JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Espresso Coffee Residues: A Valuable Source of Unextracted Compounds

Rebeca Cruz,[†] Maria M. Cardoso,[†] Luana Fernandes,[§] Marta Oliveira,[‡] Eulália Mendes,[†] Paula Baptista,[§] Simone Morais,[‡] and Susana Casal*[,†]

[†]REQUIMTE, Laboratório de Bromatologia e Hidrologia, Faculdade de Farmácia, Universidade do Porto, Rua de Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal

[§]CIMO − Centro de Investigação da Montanha, Escola Superior Agrária, Instituto Politécnico de Bragança, Campus de St. Apolónia, Apartado 1172, 5301-855 Bragança, Portugal

[‡]REQUIMTE − Instituto Superior de Engenharia do Porto, Instituto Politécnico do Porto, Rua D. António Bernardino de Almeida 431, 4200-072 Porto, Portugal

ABSTRACT: Espresso spent coffee grounds were chemically characterized to predict their potential, as a source of bioactive compounds, by comparison with the ones from the soluble coffee industry. Sampling included a total of 50 samples from 14 trademarks, collected in several coffee shops and prepared with distinct coffee machines. A high compositional variability was verified, particularly with regard to such water-soluble components as caffeine, total chlorogenic acids (CGA), and minerals, supported by strong positive correlations with total soluble solids retained. This is a direct consequence of the reduced extraction efficiency during espresso coffee preparation, leaving a significant pool of bioactivity retained in the extracted grounds. Besides the lipid (12.5%) and nitrogen (2.3%) contents, similar to those of industrial coffee residues, the CGA content (478.9 mg/100 g), for its antioxidant capacity, and its caffeine content (452.6 mg/100 g), due to its extensive use in the food and pharmaceutical industries, justify the selective assembly of this residue for subsequent use.

KEYWORDS: *spent coffee grounds, espresso coffee, coffee residues, chemical characterization*

INTRODUCTION

Coffee is one of the most popular beverages in the world, and it is the result of a long and complex technological process, from cultivation to beverage preparation. During the entire coffee-processing chain several residues are obtained. These residues can be divided in two categories: those generated in the producing countries, representing >50% of the coffee fruit mass, and those produced in the consuming countries after beverage preparation, the so-called "spent coffee".

Over the past years, several strategies have been tentatively applied, particularly in the producing countries where the direct discarding of these residues has been the cause of numerous environmental problems over decades. In particular, efforts are being made to implement adequate disposal approaches and potential reuses, including horticultural and mushroom production, animal feed, biodiesel, fuel pellets, or activated carbons.[1−5]

With regard to spent coffee, again two types should be distinguished. The soluble coffee industry gathers almost 50% of the world coffee production, with a proportional amount of spent coffee residues.[6] Despite being usually disposed of in sanitary landfills, the direct discharge of these coffee residues should be avoided as they contain high amounts of organic compounds,[1] some with established ecotoxicity such as caffeine, tannins, and polyphenols.[3,7] This residue is particularly rich in polysaccharides,[2] with a relatively low amount of soluble solids, as expected from the high extraction efficiency required during the industrial preparation of coffee extracts. Approaches to reuse them for feeding purposes display nutritional limitations, whereas their direct use as burning fuel can give rise to additional environmental problems. Recent efforts to produce biodiesel from the lipid remains (almost 15%)[8−11] or to apply diverse fermentation strategies to develop value-added products[3] are beginning to demonstrate some feasibility.

The remaining 50% of the worldwide coffee production is used for the direct preparation of beverages in cafeterias, restaurants, or homes. Depending on the brewing procedure applied, distinct coffee brews can be achieved and, consequently, the spent coffee remains will present quantitatively different amounts of unextracted coffee constituents. Regardless of the brewing process applied, the extraction efficiency will be clearly lower than the one obtained at the industrial level and, therefore, the residues will be richer in coffee constituents, opening an array of potential applications, side by side with an equally increased ecotoxicological concern.[7]

The main purpose of this study is to characterize the spent coffee grounds (SCG) obtained after beverage preparation in comparison with that defined for industrial spent coffee. Espresso coffee consumption is increasing worldwide and is the main coffee beverage consumed in Portugal, so it was selected for the present study. This characterization should provide a baseline for establishing the limits of compositional variation presented by this residue, thus allowing a better optimization of prospective applications.

Received: April 30, 2012
Revised: July 13, 2012
Accepted: July 19, 2012
Published: July 19, 2012

MATERIALS AND METHODS

Sample Preparation. Fresh SCG were collected from several coffee shops, in the Oporto metropolitan area (northwestern Portugal), serving espresso coffee on a regular basis. A total of 50 samples, from 14 trademarks, were analyzed, selected from the ones that better represented the Portuguese coffee market (according to nonofficial data from AICC, the Portuguese Industrial and Commercial Coffee Association), all corresponding to Arabica/Robusta blends in undisclosed proportions. Brands were labeled A (n = 6), B (n = 12), C (n = 3); D (n = 7), E (n = 4), F (n = 6), G (n = 4), H (n = 2), and I–N (n = 1, respectively). Most samples were obtained from manual extraction devices, except samples B9–B12, F1, and H2 that were collected from automatic espresso machines. After moisture quantification, samples were preserved by oven-drying at 80 °C (WTC Binder; Germany) to about 5% moisture.

Moisture Determination. Moisture content was determined at 103 ± 2 °C in a forced-air oven (WTC Binder), using 5 g of fresh sample, until constant weight according to AOAC method 930.04.[12] Moisture was evaluated upon arrival and after preservation by oven-drying as described above.

Total Soluble Solids (TSS) Quantification. TSS analysis procedure was based on AOAC method 973.21 for roasted coffee,[12] with slight adjustments. SCG (10 g) was weighed and boiled with deionized water (200 mL), under magnetic agitation, for 5 min. After cooling, the initial weight was readjusted with deionized water, and the suspension was filtered. Then, 25 mL of the former solution was transferred to a glass capsule, previously dried and weighed, evaporated in a boiling water bath, and oven-dried at 103 ± 2 °C (WTC Binder) for 3 h. The glass capsule was weighed after cooling in a desiccator, and the solid remains were calculated on a 100 g basis.

pH Measurement. SCG samples (5 g) were mixed with deionized water (50 mL) and boiled for 5 min with continuous shaking. The supernatant was decanted into a volumetric flask. This procedure was repeated, the supernatants were combined, and the final volume was adjusted to 100 mL with deionized water after complete cooling. This solution was used for pH determination, using a potentiometer (pH-meter GLP22, Crison, Spain), after calibration with buffer solutions at pH 4 and 7. This extract was also used for caffeine and chlorogenic acid (CGA) determination, as detailed below.

