

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

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

KEYWORDS: Amino acids; Fatty acids; Minerals; *Physalis peruviana* L.; Sterols; Tocopherols

RESUMEN: *Composición lipídica de diferentes partes del fruto del aguaymanto (Physalis peruviana L.) y valorización de residuos de semillas y cáscaras.* El consumo del aguaymanto (*Physalis peruviana* L.), fresco o procesado, está ganando popularidad en todo el mundo debido a sus beneficios nutricionales y medicinales. Este estudio se basó en el análisis de la fracción lipídica de diferentes partes de la fruta y en una mayor valorización de los desechos resultantes. Se determinó el contenido de la fracción glicéridica en semillas, cáscaras y residuos de semillas/cáscaras, así como la composición individual de ácidos grasos, esteroides y tocoferoles de los aceites. Las semillas de aguaymanto y los residuos de semillas/cáscaras fueron una rica fuente de aceite (hasta 22,93%), adecuados para un uso nutricional, debido a las altas proporciones de ácidos grasos insaturados (hasta 83,77%), esteroides (campesterol, Δ^5 -avenasterol, β -sitosterol) y tocoferoles (β -, δ - y γ -toferol). Los residuos de semillas/cáscaras y los residuos desengrasados de semillas extraídos (tortas) contenían macro y microminerales (K, Mg, Na, Fe, Zn, Mn, Cu) importantes para la nutrición humana y animal. Las tortas de semillas tenían un contenido relativamente alto de proteínas (24,32%) y celulosa (42,94%), y un perfil de aminoácidos interesante. Los resultados del estudio contribuyen a una comprensión más profunda de la composición del aguaymanto y pueden ser de relevancia práctica en el desarrollo de alimentos y alimentos funcionales.

PALABRAS CLAVE: Ácidos grasos; Aminoácidos; Esteroides; Minerales; *Physalis peruviana* L.; Tocoferoles

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1. INTRODUCTION

Cape gooseberry (*Physalis peruviana* L.), also known as goldenberry, Inca berry or Peruvian groundcherry, is the most extensively cultivated *Physalis* species, constituting an important cash crop in many countries of the tropical and subtropical regions (Puente *et al.*, 2011). Colombia is the biggest producer and exporter of fresh and dehydrated Cape gooseberry (CG) fruit worldwide, with an annual export volume of about 6000 tons, directed mainly to the European Union (the Netherlands, Germany and Belgium being the largest markets) (Olivares-Tenorio *et al.*, 2016). The fruit of CG, a berry enveloped in a protective calyx, is bright yellow to orange in color, ovoid-shaped, small and shiny (with a diameter between 1.25 and 2.50 cm and weight between 4 and 10 g), and contains about 100-300 seeds. The berries are rich in flavor, sweet and sour, resembling those of tomato, strawberry, kiwi and citrus, with a tender and juicy texture (Puente *et al.*, 2011). Ripe berries are consumed mostly fresh, but like other exotic fruits, CG is an excellent ingredient in many low calorie and dietetic products (beverages, jellies, jams, juices, yoghourts, dressings, etc.) (Ramadan, 2011; Kalugina *et al.*, 2017). Several comprehensive reviews have been published recently, which summarize the data about the chemical composition, biological activities and uses of CG fruit or whole plants (Puente *et al.*, 2011; Ramadan, 2011; Zhang *et al.*, 2013; Sharma *et al.*, 2015). The nutritional and medicinal values of CG fruit were related to the high levels of beneficial compounds such as vitamins, minerals, carotenoids, polyphenols, alkaloids, fatty acids, phytosterols, polysaccharides and others, as well as to their biological activities, such as anti-inflammatory, immunomodulatory, antioxidant, cytotoxic, antimicrobial, hepatoprotective, antiglycemic, anticholesterolemic, etc. (Ramadan and Mörsel, 2003; Ramadan *et al.*, 2008; Rodrigues *et al.*, 2009; Puente *et al.*, 2011; Ramadan 2011, 2012; Zhang *et al.*, 2013; Sharma *et al.*, 2015; Yıldız *et al.*, 2015; Kupska *et al.*, 2016; Ertürk *et al.*, 2017; Ramadan *et al.*, 2017; Mokhtar *et al.*, 2018). CG fruit was evaluated as a promising source of vegetable oil (Puente *et al.*, 2011; Ramadan, 2011), with a total oil content of 2.0, 1.8, and 0.2% (on a fresh weight basis (FW)), respectively, in the whole berries, seeds, and pulp/peel fraction of fruit of Colombian origin (Ramadan and Mörsel, 2003).

