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Phytonutrient Composition of Two Phenotypes of *Physalis alkekengi* L. Fruit

Venelina Popova [1], Nadezhda Mazova [2], Tanya Ivanova [1], Nadezhda Petkova [3], Magdalena Stoyanova [4], Albena Stoyanova [1], Sezai Ercisli [5,*], Amine Assouguem [6], Mohammed Kara [7], Samar Zuhair Alshawwa [8] and Omkulthom Al Kamaly [8,*]

[1] Department of Tobacco, Sugar, Vegetable and Essential Oils, University of Food Technologies, 4002 Plovdiv, Bulgaria; vpopova2000@abv.bg (V.P.); tantonieva@mail.bg (T.I.); aastst@abv.bg (A.S.)
[2] Department of Engineering Ecology, University of Food Technologies, 4002 Plovdiv, Bulgaria; nmihalkova11@gmail.com
[3] Department of Organic Chemistry and Inorganic Chemistry, University of Food Technologies, 4002 Plovdiv, Bulgaria; petkovanadejda@abv.bg
[4] Department of Analytical Chemistry and Physical Chemistry, University of Food Technologies, 4002 Plovdiv, Bulgaria; magdalena.stoianova@abv.bg
[5] Department of Horticulture, Atatürk University, Erzurum 25240, Turkey
[6] Laboratory of Functional Ecology and Environment, Faculty of Sciences and Technology, Sidi Mohamed Ben Abdellah University, Fez 30000, Morocco; assougam@gmail.com
[7] Laboratory of Biotechnology, Conservation and Valorisation of Natural Resources (LBCVNR), Faculty of Sciences Dhar El Mehraz, University Sidi Mohamed Ben Abdallah, BP 1796 Atlas, Fez 30000, Morocco; mohammed.kara@usmba.ac.ma
[8] Department of Pharmaceutical Sciences, College of Pharmacy, Princess Nourah bint Abdulrahman University, P.O. Box 84428, Riyadh 11671, Saudi Arabia; SZAlshawwa@pnu.edu.sa
* Correspondence: sercisli@gmail.com (S.E.); omalkmali@pnu.edu.sa (O.A.K.)

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Abstract: *Physalis alkekengi* L. is the only representative of the genus *Physalis* (Solanaceae) that is native to Bulgaria, found in wild habitats under different climatic and soil conditions. The plant is poisonous, but produces edible fruit, which are a source of functional nutrients—vitamins, phenolic antioxidants, minerals, etc. Therefore, the objective of this work was to determine the presence of certain nutrient and bioactive substances in two phenotypes of *P. alkekengi* fruit from Bulgaria, in order to better reveal the prospects of fruit use in nutrition. Different macro and micronutrients were determined in the fruit—protein, ash, lipids, fiber, natural pigments, sugars, amino acids, minerals—and the results showed differences between the phenotypes. Fruit energy values were low and identical in the samples, 43 kcal/100 g. The fruits were rich in extractable phenolics (TPC, 17.74–20.25 mg GAE/100 g FW; flavonoids, 15.84–18.03 mg QE/100 g FW) and demonstrated good antioxidant activity (DPPH, 171.55–221.26 mM TE/g; FRAP, 193.18–256.35 mM TE/g). *P. alkekengi* fruits were processed to obtain a dry extract with ethanol (yield 47.92–58.6%), and its individual composition was identified (GC-MS). The results in this study supported the presumed phytonutritive potential of *P. alkekengi* fruit, thus, opening doors for further research.

Keywords: *Physalis alkekengi* L.; nutritional composition; minerals; pigments; DPPH; FRAP; antioxidant activity; GC-MS

1. Introduction

The cosmopolitan genus *Physalis*, belonging to the family Solanaceae, includes nearly 100 species originating from Central and South America, with the single exception of *Physalis alkekengi* L., which has its natural origin from Asia and Europe. The species is the only representative of the genus *Physalis*, which is native to Bulgaria, where it is known as 'mekhunka' and is included in the list of medicinal plants administrated by national

legislation [1]. It is a wild-growing plant in the country, with natural habitats in different regions, with varying altitude, soil and climatic characteristics [2].

P. alkekengi (also known as bladder cherry, winter cherry, or Chinese lantern) is an herbaceous perennial plant, about 0.40–0.60 m in height, distinguished by the large, bright orange-to-red fruit papery fruit calyces in the period of maturity, which explains the use of dry twigs and whole plants in cut-flower bouquets and other decorations [3]. The fruit inside the calyx (the berry) is small, with a diameter less than 1.0–1.5 cm, oval, with a shiny surface and bright orange-red color [4,5].

Contemporary studies have identified about 125 bioactive individual constituents in the different aerial parts of *P. alkekengi* plants, representing various groups of chemical compounds—steroids (physalins A, B, D, F, VII; isophysalins A, B; aromaphysalin B), flavonoids (luteolin, apigenin, luteolin-7-O-β-glucopyranoside, ombuin), phenylpropanoids (ferulic acid, chlorogenic acid, 3-caffeoylquinic acid methyl ester, syringalide), alkaloids (phygrine, N-transferuloyltyramine), sucrose esters (physakengoses A–J) [4,6,7], etc.—and most of the research in the last two decades has been devoted to the disclosure of the mechanisms of *P. alkekengi's* therapeutic and pharmacological effects and their correlation with its metabolic profile [4,8,9]. A number of in vitro and in vivo models have evidenced the variety of pharmacological activities demonstrated by different extracts (ethyl acetate, methanol, ethanol, trichlormethane, etc.) and isolated individual metabolites (mainly from the groups of physalins and flavonoids), such as antidiabetic [10–15], anticholesterolemic [16], antitumor [17,18], anti-inflammatory [19,20], antioxidant [19,21,22], antimicrobial [23], vasodilative [4], vasodilative [4], spasmolytic [24], and others.

According to a recent review [4], most of *P. alkekengi's* pharmacological functions are associated with the presence of physalins, flavonoids and phenylpropanoids, acting in synergy with other groups of plant metabolites. Physalins have been identified as responsible for the established anti-inflammatory, antimicrobial, antidiabetic, anticancer, and immunosuppressive action of *P. alkekengi*-derived fractions; flavonoids—for the antidiabetic, anti-inflammatory, and anticancer effects; phenylpropanoids—for the antidiabetic, antimicrobial, and anticancer activities.

