Damian E.L. Fetzer, Luis Ricardo S. Kanda, Lorenna Alves Xavier, Pollyanna Nogueira da Cruz, Massimiliano Errico, Marcos L. Corazza,

Lipids and coumarin extraction from cumaru seeds (Dipteryx odorata) using sequential supercritical CO2+solvent and pressurized ethanol,

The Journal of Supercritical Fluids,Volume 188, 2022, 105688.

http://dx.doi.org/10.1016/j.supflu.2022.105688

Lipids and coumarin extraction from Cumaru seeds (*Dipteryx odorata)* using sequential supercritical CO_2+solvent and pressurized ethanol

Damian E. L. Fetzer[a], Luis Ricardo S. Kanda[a], Lorenna Alves Xavier[a], Pollyanna Nogueira da Cruz[a], Massimiliano Errico[b], Marcos L. Corazza[a,*]

[a]Department of Chemical Engineering, Federal University of Paraná, CEP 81531-990, Curitiba, PR, Brazil.

[b]*Department of Green Technology, University of Southern Denmark, Campusvej 55, 5230 Odense M, Denmark*

*Corresponding author: Marcos L. Corazza, PhD. E-mail: corazza@ufpr.br; Phone: 55-41-3361-3587

ABSTRACT

Lipids and coumarin were extracted from cumaru seeds using Soxhlet extraction (SE) with ethanol (EtOH) and ethyl acetate (EtOAc), $scCO_2$, $scCO_2$+EtOH and $scCO_2$+EtOAc, either in a single or in sequential stages, and with PLE with EtOH. $ScCO_2$+EtOAc provided yields from 28 to 31%, independently of the extraction conditions employed, and $scCO_2$+EtOH provide a top yield of 38%, at 25 MPa and 80 ºC. Extraction yields were enhanced by three sequential extractions, resulting in yields of 44.0% and 56.5% for $scCO_2$+EtOAc and $scCO_2$+EtOH, respectively, where these values were superior to those obtained in SE. This work also reported the kinetics of coumarin extraction using $scCO_2$+cosolvents, where in the case of $scCO_2$+EtOH the coumarin content was 30 mg g^{-1} of extract. Finally, the coumarin crystals seemed to be affected by the cosolvent nature, as indicated by the higher antioxidant activity of coumarin-rich extracts obtained by $scCO_2$+EtOAc, rather than those obtained by $scCO_2$+EtOH.

Keywords: *Dipteryx odorata*, coumarin, CO_2-cosolvent extraction, pressurized liquid extraction.

1. Introduction

Coumarins are lactones with the basic structure of 1,2 – benzopyrone. They can be found in seeds fruits, flowers, roots, leaves, and stems of over 150 different plant species distributed in over 30 families [1–3]. The study of coumarins started in 1820 when Vogel first isolated the compounds from *Dipteryx odorata* Wild; Fabaceae family, known also as cumaru. Since then, different applications have been considered. Coumarins have been extensively studied for their antiviral [4,5], antimicrobial [6,7], and antioxidant [8–10] properties. Moreover, medical evidences such as anticoagulant [11–13], anticancer [14–17], antithrombotic [18], anti-inflammatory [19], and vasodilatory activities [20] were also reported. For this reason, coumarins are of high interest for the pharmaceutical industry. Beyond the application as medical drug, due to its vanilla-like odor [21], it is used in cosmetic [22], cleaning detergents [23], and as food additive [24–26]. Although in the European Union the tolerable daily intake is lower than 0.1 mg coumarin / kg body weight /day [27], in the United States of America coumarin consumption is totally prohibited due to its toxicity [28]. However, as discussed by Cassandra Lee [29] to reach this threshold, an adult individual should consume about 2400 flavored desserts in which case intoxication from coumarins would be not the major concern. The use of coumarin derivatives is not restricted to pharmacological and food applications. In recent years researchers have explored their physical properties like the hydrophobicity and the possible application in encapsulation [30], in films [31], in dye-sensitized solar cells [32,33], and also as possible alternative to magnetic hard drives and high-capacity flash memories for information storage [34,35]. In the context of the wide spectrum of possible applications, the separation of coumarin from renewable sources is a relevant topic to be explored.

Different solvents and extraction techniques to obtain coumarin from cumaru seeds have been studied. Polar solvents are usually preferred since coumarin has a high solubility in such solvents as n-propanol, methanol, ethanol, and others. Ethanol is frequently used because it is not considered a toxic solvent as well as having a high affinity for coumarin [36]. On the other hand, Jang et al. [37] used ethyl acetate in Soxhlet extraction of cumaru seeds and indicated that the oil stimulates the enzyme quinone reductase in rat cell cultures. This represents a great protective mechanism against the onset of tumor formation, establishing a potential cancer chemopreventive feature of the cumaru seed. In addition, non-polar solvents such as n-hexane have been also studied by our research group [38]. In this case, the extract oil contains high amount of unsaturated fatty acids (~ 40 %). Compressed propane at high pressures was used by our research group and resulted in a crude oil recovery up to 98 % with low amount of coumarin and remarkable antioxidant, antimicrobial and chemical properties.

For extracts obtained from cumaru seeds, more than 190 compounds (alcohols, aliphatic and aromatic hydrocarbons, acids, carbonyl compounds, esters, lactones, terpenes, terpenoids, and other non-categorized compounds) were identified under diverse extraction methods, such as head-space solid phase micro-extraction, simultaneous distillation-extraction and supercritical fluid extraction [39]. The main constituent detected in all the previously mentioned extraction methods was coumarin and extraction with supercritical CO_2 as solvent ($scCO_2$) reported the highest selectivity to remove coumarin from the seeds [39]. Several studies with different plants demonstrated that CO_2 at supercritical conditions is a selective solvent to extract coumarin [40–42]. Lima et al. [43] used $scCO_2$ extraction with modifiers in which 20 % of coumarin was extracted from cumaru seed, but the optimization of the extraction conditions was not investigated. An alternative and innovative technique is to use CO_2 gas expanded liquid as solvents for extraction, consisting in a mixture of a compressible gas dissolved in a liquid solvent which helps to enhance the extraction yield reducing the extraction time substantially [44]. In addition, pressurized liquid extractions (PLE), in which the raw material is subjected to high-pressure liquid solvents to increase the diffusion and solubility, also have been used as a tool to facilitate the recovery of the target compounds [45]. However, the extraction of coumarin compounds using PLE methods has not been deeply studied.

In the present work, the improvement of the crude oil extraction and coumarin compounds recovery from *Dipteryx odorata* seeds using different techniques is explored. Besides the conventional extraction method using a Soxhlet apparatus, three other extractions methods were investigated: supercritical CO_2 extraction ($scCO_2$), $scCO_2$ plus organic solvents in a semi-batch mode employing ethanol and ethyl acetate ($scCO_2$ + EtOH and $scCO_2$+EtOAc), and continuous pressurized liquid extractions with ethanol (PLE-EtOH). Each sample obtained was characterized, in order to achieve a comparison between the different extraction techniques employed. In addition, the amount of coumarin in the extract was monitored over time, for the $scCO_2$+EtOH and PLE-EtOH extractions.

2. Materials and Methods

2.1 Chemicals and reagents

Carbon dioxide (99.5%, White Martins S.A., Brazil); ethanol (99.8%, Neon, Brazil); and ethyl acetate (99.5 %, Neon, Brazil) were used as solvents. Reagents used in the chemical analysis included methanol (HPLC-grade) purchased from Merck (Darmstadt, Germany), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), ABTS (≥ 98 %, Sigma-Aldrich, USA), 2,2-diphenyl-1-picrylhydrazyl), DPPH (Sigma-Aldrich, USA), gallic acid (Sigma-Aldrich, USA), Folin- Ciocalteu reagent (Sigma-Aldrich, USA), sodium carbonate (99.9 %, Merck, Germany), potassium persulfate (99 %, Neon, Brazil), and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid or Trolox. Standard of coumarin (> 99 %) purchased from Sigma-Aldrich and standard mixture of fatty acid methyl esters (FAMEs) (Supelco, MIX FAME 37, St. Louis, MO 63103, USA) were used in the chromatography analyses. All chemicals were used without any further purification.

2.2 Cumaru seeds

Cumaru (*Dipteryx Odorata* [Aubl.] Willd) seeds were collected in Altamira City, state of Pará, Brazil and a grater was used to reduce their size until an average particle size distribution of 2.0 ± 0.1 mm. The average particle size was determined using a sieve series (9-35 mesh) according to Gomide [46], under mechanical stirring. Apparent density of the bed was determined from the mass of cumaru seed particles used within the extractor vessel and the bed volume, and the real density of each particle size sample was measured by helium gas pycnometer (Micromeritics Accu Pyc II 1340), at the Laboratory of Thermodynamic and Supercritical Extraction - LATESC, Florianopolis, Santa Catarina, Brazil. Bed porosity was calculated using the apparent and real densities of the extraction bed as described in Fetzer et al. [47]. The moisture content and volatile compounds were determined according to AOAC methods 925.40 [48]. The raw material was stored in a freezer at -5 ºC inside a polypropylene plastic bag vacuum pack until its use.

2.3 Extraction Methods

2.3.1 Soxhlet extraction (SE)

A classical extraction was performed using a Soxhlet apparatus (UNIGLAS, Campinas, São Paulo, Brazil) with about 5 g of cumaru seeds and approximately 150 mL of solvent. The extractions were performed in triplicates for 6 h, under reflux conditions, temperature close to the boiling point of the solvent, according to the 920.39C method of AOAC [49], with some modifications. Then, the solvent was removed from the oil using a rotary vacuum evaporator (IKA, Model RV 10 digital, Germany). The overall extraction yield (%) was calculated as follows:

$$Yield(\%) = \frac{grams\ extracted\ oil}{grams\ cumaru\ seed\ sample} \cdot 100$$

(1)

2.3.2 Supercritical CO_2 extraction ($scCO_2$)

Extraction using $scCO_2$ were carried out in a laboratory scale apparatus, as showed in Figure 1, used for the extraction of a variety of seed oils [38]. The extraction vessel used in such experiments is made of stainless steel and has the following dimensions: length (L) 0.16 m, inner diameter (Φ) 2.52×10^{-2} m, and internal volume (V_i) 7.98×10^{-5} m^3. The pressure was maintained constant at the set point defined for the extraction condition using a 500 mL syringe pump (ISCO, model 500D, USA), and the temperature in the pump jacket set at 10 °C for all extractions controlled by a circulating thermostatic bath. Another circulating thermostatic bath was connected to the extractor jacket and was employed to control the extraction temperature. Two needle valves were used to allow the gas to pass through the extraction bed, and a micro-metering needle valve was employed to maintain a constant flow rate (2.0 ± 0.3 mL min^{-1}) during the dynamic extractions. The extracts were collected at atmospheric pressure and room temperature in the separation chamber. Two extractions were carried out using pure $scCO_2$ at 25 MPa and varying the temperature (40 and 80 °C) [50]. For this case, about 26 g of the solid matrix were used in the extraction vessel and the extract was collected every 5 min for 180 min.

Figure 1

2.3.3 $scCO_2$ + solvent extraction

Extraction using $scCO_2$ + liquid solvents (ethanol and ethyl acetate) were also performed in the apparatus presented in Figure 1. However, the experimental procedure for such extraction technique was slightly different, in the sense that the cosolvent was added into the extraction vessel mixed to the solid matrix.

Experiments for the $scCO_2$ + EtOH (or EtOAc) extraction were performed with approximately 25 g of raw material. The raw material sample was added to a pre-weighed beaker, and the organic solvent was added in a solvent to solid mass ration of (1:1). Then, the mixture (solids plus solvent) was loaded into the extraction vessel. The operating conditions were based on previous studies [51,52], in which the experimental design consisted of 7 experimental conditions, performed at two-level-two-factor, with temperatures of 40 °C and 80 °C and pressures of 2 MPa and 10 MPa, including 3 replicates at the central point.

Another set of experiments was performed with different solids to solvent ratios:(0.5:1), (1:1) and (2:1), corresponding to around 25 g, 23 g, and 18 g of raw material, respectively, to investigate the effect of this factor on the extraction kinetics and yields. After both pressure and temperature reached the set values for the experiment, a confinement time (static extraction) of 30 min was set to reach thermal and mechanical equilibration and, after that, the dynamic extraction was performed using a flow rate of 2.0 ± 0.3 mL min^{-1} for 1 h.

$ScCO_2$ + EtOH (or EtOAc) extraction were also performed in sequential stages, in which, at the end each extraction stages the extraction vessel was filled again with fresh organic solvent and another extraction run was performed. The cosolvent makeup was determined according to the solids to cosolvent mass ratio pre-defined, considering the initial weight of solids in the experiment. This approach was performed using either ethanol and ethyl acetate as cosolvents and three sequential stages were conducted at 40 °C and 15 MPa using $scCO_2$+EtOAc, and at 80 °C and 25 MPa using $scCO_2$+EtOH. The extracts were collected until the sample depletion, *i.e.* until no extract was visually observed in the extraction vessel outlet, independently of time.

2.3.4 Pressurized liquid extraction (PLE)

PLE apparatus was assembled according the experimental setup presented by Dias [53], and reported in Figure 2. Briefly, around 10.5 g of cumaru seeds were loaded into the PLE extractor vessel ($L = 0.172$ m, $\Phi = 0.0124$ m, V_i 2.044 x 10^{-5} m^3). Then, the extractor vessel was closed and filled with ethanol until the required conditions of pressure and temperature were attained. The pressure was fixed at 10 MPa using a high-pressure liquid metering pump (Eldex, model 2SM, USA) with the aid of a back-pressure regulator valve (model KPB1SOA Swagelok, UK) in the outlet of the extractor. Simultaneously, the temperature was set at 3 different conditions (at 60, 80, and 100 °C) and the heating was provided by an electric jacket resistance, controlled by a digital controller (Novus, model N480D, Brazil) coupled to a T-type thermocouple. The system was initial kept static for confinement time of 15 min. Then, the dynamic extraction was carried out at a constant flow rate of 1.9 ± 0.2 mL min^{-1}, controlled by the high-pressure liquid metering pump. Samples were collected every 2.5 min for the first 10 min, every 10 min until 30 min, subsequently, every 15 min until 60 min, and finally every 30 min until the end of the extraction fixed at 180 min. After the extraction, the solvent was evaporated using a rotary vacuum evaporator (IKA, Model RV 10 digital) at 40 °C for around

15 ± 5 min. Finally, the samples were putted into air recirculating oven (IKA, model 400-2) to remove the residual solvent, until a constant weight was obtained.

Figure 2

2.4 Characterization

2.4.1 Quantification of coumarin

Coumarin content was determined according to the methodology reported by Chu et al [54]. Analyses were carried out using an ACQUITY UPLC H-Class System with photodiode array detector (Waters, Miliford, MA, EUA) and a C18 Kinetex column (Phenomenex) (100 mm x 2.1 mm, 2.6 µm). First, 50 mg of cumaru seed oil was diluted into 1.5 mL of a mixture water:methanol (20:80), shaken and centrifuged for 10 min at 3000 rpm. Then, 2 mg mL^{-1} of the final concentration (supernatant diluted in mobile phase) was filtered through a 0.22 µm nylon filter. The elution was performed in isocratic mode at a flow rate of 0.5 mL min^{-1} using a proportion of 80% of the mixture water:acetonitrile:formic acid (95:4:1) (phase A) and 20% of the mixture acetonitrile:formic acid (99:1) (phase B). The injection volume was 5 µL and the chromatograms were recorded at 320 nm.

2.4.2 Fatty acids (FA) analysis

The fatty acid profile of the seed oil samples was obtained according to the AOCS official method (Ce 2-66), in which the oil samples were converted to fatty acid methyl esters (FAMEs) (AOCS 1997) [55]. Then, a Shimadzu gas chromatograph (GC 2010 *Plus*) coupled with a flame ionization detector (FID) was used to analyze the FAMEs. The GC was equipped with a capillary column (SH-Rtx-Wax (Shimadzu): 30 m x 0.32 mm x 0.25 µm) and a split injection mode (1:10) was used. The injector and detector temperatures were 240 °C and 250 °C, respectively. The oven temperature was set and started at 100 °C for 5 min, then the temperature was increased up to 240 °C at a rate of 4 °C min^{-1} and this temperature was maintained for 5 min. The carrier gas through the column was Helium at a constant flow rate of 32.5 cm^3 min^{-1}. The identification of FAMEs was carried out by comparison with retention times of the FAMEs standard mixture. Finally, the amount of fatty acids was determined by area normalization and the results were expressed as a percentage of each individual fatty acid present in the oils sample.

