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Determination of the chemical composition of seeds, peels, and seedcakes from two genotypes of Cape gooseberry (*Physalis peruviana* L.)

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Abstract: *Physalis peruviana* L. fruit (Cape gooseberry, CG) is a rich source of phytonutrients, including vitamins, minerals, polyphenols, polyunsaturated fatty acids (FAs), phytosterols, dietetic fibers, and others. The popularity and production areas of CG have been expanding worldwide, thus producing fruit with origin-substantiated differences in their nutrient composition.This study was based on the comparative assessment of 2 genotypes of CG produced in Bulgaria (CG-P and CG-F), through analysis of the lipid fraction of different fruit elements (seeds, peels), and further examination of the extracted seedcakes. The CG seeds reasonably yielded more oil (17.0%–22.2%) than the isolated peels (2.8%–2.9%). The main FAs in the CG-P seed oil were oleic (29.6%) and palmitic (20.6%), and in the CG-F seed oil were palmitic (20.9%) and stearic (17.5%). Both CG peel oils were dominated by palmitic acid (43.0%–60.2%), but there was a significant variation of some other FAs. The group of bioactive tocopherols was found exclusively in the oil extracted from the CG seeds, with no significant difference between the genotypes; β-tocopherol and δ-tocopherol were the most abundant. Waste from the oil extraction (the seedcakes) was found to contain high levels of macro and microminerals (K, Mg, Cu, Zn, Mn, and others), fiber (40.26%–47.62%), protein (13.73%–8.08%), and essential amino acids, with some genotype-based variations. The results demonstrated that, concerning the studied aspects of fruit composition, CG produced in Bulgaria was comparable to the fruit of other origins; hence, they might be of practical interest to national agricultural and food producers, as well as to the food industry on a wider basis, as new details are added to the knowledge about CG fruit. The outcomes from the examination of the CG seedcakes were in favor of their potential in human and animal nutrition, and might serve as grounds for the development of new products.

Key words: *Physalis peruviana*, seed oil, fatty acids, tocopherols, amino acids, minerals

1. Introduction

Cape gooseberry (CG) (*Physalisperuviana* L.) is the most important and widely spread species of the genus *Physalis* (family Solanaceae). CG has been the focus of significant research interest over the last 2 decades, and an impressive amount of data on the phytochemical composition of CG fruit has been accumulated, as well as evidence for the various aspects of its biological and pharmacological activities (Ramadan and Mörsel, 2003, 2009; Ramadan et al., 2008; Rodrigues et al., 2009; Puente et al., 2011; Ramadan, 2011, 2012; Zhang et al., 2013; Sharma et al., 2015; Yıldız et al., 2015; Ertürk et al., 2017; Mokhtar et al., 2018; El-Beltagi et al., 2019). Recently, CG has been associated with the category of superfruits or superfoods, which promote a number of less popular exotic fruit with unique nutritive and bioactive properties (Chang et al., 2019).

Awareness of the nutritional and medicinal benefits of CG, combined with plant adaptability to various climatic conditions, has extended its cultivation far outside of its native areas of origin in tropical America (the Colombian and Peruvian Andes). Currently, CG is a truly cosmopolitan species and its production areas spread over South Africa, Central and Southern Europe, Asia, the Pacific zone, and other regions (Paksi et al., 2007; Puente et al., 2011). The results from the analysis of the phytochemical composition of CG fruit of different origins has consistently suggested that the genotype and production conditions were significant factors for fruit quality and composition (Puente et al., 2011; Ramadan, 2011; Zhang et al., 2013; Sharma et al., 2015). Several studies have provided evidence for origin-based differences in CG fruit nutrients; for example, in fruit from Colombia (Fisher et

