

REVIEW

by Prof. Dr. eng. Mariyana Nedyalkova Perifanova-Nemska, Department of "Technology of Tobacco, Sugar, Vegetable and Essential Oils", UHT scientific specialty "Technology of Animal and Vegetable Fats, Soaps, Essential Oils and Perfumery and Cosmetics", UHT-Plovdiv

of a dissertation for the award of the educational and scientific degree "Doctor" in: 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 and Cosmetics

Author: Liliya Stoyanova Stoyanova

Topic: Influence of organic production on the composition of tobacco seeds and possibilities for application of glyceride oil

Scientific supervisor: Assoc. Prof. Dr. Maria Angelova-Romova, Plovdiv University "Paisiy Hilendarski" (PU)

1. General description of the submitted materials

By order No. RD-21-2253/05.12.2024 of the Rector of Plovdiv University "Paisiy Hilendarski" (PU), I have been appointed as a member of the scientific jury for ensuring a procedure for the defense of a dissertation on the topic "Influence of organic production on the composition of tobacco seeds and possibilities for the application of glyceride oil" for the acquisition of the educational and scientific degree "doctor" in the field of higher education 4. Natural sciences, mathematics and informatics, professional direction 4.2. Chemical sciences, doctoral program "Technology of animal and vegetable fats, soaps, essential oils and perfumery and cosmetic preparations". The author of the dissertation is Liliya Stoyanova Stoyanova - a full-time doctoral student at the Department of Chemical Technology with scientific supervisor Assoc. Prof. Dr. Maria Angelova-Romova, Plovdiv University "Paisiy Hilendarski".

The set of materials submitted by the doctoral student on paper is in accordance with Art. 36 (1) of the Regulations for the Development of the Academic Staff of the University of Plovdiv, and includes the following documents:

- a request to the Rector of the University of Plovdiv for the opening of the procedure for the defense of a dissertation;
- a CV in European format;
- a report from the departmental council, related to reporting the readiness for the opening of the procedure and preliminary discussion of the dissertation;
- dissertation;
- an abstract in Bulgarian and English;

- a list of scientific publications on the topic of the dissertation;
- copies of scientific publications;
- list of noted citations;
- certificate of compliance with the minimum national requirements;
- declaration of originality and authenticity of the attached documents;
- certificate of compliance with the specific requirements of the respective faculty (only for doctoral students enrolled by 04.05.2018);

The doctoral student has attached 4 full-text publications.

2. Brief biographical data about the doctoral student

Since 2019, the doctoral student has been working at the Institute of Tobacco and Tobacco Products in the research sector. She carries out research and laboratory work related to the analysis of tobacco and tobacco smoke. The accumulated experience and the provided material base enable her to carry out relevant analyses related to her dissertation work. During her doctoral studies, the doctoral student was also assigned pedagogical activity – supervision of laboratory exercises with students of the specialties "Medical Physics", and "Medical Chemistry".

3. Relevance of the topic and appropriateness of the goals and objectives

In Bulgaria and the world, tobacco (*Nicotiana tabacum* L.) is one of the most researched plants among industrial crops. In recent years, tobacco leaves and tobacco seeds, which are a waste product during production, have attracted particular interest.

Tobacco cultivation in Bulgaria is carried out according to two types of agrotechnical practices - conventional production and organic production. Since 2016, the Institute of Tobacco and Tobacco Products - Markovo village has been growing tobacco for scientific purposes on a certified organic field in an experimental station of the institute in the town of Gotse Delchev. This industrial type of tobacco is distinguished by a completely organic production technology, without the participation of modern plant protection products and soil fertilization.

The literature contains data on the chemical and lipid composition of tobacco seeds from conventionally produced tobacco seeds of varieties, some of which are no longer cultivated. There is a lack of information on the chemical and lipid composition of commercial varieties of tobacco seeds, conventionally produced at the moment in Bulgaria. So far, the composition of organically produced tobacco seeds has not been studied and there is no comparison with the same, but conventionally produced. This is the reason for the doctoral student to set her goal and formulate her tasks correctly, namely to study the influence of organic production on the biologically active components in the glyceride oil and its potential application. The inclusion of tobacco oil in various cosmetic forms is a novelty in the cosmetic industry.

Based on this, I believe that the topic of the dissertation is relevant and has a scientific and scientifically applied nature.