Total Nitrogen Quantification. Total N content was quantified according to AOAC method 920.103,[12] using 0.5 g of SCG. Total N content was converted into crude protein by using a conversion factor of 6.25, after subtraction of caffeine nitrogen.

Total Fat Determination. Total fat content was determined (6 g) with petroleum ether (40–60 °C p.a.) by Soxhlet device (Büchi B-811, extraction system, Switzerland) during 6 h. The extract was vacuum-dried in a desiccator and weighed. Because petroleum ether might extract some caffeine,[13] its amount was deducted from total fat content. The fat residues were preserved for subsequent evaluation of their fatty acid composition, by dissolution in hexane (3 mL) with 0.01% butylated hydroxytoluene (BHT, Sigma, Germany) and maintenance at 4 °C.

Fatty Acids Quantification. The fat extract was heated at 60 °C to achieve complete dissolution of the fatty esters. A portion of the supernatant was used (400 μL), being dried under a gentle nitrogen steam and redissolved in heptane (3 mL; chromatographic purity, Sigma). The fatty esters were hydrolyzed and methylated by cold alkaline transesterification, with KOH (Merck, Germany) 2 M in methanol (Sigma-Aldrich, Germany) according to ISO 5509[14] and analyzed by gas chromatography on a Chrompack CP-9001 (Netherlands) chromatograph with flame ionization detection based on ISO 5508.[15] Fatty acid methyl esters separation was accomplished on a chromatographic column CP-Sil 88 (50 m × 0.25 mm; 0.19 μm, Varian), using helium as mobile phase (110 kPa) and a temperature slope between 140 and 220 °C, at a total of 35 min. The injector temperature was at 230 °C, using split injection (1:50), and the detector temperature was at 250 °C. Results are presented as individual fatty acid relative percentage, calculated by internal standardization of the chromatographic areas between the peaks of myristic and lignoceric methyl esters. For the identification of each fatty acid retention time and calibration of the detector signals, a commercial standard mixture was used (Supelco-37 FAME Mix, Spain).

Ashes Content and Mineral Composition. Dry ashing was performed according to AOAC method 920.93,[12] with 0.5 g of SCG at 500 °C in a muffle furnace (48000 Furnace, Thermolyne, USA), until white ashes were obtained. After cooling in a desiccator, ashes were weighed for total mineral content estimation, and a portion (4.0 mg) was dissolved in water acidified with 1% HCl (37% v/v, Riedel-de Haën, Germany) (5 mL) for evaluation of the mineral composition. Depending on the studied element, proper dilutions were carried out.

Phosphorus was quantified by a standard vanadomolybdophosphoric acid colorimetric method, as described by Greenberg et al.,[16] using a double-beam UV–vis spectrophotometer (Evolution 300, Thermo Scientific, USA). Calibration curves were executed, using potassium dihydrogen phosphate (99.5%, Riedel-de Haën) standard, and all samples and standards were measured at 420 nm.

Quantification of Ca, Na, Mg, K, Mn, and Fe was performed as described by Oliveira et al.,[17] with minor adjustments, and it was achieved by high-resolution continuum source atomic absorption spectrometry (HR-CS AAS) (ContrAA 700, Analytik jena, Germany), using flame atomization with reconstituted air and acetylene, and operating with an autosampler (AS 52 S, Analytik jena). On the other hand, Cu was analyzed by the same equipment but through a graphite furnace, also equipped with an autosampler (MPE 60, Specanalitica, Germany). The apparatus presents a xenon short-arc lamp XBO 301 (GLE, Germany) with a nominal power of 300 W operating in a hot-spot mode as a continuum radiation source, with a spectral band ranging from 190 to 900 nm. Standard solutions of Ca, Mg, Fe, and Mn, were prepared from the correspondent 1000 mg/L stock solutions (Panreac, Spain). K and Na standard solutions were obtained by potassium chloride (99.5%, Riedel-de Haën) and sodium chloride (99.8%, Riedel-de Haën) dissolution in ultrapure water, respectively. For the elements determined by flame atomization 1% CsCl (p.a., Sigma-Aldrich) was also added as ionization suppressor.

Caffeine and Chlorogenic Acids Quantification. A small portion of the SCG extract, prepared for pH measurement, was centrifuged (Heraeus Instruments, Biofuge pico, Germany) at 13000 rpm for 10 min; supernatant was decanted to a new vial, and the centrifugation procedure was repeated. This new supernatant (20 μL) was directly analyzed. Chromatographic separation was performed according to the method of Chambel et al.[18] and accomplished by a HPLC equipped with a data transmitter (Jasco LC-NetII/ADC, Japan), high-pressure pumps (Jasco PU-980), refrigerated autosampler (Jasco AS-2057 Plus), and photodiode array detector (Jasco MD-2015 Plus). Control, acquisition, and data treatment system was a ChromNAV Control Center–JASCO Chromatography Data Station. A reversed-phase column (Phenomenex; 250 × 4.60 mm; C18 ODS-2; 5 μm) was used, and samples were eluted (1 mL/min) for 30 min, at room temperature, with a linear gradient from 90% 0.01 M acetate buffer (pH 3.90) [prepared with sodium acetate (p.a., Aldrich, Germany) and glacial acetic acid (p.a., Merck, Germany)] and 10% methanol (chromatographic purity, Fisher Scientific, UK) to 50% acetate buffer/methanol. Chromatograms were recorded at 276 nm for caffeine and at 325 nm for chlorogenic acid isomer determination. Calibration curves were assembled with a minimum of six concentrations for each standard. Authentic caffeine and 5-*O*-caffeoylquinic acid (5-CQA) standards were purchased from Sigma, and the calibration curve from the latter was used to quantify the remaining chlorogenic acid isomers.

To evaluate the assay precision, sample extracts were prepared on nonconsecutive days, achieving interday precisions below 1% for both compounds. The method's accuracy was assessed by the standard addition procedure, obtaining good outcomes for caffeine (98.7 ± 2.3%) and 5-CQA (95.3 ± 0.8%).

