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Phytonutrients in black nightshade fruit (*Solanum nigrum* L.) growing in Bulgaria

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Black nightshade (*Solanum nigrum* L.) belongs to the Solanaceae family and is a medicinal plant with wild populations found in different regions of Bulgaria. The aim of this study was to analyze the availability of selected phytonutrients in the fruits, and, in particular, their presence in two individual fruit structures (seeds and peels) regarded as by-products in juice production. Fresh fruits were characterized by their diameter (8.42±0.62 mm) and absolute weight (393.75±38.00 g/1000 pcs). The seeds contained 9.81% lipids, while their content in the peels was negligible. The main fatty acids in the extracted lipid fraction were linoleic (48.0%), oleic (27.8%) and palmitic (19.6%), with 76.6% share of unsaturated fatty acids. *β*-Sitosterol (70.80%) predominated in the sterol fraction, and *γ*-tocopherol (100%) – in the tocopherol fraction of the extracted oil. The main soluble carbohydrates in the seeds were glucose (2.78%) and fructose (2.42%), while the respective quantities in the peels were 4.21% and 3.82%. The cellulose content of the two fruit parts was 14.90% and 15.70%, respectively. The most abundant macro minerals in the seeds were K (15109.34 mg/kg) and Mg (1988.07 mg/kg), and the main micro minerals – Fe (53.68 mg/kg) and Zn (37.77 mg/kg). The peels contained the respective dietary minerals in substantially higher quantities; K (31373.32 mg/kg), Na (3348.46 mg/kg), Ca (2365.82 mg/kg). The results from the study contribute to a more complex appreciation of the nutritional potential of black nightshade fruit available in Bulgaria and the prospects for their processing into value-added products.

Key words: *Solanum nigrum* L., black nightshade, lipids, fatty acids, minerals

INTRODUCTION

Black nightshade (*Solanum nigrum* L.) is an annual plant belonging to the genus *Solanum* of the Solanaceae family. Nowadays it is found throughout the world, being adapted to various soil types, altitudes and climatic conditions [1, 2]. The plant usually grows as a weed in moist, shady places, bushes, forest edges, around waste and cultivated lands, along rivers and streams, *etc.* [3-5]. Black nightshade is common to Bulgaria, too, and its wild populations are found in different regions with an altitude up to 1200 m [6].

S. nigrum is a recognized medicinal plant, used in many folk medicines for thousands of years [1, 3, 7, 8]. Nowadays, it is the most extensively studied species of the genus *Solanum* [9], and has been associated with almost every possible pharmacological activity: antibacterial, antifungal, anticancer, antidiabetic, anti-obesity, antioxidant, anti-inflammatory, hepatoprotective, and many others [3, 9-14]. Various compounds responsible for these diverse activities have been identified in all plant organs, among which steroidal alkaloids, saponins, flavonoids, lignins, organic acids, volatile oils, polysaccharides, and others [2, 13-16].

The species is usually considered toxic for humans and animals, due to the presence of glycoalkaloids in all plant parts, mainly solanine (95% of the alkaloid content), solamargine, solasonine, their aglycones solanidine and solasodine, *etc.* [3, 15, 16]. However, the content of solanine, as well as the total alkaloid content significantly decreases during plant growing and maturation [2] and the ripe berries and leaves of *S. nigrum* are edible, with rarely observed toxic and side effects if consumed in normal amounts [1, 2, 17]. Indeed, apart from its undisputed medicinal value, *S. nigrum* has been reported as an ancient famine plant of China and as one of the most important traditional leafy vegetables in several African countries (being cultivated in some of them, e.g., Nigeria) [1, 3-5, 18]. In the culinary practice, ripe berries and leaves are eaten fresh (as part of traditional salads) or more often cooked (boiled,

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sauteed, steam cooked), as the process is known to destroy solanine and other antinutrient compounds [3, 4, 19, 20].

Despite all this, however, the importance of *S. nigrum* in human nutrition is far less explored, compared to its medicinal properties; still, there are data about the basic macro and micronutrients in the berries, the leaves or the whole aerial parts of the plants. The fruits, in particular, have been reported to contain sufficient amounts of dietary fibers, protein, carbohydrates, minerals, vitamins, oil, and other nutrients [1, 5, 20-23]. It is well-documented that the individual plant organs and the structural parts (seeds, pulp, peels) of different berries accumulate primary and secondary metabolites to varying degrees, and, thus, they have different biological and nutrition value. On the other hand, seed-containing berries are often processed into juice or pulp (puree) and the isolated seeds and peels (skins) remain as by-products (waste), currently underutilized, but with distinct potential, both in terms of available amounts and nutrients' concentrations [24].

