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Fractionation of spent coffee ground with tertiary amine extraction

Kaavya Rathnakumar [a], Juan C. Osorio-Arias [b,c], Padmanaban Krishnan [a], Sergio I. Martínez-Monteagudo [a,d,e,*]

[a] Dairy and Food Science Department, South Dakota State University, SD, United States
[b] BIOALI Research Group, Faculty of Pharmaceutical and Food Science. Universidad de Antioquia, Medellin, Colombia
[c] Corporación Universitaria Minuto de Dios - UNIMINUTO Sede Presencial Research Direction, Bogotá DC, Colombia
[d] Family and Consumer Sciences, New Mexico State University, Las Cruces, NM 88003, United States
[e] Department of Chemical & Materials Engineering, New Mexico State University, Las Cruces, NM 88003, United States

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ABSTRACT

Spent coffee ground (SCG) was fractionated with a tertiary amine (N,N dimethylcyclohexylamine, CyNMe2) to obtain a carbohydrate fraction rich in dietary fiber, a phenolic fraction made of chlorogenic acids (CGAs), and a lipid fraction mainly consisted of palmitic and linoleic acid. The extraction time (6–24 h) and temperature (25–45 °C) were evaluated at a constant solvent-to-sample ratio (12:1, v/w). The carbohydrate fraction obtained with CyNMe2 showed relative high values of total dietary fiber (63 to 83%). The highest concentration of phenolic compounds was obtained after 24 h at 25 °C. Seven major species of chlorogenic acids were detected within the phenolic fraction, ranging from 1.83 to 7.16 mg g^{-1} of dry matter. The lipid fraction obtained with CyNMe2 was overestimated by 186% (13.91 ± 2.3 g 100 g^{-1}) over the Soxhlet extraction (7.48 g 100 g^{-1}) due to an incomplete conversion of the amine into its respective salt.

1. Introduction

Coffee ranks highest amongst consumed beverages worldwide, with an estimate of about 2 billion cups consumed every day [28]. The brewing of coffee beans generates a large amount of wet residue – about 2 kg of wet residue per kg of brewed drink [20,23]. This wet residue is known as spent coffee ground (SCG), and it represents up to 90% of the total weight of the fresh bean [34]. Overall, SCG is made of carbohydrates (45–47%, $_{\text{d.b.}}$), proteins (13–17%, $_{\text{d.b.}}$), lipids (9–16%, $_{\text{d.b.}}$), and phenolic compounds (2–4%, $_{\text{d.b.}}$) [29,30]. Such composition makes the SCG a promising feedstock for fractionation and subsequent recovery of valuable compounds [11].

There are three main approaches used to fractionate SCG – (1) physical and chemical pre-treatments, which improves the extractability of a group of compounds; (2) novel extraction strategies, such as supercritical carbon dioxide and supramolecular solvents [36]; and (3) combinations of pretreatments and extraction techniques. State of the art regarding the processing of coffee byproducts (pulp, silver skin, and SCG) can be found elsewhere [4].

The carbohydrate fraction recovered from SCG has been used as dietary fiber in food formulations [37]. Another fraction of commercial interest is the phenolic compounds or antioxidants, where chlorogenic acids (CGAs) represent the major class of phenolic compounds [24]. A third fraction of commercial relevance recovered from SCG is the lipid portion [14], which can be used as a culinary ingredient and platform chemical.

Recently, tunable solvents or switchable solvents have been an attractive alternative for the valorization of agriculture byproducts, where the extraction and separation of compounds occur without distillation[17]. These types of solvents consist of a CO_2-responsive species that abruptly and reversible switches from polar to non-polar, high ionic strength to low ionic strength, and hydrophilic to hydrophobic in the presence or absence of CO_2 [17]. Alshamrani, Vanderveen, and Jessop [3] listed 13 CO_2-responsive compounds known for their ability to serve as switchable solvents, including amines, amidines, and guanidines. Tertiary amine such as N,N dimethylcyclohexylamine (CyNMe2) has been used as switchable hydrophilicity solvent for the extraction of phospholipids from dairy byproducts [12], lipids from freeze-dried microalgae [9], and phenol compounds from the lignin [15]. Miscibility changes of CyNMe2 are due to a reversible acid-base reaction between hydrated CO_2 or carbonic acid in the carbonated water and the amine [3]. In this paper, we report the fractionation of

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[*] Corresponding author at: Department of Chemical & Materials Engineering, New Mexico State University, NM, United States
E-mail address: Sergiomm@nmsu.edu (S.I. Martínez-Monteagudo).

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SCG using CyNMe2 as a switchable hydrophilicity solvent. The fractionation involves a one-step process to extract and separate dietary fiber, phenolic compounds, and lipids from SCG.

