits Harvested at Two Stages of Ripeness. Molecules, 28 (1), 138. doi: 10.3390/molecules28010138.

This study aimed to evaluate the influence of high-power ultrasound (HPU) technology on the stability of bioactive compounds in strawberry juices obtained from fruits at different ripeness levels (75% vs. 100%) and stored at 4°C for 7 days. The HPU parameters tested were amplitude (25, 50, 75, and 100%), pulse (50% and 100%), and treatment duration (5 vs. 10 minutes). The findings revealed that amplitude and pulse significantly influenced the content of most bioactive compounds, except for flavonols and hydroxycinnamic acids. A shorter treatment duration of 5 minutes had a statistically significant positive effect on anthocyanins, flavonols, and condensed tannins, while a longer duration of 10 minutes was more beneficial for total phenolic content. No significant effect was observed on hydroxycinnamic acids. Additionally, temperature changes during HPU treatment positively correlated with nearly all HPU parameters. These results suggest that combining strawberries at a specific ripeness level with optimal HPU settings can result in juices with highly preserved bioactive compounds, enhancing their functional properties.

Publication No. 6:

Bebek Markovinović, A., Stulić, V., Putnik, P., Birkić, A., Jambrović, M., Šaško, D., Ljubičić, J., Pavlić, B., Herceg, Z., Bursać Kovačević, D (2023) Pulsed Electric Field (PEF) and High-Power Ultrasound (HPU) in the Hurdle Concept for the Preservation of Antioxidant Bioactive Compounds of Strawberry Juice—A Chemometric Evaluation—Part I. Foods, 12 (17), 3172. doi : 10.3390/foods12173172.

This study aimed to explore the effects of combining pulsed electric field (PEF) and high-power ultrasound (HPU) within the framework of hurdle technology on the bioactive compounds and antioxidant capacity of strawberry juice stored at 4°C for 7 days. PEF treatment was conducted at 30 kV cm-1, 100 Hz, for 1.5, 3, and 4.5 minutes, while HPU was applied with an amplitude of 25%, a pulse of 50%, and treatment times of 2.5, 5.0, and 7.5 minutes. The findings indicated that total phenols and hydroxycinnamic acids were the most stable bioactive compounds throughout the PEF + HPU treatments, independent of treatment duration. In contrast, flavonols and condensed tannins showed significant variation based on the length of both treatments. The study concluded that shorter combined treatments of PEF + HPU enhanced the stability of bioactive compounds and improved antioxidant capacity in strawberry juice.

Publication No. 7:

Bebek Markovinović, A., Stulić, V., Putnik, P., Bekavac, N., Pavlić, B., Milošević, S., Velebit, B., Herceg, Z., Bursać Kovačević, D. (2024) High -Power Ultrasound (HPU) and Pulsed Electric Field (PEF) in the Hurdle Concept for the Preservation of Antioxidant Bioactive Compounds in Strawberry Juice—A Chemometric Evaluation—Part II. Foods, 13 (4), 537. doi: 10.3390/foods13040537.

The aim of this study was to assess the impact of combining high-power ultrasound (HPU) and pulsed electric field (PEF) technologies on the bioactive compound content and antioxidant capacity of strawberry juices stored at 4°C for 7 days. HPU was applied at an amplitude of 25%, pulse of 50%, and treatment times of 2.5, 5.0, and 7.5 minutes, while PEF was conducted at 30 kV cm-1, 100 Hz, for 1.5, 3, and 4.5 minutes. The results demonstrated that juices treated with the HPU + PEF combination and stored at 4°C for 7 days exhibited a statistically significant reduction in all bioactive compounds and antioxidant capacity compared to untreated control samples. Shorter HPU + PEF treatment times were associated with greater bioactive compound stability. Specifically, optimizing the process parameters revealed that a shorter HPU treatment duration of 2.5 minutes provided the best preservation of total phenols, flavonols, condensed tannins, and antioxidant capacity. These findings suggest that shorter treatment durations in the HPU + PEF combination can better maintain the functional qualities of strawberry juice, making this approach viable for producing high-quality, nutrient-rich fruit juices.

Publication No. 8:

Bebek Markovinović, A., Putnik, P., Bosiljkov, T., Kostelac, D., Frece, J., Markov, K., Žigolić, A., Kaurinović, J., Pavlić, B., Duralija, B., Zavadlav, S., Bursać Kovačević, D. (2023) 3D Printing of Functional Strawberry Snacks: Food Design, Texture, Antioxidant Bioactive Compounds, and Microbial Stability. Antioxidants, 12 (2), 436. doi: 10.3390/antiox12020436.

