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Utilization of pressurized CO₂, pressurized ethanol and CO₂-expanded ethanol mixtures for de-oiling spent bleaching earths

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Abstract

Compressed fluids were used in a two-step scheme to extract oil from rapeseed and sunflower spent bleaching earths. The first extraction, performed with CO₂ at 45°C, 23 MPa, yielded matrices depleted in neutral lipids (contents of 1% and 3.5% compared to initial 22% and 28%) and of better flowing characteristics thanks to the downsizing of particle (83% of particles had sizes below 50 µm). The second step was assisted by ethanol used either as a compressed liquid, as a co-solvent or as a CO₂-expanded mixture by changing temperature (35°, 60°C) pressure (6, 18 MPa) and ethanol percentage (10 – 100 wt%). A statistical analysis concluded that extraction yield and oligomers percentage were dependent on pressure, ethanol percentage and their interaction, whilst temperature was a significant factor only for yield. The pigments content of oils produced by CO₂ and extracts produced by pressurized ethanol was below 5 µg/g_{oil} and 32 mg/g_{extract}.

Keywords: spent bleaching earth; de-oiling; compressed fluids; carbon dioxide; expanded ethanol; pigment

1. Introduction

Bleaching is an adsorptive cleansing process used in the edible oil refinery to remove components that adversely impact the quality and the stability of oils such as carotenoids, chlorophylls, phospholipids, free fatty acids, soaps, trace metals and oxidation products [1,2]. The main types of bleaching materials used in

oil processing are natural earths and acid-activated clays, the latter showing a much higher bleaching efficiency than natural earths [1,2] especially with dark oils [3]. The clays can also be mixed with activated carbon to increase the adsorptive capacity, or with silica gel when high selectivity is required [2].

At the end of the bleaching operation, spent bleaching earths (SBE) contain 20-40% of residual oil. Due to such high oil content and to the high surface area of the materials, SBE are prone to oxidize and even spontaneously ignite in case of unsaturated oils, so they must be carefully disposed of. For many years, the conventional routes for the disposal of these wastes were farmland or burial in local landfill sites [1]. With changes in legislation and increasing costs of waste disposal, SBE disposal became an important environmental and economic problem that can be partly solved by the reuse of SBE. Several utilizations of SBE have been investigated: as animal feed supplement or low grade fuel for power and heat generation or material to assist the oil extraction when blended with seeds[4], bio organic fertilizer [5], substrate for biodiesel production [6], compact clay block for construction [7] or precursor for bricks of high insulating and mechanical properties [8]. SBE can also be subjected to oil extraction [9] with the advantage that the oil recovered can constitute a feedstock for biofuels, biolubricants or oleochemicals [5]. The treatment has the benefit of leaving the material depleted in oil so the clay can be further disposed of as a non-hazardous waste since spontaneous combustion is no longer an issue. De-oiled SBE can be reused as adsorbents provided that a heat treatment or an acid washing is applied to restore the bleaching power [10] or to recover at least the original mesoporosity [11].

Spent bleaching earths can be de-oiled by solvent extraction. The type of solvent and the extraction method will influence the de-oiling efficiency and the quality of the recovered oil. Among various alcohols and hydrocarbons tested for a SBE coming from palm oil refining [12], polar solvents with the exception of methanol were more efficient in terms of yield than non-polar hydrocarbons. However the extracted oils were of poorer quality since more impurities were co-extracted. Even oils recovered by non-polar hexane were of deteriorated quality compared to the crude oils [13], since they had higher free fatty acids content, higher degree of oxidation and a brownish or yellowish appearance. Several solvents were also screened to regenerate spent clays from corn oil by using soxhlet extraction [14]. Methyl ethyl ketone was found to be the best solvent option in terms of percentage of oil extracted (95%), whereas petroleum ether, hexane and methanol yielded lower de-oiling efficiency, namely, 66%, 62% and 53%, respectively.

The use of compressed fluids to de-oil SBE has been only marginally reported. The utilization of carbon dioxide is an environmentally acceptable solution since CO₂ is non-flammable and non-toxic, is a suitable solvent for lipids and leaves the treated matrix free of solvent residue. Waldmann and Eggers in 1991 [15] investigated the de-oiling of palm and rapeseed SBE by supercritical CO₂ (scCO₂) at 50 and 80°C and 35 to 75 MPa. An increase of pressure was found to accelerate the extraction course, whereas temperature had a more complex trend with a faster extraction at 50°C than at 80°C at 35 MPa and the opposite at 50 MPa. Whatever the operating conditions, the bleaching clays were de-oiled to approximately the same degree (> 90%) with qualities of recovered oils that did not vary notably in terms of the free fatty acid contents, peroxide and anisidine indices and colour. A high pressure / high temperature combination

(75.8 MPa/80°C) was also used to treat neutral and acid clays coming from soybean oil refining [16]. ScCO₂ extraction was found to be equally applicable to both neutral and acidic matrices and the extraction degrees approached 100% provided clays were mixed with a pelletized dispersant. Focusing on the oil quality, Kheang et al. [17] compared the characteristics of oils extracted by scCO₂ (conditions not given) or by hexane from a SBE coming from palm oil refining. Lower phosphorus content and peroxide value and light yellow colour gave a superior quality to the CO₂-recovered oil compared to the dark hexane-recovered oils. More recently, the use of sub-critical water was investigated to de-oil SBE from an Egyptian plant (oil type unknown) [18]. Sub-critical water means high temperatures, therefore the treatment was carried out at 180 - 280°C. The optimum conditions were found to be 270°C for 20 min with a feed-to-solvent ratio of 1:3 to attain the maximum extraction yield of 98.4%. The recovered oils were not characterized, except for one sample that was found to contain almost 87% of free fatty acids.

In this work, the de-oiling of bleaching earths was investigated as a two-step process using first supercritical CO₂ in mild conditions to preserve as much as possible the valuable components. Two spent bleaching earths were processed, coming from the industrial refining of rapeseed and sunflower oils, and carotenoids and chlorophylls were evaluated as potential valuable components. In the refining process, bleaching clays are expected to adsorb cationic and polar moieties [1, 2] whilst the triglycerides, the major components of the oils, should be weakly retained. Hence milder conditions for the scCO₂ treatment than those investigated in literature could be sufficient to recover significantly the residual oil. Such first step will produce a less hazardous material that can be re-used as described before or submitted to a second step of extraction to improve the overall regeneration. Several combinations of CO₂+ethanol mixtures were hence investigated to extract more polar species from the CO₂-treated SBE, and depending on the conditions, the extracting fluid was a supercritical CO₂-ethanol mixture, a pressurized ethanol (referred to as PLE in literature) or a CO₂-expanded ethanol. The use of pressurized solvents is more and more proposed for extracting polar compounds from fruits or vegetables [19, 20], however, to the authors' best knowledge, no report on the use of pressurized solvents for desorption of mineral substrates has been found in literature. The main objectives of the work can be therefore summarized as:

- to evaluate the efficiency of neat CO₂ to de-oil two spent bleaching earths that have different physico-chemical characteristics since they were used in the refining of two different oils,
- to investigate, as a second step, the use of more polar solvents obtained by various combinations of CO₂ and ethanol or by neat pressurized ethanol to further extract analytes from CO₂ de-oiled SBE
- to assess not only the yield but also the quality of extracts by monitoring the lipid classes and colour pigments

2. Materials and Methods

2.1. Materials

Spent bleaching earths were provided by Lesieur (Paris, France). SBE were removed directly from the filter at the refining plants, tightly sealed in bags and sent to Bordeaux where they were stored at 4°C. Two kinds

of samples were provided: SBE coming from the treatment of rapeseed oil, which was Tonsil 278FF (hereafter called Rapeseed SBE), and SBE coming from the treatment of sunflower oil, which was a mix of Tonsil 278FF and active charcoal (hereafter called Sunflower SBE). Tonsil® 278FF is a highly active bentonite traded by Clariant S.A. (Muttenz, Switzerland).

Solvents used for extractions (ethanol (96% w/v), hexane (96%), chloroform (99.6%), methanol (99.6%)) were supplied by Atlantic Labo ICS (Bruges, France) whereas CO₂ (99.99% purity) was supplied by Air Liquide (Floirac, France). HPLC grade solvents used for liquid chromatography (methanol 99.9% and *tert*-butyl methyl ether 99.8%) were from Atlantic Labo ICS (Bruges, France). Triethylamine (99.5%) was purchased from Sigma-Aldrich (St Quentin Fallavier, France). Tetrahydrofuran (≥99.9%) used for the analysis of lipid classes was purchased from PanReac AppliChem (Barcelona, Spain), whereas n-heptane (≥99.0%) and isopropanol (≥99.9%) used for the analysis of tocopherol were purchased from PanReac AppliChem (Barcelona, Spain) and Merck KGaA (Darmstadt, Germany), respectively. Standards of fatty acids, β-carotene, chlorophyll *a* and chlorophyll *b* were purchased at Sigma-Aldrich.

