Supercritical carbon dioxide extraction of cannabinoids from *Cannabis sativa* L.

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ABSTRACT

Cannabis sativa L. extracts with high concentration of Δ^9-tetrahydrocannabinolic acid (THCA) and Δ^9-tetrahydrocannabinol (THC) were obtained by supercritical carbon dioxide (CO_2) extraction. The objective of this work was to investigate the cannabinoid concentration of the extracts, the overall process yield under different extraction conditions, and the effect of ethanol as co-solvent. Extraction experiments were carried out with multistage pressure increments and at constant pressure of 17, 24 and 34 MPa and 328 K with flow rate of 200 g/min of CO_2. At 34 MPa apparent solubilities of extracts were determined for four different *Cannabis sativa* L. strains with variable cannabinoids initial content. Extraction yield was highly dependent on pressure and plant material starting composition. The use of ethanol as a co-solvent was investigated with two different approaches, i.e. constant co-solvent flow, and by applying pulses of ethanol at different times though the extraction procedure. The obtained extracts were fractionated in 3 separators in a cascade configuration of decreasing temperature and pressure. The cannabinoid composition of the extracts was determined with HPLC analysis. Process extraction efficiency as high as 92% was achieved.

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1. Introduction

The oldest physical evidence of cannabis use is from 2727 BC., which describes the medical and psychoactive effects on humans. Cannabis use was spread over the years in Asia and Europe and was brought to America in 1619, mainly for applications in the textile industry. During the 1800′s cannabis tinctures were common in pharmacies and its applications were described in the American pharmacopoeia for migraine, depression and pain, among other symptoms [1]. In 1937 the Marijuana Tax Act effectively banned cannabis use and sales in the United States; this document was replaced by the Controlled Substances Act in the 1970's which established cannabis as a schedule I substance (highest dangerousness and potential for addiction) and is not currently accepted for medical use by the Drug Enforcement Agency (DEA). However, in 1996 California was the first state (out of the current 23) to legalize the use of cannabis for medical purposes in the United States, and despite the controversy, the recreational consumption of cannabis is currently regulated in four states [2].

The basic material of all cannabis products is the *Cannabis sativa* L. plant, with the subspecies *sativa*, *indica* and *ruderalis.* There are almost 500 constituents of this millenary plant, classified in many chemical families, such as terpenes, amino acids, fatty acids, hydrocarbons, flavonoids, sugars, etc. There is also a family of C_{21} terpenophenolic components, highly specific and only found in cannabis plants, formed with more than 70 different cannabinoids [3–5].

The chemical phenotypes of *Cannabis sativa* L. are useful to classify the plant material as drug- or fiber-type varieties, based on quantitative differences in the content of main cannabinoids present. The key difference between these two is found in the potential content of the psychotropically active component Δ^9-tetrahydrocannabinol (THC): a high content of THC classifies as drug-type cannabis, while a low THC content (less than 0.2%) is found in fiber-type cannabis or Hemp [3,6,7]. There is a very wide range of cannabinoids composition on the hundreds of varieties or hybrid strains cultivated nowadays, the mostly employed cannabis species, *sativa* and *indica* are genetically combined at different levels in order to obtain the desired cannabinoids composition and effect [3,5]. However, the most recognized and studied active metabolites are the psychoactive Δ^9-tetrahydrocannabinol (THC),

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and cannabidiol (CBD), a non-psychoactive cannabinoid, both considered with a broad scope of potential medical applications [7–13]. Both THC and CBD are neutral form cannabinoids, obtained after a non-enizmatic decarboxylation process occurs to the acidic forms, Δ^9- tetrahydrocannabinolic acid (THCA) and Cannabidiolic Acid (CBDA) originally present in the plant material [3,7]

THC, was first isolated and synthetized in 1964 [14], since then the effect of cannabinoids over the endocannabinoid system, human behavior and health, has been subject of many investigations [6–13].

Cannabis consumption has commonly been associated with motor skills deficiency, respiratory and cardiovascular health problems; nevertheless studies on cannabis have been shown that cannabinoids have the therapeutic potential of controlling chronic pain, alleviating nausea and vomiting associated with chemotherapy, treating glaucoma and wasting syndrome associated with AIDS, and controlling muscle spasms due to multiple sclerosis and Tourette's syndrome [9–13]. These facts, together with the decriminalization and/or legalization in some regions of the world, had increased the cannabinoids (natural and synthetic) demand [2,15].

Smoked herbal cannabis is the most common method of consumption for cannabis product around the world, but can be unhealthy and dangerous due to the fact that toxins and carcinogens are released from the combustion of materials. In addition to plant material, cannabis resins commonly referred as "hashish" is primarily used in Europe, while cannabis oil is less widely used [15].

The extraction of cannabis to make other forms of concentrate is a function of the solubility of THC and other cannabinoids in different organic solvents (mainly hydrocarbons and alcohols). Solvents like methanol, ethanol, chloroform, butane, hexane, etc. are currently applied, however safety considerations related to their toxicity and flammability exist [4]. The extraction method features essentially define the quality of the final product. Among the various extraction techniques, we can highlight the use of supercritical fluids as extraction solvents.

Supercritical fluid extraction (SFE) is a technology used in large scale for extraction of essential oils and a large variety of bioactive components from vegetable matrices [16–19]. The main supercritical (SC) solvent used is carbon dioxide (CO_2), is an inexpensive GRAS (generally recognized as safe) solvent, with very well-known physicochemical properties. CO_2 reaches supercritical state at 304.25 K and 7.39 MPa and return to gas state under ambient conditions, allows for simple solute recover providing a solvent-free product. By sensitive changes in pressure and temperature the CO_2 solvent strength can be tuned, this change in the medium provides to some extent, selectivity to the extraction process. The low polarity of SC CO_2 can be overcome employing polar modifiers (alcohols, water, acids, etc.) as co-solvents, expanding in consequence the extraction range, to include more polar components.

SFE has been applied previously to cannabis by several authors, but mainly to extract oil from Hemp seeds (*Cannabis sativa* L.). Results on seed oil composition obtained at different process conditions [20,21], oil oxidation stability [22], oil antioxidant capacity [23], recovery of volatile compounds [24], and extraction and solubility parameters determination [25] had been reported.