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al., 2000), Brazil (Rodrigues et al., 2009), Egypt (El-Beltagi et al., 2019), Hungary (Paksi et al., 2007), India (Lal et al., 2019), and Turkey (Yıldız et al., 2015; Bilenler and Karabulut, 2019). A series of studies have been devoted to the lipid fraction of CG fruit, in particular, and data about the fatty acid (FA), tocopherol, and sterol composition of fruit oils of different origins are available (Ramadan and Mörsel, 2003, 2009; Ramadan et al., 2008; Rodrigues et al., 2009; Ramadan, 2012; Mokhtar et al., 2018). On the other hand, most of the annual production of CG fruit is processed into juice (pulp), which is used in many low-calorie and dietetic products (Kalugina et al., 2017); thus, considerable amounts of valuable, but unutilized waste products (i.e. seeds and peels) are generated. According to Ramadan (2011), the seed/peel pomace remaining after juice extraction constituted 27.4% of the fresh fruit weight and contained 19.3% oil, 17.8% protein, 3.10% ash, 28.7% crude fiber, and 24.5% carbohydrates. Those observations created the grounds for considering the seed and peel waste from juice production, as well as the waste remaining after oil extraction (CG seedcakes), as promising phytonutrient resources.

The sociogeographic conditions in Bulgaria are generally suitable for CG cultivation, but the popularity of the plant remains relatively limited. An original local variety (named Plovdiv) was selected and officially recognized between 2001 and 2006 (Panayotov, 2009). The fruit of the local variety was characterized as having a typical strawberry flavor, with a hint of vanilla, and a pleasant, balanced, sweet to slightly sour taste (Panayotov, 2009), and the results from the experimental production supported the potential of the variety in terms of fruit yield and quality (Panayotov and Popova, 2014a, 2014b). Some farms, mainly on a small-scale organic basis, have attempted CG production over the last 2 decades, but their endeavors were rather sporadic and practically no reliable data about sustainable production, market success, or fruit quality are available.

To the best of our knowledge, there are no previous investigations on the chemical composition of different CG genotypes cultivated in Bulgaria, or studies on the nutritive potential of different fruit parts and waste products. It was hypothesized herein that local production and genotype factors would affect the fruit chemical composition and that there would be a variation of data available for the fruit of other origins, it was further hypothesized that the byproducts of CG fruit processing (seeds, peels, seedcakes) would reveal the certain potential for nutrient use. Therefore, the objective of this work was to complete a comparative assessment of 2 genotypes of CG produced in Bulgaria (CG-P and CG-F), through analysis of the lipid fraction of different fruit parts (seeds, peels) and further examination of the resulting seedcakes [minerals, protein, fiber, amino acids (AAs)].

2. Materials and methods

2.1. Plant material

Used in this study were 2 genotypes of Cape gooseberry (*P. peruviana* L.) that had been cultivated in Bulgaria. The first was fruit from the original local variety named Plovdiv, produced in the region of Plovdiv, central south Bulgaria, 42°08′03.0″N,24°45′56.0″E (CG-P), and the second fruit was from an introduced variety, produced in a certified organic farm in the region of Mezdra, northwestern Bulgaria, 43°05′28.7″N,23°45′48.6″E (CG-F). The plants were grown by seedling and planting in the middle of May 2018 and all necessary agricultural practices were implemented. Ripe fruit was collected in October 2018 and processed within 5 days. Carefully formed within each of the genotypes was 2 individual samples, comprising seeds and peels. Seedcakes, the wastere sulting from the extraction of seed oil, were also analyzed in the study.

The absolute weight of the CG seeds was determined for 1000 randomly selected seeds (air-dried) using a Mettler-Toledo electronic precision balance (Mettler-Toledo, LLC, OH, USA; ± 0.0001 g). The initial moisture content of all of the samples was determined by drying at 103 ± 2°C to a constant weight, and all of the results were given on a dry weight (DW) basis.

2.2. Determination of oil content and fatty acids and tocopherols in the oil

Isolation of the oil (%, v/w) from the CG peels and seeds was performed using the International Organization for Standardization (ISO) (2014a) method, by extraction with n-hexane in a Soxhlet apparatus for 8 h. Determination of the FA profiles of the oils was performed according to the standard methods (ISO, 2014b; ISO, 2017). In brief, the oils were transmethylated with 2% H_2SO_4 in CH_3OH at 50 °C and analyzed on a Hewlett Packard 5890A gas chromatograph using a capillary Supelco 2560 column (Supelco Inc., Bellefonte, PA, USA), at 75 m × 0.25 mm × 18 µm (i.d.), and a flame ionization detector. The operating conditions were as follows: the column temperature increased from 130 °C (4 min) to 240 °C (5 min) at 15 °Cmin^{-1}; the injector and detector temperatures were set at 250 °C; hydrogen was used as a carrier gas, at a flow rate of 0.8 m$Lmin^{-1}$; and the split ratio was 50:1. Identification of the FAs was performed by comparison of the retention times with those of a standard mixture of FA methyl esters (FAMEs) (37 component FAME mix, Supelco Inc.).