2.2. Characterization of SBE

The **particle size distribution** was evaluated by sieving through 500, 200, 100 and 50 μm sieves (Retsch France Verder, Eragny sur Oise, France) and weighing the mass retained on each sieve.

The SBE **flowability** was characterized by the Carr index (CI) widely used in the industry as an indicator of flow properties. The Carr index was calculated using Eq.1 in which BD and TD were the powder bulk density and tapped density, respectively. The bulk density was determined by weighing the mass of a powder loaded into a 2 mL vial. The vial was then tapped in a flat shaker (HS250 Janke & Kunkel, IKA, Staufen, Germany) at 300 vibrations per minute for 3 h to ensure that no more change in the volume of the powder occurred. The lower the CI, the more flowing is the powder.

$$CI(\%) = (TD - BD)/TD \times 100 \quad \text{Eq.1}$$

The **moisture content** of SBE was evaluated by monitoring the loss of mass of a sample upon heating at 105°C in an oven (Dryline DL53, VWR, Fontenay-sous-Bois, France) until a constant weight (~24 h).

The **total lipids content** of SBE before and after the extraction treatments was measured gravimetrically by using an extraction with chloroform:methanol according to a protocol derived from the Folch method [21]. Briefly, 1.5 g SBE were extracted for 1 h with 30 mL of chloroform:methanol (2:1) supplemented with antioxidant (BHT), then washed three times with 6 mL of chloroform:methanol:potassium chloride solution 0.8% (3:48:47); the aqueous phase was removed after each washing step. The organic phase was separated from the solid phase by filtration and evaporated under vacuum, then dissolved in chloroform and filtered. Finally, chloroform was evaporated by a gentle nitrogen flux and the recovered oil was weighed ($M_{\text{ext-Folch}}$). The total lipid content, abbreviated as 'TLC%', was calculated as:

$$\text{TLC\%} = (M_{\text{ext-Folch}} / 1.5g_{\text{SBE}}) * 100 \quad \text{Eq.2}$$

The Folch extract of the unprocessed SBE is considered hereafter as the reference sample that describes the initial state of the SBEs.

The **neutral lipids content** of SBE was determined by extracting 10 g of SBE with hexane in a Soxhlet system (Soxtex system HT2 Tecator, Foss, Nanterre, France). The cartridge containing the SBE was first immersed in boiling hexane for 1 h, then the condensed solvent percolated through the cartridge for an additional hour. Hexane was then removed from the extract by evaporation and the mass of the recovered oil was weighed ($M_{\text{ext-SoxHex}}$). The content, abbreviated as 'NLC %', was calculated as:

$$\text{NLC\%} = (M_{\text{ext-SoxHex}} / 10g_{\text{SBE}}) * 100 \quad \text{Eq.3}$$

2.3. Extraction of SBE by *scCO*₂

Experiments were carried out in two home-made set-ups whose main difference came from the pump used for the CO₂ delivery (Lewa EcoFlow M210, Sartrouville, France), or Gilson 305 25SC+806 manometric module, Villiers-le-Bel, France). Reproducibility data did not discriminate which set-up was used, i.e. the effect of the set-up was included within the results deviation. The basic flow sheet comprised a cooling unit for CO₂, an extraction vessel of 98 cm³ (3 cm ID. x 14 cm L, TOP Industrie, Vaux le Penil, France) heated by electrical heating tape, a micrometering valve (Autoclave Engineers, Autoclave Maxitech, Rantigny, France) used as a restrictor at the exit of the circuit to regulate the pressure downstream and several shut-off valves (TOP Industrie, France), pressure transmitters (Delta Ohms, TC, Dardilly, France) and a safety release valve (TOP Industrie France). The supercritical CO₂ enriched with solutes passed through the heated micrometering valve and was subsequently expanded to ambient pressure in a cyclonic separator (height of 25 cm, diameter of 4.6 cm at its largest dimension) with a graduated tube attached at its bottom. The CO₂ was not recycled in the set-ups so the extraction proceeded with fresh CO₂ only. A gas volume totalizer at the separator exit allowed for determining the gaseous CO₂ flow rate that was converted in mass data via CO₂ molar volume and molar mass [22]. Unless indicated, the CO₂ mass flow rate was 0.72 ± 0.05 kg/h. The extraction vessel was loaded in standard conditions with 40 g of SBE and the CO₂ flow was from bottom to top, flushing the dissolved compounds out of a vessel held vertically. Preliminary experiments were carried out at a lower flow rate of 0.50 kg/h using 40g or 59g of SBE. The SBE sample was placed at the bottom of the vessel and the remaining volume was filled with glass beads of 2 mm in diameter. The sample was set onto a porous frit overtopped by a 0.2 μm membrane (Fluoropore™ Millipore, Merck, Fontenay sous Bois, France) and a similar filter plus membrane arrangement was placed at the vessel exit. The vessel was loaded the day before the experiment and was kept overnight at room temperature under 1.5 MPa of CO₂ after passing a gentle flux of CO₂ for several min as a precaution against sample degradation. On the day of experiment, the vessel was heated to the desired value (45°C) before introducing CO₂ up to the desired

pressure (23 MPa) and maintaining the flux for 6 h. The vessel was then gently depressurized and the treated SBE was collected and stored at 4°C. A significant change of the bed volume was observed as a void volume appeared at the top when opening the vessel. This was attributed to the large change in the particle size distribution after the CO₂ de-oiling treatment as discussed later.

2.4. Extraction by pressurized ethanol and by CO₂+Ethanol

The material obtained after the CO₂ step was then extracted using a more polar fluid. Experiments were carried out in two home-made set-ups on 5 g of SBE de-oiled by CO₂. The first set-up was the one used previously for de-oiling SBE by neat CO₂, using the Gilson pump for CO₂ (model Gilson 305 25SC+806 manometric module) and adding a second Gilson pump (model 305 5SC + manometric module) for the ethanol addition. The second set-up [23] was equipped with a Gilson analytical pump (model 305 10SC + 805 manometric module) for ethanol addition, an ISCO 260 D syringe pump for CO₂ addition, an oven (Binder, Tuttlingen, Germany) in which the extraction vessel of 10 mL was placed (TOP Industrie, Vaux le Penil, France, 1.2 cm i.d. x 9 cm L.), a micrometering valve (Autoclave Engineers, Autoclave Maxitech, Rantigny, France) used as restrictor at the exit of the circuit to regulate the pressure downstream, shut-off valves (TOP Industrie, France), pressure transmitters (Delta Ohms, TC, Dardilly, France) and a safety release valve (TOP Industrie, France). The extraction vessel, fully filled with the 5 g of solid sample, was held vertically and the solvent flowed from bottom to top. For extractions carried out with a CO₂ - EtOH mixture, the set-up was first pressurized and stabilized at the desired temperature and pressure with pure CO₂ before introducing ethanol. The composition of the extracting fluid was modified by varying mostly the ethanol flow rate and to a smaller extent, the CO₂ flow rate as well in order to minimize the increase of the overall flow rate. The overall flow rate was 3.7 ± 0.7 g/min. The extraction was carried out for 60 min before stopping the ethanol addition and pumping pure CO₂ for another 50 min to flush ethanol out of the circuit. The investigated conditions are discussed in section 2.5.

For experiments carried out with pressurized ethanol, the set-up and the extraction vessel were first pressurized with CO₂ at 4-5 MPa. Considering that the solvating ability of CO₂ is weak at this pressure level and that the most lipophilic compounds have already been extracted by the first scCO₂ step at 23 MPa, it was assumed that this pre-pressurization by CO₂ had no contribution to the extraction efficiency. Ethanol was then delivered at a flow rate of 1.48 ± 0.02 mL/min. Conditions for pressurized ethanol extraction were 60 and 36°C \pm 1°C and 6.1 ± 0.1 MPa and 60 min of dynamic extraction. At the end of the extraction, the CO₂ pump was activated to flush ethanol out of the circuit by flowing CO₂ during 50 min at 18 MPa.