2. Materials and methods

2.1. Materials

N,N-dimethylcyclohexylamine (99%, Sigma Aldrich, St. Louis, MO, USA), hexane (99.9%, Sigma Aldrich), HPLC-grade water (Sigma Aldrich), gallic acid ($\geq$98.8%, Sigma Aldrich), Folin-Ciocalteu (2.0 N) (Merck Group Darmstadt, Germany), caffeine ($\geq$99.8%, Merck Group), and standards of chlorogenic acids (CGAs) were purchased from commercial suppliers. Standards of CGAs include 5-caffeoylquinic acid (5-CQA), 3-caffeoylquinic acid (3-CQA), 4-caffeoylquinic acid (4-CQA), caffeic acid (CA), 3,4-di-caffeoylquinic acid (3,4 di-CQA), 4,5-di-caffeoylquinic acid (4,5 di-CQA), and 3,5-di-caffeoylquinic acid (3,5 di-CQA). Spent coffee ground was obtained from the university cafeteria at the South Dakota State University (Brookings, SD, USA). The SCG consisted of different coffee blends, and it was used without any pretreatment.

2.2. Composition of spent coffee ground

Spent coffee ground was analyzed for moisture content, total protein, total lipids, total carbohydrates, and ash content. The moisture content was determined gravimetrically using the vacuum oven method, according to the Association of Official Analytical Chemists (AOAC) method 968.11 [5]. Kjeldahl method (method 984.13) was used to determine total protein, while Soxhlet extraction (method 920.39) was used to determine the total fat content. Ash content was determined by incinerating the samples at 550 °C for 4 h (method 942.05). Total carbohydrates were determined by difference.

2.3. CyNMe2 extraction and fractionation

CyNMe2 extraction was carried out following the methodology reported elsewhere [12,33]. Briefly, 1 g of SCG was transferred into a 50 mL plastic tube containing 12 mL of CyNMe2. Then, the vials were kept at three different temperatures (25, 35, and 45 °C) for a predetermined extraction time (6, 12, or 24 h) under constant stirring using a magnetic stirrer (1500 rpm). At the end of the extraction time, vials were brought to room temperature and centrifugated at 4200 rpm for 10 min to obtain the carbohydrate fraction, exhausted SCG ((3) in Fig. 1). The supernatant ((4) was transferred to 20 mL glass vial, where 12 mL of water were added to maintain the stoichiometry of the reaction ((4) Fig. 1). Then, vials were bubbled with CO_2 (purity 99.9%, Praxair, Sioux Falls, SD) at room temperature for 3 h using a nitrogen evaporator (Organomation Associates Inc, Berlin, MA, USA) ((5) and (6) in Fig. 1). The bottom phase containing the water-soluble compounds was recovered and stored in the dark at −18 °C prior to HPLC analysis ((7) in Fig. 1). The lipid fraction (upper phase) was dissolved with 10 mL of hexane and transferred to a test tube for hexane evaporation at 30 °C under nitrogen flow (purity 99.9%, Praxair) ((8) in Fig. 1). Fig. 1 illustrates the fractionation of SCG with CyNMe2 to obtain carbohydrates, phenolic compounds, and lipids.

2.4. Analysis of different fractions

2.4.1. Carbohydrate fraction

The carbohydrate fraction was analyzed for total dietary fiber (TDF) according to the AOAC guidelines (method 991.43) with ANKOM^{TDF} Dietary Fiber Analyzer (ANKOM Technology, Macedon, NY).

2.4.2. Phenolic fraction

The aqueous fraction recovered after CyNMe2 extraction was analyzed for total polyphenol content (TPC) with the Folin-Ciocalteu method, following the guidelines reported elsewhere [30]. For untreated SCG, 1.0 g of SCG was mixed 12 mL of methanol: water (50:50, v/v) pH 2.0 and acetone: water (60:40, v/v) for 60 min at room temperature. Afterward, the mixture was centrifuged, and the supernatant containing the phenolic compounds was stored at −18 °C in amber flasks. In the case of CyNMe2 extracts, 50 μL of the aqueous extract was diluted in 4000 μL distilled water. Then, an aliquot of 25 μL was transferred to a test tube containing 25 μL of Folin-Ciocalteu reagent and 150 μL sodium carbonate (20% wt). The test tube was let stand for 60 min in the dark prior to reading the absorbance at 725 nm using a spectrophotometer (VARIAN Cary 50 UV–vis, Agilent Co., Santa Clara, CA). The results were expressed as mg of gallic acid equivalents per g of dry matter, according to a calibration curve of gallic acid (0 to 1000 ppm).