Tocopherols were determined directly by high performance liquid chromatography (HPLC) analysis using a Merck-Hitachi unit (Merck KGaA, Darmstadt, Germany) equipped with a 250 × 4 mm Nucleosil Si 50-5 column (Macherey-Nagel GmbH & Co. KG., Düren, Germany) and a fluorescent Merck-Hitachi F 1000 detector. The operating conditions were as follows: mobile phase n-hexane:dioxane (96:4, v/v); flow rate of 1.0 m$Lmin^{-1}$;

detector excitation at 295 nm, emission at 330 nm; and injected sample volume of 20 μL (1 g100 mL^{-1} solution of crude oil in n-hexane). Tocopherols were identified by comparison with reference tocopherol standards (DL-a-, DL-β-, DL-γ-, and DL-δ-tocopherols with purity of 98%, purchased from Merck KGaA, according to the ISO (2016).

2.3. Determination of the macro and microminerals in the seedcakes

Air-dried seedcakes were mineralized at 450 °C. The residue was first dissolved in concentrated HCl and evaporated to dryness, and then, the remainder was dissolved in 0.1 $molL^{-1}$ HNO_3 solution. Mineral contents were determined on a Perkin Elmer/HGA 500 atomic absorption spectrophotometer (AAS) (Perkin Elmer, Inc., Norwalk, CT, USA), under the following instrumental parameters for the flame AAS: 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. Identification of the metals was performed by comparison to a standard solution of metal salts, and metal concentrations were calculated from a calibration curve, built using a standard 1 μgmL^{-1} solution.

2.4. Determination of protein, AAs, and cellulose in the seedcakes

Determination of the total protein content in the CG seedcakes was performed according to the AOAC Method 976.06 (2016), using an UDK 152 System (Velp Scientifica Srl, Usmate Velate, Italy). The AccQ-Fluor kit (WATO52880, Waters Corporation, Milford, MA, USA) was used for derivatization of the free AAs resulting from protein hydrolysis. Separation of the AccQ-Fluor AA derivatives was performed on an ELITE LaChrome HPLC chromatograph (Hitachi) using a diode array detector (DAD) and a reverse phase C 18 AccQ-Tag column (3.9 × 150 mm), operated at a temperature of 37 °C. The 2 mobile phases in the gradient elution were WATO52890 buffer and 60% acetonitrile, and the detection wavelength was 254 nm.

The content of cellulose (crude fiber) in the CG seedcakes was determined using a modification of the method by Brendel et al. (2000). Hydrolysis of cellulose and hemicellulose was conducted by boiling 1 g of the seedcakes with 16.5 mL of 80% CH_3COOH and 1.5 mL concentrated HNO_3 for 1.5 h. After filtration of the suspension, the solid residue was dried at 105 °C for 24 h and weighed.

2.5. Statistics

All of the results were presented as the mean of 3 individual measurements (n = 3) with the corresponding standard deviation (mean ± SD). Significant differences ($P < 0.05$) were determined by applying ANOVA and the Tukey multiple comparison test as statistical tools.

3. Results and discussion

3.1. FA and tocopherol composition of the CG seed and peel oils

As previously described, the 2 individual CG berry structures associated with the presence of lipid fractions, i.e. the seeds and peels, were analyzed separately with the objective of completing a more detailed investigation on the CG oil composition. The results from the determination of the basic characteristics of the raw materials used in the study are presented in Table 1.

The results regarding the oil yield clearly differentiated between the 2 oil-containing CG fruit structures; reasonably, the seeds yielded significantly more oil, 22.23% (CG-P) and 17.04% (CG-F), than the isolated peels alone (2.81% and 2.88%, respectively, for CG-P and CG-F). There were significant differences between the 2 CG genotypes in terms of the seed oil yield, but not in terms of the peel oil yield. These data indicated that CG seeds from both genotypes were sufficiently rich in glyceride oil, approximating the oil content of grape seeds (8%–20%), or safflower seeds (28%)[12].

