The leaves and stems of Cape gooseberry (*Physalis peruviana* L.) as an alternative source of bioactive substances

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Abstract. The objective of this work was the investigation of the chemical composition of the leaves and stems of two Cape gooseberry (*Physalis peruviana* L.) genotypes from Bulgaria (CG-SB and CG-NB), with the view of establishing the presence of certain bioactive substances and the prospects for their use as livestock feed or cosmetic ingredients. The leaves and stems of CG-SB genotype contained 18.63% and 40.26% cellulose, respectively, and 24.83% and 13.73% protein; the respective contents in the leaves and stems of CG-NB genotype were 27.63% and 47.63% cellulose, and 9.36% and 8.07% protein. The dominant amino acids in CG-SB leaves were aspartic acid (32.04 mg.g⁻¹) and lysine (30.54 mg.g⁻¹), and in the stems – proline (46.90 mg.g⁻¹) and phenylalanine (15.42 mg.g⁻¹). The amino acid composition of the leaves of CG-NB genotype was dominated by histidine (24.88 mg.g⁻¹) and proline (21.25 mg.g⁻¹), and that of the stems – by proline (13.38 mg.g⁻¹). The main macro and micro minerals in the leaves and stems of both genotypes were K, Mg and Fe, respectively, but numerical differences were observed on a genotype and plant part basis. The leaves were processed by extraction with n-hexane and the content of volatiles was determined (by GC-MS). A total of 32 components was identified in each of the genotypes. The major volatile in both genotypes was n-pentacosanol, 17.07% in CG-SB and 12.39% in CG-NB; the dominant group of chemicals was that of oxygenated aliphatics, followed by diterpenes. The results from the study provide arguments that the leaves and stems of Cape gooseberry, currently discarded byproducts, could be regarded as alternative sources of bioactive substances.

1. Introduction

Cape gooseberry (*Physalis peruviana* L.) is the most economically important species of the genus *Physalis* (Solanaceae), together with tomatillo (*P. philadelphica* Lam., *P. ixocarpa* Brot. ex Horm); it is currently cultivated worldwide, where it can be found in numerous varieties, producing fruit with different morphology, composition and quality [1, 2]. *P. peruviana* fruit, the edible part of the plant, has been extensively investigated, and the presence of many bioactive and nutrient classes of constituents has been documented – minerals, vitamins, various types of phenolics, polysaccharides, fatty acids, phytosterols, terpenes, organic acids, and others [1, 3]. Since a substantial share of the annual fruit production is processed to juice (pulp), there are several studies on the utilization of fruit waste resulting...
from juice production, the seed/peel pomace, which has been characterized as an excellent dietary source of vitamins, minerals, essential fatty and amino acids, tocophorols, carotenoids, and other bioactive nutrients [4, 5]. Although relatively less, there are studies on the chemical composition, the ethnomedicinal use and the biological activities of Cape gooseberry leaves and stems, representing underutilized by-products of fruit production [6-9]. Cape gooseberry plants produce large amounts of vegetative biomass, discarded after fruit harvest; for example, the average leaf area per single plant of the studied “Plovdiv” variety was 17582 cm$^2$ [10], while in another study the stems constituted 34.5% of the total plant dry matter [9]. On the other hand, the investigations on the isolation of aromatic products from the leaves of the plant or on the identification of leaf volatiles are extremely limited [8], although some studies on leaf essential oil and extracts of other Physalis species are available [11]. In our previous studies, different ethanol extracts with prospective cosmetic application have been obtained from the leaves of two Cape gooseberry genotypes, characterized in terms of tannin content and extraction process parameters’ influence [12, 13].

Cape gooseberry is recently gaining popularity in Bulgaria, both as consumed fruit and profitable crop (exclusively in organic agriculture). The plant is cultivated in small organic farms in different regions of the country, although no consistent market data about fruit production are available. The original Bulgarian variety, named “Plovdiv” was selected in the early 2000s, at the Department of Horticulture of the Agricultural University, Plovdiv [14].

