GREEN EXTRACTION OF HIGH ADDED VALUE SUBSTANCES FROM SPENT COFFEE GROUNDS: PRELIMINARY RESULTS

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ABSTRACT

A supercritical fluid extraction (SFE) with pure CO_2 and ethanol as a co-solvent (10 %) of spent coffee grounds was carried out at temperatures from 313 K to 333 K, pressures up to 40.0 MPa and the results obtained were compared with those of the conventional Soxhlet method using n-hexane as a solvent. The best extraction yield (12.0 %) was achieved by SFE with CO_2+10 % ethanol, compared to 9.5 - 10.7 % by SFE with pure CO_2 and 10.4 % with n-hexane. The antioxidant potential was evaluated by DPPH method and the best antioxidant activity was exhibited by the spent coffee oil obtained by SFE, CO_2+10 % ethanol. The quantification and the identification of the oils composition were carried out by NMR. The main constituents of the extracted oils referred to the triacylglycerols (TAGs, 98.9 - 96.8 %). Minor amounts of 1,2 diacylglycerols (1,2 DAGs, 2.7 - 0.68 %), and the diterpenes cafestol (6.02 - 2.26 %), 16-O-methylcafestol (4.86 - 2.00 %) and kahweol (2.11 - 0.71 %), as well as caffeine (1.45 - 0.1 %), were also present in all the extracts.

Keywords: spent coffee ground, supercritical CO_2 extraction, antioxidants, triacylglycerols.

INTRODUCTION

According to the International Coffee Organization, total consumption of coffee in 2015/16 was estimated in 151.3 million 60 kg bags, which makes coffee one of the most consumed agricultural products and, after petroleum, the second traded product worldwide [1]. The huge consumption of coffee generates large volumes of waste, which is almost as much as the amount of coffee produced worldwide. Storage of this waste is a serious environmental problem and requires finding ways for its management and utilization.

Some applications of coffee residues have been already highlighted, such as: CO_2/mineral adsorbents, fuel pellets, source of sugars, a precursor for activated carbon production, compost and a sorbent for metal ions removal [2]. Furthermore, a number of studies confirm that spent coffee grounds (SCGs) contain large amounts of organic compounds (i.e. fatty acids, lignin, cellulose, hemicellulose, and other polysaccharides) that can be exploited as a source of value-added products [3]. Coffee residues have been investigated also for biodiesel production [4], extraction of specific components such as oil, flavor, terpenes, and as a potential source of functional ingredients for the food and cosmetic industry [5, 6]. Still, there is an urgent need for practical and innovative ideas proposing methods that will use the low cost SCGs and exploit their huge potential thus increasing the overall sustainability of the coffee agroindustry [3, 4].

The present work reports some preliminary results of

the examination and comparison of the capabilities of two techniques for extraction of SCGs in terms of global yield, principal compounds composition and antioxidant activity of the oil extracts obtained. The first technique applies to the green benign solvent CO_2 at supercritical conditions, while the second, *n*-hexane, at normal pressure.

EXPERIMENTAL

Materials and methods

Ethanol (99.5 %, PA) from Fisher Chemical (Spain), methanol (99.9 %) and Tris-Base from Fisher Chemical (United Kingdom), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and isopropanol (99.5 %) from Sigma-Aldrich (Germany) were used as purchased, while *n*-hexane (95 %) from Chem_Lab (Belgium) was distillated before used. Carbon dioxide was supplied by GASIN - Air Products (Portugal) with a purity of 99.99 %.

Raw material

The SCGs from an espresso machine were acquired from a local coffee shop, then dried at 105°C and stored in a refrigerator at 4°C until used. Before extraction, the raw material was dried again and the final moisture was 4 %. 10 g of the sample were sieved for 15 min with VEB MLW Labortechnik (Germany) vibratory sieve shaker. Moreover, 66 % of the sample was collected in the sieve with a mesh of 0.2 mm. The average particle diameter, d_p, calculated applying Eq. 1, was (0.273 ± 0.023) mm.

$$d_p = \frac{M_t}{\sum_{i=1}^{j} \frac{m_i}{d_{p_i}}} \qquad (1)$$

where M_t was the total mass of the SCG sample, m_i was the mass of particles retained below mesh size d_{pi}, while *j* was the number of mesh sizes.

