

STATEMENT

by **prof. Ginka Atanasova Antova, PhD,**
Faculty of Chemistry, University of Plovdiv „Paisii Hilendarski“

on the dissertation for awarding the educational and scientific degree “Doctor of Philosophy” (PhD)

Area of higher education: 4. Natural sciences, mathematics and informatics

Professional field: 4.2. Chemistry sciences

Doctoral program: „Technology of animal and vegetable fats, soaps, essential oils and perfumery-cosmetic preparations”

Author: *Liliya Stoyanova Stoyanova*

Thesis: *“Impact of Organic Production on the Composition of Tobacco Seeds and the Potential Applications of Glyceride Oil“*

Supervisor: *Assoc. Prof. Maria Yordanova Angelova-Romova, PhD, University of Plovdiv „Paisii Hilendarski“*

1. General overview of the procedure and the PhD student

By order № RD-21-2253/05.12.2024 of the Rector of Plovdiv University "Paisii Hilendarski" I was appointed a member of the scientific jury to provide a procedure for the defense of a dissertation on “Impact of Organic Production on the Composition of Tobacco Seeds and the Potential Applications of Glyceride Oil“ for obtaining the educational and scientific degree "Doctor" in the area of higher education 4. Natural sciences, mathematics and informatics, professional field 4.2. Chemical sciences and doctoral program “Technology of animal and vegetable fats, soaps, essential oils and perfumery-cosmetic preparations”. The author of the dissertation is Liliya Stoyanova Stoyanova - PhD student in full-time form at the Department of Chemical Technology with supervisor Assoc. Prof. Maria Angelova-Romova from University of Plovdiv "Paisii Hilendarski".

The set of materials on electronic media presented by doctoral student Liliya Stoyanova is in accordance with Article 36 (1) of the Regulations for Development of the Academic Staff of the University of Plovdiv and includes all the necessary documents.

The doctoral student has submitted a dissertation, an abstract and 4 publications, 3 of which have been published in scientific journals indexed in world-renowned databases (*Web of Science, Scopus*).

Liliya Stoyanova has been enrolled as a full-time doctoral student on March 1, 2021, and on March 1, 2024 she completed her studies with the right to defend a dissertation. During this time, she also worked at the Institute of Tobacco and Tobacco.

The dissertation consists of nine main chapters, presented on 171 standard pages including introduction, review (20.5%), main goal and objectives, materials and research methods (18.5%), results and discussion (46.2%), conclusions, contributions, used references and list of publications, and participation in scientific conferences.

2. Relevance of the topic

The topic of the presented dissertation is relevant, as research has been conducted on the search for alternative raw materials from renewable sources for the extraction of non-traditional oils that can be applied in the cosmetic, pharmaceutical, and food industries. In recent years, increasing

attention has been paid to the utilization of plant waste products to obtain natural products with biological activity. The primary significance lies in the lack of similar in-depth studies on the use and application of tobacco seeds, which are a waste product of tobacco production, as well as the glyceride oil obtained from them, revealing the possibility of using it as a raw material in the cosmetic industry.

For the first time, scientific data has been provided on the chemical and lipid composition of organically produced tobacco seeds, which have been compared with those of commercial tobacco seed varieties currently produced conventionally in Bulgaria. The potential possibility of utilizing waste tobacco seeds to extract glyceride oil, fibers, and natural antioxidants has been demonstrated. It has been established that organically produced tobacco seeds are a rich source of macronutrients, and the glyceride oil obtained from them has a higher content of biologically active substances.

The presented dissertation by Liliya Stoyanova addresses an extremely important and current problem for tobacco production in recent years, regarding research on the composition and potential of a natural resource in Bulgaria, with a view to outlining possible applications.

