

# Supercritical CO<sub>2</sub> extraction of spent coffee grounds. Influence of co-solvents and characterization of the extracts

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## Abstract

Spent coffee grounds (SCGs) were extracted with supercritical CO<sub>2</sub> at temperatures from 313 to 333 K, pressures up to 50.0 MPa and different scCO<sub>2</sub> flow rates. The addition of co-solvents isopropanol, ethanol and ethyl lactate decreased the time to achieve the maximum oil yield, to half of that with pure CO<sub>2</sub>. Analysis of the extracted oils by NMR, showed that caffeine content was (0.56-3.96) % and DUFA and MUFA contents changed within (39.8-42.0) % and (12.9-15.8) %, respectively. The analyses of fatty acids performed by GC-FID revealed the composition of the oils being 76% of the total constituted by palmitic and linoleic esters. The DPPH antioxidant capacity was improved up to 12.5 times with co-solvents. The kinetics of oil extraction from SCGs with pure scCO<sub>2</sub> was modeled successfully by combining for the first time representation of the oil mixture by a single virtual molecule with group contribution methods.

**Keywords:** Spent coffee grounds; supercritical CO<sub>2</sub> extraction; co-solvents, triacylglycerols; fatty acids; modeling.

## 1 Introduction

Spent coffee grounds (SCGs) are one of the principal biowastes in the production of instant coffee and coffee brewing. The enormous production and consumption of coffee at a global level is well documented and updated by the International Coffee Organization and in the European Coffee Report 2018/2019, as well as in the estimation of its evolution by 2024 [1–4]. In EU alone, for the period 2011–2013, 2.5 Mt of coffee were consumed. Taking into consideration that the production of coffee in 2015 was approximately 9 Mt and that from each kg of coffee 0.91 kg of solid waste is produced, the importance of a further valorization of this residue becomes obvious.

Appropriate waste supervision reduces the ecological and economic impact, the lost amounts of non-renewable resources, and the energy used in the production of new products. The social impacts are considerably smoothed as well. Attention is paid to bio-waste processing, because unsuitable treatment may have severe consequences on the environment, resulting in damage to ecosystem functions.

In recent years, the biorefinery concept has been identified as the most promising route for employment of the full potential of biomass by maximizing its conversion into high value products. Its main bottleneck, however, is how to extract the energy and non-energy compounds from the biomass without damaging one or more of the valuable components, e.g. those which are heat sensitive. SCGs contain large amounts of fatty acid esters, lignin, cellulose, hemicellulose, etc. and can be exploited as an excellent source of value-added energy and non-energy related products, like antioxidants and other functional additives [5–12].

The extraction of SCGs with the aim to obtain their lipidic fraction and important compounds like antioxidants, among others, has been studied considering conventional organic and alternative green solvents, e.g. supercritical carbon dioxide,  $\text{scCO}_2$  [13–24]. The  $\text{scCO}_2$  because of its gas-like viscosity and diffusivity, and liquid-like density and solvating power [25–29], is endorsed and commonly now accepted as an industrial solvent, particularly when the targets are thermolabile high value-added products.

This work presents for the first time the results of extraction of SCGs with pure scCO<sub>2</sub>, and with three co-solvents, and their comparison with Soxhlet *n*-hexane extraction. The co-solvents were chosen with regard to their polarity and their influence on the extraction process was studied in terms of global yield.

The composition of the extracts was examined and compared with a complementary combination of NMR, elemental analysis and gas chromatography. Moreover, DPPH free radical scavenging activities were also determined in order to understand the importance of the conditions of extraction for the antioxidant capacity.

Finally, the extraction kinetics of SCGs was simulated applying a dynamic model, advocated originally by Sovová and Stateva [30], which was planned, validated and executed using gPROMS ModelBuilder. For the first time in work on SFE, a vegetable oil (SCGs oil in our case) was represented not by one (or several) of its constituent molecules, but by a single virtual molecule that can take into full account the wide spectrum of the actual oil composition. The solubility of the virtual molecule in the pure scCO<sub>2</sub> was predicted applying the Soave-Redlich-Kwong (SRK) cubic EoS. The properties of the virtual molecule, required by the thermodynamic model, were estimated using some of the known group contribution methods with non-integer descriptors of the virtual chemical structure, which, to the best of our knowledge, has not been used before.

## **2 Materials and methods**

### **2.1 Raw Material**

The spent coffee grounds used were obtained from an espresso machine of a Bulgarian coffee shop. It was oven-dried to constant mass at 378 K and stored frozen in a refrigerator at 255 K, until used. The final moisture content ( $4.0 \pm 0.3$  %), from three replicas, was measured with a thermogravimetric balance (Kern MRS 120-3). Samples of 10 g were weighed and sieved in a mechanical system, for 15 minutes, with VEB MLW Labortechnik (Germany) vibratory sieve shaker. To calculate the average particle diameter,  $d_p$ , ( $0.273 \pm 0.023$ ) mm, Eq. 1,

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

where  $M$  is the total mass of the SCGs sample,  $m_i$  is the mass of particles retained below mesh size  $d_{p_i}$  and  $j$  is the number of mesh sizes used, was applied. The value of the apparent density of the solid matrix found was  $(0.440 \pm 0.016)$  g/cm<sup>3</sup>.

## 2.2 Reagents

Carbon dioxide was supplied by GASIN - Air Products, (Portugal) with a purity of 99.99 %. Trolox (Sigma Aldrich 98%) and ascorbic acid (Panreac 99 %), 2,2-diphenyl-1-picrylhydrazyl (DPPH), isopropanol (99.5 %), ethyl lactate from Sigma–Aldrich (Germany), ethanol (99.5 %, PA) from Fisher Chemical (Spain), methanol (99.9 %) and Tris-Base from Fisher Chemical (United Kingdom), were used as acquired, while *n*-hexane (95 %) from Chem-Lab (Belgium) was distilled before usage.

## 2.3 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 3 h at the solvent boiling point. Moreover, the same procedure was carried out with 250 mL of ethanol, the more polar co-solvent used in the supercritical CO<sub>2</sub> extraction, as a comparison to *n*-hexane.

The resulting 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 mass of extract and mass of raw material.

## 2.4 Supercritical CO<sub>2</sub> extraction

A commercial equipment (Applied Separations, Spe-ed<sup>TM</sup>SFE), equipped with a 50 cm<sup>3</sup> internal volume extractor, made from AISI 316 stainless steel tubing (32 cm long, internal diameter 1.41 cm), CO<sub>2</sub> flow meter and a totalizer from Alicat Scientific (USA), model M-5 SLPM-D are the principal parts. Pressures

and temperature in the apparatus were measured, with uncertainties associated to the measurements of  $\pm 0.1$  MPa and  $\pm 1$  K, respectively. More specifics about the apparatus are given elsewhere [31–33].

The extraction with scCO<sub>2</sub> of the SCGs was performed at  $T = (313.2 \text{ and } 333.2) \text{ K}$  and  $p = (20, 30, 40 \text{ and } 50) \text{ MPa}$  with flow rate of  $1.9 \times 10^{-3} \text{ kg} \cdot \text{min}^{-1}$ . At 313.2 K and 40 MPa three flow rates of ( $1.5 \times 10^{-3}$ ,  $1.8 \times 10^{-3}$  and  $2.2 \times 10^{-3}$ )  $\text{kg} \cdot \text{min}^{-1}$  of CO<sub>2</sub> were tested. For the extractions carried out using CO<sub>2</sub> with three different co-solvents, namely ethanol (CO<sub>2</sub> + EtOH), isopropanol (CO<sub>2</sub> + iPrOH) and ethyl lactate (CO<sub>2</sub> + EL), the two pumps were adjusted so that the final value of the CO<sub>2</sub> flow rate was  $1.9 \times 10^{-3} \text{ kg} \cdot \text{min}^{-1}$ . The values in percentage of co-solvent are in %<sub>mol</sub> fractions.

15-16 g of SCGs were used at a continuous solvent flow rate. The extract fractions were recovered at ambient pressure into a refrigerated glass tube (an ice bath) and weighted. When a co-solvent was used it was collected together with the oil in the glass tubes and then evaporated in a rotary evaporator, to accurately obtain the final mass. The samples were kept at 253 K in the dark, until analysis.

## 2.5 Analysis of the oil extracts by <sup>1</sup>H NMR

Samples of 0.0450 to 0.0550 g of oil extracts were dissolved in 500  $\mu\text{L}$  (75-100 mM solutions) of CDCl<sub>3</sub> for recording the proton NMR spectra. The <sup>1</sup>H-NMR spectra were obtained on a Bruker Avance 400 MHz NMR spectrometer (Bruker Inc., Bremen, Germany) operating at 400.13 MHz for <sup>1</sup>H-NMR, equipped with a 5 mm PABBO BB-1H probe with 90° proton pulse length of 11.8  $\mu\text{s}$  and a delay time between acquisitions of 30 s, using standard Brüker routines. All experiments were performed at 298 K and the residual signal of CDCl<sub>3</sub> (at  $\delta_{\text{H}}$  7.26 ppm) was used as the internal reference. The chemical shifts ( $\delta$ ) for the different components were assigned based on the values reported in the literature for TAGs and 1,2-DAGs [34], caffeine [35], cafestol, 16-O-methylcafestol [36] and kahweol [37]. All spectra were processed by the Brüker Topspin and MestReNova 9.0 (MestreLab Research, SL, Santiago de Compostela, Spain) software packages. Base-line correction was performed by applying a polynomial fourth-order function in order to achieve quantitative measurements upon integration of signals of interest.

