

REVIEW

by **Dr. Zhana Yuliyanova Petkova**

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On PhD Thesis for awarding the educational and scientific degree “**Doctor**”

Area of Higher education: 4. Natural science, mathematics, and informatics;

Professional field 4.2. Chemical sciences;

Doctoral Program *Technology of animal and vegetable fats, soaps, essential oils and perfumery -
cosmetic preparations*

Author: *Liliya Stoyanova Stoyanova*

Title: *„Impact of Organic Production on the Composition of Tobacco Seeds and the Potential Applications of Glyceride Oil”*

Scientific supervisor: *Assoc. Prof. Dr. Maria Angelova-Romova – University of Plovdiv „Paisii Hilendarski“*

1. General description of the submitted materials

By order No. ПД-21-2253 dated 05.12.2024, from the Rector of the University of Plovdiv "Paisii Hilendarski" (PU), I have been appointed as a member of the scientific jury to oversee the defence procedure of a dissertation titled "Impact of Organic Production on the Composition of Tobacco Seeds and the Potential Applications of Glyceride Oil" in the Area of Higher education: 4. Natural science, mathematics, and informatics, Professional field 4.2. Chemical Sciences in Doctoral Program Technology of animal and vegetable fats, soaps, essential oils and perfumery - cosmetic preparations.

The author of the dissertation is Liliya Stoyanova Stoyanova, a full-time doctoral student in the Department of Chemical Technology with a scientific supervisor Associate Professor Dr. Maria Yordanova Angelova-Romova from the University of Plovdiv "Paisii Hilendarski".

The set of paper and electronic materials presented by Liliya Stoyanova Stoyanova complies with Article 36 (1) of the Regulations for the Development of the Academic Staff at PU and includes the following documents:

- Application to the Rector of PU for the disclosure of the procedure for dissertation defence;
- CV in European format;
- Protocol from the Departmental Council addressing the reporting of the readiness to initiate the procedure and the preliminary discussion of the dissertation;

- Dissertation;
- Abstract in Bulgarian and English;
- List of scientific publications on the subject of the dissertation and a list of citations to the PhD student's work;
- Copies of the scientific publications;
- Declaration of originality and authenticity of the submitted documents;
- Statement of meeting the minimum academic requirements.

The doctoral candidate has submitted four publications: one publication in an international journal, which is refereed and indexed in the global databases Web of Science and Scopus, with a Quartile 3 (Q3) ranking; two publications in international journals, both with a Quartile 4 (Q4) ranking; one publication in a conference proceedings volume.

2. Brief Biographical data of the doctoral candidate

Liliya Stoyanova Stoyanova completed her Bachelor's program in "Biology" at the University of Plovdiv "Paisii Hilendarski" in 2008. From 2010 to 2011, she studied in the Master's program "Analysis and Control of Food" at the University of Food Technologies, obtaining a professional qualification as an "Engineer-Chemist." In 2014, she completed a Master's program in "Organic Chemistry" at the University of Plovdiv "Paisii Hilendarski". Since 2021, she has been enrolled as a full-time PhD student in the doctoral program "Technology of animal and vegetable fats, soaps, essential oils and perfumery - cosmetic preparations".

From 2019 to the present, Liliya Stoyanova has worked at the Tobacco and Tobacco Products Institute in Markovo (TTPI). Initially, she held the position of Chief Expert Chemist, and later she became an Assistant. Since 2020, she has also been appointed as the Chief Expert Chemist - Tester in the Accredited Laboratory Complex for Testing at TTPI. Between 2019 and 2024, she participated in the implementation of four projects. Two of these projects were funded by the Agricultural Academy's fund in Sofia, one was financed by the Bulgarian Science Fund, and another was under the Rural Development Program for the period 2014 - 2020, through the State Fund "Agriculture."

3. Relevance of the topic and appropriateness of the set objectives and tasks

The utilization of waste products from agriculture and the food industry is essential for both environmental protection and improving the economic efficiency of enterprises and production processes. The generated waste products can be reused or processed into various useful materials or energy resources. Over the past few decades, numerous scientific studies have focused on this topic to reduce organic waste that could find applications in different sectors. Organic production, which

relies on sustainable practices aimed at environmental protection, maintaining biodiversity, and improving product quality, is of particular interest.

In this context, the topic addressed in the present dissertation is of exceptional importance, as it provides new information regarding the chemical and lipid composition of organically produced tobacco seeds, which have not been previously studied. A comparison of their characteristics with those of conventionally produced seeds has been made, and the possibilities for the application of glyceride oil from organically produced tobacco seeds for cosmetic purposes have been investigated.