After depressurization, SBEs were collected and stored at 4°C. For extractions carried out with a concentration of ethanol equal or higher than 22%, fractions were collected during the extraction (labelled as F1 F2 F3 as the extraction proceeded, corresponding to collection times of 15 min, 15 min and 30 min respectively) whereas a single fraction was collected for experiments carried out at 11% of ethanol. Ethanol extracts were evaporated under vacuum, weighed and re-dissolved in hexane:ethanol 1:1 v:v for their transfer into small vials. Samples were stored at 4°C before analysis.

2.5. Experimental design of extraction by pressurized ethanol and by CO₂+Ethanol

The experimental design of extractions performed in various conditions of pressure, ethanol content and temperature on CO₂-deoiled SBE is shown in Fig. 1. The design was fully investigated for rapeseed SBE and only few extractions were carried out for sunflower SBE for comparison purposes. A central design was first planned with a central spot at 47°C, 12 MPa and 22 wt% ethanol to capture non-linear effects, if any.

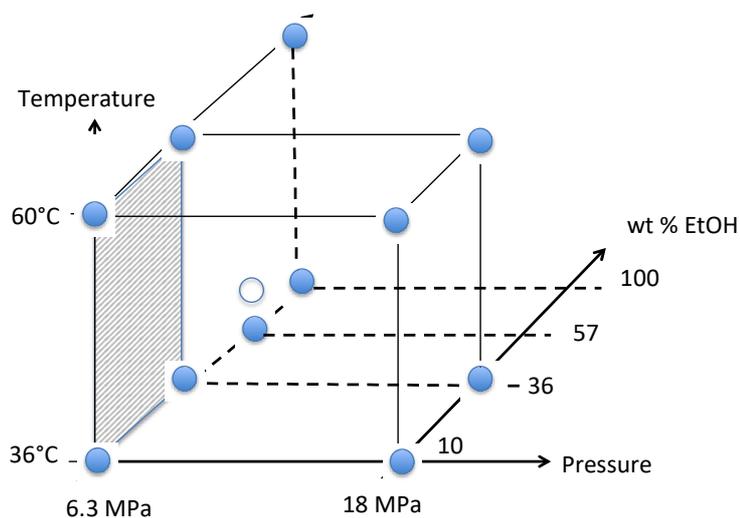


Fig. 1. Experimental design of extractions performed with various combinations of CO₂ and ethanol on de-oiled rapeseed SBE. The dashed area corresponds to conditions of two-phase mixtures.

The choice of conditions was driven by the objectives of limiting the working pressures and temperatures to reduce the energy consumption and exploring conditions that enabled to describe the effect of increasing ethanol concentration over a large range. Moreover, safety concerns of manipulating highly pressurized and heated ethanol and/or finely divided and flowing solids under high pressure were also considered so the maximum pressure was set at 18 MPa. The highest investigated temperature of 60°C was selected by considering the 70°C temperature limit of the oven housing the devices. In addition, the regeneration of spent bleaching clays by solvents was improved by an increased temperature with a maximum reached between 50 and 70°C though the clays did not come from vegetable oil refinement but from lubricating oil refining [24]. The addition of cosolvent as ethanol to the nonpolar CO₂ is a common strategy to extract polar species though percentage of co-solvent is usually set below 20% [25]. In this work, the ethanol percentage reached 57wt% in order to increase significantly the polarity of the extracting mixture, a modification of polarity that was efficient for deoiling SBE by solvent extraction [12] or that enabled the extraction of polar lipids, such as phospholipids [26]. On other hand, extractions by pressurized liquids (PLE) are usually carried out at temperatures above 50°C and under 5 MPa of pressure or more, with a general assumption that PLE conditions provide enhanced mass-transfer rate, increased solubility of the analytes and decreased solvent viscosity and surface tension [27]. Applied to oil extraction from seeds, pressurized ethanol was used in the

range of 30-90°C at 10 - 20 MPa, and it was found that pressure exerted only a little effect on the yield whereas higher temperatures promoted higher yields [29].

The response surface methodology (RSM) was used to explore the relationship between the operating variables and the responses. Pressure, temperature and ethanol percentage were used as input factors. The extracted mass normalized to the loaded amount and the percentages of each lipid class (as defined in section 2.8) were the considered responses. For the statistical analysis, each run should give a unique data. Hence, for experiments in which three fractions were collected, a unique extract was built by sampling aliquots of each fraction according to their mass contribution. The lipid analysis was carried out on that combined extract. The analysis of variance (ANOVA) was used to evaluate the significance of factors at a 95% confidence level. Multiple regression was used to calculate the fitting model and response surface. The statistical analysis was carried out using R freeware through the RStudio interface. Over the twelve explored conditions, five were duplicated for reproducibility and they were specified as 'n=2' in the relevant data Tables.

2.6. Soxhlet extraction with ethanol

For comparison purposes, SBEs were extracted with ethanol (96%) in soxhlet following the same protocol as described in § 2.2 for hexane soxhlet extraction.

2.7. Extraction yield and de-oiling efficiency

The extraction yield is defined as the extracted amount per mass of processed SBE:

$$Y \% = 100 * M_{\text{ext}} / M_{\text{SBE}} \quad \text{Eq.4}$$

The de-oiling efficiency represents the extraction yield of lipids, either for the total or the neutral lipids. It was calculated from the lipid content of SBE before and after the treatment and from the mass of SBE loaded and recovered after the treatment.

Efficiency for total lipids (TL)

$$E_{\text{TL}} (\%) = 100x (M_{\text{SBE}} * \text{TLC}_{\text{SBE}} - M_{\text{SBE after}} * \text{TLC}_{\text{SBE after}}) / (M_{\text{SBE}} * \text{TLC}_{\text{SBE}}) \quad \text{Eq.5}$$

Efficiency for neutral lipids (NL)

$$E_{\text{NL}} (\%) = 100x (M_{\text{SBE}} * \text{NLC}_{\text{SBE}} - M_{\text{SBE after}} * \text{NLC}_{\text{SBE after}}) / (M_{\text{SBE}} * \text{NLC}_{\text{SBE}}) \quad \text{Eq.6}$$

where M_{SBE} the mass of the SBE loaded in the vessel for extraction, $M_{\text{SBE after}}$ the mass of SBE recovered after the treatment, and TLC and NLC the initial content of SBE in total lipids and neutral lipids, before (subscript SBE) and after (subscript SBE after) the treatment.

2.8. Characterization of extracts

The **fatty acid composition** of oily extracts (Folch-, CO₂-, Hexane Soxhlet-, Ethanol soxhlet- extracts) was obtained by gas chromatography after transmethylation. Transmethylation was carried out in the presence of boron trifluoride-methanol complex [29]. The resulting fatty acid methyl esters were subjected to gas chromatography (GC) on a BPX 70 capillary column (60 m length, 0.25 μm film thickness, 0.25 mm i.d., SGE, nitrogen as carrier gas, split ratio of 1:80). The GC system consisted of a gas chromatograph (GC 2010 plus, Shimadzu France, Champs-sur-Marne, France) equipped with a flame ionization detector maintained at 280°C. The injector was set at 250°C. The column temperature was programmed from 150°C to 200°C (1.3°C/min) held for 30 min, from 200°C to 225°C (20°C/min), held for 30 min. Data were collected and integrated by a GC solution v2.4 integration system (Shimadzu). Fatty acids from Sigma-Aldrich France were used as standards for calibration.

Samples were characterized for their lipids classes (triacylglycerides TAG, diacylglycerides DAG, free fatty acids, FFA, polymerized species) and tocopherols.

For **lipid classes**, extracts were analysed by high-performance size-exclusion chromatography with refractive-index detection (HPSEC-RI) to evaluate the level of degradation following IUPAC standard method 2.508 [30]. Thus, polymerized triacylglycerols, i.e. dimers plus oligomers, were determined as compounds of advanced oxidative degradation. The extracts were dissolved in tetrahydrofuran at a concentration of 50 mg/mL and directly analyzed by HPSEC-RI. The chromatograph was equipped with an AT 1100 autosampler (Agilent Technologies, Palo Alto, CA, USA), a Knauer 120 HPLC pump (Knauer, Berlin, Germany), a PerkinElmer 200 Peltier column oven (PerkinElmer, Waltham, USA) set at 25 °C and a Merck L-7490 refractive index detector (Merck, Darmstadt, Germany). A volume of 10 μL of the extract solutions was analyzed. The separation was performed on two 100 and 500 Å Ultrastaygel columns (25 cm x 0.77 cm i.d., 5 μm particle size) packed with porous, highly cross-linked styrene-divinylbenzene copolymers (Agilent Technologies, Palo Alto, CA, USA) connected in series. Tetrahydrofuran at a flow rate of 1 mL/min was the mobile phase. The relative concentrations of the different components were estimated considering the same response factor for all the analytes.