In terms of nutritive functionality, *P. alkekengi* fruit complies with the concept of 'medicine food homology', a theory based on the postulates of the Traditional Chinese Medicine, for which the current interpretation endorses the incorporation of certain medicinal plants with therapeutic effects as a part of the daily healthy diet ('food as a medicine') [13,25]. It should be emphasized that the plant is poisonous and only the ripe fruits (without the calyx) are edible, while unripe fruit and other plant parts are toxic and should not be consumed. The ripe fruit of *P. alkekengi* are a functional, dietetic food, a source of vitamins (A, C), phenolic antioxidants, phytosterols, minerals (P, Ca, Fe), pectin, unsaturated fatty acids, and other valuable nutrients [13,26–28]. They are an excellent additive in the preparation of lean and meat dishes, salads, and desserts [29]. It is recommended to use only well-ripened fruit for culinary purposes, which further improve their taste qualities after freezing [23].

To the best of our knowledge, there have been no previous investigations on the phytochemical composition and the antioxidant activity of *P. alkekengi* fruit in Bulgaria. We hypothesized that there would be region-related variations in the nutritional, antioxidant and other properties of the studied fruit, compared to international data, which might add valuable supplementary information to the detailed characterization of the species. Therefore, the objective of this work was to investigate the phytochemical composition of *P. alkekengi* fruit from Bulgaria, by determining the presence of certain nutrient and bioactive substances, in order to better reveal its prospects as a functional food. Since fruit properties are sensitive to environment, and the species is encountered in different regions of the country, with varying climatic and soil conditions, a comparison between two phenotypes of *P. alkekengi* fruit was designed, in order to examine fruit variability, arising from the influence of the different habitats.

2. Materials and Methods

2.1. Plant Material

The fruits of *P. alkekengi* were collected from natural plant habitats found in two distant geomorphological zones of Bulgaria, differing in geographical characteristics—the upper Thracian plain (Southern Bulgaria) and the Danubian plain (Northern Bulgaria). The first sample (PA-SB) was collected in September 2020 from a wild plant population near the city of Plovdiv, Plovdiv region, Central Southern Bulgaria (42°14′26″ N 24°70′24″ E). The general characteristics of PA-SB habitat were: transitional continental climate, with mild winter and warm summer and small annual temperature amplitude; average annual temperature of 12.3 °C; average annual amount of precipitation about 540 mm; alluvial-meadow soil; altitude 164 m [30]. The second fruit sample (PA-NB) was collected in August 2020 from a wild population in the vicinity of Ivanski village, region Shumen, North-eastern Bulgaria (43°07′24″ N 27°04′35″ E). The general characteristics of PA-NB habitat were: temperate continental climate, with large annual temperature amplitudes (24–25 °C); annual rainfall of about 600 mm; leached chernozem soil; altitude 94 m [30]. Plant identification was performed by a botanist from the Department of Botany at the University of Plovdiv, Bulgaria. About 150 ripe fruit, by visual assessment of calyx color and development, were picked by hand from different plants in each population and transported in cloth bags to the laboratory. Before processing, whole fruits were stored briefly in the refrigerator at 5–8 °C, then were de-husked and damaged, unripe or under-developed fruit were eliminated. An illustration of selected fruits from PA-NB and PA-SB phenotypes is presented in Figure 1.

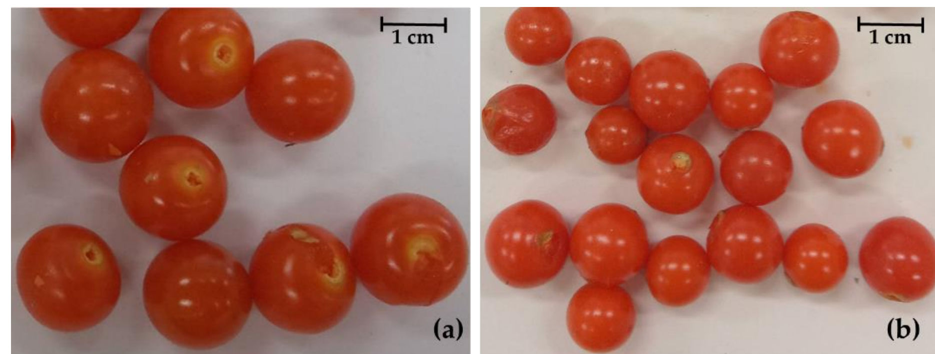

Figure 1. *Physalis alkekengi* L. fruit (de-husked): (**a**) fruit from north-eastern Bulgaria, PA-NB; (**b**) fruit from central Southern Bulgaria, PA-SB. Photos by authors.

The physical dimensions were registered for 100 fresh de-husked fruit, randomly selected; diameter (to a precision ±0.01 mm, n = 100) and weight (on a Mettler-Toledo precision weight, ±0.0001 g, n = 100). Before further processing, the fruits were quickly frozen to −18 °C temperature.

2.2. Chemical Analyses of P. alkekengi Fruit

Fresh fruit moisture content was determined by oven drying at 105 °C, until the constant weight [31].

Total protein content in fruit samples was analyzed according to the established method [31], on a UDK 152 System (Velp Scientifica Srl, Usmate Velate, Italy). Derivatization of free amino acids resulting from protein hydrolysis was performed using the AccQ-Fluor kit (WATO52880, Waters Corporation, Milford, MA, USA). The HPLC analysis of amino acids used an Elite LaChrom (Hitachi, Tokyo, Japan) instrument equipped with a DAD and a reverse phase C18 AccQ-Tag column (3.9 mm × 150 mm) operated at 37 °C temperature. Mobile phases comprised WATO52890 buffer (Waters Corporation, Milford, MA, USA) and 60% acetonitrile.

The ash content was determined through mineralization of the samples in a muffle furnace at 550 °C for 7 h [31].

The isolation of the lipid fraction in the fruit samples was carried out by Soxhlet extraction with n-hexane for 8 h [31].