2.4.3 Physicochemical characterization

Samples of cumaru seed oil samples were analyzed in terms of some physicochemical properties. The free fatty acids (FFA) were measured by the titration method according to AOCS methods Ca 5a-40 [55]. Refractive index (RI) was determined at 25 °C using an Abbe refractometer (Biobrix, Brazil) [56]. In addition, Iodine and saponification values of cumaru seed oils were calculated using the fatty acids content according to the Official methods and recommended practices of the AOCS [55].

Calorimetric analyses were performed by differential scanning calorimeter (DSC) and thermogravimetric analysis (TGA). The DSC (850 Perkin Elmer Inc. Waltham, USA) used nitrogen (N_2 99.999%) as a purge gas. Sample of the oil obtained using Soxhlet extraction with ethanol and ethyl acetate, and those obtained employing extraction with compressed fluids were evaluated. Around 4 mg of oil were weighed and sealed into a hermetic aluminum pan. Both cooling and heating tests were performed according to the following time-temperature program: equilibration at 20 °C for 5 min to ensure complete temperature homogenization of the sample; heating to 50 °C at 5 °C min^{-1}, and holding for 5 min followed by cooling to -80 °C at -5 °C min^{-1}, to induce crystallization, and holding at this temperature for 5 min. Finally, samples were subjected to a heating from -80 to 50 °C at 5 °C min^{-1} to obtain the melting profile [57].

TGA was carried out through an open aluminum pan. About 10 mg of oil samples were submitted to a heating rate of 10 °C min^{-1}, from 30 to 750 °C, at atmospheric pressure, in contact with a constant flow rate of 50 mL min^{-1} of synthetic air (White Martins S.A., 99.5% pure). Each sample was measured in duplicates. Peak and onset temperatures were calculated using PyrisTM software.

2.4.4 Antioxidant activity (AA) and total phenolic content (TPC)

The samples were prepared as follows: 100 mg of each sample was diluted into 1 mL of methanol/water (20:80), stirred and centrifuged (Edutec, Brazil) for 10 min at 3000 rpm. The AA and TPC analyses were performed in triplicates. The concentration of TPC in the cumaru seed oil was determined based on the Folin–Ciocalteu method [58] using a spectrophotometer (UV-Vis Global Analyzer; model GTA97, Brazil). For the TPC determination, 0.4 mL of the supernatant and 0.1 mL of methanol were mixed with 2.5 mL of Folin-Ciocalteu reagent (diluted 1:10 in water). Then, the mixture was held in the darkness at room temperature for 3 min. Thereafter, 2 mL of sodium carbonate (7.5 %) was added to the mixture, which was again kept in the dark not less than 2 h. Immediately after this period, the absorbance was measured at 760 nm. The results were expressed as mg of gallic acid equivalents (GAE) per 100 g of sample (mg GAE 100 g^{-1}).

The antioxidant activity (AA) was determined using ABTS and DPPH assays. The $DPPH^{\bullet}$ radical cation decolorization assay was performed according to the method described by Brand-Williams et al. [59]. An aliquot (100 μL) of sample solutions, prepared as previously described in procedure for TPC determination, was mixed with 3.9 mL of a 6×10^{-5} mol L^{-1} $DPPH^{\bullet}$. Then, the $DPPH^{\bullet}$ absorbance was measured at 515 nm after 1 h. On the other hand, the ABTS was determined from 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) ABTS radical cation ($ABTS^{\bullet+}$) scavenging. It was carried out based on the procedure described by Re et al [60]. ABTS radical cations ($ABTS^{\bullet+}$) were produced by reaction of 7 mmol L^{-1} ABTS solution with potassium persulphate solution with a concentration of 140 mmol L^{-1}. The mixture was kept in the dark at room temperature for 16 h before the analysis. The $ABTS^{\bullet+}$ working solution was prepared by diluting the $ABTS^{\bullet+}$ stock solution with ethanol until reaching an absorbance of 0.700 at 734 nm. For the analyses, 100 μL of aliquots of methanolic solutions (prepared as previously described) were added to 3.9 mL of the $ABTS^{\bullet+}$ working solution and the absorbance was determined at 734 nm after 6 min. Quantification of ABTS and DPPH was conducted using two separated Trolox analytical curves, and the results were expressed as μmol of Trolox equivalents antioxidant capacity per 100 g of sample (μmol TEAC 100 g^{-1}).

In this work, the experimental design and data analysis were performed using the Statistica® 13.5 software (StatSoft, Tulsa, OK, USA). Results were expressed as mean values ± standard deviation (SD). Analysis of variance (ANOVA) and the significant differences were evaluated by Tukey's test at a significance level of 5%.

3. Results and discussion

3.1 Extraction yields

3.1.1 Soxhlet extraction (SE)

Before presenting the extraction yields, it is important to mention that the initial moisture content of the seeds was relatively low (7.0 ± 0.3 wt %) and, thus, they were not additionally dried prior to the extraction experiments. As already mentioned, the average particle size used in all experiments was 2.0 ± 0.1 mm, which corresponds to a bed porosity of 0.64 (116 kg m^{-3} of true density and 416.7 kg m^{-3} of apparent density).

The SE results using ethanol and ethyl acetate are presented in Table 1. The extraction yield achieved using polar solvent were significantly higher when compared with SE using n-hexane, where maximum yield was around 40 %. In addition, SE results in Table 1 along with those presented by Fetzer et al. [47] have confirmed that the solvent with higher polarity (i.e., ethanol) provided higher yield, indicating the high affinity of polar compounds in the cumaru seeds besides the lipid content.

The SE results obtained with ethanol and ethyl acetate were further considered as the reference for comparison with the extraction yields obtained by compressed solvent evaluated in the present study.

Table 1

3.1.2 Extraction with supercritical CO_2 ($scCO_2$)

Preliminary tests were carried out using supercritical fluid extraction with neat CO_2. The results showed that, at conditions tested, this approach provides a low efficiency compared to SE. The $scCO_2$ extraction yields obtained were 1.7 and 1.8 % after 60 min, at 25 MPa and 40°C and 80°C, respectively. Using the same experimental conditions, after 180 min the extraction yield obtained was around 5.7 %. These results show good agreement with results reported in literature by Willd et al. [39] and Ehlers et al. [62]. In order to improve the extraction efficiency, this work was focuses on $scCO_2$ plus EtOH and EtOAc as cosolvents since the SE results with these solvents resulted in high extraction yields.

3.1.3 Extraction with $scCO_2$ + cosolvents

Results of the extraction using $scCO_2$ plus cosolvents are presented in Table 2 and 3, according to the cosolvent type along with their respective sequential extraction results. Therefore, the experimental conditions and results for extractions performed either in a single-step or following sequential stages with $scCO_2$+EtOAc are presented in Table 2, while those related to $scCO_2$+EtOH are presented in Table 3.

Table 2

Table 3

The results indicated that for $scCO_2$+EtOAc extraction using a mass ratio solid to cosolvent of (1:1) similar yields were obtained (28.5-31.5 %) after 60 min. The extractions performed at 40 °C (runs 3 and 6) showed higher yields, independently of the pressure. This response might be attributed to the higher density of CO_2 (see Table 2), which increases as the pressure is increased at a constant temperature. Figure 3(A) depicts how all the extraction curves presented high solubilities and almost equal behaviors, reaching a plateau between 17 to 23 min, except for run 6 (25 MPa and 40 °C) that presented a lower slope in the constant extraction rate (CER) period. The short time of the dynamic extractions and high yields prove the high efficiency of these extractions, since the extractions with $scCO_2$+EtOAc reached high extraction yields with a EtOAc to solids mass ratios of (1:1) and (0.5:1), as presented in Table 2. Even though all the extractions were performed for 60 min, the dynamic extraction time (DET) was assigned to the time when the extraction reached a plateau, and the measured mass of the extract was constant. The effects of pressure and temperature on the extraction may be explained by the vapor-liquid equilibrium data and by the density of the mixture $scCO_2$+EtOAc. As reported by Sima et al. [63], the mixture density does not change substantially in the pressure range considered. This nearly constant density behavior might explain why the amounts of extract do not change much as well. To investigate the extraction performance increase, different EtOAc to raw material mass ratios were evaluated in the sequential extractions. The condition at 15 MPa and 40 °C (run 3) presented the highest extraction yield and, thus, was chosen to be fixed for all the additional extractions, as showed in Figure 3(B) and Table 2. Using this approach, the results obtained for an EtOAc to cumaru seed mass ratio of (0.5:1) resulted in approximately 10 percentage points less than the yield obtained for a mass ratio of (1:1), which in terms of efficiency corresponds to 46% of the total extract available. The same tendency towards changes in the mass ratio of EtOAc in extractions with $scCO_2$ + cosolvent was reported by Santos et al. [64]. Subsequently, the second sequential stage (Run 10) for the same mass ratio (0.5:1) showed similar results to the mass ratio (1:1). Finally, the cumulative yield for the sequential extraction after three stages, using a mass ratio of (1:1), was 44%, representing 101% of the total efficiency of the cumaru seed oil in 70 min of dynamic extraction.

Figure 3

The same experimental design used for $scCO_2$+EtOAc was performed for $scCO_2$+EtOH to compare the effect of different cosolvents and the results are reported in Table 3. It can be seen that for 40 °C using an EtOH to cumaru seeds mass ratio (ECMR) of 1:1 (run 12, 15), an increase in pressure from 15 to 25 MPa had a positive effect on the total extraction yield, allowing to more than doubling this value. From the results shown in Table 3, it is evident that the extraction performance was influenced by both the pressure and the temperature, and that the major effect is due to the latter. A similar observation was reported by other researchers regarding the use of $scCO_2$+EtOH as solvent for different raw materials [65,66]. Moreover, mild extraction conditions (run 12, 15 MPa and 40 °C) showed the lowest extraction yield (13.0 %), while using severe conditions (run 16, 25 MPa and 80 °C) resulted in the highest extraction yield (37.2 %).

Figure 4(A) shows the kinetic curves obtained from the extractions using ethanol as a cosolvent, in which a different profile is observed if compared to $scCO_2$+EtOAc curves. In extractions with EtOH a sigmoidal behavior is observed, proving that the different interactions between CO_2 – solvent affect the mass transfer mechanisms. All extraction curves presented an initial "lag" phase, probability driven by diffusional mechanism, followed by the CER phase controlled by convective mass transfer mechanisms, and then a transition falling extraction rate (FER) controlled again the diffusion mechanisms. Although, all these mechanisms are observed in Figure 4(A), extraction performed at 80 °C presented the most pronounce CER period and higher overall yields for the extractions with ethanol, independently of the pressure level used. Extractions performed at 40 °C and 25 MPa (run 15) and those performed at the central point (run 14) showed almost identical curves, in which the CER period ended after 30 min. Furthermore, in both cases, the FER persisted until 40 min, meaning that these conditions resulted in more CO_2 being used, as also reported in Table 3.

Figure 4

Additional experiments with different ECMR were performed to evaluate the effect of this parameter on the extraction kinetics. As can be seen in Figure 4(B) and Table 3, for run 17 just 11.7 % of extraction yield was obtained using an ECMR of 0.5:1 ratio at 25 MPa and 80 °C (open squares in Figure 4B). It can be seen from these figures that the ethanol is the solvent for the oil solubilization and the $scCO_2$ is acting as mass transfer promoter (ethanol carrier) and then speeding up the solute extraction from the solid matrix. When a ECMR of 2:1is considered (run 18, 20 MPa and 60 °C) and it can be observed that the increasing the ethanol amount increased the extraction yield to 35.2 %. However, the initial extraction rate decreased following the increase in the ethanol load into the extractor.

Two different conditions were also chosen to show the performance of the sequential extraction for the system $scCO_2$ + EtOH, at 25 MPa, 40 °C and 80 °C with mass ratio of 1:1 and 2:1, as presented in Figure 4(C), in which 3 sequential stages were performed using higher levels of the parameters and improving the results substantially. Assuming that the extraction yield obtained in SE with ethanol corresponds to the maximum amount of extractables available in the cumaru seeds used in this work (100%), the second stage using $scCO_2$+EtOH extracted around 110 %, and the third sequential stage allowed reaching around 120 % of extraction efficiency. This result may indicate that the approach using supercritical CO_2 with ethanol is capable of to enhance the oil extraction from cumaru seeds at high pressures (25 MPa) and high temperature (80 ºC) significantly. These results are in agreement with others already presented in the literature, where small amounts of the ethanol enhanced the extraction capacity [39,67].

3.1.5 Pressurized liquid extraction (PLE) with EtOH

The extraction efficiencies previously obtained with $scCO_2$+EtOH and SE-EtOH encouraged the use of PLE with EtOH as a possibility to improve the extraction yield. The results presented in Table 4 showed that the higher the temperature, the higher the extraction yield, since this parameter has an influence over the solubility of the extract within ethanol. Thus, at 10 MPa and the highest temperature (100 °C), it was reached an extraction yield of 53.9%, corresponding to 114 % of extraction efficiency.

Moreover, Figure 5 depicts the overall extraction curves, which allows the observation of the positive influence of temperature along the PLE extractions. The experiments performed at 60 and 80 °C had similar trend until around 20 min, but after that a higher temperature results in an enhanced increase in the extraction yield. At 100 °C, the CER presented a higher apparent solubility until 60 min, where the FER period begins. Finally, the last stage, which is the diffusion-controlled period, is clearly observed from 120 min.

Table 4

Figure 5

3.2 Quantification and separation of coumarin from the cumaru seed extract

The quantity of coumarin in the cumaru seed extracts obtained by $scCO_2$ + cosolvents and PLE was evaluated and compared to SE with EtOAc and EtOH and presented in Table 5. Considering only extracts obtained by SE, the comparison between solvents indicated a lower concentration of coumarin in extracts obtained using SE-EtOAc rather than SE-EtOH. Higher coumarin concentrations in crude extracts obtained from SE-EtOH in this study were similar to those observed by Lima et al. [43]. Since coumarin is highly soluble in ethanol, SE-EtOH extracts showed a coumarin concentration of approximately 11.5 times more compared to SE-EtOAc. It is also interesting to notice that SE-EtOAc presented lower coumarin contents in contrast to a non-polar solvent (*n*-hexane), as reported in the previous work [47].

Table 5

The concentrations of coumarin in crude extracts obtained by $scCO_2$+EtAOc and $scCO_2$+EtOH were also measured and are presented in Table 5. The results demonstrated that using $scCO_2$+EtOAc similar coumarin content are observed for extraction at same temperatures, regardless the pressure. At 80 °C, higher amounts of coumarin were observed in the cumaru seed extracts, in contrast to the lower amounts observed at 40 °C. Similar behaviors of extraction pressure and temperature on the yield were reported by Rodrigues et al. [16], using emburana (*Torresea cearensis*) seeds. Finally, when compared to SE-EtOAc, $scCO_2$+EtOAc extractions resulted in significantly higher coumarin concentrations in the crude extracts.

Concerning the extractions using ethanol, even though coumarin presents high solubilities in both EtOH and CO_2 [68,69], the mix of these solvents in the $scCO_2$+EtOH mixture resulted in a lower coumarin content at 40 °C independently of the pressure compared to results obtained using SE-EtOH. On the other hand, higher coumarin contents were obtained using $scCO_2$+EtOH at 60 and 80 °C, indicating that the temperature has a positive effect in the extraction technique and the combination of high temperature and high pressure contributed achieving a coumarin content higher than those obtained by SE-EtOH. According to Torres et al. [70], the use of CO_2 as solvent under constant temperature (40 °C) and with gradual pressure increments leads to an increase in the coumarin content, which has also been observed in the present study when the pressure was varied using $scCO_2$+EtOH.

Aiming to improve the coumarin recovery, additional experiments were performed (runs 17 to 23) using both the temperature and the pressure of the central point (run 17), and at different ethanol to solid ratios. At an ECMR of (0.5:1) was obtained almost 3 times less coumarin than using an ECMR of (1:1). Furthermore, runs 19 and 20 (sequential extraction), performed at 25 MPa and 40 °C and with an ECMR of (2:1), provided a coumarin content of 20.67 mg g^{-1} in the oil, while runs 21 to 23 (3 sequential extractions), performed at 25 MPa and 80 °C with an ECMR of (1:1), allowed a higher coumarin content (30.16 mg g^{-1}). Therefore, it is possible to tune the coumarin content in the crude extracts from cumaru seeds using different process parameters for the extraction approach presented in this work, named sequential semi-batch extraction using a compressed mixture of $scCO_2$+EtOH.