The health benefits of using tobacco seed cake and the isolated glyceride oil from the seeds are still not sufficiently studied, despite their potential as a nutritional raw material and therapeutic oil for use in medicine and cosmetics. Globally, science is increasingly focusing on possible alternative uses of tobacco, aiming to maintain this technical crop as an important economic resource. This is also part of the research problem addressed in the present dissertation.

Additionally, an extended characterization of the chemical composition of tobacco seeds and glyceride oil has been conducted over a two-year period under different production conditions. This provides guidelines for the selection of varieties suitable for maximum utilization, as well as possible new directions for a closed production cycle with minimal waste products.

4. Knowledge of the problem

The literature review in the presented dissertation covers 35 pages and cites a significant number of literary sources. It examines the results obtained from numerous previous scientific studies on the relevant topic, systematized into nine separate subchapters: Origin, Cultivation, and Distribution; Botanical Characteristics; General Chemical Composition of Tobacco Seeds; Extraction of Glyceride Oil; Lipid Composition of Tobacco Seeds Oil (including biologically active components such as fatty acids, phospholipids, sterols, tocopherols, and polyphenols); Oxidative Stability of the Oil; Antioxidants and Antioxidant Activity; Applications of Tobacco Seeds and Glyceride Oil.

This comprehensive literature review demonstrates the doctoral candidate's deep understanding of the dissertation topic, as well as her ability to critically analyze the data and systematically present it. Additionally, the literature review concludes with a summary that highlights what data have already been published in the scientific literature and what aspects of the topic have not yet been explored. This provides a basis for correctly formulating the main goal of the dissertation and the tasks derived from it.

The objectives and tasks set out in the present dissertation are appropriate, well-formulated, and relevant to current issues affecting society, industry, and science in the field.

5. Research methodology

The chosen research methods are effective in achieving the goal and fulfilling the set tasks. The materials and methods section provides a detailed description of the raw materials and reagents used. It includes the analytical methods for determining the chemical composition of tobacco seeds (the content of glyceride oil, proteins, and carbohydrates, including total and reducing sugars, starch, and insoluble fibre; moisture and volatile substances, ash content, macro- and microelements, total phenolic content, and the HPLC method for the determination of polyphenols). Additionally, it outlines the analytical methods for determining the physicochemical parameters of the glyceride oil (refractive index, peroxide value, acid value, iodine value, and oxidative stability) and the methods for determining the lipid composition of the glyceride oil (including the fatty acid composition, unsaponifiable matter, total content and individual composition of tocopherols, sterols, and phospholipids).

The methods to determine the antioxidant activity of extracts from tobacco seeds, seed cake, and glyceride oil are also described. The functional properties of tobacco seed oil were assessed by calculating indicators that determine the biological value of the oils, such as the indices of atherogenicity, thrombogenicity, and hypocholesterolemic/hypercholesterolemic ratio. Furthermore, the experimental approach for preparing an emulsion cream with embedded tobacco seed oil is detailed, and the main analyses for product quality are indicated. The research was conducted using modern scientific equipment, ensuring reliable and accurate results.

6. Characteristics and evaluation of the thesis

The dissertation has a total volume of 170 pages, and it includes the main sections such as: Introduction (2 pages), Literature review (35 pages, 20.6% of the total volume of the dissertation), Main goal and tasks (1 p.), Materials and Methods (31 p., 18.2%), Results and Discussion (79 p., 46.5%), Conclusions (2 p.), Contributions (1 page), References (17 pages), Publications and participation in scientific conferences (2 pages). It includes 35 tables, 42 figures and 2 diagrams.

The literature review is well-structured and comprehensive, demonstrating the PhD student's extensive knowledge on the dissertation topic.

The "Results and Discussion" section is organized into seven subsections, highlighting the main research directions of the dissertation. The first two subsections present and discuss the results of research on the determination of primary and secondary metabolites of tobacco seeds over two consecutive growing years, 2020 and 2021, as well as the physicochemical indicators of the extracted tobacco seed oil. The research focused on tobacco seeds from the *Basmi* variety group, including *Krumovgrad 58* (organically produced), *Krumovgrad 58* (conventionally produced), and *Krumovgrad 90* (conventionally produced). The third section focuses on the changes in the

