ANOTATION OF THE MATERIALS UNDER ART. 65 OF THE REGULATIONS FOR DEVELOPMENT OF THE ACADEMIC STAFF OF THE UNIVERSITY OF PLOVDIV "PAISII HILENDARSKI", INCLUDING HABILITATION EXTENDED REFERENCE

of Ch. Assist. Prof. Zhana Yuliyanova Petkova, PhD

in connection with participation in the announced in SG, no. 39 of 02.05.2023, competition for the occupation of an academic position "Associate Professor" in the field of higher education 4. Natural sciences, mathematics and informatics, professional direction 4.2. Chemical Sciences (Organic Chemical Technology, Food Chemistry)

For participation in the competition for the academic position Associate Professor in the field of higher education 4. Natural sciences, mathematics and informatics, professional direction 4.2. Chemical Sciences (Organic Chemical Technology, Food Chemistry) are presented **18** scientific publications that are referenced and indexed in world renowned scientific databases (Web of Science and Scopus) and 1 textbook for laboratory work in the field of Food Chemistry. These materials are not the same as the previous used for occupation of academic position "Chief Assistant Profesor" and for graduating PhD and is in accordance to the National minimal requirements in the Academic Staff Register.

The numbering used reflects the number from the table of indicators of the relevant scientific work submitted for participation in the competition.

The total number of the citations of all publications (up to 2022) is **232**, with **162** in Scopus and/or Web of Science databases and **70** in other peer-reviewed journals. A total of **90** citations were noticed on the publications submitted for the participation in the competition, of which **70** were in publications referenced and indexed in Scopus and/or Web of Science databases.

In Table 1, is indicated the fulfillment of the requirements of the minimum national required number of points by group of scientific metric indicators for the academic position Associate Professor in professional direction 4.2 Chemical Sciences. Their detailed description and implementation are presented in the Reference for compliance with the minimum national requirements, supplemented by the relevant references and service notes attached to the reviewer's kits.

Table 1. Minimum national required number of points by groups of scientific metric indicators and their respective implementation

Metrics groups	Content	Associate Professor – number of points	Performance – number of points
А	Indicator 1	50	50
Б	Sum of points from indicators 3 and 4	100	122 (from indicator 4)
Г	Sum of points from indicators 5 – 10	200	252 (from indicator 7)
Д	Sum of points from indicator 11	50	324
Е	Sum of points from indicators 12 – 20	-	4 (from indicator 20)
	Total:	**400**	**752**

ANOTATIONS OF THE PUBLICATIONS

I. PUBLICATIONS UNDER INDICATOR B.4.

B.4.1. Fidan H., Stankov S., Stoyanova M., **Petkova Z.**, Petkova N., Stoyanova A., Ercisli S., Choudhary R., Karunakaran R. Chemical Composition of *Pinus nigra* Arn. Unripe Seeds from Bulgaria. *Plants*. **2022**; 11(3):245. https://doi.org/10.3390/plants11030245. **($IF_{(2021)}$ 4.658; $SJR_{(2022)}$ 0.79). Реферирано и индексирано в Web of Science (WOS) (Q1) и Scopus (Q1)**

The present paper aims to investigate the chemical composition of unripe black pine seeds obtained from Bulgaria. The lipid fraction was evaluated in unripe seeds, and the cellulose, total carbohydrates, glucose, fructose, and sucrose were evaluated in seedcakes. The major fatty acid identified in the *Pinus nigra* seed oil was unsaturated linoleic acid (44.2%), followed by the saturated palmitic acid (31.2%). The amount of unsaturated pinolenic (10.5%) and oleic acids (8.8%) was also rather high. The amino acid composition of the protein fraction of seedcakes was also determined. The amino acid composition was represented mainly by asparagine (3.92 mg/g), serine (3.79 mg/g), alanine (3.65 mg/g), arginine (3.32 mg/g), phenylalanine (2.98 mg/g), lysine (2.85 mg/g), proline (2.69 g/mg), tryptophan (2.44 mg/g), valine (2.33 mg/g), isoleucine (2.28 mg/g), and tyrosine (2.05 mg/g). The mineral content (N, P, K, Mg, Na, and Cu) of the seedcakes was evaluated, as the amount of K (8048.00 mg/kg) and Mg (172.99 mg/kg) were the highest in the samples. These findings emphasized the potential use of the unripe black pine seeds in different areas due to their chemical importance and values.

B.4.2. Stankov S., Fidan H., **Petkova Z.**, Stoyanova M., Petkova N., Stoyanova A., Semerdjieva I., Radoukova T., Zheljazkov V. **2020**, Comparative Study on the Phytochemical Composition and Antioxidant Activity of Grecian Juniper (*Juniperus excelsa* M. Bieb) Unripe and Ripe Galbuli, *Plants*, 9, 1207; https://dx.doi.org/10.3390/plants9091207. **($IF_{(2020)}$ 3.935; $SJR_{(2020)}$ 0.892). Реферирано и индексирано в Web of Science (WOS) (Q1) и Scopus (Q1)**

Grecian juniper (*Juniperus excelsa* M. Bieb.) is an evergreen tree and a rare plant found in very few locations in southern Bulgaria. The aim of this study was to evaluate the phytochemical content and antioxidant potential of *J. excelsa* unripe and ripe galbuli from three different locations in Bulgaria. The essential oil content ranged between 1.9% and 5.1%, while the lipid fraction yield was between 4.5% and 9.1%. The content of total chlorophyll was 185.4–273.4 μg/g dw. The total carotenoid content ranged between 41.7 and 50.4 μg/g dw of ripe galbuli, and protein content was between 13.6% and 16.4%. Histidine (5.5 and 8.0 mg/g content range) and lysine (4.0 and 6.1 mg/g) were the major essential amino acids. The antioxidant potential of the 95% and 70% ethanol extracts was analyzed using four different methods. A positive correlation between the antioxidant potential and phenolic content of the galbuli was found. The results obtained in this study demonstrated the differences in phytochemical composition and antioxidant capacity of *J. excelsa* galbuli as a function of maturity stage and collection locality.

B.4.3. Popova V., **Petkova Z.**, Ivanova T., Stoyanova M., Lazarov L., Stoyanova A., Hristeva T., Docheva M., Nikolova V., Nikolov N., Zheljazkov V. **2018**, Biologically active components in seeds of three *Nicotiana* species, *Industrial Crops & Products*, 117, 375-381. https://doi.org/10.1016/j.indcrop.2018.03.020. **($IF_{(2018)}$ 4.191; $SJR_{(2018)}$ 1.015). Реферирано и индексирано в Web of Science (WOS) (Q1) и Scopus (Q1)**

Tobacco seeds, available as an untitilized byproduct of the commercial leaf production in many countries around the world, can be a valuable source of natural products with biological activity. The genus Nicotiana (Solanaceae) includes more than 65 species, but the most economically important and commercially cultivated is *Nicotiana tabacum* L. (common tobacco) and to a much lesser degree *N. rustica* L. (Aztec tobacco). The objective of this study was to evaluate tobacco seed oil and cakes of three tobacco species: two genotypes of *N. alata* Link & Otto (jasmine tobacco), *N. rustica*, and *N. tabacum*, with respect to their potential uses. The glyceride seed oil content was 37.6% and 40.9% for the two genotypes of *N. alata*, 37.5% for *N. rustica*, and 30.9% for *N. tabacum*. Overall, the content of phospholipids was 0.2–0.3% in the oils. Total sterol amount in the oils was 0.35–0.48%. The main component was β-sitosterol, followed by cholesterol and Δ^5-avenasterol (in *N. alata*, white petals), cholesterol and campesterol (in *N. alata*, pink petals), campesterol and Δ^5-avenasterol (in *N. rustica*), and campesterol and stigmasterol (in *N. tabacum*). In the tocopherol fraction (101, 117, 178, and 106 $mg.kg^{-1}$ in *N.*

alata white and pink forms, *N. rustica*, and *N. tabacum*, respectively in the oils) the most predominant (higher than 97%) was the *γ*-tocopherol. Overall, the main fatty acids (FA) in the three Nicotiana species were linoleic (61.7–67.6% range), oleic (15.5–19.0%), and palmitic (9.1–12.5%). The remaining seed cakes (after Soxhlet extraction of glyceride oil), potentially valuable nutrient by-products, were characterized with regard to their content of minerals, cellulose, proteins, and amino acids. Seed cake cellulose content varied between 32.5 and 45.2%, and protein content was 26.7–34.1%. Seed cakes were rich in mineral macro- and micronutrients, with some differences between the species. Highest content of total nitrogen and protein were found in *N. rustica* (5.5% and 34.1%, respetively), and highest content of cellulose was found in *N. alata* (white petals genotype). Potassium concentration was higher in *N. tabacum* and *N. rustica* cakes, whereas the concentrations of iron and zinc were highest in *N. alata* (pink petals genotype). Amino acid composition was dominated by aspartic acid, arginine, and threonine in N. alata, and by arginine, aspartic acid, and histidine in *N. rustica*. The results suggest potential alternative uses of tobacco seeds and cake as animal feed, and possibly as feedstock for new consumer human health products. The concentration of amino acids important for animal nutrition (lysine, methionine, and cysteine) in tobacco seed cakes was low, implying a need for careful combination with other animal diet ingredients.