Data from the analysis of the individual FA composition of the seed and peel oils from the 2 CG genotypes studied are presented in Table 2.

A total of 20 FAs were identified in the oil isolated from the CG-P seeds and peels, while the number of individual FAs in the oil of the CG-F genotype was 16 in the seed oil and 23 in the peel oil, respectively. There were some numeric differences in the FA composition of the 2 CG genotypes. The main FAs in the seed oil of the CG-P genotype were oleic (29.6%) and palmitic (20.6%), while in the seed oil of the CG-F genotype, they were palmitic (20.9%) and stearic (17.5%), and the share of oleic acid was just 5.4%. In turn, both peel oils were dominated by palmitic acid (60.2% and 43.0% of CG-P and CG-F peel oil, respectively), but there were significant differences in the content of some other FAs, such as oleic, arachidonic (n-6), docosadienoic (n-6), and docosahexaenoic (n-3) (the latter 2 were identified only in the CG-F peel oil). Data regarding the ratio between the saturated FAs (SFAs) and unsaturated FAs (UFAs) showed no uniform trend, on either a genotype or fruit element basis. Interestingly, the SFA:UFA ratio was identical in the CG-F seed and peel oils (62:38), with relatively close shares of monounsaturated and polyunsaturated FAs, thus differing from the results for the CG-P seed and peel oils.

1 Heuzé V, Tran G, Chapoutot P, Renaudeau D, Bastianelli D et al. (2015). Safflower (*Carthamus tinctorius*) seeds and oil meal. Feedipedia, a programme by INRA, CIRAD, AFZ and FAO [online]. Website http://www.feedipedia.org/node/49 [accessed 20 January 2020].

[2] Heuzé V, Tran G (2017). Grape seeds and grape seed oil meal. Feedipedia, a programme by INRA, CIRAD, AFZ and FAO [online]. Website https://feedipedia.org/node/692 [accessed 20 January 2020].

Table 1. Basic indices of the structural elements of the CG fruit in the study.

Index	CG-P	CG-F
Moisture content of seeds, %	9.51 ± 0.08	8.37 ± 0.07
Moisture content of peels, %	6.26 ± 0.06	7.47 ± 0.07
Absolute weight of seeds, g/1000 seeds	1.073 ± 0.01	0.961 ± 0.01
Content of seeds, % of the fruit weight	7.28 ± 0.06	11.52 ± 0.11
Content of peels, % of the fruit weight	5.82 ± 0.04	3.93 ± 0.02
Seed oil yield, % (DW)	22.23 ± 0.21	17.04 ± 0.16
Peel oil yield, % (DW)	2.81 ± 0.02	2.88 ± 0.03

All data are given as the mean ± SD (n = 3).

A parallel between the current results and the previously published data revealed some differences in terms of the oil yield and FA composition. Most of the studies on CG oil yield and composition, however, considered the oil extracted from either whole fruit or seed/peel pomace, and reasonably, those results differed from the data presented in the current study, especially with regard to the oil yield. For example, Yıldız et al. (2015) analyzed whole CG fruit produced in Turkey and reported 0.18% oil content [on a fresh weight (FW) basis], while Ramadan and Mörsel (2003) found 2% FW oil content in whole fruit of Colombian origin. However, the yield of the oil extracted from CG seed/peel pomace (with 6.6% moisture content) in the study of Ramadan (2012) was 19.3%, which was in line with the current results for both CG genotypes studied. In terms of individual FAs, the results herein differed numerically from the data provided by Rodrigues et al. (2009) for fruit from Brazil, who identified linoleic (72.42%), oleic (10.30%), and palmitic (9.38%) acids as the main FAs. Similar data were provided by Mokhtar et al. (2018) for seed/peel waste powder of fruit from the Egyptian market, comprising linoleic (77.78%), oleic (11.32%), palmitic (7.39%), and stearic (3.51%) acids. In other studies (Ramadan and Mörsel, 2003; Ramadan, 2012), linoleic (76.1%), oleic (11.7%), palmitic (7.29%), and stearic (2.51%) acids were the dominating FAs in CG seed oil of Colombian origin, while linoleic (77.1%), oleic (10.3%), palmitic (7.95%), and stearic (2.61%) were the main FAs in the seed/peel pomace oil. In these studies, the ratio between the SFAs and UFAs was approximately 1 to 7, while the current results revealed a lower share of UFAs in both genotypes.