Total soluble carbohydrates were analyzed applying the phenol-sulfuric acid method [32], after ultrasound-assisted fruit extraction with distilled water. Carbohydrate content was determined using a calibration curve [33]. Individual sugars were quantified through HPLC analysis on an Elite LaChrom (Hitachi, Tokyo, Japan) instrument, equipped with a Shodex Sugar SP0810 column, Pb^{2+} (300 mm × 8.0 mm i.d.) and a guard Shodex SP-G column (5 µm, 6 × 50 mm), both set to 85 °C. Refractive index detector (RID, Chromaster; Hitachi, Tokyo, Japan) temperature was maintained at 35 °C [34].

The cellulose content was determined by a slight modification of a previously described method [35]. Cellulose and hemicellulose were hydrolyzed with 16.5 mL 80% CH_3COOH and 1.5 mL concentrated HNO_3 at boiling for 1.5 h; the solid residue was dried at 105 °C for 24 h and weighed.

pH values were registered in filtered water extracts from homogenized fruit, using a 7110 WTW pH meter (Weilheim, Germany) [31]. Total acidity was measured by titration with 0.1 M NaOH to pH 8.1, and the data were presented as % citric acid [36].

The sweetness index (SI) and the total sweetness index (TSI) were calculated using the data from the determination of the individual sugars (sucrose, glucose and fructose) and accounting for their contribution to sweetness perception [37,38]:

$$\mathrm{SI} = 1.00 \times [\mathrm{glucose}] + 2.30 \times [\mathrm{fructose}] + 1.35 \times [\mathrm{sucrose}], \quad (1)$$

$$\mathrm{TSI} = 1.00 \times [\mathrm{sucrose}] + 0.76 \times [\mathrm{glucose}] + 1.50 \times [\mathrm{fructose}] \quad (2)$$

The energy value of fresh fruit was calculated using the established conversion factors [39]—4 kcal/g (17 kJ/g) for protein, 9 kcal/g (37 kJ/g) for lipids, 4 kcal/g (17 kJ/g) for carbohydrates, and 2 kcal/g (8 kJ/g) for fiber.

In the analysis of natural pigments, 5.0 g fruits (FW) were extracted, in triplicate, with 80% acetone (solid to liquid ratio of 1:20, *w*/*v*) at 45 °C for 15 min, in an ultrasonic bath (VWR, Puchong, Selangor, Malaysia, 45 kHz and 30 W). Chlorophyll and carotenoid contents were determined spectrophotometrically, applying the calculation procedure described previously [40].

Sample preparation for the determination of mineral elements included mineralization at 450 °C, followed by consecutive dissolving of the residue in concentrated HCl, evaporation to dryness, and dissolving in 0.1 mol/L HNO_3 solution [41]. Atomic absorption spectrometry (AAS) was performed on a Perkin Elmer/HGA 500 instrument (Norwalk, CT, USA), 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. Metal ion identification and concentration calculation was completed by comparison to standard salt solutions and using calibration curves (1 µg/mL).

The analysis of total phenolic content and total flavonoids was carried out on acetone extracts from fresh fruits. The extraction was performed in duplicate, at solid-to-solvent ratio 1:10, *w*/*v*, in an ultrasonic bath (35 kHz; 300 W; 20 min at 75 °C). Total phenolic content was determined following the Folin–Ciocalteu method, in which 1 mL of Folin–Ciocalteu reagent (diluted 1:5) and 0.2 mL of the extract were mixed, and then combined with 0.8 mL 7.5% Na_2CO_3. The solution was incubated for 20 min at room temperature (20 ± 2 °C) and the absorbance was read at 765 nm against the blank [42]. The total flavonoid content was determined spectrophotometrically, according to the method with 10 % $Al(NO_3)_3$ reagent previously described [43]; reaction time was 40 min; wavelength 415 nm [42].

In the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, 0.15 mL of the acetone extract was mixed with 2.85 mL of freshly prepared 0.1 M solution of DPPH in methanol and reacted for 15 min at 37 °C in the dark. Absorbance was measured at 517 nm, parallel to the blank containing methanol [42]. Ferric-reducing antioxidant power (FRAP) assay was performed

following a modification of the method in [44]. The FRAP reagent was freshly prepared by mixing 0.3 M acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) (Fluka) in 40 mM HCl (Merck) and 20 mM $FeCl_3.6H_2O$ (Merck) in double-distilled water, at a ratio of 10:1:1, respectively. A portion of 0.1 mL of the acetone extract was mixed with 3.0 mL FRAP reagent and incubated for 10 min at 37 °C in the dark before measuring the absorbance (at 593 nm, relative to a FRAP reagent blank).

2.3. Extraction and GC-MS Analysis of P. alkekengi Fruit

Fresh *P. alkekengi* fruit were homogenized and double-extracted with 95% ethanol (in a solid-to-solvent ratio of 1:8, *w*/*v*) at temperature 60 °C for 2 h and 2.5 h, respectively. The combined miscellas were evaporated on a rotary vacuum evaporator at temperature 40 °C, until complete solvent removal.

Prior to the GC-MS analysis, 100.0 µL of the extract was vacuum-dried in a centrifugal vacuum concentrator (CentriVap, Labconco, Kansas City, MO, USA) at 40 °C. The dried residue was dissolved in 100 µL solution of methoxyamine hydrochloride (20 mg/mL in pyridine) and heated at 70 °C for 1 h with constant shaking (300 min^{-1}) (Thermo Shaker TS-100, Analytik Jena AG, Jena, Germany). After cooling, 100 µL of N,O-bis (trimethylsilyl)trifluoroacetamide (BSTFA, silylation agent) was added, and the mixture was heated again with stirring (70 °C, 40 min, 300 min^{-1}). The injected sample volume was 1.0 µL.

The GC-MS analysis was performed on an Agilent 7890A chromatograph interfaced with a 5975C mass selective detector (Agilent Technologies Inc., Santa Clara, CA, USA). Separations were on an HP-5ms column, 30 m × 0.25 mm (i.d.) coated with 0.25 µm film of poly (dimethylsiloxane) as the stationary phase. The instrumental parameters were as follows: carrier gas helium, maintained at constant rate of 1.0 mL/min; injector and transfer line temperature of 250 °C; MS source temperature of 230 °C; oven temperature program started from 100 °C (held for 2 min), then the temperature was increased to 180 °C at a rate of 15 °C/min (held at 180 °C for 2 min), and then to 300 °C at a rate of 5 °C/min (held at 300 °C for 10 min); total run time 42 min; split mode of 20:1; MS scans from 50 to 550 m/z. The components in the sample were identified based on their retention indices and by comparing their mass spectra with those in NIST 08 spectra library [45]. The retention (Kovats) indices were calculated using a standard calibration mixture of n-alkanes (C_8–C_{40}) in hexane. The amounts of the identified compounds were expressed as percentage of the total ion current (TIC), after normalization of the recorded peak areas.