Tocopherols were determined by HPLC with fluorescence detection following IUPAC standard method 2.411 [31]. The extracts were dissolved in n-heptane at a concentration of 50 mg/mL and directly analysed in an Agilent 1200 Infinity HPLC chromatograph (Agilent Technologies, Palo Alto, CA, USA). The chromatograph was equipped with a degasser (G1322A), a quaternary pump (G1311A), a standard autosampler (G1329A), a thermostated column compartment (TCC) (G1316A) and a fluorescence detector (FLD) (G1321A). A silica HPLC column (LiChrospher® Si 60, 250 mm x 4 mm i.d., 5 μm particle size)

(Merck, Darmstadt, Germany) was used. The volume of sample analysed was 20 μL . The temperature was set at 25 $^{\circ}\text{C}$. The separation of analytes was performed using isocratic elution with n-heptane:isopropanol (99:1, v/v) at a flow rate of 1 mL/min. The excitation and emission wavelengths were 290 and 330 nm, respectively.

Total carotenoids and chlorophyll/pheophytins contents of oily samples were evaluated by UV-Vis spectroscopy, whereas ethanol extracts were analysed by liquid chromatography. For UV spectroscopy, oily samples were dissolved in hexane:ethanol (1:1 v:v). UV-Vis spectra were recorded from 200 to 1000 nm (UH5300, Hitachi High Technologies Europe, Velizy, France) to identify the specific wavelengths for carotenoids and chlorophylls. The absorbances at 470 nm (maximal absorbance of β -carotene), 660 nm (maximal absorbance of chlorophyll a) and 642 nm (maximal absorbance of chlorophyll b) were used to quantify the total carotenoids content (expressed as β -carotene equivalent) and chlorophyll a and b contents, respectively. Calculations were done according to Lichtenthaler and Buschmann [32] from calibration curves using β -carotene, chlorophyll a and chlorophyll b solutions in hexane : ethanol (1:1).

For ethanolic extracts, liquid chromatography was used to better discriminate the pigments. Quantitative analyses were performed by reverse phase chromatography after dilution in the mobile phase and filtration of samples through 0.45 μm PVDF syringe filters (Pall France, Saint Germain en Laye, France). An Agilent 1200 system (Agilent Technologies, Les Ulis, France) equipped with a diode array detector (DAD, G1315A), an autosampler (G1329A) and a Chemstation software for data acquisition was used under the following conditions: YMC C30 column (250 x 4.6 mm ID, particle size 5 μm) coupled with a 10 x 4.0 mm ID, particle size 5 μm guard cartridge column (ImChem, Versailles, France), temperature of 25 $^{\circ}\text{C}$, flow rate of 1.0 mL/min, mobile phase consisting in a gradient of MeOH containing 50 mM of ammonium acetate (A), and methyl *tert*-butyl methyl ether (B), both solvents containing 0.1% BHT (w/v) and 0.05% of triethylamine (w/v); linear gradient from 5% B to 30% B in 30 min then to 50% B in 20 min, then back to 5% B in 0.01 min and equilibration for 10 min at the starting conditions. Chromatograms were recorded at four different wavelengths, 410 nm (pheophytin a), 439 nm (pheophytin b), 451 nm (β -carotene) and 663 nm (chlorophyll a) and UV-vis spectra were recorded in the range of 200-700 nm. The quantification was carried out using calibration curves built with standards. For pheophytins that are chlorophylls devoid of the central Mg^{2+} ion, standard solutions of pheophytin *a* and *b* were prepared by degrading chlorophyll a and b standards by addition of 1M HCl [33]. The identification of pigments in extracts was performed by comparison with the standards retention time and from their spectral characteristics as λ_{max} and peak ratios (absorbance at the maxima Soret band I, bands II, III and V) [34, 35]. Four of the produced samples were analysed twice to three times to assess the analysis reproducibility. The average deviations of peaks areas were 5% and 7% for pheophytin a and b respectively.

3. Results and discussion

3.1. First extraction step of SBE by $scCO_2$

3.1.1. Macroscopic changes and kinetic data

Characteristics of SBEs before and after the CO_2 treatment are summarized in Table 1, whereas Fig.2 shows pictures of the materials before and after the treatment and of the collected oil.



Fig.2. Sunflower and Rapeseed SBE before (left) and after (middle) the CO_2 treatment, and oils collected by the CO_2 treatment (right).

Initial sunflower SBE was a lumpy material black in colour because of the presence of active carbon mixed with the Tonsil and its particle size distribution ranged between 200 and 500 μm . Rapeseed SBE that contained only Tonsil was more brown with particles of 100- 200 μm . None of these materials was free flowing, but rapeseed SBE with a Carr Index of 33% was more flowing.

Table 1. Characteristics of SBE before and after CO_2 extraction (n=4) at 23 MPa, 45°C, $F_{CO_2} = 0.720 \pm 0.01$ kg/h; loading: 40.2 ± 0.3 g.

	Sunflower SBE initial	Sunflower SBE CO_2 -treated	Rapeseed SBE initial	Rapeseed SBE CO_2 -treated
Aspect	Black, greasy, lumpy	Grey, dry, flowing powder	Brown, greasy, lumpy	beige , dry, flowing powder
Size range	200 - 500 μm	0 – 100 μm	100 – 200 μm	0 – 100 μm
D50 diameter	-	33 μm	-	31 μm
Carr Index	66%	26%	33%	23%
TLC %	30 ± 0.3 %	11.3 ± 1.2 %	27.2 ± 0.5 %	12.5 ± 0.2 %
NLC %	28 ± 0.5 %	3.5 ± 0.5 %	22 ± 1 %	1 %
Moisture	4.3 %	2.1 %	4.4 %	1.8 %
F/S g/g	-	98 ± 9	-	83 ± 7
E_{TL} %	-	72 ± 2.5 %	-	66 ± 0.6 %
E_{NL} %	-	91 ± 1 %	-	97 %
Y %	-	21.4 ± 0.21 %	-	19.6 ± 0.36 %

F/S is the amount of CO₂ used per mass of SBE loaded; TLC%, NLC%, E_{TL}% and E_{NL}% are defined by Eq2, Eq3, Eq.5, Eq6, respectively; Y is the extraction yield (Eq. 4); □□□ : diameter at which 50% of the sample's mass is made of particles with a diameter less than this value; it is calculated by $(\sum (\text{mean sieve size} * \text{retained wt}\%)) / 100$; results are given as mean ± standard deviation for 4 extraction experiments, except for data of neutral lipids where n=2.

After the CO₂ treatment, the SBE displayed significant macroscopic changes in terms of colour and flowing characteristics that approached those of native bleaching clays. Indeed, the virgin Tonsil, i.e. before its use in the oil refining plant, is a fine, beige powder whose particle size is below 100µm and has a Carr Index of 22%. The better flowability of SBE after the CO₂ treatment (Carr Index below 26%) came from the smaller sizes of the particles that were now of 100 µm and below. More specifically, 83% and 87% (wt%) of the SBE particles presented sizes below 50 µm for sunflower and rapeseed SBE, respectively. The large decrease of particle size reflected the disintegration of the oil-bearing agglomerates that were composing the spent bleaching materials removed from the industrial units. As it will be shown later, CO₂ extracted almost all the oil from the SBE. It therefore left behind in the vessel only the solid particulate matter of the clumps that was hence recovered as a fine powder. It is worth noting that to authors' knowledge, this is the first reported example of a so marked modification of particle size during an extraction process.

The extraction courses of both SBE, expressed as volume collected as function of time, are given in Fig. 3. The total collected mass was 7.5 ± 0.4g and 8.1± 0.13g for extracts obtained from rapeseed SBE and sunflower SBE, respectively.

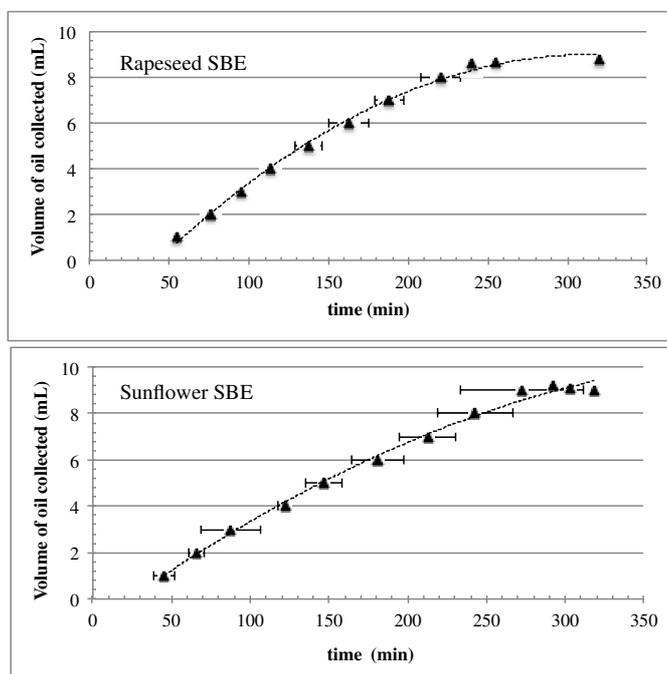


Fig. 3. Extraction kinetics of Rapeseed and Sunflower SBE by CO₂ at 45°C, 23 MPa. SBE amount = 40g. Error bars correspond to standard deviation of four extractions. Dotted lines are for eyes only.