Based on these considerations, we hypothesized that Cape gooseberry leaf and stems, waste biomasses available in large amounts, might have certain potential in circular agricultural production, as good alternative sources of functional phytochemicals. We presumed that the investigation of those discarded plant materials would be highly relevant to the contemporary tendencies in plant studies and plant-derived product development. On one hand, the last decades have been witnessing a shortage in plant resources rich in bioactive nutrients to be used in feed mixtures; due to this, there has been a constant quest for novel, alternative plant materials for feed production, such as different medicinal or essential oil bearing plants [15-17]. Similarly, the fragrance and food industries are demanding new, non-traditional aromatic products for added functionality, stability or appeal of their output [18, 19]. Therefore, the objective of this work was the investigation of the chemical composition of the leaves and stems of two Cape gooseberry genotypes from Bulgaria, with the view of establishing the presence of certain bioactive substances and the prospects for their use as livestock feed or cosmetic ingredients.

2. Materials and methods

2.1. Plant material

The leaves and stems of two Cape gooseberry genotypes were analysed in this study: the first was that of the locally selected “Plovdiv” variety, cultivated in Central South Bulgaria (in the region of Plovdiv, 42°08’03.0”N 24°45’56.0”E) (CG-SB) and the second – of an introduced South American variety, cultivated in North-West Bulgaria (the region of Mezdra, 43°05’28.7”N 23°45’48.6”E (CG-NB). The plants were grown according to the established agricultural practices. The whole plants were collected from the field in October 2018; leaves were manually detached from the stems and all plant materials were air-dried in the shade and kept at at temperature of 5-8°C to avoid deterioration.

2.2. Chemical analyses

The moisture content of the leaves and stems was determined by drying to constant weight, at 105°C, and all results from the chemical analyses were calculated on a dry weight (DW) basis.

The cellulose content was determined by a slight modification of the method described in [20]. In brief, hydrolysis of cellulose and hemicellulose was carried out with 16.5 mL of 80% CH$_3$COOH and 1.5 mL of concentrated HNO$_3$ at boiling for 1.5 h, then the solid residue was dried at 105°C for 24 h and weighed. The ash content was determined gravimetrically, after mineralization of the samples at 550°C for 5 hours [21]. Reducing sugars were determined by continuous-flow analysis, according to the standard method [22], on an AAIIc auto-analyzer (Technicon, USA). The total protein content was
determined according to [23], using an UDK 152 System (Velp Scientifica, Italy). The free amino acids from protein hydrolysis were derivatized, using the AccQ-Fluor kit (WAT052880, Waters Corporation, USA). The separation of AccQ-Fluor amino acid derivatives was performed on an ELITE LaChrom HPLC chromatograph (Hitachi) using a diode array detector (DAD) and a reverse phase C18 AccQ-Tag column (3.9 mm × 150 mm), operated at temperature 37°C; the mobile phases were WAT052890 buffer (Waters Corporation, USA) and 60% acetonitrile; the detection wavelength was 254 nm.

In the procedure for the determination of mineral elements, the plant samples were first mineralized at 450°C; the residue was then dissolved in concentrated HCl, evaporated to dryness, and the remainder was subsequently dissolved in 0.1 mol.L⁻¹ HNO₃ solution. Mineral elements were determined on a Perkin Elmer/HGA 500 (Norwalk, USA) atomic absorption spectrophotometer (AAS), under the following instrumental parameters: 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; and Mn, 257.6 nm. Metal ion identification was completed by comparison to a standard solution of metal salts, and metal concentrations were calculated from a calibration curve, built by using a standard 1 μg.mL⁻¹ salt solution.

The leaves were processed by extraction with n-hexane and the content of volatiles was determined (by GC-MS). Twofold extraction was carried out, for 60 min and 30 min each, at temperature 40°C and solid to liquid ratio of 1:10 (w/v), followed by the complete removal of the solvent from the combined extracts [19]. GC-MS analysis was performed on an Agilent 7890A chromatograph and an Agilent 5975C mass spectrometer (Agilent Technologies Inc., Santa Clara, CA, USA), under the following operational conditions: column HP-5 ms, 30 m × 250 μm × 0.25 μm; temperature increase from 35°C (3 min) to 250°C (3 min) at 5°C/min, total run time of 49 min; carrier gas helium, at a constant speed of 1 mL.min⁻¹; split ratio of 30:1. Mass spectra library data [24; NIST 08 database; own libraries] were used for volatile compound identification; components were listed according to their retention (Kovat’s) indices, calculated using a standard calibration mixture of C₆ - C₈ n-alkanes in n-hexane. Compound concentration was computed as percentage of the total ion current (TIC).