Based on these considerations, the aim of this study was to analyze the availability of selected phytonutrients in the fruits, and, in particular, their presence in two individual fruit structures (seeds and peels) regarded as by-products in juice production.

MATERIALS AND METHODS

The ripe fruits of black nightshade (*Solanum nigrum* L.) were hand-picked in July 2020 from wild plant populations in the region of Kapitan Dimitrievo village, Peshtera municipality, Central South Bulgaria (N 42°07′26″ E 24°18′52″) (Fig. 1). Species identity was confirmed at the Botany Department of "Paisii Hilendarski" University of Plovdiv.

Fig. 1. Ripe fruits of *Solanum nigrum* L. (own photo)

The physical descriptors of the fresh berries – diameter and absolute weight, were determined (n = 1000 pcs) using a precision gauge (± 0.01 mm) and an electronic precision balance (± 0.0001 g), respectively.

Fruit separation into structural elements (seeds and peels) was performed manually on frozen fruit (-18°C); the resulting samples were air-dried and stored until analysis. The seedcakes remaining after the extraction of seed oil, were also analysed in the study.

The moisture content was determined gravimetrically, by drying to constant weight at 105°C [25], and all results from the chemical analyses were presented on a dry weight (DW) basis.

The lipid fraction (the glyceride oil) was obtained by Soxhlet extraction of ground samples with *n*-hexane for 8 h [26]. All solvents and reagents used in the study were of analytical (p.a.) or higher grade, and were used without further purification.

Fatty acid composition of the lipids was determined by gas chromatography (GC) [27]; the respective fatty acid methyl esters (FAMEs) were obtained by pre-esterification with sulfuric acid in methanol [28]. FAMEs' separation was carried out on an HP 5890 instrument; capillary Supelco column, 75 m × 0.18 mm × 25 μm, and a flame ionization detector, FID. The temperature regimen was as follows: column temperature from 140°C (held 5 min) to 240°C (held 3 min) at 4°C/min; injector and detector temperatures at 250°C. A standard mixture of FAMEs (37 component FAME mix; Supelco, USA) subjected to GC under identical experimental conditions was used for the identification of fatty acids.

Tocopherols in the extracted oil were determined by HPLC, using a Merck-Hitachi chromatograph (Merck, Darmstadt, Germany); the column was Nucleosil Si 50-5, 250 mm × 4 mm; the detector – fluorescence Merck-Hitachi F 1000. The injected sample (20 μl) represented 2% crude oil solution in *n*-hexane. The mobile phase in the elution was *n*-hexane:dioxane solution, 96:4 (v/v), at a flow rate of 1 ml/min; excitation was at 295 nm, and emission at 330 nm. Tocopherol identification and quantification were achieved by comparison of retention times and peak areas, respectively, with those of a standard tocopherol solution (DL-α-, DL-β-, DL-γ- and DL-δ-tocopherol, 98% purity, purchased from Merck, Darmstadt, Germany) containing between 1 and 5 μg/mL [29].

Unsaponifiables were determined according to the standardized method [30], after saponification of the lipid fraction and extraction with *n*-hexane.

Total sterols were separated from the unsaponifiable matter by TLC. Briefly, the unsaponifiable matter was diluted in 3 mL chloroform and 1 mL of the solution was applied to

a 20×20 cm silica gel 60 G plate. The mobile phase was hexane : diethyl ether (1:1, v/v). After that, the plate was sprayed with methanol and the line of the isolated sterols was scrapped, transferred into a column and eluted with chloroform. The solution was evaporated on a rotary evaporator and the remained sterols were determined spectrophotometrically, at a wavelength of 597 nm [31], referring to an analytical calibration curve (*β*-sitosterol standard solution; 0-3000 μg/ml; R^2 = 0.9985). Sterol composition was identified by GC, on an HP 5890 unit equipped with 25 m × 0.25 mm DB - 5 capillary column and a flame ionization detector. The temperature gradient was from 90°C (held 3 min) to 290°C at a rate of 15°C/min and then up to 310°C at a rate of 4°C/min (held 10 min); the detector and injector temperatures were set at 320°C and 300°C, respectively; the carrier gas was hydrogen. A standard mixture of cholesterol (purity 95%, Acros Organics, New Jersey, USA), stigmasterol (purity 95%, Sigma-Aldrich, St. Louis, MO, USA) and *β*-sitosterol (with *ca*. 10% campesterol and *ca*. 75% *β*-sitosterol, Acros Organics, New Jersey, USA) was used in the comparison of the registered retention times [32]. The limit of detection in all GC and HPLC analyses of the lipid fraction was 0.05%.

