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Naziv studija: Poslijediplomski sveučilišni (doktorski) studij Biotehnologija i bioprocesno inženjerstvo, prehrambena tehnologija i nutricionizam
Matični broj: 350/PT
Akademska godina upisa: 2017./2018.

Zagreb, 7. listopada 2024.

Fakultetskom vijeću
Sveučilišta u Zagrebu Prehrambeno-biotehnološkog fakulteta
-putem Odbora za poslijediplomske studije-

Predmet: Zahtjev za promjenu jezika pisanja i obrane doktorskog rada Nine Marčac Duraković, mag. ing. biotechn.

Molim Fakultetsko vijeće da mi odobri promjenu jezika pisanja i obrane doktorskog rada s hrvatskog u engleski jezik, u okviru doktorskog studija Biotehnologija i bioprocesno inženjerstvo, prehrambena tehnologija i nutricionizam, naslova „Optimiranje postupaka izolacije i inkapsulacije eteričnoga ulja komorača (*Foeniculum vulgare* Mill.)“ („Optimization of isolation and encapsulation techniques of fennel (*Foeniculum vulgare* Mill.) essential oil“) u cilju pisanja doktorskog rada prema skandinavskom modelu s obzirom su objavljena 3 originalna znanstvena rada iz područja koja odgovaraju temi prijave doktorskog rada, na kojima sam koautor (prvi autor na dva rada).

Molbi prilažem:
· Odluku Senata Sveučilišta u Zagrebu o odobravanju pokretanja postupka stjecanja doktorata znanosti
· DRSC01 – PRIJAVA TEME DOKTORSKOG RADA
· DRSC02 – OCJENA TEME DOKTORSKOG RADA
· DRCS03 – ODLUKA O ODOBRAVANJU TEME DOKTORSKOG RADA
· DRSC01 – REQUEST FOR APPROVAL OF THE DISSERTATION TOPIC
· DRSC02 – EVALUATION OF THE DISSERTATION TOPIC
· DRCS03 – DECISION ON APPROVAL ON DISSERTATION TOPIC
· Kopija prve strane objavljenih radova
· Izvadak iz matice vjenčanih

Podnositelj zahtjeva:

Nina Marčac Duraković, mag. ing. biotechn.

Suglasna:

izv. prof. dr. sc. Maja Repajić - mentor

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:	Nina Marčac Duraković, M.Sc.		
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:	350/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:	Josip Marčac		
Date and place of birth:	27/10/1990, Karlovac, Croatia		
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CURRICULUM VITAE OF THE DOCTORAL CANDIDATE

Education *(in chronological order, with most recent first)*:	1. University of Zagreb Faculty of Food Technology and Biotechnology, *Molecular Biotechnology*, graduate programme, Croatia (2014/2015) 2. University of Zagreb Faculty of Food Technology and Biotechnology, *Food Technology*, undergraduate programme, Croatia (2011/2012)
Work experience *(in chronological order, with most recent first)*:	**Independent supplier of essential oils, fragrances, aromas and animal aromas, oenological and beer products** (May 1, 2017 to date) Ireks Aroma d.o.o., Trešnjevka 24, Jastrebarsko **Analytical laboratory Assistant** (July 11, 2016-May 1, 2017) Magdis d.o.o., Ul. Augusta Šenoe 37, Mala Gorica **Assistant** (October 21, 2016-September 1, 2017) Karlovac University of Applied Sciences, Department of Food Technology **Assistant** (December 10, 2015 – July 11, 2016) Microbiological laboratory of the Institute of Public Health, Department of Microbial Ecology
Bibliography and active participation in conferences:	*Articles* 1. Repajić, M., Elez Garofulić, I., **Marčac Duraković, N.**, Balun, M., Cegledi, K., Cegledi, E., Dobroslavić, E., Dragović-Uzelac, V. (2024) Physico-Chemical Characterization of Encapsulated Fennel Essential Oil under the Influence of Spray-Drying Conditions. Processes, 12 (3), 577. doi: 10.3390/pr12030577 2. **Marčac Duraković, N.**, Cegledi, E., Elez Garofulić, I., Balbino, S., Pedisić, S., Jokić, S., Dragović-Uzelac, V., Repajić, M. (2024) Recovery of Fennel Non-Polar Bioactives via Supercritical Carbon Dioxide Extraction. Processes, 12(8); 1764. doi: 10.3390/pr12081764

[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.

