



# Spent Coffee Grounds—A Coffee By-Product Abundant in Bioactive Compounds with Antioxidant Properties <sup>†</sup>

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**Abstract:** The present study concentrated on the quality assessment of spent coffee grounds (SCG) blends collected after the brewing process in local commercial cafeterias. To obtain SCG extract and oil, the ultrasound-assisted extraction with 60% hydroethanolic mixture and the conventional solid–liquid extraction with hexane were carried out, respectively. The quality of the SCG blend was assessed by performance of the ensuing analysis: total polyphenols content (TPC), antioxidant activity by using ABTS and FRAP methods, browning index (BI), caffeine and chlorogenic acids contents by using high-performance liquid chromatography as well as oxidative induction time (OIT) by using the pressure differential scanning calorimetry and fatty acids profile by using the gas chromatography. The SCG extract was characterized by a high TPC (33.79 mg GAE/g SCG), BI (0.2), caffeine (5.25 mg/g SCG), and chlorogenic acids (7.52 mg/g SCG) contents. In addition, ABTS and FRAP methods revealed the high antioxidant activity of SCG extract. The OIT of SCG oil reached 43.8 min. The SCG oil mainly contained palmitic acid (37.18%) and linoleic acid (39.69%). Overall, SCG can be regarded as a coffee by-product abundant in various chemical compounds with biological and antioxidant activity, but it is necessary to examine the opportunity for the implementation of SCG in particular forms as a new constituent of functional foodstuffs.

**Keywords:** fatty acids profile; oxidative stability; polyphenols; caffeine; chlorogenic acids; browning index; antioxidant activity



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## 1. Introduction

Recently, food processing industries have been facing ever-growing difficulties related to plant waste accumulation and environmental degradation. To overcome these problems, circular economy conceptualization (CEC) was utilized [1]. Currently, the CEC is acquiring a tremendous awareness not only of food surroundings but also scientific and legislative communities. The assumptions of the CEC concentrate on several courses of action associated with finding new sources of renewable energy, the development of agroindustry product life cycle, and the management of plant by-product utilization. The realization of CEC demands that food manufacturers and researchers implement effective and environmentally friendly investigations of plant-origin waste materials reutilization [2,3].

Spent coffee grounds (SCG) are known as coffee by-products generated during coffee infusion preparation in cafeterias, as well as industrial processes, such as instant coffee production [4]. Regarding the sustained global growth in coffee consumption [5] and annual coffee production amounting to 10 million tonnes [6], significant quantities of SCG are produced annually worldwide. The environmental pollution with SCG may be considered a great hazard if incorrectly discharged [7,8]. SCG are solid coffee waste material abundant in various chemical compounds, which are incompletely isolated from grounded

roasted coffee beans in the process of coffee brewing [9]. These compounds include the following: polyphenolic compounds, caffeine, melanoidins, and fatty acids. Additionally, these chemical compounds indicate multivariate health-beneficial biological effects, namely antioxidant, antimicrobial, antihypertensive, antiallergenic, antimutagenic, and anticarcinogenic effects. Also, the physicochemical properties of these compounds could be applied as valuable constituents of functional food products or natural preservatives [10–13].

Taking into account the above-mentioned reasons, the main goal of the present study was to evaluate the quality of the SCG blend collected after beverage preparation in local commercial coffee establishments.

## 2. Materials and Methods

### 2.1. Materials

The spent coffee grounds (SCG) were prepared in local commercial cafeterias and collected after brewing process. The collection scheme of SCG blend samples was prepared in accordance with the sampling procedure stated in the PN-ISO 3534-2:2010 standard [14]. The initial samples of SCG were gathered half dozen times every three days from selected cafeterias to generate general blends. Each day, the collected batches of SCG underwent drying process at 103.0 °C until sample weight equilibration. After drying, mixing of the samples was performed to obtain representative SCG samples. These samples were maintained in closed packages at ambient temperature without light access until more detailed examination.