Statistical Analyses. Statistical analyses were performed using GraphPad Prism 5.04 (GraphPad Software, Inc., USA). Differences of the analyzed compounds between samples were calculated using one-way analysis of variance (ANOVA) with Tukey's post hoc procedure,

Table 1. Moisture, pH, and Total Soluble Solids, Nitrogen, Protein, and Total Fat Contents in Espresso Spent Coffee Grounds

brand	moisture (%)	total soluble solids (%, DW[a])	pH	nitrogen (%, DW)	crude protein (%, DW)	total lipids (%, DW)
A1	62.2	18.9	5.89	2.70	16.9	13.8
A2	62.0	23.8	5.83	2.42	15.1	13.4
A3	65.8	20.3	5.69	2.32	14.5	13.0
A4	61.6	17.7	5.81	2.30	14.4	12.1
A5	61.6	19.8	5.68	2.32	14.5	12.5
A6	66.5	16.9	5.87	2.29	14.3	15.2
n = 6	63.3 ± 2.3a[b]	19.6 ± 2.4a	5.80 ± 0.09a	2.39 ± 0.16a	14.9 ± 1.0a	13.3 ± 1.1a
B1	66.9	13.6	5.70	2.26	14.1	13.7
B2	68.0	19.9	5.73	2.29	14.3	12.4
B3	63.4	11.6	5.89	2.25	14.0	16.2
B4	66.8	17.1	5.80	2.30	14.4	12.7
B5	64.5	15.8	5.80	2.24	14.0	11.7
B6	61.0	23.7	5.35	2.28	14.2	11.8
B7	66.6	15.8	5.77	2.37	14.8	12.8
B8	60.3	20.5	5.59	2.19	13.7	12.6
B9	61.0	23.0	5.25	2.06	12.8	13.1
B10	68.0	19.8	5.50	2.21	13.8	11.0
B11	65.0	22.3	5.40	2.52	15.8	9.5
B12	69.0	18.5	5.53	2.28	14.3	12.5
n = 12	65.0 ± 3.0a	18.5 ± 3.8a	5.61 ± 0.20ab	2.27 ± 0.11a	14.2 ± 0.7a	12.5 ± 1.6a
C1	59.0	23.9	5.23	2.29	14.3	13.5
C2	62.0	26.2	5.27	2.16	13.5	12.0
C3	61.6	20.8	5.71	2.46	15.4	13.6
n = 3	60.9 ± 1.6a	23.6 ± 2.7a	5.40 ± 0.27b	2.30 ± 0.15a	14.4 ± 1.0a	13.0 ± 0.9a
D1	65.0	21.0	5.64	2.17	13.6	10.5
D2	62.0	17.9	5.67	2.26	14.1	13.2
D3	61.1	19.0	5.70	2.33	14.6	13.4
D4	64.5	17.5	5.79	2.17	13.6	11.7
D5	64.9	16.6	5.65	2.25	14.0	11.8
D6	64.4	20.5	5.66	2.26	14.1	12.7
D7	64.8	21.8	5.72	2.32	14.5	15.1
n = 7	63.8 ± 1.6a	19.2 ± 1.0a	5.69 ± 0.05ab	2.25 ± 0.06a	14.1 ± 0.4a	12.6 ± 1.5a
E1	57.0	22.2	5.50	2.31	14.4	12.1
E2	58.1	16.3	5.58	2.38	14.9	11.6
E3	62.1	19.6	5.76	2.27	14.2	12.4
E4	66.3	15.0	5.87	2.37	14.8	12.8
n = 4	60.9 ± 4.2a	18.3 ± 3.3a	5.68 ± 0.17ab	2.33 ± 0.05a	14.6 ± 0.3a	12.2 ± 0.5a
F1	53.0	23.6	5.62	2.50	15.6	9.3
F2	53.0	27.5	5.60	2.35	14.7	11.0
F3	61.6	16.5	5.57	2.10	13.1	12.1
F4	64.7	21.1	5.63	2.18	13.6	12.1
F5	63.5	20.7	5.72	2.30	14.3	12.7
F6	69.8	18.0	5.65	2.20	13.8	11.7
n = 6	60.9 ± 4.7a	21.2 ± 4.0a	5.63 ± 0.05ab	2.27 ± 0.14a	14.2 ± 0.9a	11.5 ± 1.2a
G1	63.6	21.0	5.73	2.27	14.2	13.0
G2	66.6	17.0	5.42	2.20	13.7	12.9
G3	63.7	19.8	5.78	2.22	13.9	13.7
G4	62.5	17.7	5.59	2.13	13.3	11.0
n = 4	64.1 ± 1.7a	18.9 ± 1.8a	5.63 ± 0.16ab	2.20 ± 0.06a	13.8 ± 0.4a	12.6 ± 1.2a
H1	64.0	16.5	5.80	2.26	14.1	13.2
H2	59.0	24.7	5.41	2.21	13.8	12.6
n = 2	61.5 ± 3.5a	20.6 ± 5.8a	5.61 ± 0.28ab	2.23 ± 0.03a	14.0 ± 0.2a	12.9 ± 0.4a
I1	61.1	23.1	5.43	2.17	13.5	13.1

dx.doi.org/10.1021/jf3018854 | J. Agric. Food Chem. 2012, 60, 7777−7784

Table 1. continued

brand	moisture (%)	total soluble solids (%, DW[a])	pH	nitrogen (%, DW)	crude protein (%, DW)	total lipids (%, DW)
J1	64.7	18.2	5.69	2.33	14.6	14.5
K1	61.9	23.9	5.84	2.13	13.3	11.6
L1	55.8	19.6	5.59	2.10	13.1	10.5
M1	64.0	21.5	5.81	2.25	14.1	11.5
N1	63.3	18.8	5.87	2.19	13.7	13.1

[a]DW, dry weight. [b]Letters indicate the SCG samples that have statistically significant differences ($p < 0.05$) from the given mean.

with a level of significance at $p < 0.05$. To ensure that data came from normal distribution, standardized skewness and kurtosis were verified. Correlations between variables were measured by Spearman's rho correlation coefficient (r).

RESULTS AND DISCUSSION

Moisture Determination. Variable moisture content was observed, with values ranging from 53.0 to 69.8% (Table 1), within those reported for espresso[19] or filter SCG.[8] These amounts are lower than those observed in industrial spent coffee sludge (75−85%) from soluble coffee extraction facilities.[2,3,20] Wet SCG are highly prone to microbial growth, making it necessary to implement adequate preservation strategies for the desired final use. The water amount is also important from an economical point of view, as it increases transportation costs.