Deviations were larger for sunflower than for rapeseed SBE and this might come from the difficulties at reproducing the loading of the bed with more poly-dispersed and less flowing particles and from the larger

change of particle size experienced by sunflower SBE during the extraction. Both causes can impact the kinetics of extraction since the whole bed volume and the inter-particles pathways would change during the extraction course. Nevertheless, the overall extraction profiles were characterized by a period in which the extracted volumes varied almost linearly up to about 200 min corresponding to 2.3 kg of CO₂ for rapeseed SBE and 250-300 min (3-3.2 kg of CO₂) for sunflower. The extraction rate then slowed down because solutes must diffuse from the deeper zones of the material to the surface [15, 36,37]. Waldman and Eggers [15] also observed a linear shape of extraction curves when de-oiling clays containing rapeseed oil, with about 75% of the extractable matter collected during the constant rate period. The quantity of CO₂ used to get this 75% level of extraction was much lower than the amount used here (90 g of CO₂/g extractable matter in [15] versus 420 g_{CO₂}/g_{extract} in our case), but pressure, temperature and flow rate were higher (35 MPa, 80°C, 4 kg/h, respectively). The linear first stage of extraction is generally associated to the extraction of free solutes from the surface of particles and is therefore attributed to solubility [38-42]. When expressed in g per kg of fluid, the loadings of CO₂ were 3.4 g oil /kg CO₂ (R² = 0.995) for rapeseed SBE and 2.5 ± 0.4 g oil /kg CO₂ (R² = 0.998) for sunflower SBE. The loadings were slightly lower than those obtained in case of oil extraction from oleaginous seeds for which values of 5.5 g_{oil}/kg_{CO₂} and 3.0 g_{oil}/kg_{CO₂} were reported for canola oil extracted at 40°C/25 MPa [37] and for sunflower oil extracted at 40°C/20 MPa [39], respectively. The loadings were also lower than the solubility predicted by del Valle' model [43] (4.39 g/kgCO₂, accuracy of the model ± 40%). However, characteristics of bed and materials, and flow rate contribute to the external mass transfer and consequently to the extracted amount versus time [41]. The interactions of solutes with the sorbent also bring about an additional complexity like partition equilibrium that contributes to the desorption behaviour [41, 44-47]. For bleaching earths, there are a multitude of different physical and chemical mechanisms such as adsorption, ion-exchange, acidity and complexation that contribute to the overall sorption of components from the oils [1, 48]. The diversity of the solutes retained by the bleaching clays, their concentrations, and their various possible interactions might render the mechanism of SBE extraction more complex than extraction of oil from seeds. According to the nature of lipids left in the SBE after the CO₂ treatment (Table 1), the CO₂ mainly extracted the neutral lipids. The extraction of neutral lipids was almost completed for rapeseed (residual neutral lipid content of 1%), but was only 91% for sunflower (residual content of 3.5%) although a higher mass of CO₂ per g of SBE was used in that case. Though larger particle size could have contributed to the slower rate, the material itself was different since sunflower SBE contained active carbon in addition to Tonsil, a material of high sorption capacity that could induce a different desorption behaviour than Tonsil alone.

Two experiments were initially performed on sunflower SBE before finding the suitable conditions of the reproducibility runs. Experiments were carried out at lower CO₂ flow rate and higher mass of loaded SBE. The effects on the extraction kinetics are shown in Fig 4. The two curves at 0.72 kg/h delimited the deviations observed at this flow rate (same data as in Fig. 3).

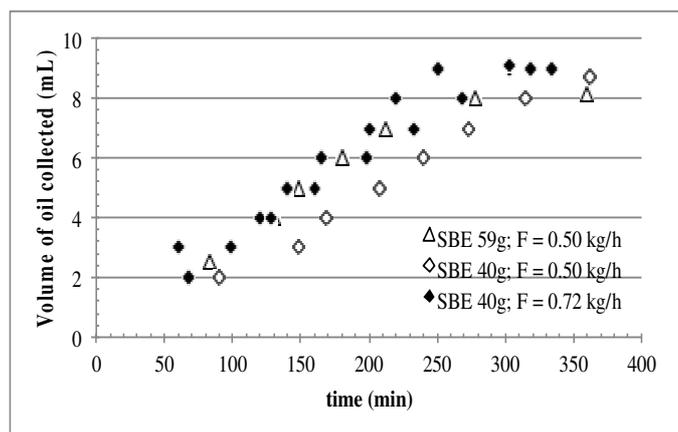


Fig. 4. Effects of CO₂ flow rate and loaded mass of sunflower SBE on the extraction course, expressed as collected volume as a function of time. T = 45°C, P= 23 MPa.

When the CO₂ flow rate was 0.5 kg/h instead of 0.72 kg/h (diamond marks in Fig. 4), the extraction was slower with less oil extracted per unit of time. However, the difference came mostly from the initial period since after 120min, the slope of the constant rate period was in the same range as the slope at 0.72 kg/h. The flow rate can influence extraction kinetics in several aspects: a lower flow rate increases the external film resistance and by consequence slows down the rate at which solute molecules enter the bulk flowing fluid and are carried out of the bed or it can decrease the dissolution of the solutes since the number of CO₂ molecules per unit volume is decreased [38,41,49,50]. On other hand, a lower flow rate increases the contact time between CO₂ and the solutes giving a higher chance to reach the solubility limit, so a higher concentration in the fluid could be expected. Here, those effects offset each other for most of the extraction duration. The linear part of the extraction curve at 0.5 kg/h lasted longer than at 0.72 kg/h (~ 310 min), indicating that free solutes were still to be extracted when the experiment was stopped. When the amount of SBE loaded was increased to 59 g (open triangles in Fig.4), the volume of oil extracted per unit of time increased, indicating that CO₂ was not totally saturated when 40 g of SBE was processed. With a longer bed, the CO₂ had more opportunities to be charged with solutes during its progression to the extractor exit provided it was not saturated earlier. The examination of the bed after the two treatments revealed that the bed was not totally de-oiled over its full height. In the experiment carried out at 0.5 kg/CO₂ - 40g of SBE, about 1 cm of the loaded SBE bed, corresponding to 12% of total mass of recovered SBE, was still black and had the same total lipids content as the loaded SBE, whereas the remaining 88% displayed the same characteristics as the SBE recovered in experiments at 0.72kg/h. For the experiment carried out with 59 g of loaded SBE, the ‘not de-oiled’ portion approached 38% (height of about 4 cm). For both experiments, the ‘not de-oiled’ parts were located at the vessel exit for a CO₂ flowing from the bottom to the top of the vessel. This can be understood by considering that the driving force for extraction is the difference of solute concentration between the fluid and the solid phases, which is the biggest at the entrance of the vessel where the fluid is fresh. Based on these results, it was decided to limit the amount of SBE to 40 g and to increase the flow rate at 0.72 kg/h for 6 h minimum to obtain the de-oiling of the whole bed height.

3.1.2. Composition of oils extracted by CO₂

The fatty acid composition of the oils extracted by CO₂ is reported in Table 2. They are compared to the composition of the oils initially contained in SBE (i.e. oils recovered by extracting SBE with hexane in a soxhlet).

Table 2. Main fatty acids of initial SBE (Hexane extraction) and of oils recovered by CO₂ extraction (45°C, 23 MPa, F_{CO₂} = 0.720 kg/h; loading: 40 g).