	3. **Marčac, N.,** Balbino, S., Tonković, P., Medved, A. M., Cegledi, E., Dragović, S., Dragović-Uzelac, V., Repajić, M. (2023) Hydrodistillation and Steam Distillation of Fennel Seeds Essential Oil: Parameter Optimization and Application of Cryomilling Pretreatment. Processes, 11(8), 2354. doi: 10.3390/pr11082354 4. Blažić, M., Pavić, K., Zavadlav, S., **Marčac, N.** (2017) The impact of traditional cheeses and whey on health. Croatian Journal of Food Science and Technology, 9 (2), 198-203. doi:10.17508/CJFST.2017.9.2.11 *Participation in conferences* 1. Cegledi, E., Repajić, M., **Marčac, N.**, Elez Garofulić, I., Cegledi, K., Dobroslavić, E., Dragović-Uzelac, V. (2022) Influence of temperature and carrier ratio on fennel essential oil powder obtained by spray drying. Book of Abstracts of the 10th International Congress of Food Technologists, Biotechnologists and Nutritionists, Zagreb, Croatia, 30th November-2nd December, 11-12. 2. Cegledi, E., Repajić, M., **Marčac, N.**, Elez Garofulić, I., Balun, M., Dobroslavić, E., Dragović-Uzelac, V. (2022) Encapsulation of fennel essential oil by spray drying. Book of Abstracts of the 19th Ružička days, Vukovar, Croatia, September 21st-23rd, 94. 3. Repajić, M., Cegledi, E., **Marčac, N.**, Balbino, S., Elez Garofulić, I., Levaj, B., Dragović-Uzelac, V. (2022) Isolation of fennel essential oil by conventional and advanced extraction techniques. Book of Abstracts of the 4th International scientific and expert conference "Natural resources, green technology and sustainable development/4-GREEN2022", Zagreb, Croatia, September 14th-16th, 103. 4. Repajić, M., Cegledi, E., Palčić, I., **Marčac, N.**, Dragović-Uzelac, V. (2021) Essential oils in wild Istrian fennel (*Foeniculum vulgare* Mill.): Variability in the content and composition. Book of Abstracts of the 13th International Scientific and Professional Conference WITH FOOD TO HEALTH, Osijek, Croatia, September 16-17th, 134.

TITLE OF THE PROPOSED TOPIC

Croatian:	Optimiranje postupaka izolacije i inkapsulacije eteričnoga ulja komorača (*Foeniculum vulgare* Mill.)
English:	Optimization of isolation and encapsulation techniques of fennel (*Foeniculum vulgare* Mill.) essential oil
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/Food Technology/Engineering

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:	Maja Repajić, PhD, Associate Professor	University of Zagreb Faculty of Food Technology and Biotechnology, Croatia	maja.repajic@pbf.unizg.hr
Second mentor:	/		

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

First mentor:	1. Maleš, I., Dragović-Uzelac, V., Jerković, I., Zorić, Z., Pedisić, S., **Repajić, M.**, Elez Garofulić, I.,Dobrinčić, A. (2022) Non-Volatile and Volatile Bioactives of *Salvia officinalis* L., *Thymusserpyllum* L. and *Laurus nobilis* L. Extracts with Potential Use in the Development of FunctionalBeverages. Antioxidants, 11(6), 1140. doi:10.3390/antiox11061140

2. Odeh, D., Oršolić, N., Berendika, M., Đikić, D., Domjanić Drozdek, S., Balbino, S., **Repajić, M.**,Dragović-Uzelac, V., Landeka Jurčević, I. (2022) Antioxidant and anti-atherogenic activities ofessential oils from *Myrtus communis* L. and *Laurus nobilis* L. in rat. Nutrients, 14(7), 1465. doi:10.3390/nu14071465

3. Malin, V., Elez Garofulić, I., **Repajić, M.**, Zorić, Z., Pedisić, S., Sterniša, M., Smole Možina, S.,Dragović-Uzelac, V. (2022) Phenolic characterization and bioactivity of fennel seed (*Foeniculum vulgare* Mill.) extracts isolated by microwave-assisted and conventional extraction.Processes, 10(3), 510. doi: 10.3390/pr10030510

4. Balbino, S., **Repajić, M.**, Obranović, M., Medved, A. M., Tonković, P., Dragović-Uzelac, V.(2021) Characterization of lipid fraction of Apiaceae family seed spices: Impact of species and extraction method. Journal of Applied Research on Medicinal and Aromatic Plants, 25, 100326. doi: 10.1016/j.jarmap.2021.100326

5. **Repajić, M.**, Cegledi, E., Zorić, Z., Pedisić, S., Elez Garofulić, I., Radman, S., Palčić, I., Dragović-Uzelac, V. (2021) Bioactive Compounds in Wild Nettle (*Urtica dioica* L.) Leaves and Stalks: Polyphenols and Pigments upon Seasonal and Habitat Variations. Foods, 10(1), 190. doi: 10.3390/foods10010190