The concentration of CGAs on the aqueous fraction obtained from CyNMe2 extraction was determined by HPLC according to the methodology reported elsewhere [30]. The HPLC instrument (Agilent Model 1260, Agilent, Santa Clara, CA, USA) was equipped with a diode array detector, and the CGAs were eluted with an Agilent column (Zorbax Eclipse XDB C18, 4.6 × 150 mm, 3.5 μm). Aliquots of 25 μL were

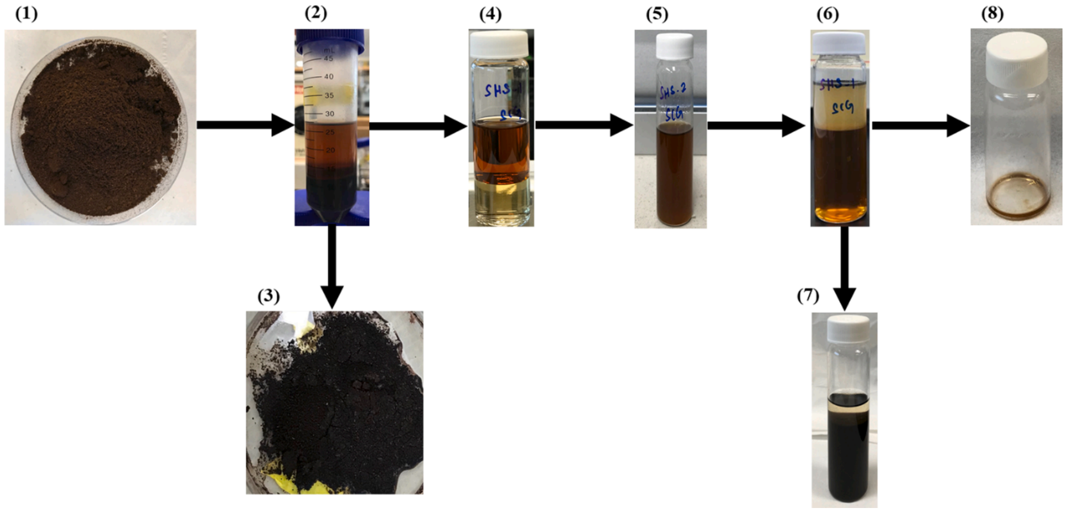


Fig. 1. Illustration of fractionation of spent coffee ground (SCG) with N,N dimethylcyclohexylamine (CyNMe2). (1) SCG prior to extraction; (2) after CyNMe2 extraction; (3) exhausted SCG containing dietary fiber; (4) addition of water to maintain the stoichiometric of the reaction; (5) after bubbling CO_2 to separate the lipid phase; (6) CyNMe2 conversion into salt; (7) phenolic fraction after centrifugation; and (8) lipid fraction after nitrogen evaporation.

injected into the HPLC system, and the identified CGAs were 5-caffeoylquinic acid (5-CQA), 3-caffeoylquinic acid (3-CQA), 4-caffeoylquinic acid (4-CQA), caffeic acid (CA), 3,4-dicaffeoylquinic acid (3,4 di-CQA); 4,5-dicaffeoylquinic acid (4,5 di-CQA), 3,5-dicaffeoylquinic acid (3,5 di-CQA). The total content of CGAs was calculated by the sum of individual quantification and reported as a dry base percentage of CGAs. The aqueous fraction was also analyzed for caffeine content according to the methodology described elsewhere [38] with some modifications. The absorbance at 273 nm was recorded, and the concentration of caffeine was calculated by an external standard with a 4-point calibration curve.

2.4.3. Lipid fraction

The lipid fraction extracted with CyNMe2 was quantified gravimetrically according to the methodology reported elsewhere [12]. The fatty acid profiles of the lipid fraction were determined by GC, according to the AOAC Official Methods [5]. Samples were analyzed by the Agricultural Experiment Station Chemical Laboratories at the University of Missouri-Columbia.

2.5. Experimental design and statistical analysis

A factorial design consisted of two variables (temperature and time) with three levels (25, 35, and 45 °C, and 6, 12, and 24 h, respectively) was used to evaluate the fractionation of SCG with CyNMe2. Each data point was performed in triplicates, and the data were expressed as mean ± standard deviation (SD). Comparison of mean values was performed with two-way analysis of variance using Tukey's post hoc ($p < 0.05$). All figures and statistical analyses were made using Sigmaplot software V11 for Windows (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Composition of spent coffee ground

Table 1 presents the gross composition of SCG prior to the CyNMe2 extraction. Carbohydrates were the most predominant group of compounds in SCG (70.57 ± 0.14%, $_{\text{d.b.}}$), followed by total protein (15.44 ± 0.03%, $_{\text{d.b.}}$) and total fat (14.31 ± 0.02%, $_{\text{d.b.}}$). Overall, the composition of SCG (Table 1) lies within the wide range of values reported in the literature, 60–82%, 10–15% $_{\text{d.b.}}$, and 6–14% $_{\text{d.b}}$ for total carbohydrates, total protein, and total fat, respectively [6,13,18,30].

3.2. CyNMe2 fractionation of SCG

3.2.1. Carbohydrate fraction

The recovered fraction after centrifugation of the SCG-CyMNe2 mixture was analyzed for TDF ((3) in Fig. 1). Overall, CyNMe2 extraction yielded values for TDF between 70 and 83% (Fig. 2). After 6 h of extraction, the TDF values from the exhausted SCG varied narrowly from 68 to 70%, regardless of the extraction temperature. Contrary, the extraction temperature become a significant factor after 12 h of CyNMe2. For instance, the TDF increased from 69.52 ± 1.98 to 83.07 ± 0.77 and 83.11 ± 0.95% at 25, 35, and 45 °C, respectively. As the extraction proceeded (up to 24 h), the TDF values decreased from about 83% to 76 and 70% at 45 and 35 °C, respectively.