2.4. Statistics

All analyses in the study were performed in triplicate (n = 3), unless for fruit physical dimensions (n = 100), and data were presented as the mean value ± the standard deviation.

3. Results

3.1. Physical Parameters of P. alkekengi Fruit

The results from the determination of the basic physical parameters, weight and diameter of the fresh fruit from the two phenotypes in the study (labelled as PA-NB and PA-SB) are presented in Table 1. As seen from the data, the fruit from the plants occurring in North-eastern Bulgaria (PA-NB) were larger and heavier than the fruit from the second phenotype (collected in Central Southern Bulgaria, PA-SB), although the differences in the mean values were not very pronounced. The deviations in fruit size (min–max) were bigger in the PA-SB phenotype.

Table 1. Physical parameters of fresh *Physalis alkekengi* fruit.

Index	PA-SB [1]	PA-NB [2]
	Fruit weight, g	
mean [3]	1.57	2.25
min	0.93	1.59
max	2.91	3.09
SD	0.56	0.52
	Fruit diameter [4], mm	
mean	13.2	16.2
min	10.9	14.3
max	16.4	17.8
SD	1.67	0.14

[1] PA-SB—fruit from Central Southern Bulgaria; [2] PA-NB—fruit from North-eastern Bulgaria; [3] all data obtained from measuring 100 fruits (n = 100); [4] measured at the widest lateral section of the fruit.

3.2. Phytonutrients and Antioxidant Activity of P. alkekengi Fruit

The indices determining the nutritional value of the studied *P. alkekengi* fruit are presented in Table 2. The data, in general, suggested slight variations between the phenotypes, except for the significant differences in the soluble carbohydrate and chlorophyll contents. Those were most probably related to the site of fruit origin and the variations in the environmental conditions of plant development in the respective habitat. Reducing sugars constituted about 50% of soluble carbohydrates in PA-SB and about 90% in PA-NB fruits. While the sucrose content was minimal and identical in both samples, the contents of monosaccharides glucose and fructose were at higher amounts in PA-NB fruit. The total chlorophyll content was about 2.5-times higher in PA-NB, but the ratio of chlorophyll *a*: chlorophyll *b* (about 1:1.2) was identical in both phenotypes.

Table 2. Indices of the nutritional composition of *Physalis alkekengi* fruit (on a fresh weight basis).

Index	PA-SB [1]	PA-NB [2]
Moisture content, %	71.87 ± 0.32 [3]	76.93 ± 0.29
Protein, %	6.44 ± 0.05	5.83 ± 0.05
Ash, %	6.18 ± 0.05	5.75 ± 0.05
Lipids, %	1.21 ± 0.01	0.75 ± 0.0
Soluble carbohydrates, %	0.21 ± 0.0	1.90 ± 0.01
Sucrose	0.02 ± 0.0	0.02 ± 0.0
Glucose	0.07 ± 0.0	1.17 ± 0.01
Fructose	0.03 ± 0.0	0.61 ± 0.0
Fiber (cellulose), %	3.00 ± 0.04	2.85 ± 0.03
Titratable acidity, % citric acid	1.12 ± 0.02	1.28 ± 0.01
pH	4.11 ± 0.03	4.05 ± 0.03
Natural pigments, µg/g		
Chlorophylls	0.09 ± 0.0	0.22 ± 0.0
Chlorophyll *a*	0.04 ± 0.0	0.10 ± 0.0
Chlorophyll *b*	0.05 ± 0.0	0.12 ± 0.0
Carotenoids	18.88 ± 0.12	22.85 ± 0.20
Energy, kcal/100 g (kJ/100 g) [4]	43.49 (181.82)	43.37 (181.96)

[1] PA-SB—fruit from Central Southern Bulgaria; [2] PA-NB—fruit from North-eastern Bulgaria; [3] data presented as the mean value ± standard deviation (n = 3); [4] calculated according to [39].

On the basis of the results from the determination of the basic macronutrients in the fresh *P. alkekengi* fruit, the sweetness index (SI), the total sweetness index (TSI), as well as the ratio of total sweetness index/titratable acidity (TSI/TA), were calculated [37,38]. Those indices reflect the balance between individual sugars and that between carbohydrates and organic acids, respectively, which is of significant importance for the sensory perception of fruit flavor. The calculated values of the indices for the two phenotypes in the study are presented in Table 3.

Table 3. Sweetness indices of two phenotypes of *Physalis alkekengi* fruit.

Index	PA-SB [1]	PA-NB [2]
Sweetness index (SI)	0.17	2.60
Total sweetness index (TSI)	0.12	1.82
Total sweetness index/Titratable acidity (TSI/TA)	0.11	1.43

[1] PA-SB—fruit from Central Southern Bulgaria; [2] PA-NB—fruit from North-eastern Bulgaria.

Protein quality is another important aspect in phytonutrient analysis, and the results from the identification of the amino acid composition (after protein hydrolysis) of *P. alkekengi* fruit are presented in Table 4. The data revealed that the dominant amino acids in both samples were aspartic acid, arginine and alanine, with consistently higher values in the PA-NB phenotype. On the other hand, amino acids, such as glutamic acid, glycine, lysine, and histidine, were found at significantly higher levels in the second phenotype, PA-SB. There were certain variations in the concentrations of the rest of the amino acids between the samples as well, probably related to the influence of plant development conditions. The share of essential amino acids was relatively low and comparable in the two samples, about 0.3–0.4:1.

Table 4. Amino acid composition of *Physalis alkekengi* fruit (on a fresh weight basis).