chemical and lipid composition of the seeds from two studied varieties of tobacco, observed over two years of vegetation. It was found that the oil from the organically produced tobacco seeds exhibited consistent results in terms of fatty acid and tocopherol composition for both harvests, contributing to its oxidative stability. The fourth subsection addresses the application and optimization of various techniques for extracting glyceride oil from tobacco seeds, a topic that has gained significant relevance in recent years. Various oil extraction methods were employed, including extraction with a Soxhlet apparatus, maceration, and ultrasound extraction, to obtain glyceride oil from the organically produced tobacco seed variety *Kr 58* (bio). Three solvents were chosen to isolate the lipid fraction from the seeds: *n*-hexane, *n*-hexane: acetone (1:1, v/v), and ethyl acetate. It was determined that the fatty acid composition of the oil is unaffected by the type of solvent or extraction technique used. However, ultrasound extraction or maceration with the polar solvent ethyl acetate effectively extracted tobacco seed oil with a high tocopherol content. The next subsection focuses on identifying biologically active substances and their antioxidant activity in tobacco seeds, oil, and seed cakes. Two solvents, 95% ethanol and 60% methanol, were used to prepare the extracts from the seeds, with studies conducted over two consecutive years. The organically grown seeds were found to have higher total phenolic content and corresponding antioxidant activity than conventionally grown seeds during both growing seasons. Three different solvents (water, 95% ethanol, and 60% methanol) were used to prepare the extracts from the seed cake obtained after isolating the tobacco seed oil. The results indicated that the total phenolic content and antioxidant activity of the extracts from the seed cake were higher than those of the seeds, suggesting potential utilization after oil extraction from the seeds. To determine the total phenolic content and antioxidant activity of glyceride oil, 80% ethanol and 80% methanol were used as solvents to obtain the extracts. The highest total phenolic content and antioxidant capacity were found in the organically grown seed oil, with 80% ethanol yielding better results among the two solvents used. The following subsection emphasizes the determination of the chemical and lipid composition of waste tobacco seeds and the oil obtained from them. The research established that waste tobacco seeds do not differ in chemical and lipid composition from cultivable seeds over 0.5 mm in size and can serve as a valuable natural source of lipids, protein, and fibre. The final subsection in this chapter explores the potential application of the oil from organically produced tobacco seeds for cosmetic purposes by preparing and analyzing emulsion creams containing tobacco seed oil. For comparison, creams were also prepared with grape seed oil, which has a similar lipid composition to tobacco oil.

Each subsection of the Results and Discussion chapter includes a written summary that highlights the most significant findings from the research.

The dissertation utilized a total of 172 literary sources, with 11 written in Cyrillic (constituting 6.4% of all sources) and 161 in Latin (93.6%). Notably, over 40% of the cited sources (75 publications) were published after 2015, underscoring the relevance of the issue addressed in the dissertation and reflecting a comprehensive understanding of contemporary scientific research on the topic.

7. Contributions and Significance of the Research for Science and Practice

The dissertation presents three scientific-applied and three applied contributions, which fully reflect the significance and originality of the research.

For the first time, the chemical composition of seeds from a Bulgarian variety of tobacco grown under organic production conditions has been investigated. A detailed study was conducted on the total phenolic content and antioxidant activity of extracts from the seeds, seed cake, and glyceride oil of both organically and conventionally produced Bulgarian tobacco varieties. Additionally, the chemical and lipid composition of waste tobacco seeds, deemed unfit for sowing, was thoroughly examined.

The research proves the potential for fully utilizing waste tobacco seeds for the production of glyceride oil, fibre, and natural antioxidants. Various techniques were employed for the extraction of glyceride oil from tobacco seeds. It was found that oil extraction by maceration and ultrasound with an *n*-hexane: acetone is a fast and cost-effective method. A method for extracting tobacco seed oil with a high tocopherol content using maceration and ultrasound extraction with an ethyl acetate has also been proposed.

Furthermore, a formulation has been developed to create an emulsion cream based on natural ingredients, incorporating tobacco seed oil and lemongrass essential oil as a natural preservative. The original contributions derived from the research align fully with the obtained results.

8. Assessment of the publications on the dissertation

Four publications are presented in the dissertation work: one is published in an international journal, indexed in the world databases Web of Science and Scopus, with quartiles 4 and 3, respectively (Q4 and Q3); one is published in an international journal, indexed in Web of Science (not ranked) and Scopus with quartile 4 (Q4); one is in an international journal, indexed in the Scopus global database with quartile 4 (Q4); and one is published in a conference proceedings.