Table 1
Gross composition of spent coffee ground.

Parameter	Value
Moisture content (%)	46.76 ± 0.12
Total protein (%, $_{\text{d.b.}}$)	15.44 ± 0.03
Total fat (%, $_{\text{d.b.}}$)	14.31 ± 0.02
Ash content (%, $_{\text{d.b.}}$)	1.56 ± 0.01
Total carbohydrates (%, $_{\text{d.b.}}$)	70.57 ± 0.14
Total dietary fiber (%)	86.30 ± 0.02
Caffeine (mg g^{-1})	4.14 ± 0.02
Total chlorogenic acids (mg g^{-1})	7.41 ± 0.04

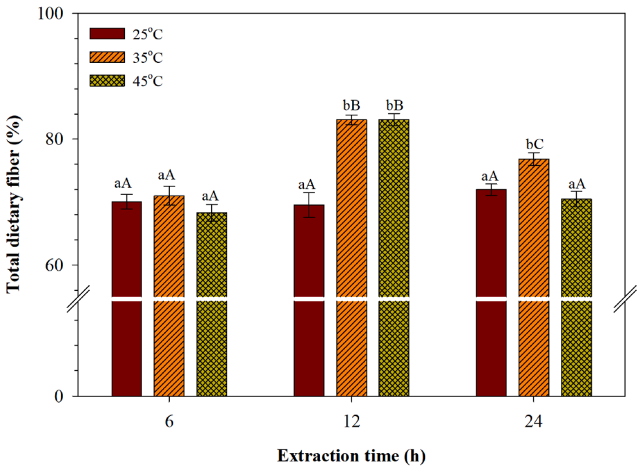


Fig. 2. Total dietary fiber obtained from the carbohydrate fraction after N,N dimethylcyclohexylamine (CyNMe2) extraction from spent coffee ground. Mean ± standard deviation within each column with different letters (a, b, c: among temperature; and A, B, C: among extraction time) are significantly different ($p < 0.05$) according to Tukey test.

3.2.2. Phenolic fraction

The remaining aqueous phase (water-soluble) after the conversion of CyNMe2 into its respective salt was analyzed for total phenolic compounds and chlorogenic acids ((7) in Fig. 1). Overall, the fraction of phenolic compounds was not significantly influenced by the temperature (25, 35, and 45 °C) after 6 and 12 h of extraction, yielding values between 488 and 526 mg g^{-1} (Fig. 3**a**). Similar values were observed after 24 h of extraction at 35 and 45 °C (484.71 ± 74.21 and 491.04 ± 49.81 mg g^{-1}, respectively). Contrary, the highest concentration of TPC (633.79 ± 34.21 mg g^{-1}) was found after 24 h of extraction at a temperature of 25 °C. Chlorogenic acids accounts for about 90 to 95% of the total phenolic compounds in SCG[30]. Fig. 3**b** shows the total CGAs extracted from SCG with CyNMe2. Overall, the highest concentration of CGAs was obtained at 25 °C (4.9–6.7 mg g^{-1}), followed by 35 and 45 °C (2.4–5.6 and 1.8–4.8 mg g^{-1}, respectively), regardless of the extraction time. At 25 °C, the concentration of CGAs decreased with increasing the extraction time, from 6.73 ± 0.33 after 6 h of extraction to 4.92 ± 0.20 and 5.53 ± 0.31 mg g^{-1} after 12 and 24 h, respectively. Similar trend but less pronounced was observed at 35 and 45 °C, where the concentration of CGAs decreased from 5.59 ± 0.59 and 4.07 ± 0.56 mg g^{-1} after 6 h to 2.45 ± 0.53 and 2.12 ± 0.22 mg g^{-1} after 24 h, respectively.

3.2.3. Lipid fraction

Fig. 4 shows the lipid fraction recovered from SCG with CyNMe2 ((8) in Fig. 1). Overall, the concentration of lipids ranged from 8.28 to 11.54%, $_{\text{d.b.}}$ without any trend with respect to temperature neither extraction time. The highest concentration of lipids (11.54 ± 1.36%, $_{\text{d.b.}}$) was obtained after 12 h at 45 °C. The concentration of lipids after CyNMe2 extraction was significantly higher than the concentration obtained with Folch and Soxhlet extraction (7.22 ± 0.95 and 7.48 ± 1.01%, $_{\text{d.b}}$, respectively). An incomplete conversion of CyNMe2 into its respective salt can lead to an overestimation of the total lipids. The lipid fraction was also analyzed to determine the fatty acid profile (Table 2), where the predominant fatty acid was linoleic acid (C18:2 *n6*) accounting for 44.50 ± 0.11% of the total fat, followed by palmitic acid (C16:0) and oleic acid (C18:1), representing 33.24 ± 0.02 and 8.04 ± 0.04% of the total fat, respectively. The concentration of these major fatty acids was within the range reported elsewhere [19].