## **2.6 Elemental analysis**

Analysis of carbon, hydrogen, nitrogen and other elements is essential for characterization and/or proving the composition of an organic sample. For substances, like the biomass studied, which contain mainly C, H, N and oxygen, the latter may be determined by the mass balance of the elements to 100 %.

The experimental determination of C/H/N in our samples was done in the certificated laboratory LAIST, Lisbon, Portugal by the method: M.M. 8.6 (A.E) (2009-05-06). The concentration of oxygen in the samples was calculated by difference. Mass balances of the extractions were employed to complement the experimental elemental compositions of the extracts and residues with estimated values.

## **2.7 Quantitative analysis of fatty acid methyl esters (FAMES).**

A gas chromatographic method was employed to characterize the fatty acid ester profile of the SCGs oils attained. The analyses were performed with reference to the parameters in Annex I to Commission Regulation (EEC) No 2568/91(1), CELEX\_01991R2568 published 04.12.2016, with the necessary adaptations. The transesterification of the oil into fatty acid methyl esters (FAMES) was carried out in methanol solution of KOH (2M). The separation was performed in a fused-silica capillary column (SP-2380; stabilized; poly (90 % biscyanopropyl/10 % cyanopropylphenyl siloxane, phase) 60 m length, 0.25 mm of internal diameter, 0.20  $\mu\text{m}$  film thickness as described: oven temperature of 438 K for 25 min then, programmed heating from (438 to 483) K at 5 K/min and subsequent holding at 483 K for 10 min. The temperatures of the injector and detector were kept constant at (523 and 553) K, respectively. The carrier gas employed was helium with a flow rate of 1.0 mL/min. FAMES were identified by relating their retention times with those of a reference solution run at identical GC conditions in the GC apparatus. Two replicas of the GC analyze were done. The quantification was obtained through the calculation of the chromatographic relative percentage areas.

## **2.8 Free radical scavenging activity**

The DPPH free radical activity is used widely to estimate the activity of antioxidant essays. Despite the simplicity of the method some differences in the reactions conditions [38], make the comparison of the

results of different laboratories difficult. The DPPH assays of the SCG extracts obtained by scCO<sub>2</sub>, and by Soxhlet were determined by the method described by Prevc et al. [39], following the microplates system used before [40]. 30 µL of the extract were dissolved in mixtures of methanol and isopropanol (propan-2-ol) 1:1 (V/V) (MP), containing the acid–base pair tris-(hydroxymethyl)aminomethane (Tris\_Base, 30.0mM) and acetic acid (37.8 mM) in molar ratio 1:1.26 (MP-AB) in a microplate (Nunc) with 270 µL of DPPH solution (100 µM). The solutions were kept at 298 K in the dark. The absorbance was measured at 517 nm after 1 hour in a microplate reader (BioTek Synergy 2)

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

$$IC = \left[ 1 - \left( \frac{A_s - A_b}{A_c - A_b} \right) \right] \times 100 \quad (2)$$

Where  $A_s$ ,  $A_b$  and  $A_c$  are the measured absorbance of the sample, the blank with pure solvent and the control with the solution of DPPH, respectively. All measurements were done in triplicate. Solutions of trolox and ascorbic acid were used as references.

### 3 Modeling the kinetics of the scCO<sub>2</sub> extraction of SCGs

Modeling the extraction kinetics of oil from SCGs requires a suitable framework, which comprises a kinetic model and a model to simulate the phase behavior of the complex system (oil +scCO<sub>2</sub>).

In the present study, a kinetic model advocated by Sovová and Stateva [30] was used. It requires knowledge of the solubility of the oil in the scCO<sub>2</sub>, at the temperatures and pressures of interest to the experiment. In what follows, firstly a very concise description of the kinetics model will be given.

#### *Kinetics model*

The model proposed by Sovová and Stateva [30] allows the calculation of the evolution of the concentration in the solid and fluid phases inside the extractor, considering homogeneous distribution at both phases. It assumes that the extracts are located on the surface of the solid particles which allows neglecting internal diffusion. Although this assumption may limit the application of the model when coarsely ground substrates are used, it has proved to be adequate for finely ground substrates, as grape seeds [41], where the diffusion

path in the particles is short and the extract is easily accessible. The equations of the model are not reproduced here for a matter of space and can be found in the original document [30].

gPROMS ModelBuilder [42], an equation-oriented modeling and optimization platform for steady-state and dynamic systems, was used to solve the model. gPROMS includes a parameter estimation tool, that uses the available experimental data and, through a maximum likelihood parameter estimation problem, finds the parameter values that maximize the probability of adequately describing the process.

The model has four unknown parameters:  $b$  - a coefficient, with a value that should be much higher than one [30],  $K$  (kg solid matrix / kg CO<sub>2</sub>) - the partition coefficient,  $k_f$  (m/min) – the external mass transfer coefficient, and  $w_t$  (kg/kg solid matrix) - the monolayer adsorption maximum content. To adequately describe the extraction system, the missing parameters have to be determined. Considering the experimental data available, we opted to estimate only the value of  $K$ , in order to not compromise the confidence interval for the estimation results. Thus, predefined fixed values were assigned to remaining parameters [41]. The value of parameter  $b$ , was determined after preliminary analysis, including a sensitivity analysis of its influence on the extraction kinetics modeling. The best value obtained was  $b = 40$ , and hence it was used for all cases examined. The value of  $k_f$ , following Coelho et al. [41], was estimated applying the relation of Wilke and Chang [43].

The fitting accuracy was evaluated using absolute average relative deviation, AARD, a widely used standard deviation measure, calculated using Eq.(3), where  $N$  is the total number of experimental points, and  $e_i^{exp}$  and  $e_i^{est}$  are the  $i$ -th experimental and estimated point, respectively.

$$AARD = \frac{100}{N} \sum_{j=1}^N \frac{|e_i^{exp} - e_i^{est}|}{e_i^{exp}} \quad (3)$$

Following the algorithm advocated by Sovová and Stateva [30], second order polynomial functions are fitted to the solubility data points and used in the kinetics model.

To reliably simulate the solubility of the SCGs oil in the scCO<sub>2</sub> two important issues should be addressed: how to adequately represent the complex chemical structures and composition of the oil



extracted, and which model to use to reliably simulate the phase equilibria of the system studied at the temperature and pressures of interest to the experiments.

***Representation of the SCGs oil by a virtual molecule and non-integer group contribution.***

The extracted SCGs oil, as any other extracted oil, is a very complex mixture of many compounds, mainly triacylglycerols (TAGs). In order to reduce the size of the kinetics modeling task, the usual procedure is to represent the oil examined either by a single TAG [13,44], or as a binary mixture of triolein and oleic acid [45]. Recently, there were attempts to represent some vegetable oils as a mixture of several TAGs. The more detailed presentation considerably increased computational effort while achieving a varied result – from a failure to predict the phase equilibrium of the model multicomponent mixture examined [46] to an acceptable quantitative and qualitative representation of the kinetic curves measured [41].

In this work, we have tested an innovative combination of known methods - representation of the chemical structures in the oil mixture by a single virtual molecule, and group contribution methods. The SCGs extracts were treated as consisting only of TAGs, each of which contributes to the values of the chemical structure descriptors of the respective single virtual molecule, proportionally to its concentration. We believe this presentation is fundamental and with a significant potential for modeling of both linear and non-linear quantitative structure – property relationships for mixtures. For a given descriptor of the chemical structure of a virtual molecule:

$$DVM = \sum_{i=1}^n \alpha_i d_i \quad (4)$$

where  $DVM$  is the value of the descriptor of the virtual molecule,  $n$  – the number of representative or all mixture components (individual molecules),  $d_i$  - the value of the descriptor for the  $i$ -th individual component of the multicomponent mixture and  $\alpha_i$  - its concentration (mass parts) in the particular mixture. In group contribution methods, the chemical structure descriptors are integer numbers of appearance of structural groups, defined by the method. Eq. 4, however, might provide integer and/or non-integer numbers. The use of the latter with group contribution methods for estimating properties of mixtures, to the best of our knowledge, has not been suggested previously.

The respective descriptors of the virtual coffee oil molecule have been calculated with all its components, experimentally identified by gas chromatography. With UNIFAC groups, the extracted oil is presented as  $\text{CH} (\text{CH}_2)_{39.2798} (\text{CH}_3)_3 (\text{CH}=\text{CH})_{3.0030} (\text{CH}_2\text{COO})_3$ .

### ***Thermodynamic model.***

In general, equations of state (EoSs) are the usual choice for calculation of solubility of a compound (mixture of compounds) in scCO<sub>2</sub>. In the present study we have chosen the Redlich-Kwong-Soave cubic equation of state (SRK EoS), the application of which requires knowledge of the thermophysical parameters of the virtual molecule representing the SCGs oil, namely its critical temperature and pressure.

### ***Parameters of the virtual molecule.***

TAGs with chains longer than C14:0 degrade before reaching critical points and their parameters must be estimated, which is usually done applying group contribution methods.

The critical parameters of the virtual molecule were estimated with the well-known methods [47] of Ambrose, of Joback and Reid, of Constantinou and Gani, and of Marrero and Gani. The correct selection of their groups was validated with predictions for esters, published by other authors.