Total Soluble Solids Quantification. This parameter is a standard evaluation in roasted coffees. TSS represents the substances that are potentially extracted during brewing, being therefore of utmost importance for beverage sensorial properties, including body, flavor, and aroma. TSS in roasted coffees ranges between 29 and 37% for Robusta, whereas Arabica usually presents values from 26 to 32%, depending also on the analytical method used.[21] It can also be used to detect fraud, both by the addition of foreign soluble materials and by dilution with dried SCG.[1]

In the present work TSS values ranged from 11.6 to 27.5% (dry weight, DW) (Table 1). When compared to roasted coffee, these figures are still very high, although in accordance with the reduced extraction efficiency described for most coffee brews (ranging from 14 to 30%) and with espresso coffee attaining around 24%.[22] The extractive efficiency is known to be dependent on several variables, including the coffee/water ratio, roasting grade, grinding degree, and percolation temperature; the higher the roasting temperature, grinding degree, or percolation temperature,[22] the greater is the TSS content transferred into the brew and the lesser is left in the spent coffee. Being a small beverage (30−50 mL), extracted in a reduced time (30 s),[22] espresso coffee spent grounds are still rich in soluble components. The soluble coffee industries usually prefer Robusta coffee, due to its higher amounts of TSS and lower market price, but the extraction efficiency imposed by the industrial process results in spent grounds with reduced water-soluble components (6.6%).[23] The possibility of further extraction of soluble coffee components from spent grounds is therefore greater with commercial coffee beverages than with industrial ones.

pH Measurement. In general, green coffee acids represent about 11% of their mass,[24] being responsible for the known characteristic acidity of this raw material. These are reduced to about 6% (DW) during roasting, sustaining a clear perceived acidity in the brew. Beyond phenolic acids, it is relevant to mention other nonvolatile aliphatic acids (such as citric, malic, and quinic acids) and volatile acids (such as acetic, propanoic, butanoic, isovaleric, hexanoic, and decanoic acids) that are also present in coffee. Coffee origins and species, growth conditions, processing method, roasting degree, and beverage extraction type influence the brew acidity, affecting the coffee's aroma and flavor. The pH estimation can give a fast impression of total acid amounts in coffee despite being more associated with the solution ionization degree, rather than with perceived sourness as would be total acidity.[24] In Arabica coffee, the pH usually ranges from 5.02 to 5.45, whereas Robusta presents a pH of 5.32−5.49.[25]

Table 2. Average Fatty Acids Composition in Espresso Spent Coffee Grounds ($n = 50$)

fatty acid	rel percentage (%)	min value (%)	max value (%)
C14:0	0.1 ± 0.0	0.0	0.2
C16:0	32.8 ± 0.9	30.2	33.9
C16:1	0.1 ± 0.0	0.0	0.1
C18:0	7.1 ± 0.2	6.8	7.9
C18:1	10.3 ± 0.5	9.3	11.4
C18:2	44.2 ± 0.7	42.3	45.7
C18:3	1.5 ± 0.2	0.9	1.9
C20:0	2.6 ± 0.1	2.4	3.3
C22:0	0.5 ± 0.1	0.4	0.9
C24:0	0.2 ± 0.0	0.2	0.4

All SCG samples analyzed revealed a close variation range of 5.23−5.89 (Table 1), higher than the figures given for industrial spent coffee (4.9 ± 0.9).[5] Such acidity may impose some precautions when used, for instance, as direct fertilizer, except for plants that are favored by acid soils.[4]

Total Nitrogen Amounts. Total coffee nitrogen compounds are relatively stable between species or even during roasting, ranging from 8.5 to 13.6% (DW).[26,27] In the present study, crude protein amounts were higher, varying between 12.8 and 16.9% (DW) (Table 1). These results are in accordance with the mean 13.6% reported for SCG obtained after soluble coffee preparation[3,28] and slightly higher than those described by Tokimoto et al.[29] (1.7−2.0% of organic nitrogen and 10.9−12.9% of total protein). SCG presents a total C/N ratio of 22:1,[3] making it seemingly interesting for use as a source of nitrogen for fertilizing. Even so, it should be remembered that not all SCG nitrogen is "free", and composting should be considered to increase its availability.[30]

Total Fat and Fatty Acids Contents. Roasted coffee is composed by 11−20% lipids, with higher amounts in Arabica (14−20%) than Robusta coffee (11−16%).[13] These are relatively stable to roast, except when intensive roast is practiced and the lipids are expelled to the bean surface. Such lipids include mainly triacylglycerols (75%), sterols (5%), and diterpenes of the kaurene family (19%), besides a small fraction of tocopherols. Coffee brews are generally poor in lipids, as these are not efficiently extracted in an aqueous environment.[31]

Table 3. Total Ashes (Grams per 100 g, Dry Weight (DW)) and Mineral Composition (Milligrams per 100 g, DW) of Espresso Spent Coffee Grounds