B.4.4. **Petkova Z.**, Antova G., Angelova-Romova M., Todorova I., Stoyanova M., Stoyanova A. **2022**, *Lupinus angustifolius* L. cultivar "Boregine" from South of Bulgaria: a source of nutrients and natural biologically active components, *OCL-Oilseeds and fats, Crops and Lipids*, 29, 10. https://doi.org/10.1051/ocl/2022003. **($IF_{(2022)}$ -; $SJR_{(2022)}$ 0.356). Реферирано и индексирано в Web of Science (WOS) и Scopus (Q2)**

Nowadays, the requirements of new sources of natural food components are constantly expanding worldwide. On one hand, the constituents derived fromthe common agriculture plants satisfy the needs of the body to function properly. On the other hand, the price of producing ordinary foods is gradually increasing. For that reason, it is necessary to find a cheaper alternative industrial crops, such as a specific variety of lupin (*Lupinus angustifolius* L. cultivar "Boregine"). The chemical and lipid composition of lupin seeds as well as the physicochemical characteristics of the oil were examined. The seeds are rich in proteins and carbohydrates, mainly starch, but have low oil content. Sucrose was the main soluble sugar and the major amino acids were phenylalanine, arginine, tyrosine and serine. Linoleic and oleic acids were predominant in the oil; *β*-sitosterol and *γ*-tocopherol were the main components in the sterol and tocopherol fractions, respectively. Phosphatidylinositol and phosphatidylcholine represented more than 50% of all phospholipids and oleic acid was in the highest amount in all phospholipid classes. All physicochemical characteristics of lupin seed oil were in agreement with the requirements for edible oils and its oxidative stability at 100°C and an air flow rate of 20 L/h was extremely high (more than 100 h).

Lupin seeds have high nutritional value and their oil depicts to be stable, which makes them a possible source of high quality lipids with long shelf life.

B.4.5. Popova V., **Petkova Z.**, Ivanova T., Stoyanova M., Mazova N., Stoyanova A. **2021**, Lipid composition of different parts of Cape gooseberry (*Physalis peruviana* L.) fruit and valorization of seed and peel waste, *Grasas y Aceites*, 72 (2), e402. https://doi.org/10.3989/gya.1256192. **(IF$_{(2012)}$ 1.416; SJR$_{(2022)}$ 0.311). Реферирано и индексирано в Web of Science (WOS) (Q4) и Scopus (Q3)**

The consumption of Cape gooseberry (*Physalis peruviana* L.) fruit (CG), fresh or processed, is gaining popularity worldwide, due to its nutritional and medicinal benefits. This study was based on the analysis of the lipid fraction of different parts of CG fruit and on further valorization of the resulting CG waste. The content of glyceride oil in CG seeds, peels and seed/peel waste, as well as the individual fatty acid, sterol and tocopherol composition of the oils was determined. CG seeds and seed/peel waste were a rich source of oil (up to 22.93%), which is suitable for nutritional application, due to its high proportions of unsaturated fatty acids (up to 83.77%), sterols (campesterol, Δ^5-avenasterol, β-sitosterol) and tocopherols (β-, δ- and γ-tocopherols). Seed/peel waste and the extracted seed cakes contained macro- and microminerals (K, Mg, Na, Fe, Zn, Mn, Cu) which are important for human and animal nutrition. Seed cakes had relatively high protein (24.32%) and cellulose (42.94%) contents, and an interesting amino acid profile. The results from the study contribute to a deeper understanding of the composition of CG fruit, and might be of practical relevance in the development of functional foods and feeds.

B.4.6. **Petkova Z.**, Stefanova G., Girova T., Antova G., Stoyanova M., Damianova S., Gochev V., Stoyanova A., Zheljazkov V. D., **2019**, Phytochemical investigations of laurel fruits (*Laurus nobilis*). *Natural Product Communications*, 1-10. https://doi.org/10.1177/1934578X19868876. **(IF$_{(2019)}$ 0.468; SJR$_{(2019)}$ 0.199). Реферирано и индексирано в Web of Science (WOS) (Q4) и Scopus (Q4).**

Bay laurel (*Laurus nobilis* L.) is an evergreen tree. The objective of this study was to determine the chemical composition (polyphenols, essential oil [EO], lipid fraction, cellulose, and protein content) of laurel fruits collected from Greece (Mount Athos) and Georgia (the village of Meria), and to evaluate the antimicrobial activity of laurel fruit EOs. The major phenolic acids in the fruits from Greece were *p*-coumaric acid (free 261.6 μg/g) and vanillic acid (free 253.1 μg/g and conjugated 925.8 μg/g). The major phenolic acids in fruits from Georgia were vanillic acid (free 105.6 μg/g and caffeic acid [conjugated 439.2 μg/g], and syringic acid [conjugated 390.7 μg/g]). The laurel fruit EOs from Greece (1.4% content) and Georgia (1.6%) had distinct composition. Monoterpene hydrocarbons were the dominant group of compounds in the EOs, with

49.7% in the EO from Greece and 68.7% in the EO from Georgia. The major constituents of the fruit EO from Greece were 1,8-cineole (18.2%), *α*-phellandrene (15.0 %), *β*-pinene (9.4%), and *α*-pinene (9.1%), whereas the ones from Georgia were trans-*β*-ocimene (59.4%) and 1,8-cineole (7.6%). Laurel fruit EO from Greece and Georgia demonstrated low to moderate antimicrobial activity against pathogenic and spoilage microorganisms and the dimorphic yeast *Candida albicans*. The main fatty acids (FAs) in the lipid fractions were oleic, palmitic, and linoleic; there were differences in FA composition between the shells and the seeds of the fruits from the two countries. *γ*-Tocopherol predominated in the tocopherol fraction of the lipids from fruits shells and seeds from Greece (65.3% and 54.4%, respectively), whereas *β*-tocopherol predominated in fruits shells and seeds from Georgia (93.7% and 45.6%, respectively). Currently underutilized, the laurel fruits from both Greece and Georgia contain various valuable compounds that may potentially be used for perfumery, cosmetic, and pharmaceutical applications.

II. PUBLICATIONS UNDER INDICATOR Г.

Г.1. Zhelev I., **Petkova Z.**, Kostova I., Damyanova S., Stoyanova A., Dimitrova-Dyulgerova I., Antova G., Ercisli S., Assouguem A., Kara M., Almeer R., Sayed A.A., **2022**, Chemical Composition and Antimicrobial Activity of Essential Oil of Fruits from *Vitex agnus-castus* L., Growing in Two Regions in Bulgaria, *Plants*, 11 (7), 896. https://doi.org/10.3390/plants11070896. **(IF$_{(2021)}$ 4.658; SJR$_{(2022)}$ 0.79). Реферирано и индексирано в Web of Science (WOS) (Q1) и Scopus (Q1)**

The chemical composition of *Vitex agnus-castus* L. (Verbenaceae family) fruits, collected from two regions in Bulgaria (south-central and north-east Bulgaria), was investigated. The content of proteins (5.3–7.4%), carbohydrates (73.9–78.8%), fiber (47.2–49.9%), ash (2.5–3.0%), essential oils (0.5%), and vegetable oil (3.8–5.0%) were identified in the fruits. The composition of the essential oils (EOs) of Vitex fruits from both regions was determined; the main compounds were 1,8-cineole (16.9–18.8%), *α*-pinene (7.2–16.6%), sabinene (6.7–14.5%), and bicyclogermacrene (7.3–9.0%), but significant differences in the quantitative and qualitative composition of EOs between the regions were found. The EOs of plants from north-east Bulgaria demonstrated antimicrobial activity against the pathogenic species *Salmonella abony*, *Staphylococcus aureus*, and *Bacillus subtilis*, but the Gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa* exhibited resistance to the oil. Linoleic acid predominated in vegetable oil from both regions, followed by oleic acid. *β*-sitosterol and *γ*-tocopherol were the main components in the sterol and tocopherol fraction of the lipids. Phosphatidic acids were the main components in the vegetable oil from north-east Bulgaria, while in the vegetable oil from south-central Bulgaria, all phospholipids were found in almost the same quantity. Overall, significant differences were observed in the chemical composition (proteins, carbohydrates, ash and moisture)

of the fruits from the two regions of Bulgaria, as well as in the content of the main components of their essential and vegetable oils.

Г.2. Ognyanov M., Denev P., Petkova N., **Petkova Z.**, Stoyanova M., Zhelev P., Matev G., Teneva D., Georgiev Y. **2022**, Nutrient Constituents, Bioactive Phytochemicals, and Antioxidant Properties of Service Tree (*Sorbus domestica* L.) Fruits, *Plants*, 11 (14): 1832. https://doi.org/10.3390/plants11141832. **(IF$_{(2021)}$ 4.658; SJR$_{(2022)}$ 0.79). Реферирано и индексирано в Web of Science (WOS) (Q1) и Scopus (Q1)**

The current study aimed to determine the major and minor nutritional constituents of *Sorbus domestica* L. fruits. It was revealed that palmitic acid was the most commonly occurring saturated fatty acid, while linoleic acid represented the major polyunsaturated fatty acid. The sterol fraction consisted mainly of *β*-sitosterol. Small amounts of lipophilic pigments were quantified. Potassium, iron, and boron were the most abundant macro-, micro-, and ultra-trace elements. The amino acid composition analysis suggested that the non-essential amino acids predominated over the essential ones. Soluble sugars (fructose and glucose) represented a large part of the total carbohydrate content, but pectin formed the major part of polysaccharides. Malic acid was the most abundant organic acid whereas quercetin-3-*β*-glucoside, neochlorogenic, and 3,4-dihydroxybenzoic acids were the major phenolic constituents. Fruits exhibited free-radical scavenging and protecting ability against peroxyl and hydroxyl radicals. Service tree fruits provided valuable bioactive constituents having a high nutritional value and potential health benefits.