Second mentor:	/

TOPIC OUTLINE

Summary in Croatian *(no more than 1000 characters with spaces):*	Sjemenke komorača predstavljaju bogat izvor eteričnog ulja (ETU), ali i drugih biološki aktivnih spojeva (BAS). U svrhu poboljšanja učinkovitosti, nedostataka i ograničenja konvencionalnih metoda izolacije ETU poput vodene (VD) i parne destilacije (PD), sve je veći fokus u primjeni kriogene meljave (KM) kao predtretmana ili novijih metoda poput ekstrakcije superkritičnim CO_2 (SCO_2). Nadalje, za stabilizaciju ETU primjenjuju se različite tehnike inkapsulacije poput sušenja raspršivanjem. Stoga je cilj ovog istraživanja utvrditi utjecaj KM kao predtretmana u unaprijeđenju konvencionalnih metoda izolacije ETU sjemenki komorača (VD i PD) te utjecaj SCO_2 na prinos i BAS ETU sjemenki komorača. Također, u svrhu stabilizacije, zaštite i očuvanja BAS ETU sjemenki komorača optimirat će se postupak inkapsulacije metodom sušenja raspršivanjem te ispitati fizikalno-kemijska svojstva prahova s ciljem nadogradnje spoznaja o primjeni inkapsulacijskih tehnika u proizvodnji prahova ETU komorača.
Summary in English *(no more than 1000 characters with spaces):*	Fennel seeds are a rich source of essential oil (EO), but also of other biologically active compounds (BAC). To improve the efficacy, drawbacks, and limitations of conventional EO isolation methods such as hydro- (HD) and steam distillation (SD), there is an increasing focus on the use of cryogenic milling (CM) as a pretreatment or on newer methods, e.g., supercritical CO_2 extraction (SCO_2). Moreover, various encapsulation techniques such as spray drying are used to stabilize EO. Thus, the aim of this research is to determine the influence of CM as a pretreatment to improve the conventional isolation methods of fennel seeds EO (HD and SD) and the influence of SCO_2 on the yield and BAC of fennel seeds EO. To stabilize, protect and preserve BAC of fennel seeds EO, encapsulation using spray drying will be optimized and physicochemical properties of the powders will be tested to improve the knowledge on the application of encapsulation techniques in the production of fennel EO powders.

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

EO is a secondary plant metabolite that represents a mixture of various volatile compounds that carry a characteristic aroma and is also gaining importance as an alternative to synthetic chemicals due to its antioxidant and antimicrobial properties and its widespread use in the pharmaceutical and food industries. Among the many plant sources, fennel (Foeniculum vulgare Mill.) and its seeds are characterized by their high EO content. Fennel is a wild or cultivated, aromatic, perennial herb that belongs to the Apiaceae family (lat. Apiaceae) and is widely distributed (Badgujar et al., 2014). The proportion of fennel EO and its chemical composition may vary due to differences in cultivar (Sayed-Ahmad et al., 2017), growing conditions (Javed et al., 2020), geographical origin (Anka et al., 2020) and vegetation stage (Belabdelli et al., 2020). The most abundant volatile constituents of the EO of fennel seeds are trans-anethole, estragole, fenchone and limonene, and only the EO showed antioxidant, antimicrobial and anticancer activities (Ahmed et al., 2019; Ghasemian et al., 2020). In addition to volatile compounds, fennel seeds also contain other bioactive compounds (BAC) such as phenols, of which hydroxycinnamic acids and flavonols are the most abundant (Malin et al., 2022), fatty acids, of which oleic acid and petroselinic acid account for the largest proportion, and phytosterols such as β-sitosterol and stigmasterol (Balbino et al., 2021).

EO is usually isolated by conventional methods such as hydrodistillation (HD) and steam distillation (SD). In HD, the material is emerged directly into water and heated to boiling, releasing EO from the oil glands of the plant tissue and vaporizing volatile components whose property of hydrophobicity leads to separation into the oil phase during condensation. For this type of plant material, the effectiveness of HD depends mainly on the duration of distillation and the amount of plant material (Timung et al., 2016; Perović et al., 2021). In contrast to HD, the plant material comes into contact with water vapor in SD, where parameters such as distillation time and water vapor flow rate influence the EO yield (Galadima et al., 2012; Božović et al., 2017). Although HD and SD are frequently and most commonly used for EO isolation, the main disadvantages of the mentioned techniques are the long extraction time, high energy consumption and loss or degradation of volatile compounds (Gavahian et al., 2015).

In general, the yield of EO is relatively low when using conventional isolation techniques, but efficiency can be improved by a pretreatment such as cryomilling (CM). CM is a grinding technique carried out at a temperature lower than -150 °C using cryogenic liquids that affect the stability of BAC, which in turn leads to its higher proportion of EO. The advantages of CM also include the retention of highly volatile compounds due to the application of extremely low temperatures during the rapid pulverization of the particles, shorter milling time, less powder agglomeration and less possibility of fouling and oxidation (Katiyar et al., 2020). Mékaoui et al. (2016) showed that CM as a pretreatment for HD and SD with the support of microwaves significantly increased the EO yield of cumin seeds with shorter extraction time and reduced energy consumption. However, the use of CM as a pretreatment for the isolation of EO from fennel seeds was not reported in the available literature.