Finally, since ethanol has a high capability to extract coumarin, additional extractions were performed at 10 MPa and temperatures ranging from 60 to 80 °C (runs 24 to 26). The highest amount of coumarin was obtained at 10 MPa and 80 °C, while at 10 MPa and 100 °C the concentration of coumarin was slightly lower. In the literature, Doctor et al. [71] described the use of pressurized water at 50 bar and observed the extraction reached the maximum yield for temperatures exceeding 150 °C. In contrast, results obtained in this work showed that a maximum extraction yield can be obtained at 80 °C, which is a comparatively lower temperature and could help to avoid the degradation of the thermolabile compounds present in cumaru seed extracts.

3.2.1 Kinetics of coumarin extraction from cumaru seeds

Coumarin content was monitored during the extraction using $scCO_2$+EtOAc. The experimental conditions employed in run 7 were chosen since such condition was associated to the highest cumarin extraction content. Figure 6 shows that coumarin concentration in the crude extract was low at the beginning of the extraction and increased along the extraction curve (extraction time). When a large amount of oil was extracted (aided by the oil's affinity with ethyl acetate), the coumarin concentration was low probably by an “dilution” effect of coumarin in higher amounts of oil and other compounds in the extract.

Figure 6

In the case of extractions with $scCO_2$+EtOH and PLE+EtOH, all extraction curves obtained from different experimental conditions were analyzed because using these solvents a separation of coumarin from the bulk phase was observed. $ScCO_2$+EtOH curves could be divided into 3 tendency groups. Figures 7(A) and 7(B) present the results obtained at 40 °C, in which nearly constant and indeed low coumarin contents in crude extracts were observed, regardless the pressure and independently from the extraction yield. The second group was composed by the results obtained for extractions performed at 80 °C (runs 14 and 17), shown in Figures 7(C) and 7(D). These extractions presented higher diffusivity at the beginning of the extraction, probably because of high apparent solubility under these conditions during the confinement time. Then, both runs showed decreases in the coumarin content, with the values reaching low-concentration values after 5 min at 25 MPa (6 mg g^{-1}) and after 7.5 min at 15 MPa (9 mg g^{-1}). Furthermore, in both cases the coumarin content remained approximately constant until 20 min and 15 min, respectively. After these extraction times, the concentration has dramatically increased, coinciding to the complete withdrawal of ethanol from the extractor and, consequently, approaching the depletion of oil from the seeds. The last group of results is related to the extractions performed at the central point conditions (20 MPa and 60 °C), shown in Figure 7(E). The first sample obtained after the static extraction period (2.5 min) presented a coumarin concentration comparable to the samples obtained at 40 °C. However, at the beginning of the dynamic extraction, a high solubility was observed until it reached a high-concentration peak at 7.5 min (CER extraction stage). The remaining samples presented the same behavior as samples obtained at 80 °C, in which the coumarin concentration decreased, in this case reaching a low-concentration peak after 12.5 min. Then, the extraction stabilized and the coumarin concentration sustained between 12 and 14 mg g^{-1}, up to 30 min, and then another increase in the coumarin concentration is observed at the ending stage of the extraction. In summary, as the extractions proceeded and the solvent mixtures became richer in CO_2, coumarin contents tended to increase, probably due to a high coumarin-selectivity shown by neat CO_2, although this behavior is less pronounced at 40 °C.

Figure 7

Finally, coumarin extraction kinetics using PLE+EtOH was assessed, and the results are presented in Figure 8. High coumarin concentrations were observed at the beginning of the extractions and then the values decreased for all experimental conditions evaluated. However, at 60 °C and 80 °C, Figures 8.A and 8.B, after 15 min of extraction, coumarin contents were 15 mg g^{-1} and 20 mg g^{-1}, respectively. On the other hand, the coumarin content decreased rapidly at 100 °C, as shown in Figure 8(C), in which is possible to notice that after the same time of extraction the concentration felt to 9 mg g^{-1}. Nevertheless, the results presented in Table 5 indicate that at higher temperatures, the absolute mass of coumarin extracted increased monotonically, from 27.48 mg at 60 °C to 39.17 mg and 46.39 mg, at 80 °C and 100 °C, respectively. Figure 8 also allowed the observation of similar tendencies among higher temperatures. Taking the previous information into account, it is possible to conclude that the melting point of coumarin (68-72 °C), combined with the solvent diffusion into the matrix, resulted in the removal of large amounts of these compounds from the cumaru seeds. This behavior was also reported by Trabelsi et al. [72] for processes of oil extraction from oilseeds. Furthermore, the possibility to obtain different coumarin contents according to the time of extraction may encourage further investigations and optimization cumarin extraction from cumaru seeds using compressed fluid processes. In this sense, diverse coumarin contents have been found in different edible products, although it is important to notice that only cinnamon and dietary supplement/commercial rose oil show similarly high values compared to cumaru [24].

Figure 8

3.2.2 Visual aspect of extracts obtained by different methods

When working with supercritical fluid CO_2 extraction, Ehlers [62] reported that the cumaru seed oil had two phases, a lipophilic top phase (liquid), and a coumarin-rich bottom phase (solid crystals). This phenomenon was somehow also observed in this work when using pure CO_2. However, since a portion of coumarin flakes remained adhered to the assay tubes internal wall, the separation was not complete and, rather than that, there was a mixture of lipids with coumarin crystals. On the other hand, in the extracts obtained using $scCO_2$+EtOH technique, the crystals clumped together and, as a result, settled to the bottom of the tube. Thus, the separation between the lipid phase and coumarin crystals was observed, as described in Figure 9. In other words, aggregation and separation of coumarin from the extracts may be achieved and enhanced by means of the amount of ethanol present in the extract. The same behavior may have happened during the PLE, where the coumarin affinity with ethanol causes the separation of coumarin solids from the liquid extract during and after extraction. Moreover, the mass transfer coefficient of the cumaru seed extract during the extraction process increases with increasing temperature, at constant pressure.

Figure 9

The extract obtained by $scCO_2$+EtOAc technique presented turbidity, indicating that coumarin crystals were well dissolved in the lipid-phase, which may have been caused by the affinity of cumaru seed oil with ethyl acetate and the prevention of agglomeration of coumarin solids. The observations regarding the affinities of coumarin with different solvents could be useful for the development of processes related to bio-packing, encapsulation, and other material purposes.

3.3 Fatty acid profile

Samples of cumaru seed oil obtained by $scCO_2$+EtOAc, $scCO_2$+EtOH (both at 60 °C and 20 MPa) and SE using EtOAc, and EtOH were analyzed by gas chromatography in order to determine the fatty acids composition. The results are presented in Table 6, in which one can observe that the fatty acid composition is very similar between all extracts obtained, regardless of the extraction technique and conditions. Oleic acid was the major component found in all extracts obtained, whose values ranged from 51.32 % (SE-EtOH) to 53.62 % ($scCO_2$+EtOAc). It is also possible to notice that the oils had chemical profiles similar to those obtained by other authors for the cumaru seeds oil extracted using different methods [73,74].

The results also revealed a good nutritional value for the oil samples, since the unsaturated fatty acids (UFA) represent the major fatty acid group in the crude oil samples (from 80.03 % to 82.41 %), with polyunsaturated fatty acids (PUFA) contents between 21.64

% and 22.38 %, which are higher values than other renowned seed oils, such as avocado [52], blackberry [75] and hempseed [76] oils. Moreover, saturated fatty acids (SFA) contents ranged from 17.92 % and 19.96 %. The slight differences between the values obtained using different extraction techniques can be attributed to deviations inherent to the analytical method. In general, *Dipteryx odorata* fatty acid profiles presented coherence with values reported in literature [77].

Table 6

3.4 Physicochemical and thermal behavior of cumaru seed extracts

Crude oils samples obtained from cumaru seeds were found to have different tones of dull yellow color, as shown in Figure 10, and had a strong vanilla-like odor. Moreover, the physicochemical parameters of each condition evaluated in this study (compressed fluids and Soxhlet extractions) are presented in Table 7. Since the free fatty acid values in extracted oils are in the range of non-drying oils, cumaru seeds oil obtained in this work may be regarded to the same use as other conventional oils. Refraction index, iodine value and saponification value are comparable with other vegetable oils, such as rubber [78], perilla [79], *Sapindus mukorossi* [80], *Nigella sativa L.* [81], and *Acacia nilotica* [82] seed oils. In addition, the values of all physical properties of the extracts obtained are in the range required by the AOCS standards.

Figure 10

Table 7

In terms of thermal analysis, the TGA curves (Figure 11) showed that the thermal behavior of the oil samples obtained by SE+EtOAc (run 1), SE+EtOH (run 2), $scCO_2$+EtOAc (run 5) and $scCO_2$+EtOH (run 14) are similar. Thermal decomposition events were more or less pronounced, according to the sample, although three events can be considered for all the samples. In summary, the extracts showed thermal stabilities up to approximately 200 °C, while the complete decomposition was observed near to 600 °C. The first step started around 200 °C, probably due the presence of lipid oxidation products and molecules from triacylglycerols hydrolysis [75]. In the second step, a maximum weight loss was observed and can be related to the polyunsaturated fatty acids decomposition (around 250–450 °C) [83–85]. Furthermore, the coumarin compounds present in the cumaru seed crude oil probably degraded in this step as described by other authors [86–88]. It is interesting to notice that for the samples obtained by SE+EtOH and $scCO_2$+EtOH, the second thermal event was more pronounced than for the samples obtained using ethyl acetate, which can be attributed to the heterogeneity observed by the samples obtained with ethanol. Finally, the third degradation step occurred in a temperature ranging from around 450 to 600 °C, which corresponds to the decomposition of monounsaturated and saturated fatty acids, such as palmitic acid [75].

Figure 11

In addition, cooling and heating curves of cumaru seed oil samples obtained by compressed solvent (at the conditions of central point) and Soxhlet extractions were determined by differential scanning calorimetry (DSC) method. All the samples of cumaru seeds crude oil showed similar behaviors during cooling and heating procedures. As can be seen from Figure 12(A), the nucleation phenomenon for these samples showed similar patterns, with slightly major intensities shown for the curves of samples obtained by $scCO_2$+EtOH and SE+EtOAc.

The crystallization curves indicate the cooling behavior with a severe amount of broadening over the exothermic peaks. During cooling, all samples presented onset temperatures starting around 18 °C. On the other hand, the second peak occurred in the temperature range from 10 °C to 1 °C. The last event was either a shoulder or a set of peaks ending around 0.0 to -20.0 °C, where occurred the total crystallization of these oil samples. They may co-crystallize with low melting point components, and this causes a change in the crystallization temperatures and, hence, shifting the crystallization peaks to even lower temperatures. Moreover, these results are suggesting the presence of different kinds of compounds in the oil samples in addition to the coumarin compounds.

Figure 12(B) represents crystallization curves, in which two separated exothermic peaks are observed. The first event, indicating the transition to the liquid state, exhibited similar peaks for all oils, around 5 °C. The second event was also observed at nearly the same temperature for all samples, which ranged between -50 °C and -55 °C. The slight differences between these values might be due to the saturated and unsaturated fatty acids content, as also reported by Xu et al. [89].

Figure 12

3.5 Antioxidant activity (AA) and total phenolic content (TPC)

The antioxidant activities by DPPH and ABTS in cumaru seed oils were carried out following the methods presented in Section 2.4. and the results are presented in Table 8. In the case of extracts obtained from $scCO_2$+EtOH, the upper phase (oil) was analyzed. The higher DPPH achieved by SE+EtOH was 901.81 mmol TE 100 g^{-1}, which is much higher than the values of the extracts obtained by $scCO_2$+EtOH and $scCO_2$+EtOAc.

On the other hand, the lowest value of DPPH (20.41 mmol TE 100 g^{-1}) was obtained by $scCO_2$+EtOH at 40 °C. Moreover, the temperature presents a positive effect, showing that higher temperatures lead to higher DPPH extractions. The highest ABTS value was attained for the oil obtained at 80 °C and 25 MPa (471.73 mmol TE 100 g^{-1}). In general, the extracts obtained at higher temperatures using $scCO_2$+EtOAc showed the highest antioxidant activities. Therefore, all $scCO_2$+EtOAc extraction runs presented superior antioxidants capacity than the observed for $scCO_2$+EtOH extracts. According to Kassim et al. [90] extracts obtained with EtAOc exhibit potent inhibition against the DPPH radical. Moreover, the results can be explained by the higher solubility of coumarin into the oil when using $scCO_2$+EtOAc extractions, in contrast to the cumaru seed oils obtained by $scCO_2$+EtOH. Since coumarin crystals agglomerate and precipitate to the bottom in the presence of ethanol, the resulting oil presents a lower amount of this compound, which has an important antioxidant activity. Nonetheless, all the oils showed remarkable antioxidant activities, as also reported by other authors [91,92].

TPC analyses were also carried out following the methods presented in Section 2.4.4, and the values are also presented in Table 8. The extract obtained by SE+EtOAc showed lower value, in contrast to those obtained in the $scCO_2$+EtOAc experimental design. Run 7 (25 MPa and 80 °C) showed the highest TPC values, with 117.84 mg GAE 100 g^{-1}. In general, for high-pressures, the higher temperature resulted in higher TPC values. For instance, the extraction performance using CO_2 at the higher temperature presented 5 to 6 times more TPC than other extracts obtained at 40 °C. It is also important to mention that the use of CO_2 improves the extraction of TPC in case of using EtOAc as cosolvent, while the opposite was observed when using EtOH. The SE+EtOH showed 5 to 10 times more TPC in contrast to the $scCO_2$+EtOH. That occurred because the separation between the oil and coumarin employing $scCO_2$+EtOH was almost complete, describing the influence of the coumarin compound in antioxidant activity (AA). In summary, broad values were obtained, to multiple extracts obtained though the supercritical fluid extraction method using also a cosolvent, confirming the high efficiency of this methods to extract phenolic compounds as also was reported by Týskiewicz [93].

Table 8

4. Conclusions

This work investigated the extraction of cumaru seeds oil using supercritical CO_2associated with organic polars solvents (ethanol and ethyl acetate) in a semi-batch extraction approach. The results in terms of extraction yield were compared to Soxhlet extraction with the same solvents as a reference point. The best yield of 37.4 % was obtained at the highest conditions of pressure and temperature investigated (25 MPa and 80 °C) using $scCO_2$+EtOH with a cosolvent to solids mass ratio (ECMR) of (1:1), reaching the maximum extraction condition in less than 25 min. Sequential extraction maximized both crude oil extraction and coumarin extraction for a ECMR of (1:1). Moreover, when using $scCO_2$+EtOH, after 3 stages almost 120 % of extraction efficiency was observed and 30.16 mg g^{-1} of coumarin was recovered.

In the case of $scCO_2$+EtOAc it was not observed significant difference between the yields obtained for all conditions investigated. Employing $scCO_2$+EtAOc in 2 sequential stages with a 1:1 ratio, more than 100% of efficiency was obtained. Rather unexpected results were obtained with $scCO_2$+EtOAc in which besides the extraction of seed oil, the extractions also recovered coumarin (26.55 mg g^{-1} of oil), in a slightly higher concentration than the obtained by $scCO_2$+EtOH at 25 MPa and 80 °C with a ratio of 1:1. Moreover, PLE+EtOH also showed a great capacity to remove oil (~54%) after 180 min of extraction. In addition, the coumarin concentration reached steady values up to 80 °C.

The study also proves that the CO_2 is selective in removing coumarin and EtOH helps to separate it from the oil, occurring two processes in one (extraction and separation). Kinetic of coumarin extraction showed that employing $scCO_2$+EtOH and the PLE with EtOH high amount of coumarin is removed in the first part of the extraction while extractions performance with $scCO_2$+EtAOc, due to significant affinity to the oil, the coumarin is increasing during the extraction. Furthermore, the possibility to obtain different coumarin contents along the time of extraction may encourage a wider range of applications for these processes herein investigated. The characterization of the different extracts obtained in this work with different coumarin content has indicated its promising use in different areas.

Finally, extracts obtained from compressed solvents showed good TPC and AA. Furthermore, the physicochemical and FA profiles present similar values in comparison to other literature. As a final conclusion, the employed compressed solvent extraction on sequential extraction methodology, led to potentially viable extracts for food, pharmaceutical, medical and materials industries, from a raw material native to Amazon region applying scCO2-based extraction and separation processes.

Acknowledgements

The authors would like to thank the Brazilian funding agencies (M.L.C thanks the CNPq, grant numbers 435873/2018-0 and 310038/2020-0, and Fundação Araucária – grant agreement 004/2019; D.L.F thanks the CAPES, grant number 40001016019P6).

M.E and M.L.C. thank the funding from the European Union's Horizon 2020 research and innovation programme under Marie Skłodowska-Curie grant agreement No 778168.

References

[1] M.J. Matos, L. Santana, E. Uriarte, O.A. Abreu, E. Molina, E.G. Yordi, Coumarins — An Important Class of Phytochemicals, in: Phytochem. - Isol. Characterisation Role Hum. Heal., InTech, 2015: pp. 113–140. https://doi.org/10.5772/59982.