Some other studies investigated the triacylglycerol, tocopherol and phytosterol composition of CG pomace oil extracted from the seed and peel waste resulting from juice processing (Ramadan *et al.*, 2008; Ramadan, 2012; Mokhtar *et al.*, 2018). The seed/peel pomace, as outlined by Ramadan, (2011), constituted the largest portion of the waste generated from juice production, 27.4% of fruit weight, and contained 19.3% oil, 17.8% protein, 3.10% ash, 28.7% crude fiber, and 24.5% carbohydrates. Therefore, the seed/peel waste remaining after juice extraction, as well as the seed cakes remaining after oil extraction, can be considered an integral element in the sustainable CG usability, as they constitute a significant amount of fruit weight and contain compounds which are important for human and animal nutrition. These considerations create reasonable grounds for targeted analysis of these by-products, e.g. the determination of macro- and microminerals, fibers, protein, amino acids, vitamins, and other constituents with nutritional value. However, to the best of our knowledge, research data based on fractionization by fruit structural parts and processing by-products (seeds, peels, seed/peel waste, and seed cakes) are far from exhaustive, despite the intensive marketing promotion of CG fruit worldwide and the growing awareness of CG's nutritional benefits.

Therefore, the objective of this work was to study the lipid composition of the different parts of CG fruit and the valorization of the resulting CG waste. We hypothesized that the results from the analysis of the lipid fraction and other micro- and macronutrients (in terms of oil content; sterol, tocopherol, and fatty acid composition; protein, cellulose, amino acids, minerals) of CG seeds, peels, seed/peel waste and extracted seed cakes, respectively, would reveal the individual contribution of each element to the properties of the whole fruit, as well as provide further arguments in favor of CG waste assessment and its potential for wider use. Therefore, the outcomes from this study may contribute to the better understanding and evaluation of this exotic tropical fruit worldwide, and could be of interest to the food industry and nutrition.

2. MATERIALS AND METHODS

2.1. Plant material

CG fruit (*P. peruviana* L.) of Colombian origin was purchased from a local supermarket in January 2019.

Undamaged fruit of uniform ripeness were selected, then two separate sub-samples were formed, seeds and berry peels. Those were obtained by careful manual isolation of peels and seeds from individual berries in order to make distinguishable samples of berry structural elements. Fruit samples then were kept in the refrigerator at a temperature of $-18\text{ }^{\circ}\text{C}$ until analysis. Another sample constituted air-dried seed and peel mixture (seed/peel waste, seed/peel pomace) remaining after a high-speed vacuum separation of fruit juice. Seed cakes, in turn, were taken after the Soxhlet extraction of seed oil with n-hexane and air dried. The moisture content of the samples was determined by oven-drying at $103 \pm 2\text{ }^{\circ}\text{C}$ to constant weight.

2.2. Fatty acids (FAs), sterols and tocopherols in CG seed and peel oils

The isolation of the oil (% v/w) from fruit peels and seeds was carried out by extraction with n-hexane in a Soxhlet apparatus for 8 h. The solvent was then completely evaporated in a rotary vacuum evaporator at a temperature of $40\text{ }^{\circ}\text{C}$ (ISO 659:2014). The FA composition of the oils was determined by GC analysis after transmethylation of the sample with 2% H_2SO_4 in CH_3OH at $50\text{ }^{\circ}\text{C}$ (ISO 12966-1:2014, ISO 12966-2:2011). Determination was performed on a Hewlett Packard 5890 A gas chromatograph equipped with a capillary Supelco 2560 column, $75\text{ m} \times 0.25\text{ mm} \times 18\text{ }\mu\text{m}$ (i.d.), and a flame ionization detector (FID). The column temperature was programmed from $130\text{ }^{\circ}\text{C}$ (4 min) to increase at $15\text{ }^{\circ}\text{C}/\text{min}$ to $240\text{ }^{\circ}\text{C}$ (5 min); injector and detector temperatures were $250\text{ }^{\circ}\text{C}$; hydrogen was the carrier gas at a $0.8\text{ mL}/\text{min}$ flow rate; the split ratio was 50:1. The identification of FAs was performed by comparison of the retention times with those of a standard mixture of fatty acid methyl esters (FAME) (37 component FAME mix, Supelco, USA), according to ISO 12228-1:2014.