Amino Acids, mg/g	PA-SB [1]	PA-NB [2]
Aspartic acid	7.29 ± 0.06 [3]	8.36 ± 0.06
Serine	2.53 ± 0.01	1.86 ± 0.01
Glutamic acid	1.95 ± 0.01	0.77 ± 0.01
Glycine	1.20 ± 0.01	0.26 ± 0.0
Histidine [4]	4.50 ± 0.03	0.02 ± 0.0
Arginine	6.11 ± 0.06	7.55 ± 0.06
Threonine [4]	2.29 ± 0.01	1.72 ± 0.01
Alanine	5.49 ± 0.04	7.80 ± 0.05
Proline	4.47 ± 0.03	3.48 ± 0.03
Cysteine	3.16 ± 0.03	0.11 ± 0.0
Tyrosine	3.28 ± 0.02	2.12 ± 0.01
Valine [4]	1.96 ± 0.01	1.15 ± 0.01
Methionine [4]	0.31 ± 0.0	0.85 ± 0.0
Lysine [4]	1.28 ± 0.0	0.44 ± 0.0
Isoleucine [4]	2.47 ± 0.01	2.59 ± 0.01
Leucine [4]	0.67 ± 0.0	0.93 ± 0.0
Phenylalanine [4]	3.26 ± 0.02	2.42 ± 0.02

[1] PA-SB—fruit from Central Southern Bulgaria; [2] PA-NB—fruit from North-eastern Bulgaria; [3] data presented as the mean value ± standard deviation (n = 3); [4] essential amino acid.

In view of the importance of the balanced intake of digestible minerals in modern nutrition and in the prevention of various metabolic disorders, the individual macro- and micromineral composition of the fruits from the compared *P. alkekengi* phenotypes were analyzed; the results are given in Table 5.

As a part of the complex characterization of the nutrient potential of *P. alkekengi* fruit, the total content of the biologically active phenols and flavonoids in acetone extracts was determined, as well as the antioxidant activity, evaluated by two in vitro assays (DPPH and FRAP). The results of those analyses are presented in Table 6.

Table 5. Macro- and microminerals in *Physalis alkekengi* fruit (on a fresh weight basis).

Minerals, mg/kg	PA-SB [1]	PA-NB [2]
Potassium (K)	5564.00 ± 43.08 [3]	12,641.00 ± 98.12
Sodium (Na)	5.93 ± 0.01	35.58 ± 0.17
Calcium (Ca)	nq [4]	1.37 ± 0.01
Magnesium (Mg)	29.20 ± 0.17	33.73 ± 0.18
Iron (Fe)	4.70 ± 0.01	5.26 ± 0.01
Manganese (Mn)	1.25 ± 0.01	1.47 ± 0.01
Copper (Cu)	1.74 ± 0.01	2.25 ± 0.0
Zinc (Zn)	3.62 ± 0.01	3.95 ± 0.01
Lead (Pb)	nq	nq
Cadmium (Cd)	nd [5]	nd
Chromium (Cr)	nq	nq

[1] PA-SB—fruit from Central Southern Bulgaria; [2] PA-NB—fruit from North-eastern Bulgaria; [3] data presented as the mean value ± standard deviation (n = 3); [4] below the limit of quantification, LOQ = 0.1 mg/kg; [5] not detected.

Table 6. Total phenolic and flavonoid content and antioxidant activity of acetone extracts from *Physalis alkekengi* fruit.

Fruit Sample	Total Phenolic Content, mg GAE/100 g FW	Total Flavonoids Content, mg QE/100 g FW	Antioxidant Activity, mM TE/g FW	
			DPPH	FRAP
PA-SB [1]	17.74 ± 0.15 [3]	15.84 ± 0.12	171.55 ± 1.26	193.18 ± 2.12
PA-NB [2]	20.25 ± 0.01	18.03 ± 0.21	221.26 ± 2.12	256.35 ± 3.56

[1] PA-SB—fruit from Central Southern Bulgaria; [2] PA-NB—fruit from North-eastern Bulgaria; [3] data presented as the mean value ± standard deviation (n = 3).

3.3. Extraction and CG-MS Analysis of Fruit Dry Extracts

In the next step of the study, the fruits were processed by ethanol extraction to obtain a concentrated extract, potentially suitable as an additive in foods and other products. The basic characteristics of the obtained extracts are presented in Table 7. As seen from the data, both extracts were practically identical, in terms of yield, appearance and odor.

Table 7. Characteristics of dry ethanolic extracts obtained from *Physalis alkekengi* fruit.

Index	Description	
	PA-SB [1]	PA-NB [2]
Yield, % DW (w/w)	58.64 ± 0.38 [3]	47.92 ± 0.41
Appearance	Thick, viscous mass	
Color	Dark yellow	
Odor	Intense green, with fruit accords and faint woody notes	

[1] PA-SB—fruit from Central Southern Bulgaria; [2] PA-NB—fruit from North-eastern Bulgaria; [3] data presented as the mean value ± standard deviation (n = 3).

The results from the GC-MS analysis of the metabolite profile of the extracts are presented in Table 8. Data revealed the identity of 51 individual plant metabolites in the extract from PA-SB fruit and, respectively, 48 components in the fruit from PA-NB phenotype. Those compounds accounted for 98.66% and 98.83% of the total ion current (TIC) in the chromatograms obtained by the applied GC-MS analysis of the extracts from PA-SB and PA-NB fruit, respectively. The major constituents (i.e., those with a share above 3%) were identical in the analyzed extracts, with only slight numerical variations: 4-O-methyl-myo-inositol (15.44% and 16% in PA-SB and PA-NB, respectively), glucose methoxyamine (two isomers, 13.78% and 5.84% in PA-SB; 5.61% and 14.00% in PA-NB, respectively), 1-methyl-α-D-glucopyranoside (13.86% and 13.20% in PA-SB and PA-NB, respectively), methylcitric acid (7.92% and 6.75% in PA-SB and PA-NB, respectively), sucrose (two isomers, 6.53% and 2.97%), melibiose (two isomers, 4.89% and 1.28% in PA-SB; 7.41% and 3.05% in

PA-NB, respectively), glyceric acid-3-phosphate (4.56% and 4.00% in PA-SB and PA-NB, respectively), and fructose methoxyamine (two isomers, 2.91% and 2.41% in PA-SB; 6.00% and 4.50% in PA-NB, respectively).

Table 8. Identification of the chemical composition (GC-MS) of dry ethanolic extract from *Physalis alkekengi* fruit.

