JMBFS

Journal of Microbiology, Biotechnology and Food Sciences

International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

EXPLORING THE POTENTIAL OF SPENT COFFEE GROUNDS 100 % *COFFEA ARABICA* REGARDING CHEMICAL COMPOUNDS

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https://doi.org/10.55251/jmbfs.12112

ARTICLE INFO

Received 28. 11. 2024
Revised 29. 5. 2025
Accepted 8. 12. 2025
Published 1. 2. 2026

Regular article

OPEN ACCESS

ABSTRACT

Worldwide coffee consumption continues to grow, resulting in the production of co-products generated during its processing. One of these co-products is spent coffee grounds (SCG), which was the subject of our research, focusing on the quantitative determination and evaluation of selected physicochemical parameters in coffee samples and the SCG derived from them. The samples consisted of 100 % *C. arabica* from Peru, Kenya, Colombia, Cuba, and Vietnam, roasted to a medium level. First, we analyzed 5 extract samples prepared from roasted coffee beans, and subsequently 5 extract samples of SCG obtained from these beans. The analysis included determining pH, dry matter content, total antioxidant capacity (TAC), total polyphenol content (TPC), caffeine, and chlorogenic acids. The pH values measured in coffee beans ranged from 4.733 to 5.113, and in SCG from 4.808 to 5.138, with both being slightly acidic. The highest dry matter content was observed in sample 5B, at 98.280 %, while the lowest was in 4G, at 93.030 %, related to the combination of temperature and time applied during the SCG drying process. The percentage inhibition of DPPH showed the lowest value in coffee bean sample 4B (81.914 %), while the highest was found in SCG sample 2G (88.021 %). In contrast, the total polyphenol content (TPC) in the coffee beans samples was higher (40.024 to 51.074 g GAE. kg^{-1} (GAE – gallic acid equivalent)) compared to the SCG samples (6.985 to 14.569 g GAE. kg^{-1}). The highest caffeine content was found in coffee bean sample 5B (9.826 $mg.g^{-1}$). In the case of SCG, the average caffeine content decreased due to leaching into the coffee extract, with the highest value recorded in sample 4G at 3.438 $mg.g^{-1}$. The average chlorogenic acids (CGAs) content in coffee beans ranged from 20.745 to 25.741 $mg.g^{-1}$, and in SCG from 6.153 to 10.867 $mg.g^{-1}$. Our results suggest that SCG represent a natural source of bioactive compounds that could be reused, thereby contribute to waste minimization disposal and environmental impact.

Keywords: coffee co- product, caffeine, chlorogenic acids, polyphenols

INTRODUCTION

The coffee industry is one of the largest food industries in the world. The high demand for coffee beans leads to the production of excessive amounts of co-products throughout all stages of its processing **(Oliveira *et al.*, 2021)**. The coffee industry produces more than 2 billion tons of coffee co-products annually. The global amount of SCG is estimated to be 6 million tons per year **(Janissen and Huynh, 2018)**.

Spent coffee grounds (SCG) represent roasted and ground coffee beans that have been depleted of certain water-soluble compounds they previously contained **(Franca and Oliveira, 2022)**. SCG is generated during the extraction of coffee beans with hot water (coffee preparation) as well as in the production of instant coffee **(Klingel *et al.*, 2020; Poláková *et al.*, 2024)**. It is a raw material rich in macronutrients. Recent studies have shown that the most abundant components of SCG are polysaccharides such as hemicellulose and cellulose (45 %), as well as proteins and lipids **(Yusufoğlu *et al.*, 2024)**. The oil obtained from SCG consists mainly of linoleic and palmitic acids, as well as diterpenes, specifically kahweol, cafestol, and 16-O-methylcafestol **(Klingel *et al.*, 2020)**. The primary group of phenolic compounds found in SCG are chlorogenic acids (CGAs) and their derivatives, such as 5-CQA, 4-CQA, 3-CQA, and also caffeic acid **(Oliveira *et al.*, 2021)**. Caffeine, the most represented methylxanthine compound, constitutes 1-2 % of dried SCG. Due to caffeine's good water solubility, its content in SCG is lower than in roasted coffee. Both caffeine and CGAs offer notable health benefits associated with their biological properties, including strong antioxidant capacity, which provides protection against damage caused by free radicals and oxidative stress **(Klingel *et al.*, 2020)**. Caffeine is the main alkaloid present in SCG **(Saud and Salamatullah, 2021)**. It is a bioactive compound that is thermally stable and acts as a stimulant of the central nervous system (CNS) by blocking adenosine receptors **(Awwad *et al.*, 2021)**. It is associated with a reduced risk of type 2 diabetes and neurodegenerative diseases such as Alzheimer's and Parkinson's **(Saud and Salamatullah, 2021)**. According to the European Food Safety Authority (EFSA), daily caffeine intake for healthy adults should not exceed 400 mg **(Olechno *et al.*, 2021)**. Excessive caffeine intake can lead to adverse effects such as dependence, depression, insomnia, headaches, psychomotor agitation, and cardiovascular diseases **(Girma *et al.*, 2020; Cavanagh *et al.*, 2023)**.