Due to the above-mentioned disadvantages of conventional methods, new isolation methods are being developed to shorten the extraction time, achieve a higher yield and a better chemical composition of the EO while being environmentally friendly. Examples of such methods are microwave-assisted extraction (MAE) and ultrasound-assisted extraction (UAE), subcritical water extraction (SWE) and supercritical fluid extraction (SFE), with supercritical CO_2 extraction (SCO_2) being the most commonly used. SCO_2 is based on extraction with CO_2 whose pressure and temperature are above the critical values (31.1 °C and 73.8 bar). The advantages of this technique are inertness, non-toxicity and easy availability of CO_2, suitability for the extraction of thermosensitive compounds due to moderate working conditions, high solubility and selectivity (Chemat et al., 2020). Precisely for this reason, the specificity of CO_2 extracts includes the presence of other BAC such as phenols, pigments, fatty acids and phytosterols in addition to volatile compounds. So far, the knowledge on the application of SCO_2 in fennel seeds mainly refers to the isolation of volatile compounds and fatty acids (Rodríguez-Solana et al., 2014; Mokhtari and Ghoreishi, 2019; Maitusong et al., 2021), while the application of SCO_2 in the isolation of other BAC present is poorly explored in the available literature.

EO is unstable in the presence of oxygen, light, heat, moisture and metals, so a stabilization process is required to preserve the bioactive volatiles during storage and to extend shelf life, increase their bioavailability and control their discharge. Various encapsulation techniques are used to stabilize EO by incorporating the volatiles into a protective carrier layer, thus preventing the loss of their biological activity (Osorio-Tobón et al., 2016). Spray drying is the leading encapsulation technique in the food industry, in which the liquid medium is converted into powder form and a very short thermal contact time and the high water evaporation rate result in a high-quality, functional and stable product with a low moisture content (Fernandes et al., 2013). The aim is to achieve a high encapsulation efficiency with a suitable carrier type and drying temperature. When selecting a carrier, important properties are high solubility, good emulsifying, drying and film-forming properties, non-toxicity, low price and biodegradability. Maltodextrins, gum arabic and cyclodextrins and their combinations are most commonly used for the encapsulation of EO (Jafari and et al., 2008). For example, Fernandes et al. (2013) used gum arabic to encapsulate EO rosemary, while the combination of gum arabic and maltodextrin was found to be the most effective for encapsulating ginger EO (de Barros Fernandes et al., 2016). With regard to the available literature, the encapsulation of fennel EO by the spray-drying method has not yet been investigated.

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

Objectives:

1. to determine the influence of CM as a pretreatment on the yield and BAC of fennel seed EO produced with HD and SD

2. to determine the influence of SCO_2 on the yield and BAC of fennel seed EO/extract

3. to optimize the encapsulation process of fennel seed EO using the spray drying method and to examine the physical and chemical properties of the powders

Hypotheses:

1. EO yield depends on the applied technique and distillation parameters, and the application of CM as a pretreatment increases the efficiency of distillation

2. under optimal SCO_2 conditions it is possible to produce fennel seed EO/extracts with a high BAC content

3. encapsulation results in better retention of the EO components in the powders and their stability during storage

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

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

For research purposes, dried seeds of bitter fennel (*Foeniculum vulgare* Mill.) will be used, obtained from conventional cultivation, which includes the implementation of the following agrotechnical measures: plowing, pre-sowing preparation (plate and roto-harrowing), sowing with grain using a seed drill, weed control by mowing and harvesting with a combine harvester. The seeds will be dried on floor dryers in a 30 cm thick layer at 42 °C for 20 h using gas as an energy source. Immediately before the experiment, the fennel seeds will be ground and the ground sample will be used for extraction.

In order to maximize the yield of EO with suitable composition and quality, the following work will be carried out in the first phase of the research: (i) optimization and comparison of the efficiency of EO isolation by conventional extraction techniques, i.e., hydrodistillation (HD) and steam distillation (SD), and (ii) testing the influence of CM as a pretreatment to shorten the extraction time while achieving the same or higher yield of EO.