sample	total ashes	K	Mg	P	Ca	Na	Fe	Mn	Cu
A1	0.82	312.9	87.2	64.6	16.7	8.4	2.4	1.4	1.2
A2	3.24	1642.1	413.7	276.9	59.8	25.4	7.5	4.0	6.3
A3	1.73	714.0	226.0	139.1	30.8	75.7	5.0	3.5	2.1
A4	2.08	726.0	181.7	137.7	36.0	55.1	3.7	2.3	1.7
A5	1.66	848.9	185.1	149.2	32.3	13.6	3.8	2.5	1.8
A6	1.57	453.4	185.7	138.5	29.1	26.4	4.1	2.6	2.2
n = 6	1.85 ± 0.80a[a]	782.9 ± 464.8a	213.2 ± 108.4a	151.0 ± 69.0a	34.1 ± 14.2a	34.1 ± 26.0a	4.4 ± 1.7a	2.7 ± 0.9aa	2.6 ± 1.9a
B1	1.14	389.0	115.9	99.6	25.0	10.1	3.7	2.1	1.7
B2	3.20	1509.4	332.4	275.1	70.8	23.2	9.0	5.5	6.6
B3	1.33	484.8	161.8	125.3	33.4	22.7	5.8	2.5	1.8
B4	2.00	678.7	218.6	159.6	41.0	16.7	4.7	2.9	2.6
B5	1.61	587.3	155.5	145.7	29.8	19.8	3.3	2.5	1.8
B6	3.23	2106.0	395.0	233.5	50.2	13.4	6.0	4.1	2.8
B7	1.43	500.4	179.9	141.9	29.3	19.2	3.5	2.5	1.6
B8	1.84	671.6	172.5	128.5	29.5	26.0	3.8	2.1	1.6
B9	1.60	708.3	181.3	125.7	26.0	6.7	2.9	2.4	2.2
B10	2.87	1690.7	676.1	251.6	61.8	37.3	8.8	4.7	4.9
B11	1.99	989.5	223.7	177.4	35.9	11.6	6.8	2.7	2.3
B12	1.73	870.6	209.1	139.9	29.5	22.1	4.1	2.5	2.7
n = 12	2.00 ± 0.71a	932.2 ± 545.8a	251.8 ± 154.6a	167.0 ± 56.2a	38.5 ± 14.8a	19.1 ± 8.2abc	5.2 ± 2.1a	3.0 ± 1.1a	2.7 ± 1.5a
C1	3.46	2081.9	542.3	245.0	55.4	39.8	14.1	5.4	5.2
C2	3.52	1717.5	292.1	223.7	61.0	26.0	6.8	4.6	5.4
C3	1.25	573.3	128.0	113.5	28.2	10.8	3.0	2.3	1.7
n = 3	2.84 ± 1.13a	1457.6 ± 787.2a	320.8 ± 208.6a	194.1 ± 70.6a	48.2 ± 17.5a	25.5 ± 14.5abc	8.0 ± 5.6a	4.1 ± 1.6a	4.1 ± 2.1a
D1	3.13	1808.0	556.5	253.4	54.8	29.8	8.9	4.8	4.6
D2	0.98	402.5	114.5	81.4	16.5	8.3	2.9	1.8	1.5
D3	1.75	1076.8	153.6	124.3	31.5	29.1	4.5	2.7	1.9
D4	1.70	842.2	186.0	151.0	32.0	31.3	4.0	2.1	2.1
D5	1.68	768.1	216.2	140.9	31.8	28.9	3.6	2.8	3.5
D6	1.58	773.9	142.5	117.2	30.0	12.3	3.1	2.0	1.8
D7	2.55	851.3	197.7	175.3	38.4	11.3	4.5	2.9	1.8
n = 7	1.91 ± 0.71a	931.8 ± 435.0a	223.9 ± 150.7a	149.1 ± 54.5a	33.6 ± 11.5a	21.6 ± 10.3abc	4.5 ± 2.0a	2.7 ± 1.0a	2.5 ± 1.1a
E1	3.42	2188.6	684.2	262.0	60.6	49.4	6.4	4.7	2.9
E2	2.08	961.4	267.4	174.2	38.0	55.6	3.8	3.1	2.9
E3	1.73	459.1	161.1	114.3	27.6	25.1	2.8	1.9	1.6
E4	1.45	593.5	173.6	133.4	34.1	28.4	3.8	2.9	2.9
n = 4	2.17 ± 0.88a	1050.7 ± 787.8a	321.6 ± 246.4a	171.0 ± 65.6a	40.1 ± 14.3a	39.6 ± 15.1ab	4.2 ± 1.5a	3.2 ± 1.2a	2.6 ± 0.7a
F1	1.77	795.4	171.3	127.3	24.0	5.3	6.3	2.0	1.5
F2	2.27	1202.4	247.7	165.7	28.6	7.2	4.0	2.9	2.0
F3	1.41	524.9	132.3	119.0	27.1	7.1	4.5	1.8	1.8
F4	1.42	630.7	147.8	166.6	27.7	7.5	2.4	1.8	2.1
F5	1.83	549.5	177.6	133.2	33.3	18.8	4.2	2.0	2.5
F6	1.71	823.5	182.5	130.5	35.6	10.3	3.6	2.2	2.3
n = 6	1.74 ± 0.32a	754.4 ± 251.8a	176.5 ± 39.8a	140.4 ± 20.5a	29.4 ± 4.3a	9.4 ± 4.9c	4.2 ± 1.3a	2.1 ± 0.4a	2.0 ± 0.4a
G1	1.40	667.8	129.9	115.0	25.3	6.7	3.8	1.5	2.2
G2	1.93	841.4	207.0	159.5	35.0	12.9	4.6	2.0	2.7
G3	1.66	631.2	146.9	126.8	31.7	14.7	4.4	2.4	2.0
G4	2.38	1129.4	218.1	184.3	51.6	11.9	6.2	2.5	2.6
n = 4	1.84 ± 0.42a	817.5 ± 227.3a	175.5 ± 43.6a	146.4 ± 31.5a	35.9 ± 11.2a	11.6 ± 3.4abc	4.7 ± 1.0a	2.1 ± 0.5a	2.4 ± 0.3a
H1	1.47	583.3	158.1	142.0	30.8	8.2	3.5	2.8	2.3
H2	2.39	1207.2	322.2	195.0	41.2	8.2	4.4	2.8	3.1
n = 2	1.93 ± 0.65a	895.3 ± 441.2a	240.2 ± 116.0a	168.5 ± 37.5a	36.0 ± 7.4a	8.2 ± 0.0abc	4.0 ± 0.6a	2.8 ± 0.0a	2.7 ± 0.6a
I1	1.59	803.6	135.5	167.8	31.7	9.5	3.3	2.1	2.3
J1	1.33	615.3	130.8	113.5	27.5	8.7	3.0	2.4	1.8

dx.doi.org/10.1021/jf3018854 | J. Agric. Food Chem. 2012, 60, 7777−7784

Table 3. continued

sample	total ashes	K	Mg	P	Ca	Na	Fe	Mn	Cu
K1	1.93	902.0	149.3	152.7	32.1	7.5	3.6	1.9	2.3
L1	1.65	577.8	132.3	123.8	26.0	32.1	3.0	1.9	1.7
M1	1.64	662.3	130.9	129.1	26.0	7.4	4.4	1.8	2.1
N1	1.69	608.2	163.6	132.1	31.1	30.9	4.0	2.7	2.1

[a]Letters indicate the SCG samples that have statistically significant differences ($p < 0.05$) from the given mean.

Herein, SCG total lipids ranged from 9.3 to 16.2% (Table 1). These values are within those described for roasted coffee, confirming that the majority of lipids are retained in spent coffee. When compared with the lipid amounts described by Kondamudi et al.[8] for U.S. filter coffee spent grounds (10−15%), our values are very similar, as well as with Tokimoto et al.[29] at 6.2−14.5%. Our values are also similar to those described for industrial soluble coffee SCG, namely, the 15.4% reported by Couto et al.[10] or the 14% reported by Calixto et al.[11] These data support that, despite the high temperatures and pressures used during industrial extractions, lipids are mostly retained in the spent grounds. Therefore, with regard to fat amounts, no differences were found between both coffee residues, yet all SCG should be dried as soon as possible with the aim of preventing lipid hydrolysis that might reduce the transesterification efficiency when these lipids are intended to be used for the preparation of biodiesel.