Compound	RT [1]	RI [2]	Content, % of TIC [3]	
			PA-SB [4]	PA-NB [5]
Lactic acid 2TMS [6]	4.98	1057	0.10 ± 0.0 [7]	0.18 ± 0.0
Alanine 2TMS	5.61	1098	0.30 ± 0.0	0.58 ± 0.0
Oxalic acid 2TMS	5.96	1127	0.09 ± 0.0	0.11 ± 0.0
2-Aminobutyric acid 2TMS	6.21	1162	0.11 ± 0.0	0.09 ± 0.0
Malonic acid 2TMS	6.43	1186	0.17 ± 0.0	0.13 ± 0.0
n-Dodecane	6.72	1200	0.11 ± 0.0	0.16 ± 0.0
Valine 2TMS	6.81	1209	0.07 ± 0.0	0.10 ± 0.0
Glycerol 3TMS	7.36	1278	0.32 ± 0.0	0.03 ± 0.0
Proline 2TMS	7.66	1301	0.52 ± 0.0	0.13 ± 0.0
Succinic acid 2TMS	7.82	1320	0.37 ± 0.0	0.23 ± 0.0
Fumaric acid 2TMS	8.19	1345	0.06 ± 0.0	0.14 ± 0.0
Serine 3TMS	8.45	1376	0.29 ± 0.0	0.24 ± 0.0
Threonine 3TMS	8.62	1392	0.10 ± 0.0	0.26 ± 0.0
Malic acid 3TMS	8.99	1470	0.52 ± 0.0	0.45 ± 0.0
Aspartic acid 3TMS	9.33	1519	0.17 ± 0.0	0.21 ± 0.0
Pyroglutamic acid 2TMS	9.47	1525	0.19 ± 0.0	0.21 ± 0.0
4-Aminobutyric acid 3TMS	9.78	1534	0.10 ± 0.0	0.16 ± 0.0
N-Acetylglutamic acid 2TMS	10.02	1542	0.18 ± 0.0	0.19 ± 0.0
Threonic acid 4TMS	10.33	1567	0.27 ± 0.0	0.24 ± 0.0
2-Ketoglutaric acid methoxyamine 2TMS	10.47	1579	0.14 ± 0.0	0.09 ± 0.0
L-Glutamic acid 3TMS	11.89	1640	0.30 ± 0.0	0.14 ± 0.0
Xylose methoxyamine 4TMS isomer	12.01	1655	0.26 ± 0.0	0.30 ± 0.0
Xylose methoxyamine 4TMS isomer	12.12	1668	0.83 ± 0.0	0.94 ± 0.0
Glycerol-3-phosphate 4TMS	12.98	1773	0.23 ± 0.0	0.10 ± 0.0
Glyceric acid-3-phosphate 4TMS	13.17	1809	4.56 ± 0.02	4.00 ± 0.04
Citric acid 4TMS	13.34	1826	0.12 ± 0.0	0.08 ± 0.0
Fructose methoxyamine 5TMS isomer	13.45	1851	2.91 ± 0.02	6.00 ± 0.05
Fructose methoxyamine 5TMS isomer	13.55	1860	2.41 ± 0.01	4.50 ± 0.03
Galactose methoxyamine 6TMS	13.61	1868	0.30 ± 0.0	0.33 ± 0.0
1-Methyl-α-D-glucopyranoside 4TMS	13.70	1874	13.86 ± 0.11	13.20 ± 0.12
Galactose methoxyamine 6TMS	13.98	1882	1.24 ± 0.01	0.79 ± 0.0
Glucose methoxyamine 6TMS isomer	14.70	1905	5.84 ± 0.04	5.61 ± 0.04
Glucose methoxyamine 6TMS isomer	14.88	1911	13.78 ± 0.12	14.00 ± 0.13
Hexitol 6TMS	15.37	1920	1.07 ± 0.01	0.71 ± 0.0
Methylcitric acid 4TMS	15.54	1932	7.92 ± 0.05	6.75 ± 0.05
Ascorbic acid 5TMS	15.69	1944	0.49 ± 0.0	0.50 ± 0.0
4-O-Methyl-*myo*-inositol 5TMS	16.26	1962	15.44 ± 0.14	16.00 ± 0.15
Palmitic acid TMS	17.33	2041	0.13 ± 0.0	nd [8]
Stearic acid TMS	18.11	2230	0.46 ± 0.0	0.24 ± 0.0
Melibiose 8TMS isomer	24.34	2455	1.28 ± 0.01	1.30 ± 0.01
n-Docosanoic acid 1TMS	26.41	2649	0.84 ± 0.01	0.40 ± 0.0
Sucrose 8TMS isomer	26.67	2656	2.97 ± 0.01	3.05 ± 0.02
Maltose 8TMS isomer	26.81	2703	1.52 ± 0.01	1.30 ± 0.01
Melibiose 8TMS isomer	26.93	2721	4.89 ± 0.02	4.70 ± 0.04
Sucrose 8TMS isomer	27.15	2742	6.53 ± 0.04	7.41 ± 0.06
Maltose 8TMS isomer	27.66	2790	0.85 ± 0.0	0.90 ± 0.0
Squalene	27.97	2820	1.99 ± 0.01	1.50 ± 0.01
Tetracosanoic acid 1TMS	28.33	2853	0.22 ± 0.0	nd
Pentacosanoic acid 1TMS	29.40	2939	0.45 ± 0.0	0.10 ± 0.0

Table 8. *Cont.*

Compound	RT [1]	RI [2]	Content, % of TIC [3]	
			PA-SB [4]	PA-NB [5]
n-Triacontane	29.88	3000	0.10 ± 0.0	nd
Hexacosanoic acid 1TMS	30.15	3036	0.70 ± 0.0	0.21 ± 0.0
Total identified, %			98.66	98.83

[1] RT—retention time, min; [2] RI—retention index (Kovat's index); [3] TIC—total ion current; [4] PA-SB—fruit from Central Southern Bulgaria; [5] PA-NB—fruit from North-eastern Bulgaria; [6] *n*TMS—trimethylsilyl derivate with *n* substituted H-atoms; [7] data presented as the mean value ± standard deviation (*n* = 3); [8] not detected.