Total phospholipids were determined spectrophotometrically at 700 nm after mineralization of the glyceride oil with sulfuric and perchloric acid (1:1, v/v) and reaction of the remaining phosphorus with sulfate–molybdate reagent.

The total content of soluble carbohydrates was determined by the phenol-sulfuric acid method [33], in which 100 μl of aqueous fruit extract were hydrolyzed with 1 ml of 5% phenol and 5 ml of concentrated sulfuric acid, in a water bath at 30°C for 20 min. The absorbance was read at 490 nm against a blank, and the carbohydrate content was obtained from the calibration curve built for glucose. The individual sugar contents were determined by HPLC-RID analysis of water extracts, using an Elite LaChrome Hitachi instrument with a Chromaster 5450 refractive index detector (RID) coupled with Shodex® Sugar SP0810 with Pb^{2+} (300 mm × 8.0 mm) and Shodex SP - G (5 μm, 6 mm × 50 mm) columns operated at 85°C; the elution was with distilled water at a flow rate of 1.0 ml/min [34]. The identification and quantification of sugars was based on their retention times and the respective peak areas, as previously described [34].

Cellulose content was determined following the procedure described by Brendel *et al.* [35], in which the acidic hydrolysis of cellulose and hemicellulose was carried out by boiling 1 g of fruit samples with 16.5 ml of 80% CH_3COOH and 1.5 ml of concentrated HNO_3 for 1.5 h. The suspension was filtered, and the solid residue was dried at 105°C for 24 h and weighed.

Mineral elements' contents in the fruit samples were determined after mineralization at 450°C, after which the solid residue was dissolved in concentrated HCl, evaporated to dryness, and redissolved in 0.1 mol/l HNO_3. Atomic absorption spectrophotometer Perkin Elmer/HGA 500 (Norwalk, USA) was used for the analysis of mineral elements, at the following wavelengths: Na, 589.6 nm; K, 766.5 nm; Mg, 285.2 nm; Ca, 317.0 nm; Zn, 213.9 nm; Cu, 324.7 nm; Fe, 238.3 nm; Mn, 257.6 nm; Pb, 283.3 nm; Cd, 228.8 nm; Cr, 357.9 nm. The identification of metal ions referred to a standard solution of metal salts. The respective metal contents were calculated from calibration curves for standard 1 μg/ml solutions. The measurements were carried out in triplicate, unless stated otherwise. The results were expressed as the mean value ± SD.

RESULTS AND DISCUSSION

The fresh berries of *S. nigrum* are matted dark blue in color, with an average diameter (measured at the widest lateral section) of 8.42 ± 0.62 mm (min 6.93 mm; max 9.62 mm). The absolute weight of the fruits was 393.75 ± 38.00 g/1000 pcs. Those basic physical characteristics of the fruit from Bulgaria did not differ from the range typical for the species [2].

As observed in previous studies for other berries [24], the seeds and, to a lesser extent, the peels are the structural elements that concentrate fruit lipids. Besides, those fruit parts, regarded as by-products in juice/pulp production, have been shown to contain sufficient amounts of valuable phytonutrients [36, 37]. Based on these grounds, and in compliance with study objectives, the air-dried seeds and peels of black nightshade fruit (with moisture content 3.25 ± 0.03%) were further characterized in this study.

The total lipid fraction (glyceride oil) content in the seeds was 9.81 ± 0.09%, while in the isolated peels alone its presence was practically negligible, below 1%.

The results about the content of the extracted lipid fraction were considerably below the data provided by previous studies for black nightshade seed oil; for instance, 34.5% [38], 36.5% [5], 38% [39]. This could be explained by variations in the analyzed fruit parts and the applied extraction procedures between the studies. Nevertheless, the oil content in the studied by-product was comparable with that found in maize bran (below 10%), or in some fruit by-products, such as grape seeds, date

palm seeds, guava seeds, mango seeds, and others [40].