HD will be carried out using a Clevenger apparatus in which the ratio of plant material to water (1:10 to 1:30) and the distillation time (40 to 120 min) will be varied, while SD will be carried out in a laboratory distillation unit (Darkol d.o.o., Varaždin, Croatia) with varying pressure (0.15 to 0.85 bar) and distillation time (40 to 120 min). CM will be performed in time intervals from 1 to 9 min in the cryogenic laboratory mill (6875, Spex Sample Prep, New Jersey, USA) using liquid nitrogen, after which EO isolation will be performed using the previously established optimal parameters of selected conventional technique for EO isolation. In all experiments, the yield of EO obtained and the identification and quantification of volatile compounds will be determined by gas chromatography (GC) using mass detector (MS) (Agilent 6890N Network GC System, Agilent Technologies, USA). The experimental design for optimizing the conditions for EO isolation in each technique will include a central-composite design in which each extraction parameter will be examined as an independent factor at five levels ($-\alpha$, -1, 0, 1, α) including factorial, axial and central points with five replicates. The experimental design for the influence of CM will be set as a full factorial design (2 factors at 3 levels) with milling time and distillation time as independent factors.

In the second phase of the research, supercritical CO_2 extraction (SCO_2) will be used as an advanced extraction technique whose efficiency will be compared with that of conventional extraction techniques. When performing SCO_2 in a SCO_2 laboratory extractor, pressure (120 to 320 bar) and temperature (40 to 60 °C) will be tested to maximize process efficiency and high yield of the desired BAC. The yield and composition of the EO will be determined in the same way as described in the first phase of the research. Due to the presence of non-volatile components in the CO_2 extract samples, the identification and quantification of phenolic compounds will be performed using ultra-performance liquid chromatography (UPLC) with tandem mass spectrometry (MS/MS) (Agilent 6430 Triple Quad LC/MS, Agilent Technologies, USA), pigments and tocopherols by high-performance liquid chromatography (HPLC) using a diode array detector (DAD) or fluorescence detector (FD) (Agilent 1260 Infinity, Agilent Technologies, USA), while the presence of fatty acids and phytosterols will determined by GC with flame ionization (FID) and MS detector. The experimental design will be set in the same way as for the optimization of conventional extraction techniques.

The third phase of the research will include the optimization of the conditions for the encapsulation of fennel seed EO by spray drying in a Mini Spray Dryer B-290 (Büchi, Flawil, Switzerland) device during which the type of carrier will be varied (two-component combination of maltodextrin, gum arabic and β-cyclodextrin) as well as their ratio to each other (1:1, 1:3 and 3:1) and the drying temperature (120, 160 and 200 °C). Process yield, retention of EO using HD and efficiency of the process will be determined, and the moisture content of the resulting powders will be tested by drying at 105 °C to constant mass, particle size by laser light scattering, solubility, hygroscopicity and chemical composition by GC-MS analysis. The experimental design of the experiment will be a full factorial design (3 factors at 3 levels) with the type and ratio of carriers and drying temperature as independent factors.

The statistical analysis of the results obtained to determine the optimal conditions for EO isolation will be carried out using the Response Surface Methodology (RSM) with the desirability method in the program Design Expert 11.0 (Stat-Ease Inc., Minneapolis, USA). The statistical analysis will also include a multifactorial analysis of variance and a Tukey HSD post-hoc test with a significance level of $p \leq 0.05$ (95% confidence interval) to investigate the differences in the yield and composition of EO under the applied conditions and iwill be performed with the Statsoft program STATISTICA v.12 (Statsoft Inc., Tulsa, USA).

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

The scientific contribution manifests in: (i) the application of CM as a pretreatment in the improvement of conventional techniques (HD and SD) for the isolation of EO from fennel seeds, (ii) the optimization of the fennel seeds extraction conditions using advanced techniques (SCO_2), all with the aim of increasing the yield and obtaining an oil with a high content of volatile and non-volatile BAC. Additional contribution is to increase knowledge on the application of encapsulation techniques in the production of fennel seed EO powders to stabilize, protect and preserve BAC.

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

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11. Gavahian, M., Farhoosh, R., Farahnaky, A., Javidnia, K., Shahidi, F. (2015) Comparison of extraction parameters and extracted essential oils from *Mentha piperita* L. using hydrodistillation and steamdistillation. International Food Research Journal, 22(1), 283–288.
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Total cost estimate of proposed research *(in kuna)*

167 000

Proposed sources of funding for research

Type of funding	Title of project	Project leader	Signature
National funding	Isolation and encapsulation of bioactive molecules of wild and cultivated nettle and fennel and effects on organism physiology (IP-2018-01-4924)	Verica Dragović-Uzelac, PhD, Full Professor	
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

Maja Repajić, PhD, Associate Professor

[3] Fill out only if needed

Signature

_____

Nina Marčac Duraković, M.Sc.

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, October 7, 2024

Signature

_____

Nina Marčac Duraković, M.Sc.

