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Recovery of Natural Antioxidants from Spent Coffee Grounds

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ABSTRACT: Spent coffee grounds (SCG) were extracted with an environmentally friendly procedure and analyzed to evaluate the recovery of relevant natural antioxidants for use as nutritional supplements, foods, or cosmetic additives. SCG were characterized in terms of their total phenolic content by the Folin−Ciocalteu procedure and antioxidant activity by the DPPH scavenging assay. Flavonoid content was also determined by a colorimetric assay. The total phenolic content was strongly correlated with the DPPH scavenging activity, suggesting that phenolic compounds are mainly responsible for the antioxidant activity of SCG. An UHPLC-PDA-TOF-MS system was used to separate, identify, and quantify phenolic and nonphenolic compounds in the SCG extracts. Important amounts of chlorogenic acids (CGA) and related compounds as well as caffeine (CAF) evidenced the high potential of SCG, a waste material that is widely available in the world, as a source of natural phenolic antioxidants.

KEYWORDS: *phenolic compounds, antioxidants, solvent extraction, spent coffee grounds, UHPLC-PDA-TOF-MS*

INTRODUCTION

Coffee is one of the most popular and consumed beverages in the world, as attested by the over 7 million tons of green coffee beans produced in 2011.[1] *Coffea arabica* (Arabica) and *Coffea canephora* var. *robusta* (Robusta) are the two main species of the genus *Coffea* that are cultivated for commercial production. Currently, Arabica accounts for about 75% of the world production. It is grown at altitudes over 1000 m and is considered to be superior to Robusta because of its milder and more flavorful taste.[2] Robusta is mostly used by the instant coffee industry for the manufacturing of soluble coffee.[3]

From the production of espresso beverages or soluble coffee, a solid residue known as spent coffee grounds (SCG) is generated. SCG have no commercial value and are currently disposed of as a solid waste or, in some cases, used as fertilizers or burned. Due to their high organic material content[4] and the presence of compounds[5] such as caffeine, tannins, and polyphenols, which can have negative effects on the environment, the disposal of SCG needs to be properly managed. Similarly, burning of SCG can result in the release of greenhouse gases into the atmosphere.[6] This has stimulated efforts to find ways of reducing their environmental impact and/or transforming them into value-added products. The production of biofuels such as ethanol[7] and biodiesel[8,9] the use as a substrate for the cultivation of mushrooms[10] and use as an adsorbent for the removal of basic dyes[11] or heavy metals[12] from wastewater are some of the applications under consideration. Another promising but still relatively unexplored approach is the use of SCG as a raw material for the recovery of functional compounds of potential interest to the food and pharmaceutical industries.[13−16]

As is known, coffee beans contain several classes of health-related chemicals such as phenolic compounds, melanoidins, diterpenes, xanthines, and vitamin precursors.[17,18] Caffeine is the most studied coffee component because of its well-established psychoactive effects and promotion of energy metabolism.[19] Coffee phenolics have attracted much interest in recent years due to their strong antioxidant and metal-chelating properties. These properties are believed to provide in vivo protection against free radical damage and reduce the risk of degenerative diseases associated with oxidative stress.[20]

Chlorogenic acids (CGA) are the main components of the phenolic fraction of green coffee seeds.[21] Several studies demonstrate that the consumption of CGA-rich beverages may result in remarkable health benefits including reduced incidence of atherosclerosis, diabetes, and various types of cancer.[22−24] In addition, the main CGA present in coffee are highly bioavailable, being easily absorbed and/or metabolized throughout the gastrointestinal tract.[25]

CGA are formed by esterification of one molecule of quinic acid and one to three molecules of *trans*-hydroxycinnamic acids, mainly caffeic, ferulic, and *p*-coumaric.[26] During coffee processing, CGA may undergo chemical transformations such as isomerization, hydrolysis, or degradation into lower molecular weight compounds; the high temperature of roasting also leads to a reduction of the amount of CGA by transformation into quinolactones[27,28] and melanoidins.[29,30] Melanoidins are high molecular weight brown-colored compounds originating from the Maillard reaction between amino groups and reducing sugars.[31] They may account for up to about 25% of the dry weight of roasted coffee beans, but their chemical structure remains largely unknown. Coffee melanoidins have high antioxidant activity, which is due, at least in part, to their ability to incorporate or bind noncovalently CGA.[32,33] Evidence to date also suggests

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that they can modulate bacterial growth in the colon, exert anti-inflammatory and antiglycative effects, and, more importantly, inhibit matrix metalloproteinases, a family of *endo*-peptidases that are thought to play a key role in tumor growth and metastasis.[31,34]

Other biologically active coffee components with potential beneficial health effects are nicotinic acid, trigonelline, quinolinic acid, tannic acid, and pyrogallic acid.[35] Antioxidant properties such as reactive oxygen species (ROS) scavenging have also been recently proposed for caffeine, the most abundant alkaloid present in coffee beans.[36−38]

From the above, it follows that the presence of both native and roasting-derived components imparts a wide range of beneficial properties to coffee. Because these compounds are only partially extracted during brewing, the resulting SCG can be considered as a source of potentially valuable bioactive compounds. However, although considerable attention has been devoted to the chemistry and pharmacological effects of coffee, very few studies have been made on the characterization of extracts from SCG.