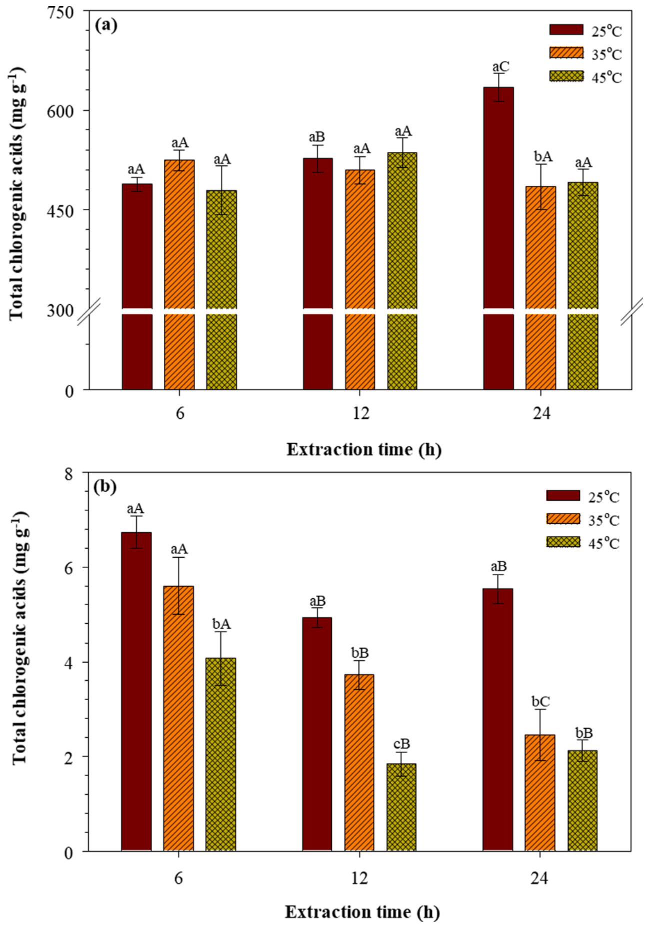


Fig. 3. Total phenolic compounds (a) and total chlorogenic acids (b) obtained after N,N dimethylcyclohexylamine (CyNMe2) extraction from spent coffee ground. (a) and (b). Mean ± standard deviation within each column with different letters (a, b, c: among temperature; and A, B, C: among extraction time) are significantly different ($p < 0.05$) according to Tukey test.

4. Discussion

The gross composition of SCG (Table 1) is within the range of that reported in the literature [6]. A quite diverse composition of SCG can be found in the literature due to the type of beans, origin, roasting and grinding conditions, storage, and brewing practices [10]. Detailed composition of SCG allows interpretation and comparison of the recovered fractions reported in the literature.

Fractionation and re-use of SCG is an issue that has come to the forefront globally. In this work, SCG was fractionated with a tertiary amine (CyNMe2) to produce three fractions – 1) carbohydrate fraction that was rich in dietary fiber; 2) phenolic fraction that was mainly made of CGAs; and 3) lipid fraction predominantly made of linoleic and palmitic acid. Overall, CyNMe2 is a commercially available amine having low miscibility with water at atmospheric conditions. This amine is converted into bicarbonate salt, water-soluble, in the presence of CO_2, switching its hydrophilicity. Conversion of CyNMe2 into bicarbonate salt is reversible upon removal of CO_2, which allows it to fractionate SCG. The conversion of CyNMe2 into bicarbonate salt is due to an acid-base reaction, where uncharged basic group is converted to a protonated cationic state, according to Scheme 1. The ions create an ionic field that

Table 2
Fatty acid composition (% of the total fatty acids) profile of the lipid fraction extracted from spent coffee ground using N,N dimethylcyclohexylamine (CyNMe2).

Name	Generic formula	% Fatty acid
Myristic acid	C14:0	0.43 ± 0.10
Pentadecanoic acid	C15:0	0.03 ± 0.01
Palmitic acid	C16:0	33.24 ± 0.02
Palmitoleic Acid	C16:1 *n7*	0.02 ± 0.01
Heptadecanoic acid	C17:0	0.11 ± 0.01
Stearic acid	C18:0	7.31 ± 0.04
Oleic acid	C18:1	8.04 ± 0.04
Cis-vaccenic acid	C18:1 *n7 c*	0.48 ± 0.01
Linoleic acid	C18:2 *n6*	44.50 ± 0.11
α-Linolenic acid	C18:3 *n3*	1.39 ± 0.01
Stearidonic acid	C18:4 *n3*	0.05 ± 0.01
Arachidic acid	C20:0	2.92 ± 0.02
Eicosenoic acid	C20:1 *n9*	0.33 ± 0.01
Heneicosanoic acid	C21:0	0.09 ± 0.01
Behenic acid	C22:0	0.64 ± 0.07
Tricosanoic acid	C23:0	0.09 ± 0.02
Lignoceric acid	C24:0	0.21 ± 0.02

