ISSN: 2347-467X, Vol. 12, No. (1) 2024, Pg. 374-383

Current Research in Nutrition and Food Science

www.foodandnutritionjournal.org

Bioactive Compounds and Nutritive Composition of Waste Seeds from *Nicotiana tobacum* L. (Solanaceae)

LILIYA STOYANOVA STOYANOVA[1,2]* and MARIA YORDANOVA ANGELOVA-ROMOVA[1]

[1]Department of Chemical Technology, University of Plovdiv "Paisii Hilendarski", 24 Tsar Asen Str., 4000 Plovdiv, Bulgaria.

[2]Tobacco and Tobacco Products Institute, Agricultural academy, Markovo 4108, Bulgaria.

Abstract

The investigation aims to elucidate the bioactive constituents present in waste seeds derived from *Nicotiana tobacum* L., there by contributing to a more holistic comprehension of the health-related implications associated with tobacco plants beyond conventional consumption concerns. Waste seeds from tobacco plants *N. tobacum* L. were grown during harvest 2021-2022 at the Tobacco and Tobacco Products Institute (part of Bulgarian Agriculture Academy). Chemical analysis of tobacco waste seeds (TWS) encompassed the examination of primary metabolites including lipids (32.1±1.0%), proteins (29.4±1.4 %), and carbohydrates (27.6±0.5 %). Furthermore, various lipid indices (Atherogenicity, Thrombogenicity, Hypocholesterolemic/hypercholesterolemic, etc.) were computed utilizing the fatty acid composition of the oil extracted from tobacco waste seeds. The results showed that TWS could be utilized with health benefits – seeds are a good resource for n-6 fatty acids (linoleic acid - 71.94±1.05 %) with good atherogenicity and thrombogenicity qualities and natural antioxidants.

Article History

Received: 29 February 2024

Accepted: 15 April 2024

Keywords

Bioactive Compounds;
Lipid Indices;
Nutrition Value;
Tobacco Waste Seeds.

Introduction

Nicotiana tobacum L. is a widespread non-food crop all over the world. The tobacco plant has many applications – as a substance of abuse and tobacco products manufacturing, medical plant, and industrial crop good for obtaining seed oil, biodiesel, and biomass.[1, 2, 3] The tobacco leaves are an economically important part of the plant, but tobacco seeds are also part of its cultivation process. Tobacco seeds are gaining popularity as they are not sufficiently studied as part of the plant with good potential for utilization.[4, 5] Tobacco seeds can be considered waste under certain circumstances, typically when they are no longer needed for cultivation and are discarded. From that point tobacco, waste seeds can be obtained when there is a surplus of seeds that exceeds the planting needs for a specific season or if a specific line or

CONTACT Liliya Stoyanova Stoyanova liliyastoyanova@uni-plovdiv.bg Tobacco and Tobacco Products Institute, Agricultural academy, Markovo 4108, Bulgaria.

Doi: https://dx.doi.org/10.12944/CRNFSJ.12.1.30

batch of seeds is no longer needed for ongoing studies or breeding programs. After the harvesting and processing of tobacco leaves, any remaining seeds that are separated during the manufacturing process may be treated as waste. The aerodynamic characteristics of seeds are significant for their further utilization.[6] The tobacco seeds are classified by their size as small (less than 0.5 mm) and large (over 0.5 mm). Only large and suitable seeds are used for planting material. The aerodynamic characteristics of tobacco seeds, techniques, and principles for separation for granulation purposes could identify tobacco seeds less than 0.5 mm in size as waste.[7]

The chemical characterization of *Nicotiana tabacum* L. seeds is important in the search for alternatives to oil for food and cosmetics purposes. Tobacco seeds are known to generate between 28.0 and 45.7% glyceride oil.[8-10] After investigating seven tobacco species, it was found that they contain high levels of glyceride oil (ranging from 30.1% to 41.3%).[11]

The phospholipids and sterols in the tobacco seed oils had a closer value to those of other oil-bearing seeds such as sunflower, soya, and corn.[12, 13] While tobacco seeds are typically discarded or used in limited applications, other studies also suggest that they contain a diverse array of bioactive compounds - polyphenols, tocopherols, and essential fatty acids. Although tobacco oil is not rich in tocopherols (2–195 mg/kg), the seeds are rich in protein and fiber and can be used as an animal protein source.[8,11,14] Previous research shows that palmitic (12.6–36.7%), oleic (15.2–26.3%), and linoleic acid (17.6–62.7%) predominated in tobacco seed oils are potential sources of omega fatty acids.[8, 11]

Understanding the composition of tobacco waste seeds and unraveling the potential health benefits could open new avenues for sustainable utilization and contribute to the broader field of natural products with health applications. As global concerns about sustainable agriculture and waste reduction continue to grow, this research seeks to shed light on the often-overlooked value residing in the waste seeds of *Nicotiana tobacum* L. The study is directed to investigate bioactive compounds of waste seeds from *Nicotiana tobacum* L. that contribute to a more comprehensive understanding of the health-related aspects of tobacco plants beyond the conventional concerns associated with tobacco consumption.