SCG also contains other bioactive compounds such as trigonelline, lignin, and melanoidins **(Balzano *et al.*, 2020)**. It also contains minerals in smaller quantities (less than 1 %). Micronutrients include folic acid and vitamins E and B **(Yusufoğlu *et al.*, 2024)**. **Hechmi *et al.* (2023)** reported the presence of calcium (Ca) and magnesium (Mg). It is known that SCG contains fiber, which constitutes 19.7 - 22.1 % **(Balzano *et al.*, 2020)**. Depending on the conditions of coffee extraction and the composition of roasted coffee beans, deviations in the chemical composition of SCG may occur **(Oliveira *et al.*, 2021)**.

SCG also contains antinutritional substances, such as acrylamide and hydroxymethylfurfural, about 70 % - 80 % of which are transferred into the coffee. In humans, they are metabolized into corresponding carboxylic acids and are almost completely excreted by the kidneys **(Klingel *et al.*, 2020)**. Since SCG is prone to contamination by filamentous fungi, **Iriondo-DeHond *et al.* (2019)** investigated the presence of mycotoxins in it. They did not detect aflatoxin B1 but found ochratoxin A.

Due to the high content of organic matter, which requires a large amount of oxygen for decomposition, the disposal of SCG presents a significant environmental issue **(Franca and Oliveira, 2022)**. This process results in the release of carbon dioxide and other greenhouse gases, as well as the release of chemical compounds into the environment **(Crossley *et al.*, 2020)**. Additionally, burning this waste is hazardous. With the worsening effects of climate change on the environment, international organizations are increasingly encouraging the valorization of materials previously considered waste **(Mata *et al.*, 2018*)***. Despite SCG's nutritional value, it is unlikely to become a significant source of nutrients in our diet. However, it has potential for use in various industries **(Yusufoğlu *et al.*, 2024; Poláková *et al.*, 2023)**. SCG is gaining increasing recognition in the production of bioethanol, biodiesel, animal feed, fertilizers, and wastewater treatment (e.g., reducing cationic dyes due to its adsorption properties). These applications contribute to the circular economy and sustainability efforts **(Benincá *et al.*, 2023)**. Many recent studies focus on finding alternative uses for SCG **(Franca and Oliveira, 2022)**. Processing SCG could be a promising approach for developing value-added food products **(Benincá *et al.*,**

2023). **Iriondo-DeHond *et al.* (2019)** propose using the chemical profile and antioxidant activity of SCG as an ingredient in healthy food products. Antioxidant compounds such as polyphenols, caffeine, and melanoidins found in SCG's aqueous extract can act as antimicrobial agents and extend food shelf life **(Campos-Vega *et al.*, 2015)**.

MATERIAL AND METHODS

In this research, 10 samples were examined, consisting of 5 extracts of 100 % *C. arabica* and their corresponding 5 extracts of spent coffee grounds (SCG). For better clarity, the coffee samples were labeled with the letter "B" (bean), and the SCG samples with the letter "G" (grounds). Detail description of samples is provided in Table 1.

Table 1 List of analyzed samples

Samples	**1 B** **1 G**	**2 B** **2 G**	**3 B** **3 G**	**4 B** **4 G**	**5 B** **5 G**
Botanical origin	100 % C. *arabica*				
Country of origin	Peru	Kenya	Colombia	Cuba	Vietnam
Variety	Typica	SL 34	Bourbon	Bourbon	Typica
Altitude	1900 mamsl	1800 mamsl	1750 - 1900 mamsl	900 mamsl	1000 - 1600 mamsl
Processing	Wet				
Roast level	Medium light	Medium	Medium light	Medium light	Medium

Notes: Samples: B – roasted coffee beans, G – spent coffee grounds; mamsl - meters above mean sea level

Methodology

Extract preparation of samples

Prior the analysis, coffee samples were homogenized using electrical grinder (Grindomix GM 200, Retsch, Haan, Germany) at a rotational speed of 10,000 rpm for a duration of 60 seconds. Subsequently, 7 g from each sample was weighed using a laboratory scale (Kern, PCB 1000-1, Kern&Sohn GmbH, Germany) and poured with 120 mL of hot distilled water heated to 98 °C. Extraction time was 5 minutes with occasional stirring. Then were samples filtered through Sartorius filter paper (Grade 390, Germany) to obtain extract for the analysis. Remained SCG were transferred to drying containers and dried at a temperature of 110 °C for 5 hours **(Franca *et al.*, 2009)**. After the drying process, the SCG extracts were prepared in the manner described above.

Determination of pH

The pH of the samples was measured using a pH meter (SI Analytics Lab 845, Germany) after cooling to a standardized temperature of 25 °C. Hamilton Buffer Solutions (Hamilton Bonaduz AG, Bonaduz, Switzerland) with pH values of 4.01, 7.00, and 10.01 were used to calibrate the pH meter.

Determination of dry matter content

The dry matter content was determined using Kern DAB 100-3 analytical scales (Kern&Sohn GmbH, Balingen, Germany) at a temperature of 110 °C until the sample weight stabilized. The results are expressed as a percentage of dry matter.