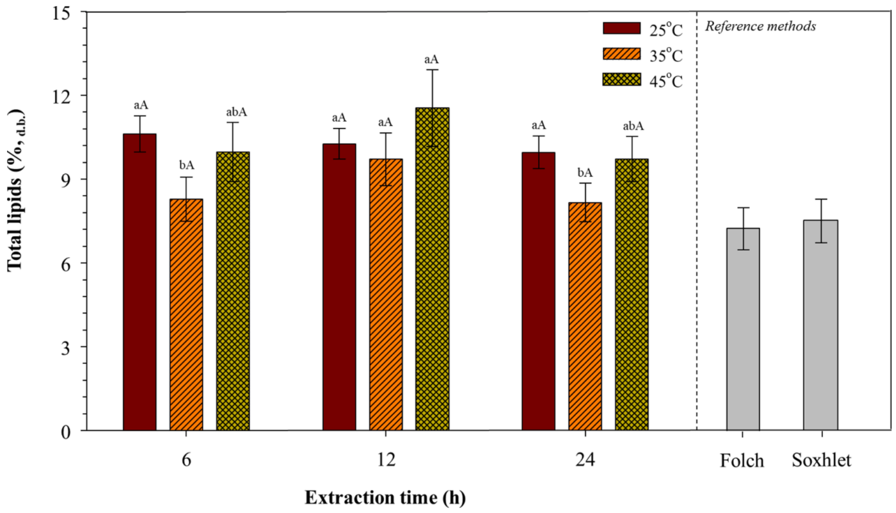


Fig. 4. Total lipids (%) obtained from the carbohydrate fraction after N,N dimethylcyclohexylamine (CyNMe2) extraction from spent coffee ground. Mean ± standard deviation within each column with different letters (a, b, c: among temperature; and A, B, C: among extraction time) are significantly different ($p < 0.05$) according to Tukey test.

attracts the hydrophilic molecules and isolates the less polarized species in the solution. Fundamentals of CO_2-responsive species that can be used as switchable solvents can be found elsewhere [3].

The first fraction recovered from SCG after CyNMe2 extraction was a fraction rich in carbohydrate that showed relative high values of total dietary fiber (63 to 83%, Fig. 2). SCG is made of a number of carbohydrates, including cellulose, hemicellulose, lignin, pectins, and gums. Such components represent the edible portion of the beans, and collectively refer to TDF [8]. The utilization of the carbohydrates in SCG was exemplified by Ballesteros et al. [6], who recovered a lignocellulosic fraction from SCG using Soxhlet extraction, where the total dietary fiber was about 60%. Similarly, Murthy and Naidu [22] used Soxhlet extraction to recover a carbohydrate fraction from SCG, containing about 43% of the total dietary fiber. Vazquez-Sanchez et al. [37] used Ohmic heating prior to water extraction to recover a fraction of polysaccharides from SCG, containing 57% of dietary fiber. Simoes, Nunes and Coimbra [27] obtained a fraction containing 56% of total dietary fiber from SCG using NaOH extraction.

The second fraction obtained from SCG with CyNMe2 was the water-soluble compounds containing phenolic compounds (Fig. 3**a and 3b**). Extraction of phenolic compounds from SCG with different extraction methods has been reported in the literature. Zuorro and Lavecchia [41] extracted about 90% of phenolic compounds from SCG with a mixture of ethanol/water. Panusa, Zuorro, Lavecchia, Marrosu, and Petrucci [31] used ethanol/water (60:40, v/v) as solvent to extract about 70–80% of the total phenolic compounds from SCG made of different coffee blends. Pettinato, Casazza, and Perego [32] improved the extraction of phenolic compounds from SCG by using microwave prior to ethanol/water extraction. Phenolic compounds have also been extracted from espresso SCG using ultrasound prior to methanol/water extraction [35].

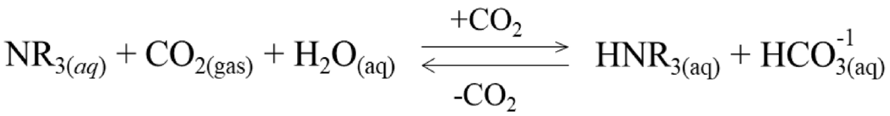
Fig. 3**a** shows that temperatures within the lower range (25 °C) produced higher concentration of phenolic compounds, including CGAs, where seven major species of chlorogenic acids were detected ranging from 1.83 to 7.16 mg g^{-1} of dry matter. Similarly, Boyd et al. [9] reported higher extraction yield from algae using CyNMe2 at room temperature compared to 60–80 °C. There are two reasons that can explain such observation. Firstly, amines become unstable at higher temperatures (>60 °C), impacting their ability to extract [3]. Secondly, CyNMe2 ability to be converted into its respective salt is favored at room temperature. Zhao et al. [40] determined some physical properties (molar volume, thermal expansion coefficient, and excess molar volume) of binary systems containing CyNMe2, and found that these parameters decreased with temperature, reaching a minimum value at 25 °C. Similar observations have been reported in a model system made of CyNMe2, water, and CO_2 [39]. These observations indicate that the process of capturing CO_2 by CyNMe2 is enhanced at 25 °C compared to higher temperatures (40 °C). It is worth mentioning that the solubility of CO_2 increased marginal with increasing the temperature within the region of interest, from 25 to 45 °C. The behavior of CO_2 in water has been studied using parameters derived from Hnery's Law [3].