4. Knowledge of the problem

The doctoral student makes a very extensive literature review. The conclusions drawn from the review show that she has become acquainted in detail with the state of the problem, which gives her the opportunity to correctly formulate the goal and objectives of her dissertation.

There is a very thorough knowledge of the scientific literature, with 172 sources cited, of which 11 in Cyrillic and 161 in Latin, with 61% of them being after 2010.

This, in my opinion, is an indicator of the doctoral student's very good awareness of the chosen topic of the dissertation.

5. Research methodology

The object of research in the dissertation is seeds of oriental tobacco, ecotype Krumovgrad, variety group Basmi, Bulgaria - usable and waste (unusable) from different varieties of oriental tobacco and different years of vegetation. Tobacco plants were grown and provided by the Institute of Tobacco and Tobacco Products - village of Markovo.

This section describes in detail the raw materials and methodologies used in the dissertation for analysis and preparation of the final products. The methodologies used can also be used by other researchers.

During the research work, the doctoral student has mastered various methodologies for analysis, has become familiar with modern analytical methods, therefore, I believe that the educational task in developing the dissertation has also been fulfilled. The chosen research methodology allows achieving the set goal and obtaining an adequate answer to the tasks solved in the dissertation.

6. Characteristics and evaluation of the dissertation work

The analyses were conducted in the laboratory of the Department of Chemical Technology at the Paisiy Hilendarski University and in the chemical laboratory of the Institute of Tobacco and Tobacco Products - Markovo village. A large number of experiments were performed. The obtained data were processed as the arithmetic mean value between three parallel measurements, with the calculation of the standard deviation between them (Mean \pm SD, n=3). The results were evaluated using general linear models with differences between the groups, determined using the least squares mean, which makes the obtained data reliable. ANOVA analysis of variance and comparison of the mean values with Duncan's test were used. The difference was considered statistically significant at $\alpha < 0.05$.

The software programs used for processing the results were Microsoft Excel 2016 and IBM SPSS Statistics (version 25, IBM Corp., 2017).

The dissertation is written on 170 standard typewritten pages and includes 34 tables, 40 figures, two diagrams and 2 photographs. The diverse graphical presentation of results makes a very good impression. The material is structured in the following sections: Introduction – 2 pages, Literature review and conclusion – 35 pages, Main goal and tasks – 1 page, Material and methods of analysis – 30 pages, Results and discussion – 78 pages, Conclusions – 2 pages, Contributions – 1 page and Literature – 22 pages.

The sections in the dissertation are very well structured and provide an opportunity for a detailed presentation of the obtained results and drawing conclusions that fully correspond to the tasks set and correctly reflect the work done in the individual parts of the Results and Discussions section.

The obtained results are thoroughly discussed and also compared with the studies conducted by other authors.

A detailed characterization of the chemical and lipid composition of two varieties of Bulgarian tobacco seeds – varieties Krumovgrad 58 and Krumovgrad 90 grown under conditions of organic and conventional production was carried out. It was established that organically produced tobacco seeds and the glyceride oil obtained from them are distinguished by a higher content of biologically active substances – phospholipids, tocopherols and polyphenolic compounds. The glyceride oil obtained from them also has good oxidative stability and good atherogenic and thrombogenic properties, compared to conventional production. Regardless of the method of cultivation of the tobacco plant, linoleic acid predominates in the glyceride oil from tobacco seeds – 65%.

- The chemical composition of two varieties of tobacco seeds from the Basmi variety group was monitored in two consecutive growing seasons. Based on the results obtained, it was concluded that they are a sustainable crop regardless of the production method. Tobacco seeds of the Krumovgrad 58 and Krumovgrad 90 varieties can be successfully used as a source of fiber and raw material for the production of glyceride oil.

- The doctoral student found that the extraction method and the extractant used affect the yield of glyceride oil and its tocopherol composition. The fatty acid composition of the oil remains unsaturated, regardless of the extraction method used.

- Extracts from seeds, meal and oil can be a source of polyphenolic compounds. The most suitable extractant for extracting polyphenolic compounds from tobacco seeds and tobacco meal is 60% methanol, and for extracting them from tobacco oil – 80% ethanol and 80% methanol. The seed extracts show higher antioxidant activity by the FRAP electron transfer method (ability to reduce Fe³⁺ to Fe²⁺) compared to the hydrogen atom transfer methods (ABTS method and DPPH method). The antioxidant activity of the meal extracts was found to be higher than those from the seeds and glyceride oil. The extracts from the studied tobacco oil have the highest relative antioxidant capacity. The extracts from organically produced tobacco seeds, meal and oil have a better total phenolic content and a higher relative antioxidant capacity than those produced conventionally.