The extracted lipids were also analyzed for their fatty acid composition. Despite the variations in the quantified total lipid amounts, as discussed, the relative fatty acid percentages were very constant, making it senseless to report the individual data for each sample (Table 2). The major fatty acid was linoleic acid (C18:2) at 44.2%, followed by palmitic acid (C16:0) at 32.8%, oleic acid (C18:1) at 10.3%, and stearic acid (C18:0) at 7.1% (Table 2), as reported for industrial spent coffee.[20] All of the reported fatty acids are totally overlapped with the ones presented by Martin et al.[32] for standard roasted coffees, supporting that the lipid fraction is unaffected by beverage preparation. This high homogeneity in the lipid fraction is relevant for a consistent optimization of strategies and processes for reuse of this residue, as in biodiesel production.[10]

Ashes Content and Mineral Composition. Roasted coffee beans usually present around 4.6% of total minerals,[26] easily extracted by hot water, leading to reduced total mineral amounts in soluble coffee residues (0.25−1.6%).[3,5,20,28] In our work, total mineral amounts, estimated by dry-ashing, varied from 0.82 to 3.52% (Table 3), supporting the mineral leaching during espresso coffee preparation, although not as exhaustive as with soluble coffee.

Eight major and minor elements were detailed (K, Mg, P, Ca, Na, Fe, Mn, and Cu), and the results are presented in Table 3, by decreasing order of content. In general, a high variability is observed, but if expressed on a percentage basis, from total ashes, the variations reduce widely, highlighting that all minerals behave similarly during espresso coffee extraction. As observed in roasted beans,[33] potassium is also the most profuse element in espresso spent coffee, corresponding to 40% of the oxide ash, ranging from 312 to 2188 mg/100 g (Table 3). The industrial spent grounds are described to contain lower absolute (355 mg/100 g) and relative amounts (22%) of this element.[2] Coffee is regarded as an important source of Mg, comprising 11% of the SCG minerals, again higher than the amounts described for industrial spent coffee.[2] With regard to P, the third major element, and Cu, the minor element, our values are similar to those reported for soluble coffee residues.[2] In opposition, the experimental values for Ca, Fe, and Mn in the espresso SCG samples are slightly lower than the values reported by Mussatto et al.,[2] and no reported values for Na were found.

The higher mineral amounts in espresso SCG can be of some significance if used as soil amendment.[8]

Caffeine and Chlorogenic Acids Quantification. The purine caffeine is the main alkaloid in coffee beans. In raw Arabica coffee, caffeine can be found in values varying between 0.8 and 1.4% (w/w), whereas for Robusta these amounts vary between 1.7 and 4.0% (w/w).[2] Phenolic compounds are mainly found in green coffee beans as CGA (up to 12% of solids).[34] Whereas caffeine is relatively stable to roasting, the phenolic compounds are partially degraded, representing only around 3% in roasted coffee.[25]

Espresso SCG samples exhibited high variance with regard to both caffeine and CGA values (Table 4). Caffeine amounts ranged from 194.0 to 787.7 mg/100 g (DW), with a mean amount of 452.6 mg/100 g (DW). The caffeine extractability coefficient in espresso coffee is 75−85%,[22] so these figures correspond to a predicted mean caffeine content of 2250 mg/100 g (DW) in the original roasted beans, which is in accordance with the literature[25,35] and consistent with the use of Arabica/Robusta blends, as usual in Mediterranean espresso coffee mixtures. With regard to caffeine residual amounts in industrial spent coffee, no data were found in the literature. Still, lower contents are expected due to the enhanced industrial coffee extraction efficiency.

The main compound from the CGA phenolic family, 5-CQA, was quantified individually. Total CGA isomers were estimated on the basis of their chromatographic spectral characteristics, being expressed on a 5-CQA equivalent mass basis (Table 4). Once again, SCG samples revealed high variability, with 5-CQA ranging from as low as 39.7 to 264.2 mg/100 g (DW) and total CGA varying from 212.1 to 765.6 mg/100 g (DW) (Table 4). These amounts are in the lower range reported for roasted coffees[25,36] as expected after beverage preparation. Yen and co-workers[23] found equivalent amounts of phenolics in simulated coffee residues. Mussatto et al.[37] analyzed industrial spent coffee grounds and verified the presence of lower CGA contents (57 mg/100 g, DW). A strong linear correlation between caffeine and total CGA was verified ($r = 0.727$, $p < 0.001$). This could be a direct consequence of the blend used, with Robusta presenting simultaneously higher amounts of both compounds, in contrast to Arabica coffee. As both compounds are highly water-soluble, this correlation is maintained after brewing.

The remaining caffeine and phenolic compounds in espresso SCG should be taken into account if this residue is simply discharged in landfills or when used as soil amendment, possibly leading to higher ecotoxicity than the industrial SCG.[3,7] Their extraction for further use, in contrast, could

Table 4. Caffeine, Total Chlorogenic Acids, and 5-Chlorogenic Acid Contents in Espresso Spent Coffee Grounds