The reliability of the values of the virtual molecule parameters estimated was accessed applying the generalized semi-theoretical expression advocated by Zbogor et al. [48], which correlates  $T_c/p_c$  with the van der Waals surface area,  $Q_k$ . UNIFAC was used with PSRK values of  $Q_k$  [41]. For the virtual molecule the  $T_c/p_c$  ratio is 393.6. The closest to that  $T_c/p_c$  ratio (namely 386.0) was obtained with  $T_c = 968.8$  K, estimated with the method of Constantinou and Gani, and  $p_c = 2.51$  bar estimated with the method of Joback and Reid. Those values of the critical parameters were used by the thermodynamic model to calculate the solubility of the virtual molecule in the scCO<sub>2</sub>.

## 4 Results and discussion

### 4.1 Supercritical fluid extraction curves

Figure 1 shows the cumulative extraction curves plotted to evaluate the effect of pressure, temperature and flow rate in the process of scCO<sub>2</sub>, as well as for comparison with the yield, obtained after 180 min *n*-hexane Soxhlet extraction (the continuous line), parallel to the abscissa.

The assessment of yield extraction, obtained under different conditions of temperature and pressure, shows the effect of parameters like solubility of coffee oil in the solvent and consequently -their influence on the process yields.

The Soxhlet extraction of SCGs with hexane obtained a yield of 10.4 % on a dry mass basis (% g oil/100 g dry SCGs). The lowest yield was obtained by scCO<sub>2</sub> at 20 MPa and 333 K, and only up to pressures of 30 MPa it is possible to achieve, in a reasonable time (240 min), similar yields compared with the hexane extraction. For both temperatures it is observed that the extraction efficiency increases when pressure is raised, while, at 313 K and up to (40 and 50) MPa no such behavior is distinguished. This experimental observation is explained by the increase of solvent density with the pressure, which boosts the solvation power of scCO<sub>2</sub> [13,49]. The impact of the temperature on the extraction yield of the oils is more intricate than the pressure effect. At 20 and 30 MPa, extraction yield decreases with the rise of temperature while at 40 MPa, the effect is insignificant. In part, this tendency can be due to the balance between two reverse effects: increasing the temperature decreases the density of the scCO<sub>2</sub> and thus its solubility capacity; but at same time, it upsurges the vapor pressure of the compounds, consequently enhancing their solubility in the supercritical fluid [18,20–22]. Finally, similar maximum yields, after 120 min of scCO<sub>2</sub> and comparable with the hexane extraction, were obtained at 40 and 50 MPa, 313 K and 40 MPa, 333 K. Considering the percentage error of the methods used (4 %), the results show insignificant differences.

The behaviors described complement previous results of other authors. The results from Couto *et al.* [13] show that at 313 K the oil yields, obtained from SCGs at 20, 25 and 30 MPa, are almost the same. Moreover, at temperature of 323 K the increase of pressure from 15 to 20 MPa increases significantly the yield, a decrease happens at 25 MPa, then it rises again at pressure of 30 MPa. The experimental data from Andrade *et al.* [17] show that at 313 and 333 K the increase of pressure improves the yield of SCG oil, but at

323 K the change of pressure from (20 to 30) MPa, decreases it. The same authors achieved a maximum SCGs oil yield at 30 MPa (maximum pressure in the study) and 313 K, and denoted a crossover isotherm region, unclear, at 20 MPa. Manna *et al.* [49] present a maximum extraction yield of SCGs oil at 50 MPa and 333 K, the only isotherm shown, where authors compare with other food waste residues. An experimental design has been used to optimize the extraction conditions of oil from SCG [50] in the range of temperature 306-340 K, pressure 11.6-28.4 MPa and time from 19 to 221 minutes. They conclude that the best conditions for the extractions are respectively 306 K, 28.4 MPa and 221 min. duration. The authors have not explored higher pressures.

Figure 2 shows the cumulative extraction curves plotted to evaluate the effect of addition of co-solvents in scCO<sub>2</sub> at the previously used pressures and temperatures, as well as for comparison with the *n*-hexane and ethanol Soxhlet extraction (continuous lines, parallel to the abscissa).

The main observation from the Figure is that the cumulative experimental extraction curves with scCO<sub>2</sub> +co-solvent, exhibit a maximum of (11–12) % in the yield. These values are higher than that achieved with a 180 minutes of Soxhlet extraction with *n*-hexane, and just very slightly higher or commensurable with the yield of Soxhlet extraction with ethanol, however realized just for half of the time required with pure CO<sub>2</sub>, at the same flow rate ( $1.8 \times 10^{-3}$  kg·min<sup>-1</sup>). Couto *et al.* [13] found a similar trend when changing from pure scCO<sub>2</sub> to co-solvent ethanol with CO<sub>2</sub> (mass ratio of 6.5:93.5 (w/w)). A 60 % reduction of the time needed

to reach the maximum yield of oil from SCGs was observed. An explanation for this behavior has been reported previously [51]. The addition of a co-solvent has the consequence of increasing the local density around the solute molecule, leading to the increase of physical and chemical interactions between co-solvent and solute molecules, such as H-bonding interactions, which outcomes in a solubility improvement of the lipids in the solvent phase.

At 20 MPa and 313 K, the increase of the concentration of ethanol added within (5 to 10) %, not only increases the final yield, from 10.7 to 11.4, but also decreases significantly the extraction time from 100 to 58 min, (if we do not consider the last point in Figure 2). At the higher temperature (333 K) applied, for the same concentration of the polar co-solvent (10 % EtOH), the increase of pressure from (20 to 30) MPa, not only gives a higher yield of oil (11.5 to 12 %), but also diminishes the extraction time from (96 to 57) min.

For the other temperatures and co-solvents (isopropanol and ethyl lactate) added, the increase of pressure from (20 to 30) MPa does not change significantly the oil yield and the time of extraction.

A maximum yield, comparable to that achieved at 30 MPa, 333 K and 10 % of EtOH, has been obtained in the experiments with 5 % of EL, independently of the temperature and pressure tested. Phase behavior of binary CO<sub>2</sub> + EL mixtures has been studied and reported by other authors [52]. Ethyl lactate is an agrochemical solvent recently studied for the extraction of caffeine from green coffee beans and green tea leaves [53–57]. Extraction with scCO<sub>2</sub> of caffeine from green tea leaves with three co-solvents, ethyl lactate, ethyl acetate and ethanol, has shown that the maximum yields of caffeine were obtained with ethyl lactate in static or dynamic mode of extraction [56]. The authors ranked the co-solvent effect in the order: ethyl lactate > ethanol > ethyl acetate, which corresponds to our results. To the best of our knowledge, the present study is the first to report the application of ethyl lactate as a co-solvent in the SCE of SCGs.

#### **4.2. Analysis of the oil extracts by proton NMR.**

The oil extracts obtained from SCGs by Soxhlet extraction with *n*-hexane and by scCO<sub>2</sub> extractions using different conditions and co-solvents, were analyzed by <sup>1</sup>H-NMR, which has demonstrated to be a fast and useful tool for analyzing oils from different vegetal matrices [21,41]. As an example, the <sup>1</sup>H NMR spectrum of the SCG extract obtained by scCO<sub>2</sub> extraction is shown in Figure 3, where the signals used to quantify the different components of the oil are highlighted.

The SCGs oil has a complex chemical composition, in which the presence of triacylglycerols (TAGS), 1,2-diacylglycerols (1,2-DAGS) and caffeine, as well as the pentacyclic diterpenes of the kaurene family cafestol, 16-O-methylcafestol and kahweol, has been confirmed by the correspondent signals in the proton NMR spectra. In fact, the presence of these minor diterpenic components was already reported [37,58] and can be used to characterize the coffee beans variety. Cafestol and kahweol are found in Arabica and Robusta beans, while the 16-O-methylcafestol is found exclusively in Robusta beans [59]. Both positive and negative physiological effects have been assigned to the three of them, in addition to their antioxidant capacity [59-61].

The amount of TAGs in the samples was determined using the signal at 4.31 ppm attributed to the glyceryl methylene protons in the sn-3 position, while the presence of 1,2-DAGs was confirmed by the signal at  $\delta_{\text{H}}$  3.72 ppm attributed to the glyceryl methylene protons in the same position (Figure 3). Due to the higher presence of TAGs, the NMR spectra are dominated by the resonances attributable to the triglyceride component. However, a more detailed analysis of the spectra revealed the presence of small resonances attributed to the additional species in coffee oil, namely caffeine and the diterpenic alcohols cafestol, 16-O-methylcafestol and kahweol, in accordance with the assignments reported before [36,37]. Thus, for the contents determination of these minor constituents of the SCG oil samples, isolated signals with adequate intensity and location outside of the range of the chemical shifts attributed to the major components TAGs and 1,2-DAGs were selected for quantification (see Figure 3, regions **A** and **B**). For the caffeine's content, the singlet signal at 3.41 ppm was selected, corresponding to one of the N-methyl groups in the pyrimidine ring.

For the diterpenic alcohols cafestol, 16-O-methylcafestol and kahweol, the isolated signals at 3.17 ppm (16-O-methylcafestol), 6.21 ppm (cafestol and 16-O-methylcafestol) and 6.30 ppm (kahweol) were selected. The discrimination between the signals of cafestol and kahweol in the diterpenes region is a clear example of the potential of  $^1\text{H}$  NMR in SCGs oil analysis, which in the present work has been used to quantify the corresponding amounts in the SCGs oil samples. Since these diterpenoids are present in the free form (vestigial), but mostly as fatty acid esters, their content has been determined as relative to the total fatty acid ester composition instead of being included in the calculation of the total of the SGCs oil components. Thus, the diterpenoids content was obtained from the relative areas between the selected signal of each diterpenoid and the carbonyl  $\alpha$ -methylene signal at  $\delta_{\text{H}}$  2.30 ppm and present in all the fatty acid derivatives.