Supercritical fluid extraction

Supercritical fluid extraction (SFE) has several advantages over traditional extraction techniques: it is a flexible process due to the possibility of a continuous modification of the solvent power/selectivity of the supercritical fluid (SCF) solvent; allows the elimination of polluting and hazardous organic solvents as well as of expensive post-processing of the extracts for solvent elimination. Carbon dioxide is the most popular and widely used SCF solvent because it is safe, non-mutagen, non-flammable, readily available and has a low cost [7 - 9].

In our study SCE was performed in a laboratory apparatus Applied Separations with some modifications (shown schematically in Fig. 1). It was equipped with a 50 cm³ internal volume extractor built from AISI 316 stainless steel tubing (32 cm long and an ID of 1.41 cm).

The high-pressure equipment [10, 11] was modified by the insertion of a co-solvent pump, which was connected to the extraction line. The pre-established flow rate of this pump allowed ensuring the correct ratio of the solvent:co-solvent in the solvent mixture before it enters the extraction vessel. Ethanol (EtOH) was used as a co-solvent in concentrations of 10 % (mole fraction) relative to the CO_2 amount.

Temperatures and pressures in the extraction apparatus were measured by thermocouples and test gauges; the uncertainties associated with the measurements were ± 1°C and ± 0.1 bar, respectively. In each experiment, the
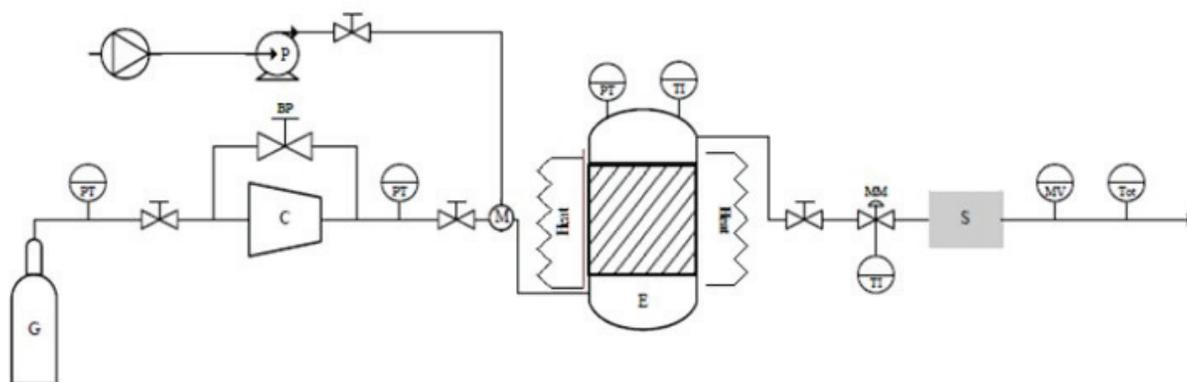

Fig. 1. Scheme of the supercritical CO_2 extraction equipment: G-bottle CO_2; C-compressor; E-extractor; S-separator; BP-regulator of back pressure; MM-micrometer valve; MV-flow meter; Tot-totalizer; TI-temperature indicator; PT-pressure indicator.

amount of the SCGs introduced in the extraction vessel was 15 g in average. The SCE process started as soon as the desired pressure and temperature were reached by letting the carbon dioxide flow through the extraction vessel at a constant flow rate of 1.8 g min⁻¹. Mass flow, temperature and pressure readings were taken at identical time intervals. The extraction was performed at $T =$ (40 and 60)°C and $p =$ (300 and 400) bar, respectively. The extracts were collected into a glass trap immersed in an ice bath.

Organic solvent extraction

The organic extraction method applied in our study was a conventional solid/liquid extraction with a Soxhlet extractor. 25 g of coffee samples were extracted with 250 mL of *n*-hexane for 4 h at the solvent boiling point. The result solution (solvent+oil) was filtered and dried over anhydrous sodium sulfate. The solvent was subsequently evaporated from the extracted oil in a rotary evaporator (Büchi, model R-205). The global yield was calculated as the mean value from duplicate experiments taking into consideration the ratio between the extract mass and that of the raw material.

Antioxidant capacity (AOC)

The antioxidant activity (AOC) of SCG extracts obtained by SFE and Soxhlet was determined by DPPH method, which was largely used because of its simplicity and reproducible results [12, 13]. DPPH assay was performed at 517 nm according to the method described by Prevc et al. [13].