Determination of total polyphenolic content (TPC) by modified method using Folin-Ciocalteu reagent

The basis of the chosen method is the reaction of the Folin-Ciocalteu reagent with polyphenols present in the sample, leading to the formation of a blue complex. The intensity of the color of this complex depends on the amount of polyphenols present **(Raposo *et al.*, 2024)**. In preparing the samples for measurement, a specified volume of distilled water was pipetted into 50 mL volumetric flasks. Subsequently, 0.05 mL of the sample and 2.5 mL of diluted Folin-Ciocalteu reagent solution (1:2) (Sigma-Aldrich, Switzerland) were added. After a three-minutes, 5 mL of a 20 % aqueous Na_2CO_3 solution (Centralchem, Bratislava, Slovakia) was added and thoroughly mixed. The volume was made up to the mark with distilled water to a volume of 50 mL. The prepared solutions were allowed to stand for 2 hours. The absorbance of the solutions was measured using a double-beam UV/VIS spectrophotometer (T-80 UV/VIS; PG Instruments Ltd; Czech Republic) at a wavelength of 765 nm. The measurement was repeated four times for each sample. The TPC results were expressed as gallic acid equivalents in g GAE. kg^{-1} of dry matter.

Determination of total antioxidant capacity (TAC) spectrophotometrically using the DPPH method according to Brand-Williams

We assessed the total antioxidant capacity (TAC) following the method by **Brand-Williams *et al.* (1995)**, with modifications by **Bobková *et al.* (2020)**, based on the principle of free radical scavenging. This approach involves the conversion of the DPPH radical (2,2-diphenyl-1-picrylhydrazyl) in solution to a stable form due to the action of antioxidants in the sample, resulting in decolorization of the radical in the cuvette. To measure the TAC in the samples, a stock solution was prepared by dissolving 0.025 g of DPPH (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) in 100 mL of ethanol (Centralchem, Bratislava, Slovakia). A working solution was then made by diluting the stock solution with ethanol in a 1:9 ratio. Absorbance was measured using a Biotek Epoch 2 Microplate UV/VIS spectrophotometer (Winooski, Vermont) at a wavelength of 515.6 nm, the absorption peak of the DPPH solution. For each sample's absorbance measurement, 3.9 mL of the diluted DPPH solution was pipetted into a cuvette, and the initial absorbance value (A_0) was recorded. Then, 0.1 mL of the sample extract was added and thoroughly mixed using a glass stirrer. After 10 minutes, the absorbance (At) was measured again. The antioxidant activity of each extract was expressed as the percentage inhibition of the DPPH radical. We determined the antioxidant activity of individual extracts as the percentage of inhibition:

$$\%\ inhibition\ DPPH = \frac{((A_0 - A_s) - (A_t - A_s))}{(A_0 - A_s)}\ x\ 100$$

where:

A_0 – initial absorbance at time t = 0 minutes
At – absorbance at retention time t = 10 minutes
As – absorbance of the blank (ethanol)

Qualitative and quantitative determination of caffeine and chlorogenic acids in samples using HPLC

For the analysis of caffeine and chlorogenic acid content, HPLC analysis was performed according to the methodology described by **Bobková *et al.* (2021).** The HPLC-DAD chromatograph Agilent Infinity 1260 (Agilent Tech. GmbH, Germany) was used. Before HPLC analysis, pre-filtered coffee extracts and coffee grounds were re-filtered through a Frisenette syringe microfilter (25 mm, 0.45 µm; Q-Max, Syringe Filters, Germany), and the resulting filtrate was transferred to HPLC vials for analysis. Separation was performed on a C-18 Poroshell 120 column (150 mm × 3 mm × 2.7 µm; Agilent Technologies, Waldbronn, Germany). The mobile phases used were acetonitrile (A) and 0.1 % H_3PO_4 in ddH_2O (v/v) (B). The gradient elution was programmed as follows: 0 - 1 min isocratic elution with 20 % A + 80 % B, followed by linear gradient elution: 1 - 5 min with 25 % A + 75 % B, 5 - 15 min with 30 % A + 70 % B, and 15 - 25 min with 40 % A + 60 % B. A 3-minute equilibration time was set prior to each injection. The mobile phase flow rate was 1 mL/min, the injection volume was 10 µL, and the separation temperature was maintained at 30 °C. Samples were stored at 4 °C prior to analysis. Detection was carried out at 276 nm for caffeine and 320 nm for chlorogenic acids, with the full wavelength range for data collection set to 210 - 400 nm. The caffeine and CGA content values in the analyzed samples were expressed in $mg.g^{-1}$ of dry matter. In our analyses, we determined CGAs content as the sum of 3-CQA, 4-CQA, 5-CQA, 3,5-diCQA, and 4,5-diCQA. Further referred to as CQAs.

Statistical analysis

Statistical analysis was done using Microsoft Office Excel 365 for iOS and Addinsoft 2022, XLSTAT statistical and data analysis solution, New York, USA and subsequently evaluated using descriptive statistical indicators (arithmetic mean, minimum, maximum). Differences between the values of the analyzed compounds in roasted coffee beans and spent coffee grounds (SCG) were calculated using the analysis of variance (ANOVA) (one-way ANOVA) with the Duncan test, REGWQ test, and Tukey range test at a significance level of $\alpha = 0.05$. The correlation between TAC, TPC, CQAs, and caffeine values was determined using Pearson's correlation test, separately for roasted coffee beans and SCG.

RESULTS AND DISCUSSION

The values of dry matter content and pH in roasted coffee beans and SCG are presented in Table 2.