Tocopherols were determined directly by HPLC analysis using a Merck-Hitachi unit equipped with a $250\text{ mm} \times 4\text{ mm}$ Nucleosil Si 50-5 column and a fluorescent Merck-Hitachi F 1000 detector. The operating conditions were as follows: mobile phase n-hexane:dioxan (96:4, v/v); flow rate of $1.0\text{ mL}/\text{min}$; detector excitation at 295 nm , emission at 330 nm ; injected sample volume of $20\text{ }\mu\text{L}$ (1 g/100 mL solution of crude oil in n-hexane). Tocopherols were

identified by comparison with the retention times of reference tocopherol standards (DL- α -, DL- β -, DL- γ - and DL- δ -tocopherols with 98% purity, purchased from Merck, Darmstadt, Germany), according to ISO 9936:2016.

For the determination of sterols, the unsaponifiable fraction was isolated after saponification of the oil and extraction with n-hexane, according to ISO 18609:2000. Sterols were identified by comparison of the retention times with those of a standard sterol mixture containing cholesterol (stabilized, purity 95%, Across Organics, New Jersey, USA), stigmasterol (purity 95%, Sigma-Aldrich, St. Louis, MO, USA) and β -sitosterol (with ca 10% campesterol, ca 75% β -sitosterol, Across Organics, New Jersey, USA), according to ISO 12228-1:2014.

The phospholipid content was determined by spectrophotometry, measuring the content in phosphorus at 700 nm after mineralization of the oil with a solution of HClO_4 and H_2SO_4 (1:1, v/v) (ISO 10540-1:2014).

2.3. Mineral elements in seed/peel waste and seed cakes

The seed/peel fraction and seed cakes were mineralized at $450\text{ }^{\circ}\text{C}$; the residue was first dissolved in concentrated HCl, evaporated to dryness and then dissolved again in $0.1\text{ mol}/\text{L}$ HNO_3 . The concentrations of mineral elements were determined by using an atomic absorption spectrophotometer (AAS) Perkin Elmer/HGA 500 (Norwalk, USA). The respective wavelengths for the AAS detection were: 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 . Elemental identification was carried out by comparing with a standard solution of metal salts, and the calculation of the respective metal concentrations were based on a standard solution of $1\text{ }\mu\text{g}/\text{mL}$, using a calibration curve.

2.4. Protein, amino acids and cellulose in seed cakes

The total protein content in CG seed cakes was determined by the Kjeldahl method, as described in AOAC Method 976.06 (2016), on a UDK 152 System (Velp Scientifica, Italy).

The hydrolysis of protein to free aminoacids was completed by reacting 30 mg dried seed cake with 3

mL 6N HCl in a sealed glass ampule at 105 °C for 24 h. The solvent was evaporated in a vacuum chamber at 40 °C and the residue was fully diluted in 20 mM HCl (2 mL). After filtration, 20 µL of the solution were derivatized with AccQ-Fluor kit (WATO52880, Waters Corporation, USA). Finally, the solution was heated to 55 °C and 20 µL were injected into an ELITE LaChrome HPLC chromatograph (Hitachi) equipped with a diode array detector (DAD) and a reverse phase C 18 AccQ-Tag (3.9 mm × 150 mm) column. The mobile phases in the gradient elution were WATO52890 buffer (Waters Corporation, USA) and 60% acetonitrile. The detection wavelength was 254 nm and column temperature was 37 °C.

The content of cellulose (crude fiber) in the CG seed cakes was determined by a modification of the method of Brendel *et al.*, (2000). The hydrolysis of cellulose and hemicellulose was carried out by boiling 1 g of seed cakes in 16.5 mL of 80% CH₃COOH and 1.5 mL concentrated HNO₃ 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

The measurements were made in triplicate (n = 3), and the results were presented as mean values of the individual measurements with the corresponding standard deviation (mean ± SD). Statistical tools, such as ANOVA and Tukey's multiple comparison test, were used to determine significant differences (p < 0.05).

3. RESULTS AND DISCUSSION

3.1. Lipid fraction of different CG fruit elements (seed, peel and seed/peel waste)

As stated above, CG seeds and seed/peel waste have been reported as promising sources for obtaining edible oil (Ramadan and Mörsel, 2003;

Ramadan, 2012). In turn, the shiny surface of the CG fruit is also associated with the presence of lipid fractions in the peel, so in this study the two oil-containing fruit elements – seeds and peels, were evaluated separately in order to trace their individual contributions. The pulp (juice) was not analyzed alone, as previous studies showed that it contained considerably smaller amounts of oil (0.2% FW) (Ramadan, 2011). Results on the total oil content and the general composition of the oil isolated from the respective fruit elements are presented in Table 1.