sample	caffeine (mg/100 g, DW)	total CGA[a] (mg/100 g, DW)	5-CQA[b] (mg/100 g, DW)	sample	caffeine (mg/100 g, DW)	total CGA[a] (mg/100 g, DW)	5-CQA[b] (mg/100 g, DW)
A1	nd[c]	nd	nd	n = 7	419.0 ± 81.1a	463.6 ± 72.2a	146.0 ± 28.3a
A2	475.3	371.3	39.7				
A3	464.1	435.7	126.3	E1	464.3	382.1	102.6
A4	666.1	573.7	173.3	E2	420.6	335.0	91.4
A5	527.6	525.4	157.3	E3	446.9	343.4	98.7
A6	250.3	227.0	61.6	E4	237.6	212.1	53.7
n = 6	414.3 ± 203.4a[d]	426.6 ± 136.4a	111.6 ± 58.7a	n = 4	392.3 ± 104.7a	318.1 ± 73.6a	86.6 ± 22.4a
B1	264.0	307.0	95.9	F1	709.2	715.2	141.7
B2	444.0	508.9	144.2	F2	787.7	765.6	240.4
B3	194.0	241.1	67.3	F3	467.9	528.9	170.3
B4	417.1	335.6	90.7	F4	493.7	556.8	180.3
B5	497.7	524.9	158.4	F5	395.6	364.0	101.2
B6	495.8	563.5	158.2	F6	549.9	659.5	216.1
B7	337.9	371.1	111.1	n = 6	567.3 ± 150.9a	598.3 ± 146.2b	175.0 ± 50.2a
B8	546.7	638.0	218.1				
B9	436.4	586.9	130.0	G1	444.9	n.d.	n.d.
B10	254.5	538.8	144.9	G2	589.8	504.1	146.9
B11	445.5	706.1	221.4	G3	389.3	445.7	142.3
B12	305.9	516.6	142.7	G4	772.4	684.1	206.5
n = 12	386.6 ± 112.2a	486.6 ± 141.9a	140.2 ± 46.7a	n = 4	549.1 ± 171.2a	544.6 ± 124.3ab	165.3 ± 35.8a
C1	450.7	304.5	53.8	H1	324.9	279.5	79.6
C2	432.4	471.5	136.7	H2	592.4	625.2	188.0
C3	389.9	438.0	137.6				
n = 3	424.3 ± 31.2a	404.6 ± 88.3a	109.4 ± 48.1a	n = 2	458.6 ± 189.2a	452.3 ± 244.5ab	133.8 ± 76.7a
D1	358.7	389.4	107.9	I1	550.4	758.6	264.2
D2	356.7	487.7	156.5	J1	351.2	412.7	137.0
D3	480.3	448.5	137.1	K1	693.7	625.2	187.7
D4	478.5	562.7	180.1	L1	280.5	446.7	146.6
D5	297.9	355.5	111.5	M1	576.2	506.2	139.8
D6	446.2	481.2	156.9	N1	407.3	407.2	133.1
D7	514.9	520.2	172.1				

[a]CGA, chlorogenic acid. [b]5-CQA, 5-caffeoylquinic acid. [c]nd, not determined. [d]Letters indicate the SCG samples that have statistically significant differences ($p < 0.05$).

represent important revenue for this residue, with potential applications in the pharmaceutical and food industries.[38]

Overall Variability. Sampling was designed to ascertain the limits of compositional variation presented by espresso spent coffee, including therefore several brands, from diversified coffee shops, and prepared with distinct coffee machine types. In general, a high variability was verified, inclusive within brands, thus reducing the significance of any apparent interbrand difference (Tables 1, 2, and 4). Significant differences were achieved for pH levels only between brands A and C (Table 1), which might be related to the blend used. As expected, nitrogen, total lipids (Table 1), and fatty acids (Table 2) were not affected by brand, because most of their changes occur during the roasting process and not during beverage extraction. In contrast, a high variability was observed for the water-soluble components, namely, TSS, acids (pH), total minerals, caffeine, and total CGA. This is a direct consequence of the extraction efficiency, as confirmed by the existence of highly significant correlations between all of these variables. Positive linear correlations were established between TSS and total ashes ($r = 0.531$, $p < 0.001$), caffeine ($r = 0.471$, $p < 0.001$), and total CGA ($r = 0.459$, $p < 0.001$). On the other hand, a negative linear correlation was verified between TSS and pH ($r = -0.429$, $p < 0.005$), indicative of higher amounts of acidic compounds in the TSS.

On the basis of these observations, in the particular case of espresso coffee, the brewing method itself should be the main contributing factor to the compositional variance. The incomplete extraction during brew preparation, even on soluble compounds, gives rise to a diversified pool of bioactivity retained in the extracted grounds, as summarized in Table 5.

In conclusion, this was the first study dealing with the chemical characterization of espresso SCG, revealing distinct features from the industrial ones. Depending on the intended reuse, espresso spent coffee revealed a similar or even greater reuse potential than the one expected from spent grounds obtained from the soluble coffee industry, exhausted of most of its soluble components. Espresso SCG's greater richness in highly pursued natural compounds, such as caffeine and CGA, may represent a considerable economical return, requiring well-defined re-collection and transportation logistics for economic feasibility. Such strategies are already in progress in some countries for spent coffee capsules. SCG's high lipid content and homogeneous fatty acid composition is also an

Table 5. Chemical Composition of Espresso Spent Coffee Grounds

component	av content[a] (± SD)
moisture (g/100 g)	63.0 ± 3.6
total soluble solids (g/100 g)	19.7 ± 3.2
pH	5.7 ± 0.2
nitrogen (g/100 g)	2.3 ± 0.1
crude protein (g/100 g)	14.2 ± 0.7
total fat (g/100 g)	12.5 ± 1.3
caffeine (mg/100 g)	452.6 ± 134.0
5-cafeoylquinic acid (mg/100 g)	140.8 ± 49.5
total chlorogenic acids (mg/100 g)	478.9 ± 138.6
total ashes (g/100 g)	1.9 ± 0.7
K (mg/100 g)	882.4 ± 466.2
Mg (mg/100 g)	220.1 ± 134.1
P (mg/100 g)	153.4 ± 50.3
Ca (mg/100 g)	34.9 ± 12.2
Na (mg/100 g)	20.1 ± 15.0
Fe (mg/100 g)	4.6 ± 2.1
Mn (mg/100 g)	2.7 ± 1.0
Cu (mg/100 g)	2.5 ± 1.2

[a]All components are expressed on a dry basis, except for moisture.

important issue for its potential applications in the energy field, particularly for biodiesel production. Depleted of those components, and along with its richness in N, K, P, and Mg, among others, it can also be regarded as a soil amendment,[8] ensuring that adequate pretreatments are applied, as substrate or solid support in fermentative processes,[2] or in production of low-cost adsorbents for environmental remediation.[1]

AUTHOR INFORMATION

Corresponding Author

*Phone: +351-222 042 8638. Fax: +351-226 093 390. E-mail: sucasal@ff.up.pt.

Funding

We acknowledge FCT (Fundação para a Ciência e a Tecnologia) and FEDER through the COMPETE program under Projects FCOMP-01-0124-FEDER-008703 (FCT/PTDC/AGR-AAM/102447/2008) and PEst-C/EQB/LA0006/2011. R.C. acknowledges a research scholarship within the same project. Marta Oliveira is grateful to FCT for her fellowship SFRH/BD/80113/2011.

Notes

The authors declare no competing financial interest.

REFERENCES

(1) Franca, A. S.; Oliveira, L. S. In *Agricultural Wastes*; Ashworth, G. S., Azevedo, P., Eds.; Nova Science Publishers: New York, USA, 2009; pp 155−189.

(2) Mussatto, S. I.; Carneiro, L. M.; Silva, J. P.; Roberto, I. C.; Teixeira, J. A. *Carbohydr. Polym.* **2011**, *83*, 368−374.