In a previous study a solvent extraction procedure to obtain phenolic extracts from SCG based on the use of aqueous ethanol as the solvent was developed.[15] By applying this method to the wastes collected from espresso machines and coffee capsules, >90% of the phenolic compounds present in the waste were recovered. In view of these findings, the present study was undertaken to further assess the potential of this approach as a way of adding value to SCG extracts from Arabica and Robusta species by the recovery of valuable antioxidants with beneficial effects on human health.

More specifically the objectives of the present study were (1) to characterize the SCG extracts in terms of their total phenolic content and antioxidant activity, (2) to outline the UHPLC-PDA-TOF-MS profile of CGA and related compounds in the same extracts, (3) to quantify by UHPLC-PDA the main CGA together with the bioactive alkaloid caffeine (CAF), and (4) to evaluate possible changes that might occur in terms of composition and antioxidant activity when replacing aqueous ethanol with pure water as extraction solvent.

MATERIALS AND METHODS

Chemicals and Reagents. Ethanol (CAS Registry No. 64-17-5), methanol (CAS Registry No. 67-56-1), sodium carbonate (CAS Registry No. 497-19-8), hydrochloric acid (CAS Registry No. 7647-01-0), sodium acetate (CAS Registry No. 127-09-3), and aluminum chloride (CAS Registry No. 7446-70-0) were obtained from Carlo Erba (Milano, Italy). Folin−Ciocalteu's phenol reagent, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH, CAS Registry No. 1898-66-4), gallic acid (CAS Registry No. 149-91-7), quercetin (CAS Registry No. 117-39-5), caffeine (CAF, CAS Registry No. 58-08-2), caffeic acid (CA, CAS Registry No. 331-39-5), and 5-caffeoylquinic acid (5-CQA, CAS Registry No. 327-97-9) were purchased from Sigma-Aldrich (Milano, Italy). All chemicals were of reagent grade and used without further purification.

Hypergrade acetonitrile for LC-MS was purchased from Merck (Darmstadt, Germany) and HPLC grade methanol from Sigma-Aldrich. HPLC grade water was prepared with a Direct-Q 3 (Millipore, Vimodrone, Italy) water purification system. Formic acid (98%) was purchased from J. T. Baker. Leucine enkephalin, reference compound, was from Sigma-Aldrich.

Analytical Methodologies. *UHPLC-PDA-TOF-MS Analysis.* Chromatographic analyses were performed on a Waters Acquity UPLC system (Milford, MA, USA) including a binary pumping system, an autosampler with thermostat control set at 10 °C, a high-temperature column heater set at 30 °C, and a photodiode array (PDA) detector.

Separations were carried out on a Waters Acquity BEH C_{18} (50 × 2.1 mm i.d., 1.7 μm) analytical column with A = 0.02% water−formic acid and B = 0.02% acetonitrile−formic acid as mobile phase at a flow rate of 0.6 mL min^{-1}. The elution gradient was as follows: 0−6 min, 5−42% B; 6−7 min, 42−100% B; 7−7.5 min, 100% B; 7.5−8 min, 100−5% B; 8−8.5 min, 5% B. The PDA detector was set to scan in the 210−500 nm range with a frequency of 20 Hz.

The Acquity UPLC system was coupled with a high-resolution (HR) Waters Micromass LCT Premier XE time-of-flight (TOF) mass spectrometer (MS) with an electrospray ionization (ESI) source. Data acquisition was carried out in negative ionization and centroid modes with a m/z 100−1000 mass range; the analyzer was in W mode with a scan time of 0.2 s, and dynamic range enhancement (DRE) was enabled. A solution of leucine enkephalin, as lock mass (C_{12} $[M - H]^-$ at m/z 554.2615) at a concentration of 2 μg mL^{-1} was infused through the lock spray ion source at a flow rate of 5 μL min^{-1}. The lock spray frequency scan was set at 50. Resolution calculated at $[M - H]^-$ at m/z 554.2615 was 10000 at fwhm. The ESI source parameters were as follows: capillary voltage, 1300 V; cone voltage, 30 V; source temperature, 120 °C; desolvation temperature, 450 °C; cone gas flow, 40 L h^{-1}; desolvation gas flow, 800 L h^{-1}. Data acquisition, data handling, and instrument control were performed by MassLynx Software 4.1v.