As anticipated, the data revealed considerable numerical differences in the lipid fractions of CG fruit elements. The oil content in the peels was about seven times lower than that in the seeds and seed/peel waste; the latter two showing insignificant variation. The content of the oil extracted from CG seeds and seed/peel waste (22.93% and 21.03%, respectively) was higher than that of some common oilseed crops, e.g. corn (3-5%), cotton (16%) and soybean (18%) (Popov and Ilinov, 1986), or some prospective fruit seeds, such as grape seeds (8-20%) (Heuzé and Tran, 2017). The phospholipid concentration in the peel oil was about twice as high as that in the seed/peel oil, and about four times higher than that in seed oil. The contents in phospholipids in the three CG oils were higher than the respective data for sunflower, corn germ and safflower oil (0.4-0.9%) or for soybean oil (1.0-3.0%) (Popov and Ilinov, 1986). The contents in sterols, a known group of biologically active dietary cholesterol-reducing agents, were similar in the three fruit fractions, and higher than many seed oils, such as sunflower, soybean, cotton, safflower, and others (0.24-0.64%) (Popov and Ilinov, 1986; FAO/WHO, 1999). The total amount of biologically active tocopherols in the two seed-containing CG fruit samples (5096 and 5634 mg/kg, respectively) was twice as high as that in the peel oil (2648 mg/kg). These results were comparable to the tocopherol

TABLE 1. Contents in glyceride oil and sterols, tocopherols and phospholipids in Cape gooseberry oil

Index	Seed/peel waste ^a	Peels	Seeds
Oil (%)	21.03 ± 0.20 ^b	3.21 ± 0.03 ^c	22.93 ± 0.21 ^b
Phospholipids (%)	4.38 ± 0.04 ^b	10.72 ± 0.09 ^c	2.69 ± 0.02 ^d
Sterols (%)	1.42 ± 0.01 ^b	1.29 ± 0.01 ^b	1.31 ± 0.01 ^b
Tocopherols (mg/kg)	5634.00 ± 54.00 ^b	2648.00 ± 20.00 ^c	5096.00 ± 50.00 ^d

^a All data are presented as mean value ± standard deviation (from a three-fold repetition, n=3).

^{b-d} Values with different superscripts within a row differed significantly (by Tukey's test at 5% probability).

values for common oils, such as soybean (600-3370 mg/kg), maize (330-3720 mg/kg) and rapeseed (430-2680 mg/kg) (FAO/WHO, 1999).

The FA composition of the analyzed GC oil is presented in Table 2. The results revealed significant differences in the FA composition of CG peel oil compared to the seed and seed/peel oils. The most abundant FAs in the CG peel oil, out of 14 identified, were capric, palmitic and oleic acids. The ratio between saturated (SFA) and unsaturated (UFA) FAs was 67.7:32.3, and the ratio between monounsaturated (MUFA) and polyunsaturated (PUFA) FAs was 23.7:8.6. Linoleic, oleic and palmitic acids were the dominating FAs in the seed and seed/peel waste oils. These results suggested that both CG wastes from juice production could be used as alternative sources of vegetable oils rich in linoleic (63.19-67.89%) and oleic (14.69-16.56%) acids, which are important for the prevention of cardiovascular diseases – as are the seed oils of grapes, melon, tobacco, poppy, and others (Popov and Ilinov, 1986). The ratios of SFA to UFA

were similar in the two oils, 17.7:82.3 and 16.2:83.8, respectively, as well as the PUFA-to-MUFA ratios (63.6:18.7 and 68.3:15.5, respectively).

The comparative analysis of our results and literature data about CG fruit oil revealed that there were some differences in oil yield and FA composition. It should be noted that most of the available studies on CG oil and its FAs were carried out either on whole (intact) fruit, seeds or pomace (the combined seed and peel waste from juice separation), so it was difficult to compare our results for CG peel alone. In the study by Yıldız *et al.*, (2015) the n-hexane extracted oil from whole fruits harvested at Bursa, Turkey, was 0.18% (on a fresh weight basis, FW), while the yield reported by Ramadan and Mörsel, (2003) was 2.0% oil of berry weight, in which seed oil comprised 1.8% and pulp/peel oil, 0.2%. In another study, the n-hexane extracted oil from seed/peel pomace was 19.3% (Ramadan, 2012), which was very close to our results.

In terms of FA profiles, our results differed only numerically from the data provided by previous studies.