All analyses were performed in a threefold repetition and data were presented as mean value ± standard deviation.

### 3. Results and discussion

The two plant parts of Cape gooseberry were analysed individually in order to identify the content of macro and micro components important in human and animal nutrition, in a direct comparison between the two genotypes.

#### 3.1. Cellulose, ash, minerals, protein, and amino acids in Cape gooseberry leaves and stems

Data from the analyses of the macro components of Cape gooseberry leaves and stems are presented in Table 1.

| Chemical Index       | CG-SBa | CG-NB |
|----------------------|--------|-------|
| Moisture (wt%)       | Leaves | Stems | Leaves | Stems |
| 8.32±0.08b           | 9.30±0.08 | 8.79±0.07 | 8.84±0.07 |
| Cellulose (wt%)      | 18.63±0.18 | 40.26±0.39 | 27.63±0.26 | 47.63±0.45 |
| Protein (wt%)        | 24.83±0.23 | 13.73±0.12 | 19.36±0.19 | 8.08±0.08 |
| Reducing sugars (wt%)| 16.71±0.15 | 15.61±0.14 | 12.37±0.11 | 11.81±0.10 |
| Ash (wt%)            | 12.60±0.12 | 11.82±0.11 | 13.57±0.12 | 12.27±0.11 |

|       | CG-SBa | CG-NB |
|-------|--------|-------|
|       | Leaves | Stems | Leaves | Stems |
|       | 8.32±0.08b | 9.30±0.08 | 8.79±0.07 | 8.84±0.07 |
|       | 18.63±0.18 | 40.26±0.39 | 27.63±0.26 | 47.63±0.45 |
|       | 24.83±0.23 | 13.73±0.12 | 19.36±0.19 | 8.08±0.08 |
|       | 16.71±0.15 | 15.61±0.14 | 12.37±0.11 | 11.81±0.10 |
|       | 12.60±0.12 | 11.82±0.11 | 13.57±0.12 | 12.27±0.11 |

a CG-SB – genotype from the region of Plovdiv, Central South Bulgaria; CG-NB – genotype from the region of Mezdra, North-West Bulgaria.

b All data are presented as mean value ± standard deviation (n=3).
The results from the chemical analyses revealed that Cape gooseberry leaves and stems were rich in functional phytonutrients. There were some genotype based variations in the chemical indices of the studied plant parts. The leaves and stems of CG-NB genotype contained significantly higher amounts of cellulose and lower – of protein and sugars, than the second CG-SB genotype; those differences were obviously due to the influence of production and genetic specifics, therefore, plant origin might be considered a factor affecting biomass nutrition quality. On a plant part basis, leaves reasonably contained substantially more protein and less cellulose (about 2-time difference) than the stems; no significant differences were found in sugar and ash contents. Despite those differences, the results suggested that both aerial parts studied can equally be considered (as well as their combination) as relevant diet ingredients in animal feed composition. It is known that protein content in plant materials, in particular, is decisive in the feeding of different animal species [15, 16]. Therefore, with regard to the observed protein content the dry leaves of Cape gooseberry approximated established plant feeds, such as alfalfa before flowering (224 g.kg\(^{-1}\)) and fodder peas wintering at flowering (221 g.kg\(^{-1}\)); in turn, the cellulose content was also close to that in alfalfa before flowering (203 g.kg\(^{-1}\)) [16].

As the dietary quality of feed ingredients is closely related to protein amino acid composition, the next step in the study was the identification of the amino acid content of Cape gooseberry leaves and stems; the results from the analysis are presented in Table 2.

