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Optimization of the supercritical fluid coextraction of oil and diterpenes from spent coffee grounds using experimental design and response surface methodology

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ABSTRACT

The reported work aimed at the optimization of operating conditions of the supercritical fluid extraction (SFE) of spent coffee grounds (SCG) using pure or modified CO_2, with particular emphasis on oil enrichment with diterpenes like kahweol, cafestol and 16-*O*-methylcafestol. The analysis comprised the application of Box–Behnken design of experiments and response surface methodology, and involved three operating variables: pressure (140–190 bar), temperature (40–70 °C) and cosolvent (ethanol) addition (0–5 wt.%). The best conditions to maximize total extraction yield are 190 bar/55 °C/5 wt.% EtOH, leading to 11.97% (g_{oil}/100 g_{SCG}). In terms of the concentration of diterpenic compounds in the supercritical extracts, the best operating conditions are 140 bar/40 °C/0 wt.% EtOH, providing 102.90 mg g^{-1}_{oil}. The measurement of extraction curves near optimized conditions (140 bar/55 °C/0 wt.% EtOH and 190 bar/55 °C/0 wt.% EtOH) confirmed the trends of the statistical analysis and revealed that SFE enhances diterpenes concentration by 212–410% at the expenses of reducing the extraction yield between 39% and 79% in comparison to *n*-hexane extraction.

1. Introduction

Coffee is one of the most consumed beverages in the world and gives rise to around 6 million tons of spent coffee grounds (SCG) every year [1,2]. The large variability of SCG composition in terms of carbohydrates, proteins and phenolic compounds makes this residue a potential raw material for industrial processes [3,4]. Some years ago, SCG used to find application as animal feed or farming fertilizers, but recent studies showed its potential as a source of green energy like biofuel, and oil or isolated molecules for the pharmaceutical, cosmetic and food industries [5–8]. In fact, SCG contain several human health related compounds, such as phenolics, which have demonstrated bioactivities at antioxidant, anti-bacterial, antiviral, anti-inflammatory and anti-carcinogenic levels [9,10].

Almost all coffee production originates from the exploration of two species, *Coffea arabica* and *Coffea robusta*, yielding between 7 and 17 wt.% of oil [7,8,11]. The average lipid content of green *C. arabica* is higher than *C. robusta*, 15% *vs.* 10%, respectively [12]. In roasted coffee, oil is composed of fatty acids esterified with glycerol (triacylglycerols, around 78 wt.%) and diterpenes (around 15 wt.%), and only a small fraction is in sterol esters form [12,13].

Diterpenes belong to a group of compounds with important physiological activities, which also present beneficial effects to human health. Even though they are related with the increase of serum cholesterol, they manage to enhance glutathione S-transferase activity and to protect against benzo[a]pyrene and aflatoxin B1 – induced genotoxicity [14–17]. The main diterpenes found in SCG are cafestol, kahweol and 16-*O*-methylcafestol (see Fig. 1), which are mainly sterified by fatty acids such as palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2), α-linolenic (C18:3) and arachidic (C20:0) [18]. The total amount of these compounds in SCG depends on coffee species and on the brewing method [19,20]. For instance, in filtered coffee the kahweol levels range between 0.06 mg L^{-1}_{oil} to 2.66 mg L^{-1}_{oil}, while cafestol levels are between 0.26 mg L^{-1} and 5.30 mg L^{-1}. When dealing with *espresso* coffee, these levels are 1.2–8 and 4–16 mg L^{-1}_{oil}, respectively [21]. Kurzrock et al. [12] reported different levels on *espresso* coffee samples – 26 mg L^{-1}_{oil} for cafestol and 10 mg L^{-1}_{oil} for kahweol – when prepared from *C. arabica* species. Concerning SCG it has been reported that samples can lead to very distinct extraction yields and also to distinct extract composition values, that vary even within the same commercial brand [8,22].

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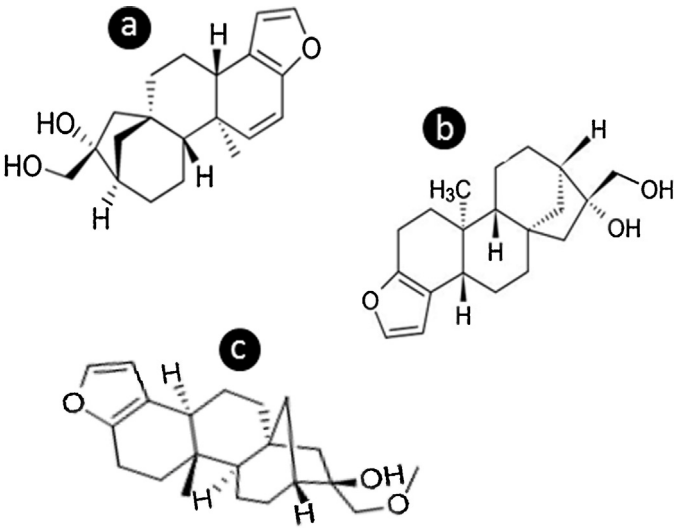

Fig. 1. Main diterpenes found in spent coffee grounds: (a) kahweol, (b) cafestol, and (c) 16-*O*-methylcafestol.