To the best of our knowledge, there are only two studies on SFE applied to drug-type *Cannabis sativa* L.; the PhD thesis of Perrotin [26] who explored some process conditions for the production (extraction and isolation) of cannabinoids including THC, CBD, cannabinol (CBN) and cannabigerol (CBG). Perrotin concluded that the SFE process is preferred over traditional solvent (hexane) extraction from an economic and ecological point of view. The highest yield was reported for the pilot scale set-up at 313 and 23 MPa with a 6 kg/h (100 g/min) CO_2 flow using samples of 45 g, with a minimum solvent to feed ratio (S/F) of 400:1. The extraction conditions were explored based on solubility measurements of pure THC, CBD, CBN and CBG in SC CO_2 [27,28] also reported in the thesis.

A most recent work of Omar et al. [29], compare the efficiency of two different techniques, focused ultrasound extraction (FUSE) and SFE for the deterpenation of the plant material and a subsequent extraction of cannabinoids. The SFE was carried out in a Method Station Supercritical Fluid Chromatography (SFC) System, with 100 mg of material placed in a 1 ml high-pressure extraction vessel; the operating variables were explored in the following ranges: pressure 10–25 MPa, temperature 308–328 K, flow 1–2 ml/min and ethanol as co-solvent between 0 and 40%. On Omar's optimization experiments two opposite trends (in terms of values of the most favorable extraction parameters) were observed for terpenes and cannabinoids respectively, concluding that two different optimum extraction conditions were required depending on the nature of the target compound. Briefly, 100 bar, 308 K and 1 ml/min were arbitrary chosen as the optimum conditions for the extraction of both terpenes and cannabinoids, with the addition of 20% of ethanol for the highest yields of cannabinoids.

Regardless of the rising popularity and usage of SC CO_2 extraction, there is very limited reported information about the efficiency of the extraction process for this plant material; there is not conclusive evidence neither on the most favorable extraction conditions nor cannabinoids concentration on the extracts. Studies of supercritical extraction of cannabinoids from drug-type *Cannabis sativa* L. has not been satisfactorily explored in the literature. Therefore, in this context the aim of this work is to evaluate different extraction conditions from *Cannabis sativa* L. exploring the effect of pressure, initial cannabinoids plan material composition, time and the use of ethanol as co-solvent to obtain extracts with high cannabinoids content, in a pilot plant scale equipment.

Coupled with the latest progress in legislation, there is a wide-reaching growing interest in a clean, efficient and reliable extraction method of these target components.

2. Materials and methods

2.1. Chemicals

Carbon dioxide used (instrument grade, purity >99.99%) was supplied by General Air. Absolute, Anhydrous Ethanol 200 proof and all other solvents and reagents used in HPLC analytical determinations were provided by Rocky Mountain Reagents, Colorado. Cannabinoids standards of 1.0 mg/ml for HPLC calibrations were purchased from Cerilliant.

2.2. Sample preparation

Different strains of *Cannabis sativa* L. were purchased from local growers in the state of Colorado (USA). HPLC analysis was performed to quantify the cannabinoids composition of all the samples (samples A–D) and the results are listed in Table 1. Prior to extraction, plant material (leaves and buds) used in the experiments is ground in a commercial grinder Dade Model DF-20 to decrease the particle size and therefore enhance the extraction efficiency. The plant material size distribution was determined using a nest of 6 sieves of aperture 63, 125, 250, 500, 2000 and 4000 μm. The mass remaining on each sieve after 20 min sieving was used to calculate the distribution of fragments, which was then normalized respect to the sample size. Particle size distribution (PSD) was quantitatively analyzed by Rosin-Rammter-Bennett (RRB) distribution function [31]. PSD determination was done by triplicate with a maximum standard deviation (SD) of 1.5 for the retained material percentage. The model fitted well the particle size distribution data over the entire range of the size distribution with high coef-

Table 1

Cannabinoid composition of different *Cannabis Sativa* L. strains used in the experiments. Analysis were done by duplicate and values expressed as cannabinoid percentage as mean ± SD. For total THC % (*potency*) calculation refer to Section 2.4.

Sample identification	*Cannabis Sativa* L. strain	Cannabinoids concentration			
		CBDA %	THCA %	THC %	Total THC % (*potency*)
Sample A	HashBerry (Sativa 50%- Indica 50%)	0.48 ± 0.02	12.95 ± 0.52	5.28 ± 0.20	16.63 ± 0.80
Sample B	Sour Alien OG (Sativa 40%- Indica 60%)	0.66 ± 0.17	12.81 ± 1.40	1.05 ± 0.31	14.03 ± 1.54
Sample C	White Widow (60% Sativa −40% Indica)	n.d.	9.05 ± 0.01	2.18 ± 0.01	10.11 ± 0.02
Sample D	Abusive OG (Indica)	0.46 ± 0.01	6.90 ± 0.31	n.d.	6.05 ± 0.27

n.d. = no detectable.

ficient of determination ($R^2 = 0,98$). The characteristic particle size describing the particle fineness D is equals to 1216 μm and uniformity coefficient describing the distribution width n is equal to 1,46.

2.3. Supercritical extraction equipment and procedure

The Waters Co. Bio-botanical extraction system [30] used in these experiments is represented schematically in Fig. 1. The actual configuration included two extraction vessels of 5 liters each (suitable for a series configuration) with a maximum operating pressure up to 60 MPa; the extraction pressure is maintained constant by an automatically actuated needle valve acting as a back pressure regulator (ABPR). The extraction vessel temperature is controlled by an electrical jacket. There are three collection vessels or cyclonic separators (CS) of 1 L, each independently heated and provided with manual pressure control (back pressure regulators). For all the experiments the sample is placed in an extraction vessel and pressurized with CO_2. The CO_2 is cooled down to approximately 276 K to ensure liquid state and proper density before reaching the high pressure pump which delivers a maximum mass flow rate of 200 g/min. Depending on the experiment, a determined amount of ethanol is provided by the co-solvent pump (flow up to 50 g/min) and mixed with the main CO_2 stream. The solvent current flows through an electrical heat exchanger to bring the liquid CO_2 (and co-solvent) to supercritical state before entering into the extractor vessel. The supercritical stream, dissolve the target components from the vegetable matrix and carry them from the extraction vessel to the cyclonic separators for a controlled depressurization process. The separators conditions were set up in a temperature-pressure cascade configuration, at levels below the operating conditions used during the SFE, to separate different fractions in all experimental assays: CS1: 13 MPa and 328 K; CS2: 9 MPa and 328 K; CS3: 6 MPa and 298 K. The chosen configuration induce a stepwise decrease of SC CO_2 density from 571 down to 190 kg/m³; allowing the separation from the supercritical solvent current, of heavy, middle weight and light components respectively. Each cyclonic separator allows for periodical discharge of the extracted material collected during the SFE process.