Total Phenolics Determination. Total phenolic content was determined by using Folin−Ciocalteu's method according to the procedure described by Singleton et al.[39] with some modifications. Briefly, 5 mL of 0.1 M HCl, 150 μL of Folin−Ciocalteu's reagent, and 200 μL of the sample to be tested were poured into a graduated glass vial and an aqueous Na_2CO_3 solution (20% w/v) was added to a final volume of 10 mL. The vial was thoroughly shaken and kept at room temperature in the dark for 1 h. Then, the absorbance at 525 nm was measured with a colorimeter (HI83742, Hanna Instruments, Italy). The total phenolic content was expressed as gallic acid equivalents (GAE) on a dry weight basis (mg GAE per g of solid), using a calibration curve obtained with gallic acid standards.

Flavonoids Determination. Flavonoids were determined using the colorimetric assay described by Chang et al.[40] with some modifications. A volume of 300 μL of the sample to be tested was poured into an optical glass cuvette together with 900 μL of methanol, 60 μL of aluminum chloride at 10% (w/v), 60 μL of sodium acetate (1 M), and 1.7 mL of distilled water. The cuvette was shaken and maintained at room temperature in the dark for 30 min. After this time, the absorbance of the mixture was measured at 415 nm against a blank of distilled water. The total flavonoid content was expressed as quercetin equivalents (QE), using a calibration curve obtained with standard solutions of quercetin.

Antioxidant Activity Determination by DPPH Scavenging Assay. Antioxidant activity was evaluated by using the DPPH assay.[41] Briefly, a DPPH stock solution at 1 g L^{-1} in methanol was first prepared and stored at −20 °C. Prior to performance of the test, this solution was diluted with methanol so as to obtain a working solution at 0.05 g L^{-1}. A volume of 50−300 μL of the sample to be assayed was then poured into an optical glass cuvette, and the working DPPH solution was added to a final volume of 4 mL. After 30 min of incubation at room temperature in the dark, the absorbance at 517 nm was measured against a blank of pure ethanol. The antioxidant activity was expressed as the EC_{50} value, which represents the concentration (% v/v) of the sample in the cuvette necessary to decrease the initial DPPH concentration by 50%.

Preparation and Characterization of SCG. SCG were collected from bars in the city of Rome (SCG-b) and recovered from used coffee capsules (SCG-c). The first type of waste was produced from two different coffee blends, one richer in Robusta (SCG-b1) and the other richer in Arabica (SCG-b2). The second was obtained from an automatic espresso machine and consisted of either regular (SCG-c1) or decaffeinated (SCG-c2) coffee.

Appropriate amounts of SCG were collected on different days, pooled together, and dried to 7−10% residual moisture to prevent microbial growth. Drying was carried out at 40 °C for 12−15 h in an electric forced-air food dehydrator (Stöckli, Switzerland). Dried SCG were stored in the dark at room temperature until use. All of the materials were characterized for moisture and total phenolic content.

Moisture content was determined by an electronic moisture analyzer (model MAC 50/1, Radwag, Poland). A three-stage extraction procedure was used to evaluate the total phenolic content.[42] The extraction was carried out with aqueous ethanol (50% v/v) as the solvent under conditions allowing an almost complete exhaustion of the solid. More specifically, 0.2 g of coffee powder and the appropriate amount of solvent (20, 10, and 5 mL in the first, second, and third stages, respectively) were poured into a glass flask thermostated at 60 °C and stirred for 30 min. After this time, the liquid was separated from the solid, filtered at 0.45 μm, and assayed for total phenolic content.

Sample Preparation. Dried SCG were extracted under conditions close to those found as optimal in previous studies.[15,42] In particular, 2 g of dried SCG and 100 mL of the extraction solvent (pure water or ethanol/water 60:40, v/v) were transferred into thermostated and magnetically stirred glass flasks. The extraction temperature was 60 °C. After a time period of 30 min under continuous stirring, a sample of the liquid was taken, filtered at 0.45 μm, and submitted to analytical determinations.

Sample Preparation for UHPLC-PDA-TOF-MS Analysis. Samples were diluted 1:3 and 1:20 with mobile phase A:B (95:5, v/v) for UHPLC-PDA and UHPLC-TOF-MS analyses, respectively, and filtered (0.22 μm, Millipore, Milan, Italy), and the filtrates were transferred to vials for analysis (1 μL injected). All samples were analyzed in duplicate in two independent runs.