Official stamp here

[4] Not required in case of dual doctorate (*Cotutelle de these*)

PhD-02 EVALUATION 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:		Nina Marčac Duraković, M.Sc.
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:		350/PT
Title of proposed topic in	*language of the dissertation:*	english
	Croatian	Optimiranje postupaka izolacije i inkapsulacije eteričnoga ulja komorača (*Foeniculum vulgare* Mill.)
	English	Optimization of isolation and encapsulation techniques of fennel (*Foeniculum vulgare* Mill.) essential oil
Area/field/branch (if the doctoral study is performed in a branch)**:**		Biotechnology/Food Technology/Engineering

MENTOR(S)

	First and last name, and title:	**Institution, country:**	**E-mail:**
First mentor:	Maja Repajić, PhD, Associate Professor	University of Zagreb Faculty of Food Technology and Biotechnology, Croatia	maja.repajic@pbf.unizg.hr
Second mentor:	/	/	/
The committee appointed for topic evaluation and mentor appointment proposal	**First and last name, and title:**	**Institution, country:**	**E-mail:**
	1. Verica Dragović-Uzelac, PhD, Full Professor	University of Zagreb Faculty of Food Technology and Biotechnology, Croatia	vdragov@pbf.hr
	2. Maja Repajić, PhD, Associate Professor	University of Zagreb Faculty of Food Technology and Biotechnology, Croatia	maja.repajic@pbf.unizg.hr
	3. Sanda Vladimir-Knežević, PhD, Full Professor	University of Zagreb Faculty of Pharmacy and Biochemistry, Croatia	svladimir@pharma.hr
	4./	/	/
	5./	/	/

[1] Please name file as: DR.SC.-02 – Last name and first name of doctoral candidate.doc
Please send the filled-out form DR.SC.-02, in electronic and written format, and signed, to the appropriate Registrar's Office.

Session of the competent body at which the Committee was appointed, and number of item on the agenda	Faculty Council of the University of Zagreb Faculty of Food and Biotechnology, 10th regular session for acad. year 2021/2022. held on July 15, 2022, item 41 of the agenda
Session of the Ethics Committee at which consent was given to the research proposal	/

A. Report on the Public Defence of the Dissertation Topic

The Faculty Council of the University of Zagreb Faculty of Food and Biotechnology at the 10th regular session of the academic year 2021/2022 on July 15, 2022, appointed us as members of the Commission for the evaluation of the topic and the appointment of a supervisor for the doctoral thesis of the applicant Nina Marčac Duraković, M.Sc., as part of the doctoral studies in Biotechnology and Bioprocess Engineering, Food Technology and Nutrition, specialization Food Technology, entitled "Optimization of isolation and encapsulation techniques of fennel (*Foeniculum vulgare* Mill.) essential oil". On the basis of Art. 29 of the Doctoral Degree Regulations of the University of Zagreb Faculty of Food and Biotechnology, Nina Marčac Duraković, M.Sc. started the public defense of the dissertation topic on September 14, 2022.
The applicant Nina Marčac Duraković, M.Sc. explained the role and purpose of optimizing the methods of isolation of the essential oil/CO_2 extract from fennel seeds and encapsulation of the essential oil from fennel seeds for the purpose of isolation and stabilization, and she presented the research plan and explained the methodology that will be used for the purpose of preparing the doctoral thesis. The PhD student systematically presented the objectives and hypotheses of the research and the expected future scientific contribution. The oral defense of the dissertation topic proposal took place in the presence of the members of the Postgraduate Studies Committee, the members of the selected topic evaluation committee and the proposing supervisor: Verica Dragović-Uzelac, PhD, Full Professor, the PhD student's supervisor Maja Repajić, PhD, Associate Professor and Sanda Vladimir-Knežević, PhD, Full Professor as well as other interested faculty members and students.

B. Evaluation of the Dissertation Topic

(original scientific contribution and evaluation of viability)

The proposed topic of the PhD thesis of Nina Marčac Duraković, M.Sc., belongs to the scientific field of biotechnical sciences, the scientific field of food technology, and will be carried out in the Laboratory for Chemistry and Technology of Fruits and Vegetables and Laboratory for Drying Processes and Stability Monitoring of Biologically Active Compounds, Faculty of Food and Biotechnology in Zagreb in cooperation with other laboratories of Faculty of Food and Biotechnology and other faculties and institutions.

The aim of this PhD thesis is to determine the influence of cryomilling as a pretreatment on the yield and biologically active compounds of fennel seed essential oil produced by hydrodistillation and steam distillation, to determine the influence of supercritical CO_2 extraction on the yield and biologically active compounds of the essential oil/CO_2 extracts of fennel seeds and to optimize the fennel seed essential oil encapsulation process by the spray drying method, and to investigate the physical and chemical properties of the powders.