| Amino acid | CG-SB\(^a\) | CG-NB |
|------------|-------------|-------|
|            | Leaves | Stems | Leaves | Stems |
| Asp        | 32.04±0.31\(^b\) | 6.11±0.06 | 13.61±0.12 | 2.59±0.02 |
| Ser        | 3.36±0.03 | 5.69±0.05 | 15.33±0.14 | 1.43±0.01 |
| Glu        | 5.38±0.05 | 14.06±0.13 | 6.94±0.06 | 6.02±0.06 |
| Gly        | 2.86±0.02 | 2.77±0.02 | 2.08±0.01 | 0.67±0.01 |
| His\(^c\)  | 19.01±0.18 | 13.21±0.12 | 24.88±0.30 | 6.09±0.06 |
| Arg\(^c\)  | 14.13±0.13 | 0.62±0.01 | 0.98±0.01 | 3.17±0.03 |
| Thr\(^c\)  | 5.17±0.04 | 5.92±0.05 | 5.34±0.05 | 2.46±0.02 |
| Ala        | 16.72±0.15 | 14.03±0.13 | 20.75±0.19 | 5.32±0.05 |
| Pro        | 22.27±0.21 | 46.90±0.46 | 21.25±0.20 | 13.38±0.13 |
| Cys        | 5.46±0.05 | 2.07±0.02 | 1.35±0.01 | 0.38±0.00 |
| Tyr        | 7.96±0.06 | 13.32±0.12 | 9.72±0.08 | 2.15±0.02 |
| Val\(^c\)  | 10.10±0.09 | 8.94±0.08 | 12.38±0.11 | 2.79±0.02 |
| Met\(^c\)  | 16.13±0.15 | 7.95±0.07 | 0.45±0.01 | 0.83±0.01 |
| Lys\(^c\)  | 30.54±0.29 | 14.63±0.14 | 19.91±0.18 | 4.33±0.04 |
| Ile\(^c\)  | 17.69±0.16 | 9.64±0.09 | 11.62±0.10 | 2.80±0.02 |
| Leu\(^c\)  | 3.11±0.03 | 1.47±0.01 | 1.96±0.01 | 0.49±0.01 |
| Phe\(^c\)  | 16.66±0.15 | 15.42±0.15 | 10.75±0.09 | 2.98±0.02 |

\(^a\) CG-SB – genotype from the region of Plovdiv, Central South Bulgaria; CG-NB – genotype from the region of Mezdra, North-West Bulgaria.

\(^b\) All data are presented as mean value ± standard deviation (n=3).

\(^c\) Essential amino acid.
According to the data in Table 2, the amino acid composition of the two studied genotypes differed considerably, as already observed for their protein content; additionally, there were substantial variations on a plant part basis within each of the genotypes. The dominant amino acids in the leaves of CG-SB were aspartic acid (32.04 mg.g\(^{-1}\)) and lysine (30.54 mg.g\(^{-1}\)), and in the stems – proline (46.90 mg.g\(^{-1}\)) and phenylalanine (15.42 mg.g\(^{-1}\)). The amino acid composition of the leaves of CG-NB genotype was dominated by histidine (24.88 mg.g\(^{-1}\)), proline (21.25 mg.g\(^{-1}\)), alanine (20.75 mg.g\(^{-1}\)), and lysine (19.91 mg.g\(^{-1}\)), and that of the stems – exclusively by proline (13.38 mg.g\(^{-1}\)). Amino acids, such as asparagaine, valine, lysine, leucine, and isoleucine were predominantly in the leaves of both genotypes, while the ratio was reversed for amino acids like glutamine, proline and tyrosine (in CG-SB), and arginine (in CG-NB). The rest of the amino acids also varied between the genotypes, explicable by the different production conditions, as well as between the two individual plant parts. The ratio of essential to non-essential amino acids took values of 1.3:1 (CG-SB) and 0.9:1 (CG-NB) in the studied leaves, and about 0.8:1 in the stems of both genotypes; thus, Cape gooseberry leaves and stems can be classified as plant materials with substantial protein quality [17], a very important aspect in livestock feed. In that course, it might be worth noticing the high relative concentrations of lysine, especially in the leaf fractions, which, together with methionine and cysteine, are the common limiting amino acids in swine and poultry nutrition.

The results from the analysis of mineral elements in Cape gooseberry leaves and stems are presented in Table 3.