The CO_2 gas is afterwards re-circulated to the system. A view cell is connected after the third separator and before the gas recycler to visually verify flow conditions and correct operation. The whole system is controlled by the software ChromScope V1.5 (Waters Corp.) [30].

In a typical experimental run, 500 ± 2 g of ground material is placed in the 5 L extraction vessel. Because the total capacity of the 5 L extractor is about 1,7–2 kg of material, the void space of the vessel is filled with stainless steel wood whereas the plant material is packed in the central area of the extraction vessel. The vessel is heated up to the desired temperature while pumping the supercritical solvent phase into the extractor to reach the system set values. When pressure is near the set value, the extraction process

starts by automatic opening of the ABPR allowing the solvent to flow continuously through the extractor and carrying the solubilized components to the separators. This is considered the initial time of the extraction procedure. The pressure and temperature configuration of the separators enable a partial fractionation of the extract; those fractions precipitate from the SC CO_2 (and SC CO_2 plus ethanol) stream into the CS1, CS2 and CS3 respectively. The extracts collected in each cyclonic separator are recovered by opening the drain valve of each separator into a stainless steel container provided with a lid to avoid splits and material loss. Those containers are weigh at different time intervals during extraction procedures to determine the extraction kinetics and evaluate final yield. Extracts *potency* (THC and THCA% content) is determined by HPLC analysis, sampling the material from the separators and following the procedure described in the following section.

2.4. HPLC analysis

HPLC analysis is performed to determine cannabinoids composition of obtained extracts, starting plant material and exhausted material after extraction to corroborate the process efficiency. Mainly THC, THCA and CBDA were quantitated, other cannabinoids were not found in the plant material used or obtained extracts. The material "*potency*" is generally expressed by the total percentage of neutral cannabinoids present in the sample. THC total percentage is calculated as the sum of THC percentage plus THCA percentage, this THCA value is multiplied by a conversion factor that counts for the difference in molecular weight between the acidic and neutral form.

Based on molar conservation, the conversion factor is calculated by the molecular weight relationship in the transformation from the acidic (THCA) to the neutral from (THC) through the loss of a carbon dioxide molecule (CO_2). THCA weighs 358 g/mol, while THC weighs 314 g/mol, thus the conversion factor is 358/314 = 0.877 [31]. Therefore, the correct way of calculating the total percentage of THC, or in other words material "*potency*" in HPLC is: % of THC Total = % of THC + (% of THCA × 0.877).

No further compositional analysis of the co-extracted plant material was carried out on these studies. All chromatographic analysis were carried out using Agilent 1200 HPLC System, consisting of a G1311A binary solvent pump, a G1322A solvent degasser, a G1313A autosampler and a G1316A column compartment. An Agilent 1200 series (G1315D) photodiode-array detector (DAD) was used for detection and recorded at UV/Vis 220 nm. Cannabinoids chromatographic separations were achieved using a Restek Raptor™ ARC-18 column (150 mm × 4,6 mm ID and 2,7 μm particle size). Equipment control, data acquisition and integration were performed with ChemStation (2001–2007) software.

Depending of the type of material to be analyzed (vegetable matrix or extract) a different sample preparation must be carried out [32]. Plant material is dissolved with 9:1 Methanol:Chloroform, and the filtered solution is then diluted with 1:1 Methanol:Water.
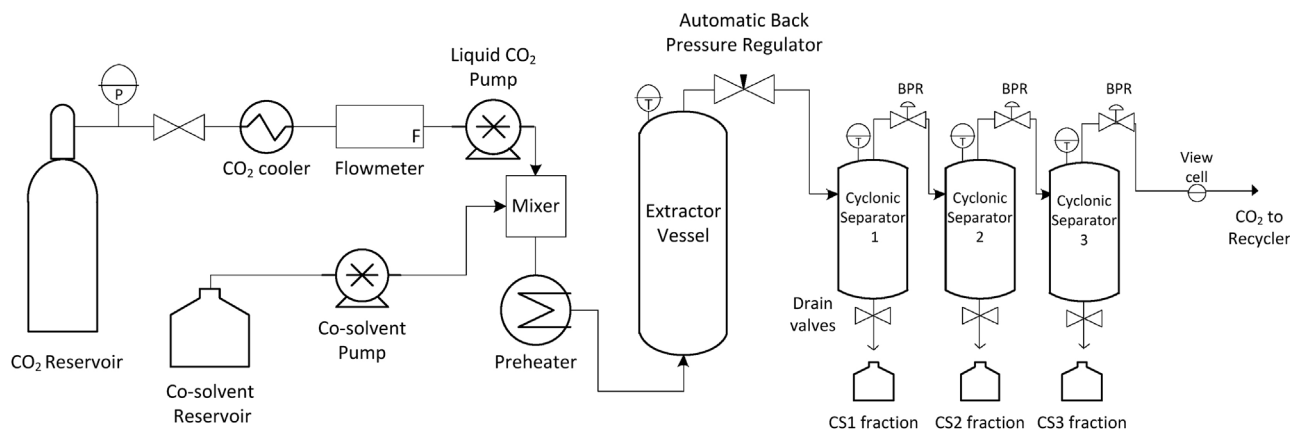
Fig. 1. Schematic representation of the SFE pilot plat scale experimental set up.

The dilution factor for the plant material before extraction (raw plant material) is 400:1 and for the plant material after extraction (exhaust plant material) is 20:1. For the extracts obtained by SFE, a sample of approximately ~0.07 g of material is diluted in 10 ml of methanol (120 rpm of stirring and heat up to 40 °C was applied in some cases to achieve full dissolution of the extract), then a 40:1 dilution is performed with 1:1 Methanol:Water. The chromatographic methodology applied is described elsewhere [33]. The identification and quantification of cannabinoids was based on external standards using a calibration of commercial reference compounds. Intermediate dilutions of the 1.0 mg/ml standards were prepared to build calibration curves between 10 and 100 μg/ml. A good linearity was assessed using the regression method, with a coefficient of determination (R^2) greater than 0.99 in all cases. In addition to the instrument precision, for single compounds the coefficient of variation, RSD <0.80% was obtained in all cases.