Calibration Standards Preparation. Standard stock solutions of CAF, CA, and 5 CQA were prepared separately in methanol at the concentration of 1 mg mL^{-1} and stored at 4 °C.

Working solutions of each standard at the different concentrations of 5, 10, 20, 50, and 100 μg mL^{-1} were prepared by diluting the stock solutions with the mobile phase A:B (95:5, v/v). The limit of 5 μg mL^{-1} was chosen for quantification, as a lower content of the analytes in coffee solid wastes was not attractive for recovery purposes.

Calibration samples were prepared in triplicate and analyzed in duplicate in two independent runs. Calibration curves were calculated with equal weighted least-squares linear regression analysis of peak area against standard nominal concentration.

RESULTS AND DISCUSSION

SCG collected from bars and recovered from used capsules were extracted, according to the procedures described under Sample Preparation, with pure water and with ethanol/water 60:40 (v/v), and the extracts were analyzed for total phenolic and flavonoid contents and for radical scavenging activity.

The extracts were then analyzed by UHPLC to identify and to quantify the main phenolic and nonphenolic compounds such as CGA and CAF present in coffee beans.

Total Phenolic and Flavonoid Contents. Table 1 shows the total phenolic content of SCG collected from bars and recovered from used capsules. It can be seen that all of the wastes contained large amounts of total phenolics, the measured phenolic content ranging from about 17 to 35 mg GAE g^{-1} dry waste. These values are in line with those reported previously for SCG[15,16,43,44] and are higher than those found for other agroindustrial wastes such as grape pomace, carrot peels, and apple peels, which were below 15 mg GAE g^{-1} dry matter.[45−47] This supports the suitability of SCG as a source of phenolic antioxidants. We also note that the phenolic content of SCG from coffee bars (SCG-b1, SCG-b2) was almost twice that from coffee capsules (SCG-c1, SCG-c2). This may be due to differences in the coffee blends used and/or in the brewing procedure, which is known to affect the amount of phenolics released into the coffee beverage.[16] Examination of the results also reveals that the waste richer in Robusta had a higher phenolic content, in agreement with what is usually observed for this coffee variety.[48,49] In contrast, there were only marginal differences between regular and decaffeinated coffees of the same coffee variety (100% Arabica).

The characteristics of the extracts obtained from SCG using pure water or 60:40 ethanol/water mixture as the solvent are presented in Table 2. The total phenolic content ranged from 6.33 to 28.26 mg GAE g^{-1} dry waste, whereas the total flavonoid content ranged from 2.11 to 8.03 mg QE g^{-1} dry waste. Again, the extracts from bar wastes were richer in phenolics than those from used capsules. The same was observed for flavonoids (Table 2). Examination of the data also reveals that the solvent had a significant effect on the extraction efficiency. On average, the phenolic and flavonoid contents were increased by about 1.5- and 2.2-fold, respectively, when water was replaced by aqueous ethanol. These results indicate that the ethanol/water mixture can dissolve a wider range of phenolic and flavonoid components, which can be attributed to an on-average higher affinity of these compounds for aqueous ethanol than for pure water. However, other effects could also be involved, such as the solvent-induced swelling of the solid matrix or perturbations of the solute−matrix interactions capable of favoring the release of bound solutes.[50]

Radical Scavenging Activity. As indicated by the EC_{50} values in Table 2, for each waste material the use of aqueous ethanol as extraction solvent resulted in higher antioxidant activity. In addition, plotting the EC_{50} value of the various samples against their corresponding total phenolic content showed that these quantities were highly correlated (Figure 1),

Table 1. Moisture and Total Phenolic Content of SCG[a]

waste material	coffee type	moisture content (% w/w)	total phenolics (mg GAE g^{-1})
SCG-b1	40% Arabica + 60% Robusta	7.84 ± 0.18	35.52 ± 1.42
SCG-b2	70% Arabica + 30% Robusta	7.12 ± 0.22	31.92 ± 1.93
SCG-c1	100% Arabica	9.50 ± 0.09	17.45 ± 1.23
SCG-c2	100% Arabica, decaffeinated	9.23 ± 0.25	17.07 ± 0.91

[a]Total phenolics are expressed per gram dry weight. Values are given as the mean ± standard deviation of triplicate determinations.