The aim of this work was to apply 3D printing technology to create functional strawberry-based 3D printed products using starch carrier (wheat and corn) in varying proportions (10%, 15% and 20%) and two different 3D printing programs (Program 1 and Program 2). The research examined how process parameters (type and proportion of starch carrier and printing program) affected the physicochemical and textural properties, bioactive compound content, antioxidant potential, and microbiological stability of the printed products. Additionally, the study explored the impact of adding natural antimicrobial agents, such as citral and vanillin.

The results showed that the type of starch significantly influenced all tested bioactive compounds as well as starch content, except for total phenolic compounds and hydroxycinnamic acids. In terms of bioactive compound content and antioxidant capacity, Program 2 proved to be more effective than Program 1. All samples exhibited good textural properties, high stability, and minimal geometric deviations. Regarding microbiological safety, no pathogenic bacteria were found in the 3D-printed products after 10 days of storage at 4°C. The 3D-printed sample containing citral at a concentration of 75 mg L-1 demonstrated the best microbiological quality.

Publication No. 9:

Bebek Markovinović, A., Brdar, D., Putnik, P., Bosiljkov, T., Durgo, K., Huđek Turković, A., Brčić Karačonji, I., Jurica, K., Pavlić, B., Granato, D., Bursać Kovačević, D. (2024) Strawberry tree fruits (Arbutus unedo L.): Bioactive composition, cellular antioxidant activity and 3D printing of functional foods. Food Chemistry, 433, 137287. doi: 10.1016/j.foodchem.2023.137287.

The aim of this work was to use 3D printing technology to create functional 3D products based on Arbutus unedo L. by incorporating a starch carrier (wheat and corn) in varying proportions (4%, 6% and 8%) across two different printing programs (Program 1 and Program 2). The study investigated the impact of process parameters, including starch type and proportion, and printing program, on the physicochemical and textural properties, as well as on the bioactive and antioxidant potential of the products. The findings indicated that both the starch type and proportion, along with the selected printing program, had significant effects on the physicochemical properties, bioactive composition, and antioxidant potential of the 3D-printed products. Additionally, the rheological properties (textural properties, particle size distribution) of the products were influenced by these process parameters. The optimal yield of bioactive compounds was observed in samples containing 4% and 6% starch, depending on the specific group of bioactive compounds analyzed.

Publication No. 10:

Bebek Markovinović, A., Bosiljkov, T., Janči, T., Kostić, M., Dedović, N., Lučić, E., Bavrka, K., Pavlić, B., Bursać Kovačević, D. (2024) Characterization of Antioxidant Bioactive Compounds and Rheological, Color and Sensory Properties in 3D-Printed Fruit Snacks. Foods, 13, 1623. doi: 10.3390/foods13111623.

The aim of this work was to use 3D printing technology to print functional 3D products based on strawberries and strawberry tree fruits with the addition of wheat starch carrier in proportions of 6%, 8% and 10% using two 3D printing programs (Program 1 and Program 2) and to investigate their effects on the content of bioactive compounds, antioxidant capacity, color parameters, rheological and sensory properties. The findings revealed that increasing the starch content led to a reduction in the concentration of nearly all bioactive compounds, though this did not negatively impact antioxidant capacity. The choice of 3D printing program did not significantly affect the levels of bioactive compounds (except for hydroxycinnamic acids), antioxidant capacity, or color parameters. A

higher starch content had no effect on the textural properties, but it did have an effect on the particle size distribution parameters. For sensory evaluation, the 3D-printed product with the highest bioactive potential was chosen. The study also tested the effects of eight different sweeteners at two concentrations to assess product acceptability in the context of personalized diets. The results indicated that only variations in sweetener content affected the sensory attributes of sweetness and taste harmony.