The concentration of CGAs was relatively high considered that the acidity of CGAs and the complexity of the matrix that make it difficult to extract via CyNMe2 (Fig. 3**b**). Phenolic compounds in SCG can be found free and bound within the matrix attached to proteins and carbohydrates through hydrogen bonds, ionic and covalent bonds [21]. The distinction between free and bound phenolic compounds is not clearly defined within the literature since the degree of attachment strongly depends on the roasting conditions[27]. Pre-treatments such as alkaline solution, mechanical forces, acid hydrolysis, and saponification have been used with the idea to break the linkage between bound phenolic compounds and the SCG matrix [7,25,27]. The concentration of TPC (Fig. 3**a**) mainly accounts for the phenolic compounds that are free and easily extracted in water. On the other hand, the concentration of total CGAs (Fig. 3**b**) after CyMNe2 was slightly higher than the concentration in untreated SCG (Table 1). This observation suggests that the CyNMe2 treatment might be a suitable method to extract the bound phenolic compounds. The formation of the salt increases the ionic strength of the solution, which might help to break the linkage of bound phenolic compounds. However, additional experimental evidence is needed to support such claims. The resulting pH of the mixture (CyNMe2 and SCG containing about 46% moisture content) is within the basic region, pH 8–10. Under such circumstances, the percentage of protonation in the CyNMe2 is expected to be very low [3]. Upon addition of CO_2, the pH of the mixture significantly decreased to about 6–8, where the percentage of amine protonation is expected to be high. Overall, alkaline conditions benefited the extraction of bound phenolic compounds [21]. However, the degradation of CGAs cannot be ignored. For instance, caffeic acid might undergo hydrolysis under alkaline conditions [26].

Seven major classes of CGAs were identified within the phenolic fraction obtained with CyNMe2. An interesting observation was that the relative distribution of CGAs changed with the extraction conditions (time and temperature). Fig. 5 shows the relative distribution of the major classes of CGAs, where 5-CQA was the most predominant compound (59–97%) regardless of the extraction conditions. The relative distribution of other CGAs strongly depended on the extraction temperature. For instance, the percentage of 3-CQA varied between 37 and 16% at 25 °C, while at 35 and 45 °C the concentration varied from 28 to 0% and 30–0%, respectively. The final distribution of CGAs within the phenolic fraction can be modulated through the extraction conditions, including temperature, time, solvent to sample ratio, and concentration.

The third fraction obtained from SCG with CyNMe2 was the lipid fraction (Fig. 4), where the concentration of lipids was considerable higher with CyNMe2 compared with the traditional extraction methods (8.28 to 11.54%, $_{\text{d.b.}}$ and 7.22 to 7.48, respectively). It appears the CyNMe2 overestimated the concentration of lipids due to an incomplete conversion of CyNMe2 into its respective salt. Already, Boyd et al. [9] reported about 18–24% of residual CyNMe2 due to an incomplete conversion of CyNMe2 during the extraction of lipids from microalgae. Similarly, Rathnakumar et al. [33] estimated a 25% of residual CyNMe2 during the extraction of lipids from dairy byproducts. A number of factors influence the conversion of CyNMe2 into its respective salt, including temperature, CO_2 concentration, molar concentration of the amine, and the composition of the matrix. All these factors impact the equilibrium of the acid-base reaction, according to the Le Chatelier principle. Research strategies aimed at understanding the equilibrium of the acid-base reaction is of great relevance that will improve the extraction yields while minimizing the amine losses.

The fatty acid profile of the lipid fraction was not significantly different among the extraction conditions. Lipids in the form of triacylglycerides are methylated prior to the analysis of the fatty acid profile, where the CyNMe2 does not interfered. Similar fatty profile has been reported in lipids extracted from SCG. A lipidic fraction rich in palmitic and linoleic acid was produced from SCG using supercritical carbon dioxide extraction [2]. Abdullah and Bulent Koc [1] extracted 98% of available lipids from SCG using ultrasound prior to methanol extraction. Ahangari and Sargolzaei (2013) extracted about 98% of the available oil from SCG with supercritical carbon dioxide. Go, Conag, and Cuizon [16] recovered sugars and lipids through hydrolysis of SCG with



$$NR_{3(aq)} + CO_{2(gas)} + H_2O_{(aq)} \underset{-CO_2}{\overset{+CO_2}{\rightleftarrows}} HNR_{3(aq)} + HCO^{-1}_{3(aq)}$$

Scheme 1. Conversion of N,N dimethylcyclohexylamine (CyNMe2) into bicarbonate salt in the presence of water and CO_2.