	C16:0	C18:0	C18:1	C18:2	C18:3	C20:1	others
Rapeseed SBE initial (hexane extract)	5.7 ± 0.3	1.8 ± 0.1	72.7 ± 0.2	5.4 ± 1.4	< 1	1.1 ± 0	11.1 ± 1.1
CO ₂ -extracted oil from Rapeseed SBE	4.6 ± 0.3	1.4 ± 0.1	66.7 ± 3.2	16.1 ± 2.7	5.5 ± 2.1	< 1	4.0 ± 1.1
Sunflower SBE initial (hexane extract)	9.1	4.4	23.7	41.8	0	1.2	16.7
CO ₂ -extracted oil from Sunflower SBE	7.3 ± 0.1	3.2 ± 0	28.7 ± 1.0	56.4 ± 1.6	0	0	3.4 ± 0.3

Others corresponds to fatty acids accounting for less than 1% or unidentified species. Results are given as mean ± standard deviation (*n*=2).

The crude oils in the native seeds are characterized by a high level of C18:1 for rapeseed oil [51] and C18:1 and C18:2 for sunflower oil [52]. The oils trapped in the bleaching earths exhibited similar fatty acid compositions to the corresponding processed oils since a predominance of C18:1 and of C18:1 and C18:2 was found in the hexane extracts of rapeseed and sunflower SBEs, respectively. The extraction by CO₂ induced some selectivity towards the lipid composition. Indeed, the CO₂ extracted oils had a richer composition in unsaturated C18 acids than hexane extracts, with values of 88% and 85% for rapeseed and sunflower CO₂ extracts, respectively, compared to 78% and 65% for rapeseed and sunflower hexane extracts. The enrichment came mostly from the depletion in *Others* species that accounted only for 4% in the CO₂ extracts.

The level of oxidative degradation was assessed by the analysis of dimers and oligomers (Table 3). Along with the polymerized triacylglycerols, the direct analysis of the oils by HPSEC also enabled the determination of other lipid classes such as TAG, and free fatty acids. At the applied analytical conditions, the TAG peak included TAG, oxidized TAG and diacylglycerols (DAG), the latter appearing as a shoulder. The Folch samples from Rapeseed and Sunflower SBEs were indicators of the SBE state before the CO₂ treatment.

Table 3. Analysis of polymers and lipids classes in oils produced by neat CO₂ and in the SBE before the CO₂ treatment (HPSEC method).

	Oligomers %	Dimers %	TAG+DAG %*	FFA %**
Rapeseed SBE initial (Folch extract)	4.6	5.4	83.2	6.9
CO ₂ -extracted oil from rapeseed SBE	0.05 ± 0.05	0.85 ± 0.5	96.9 ± 0.1	2.15 ± 0.15
Sunflower SBE initial (Folch extract)	4.4	5.1	86.5	2.3
CO ₂ -extracted oil from sunflower SBE	0.5 ± 0.1	3.85 ± 0.35	94.1 ± 0.6	1.55 ± 0.15

*group of compounds formed by TAG and DAG; **group of compounds formed by FFA and compounds of lower molecular weight than FFA; Results are given as mean ± standard deviation of 2 extraction experiments ($n=2$).

In the initial SBEs, polymerized species (dimers plus oligomers) represented about 10% of the total lipids. In contrast, the oils produced by CO₂ were depleted in such degradation products, which accounted for less than 1% in the rapeseed extract and below 5% for sunflower extract. The depletion was also noticeable for the class of lower molecular weight compounds (FFA) so the recovered oils appeared to be purer in {TAG+DAG}, a class that accounted for more than 93%. DAG, eluted as a shoulder of the peak, was less than 2% in the oil. The proposed step of extraction by CO₂ thus allowed to recover the TAG and left the polymerized species on the sorbent.

Regarding minor components, tocopherols were detected in the recovered rapeseed and sunflower oils, at levels of 6-10 ppm for α -Toc and 2-5 ppm for γ -Toc. The presence of chlorophylls or more likely the pheophytins (their degradation products) and carotenoids or equivalent was evidenced in the CO₂-produced oils at levels below 1-2 mg/kg oil (Table 4).

Table 4. Colour pigments in processed SBE and recovered oils (spectrophotometric method). Values expressed as equivalents of β -carotene, chlorophyll-a, chlorophyll-b.

	Chlorophyll –a mg/kg oil	Chlorophyll –b mg/kg oil	Carotenoids mg. /kg oil
Rapeseed SBE initial (Folch extract)	25	12	2
CO ₂ -extracted oil from Rapeseed SBE	0.3 ± 0.4	1.7 ± 2.5	0.5 ± 0.2
Sunflower SBE initial (Folch extract)	2.6 ± 0.9	12 ± 5	0.9 ± 0.2
CO ₂ -extracted oil from Sunflower SBE	0.1 ± 0.1	0.8 ± 0.7	0.4 ± 0.2

To summarize, the treatment of SBE with pure CO₂ produced oils that contained TAG as predominant lipids with less than 5% of polymerized lipids. Tocopherols and colouring compounds were present in small concentration so the added value of the recovered oil for food-grade applications might be questionable. The product could be better valorised for industrial use or as a source of non-petrochemical synthons for chemistry, especially owing to the enrichment in unsaturated species. Conversely, the CO₂ step generated a partly de-oiled residue that still contained polar and polymerized lipids and coloured pigments that can be recovered by a second treatment.

3.2. Extraction of de-oiled SBE by pressurized EtOH and CO₂+EtOH mixtures

3.2.1. Extraction of de-oiled rapeseed and sunflower SBE under selected conditions and comparison with soxhlet extraction using ethanol

The second stage of SBE extraction was first carried out at 60°C using ethanol as a co-solvent with CO₂ (EtOH content of 11 wt%) at 18 MPa or using neat ethanol pressurized at 6.1 MPa. Ethanol was selected because of its ability to enhance the solubility of polar species when mixed with CO₂ [26, 53,54] or to extract oils [55]. Pure ethanol under pressure is also being more and more investigated as a GRAS solvent for extracting not only polar species [19,20] but also oil from seeds [28,56]. The extraction yield, total lipid content after the treatment and the lipid composition of the extracts are given in Table 5, whereas the kinetics of extraction by pressurized ethanol are displayed in Fig. 5.

Table 5. Second step of SBE extraction using 100% ethanol or CO₂ + ethanol (EtOH = 11wt%) as extracting fluid. SBE amount: 5g. Extraction time = 60 min.

Samples	TLC (%)	Yield (%)	fraction	Olig %	Dim %	TAG+DAG %*	FFA %**
<i>De-oiled Rapeseed SBE</i>							
Extraction CO ₂ - EtOH 60°C – 18 MPa	4.4	6.8	single	1.2	19.8	67.3	11.7
Extraction ethanol 60°C – 6.1 MPa	0.92 ± 0.15	12 ± 0.2	F1	22.7 ± 0.9	20.8 ± 0.2	50.5 ± 1.0	6.0 ± 0.2
			F2	36.1 ± 3.3	20.2 ± 1.2	40.5 ± 0.5	3.1 ± 1.6
			F3	50.2 ± 4.2	19.7 ± 0.2	27.7 ± 4.8	2.3 ± 0.3
Extraction ethanol Soxhlet	nd	10.5	single	30.1	18.0	44.8	7.1
<i>De-oiled Sunflower SBE</i>							
Extraction CO ₂ - EtOH 60°C – 18 MPa	5.7	5.1 ± 0.2	single	3.1 ± 3.1 ^a	21.2 ± 0.85	70.6 ± 1.8	5.05 ± 0.45
Extraction Ethanol 60°C – 6 MPa	1.6	8.4	F1	23.9	21.9	51.6	2.6
			F2	36.0	19.4	41.6	3.0
			F3	32.6	18.2	45.0	4.2
Extraction ethanol Soxhlet	nd	9.7	single	54.8	12.7	28.5	3.9

* group of compounds formed by TAG and unresolved DAG; ** group of compounds formed by free fatty acids and minor components; ^a: in one sample, oligomers were eluted as a shoulder of dimer group; nd: not analysed; TLC% and Y are given by Eq2 and Eq. 4, respectively. Data are mean ± standard deviation of 2 extraction experiments (n=2).

When ethanol was used as a co-solvent in CO₂ (i.e. at 60°C, 18 MPa), the extraction yields stood in the range of 5-7% depending on the SBE samples with a significant extraction of the lipids since the TLC of SBE was below 6% after the treatment (to be compared to 11% range of the SBE before this second extraction step) . The use of pure pressurized ethanol improved the de-oiling since the TLC decreased to 1.6% or below. The extraction kinetics showed that 78-82% of the total extracted matter was collected during the first 20 min (Fig. 5). In the CO₂-EtOH extractions, the fluid-to-SBE ratio was in the range of 50, a range that was larger than the 15 to 20 ratio used in the extractions with pressurized ethanol. However the extracted amounts were lower. The behaviour could come from a possible lower solubility of the polar species that constitute the main lipids of the SBE after the first CO₂ extraction in CO₂-11%Ethanol than in 100%Ethanol. Compared to a soxhlet extraction, pressurized ethanol gave similar efficiencies with an extraction yield better for rapeseed SBE but lower for sunflower SBE. The difference was about 2%, a range that was consistent with the yields reported for oil extraction from seeds when comparing pressurized ethanol and soxhlet extraction [28].