3. Results and discussion

This work presents results on the use of a pilot plant scale SFE bio-botanical extractor to obtain extracts with high cannabinoids content (mainly THC and THCA). To explore different extraction parameters (pressure, time and co solvent effect) on extraction yield and composition, solvent mass flow rate was kept constant at the equipment maximum capacity of 200 g/min (12 kg/h); this flow was chosen based on the criteria of reducing process time for real extraction operation.

Preliminary investigation on the effect of temperature was carried out in the range of 313–333 K (not reported). Although the initial obtained results suggested that at a constant pressure of 34 MPa, a faster extraction rate can be achieved at higher temperature, there were no conclusive results on this regards and further investigation at isothermal conditions is required. Therefore a constant temperature of 328 K was arbitrary chosen for the experiments in this study.

During each supercritical extraction process, the obtained extracts precipitate into the different separators vessels due to the solvent density decrease; discharge and immediate weigh of the extracted material from each separator at defined time intervals was performed, in order to follow the gradual progression of the extraction process. The total accumulated extract mass (g) reported for each interval point is obtained by adding the mass of the three collection vessels (g CS1 + g CS2 + g CS3). The extraction curves are plotted with the accumulated mass versus the CO_2 consumption during the extraction.
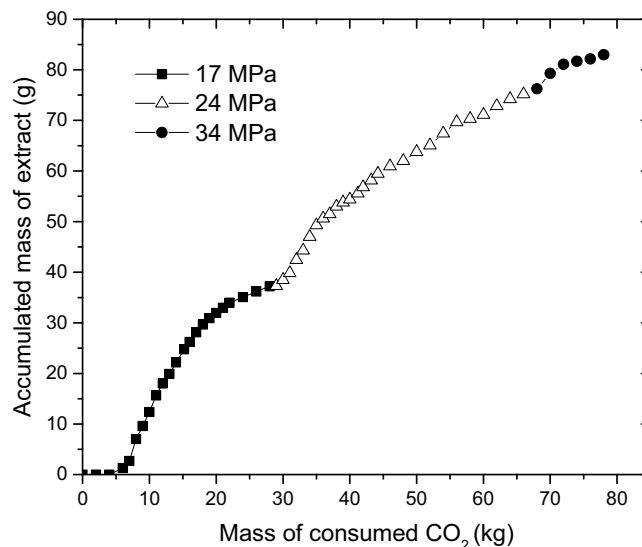

Fig. 2. Total accumulated mass of extract from *Cannabis Sativa* L. sample A at 328 K in sequential extraction steps of 17, 24 and 34 MPa. Data refers to duplicated experimental run.

Extraction yield is calculated as the total mass of extract (g) obtained divided by the mass (g) of plant material fed into the extractor (500 ± 2 g in all cases). As a reference value, a traditional solvent extraction was carried out by triplicate with plant material of sample A, using ethanol as a solvent and following the protocol described elsewhere [4]. Results are included for comparison in Table 2.

3.1. Effect of pressure

The effect of pressure on the extraction rate and total amount of obtained cannabinoids extract was studied at constant temperature of 328 K and pressure in the range of 17–34 MPa using sample A plant material. Fig. 2 shows a multistage extraction procedure results with consecutive steps at 17, 24 and 34 MPa. This extraction procedure was carried out by duplicate; the average obtained values were used to plot the accumulated mass of extract obtained at different times represented by the amount of CO_2 consumed. The final time of each extraction step was determined by the decrease in the extracted mass increments between consecutive weight measurements. By varying pressure at the same temperature, solvent density and strength change [16,34]; this may affect cannabinoids solubility and co-extraction of different components from the same

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Table 2
Cannabinoids extracts yield and composition from sample A under different extraction conditions. Comparison between multistage pressure and constant pressure extractions. Extractions were done by duplicate and values are expressed as mean ± SD. For total THC % (potency) calculation refer to Section 2.4.

Experiment	Pressure (MPa)	Yield (g extract /g feed)	Cannabinoids concentration			Total THC % (potency)	Cannabinoid extract (g)	THC (g)
			CBDA %	THCA %	THC %			
Ethanol extraction	0.1[c]	0.132 ± 0.008	1.49 ± 0.05	48.90 ± 3.45	11.32 ± 0.62	58.83 ± 2.90	n.a.	n.a.
Multistage pressure	17[a]	0.074 ± 0.005	2.92 ± 1.05	70.56 ± 3.45	6.47 ± 0.62	76.23 ± 2.90	37.21 ± 1.98	28.37 ± 1.48
	24	0.150 ± 0.003[b]	1.69 ± 0.56	46.20 ± 2.33	20.50 ± 1.43	64.17 ± 1.98	37.98 ± 2.05	24.37 ± 2.39
	34	0.166 ± 0.006[b]	1.08 ± 0.79	45.26 ± 1.12	22.87 ± 1.64	65.31 ± 1.53	7.80 ± 2.34	5.09 ± 1.98
							Tot=82.99 ± 1.87	Tot=57.83 ± 2.14
Independent pressure	17[a]	0.074 ± 0.005	2.92 ± 1.05	70.56 ± 3.45	6.47 ± 0.62	76.23 ± 2.90	37.21 ± 1.19	28.37 ± 1.48
	24	0.171 ± 0.006	1.48 ± 0.38	62.51 ± 1.80	9.26 ± 1.50	70.63 ± 3.11	85.83 ± 1.57	60.62 ± 3.69
	34	0.185 ± 0.005	1.09 ± 0.93	49.75 ± 2.54	25.78 ± 1.82	69.41 ± 2.87	92.57 ± 2.14	64.25 ± 2.54

n.a. No available.
[a] Same experiment.
[b] Accumulated yield.
[c] Ethanol extraction procedure from Ref. [4].

vegetable matrix, which influence the final THC content in the extracts.