(3) Mussatto, S. I.; Machado, E. M. S.; Martins, S.; Teixeira, J. A. *Food Bioprocess. Technol.* **2011**, *4*, 661−672.

(4) Cruz, R.; Baptista, P.; Cunha, S.; Pereira, J. A.; Casal, S. *Molecules* **2012**, *17*, 1535−1547.

(5) Kostenberg, D.; Marchaim, U. *Environ. Technol.* **1993**, *14* (10), 973−980.

(6) Ramalakshmi, K.; Rao, L. J. M.; Takano-Ishikawa, Y.; Goto, M. *Food Chem.* **2009**, *115*, 79−85.

(7) Buerge, I. J.; Poiger, T.; Müller, M. D.; Buser, H.-R. *Environ. Sci. Technol.* **2003**, *37*, 691−700.

(8) Kondamudi, N.; Mohapatra, S. K.; Misra, M. *J. Agric. Food Chem.* **2008**, *56* (24), 11757−11760.

(9) Oliveira, L. S.; Franca, A. S.; Camargos, R. R. S.; Ferraz, V. P. *Bioresour. Technol.* **2008**, *99*, 3244−3250.

(10) Couto, R. M.; Fernandes, J.; Gomes da Silva, M. D. R.; Simões, P. C. *J. Supercrit. Fluids* **2009**, *51*, 159−166.

(11) Calixto, F.; Fernandes, J.; Couto, R.; Hernández, E. J.; Najdanovic-Visak, V.; Simões, P. C. *Green Chem.* **2011**, *13*, 1196−1202.

(12) AOAC, Association of Official Analytical Chemists. *Official Methods of Analysis*; Horwitz, W., Ed.; AOAC International: Gaithersburg, MD, USA, 2000.

(13) Folstar, P. In *Coffee: Vol. 1 − Chemistry*; Clarke, R. J., Macrae, R., Eds.; Elsevier Science Publishers: New York, USA, 1985; pp 203−222.

(14) International Organization of Standardization. *ISO 5509:2000, Animal and vegetable Fats and Oils Preparation of Methyl Esters of Fatty Acids*; Geneve, Switzerland, 2000.

(15) International Organization of Standardization. *ISO 5508:1990, Animal and Vegetable Fats and Oils − Analysis by Gas Chromatography of Methyl Esters of Fatty Acids*; Geneve, Switzerland, 1990.

(16) Greenberg, A. E.; Clesceri, L. S.; Eaton, A. D. *Standard Methods for the Examination of Water and Wastewater (4500-P)*; American Public Health Association: Washington, DC, USA, 1992; pp 4108−4113.

(17) Oliveira, M.; Casal, S.; Morais, S.; Alves, C.; Dias, F.; Ramos, S.; Mendes, E.; Delerue-Matos, C.; Oliveira, M. B. P. P. *Food Chem.* **2012**, *130*, 702−709.

(18) Chambel, P.; Oliveira, M. B.; Andrade, P. B.; Seabra, R. M.; Ferreira, M. A. *J. Liq. Chromatogr. Relat. Technol.* **1997**, *20* (18), 2949−2957.

(19) Zuorro, A.; Lavecchia, R. J. *Clean. Prod.* **2012**, DOI: 10.1016/j.jclepro.2011.12.003.

(20) Adams, M. R.; Dougan, J. In *Coffee Technology*; Clarke, R. J., Macrae, R., Eds.; Elsevier Science Publishers: New York, USA, 1987; pp 257−287.

(21) Illy, A.; Viani, R. *Espresso Coffee: The Chemistry of Quality*; Academic Press: London, UK, 1995.

(22) Petracco, M. In *Espresso Coffee: The Science of Quality*; Illy, A., Viani, R., Eds.; Elsevier Academic Press, London, UK, 2005; pp 290 − 313.

(23) Yen, W.; Wang, B.; Chang, L.; Duh, P. *J. Agric. Food Chem.* **2005**, *53*, 2658−2663.

(24) Balzer, H. H. In *Coffee: Recent Developments*; Clarke, R. J., Vitzthum, O. G., Eds.; Blackwell Science: London, UK, 2001; pp 18−32.

(25) Bicho, N. C.; Leitão, A. E.; Ramalho, J. C.; Lidon, F. C. *Eur. Food Res. Technol.* **2011**, *233*, 303−311.

(26) Belitz, H.-D.; Grosch, H.; Schieberte, P. In *Food Chemistry*; Springer: Berlin, Germany, 2004; pp 939−969.

(27) Casal, S.; Mendes, E.; Oliveira, M. B. P. P.; Ferreira, M. A. *Food Chem.* **2005**, *89* (3), 333−340.

(28) Silva, M. A.; Nebra, S. A.; Silva, M. J. M.; Sanchez, C. G. *Biomass Bioenerg.* **1998**, *14*, 457−467.

(29) Tokimoto, T.; Kawasaki, N.; Nakamura, T.; Akutagawa, J.; Tanada, S. *J. Colloid Interface Sci.* **2005**, *281*, 56−61.

(30) Morikawa, C. K.; Saigusa, M. *Plant Soil* **2008**, *304*, 249−255.

(31) Ratnayake, W. M. N.; Hollywood, R.; O'Grady, E.; Stavric, B. *Food Chem. Toxicol.* **1993**, *31* (4), 263−269.

(32) Martin, M. J.; Pablos, F.; Gonzalez, A.; Valdenebro, M.; Leon-Camacho, M. *Talanta* **2001**, *54*, 291−297.

(33) Grembecka, M.; Malinowska, E.; Szefer, P. *Sci. Total Environ.* **2007**, *383* (1−3), 59−69.

(34) Esquivel, P.; Jiménez, V. M. *Food Res. Int.* **2012**, *46*, 488−495.

(35) Casal, S.; Oliveira, M. B. P. P.; Alves, M. R.; Ferreira, M. A. *J. Agric. Food Chem.* **2000**, *48* (8), 3420−3424.

(36) Clifford, M. N.; Wilson, K. C. *Coffee: Botany, Biochemistry and Production of Beans and Beverage*; Croom Helm: New York, USA, 1987.

(37) Mussatto, S. I.; Ballesteros, L. F.; Martins, S.; Teixeira, J. A. *Sep. Purif. Technol.* **2011**, *83*, 173−179.

(38) Sung, J.-S.; Go, E.-B.; Shin, H.-S. *Food Sci. Biotechnol.* **2012**, *21* (1), 137−143.