Table 2. Total Phenolics, Flavonoids, and DPPH Antioxidant Activity (Expressed as EC_{50}) of Extracts Obtained from SCG Using Pure Water or Ethanol/Water 60:40 (v/v) as Extraction Solvent[a]

waste material	extraction solvent (ethanol/water % v/v)	total phenolics (mg GAE g^{-1})	flavonoids (mg QE g^{-1})	EC_{50} (% v/v)
SCG-b1	0:100	19.62 ± 0.02	3.24 ± 0.43	2.33 ± 0.30
	60:40	28.26 ± 0.49	5.63 ± 0.60	1.47 ± 0.07
SCG-b2	0:100	17.43 ± 0.21	3.31 ± 0.11	2.47 ± 0.14
	60:40	23.90 ± 0.22	8.03 ± 0.54	1.71 ± 0.07
SCG-c1	0:100	7.43 ± 0.15	2.11 ± 0.09	5.52 ± 0.32
	60:40	12.58 ± 0.21	5.21 ± 0.35	3.37 ± 0.12
SCG-c2	0:100	6.33 ± 0.36	2.24 ± 0.15	6.74 ± 0.36
	60:40	11.83 ± 0.29	5.01 ± 0.34	3.67 ± 0.13

[a]Total phenolics and flavonoids are expressed per gram dry weight. Values are given as the mean ± standard deviation of triplicate determinations.

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thus suggesting that phenolic compounds are mainly responsible for the antioxidant activity of SCG.

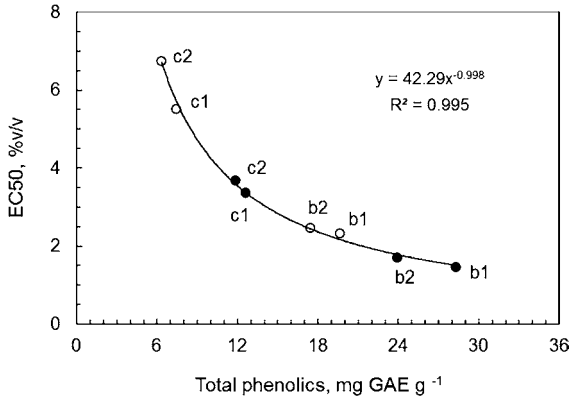

Figure 1. Observed dependence of antioxidant activity, expressed as EC_{50} values, on the total phenolic content of extracts obtained from SCG using pure water (open circles) or ethanol/water 60:40 (solid circles) as extraction solvent. Each data point is labeled with the sample identifier given in Table 1.

UHPLC-PDA-TOF-MS Profiling of SCG Extracts. The simultaneous use of an UHPLC system and a sub-2-μm particle column permitted the entire profile of the SCG extract to be obtained with a 6 min gradient, much less time-consuming than 50−60 min gradients previously reported in the literature for the chromatographic analyses of coffee.[51−55]

As a result, the PDA and the CGA characteristic absorption (λ = 324 nm) chromatograms of the aqueous ethanol extract of SCG-b1 are shown in panels a and b, respectively, of Figure 2.

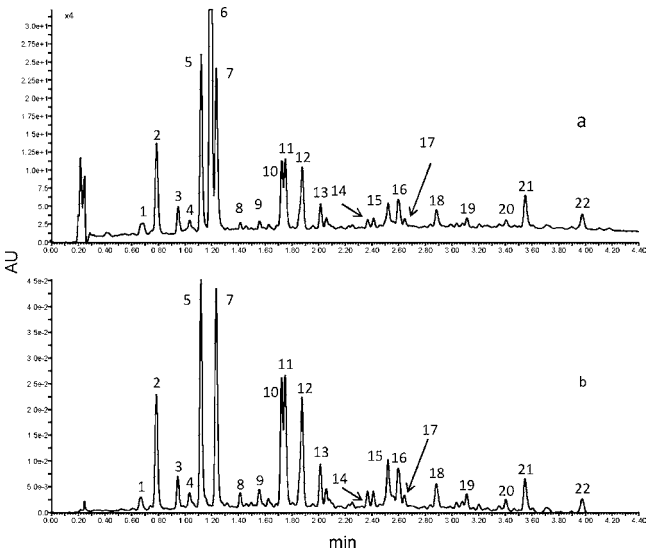

Figure 2. UHPLC profile of the aqueous ethanol extract of SCG-b1: (a) PDA chromatogram (210−500 nm); (b) chromatogram extracted at λ = 324 nm.

5-CQA, CAF, and CA in the SCG extracts were identified by comparison of retention times (t_R) and UV absorbance spectra with those of the corresponding analytical standards. Therefore, peaks 5 and 6 (Figure 2a) were identified as 5-CQA and CAF, respectively, whereas CA (t_R = 1.36 min, λ_{max} = 324 nm) was hardly detectable in all of the samples.