At 328 K, CO_2 density values are 708 kg/m^3, 801 kg/m^3 and 875 kg/m^3 at 17, 24 and 34 MPa respectively. Components that are soluble in low pressure CO_2 are anticipated to be extracted at lower density, changing with time the vegetable matrix composition, as the pressure increases the solubilization of other components (soluble at high CO_2 pressure) can be achieved; this overall matrix modification through the extraction process may influence the solute-matrix interaction and the consequent extraction of target components. The obtained mass and yield values at the end of each pressure step, final yield and cannabinoids extracts composition are reported in Table 2 (traditional ethanol extraction yield and composition is also included for comparison).

Total mass of cannabinoid extract and calculated amount of total THC recovered on each step is also reported. Fig. 2 show the kinetics of the extraction. As expected, an increase of the extraction rate and extract yield is observed with consecutive pressure increments. At the initial extraction pressure, an extract with the highest cannabinoid concentration (expressed as total THC%) is obtained, however the calculated yield at this point is even lower than the traditional ethanol extraction. The subsequent raises in pressure enhance the final yield achieved, keeping the final total THC concentration around 65%; almost 10% of the total obtained mass was collected during the final pressure increment up to 34 MPa.

From Fig. 2, it can been seen that even when the mass increments decreases towards the end of the experiment a total extraction was not achieved yet; in fact the HPLC analysis of the exhausted material evidence a remaining THC total content of 1,87 ± 0,53%.

In order to compare the solely effect of pressure over a vegetable matrix without compositional changes due to prior pressure extraction (as when the sequential method was applied) sample A material was used to perform extractions at the 3 different pressures previously measured. Fig. 3(a) shows the extraction curve as total mass of cannabinoids extract obtained as a function of the mass of CO_2 consumed at 17, 24 and 34 MPa. The extraction curves presented in Fig. 3(a) show the classic behavior of extraction with supercritical fluids [35]; the typical periods are observed: the constant extraction rate period (CER) were the linear slope value is close to the value of the extract solubility in the solvent phase and the falling extraction rate period (FER) where the rate of extraction drops rapidly; towards the end of the process the beginning of the diffusion-controlled period is observed.

As a general rule, the higher is the pressure, the larger is the solvent power and the smaller is the extraction selectivity [16]. In our experiments, at the same extraction time or amount of CO_2 consumed, the expected increment in yield is observed at higher pressures. As an example, when 20 kg of CO_2 passed through the plant material (equivalent to a solvent to feed ratio S/F = 40), the yield of 0.0638 at 17 MPa is almost duplicated to 0.1262 at 24 MPa. At the same S/F ratio and 34 MPa the extraction yield rises 35% reaching 0.1707. In other words, a S/F > 70 is required at 24 MPa for the same extraction performance than at 34 MPa (yet, an order of magnitude less than the work of Perrotin [26] with S/F = 400 reported).

At the end of the extraction process, although at 34 MPa the amounts of collected cannabinoid extract and total THC extracted are slightly higher, those values are comparable with the extraction at 24 MPa. Nevertheless, even if the final values reached at the end of the process for the 24 and 34 MPa extractions are alike, operating at 34 MPa is recommended due to the higher extraction rate and consequent shorter processing time.

The extract apparent solubility in CO_2 [36] increases with pressure increments; it can be estimated from the initial slope of a linear fit when yield or e (mass of extract/mass of feed) is plotted versus q (mass of CO_2 passed through the vessel/mass of feed) during the
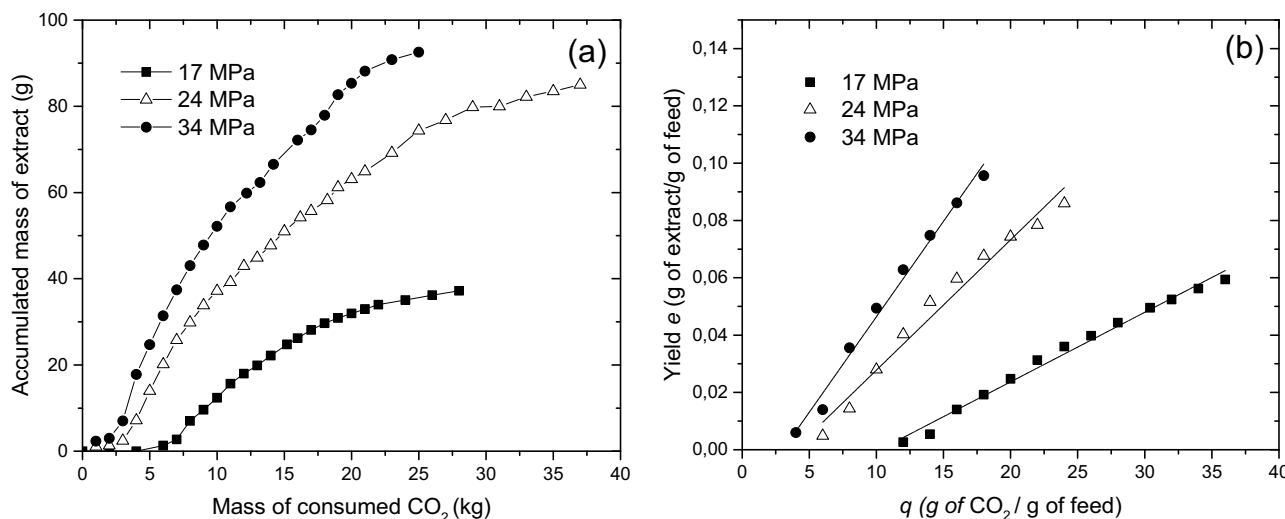
Fig. 3. (a) Extraction curves from CO_2 supercritical extraction of *Cannabis Saltiva* L. sample A at 328 K and constant pressures of 17, 24 and 34 MPa. Data refers to duplicated experimental run (Table 2). (b) Effect of pressure on extract solubility. Dashed lines correspond to linear regression in Table 3.

Table 3
Apparent solubility estimated by linear regression for different experiments at 328 K.