The data of DPPH assays were recorded on a VWR UV 1600PC UV–vis spectrophotometer. They were performed in mixtures of methanol and propan-2-ol 1:1 (v/v) (MP) containing the acid-base pair tris-(hydroxymethyl) aminomethane (Tris_Base, 30.0 mM) and acetic acid (37.8 mM) in a molar ratio of 1:1.26 (MP-AB). Tris is more readily soluble in methanol than in MP mixture, therefore the stock solution for MP-AB was prepared in methanol. Solutions were prepared daily and used fresh [13]. For the analysis of the oils, several samples were prepared with a different oil concentration. 1 ml of each sample was mixed with 4 ml of DPPH solution. After mixing, all solutions were kept at 25°C in the dark. The absorbance was measured at 517 nm after 1 h.

The inhibition capacity (IC) of the sample was calculated by the expression:

$$IC_{50}(\%) = \left[1 - \left(\frac{A_s - A_b}{A_c - A_b}\right)\right] \times 100 \qquad (2)$$

where A_s, A_b and A_c were the measured absorbances of the sample, the blank one containing the pure solvent and the control one containing the solvent and DPPH, respectively, at 517 nm, under identical conditions in respect to time and temperature.

The antioxidant capacity was expressed as IC_{50} value, which represents the concentration of a sample that inhibits 50 % of the free radicals added to the system. IC_{50} value could be determined from the chart that expressed IC as a function of the extract concentration Cs. A smaller value of Cs corresponded to higher AOC, i.e. a smaller quantity of this extract was needed for neutralization of 50 % of the free radicals.

Analysis of the crude oil extracts by ¹H NMR

The ¹H-NMR spectra of the hexane and supercritical oils were recorded on a Bruker Avance 400 MHz NMR spectrometer (Bruker Inc., Bremen, Germany). It operated at 400.13 MHz for ¹H-NMR, equipped with a 5 mm PABBO BB-1H probe with 90° proton pulse length of 11.8 μs and a delay time between acquisitions of 30 s. All spectra were taken at 298 K in CDCl₃ (500 μL, 75 - 100 mM) solutions using standard Bruker routines and the residual signal of CDCl₃ (at δ_H 7.26 ppm) was used as the internal reference. The chemical shifts (δ) were assigned based on previous reports [14-18]. Data analysis and integration of the signals were processed with MestReNova 9.0 (MestreLab Research, SL, Santiago de Compostela, Spain).

RESULTS AND DISCUSSION

The Soxhlet extraction with *n*-hexane of SCGs achieves a yield of (10.37 ± 0.82) % on a dry weight basis. This yield falls in the range from (4 to 21) % on a dry weight basis reported in the literature [19 - 21]. It should be noted that the considerable differences in the oil yields obtained by the different research groups can be explained by the differences in the varieties of the coffee used.

SFE with CO_2 and CO_2+10 % of ethanol

Fig. 2 shows the cumulative SCE curves plotted to assess the effects of pressure, temperature and co-solvent addition on the oil yield. The maximum oil yields

achieved by the SCE are in the range (9.45 - 10.72) %, while this obtained by n-hexane extraction is 10.37 % as denoted by the continuous line, parallel to the abscissa.

In order to study the effect of SCE operating parameters, two sets of temperatures and pressures were examined, namely T = (40 and 60)°C, and p = (300 and 400) bar. The results obtained demonstrate that the oil yield is slightly influenced by the pressure (Fig. 2). However, the higher pressure affects the extraction time. Thus, the extraction plateau at p = 400 bars is reached for 60 min less than in case of extraction performed at p = 300 bar. This behavior is explained by the solvent density increase, which leads to enhancing the solvation power of CO_2. Furthermore, higher pressures lead to disruptions of the plant cells facilitating the release of compounds that are not previously available and thus increasing the yield of the process [7].

At the lower pressure of 300 bar, the effect of temperature on the extraction yield is clearly demonstrated - the temperature increase leads to a 10 % of diminution in the oil yield. However, at the higher pressure (400 bar) the temperature doesn't have any significant influence. This trend is a result of the balance between two opposite effects: on the one hand, increasing the temperature decreases the density of the supercritical fluid and thus its solvation capacity; on the other hand, it increases the vapor pressure of the solutes, therefore increases their solubility in the supercritical solvent [7]. At lower pressures, the first

effect prevails and thus the extraction yield decreases with temperature. In this case, the density of carbon dioxide decreases moderately with temperature increase.

Comparing the yields obtained by SCE with the conventional Soxhlet extraction, a slightly higher (but in the range of the experimental error - 5 %) yield is achieved by SCE at T = 40°C and p = 300 bar, while for the other conditions the SCE yield is very close to that of *n*-hexane extraction. Still, taking into consideration the experimental errors involved, it could be accepted that the performance of SCE with regard to the yield is similar to that of *n*-hexane extraction.