**Table 3. Minerals in Cape gooseberry leaves and stems (two genotypes).**

| Mineral element (mg.kg\(^{-1}\)) | CG-SB\(^a\) | CG-NB | Leaves | Stems | Leaves | Stems |
|---|---|---|---|---|---|---|
| K | 23323.00±232.30\(^b\) | 34473.00±340.05 | 25053.00±240.00 | 37532.00±370.50 | | |
| Mg | 13314.00±131.00 | 2955.00±28.40 | 3846.00±37.00 | 1999.00±19.05 | | |
| Ca | 922.50±9.00 | 3800.00±37.00 | 1066.00±10.00 | 173.23±1.68 | | |
| Na | 201.94±2.00 | 133.56±1.30 | 92.71±0.90 | 225.71±2.20 | | |
| Fe | 427.00±4.10 | 40.43±0.39 | 69.03±0.68 | 69.01±0.64 | | |
| Mn | 33.28±0.30 | 11.10±0.10 | 19.99±0.18 | 16.66±0.15 | | |
| Zn | 28.65±0.27 | 22.70±0.21 | 30.10±0.30 | 31.80±0.30 | | |
| Cu | 25.85±0.25 | 9.81±0.09 | 19.64±0.19 | 8.08±0.07 | | |
| Pb | <0.10\(^c\) | <0.10 | <0.10 | <0.10 | | |
| Cd | <0.01\(^d\) | <0.01 | <0.01 | <0.01 | | |
| Cr | <0.10 | <0.10 | <0.10 | <0.10 | | |

\(^a\) CG-SB – genotype from the region of Plovdiv, Central South Bulgaria; CG-NB – genotype from the region of Mezdra, North-West Bulgaria.

\(^b\) All data are presented as mean value ± standard deviation (n=3).

\(^c\) Not quantified.

\(^d\) Not detected.

Data in Table 3 suggested that Cape gooseberry leaves and stems contained functional macro and micro minerals, which substantiated their consideration as potential components in livestock feed. The main macro minerals in the leaves and stems of both genotypes were K and Mg, with contents varying from 23323 mg.kg\(^{-1}\) to 37532 mg.kg\(^{-1}\) (K) and from 1999 mg.kg\(^{-1}\) to 13314 mg.kg\(^{-1}\) (Mg). There was no uniform distribution trend of the macro minerals between the two plant parts. The group of micro minerals was dominated by Fe and Zn, but numerical differences in micro mineral contents were observed on a genotype and plant part basis. The heavy metals Pb, Cd and Cr were not identified in
either of the genotypes. It was hard to make parallels between our results and data about the mineral content in other Cape gooseberry varieties, as, to the best of our knowledge, no previous studies in that aspect were announced. Therefore, a comparative analysis of the obtained data with the macro mineral content in different groups of plant materials used in animal feed was performed [16]. Regardless of plant part or genotype, the content of K in Cape gooseberry was comparable to that in silage, such as alfalfa (19.4-23.8 g.kg⁻¹), broad beans (20.0-324.5 g.kg⁻¹), maize-pea combination (2.2-2.5 g.kg⁻¹), and others, and in hay fodder, such as alfalfa (24.4-25.8 g.kg⁻¹) or natural meadow (22.0-23.4 g.kg⁻¹). The content of Mg approximated that in seed fodder, for example cottonseed (3.7 g.kg⁻¹) or flaxseed (4.1 g.kg⁻¹), and in industrial grain waste, such as wheat (13.6 g.kg⁻¹) and rice (9.2 g.kg⁻¹) bran. In turn, the content of Ca was comparable to that found in common grain fodder, barley (0.9 g.kg⁻¹), millet (1.1 g.kg⁻¹), and others; that of Na – close to grain fodder (0.1-0.3 g.kg⁻¹), seed fodder (0.1-0.5 g.kg⁻¹) and industrial grain waste (0.1-0.6 g.kg⁻¹).

Finally, it should be outlined that, although the foliage of Cape gooseberry and other Physalis species is considered “somewhat poisonous” [25], as they may contain solanine-type glycoalkaloids, the conclusion is that “overall there is little reason to consider the plants toxic” [25]. Therefore, the above discussion of the prospective use of Cape gooseberry leaf and stem was carried, but only in view of their incorporation as minor ingredients in animal feed, in combination with other dietary supplements.