Table 2 Average Values of pH and Dry Matter Content

Sample	pH	dry matter %
1B	4.900 ^{f}	98.233 ^{b}
2B	4.733 ^{h}	98.040 ^{d}
3B	4.825 ^{g}	97.760 ^{e}
4B	5.113 ^{b}	98.170 ^{c}
5B	4.958 ^{d}	98.280 ^{a}
1G	4.933 ^{e}	93.180 ^{i}
2G	4.808 ^{g}	93.500 ^{h}
3G	5.015 ^{c}	93.650 ^{g}
4G	5.138 ^{a}	93.030 ^{j}
5G	5.018 ^{c}	93.873 ^{f}
Significant	Yes	Yes

Notes: a, b, c, d, e, f, g, h, i, j = groups within a column with different superscripts differ significantly at $p \leq 0.05$; ANOVA Duncan test

The pH values measured in our samples range from 4.733 in coffee beans sample 2 B (Kenya) to 5.138 in SCG sample 4 G (Cuba). The pH values we measured in coffee beans were like those in SCG, and in some cases, they were significantly higher. We can conclude that the pH was mildly acidic in all samples. Similar pH values were reported by **Laukalçja *et al.* (2022)** for medium-roasted *C. arabica* coffee (Colombia), specifically 5.170. **Kim, M. S. and Kim, J. G. (2020)** analyzed the pH of SCG solutions and determined an average pH of 5.2, which is consistent with our findings. **Lee *et al.* (2022)** identified slightly higher pH values in their study of SCG samples, reporting an average pH value of 5.411. Similar results were reported by **Mussatto *et al.* (2011)**, who suggest applying SCG to soil because it is acidic and can lower the pH. **Belviso *et al.* (2014)** report that fresh SCG shows an average pH value of 5.92, which is higher than the average pH they measured in roasted coffee, which was 5.46. Based on our results, we tend to agree with this assertion.

Based on the ANOVA, we found significant differences between the coffee beans and SCG samples at a significance level of $\alpha = 0.05$ (Table 2).

Arulrajah *et al.* (2014) state that SCG are biomass residues with a high moisture content due to the addition of hot water during coffee preparation. Moisture is an important attribute and an indicator of quality for coffee beans and SCG **(Suleiman *et al.*, 2018)**. Drying is a common process used for their preservation. The dry matter content in the final product should be more than 90 % to ensure safe storage and to prevent microbial growth, particularly filamentous fungi **(Arulrajah *et al.*, 2014)**.

The dry matter content of SCG (93.030 – 93.873 %) is significantly lower compared to coffee beans (97.760 – 98.280 %). This is due to the combination of time and temperature during the drying process of SCG we chose (110 °C for 5 hours). **Lee *et al.* (2022)** indicate that with increasing temperature and drying time of SCG, moisture decreases and the proportion of dry matter increases. **Tun *et al.* (2020)** employed various drying methods (air drying, solar drying, and oven drying) and reached the following conclusions: The results show that the moisture content in SCG decreased from 65 % to 37 % with air drying over approximately 10 hours. With solar drying, the moisture in SCG decreased to 10 % within 10 hours. SCG dried the most in the oven, where their moisture content dropped to 7 % within 6 hours (93 % dry matter), which aligns with our values.

By mutually comparing coffee bean and SCG samples using ANOVA, we found statistically significant differences in selected parameters such as TPC, TAC, caffeine, and CQAs (Table 3).

Table 3 ANOVA of chemical compounds (Duncan test, REGWQ test and Tukey range test)

Sample	TPC	TAC	Caffeine	CQAs
1B	50.104 ^{h}	84.764 ^{abc}	7.620 ^{d}	20.745 ^{f}
2B	51.074 ^{h}	82.340 ^{ab}	8.283 ^{e}	23.969 ^{h}
3B	40.024 ^{e}	87.713 ^{c}	8.677 ^{f}	25.505 ^{i}
4B	41.290 ^{f}	81.914 ^{a}	9.397 ^{g}	25.741 ^{i}
5B	43.317 ^{g}	84.390 ^{abc}	9.826 ^{h}	21.118 ^{g}
1G	7.766 ^{a}	84.544 ^{abc}	3.038 ^{a}	9.025 ^{b}
2G	14.569 ^{d}	88.021 ^{c}	3.288 ^{b}	10.867 ^{e}
3G	11.349 ^{c}	86.270 ^{bc}	3.318 ^{b}	10.368 ^{d}
4G	9.732 ^{b}	82.708 ^{ab}	3.438 ^{c}	9.876 ^{c}
5G	6.985 ^{a}	82.979 ^{ab}	3.046 ^{a}	6.153 ^{a}
Significant	Yes	Yes	Yes	Yes

Notes: a, b, c, d, e, f, g, h, i, j = groups within a column with different superscripts differ significantly at $p \leq 0.05$; ANOVA Duncan test; TPC - total polyphenol content (g GAE.100 g^{-1}); TAC - DPPH total antioxidant capacity (%); chlorogenic acids and caffeine (mg.g^{-1}).