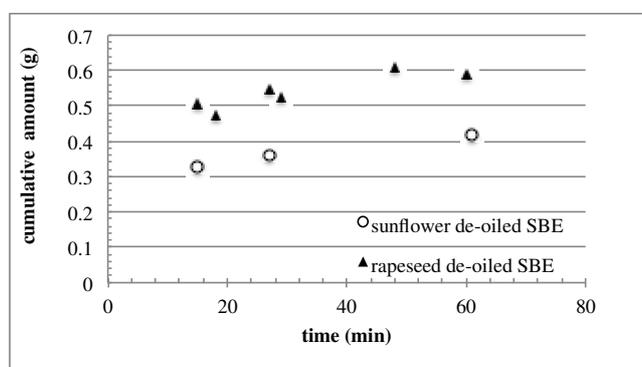


Fig. 5. Extraction kinetics of de-oiled rapeseed (n=2) and sunflower SBE by pressurized ethanol at 60°C, 6.1MPa. SBE amount = 5g.

The examination of the lipid classes gave an indication of the selectivity provided by the extracting fluids (Table 5). For both SBE, pressurized ethanol or soxhlet extraction gave extracts in which polymerized species accounted for more than 42% of the lipids, with a significant contribution of oligomers (above 22%), whereas the CO₂-ethanol mixture extracted oligomers to a much lower extent and was therefore more selective. The data of kinetics showed that the dimers contribution did not change notably as the extraction proceeded so the change of composition in the extracted fractions came mostly from the balance between oligomers and triglycerides. The increasing percentage of oligomers in fractions might come from the oligomers extraction from the matrix along with the SBE exhaustion in TAG.

3.2.2. Effect of temperature, pressure and fluid composition on the extraction of de-oiled rapeseed SBE

In order to widen the conditions and identify parameters responsible for the yield and extract composition, pressure, ethanol content and temperature were varied according to the experimental design shown in Fig. 1. It is relevant to be more specific about the extracting mixture since the various conditions led to different states of the CO₂-ethanol mixture according to the vapour-liquid equilibria (VLE). The P-T-x bubble point

curves, obtained from literature at temperatures close to the ones used in this work, are reported in Fig. 6 [57-59]. At 18 MPa, the extracting fluid is a supercritical $\{\text{ethanol} + \text{CO}_2\}$ mixture whatever the temperature and ethanol percentage, since the pressure of 18 MPa is far above the critical pressure of the CO_2 -ethanol mixture at the three considered isotherms [59]. At 6.3 MPa, the situation is more complex since the mixture can exist as a two-phase system in which a liquid and a vapour co-exist. In a VLE diagram, the composition of each phase is given by the abscissa of the bubble point curve for the liquid phase composition and of the dew point curve for the gaseous phase composition, at the considered pressure. The dew point curves, here constricted to the interval 0.95 - 0.99 molar in CO_2 for the considered isotherms, are not shown in Fig 6. In our experiments, when the percentage of ethanol increased from 10 to 36 wt%, the CO_2 mole fraction decreased accordingly, from A to B in Fig. 6. According to the VLE, the liquid phase is richer in CO_2 at 35°C than at 60°C since values close to 0.6 and 0.36 molar in CO_2 are obtained (labels X AB at 35°C and at 60°C in Fig.6), whereas the vapour phase is only of 0.02 molar in ethanol. When the extracting mixture is made of 57 wt% of ethanol (point C in Fig 6), the fluid is a monophasic system at 35°C since it is outside the VLE envelope, but in a two-phase condition for the 60°C experiment. Looking back to the experimental design provided in Fig. 1, conditions that correspond to a biphasic extracting fluid were hence located on the left side of the primary cube, as indicated by a shaded area.

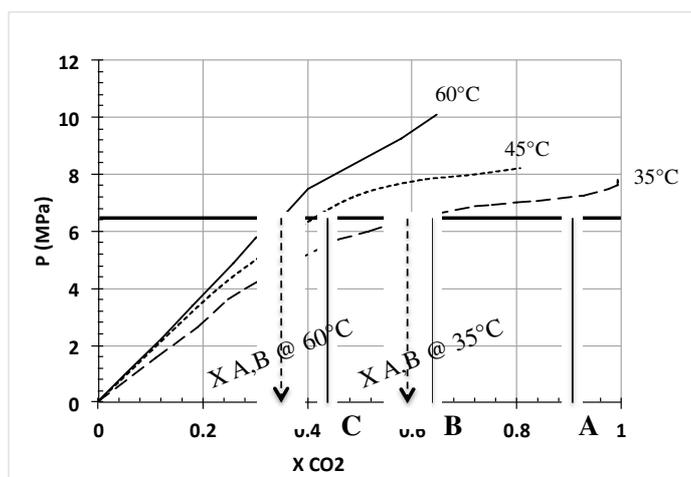


Fig. 6. Diagram P-T-x (bubble points) of the CO_2 -ethanol system. Data are from Tanaka and Kato at 35°C [57], Mehl et al at 45°C [58] and Secuianu et al at 60°C [59]. P: pressure, in MPa, T: temperature, in $^\circ\text{C}$, X_{CO_2} : composition in CO_2 of the liquid phase, in molar fraction. A, B and C illustrate the three overall compositions investigated in this work, corresponding to 10 wt%, 36 wt% and 57 wt% in ethanol, respectively. XA,B is the composition of the liquid phase at 6.4 MPa and 35°C or 60°C when the overall composition correspond to A or B.

The statistical analysis for yield has identified all factors as significant contributors and has detected also a significant interaction between pressure and ethanol percentage. The resulting equation, written in un-coded variables is given hereafter (Eq. 7; $R^2 = 0.73$) and a response surface, calculated for an extraction temperature

of 60°C is presented in Fig. 7a. The analysis performed on the lipid classes as response (Eq. 8) has identified pressure, ethanol percentage and Pressure-Ethanol interaction as significant contributors for oligomers and TAG+DAG percentage whereas none of the studied factors were significant for dimers. A response surface of oligomers is shown in Fig. 7b.

$$\text{Yield (\%)} = 10.98 + 0.06 * T - 0.51 * P - 0.10 * E + 0.02 * P * E \quad \text{Eq.7}$$

$$\text{Oligomers (\%)} = 40.20 - 2.61 * P - 0.40 * E + 0.07 * P * E \quad \text{Eq.8}$$

with T, temperature in °C, P pressure in MPa and E, ethanol wt%.

The models showed that high temperature promoted the extracted amount but had no effect on oligomer content of the extracts. At a given temperature and for an overall composition of 10 wt% in ethanol (e.g. 90 wt% CO₂), the increase of pressure decreased the yield and the oligomers content (Fig. 7), a trend that could be related to the progressive depletion of the liquid phase in ethanol according to the VLE (Fig.6). Indeed, as the extracting fluid moved from a biphasic system at low pressure, characterized by a high X_{etoh} content, to a monophasic supercritical phase at 18 MPa, the ethanol content of the extracting phase decreased. One can assume that the polarity of the fluid decreased accordingly so that a low yield and a low content of polymerized species were obtained. At higher overall percentage of ethanol, the negative effect of pressure became overridden by the positive effect of the P-E interaction so that the highest yield and the highest content of extract in oligomers would be obtained by pressurizing pure ethanol at 18 MPa. Data were also computed by considering the state of the CO₂-ethanol mixture, setting either biphasic / monophasic state as a qualitative factor or the estimated composition of the liquid phase. None of these factors provided a satisfactory regression of data. It is worth noting that a deep interpretation of the results not only relies on the accuracy of phase equilibrium data, but should also consider and elucidate the behaviour of a two-phase mixture upon contact with the matrix to be extracted.