The addition of ethanol as a co-solvent in the SCE process leads to a slight increase in the extract yield. When 10 % ethanol is used, a maximum yield of the oil is obtained, 12 %, under the following operating conditions: p = 300 bar and T = 60°C. This represents an increase of 18 % in the yield compared to that achieved under the best conditions with pure CO_2, and an increase of 13 % in the yield obtained by the Soxhlet extraction. Our results are in a good agreement with the results of other authors [7, 22] and the behavior observed can be explained with the solubility enhancement of polar compounds in the ethanol/CO_2 mixture, compared to the solubility in pure CO_2 [8]. The results also suggest that solvents of higher polarity provide higher extraction yields, indicating that the compounds present in the plant matrix have intermediate to high polarity. The other significant effect of the
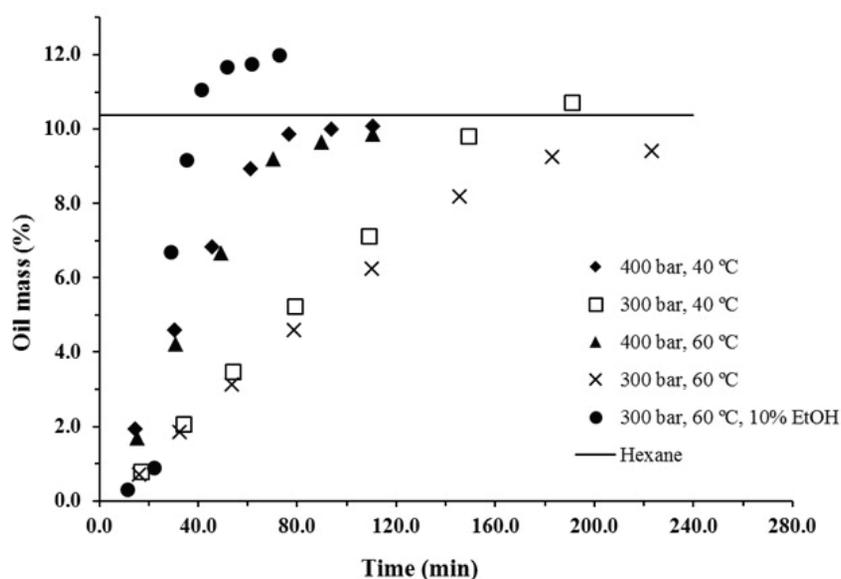

Fig. 2. Comparison between the coffee oil yields obtained for different SCE conditions and *n*-hexane.

co-solvent used, in addition to the higher yield achieved, refers to extraction time shortening by about 50 %.

Antioxidant activity

The results of the antioxidant activity obtained for all extract samples analyzed are presented in Table 1. The highest antioxidant capacity is found for the oil extracted by SCE at 300 bar, 60ºC and with 10 % of the co-solvent ethanol. It is followed by the n-hexane extract. Furthermore, the oils obtained by SFE without a co-solvent have a light brown color, while those obtained with the addition of the co-solvent, as well as the *n*-hexane extract, show a dark brown color, which could be indicative of the different extract compositions. Also, the higher capacity of the oil obtained by SFE+10 % ethanol substantiates the well-known fact that the solvent polarity increase is essential for extraction of polar compounds responsible for the antioxidant capacity.

Quantitative analysis of the crude oil extracts

The composition of SCG oils obtained by n-hexane and scCO$_2$ extraction without/with a co-solvent present, established by ^{1}H-NMR quantitative analyse is displayed in Table 2. The values obtained are in agreement with those in the literature depending on the extraction conditions and the vegetable matrix origin [7].

It is evident that the extracts are largely dominated by triacylglycerols (TAGs, 98.9 - 96.8 %) with minor amounts of 1,2 diacylglycerols (1,2 DAGs, 2.7 - 0.68 %). Caffeine (1.45 - 0.1 %), with some values below the NMR detection (limit ≤ 0.5 %), represents only a minor contribution to the overall composition of these oil extracts. The other detected compounds refer to Cafestol (6.02 - 2.26 %),

Table 1. IC$_{50}$ % values of the hexane and SCE extracts using the DPPH as-

Sample (bar/ºC)	IC$_{50}$ (mg/ml)
300/40	163.1±6.7
400/40	144.9±3.8
300/60	109.9±5.3
400/60	96.23±2.77
300/60 10 % Ethanol	12.39±0.52
n-hexane	25.07±1.37

its methylated derivative: 16-O-methylcafestol (4.86 - 2.00 %) and Kahweol (0.71 - 2.11%). A lower value of TAGs is obtained with hexane when compared with that of the SCE extracts.