[2] C. Gleye, G. Lewin, A. Laurens, J.C. Jullian, P. Loiseau, C. Bories, R. Hocquemiller, Acaricidal activity of tonka bean extracts. Synthesis and structure-activity relationships of bioactive derivatives, J. Nat. Prod. 66 (2003) 690–692. https://doi.org/10.1021/np020563j.

[3] I.A. Stringlis, R. De Jonge, C.M.J. Pieterse, The Age of Coumarins in Plant-Microbe Interactions, Plant Cell Physiol. 60 (2019) 1405–1419. https://doi.org/10.1093/pcp/pcz076.

[4] M.Z. Hassan, H. Osman, M.A. Ali, M.J. Ahsan, Therapeutic potential of coumarins as antiviral agents, Eur. J. Med. Chem. 123 (2016) 236–255. https://doi.org/10.1016/j.ejmech.2016.07.056.

[5] S. Mishra, A. Pandey, S. Manvati, Coumarin: An emerging antiviral agent, Heliyon. 6 (2020). https://doi.org/10.1016/j.heliyon.2020.e03217.

[6] H.M. Alshibl, E.S. Al-Abdullah, M.E. Haiba, H.M. Alkahtani, G.E.A. Awad, A.H. Mahmoud, B.M.M. Ibrahim, A. Bari, A. Villinger, Synthesis and evaluation of new coumarin derivatives as antioxidant, antimicrobial, and anti-inflammatory agents, Molecules. 25 (2020) 1–28. https://doi.org/10.3390/molecules25143251.

[7] H.L. Qin, Z.W. Zhang, L. Ravindar, K.P. Rakesh, Antibacterial activities with the structure-activity relationship of coumarin derivatives, Eur. J. Med. Chem. 207 (2020) 112832. https://doi.org/10.1016/j.ejmech.2020.112832.

[8] W.B. Li, X.P. Qiao, Z.X. Wang, S. Wang, S.W. Chen, Synthesis and antioxidant activity of conjugates of hydroxytyrosol and coumarin, Bioorg. Chem. 105 (2020) 104427. https://doi.org/10.1016/j.bioorg.2020.104427.

[9] G. Wang, Y. Liu, L. Zhang, L. An, R. Chen, Y. Liu, Q. Luo, Y. Li, H. Wang, Y. Xue, Computational study on the antioxidant property of coumarin-fused coumarins, Food Chem. 304 (2020) 1–7. https://doi.org/10.1016/j.foodchem.2019.125446.

[10] B.M. Geetha, J.G. Małecki, M. Alwarsamy, R.S. Keri, V.S. Betageri, S. Budagumpi, Coumarin substituted 4–aryl–1,2,4–triazolium salts and their silver(I) N–heterocyclic carbene complexes: Effects of counterions on the antioxidant and antihaemolytic properties, J. Mol. Liq. 316 (2020) 1–9. https://doi.org/10.1016/j.molliq.2020.113809.

[11] S. Weigt, N. Huebler, R. Strecker, T. Braunbeck, T.H. Broschard, Developmental effects of coumarin and the anticoagulant coumarin derivative warfarin on zebrafish (Danio rerio) embryos, Reprod. Toxicol. 33 (2012) 133–141. https://doi.org/10.1016/j.reprotox.2011.07.001.

[12] J.P. Salvador, D. Tassies, J.C. Reverter, M.P. Marco, Enzyme-linked immunosorbent assays for therapeutic drug monitoring coumarin oral anticoagulants in plasma, Anal. Chim. Acta. 1028 (2018) 59–65. https://doi.org/10.1016/j.aca.2018.04.042.

[13] M.L. Orme, A.M. Breckenridge, Coumarin anticoagulants, in: Meyler’s Side Eff. Drugs, Elsevier, Dordrecht, 2016: pp. 702–737. https://doi.org/10.1016/B978-0-444-53717-1.00556-4.

[14] A. Thakur, R. Singla, V. Jaitak, Coumarins as anticancer agents : A review on synthetic strategies, mechanism of action and SAR studies, Eur. J. Med. Chem. 101 (2015) 476–495. https://doi.org/10.1016/j.ejmech.2015.07.010.

[15] H. Perumalsamy, K. Sankarapandian, K. Veerappan, S. Natarajan, N. Kandaswamy, L. Thangavelu, S.R. Balusamy, In silico and in vitro analysis of coumarin derivative

induced anticancer effects by undergoing intrinsic pathway mediated apoptosis in human stomach cancer, Phytomedicine. 46 (2018) 119–130. https://doi.org/10.1016/j.phymed.2018.04.021.

[16] R.F. Rodrigues, A.K. Tashima, R.M.S. Pereira, R.S. Mohamed, F.A. Cabral, Coumarin solubility and extraction from emburana (*Torresea cearensis*) seeds with supercritical carbon dioxide, J. Supercrit. Fluids. 43 (2008) 375–382. https://doi.org/10.1016/j.supflu.2007.07.014.

[17] E.H. Maleki, A.R. Bahrami, H. Sadeghian, M.M. Matin, Discovering the structure–activity relationships of different O-prenylated coumarin derivatives as effective anticancer agents in human cervical cancer cells, Toxicol. Vitr. 63 (2020). https://doi.org/10.1016/j.tiv.2019.104745.

[18] C.L. Marvig, T.I. Verhoef, A. De Boer, F. Kamali, K. Redekop, M. Pirmohamed, A.K. Daly, V.G. Manolopoulos, M. Wadelius, M. Bouvy, A.H. Maitland-Van Der Zee, Quality of life in patients with venous thromboembolism and atrial fibrillation treated with coumarin anticoagulants, Thromb. Res. 136 (2015) 69–75. https://doi.org/10.1016/j.thromres.2015.04.026.

[19] K.C. Fylaktakidou, J. Hadjipavlou-Litina, K.E. Litinas, D.N. Nicolaides, Natural and Synthetic Coumarin Derivatives with Anti-Inflammatory/ Antioxidant Activities, 2004.

[20] T. Bor, S.O. Aljaloud, R. Gyawali, S.A. Ibrahim, Antimicrobials from herbs, spices, and plants, in: Fruits, Veg. Herbs Bioact. Foods Heal. Promot., Elsevier, NC, United States, 2016: pp. 551–578. https://doi.org/10.1016/B978-0-12-802972-5.00026-3.

[21] C.M. Moreno-Ley, D.M. Hernández-Martínez, G. Osorio-Revilla, A.P. Tapia-Ochoategui, G. Dávila-Ortiz, T. Gallardo-Velázquez, Prediction of coumarin and ethyl vanillin in pure vanilla extracts using MID-FTIR spectroscopy and chemometrics,

Talanta. 197 (2019) 264–269. https://doi.org/10.1016/j.talanta.2019.01.033.

[22] Q. Ma, H. Xi, H. Ma, X. Meng, Z. Wang, H. Bai, W. Li, C. Wang, Simultaneous Separation and Determination of 22 Coumarin Derivatives in Cosmetics by UPLC-MS/MS, Chromatographia. 78 (2015) 241–249. https://doi.org/10.1007/s10337-014-2841-3.

[23] S.C. Rastogi, J.D. Johansen, P. Frosch, T. Menné, M. Bruze, J.P. Lepoittevin, B. Dreier, K.E. Andersen, I.R. White, Deodorants on the European market: Quantitative chemical analysis of 21 fragrances, Contact Dermatitis. 38 (1998) 29–35. https://doi.org/10.1111/j.1600-0536.1998.tb05633.x.

[24] M. Lončar, M. Jakovljević, D. Šubarić, M. Pavlić, V. Buzjak Služek, I. Cindrić, M. Molnar, Coumarins in Food and Methods of Their Determination, Foods. 9 (2020) 645. https://doi.org/10.3390/foods9050645.

[25] M. Raters, R. Matissek, Analysis of coumarin in various foods using liquid chromatography with tandem mass spectrometric detection, Eur. Food Res. Technol. 227 (2008) 637–642. https://doi.org/10.1007/s00217-007-0767-9.

[26] N.Z. Ballin, A.T. Sørensen, Coumarin content in cinnamon containing food products on the Danish market, Food Control. 38 (2014) 198–203. https://doi.org/10.1016/j.foodcont.2013.10.014.

[27] EFSA, Regulation (EC) no 1334/2008 of the European Parliament and of the council of on flavourings and certain food ingredients with flavouring properties for use in and on foods and amending Council Regulation (EEC) No 1601/91, Regulations (EC, Off. J. Eur. Union. 354 (2008) 34–50.

[28] D. Ratanasavanh, D. Lamiable, M. Biour, Y. Guédès, M. Gersberg, E. Leutenegger, C. Riché, Metabolism and toxicity of coumarin on cultured human, rat, mouse and rabbit hepatocytes, Fundam. Clin. Pharmacol. 10 (1996) 504–510.

https://doi.org/10.1111/j.1472-8206.1996.tb00607.x.

[29] C. Lee, Coumarin, the illegal chemical causing Americans to miss out on a sweet treat., Off. Sci. Soc. Separating Sense from Nonsense. (2017).

[30] C.C. Ruiz, J.M. Hierrezuelo, J.A. Molina-Bolivar, Analysis of the photophysical behavior and rotational-relaxation dynamics of coumarin 6 in nonionic micellar environments: The effect of temperature, Molecules. 20 (2015) 19343–19360. https://doi.org/10.3390/molecules201019343.

[31] K. Xu, J.L. Wang, M.P. Chu, C. Jia, Activity of coumarin against *Candida albicans* biofilms, J. Mycol. Med. (2019). https://doi.org/10.1016/j.mycmed.2018.12.003.

[32] M. Souilah, M. Hachi, A. Fitri, A.T. Benjelloun, S. El Khattabi, M. Benzakour, M. Mcharfi, H. Zgou, Coumarin-based D–π–A dyes for efficient DSSCs: DFT and TD-DFT study of the π-spacers influence on photovoltaic properties, Res. Chem. Intermed. (2020). https://doi.org/10.1007/s11164-020-04302-9.

[33] P. Wang, S. Guo, H.J. Wang, K.K. Chen, N. Zhang, Z.M. Zhang, T.B. Lu, A broadband and strong visible-light-absorbing photosensitizer boosts hydrogen evolution, Nat. Commun. 10 (2019). https://doi.org/10.1038/s41467-019-11099-8.

[34] D. Gindre, K. Iliopoulos, O. Krupka, M. Evrard, E. Champigny, M. Sallé, Coumarin-containing polymers for high density non-linear optical data storage, Molecules. 21 (2016) 1–13. https://doi.org/10.3390/molecules21020147.

[35] P. Richomme, Coumarins, Xanthones and Related Compounds, Molecules. 21 (2016) 341. https://doi.org/10.3390/molecules21030341.

[36] X. Huang, J. Wang, H. Hao, J. Ouyang, Y. Gao, Y. Bao, Y. Wang, Q. Yin, Determination and correlation of solubility and solution thermodynamics of coumarin in different pure solvents, Fluid Phase Equilib. 394 (2015) 148–155. https://doi.org/10.1016/j.fluid.2015.03.022.

[37] D.S. Jang, E.J. Park, M.E. Hawthorne, J.S. Vigo, J.G. Graham, F. Cabieses, B.D. Santarsiero, A.D. Mesecar, H.H.S. Fong, R.G. Mehta, J.M. Pezzuto, A.D. Kinghorn, Potential cancer chemopreventive constituents of the seeds of *Dipteryx odorata* (tonka bean), J. Nat. Prod. 66 (2003) 583–587. https://doi.org/10.1021/np020522n.

[38] D.L. Fetzer, P.N. Cruz, F. Hamerski, M.L. Corazza, Extraction of baru (*Dipteryx alata* vogel) seed oil using compressed solvents technology, J. Supercrit. Fluids. 137 (2018) 23–33. https://doi.org/10.1016/j.supflu.2018.03.004.

[39] A. Willd, T. Bajer, S. Surmová, A. Eisner, K. Ventura, P. Bajerová, Industrial Crops & Products Use of simultaneous distillation-extraction , supercritical fl uid extraction and solid-phase microextraction for characterisation of the volatile pro fi le of *Dipteryx odorata* (Aubl.) Willd., (2018). https://doi.org/10.1016/j.indcrop.2018.01.055.

[40] B. Medeiros-Neves, K.A.P. Diel, V.L. Eifler-Lima, H.F. Teixeira, E. Cassel, R.M. Figueiró Vargas, G.L. Von Poser, Influence of the supercritical CO2 extraction in the stability of the coumarins of *Pterocaulon lorentzii* (Asteraceae), J. CO2 Util. 39 (2020). https://doi.org/10.1016/j.jcou.2020.101165.

[41] M. Molnar, I. Jerković, D. Suknović, B. Bilić Rajs, K. Aladić, D. Šubarić, S. Jokić, Screening of Six Medicinal Plant Extracts Obtained by Two Conventional Methods and Supercritical CO_2 Extraction Targeted on Coumarin Content, 2,2-Diphenyl-1-picrylhydrazyl Radical Scavenging Capacity and Total Phenols Content, Molecules. 22 (2017) 348. https://doi.org/10.3390/molecules22030348.

[42] E. Gracia, M.T. García, A.M. Borreguero, A. De Lucas, I. Gracia, J.F. Rodríguez, Functionalization and optimization of PLA with coumarin via click chemistry in supercritical CO2, J. CO2 Util. 20 (2017) 20–26. https://doi.org/10.1016/j.jcou.2017.04.008.

[43] J.C. Lima, M. Traczynski, W.M. Giufrida, A.C. Feihrmann, L.S. Freitas, L. Cardozo-

Filho, Modifier-enhanced supercritical CO2 extraction with GRAS solvents of coumarin from cumaru seeds (*Dipteryx odorata*), Chem. Eng. Trans. 57 (2017) 1801–1806. https://doi.org/10.3303/CET1757301.

[44] M. V. Calvo, D. Villanueva-Bermejo, P. Castro-Gómez, T. Fornari, J. Fontecha, Appraisal of the suitability of two-stage extraction process by combining compressed fluid technologies of polar lipid fractions from chia seed, Food Res. Int. 131 (2020) 109007. https://doi.org/10.1016/j.foodres.2020.109007.

[45] D. Ballesteros-Vivas, J.P. Ortega-Barbosa, A. del P. Sánchez-Camargo, L.I. Rodríguez-Varela, F. Parada-Alfonso, Pressurized Liquid Extraction of Bioactives, in: Compr. Foodomics, Elsevier, 2021: pp. 754–770. https://doi.org/10.1016/B978-0-08-100596-5.22817-5.

[46] R. Gomide, Operação com sistemas sólidos granulares, Catalogaçã, São Paulo, 1983.

[47] D.L. Fetzer, F. Hamerski, M. Errico, M.L. Corazza, Extraction of cumaru seed oil using compressed propane as solvent, J. Supercrit. Fluids. 169 (2021) 105123. https://doi.org/10.1016/j.supflu.2020.105123.

[48] AOAC, (Official Methods of Analysis) -Association of Official Analytical Chemests International, 18th ed., AOAC, Maryland, 2005.

[49] K. Helrich, Official Methods of Analysis of the Association of afficial Analytic Chemists, 15 edition, Aoac intl., Arlington, Va., 1990.

[50] A. Jamila dos Santos, T.C. Confortin, I. Todero, A.S. Rodrigues, S.R. Ribeiro, S.R. Sasso, N.I. Canabarro, R. Wagner, A.J. Cichoski, M.A. Mazutti, C.S. da Rosa, Use of compressed fluids in the recovery of pecan nut cake oil: Influence of extraction conditions on yield and extract quality, J. Supercrit. Fluids. 161 (2020) 104820. https://doi.org/10.1016/j.supflu.2020.104820.

[51] P.N. Cruz, D.L. Fetzer, W. do Amaral, E.F. de Andrade, M.L. Corazza, M.L. Masson,

Antioxidant activity and fatty acid profile of yacon leaves extracts obtained by supercritical CO_2 + ethanol solvent, J. Supercrit. Fluids. 146 (2019) 55–64. https://doi.org/10.1016/j.supflu.2019.01.007.

[52] P.R.S. Páramos, J.F.O. Granjo, M.L. Corazza, H.A. Matos, Extraction of high value products from avocado waste biomass, J. Supercrit. Fluids. 165 (2020). https://doi.org/10.1016/j.supflu.2020.104988.

[53] I.P. Dias, S.F. Barbieri, D.E.L. Fetzer, M.L. Corazza, J.L.M. Silveira, Effects of pressurized hot water extraction on the yield and chemical characterization of pectins from *Campomanesia xanthocarpa* Berg fruits, Int. J. Biol. Macromol. 146 (2020) 431–443. https://doi.org/10.1016/j.ijbiomac.2019.12.261.