Tocopherols, known as potent antioxidants and important contributors with vitamin E activity in the human diet, were also analyzed in this study, and the results for the 2 types of CG oils are presented in Table 3.

As seen in Table 3, tocopherols were found exclusively in the oil extracted from the CG seeds, with no significant difference between the 2 genotypes (2833 and 2423 $mgkg^{-1}$, respectively). In both seed oils, β-tocopherol was the main representative (54.7% and 62.9% of the total tocopherol content), followed by δ-tocopherol (25.8% and 20.5%). The only representative of the tocopherol fraction in the peel oil of the CG-F fruit was α-tocopherol (97 $mgkg^{-1}$), which was not identified in the rest of the samples; no tocopherols at all were identified in the CG-P peel oil. These results corresponded well with previous data about CG oil tocopherols. For example, Ramadan and Mörsel (2003, 2009) also identified β-tocopherol and γ-tocopherol as the main components in CG whole berry and seed oils, and γ-tocopherol and α-tocopherol in the pulp/peel oil. Additionally, the results from this study were very close to the tocopherol contents in the CG seed/peel pomace oil reported by Ramadan (2012), while β-tocopherol (47% of the tocopherol fraction), *γ*-tocopherol (26%), δ-tocopherol (18.5%), and α-tocopherol (6%), as well as α-tocopherol (7.34 mg100 g^{-1}) were reported in CG whole fruit oil by Paksi et al. (2007).

3.2. Examination of the CG seedcakes

The seedcakes resulting from oil extraction were further analyzed, as it was assumed that they represented an underutilized part of the CG fruit, with a potential forhuman and animal nutrition. In this study, the seeds were responsible for 7.28% (CG-P) and 11.52% (CG-F) of the fresh fruit weight (Table 1), which additionally supported the objective of a more detailed analysis of these byproducts and,in particular,the determination of constituents with a nutritional value, such as minerals, fiber, protein, and AAs.

3.2.1. Minerals in the CG seedcakes

The results from the analysis of the macro and microminerals in the seedcakes of the 2 CG genotypes produced in Bulgaria are presented in Table 4.

Data in Table 4 revealed that both CG seedcakes were rich in minerals, although some numerical differences

Table 2.FA composition of the seed and peel oil of the 2 CG genotypes.