3. Knowledge of the problem

The review covers the botanical characteristics of tobacco, the chemical composition of the seeds, the lipid composition of tobacco glyceride oil, and its oxidative stability. It also discusses the applications of the seeds and the extracted oil. Additionally, methods for studying the antioxidant activity of tobacco seed extracts and glyceride oil are described. The 172 literary sources used, of which 11 are in Cyrillic and 162 are in Latin script, demonstrate the doctoral student's good literary awareness. The bibliographic reference shows that a large part of the literary sources, about 90%, are from after the year 2000, and about 46% are from after 2015, indicating the use of contemporary scientific research on the dissertation topic. The Review section concludes with a summary that has allowed the doctoral student to correctly formulate the objective and the resulting tasks, which correspond to the dissertation's topic.

4. Research methodology

The subject of the study is tobacco seeds grown under organic production conditions and the glyceride oil obtained from them, as well as its application in cosmetic products. The materials and methods section describes the raw materials used, the methods for determining the chemical composition of the seeds, the methods for determining the physicochemical properties of the glyceride oil, and the methods for determining the lipid composition of the tobacco oil. The methods for determining the antioxidant activity of extracts from tobacco seeds, meal, and glyceride oil, as well as for determining the functional properties of lipids from tobacco seeds, are detailed. Standardized methods have been used to analyze the raw materials, as well as the glyceride oil. The formulation for obtaining an emulsion cream based on natural ingredients, containing tobacco oil, is described. The chosen research methods allow the achievement of the set goal and provide answers to the tasks set for solving in the dissertation. During the research work, the doctoral student has mastered various analytical methods and has become acquainted with modern analytical techniques. Therefore, I believe that the educational task of developing the dissertation has also been fulfilled.

5. Characteristics and evaluation of the dissertation and contributions

The dissertation is very well structured, with the nine chapters presented in chronological order across 171 standard pages. A vast amount of work has been done on the dissertation, which includes 42 figures, 35 tables, and 2 diagrams. In the 'Results and Discussion' section, the original results are presented in 32 tables and illustrated in 21 well-formatted figures. The research was conducted with modern scientific equipment, ensuring the obtaining of objective results. The doctoral student has mastered new methods for analyzing both the raw materials and for characterizing the lipid and lipid-containing products. The obtained data have been correctly discussed, which indicates that the doctoral student has acquired skills for presenting results. It is impressive that each part of the experimental research concludes with a summary-conclusion, which has helped derive 7 summarized conclusions and 3 original scientific-applied and applied contributions that are genuinely significant for the oil-producing and cosmetic industries. The conclusions and contributions fully correspond to the obtained results.

I accept the contributions presented by the doctoral student and their classification into scientific-applied and applied.

For the first time, data on the chemical and lipid composition of two Bulgarian tobacco seed varieties (Krumovgrad 58 and Krumovgrad 90) grown under organic production conditions have been obtained and compared with those from conventional production. It has been established that organically produced tobacco seeds are a rich source of macronutrients and the glyceride oil obtained from them is distinguished by a higher content of biologically active substances and has better oxidative stability and good atherogenic and thrombogenic properties.

Various techniques have been used for the extraction of glyceride oil from tobacco seeds. Oil extraction by maceration methods with extractant n-hexane:acetone and ultrasound were found to be rapid and affordable methods for oil extraction.

For the first time, a study has been conducted on the total content of phenolic compounds and antioxidant activity of extracts from seeds, meal and oil of organically and conventionally produced Bulgarian tobacco varieties. It has been proven that the most suitable extractant for extracting polyphenolic compounds from tobacco seeds and meal is 60% methanol, and for extracting them from tobacco oil is 80% ethanol and 80% methanol. Higher antioxidant activity was found in the extracts from the meal compared to those from the seeds and glyceride oil. Extracts from organically produced tobacco seeds, meal and oil had better total phenolic content and higher relative antioxidant capacity than conventionally produced ones.

For the first time, the chemical and lipid composition of waste tobacco seeds unfit for sowing has been studied in detail. The possibility of fully utilizing waste tobacco seeds for the production of glyceride oil, fiber, and natural antioxidants has been proven.