Г.3. Popova V., **Petkova Z.**, Mazova N., Ivanova T., Petkova N., Stoyanova M., Stoyanova A., Ercisli S., Okcu Z., Skrovankova S., Mlcek J. **2022**, Chemical Composition Assessment of Structural Parts (Seeds, Peel, Pulp) of *Physalis alkekengi* L. Fruits, *Molecules*, 27 (18): 5787. https://doi.org/10.3390/molecules27185787. **(IF$_{(2021)}$ 4.927; SJR$_{(2022)}$ 0.704). Реферирано и индексирано в Web of Science (WOS) (Q2) и Scopus (Q1)**

In recent years there has been an extensive search for nature-based products with functional potential. All structural parts of *Physalis alkekengi* (bladder cherry), including fruits, pulp, and less-explored parts, such as seeds and peel, can be considered sources of functional macro- and micronutrients, bioactive compounds, such as vitamins, minerals, polyphenols, and polyunsaturated fatty acids, and dietetic fiber. The chemical composition of all fruit structural parts (seeds, peel, and pulp) of two phenotypes of *P. alkekengi* were studied. The seeds were found to be a rich source of oil, yielding 14–17%, with abundant amounts of unsaturated fatty acids (over 88%) and tocopherols, or vitamin E (up to 5378 mg/kg dw; dry weight). The predominant fatty acid in the seed oils was linoleic acid, followed by oleic acid. The seeds contained most of the

fruit's protein (16–19% dw) and fiber (6–8% dw). The peel oil differed significantly from the seed oil in fatty acid and tocopherol composition. Seed cakes, the waste after oil extraction, contained arginine and aspartic acid as the main amino acids; valine, phenylalanine, threonine, and isoleucine were present in slightly higher amounts than the other essential amino acids. They were also rich in key minerals, such as K, Mg, Fe, and Zn. From the peel and pulp fractions were extracted fruit concretes, aromatic products with specific fragrance profiles, of which volatile compositions (GC-MS) were identified. The major volatiles in peel and pulp concretes were *β*-linalool, *α*-pinene, and *γ*-terpinene. The results from the investigation substantiated the potential of all the studied fruit structures as new sources of bioactive compounds that could be used as prospective sources in human and animal nutrition, while the aroma-active compounds in the concretes supported the plant's potential in perfumery and cosmetics.

Г.4. Stefanova G., Girova T., Gochev V., Stoyanova M., **Petkova Zh.**, Stoyanova A., Zheljazkov V. D., 2020, Comparative study on the chemical composition of laurel (*Laurus nobilis* L.) leaves from Greece and Georgia and the antibacterial activity of their essential oil, *Heliyon*, 6 (12), e05491, https://doi.org/10.1016/j.heliyon.2020.e05491. **($IF_{(2020)}$ -; $SJR_{(2020)}$ 0.455). Реферирано и индексирано в Web of Science (WOS) и Scopus (Q1).**

Laurel (*Laurus nobilis* L.) is a plant species from Lauraceae family, and is native to the Mediterranean region. The goal of this study was to compare chemical composition of laurel leaves and antibacterial activity of its essential oil (EO) from wild-grown trees in Greece and Georgia. The laurel leaves from the two native habitats had dissimilar concentrations of phenolic acids. Of the conjugated flavonols and flavons, kaempferol (1981.3 μg/g) and apigenin (1433.6 μg/g) were the major representatives in the leaves from Greece, while luteolin (839.1 μg/g) and kaempferol (688.1 μg/g) were the major ones in the leaves from Georgia, respectively. The EO content was 1.42% and 4.54% in the leaves from Greece and Georgia, respectively. The main EO constituents of the Greek laurel plants were 1,8-cineole (30.8%), *α*-terpinyl acetate (14.9%), *α*-terpineol (8.0%), sabinene (7.9%), and terpinen-4-ol (6.0%). The main EO constituents of the Georgian laurel plants were 1,8-cineole (29.2%), *α*-terpinyl acetate (22.6%), sabinene (12.2%), and methyleugenol (8.1%). The EO antimicrobial activities against 20 microorganisms were determined. Among the Gram-positive bacteria, the *Enterococcus faecalis* strain was the most sensitive, followed by *Staphylococcus aureus* ATCC 6538. Among the Candida species, *C. albicans* ATCC 10231 was the most sensitive to the laurel leaf EOs.

Г.5. Antova G., Gerzilov V., **Petkova Z.**, Boncheva V., Bozhichkova I., Penkov S., Petrov P. **2019**, Comparative analysis of nutrient content and energy of eggs from different chicken genotypes, *Journal of the Science of Food and Agriculture*, 99 (13), 5890–5898. https://doi.org/10.1002/jsfa.9863. **($IF_{(2019)}$ 2.614; $SJR_{(2019)}$ 0.718). Реферирано и индексирано в Web of Science (WOS) (Q1) и Scopus (Q1)**

BACKGROUND: Eggs are important foods in the daily diet of humans and have great biological activity and a high digestibility. Egg yolk is a good source of biologically active substances such as fatty acids, phospholipids, sterols and tocopherols. The eggs of seven chicken genotypes were analyzed for their chemical composition, and a detailed study of the lipids in egg yolk was conducted.

RESULTS: Energy composition of the egg yolk and egg albumen was 29.06–30.51 $MJ.kg^{-1}$ and 19.77–20.93 $MJ.kg^{-1}$ respectively. Regarding their chemical composition: water ranged from 471.7 to 515.4 $g.kg^{-1}$ and 878.3–885.9 $g.kg^{-1}$; fat content in dry matter ranged from 607 to 647 $g.kg^{-1}$ and 6.7–11.6 $g.kg^{-1}$; protein varied from 302 to 331.7 $g.kg^{-1}$ and 823.6–892.5 $g.kg^{-1}$; ash ranged from 33.7 to 37.7 $g.kg^{-1}$ and 63.8–74.0 $g.kg^{-1}$; and nitrogen-free extracts ranged from 12.7 to 36.5 $g.kg^{-1}$ and 35.0–96.2 $g.kg^{-1}$. The sterols and phospholipids in the yolk lipids were 16–26 $g.kg^{-1}$ and 59–127 $g.kg^{-1}$. The main fatty acids in the lipids were oleic (39.1–47.3%) and palmitic (26.0–35.5%) acids. Cholesterol in the yolk lipids ranged from 15.9 to 25.9 $g.kg^{-1}$. Phosphatidylcholine (389–573 $g.kg^{-1}$), phosphatidylethanolamine (219–355 $g.kg^{-1}$) and phosphatidylinositol (112–284 $g.kg^{-1}$)were themain phospholipids. The content of saturated fatty acids inthe phospholipidswas significantlyhigher than that in triacylglycerols.

CONCLUSION: Small variations in the chemical composition of eggs from seven different genotypes were observed. Significant differences in the fatty acid compositions of the main classes of phospholipids and the triacylglycerol fractionwere established.

Г.6. Popova V., **Petkova Z.**, Ivanova T., Stoyanova M., Panayotov N., Mazova N., Stoyanova A., **2020**, Determination of the chemical composition of seeds, peels, and seedcakes from two genotypes of Cape gooseberry (*Physalis peruviana* L.), *Turkish Journal of Agriculture and Forestry*, 44, 642-650. doi:10.3906/tar-2003-66. **($IF_{(2020)}$ 2.585; $SJR_{(2020)}$ 0.624). Реферирано и индексирано в Web of Science (WOS) (Q2) и Scopus (Q2)**

Physalis peruviana L. fruit (Cape gooseberry, CG) is a rich source of phytonutrients, including vitamins, minerals, polyphenols, polyunsaturated fatty acids (FAs), phytosterols, dietetic fibers, and others. The popularity and production areas of CG have been expanding worldwide, thus producing fruit with origin-substantiated differences in their nutrient composition.This study was based on the comparative assessment of 2 genotypes of CG produced in Bulgaria (CG-P and CG-F), through analysis of the lipid fraction of different fruit elements (seeds, peels), and further examination of the extracted seedcakes. The CG seeds reasonably yielded more oil (17.0%–22.2%)

than the isolated peels (2.8%–2.9%). The main FAs in the CG-P seed oil were oleic (29.6%) and palmitic (20.6%), and in the CG-F seed oil were palmitic (20.9%) and stearic (17.5%). Both CG peel oils were dominated by palmitic acid (43.0%–60.2%), but there was a significant variation of some other FAs. The group of bioactive tocopherols was found exclusively in the oil extracted from the CG seeds, with no significant difference between the genotypes; *β*-tocopherol and *δ*-tocopherol were the most abundant. Waste from the oil extraction (the seedcakes) was found to contain high levels of macro and microminerals (K, Mg, Cu, Zn, Mn, and others), fiber (40.26%–47.62%), protein (13.73%–8.08%), and essential amino acids, with some genotype-based variations. The results demonstrated that, concerning the studied aspects of fruit composition, CG produced in Bulgaria was comparable to the fruit of other origins; hence, they might be of practical interest to national agricultural and food producers, as well as to the food industry on a wider basis, as new details are added to the knowledge about CG fruit. The outcomes from the examination of the CG seedcakes were in favor of their potential in human and animal nutrition, and might serve as grounds for the development of new products.

Г.7. Fidan H., Stankov St., Petkova N., **Petkova Zh.**, Iliev A., Stoyanova M., Ivanova T., Zhelyazkov N., Ibrahim S., Stoyanova A., Ercisli S., **2020**, Evaluation of chemical composition, antioxidant potential and functional properties of carob (*Ceratonia siliqua* L.) seeds, *Journal of Food Science and Technology*, https://doi.org/10.1007/s13197-020-04274-z. **($IF_{(2020)}$ 2.701; $SJR_{(2020)}$ 0.656). Реферирано и индексирано в Web of Science (WOS) (Q3) и Scopus (Q2)**

The interest in carob pod as an ingredient of functional foods is constantly increasing due to its beneficial health effect and functional properties. The aim of this study was to evaluate the chemical composition and antioxidant potential of carob seeds, as well as the functional properties of its isolated galactomannan fraction. The lipid, protein, carbohydrate and phenolic composition were analysed. The obtained results demonstrated that the main detected fatty acids were oleic (45.0%), linoleic (32.4%), and palmitic (16.6%) acid. The primary tocopherol in the tested tocopherol fraction was *γ*-tocopherol (53.1%). It was established that *β*-sitosterol (74.2%) and stigmasterol (12.8%) predominated in the sterol fraction. Carob seed was characterized by high protein (25.7%) content, while mannose and galactose were the dominating monosaccharides. Moreover, the isolated galactomannan from carob seed demonstrated good swelling properties – 30.1 ml per g sample and oil-holding capacity (27.9 g/g sample). The total polyphenolic and flavonoids content of carob seeds was 1.76 mg Gallic acid equivalent/g dry weight and 0.30 mg quercetin equivalents/g dry weight, respectively. The carob seeds showed the highest antioxidant potential by cupper reduction assay—15.71 mM Trolox® equivalent/g dry weight. The mineral composition was also defined as the macroelements Ca and Mg were the predominant minerals in the seed. The obtained results showed that carob seeds were a valuable source not only of phenolic

compounds and antioxidants, but also of proteins, lipids, galactomannan with functional properties that could improve the nutritional value of foods in which are incorporated.