TABLE 2. Fatty acid (FA) composition of Cape gooseberry oil

Fatty acids		Contents (% of the oil)		
		Seed/peel waste oil ^a	Peel oil	Seed oil
C _{8:0}	Caprylic	nd ^b	nd	0.11 ± 0.00
C _{10:0}	Capric	0.11 ± 0.00	32.17 ± 0.28	nd
C _{12:0}	Lauric	0.12 ± 0.00	6.21 ± 0.05	nd
C _{14:0}	Myristic	0.11 ± 0.00	1.26 ± 0.01	0.62 ± 0.00
C _{14:1}	Myristoleic	0.09 ± 0.00	0.52 ± 0.00	nd
C _{15:0}	Pentadecanoic	nd	0.44 ± 0.00	nd
C _{16:0}	Palmitic	12.48 ± 0.11	24.51 ± 0.23	11.81 ± 0.10
C _{16:1}	Palmitoleic	0.64 ± 0.00	0.79 ± 0.00	0.78 ± 0.00
C _{17:0}	Margaric	0.18 ± 0.00	0.28 ± 0.00	0.21 ± 0.00
C _{17:1}	Heptadecenoic	1.37 ± 0.01	3.08 ± 0.02	nd
C _{18:0}	Stearic	4.32 ± 0.03	2.52 ± 0.01	3.48 ± 0.03
C _{18:1}	Oleic	16.56 ± 0.15	19.31 ± 0.18	14.69 ± 0.13
C _{18:2} (ω-6)	Linoleic (<i>cis</i>)	63.19 ± 0.60	7.02 ± 0.06	67.89 ± 0.65
C _{18:3} (ω-3)	Linolenic	0.41 ± 0.00	1.56 ± 0.01	0.41 ± 0.00
C _{20:0}	Arachidic	0.42 ± 0.00	0.33 ± 0.00	nd
Saturated FAs		17.74	67.72	16.23
Unsaturated FAs		82.26	32.28	83.77
Monounsaturated FAs		18.66	23.70	15.47
Polyunsaturated FAs		63.60	8.58	68.30

^a All data are presented as mean value ± standard deviation (from a three-fold repetition, n=3). Standard deviation values below 0.01 were equal to ±0.00.

^b nd: not detected.

For example, Rodrigues *et al.*, (2009) identified linoleic acid (72.42%), oleic acid (10.03%) and palmitic acid (9.38%) as the main FAs in the lipid fraction of fruit from Brazil; the SFAs were 12.87% of total FAs. Similar results were reported by Mokhtar *et al.*, (2018) for seed/peel waste powder, in which four FAs were quantified, linoleic (77.78%), oleic (11.32%), palmitic (7.39%), and stearic (3.51%), at a SFA:UFA ratio of 10.9:89.1. In the study by Ramadan and Mörsel, (2003) the contents of the main FAs of CG seed oil were: linoleic (76.1%), oleic (11.7%), palmitic (7.29%), and stearic (2.51%), with a SFA:UFA ratio of 12.8:87.2. The FA profile of the seed/peel extracted oil was also dominated by linoleic (77.1%), oleic (10.3%), palmitic (7.95%), and stearic (2.61%), at a SFA:UFA ratio of 12.8:88.2 (Ramadan, 2012). As it can be seen, the ratio of linoleic to oleic acid in these studies was about 7:1. While our results suggested a lower value, about 4:1; the relative share of UFA was slightly lower in this study, too (SFA:UFA ratios of 17.7:82.3 and 16.2:83.8, for seed/peel and seed oils). Nevertheless, the results from this study supported the evaluation of CG fruit oil as especially suitable for nutritional application, due to its high proportions of PUFAs.

The individual sterol composition of the oils is presented in Table 3. The results revealed that the total sterol content in the unsaponifiable fraction

was comparable in seed and seed/peel oils (33.33-44.59%), but that it was considerably lower in the peel oil (2.14%). The total amount of unsaponifiables was in a reversed order, i.e. higher in the peel oil (61.33%) compared to seed and seed/peel oils (3.02-4.21%). With a share of 80.23% in the total sterol content, β -sitosterol was the dominating sterol in the oil of CG peels, while the other two oils contained campesterol, Δ^5 -avenasterol and β -sitosterol as their main sterols (constituting 67.65% and 77.80% of the sterol fraction in seed and seed/peel oil, respectively). These results were very close to the findings by Ramadan, (2012), who also reported high levels of unsaponifiables in CG pomace oil (22.0 g/kg), and campesterol (4.70 g/kg), Δ^5 -avenasterol (2.63 g/kg) and lanosterol (1.60 g/kg) as responsible for about 75% of total sterols.