### 3.2. Volatile composition of Cape gooseberry leaves

In compliance with the objectives of the study, the dry leaves of Cape gooseberry were subjected to extraction and identification of volatile compounds. The obtained concentrated n-hexane extracts (leaf concretes) [18, 19] were with identical yields, 2.66±0.02% (CG-SB) and 2.96±0.02% (CG-NB); both were waxy yellow-green masses, with specific odor. The results from the identification of the leaf volatile composition (% of TIC) are presented in Table 4, and the distribution of the identified compounds by chemical classes – on Figure 1.

Data in Table 4 reveal that the contents of the identified volatile compounds were comparable in both genotypes, with single individual exceptions, such as methyl linoleate, 3α-acetoxy-manool and vitamin E content. The GC-MS analysis identified a total of 32 components in each of the genotypes, representing 98.44% and 98.46% of the total volatile content, respectively in CG-SB and CG-NB. As seen from Table 4, nearly half of the identified constituents in either of the genotypes were in concentrations over 1%. The major volatiles (over 3%) in CG-SB genotype leaves, thirteen by number, were: n-pentacosanol (17.07%), vitamin E (10.03%), methyl hexadecanoate (8.89%), phytol (6.65%), (2E,6E)-farnesioic acid (5.32%), n-pentacosane (5.79%), n-hexacosane (5.50%), methyl octadecanoate (5.28%), monoethylhexyl phthalate (4.05%), n-heptacosane (4.04%), 3α-acetoxy-manool (3.48%), n-octacosane (3.47%), and 4,8,12,16-tetramethylheptadecan-4-olide (3.04%). Eleven major compounds (over 3%) were identified in the second genotype, CG-NB: n-pentacosanol (12.39%), methyl hexadecanoate (11.76%), methyl linoleate (10.07%), n-pentacosane (6.31%), n-hexacosane (6.07%), vitamin E (5.91%), (2E,6E)-farnesioic acid (5.79%), phytol (5.32%), methyl octadecanoate (5.74%), n-heptacosane (3.41%), and 4,8,12,16-tetramethylheptadecan-4-olide (3.31%).

The dominant group of aroma substances in the extracts (Figure 1) was that of oxygenated aliphatics (alcohols, acids and esters), followed by diterpenes. The presence of extracted aliphatic hydrocarbons was in agreement with the visual assessment of the extraction concentrates, waxy colored masses, as described above.
Table 4. Volatiles (by GC-MS) in Cape gooseberry leaves (two genotypes).

| No | Compound                             | RIb | CG-SBc | CG-NB |
|----|--------------------------------------|-----|--------|-------|
| 1  | Geranyl acetone                      | 1423| 1.03±0.01d | 1.12±0.01 |
| 2  | Methyl dodecanoate                   | 1524| 0.88±0.01 | 0.95±0.01 |
| 3  | Dihydroactinidiolide                 | 1531| 1.53±0.01 | 1.66±0.01 |
| 4  | Butyl laurate                        | 1772| 2.05±0.02 | 2.23±0.02 |
| 5  | (2E,6E)-Farnesic acid                | 1816| 5.32±0.05 | 5.79±0.05 |
| 6  | (2Z,6E)-Farnesyl acetate             | 1821| 1.39±0.01 | 1.51±0.01 |
| 7  | n-Hexadecanol                        | 1874| 1.62±0.01 | 2.75±0.02 |
| 8  | Methyl hexadecanoate                 | 1921| 8.89±0.08 | 11.76±0.11 |
| 9  | n-Hexadecanoic acid                  | 1958| 0.36±0.00 | 0.39±0.00 |
| 10 | n-Eicosane                           | 2000| 0.14±0.00 | 0.15±0.00 |
| 11 | Phytol                               | 2104| 6.65±0.06 | 5.32±0.05 |
| 12 | Methyl linoleate                     | 2095| 0.80±0.01 | 10.07±0.10 |
| 13 | n-Heneicosane                        | 2100| 0.20±0.00 | 0.22±0.00 |
| 14 | cis-Vacceninc acid                   | 2111| 1.03±0.01 | 0.11±0.00 |
| 15 | Methyl octadecanoate                 | 2117| 5.28±0.05 | 5.74±0.05 |
| 16 | 4,8,12,16-Tetramethylheptadecan-4-olide | 2124| 3.04±0.03 | 3.31±0.03 |
| 17 | Linoleic acid                        | 2133| 0.20±0.00 | 0.32±0.00 |
| 18 | Oleic acid                           | 2140| 0.48±0.00 | 0.52±0.00 |
| 19 | Monoethylhexyl phthalate             | 2163| 4.05±0.04 | 2.41±0.02 |
| 20 | 1-Docosene                           | 2182| 0.38±0.00 | 0.41±0.00 |
| 21 | Ethyl octadecanoate                  | 2196| 0.53±0.00 | 0.58±0.00 |
| 22 | n-Docosane                           | 2200| 0.45±0.00 | 0.49±0.00 |
| 23 | n-Tricosane                          | 2300| 0.21±0.00 | 0.23±0.00 |
| 24 | 3α-acetoxy-Manool                    | 2357| 3.48±0.03 | 1.78±0.01 |
| 25 | n-Tetraoxylanol                      | 2400| 0.51±0.00 | 0.56±0.00 |
| 26 | n-Tetraoxylanol                      | 2422| 2.05±0.02 | 1.22±0.01 |
| 27 | n-Pentacosane                        | 2500| 5.79±0.05 | 6.31±0.06 |
| 28 | n-Pentacosanol                       | 2525| 17.07±0.17 | 12.39±0.12 |
| 29 | n-Hexacosane                         | 2600| 5.50±0.05 | 6.07±0.06 |
| 30 | n-Heptacosane                        | 2700| 4.04±0.04 | 3.41±0.03 |
| 31 | n-Octacosane                         | 2800| 3.47±0.03 | 2.77±0.02 |
| 32 | Vitamin E                            | 2877| 10.03±0.10 | 5.91±0.05 |
|    | Total identified (%)                 |     | 98.44   | 98.46  |