The HR-TOF-MS analyzer was used as a powerful tool for the identification of CGA and their derivatives as deprotonated molecules $[M - H]^-$. The extracted ion chromatograms (tolerance 0.05 Da) of the CGA and related compounds in the aqueous ethanol extract of SCG-b1 are shown in Figure 3.

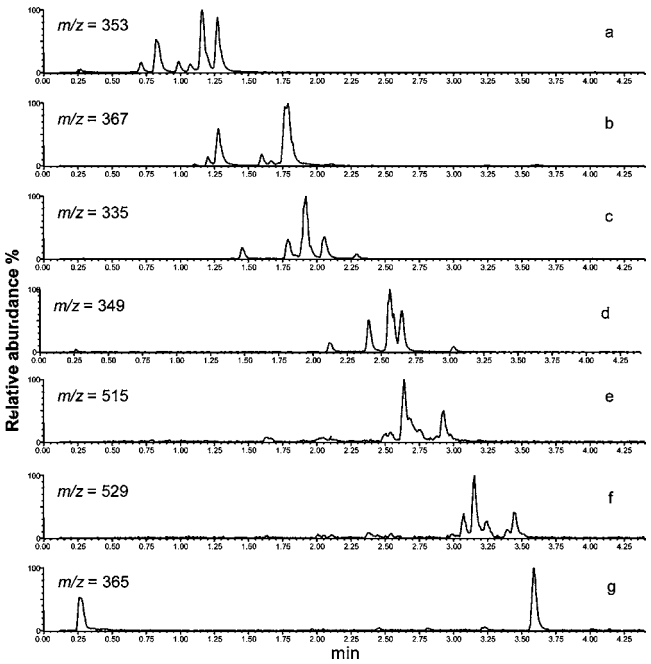

Figure 3. $[M - H]^-$ extracted ion chromatograms of the CGA and related compounds in the aqueous ethanol extract of SCG-b1.

The assignment of the peaks was supported also by UV absorbance spectra that may be grouped into two types in agreement with those reported in the literature:[53] profile A, with λ_{max} between 322 and 326 nm, characteristic of all CGA, di-CGA, quinolactones (CQL and FQL, respectively); and profile B, characteristic of cinnamoyl-tryptophans (Figure 4A,B).[54,56] The UV spectrum of CAF is also reported in Figure 4C.

In Figure 3a all isobaric compounds with $[M - H]^-$ at m/z 353.0873 were assigned to caffeoylquinic acids (CQA), the most abundant group of CGA, which correspond to peaks 1, 2, 3, 4, 5, and 7 in Figure 2b; peak 5 was assigned to 5-CQA as previously mentioned, and peaks 2 and 7 may be assigned to the isomers 3-CQA and 4-CQA, on the basis of their elution order under similar experimental conditions reported in the literature,[26] whereas peaks 1, 3, and 4 are due to other isomers.[57] All compounds showed an absorbance spectrum of profile A.

In Figure 3b all isobaric compounds with $[M - H]^-$ at m/z 367.1029 were assigned to feruloylquinic acids (FQA), which correspond to peaks 9, 10, and 11 in Figure 2b; peak 10 may be assigned to 5-FQA on the basis of data reported in the literature;[26] another FQA isomer coelutes with a CQA isomer (peak 7, Figure 2b). All compounds had also very similar absorbance spectra of profile A.

The third most abundant group of chromatographic peaks was assigned to quinolactones: in Figure 3c all isobaric compounds with $[M - H]^-$ at m/z 335.0767 were assigned to CQL; three of these correspond to peaks 8, 12, and 13 in Figure 2b, whereas another one coelutes with a FQA isomer (peak 11, Figure 2b). In Figure 3d all isobaric compounds with $[M - H]^-$ at m/z 349.0923 were assigned to FQL, corresponding to peaks 14, 15,

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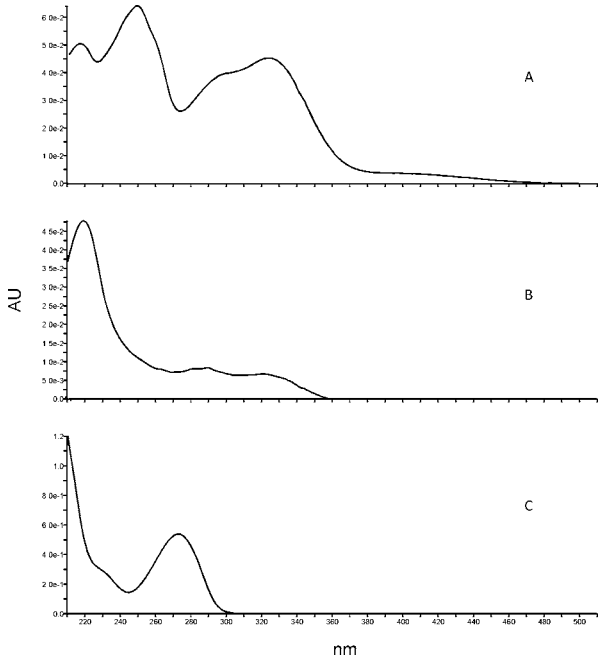