[54] J. Chu, S.L. Li, Z.Q. Yin, W.C. Ye, Q.W. Zhang, Simultaneous quantification of coumarins, flavonoids and limonoids in Fructus Citri Sarcodactylis by high performance liquid chromatography coupled with diode array detector, J. Pharm. Biomed. Anal. 66 (2012) 170–175. https://doi.org/10.1016/j.jpba.2012.03.041.

[55] AOCS, American Oil Chemist's Society, 1997.

[56] L.R.S. Kanda, C.I. Yamamoto, A.R. Lopes, F.A.P. Voll, M.L. Corazza, F. Wypych, Density, refractive index and viscosity as content monitoring tool of acylglycerols and fatty acid methyl esters in the transesterification of soybean oil, Anal. Methods. 8 (2016) 5619–5627. https://doi.org/10.1039/C6AY00663A.

[57] A.M.M. Guedes, R. Antoniassi, M.C. Galdeano, R. Grimaldi, M.G. de Carvalho, A.E. Wilhelm, A.G. Marangoni, Length-scale specific crystalline structural changes induced by molecular randomization of pequi oil, J. Oleo Sci. 66 (2017) 469–478. https://doi.org/10.5650/jos.ess16192.

[58] V.L. Singleton, J.A. Rossi, Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents, Am. J. Enol. Vitic. 16 (1965) 144 LP – 158.

[59] W. Brand-Williams, M.E. Cuvelier, C. Berset, Use of a free radical method to evaluate antioxidant activity, LWT - Food Sci. Technol. 28 (1995) 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5.

[60] R. Re, N. Pellegrini, A. Pannala, M. Yang, C. Rice-Evans, Antioxidant activity applying an improved ABTS radical cation decolorization assay, Free Radic. Biol. Med. 26 (1999) 1231–1237.

[61] C. Reichardt, T. Welton, Solvents and Solvent Effects in Organic Chemistry: Fourth Edition, Solvents Solvent Eff. Org. Chem. Fourth Ed. (2010). https://doi.org/10.1002/9783527632220.

[62] D. Ehlers, M. Pfister, D. Gerard, K.W. Quirin, W.R. Bork, P. Toffel-Nadolny, Reducing the coumarin content of tonka bean extracts using supercritical CO_2, Int. J. Food Sci. Technol. 31 (1996) 91–95. https://doi.org/10.1111/j.1365-2621.1996.19-319.x.

[63] S. Sima, V. Feroiu, D. Geană, New high pressure vapor-liquid equilibrium data and density predictions for carbon dioxide+ethyl acetate system, Fluid Phase Equilib. 325 (2012) 45–52. https://doi.org/10.1016/j.fluid.2012.03.028.

[64] K.A. Santos, P.C. Frohlich, J. Hoscheid, T.S. Tiuman, J.E. Gonçalves, L. Cardozo-Filho, E.A. da Silva, Candeia (*Eremanthus erythroppapus*) oil extraction using supercritical CO_2 with ethanol and ethyl acetate cosolvents, J. Supercrit. Fluids. 128 (2017) 323–330. https://doi.org/10.1016/j.supflu.2017.03.029.

[65] A.A. da Cruz Lima, D. Szczerbowski, A. Zandoná Filho, R.B. Derner, M.L. Corazza, L.P. Ramos, Choricystis minor var. minor lipids: Extraction using conventional and pressurized solvents and assessment of their potential to produce fatty acid methyl esters, Algal Res. 33 (2018) 28–35. https://doi.org/10.1016/j.algal.2018.04.017.

[66] M.N. Araújo, A.Q.P.L. Azevedo, F. Hamerski, F.A.P. Voll, M.L. Corazza, Enhanced

extraction of spent coffee grounds oil using high-pressure CO_2 plus ethanol solvents, Ind. Crops Prod. 141 (2019) 111723. https://doi.org/10.1016/j.indcrop.2019.111723.

[67] G. Sodeifian, N. Saadati Ardestani, S.A. Sajadian, H. Soltani Panah, Experimental measurements and thermodynamic modeling of Coumarin-7 solid solubility in supercritical carbon dioxide: Production of nanoparticles via RESS method, Fluid Phase Equilib. 483 (2019) 122–143. https://doi.org/10.1016/j.fluid.2018.11.006.

[68] E.M. Calvey, S.W. Page, L.T. Taylor, Apparent solubility threshold densities of substituted coumarins, J. Supercrit. Fluids. 3 (1990) 115–120. https://doi.org/10.1016/0896-8446(90)90035-K.

[69] Y.H. Choi, J. Kim, M.J. Noh, E.S. Choi, K.P. Yoo, Effect of Functional Groups on the Solubilities of Coumarin Derivatives in Supercritical Carbon Dioxide, ACS Symp. Ser. 670 (1997) 110–118. https://doi.org/10.1021/bk-1997-0670.ch009.

[70] F.C. Torres, B. Medeiros-Neves, H. Ferreira Teixeira, D. Kawanoa, V.L. Eifler-Lima, E. Cassel, R.M.F. Vargas, G.L. Von Poser, Supercritical CO_2 extraction as a selective method for the obtainment of coumarins from *Pterocaulon balansae* (Asteraceae), J. CO2 Util. 18 (2017) 303–308. https://doi.org/10.1016/j.jcou.2017.02.008.

[71] N. Doctor, G. Parker, K. Vang, M. Smith, B. Kayan, Y. Yang, Stability and extraction of vanillin and coumarin under subcritical water conditions, Molecules. 25 (2020) 1–9. https://doi.org/10.3390/molecules25051061.

[72] D. Trabelsi, A. Aydi, A.W. Zibetti, G. Della Porta, M. Scognamiglio, V. Cricchio, E. Langa, M. Abderrabba, A.M. Mainar, Supercritical extraction from *Citrus aurantium* amara peels using CO_2 with ethanol as co-solvent, J. Supercrit. Fluids. 117 (2016) 33–39. https://doi.org/10.1016/j.supflu.2016.07.003.

[73] E.W. Eckey, Vegetable Fats and Oils, New York, 1954. https://doi.org/https://www.worldcat.org/title/vegetable-fats-and-oils/oclc/665924.

[74] D. Rodrigues Valente, I. Valente de Oliveira, M. Valente da cunha, C. Sousa da Sá Leitão, Extração da essência do cumaru para utilização na gastronomia, CIESA. 3 (2016). https://doi.org/https://docplayer.com.br/62867996-Extracao-da-essencia-do-cumaru-para-utilizacao-na-gastronomia.html.

[75] M.S. Correa, D.L. Fetzer, F. Hamerski, M.L. Corazza, A.P. Scheer, R.H. Ribani, Pressurized extraction of high-quality blackberry (*Rubus spp. Xavante* cultivar) seed oils, J. Supercrit. Fluids. 169 (2021) 105101. https://doi.org/10.1016/j.supflu.2020.105101.

[76] T. Bavaro, I. Benucci, A. Pedrali, G. Marrubini, M. Esti, M. Terreni, G. Massolini, D. Ubiali, Lipase-mediated hydrolysis of hempseed oil in a packed-bed reactor and in-line purification of PUFA as mono- and diacylglycerols, Food Bioprod. Process. 123 (2020) 345–353. https://doi.org/10.1016/j.fbp.2020.07.009.

[77] T.P.. S. Hilditch, The Chemical Composition of Natural Fats, W. J. J. SOC.Chem. Ind, London, 1934.

[78] A.K. Paul, V.B. Borugadda, A.S. Reshad, M.S. Bhalerao, P. Tiwari, V. V. Goud, Comparative study of physicochemical and rheological property of waste cooking oil, castor oil, rubber seed oil, their methyl esters and blends with mineral diesel fuel, Mater. Sci. Energy Technol. 4 (2021) 148–155. https://doi.org/10.1016/j.mset.2021.03.004.

[79] K.Y. Lee, M. Shafiur Rahman, A.N. Kim, E.J. Jeong, B.G. Kim, M.H. Lee, H.J. Kim, S.G. Choi, Oil yield, physicochemical characteristics, oxidative stability and microbial safety of perilla seeds stored at different relative humidity, Ind. Crops Prod. 165 (2021) 113431. https://doi.org/10.1016/j.indcrop.2021.113431.

[80] B. Hu, X. Xi, H. Li, Y. Qin, C. Li, Z. Zhang, Y. Liu, Q. Zhang, A. Liu, S. Liu, Q. Luo, A comparison of extraction yield, quality and thermal properties from *Sapindus*

mukorossi seed oil between microwave assisted extraction and Soxhlet extraction, Ind. Crops Prod. 161 (2021). https://doi.org/10.1016/j.indcrop.2020.113185.

[81] M. Alrashidi, D. Derawi, J. Salimon, M. Firdaus Yusoff, An investigation of physicochemical properties of *Nigella sativa L*. Seed oil from Al-Qassim by different extraction methods, J. King Saud Univ. - Sci. 32 (2020) 3337–3342. https://doi.org/10.1016/j.jksus.2020.09.019.

[82] B.M. Abdullahi, A. Garba, A. Salihu, M.A. Saliu, Effect of degumming on physicochemical properties of fatty acid ethyl esters obtained from *Acacia nilotica* seed oil, Bioresour. Technol. Reports. 14 (2021) 100678. https://doi.org/10.1016/j.biteb.2021.100678.

[83] J.C.O. Santos, I.M.G. Dos Santos, A.G. De Souza, S. Prasad, A. V. Dos Santos, Thermal stability and kinetic study on thermal decomposition of commercial edible oils by thermogravimetry, J. Food Sci. 67 (2002) 1393–1398. https://doi.org/10.1111/j.1365-2621.2002.tb10296.x.

[84] A. Gouveia De Souza, J.C. Oliveira Santos, M.M. Conceição, M.C. Dantas Silva, S. Prasad, A thermoanalytic and kinetic study of sunflower oil, Brazilian J. Chem. Eng. 21 (2004) 265–273. https://doi.org/10.1590/s0104-66322004000200017.

[85] F. Gao, J. Birch, Oxidative stability, thermal decomposition, and oxidation onset prediction of carrot, flax, hemp, and canola seed oils in relation to oil composition and positional distribution of fatty acids, Eur. J. Lipid Sci. Technol. 118 (2016) 1042–1052. https://doi.org/10.1002/ejlt.201500208.

[86] M.S. Khan, R. Agrawal, M. Ubaidullah, M.I. Hassan, N. Tarannum, Design, synthesis and validation of anti-microbial coumarin derivatives: An efficient green approach, Heliyon. 5 (2019) e02615. https://doi.org/10.1016/j.heliyon.2019.e02615.

[87] Y. Erdogdu, U.C. Baskose, S. Saglam, M. Erdogdu, H. Ogutcu, S. Özçelik, Structural,

thermal, spectroscopic, electronic and biological activity properties of coumarin-153 dyes for DSSCs: A DFT benchmark study, J. Mol. Struct. 1221 (2020) 128873. https://doi.org/10.1016/j.molstruc.2020.128873.

[88] S. Venkatesan, B. Ranjithkumar, S. Rajeshkumar, K. Anver Basha, Synthesis, characterization, thermal stability and antibacterial activity of coumarin based methacrylate copolymers, Chinese J. Polym. Sci. (English Ed. 32 (2014) 1373–1380. https://doi.org/10.1007/s10118-014-1515-9.

[89] Y. Xu, X. Zhu, X. Ma, H. Xiong, Z. Zeng, H. Peng, J. Hu, Enzymatic production of trans-free shortening from coix seed oil, fully hydrogenated palm oil and Cinnamomum camphora seed oil, Food Biosci. 22 (2018) 1–8. https://doi.org/10.1016/j.fbio.2017.12.010.

[90] N.K. Kassim, M. Rahmani, A. Ismail, M.A. Sukari, G.C.L. Ee, N.M. Nasir, K. Awang, Antioxidant activity-guided separation of coumarins and lignan from *Melicope glabra* (Rutaceae), Food Chem. 139 (2013) 87–92. https://doi.org/10.1016/j.foodchem.2013.01.108.

[91] A. Witaicenis, L.N. Seito, A. Da Silveira Chagas, L.D. De Almeida, A.C. Luchini, P. Rodrigues-Orsi, S.H. Cestari, L.C. Di Stasi, Antioxidant and intestinal anti-inflammatory effects of plant-derived coumarin derivatives, Phytomedicine. 21 (2014) 240–246. https://doi.org/10.1016/j.phymed.2013.09.001.

[92] H. Niu, W. Wang, J. Li, Y. Lei, Y. Zhao, W. Yang, C. Zhao, B. Lin, S. Song, S. Wang, A novel structural class of coumarin-chalcone fibrates as PPARα/γ agonists with potent antioxidant activities: Design, synthesis, biological evaluation and molecular docking studies, Eur. J. Med. Chem. 138 (2017) 212–220. https://doi.org/10.1016/j.ejmech.2017.06.033.

[93] K. Týskiewicz, M. Konkol, E. Rój, The application of supercritical fluid extraction in

phenolic compounds isolation from natural plant materials, Molecules. 23 (2018).

https://doi.org/10.3390/molecules23102625.

Figure Captions

Figure 1. Schematic diagram of the supercritical extraction setup (SFE). V1: cylinder valve; V2: ball valve; V3: needle valve; V4: micro-metering needle valve. The transversal lines identify the water supply of the baths.

Figure 2. Schematic diagram of pressurized liquid extraction (PLE) setup. V1 and V2: needle valves; V3: back-pressure regulator valve. The transversal lines identify the transport of fluids at high temperatures.

Figure 3. Overall extraction curves of cumaru seeds obtained by $scCO_2$+EtOAc: (A) single stage, solvent to solids mass ratio of (1:1) and different conditions of pressure and temperature; and (B) sequential extractions, cosolvent to solids mass ratio of (1:1) (3 stages) and (0.5:1) (2 stages), at 40 °C and 15 MPa.

Figure 4. Overall extraction curves of cumaru seeds obtained by $scCO_2$+EtOH: (A) single stages at different conditions of pressure, temperature and cosolvent to solid mass ratio of (1:1); (B) single stages extraction varying the cosolvent to solid mass ratio of 1:1; and (C) sequential extractions, different conditions of pressure, temperature and cosolvent to solid mass ratio (1:1, and 2:1).

Figure 5. Overall extraction curves of cumaru seeds obtained using pressurized liquid extraction (PLE) with ethanol at 100 bar and 60, 80 and 100 °C.

Figure 6. Coumarin content associated to the extraction curves obtained from cumaru seeds extraction by $scCO_2$+EtOAc to solids mass ratio of (1:1), at 25 MPa and 80 ºC. Bars represent the extraction yield measured each time and lines represent the coumarin content in the crude extracts.

Figure 7. Coumarin content associated to the extraction curves obtained from cumaru seeds extraction by $scCO_2$+EtOH at different EtOH to solids mass ratio of (1:1), and (A) 15 MPa and 40 °C; (B) 25 MPa and 40 °C; (C) 15 MPa and 80 °C; (D) 25 MPa and 80 °C; and (E) 20 MPa and 60 °C. Bars represent the extraction yield measured each time and lines represent the coumarin content in the crude extracts.

Figure 8. Coumarin extraction curves from cumaru seeds extraction by pressurized liquid extraction with ethanol at: (A) 10 MPa and 60 °C; (B) 10 MPa and 80 °C; and (C) 10 MPa and 100 °C. Bars represent the extraction yield measured each time and lines represent the coumarin content in the crude extracts.

Figure 9. Infographic of the separation process between coumarin and extract obtained using $scCO_2$+EtOAc and $scCO_2$+EtOH.

Figure 10. Visual aspects of cumaru seed extracts obtained by: (A) CO_2 extraction; (B) SE+EtOH; (C) SE+EtOAc; (D) $scCO_2$+EtOAc; and (E) $scCO_2$ + EtOH.

Figure 11. TGA curves of cumaru seed extracts obtained by scCO2+EtOAc, scCO2+EtOH, SE+EtOAc, and SE+EtOH.

Figure 12. DSC of cumaru seed extract: (A) melting curves (-80 to 50 °C); and (B) crystallization curves (50 to -80 °C).