- It has been established that waste, unfit for sowing tobacco seeds are a valuable source of glyceride oil and energy. They have identical chemical and lipid composition with usable tobacco seeds and can also be successfully used as a raw material.
- Relative antioxidant capacity (RACI) and phenolic antioxidant capacity (PAC) have been determined
- Functional properties of tobacco seed lipids have been determined such as – Atherogenic index (AI), Thrombogenic index (TI), Hypocholesterolemic/hypercholesterolemic index (h/H), Oxidant stability index (OSI) and Peroxidation index (PI).
- It has been established that tobacco oil is a suitable ingredient for the preparation of moisturizing cosmetic products. A recipe for the preparation of an emulsion cream based on tobacco oil has been developed.

7. Contributions and significance of the work for science and practice

The presented contributions are very well summarized in two groups – scientific-applied and applied, which I accept in full.

Scientific-applied

- For the first time, the chemical composition of seeds of a Bulgarian tobacco variety grown under organic production conditions has been studied.
- For the first time, a study has been conducted on the total content of phenolic compounds and antioxidant activity of extracts of seeds, meal and oil from organically and conventionally produced Bulgarian tobacco varieties.
- 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.

Applied

- Various techniques have been used for the extraction of glyceride oil from tobacco seeds. It has been established that oil extraction by maceration and ultrasound with an extractant n-hexane: acetone are fast and affordable methods for oil production.
- A method for extracting glyceride tobacco oil with a high content of tocopherols has been proposed - maceration and ultrasound extraction with an extractant ethyl acetate.
- 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.

In my opinion, some of the scientific and applied contributions are of a scientific nature.

8. Assessment of the publications on the dissertation work

Four scientific publications in foreign and our journal in English are presented. Three of them are referenced in the Scopus and Web of Science databases, reflecting the research from the dissertation work. One of the articles was presented at an international scientific conference.

The doctoral student is in first place in all the publications, which gives me reason to believe that her contribution is significant.

The total number of points received from the presented publications is 39, which exceeds the scientometric requirements of the Regulations for the Development of the Academic Staff at the University of Plovdiv.

9. Personal participation of the doctoral student

Based on the dissertation work presented to me, its design, setting goals and objectives and their implementation, and the conclusions drawn, I can categorically state that its implementation is the merit of the doctoral student.

10. Abstract

The abstract accurately and completely describes the purpose and tasks, materials and methods of work, the experimental data obtained and their discussion, the conclusions and contributions of the dissertation, and the publications. Its format is in accordance with the requirements of the Regulations for the Development of Academic Staff at the University of Plovdiv.

11. Critical remarks and recommendations

The doctoral student and the scientific supervisor have taken into account the preliminary recommendations and remarks made, which is why I do not have any in the final version of the work. I think that the determination of the relative antioxidant capacity (Relative antioxidant Capacity Index – RACI), phenolic antioxidant capacity (Phenolic Antioxidant coefficient – PAC) and the determination of the functional properties of tobacco seed lipids such as – Atherogenic index (AI), Thrombogenic index (TI), Hypocholesterolemic/hypercholesterolemic index (h/H), Oxidant stability index (OSI) and Peroxidation index (PI) should also be reflected as a contribution.

12. Personal impressions

I do not know the doctoral student personally, but the dissertation material provided to me and its presentation to the extended departmental council form the impression, that Lilia Stoyanova Stoyanova is a precise, conscientious, ambitious researcher.

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

I recommend the author to continue his research on other waste products from the food industry, with a view to extracting and utilizing the vegetable oil contained in them.

CONCLUSION

The dissertation contains scientific and applied scientific results that represent an original contribution to science and meet 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 Paisii Hilendarski University.

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

Due to the above, I confidently give my positive assessment of the conducted research, presented by the above-reviewed dissertation, auto-abstract, achieved results and contributions, and I propose to the esteemed scientific jury to award the educational and scientific degree "doctor" to Lilia Stoyanova Stoyanova in the field of higher education 4. Natural sciences, mathematics and computer science; Professional field 4.2. Chemical sciences; Doctoral program "Technology of animal and vegetable fats, soaps, essential oils and perfumery and cosmetic preparations".

20.01.2025

Reviewer:

(Prof. Dr. M. Perifanova-Nemska)