The study aimed to determine the bioactive compounds in waste seeds from *Nicotiana tobacum* L. and to evaluate their nutritive value and health benefits.

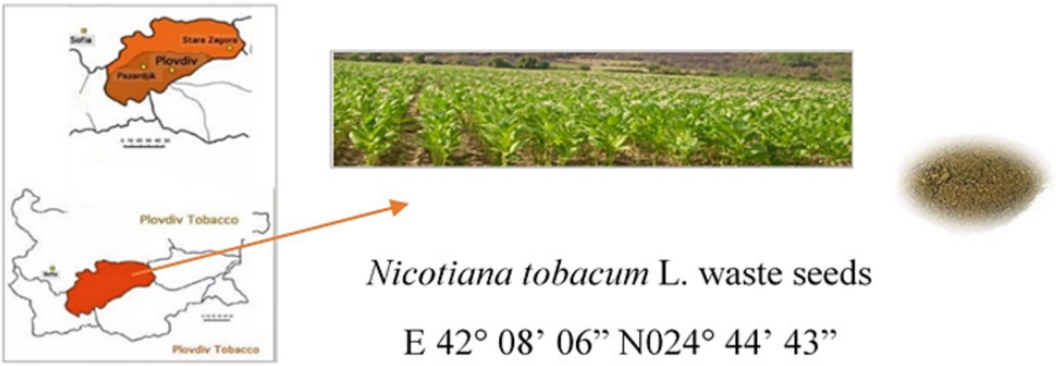

Fig. 1: *Nicotiana tobacum* L. waste seeds cultivation region - the Tobacco and Tobacco Products Institute experimental field

Materials and Methods

Plant material

The tobacco plants were produced by the Tobacco and Tobacco Products Institute (part of Bulgarian Agriculture Academy) in geographic area 42° 08’ 06” N024° 44’ 43” E - Figure 1. Waste seeds - 250g, (harvest 2021) of oriental tobacco variety, were used for the study. Seeds were determined as waste after separation based on their size – under 0.5 mm. They were stored in a warehouse at a temperature of 10 – 20°C and standard humidity (40 – 60%). Seeds were ground before the analysis. The analysis of the chemical composition of tobacco seeds was conducted in the 2021-2022 year.

Chemical Composition

Tobacco waste seeds (TWS) were analyzed for chemical primary metabolites – lipids, proteins, and carbohydrates. Glyceride oil was obtained from 50 g of tobacco waste seeds by laboratory Soxhlet apparatus.[15] All the analyses (ash, moisture, carbohydrate content, fiber, and energy value) were determined according to the procedures described in AOAC (2016) and FAO.[12, 16] Total protein content was examined according to ISO 1871:2009.[17]

Lipid Composition

The lipid fraction of tobacco waste seeds consists of biologically active compounds such as sterols, phospholipids, tocopherols, and fatty acids. Standard methods were used according to the International Organization for Standardization.[18-25]

Table 1. Description of lipid indices and their calculation method

Lipid index	Formula
Index of atherogenicity (IA)[26]	$IA=\frac{(C_{12:0}+4\times C_{14:0}+C_{16:0})}{(\sum MUFA+\sum PUFA)}$
Index of thrombogenicity (IT)[26]	$IT=\frac{(C_{14:0}+C_{16:0}+C_{18:0})}{(0.5\times\sum MUFA)+(0.5\times\sum n\text{-}6\ PUFA)+(3\times\sum n\text{-}3\ PUFA)+\left(\frac{\sum n\text{-}3\ PUFA}{\sum n\text{-}6\ PUFA}\right)}$
Hypocholesterolemic /hypercholesterolemic (HH)[26]	$HH=\frac{C_{18:1}(n\text{-}9)+C_{18:2}(n\text{-}6)+C_{18:3}(n\text{-}6)+C_{18:3}(n\text{-}3)+C_{20:2}(n\text{-}6)+C_{20:3}(n\text{-}3)+C_{20:4}(n\text{-}6)+C_{20:5}(n\text{-}3)+C_{22:6}(n\text{-}3)}{(C_{12:0}+C_{14:0}+C_{16:0})}$
The peroxidability index (PI)[27]	PI=(monoenoic FA×0.025)+(dienoic FA×1)+(trienoic FA×2)+(tetraenoic FA×4)+(pentaenoic FA×6)+(hexaenoic FA×8)
Allylic Position equivalent (APE)[28]	APE=2×(C18:1+ C18:2+ C18:3)
Bis-Allylic position equivalent (BAPE)[28]	BAPE=C18:2+(2× C18:3)
Oxidation Stability Index (OSI)[29]	OSI=3.91–(0.045×BAPE)