Figure 1

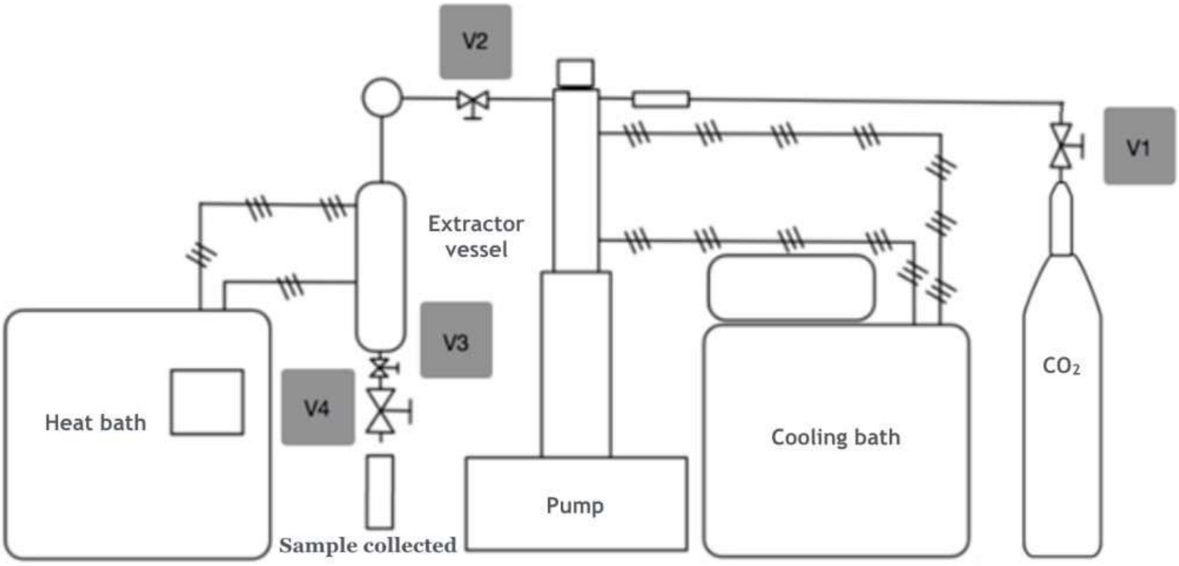


Figure 2

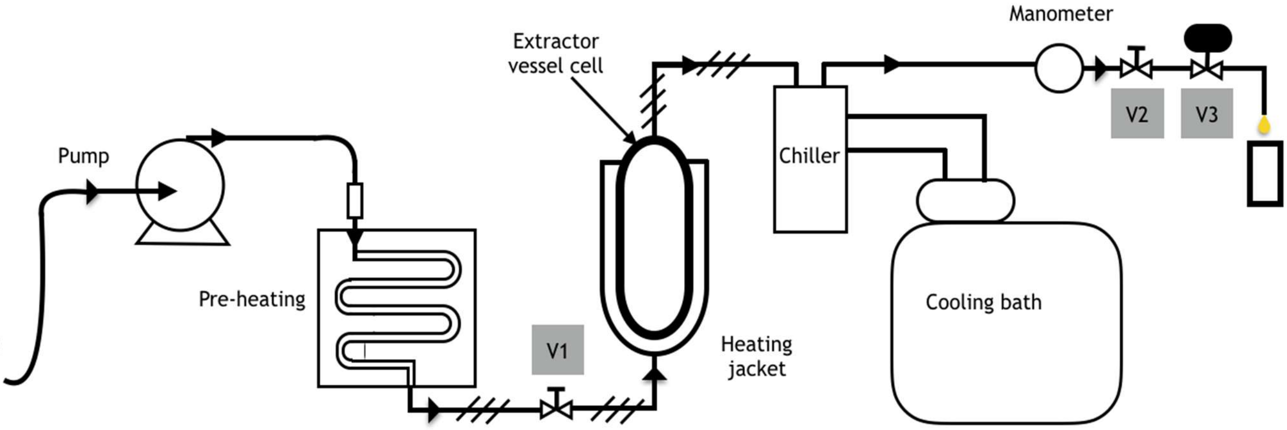


Figure 3

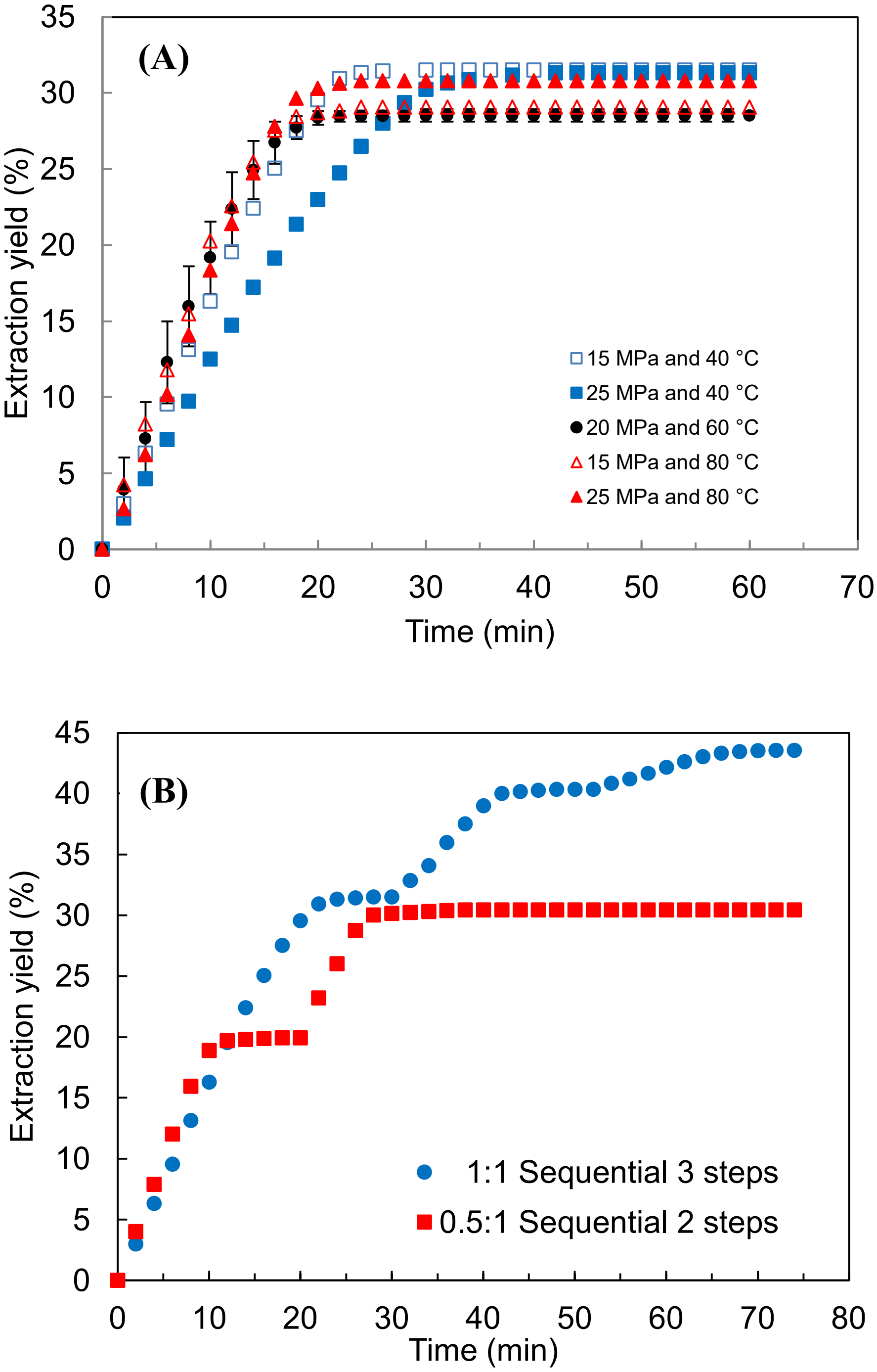
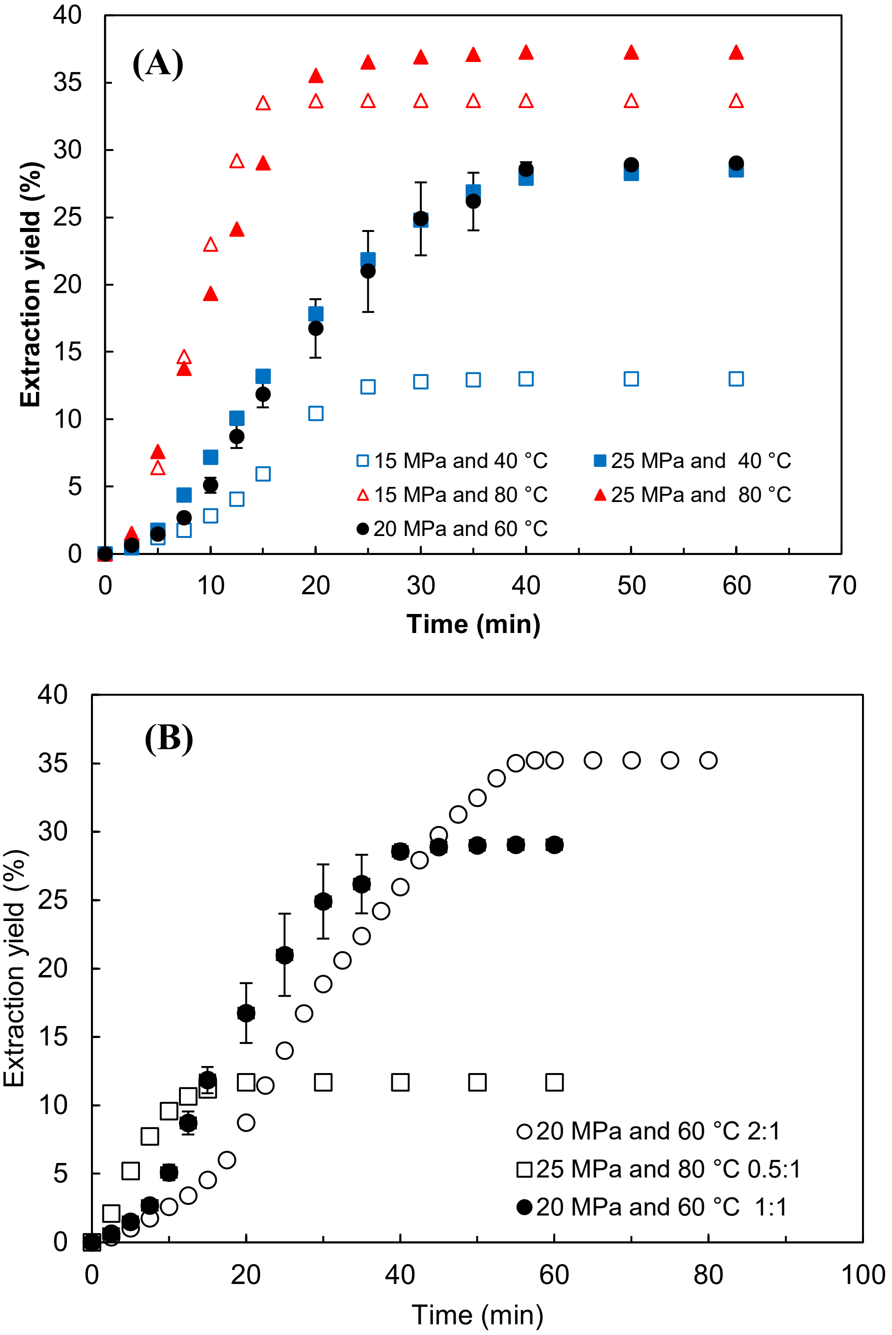

(A)
Extraction yield (%)
0
5
10
15
20
25
30
35
Time (min)
0
10
20
30
40
50
60
70
□ 15 MPa and 40 °C
■ 25 MPa and 40 °C
● 20 MPa and 60 °C
△ 15 MPa and 80 °C
▲ 25 MPa and 80 °C
(B)
Extraction yield (%)
0
5
10
15
20
25
30
35
40
45
Time (min)
0
10
20
30
40
50
60
70
80
● 1:1 Sequential 3 steps
■ 0.5:1 Sequential 2 steps

Figure 4


(A)
Extraction yield (%)
0
5
10
15
20
25
30
35
40
Time (min)
0
10
20
30
40
50
60
70
□ 15 MPa and 40 °C
■ 25 MPa and 40 °C
△ 15 MPa and 80 °C
▲ 25 MPa and 80 °C
● 20 MPa and 60 °C
(B)
Extraction yield (%)
0
5
10
15
20
25
30
35
40
Time (min)
0
20
40
60
80
100
○ 20 MPa and 60 °C 2:1
□ 25 MPa and 80 °C 0.5:1
● 20 MPa and 60 °C 1:1

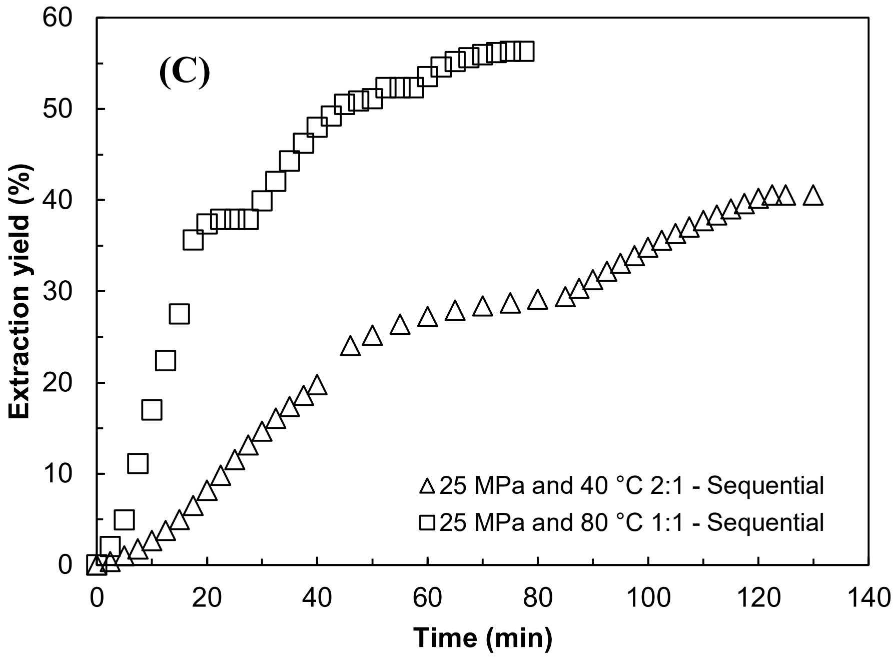


Figure 4. Overall extraction curves of cumaru seeds obtained by $scCO_2$+EtOH: (A) single stages at different conditions of pressure, temperature and cosolvent to solid mass ratio of (1:1); (B) single stages of extraction varying the cosolvent to solid mass ratio of 1:1; and (C) sequential extractions, different conditions of pressure, temperature and cosolvent to solid mass ratio (1:1, and 2:1).