Г.8. Marudova M., Momchilova M., Antova G., **Petkova Z.**, Yordanov D., Zsivanovits G., **2018**, Investigation of fatty acid thermal transitions and stability in poultry pates enriched with vegetable components, *Journal of Thermal Analysis and Calorimetry*, 133 (1), 539-547. https://doi.org/10.1007/s10973-017-6841-z. **($IF_{(2018)}$ 2.471; $SJR_{(2018)}$ 0.634). Реферирано и индексирано в Web of Science (WOS) (Q2) и Scopus (Q2)**

The aim of the study was to describe the thermal characteristics of poultry pates enriched with vegetable components in relation to their chemical composition and technological process. Two poultry pates from chicken liver, chicken or turkey meat with vegetables were developed. The thermal characteristics of the raw materials and the ready pates were examined by differential scanning calorimetry; fatty acid profiles were detected by gas chromatography analysis. The study investigated the effect of such factors as heating/cooling rate and matrix effect of other components (e.g., proteins) in the raw materials and in the pates. It was observed that the cooling rate has a considerable effect on melting/crystallization temperature, enthalpy, and height of peaks in the process of pates fat crystallization, as well as peak height and enthalpy in the melting process. The first peaks formed during the crystallization were characterized by high instability, demonstrated by various peak shapes. The rapid cooling led to lowering of the melting point, assigned to the presence of unstable α crystals. The slow cooling led to mainly stable β' crystals. The fraction of unsaturated fatty acids present in the fat was important for both crystallization rate and melting points in the raw materials and in the products as well. This effect was stronger in the pate products because of the presence of diverse components such as proteins. The results obtained could be used for the evaluation of thermal stability of pate fatty acids and further optimization of the pate thermal treatment.

Г.9. Antova G, Angelova-Romova M., **Petkova Z.**, Teneva O., Marcheva M., Zlatanov M., **2017**, Biologically active components in *Madia sativa* seed oil, *Journal of Food Science and Technology*, 54, 3044-3049. DOI 10.1007/s13197-017-2739-9. **($IF_{(2017)}$ 1.797; $SJR_{(2017)}$ 0.689). Реферирано и индексирано в Web of Science (WOS) (Q2) и Scopus (Q2)**

Biologically active components in lipids (fatty acids, phospholipids, sterols and tocopherols) from three varieties of *Madia sativa* seeds introduced in Bulgaria (BGR 457 and BGR 458 with German origin, and BGR 459 with US origin) were investigated. Glyceride oil in the seeds was found to be 36.6, 34.2 and 35.4%, respectively. Total phospholipid content was 2.4, 1.7 and 2.6% and the main classes were phosphatidylcholine, phosphatidylinositol and

phosphatidylethanolamine. The amount of sterols in the oil was 0.3% for all samples and the major component was β-sitosterol, followed by campesterol and stigmasterol. Total tocopherols in the oils were 768, 795 and 856 $mg.kg^{-1}$, respectively and α-tocopherol predominated (more than 70.0%). Fatty acid composition of triacylglycerols and sterol esters was also established. Main fatty acids in triacylglycerols were linoleic (47.5–50.5%), oleic (30.2–32.4%) and palmitic acids (13.0–13.5%). The content of saturated fatty acids (palmitic and stearic) in sterol esters (40.1–50.9%) was significantly higher than in triacylglycerols (18.3–19.4%).

Г.10. **Petkova Z.**, Antova G., Angelova-Romova M. **2020**, Biologically active components and health benefits of nettle seed oil, *Grasas y Aceites*, 71 (1), p. e347. doi: http://dx.doi.org/10.3989/gya.0108191. **($IF_{(2020)}$ 1.650; $SJR_{(2020)}$ 0.384). Реферирано и индексирано в Web of Science (WOS) (Q4) и Scopus (Q3)**

The biologically active components of nettle seed oil and important lipid indices, which are criteria for the health benefits of the oil, have been examined. Linoleic acid predominates in triacylglycerols (77.7%), followed by oleic (16.2%). Sterols in the lipids are present at 1.1% and β-sitosterol is the main component (90.1%). The oil contains 711 mg/kg tocopherols and γ-tocopherol predominates (36.1%), followed by α-tocopherol (28.9%) and δ-tocopherol (26.9%). Atherogenicity and thrombogenicity index values are significantly low, which determine the best anti-atherogenic and anti-thrombogenic properties of the oil. The cholesterolemic index and the ratio of polyunsaturated and saturated fatty acids are considerably higher than 1.0 and reveal good hypo-cholesterolemic potential and nutritional value. The content of biologically active components of nettle seed oil indicates that it is a rich source of essential fatty acids, sterols and tocopherols and this oil can be used in food, cosmetics, and pharmaceutical products.

Г.11. **Petkova Z.,** Antova G. **2019**, A comparative study on quality parameters of pumpkin, melon and sunflower oils during thermal treatment, *OCL-Oilseeds and fats, Crops and Lipids*, 26, 32. DOI: https://doi.org/10.1051/ocl/2019028. **($IF_{(2019)}$ -; $SJR_{(2019)}$ 0.365). Реферирано и индексирано в Web of Science (WOS) и Scopus (Q2)**

Current paper reveals the impact of thermal treatment on the quality of two seed oils – pumpkin and melon compared to the quality of the most used oil – sunflower oil. Conventional and microwave heating were used for processing the oils. The duration of the thermal treatment was 9, 12 and 18 min for the conventional heating. The microwave heating was performed with two microwave powers of the equipment (600Wand 900 W) for 3, 6, 9 and 12 min. At every stage of the thermal processing were determined acid and peroxide value, the absorbance of the oils at 232 and 268 nm, tocopherol and fatty acid composition. It was observed that the degree of oxidation of the examined oils during microwave and conventional heating increased with the

duration of the thermal process and the power of the microwaves. Also, the two methods of heating had a little impact on the processes leading to the formation of free fatty acids. Total tocopherols of the melon seed oil were more stable to thermal treatment. The amount of linoleic acid decreased in the pumpkin and sunflower oils during microwave treatment, while that of oleic and palmitic acid relatively increased. The biggest change in the fatty acid composition of both oils was found during microwave heating at 900W. The changes in fatty acid composition of thermally treated melon seed oil were insignificant. Overall, melon seed oil was observed to be more thermally stable than pumpkin and sunflower oils.

Г.12. **Petkova Z.**, Antova G., Angelova-Romova M., Petrova A., Stoyanova M., Petrova S., Stoyanova A. **2020**, Bitter vetch seeds (*Vicia ervilia* L.) – A valuable source of nutrients, *Bulgarian Chemical Communications*, 52 (B), 12 – 15. DOI: 10.34049/bcc.52.B.0003. **($IF_{(2020)}$ -; $SJR_{(2020)}$ 0.179). Реферирано и индексирано в Scopus (Q4).**

The main nutrients of seeds from bitter vetch (*Vicia ervilia* L.) were determined. The seeds were with Russian origin, introduced in Bulgaria. They possessed high carbohydrate (66.2%) and protein (20.1%) content, but their oil content was extremely low (1.4%). The starch and dietary fibers were found to be 20.4 and 3.1%, respectively. The individual composition of water-soluble carbohydrates was determined by high performance liquid chromatography on an Agilent® LC 1220 instrument equipped with a refractive index detector. Total content of water-soluble carbohydrates was found to be 4279.0 mg/100 g. It was established that the only disaccharides in the seeds were sucrose (2649.8 mg/100 g) and cellobiose (149.4 mg/100 g), while the main monosaccharides were glucose (600.9 mg/100 g) and fructose (540.7 mg/100 g). The content of rhamnose and xylose was 198.3 and 140.1 mg/100 g, respectively. Amino acid composition of the seeds from bitter vetch was also determined and 17 amino acids were identified. The major amino acid was phenylalanine (46.2 mg/g), followed by lysine (34.8 mg/g) and histidine (30.0 mg/g). The moisture of the seeds was found to be 9.7% and the ash content was 2.6%. The content of the main nutrients in bitter vetch seeds is relatively high which determines their satisfying energy value – 357 kcal/100 g (1520 kJ/100 g) that corresponds to the energy value of the most commonly used legume seeds.

III. EDUCATION TEXTBOOK

E.1. Antova G., Angelova-Romova M., **Petkova Zh.**, Teneva O., Simeonova Zh., Textbook for Laboratory work in Food Chemistry, Plovdiv University Publishing House “Paisii Hilendarski”, Plovdiv, 2023. eISBN 978-619-202-859-6.

The textbook for the discipline "Food Chemistry" is intended for students in the "Master's" degree in "Food Chemistry", as well as for students in "Bachelor" degree in "Chemical Analysis and Quality Control" at University of Plovdiv "Paisii Hilendarski".

The guide examines the principles of existing modern methods for the analysis of the main components of the food products – proteins, carbohydrates, lipids, vitamins and water content, as well as the methods for their quantitative determination. The experiments included in the textbook are in accordance with the curriculum of the discipline "Food Chemistry". Each topic begins with a short theoretical introduction on the composition, properties and importance of the main nutrients of food. The method of work and the equipment used in the research are indicated. The exercises in the manual are aimed at imparting knowledge and creating skills for solving practical problems from everyday chemical practice for the quality of food products in terms of determining the content of the main nutrients and biologically active components, as well as determining the main physicochemical indicators of lipids and of food products. Students will become familiar the basic modern methods for food analysis

HABILITATION EXTENDED REFERENCE

The habilitation reference was prepared on the basis of **6** publications in journals, referenced and indexed in databases Scopus and/or Web of Science (Annex 5; indicator B.4).