The experimental results for the composition of the extracts are displayed in Tables 1 and 2. Table 1 demonstrates that they are largely dominated by triacylglycerols (TAGs, 90.56–99.03 %<sub>mol</sub>) with minor amounts of 1,2-diacylglycerols (0.56–5.75%<sub>mol</sub>) and caffeine (0.15–3.96 %<sub>mol</sub>). The values are in agreement with those published in the literature, having in mind that they depend on the extraction conditions and the matrix origin [19,35,36]. The presence of 1,3-DAGs was not detected. Similar values for TAGs content were found for the *n*-hexane extract and high pressure scCO<sub>2</sub> extractions. The co-solvent effect depends on the

solvent nature: e.g. the addition of ethanol decreases the TAGs content, while isopropanol and ethyl lactate increase the values obtained. For the 1,2-DAGs content, a lower value is obtained with hexane when compared with the values for scCO<sub>2</sub> extracts. The addition of co-solvents increases the value in the case of ethanol and decreases those values for the other two co-solvents. Concerning the minor components, the caffeine amount increases in the scCO<sub>2</sub> extracts obtained at low pressure. The addition of co-solvents follows the same trend observed for 1,2-DAGs. The low values found in the ethyl lactate case can be explained by the losses during the solvent evaporation process.

The diterpenes vary within the following ranges (Table 2): cafestol (0.53 - 2.05 %<sub>mol</sub>), its methylated derivative 16-O-methylcafestol (1.16 - 2.20 %<sub>mol</sub>) and kahweol (0.30 - 1.09 %<sub>mol</sub>). The amount of the last one is generally lower than the others and the presence of the three diterpenes is indicative that the spent coffee sample is a mixture of both Arabica and Robusta blends. The low pressure scCO<sub>2</sub> extracts generally present higher contents when compared with the hexane extract, but the addition of co-solvents generally has the opposite effect. The same trend is followed by the total diterpene content, where the values for scCO<sub>2</sub> oils are slightly higher when compared with hexane and the addition of co-solvents causes a decrease of up to 40 %.

The NMR data has also been used for the determination of fatty acid profiles, namely the saturated (SFA), mono-unsaturated (MUFA) and di-unsaturated (DUFA) acyl chains distribution on the glycerol backbone. The di-unsaturated acyl chains are more abundant than the mono-unsaturated ones, this difference being evident when considering the intensities of the signals due to bis-allylic and allylic methylenes (at 2.76 ppm and 2.04 ppm, respectively). Furthermore, the integration of these signals together with the signal at  $\delta_H$  2.30 ppm, present in all the fatty ester derivatives, and attributed to the carbonyl  $\alpha$ -methylene group can be used to determine the amount of hydrocarbon chains with different unsaturation. Thus, the DUFA content (mainly linoleic acid ester) was obtained by the relative areas between the signal of the bis-allylic protons (at  $\delta_H$  2.76 ppm, 2H) and the carbonyl  $\alpha$ -methylene signal (at  $\delta_H$  2.30 ppm, 2H), while the MUFA content (mainly 18:1, oleic acid ester) was determined by a similar procedure involving the signals of the allylic protons (at  $\delta_H$  2.04 ppm, 4H) and the carbonyl  $\alpha$ -methylene protons. The saturated chains (SFA) content was obtained from the difference between the total fatty acid and all the unsaturated

(MUFA+DUFA) fatty acid esters, as previously reported [41]. The DUFA (39.8 – 42.0), MUFA (12.9 – 15.8) and UI values (0.94 – 0.98) are very similar for all extracts. Considering the DUFA content, the low pressure scCO<sub>2</sub> extracts showed a slight increase when compared with the hexane extract while the introduction of co-solvent generates a slight decrease of the values. MUFA percentages remain almost constant as well as the UI variations, the last one showing a decrease with the co-solvent addition.

### 4.3. Elemental analysis of the SCG

The results from the elemental analysis are presented in Table 3. It contains data from experimental measurements, given in bold, and data calculated from experimental measurements, the material and the element balances of the respective extractions, given in italic.

For seven extractions measured data have been obtained both for the extracts and for the respective residues. These have been used to calculate the compositions of the residues and compare them with the measured compositions, thereby estimating the uncertainties of all calculated data in Table 3.

The results obtained for the average absolute relative deviations - 3.78 % for N, 2.32 % for C and 2.86 % for H, respectively, are higher than those from the parallel measurements of the elements, 1.18 % for N, 0.32 for C and 2.51 % for H. This is not surprising since the latter are only repeatability errors, while the former on top of those incorporate the errors in the yields determination which are presented above with distributed losses.

It should be noted that four of the seven calculated results for the N content in the extracts show significant overestimation, which contradicts the experimentally determined nitrogen as below 0.5 %.

Both results, however, allow for comparison of the element ratios of the extracts and residues with the requirements for their application in biofuels (e.g., high C and H content), adsorbents (low H/C and low polar coefficient), composts (e.g. low C/N ratio), etc.

The results in Table 3 complement relevant observations and tendencies established in previous publications [62–64]. For instance, they show that the extracts contain significant amounts of nitrogen, which would be transformed to air-polluting nitrogen oxides in combustion. For application in compost the nitrogen in the residues needs to be complemented with such from another component. Similarly, they



support previous results for the semi-coking of the residuals and the need to enhance the properties of the obtained active carbons with a coal component [63].

The experimental results from the element analysis provide also an opportunity for corroboration of the content of glycerides and caffeine determined experimentally by H-NMR. In order to test that, we assumed that the SCF extracts contain TAGs, presented with a virtual molecule, and caffeine. The concentrations of these from Table 1 and the contents of the elements in them were used to calculate and compare the elemental compositions, estimated with H-NMR with the experimental data for the elements in the extracts (Table 3). The results obtained for C and H in the six extracts, which have data from both methods, differed within the experimental error of the elemental analysis; all nitrogen concentrations calculated from H-NMR were below 0.5 % - the lower experimental limit of the elemental analysis.

Thus, elemental analysis has been confirmed to be a relatively inexpensive tool, providing quickly useful information for all materials in the extraction technologies, which is why it is increasingly been included in the general matrix of their research.

#### **4.4. Analysis of the fatty acid methyl esters (FAMES).**

Tables 4 and 5 show the fatty acid ester composition of the oils obtained by scCO<sub>2</sub>, scCO<sub>2</sub> plus co-solvent and by hexane Soxhlet extraction. The main fatty acid moieties present in all the samples are of palmitic acid (C16:0) and of linoleic acid (C18:2), followed by oleic acid (C18:1) and stearic acid (C18:0). Similar fatty acid ester profiles (FA) have been found in a previous work [19]. In the FA profiles of oils obtained by scCO<sub>2</sub> with or without co-solvent, there are not visible differences. Although, the polyunsaturated/saturated ratio is slightly lower for the experiments carried out with pure scCO<sub>2</sub>, due to higher content of linoleic acid in the oils with co-solvents, the results do not present significant differences. A similar conclusion was obtained in the extraction of oil with scCO<sub>2</sub> from green coffee beans for the FA profile applying the Duncan test [46].

The comparison of the GC results obtained for the total DUFA, MUFA and SFA with the results of <sup>1</sup>H-NMR shows a good agreement between both analytical techniques.

The fatty acid ester composition confirms the typical profile for SCGs, reported by most of the previous authors, namely domination of esters of palmitic and linoleic acids, which together with oleic esters constitute around 87 % of the total. Couto *et al.* [13] reported higher values for the total saturated fatty acid ester content in the scCO<sub>2</sub> coffee oils (46.2-58.1 %<sub>mass</sub>), namely in the values of C12:0 (3.57-7.41 %<sub>mass</sub>) and in C20:0 where a value of 4.28 %<sub>mass</sub> was determined. This composition can be explained mainly by the preferences of the respective local coffee drinking habits. SCGs in Turkey, for instance, are reported to have much more nitrogen and sulfur, with substantially different fatty acid esters composition [65].

The analysis of the fatty acid ester profiles in the hexane Soxhlet extraction from green and roasted coffee and from Arabic and Robusta varieties, allows for identification and differentiation of the coffees [66]. However, when SCGs are studied, the residuals collected from local coffee shops make this characterization almost impossible, given the variability in the fatty acid ester profiles.

#### **4.5 Free radical scavenging activity**

The results for the DPPH assays, obtained for the oil samples analyzed, and compared to the results for the trolox and ascorbic acid, as standards, are presented in Table 6. The oils were extracted from SCGs using hexane, and scCO<sub>2</sub> without and with co-solvents. The results show that for pure scCO<sub>2</sub> the values obtained are higher than those for the hexane extraction and consequently a poorest antioxidant capacity was found, since lower values correspond to better DPPH assays.

The influence of pressure and temperature on the antioxidant capacity is not completely clear, since only two levels of variation of these parameters were studied. The results obtained are in partial agreement with those previously reported [17], since at  $p = 10$  MPa, the increase of temperature (313, 323 and 333) K gives better values for the IC<sub>50</sub> of the oils, but for the other pressures, namely  $p = (20$  and  $30)$  MPa, respectively, no specific trends were observed.