The component distribution in *P. alkekengi* fruit extracts by groups of chemicals is presented in Figure 2. As seen from the data, the profile of the metabolites in the extracts, which were possible to be identified with GC-MS, clearly dominated, regardless of the site of fruit collection, by carbohydrates (77.1–82.0% of the identified content), followed by organic acids (13–15%). Representatives of the groups of fatty acids (1.0–2.8%), amino acids (2.3–2.4%) and triterpenes (1.5–2.0%), together with some minor miscellaneous chemicals (hydrocarbons, alcohols, etc., with a share of 0.1–0.7%), accounted for the remaining part of extract composition.

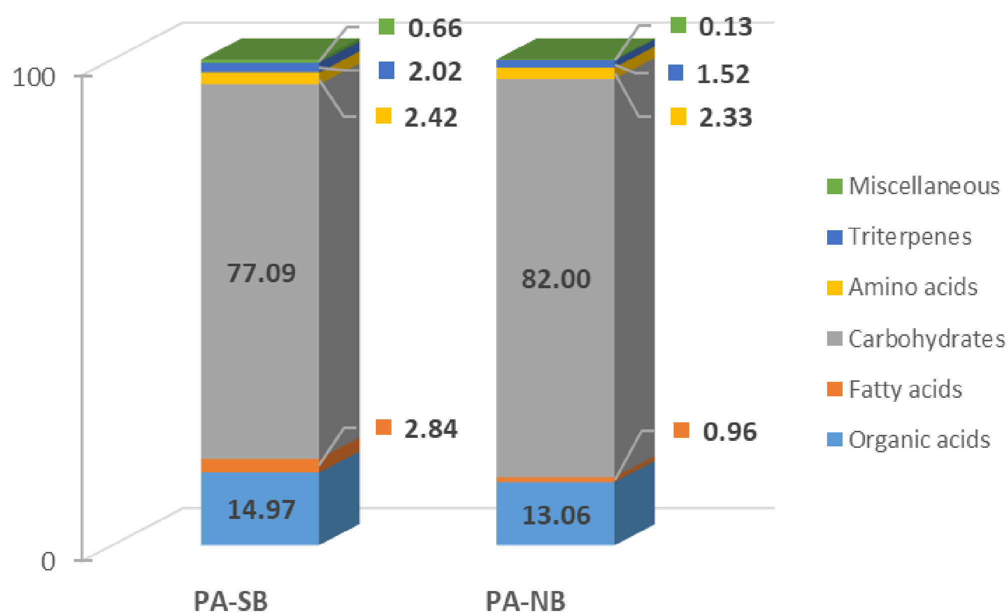

Figure 2. Distribution of compounds (GC-MS) in dry ethanolic extracts from *Physalis alkekengi* fruit: PA-SB—fruit from Central Southern Bulgaria; PA-NB—fruit from North-eastern Bulgaria.

4. Discussion

The values obtained from the measurement of the physical dimensions of the fruit from the studied Bulgarian phenotypes (Table 1) were significantly lower than the data provided previously for fresh fruit cultivated in three provinces in China; diameter, 2.1–2.2 cm; weight, 5.3–5.8 g [27]. In that comparison, the formation of smaller fruits in the studied local phenotypes reflected plant development in natural wild populations, as opposed to the cultivated farmers' produce [3], as well as the impact of the geographical characteristics of Bulgaria. Probably, there were also genotype-related differences between the local and Chinese fruits, but such speculation could be confirmed only by additional data. The smaller and less varying sizes of the fruits from the PA-SB phenotype were most probably affected by the climatic and other specifics of the site of fruit collection, which was much shadier and less humid than that of PA-NB.

The results for the total sugar concentration in the studied fruits, as well as those for glucose and fructose (Table 2), were considerably below the values associated with three genotypes of cultivated *P. alkekengi* fruit from China (glucose, 2.25–2.75 g/100 g FW; fructose, 2.53–3.13 g/100 g FW) [27]; still, they were in compliance with the previously observed practical absence of sucrose in species fruit. The sugar contents in *P. alkekengi* fruit, regardless of phenotype, were significantly lower than those found in other *Physalis* species; Cape gooseberry (*P. peruviana* L.): glucose, 1.15–2.15 g/100 g FW; fructose, 1.12–1.87 g/100 g FW; sucrose, 1.0–2.09 g/100 g FW [46], or tomatillo, *P. philadelphica* Lam.: total sugars, 3.93 g/100 g FW [47]. The cellulose content in fresh *P. alkekengi* fruit supported their consideration as a relatively good source of fiber [48], with no differences observed between the phenotypes. Fruit protein content was relatively high, especially in the PA-SB phenotype, exceeding that found in other fruits with similar structure, such as different species of passion fruit [49] or tomatillo [47], which is most likely related to the considerable share of seeds in the berries. The lipid content in the fruits of both phenotypes was generally low, but comparatively higher values were registered in PA-SB fruit. There were no significant variations in the ash content of the studied phenotypes. The results about fruit acidity and pH corresponded well with previous data for *P. alkekengi* fruit from China [27]—titratable acidity 1.55–1.84% and pH 3.5, as well as with data characterizing the fruit of *P. peruviana* [50–53].

The results from the calculation of the sweetness indices (Table 3) suggested significant differences in the taste and flavor balance between the studied phenotypes, which fully corresponded to the observed differences in fruit mono- and disaccharide contents. The numerical index values (SI, TSI) were low compared to those found in other small fruits, such as strawberries, blueberries, and others [38,54]. The very low values of TSI/TA (far below 3) supported the description of the taste profile of the studied wild-growing *P. alkekengi* fruit as rather sour.

The data from the determination of fruit macronutrients (sugars, protein, lipids, fibers) were used for the calculation of their energy (caloric) value, applying the recommended conversion factors [39]. The energy values, as shown in Table 2, were low, especially in comparison with those found in some higher-caloric popular fruits, including bananas, grapes or apples [55], as well as in comparison with the energy values associated with *P. peruviana* fruit [46,56,57]. The calculated energy values were identical in the two phenotypes studied, 43.49 kcal/100 g FW (PA-SB) and 43.37 kcal/100 g FW (PA-NB). The greatest contribution in the total energy intake was that of soluble carbohydrates (59% and 54% in PA-SB and PA-NB, respectively) and protein (25% and 16%, respectively).