It has been found that tobacco oil is a suitable ingredient for the preparation of moisturizing cosmetic products. A recipe has been developed for the preparation of an emulsion cream based on natural ingredients, containing tobacco oil and lemongrass essential oil as a natural preservative.

The detailed characterization of the chemical composition of tobacco seeds and glyceride oil, tracked over time and under different production conditions, can provide guidelines for selecting varieties suitable for maximum utilization, as well as provide new directions for a closed production cycle with minimal waste products.

6. Evaluation of the publications and the personal contribution of the PhD student

The list of publications by Liliya Stoyanova in connection with her dissertation includes 4 articles: 3 of them are in journals, referenced and indexed in *Web of Science* and *Scopus*. Specifically, 1 of the publications is in a journal with a Q3 quartile (*Current Research in Nutrition and Food Science*) and 2 are in journals with a Q4 quartile (*International Journal of Secondary Metabolite* and *Bulgarian Chemical Communications*). Additionally, 1 publication is in the proceedings of a national scientific conference with international participation “Ecology and Health, 2023 autumn”.

Two of the publications are in international journals, while the other two are in Bulgarian editions. Of the presented publications, three are in English. Liliya Stoyanova's personal involvement in the mentioned scientific works is illustrated by the fact that in all four publications, she is listed as the first author.

The results of the conducted research have been reported at three national scientific forums with international participation and at two seminars organized by ACM2 and the Faculty of Chemistry. The doctoral student is listed as the first author in the presented four posters and one report.

The quality of the conducted research is further validated by the citation of one of the publications by foreign authors. The publication activity meets the requirements for acquiring the educational and scientific degree "Doctor". The minimum national requirements and the minimum requirements of the Regulations of the PU for the Academic Staff Development are over fulfilled, as the PhD student has submitted 3 publications in refereed and indexed journals in *Web of Science* and *Scopus*, accumulating 39 points out of the required 30.

7. Abstract on dissertation

The abstract, which is 39 pages, corresponds to the content of the dissertation and is an abbreviated version of its essence, fully and accurately reflects the results achieved in the dissertation, and their analysis. It is also presented in English (35 pages).

8. Recommendations for future use of dissertation contributions and results

The presented dissertation contains a sufficient amount of material and is written in accordance with the requirements for such types of work. I do not have critical remarks and recommendations for the conducted research and the presented materials.

I have no critical remarks or recommendations regarding the conducted research and presented materials. However, I have noticed several technical errors: there is an omission in the numbering of the figures: number 31 is missing; one of the articles is in Bulgarian and should be presented in the list of publications in Bulgarian. The comments made are of an editorial nature and do not diminish the scientific value of the dissertation. I recommend that the doctoral student continues to work just as hard and even more deeply in the fields of phytochemistry and lipid chemistry, as well as on the utilization of biological waste from the tobacco industry.

CONCLUSION

The dissertation *contains scientific-applied and applied results that represent an original contribution to science* and, in terms of volume and number of publications, *fully meets* all the requirements of the Academic Staff Development Act in the Republic of Bulgaria (ASDARB) and the Regulations for application of ASDARB, and the Regulations of University of Plovdiv “Paisii Hilendarski”.

The dissertation shows that the doctoral student Liliya Stoyanova *has* in-depth theoretical knowledge and professional skills in the doctoral program “Technology of animal and vegetable fats, soaps, essential oils and perfumery-cosmetic preparations”, *demonstrating* qualities and skills for independent conduct of scientific research.

Due to the above, I confidently give my *positive assessment* of the conducted research and *I propose to the esteemed scientific jury to award the educational and scientific degree "Doctor" to Lilia Stoyanova Stoyanova* in the area of higher education: 4. Natural Sciences, Mathematics and Informatics, in professional field 4.2 Chemical Sciences, in doctoral program “Technology of animal and vegetable fats, soaps, essential oils and perfumery-cosmetic preparations”.

17.01.2025

Statement prepared by:

Prof. Ginka Antova, PhD