The importance of the application of a co-solvent with scCO<sub>2</sub> is obvious when the antioxidant capacity is compared - the best antioxidant capacity increases 12.5 times (155.2 to 12.39 mg/mL). The use of co-solvents with scCO<sub>2</sub> gives better results for the antioxidation capacity as compared to hexane extraction, except for two of the cases studied: 20/313 (10 % iPrOH) and 20/313 (5 % EtOH). The best result - 12.39

mg/mL - was obtained with 10 % of ethanol, as a co-solvent, at 30 MPa and 333 K. The improvement can be explained by the increase of the solvent polarity and consequently the affinity and capacity to extract more phenolic compounds with intermediate to high polarity [17].

These results are partially in agreement with the values obtained from Andrade *et al.* [17], since when scCO<sub>2</sub> with 4 % of ethanol as a co-solvent was used, they obtained a lower and better value for the IC50 when compared with pure scCO<sub>2</sub>. However, when the co-solvent concentration was increased to 8 % a worse result was observed.

Finally, our results show higher absolute values of IC50 when compared to those of other authors, namely Andrade *et al.* [17], who report values between (0.4-2.3) mg/mL. The difficulties associated with the determination of DPPH values in vegetable oils and the solvents used to dissolve the samples and analysis have been previously discussed [39], where the authors conclude that performing DPPH assays of vegetable extracts is far from trivial and that it is not straightforward to name the best method. In this work we have chosen the method described in section 3, since it is possible to have complete miscibility of the oil with the solvent used and consequently, guarantee that all components of the extract have been quantified.

## **5. Results of the SCE kinetics modelling**

Table 7 shows the values obtained for  $K$  at  $T = 40$  C and at different operating conditions of pressure and flow rate. The agreement between the simulated and experimentally measured extraction curves, represented by AARD, is very good, considering the complex nature of the system and the innovative virtual molecule methodology introduced.

As seen in the Table 7, the value of  $K$  is influenced by the pressure and the flow rate: when the pressure increases,  $K$  increases; while, when the flow rate increases -  $K$  decreases. The variation observed when pressure increases is due to the higher mass of scCO<sub>2</sub> present in the extractor that results in more oil being transferred to the gas phase. As for the flow rate, the reduction in the residence time when the flow rate increases, results in a reduced amount of oil being present in the gas phase during extraction.

Figure 4 shows the experimental and simulated profiles. The simulation results reproduce closely the trend of the experimental data and, as demonstrated by the AARD values shown in Table 7, there is, generally, a good agreement between the experimental and simulated values.

The success of the representation of complex systems (e.g. vegetable oils), by a single virtual molecule and its combination with group contribution methods, being demonstrated for the first time in the present work, offers potential advantages, some of which are summarized below.

- The kinetics of vegetable oil SCE can be successfully modeled, representing the extract with a single surrogate molecule. However, the selection of the latter is based on intuition rather than on some sound principles. There could always be oils, for which it would not be so easy to select surrogate molecules. Typical examples are mixtures of oils, used increasingly in biodiesel production (e.g., 50:50 sunflower and coconut oils).

- Minor composition factors like content of free fatty acids, mono- and diglycerides, chain lengths, caffeine, etc. can significantly influence the oil solubility in scCO<sub>2</sub>. The proposed presentation of mixtures allows for including these and other components into the virtual molecule for developing more detailed models, without increasing the dimensionality of the phase equilibria calculations, while extending the use of group contribution methods to mixtures.

## 6. Conclusions

Results presented in this work demonstrate that extraction with scCO<sub>2</sub> and a co-solvent decrease in half the time necessary to obtain the maximum oil yield. Moreover, the oil extracts obtained have higher antioxidant capacity (12.5 higher at the optimum conditions applied) when compared to those obtained with pure scCO<sub>2</sub>. Different analytical techniques like NMR, elemental analysis and GC-Fid have been used with success to characterize the principal compounds in the extracts. The esters of palmitic and linoleic acids were identified as main components in its lipid profile and TAGs as well as the proportion of SFA, MUFA and DUFA were shown to have no significant differences in the supercritical conditions applied.

In the present paper, for the first time, the kinetics of oil extraction from SCGs with pure scCO<sub>2</sub> was modelled successfully by representation of the oil mixture as a single virtual molecule, whose critical

properties were estimated with group contribution methods. The agreement between the experimentally measured and simulated cumulative extraction yield curves is acceptable ( $5\% < \text{AARD} < 9\%$ ) considering the complexity of the system studied.

Hence, it is the authors' belief that the representation of any natural composite system by a single virtual molecule is a perspective new direction that should be further explored and tested when modeling the kinetics of other SCE processes for obtaining high value added substances from biomass and biowaste.

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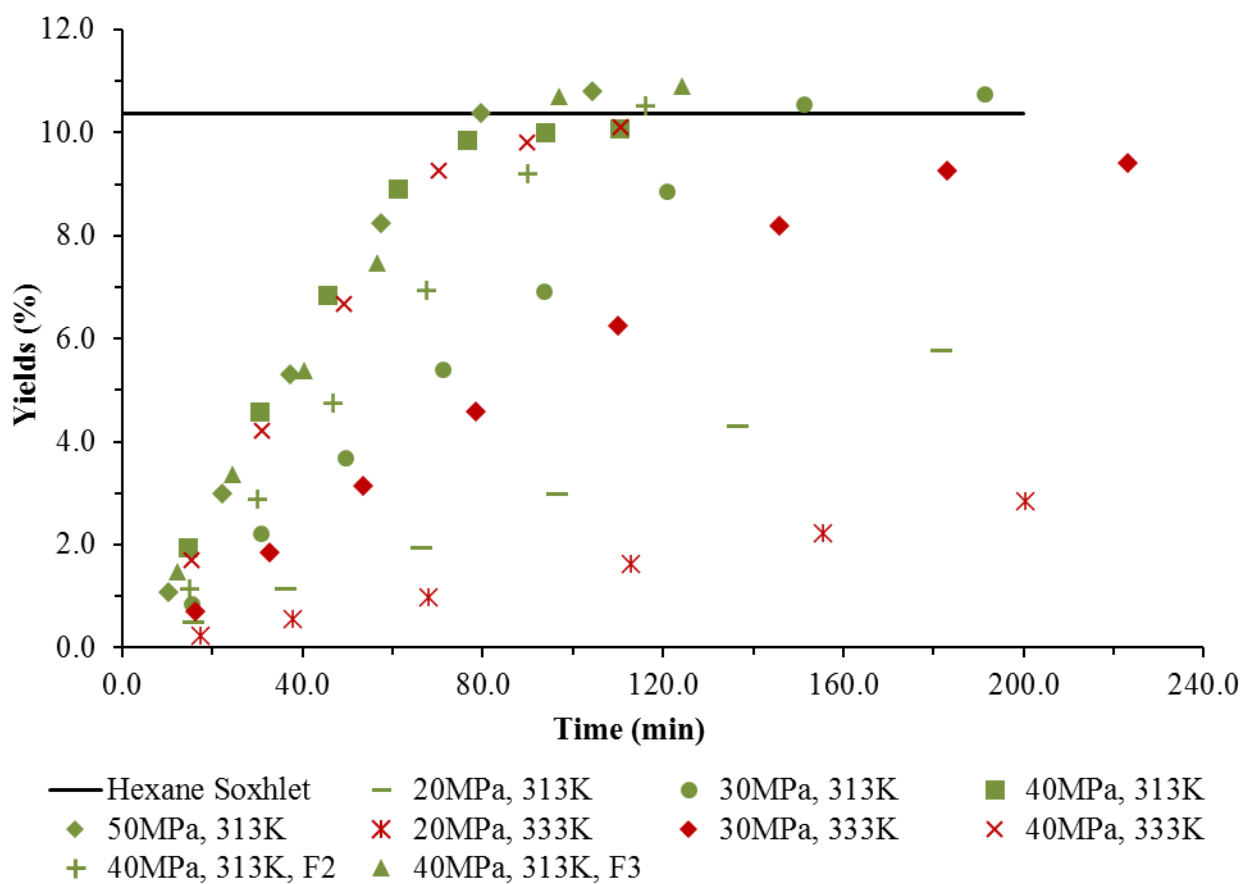
## Captions Figure

**Figure 1.** Experimental cumulative extraction yield curves plotted *vs* the extraction time, at scCO<sub>2</sub> flow rate of  $1.8 \times 10^{-3}$  kg/min for different pressures (MPa) and temperatures (K). F2 and F3 in the legend correspond to scCO<sub>2</sub> flow rates of F2= $1.5 \times 10^{-3}$  kg/min and F3= $2.2 \times 10^{-3}$  kg/min.