MUFA – monounsaturated fatty acids
PUFA – polyunsaturated fatty acids
n-6 PUFA – polyunsaturated fatty acids (n-6)
n-3 PUFA – polyunsaturated fatty acids (n-3)
C12:0 – lauric acid
C14:0 – myristic acid
C16:0 – palmitic acid
C18:0 – stearic acid
C18:1 (n-9) – oleic acid
C18:2 (n-6) – linoleic acid
C18:3 (n-3) – α-linolenic acid
C18:3 (n-6) – γ-linolenic acid
C20:2 (n-6) – eicosadienoic acid
C20:3 (n-6) – di-homo-gamma-linolenic acid
C20:4(n-6) – arachidonic acid

Lipid Indices

Different lipid indices were calculated mathematically as described in Table 1.

Statistic

The MS Excel was used for data processing and the result is given as Means ± Standard deviation (SD) provided in triplicate (n=3, p<0.05).

Table 2. Chemical composition and energy value of *Nicotiana tobacum* L. waste seeds, Mean ± SD (n=3, p<0.05)

Chemical composition	*Nicotiana tobacum* L. waste seeds
Oil content, %	32.1±1.0
Proteins, %	29.4±1.4
Carbohydrates, %	27.6±0.5
Fibers, %	26.6±0.6
Ash, %	4.3±0.3
Moisture, %	6.6±0.03
Energy value, kcal/100 g	517.0±3.1

Results and Discussion

The chemical composition of TWS was investigated to help better understand the nutritional value of the waste seeds and their potential for utilization. The obtained results are presented in Table 2.

Plant seeds are mainly formed by primary metabolites such as lipids, proteins, carbohydrates, and water content. Lipids were extracted from the seeds and quantified as glyceride oil content. The tobacco waste seeds were found to be rich in glyceride oil (32.1±1.0%), which is consistent with previous research indicating a range of 30.1 - 49.2%.[8, 11, 30] It was found that the amount of oil in tobacco seeds was higher than the other traditionally used technical crops such as maize and amaranth.[31] Tobacco seeds from India, Manasi variety, were reported to have oil content - 32.79 %, and seeds were not considered as waste.[32] Research on the chemical composition of Plovdiv 7 (oriental tobacco) from the same region as TWS reported 30.9±1.3% oil content.[10] Seeds were viable. The TWS reflects a respectable outcome for oil content, even though climate, genotype, and other factors influence the production of common tobacco seed oil.[1, 14, 33]

Apart from being high in oil, the TWS was characterized by high protein content – 29.4±1.4%, which is an average value for tobacco seeds as other researchers reported between 18.0 - 41.0 %.[10, 33] The TWS protein content was lower than the soybean meal, the most commonly used plant protein (35 – 45 %), but it is considered a good protein resource in swine nutrition.[14, 34] For comparison with popular edible seeds such as pumpkin – 21.413±0.004 g/100g and sunflower – 27.531±0.0018 g/100 g, the TWS was with better protein content.[35]

Concerning the carbohydrates, the TWS showed a result of 27.6±0.5%, from which fiber content is very high (26.6±0.6%), making this waste a valuable raw material for insoluble carbohydrates. The TWS fibers value was near the reported content of tobacco seed flour (22.1 %) suitable for flour mixture with rye flour for bread.[30]

The content of protein and crude fiber in TWS were higher than in some previous studies: 19.21 – 21.05% and 14.58 – 16.89%, respectively.[8] The ash content, as an indicator of the amount of mineral elements, is relatively high (4.3±0.3%) and the moisture was 6.6±0.03%. The data corresponded to the moisture and ash content of tobacco seeds in the literature.[32]

The energy value of the seeds is an important characteristic, in determining their nutritional qualities. Numerous health issues, including serious non-communicable diseases, can be prevented by leading a healthy lifestyle and avoiding stress, bad eating habits, and physical inactivity.[3] The energy value was calculated as 517.0±3.1 kcal/100g, higher than corn (437.36 kcal/100g) and chickpea (423.54 kcal/100g).[35] The results about the content of biologically active substances in tobacco waste seeds and glyceride oils are presented in Table 3.