In the analysis of chemical compounds, we determined the total polyphenol content (TPC) in our samples. Table 3 shows that the coffee beans sample 2B had the highest polyphenol content (51.074 g GAE.kg^{-1}). Similar findings were presented by **Duangjai *et al.* (2021)**. Authors reported an average TPC of 50.39 g GAE.kg^{-1} in medium roasted coffee samples after 5 minutes of brewing. Moreover, **Bobková *et al.* (2020)**, obtained similar concentrations of TPC in medium roasted coffee samples, ranging from 43.90 to 56.06 g GAE.kg^{-1}. However, authors further stated that these values can vary based on roasting conditions and coffee origin. **Endeshaw and Belay (2020)** present that the content of phenolic compounds in coffee depends on the variety, roasting degree, and preparation method. **Lapčíková *et al.* (2023)** monitored TPC in five varieties of *C. arabica* prepared by various methods (cold brew, espresso, French press, and aeropress). They found that the most effective extraction method for this parameter is cold brew coffee, with an average TPC value ranging from 36.89 to 60.06 g GAE.kg^{-1}, and espresso with an average TPC value of 44.41 g GAE.kg^{-1}. The lowest polyphenol content was measured in SCG sample 5G (6.985 g GAE.kg^{-1}). From the values presented in Table 3, it is evident that the TPC in SCG is lower compared to the TPC in coffee beans samples. **Ballesteros *et al.* (2017)** indicate that the main reason is the sensitivity of phenolic compounds to oxidative environments (e.g., temperature, light, oxygen, moisture). Similar findings are presented by **Brezová *et al.* (2009)**. They established TPC values in Arabica and Robusta coffee beans samples ranging from 41 to 58 g GAE.kg^{-1}. Authors observed that after coffee preparation, the TPC of fresh SCG decreased to 25.17 g GAE.kg^{-1}. **Mussatto *et al.* (2011)** indicated that the TPC values in SCG are comparable to other important sources of antioxidants, such as ripe raspberries (12.0 – 15.3 g GAE.kg^{-1} dry matter), blackberries (12.1 – 14.8 g GAE.kg^{-1} dry matter), and almond shells (22.0 g GAE.kg^{-1} dry matter). In the food industry, natural polyphenols can be used as substitutes for synthetic antioxidants, such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and tertiary butylhydroquinone (TBHQ), which are reported to pose health risks **(Samarin *et al.*, 2012)**.

There are statistically significant differences in the TPC parameter between coffee beans samples and SCG.

Antioxidants are substances necessary for neutralizing free radicals **(Yust *et al.*, 2024)**. **Seninde and Chambers (2020)** identify coffee as a primary source of antioxidant intake, followed by fruits and vegetables. The average values of total antioxidant capacity (TAC), specifically the inhibition of DPPH radicals, in the analyzed samples ranged from 81.914 % (sample 4B) to 88.021 % (sample 2G). Our findings suggest that there are no significant differences (Table 3) in the TAC parameter between some SCG and coffee beans samples. In some cases, SCG samples even exhibit higher TAC values than the coffee beans samples. This may be due to the subsequent drying process, during which some compounds likely degraded and new compounds were formed, which also exhibit antioxidant activity, as captured by the DPPH method. **Tun *et al.* (2020)** note that during the drying process of SCG at temperatures ranging from 40 to 105 °C, the content of certain compounds with antioxidant potential, such as lactones, alcohols, or pyrans, increases. Conversely, there is a decrease in phenolic compounds and phytosterols. According to **Yust *et al.* (2024)**, SCG contains a number of antioxidants that remain after coffee extraction, such as chlorogenic acids, their derivatives, and melanoidins induced by roasting. **Bobková *et al.* (2020)** monitored TAC in Arabica and Robusta coffees at different roasting stages. Their results revealed that all samples roasted to a light degree exhibited the highest TAC levels (from 69.08 % to 78.55 %), and subsequent roasting at medium and dark levels resulted in a decrease in TAC levels (down to 37.44 % for *C. arabica*). The average TAC value measured by **Bobková *et al.* (2020)** in medium-roasted coffee bean samples was 65.22%, which is lower than our findings. These differences could be attributed to varying sample origins.

Várady *et al.* (2022) state that the antioxidant activity of coffee depends on many factors, including the type and variety of coffee beans as well as their origin. Comparable TAC values are reported by **Machado *et al.* (2012)**, who evaluated the ability of seven different genera of filamentous fungi from the genera *Aspergillus*, *Mucor*, *Penicillium*, and *Neurospora* to grow and release antioxidant compounds from SCG on which they were cultivated. The highest DPPH inhibition values were exhibited by extracts obtained from cultures of Mucor (81.9 %) and Aspergillus (81.6 %). The findings of these authors indicate that cultivating filamentous fungi on SCG can be a useful and ecological alternative for extracting bioactive compounds from it. Additionally, obtaining these substances through fermentation positively impacts the environment by avoiding the use of chemical solvents.

A mutual statistical comparison of the TAC results between coffee and SCG samples showed significant differences ($\alpha = 0.05$).