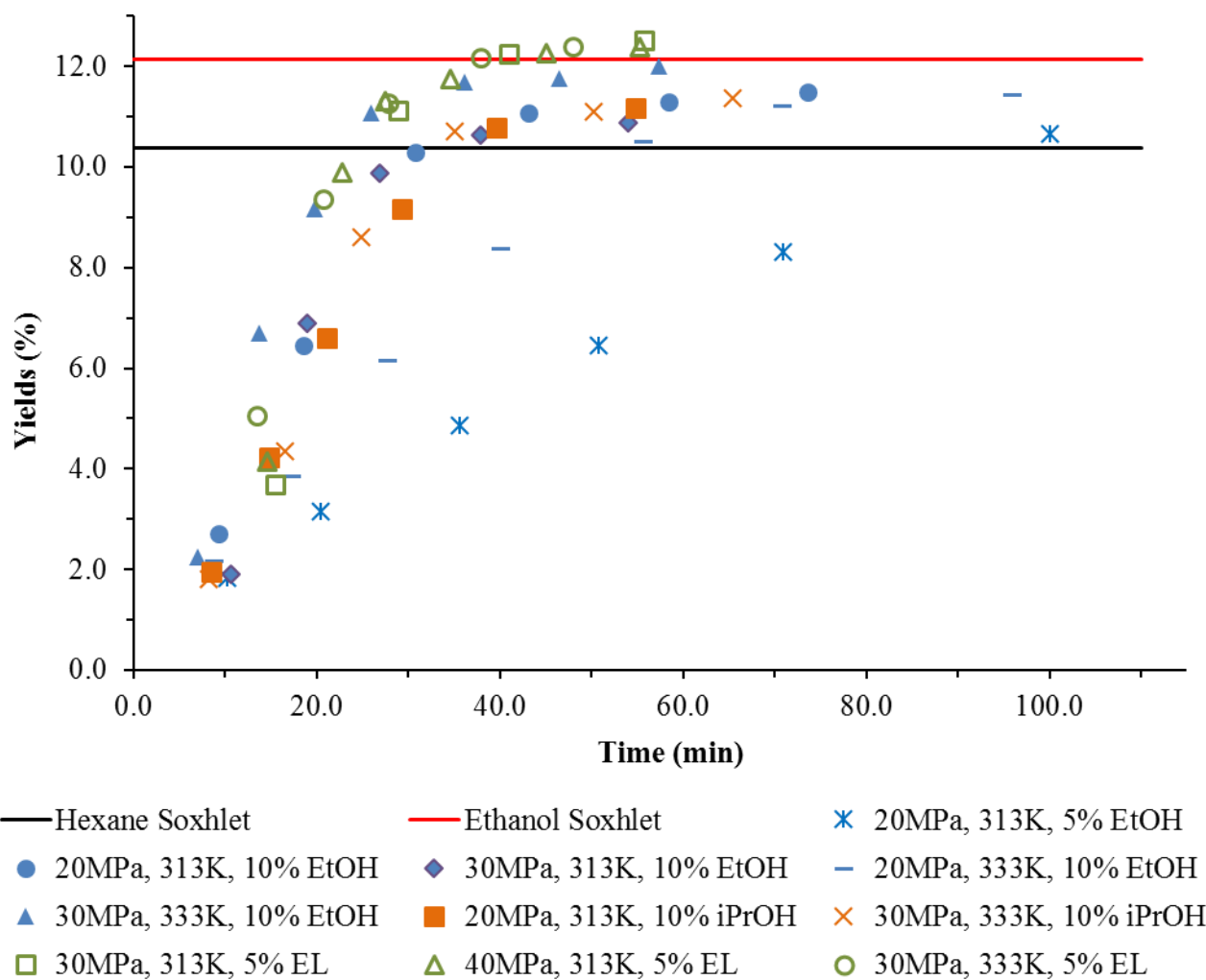
**Figure 2.** Experimental cumulative extraction curves plotted of recovery oil from SCG *vs* the extraction time, at scCO<sub>2</sub> flow rate of  $1.8 \times 10^{-3}$  kg·min<sup>-1</sup> for different pressures (MPa), temperatures (K) and different percentage of co-solvents: ethanol (EtOH), isopropanol (iPrOH) and ethyl lactate (EL).

**Figure 3.** <sup>1</sup>H NMR spectrum of SCGs oil obtained by scCO<sub>2</sub> extraction (30 MPa, 333 K, 10 % ethanol) in CDCl<sub>3</sub>, showing the attribution of the resonances to specific protons in the linoleic chain, used to determine the TAGs, DUFA and MUFA contents; (bottom) expanded views of the regions **A** (3.0 – 4.0 ppm) and **B** (6.0 – 7.0 ppm) used to determine the 1,2-DAG, caffeine, cafestol, 16-O-methylcafestol and kahweol (Caf = Caffeine; 16O-Me = 16-O-methylcafestol, C = Cafestol, K = kahweol)

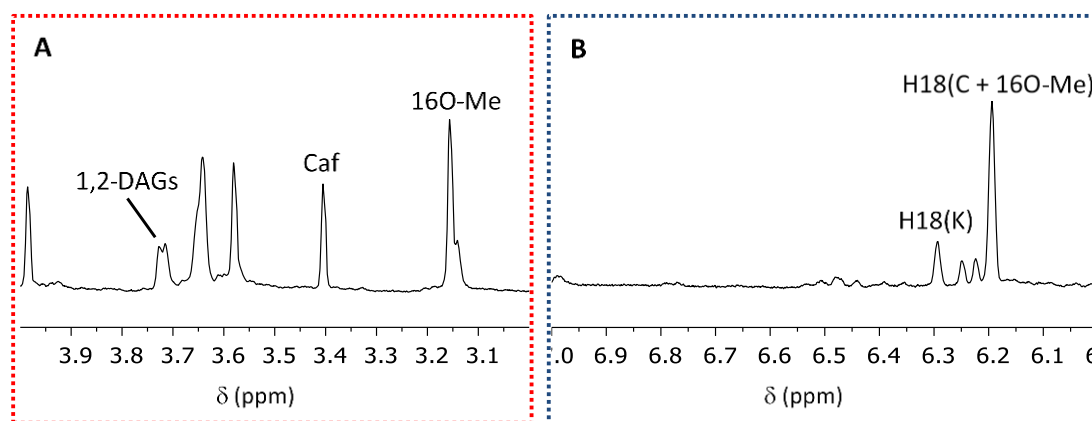
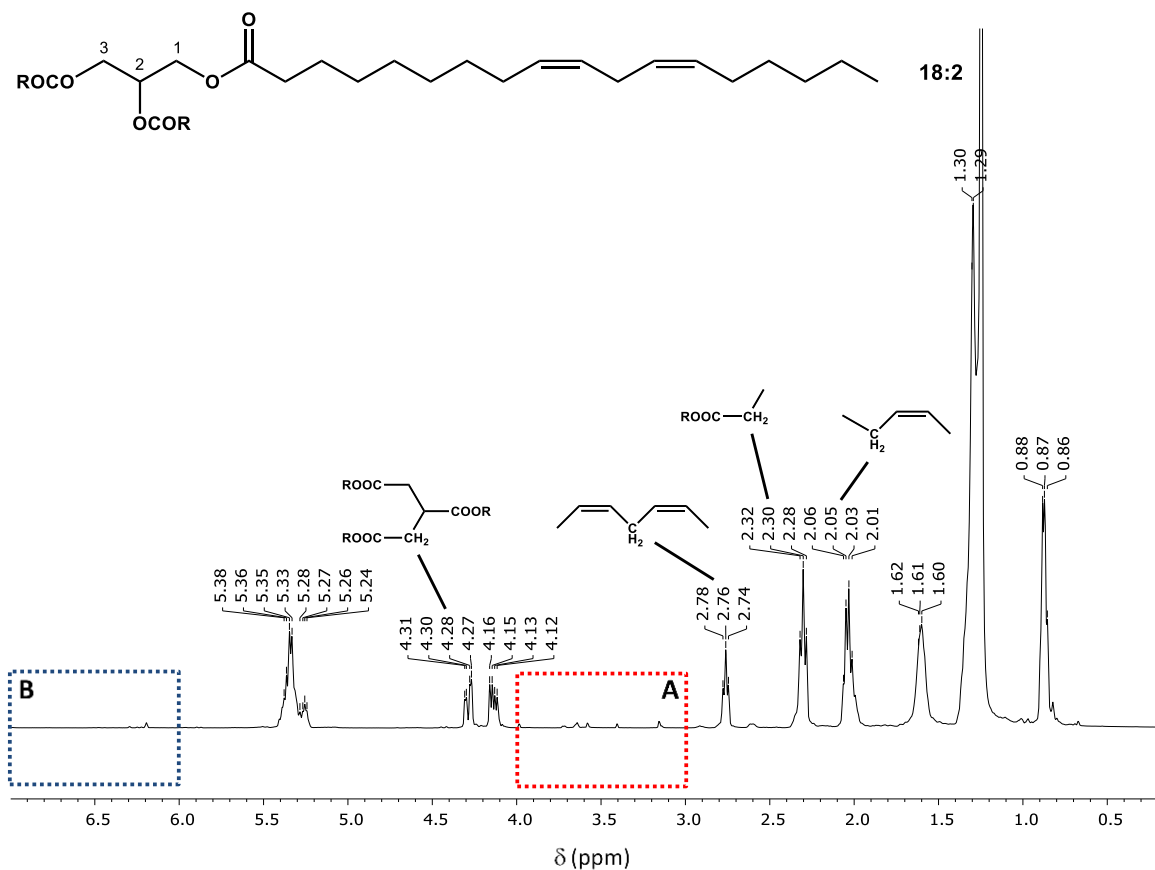
**Figure 4.** Experimental (Exp) and simulated (Sim) cumulative extraction yield curves plotted *vs* the extraction time, at scCO<sub>2</sub> flow rate of  $1.8 \times 10^{-3}$  kg/min for different pressures (MPa). F2 and F3 in the legend correspond to scCO<sub>2</sub> flow rates of F2 =  $1.5 \times 10^{-3}$  kg/min and F3 =  $2.2 \times 10^{-3}$  kg/min.