Scientific publications of Ch. Assist. Prof. Zhana Yuliyanova Petkova, PhD according to indicator B.4., in connection with participation in the announced in SG, no. 39 of 02.05.2023, competition for the occupation of an academic position "Associate Professor" in the field of higher education 4. Natural sciences, mathematics and informatics, professional direction 4.2. Chemical Sciences (Organic Chemical Technology, Food Chemistry).

The numbering of the publications is according to **Appendix 5** (indicator **B.4.**):

B.4.1. Fidan H., Stankov S., Stoyanova M., **Petkova Z.**, Petkova N., Stoyanova A., Ercisli S., Choudhary R., Karunakaran R. Chemical Composition of *Pinus nigra* Arn. Unripe Seeds from Bulgaria. *Plants*. **2022**; 11(3):245. https://doi.org/10.3390/plants11030245. **($IF_{(2021)}$ 4.658; $SJR_{(2022)}$ 0.79). Referenced and indexed in Web of Science (WOS) (Q1) and Scopus (Q1)**

B.4.2. Stankov S., Fidan H., **Petkova Z.**, Stoyanova M., Petkova N., Stoyanova A., Semerdjieva I., Radoukova T., Zheljazkov V. **2020**, Comparative Study on the Phytochemical Composition and Antioxidant Activity of Grecian Juniper (*Juniperus excelsa* M. Bieb) Unripe and Ripe Galbuli, *Plants*, 9, 1207; https://dx.doi.org/10.3390/plants9091207. **($IF_{(2020)}$ 3.935; $SJR_{(2020)}$ 0.892). Referenced and indexed in Web of Science (WOS) (Q1) and Scopus (Q1)**

B.4.3. Popova V., **Petkova Z.**, Ivanova T., Stoyanova M., Lazarov L., Stoyanova A., Hristeva T., Docheva M., Nikolova V., Nikolov N., Zheljazkov V. **2018**, Biologically active components in seeds of three *Nicotiana* species, *Industrial Crops & Products*, 117, 375-381. https://doi.org/10.1016/j.indcrop.2018.03.020. **($IF_{(2018)}$ 4.191; $SJR_{(2018)}$ 1.015). Referenced and indexed in Web of Science (WOS) (Q1) and Scopus (Q1)**

B.4.4. **Petkova Z.**, Antova G., Angelova-Romova M., Todorova I., Stoyanova M., Stoyanova A. **2022**, *Lupinus angustifolius* L. cultivar "Boregine" from South of Bulgaria: a source of nutrients and natural biologically active components, *OCL-Oilseeds and fats, Crops and Lipids*, 29, 10. https://doi.org/10.1051/ocl/2022003. **($IF_{(2022)}$ -; $SJR_{(2022)}$ 0.356). Referenced and indexed in Web of Science (WOS) and Scopus (Q2)**

B.4.5. Popova V., **Petkova Z.**, Ivanova T., Stoyanova M., Mazova N., Stoyanova A. **2021**, Lipid composition of different parts of Cape gooseberry (*Physalis peruviana* L.) fruit and valorization of seed and peel waste, *Grasas y Aceites*, 72 (2), e402. https://doi.org/10.3989/gya.1256192. **($IF_{(2012)}$ 1.416; $SJR_{(2022)}$ 0.311). Referenced and indexed in Web of Science (WOS) (Q4) and Scopus (Q3)**

B.4.6. **Petkova Z.**, Stefanova G., Girova T., Antova G., Stoyanova M., Damianova S., Gochev V., Stoyanova A., Zheljazkov V. D., **2019**, Phytochemical investigations of laurel fruits (*Laurus nobilis*). *Natural Product Communications*, 1-10. https://doi.org/10.1177/1934578X19868876. **(IF$_{(2019)}$ 0.468; SJR$_{(2019)}$ 0.199). Referenced and indexed in Web of Science (WOS) (Q4) and Scopus (Q4).**

10 independent positive citations in Scopus/Web of Science were found for the publications (Annex 6.1; indicator Д.12).

The research and processing of the results of the presented publications were carried out in various structural units of University of Plovdiv „P. Hilendarski“, University of Food Technologies (Plovdiv), Tobacco and Tobacco Products Institute (Markovo), “Angel Kanchev” University of Russe, Oregon State University (USA).

Unconventional plant sources of biologically active substances and their potential application for food purposes

INTRODUCTION

Over ten thousands of edible plants are present in the world. However, only 150 plant species have found a niche in the market, twelve of which supply approximately 80% of the food energy needed by humans; and only four types meet the necessary requirements for energy value and protein content, including rice, wheat, corn and potatoes. Therefore, in recent years, the focal point of various researches are plants that have not been sufficiently studied, but are a source of valuable nutritional ingredients and can successfully replace established plant species in the human diet. On the other hand, these plants can contain in larger quantities other biologically active components, such as essential fatty acids and amino acids, tocopherols, carotenoids, phospholipids, sterols and polyphenols, which are not synthesized in the human body. They are taken only with food and their balanced intake is an important for the health.

For that reason, my studies in the recent years has been focused on the determination of these components in a number of unconventional plant sources, with the aim of establishing their potential application in various food products with improved functional properties and oxidative stability. A small number of such plants with potential use in the food industry are: black pine (*Pinus nigra* Arn.), juniper (*Juniperus excelsa* M. Bieb), tobaco (*Nicotiana alata* Link & Otto, *N. rustica* and *N. tabacum*), lupin (*Lupinus angustifolius* L. cultivar “Boregine”), cape gooseberry (*Physalis peruviana* L.) and bay laurel (*Laurus nobilis*).

1. Chemical and lipid composition of unripe black pine seeds (*Pinus nigra* Arn.)

The Pinaceae family consists of 11 genera (Delipavlov и Chechmedzhiev, 2003). With more than 100 existing species, Pinus is the largest genus of conifers and the most widespread in the Northern Hemisphere. There are five species naturally growing in Bulgaria: *Pinus sylvestris* L., *Pinus nigra* Arn., *Pinus peuce* Grab., *Pinus holdreichii* H. and *Pinus mugo* Turra. Species of

the genus Pinus are a source of seeds that contain a wide variety of nutrients (Nergiz и Dönmez, 2004). Examples of such are cedar seeds, which are obtained from the species *P. pinea* L., and are used as delicacies. The content of monounsaturated and polyunsaturated fatty acids in the composition of seeds of the genus Pinus is high, and it is considered a prerequisite for preventing cardiovascular disease (Savage, 2001). Many studies on the composition of other species of the genus Pinus showed that their composition varies depending on geographical and climatic conditions (Wolff et al. 2001; Cheikh-Rouhou et al., 2006; Kadri et al., 2014; Macchioni et al., 2003). The studies showed that pine seeds contain α-linolenic acid, antioxidants, and other biologically active components (Kadri et al., 2015; Cheikh-Rouhou et al., 2006; Kadri et al., 2014).

The chemical composition of the various anatomical parts of *P. sylvestris* and *P. nigra*, and the period of their vegetation, determine the composition of many components in their composition. The species' quantitative and qualitative composition depends on the soil, climatic, geographical, and species characteristics, to no small extent. Both low and high levels of nutrients in plant cells can indicate the species' resilience and the possibility of its development (Parzych and Sobisz, 2012). The use of unripe cones in various food products such as jams, jellies, and infusions in traditional Bulgarian folk medicine necessitates the further evaluation of their chemical characterization. Seeds are a source of biologically active components and may have applications in nutrition or food technology. Therefore, the fatty acid and tocopherol composition of the isolated glyceride oil from the unripe black pine seeds was investigated, as well as the content of carbohydrate, cellulose, protein and amino acid composition, total ash content and mineral composition of the residual meal after the extraction of the vegetable oil were determined.

The lipids of the unripe seeds of *P. nigra* was 1.68%. The amount of essential linoleic (n-6) acid (44.2%) was the highest in the composition of the oil from the unripe seeds. Among saturated fatty acids, palmitic acid had the highest content (31.2%). Pinolenic acid is a polyunsaturated fatty acid that occurs mainly in plants, especially in gymnosperms, and has a high content in the analyzed seeds - 10.5%. Oleic acid content was lower (8.8%), followed by a small amount of linolenic acid (3.0%). The content of the other fatty acids varied from 0.1 to 0.6%. Black pine seed oil contained higher levels of unsaturated fatty acids (67.2%) compared to the lower amount of saturated fatty acids (32.8%). The amount of polyunsaturated fatty acids prevailed (57.8%) in the oil, constituting 86.01% of the amount of unsaturated ones. The content of monounsaturated fatty acids was low (9.4%) and constituted 13.99% of the share of the total unsaturated fatty acids. Regarding the tocopherol composition of the studied oil, α-tocopherol (53.1 0.4%) and γ-tocopherol (46.9 ± 0.2%) were found in the tocopherol fraction. The total content of tocopherols in the examined oil from unripe seeds was significantly high – 1290 mg/kg.

The main carbohydrate determined in black pine seed cake was cellulose (28.58 ± 0.27%), and the content of water-soluble sugars was 0.20 ± 0.02%, represented mainly by glucose (0.09%). fructose (0.02%) and sucrose (0.03%), while sorbitol was not detected in the samples. The protein content of the seed cake was determined to be around 38.42 ± 0.37%. The main amino acids were asparagine (3.92 mg/g), serine (3.79 mg/g), alanine (3.65 mg/g), arginine (3.32 mg/g), phenylalanine (2.98 mg/ g), lysine (2.85 mg/g), tryptophan (2.44 mg/g), valine (2.33 mg/g),

isoleucine (2.28 mg/g), and tyrosine (2.05 mg /g). The ash content in the residual black pine seed cakes amounted to 2.99 ± 0.02%. The results exhibit high values of the macroelements in the composition of the seed cake. Potassium had the highest concentration (8048.00 mg/kg), followed by magnesium (172.99 mg/kg). The lower content of nitrogen (1.96 mg/kg) and phosphorus (0.08 mg/kg) can be explained by the slower rate of plant development as well as the lower levels of macronutrients in the soil.