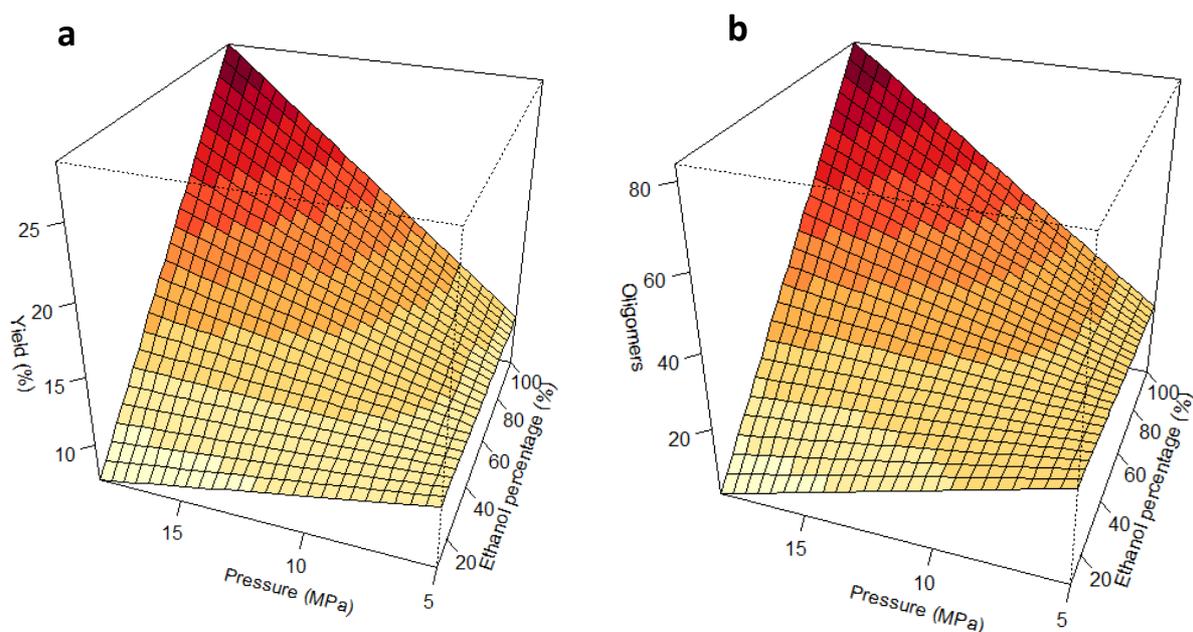


Fig. 7. Response surface plot of (a) extraction yield as a function of pressure and ethanol content (wt%) at 60°C, (b) oligomers content as function of pressure and ethanol content.

3.2.3. Effect of temperature, pressure and fluid composition on pigments extraction

Extracts obtained from de-oiled rapeseed SBE were analysed with priority owing to their more intense colour and to the fact that rapeseed/canola oils are commonly rich in chlorophylls and pheophytins (level of 5-30 ppm [1]). Pheophytins are moreover formed in acid media so the acid-activated clays can convert chlorophyll to pheophytins, which are subsequently adsorbed on the clay [1, 60]. In our extracts, pheophytins *-a* and *-b* were detected rather than chlorophylls. The chromatographic separation used for analysing the extracts produced at this step allowed for distinguishing pheophytins from chlorophylls, a distinction that was less obvious by the spectrophotometric method used when characterizing oils (Table 4). Few samples obtained from de-oiled sunflower SBE were analysed as well and it included the sample obtained by pressurized ethanol extraction at 6 MPa and 60°C. For these samples, the measured concentration was below the threshold limit of 5 mg/L, which led to the conclusion that either the pheophytin content of sunflower SBE was below 10 mg/kg_{de-oiled SBE}, or the binding between species and the clay was too strong to allow their extraction. For extracts obtained from rapeseed SBE, β -carotene was not detected whereas pheophytins were present in several extracts. The highest extracted amounts were obtained in the cases of extraction by pressurized ethanol (3.0 - 3.8 mg of total pheophytins from 5 g of CO₂- de-oiled SBE), which led to a SBE content of 600-760 mg/kg_{de-oiled SBE}. The kinetic of pheophytin extraction by pressurized ethanol at 36°C and 60°C is provided in Fig. 8. The samples obtained at 60°C are the same samples whose collected amount was given in Fig 5.

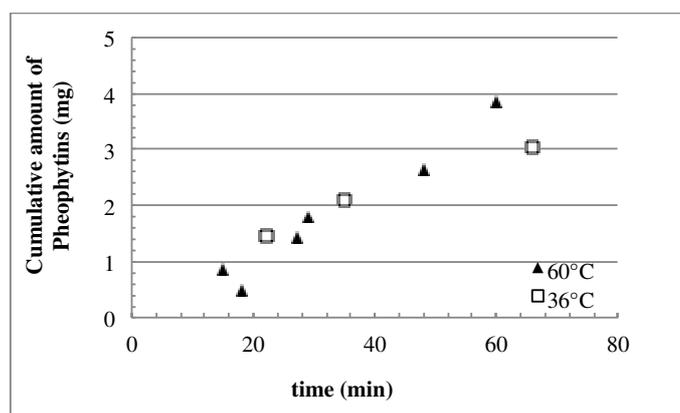


Fig. 8. Extraction kinetics of pheophytins by pressurized ethanol at 36°C and 60°C, 6.3 MPa from 5g of de-oiled rapeseed SBE.

Contrary to the collected amount, the extraction of pheophytins progressed regularly with time and was only slightly affected by temperature for most of the extraction duration. The highest concentration in pheophytin was thus obtained for the third collected fraction and was of 32 mg/g_{extract}. All other conditions of extraction led to lower extracted amounts of pheophytins, with even no extraction at 18 MPa, 10 wt% EtOH, 36°C and 60°C. This result was consistent with the low concentration of colouring pigments of the oils obtained during the first extraction step by pure CO₂ and therefore confirmed the poor solubilizing or desorbing strength of CO₂ for these species. The statistical analysis of samples obtained under conditions of the Design Of Experiments given in Fig 1 identified the ethanol percentage as the sole factor influencing the extraction of pheophytins (Eq. 9, R² = 0.93).

$$\text{Pheophytins extracted amount (mg per g of de-oiled SBE)} = 6.3 \cdot 10^{-5} \cdot E^2 \quad \text{Eq.9}$$

with E, ethanol in wt%.

4. Conclusions

In this work, two spent bleaching earths coming from the refining of sunflower and rapeseed oils were treated by pressurized fluids as green solvents, targeting milder conditions of extraction than those described in literature. Supercritical CO₂, used as a first step of de-oiling, removed 92-97% of the neutral lipids and 66-75% of the total lipids and left a matrix of better flowing characteristics due to a significant decrease of particle sizes that after the extraction were below 100 µm. The oils produced at this stage did not contain polymerized triacylglycerols known as oligomers, and presented a fatty acid composition slightly richer in polyunsaturated fatty acids than oils obtained by hexane extraction. Valuable compounds such as tocopherols or colour pigments were present in too small quantities to confer an added value to the oils, but the oils could be valorised as non-petroleum feedstock for industrial applications. The extraction process was mostly limited by solubility, probably because of the high oil content of SBE. For industrial perspectives, the

extraction course hence could be accelerated by increasing the pressure and additionally temperature at pressures above 40 MPa due to the effect of those parameters on lipid solubility. The CO₂-treated SBE was then submitted to a second stage of extraction using ethanol either as a pressurized solvent, or as a co-solvent with CO₂ or as a CO₂-expanded solvent. Though pressurized ethanol yielded better efficiencies (SBE after treatment contained less than 2% of lipids, polymerized and colouring species were better extracted), the use of CO₂ + ethanol mixture opens opportunities to produce extracts of different characteristics. Finally, among the two bleaching samples, sunflower SBE was more difficult to de-oil since extraction efficiencies were lower than those obtained with the rapeseed SBE. The difference was attributed to the presence of activated carbon that was added to Tonsil when the sunflower oil was bleached.

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Spent Bleaching Earth

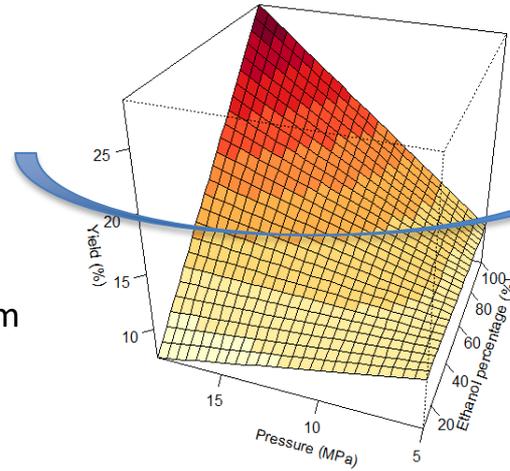


CO₂
45°C – 23 MPa



Particle size : 100 – 500 μm -> below 100 μm
Lipid content : 27-30 % -> 12 %

Pressurized CO₂-EtOH or EtOH



- Yellow to brown extracts
- SBE lipid content below 2%
- with pressurized EtOH