### 2.2. Procedure of Preparation of SCG Extract

Procedure of SCG extract preparation was performed by using extraction parameters described by Brzezińska et al. [15]. Ultrasound-assisted extraction with 60% hydroethanolic solution and one gram of dried SCG blend sample was used. Emmi-D60 ultrasonic heater bath (Salach, Germany) set at 60 °C for 30 min was used. The filtrated supernatant of SCG was kept at the storage temperature between two and eight degrees Celsius in the dark for further analysis.

### 2.3. Procedure of Preparation of SCG Oil

Procedure of SCG oil preparation was performed in accordance with the Górska et al. [16] method. The conventional solid–liquid extraction with hexane (150 mL) and 30 g of dried SCG blend sample was applied. The SCG oil extraction was conducted at ambient temperature for 60 min by means of a laboratory water bath with agitation Elpin Plus type 357 (Lubawa, Poland). After filtration, 3 g of anhydrous magnesium sulphate was added and kept for 30 min. Subsequently, hexane was evaporated from the SCG filtrate samples. Finally, the obtained SCG samples were dried under nitrogen atmosphere to remove hexane residues. The SCG oil was kept at sub-zero temperature (−20 °C).

### 2.4. Total Polyphenols Content (TPC)

The modified colorimetric method with Folin–Ciocalteu reagent [17] was used for the TPC determination. Distilled water was used to dilute the SCG extract (50 µL) to acquire 3.2 mL of solution. After shaking, 200 µL of Folin–Ciocalteu reagent and 600 µL 20% (*w/v*) Na<sub>2</sub>CO<sub>3</sub> solution were mixed with the diluted SCG extract and vortexed thoroughly. The sample was stored in the dark for 2 h. Then, the absorbance measurements at 765 nm were carried out with the use of UV-1280 Shimadzu spectrophotometer (Kyoto, Japan). The solutions of gallic acid were applied to plot the standard curve.

### 2.5. Antioxidant Activity—ABTS Assay

Antioxidant activity of SCG extract was measured based on the procedure reported by Re et al. [18]. Chemical reagents such as 2.4 mM of potassium peroxydisulphate and 7.5 mM of ABTS were used to prepare the ABTS working solution. The ABTS working solution was kept in the dark for 12 h. The reaction of SCG extract (40 µL) and properly

diluted working ABTS solution was carried out in the dark. The reaction time was 6 min, and the absorbance of the resulting solution was measured at 734 nm. For calibration, the solutions of Trolox in PBS buffer were applied.

#### 2.6. Antioxidant Activity—FRAP Assay

The modified method presented by Benzie and Strain [19] was used for FRAP assay. The three solutions, 10 mM TPTZ in 40 mM HCl, 20 mM FeCl<sub>3</sub>, 0.3 M acetate buffer (pH 3.6), were mixed in a ratio of 1:1:10 (v/v/v) for preparation of working solution of FRAP reagent. The working solution of FRAP reagent was mixed with the diluted SCG extract. The resulting sample solution was incubated at 37 °C for 30 min, and its absorbance was registered at 593 nm. The aqueous solutions of ferrous sulphate (FeSO<sub>4</sub>·7H<sub>2</sub>O) were used to plot the external curve.

#### 2.7. Browning Index (BI)

The spectrophotometric measurements of BI were performed in accordance with the method described by Bravo et al. [20]. Moreover, 2 mL of distilled water was mixed with 50 µL of SCG extract, and then the absorbance of the resulting sample was measured at 420 nm.

#### 2.8. High-Performance Liquid Chromatographic Determination of Caffeine and Chlorogenic Acid

Analytical method described by Głowacka et al. [21] was performed for the determination of the chlorogenic acid and caffeine content. The high-performance chromatograph Dionex (Germering, Germany) coupled with UVD 170S detector and Supelco Discovery C18 column was used for analytes separation. The mobile phase was pumped at a flow rate of 0.8 mL/min and composed of eluent A—0.3% (v/v) acetic acid solution and eluent B—methanol. The elution program was as follows: 80% A, 0 min; 50% A, 15–24 min; and finally, 80% A, 27–29 min.

#### 2.9. Gas Chromatographic Determination of Fatty Acids Profile

The YL6100 GC-FID (Young Lin Bldg., Anyang, Hogue-dong, Republic of Korea) equipped with BPX 70 capillary column (SGE Analytical Science, Milton Keynes, UK) was used to determine fatty acids in SCG oil. The procedure of derivatization of fatty acids to methyl esters and parameters used during chromatographic separation was shown in the paper described by Górska et al. [16].