The obtained results highlight the potential use of unripe black pine seeds in various fields due to their nutritional content and presence of biologically active substances.

2. Phytochemical, lipid composition and antioxidant activity of ripe and unripe juniper galbuli

The genus Juniperus (Cupressaceae) contains more than 60 species, widespread, mainly in the northern hemisphere including North America, Europe, and Asia (Adams, 2004). *Juniperus excelsa* is an evergreen tree species up to a height of 15 m, with medicinal and landscaping importance. The galbuli (cones) are spherical with a diameter of 7 to 12 mm and are covered with a grayish-gray coating (Yordanov, 1963). The habitats of *J. excelsa* form endemic juniper forests, and they are very rare in the European countries. The species is included in the IUCN Red list (Farjon, 2017) and Red Data Book of the Republic of Bulgaria under the "Critically Endangered" category (Peev et al., 2015). In Bulgaria, *J. excelsa* is a rare plant species protected by the Biological Diversity Law in Bulgaria (Peev et al., 2015). In Bulgaria, the plant grows in places with Mediterranean and temperate continental climate on the steep slopes of deep gorges in the Western Rhodopes (the reserve "Izgoryaloto Gyune"), and more commonly occurs in the valley of the Struma river (the reserve "Tisata"). The latter reserve includes thousands of *J. excelsa* trees, making it the most representative and numerous population of this species in Bulgaria.

Because of its phytochemical composition, Juniperus species are used in folk medicine; they are widely used in the treatment of various diseases such as cough, cold, hemorrhoids, fungal infections, *etc.* (Filipowic et al., 2003; Güvenç et al., 2012). Some previous reports have shown diversities in the chemical composition of the *J. excelsa* galbuli essential oils from different parts of the world (Hafi et al., 2015; Topçu et al., 2005). The diversities in the quantity and quality of the volatile oils are the function of genetic and nongenetic variables such as climatic, edaphic conditions, the season and time of the harvest, and even the duration of the sunlight exposure (Adams, 2004).

Knowledge of the antioxidant properties of many plant species allows their usage as a means of preserving food quality by slowing down or preventing lipid oxidation processes (Brewer, 2011; Asif, 2015].

However, there is no information on the composition and the antioxidant activity of unripe and ripe galbuli of *J. excelsa*. Therefore, the goal of this study was to investigate the phytochemical composition and to assess the content of phenolic compounds, flavonoids, as well as the antioxidant capacity of *J. excelsa* galbuli extracts. The hypothesis was that the phytochemical

composition, phenolic compounds, and antioxidant activity of *J. excelsa* galbuli will depend on their maturity phase (unripe and ripe) and collection location.

Ripe and unripe juniper galbuli collected from two protected areas in Bulgaria were used for the experiment:

1) location 1 - the reserve “Izgoryaloto Gyune”, above the town of Krichim, Bulgaria (part of the Rodopi Mountain); and

2) the reserve „Tisata”, consisted of two sections: location 2 – east and west-located in the Maleshevska Planina; and location 3 – west, located in Pirin Mountain.

The protein content was found to be the highest (16.4%) in the unripe galbuli of *J. excelsa* collected from location 2. The protein content of the unripe galbuli (location 1 and 2) was higher than that of the fully ripe ones from the same regions. On the other hand, the ripe galbuli from location 3 had a higher protein content (15.4%) than unripe ones. The differences in the amount of the protein fraction in the unripe and ripe galbuli of *J. excelsa* may be due to the difference in the content of moisture of the samples, as there is a relationship between the amount of moisture and the protein content of the samples.

Chlorophyll content was higher in the ripe galbuli from location 3 (273,4 μg/g d.w.), and the levels of chlorophyll *a* and chlorophyll *b* were 193,1 and 80,3 μg/g d.w., respectively. The lowest concentrations of total chlorophyll were found in the sample of unripe galbuli from the same location. Total carotenoid content varied between 41.7 (ripe galbuli from location 2) and 50.4 μg/g d.w. (ripe galbuli from location 3). Ripe galbuli from *J. excelsa* (location 1) had the highest essential oil yield of 5.1%, followed by the sample of ripe galbuli from locations 2 and 3 (2.6% and 2.5%, respectively). In general, the ripe galbuli were characterized by a higher essential oil content than unripe samples. Lipid content (ranging between 4.5% and 9.1%) was higher in the ripe galbuli from all locations compared to unripe ones.

The results for the fatty acid composition of glyceride oil from ripe and unripe juniper galbuli from different geographical locations showed no dependence between individual samples. The amount of linoleic acid (26.2%) was the highest in unripe galbuli from location 1 compared to the other samples. The content of saturated fatty acids (palmitic acid) in the sample of unripe galbuli (location 2) was the highest (41.3%) compared to the other samples. Palmitic acid was the major fatty acid in lipids from almost all galbuli except those from location 3. Oleic acid content was lowest in the glyceride oil from unripe galbuli from location 2 (8.4%) and highest in the ripe ones from location 3 (32.6%). The amount of stearic acid varied between 4.5% and 8.4%. A small amount of linolenic (1.3–4.8%), eicosatrienoic (1.7–8.8%) and lignoceric (1.8–6.4%) acids were also detected in all samples.

The highest unsaponifiable matter content was found in the lipid fraction of ripe galbuli (13.5%) from location 3, followed by ripe (11.4%) and unripe (11.6%) galbuli samples from location 2. The content of sterols was also the highest in the lipid fraction of ripe galbuli from location 2 and 3 (0.3%). The total tocopherol composition of the glyceride oil of ripe galbuli from location 1 was the highest (1894 mg/kg), while the lowest levels (721 mg/kg) were found in unripe galbuli from location 2. Overall, it was observed that the total amount of tocopherols increased

with the ripening of the juniper pods. For all investigated samples, it was found that the main component in the sterol fraction was β-sitosterol (64.8 – 91.7%), and in the tocopherol fraction were α-tocopherol (19.0 – 88.5%) and α-tocotrienol (6.1 – 66.2%).

Histidine was the main essential amino acid (5.5–8.0 mg/g) in all samples, followed by lysine (4.0–6.1 mg/g). Among non-essential amino acids, asparagine (3.4–8.6 mg/g), alanine (4.6–7.1 mg/g) and glutamic acid (2.8–6.7 mg/g) had the highest concentrations in all tested samples. The results showed that the ripening of juniper galbuli had a significant effect on their amino acid composition. The antioxidant potential of the 95% and 70% ethanol extracts was analyzed using four different methods. A positive correlation was found between antioxidant potential and phenolic content in juniper galbuli.

The results obtained in this study demonstrate the differences in the phytochemical composition and antioxidant activity of *J. excelsa* galbuli as a function of the stage of maturity and the location of their collection.

3. Biologically active components in seeds of three Nicotiana species

Tobacco is a perennial plant from the Solanaceae family, grown as an important cash crop for over 350 years in many countries around the world. Although the genus includes more than 65 species, *Nicotiana tabacum* L. (common tobacco) is the only commercially cultivated and economically important species. *Nicotiana rustica* L. (also known as Aztec or wild tobacco), is characterized by up to nine times more nicotine than common tobacco, and has been and continues to be used in very limited amounts in Mexico, Russia, South America, Vietnam, and other Asian countries (Kishore, 2014; Yadav et al., 2016).

Tobacco has long been a cash crop for Bulgaria, with great impact on the national economy. Three types of *N. tabacum* (common tobacco) are grown in Bulgaria: sun-cured Oriental (basma and Kaba Koulak), flue-cured Virginia bright, and air-cured Burley. Cured and fermented tobacco leaves are used for the production of various smoking and smokeless tobacco products, and their chemical composition and biological activity have been studied extensively (Rodgman and Perfetti, 2013). The tobacco plant, however, offers a wide range of alternative applications, such as production of biopellets from tobacco stalks as a renewable energy source, biodiesel from tobacco seeds, seed oil for the nutraceutical market, seed oil and cake for animal feeds, silage mixes, and more (Grisan et al., 2016; Rossi et al., 2013; Xie et al., 2011).

Common tobacco seeds have been considered as a source of glyceride oil, and its chemical composition has been studied previously (Ali et al., 2008; Xie et al., 2011; Zlatanov et al., 2007a).

Despite some previous studies on the chemical and lipid composition of the seeds from traditional types of tobacco, there is limited information about the chemical composition of seeds and seed oils in other tobacco species grown side by side (Koiwai et al., 1983). In 2015, the Tobacco and Tobacco Products Institute in Bulgaria started experimentally growing two (uncommon for the country) tobacco species, *N. alata* Link. & Otto and *N. rustica* L., with the intention of widening the scope of biologically active products obtained from tobacco leaves and seeds. It was hypothesized that the chemical composition of *N. alata*, *N. rustica*, and *N. tabacum*

grown side by side would differ, and would depend on genotype. The objective of this study was to present a comparative analysis of biologically active compounds in seeds, seed oils, and seed cakes from two tobacco species introduced in Bulgaria (*N. alata* and *N. rustica*), and compare those with *N. tabacum* (common tobacco).

The glyceride oil content of the seeds varied between 30.9% (*N. tabacum*) and 40.9% (*N. alata*, pink petals). The phospholipid content was low (0.2–0.3% in the oils) and the total sterol content was 0.35–0.48%. The main component in the sterol fraction was *β*-sitosterol (62.1 – 73.6%) of all seed oils of the investigated tobacco varieties; followed by cholesterol (13.0%) and Δ^5-avenasterol (9.3%) (in *N. alata* white petals); cholesterol (14.5%) and campesterol (7.9%) (in *N. alata* pink petals); campesterol (11.8%) and Δ^5-avenasterol (8.3%) (in *N. rustica*); campesterol (15.0%) and stigmasterol (10.7%) (in *N. tabacum*). The content of tocopherols varied between 101 and 178 mg/kg, being lowest in the seed oil of tobacco seeds *N. alata*, white petals, and *N. tabacum*, and the highest in that of the seeds from *N. rustica*. The main component in the tocopherol fraction in all samples examined was *γ*-tocopherol (higher than 97%). The main fatty acids in the three Nicotiana species were linoleic (61.7–67.6%), oleic (15.5–19.0%) and palmitic acids (9.1–12.5%).