Figure 5

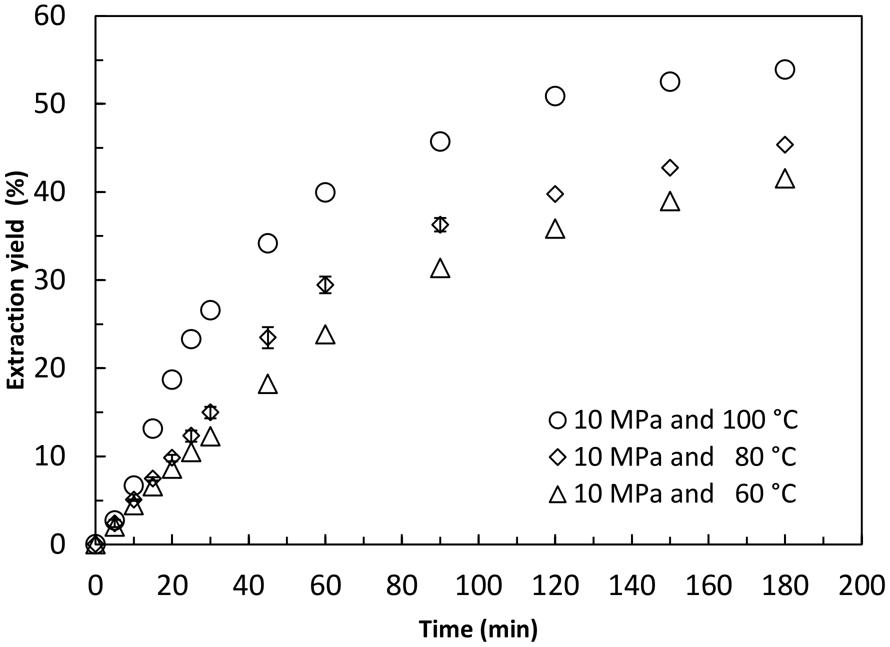


Figure 6

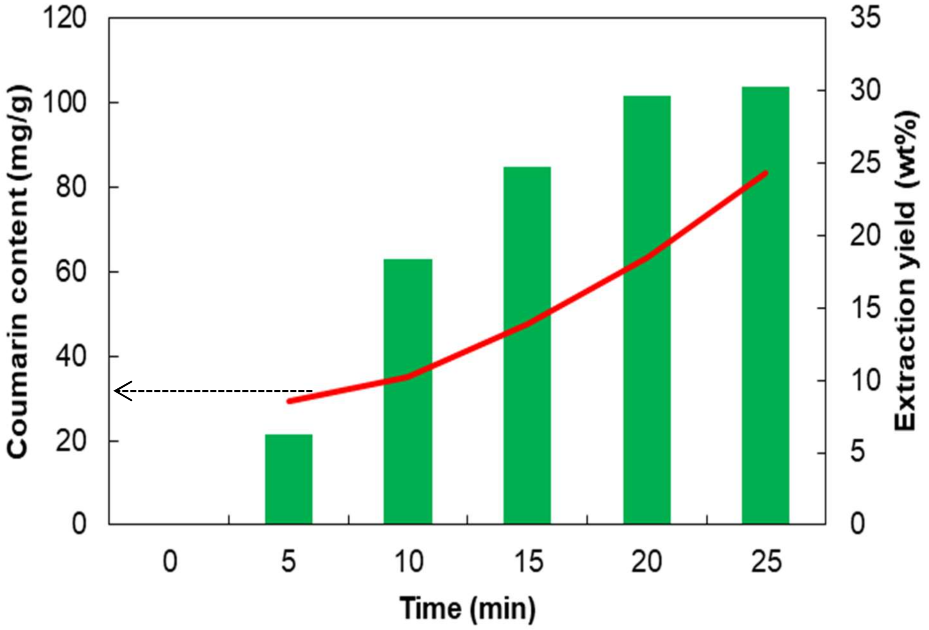


Figure 7

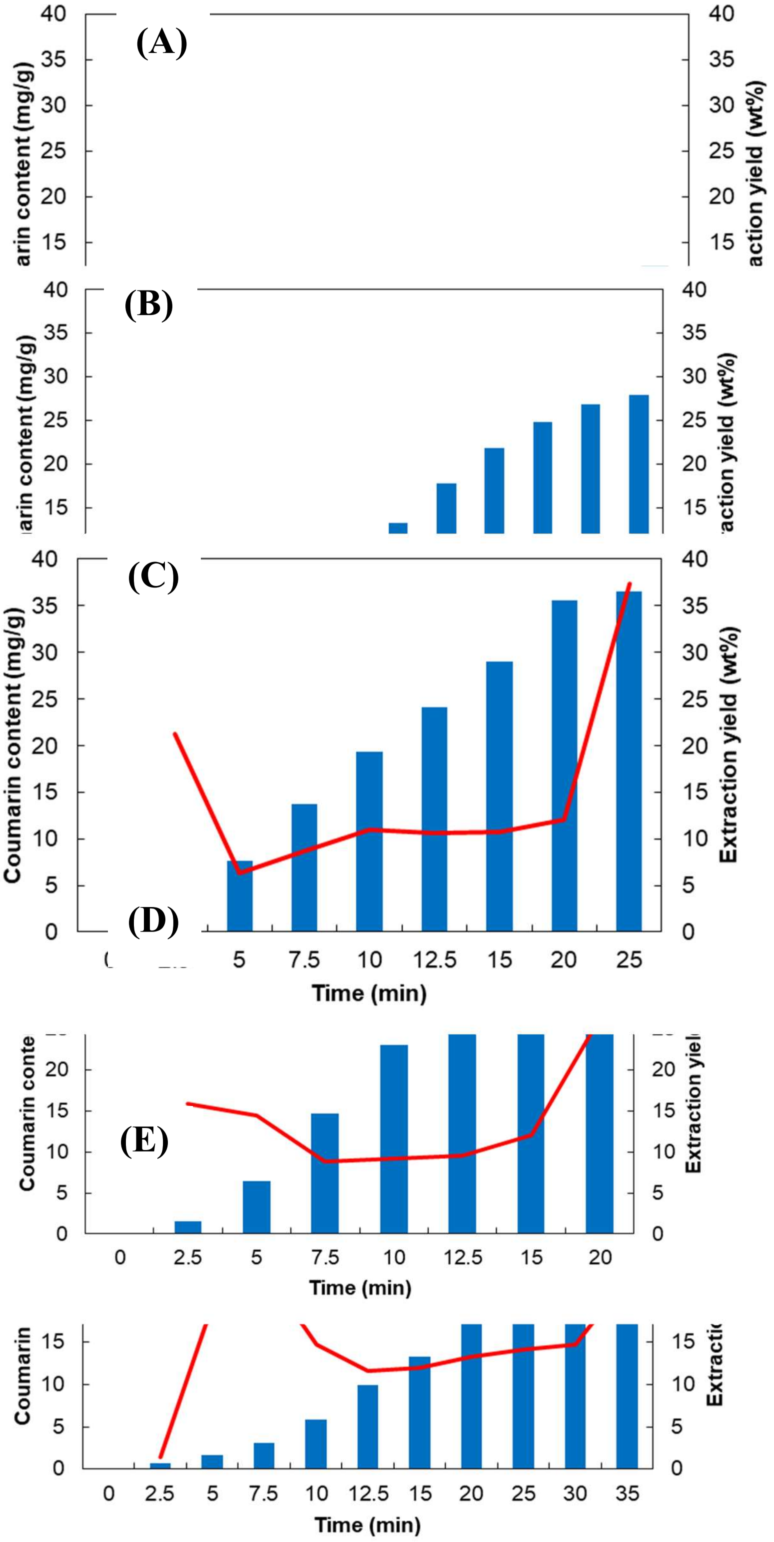
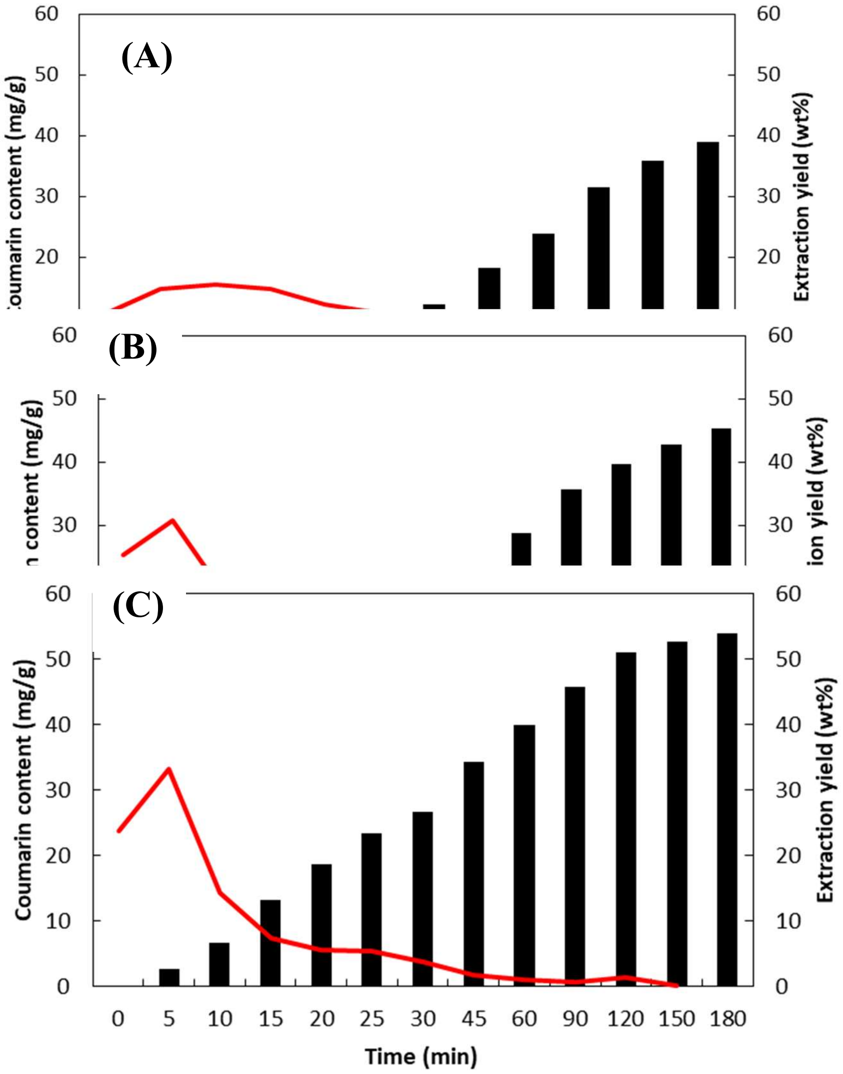

(A)
(B)
(C)
(D)
(E)
Coumarin content (mg/g)
Extraction yield (wt%)
0
5
10
15
20
25
30
35
40
0
2.5
5
7.5
10
12.5
15
20
25
30
35
Time (min)

Figure 8



(A)
(B)
(C)
Coumarin content (mg/g)
Extraction yield (wt%)
0
10
20
30
40
50
60
Time (min)
0
5
10
15
20
25
30
45
60
90
120
150
180

Figure 9

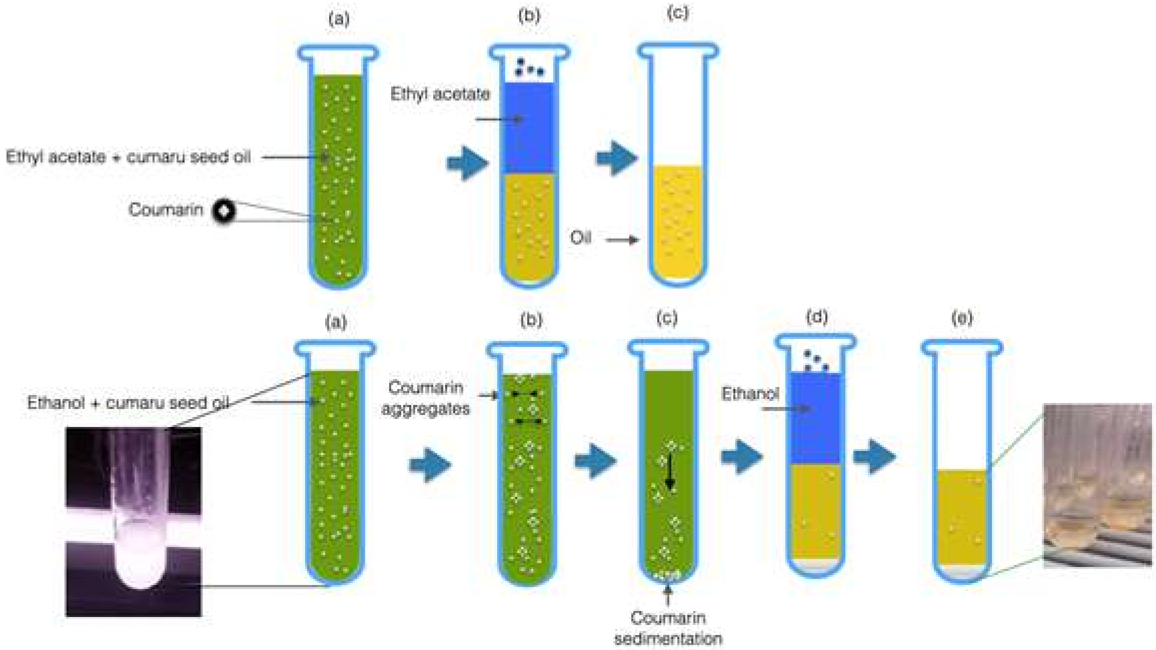


Figure 10

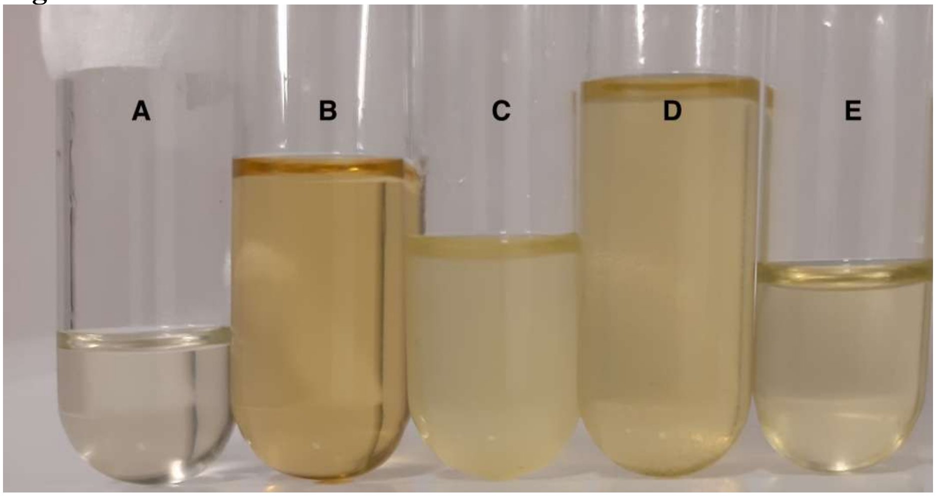
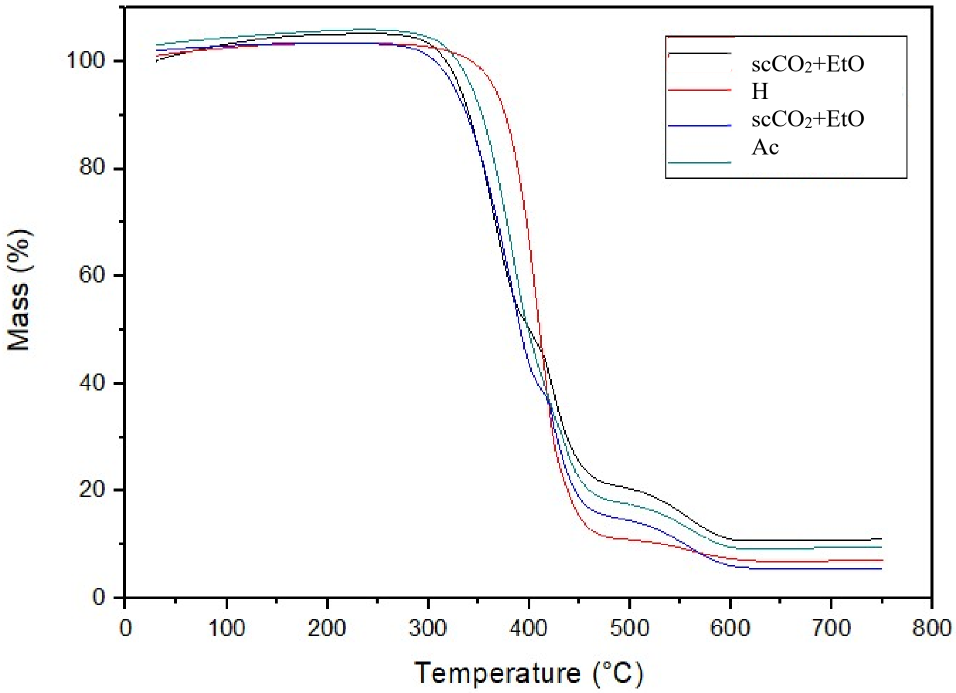
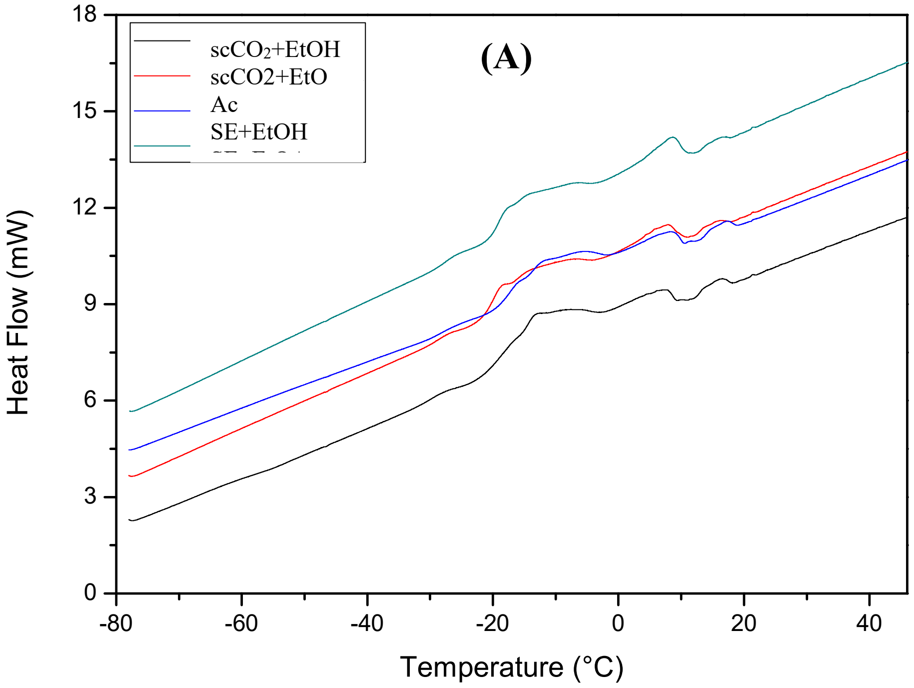