#### 2.10. Determination of Oxidation Induction Time of SCG Oil

Oxidation induction time of SCG oil was determined in accordance with the pressurized differential scanning calorimetric (PDSC) method reported by Brzezińska et al. [22]. Isothermal (120 °C) PDSC analysis was performed by means of DSC Q20 TA Instrument (TA Instruments, New Castle, DE, USA). SCG oil (3–4 mg) was placed into an open aluminum crucible in the heating chamber under oxygen atmosphere with an initial pressure of 1400 kPa.

### 3. Results and Discussion

The quality assessment of SCG extract by using spectrophotometric and chromatographic analysis was summarized in Table 1. The antioxidant activity of the SCG extract was determined using the FRAP and ABTS tests. These methods exploit different reaction mechanisms. The FRAP assay involves a reaction mechanism based on a single electron transfer, namely SET, whereas the ABTS assay is considered to be a more complex reaction, like mixed mode [23]. The ABTS value of SCG extract was higher compared to those described by Bravo et al. [24]. The differences in the results may be caused by the application of various SCG sample types and extraction procedures. The FRAP assay results are in agreement with the data presented by Mussatto et al. [25]. It is also apparent from the data

in Table 1 that the antioxidant activity of SCG extract obtained by the ABTS assay indicated a higher value in comparison to the FRAP assay value.

**Table 1.** The quality evaluation of SCG extract by using following spectrophotometric and chromatographic determinations: TPC (total polyphenols content), antioxidant activity—ABTS/FRAP, BI (browning index), caffeine and chlorogenic acids (CQA) contents. Data are shown as mean value  $\pm$  standard deviation. Abs<sub>420</sub>—spectrophotometric measurements of absorbance at 420 nm wavelength. \*—analysis performed using a high-performance liquid chromatograph.

Type of the Sample	Type of the Analysis	Value of the Obtained Result
SCG extract	TPC (mg GAE/g SCG d.m.)	33.79 $\pm$ 0.07
	ABTS (mg Trolox/g SCG d.m.)	72.83 $\pm$ 0.10
	FRAP ( $\mu$ mol Fe(II)/g SCG d.m.)	71.39 $\pm$ 0.10
	BI (Abs <sub>420</sub> )	0.20 $\pm$ 0.01
	Caffeine content * (mg/g SCG d.m.)	9.06 $\pm$ 0.07
	CQA content * (mg/g SCG d.m.)	7.52 $\pm$ 0.05

The total polyphenols content in the SCG extract was higher than reported in other papers. Chatzimitakos et al. [26] conducted the extraction process for 120 min at 65 °C with the use of 50% hydroethanolic solution as a solvent and 35 mL/g solvent to solid ratio, reaching TPC levels above 19 mg GAE/g SCG d.m.