Lipid fraction of TWS, as all plant seeds contain unsaponifiable, sterols, phospholipids, and tocopherols. The content is shown in Table 3. The unsaponifiable matter in TWS oil was 3.4±0.9%, which is lower compared to other oil crops, such as sunflower and rapeseed.[31] Sterols are naturally occurring organic molecules that play a significant role in the lipid bilayer of the membrane and are engaged in various plant functions, including control of development and growth to minimize stress. Sterols are formed via the mevalonic acid pathway and the most common sterols in plants are campesterol and β-sitosterol.[37] TWS oil has a total sterol content of 0.7±0.4% and 0.2±0.1%, respectively. The latter outcome was in line with the sterol content-which varied from 0.1 to 1.3%-that was found in a previous study on common vegetable oils.[13] The main sterol in the oil fraction was β-sitosterol (61.2±0.5%) followed by stigmasterol (15.2±0.5%) and campesterol (10.2±0.04 %). Comparison between grape seed oil and TWS oil could be provided for sterol content in which β- sitosterol was 66.6 – 67.4 mg/kg/oil and stigmasterol 10.2 – 10.8 mg/kg/oil. Grape seed oil is known as beneficial to human health.[38] The cholesterol content in the TWS oil was 4.5±0.3%. Similar cholesterol content in tobacco seed oil has been reported by other authors.[11] The relatively high amount of cholesterol in TWS oil is typical for animal lipids while in vegetable oils, this content was from 0.1 – 0.5%.[12, 13] High cholesterol levels are known as a risk factor for cardiovascular diseases, but plant cholesterol application by 2 g per day could reduce serum cholesterol by about 10%.[39]

Table 3. Content of bioactive compounds in *Nicotiana tobacum* L. waste seeds and in the oil, Mean ± SD (n=3, p<0.05)

Lipid composition	*Nicotiana tobacum* L. waste seeds	*Nicotiana tobacum* L. seed oil
Unsaponifiable mater, %	0.7±0.1	3.4±0.9
Sterols, %	0.2±0.1	0.7±0.4
Tocopherols, mg/kg	4.0±1.0	144.0±1.5
γ- Tocopherol, mg/kg	2.3±0.2	81.5±2.1
δ- Tocopherol, mg/kg	1.7±0.7	62.5±2.4
Phospholipids, %	0.2±0.06	1.5±0.2

The phospholipids content of the TWS was closed and similar to those of other common oil-bearing seeds such as sunflower.[12, 13] Total phospholipid content was also conducted in the study shown in Table 3. The individual phospholipid content shows that phosphatidylcholine was in the greatest quantity 35.7±0.4%. Phosphatidylinositol and lysophosphatidylcholine were 23.8±0.4% and 11.9±0.3%, respectively. Phospholipids in other tobacco varieties have been reported in the range of 0.2 – 1.7%.[11]

Plant oils are rich in tocopherols as well as oilseeds and nuts. Tocopherols are known as saturated forms of vitamin E, known as natural antioxidants. The tocopherol content in different plant oils varied between 11 and 3468 mg/kg.[40] The total tocopherols of the TWS oil was 144 mg/kg, which was close to the results by Zlatanov *et al.*, 2007 (2 – 195 mg/kg).[11] Tocopherol content of the TWS and TWS oil is presented in Table 3. γ- tocopherol and δ - tocopherol were identified both in the TWS and oil. The total tocopherol content was 144.0±1.5 mg/kg, which is close to seed oils used in food, pharmaceutics, or cosmetics – red palm oil - 121.6 mg/kg, poppy oil 123.5 mg/kg and pistacia oil 159.6 mg/kg.[40]

To analyze the fatty acid make up of tobacco seed oil, a comprehensive study of the literature was undertaken. The dominant fatty acid identified was linoleic between 60 – 80%.[8,41-43] Fatty acid composition of the TWS identified ten fatty acids but three predominated: linoleic acid (71.94±1.05%), followed by oleic (13.70±0.10%) and palmitic (12.86±0.05%) acids. These acids formed the oil as rich in polyunsaturated fatty acids (PUFAs – 71.94±1.05%) and with almost equal levels of saturated (SFA – 14.30±0.02%) and monounsaturated fatty acids (MUFA – 13.76±0.11%) (Table 4). The two primary functions of linoleic acid (n-6 fatty acid) in the body are first as a precursor of eicosanoids, which regulate renal and pulmonary function, vascular tone, and inflammatory responses, and second as a structural component of membranes that influence membrane function.[44]

The fatty acid composition of the TWS oil is comparable to the grape seed oil – (SFA – 10.4%, MUFA – 14.8%, and PUFA – 74.9%).[38] This fatty acids composition of the examined seeds was probably due to the geographical area where the tobacco was grown.[45]