Experiment type		Linear fitting parameters	
		Slope: apparent solubility of linear fittingof linear fitting (g extract/g solvent) $\pm$ SE	R^2
Sample A	P = 17 MPa	0.00243 $\pm$ 7.76247E-5	0.988
	P = 24 MPa	0.00455 $\pm$ 2.49919E-4	0.973
	P = 34 MPa[a]	0.00666 $\pm$ 2.77282E-4	0.988
34 MPa	Sample A[a]	0.00666 $\pm$ 2.77282E-4	0.988
	Sample B	0.01361 $\pm$ 9.42354E-4	0.981
	Sample C	0.00431 $\pm$ 2.2355E-4	0.989
	Sample D	0.00186 $\pm$ 7.7641E-5	0.990

[a] Same experiment.

linear behavior of the extraction curve [37]. Using the linear range of the obtained data plotted in Fig. 3(b), the apparent solubilities calculated at 328 K and each pressure condition, are summarized in Table 3. By working at high pressure a shorter extraction time is required for an efficient extraction due to an increase in extract apparent solubility, therefore, this condition is chosen for the next experiments.

From the experimental results presented in Tables 2 and 3, a higher pressure offers the most efficient kinetic condition for cannabinoids extraction using SC CO_2, with a high initial extraction rate, apparent solubility, and total yield.

Plant material after extraction at 34 MPa was also analyzed by HPLC to establish the final THC concentration remaining; a residual THC *potency* of 1.68 $\pm$ 0.87% was found in the exhausted vegetal matrix.

3.2. Effect of composition variability on plant material

Keeping extraction conditions at 34 MPa and 328 K, other three different strains of *Cannabis Sativa* L. plant material (Samples B–D, listed in Table 1) with diverse cannabinoids concentration were also investigated and compared with Sample A results. From the information available in Table 1, the total THC% (*potency*) of the analyzed plant material shows the following decreasing order: A > B > C > D.

The main objective of this experiment was to compare final yields and extracts concentration, when same extraction conditions are applied to different plant material of *Cannabis Sativa* L., in order to evaluate the potential extrapolation of previous obtained

results (i.e. extraction rate, maximum yield achieved at certain S/F ratio, etc). Fig. 4(a) shows the obtained extraction curves for all the samples (including for comparison results from sample A already depicted in Fig. 2); the extraction rate of each sample differ considerably between them at the current operating conditions. Table 4 summarized the obtained results for these experiments.

Sample B plant material with THC *potency* equals to 14.03 $\pm$ 1.54%, shows the highest extraction rate and total mass of obtained cannabinoids extract; when comparing with sample A, the plant material with maximum total THC content of 16.63 $\pm$ 0.70%, this last one shows a lower extraction rate and total extracted mass. However, final extraction yield of both samples are similar in value: sample A 0.181 and sample B 0.191 g of extract/g of feed, but different total THC content. The cannabinoid extract with the highest THC *potency* of 69.41 $\pm$ 2.87% was measured for Sample A, corresponding to the plant material with highest cannabinoid concentration, yielding as a consequence a larger total amount of THC extracted. Sample D plant material has only 6.05 $\pm$ 0.27% of total THC, and yields the less amount of obtained extract with the lowest THC *potency* of 56.06%. From Fig. 4(a), it can be observed that a larger S/F ratio is required for an efficient extraction from plant material with lower cannabinoids concentration.

Although some of the obtained final extraction and composition values are comparable, samples corresponding to plant material with lower cannabinoid content show slower extraction rates and lower extract yields, when compared with high cannabinoids concentration plant material samples. Nevertheless, all the obtained extracts have high total THC content, between 56 and 67%. An

overall higher THCA concentration on the obtained cannabinoids extracts is observed in all the samples; this is expected from a non decarboxylated plant material. However, an increase on the THC/THCA ratio is observed in all cases for the extract composition when comparing with the starting plant material. Temperature and exposure time are factors that promote decarboxylation of acidic cannabinoids, these results suggests that a partial decarboxylation takes place during the extraction procedure.

The apparent solubility of cannabinoids extracts was calculated as previously described, from data depicted in Fig. 4(b); the obtained values are listed for all the samples at 34 MPa and 328 K in Table 3. Sample B calculated extract solubility shows a higher value than the other samples, whereas Sample D (the plant material with lower cannabinoid concentration) is the one with the lowest extract apparent solubility value. Plant material cannabinoids composition strongly influences the extraction rate and the apparent solubility of the extract in supercritical CO_2. A preliminary determination of extraction rate and final obtained yield is advised for each type of plant material to be used for cannabinoids extraction. The total amount of extracted cannabinoids is function of the quality (i.e cannabinoids concentration) of the plant material used.

HPLC analysis of the plant material after extractions was performed; the remaining total cannabinoids concentration in the exhausted material and estimated extraction efficiencies are listed in Table 4.

3.3. Cannabinoid profile in separators

As mentioned before, cannabinoids extract total yield is calculated adding the collected mass of the three collection vessels divided by initial mass of plant material used in the extraction (g/g). The mass amount and cannabinoid composition, collected from each separator depend on the following conditions, CS1: 13 MPa and 328 K; CS2: 9 MPa and 328 K; CS3: 6 MPa and 298 K. From all the carried out experiments, a significant higher amount of mass (>60%) was always collected from CS1, and only a few grams in CS3. As a reference, Fig. 6(a) shows the collected mass from each separator (CS) and the total accumulated mass through the extraction process for sample B at 34 MPa and 328 K. The CO_2 density drop, from extraction conditions (875 kg/m³) down to separators values; CS1: 571 kg/m³ and CS2: 255 kg/m³ enables most of the cuticular waxes and cannabinoids, (together with some other low solubility co-extracted material) to precipitate mainly in CS1. In general, the

material recovered in CS1 is waxy, pasty and darker in color, the fraction in CS2 is a more fluid yellow in color extract, but still with very high density; the oiliest and most light material is recovered in CS3. These physical characteristic are common to all the samples in this study for supercritical extraction with CO_2.

The final cannabinoid concentration (expressed as total THC%) was found to be higher in separator CS1 for the analyzed samples B, C and D. The calculated ratios CS1:CS2 of total THC% or *potency* >1.5 for all cases, are listed in Table 4.

Cannabinoid profile of extracts from each separator was analyzed during the extraction process for samples B, C and D, mainly to evaluate THCA and THC content and distribution. HPLC analyses were performed to samples taken during the extraction runs. Fig. 5 illustrates the relative cannabinoids (THCA and THC) profile change during the extraction process at different time intervals for CS1 and CS2 for the three samples. A higher THCA concentration is observed in CS1 for all the samples until extraction time is completed. Furthermore, at each time interval analyzed for all the samples, the THCA content is higher in CS1 than in CS2. The THC concentration does not show a significant variation in the separators at the time intervals evaluated through the extraction processes, except for sample D, the sample analyzed for the longest time, in which the THC concentration increases with time (after 120 min) in CS2. This observation is consistent with the idea of a possible partial decarboxylation when exposed at temperature for longest time during the extraction process.