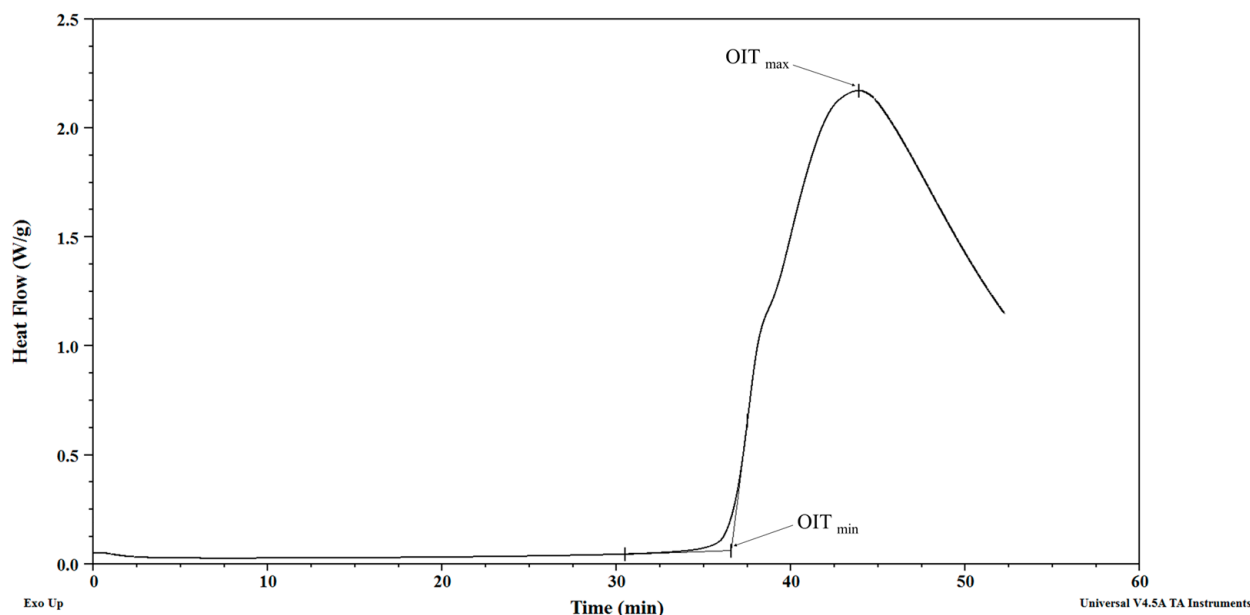
Our results associated with the caffeine content, CQA content, and BI in the SCG extract are in accordance with those reported in the literature [27–29].

In Table 2, the SCG oil fatty acids composition is shown. The lipid fraction of oil extracted from SCG was characterized by the share of SFA acids at the level of 46.92%, PUFA—41.64%, and MUFA—11.08%. Similar results were obtained by de Melo et al. [30] and Cruz et al. [31]. Based on the fatty acid profile of oil from spent coffee grounds, it was found that the fatty acids with the highest share were palmitic acid (37.18%) and linoleic acid (39.69%). Among monounsaturated fatty acids, only oleic acid has been identified. The share of this fatty acid was 11.08%. Moreover, the ratio of fatty acids belonging to the n-6 group to fatty acids belonging to the n-3 group was determined, the value of which reached 20:1.

**Table 2.** Fatty acids profile present in SCG oil (SFA—saturated fatty acids, MUFA—monounsaturated fatty acids, PUFA—polyunsaturated fatty acids). Data are shown as mean value  $\pm$  standard deviation.

Group of Fatty Acids	Fatty Acid	Share of a Given Fatty Acid (%)	The Total Share of the Fatty Acid Group (%)
SFA	C16:0	37.18 $\pm$ 0.50	46.92 $\pm$ 0.43
	C18:0	8.21 $\pm$ 0.16	
	C20:0	1.53 $\pm$ 0.01	
MUFA	C18:1 n-9c	11.08 $\pm$ 0.22	11.08 $\pm$ 0.22
PUFA	C18:2 n-6c	39.69 $\pm$ 0.52	41.64 $\pm$ 0.34
	C18:3 n-3c	1.95 $\pm$ 0.02	

Oxidation induction time is considered to be a significant parameter of oil oxidative stability. This parameter is used for the evaluation of the analysed oil resistance to its thermal degradation in an oxygen atmosphere. The PDSC curve of SCG oil is presented in Figure 1. The maximum oxidation induction time of SCG oil reached 43.8 min, and the initial oxidation induction time (onset point) reached 36.45 min. The oxidative stability of SCG oil is related to the groups of bioactive compounds that have a significant contribution to the overall activity of SCG. The SCG oil indicates relatively low stability in comparison to other vegetable oils but higher stability compared to coffee silverskin [16].



**Figure 1.** PDSC curve of oxidation induction time (OIT) of oil extracted from SCG blend.

#### 4. Conclusions

Our investigation clearly demonstrates that spent coffee grounds collected from cafeterias can be recognized as valuable coffee waste material rich in compounds with antioxidant activity. Therefore, spent coffee grounds indicate the possibility of implementation in various branches of the food processing industry. Additionally, it is recommended to investigate further experiments which can contribute to identifying the opportunity of the SCG application in different forms as a beneficial constituent of innovative food comestibles.

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