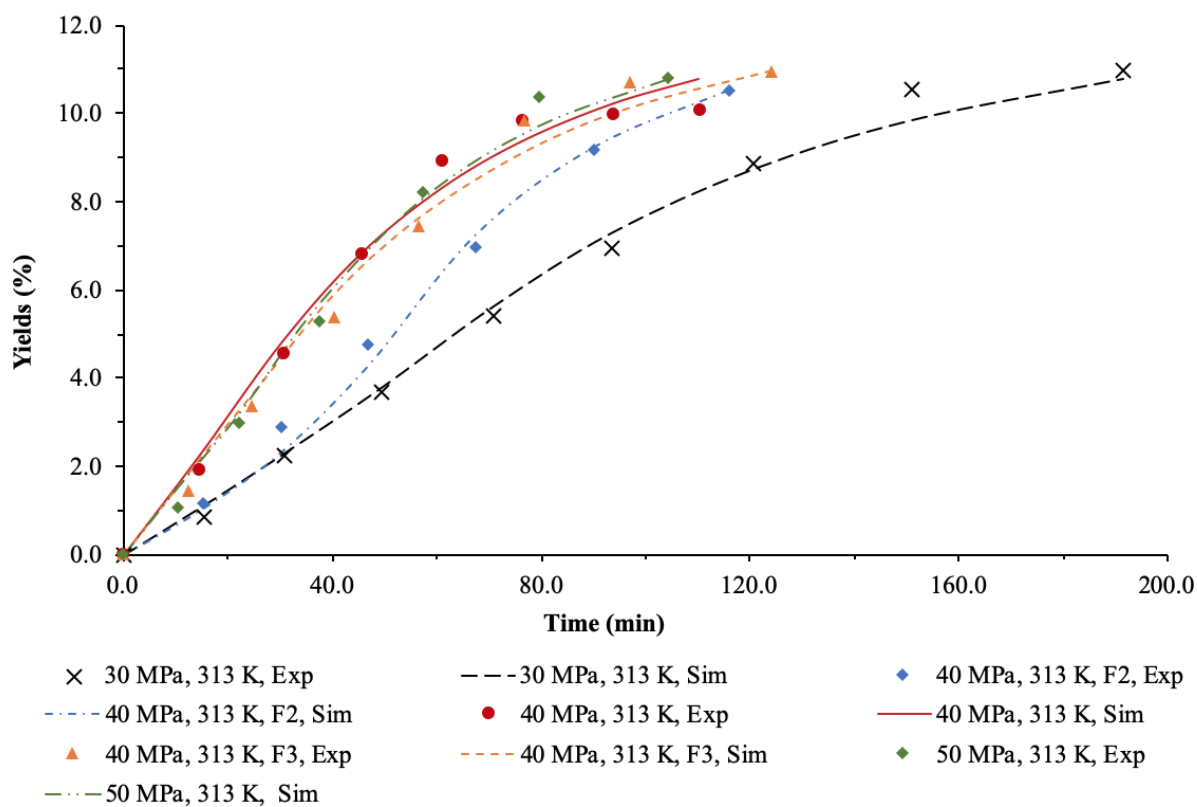
**Figure 1.** Experimental cumulative extraction yield curves plotted vs the extraction time, at scCO<sub>2</sub> flow rate of  $1.8 \times 10^{-3}$  kg/min for different pressures (MPa) and temperatures (K). F2 and F3 in the legend correspond to scCO<sub>2</sub> flow rates of  $F2 = 1.5 \times 10^{-3}$  kg/min and  $F3 = 2.2 \times 10^{-3}$  kg/min.



**Figure 2.** Experimental cumulative extraction curves plotted of recovery oil from SCG vs the extraction time, at  $\text{scCO}_2$  flow rate of  $1.8 \times 10^{-3}$  kg/min for different pressures (MPa), temperatures (K) and different percentage of co-solvents: ethanol (EtOH), isopropanol (iPrOH) and ethyl lactate (EL).



**Figure 3.** <sup>1</sup>H NMR spectrum of SCGs oil obtained by scCO<sub>2</sub> extraction (30 MPa, 333 K, 10 % ethanol) in CDCl<sub>3</sub>, showing the attribution of the resonances to specific protons in the linoleic chain, used to determine the TAGs, DUFA and MUFA contents; (bottom) expanded views of the regions **A** (3.0 – 4.0 ppm) and **B** (6.0 – 7.0 ppm) used to determine the 1,2-DAG, caffeine, cafestol, 16-O-methylcafestol and kahweol contents (Caf = Caffeine; 16O-Me = 16-O-methylcafestol, C = Cafestol, K = kahweol)



**Figure 4.** Experimental (Exp) and simulated (Sim) cumulative extraction yield curves plotted vs the extraction time, at scCO<sub>2</sub> flow rate of  $1.8 \times 10^{-3}$  kg/min for different pressures (MPa). F2 and F3 in the legend correspond to scCO<sub>2</sub> flow rates of F2 =  $1.5 \times 10^{-3}$  kg/min and F3 =  $2.2 \times 10^{-3}$  kg/min.

**Table 1.** Lipids composition of spent coffee oils obtained by hexane and scCO<sub>2</sub> extraction at a flow rate of scCO<sub>2</sub> F = 1.8x10<sup>-3</sup> kg·min<sup>-1</sup>, as established by <sup>1</sup>H-NMR quantitative analysis. All values represent % molar fractions. Unsaturation index (UI) is defined by UI = (2×DUFA % molar fraction + MUFA % molar fraction)/100.

Lipids (% <sub>mol</sub> )	scCO <sub>2</sub> conditions: p(MPa)/T (K)													
	Hexane	% / Co-solvent												
		30/313	40/313	30/333	40/333	20/313 5/EtOH	20/313 10/EtOH	20/333 10/EtOH	30/313 10/EtOH	30/333 10/EtOH	20/313 10/iPrOH	30/333 10/iPrOH	30/313 5/EL	30/333 5/EL
TAG	96.25	94.36	96.44	95.37	96.68	93.15	91.07	98.66	90.56	91.14	95.99	96.25	99.03	98.02
1,2 DAG	2.42	3.81	3.00	3.12	2.72	4.06	5.40	0.56	5.75	4.90	2.95	2.55	--	1.83
Caffeine	1.33	1.83	0.56	1.51	0.60	2.79	3.53	0.78	3.69	3.96	1.06	1.20	0.97	0.15
DUFA	41.5	42.0	41.1	41.8	41.1	40.8	41.5	39.8	41.3	41.8	40.1	40.5	40.3	40.3
MUFA	14.1	12.9	13.9	13.0	14.1	13.6	13.8	14.8	13.7	14.8	14.9	14.9	13.9	15.8
SFA	44.4	45.1	45.0	45.2	44.8	45.6	44.7	45.4	45.0	43.4	45.0	44.6	45.8	43.9
UI	0.97	0.97	0.96	0.97	0.96	0.95	0.97	0.94	0.96	0.98	0.96	0.96	0.94	0.96



**Table 2.** Diterpenes contents obtained by hexane and scCO<sub>2</sub> extraction at a flow rate of scCO<sub>2</sub> F = 1.8x10<sup>-3</sup> kg·min<sup>-1</sup>, established quantitatively by <sup>1</sup>H-NMR.

Compounds (% <sub>mol</sub> )	scCO <sub>2</sub> conditions: p(MPa)/T (K)													
	Hexane	% / Co-solvent												
		30/313	40/313	30/333	40/333	20/313 5/EtOH	20/313 10/EtOH	20/333 10/EtOH	30/313 10/EtOH	30/333 10/EtOH	20/313 10/iPrOH	30/333 10/iPrOH	30/313 5/EL	30/333 5/EL
Cafestol	1.32	1.52	2.04	1.75	2.05	1.42	0.65	1.12	0.86	0.53	1.68	1.36	1.21	1.67
16-O-Methyl- Cafestol	1.81	1.96	1.41	1.80	1.39	1.89	2.03	1.30	1.97	2.20	1.26	1.34	1.16	1.33
Kahweol	0.83	0.84	0.71	1.09	0.75	0.74	0.65	0.53	0.30	0.81	0.67	0.69	0.34	0.56
Diterpene content (%)	3.96	4.32	4.16	4.64	4.19	4.05	3.33	2.95	3.13	3.54	3.61	3.39	2.71	3.56