For research purposes, dried seeds of bitter fennel (Foeniculum vulgare Mill.) obtained by conventional cultivation will be used. Chopped parts of fennel seeds will be subjected to conventional extraction techniques (hydrodistillation and steam distillation) which parameters will be optimized in aim to reach maximum yield EO and their accepted quality and quantity and the impact of cryomilling as a pre-treatment will be tested in order to reduce the extraction time while achieving the same or higher yields of essential oil. The optimization of hydrodistillation and steam distillation will be carried out according to the defined experiment plan, where in hydrodistillation the ratio of plant material and water and the time of distillation are varied, while in steam distillation the pressure and time of distillation are varied. With cryomilling, the time interval of grinding will be varied, after which essential oil isolation will be carried out on previously defined optimal parameters of the selected conventional technique for essential oil isolation. When conducting all experiments, the yield of the obtained essential oil will be determined, and the identification and quantification of volatile compounds will be carried out by gas chromatography (GC) with the use of a mass detector (MS). Also crushed fennel seeds will be subjected to supercritical CO_2 extraction (SCO_2) – an advanced extraction technique, and the efficiency of this technique will be compared with conventional extraction techniques. When conducting SCO_2, it will be examined pressure and temperature in order to achieve maximum process efficiency and high yield of targeted BAC will be performed. The yield and composition of the essential oil will be determined, and due to the presence of non-volatile components, the identification and quantification of phenolic compounds in CO_2 extract samples will be carried out using ultra high performance liquid chromatography (UPLC) with tandem mass spectrometry (MS/MS), pigments and tocopherols using high-performance liquid chromatography (HPLC) with the use of a diode array detector (DAD) or fluorescent detector (FD), while the presence of fatty acids and phytosterols will be determined using gas chromatography with a flame-ionization detector/ by mass spectrometry (GC-FID, GC-MS).

For research purposes, dried seeds of bitter fennel (*Foeniculum vulgare* Mill.) obtained by conventional cultivation will be used. The ground fennel seeds will be subjected to conventional extraction techniques (hydrodistillation and steam distillation) whose parameters will be optimized to achieve maximum yield of essential oil of suitable quality and quantity, and the effect of cryomilling will be tested as a pretreatment to shorten the extraction time while achieving the same or higher yield of essential oil. The optimization of hydrodistillation and steam distillation will be carried out according to the defined experiment plan, whereby the ratio of plant material and water and the distillation time will be varied in hydrodistillation, while the pressure and distillation time will be varied in steam distillation. In cryomilling, the time interval for milling will be varied, after which the isolation of the essential oil will be carried out with the previously determined optimal parameters of the selected conventional technique for isolating the essential oil. In all experiments, the yield of the essential oil obtained will be determined and the identification and quantification of the volatile compounds will be carried out by gas chromatography (GC) using a mass detector (MS). In addition, the ground fennel seeds will be subjected to supercritical CO_2 extraction (SCO_2), as an advanced extraction technique, whose efficiency will be compared with that of conventional extraction techniques. When performing SCO_2, pressure and temperature will be examined to maximize process efficiency and achieve a high yield of the targeted BAC. The yield and composition of the essential oil will be determined, and due to the presence of non-volatile components, the identification and quantification of phenolic compounds in the CO_2 extract samples will be performed using ultra-performance liquid chromatography (UPLC) with tandem mass spectrometry (MS/MS), pigments and tocopherols by high-performance liquid chromatography (HPLC) using a diode array detector (DAD) or fluorescence detector (FD), while the presence of fatty acids and phytosterols will be determined by gas chromatography with flame ionization detector/mass spectrometry (GC-FID, GC-MS).

Since the essential oils become unstable over time, it is desirable to stabilize them further. For this purpose, spray drying encapsulation technique will be applied to encapsulate fennel seed essential oil in a protective layer of the carrier. To obtain a product with high stability and desirable properties with a longer shelf life, optimization of spray drying conditions will be carried out by varying the type and proportion of carrier and the temperature. The process yiled, essential oil retention and efficiency of the process will be determined, while the resulting spray-dried powders will be tested for physico-chemical properties (moisture content, particle size, solubility, hygroscopicity and chemical composition by GC-MS analysis).

The statistical evaluation of the results will be carried out using statistical tests such as the response surface methodology (RSM), the desirability method and the multifactorial analysis of variance with Tukey HSD post-hoc test.

Opinion and recommendation:

Proposed topic of the PhD thesis of Nina Marčac Duraković, M.Sc., represents an original scientific contribution in the field of Food Technology and Engineering. The PhD student presented an innovative approach to the scientific problem and its solution, and the planned research are applicable, and the procedures and analyzes that will be used to conduct the research are repeatable. The applicant has demonstrated a good knowledge in the field of her PhD dissertation and clearly defined research objectives and hypotheses. Therefore, we propose to the Council of the University of Zagreb Faculty of Food and Biotechnology to accept the topic of PhD student Nina Marčac Duraković, M.Sc. under the title "Optimization of isolation and encapsulation techniques of fennel (*Foeniculum vulgare* Mill.) essential oil". We also propose the approval of the further procedure for obtaining the title of Doctor of Biotechnical Sciences, in the field of Food Technology, and the appointment of the Commission for the Defense and Evaluation of the Dissertation.