With regard to the fatty acids content of the SCG extracts, the distribution of the di-unsaturated (DUFA), mono-unsaturated (MUFA) and saturated (SFA) acyl chains on the glycerol backbone is determined by the relative integration of the ^{1}H-NMR signals at 2.30 ppm (methylene group in α position to the carbonyl group) and 2.76 (bis-allylic protons) and 2.04 ppm (allylic protons), attributed to hydrocarbon chains with different number of unsaturations, as previously reported [15]. The DUFA (41.3 - 43.6 %), MUFA (13.8 - 16.8 %) and UI values are very similar for all the extracts.

The pentacyclic diterpenes based on the kaurane skeleton and generally present in different coffee products are cafestol, kahweol and 16-O-methylcafestol [23 - 25]. Due to the higher composition of TAGs, the NMR spectra are dominated by the resonances attributable to the triglyceride component of the lipophilic extract. However, from small resonances arising from the extract, minor components can be distinguished and their contents determined in absence of triglyceride signals overlapping. The NMR signal at 3.59 ppm is used for caffeine content determination. For the diterpenes cafestol, kahweol and 16-O-methylcafestol the isolated peaks at 3.17 ppm (16-O-methylcafestol), 6.21 ppm (cafestol and 16-O-methylcafestol) and 6.30 ppm (kahweol) were used in accordance with the assignments reported before [18, 26]. It is worth noting that there is no significant difference in respect to diterpenes content of the *n*-hexane and SCE oils. However, the extract obtained by SFE with CO$_2$ + 10 % of ethanol at 300 bar and 60ºC shows a lower content of these diterpenes but a higher content of caffeine, which can be associated with the increase of the supercritical solvent polarity.

CONCLUSIONS

Supercritical fluid extraction of oil from spent coffee grounds was studied in this work by measuring extraction curves at different conditions and subsequent comparison with n-hexane Soxhlet extraction. Moreover, the oils obtained were characterized by NMR and further studies of their antioxidant activity were also performed.

The highest oil yields achieved by the SCE without a co-solvent were in the range 10.4 - 10.7 %, while with

Table 2. Composition of spent coffee oils obtained by hexane and SCE established by 1H-NMR quantitative analysis. All values represent % molar fractions. Unsaturation index (UI) is defined by UI = (2×DUFA % molar fraction + MUFA % molar fraction)/100.)

Compounds	Hexane	scCO$_2$ conditions: p(bar)/T (ºC)				
		300/40	400/40	300/60	400/60	300/60 10 % EtOH
TAGs	96.8	98.9	98.1	98.4	98.6	97.9
1,2 DAGs	2.66	0.67	1.83	1.46	1.38	0.68
Caffeine	0.55	0.41	0.10	0.13	0.04	1.45
Cafestol	5.46	5.13	6.02	5.30	5.58	2.26
16-OMethyl-cafestol	4.86	2.84	3.73	3.70	3.47	2.00
Kahweol	1.56	2.11	1.32	0.86	0.71	1.20
DUFA	43.6	42.8	43.3	43.4	43.1	41.3
MUFA	13.9	14.9	13.8	14.5	14.1	16.8
SFA	42.5	42.3	42.9	42.1	42.1	41.9
UI	1.01	1.01	1.00	1.01	1.00	0.994

a co-solvent - 12.0 %. They are higher than that of 10.4 % obtained by the conventional *n*-hexane extraction. The best antioxidant activity, IC$_{50}$ (mg/ml) = 12.4, was found in the oil obtained by SCE with a co-solvent. It is equal to the half of the *n*-hexane oil value. The SCG oils composition, determined by ^{1}H-NMR, showed to be dominated by triacylglycerides, with minor amounts of 1,2-DAGs, caffeine and the pentacyclic diterpenes cafestol, kahweol and 16-O-methylcafestol. The values of triacylglycerides, obtained by SCE, were generally higher than those obtained by *n*-hexane oil extraction.

The oil extract obtained by SCE with CO$_2$ and 10 % of ethanol at 300 bar and 60ºC showed a lower content of pentacyclic diterpenes but a higher content of caffeine. This can be attributed to the increase of the supercritical solvent polarity

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