Based on these observations, CS1 shows higher total THC% or potency and separator yield.

3.4. Effect of ethanol as co-solvent

A co-solvent can be added to supercrtical CO_2 to increase its solvent power towards polar molecules. In this case, the effect ethanol (GRAS) as the most commonly used co-solvent is evaluated [16]. Reported data in literature suggest that the use of ethanol enhance the cannabinoids extraction efficiency [29], however a larger solvent power could also mean lower process selectivity [16]. In some cases this strategy has the drawback that, being the co-solvent liquid at atmospheric pressure, it will be collected together with the extracted compounds and subsequent processing for solvent elimination is required. However, in this particular case, the cannabinoids extract goes through a wax removal process called "*winterization*" [26] that includes a solubilization step in ethanol
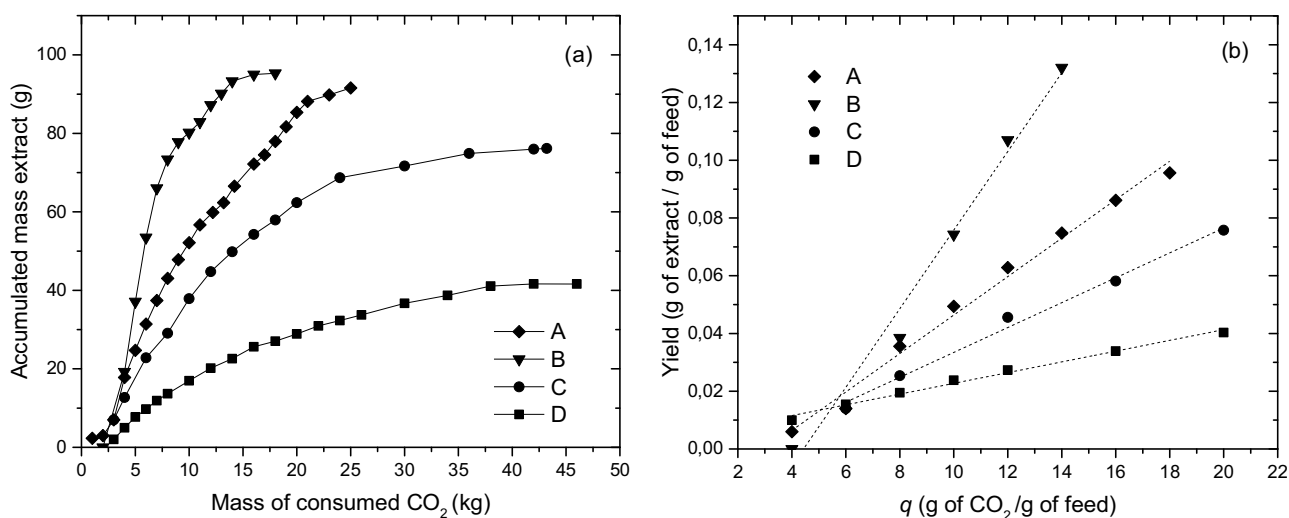
Fig. 4. (a) Extraction curves at 328 K and 34 MPa from different samples of *Cannabis sativa* L. plant material listed in Table 1. Extraction results reported in Table 4. (b) Different samples extract solubility. Dashed lines correspond to linear regression in Table 3.

Table 4
Cannabinoid extract composition and process results from plant material listed in Table 1 at 34 MPa and 328 K.

Sample	Yield (g extract/g feed)	Cannabinoids concentration				THC %CS1/CS2	THC (g)	Cannabinoid extract (g)	Exhaust total THC %	Extraction efficiency (%)
		CBDA %	THCA %	THC %	Total THC % (potency)					
Sample A[a]	0.185 ± 0.005	1.09 ± 0.93	49.75 ± 2.54	25.78 ± 1.82	69.41 ± 2.87	n.a.	64.25 ± 2.54	92.57 ± 2.14	1.68 ± 0.87	89.89 ± 1.07
Sample B	0.191	1.66	46.37	20.55	61.21	1.53 ± 0.11	58.33	95.30	1.52	89.17
Sample C	0.152	0.97	39.68	23.07	57.86	1.81 ± 0.20	43.97	75.98	0.98	90.31
Sample D	0.088	0.01	32.35	27.69	56.06	1.68 ± 0.94	24.57	43.82	0.47	92.23

n.a. No available.

[a] Average value and standard deviation of duplicate run, also reported in Table 2.
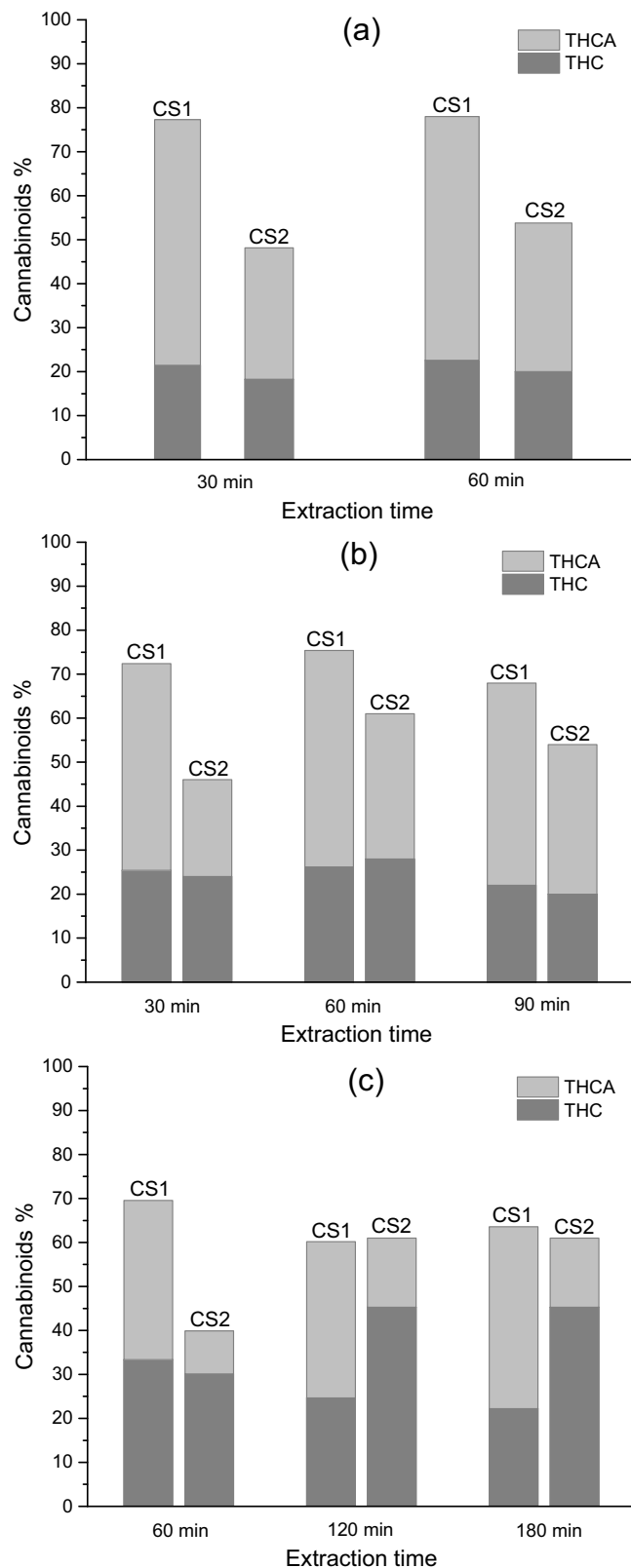