Therefore, the results of the study provide objective grounds for characterizing the fruit of *P. alkekengi* occurring in Bulgaria, despite the observed phenotype-related differences, as a comparatively low-caloric and low-lipid food, and a source of protein [48,55].

The micronutrient analysis (Tables 2 and 4) also spoke in favor of the feasibility of regarding the species as a source of functional nutrients. Carotenoids were accumulated in a significant amount, regardless of phenotype, thus, characterizing *P. alkekengi* fruit as a good source of that group of bioactive nutrients. Our data (18.88–22.85 µg/g FW) were substantially lower than the results for the total carotenoid content in cultivated Chinese fruits (19.8–21.6 mg/100 g FW) [58], but very close to the carotenoid content in *P. peruviana* (13.91–22.36 µg/g FW) [46]; 1.53 mg/100 g FW [59]. The higher carotenoid concentration in the fruit of PA-NB correlated with the visual perception of a more intensive orange-red coloration compared with the fruit of the second phenotype. The amino acid composition of fruit protein was more difficult to compare with previous data, as there is rather scarce reference information, not only for *P. alkekengi*, but also for other closely related *Physalis* species [57]. Still, the amino acid profile of fruit protein in this study was close to that found in dried seed/peel powder from *P. peruviana* fruit, with glutamic acid, arginine and aspartic acid being the major amino acids [60] and in fresh *P. pruinosa* fruit, glutamic acid, aspartic acid and arginine [61]. The share of essential amino acids was comparable to the reported

presence of 31.8% essential amino acids in *P. pubescens* L. fruit juice [62] and 38.71 g/100 g protein essential amino acids in the fresh fruit of *P. pruinosa* [61].

The data presented in Table 5 suggested that *P. alkekengi* fruit contained various macro and microminerals, important for the proper functioning of the human body, with some deviations between the phenotypes. Generally, the concentrations of the identified elements were lower in PA-SB compared with PA-NB phenotype, and the greatest differences were with respect to potassium (about 2-times) and sodium (about 6-times) contents. Similar to many other fruits, potassium was the dominant macromineral in *P. alkekengi* fruit. Despite the region-based differences, potassium concentration was considerable [39], thus, outlining the species as a rich source of the mineral, surpassing many popular fruits and with the potential to provide over 21% of the reference dietary intake (DRI) for men (2700 mg) and over 16% for women (3500 mg), in the 19–30 years age group [63]. Interestingly, the content of other macrominerals, Na and Mg, was low, while Ca was practically not present in the studied fruit. Contrasting to the observed variation in macromineral content, the identified microminerals were at nearly identical levels in both phenotypes. Although the numerical values were relatively small, the results showed that *P. alkekengi* fruit could be considered a source of microelements, valuable for the metabolic and regulatory processes in the human body (Cu, Zn, Fe, and Mn). The trace metals, Pb, Cd and Cr, were not identified in either of the phenotypes.

The results from the determination of the total phenolic and total flavonoids content in the acetone extracts from *P. alkekengi* fruit supported the assumption that the species was rich in extractable phenolic compounds (Table 6). The data revealed a higher level of the total phenolic and flavonoids contents in the PA-NB sample, but the differences between the two phenotypes were not substantial. The acetone extracts obtained from the fruits demonstrated good antioxidant activity in the DPPH and FRAP assays. In correspondence with the highest content of total phenols and flavonoids, the higher antioxidant activity in both assays was revealed by the fruit from the PA-NB phenotype. Our results were in compliance with the documented radical-scavenging activity of different extracts and fruit fractions [4,15], and confirmed that the studied extracts from *P. alkekengi* fruit had comparable or higher antioxidant activity than many other fruits (plum, peach, grape, pear, cherry tomatoes, and others) [64,65].

In accordance with the objectives of the study, an attempt was made to provide another argument in favor of the prospective use of the studied fruits, by processing them into dry extracts and identifying their individual composition. Similar concentrated extracts from different berries and other fruits have the potential to be used in foods, as flavor enhancers and nutritional supplements. The specific odor description and the high yield (47.92–58.6% DW) (Table 7) supported the effectiveness of prospective fruit processing. The applied parameters of the GC-MS analysis and component analysis of the extracted polar phase from *P. alkekengi* fruit resulted in the identification of various classes of chemicals (Figure 2), although they represented mostly primary metabolites, such as carbohydrate derivatives (hexose sugars, disaccharides), organic, amino and fatty acids. Those results were in full compliance with the findings from previous analysis of *Melissa officinalis* plant extracts, under similar metabolite identification procedures, in which sugars, amino acids and organic acids were the most abundant extract constituents [66]. Other compounds found to be present in the analyzed fruit, such as flavonoids, were not volatile, even after derivatization (metoxyamination and silylation) and could not be determined in the applied GC-MS procedure. Still, an interesting finding was the presence of squalene (1.50–1.99%), a triterpene characteristic for shark liver oil, but also present in many glyceride oils from plant species (olive, palm, wheat germ, rice bran, amaranth, etc.). Since squalene is highly appreciated in cosmetics due to its beneficial effect on the skin, having chemopreventive (anticancer), antioxidant, hydrating, moisturizing, and emulsifying properties [67], its identification could motivate further research in that direction, as well as the application of targeted extract fractionization and metabolite separation, in order to reveal the volatile profile of *P. alkekengi* fruit (terpenoids, carotenoid derivatives, aromatics, etc.).

5. Conclusions

The results obtained in this first study of the phytochemical indices of *P. alkekengi* fruit from Bulgaria supported our assumption that the fruit of the regarded wild-growing species of medicinal plants are a rich source of macro and micronutrients, thus, having a tangible potential in human nutrition, in a fresh or processed form. The direct comparison between two fruit phenotypes confirmed the hypothesis that fruit phytochemical constituents were affected by the geographic and environmental characteristics of plant habitats—carbohydrates, natural pigments, K, Na—while others were less dependent (protein, lipids, fiber, microminerals). Of course, in order to draw a more definite conclusion on a country basis, a wider set of phenotypes and sampling sites should be involved. The sufficient yield and the identified metabolite composition of the concentrated ethanolic extract, obtained and analyzed for the first time in this study, provide grounds for a prospective use of similar *P. alkekengi* products in food and other areas, which could also be the object of further research.

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