To determine the positive effects of oils on health,

Table 4. Fatty acids composition of the *Nicotiana tobacum* L. waste seed oil

Fatty acids, %	*Nicotiana tobacum* L.waste seeds oil
$C_{8:0}$ Caprylic	0.05±0.01
$C_{11:0}$ Undecylic	0.04±0.01
$C_{12:0}$ Lauric	0.06±0.01
$C_{15:0}$ Pentadecanoc	0.02±0.01
$C_{16:0}$ Palmitic	12.86±0.05
$C_{17:0}$ Margaric	0.14±0.01
$C_{17:1}$ Heptadecanoic	0.06±0.01
$C_{18:0}$ Stearic	1.13±0.01
$C_{18:1}$ Oleic	13.70±0.10
$C_{18:2}$ Linoleic	71.94±1.05
SFA	14.30±0.02
MUFA	13.76±0.11
PUFA	71.94±1.05

*SFA - saturated fatty acids, MUFA - monounsaturated fatty acids, PUFA - polyunsaturated fatty acids, Mean ± SD (n=3, p<0.05)

Table 5. Lipid indices of tobacco oil obtained from *Nicotiana tobacum* L. waste seeds, Mean ± SD (n=3, p<0.05)

Lipid indices	*Nicotiana tobacum* L. waste seeds oil
Index of atherogenicity (IA)	0.2±0.0
Index of thrombogenicity (IT)	0.3±0.0
Hypocholesterolemic/hypercholesterolemic (HH) ratio	7.0±0.1
Peroxidability index (PI)	72.0±1.0
Allylic Position equivalent (APE)	171.0±1.9
Bis-Allylic position equivalent (BAPE)	72.0±1.0
Oxidation Stability Index (OSI)	1.0±0.1

lipid levels can be assessed by analyzing the types of fatty acids present (Table 1). Seven indices were provided for TWS oil evaluation (Table 5). The index of atherogenicity (IA) and Index of thrombogenicity (IT) are some of the most well-known and used indices that can be used to assess the potential effects of fatty acids on cardiovascular disease.[26] The atherogenic index shows the relationship between the sum of the main saturated fatty acids that are considered proatherogenic and essential unsaturated fatty acids having an antiatherogenic effect. Proatherogenic acids (SFA) cause an increase in cholesterol in the blood because they are easily deposited on the walls of the arteries.[26] The IT indicates the propensity of FAs to form clots in blood arteries and characterizes their thrombogenic potential.[26] The IA and IT for TWS oil were 0.2±0.0 and 0.3±0.0, respectively. These two indices are below 1.0, which means that lipids in TWS oil could reduce the content of total cholesterol as well as the LDL cholesterol in blood and blood plasma.

The HH index for the examined oil was 7.0±0.1, considering that it is recommended to be higher than 1.0, the oil lipids have a positive effect on cardiovascular diseases. Peroxidability index (PI) indicates oxidation stability and was reported in vegetable oils in the range from 7.10 to 111.87 with the lowest value found in olive oils and the highest in perilla oils.[27] The PI index for TWS oil was 72.0±1.0, which means a tendency to oxidation.

The Oxidation Stability Index (OSI) can be used to anticipate the oil's respective shelf-life and the efficiency of antioxidants or determine how much the oil lasts.[29] The number of double bonds per mole and their respective positions, APE (presence of $-H_2C=CH-CH_2-$) and BAPE (presence of $R-CH=CH-CH_2-CH=CH-R$) determine the rate of oxidation of fatty compounds.[28] The APE value for TWS oil - 171.0±1.9 and BAPE - 72.0±1.0, are expected due to its high-unsaturated composition. The higher the results for these two indices are, the higher the susceptibility of the oil to oxidation. The OSI of TWS oil was calculated as 1.0±0.1, which correlated to the APE value.

Conclusion

The evaluation of TWS nutritive composition represents a good source of protein and carbohydrate content of the seeds, which may be used for animal feed or as flour in addition to traditional crops flour. According to the previous, reported data for tobacco seeds and oil, the TWS and oil do not show any deterioration in quality. The results obtained in the study confirm the hypothesis that TWS can be utilized with health benefits – seeds are a good resource for n-6 – fatty acids with good atherogenicity and thrombogenicity qualities and natural antioxidants. The oil may find use as a therapeutic one, similar to other vegetable oils like grape seed oil. The outcomes of this research may have implications for the development of functional foods, nutraceuticals, or pharmaceuticals, thus fostering a more sustainable and diversified approach to the agricultural legacy of *Nicotiana tobacum* L. Further analyses can be performed to assess its benefits to determine any potential benefit for human health.