Figure 11

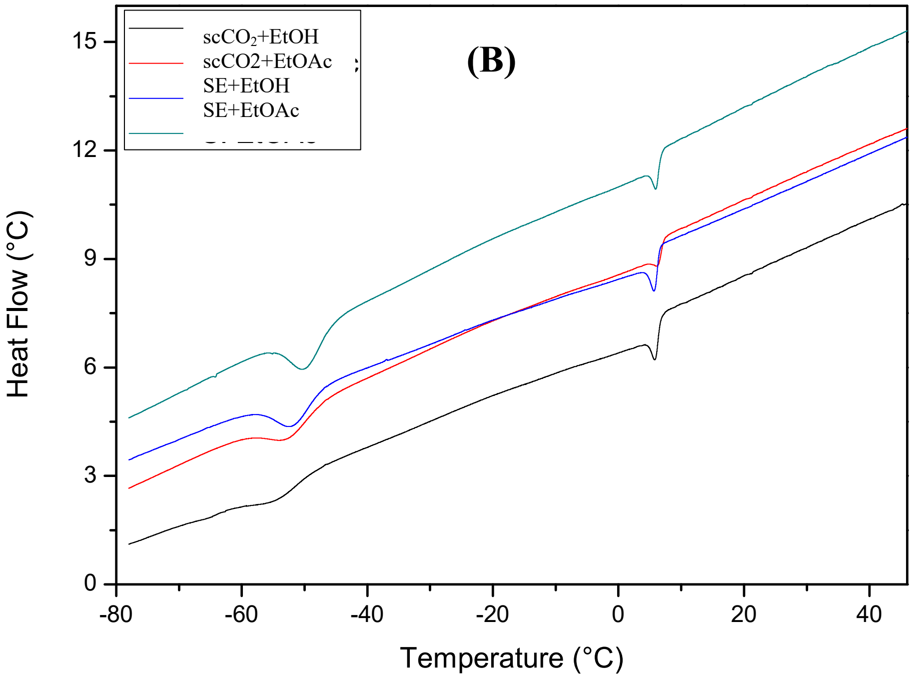


scCO2+EtO
H
scCO2+EtO
Ac
Mass (%)
0
20
40
60
80
100
Temperature (°C)
0
100
200
300
400
500
600
700
800

Figure 12



(A)
scCO$_2$+EtOH
scCO2+EtO
Ac
SE+EtOH
Heat Flow (mW)
Temperature (°C)
0
3
6
9
12
15
18
-80
-60
-40
-20
0
20
40



(B)
scCO$_2$+EtOH
scCO2+EtOAc
SE+EtOH
SE+EtOAc
Heat Flow (°C)
Temperature (°C)
0
3
6
9
12
15
-80
-60
-40
-20
0
20
40

Table 1 - Results of Soxhlet extractions performed with cumaru seeds and using ethanol, and ethyl acetate as solvents.

Run	Solvent	Temperature (°C)[b]	Polarity index[c]	Extraction yield (wt%)[a]
1	Ethyl Acetate	77.10	0.228	43.6 ± 0.3
2	Ethanol	78.37	0.654	47.2 ± 1.2
Lit.	n-Hexane	68.7	0.009	39.2 ± 0.5[d]

* All the experiments were performed for 360 min and at the local atmospheric pressure (91.6 kPa). [a] Average and standard deviation values of three extractions. [b] Boiling point temperature [61]. [c] Polarity index [61]. [d] Fetzer et al. [47].

Table 2 – Experimental conditions and results for cumaru seed oil extraction yield using scCO_2+EtOAc as solvent*.

Run	Solvent	P (MPa)	T (°C)	DET (min)	$m_{0,\mathrm{EtOAc}}$ (g)[a]	$m_{CT,\mathrm{CO_2}}$ (g)[b]	$m_{D,\mathrm{CO_2}}$ (g)[c]	$\rho_{\mathrm{CO_2}}$** (kg m^3)	Extraction yield (wt%)	Extraction efficiency (%)
Singles stages										
3	CO_2+EtOAc[e][1]	15 (- 1)	40 (- 1)	22	26.00	53.32	41.98	780.23	31.5	72.4
4	CO_2+EtOAc[e]	15 (- 1)	80 (+1)	20	25.25	53.57	38.18	427.15	29.1	66.7
5	CO_2+EtOAc[e]	20 (0)	60 (0)	22	25.80	53.22	39.22	723.68	28.5 ± 0.4[d]	65.4
6	CO_2+EtOAc[e]	25 (+1)	40 (- 1)	32	25.59	73.72	64.11	879.49	31.3	71.9
7	CO_2+EtOAc[e]	25 (+1)	80 (+1)	20	26.07	58.10	40.07	686.22	30.8	70.7
Sequential extractions										
8	CO_2+EtOAc[f][1]	15	40	12	12.92	58.52	27.40	780.23	19.9	45.7
9	CO_2+EtOAc[f][2]	15	40	8	10.66	70.25	15.68	780.23	10.5	24.1
					Overall yield of sequential extractions				30.4	69.9
10	CO_2+EtOAc[e][2]	15	40	12	18.17	82.76	48.04	780.23	9.2	21.2
11	CO_2+EtOAc[e][3]	15	40	14	14.90	78.58	25.04	780.23	3.2	7.4
					Overall yield of sequential extractions				44.0	100.9

*Factors and levels, and 2^2 experimental design with independent variables. Confinement time of 30 min in all the extractions. Solvent flow rate of 2 mL min^{-1}.

** Density of CO_2 at the extraction conditions obtained from the NIST database. DET: dynamic extraction time.

[a] Mass of ethyl acetate used in the confinement extraction. [b] Mass of CO_2 used in the confinement extraction; [c] Mass of CO_2+EtOAc mixture in the dynamic extraction

[d] Average and standard deviation values of three extractions. [e] Ethyl acetate to solids mass ratio of (1:1). [f] Ethyl acetate to solids mass ratio of (0.5:1).

[1] 1st stage of sequential extraction; [2] 2nd stage of sequential extraction. [3] 3rd stage of sequential extraction.

Table 3 – Experimental conditions and result for cumaru seed oil extraction yield using $scCO_2$+EtOH.

Run	Solvent	P (MPa)	T (°C)	DET (min)	$m_{0,EtOH}$ (g)[a]	m_{CT,CO_2} (g)[b]	m_{D,CO_2} (g)[c]	ρ_{CO_2}** (kg/m^3)	Extraction yield (wt%)	Extraction efficient (%)
Single stages										
12	CO_2+ EtOH[e]	15 (- 1)	40 (- 1)	25	26.50	26.09	47.76	780.23	13.0	27.5
13	CO_2+ EtOH[e]	15 (- 1)	80 (+1)	15	29.00	20.21	30.00	427.15	33.7	71.3
14	CO_2+ EtOH[e]	20 (0)	60 (0)	45	28.37	29.67	58.80	723.68	29.1 ± 0.2[d]	61.6
15	CO_2+ EtOH[e]	25 (+1)	40 (- 1)	45	27.75	27.47	70.09	879.49	28.7	60.7
16	CO_2+ EtOH[e]	25 (+1)	80 (+1)	20	26.38	25.99	40.12	686.22	37.2	78.9
Additional experiments with different EtOH to solids ratios										
17	CO_2+EtOH[f]	25	80	15	13.19	36.25	29.07	686.22	11.7	24.8
18	CO_2+EtOH[g]	20	60	55	50.51	42.64	107.82	723.68	35.2	74.7
Sequential extractions										
19	CO_2+EtOH[g1]	25	40	55	50.55	35.83	110.30	879.49	29.4	62.4
20	CO_2+EtOH[g2]	25	40	37.5	49.97	28.16	75.15	879.49	11.7	24.1
					Overall yield of sequential extractions				40.8	86.4
21	CO_2+EtOH[e1]	25	80	20	29.41	22.67	40.07	686.22	37.9	80.3
22	CO_2+EtOH[e2]	25	80	20	27	27.05	40.07	686.22	14.4	30.6
23	CO_2+EtOH[e3]	25	80	15	29.15	28.33	30.07	686.22	4.1	8.7
					Overall yield of sequential extractions				56.5	119.6

*Factors and levels, and 2^2 experimental design with independent variables. Confinement time of 30 min in all the extractions. Solvent flow rate of 2.0 ± 0.3 mL min^{-1}.

** Density of CO_2 at the extraction conditions obtained from the NIST database. DET: dynamic extraction time.

[a] Mass of ethanol used in the continent extraction. [b] Mass of CO_2 used in the confinement extraction; [c] Mass of CO_2+EtOH mixture in the CXL in the dynamic extraction

[d] Average and standard deviation values of three extractions. [e] Ethanol to solids mass ratio of (1:1). [f] Ethanol to solids mass ratio of (0.5:1). [g] Ethanol to solids mass ratio of (2:1). [1] 1st stage of sequential extraction; [2] 2nd stage of sequential extraction. [3] 3rd stage of sequential extraction.

Table 4 – Experimental conditions and results for cumaru seed oil extraction yield using PLE-EtOH.

Run	Solvent	P (MPa)	T (°C)	DET (min)	ρ_{EtOH}*	Extraction yield (wt%)	Extraction efficient (%)
Pressurized Liquid Extraction							
24	EtOH	10	60	180	761.28	41.5	88.0
25	EtOH	10	80	180	742.34	45.4 ± 0.7[a]	96.1
26	EtOH	10	100	180	723.40	53.9	114.2

[a] Average and standard deviation values from extractions performed in triplicates. * Extraction times were 180 min for all the experiments.

Table 5 – Concentration and mass of coumarin in the cumaru seed extracts.*

Run	Solvents	Extraction conditions	Ratio[a]	Coumarin (mg/g)	Mass of extract (g)	Mass of Coumarin in extract (mg)
Soxhlet						
1	SE+EtOAc	B.P./A.P.**	30:1	0.67	2.20	1.47
2	SE+EtOH	B.P./A.P.**	30:1	7.76	2.29	17.77
scCO$_2$+EtOAc						
3	scCO$_2$+EtOAc	15 MPa/40 °C	1:1	2.25	7.82	17.59
4	scCO$_2$+EtOAc	15 MPa/80 °C	1:1	18.69	7.17	134.01
5	scCO$_2$+EtOAc	20 MPa/60 °C	1:1	8.36	7.09	59.27
6	scCO$_2$+EtOAc	25 MPa/40 °C	1:1	2.00	7.75	15.50
7	scCO$_2$+EtOAc	25 MPa/80 °C	1:1	26.55	7.43	197.26
scCO$_2$+ EtOH						
12	scCO$_2$+ EtOH	15 MPa/40 °C	1:1	2.25	3.21	0.70
13	scCO$_2$+ EtOH	15 MPa/80 °C	1:1	12.50	7.33	91.62
14	scCO$_2$+ EtOH	20 MPa/60 °C	1:1	14.65	6.78	99.62
15	scCO$_2$+ EtOH	25 MPa/40 °C	1:1	0.93	6.75	6.28
16	scCO$_2$+ EtOH	25 MPa/80 °C	1:1	20.25	8.18	165.64
17	scCO$_2$+ EtOH	25 MPa/80 °C	0.5:1	1.90	2.83	5.37
19	scCO$_2$+ EtOH	25 MPa/40 °C	1st Stage 2:1	15.67	7.26	113.76
20	scCO$_2$+ EtOH	25 MPa/40 °C	2nd Stage 2:1	5.00	2.80	14.00
	Cumulative coumarin in sequential extraction			20.67		133.13
21	scCO$_2$+ EtOH	25 MPa/80 °C	1st Stage 1:1	18.65	8.70	162.25
22	scCO$_2$+ EtOH	25 MPa/80 °C	2nd Stage 1:1	8.53	3.31	28.23
23	scCO$_2$+ EtOH	25 MPa/80 °C	3rd Stage 1:1	2.16	1.67	3.60
	Cumulative coumarin in sequential extraction			30.16		194.08
PLE+EtOH						
24	PLE + EtOH	10 MPa/60 °C	13:1	6.19	4.44	27.48
25	PLE + EtOH	10 MPa/80 °C	13:1	8.17	4.79	39.17
26	PLE + EtOH	10 MPa/100 °C	13:1	7.85	5.91	46.39

* All the analyses were performed in duplicate. [a] Mass of sample to mass of solvent. ** Boiling point/atmospheric pressure.

Table 6 - Fatty acid composition (wt.%) of cumaru seed oil extracted by different methods.

Fatty acids	**$scCO_2$+EtOAc**	**$scCO_2$+EtOH**	**SE+EtOAc**	**SE+EtOH**
Palmitic acid (C16:0)	6.33	6.66	6.01	7.26
Stearic acid (C18:0)	3.86	4.66	3.54	4.88
Oleic acid (C18:1)	53.62	53.12	53.44	51.32
Linoleic acid (C18:2)	17.64	17.80	17.61	18.67
Linolenic acid (C18:3)	3.60	3.09	3.82	2.64
Cis-11-eicosenoic acid (20:1)	1.11	1.38	1.07	1.64
Arachidonic acid (20:4)	0.72	0.63	0.95	0.33
Tricosanoic acid (23:0)	8.22	8.01	8.37	7.75
Nervonic acid (24:1)	4.89	4.70	5.10	5.53
SFA	18.42	19.33	17.92	19.89
UFA	81.58	80.67	82.08	80.11
PUFA	21.96	21.52	22.38	21.64

The standard deviations for all fatty acids were lower than 0.003.
SFA: saturated fatty acids.
UFA: unsaturated fatty acids.
PUFA: polyunsaturated fatty acids.

Table 7 - Physicochemical parameters values of oil seed cumaru.

Run	Extraction methods	Solvent	Free fatty acid (wt.%)**	Refraction index*	Saponification value (mg KOH g^{-1})	Iodine value
2	Soxhlet	Ethanol	0.58 ± 0.04	1.4675	205.48	83.38
7	Soxhlet	Ethyl Acetate	0.60 ± 0.03	1.4670	205.26	86.54
18	20 MPa/60 °C	$scCO_2$+EtOAc	0.45 ± 0.04	1.4700	205.59	86.08
22	20 MPa/60 °C	$scCO_2$+EtOH	0.40 ± 0.02	1.4680	205.99	84.42

*Average and standard deviation value of duplicates (standard deviation= ± 0.0005).
** Average and standard deviation values of three analyses.

Table 8 - Total phenolic content (TPC) and antioxidant activities (AA) by DPPH and ABTS methods for the extracts.

Run	Extraction methods	Solvent	TPC (mg GAE/ 100 g extract)	DPPH (mmol TE 100 g$^{-1)}$	ABTS (mmol TE 100 g^{-1})
1	Soxhlet	EtOAc	16.96 ± 1.54	26 ± 1	82 ± 5
2	Soxhlet	EtOH	111.61 ± 4.26	902 ± 23	443 ± 32
3	15 MPa/40 °C	CO$_2$+EtOAc	29.97 ± 1.71	38 ± 1	159 ± 9
4	15 MPa/80 °C	CO$_2$+EtOAc	105.41 ± 4.45	89 ± 1	413 ± 6
5	20 MPa/60 °C	CO$_2$+EtOAc	63.19 ± 2.35	59 ± 2	251 ± 7
6	25 MPa/40 °C	CO$_2$+EtOAc	19.73 ± 1.39	36 ± 2	86 ± 6
7	25 MPa/80 °C	CO$_2$+EtOAc	117.84 ± 5.31	177 ± 2	472 ± 11
13	15 MPa/40 °C	CO$_2$+EtOH	7.98 ± 0.25	22 ± 2	11 ± 3
14	15 MPa/80 °C	CO$_2$+EtOH	20.58 ± 0.55	34 ± 3	44 ± 4
15	20 MPa/60 °C	CO$_2$+EtOH	10.66 ± 0.60	26 ± 3	14 ± 4
16	25 MPa/40 °C	CO$_2$+EtOH	6.18 ± 0.76	20 ± 3	3 ± 1
17	25 MPa/80 °C	CO$_2$+EtOH	19.38 ± 0.23	29 ± 1	25 ± 5

Total Phenolics Content Expressed as mg of Gallic Acid Equivalents per 100 g of sample.
Antioxidant Activity by DPPH●. Expressed as mmol of Trolox Equivalents per 100 g of sample.
Antioxidant Activity by ABTS●. Expressed as mmol of Trolox Equivalents per 100 g of sample.