Caffeine is a methylxanthine alkaloid (1,3,7-trimethylxanthine) that stimulates the central nervous system (CNS). It is the most widespread psychoactive, legal, and unregulated drug in almost all parts of the world **(Renda and De Caterina, 2020)**. Since coffee is the main source of caffeine, determining this parameter is crucial for quality and safety **(Hečimović *et al.*, 2011)**. We determined the caffeine content ranging between 7.620 mg.g^{-1} (sample 1B) and 9.826 mg.g^{-1} (sample 5B). **Cwiková *et al.* (2022)** evaluated the impact of processing methods (dry and wet) and roasting degrees (light, medium, and dark) on the caffeine content in 15 samples of *C. arabica*. The average values determined for medium-roasted coffees were 11.9 mg.g^{-1} (dry) and 12.9 mg.g^{-1} (wet). These values are significantly higher

than what we found in our Arabica coffees. The difference may be attributed to the varied origins of the coffee samples, as the authors found that neither processing nor roasting affected caffeine content. This viewpoint is contested by **Franca *et al.* (2005)**, who concluded that roasting reduces caffeine content by approximately 30 %, as its solubility in water increases with temperature, leading to its release through steam. This opinion is also supported by **Król *et al.* (2020)**, who measured higher caffeine levels in light-roasted *C. arabica* (6.42 $mg.g^{-1}$) compared to medium (5.77 $mg.g^{-1}$) and dark (2.63 $mg.g^{-1}$) roasts. Regardless of the aforementioned differences, the caffeine content reported by these authors was lower compared to our data, possibly due to different geographical origins.

Extractable compounds from SCG that are interesting in terms of food-grade components include bioactive substances such as caffeine **(Arya *et al.*, 2022)**. Although caffeine is water-soluble, certain amounts remain in the SCG **(Cavanagh *et al.*, 2023)**. We measured the highest values in sample SCG 4G, at 3.438 $mg.g^{-1}$. Caffeine levels vary not only by species but also by extraction conditions and the type of solvent used **(Franca and Oliveira, 2022)**. **Chatzimitakos *et al.* (2023)** utilized various solvents (water, ethanol, and their mixtures) for extraction and confirmed statistically significant differences. They reported that caffeine extracted best when water was used, likely due to caffeine's hydrophilic nature, which allows it to form hydrogen bonds with water molecules, whereas the number of hydrogen bonds formed with ethanol is lower **(Tavagnacco *et al.*, 2011)**. They determined the average caffeine content in SCG to be 6.6 $mg.g^{-1}$, which is higher compared to our measured value. This could be due to the different samples they analyzed, as they used spent coffee grounds (60 % Arabica and 40 % Robusta), where it is confirmed that Robusta generally contains twice as much caffeine as Arabica. However, **El-Chaghaby *et al.* (2024)** state that the yield of extracted caffeine using ethanol was higher than that using water. Similarly, the study by **Lauberts *et al.* (2023)** confirmed that ethanol is the most suitable solvent for extracting caffeine from SCG. Contrarily, **Vandeponseele *et al.* (2021)** reported different findings. They tested the effect of solvents, such as ethanol-water solutions (20, 40, 60, 80 %), pure ethanol, water, dichloromethane, and ethyl acetate, on the extraction of caffeine from SCG (a blend of 80 % *C. arabica* and 20 % *C. canephora*). They found the highest caffeine content when using an ethanol-water solution (2:3) (4.32 $mg.g^{-1}$) compared to pure water (3.63 $mg.g^{-1}$) or 100 % ethanol (0.26 $mg.g^{-1}$). The value they measured for water matches our findings. **Andrade *et al.* (2022)** quantified caffeine content in SCG samples from various geographical origins. They analyzed samples of 100 % Arabica and found caffeine content ranging from 1.94 $mg.g^{-1}$ to 3.92 $mg.g^{-1}$, corresponding to the values we established as well. Our results indicate that SCG samples exhibited lower caffeine values than the coffee samples. These findings confirm those of **Franca and Oliveira (2022)**, who noted that the caffeine concentration in SCG is lower than in coffee samples due to extraction that occurs during beverage preparation.

Through statistical evaluation of our results, we concluded that significant differences exist between coffee and SCG samples.

Chlorogenic acids (CGAs) are phenolic compounds widely found in various plant sources such as fruits, vegetables, coffee, tea, and wine. They are present in coffee as a complex mixture of positional and geometric isomers. Caffeoylquinic acids (CQAs) and dicaffeoylquinic acids (diCQAs) are the main CGAs found in nature **(Yeager *et al.*, 2023)**. CQAs are reported to be the most abundant compounds in green coffee beans and spent coffee grounds (SCG) **(Belviso *et al.*, 2014)**. In our analyses, we determined the total content of CQAs as the sum of 3-CQA, 4-CQA, 5-CQA, 3,5-diCQA, and 4,5-diCQA. The average values we measured for coffee beans samples ranged from 20.745 $mg.g^{-1}$ (1B) to 25.741 $mg.g^{-1}$ (4B), and for SCG from 6.153 $mg.g^{-1}$ (5G) to 10.867 $mg.g^{-1}$ (2G). **Badmos *et al.* (2020)** analyzed 68 samples of medium-roasted coffee from various regions in Brazil, grown using organic, conventional, and biodynamic agricultural practices, using HPLC methods. They established an average value for CQAs at 21.59 $mg.g^{-1}$, which is consistent with our findings. Among the three cultivation systems, the highest content of these acids was measured in conventional agriculture.