The results from the analysis of the individual tocopherol composition of CG oil are presented in Table 4. Four tocopherols were identified in each of the oils, and the data revealed that γ -tocopherol was noticeably the dominating structure in the peel oil, while β -tocopherol, δ -tocopherol and γ -tocopherol had similar individual shares (31.40-34.15%) in both seed and seed/peel waste oils. As tocopherols are known to be potent antioxidants, it can be suggested that the high amount of tocopherols in the studied CG oils, and especially the high γ -tocopherol levels,

TABLE 3. Sterol composition of Cape gooseberry oil

Sterols (% of total)	Seed/peel waste oil ^a	Peel oil	Seed oil
Cholesterol	1.39 ± 0.01 ^b	2.52 ± 0.02 ^c	0.31 ± 0.00 ^d
Ergosterol	nd ^e	nd	1.62 ± 0.01
Campesterol	57.23 ± 0.56 ^b	5.56 ± 0.05 ^c	22.56 ± 0.21 ^d
Stigmasterol	6.39 ± 0.06 ^b	0.47 ± 0.00 ^c	9.03 ± 0.08 ^d
Δ^7 -Campesterol	8.74 ± 0.08 ^b	6.45 ± 0.06 ^c	nd
β -Sitosterol	9.36 ± 0.09 ^b	80.23 ± 0.79 ^c	18.32 ± 0.17 ^d
Lanosterol	nd	nd	2.11 ± 0.01
Δ^5 -Avenasterol	11.21 ± 0.10 ^b	1.52 ± 0.01 ^c	26.77 ± 0.25 ^d
$\Delta^{7,25}$ -Stigmastadienol	0.89 ± 0.00 ^b	0.44 ± 0.00 ^b	nd
Δ^7 -Stigmasterol	3.92 ± 0.03 ^b	2.08 ± 0.01 ^c	14.72 ± 0.14 ^d
Δ^7 -Avenasterol	0.87 ± 0.00 ^b	0.73 ± 0.00 ^b	4.56 ± 0.04 ^c
Total sterols in the unsaponifiable fraction (%)	33.33	2.14	44.59
Unsaponifiables (% in the oil)	4.21	61.33	3.02

^a All data are presented as mean value ± standard deviation (from a three-fold repetition, n=3). Standard deviation values below 0.01 were equal to ±0.00.

^{b-d} Values with different superscripts within a row differed significantly (by Tukey's test at 5% probability).

^e nd: not detected.

TABLE 4. Tocopherol composition of Cape gooseberry oil

Tocopherols (% of total)	Seed/peel waste oil ^a	Peel oil	Seed oil
α -Tocopherol	1.72 \pm 0.01 ^b	19.30 \pm 0.18 ^c	1.14 \pm 0.01 ^b
β -Tocopherol	33.04 \pm 0.30 ^b	nd ^d	34.15 \pm 0.33 ^b
γ -Tocopherol	31.46 \pm 0.30 ^b	72.78 \pm 0.70 ^c	31.40 \pm 0.30 ^b
γ -Tocotrienol	nd	3.59 \pm 0.03	nd
δ -Tocopherol	33.78 \pm 0.31 ^b	4.33 \pm 0.04 ^c	33.31 \pm 0.32 ^b

^a All data are presented as mean value \pm standard deviation (from a three-fold repetition, n=3).

^{b-c} Values with different superscripts within a row differed significantly (by Tukey's test at 5% probability).

^d nd: not detected.

would be a preventive factor against lipid oxidation processes during the storage of oil and oil-containing products.

These results were in compliance with the findings by Ramadan and Mörsel, (2003), who also identified γ - and α -tocopherols as the main constituents in the pulp/peel oil of CG fruit (45.5 mg/kg and 28.3 mg/kg, respectively), and β -tocopherol and γ -tocopherol in the seed oil. In another study (Ramadan, 2012), β -tocopherol comprised 47% of the tocopherol fraction in the CG seed/pulp oil (2.10 g/kg), γ -tocopherol was 26% (1.08 g/kg), δ -tocopherol 18.5% (0.85 g/kg), and α -tocopherol about 6% (0.34 g/kg). There were no sufficient data in the literature about the tocopherol content in CG fruit peel alone, so it was difficult to make a more detailed comparison of our results with previous findings.

3.2. Minerals, fiber, protein, and amino acids in CG waste

As already stated, the two by-products obtained from CG fruit, seed cakes and seed/peel waste, represent raw materials which are underutilized, but valuable in

terms of both quantity and functionality. In this study, the peels were found to constitute 11.4% of fresh fruit weight, and the seeds 13.2%. For example, the combined seed/peel fraction made up nearly a quarter of the fruit weight. Our results were very close to the findings by Ramadan, (2011) for CG seed/peel pomace at 27.4% of fruit weight. On the other hand, CG waste fractions were previously indicated as potential sources of nutrients, e.g. vitamins, minerals, carbohydrates, protein, etc. (Ramadan *et al.*, 2008; Ramadan, 2011, 2012). On these grounds, the two waste products from fruit processing in this study were considered worthy of further analysis in order to determine the contents in some components with nutritional value, e.g. minerals, fiber, protein, and amino acids.