Taking into account the results about the lipid content in the two separate waste fractions, it was considered rational to analyze and discuss further only the composition of the lipid fraction extracted from black nightshade seeds.

The fatty acid (FA) composition of the lipid fraction obtained from *S. nigrum* seeds is presented in Table 1. The data showed that three fatty acids dominated in the oil composition, linoleic (48.0%), oleic (27.8%) and palmitic (19.6%) acids. The ratio between saturated and unsaturated FAs was 1:3.27, while that between monounsaturated and polyunsaturated FAs was 1:1.72. Our results were fully consistent with previous data characterizing the seed oil of black nightshade as a rich source of polyunsaturated FAs, and, in particular, of linoleic acid. For example, the share of polyunsaturated FAs varied from 61.0% [22] to 68.4% [39] and 68.9% [38], and that of linoleic acid – from 47.9% [22] to 65.5% [5] and 67.6-67.8% [38, 39]. The numerical differences observed could readily be explained by the influence of ecological factors, fruit maturity and oil extraction methods [22, 38, 39].

Table 1. Fatty acid composition of the lipid fraction of *S. nigrum* seeds, %.

Fatty acids		Content, %
Lauric acid	C 12:0	0.2 ± 0.0 [a]
Palmitic acid	C 16:0	19.6 ± 0.15
Palmitoleic acid	C 16:1	0.2 ± 0,0
Margaric acid	C 17:0	0.1 ± 0.0
Heptadecenoic acid	C 17:1	0.2 ± 0.0
Stearic acid	C 18:0	3.3 ± 0.03
Oleic acid	C 18:1	27.8 ± 0.25
Linoleic acid	C 18:2	48.0 ± 0.40
Linolenic acid	C 18:3	0.2 ± 0.0
Eicosadienoic acid	C 20:2	0.2 ± 0.0
Behenic acid	C 22:0	0.2 ± 0.1
Saturated fatty acids, %		23.4
Unsaturated fatty acids, %:		76.6
Monounsaturated fatty acids, %		28.2
Polyunsaturated fatty acids, %		48.4

[a] All data are presented as mean value ± standard deviation (n=3)

Table 2 presents the content of biologically active substances – unsaponifiable matter, phospholipids, sterols and tocopherols, in the extracted seed oil of *S. nigrum* fruits. The total quantity of the biologically active sterols in the unsaponifiable fraction (0.70%) was higher than that found in many common seed oils, for example, sunflower, soybean, cottonseed, saffron, and others (0.24-0.64%) [40, 41]. The phospholipid content in *S. nigrum* seed oil was also sufficiently high (6.61%), approximating that in many plant oils used in the food industry [40].

Table 2. Biologically active substances in the lipid fraction of *S. nigrum* seeds.

Compound	Content
Unsaponifiables, %	12.01 ± 0.11 [a]
Sterols, %	0.70 ± 0.0
Phospholipids, %	6.61 ± 0.06
Tocopherols, mg/kg	121.00 ± 1.11

[a] All data are presented as mean value ± standard deviation (n=3)

The sterol composition of the lipid fraction from *S. nigrum* seeds is presented in Table 3. Six individual sterols were identified, among which *β*-sitosterol was clearly predominating (70.8%), followed by stigmasterol, cholesterol and campesterol in nearly identical shares (8.40-9.91%).

Table 3. Sterol composition of the lipid fraction of *S. nigrum* seeds.

Compound	Content, %
Cholesterol	9.50 ± 0.08 [a]
Brassicasterol	0.70 ± 0.0
Campesterol	8.40 ± 0.08
Stigmasterol	9.91 ± 0.09
Δ^7-Campesterol	0.70 ± 0.0
β-Sitosterol	70.80 ± 0.70

[a] All data are presented as mean value ± standard deviation (n=3)

With regard to oil's tocopherols, only *γ*-tocopherol was identified (100%; 121 mg/kg). Seed oils rich in *γ*-tocopherol are soybean (60-85 mg%) and corn (50-62 mg%) oils, which are the main dietary sources in the American diet, while the European diet mainly utilizes olive and sunflower oils, rich in *α*-tocopherol [40, 41].

The seedcakes remaining after oil extraction, as well as the peels of *S. nigrum* fruit, were further analyzed to determine other phytochemical indices. The results for the carbohydrate content in the two fruit elements are presented in Table 4.