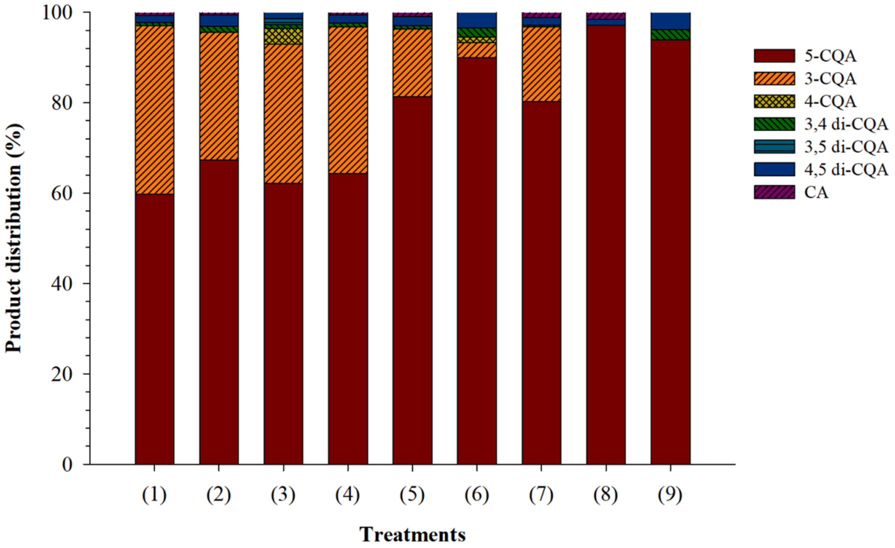


Fig. 5. Relative distribution of chlorogenic acids obtained after N,N dimethylcyclohexylamine (CyNMe2) extraction from spent coffee ground. (1) 6 h at 25 °C, (2) 6 h at 35 °C, (3) 6 h at 45 °C, (4) 12 h at 25 °C, (5) 12 h at 35 °C, (6) 12 h at 45 °C, (7) 24 h at 25 °C, (8) 24 h at 35 °C, (9) 24 h at 45 °C. 5-CQA – 5-caffeoylquinic acid, 3-CQA – 3-caffeoylquinic acid, 4-CQA – 4-caffeoylquinic acid, 3,4 di-CQA – 3,4-dicaffeoylquinic acid, 3,5 di-CQA – 3,5-dicaffeoylquinic acid, 4,5 di-CQA – 4,5-dicaffeoylquinic acid, CA – caffeic acid.

dilute (3–5%) sulfuric acid.

Overall, the yield of carbohydrates, phenolic compounds, and lipids was influenced by extraction conditions. Nevertheless, 24 h of extraction within a temperature range of 35–25 °C produced relative high yields of carbohydrates (68–83%), phenolic compounds (479–535 mg g-1), and lipids (9.97–11.54%). This is observation is of technological relevance since it suggests that CyMNe2 can produce satisfactory yields of each fraction at once.

The toxicity and economic analysis are of paramount importance to develop rational strategies to scale-up the CyNMe2 extraction. Currently, the Food Drug Administration (FDA) authorizes the use of rubbers and polymers containing CyNMe2 as an indirect Food Additive (CFR:177.2600). Samori et al. (2014) evaluated the influence of CyNMe2 on the growth of algae (*N. gaditana*), and reported EC_{50} (concentration that reduces 50% of algae growth) values of above 100 mg L^{-1}, belonging to the Category 4 (least hazardous substance to the aquatic environment). However, the toxicity of residual CyNME2 in humans is largely unknown. In addition to the toxicity, a technoeconomic analysis of the fractionation of SGC with CyNMe2 is needed

5. Conclusions

This work demonstrates the feasibility of CyNMe2 to obtain three value-added fractions from SCG. Moreover, the fractionation was carried out within one-step extraction by switching the amine into its respective salt. Optimization of the CyNMe2 extraction is needed to maximize the extraction yield of a given fraction and achieve a satisfactory conversion of CyNMe2. The fractionation of SCG with CyNMe2 can be economically feasible due to reducing the overall cost of the process. However, further development of CyNMe2 would require safety and techno-economic analysis.

CRediT authorship contribution statement

Kaavya Rathnakumar: Formal analysis, Writing - original draft. **Juan C. Osorio-Arias:** Formal analysis, Writing - original draft. **Padmanaban Krishnan:** Project administration, Supervision, Writing - review & editing. **Sergio I. Martínez-Monteagudo:** Conceptualization, Funding acquisition, Project administration, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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