Supercritical fluid extraction (SFE) has been widely studied within the scope of valorization of waste and side products from industrial processes (biorefinery concept), being examples of this role works concerning grape seed [23–26], eucalypt bark [27–30], tomato [31,32], pumpkin [33,34], apricot [35,36], rice [37,38]. The SFE of coffee has also been reported in the literature, either in green [39,40], roasted [39] or spent [7,8,41,42] forms. Carbon dioxide is the commonest solvent adopted for SFE processes, a fact that is owed to the matching of several valuable characteristics. To begin with, the green character ensured by this species (innocuous to human health and to environment) respects the sustainability criteria that increasingly govern the adequacy of chemical processes. Secondly extraction processes involving supercritical carbon dioxide (SC-CO_2) can be operated at moderate temperatures which is a key issue for the preservation of labile compounds in extracts; preserved from air, light oxidation reactions are avoided. Finally significant selectivity enhancements may be obtained when targeting specific compounds through SFE. Nevertheless, the latter is only achievable upon an optimized combination of the SFE operating conditions with the intrinsic physical characteristics and chemical composition of the vegetable matrices under study. Taking this into account, to the best of our knowledge, no work has been published yet covering the optimization of operating conditions for diterpenes extraction from spent coffee grounds. For this purpose both design of experiments (DoE) and response surface methodology (RSM) can be applied with advantage to disclose the individual an crossed influence of operating conditions of SFE processes, as well as to identify optimum regions for extraction [28,43–45].

In the whole, this work aims to study the influence of pressure, temperature and cosolvent (ethanol) in the extraction yield of SCG oil, as well as to identify the operating conditions that better enhance diterpenes concentration in extracts, for which design of experiments and response surface methodology are implemented. Supercritical extraction curves are also measured and analyzed in order to disclose the trends of the process along time.

Concerning the structure of this document, Section 2 provides all information regarding experimental and statistical modeling features. Section 3 is devoted to the presentation and discussion of experimental results, namely the optimization of conditions such as pressure, temperature and ethanol content (by DoE and RSM), and extraction curves. The final section compiles the main conclusions of the work.

2. Materials and methods

2.1. Material and reagents

Individual standards of kahweol, cafestol and 16-*O*-methylcafestol were purchased from LKT Laboratories Inc. All other reagents used were of analytical grade or higher available purity. Carbon dioxide was supplied with a purity of 99.95% from Praxair (Porto, Portugal).

Espresso spent coffee grounds (SCG) were obtained from a commercial batch of Delta Cafés Platina (Portugal) at the Chemistry Department of University of Aveiro. The SCG samples were dried according to the ISO/DIS 11294-1993, following the method of oven drying at 105 °C for 8 h [46].

2.2. Soxhlet extraction

A sample of 45 g of SCG was loaded in a Soxhlet cartridge and extracted with *n*-hexane for 4 h and 80 °C. At the end of extraction the solvent was recovered by rotary evaporation at 40 °C and the resulting oil was weighed. The results were expressed in mass percentage of dry residue and were used as reference for the supercritical fluid extractions. The extraction yield (η_{Total}) is expressed in weight percentage as the quantity of oil ($w_{SCG\ oil}$) obtained from dried SCG (w_{SCG}), and were used as reference for the supercritical fluid extractions:

$$\eta_{Total}(\text{wt.\%}) = 100 \times \frac{w_{SCG\ oil}}{w_{SCG}} \quad (1)$$

2.3. Supercritical fluid extraction

Supercritical fluid extractions were performed in an apparatus developed at Department of Chemistry of University of Aveiro. The scheme of process can be visualized on the publications of Passos et al. [23,47], together with the corresponding full description of the set up. Concisely, the CO_2 withdrawn from a container is primarily liquefied in a refrigerated bath and then pressurized by an air driven liquid pump to a high-pressure vessel. The solvent is brought to the extraction temperature by means of a long tubing coil placed inside the oven and the pressure is fixed in a forward pressure regulator. After percolating the seed bed, the extract stream passes through micrometering valves. The valves and the adjoining line are heated to prevent blocking up due to oil and CO_2 freezing, enabling the safe collection of extract in a separator.

In each run 60 g of SCG were introduced in the extraction vessel and a constant CO_2 mass flow rate of 12 g min^{-1} was applied. Extracts were collected in a recovery vessel with ethanol, where the effluent stream is submerged after extraction to avoid the loss of compounds. At the end of experiments, the ethanol of the recovery vessel was evaporated in a rotary evaporator. The results were expressed in weight percentage of dry biomass by Eq. (1).

2.4. Diterpenes profile

Extracts were analyzed in a HPLC equipment with a UV detector (Gilson) and a reverse-phase column (Spherisorb S10 ODS2 (C18), 25 cm × 4.6 mm). The mobile phase was a mixture of methanol/water (85:15, v/v) using a flow rate of 0.7 mL min^{-1} and detection wavelength of 220 nm; in agreement with the setting reported by Amorim et al. [48].

The total diterpenes content was determined by HPLC after saponification with KOH/Ethanol, using diethyl ether for the solvent extraction of a 40 mg sample. Two additional washing steps with water were added to the procedure reported by Rafael et al. [49]. The identity of individual diterpenes was ensured by