Recommendation on change or revision of dissertation title:

/

Proposal for change of mentor and/or appointment of another mentor *(specify title, first and last name, institution):*

/

Dissertation defence planned for (*specify year and semester):*

2024/2025, summer semester

Separate opinion *(only if any of the members of the Committee for evaluation of dissertation topic and mentor appointment proposal has a separate opinion)*

/

Signature

(*First name and last name of committee member)*

ADDITIONAL COMMENTS *(as needed):*

	First and last name, title, institution, country:	Signature:
The committee appointed for topic evaluation and mentor appointment proposal	1. Verica Dragović-Uzelac, PhD, Full Professor, University of Zagreb Faculty of Food Technology and Biotechnology, Croatia	
	2. Maja Repajić, PhD., Associate Professor, University of Zagreb Faculty of Food Technology and Biotechnolgy, Croatia	
	3. Sanda Vladimir-Knežević, PhD, Full Professor, University of Zagreb Faculty of Pharmacy and Biochemistry, Croatia	
	4./	/
	5./	/

Zagreb, October 7, 2024 **Official stamp here**

DR.SC-03 DECISION ON 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:	Nina Marčac Duraković, M.Sc.	
Provider of the study:	University of Zagreb Faculty of Food Technology and Biotechnology	
Name of the study:	Biotechnology and Bioprocess Engineering, Food Technology and Nutrition	
Scientist ID of the doctoral candidate:	350/PT	
Approval of the dissertation topic within dual doctorate (cotutelle de these) *(please fill in an appropriate box)*:	☐ YES	☒ NO
Title of proposed topic in *language of the dissertation:*	english	
Croatian:	Optimiranje postupaka izolacije i inkapsulacije eteričnoga ulja komorača (Foeniculum vulgare Mill.)	
English:	Optimization of isolation and encapsulation techniques of fennel (Foeniculum vulgare Mill.) essential oil	
Area/field/branch (if the doctoral study is performed in a branch)**:**	Biotechnology/Food Technology/Engineering	

MENTOR(S)

First mentor:	**First and last name, title:**	**Institution, country:**	**E-mail:**
	Maja Repajić, PhD, Associate Professor	University of Zagreb Faculty of Food Technology and Biotechnology, Croatia	maja.repajic@pbf.unizg.hr
Second mentor:	**First and last name, title:**	**Institution, country:**	**E-mail:**
	/	/	/
The committee appointed for topic evaluation and mentor appointment proposal:	**First and last name, title:**	**Institution, country:**	**E-mail:**
	1. Verica Dragović-Uzelac, PhD, Full Professor	University of Zagreb Faculty of Food Technology and Biotechnology, Croatia	vdragov@pbf.hr
	2. Maja Repajić, PhD, Associate Professor	University of Zagreb Faculty of Food Technology and Biotechnology, Croatia	maja.repajic@pbf.unizg.hr
	3. Sanda Vladimir-Knežević, PhD, Full Professor	University of Zagreb Faculty of Pharmacy and Biochemistry, Croatia	svladimir@pharma.hr

[1] Please name file as: DR.SC.-03 – Last Name and First Name of Doctoral Candidate.doc
Please send the filled-out form DR.SC.-03 along with the forms DR.SC-01 i DR.SC.-02and the Opinion of the Ethics committee of the constituent part (if needed), in electronic and written format, signed and with accompanying documentation, to the appropriate Registrar's Office. The Registrar's Office forwards the filled-out form in electronic form (e-mail: dr.sc.obrasci@unizg.hr) and in written form to the Mail Office of the University of Zagreb (Trg maršala Tita 14).

	4./	/	/
	5./	/	/
Date of the public defence of the topic: *(day/month/year)*	September 14, 2022		
Date of enrolment to the doctoral study programme *(day/month/year)*:	May 2, 2018		
Dissertation defence planned for (*specify year and semester)*:	2024/2025, summer semester		
Session of the competent body at which the decision on topic approval was made, and number of item on the agenda:	Faculty Council of the of Faculty Food Technology and Biotechnology of the University of Zagreb, 1st regular session for acad. year 2022/2023. held on October 21, 2022, item 29 of the agenda.		
Comments (as needed):			
LIST OF ATTACHMENTS *(to the Form)*:	**1.** Form DR.SC.-01 Request for approval of the dissertation topic **2.** Form DR.SC.-02 Evaluation of the dissertation topic **3.** Opinion of the Ethics committee (for topic proposals requiring it)		
Date and authorization—stamp and signature of the person in charge:			
Zagreb, (date) October 7, 2024 Signature *(first name and last name)* **Official stamp here**			