3.2.1. Mineral elements in seed cakes and seed/peel waste

The data presented in Table 5 revealed that CG seed cakes and seed/peel waste were a source of minerals, with some differences in both macro- and microelement concentrations between the two waste products. K and Mg dominated in the group of macroelements, and Zn, Cu and Fe in the group

TABLE 5. Mineral elements (mg/kg) in Cape gooseberry seed/peel waste and seed cakes

Fruit sample	Mineral elements (mg/kg)										
	K	Na	Ca	Mg	Fe	Mn	Cu	Zn	Pb	Cd	Cr
Seed/peel waste	4527.00 \pm 12.31 ^a	112.63 \pm 1.09	<0.10 ^b	1750.00 \pm 8.22	42.85 \pm 0.39	17.77 \pm 0.09	10.71 \pm 0.08	34.65 \pm 0.12	<0.10	<0.01 ^c	<0.10
Seed cake	3911.00 \pm 11.78	124.44 \pm 1.06	<0.10	2095.00 \pm 8.67	52.36 \pm 0.48	24.44 \pm 0.11	114.62 \pm 0.81	130.60 \pm 0.39	<0.10	<0.01	0.75 \pm 0.02

^a All data are presented as mean value \pm standard deviation (from a three-fold repetition, n=3).

^b Not quantified.

^c Not detected.

of microelements. These results supported the assumption that CG waste products have possible use as ingredients in functional foods or as additives in forage mixtures for animal nutrition, as they are carriers of macro- and microelements with nutritional value. There were some interesting variations in the distribution of minerals between the two waste products. For example, Cu and Zn were in considerably lower amounts in the seed/peel waste compared to the seed cakes, but the opposite situation was observed for K (Table 5). This variance could be attributed to the different mobility and accumulation of those minerals in the different fruit parts (pulp, peel, seeds). Therefore, the differences were conditioned by the nature of the two analyzed CG waste samples in the study, reflecting the presence of peels in the seed/peel waste (and partly juice, as the waste was not additionally rinsed). Such an assumption is in compliance with the observations by Morais *et al.*, (2017) for the distribution of K, Cu and Zn among the seeds, pulp and peels of fruits like papaya, passion fruit, watermelon, and melon. K had the highest contents in the peels and pulps compared to the seeds, while the microminerals tended to be higher in the seeds.

The results from the study were in full compliance with previous findings about high K contents in CG, and exceeded those found in many other fruits (Olivares-Tenorio *et al.*, 2016; Mayorga *et al.*, 2001; Rodrigues *et al.*, 2009; Ozturk *et al.*, 2017; Zhang *et al.*, 2013; Mokhtar *et al.*, 2018). This was a certain asset of the studied CG fruit wastes, as K represents an intracellular element involved in neural and muscle electrochemical processes and in the acid-base regulation in the body. The levels of Na and Mg were higher than the results achieved by Rodrigues *et al.*, (2009) (Na - 1.1 mg/100g DW, Mg - 34.7 mg/100g whole fruit), Ozturk *et al.*, (2017) (Mg - 102.5 mg/100 g), Eken *et al.*, (2014) (Mg - 145 mg/100g fruit) or Leterme *et al.*, (2006) (Mg - 19 mg/100 g, Na - 6 mg/100 g fruit pulp). Ca was practically absent, similar to the previously observed low levels of this macromineral in CG fruit (values between 7.0 and 43.65 mg/100 g in whole fruit or fruit pulp) (Leterme *et al.*, 2006; Rodrigues *et al.*, 2009; Puente *et al.*, 2011; Zhang *et al.*, 2013; Eken *et al.*, 2014; Olivares-Tenorio *et al.*, 2016; Ozturk *et al.*, 2017). In general, the contents of microelements registered in this study, Fe, Zn, Mn, and Cu, were

higher than the data reported by most of the above mentioned authors (Rodrigues *et al.*, 2009; Puente *et al.*, 2011; Olivares-Tenorio *et al.*, 2016), although some variation also existed. The contents of Fe and Zn were lower than those reported by Eken *et al.*, (2014) for whole fruit (Fe - 36 mg/100 g fruit, Zn - 11.4 mg/100 g fruit). Our results varied from the data for CG seed/peel waste analyzed by Mokhtar *et al.*, (2018), regarding Fe (13.04 mg/100 g), Zn (0.88 mg/100 g), and Mn (0.67 mg/100 g). The heavy metals Pb, Cd and Cr were practically undetected in CG seed cakes and seed/peel waste, similar to the findings by Rodrigues *et al.*, (2009) and Eken *et al.*, (2014). All those numerical variations could be explained by the influence of fruit origin (production conditions) and sample preparation (the analyzed fruit fraction).