a RI – retention (Kovats’) index.
b TIC – total ion current.
c CG-SB – genotype from the region of Plovdiv, Central South Bulgaria; CG-NB – genotype from the region of Mezdra, North-West Bulgaria.
d All data are presented as mean value ± standard deviation (n=3).
Figure 1. Distribution of identified volatiles in Cape gooseberry leaves, by chemical classes (total of identified=100%). CG-SB – genotype from the region of Plovdiv, Central South Bulgaria; CG-NB – genotype from the region of Mezdra, North-West Bulgaria.

In general, it can be summarized that genotype, at least on a limited regional basis, was not a factor affecting the volatile composition of Cape gooseberry leaves. As stated earlier, parallels to other results about Cape gooseberry leaf volatile composition were hard to make, due to the absence of previously published data. In a single study [11], the chemical composition of the essential oil obtained by hydrodistillation from the leaves of another Physalis species, *P. angulata* L., was identified; the major oil components belonged to the groups of diterpenes (31.7%), fatty acids (22.8%) and oxygenated sesquiterpenes (22.3%), but the individual composition was greatly different.

As seen from Table 4 and Figure 1, the aliphatic-derived aroma volatiles represented several chemical classes as well as respective individual compounds known to contribute to the aroma of various fragrance products and foods, for example: esters – methyl octadecanoate, methyl hexadecanoate, methyl dodecanoate, ethyl octadecanoate, butyl laurate, and methyl linoleate; alcohols – n-tetracosanol and n-pentacosanol; acids – n-hexadecanoic acid and the different members of C18-chained acids. In turn, the identified terpene-derived aroma volatiles, grouped into several classes, also contained specific odor-contributing compounds: esters – farnesyl acetate, with floral type odor; ketones – geranyl acetone, with a fresh, floral, rose, green magnolia, aldehyde, fruity odor; acids – farnesoic acid.

The dominant share of aroma-active compounds, oxygenated aliphatics and diterpenes, as well as that of some individual constituents, provide arguments in favor of the possible use of Cape gooseberry leaf extracts in perfumery and cosmetics. Moreover, none of the recognized cosmetic allergens were identified in the extracts [18].

4. Conclusions

The results from the study confirmed the assumption that the leaves and stems of Cape gooseberry, currently discarded by-products, could be regarded as alternative sources of bioactive substances. Based on the obtained phytoneutrient composition data, the two Bulgarian Cape gooseberry genotypes reveal certain potential for use as minor supplementary ingredients in livestock feed. The presence of aroma-
active volatiles and the specific profiles of the extracts from the leaves substantiate their prospective use in perfumery and cosmetics.

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