Two of the articles were published in foreign journals (*Current Research in Nutrition and Food Science* and *International Journal of Secondary Metabolite*), and two were published in Bulgarian journals (*Bulgarian Chemical Communications* and *Proceedings of the National Technical Conference with International Participation "Ecology and Health"*). Three of the publications are written in English, and one is in Bulgarian.

Half of the publications are co-authored with the PhD student's supervisor, and the others are co-authored by more than three authors. One of the publications has been cited by foreign authors.

The total score of the presented publications is 39, which meets the national minimum requirements for acquiring the educational and scientific degree "Doctor" and the requirements of the Regulations of the University of Plovdiv "Paisii Hilendarski" for the development of academic staff (minimum required: 30 points). The PhD student is the primary author in all the presented publications, indicating her significant contribution to their development. The publications fully reflect the essence of the dissertation work.

9. Personal Contribution of the PhD student

The PhD student Liliya Stoyanova is the first author of all four presented publications, indicating her primary and active involvement in conducting the studies, analyzing the results, and shaping the dissertation thesis. She has also participated in five conferences, presenting the research findings through a report at one conference and through poster presentations at the others. This demonstrates her active role in disseminating the results of her dissertation work.

10. Abstract

The presented abstract covers 39 pages, containing 30 tables and 20 figures. It reflects all the main results described in the dissertation. It is presented both in Bulgarian and in English. The author's abstract fully meets the requirements of the Regulations of the University of Plovdiv "Paisii Hilendarski" for the development of the academic staff.

11. Critical remarks and recommendations

In essence, I have no critical remarks on the presented dissertation. The PhD student has addressed all the comments and recommendations provided by the expanded departmental council, which has significantly improved the quality of the dissertation.

However, I have two minor remarks about the thesis:

1) On page 59, in formula (16) for the calculation of the total content of tocopherols, " λ " should be corrected to " γ ."

2) On page 142, the quality of Diagram 2 should be enhanced to make the labels easier to read.

I have a question for the doctoral student:

1) Two solvents (95% ethanol and 60% methanol) were used to determine the total phenolic content and antioxidant activity of the tobacco seed extracts. Additionally, water was used to determine the same parameters for the waste seeds, along with the aforementioned solvents. What

prompted the necessity to analyze the aqueous extracts of the waste seeds for their total phenolic content and antioxidant activity?

12. Personal Impressions

I have known Liliya Stoyanova Stoyanova since the beginning of her doctoral studies in 2021. My impression of her is that she approaches her assigned tasks with great responsibility, demonstrating meticulous attention to detail and a high level of accuracy. Liliya Stoyanova exhibits a strong sense of ethics and professionalism in the workplace. She systematically and methodically addresses problems that arise, enhancing the efficiency and success of the work process.

13. Recommendations for future use of the dissertation contributions and results

I believe that the results obtained in this dissertation could have significant industrial applications. It has been demonstrated that tobacco seeds grown under both organic and conventional production conditions are excellent sources of macronutrients with high energy value and are rich in biologically active substances. Research on the chemical and lipid composition of waste tobacco seeds, which are unsuitable for planting, confirms the potential for fully utilizing these seeds to extract glyceride oil, fibre, and natural antioxidants. These extracts can be directly used in agriculture and the food industry.

Additionally, the study highlights the potential application of the glyceride oil from tobacco seeds in creating stable emulsion creams. These creams exhibit characteristics similar to, and in some cases even surpass, those of products made with grape seed oil, which is traditionally used for cosmetic purposes.

CONCLUSION

The dissertation presents scientific-applied and applied results, representing an original contribution to science and meeting all the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria (ZRASRB), the Regulations for the Implementation of ZRASRB, and the relevant Regulations of the University of Plovdiv "Paisii Hilendarski."

The dissertation demonstrates that the doctoral candidate, Liliya Stoyanova Stoyanova, possesses in-depth theoretical knowledge and professional skills in the doctoral program "*Technology of animal and vegetable fats, soaps, essential oils and perfumery - cosmetic preparations*" by showcasing qualities and skills to independently conduct scientific research.

Consequently, I confidently give my positive assessment of the conducted research, presented in the above-reviewed dissertation, abstract, achieved results, and contributions. I propose that the honorable scientific jury award the educational and scientific degree of "Doctor" to Liliya Stoyanova Stoyanova in the field of higher education: 4. Natural Sciences, Mathematics, and

Informatics, professional field 4.2. Chemical Sciences, doctoral program "*Technology of animal and vegetable fats, soaps, essential oils and perfumery - cosmetic preparations.*"

20.01.2025

Reviewer:

Assoc. Prof. Zhana Petkova