Some important nutritional components in the seed cakes (after extraction of the glyceride oil) have also been determined: minerals, cellulose, proteins and amino acids. The cellulose content of the cakes varied between 32.5 and 45.2%, and the protein content was 26.7–34.1%. Seed cake was rich in macro- and micronutrients, with differences in their content observed between the varieties. The highest nitrogen and protein content was found in *N. rustica* (5.5% and 34.1%, respectively) and the highest cellulose content was found in *N. alata* (white petals) (45.2 %). The main amino acids were aspartic acid (0.190 g/100g), threonine (0.104 g/100g) and arginine (0.096 g/100g) in *N. alata* (pink petals), and in *N. rustica* seed cake – arginine (0.187 g/100g), aspartic acid (0.100 g/100g) and histidine (0.087 g/100g). The amount of potassium was higher in the seed cake from *N. tabacum* and *N. rustica* (11890.5 and 11378.2 mg/kg, respectively), while that of iron and zinc was the highest in *N. alata* (pink petals) (208.4 and 112.6 mg/kg, respectively).

Based on the obtained results, an assumption can be made about the potential alternative use of tobacco seeds and seed cakes as animal feed and possibly as a raw material for the preparation of new food products beneficial to human health. The relatively low concentration of lysine, methionine, and cysteine, being limiting amino acids in pig and poultry nutrition, suggests that tobacco seed cakes should be combined with other diet ingredients in livestock feed.

4. Nutritional components and natural biologically active substances in lupin seeds (*Lupinus angustifolius* L. cultivar "Boregine")

In recent years, there has been a growing interest in new alternative crops that could be a source of valuable products for people. Lupin can be offered as an alternative to the main legume crops in our country. Lupin belongs to the Fabaceae family, it is similar in composition to chickpeas and peanuts. It is believed to contain all 8 essential amino acids, making it a preferred protein, although the percentage is not the highest compared to other plant sources. After many

years of research, lupin has become a source of important proteins in the food industry and may even replace soybeans in some markets, and according to some studies, the seeds are useful in the prevention of cardiovascular diseases.

The soil and climate conditions in Bulgaria are suitable for growing lupin, especially in areas where the soils have an acidic reaction and other leguminous crops (soy and peas) do not develop normally in these circumstances. There are no selected varieties of this crop in our country; seeds of foreign varieties are imported by companies in limited quantities, which is why there is a lack of research on their chemical composition.

In connection with this, the aim of the present study is to conduct research on the chemical composition of lupin seeds (*Lupinus angustifolius* L. cultivar "Boregine") with German origin, as well as to characterize the isolated lipids in terms of fatty acid composition and content of biologically active substances in order to be performed full assessment of their nutritional qualities.

The chemical and lipid composition of the lupin seeds were investigated, and the physicochemical characteristics of the oil were determined: acid, peroxide, saponification and iodine values, relative density, refractive index and oxidative stability using the accelerated method with "Rancimat" apparatus. The seeds were rich in protein (23.9%) and carbohydrates (57.1%), mostly starch (24.1%), but there was relatively low content of glyceride oil (7.4%). Sucrose (2341.4 mg/100 g) was found to be the major disaccharide and the major amino acids were phenylalanine (24.8 mg/g), arginine (13.6 mg/g), tyrosine (12.8 mg/g) and serine (12.6 mg/g).

Linoleic (41.0%) and oleic (32.9%) acids predominated in the glyceride oil, followed by saturated palmitic (11.9%) and stearic (7.4%) acids. A small amount of linolenic acid was also found - about 4.4%. Unsaturated fatty acids prevailed in lupin seed oil (79.6%), with the amount of polyunsaturated acids constituting of 46.1% of the fatty acid composition.

Total content of sterols in the lipid fraction was about 1.0%, and that of tocopherols was significantly high (1585 mg/kg). β-Sitosterol (71.3%) was the major component in the sterol fraction of lupin seed oil, followed by campesterol (24.3%). In the tocopherol fraction, γ-tocopherol was the main component, which constituted 92.9% of the total amount of tocopherols. The oil also containsed a small amount of α-tocopherol (5.2%) and γ-tocotrienol (2.6%).

Phosphatidylinositol and phosphatidylcholine made up more than 50% of the total phospholipids, with oleic acid being the most abundant of all phospholipid classes. All of the physicochemical characteristics of lupin seed oil met the requirements for edible oils, and its oxidative stability at 100°C and air flow rate of 20 L/h was extremely high (the induction period was more than 100 h).

Based on the obtained results, it can be concluded that lupin seeds have a high nutritional value and their oil is significantly stable, making them a possible source of high-quality lipids with a long shelf life.

5. Lipid composition of different parts of Cape gooseberry (*Physalis peruviana* L.) fruit and valorization of seed and peel waste

Cape gooseberry (*Physalis peruviana* L.), also known as goldenberry, Inca berry or Peruvian groundcherry, is the most extensively cultivated *Physalis* species, constituting an important cash crop in many countries of the tropical and subtropical regions (Puente *et al.*, 2011). Colombia is the biggest producer and exporter of fresh and dehydrated Cape gooseberry (CG) fruit worldwide, with an annual export volume of about 6000 tons, directed mainly to the European Union (the Netherlands, Germany and Belgium being the largest markets) (Olivares-Tenorio *et al.*, 2016). The fruit of CG, a berry enveloped in a protective calyx, is bright yellow to orange in color, ovoid-shaped, small and shiny (with a diameter between 1.25 and 2.50 cm and weight between 4 and 10 g), and contains about 100-300 seeds. The berries are rich in flavor, sweet and sour, resembling those of tomato, strawberry, kiwi and citrus, with a tender and juicy texture (Puente *et al.*, 2011). Ripe berries are consumed mostly fresh, but like other exotic fruits, CG is an excellent ingredient in many low calorie and dietetic products (beverages, jellies, jams, juices, yoghourts, dressings, etc.) (Ramadan, 2011; Kalugina *et al.*, 2017). Several comprehensive reviews have been published recently, which summarize the data about the chemical composition, biological activities and uses of CG fruit or whole plants (Puente *et al.*, 2011; Ramadan, 2011; Zhang *et al.*, 2013; Sharma *et al.*, 2015). The nutritional and medicinal values of CG fruit were related to the high levels of beneficial compounds such as vitamins, minerals, carotenoids, polyphenols, alkaloids, fatty acids, phytosterols, polysaccharides and others, as well as to their biological activities, such as anti-inflammatory, immunomodulatory, antioxidant, cytotoxic, antimicrobial, hepatoprotective, antiglycemic, anticholesterolemic, etc. (Ramadan and Mörsel, 2003; Ramadan et al., 2008; Rodrigues et al., 2009; Puente et al., 2011; Ramadan 2011, 2012; Zhang et al., 2013; Sharma et al., 2015; Mokhtar et al., 2018).

The seed/peel waste remaining after juice extraction, as well as the seed cakes remaining after oil extraction, can be considered an integral element in the sustainable CG usability, as they constitute a significant amount of fruit weight and contain compounds which are important for human and animal nutrition. These considerations create reasonable grounds for targeted analysis of these by-products, e.g. the determination of macro- and microminerals, fibers, protein, amino acids, vitamins, and other constituents with nutritional value. However, to the best of our knowledge, research data based on fractionization by fruit structural parts and processing by-products (seeds, peels, seed/peel waste, and seed cakes) are far from exhaustive, despite the intensive marketing promotion of CG fruit worldwide and the growing awareness of CG's nutritional benefits.

Therefore, the aim of this study was based on the analysis of the lipid fraction of different parts of cape gooseberry fruits and on the further characterization and evaluation of the resulting waste products from the plant material. A detailed study was made on the lipid composition of seeds, peels and the seed/peel wastes after high speed vacuum separation of fruit juice from cape gooseberry fruits. The glyceride oil content of the peel was found to be relatively lower (3.21%) than that of the seed (22.93%) and the seed/peel wastes (21.03%). The total phospholipid content

of the peel was almost 2 to 3 times higher (10.72%) than that of the other two fractions (2.69 – 4.38%), and the sterol content was found to be between 1.29 and 1.42%. The total amount of tocopherols in the oil from the peel was about two times lower (2648 mg/kg) than that of the other two fractions (5096 – 5634 mg/kg).

The predominant fatty acid in cape gooseberry seed oil was linoleic (67.89%), followed by oleic (14.69%) and palmitic (11.81%) acid. In the oil of the peels, the main fatty acid was capric acid (32.17%), followed by palmitic acid (24.51%) and oleic acid (19.31%). On the other hand, the fatty acid composition of the mixed fraction of seed/peel waste products approached that of the seeds, with the predominant fatty acid again being linoleic (63.19%), followed by oleic (16.56%) and palmitic acid (12.48%). Saturated fatty acids predominated in the fatty acid composition of cape gooseberry peel oil (67.72%), while the seed oil and the glyceride oil from seed/peel wastes are dominated by unsaturated fatty acids (82.26 – 83.77%).

The total sterol content of the studied samples varied from 1.29% (in the fruit peels) to 1.42% (in the mixed fraction of seed/peel waste), with the main components in the sterol fraction of all studied samples being β-sitosterol, campesterol and Δ^5-avenasterol. The main representatives in the tocopherol fraction were β-, γ- and δ-tocopherol, and in the oil of the peel of the fruit was γ-tocopherol (72.78%), while in the remaining two samples the amount of β-, γ- and δ-tocopherol was approximately the same (33.04 – 34.15%, 31.40 – 31.46% and 33.31 – 33.78%, respectively).