Table 4. Carbohydrates in the by-products of *S. nigrum* fruit.

Index, %	Seedcakes	Peels
Total soluble carbohydrates	5.39 ± 0.50 [a]	8.21 ± 0.80
Sucrose	0.19 ± 0.00	0.18 ± 0.00
Glucose	2.78 ± 0.20	4.21 ± 0.40
Fructose	2.42 ± 0.20	3.82 ± 0.30
Sorbitol	nd	nd
Cellulose	14.90 ± 0.13	15.70 ± 0.17

[a] All data are presented as mean value ± standard deviation (n=3); [b] Not detected.

Reasonably, the peels contained considerably higher (nearly 2-times) concentrations of total soluble carbohydrates, as well as glucose and fructose, than the seedcakes, which was observed for other berries, too [37]. The results corresponded well with the carbohydrate content of dried *S. nigrum* seeds, 5.45% [5], but, expectedly, they were significantly lower than the data achieved for dried whole berries, containing the fruit pulp sugars; 34.36% [39], 40.4% [23], 55.85% [20].

The cellulose content in the seedcakes was 14.90%, and slightly higher in the isolated peels fraction, 15.70%. Although it was not possible to make any direct comparison, as there were no previous data for *S. nigrum* fruit cellulose content, our results supported the findings by other studies suggesting that the fruit and the seeds are a good source of dietary fiber [5, 20, 23].

The data for the structure of the identified mineral elements in the studied *S. nigrum* fruit fractions, the seedcakes and the peels, are presented in Table 5.

The elemental composition revealed significant amounts of K in both by-products, with about twice as higher concentration in the isolated peels.

Table 5. Minerals in the by-products from *S. nigrum* fruit.

Minerals, mg/kg	Seedcakes	Peels
Macro minerals		
Potassium (K)	15109.34 ± 150.00 [a]	31373.32 ± 300.00
Calcium (Ca)	813.12 ± 8.00	2365.82 ± 21.11
Magnesium (Mg)	1988.07 ± 18.11	1578.53 ± 14.00
Sodium (Na)	103.18 ± 1.10	3348.46 ± 30.00
Micro minerals		
Copper (Cu)	11.93 ± 0.10	11.84 ± 0.10
Iron (Fe)	53.68 ± 0.50	88.79 ± 0.85
Zinc (Zn)	37.77 ± 0.36	27.62 ± 0.26
Manganese (Mn)	23.86 ± 0.22	19.73 ± 0.18
Lead (Pb)	< 0.01 [b]	< 0.01
Cadmium (Cd)	< 0.01	< 0,01
Chromium (Cr)	< 0.01	< 0.01

[a] All data are presented as mean value ± standard deviation (n=3); [b] Not detected.

The complementary macro minerals in the seedcakes were Mg and Ca, while the content of Na was very low. The distribution of the same macro minerals was completely different in the peels; Na was the second-most abundant mineral, followed by Ca, both considerably exceeding the respective concentrations found in the seedcakes. The contents of the identified micro minerals in the two fruit structural elements were comparable, with the exception of Fe, found at about 1.5-time higher level in the peels. The heavy metals, Pb, Cd and Cr, were practically not detected in the samples. The differences in the mineral composition of the seedcakes and the peels could be explained with the different nature of the metabolic biochemical processes during fruit maturation [21, 22]. Our results differed to some extent from previous reports, which identified Mg as the predominant mineral in the dried seeds of black nightshade fruits of different origin, followed by K, but agreed very well with the established values; Mg, 201.3 mg/100 g [20], 182.3 mg/100 g [5], 426 mg/100 g [39]. The high mineral concentrations in the analyzed fruit structures, as well as the presence of important dietary micro elements, such as Cu, Zn and Mn, support the nutritive value of the regarded by-products and the viability of seeking alternatives for their incorporation in different food and feed products.

CONCLUSIONS

The study provides new data from the determination of basic macro and micronutrients in two structural elements of black nightshade (*S. nigrum*) fruit, the seeds and the peels – glyceride oil and the profile of saturated and unsaturated fatty acids, sterols and tocopherols in the oil; carbohydrates and minerals. The results from the study contribute to a more complex appreciation of the nutritional potential of black nightshade fruits available in Bulgaria and the prospects for their processing into value-added products.

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