FAs, % of the oil		CG-P		CG-F	
		Seed oil	Peel oil	Seed oil	Peel oil
$C_{10:0}$	Capric	nd	nd	0.5 ± 0.00[a]	3.1 ± 0.03[b]
$C_{12:0}$	Lauric	0.2 ± 0.00[a]	3.9 ± 0.01[b]	nd	6.1 ± 0.06[c]
$C_{14:0}$	Myristic	1.6 ± 0.01[a]	5.5 ± 0.01[b]	1.7 ± 0.01[a]	3.0 ± 0.03[c]
$C_{14:1}$	Myristoleic	0.2 ± 0.00[a]	0.7 ± 0.00[a]	nd	0.4 ± 0.00[a]
$C_{15:0}$	Pentadecanoic	2.7 ± 0.02[a]	2.4 ± 0.02[a]	6.6 ± 0.06[b]	1.2 ± 0.01 [c]
$C_{15:1}$	Pentadecenoic	0.5 ± 0.00[a]	1.6 ± 0.01[b]	0.9 ± 0.00[a]	0.2 ± 0.00 [a]
$C_{16:0}$	Palmitic	20.6 ± 0.19[a]	60.2 ± 0.59[b]	20.9 ± 0.19[a]	43.0 ± 0.42[c]
$C_{16:1}$	Palmitoleic	3.1 ± 0.03[a]	0.7 ± 0.00 [b]	0.6 ± 0.00[b]	1.2 ± 0.01[c]
$C_{17:0}$	Margaric	8.7 ± 0.08[a]	0.8 ± 0.00[b]	14.2 ± 0.13[c]	1.1 ± 0.01[d]
$C_{17:1}$	Heptadecenoic	0.5 ± 0.00[a]	3.0 ± 0.02[b]	0.5 ± 0.00[a]	0.2 ± 0.00[a]
$C_{18:0}$	Stearic	13.0 ± 0.12[a]	8.6 ± 0.08[b]	17.5 ± 0.16[c]	3.4 ± 0.03[d]
$C_{18:1}$	Oleic	29.6 ± 0.28[a]	1.4 ± 0.01[b]	5.4 ± 0.05[c]	11.6 ± 0.10[d]
$C_{18:2}$(n-6)	Linoleic	5.3 ± 0.05[a]	1.3 ± 0.01[b]	11.3 ± 0.10[c]	1.1 ± 0.01[b]
$C_{18:3}$(n-3)	Linolenic	5.4 ± 0.05[a]	2.4 ± 0.02[b]	9.2 ± 0.08 [c]	1.3 ± 0.01[d]
$C_{20:0}$	Arachidic	nd	0.2 ± 0.00	nd	nd
$C_{20:1}$	Eicosenoic	1.2 ± 0.01[a]	0.9 ± 0.00[a]	nd	0.9 ± 0.00[a]
$C_{20:2}$(n-6)	Eicosadienoic	3.0 ± 0.02[a]	0.6 ± 0.00[b]	5.2 ± 0.05[c]	0.5 ± 0.00[b]
$C_{20:3}$(n-3)	Eicosatrienoic	nd	nd	nd	0.8 ± 0.00
$C_{20:4}$(n-6)	Arachidonic	1.3 ± 0.01[a]	3.2 ± 0.03[b]	3.7 ± 0.03[b]	0.6 ± 0.00[c]
$C_{20:5}$(n-3)	Eicosapentaenoic	1.1 ± 0.01[a]	1.8 ± 0.01[b]	1.4 ± 0.01[a]	2.8 ± 0.02[c]
$C_{22:0}$	Behenic	0.8 ± 0.00[a]	0.6 ± 0.00[a]	0.4 ± 0.00[a]	0.9 ± 0.00[a]
$C_{22:2}$(n-6)	Docosadienoic	nd	0.2 ± 0.00[a]	nd	9.4 ± 0.09[b]
$C_{22:6}$(n-3)	Docosahexaenoic	nd	nd	nd	7.1 ± 0.07
$C_{23:0}$	Tricosylic	0.6 ± 0.00	nd	nd	nd
$C_{24:0}$	Lignoceric	0.6 ± 0.00	nd	nd	0.1 ± 0.00
Saturated FAs		48.8	82.2	61.8	61.9
Unsaturated FAs		51.2	17.8	38.2	38.1
Monounsaturated FAs		35.1	8.3	7.4	14.5
Polyunsaturated FAs		16.1	9.5	30.8	23.6

nd: Below 0.01% or not detected.
All data are given as the mean ± SD (n = 3).
[a-d]: Values with a different superscript in a row differed significantly ($P < 0.05$).

were observed between the 2 genotypes in the study, as well as between the current results and previous data. Generally, seedcakes of the CG-F genotype had relatively higher contents of the identified macro and microminerals when compared to the CG-P genotype. In view of the individual mineral composition, the current results were in good agreement with the previous findings, which indicated that CG fruit had a high K content, which is one of the important intracellular elements involved in physiological functions in the body (Puente et al., 2011; Olivares-Tenorio et al., 2016). For example, the K content in this study was very close to the values reported by Rodrigues et al. (2009) at 347 mg100 g^{-1}in whole fruit, Leterme et al. (2006) at 467 mg100 g^{-1} in fruit pulp, and Mokhtar et al. (2018) at 560 mg100 g^{-1}in seed/peel waste powder. The other macrominerals, Na, Ca, and Mg, showed no common variation trend when compared to previous reports. For example, Na in the studied seedcakes

Table 3. Tocopherol composition of the seeds and peel oil of the 2 CG genotypes.