The seed cake, after the extraction of the glyceride oil from the cape gooseberry seeds, had a relatively high content of protein (24.32%) and cellulose (42.94%). Potassium (3911.00 – 4527.00 mg/kg), magnesium (1750.00 – 2095.00 mg/kg) and sodium (112.63 – 124.44 mg/kg) were the main macronutrients in the seed cake and waste fraction of seeds and peels from the fruits of cape gooseberry. Iron (42.85 – 52.36 mg/kg), manganese (17.77 – 24.44 mg/kg), copper (10.71 – 114.62 mg/kg) and zinc (34, 65 – 130.60 mg/kg) were also established in the samples. The main amino acids found in cape gooseberry seed cakes were aspartic acid (32.11 mg/g), lysine (27.22 mg/g) and alanine (17.96 mg/g).

The results of the study on the composition of cape gooseberry fruits may be of practical importance in the development of various functional foods and feeds.

6. Phytochemical investigations of Laurel fruits (*Laurus nobilis*)

Bay laurel (*Laurus nobilis* L.), family Lauraceae, is native to the southern Mediterranean region and widely cultivated in Asia, Europe, and America as a spice, medicinal, and ornamental plant. Turkey, Spain, Portugal, and Iran have traditionally been some of the largest exporters of dried laurel leaves (bay leaves). Laurel dried leaves, fruits, and essential oils (EOs) are used extensively in the food industry (Kumar et al., 2001; Parthasarathy et al., 2008).

Laurel berries are one-seeded ovoid fruits with a dark purple, thin, brittle, wrinkled pericarp, which, when broken, discloses a kernel whose seed coat adheres to the inner surface of the pericarp. Both EOs (about 1%) and lipids (up to 30% fixed, lipid fraction) are present in the fruit. The EO is used in the food and perfume industries and the lipid fraction for soap production (Parthasarathy et al., 2008; Patrakar et al., 2012; Uysal et al., 2010). However, laurel fruit (berry)

EO also has commercial value and has been investigated (Sangun et al., 2007). Interest in laurel fruit EO surged the following recent reports on its bioactivity. The demonstrated bioactivity of laurel fruit EO may be due to differences in its composition compared with those of laurel leaf and flowers (Parthasarathy et al., 2008). Studies on the chemical composition of *L. nobilis* reveal opportunities to use it not only as a spice but also as a source of natural bioactive compounds.

Laurel fruits offer an opportunity for dual utilization: for high-valued EOs and for FAs. The hypothesis of this study was that laurel fruit EO from Greece and Georgia would differ in composition and bioactivity. Therefore, the specific objectives of this study were to determine the chemical composition (polyphenols, EO, lipid fraction, cellulose, and protein content) of laurel fruits collected from Greece and Georgia and to evaluate the antimicrobial activity of fruit EOs against pathogenic microorganisms and those that cause food spoilage.

The major phenolic acids in the fruits from Greece were *p*-coumaric acid (free 261.6 μg/g) and vanillic acid (free 253.1 μg/g and conjugated 925.8 μg/g). The major phenolic acids in fruits from Georgia were vanillic acid (free 105.6 μg/g and caffeic acid [conjugated 439.2 μg/g], and syringic acid [conjugated 390.7 μg/g]). The laurel fruit EOs from Greece (1.4% content) and Georgia (1.6%) had distinct composition. Monoterpene hydrocarbons were the dominant group of compounds in the EOs, with 49.7% in the EO from Greece and 68.7% in the EO from Georgia. The major constituents of the fruit EO from Greece were 1,8-cineole (18.2%), α-phellandrene (15.0 %), β-pinene (9.4%), and α-pinene (9.1%), whereas the ones from Georgia were trans-β-ocimene (59.4%) and 1,8-cineole (7.6%). Laurel fruit EO from Greece and Georgia demonstrated low to moderate antimicrobial activity against pathogenic and spoilage microorganisms and the dimorphic yeast *Candida albicans*.

The main fatty acids in the lipid fraction of Georgian laurel fruit shells were lauric, oleic, linoleic and palmitic acids. The ratio between saturated and unsaturated fatty acids was 61.1:38.9. The main fatty acids in the lipid fraction from the seeds were oleic, palmitic and linoleic acids, the ratio between saturated and unsaturated fatty acids being 30.1:69.9. The content and individual composition of some lipid-soluble components (tocopherols, sterols and phospholipids) in the lipids of laurel seeds and fruit shells were also determined. The lipid content of the seeds varied from 24.5 to 53.3% and that of the fruit shells – from 7.9 to 37.8%. The amount of sterols and phospholipids in all samples was found to be 0.3 – 0.5% and 1.5 – 2.1%, respectively. The main component in the sterol fraction was β-sitosterol (79.8 – 94.3%), and there was a presence of campesterol (4.8 – 11.5%), stigmasterol (0.2 – 6.7%) and small amounts of cholesterol (0.4 – 2.1). The total content of tocopherols was higher in the lipid fraction of the fruit shells (1945.1 – 2036.2 mg/kg) than in the seeds (314.0 – 531.0 mg/kg), and in all investigated samples it was found presence of α-, β- and γ-tocopherol.

Based on the detailed studies carried out on the chemical and lipid composition of laurel fruits from Greece and Georgia, it can be concluded that they are rich in various valuable compounds that can potentially be used for the needs of the perfumery, cosmetics and pharmaceutical industries.

CONTRIBUTIONS

The main contributions in the presented publications can be summarized as being scientific and scientific-applied.

1. Scientific contributions

1.1. Chemical composition of different types of unconventional cultivated and wild-growing oil-bearing plants in Bulgaria with possibilities for application in food products or supplements has been investigated for the first time:

- Black pine seeds (*Pinus nigra* L.) – the fatty acid and tocopherol composition of the isolated glyceride oil from the seeds were determined, as well as the carbohydrate, cellulose, protein and amino acid composition, total ash content and mineral composition of the residual seed cakes after the extraction of the vegetable oil were determined [**B.4.1**].
- Ripe and unripe galbuli of Greek juniper (*Juniperus excelsa* M. Bieb) – a comparative study of the chemical and lipid composition of ripe and unripe galbuli of Greek juniper growing in three different locations was performed. Moisture, protein, chlorophyll, carotenoids, essential and glyceride oil contents were determined. The individual fatty acid, tocopherol and sterol composition in the lipid fraction of the samples, the amino acid composition of the protein fraction, as well as the total phenolic and flavonoid content were determined, and the antioxidant capacity of the extracts from the galbuli was determined by four methods – DPPH, ABTS, FRAP и CUPRAC [**B.4.2**].
- Seeds of three *Nicotiana* species in experimental cultivation of *N. alata* Link & Otto (two genotypes), *N. rustica* var. *rustica* and *N. tabacum* (variety Plovdiv 7“). For the first time, the content and composition of biologically active substances in the seeds of the mentioned varieties of tobacco plants were investigated, and it was found that they were rich in glyceride oil. The content of sterols, tocopherols and fatty acids in the glyceride oil was determined. The content of various macro- and micronutrients (protein, cellulose, macro- and microelements) in the seed cake remaining after the extraction of the glyceride oil was investigated in order to be made a detailed assessment of the possibilities for full utilization of the waste products. On the other hand, low concentrations of amino acids important for animal nutrition, such as lysine, methionine and cysteine, have been found in the seed cakes. For this reason, it is recommended to use the seed cake in the form of a suitable combination with other nutritional ingredients in mixtures [**B.4.3].**
- Lupin seeds (*Lupinus angustifolius* L.) cultivar “Boregine”. For the first time, detailed studies were carried out on the chemical and lipid composition of lupin seeds from the cultivar "Boregine", as well as the more important physico-chemical characteristics of the glyceride oil (peroxide, acid, iodine and saponification value, relative density at 20 °C, refractive index and oxidative stability) were determined. The chemical composition of the seeds was established, including the content of proteins, glyceride oil, carbohydrates (including starch, water-soluble sugars and fiber), moisture, and the

energy value was calculated. The individual amino acid composition and major mono- and disaccharides in lupin seeds were also determined. The total content and individual composition of the lipid-soluble biologically active compounds – sterols, tocopherols, carotenoids, chlorophyll, phospholipids, were examined. The fatty acid composition of triacylglycerols and the main classes of phospholipids was determined. Based on the obtained results, it was concluded that lupin seeds from the cultivar "Boregine" are a promising industrial crop that has nutritional value. Lupin seed oil can be presented as an alternative source of high-quality lipids with nutritional value and a long shelf life due to its extremely high oxidative stability [**B.4.4**].

- A detailed study was performed on the lipid composition of seeds, fruit shell and the seeds/peel wastes after high-speed vacuum separation of the fruit juice of cape gooseberries with Colombian origin. Their oil content, the total content of phospholipids, sterols and tocopherols in the oils, as well as their individual composition, were determined. The fatty acid composition of the different fractions of cape gooseberries after the separation of the fruit juice was determined. The results of the study of the composition of capegooseberriy fruits may be of practical importance in the development of various functional foods and feeds [**B.4.5].**

2. Scientific and applied contributions

2.1. The possibilities for utilizing the biologically active substances from various unconventional plants (laurel bay in particular) have been studied:

- Glyceride and essential oils from laurel fruits (*Laurus nobilis* L). The chemical composition (polyphenols, essential oil, lipid fraction, cellulose and protein content) of laurel fruits collected from Greece (Athos) and Georgia (Meria village) were determined, and the antimicrobial activity of the isolated essential oil from the fruits was evaluated. The content and composition of some lipid-soluble biologically active components – fatty acids, tocopherols, sterols and phospholipids, were determined in the lipid fraction. Based on the detailed research on the chemical and lipid composition of laurel fruits from Greece and Georgia, it was concluded that they are rich in various valuable compounds that can potentially be used for the needs of the perfumery, cosmetics and pharmaceutical industries **[B.4.6]**.

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/Ch. Assist. Prof. Zhana Petkova/