General discussion

The general discussion section of this dissertation is divided into three parts: (i) Investigation of strawberry quality and juice processing: This section focuses on the ripeness levels of strawberries (75% and 100%) and their impact on juice quality. It highlights the differences in the quality of the produced juice compared to by-products. It also emphasizes the importance of proper control over ripeness and storage conditions to produce high-quality functional strawberry juice; (ii) Effects of non-thermal technologies (PEF and HPU): The section details how pulsed electric field (PEF) and high-power ultrasound (HPU) technologies influence the stability of bioactive compounds in strawberry juices. The chapter highlights that optimizing these technologies can significantly improve the preservation of bioactive compounds and antioxidant capacity. It further explains how a combination of PEF and HPU (hurdle technology) enhances juice stability and nutrient retention; (iii) 3D printing technology for strawberry-based functional foods: This part explores the role of 3D printing in creating functional food products using strawberries and strawberry tree fruits. The results show how the starch content in the food material and printing parameters affect the yield of bioactive compounds and product stability. Optimal parameters (e.g., 6% wheat starch) were identified for producing 3D-printed foods with high bioactive compound content.

Conclusions and perspectives:

This chapter summarizes the main conclusions of the research. It was found that strawberry fruits at both ripeness levels (75% and 100%) are suitable for the production of functional fruit juices, with significant differences in quality between the juices and by-products. The process parameters of pulsed electric field (PEF) and high-power ultrasound (HPU) were shown to significantly influence the stability of most tested bioactive compounds in strawberry juices. Through the optimization of the results from the individually applied non-thermal technologies, optimal process parameters were determined, and hurdle technology was employed by combining PEF + HPU and HPU + PEF. It was discovered that, in the PEF + HPU combination, shorter PEF treatment times and longer HPU treatment times resulted in greater bioactive compound stability and higher antioxidant capacity in strawberry juices. Conversely, the optimization of HPU + PEF parameters showed that shorter treatment times for both technologies led to better preservation of bioactive compounds. In the final part of the study, focusing on 3D printing for producing functional 3D products from strawberries and strawberry tree fruits, it was found that wheat starch content significantly affected the yield of bioactive compounds and the preservation of antioxidant capacity in the printed products. The highest yield of bioactive compounds in the 3D-printed products was achieved with the addition of 6% wheat starch. The texture analysis showed that all 3D-printed products exhibited good textural properties, a high degree of stability and minimal geometric deviations. At the conclusion of this chapter, the dissertation emphasizes the significant scientific contribution in enhancing the understanding of the influence of non-thermal technologies (PEF and HPU) in hurdle technology, as well as the impact of additive 3D printing on the quality and stability of strawberry and strawberry tree fruit-based functional products.

Literature:

The literature used in the preparation of this dissertation relates to the research areas of this thesis and includes the latest findings in the field.

In conclusion, the dissertation confirms the hypotheses put forward and clearly and argumentatively substantiates and underpins the objectives of the research.

Opinion and recommendation:		
The Commission has concluded that Anica Bebek Markovinović's dissertation has been thoroughly prepared in alignment with the proposed hypotheses, objectives, and the overall research topic. The applicant systematically and clearly presented her results, drawing logical conclusions based on thorough discussions.The research introduces valuable new insights into the impact of non-thermal technologies, specifically the pulsed electric field (PEF) and high-power ultrasound (HPU), both applied individually and in combination as part of hurdle technology. These techniques were shown to influence the physicochemical properties, bioactive compound content, and antioxidant activity in strawberry juices. The optimal process parameters for these non-thermal technologies were defined, both when applied individually and in combination (PEF + HPU and HPU + PEF), to maximize the bioactive compound content and antioxidant activity in strawberry juices. Moreover, the research deepens our understanding of the impact of 3D printing parameters, such as the type and proportion of starch, as well as the 3D printing program on the content of bioactive compounds, antioxidant capacity, color parameters, and rheological properties in 3D-printed functional products made from strawberries, strawberry tree fruits, and combinations of these raw materials. The optimal parameters for 3D printing were determined, aiming to produce 3D-printed functional products with maximum bioactive compounds and antioxidant capacity. Based on this assessment, the Commission recommends that the Faculty Council of the University of Zagreb, Faculty of Food Technology and Biotechnology, approve Anica Bebek Markovinović's doctoral thesis, titled "Application of hurdle technology and 3D printing in the development of strawberry-based functional food," and advance it for the awarding of the academic degree of Doctor of Science in the field of Biotechnical Sciences, filed of Food Technology.		
The committee appointed for dissertation evaluation	**First and last name, title, institution, country:**	**Signature:**
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Session of the competent body at which the Committee was appointed, and number of item on the agenda:	11st regular session of the Faculty Council of the Faculty of Food Technology and Biotechnology in Zagreb in the academic year 2024/2025 held on September 25th, 2024 (ad 33).	
Comment (as needed):		
Zagreb, 10.10.2024.		**Official stamp here**