Tocopherols	CG-P		CG-F	
	Seed oil	Peel oil	Seed oil	Peel oil
Tocopherols, mgkg^{-1}	2833 ± 27.50^{a}	nd	2423 ± 23.10^{a}	97 ± 0.89^{b}
Tocopherols, % of the total content				
α-Tocopherol	nd	nd	nd	100 ± 0.00
β-Tocopherol	54.7 ± 0.52^{a}	nd	62.9 ± 0.60^{a}	nd
γ-Tocopherol	19.5 ± 0.18^{a}	nd	16.6 ± 0.15^{a}	nd
δ-Tocopherol	25.8 ± 0.24^{a}	nd	20.5 ± 0.19^{a}	nd

All data are given as the mean ± SD (n = 3).
nd: below 0.05 mg.kg^{-1} or not detected.
$^{a-b}$: Values with a different superscript in a row differed significantly ($P < 0.05$).

was higher than that found in the whole fruit at 1.1 mg100 g^{-1}DW (Rodrigues et al., 2009), or fruit pulp at 6.0 mg100 g^{-1} (Leterme et al., 2006), but lower than that in the seed/peel waste at 170 mg100 g^{-1} (Mokhtar et al., 2018). The Ca content in CG fruit is known to be generally low (Puente et al., 2011); the current results were in compliance with that, although numerically, the Ca levels were lower than those reported previously for different parts of the CG fruit, varying in the range of 9.0 to 43.6 mg100 g^{-1} (Leterme et al., 2006; Rodrigues et al., 2009; Eken et al., 2014; Ozturk et al., 2017). Seedcakes were rich in Mg, an essential structural element in the human body, and the current results were very close to some previous findings reporting 102.5–122.5 mg100 g^{-1} (Ozturk et al., 2017) or 145 mg100 g^{-1} (Eken et al., 2014), and considerably higher than some other data, such as 19 mg100 g^{-1} (Leterme et al., 2006) or 34.7 mg100 g^{-1} (Rodrigues et al., 2009). CG seedcakes contained microminerals important in human nutrition; as a general observation, the Cu, Zn, Fe, and Mn contents were close or higher than the respective results from previous studies on different CG fruit fractions (Leterme et al., 2006; Rodrigues et al., 2009; Ramadan, 2011; Eken et al., 2014; Ozturk et al., 2017; Mokhtar et al., 2018). It might be worth outlining the high content of Cu and Zn, which are elements with expressed antioxidant and radical-scavenging activities, in this study, as well as the levels of Mn, an essential element in bone formation and macronutrient metabolism. Despite the numerical variations, origin, or methodology related, the results of this study further demonstrated the potential of CG seedcakes in human and animal nutrition.

3.2.2. Protein, cellulose, and AAs in the CG seedcakes

The protein content of the analyzed seedcakes was 13.73 ± 0.12% DW (CG-P) and 8.08 ± 0.07% (CG-F), thus showing significant variation between the 2 CG genotypes, probably due to the influence of genetic and production factors. The seedcakes contained similar amounts of cellulose, at 40.26 ± 0.39% DW (CG-P) and 47.62 ± 0.46% DW(CG-F). In a brief comparison, the CG protein content in the current study, although lower, was close to the reported values of 17.8% (Ramadan, 2011) and 15.89% (Mokhtar et al., 2018) protein in CG seed/peel waste, and approximated that of some alternative seed oil sources, such as grape seeds (9%–11%)[2], Jatropha seeds (16%–18 %)[3], or safflower seeds (17%–18%)[1]. In turn, the cellulose content was significantly high, and the relatively higher cellulose content in the CG-F seedcakes corresponded well with the lower seed absolute weight value (Table 1), reflecting the different shares of the seed layers. The content of cellulose in the studied CG seedcakes was close to that in grape seeds, Jatropha seeds (both over 33%), or safflower seeds (about 30%–40%)[1,2,3].

The AA composition of the 2 seedcakes is presented in Table 5.

Arginine was the dominant AA in both CG genotypes, at 13.90 mgg^{-1} (CG-P) and 15.51 mgg^{-1} (CG-F), but the rest of the AAs showed different distribution on a genotype basis. The most significant variations were found with regard to tyrosine, aspartic acid, serine, glutamic acid, and valine. Although detailed data on the AA composition of CG fruit is generally limited (Puente et al., 2011), the current results were in agreement with the findings of Mokhtar et al. (2018), who also identified glutamic acid, arginine, and aspartic acid as the most abundant AAs in dehydrated CG seed/peel powder. The current results clearly suggested that CG seedcakes could be regarded as a valuable source of AAs, both essential and nonessential, if used in food and livestock feed.