Figure 4. UV spectra profiles of the compounds identified in SCG extracts (A and B) and of CAF (C).

and 16 in Figure 2b. All compounds showed an absorbance spectrum of profile A.

The last group of chromatographic peaks was assigned to di-CGA: all isobaric compounds with $[M - H]^-$ at m/z 515.1190 (Figure 3e) were assigned to di-CQA, which correspond to peaks 17 and 18 in Figure 2b, whereas another one coelutes with a FQL isomer (peak 16). All isobaric compounds with $[M - H]^-$ at m/z 529.1346 (Figure 3f) were assigned to caffeoyl-feruloylquinic acid (CFQA),[26] which correspond to peaks 19 and 20 in Figure 2b. All of these compounds showed an absorbance spectrum of profile A.

Peak 21, with a different UV spectrum (profile B) and with $[M - H]^-$ at m/z 365.1137 (Figure 3g), was assigned to one caffeoyltryptophan, according to the literature.[54,57]

Peak 22, with $[M - H]^-$ at m/z 497.1084 (data not shown), might be assigned to a dicaffeoylquinic lactone (di-CQL).[51]

No significant peaks with $[M - H]^-$ at m/z 337.092 were observed, suggesting that only traces of coumaroylquinic acids (CoQA) may be present in SCG extracts (data not shown).

All retention times, monoisotopic deprotonated molecules, and UV profiles data are summarized in Table 3.

From a qualitative point of view, UHPLC profiles of all the samples are very similar with the exception of the decaffeinated SCG-c2, lacking peak 6 of CAF as expected, and they are also quite similar to HPLC profiles of CGA in green or roasted coffee beans reported in the literature.[51,54] In addition, no differences with the exception of the amounts were found in the profile of the extracts of coffee capsules before and after beverage preparation (data not shown).

In general, by comparing the chromatograms of all the samples, in both aqueous and aqueous ethanol media, only slight differences were evidenced: (a) the extracts of used capsules (SCG-c) appear lightly richer in quinolactones and di-CQA than SCG-b extracts, which might be due to differences in the degree of roasting of coffee that was demonstrated to highly affect the isomerization and transformation of CGA in quinolactones;[28] (b) the aqueous ethanol extracts appear lightly richer in quinolactones and di-CGA than the aqueous extracts, due to the higher affinity of these compounds for aqueous ethanol than for pure water.

Table 3. Peak Number, Retention Time (t_R), Monoisotopic Deprotonated Molecule ($[M - H]^-$), and UV Profile of Compounds Identified in SCG Extracts

peak	t_R (min)	$[M - H]^-$ (m/z)	UV	compound
1	0.67	353.0873	A	CQA
2	0.79	353.0873	A	CQA
3	0.95	353.0873	A	CQA
4	1.03	353.0873	A	CQA
5	1.12	353.0873	A	5-CQA
6	1.19		C	CAF
7	1.23	353.0873 + 367.1029	A	CQA + FQA
8	1.41	335.0767	A	CQL
9	1.56	367.1029	A	FQA
10	1.72	367.1029	A	FQA
11	1.75	367.1029 + 335.0767	A	FQA + CQL
12	1.88	335.0767	A	CQL
13	2.01	335.0767	A	CQL
14	2.37	349.0923	A	FQL
15	2.52	349.0923	A	FQL
16	2.60	349.0923 + 515.1190	A	FQL + di-CQA
17	2.64	515.1190	A	di-CQA
18	2.88	515.1190	A	di-CQA
19	3.11	529.1346	A	CFQA
20	3.40	529.1346	A	CFQA
21	3.55	365.1137	B	caffeoyltryptophan
22	3.98	497.1084	A	di-CQL

UHPLC-PDA Quantitative Analysis. 5-CQA and CAF were quantified by comparing their chromatographic peak areas at 324 and 275 nm, respectively, with the calibration curves of the corresponding analytical standards recorded at the same wavelengths. CA, detected only in SCG-b2 and SCG-c1, was always under the LOQ.