Pedan *et al.* (2020) found in their study that longer roasting times or higher temperatures lead to the degradation of CGAs, with a loss of about 60 % under moderate roasting conditions and nearly 100 % after intense roasting. **Belviso *et al.* (2014)** prepared espresso from capsule coffee of Arabica and measured the content of CQAs at 8.29 $mg.g^{-1}$. This value is lower compared to our findings, which may be due to the fact that the mentioned authors calculated the total content of CQAs as the sum of 3-CQA, 4-CQA, and 5-CQA. Differences may also arise from analyzing samples with different roasting types, preparation methods, or varying extraction conditions (ethanol/water, 60:40). The content of CGA in SCG is highly dependent on the extraction process and the source of SCG **(Mussatto *et al.*, 2011)**. According to the results of **Torres-Valenzuela *et al.* (2019)**, the maximum extraction of CGA from SCG (Arabica) was 4.3 $mg.g^{-1}$ (using 24 % hexanol, 30 % ethanol, and 46 % water). The content of CQAs was determined using the HPLC method. The average value reported by these authors (3.05 - 5.48 $mg.g^{-1}$) does not agree with our higher values. This discrepancy may arise from the fact that we used distilled water for extraction, and the mentioned authors report the CQAs content only as the content of 5-CQA.We can conclude that the content of the studied CQAs in SCG samples is at least half lower compared to coffee. This is due to the leaching of these compounds into the coffee extract. Nevertheless, we have determined a certain amount of CQAs in SCG that could be extracted and utilized.

Statistical comparisons between coffee and SCG samples revealed significant differences regarding the content of CQAs.

Further statistical analysis was focused on Pearson's correlation. The correlation coefficient can range from -1 to 1 and determines the strength of the correlation between individual variables. The closer the correlation coefficient is to (-) 1, the stronger the (negative) positive correlation between the analyzed parameters. If its value is between -0.09 and 0.09, no correlation is observed. Correlation matrix of (Figure 1), showed that only caffeine and CQAs show a positive correlation with each other in coffee beans. All other parameters show a weak to strong negative correlation with each other. In contrast, in SCG (Figure 2), all parameters exhibit a positive linear relationship with each other. We can also observe that caffeine and TAC have the weakest correlation (r = 0.163).

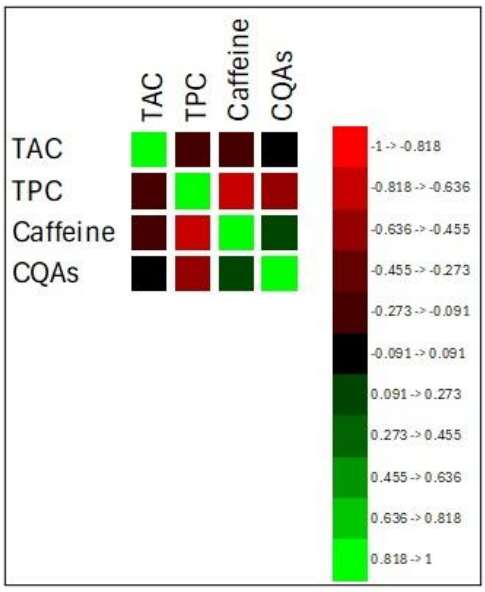

Figure 1 Correlation matrix of selected parameters in coffee

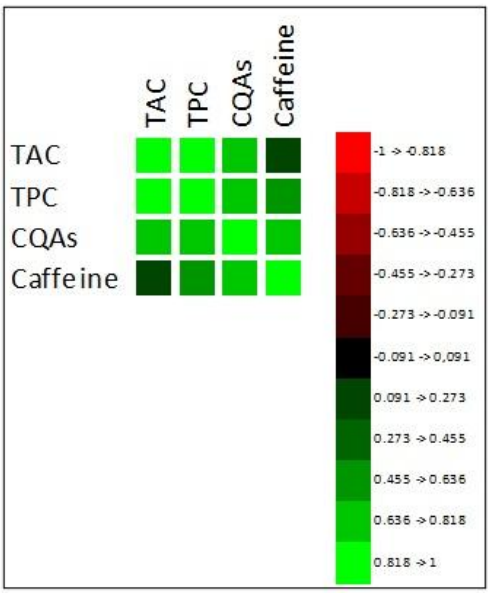

Figure 2 Correlation matrix of selected parameters in SCG

Jung *et al.* (2021) studied the effect of roasting degree on the antioxidant properties of *C. arabica*, reported a moderately negative correlation between caffeine and DPPH radical-scavenging activity, while we found a weak negative correlation between these parameters (r = -0.111). The reason for this negative correlation may lie in the DPPH method, which we used to determine TAC. **De Almeida *et al.* (2019)** found that polyphenols exhibited antioxidant activity using the DPPH, FRAP, and ORAC methods, but caffeine showed such activity only in the ORAC method, and even then, it was weak.

Brezová *et al.* (2009) focused on a correlation between TAC and TPC values in coffee. **Andrade *et al.* (2022)** state that a correlation between TPC values and

antioxidant capacity can be expected, as phenolic compounds are strong antioxidants. However, this was not observed in their study results. They explain this by the presence of non-phenolic compounds in the analyzed extracts, which have antioxidant capacity and help reduce free radicals. A similar finding was made by **Lapčíková *et al.* (2023)**, who reported that there was no correlation between antioxidant activity and polyphenol content in their coffee results. The authors further state that melanoidins, which are formed during roasting, play a key role in the antioxidant activity of roasted coffee. These substances are highly water-soluble and are easily extracted during coffee preparation. These findings are consistent with our observations, where a weak negative correlation was found between the given parameters (r = -0.245).

Zainol *et al.* (2020) identified the physicochemical and antioxidant properties of three different types of SCG, specifically Robusta, Arabica, and Liberica, extracted using ultrasonic techniques and methanol. A positive correlation between TAC and TPC was observed. These findings are consistent with our results. Similarly, **Panusa *et al.* (2013)** report that the total phenolic content correlated with DPPH scavenging activity (TAC). They further state that, based on their findings, phenolic compounds are responsible for the antioxidant activity of SCG.