The results confirmed the studied CG waste products to be a potent source of macro- and microminerals for human and animal nutrition, which might be of practical importance in the development of functional foods and feeds.

3.2.2. Amino acids, protein and fiber in seed cakes

CG seed cakes had a protein content of $24.32 \pm 0.22\%$ DW, total nitrogen content of $3.89 \pm 0.03\%$, DW and cellulose contents (crude fiber) of $42.94 \pm 0.31\%$ DW. Thus, CG seed cakes could be considered a relatively rich source of protein, similar to linseeds (20-28% DW) (Heuzé *et al.*, 2015b) and rapeseeds (17-24% DW) (Heuzé *et al.*, 2019), and richer than grape seeds (9-11%) (Heuzé and Tran, 2017), sunflower and safflower (about 17-17.5%) (Heuzé *et al.*, 2015a, 2016). In turn, the cellulose content was also significantly high, approximating that of seed cakes from safflower (30-40%), sunflower (27-31%) or grape seeds (over 33%) (Heuzé *et al.*, 2015a, 2016; Heuzé and Tran, 2017).

The amino acid composition of CG seed cake is presented in Table 6. Surprisingly, lysine (27.22 mg/g) was the second most abundant amino acid, next to aspartic acid (32.11 mg/g). Relatively high levels of alanine (17.96 mg/g), arginine (14.67 mg/g), histidine (12.32 mg/g), and threonine (12.30 mg/g) were also found. Our results suggested an amino acid profile of CG seed cakes which differed from the data by Mokhtar *et al.*, (2018), who found glutamic acid (18.09 g/100 g protein), arginine (11.57 g/100 g protein), aspartic acid (7.82 g/100 g protein), and leucine (5.87 g/100

TABLE 6. Amino acids in Cape gooseberry seed cakes

Amino acids	Content (mg/g)
Cysteine	1.55 ± 0.01 ^a
Methionine	2.30 ± 0.02
Valine	8.73 ± 0.07
Isoleucine	11.43 ± 0.09
Leucine	2.13 ± 0.02
Lysine	27.22 ± 0.22
Histidine	12.23 ± 0.11
Threonine	12.30 ± 0.11
Tyrosine	9.26 ± 0.09
Phenylalanine	10.50 ± 0.08
Aspartic acid	32.11 ± 0.23
Serine	11.90 ± 0.09
Glutamic acid	7.65 ± 0.08
Glycine	2.46 ± 0.02
Arginine	14.67 ± 0.12
Alanine	17.96 ± 0.14
Proline	7.17 ± 0.05

^a All data are presented as mean value ± standard deviation (from a three-fold repetition, n=3).

g protein) as the dominant amino acids in dehydrated CG waste powder. Further comparisons to previous data were hard to make, as there were no detailed records of CG amino acids or protein quality (Puente *et al.*, 2011). The dominant share of lysine in CG seed cake was an interesting finding, as its low levels (limiting amino acid) are an issue which is common to many oil seed cakes, such as sunflower, safflower and others. Regarding the potential use in livestock feed, it should be noted that the levels of the two other limiting amino acids (beside lysine) in pig and poultry nutrition, methionine and cysteine, were relatively low, suggesting that CG seed cakes must be carefully combined with other animal feed ingredients.

4. CONCLUSIONS

The results from the study demonstrated that the seeds and peels of CG fruit, a waste from juice production, could be a valuable source of functional nutrients for human and animal nutrition. The yield of edible oil from CG seed and seed/peel fractions was sufficiently high, over 21%, making oil extraction feasible. The composition of the analyzed oils was favorable, too, as they were rich in bioactive compounds (such as campesterol, Δ^5 -

avenasterol, β -sitosterol, β -, δ - and γ -tocopherols) and unsaturated FAs (over 82% of the oil, mainly linoleic and oleic). The further valorization of the obtained CG wastes (seed cakes, seed/peel pomace) revealed them to be fully fit for incorporation in food and feed products. The studied wastes contained high levels of important macro- and microminerals (K, Mg, Na, Zn, Cu, Fe), they were rich in protein (about 24% in seed cakes), cellulose (about 42%) and essential AAs (especially lysine, threonine and histidine).

This study provides new data on Cape gooseberry (*P. peruviana* L.) composition, from the perspective of the contribution of the different parts of the fruit and their potential uses. Thus, the outcomes from the study might be of practical relevance in the development of functional foods and feeds, as they add new details to the existing knowledge about CG fruit. They might also serve as grounds for the development of more efficient approaches to the circular processing of CG fruit worldwide.

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