**Table 3.** Experimental and estimated results from the elemental analysis.\*

Sample of spent coffee grounds (SCG)	Yield, % of SCG	Elements, % in sample			
		N**	C	H	O
Initial SCG	<b>100.00</b>	<b>2.11</b>	<b>51.02</b>	<b>7.04</b>	<i>39.83</i>
Soxh hexane, Residue	<b>89.63</b>	<i>2.35</i>	<i>48.01</i>	<i>6.50</i>	<i>43.14</i>
Soxh hexane, Extract	<b>10.37</b>	<b>&lt;0.5/0.04</b>	<b>77.00</b>	<b>11.74</b>	<i>11.26</i>
SCO <sub>2</sub> (30/313), Residue	<b>89.28</b>	<b>2.35</b>	<b>48.12</b>	<b>6.38</b>	<i>43.15</i>
SCO <sub>2</sub> (30/313), Extract	<b>10.72</b>	<b>&lt;0.5/0.11</b>	<b>77.03</b>	<b>12.14</b>	<i>10.83</i>
SCO <sub>2</sub> (40/313), Residue	<b>89.20</b>	<b>2.26</b>	<b>47.46</b>	<b>6.30</b>	<i>43.98</i>
SCO <sub>2</sub> (40/313), Extract	<b>10.80</b>	<b>&lt;0.5/0.87</b>	<b>76.96</b>	<b>11.54</b>	<i>11.50</i>
SCO <sub>2</sub> (40/333), Residue	<b>89.40</b>	<b>2.33</b>	<b>48.08</b>	<b>6.31</b>	<i>43.28</i>
SCO <sub>2</sub> (40/333), Extract	<b>10.60</b>	<b>&lt;0.5/0.25</b>	<b>77.07</b>	<b>12.07</b>	<i>10.86</i>
SCO <sub>2</sub> (30/333, 10% iPrOH), Residue	<b>88.63</b>	<b>2.28</b>	<b>45.93</b>	<b>6.15</b>	<i>45.64</i>
SCO <sub>2</sub> (30/333, 10% iPrOH), Extract	<b>11.37</b>	<b>&lt;0.5/0.78</b>	<b>76.51</b>	<b>11.38</b>	<i>12.11</i>
SCO <sub>2</sub> (30/313, 10% EtOH), Residue	<b>88.00</b>	<b>2.30</b>	<b>45.59</b>	<b>6.28</b>	<i>45.83</i>
SCO <sub>2</sub> (30/313, 10% EtOH), Extract	<b>12.00</b>	<b>&lt;0.5/0.72</b>	<b>76.63</b>	<b>11.49</b>	<i>11.88</i>
SCO <sub>2</sub> (30/333, 5% EL), Residue	<b>87.60</b>	<b>2.23</b>	<b>45.57</b>	<b>6.12</b>	<i>46.08</i>
SCO <sub>2</sub> (30/333, 5% EL), Extract	<b>12.40</b>	<b>&lt;0.5/1.26</b>	<b>76.49</b>	<b>11.26</b>	<i>12.25</i>
Initial SCG, Cholakov et al.[67]	<b>100.00</b>	<b>2.56</b>	<b>51.31</b>	<b>7.21</b>	<i>38.92</i>
Soxh hexane, Residue, Cholakov et al.[67]	<b>87.80</b>	<i>2.91</i>	<i>47.85</i>	<i>6.64</i>	<i>42.60</i>
Soxh hexane, Extract, Cholakov et al.[67]	<b>12.20</b>	<b>0.00/0.04</b>	<b>76.21</b>	<b>11.32</b>	<i>12.47</i>
Initial, Moser et al. [68]	<b>100.00</b>	<b>2.40</b>	<b>56.30</b>	<b>7.20</b>	<i>34.10</i>
Soxh hexane, Residue, Moser et al.[68]	<b>84.10</b>	<b>2.80</b>	<b>52.00</b>	<b>6.30</b>	<i>38.90</i>
Soxh hexane, Extract, Moser et al.[68]	<b>15.90</b>	<i>0.28</i>	<i>79.04</i>	<i>11.96</i>	<i>8.71</i>

\*Experimental results are given in boldface, estimated – in italic. Estimates were calculated from the mass balances of the extractions and the elements in the samples.

\*\*The lowest limit under which the nitrogen concentration could not be determined quantitatively by the method used was 0.5.

**Table 4. Composition of the esters of fatty acids, obtained with scCO<sub>2</sub>, %<sub>mass</sub>\*.**

Fattyacid ester	Hexane	scCO <sub>2</sub> conditions: <i>p</i> (MPa)/ <i>T</i> (K)				
		20/313	30/313	40/313	30/333	40/333
C12:0 - Lauric	0.00	0.00	0.00	0.00	0.00	0.00
C14:0 - Myristic	0.09	0.06	0.09	0.09	0.05	0.09
C16:0 - Palmitic	33.21	33.69	33.22	33.87	33.19	33.34
C16:1 - Palmitoleic	0.06	0.02	0.1	0.1	0.02	0.11
C18:0 - Stearic	7.51	7.49	7.28	7.32	7.48	7.51
C18:1 - Oleic	11.22	11.12	11.1	11.11	11.5	11.12
C18:2 -Linoleic	42.59	42.7	42.6	42.98	42.76	42.88
C18:3 -Linolenic	0.91	1.11	1.67	0.85	0.98	0.85
C20:0 -Arachidic	3.14	2.86	2.89	2.74	3.2	3.01
C20:1 -Gadoleic	0.45	0.43	0.38	0.39	0.27	0.38
C22:0 -Behenic	0.54	0.5	0.44	0.39	0.36	0.46
C22:1 - Erucic	0.00	0.02	0.00	0.00	0.00	0.00
C24:0 -Lignoceric	0.27	0.00	0.23	0.16	0.18	0.23
C24:1 -Nervonic	0.01	0.00	0.00	0.00	0.01	0.02
DUFA	42.59	42.7	42.6	42.98	42.76	42.88
MUFA	11.74	11.59	11.58	11.6	11.8	11.63
SFA	44.76	44.6	44.15	44.57	44.46	44.64
UI	0.97	0.97	0.97	0.98	0.97	0.97

\*The lowest limit under which concentrations could not be determined quantitatively by the method used was 0.003. These concentrations were assumed to be 0.00 %<sub>mass</sub>

Table 5. Composition of the esters of fatty acids obtained with scCO<sub>2</sub> and co-solvents %<sub>mass</sub>\*.

Fatty acid ester	scCO <sub>2</sub> conditions: <i>p</i> (MPa)/ <i>T</i> (K)							
	% / Co-solvent							
	20/313	20/313	30/313	30/333	20/313	30/333	30/313	30/333
	5/EtOH	10/EtOH	10/EtOH	10/EtOH	10/iPrOH	10/iPrOH	5/EL	5/EL
C12:0 - Lauric	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00
C14:0 - Myristic	0.03	0.05	0.09	0.09	0.07	0.09	0.09	0.03
C16:0 - Palmitic	33.60	33.02	33.05	33.18	33.54	33.27	33.2	33.03
C16:1 - Palmitoleic	0.03	0.09	0.06	0.06	0.02	0.05	0.10	0.04
C18:0 -Stearic	7.52	7.61	7.68	7.60	7.74	7.66	7.43	7.34
C18:1 - Oleic	11.4	11.29	11.23	11.07	11.08	11.07	11.20	11.66
C18:2 -Linoleic	42.90	42.85	42.79	42.78	42.89	42.78	42.86	42.48
C18:3 -Linolenic	0.94	0.96	0.85	0.89	0.54	0.85	0.88	0.86
C20:0 – Arachidic	2.95	3.10	3.10	3.11	3.10	3.12	3.03	3.35
C20:1 – Gadoleic	0.27	0.38	0.42	0.39	0.35	0.44	0.41	0.58
C22:0 –Behenic	0.36	0.39	0.53	0.53	0.44	0.52	0.48	0.36
C22:1 – Erucic	0.00	0.00	0.04	0.00	0.00	0.03	0.00	0.00
C24:0 -Lignoceric	0.00	0.12	0.08	0.21	0.14	0.06	0.21	0.17
C24:1 -Nervonic	0.00	0.14	0.08	0.09	0.07	0.06	0.11	0.10
DUFA	42.90	42.85	42.79	42.78	42.89	42.78	42.86	42.48
MUFA	11.70	11.9	11.79	11.61	11.52	11.62	11.82	12.38
SFA	44.46	44.29	44.53	44.72	45.05	44.72	44.44	44.28
UI	0.98	0.98	0.97	0.97	0.97	0.97	0.98	0.97

\*The lowest limit under which concentrations could not be determined quantitatively by the method used was 0.003. These concentrations were assumed to be 0.00 %<sub>mass</sub>.

Table 6. Antioxidation capacity of the extracts.

<b>scCO<sub>2</sub>: <i>p</i>(MPa)/<i>T</i> (K) (% / Co-solvent)</b>	<b>IC<sub>50</sub> (mg/ml)</b>
30/313	155.2±7.3
40/313	144.9±3.8
30/333	109.9±5.3
40/333	96.23±2.77
20/313 (5 % EtOH)	114.2±5.2
20/313 (10 % EtOH)	24.58±1.25
30/313 (10 % EtOH)	14.43±0.69
20/333 (10 % EtOH)	19.04±0.67
30/333 (10 % EtOH)	12.39±0.56
20/313 (10 % iPrOH)	64.00±1.86
30/333 (10 % iPrOH)	22.77±0.93
30/313 (5 % EL)	15.86±0.59
30/333 (5 % EL)	14.04±0.58
<i>n</i> -hexane	25.07±1.37
Trolox	0.0742±0.0046
Ascorbic acid	0.0619±0.0032

Table 7. Estimated values of  $K$  at different experimental conditions ( $T = 313 \text{ K}$ )

P (MPa)	Flow rate ( $10^3 \text{ kg/min}$ )	$k_f$ ( $10^4 \text{ m/min}$ )	$K$ (kg solid matrix / kg $\text{CO}_2$ )	AARD (%)
30	1.80	2.23	0.160	6.30
40	1.50	1.55	0.536	6.27
40	1.80	1.87	0.294	5.36
40	2.20	2.16	0.202	7.08
50	1.80	1.73	0.321	8.96