Acknowledgements

We acknowledge the Department of Chemical Technology, University of Plovdiv “Paisii Hilendarski for providing the infrastructure, chemicals, and continuous support during the research. In addition, the Tobacco and Tobacco Products Institute, Agricultural academy, Bulgaria for providing the tobacco waste seeds for that research.

Funding Sources

The authors received no financial support for the research, authorship, and/or publication of this article.

Conflict of interest

The authors declares no conflict of interest.

Author’s contributions

Liliya Stoyanova Stoyanova: Conceptualization, Methodology, Formal analysis and investigation, Writing – original draft preparation.Maria Yordanova Angelova-Romova: Writing – review and editing, Supervision.

Data Availability Statement

Not applicable.

Ethics Statement

The document accurately and thoroughly presents the authors; original research and analysis.

Informed Consent Statement

Not applicable.

References

1. Grisan S., Polizzotto R., Raiola P., Cristiani S., Ventura F., di Licia F., Zuin M., Tommasini S., Morbidelli R., Damiani F., Pupilli F., Bellucci M. Alternative use of tobacco as a sustainable crop for seed oil, biofuel, and biomass. *Agron Sustain Dev.* 2016; 36(55). DOI: https://doi.org/10.1007/s13593-016-0395-5
2. Sanchez-Ramos J. R. The rise and fall of tobacco as a botanical medicine. *J Herb Med.* 2020; 22:100374. DOI: https://doi.org/10.1016/j.hermed.2020.100374
3. Ramachandran B., Solomon R., Sangwan P., Samuel C. E., Fernandez-Gamiz U., Santra S. S., Altanji M., Govindan V. An experimental

analysis on performance of tobacco seed oil as an alternative fuel for diesel engine. *Alexandria Eng J.* 2023; 80:408-416. DOI: https://doi.org/10.1016/j.aej.2023.08.070

4. Usta N., Aydoğan B., Çon A. H., Uğuzdǒan E., Özkal S. G. Properties and quality verification of biodiesel produced from tobacco seed oil. *Energy Convers Manag.* 2011; 52(5):2031-2039. DOI: https://doi.org/10.1016/j.enconman.2010.12.021
5. Onorevoli B., Machado M. E., Polidoro A. D. S., Corbelini V. A., Caramão E. B., Jacques R. A. Pyrolysis of Residual Tobacco Seeds: Characterization of Nitrogen Compounds in Bio-oil Using Comprehensive Two-Dimensional Gas Chromatography with Mass Spectrometry Detection. *Energy and Fuels*. 2017; 31(9):9402-9407. DOI: https://doi.org/10.1021/acs.energyfuels.7b00405
6. Chavoshgoli Es., Abdollahpour Sh., Abdi R., Babaie A. Aerodynamic and some physical properties of sunflower seeds as affected by moisture content. *Agric Eng Int: CIGR J.* 2014; 16(2):136-142.https://cigrjournal.org/index.php/Ejounral/article/view/2662. Published:2014-06-30
7. Kochev Y. Investigation of the influence of the parameters in the separation of tobacco seeds in an inclined air duct. *USB-Plovdiv, Jub Sci Sess.* 2008; 7:119-122.
8. Abbas Ali M., Abu Sayeed M., Kumar Roy R., Yeasmin S., Mohal Khan A. Comparative Study on Characteristics of Seed Oils and Nutritional Composition of Seeds from Different Varieties of Tobacco (*Nicotiana tabacum* L.) Cultivated in Bangladesh. *Asian J Biochem.* 2008; 3(4):203-212. DOI: https://doi.org/10.3923/ajb.2008.203.212
9. Xie Z., Whent M., Lutterodt H., Niu Y., Slavin M., Kratochvil R., Yu L. L. Phytochemical, Antioxidant, and Antiproliferative Properties of Seed Oil and Flour Extracts of Maryland-Grown Tobacco Cultivars. *J Agric Food Chem.* 2011; 59(18):9877-9884. DOI: https://doi.org/10.1021/jf202069g
10. Popova V., Petkova Z., Ivanova T., Stoyanova M., Lazarov L., Stoyanova A., Hristeva T., Docheva M., Nikolova V., Nikolov N., Zheljazkov V. Biologically active components in seeds of three Nicotiana species. *Ind Crops & Products.* 2018; 117:375-381. DOI: https://doi.org/10.1016/j.indcrop.2018.03.020
11. Zlatanov M., Angelova M., Antova G. Lipid composition of tobacco seeds. *Bulg J Agric Sci*. 2007a; 13:539-544. https://www.agrojournal.org/13/05-07-07.pdf .
12. FAO Food and Nutrition Paper 77, Food energy – methods of analysis and conversion factors, *Report of a Technical Workshop, Rome*, 2003. https://www.fao.org/3/Y5022E/Y5022E00.htm
13. Food and Agriculture Organization of the United Nations, Codex Revisions: 2001, 2003, 2009. *Standard for named vegetable oils Codex Stan* 210 – 1999. Codex Alimentarius, Amendments: 2005, 2011, 2013 and 2015.
14. Rossi L., Fusi E., Baldi G., Fogher C., Cheli F., Baldi A., Dell'Orto V. Tobacco Seeds By-Product as Protein Source for Piglets. *Open J Vet Med.* 2013; 3(1):73-78. DOI: https://doi.org/10.4236/ojvm.2013.31012
15. ISO 659:2009; Oilseeds. Determination of oil content (Reference method). ISO: Geneva, Switzerland
16. Latimer, Jr. G. W. Official Methods of Analysis of AOAC INTERNATIONAL. 22nd ed. Rockville, MD, USA; 2023. https://doi.org/10.1093/9780197610145.001.0001
17. ISO 1871:2009; Food and feed products. General guidelines for the determination of nitrogen by the Kjeldahl method. ISO: Geneva, Switzerland
18. ISO 18609:2000; Animal and vegetable fats and oils. Determination of unsaponifiable matter. Method using hexane extraction. ISO: Geneva, Switzerland
19. Ivanov S., Bitcheva P., Konova B. Méthode de détermination chromatographyque et colorimétrique des phytosterols dans les huiles végétales et les concentres steroliques. *Rev Fr Corps Gras.* 1972; 19:177-180.
20. ISO 12228-1:2014; Determination of individual and total sterols contents. Gas chromatographic method. Part 1: Animal and vegetable fats and oils. ISO: Geneva, Switzerland
21. ISO 9936:2016; Animal and vegetable fats and oils. Determination of tocopherol and tocotrienol contents by high-performance liquid chromatography. ISO: Geneva, Switzerland