Linear Discriminant Analysis (LDA) is a method used for data classification and dimensionality reduction in statistical research **(Huang and Guan, 2015)**. It aims to find an optimal linear transformation that projects the original classes or data groups into a lower-dimensional space **(Park and Haesun, 2008)**. The goal of LDA is to learn an optimal projection matrix so that the projection points of dissimilar categories are as far apart as possible, while similar categories are brought closer together. This method is often used in areas such as machine learning, pattern recognition, and data analysis **(Huang and Guan, 2015)**. We used LDA to classify our samples into two categories (Coffee Bean and Coffee Co-product – Grounds) based on input values of the evaluated parameters.

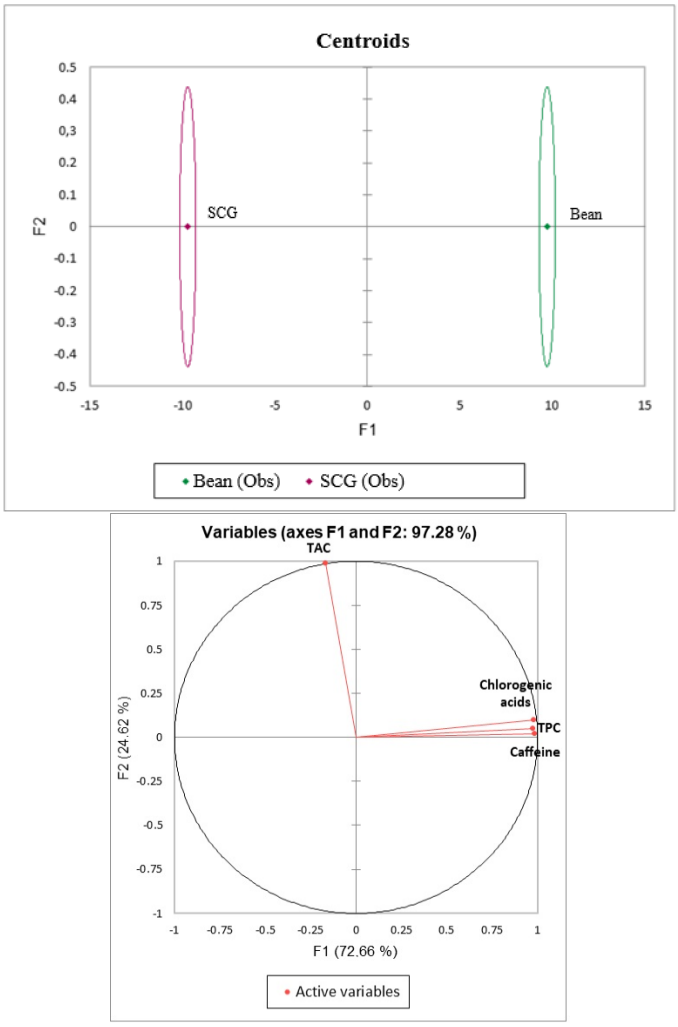

Figure 3 LDA map of selected parameters in bean and SCG

The application of multivariate statistical analysis LDA demonstrated that there are statistically significant differences between the observed samples, or groups, based on the values of specific parameters (TAC, TPC, chlorogenic acids, and caffeine). The resulting Wilks' Lambda test values confirmed that there are statistically significant differences between the observed groups – the coffee bean and the coffee co-product (SCG) as we can see on Figure 3.

Based on factors F1 and F2, LDA is able to explain 97.28 % of the differences or variances between the observed samples, with regard to the parameters we measured in them. Furthermore, we can see that chlorogenic acids, TPC, and caffeine correlate with factor F1, meaning these parameters contribute the most to explaining the differences between the group's coffee bean and SCG. From the Figure 4, we can conclude that in the case of the coffee beans and spent coffee grounds group, there is no overlap in the data within these groups, which indicates that they are sufficiently distinct from each other.

CONCLUSION

Based on our findings, both coffee and spent coffee grounds (SCG) are valuable sources of bioactive compounds, such as chlorogenic acids, caffeine, and polyphenols with antioxidant properties. In some SCG samples, antioxidant capacity was higher than in the original coffee, despite a decrease in caffeine, TPC, and CQA content due to extraction and heat exposure. These bioactive compounds offer promising applications across various sectors, from food additives and packaging materials to biofuels and composting, aligning with consumer demand for safe, health-beneficial products. Utilizing SCG supports sustainability and exemplifies the potential for waste valorization within the circular economy. We recommend further research focusing on microbiological and nutritional parameters, such as fiber, which could make SCG an attractive additive for food products. Identifying optimal conditions for isolating and applying these bioactive compounds in the food industry is also essential, with an emphasis on their quality and safety.

Acknowledgments: This research has been supported by The Ministry of Education, Science, Research and Sport of the Slovak Republic, grant VEGA 1/0412/25, grant KEGA 001SPU-4/2023 and by the Grant Agency of the Slovak University of Agriculture (GA SPU), project No. 03-GA-SPU-2024 and by the Slovakia's recovery and resilience plan, project No. 09I03-03-V05-00018 – Early Stage Grants at SUA in Nitra.

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