[3] Heuzé V, Tran G, Edouard N, Renaudeau D, Bastianelli D et al. (2016). Jatropha (*Jatropha*sp.) kernel meal and other jatropha products. Feedipedia, a programme by INRA, CIRAD, AFZ and FAO [online]. Website https://www.feedipedia.org/node/620 [accessed 20 January 2020].

Table 4. Macro and microminerals in seedcakes of the 2 CG genotypes.

Minerals, mgkg^{-1}	CG-P	CG-F
K	4290.00 ± 18.33^{a}	3485.00 ± 12.11^{b}
Na	76.00 ± 0.66^{a}	124.15 ± 0.81^{b}
Ca	16.56 ± 0.12^{a}	15.32 ± 0.11^{a}
Mg	952.10 ± 7.22^{a}	1205.00 ± 10.01^{b}
Fe	49.69 ± 0.35^{a}	37.52 ± 0.29^{a}
Mn	9.35 ± 0.08^{a}	10.83 ± 0.08^{a}
Cu	182.00 ± 1.03^{a}	348.19 ± 2.55^{b}
Zn	121.61 ± 0.99^{a}	308.64 ± 1.82^{b}
Pb	nd	1.30 ± 0.01
Cd	12.92 ± 0.08^{a}	23.07 ± 0.13^{b}
Cr	nd	6.60 ± 0.02

All data are given as the mean ± SD (n = 3).
nd: below 0.01 mg.kg^{-1}.
^{a,b}:Values with a different superscript in a row differed significantly ($P < 0.05$).

Table 5.AAs in the seedcakes of the 2 CG genotypes.

AAs	Content, mgg^{-1}	
	CG-P	CG-F
Aspartic acid	10.11 ± 0.09^{a}	3.26 ± 0.02^{b}
Serine	2.81 ± 0.02^{a}	7.19 ± 0.07^{b}
Glutamic acid	7.42 ±0.06^{a}	3.29 ± 0.03^{b}
Glycine	1.47 ± 0.01^{a}	1.07 ± 0.01^{a}
Histidine	4.03 ±0.03^{a}	3.41 ±0.03^{b}
Arginine	13.90 ±0.12^{a}	15.51 ± 0.13^{b}
Threonine	2.02 ±0.02^{a}	2.34 ± 0.02^{a}
Alanine	4.41 ± 0.03^{a}	5.13 ±0.05^{b}
Proline	2.13 ± 0.02^{a}	2.39 ±0.02^{a}
Cysteine	4.68 ± 0.04	nd
Tyrosine	10.91 ± 0.10^{a}	1.39 ±0.01^{b}
Valine	2.68 ± 0.02^{a}	4.27 ±0.03^{b}
Methionine	0.52 ± 0.00^{a}	0.10 ± 0.00^{a}
Lysine	3.07 ± 0.02^{a}	3.58 ± 0.03^{a}
Isoleucine	3.23 ±0.03^{a}	3.58 ±0.02^{a}
Leucine	0.50 ±0.00^{a}	0.55 ±0.00^{a}
Phenylalanine	3.15 ± 0.03^{a}	3.49 ± 0.02^{a}

All data are given as the mean ± SD (n = 3).
nd: not detected.
$^{a, b}$: Values with a different superscript in a row differed significantly ($P < 0.05$).

In conclusion, the results from the analyses of the lipid fraction of the CG seeds and peels and the examination of CG seedcakes in this study revealed some new aspects in CG phytonutrient composition. For the first time, data about CG fruit from 2 genotypes cultivated in Bulgaria were obtained and compared to fruit of other origins. The seeds, peels, and seedcakes, which are underutilized byproducts in CG juice production, were rich resources of edible oil (seeds), FAs (about half of which were unsaturated), tocopherols, minerals (especially K, but also Mg, Cu, Zn, Fe, and Mn), protein, cellulose, and essential AAs. The results from the study supported the assumption that the CG genotype influenced fruit composition; thus, if possible, individual assessment of the CG fruit of different origins should be applied. The accumulated new data about lipid distribution between fruit structural elements, as well as those about the nutritive potential of seedcake waste might be of practical interest, not only to national agricultural and food producers, but also to those generally involved in the development of functional foods and feeds.

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