22. Schneiter R., Daum G. Extraction of yeast lipids. *Methods Mol Biol.* 2006; 313:41-45 DOI: https://doi.org/10.1385/1-59259-958-3:041
23. ISO 10540-1:2003; Animal and vegetable fats and oils. Determination of phosphorus content. Part 1: Colorimetric method. ISO: Geneva, Switzerland
24. ISO 12966-1:2014; Animal and vegetable fats and oils. Gas chromatography of fatty acid methyl esters. Part 1: Guidelines on modern gas chromatography of fatty acid methyl esters. ISO: Geneva, Switzerland
25. ISO 12966-2:2017; Animal and vegetable fat and oils. Gas chromatography of fatty acid methyl esters. Part 2: Preparation of methyl esters of fatty acids. ISO: Geneva, Switzerland
26. Chen J., Liu H. Nutritional Indices for Assessing Fatty Acids: A Mini-Review. *Int J Mol Sci.* 2020; 21(16):5695. DOI: https://doi.org/10.3390/ijms21165695
27. Yun J.M., Surh J. Fatty Acid Composition as a Predictor for the Oxidation Stability of Korean Vegetable Oils with or without Induced Oxidative Stress. *Prev Nutr Food Sci*. 2012; 17(2):158-165. DOI: https://doi.org/10.3746/pnf.2012.17.2.158
28. Kumar M., Sharma M. P. Potential assessment of microalgal oils for biodiesel production: A review. *J Mater Environ Sci.* 2014; 5(3):757-766. https://www.jmaterenvironsci.com/Document/vol5/vol5_N3/95-JMES-724-2014-Kumar.pdf Received: 2013-11-10, Revised: 2013-10-11, Accepted: 2013-10-11
29. Pinto T. I., Coelho J. A., Pires B. I., Neng N. R., Nogueira J. M., Bordado J. C., Sardinha J. P. Supercritical Carbon Dioxide Extraction, Antioxidant Activity, and Fatty Acid Composition of Bran Oil from Rice Varieties Cultivated in Portugal. *Separations.* 2021; 8(8):115. DOI: https://doi.org/10.3390/separations8080115
30. Lazova-Borisova I., Ivanova P., Taxin N. Opportunities for the Use of Tobacco Flour in Organic Flour for Diabetic Bread. *J Mount* Agri Balkans. 2020; 23(2):1-8.
31. Petkova Zh. Y., Antova G. A., Angelova-Romova M. I., Vaseva I. Ch. A comparative study on chemical and lipid composition of amaranth seeds with different origin. *Bul Chem Comm.* 2019; 51(D): 262-267. http://www.bcc.bas.bg/bcc_volumes/Volume_51_Special_D_2019/BCC-51-D-2019-262-267-Petkova-54.pdf . Received: 2018-10-13, Revised: 2019-01-4.
32. Raju K. S., Reddy D. D., Rao C. V. N. Comparative Study on Characteristics of Seed Oil and Nutritional Composition of Seed Cake in Different Tobacco Types Cultivated in India. *Tobacco Res.* 2015; 41(1):6-14. https://krishi.icar.gov.in/jspui/bitstream/123456789/16593/1/TR41%281%296-14.pdf . Received: 2015-01-10, Accepted: 2015-03-12.
33. Mohamad T.M., Tahir N.A. Evaluation of Chemical Compositions of Tobacco (*Nicotiana tabacum* L) Genotypes Seeds.*Annual Research and Review in Biology*.2014; 4(9):1480-1489. DOI: https://doi.org/10.9734/ARRB/2014/8211. Published: 2014-01-10.
34. Sharma S., Kaur M., Goyal R., Gill B. S. Physical characteristics and nutritional composition of some new soybean (Glycine max (L.) Merrill) genotypes. *J Food Sci Technol.* 2014; 51(3):551-557. DOI: https://doi.org/10.1007/s13197-011-0517-7
35. Saad S. S., Elmabsout A. A., Alshukri A., El-Mani S., Al Mesmary E., Alkuwafi I., Almabrouk O., Buhagar S. A. M. Approximate composition analysis and nutritive values of different varieties of edible seeds. *Asian J Med Sci,* 2021. 12(6):101-108. DOI: https://doi.org/10.3126/ajms.v12i6.33792
36. Kakkar S., Tandon R., Tandon N. The rising status of edible seeds in lifestyle related diseases: A review. *Food Chemistry.* 2023; 402(3):134220. DOI: https://doi.org/10.1016/j.foodchem.2022.134220
37. Rogowska A., Szakiel A. The role of sterols in plant response to abiotic stress. *Phytochem Rev.* 2020; 19(6):1525-1538. DOI: https://doi.org/10.1007/s11101-020-09708-2
38. Garavaglia J., Markoski M. M., Oliveira A., Marcadenti A. Grape Seed Oil Compounds: *Biological and Chemical Actions for Health. Nutr Metab Insight*. 2016; 9:59-64. DOI: https://doi.org/10.4137/NMI.S32910
39. Weingärtner O., Baber R., Teupser D. Plant sterols in food: No consensus in guidelines. *Biochem Biophys Res Commun.* 2014; 446(3):811-813. DOI: https://doi.