CGA and Derivatives. CGA and their derivatives identified in the SCG as previously described were quantified as CQAE by comparing their total chromatographic peak area at 324 nm with the standard 5-CQA calibration curve. In particular, all of the peaks assigned to CQA (peaks 1, 2, 3, 4, 5, 7), FQA (peaks 9, 10, 11), CQL (peaks 8, 12, 13), and FQL (peaks 14, 15, 16) were integrated. Quantitative data are summarized in Table 4.

Their total content ranged from 1.65 to 6.09 mg CQAE g^{-1} dry waste: no significant differences were found between SCG-b1 and SCG-b2 from coffee bars (5.67−6.09 mg CQAE g^{-1} dry waste), having CQAE content that is more than twice the one in SCG-c from used capsules (1.65−2.26 mg CQAE g^{-1} dry waste), in agreement with the total phenolics data discussed above. On the other hand, a difference between SCG-c1 and SCG-c2 was found, the content of CQAE being about 20% lower in the SCG of decaffeinated capsules, in agreement with the literature for decaffeinated coffee samples.[57,58]

No significant advantages were found upon the change from pure water to aqueous ethanol as extraction solvent, indicating that predominant CGA are well extracted with water. In fact, only a very small increase in CQAE was found in the aqueous ethanol extracts (Table 4), probably due to the higher fraction of quinolactones extracted. In contrast, an increase of about 8% of CQAE in the aqueous ethanol extract with respect to the aqueous extract was found in the decaffeinated sample SCG-c2, in

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Table 4. Total CGA and CAF Determined by UHPLC-PDA in the Extracts Obtained from SCG Using Pure Water or Ethanol/Water 60:40 v/v) as Extraction Solvent[a]

waste material	extraction solvent (ethanol/water % v/v)	total CGA (mg CQAE g^{-1})	CAF (mg g^{-1})
SCG-b1	0:100	5.67 ± 0.14	11.23 ± 0.11
	60:40	5.97 ± 0.11	11.50 ± 0.21
SCG-b2	0:100	6.00 ± 0.11	6.00 ± 0.05
	60:40	6.09 ± 0.11	5.99 ± 0.16
SCG-c1	0:100	2.15 ± 0.06	0.97 ± 0.01
	60:40	2.26 ± 0.06	0.96 ± 0.03
SCG-c2	0:100	1.65 ± 0.06	0.00 ± 0.00
	60:40	1.81 ± 0.05	0.00 ± 0.00

[a]Values are expressed per gram dry weight and given as the mean ± standard deviation of triplicate determinations.

agreement with the higher content in quinolactones of decaffeinated coffee samples, as also reported in the literature.[59]

In general, by comparison of data in Tables 2 and 4, CGA and derivatives represent almost 30% of the total phenolic content found with the FC assay for SCG aqueous extracts, even if the amounts are not severely comparable. These data suggest that an interesting amount of remarkable bioactive and high bioavailable compounds such as CGA can be recovered under environmentally friendly conditions from SCG.

Caffeine. Amounts of CAF in the range of 5.99−11.50 mg g^{-1} dry matter were found in the extracts of SCG from coffee bars: the higher amount was found in SCG-b1, and it was nearly twice that found in SCG-b2, the difference likely due to the higher content of Robusta (see Table 1), richer in CAF than Arabica,[60] in the coffee blends that produced SCG-b1. No significant differences were found between aqueous and aqueous ethanol extracts (see Table 4).

On the contrary, a very low level of CAF was found in the extracts SCG-c1 from spent coffee capsules (0.96−0.97 mg g^{-1} dry sample), whereas no CAF was detected in the extracts of decaffeinated samples SCG-c2, as expected.

In conclusion, the results of the present study provide clear evidence of the potential of SCG, a waste material that is widely available in the world, as a source of natural phenolic antioxidants. In particular, analysis of the extracts showed that a relatively high percentage of the recovered phenolic compounds consisted of residual chlorogenic acids, whose beneficial effects on human health have received increasing attention in recent years. Another interesting result is that the extraction solvent has a significant effect on the amount of total phenolics and flavonoids obtained from SCG, but not on total chlorogenic acids, thus supporting the idea of using pure water to produce extracts rich in chlorogenic acids.

The presence of important amounts of caffeine in the extracts should be considered in the evaluation of possible fields of application of these extracts, which could include, among others, the energy drink industry and the cosmetic sector. On the other hand, caffeine-free SCG, as are those from the manufacturing of decaffeinated soluble-coffee products, could be used to produce phenolic-rich extracts for a variety of food and nutraceutical applications.

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Notes

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