org/10.1016/j.bbrc.2014.01.147

40. Aksoz E., Korkut O., Aksit D., Gokbulut C. Vitamin E (α-, β + γ- and δ-tocopherol) levels in plant oils. *Flavour Fragr J.* 2020; 35(177):504-510. DOI: https://doi.org/10.1002/ffj.3585
41. Srbinoska M., Filiposki K., Rafajlovska V. Fatty acid composition of tobacco seed oil and its potential as a source of Linoleic acid. Int. Conf. Biosci. Biotechnol. *Biodivers.* 2012; 164-167. https://www.academia.edu/3181298/Fatty_Acid_Composition_of_Tobacco_Seed_Oil_and_its_Potential_as_Source_of_Linoleic_Acid
42. Ashirov M. Z., Datkhayev U. M., Myrzakozha D. A., Sato H., Zhakipbekov K. S., Rakhymbayev N. A., Sadykov B. N. Study of Cold-Pressed Tobacco Seed Oil Properties by Gas Chromatography Method. *Sci World J.* 2020; 8852724-5. DOI: https://doi.org/10.1155/2020/8852724.
43. Özcan M. M., Uslu N., Lemiasheuski V., Kulluk D. A., Gezgin S. Effect of roasting on the physico-chemical properties, fatty acids, polyphenols and mineral contents of tobacco (*Nicotiana tabacum* L.) *seed and oils. JAOCS.* 2023; 100(5):403-412. DOI: https://doi.org/10.1002/aocs.12680
44. Mori T. A., Hodgson J. M. Fatty acids: Health Effects of Omega-6 Polyunsaturated Fatty Acids. *Encyc Hum Nutrition*. 2013; 2:209-214. DOI: https://doi.org/10.1016/B978-0-12-375083-9.00100-8
45. Stoyanova A., Perifanova-Nemska M., Georgiev E. Raw Material Science about glyceride and essential oils. *Agency* 7D Publishing, Plovdiv; 2006.