Fig. 5. Quantitated distribution profile of cannabinoids (THCA and THC) of extracts obtained in cyclonic separators CS1 (13 MPa; 328 K) and CS2 (9 MPa; 328 K) from Sample B (a) Sample C (b) and Sample D (c).
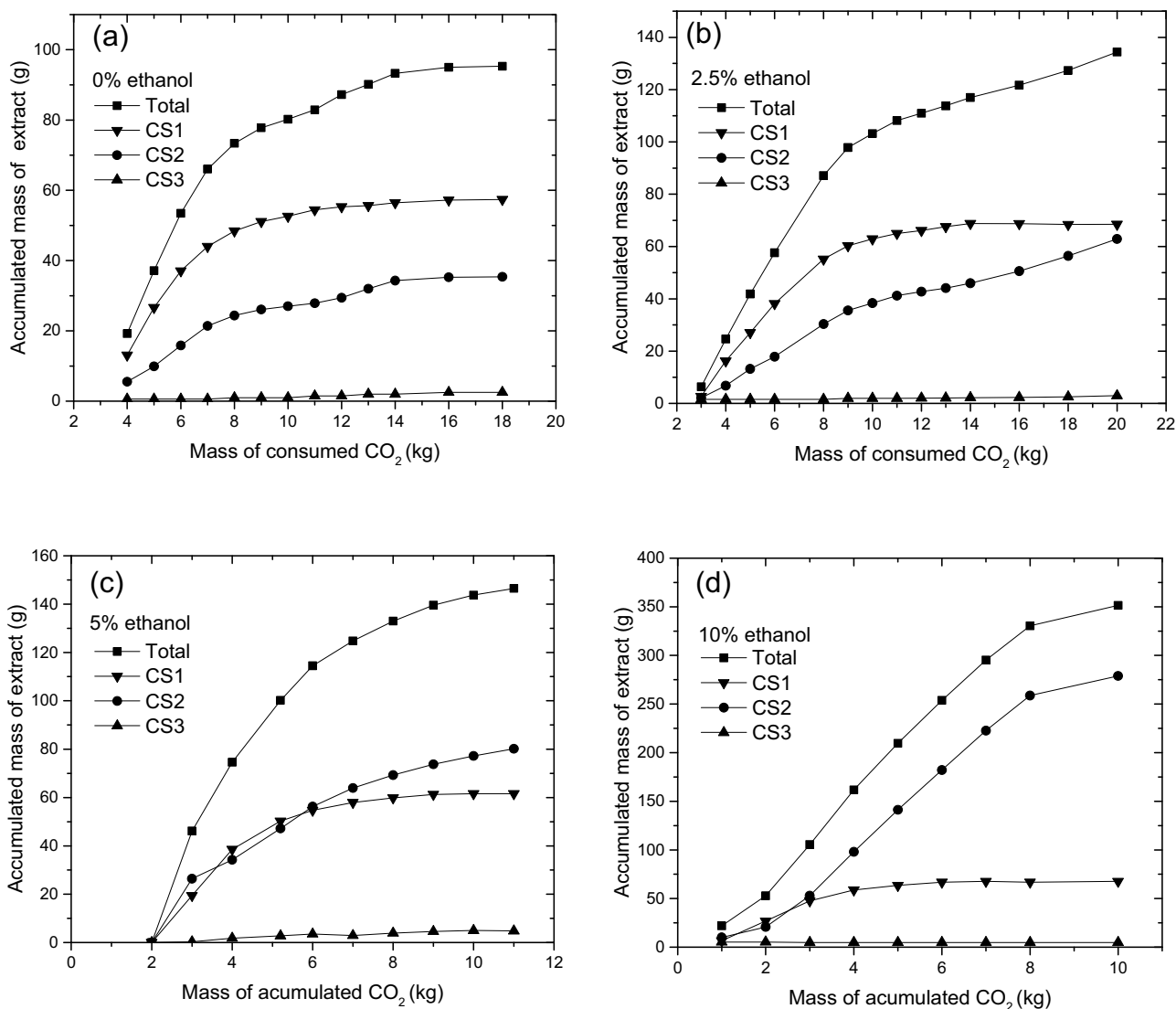
Fig. 6. Effect of ethanol addition as co-solvent for supercritical CO_2 extraction of cannabinoids from sample B at 34 MPa and 328 K. Mass of extract collected from the three separators and total accumulated mass (a) Pure CO_2 (b) CO_2 + 2.5wt% of ethanol. (c) CO_2 + 5wt% ethanol. (d) CO_2 + 10wt% ethanol.

for further purification of the final product; hence a small addition of ethanol as co-solvent does not modify the overall process.

Based on the separator conditions most of the ethanol is recovered in the CS2, at 9 MPa and 328 K. The ethanol present in the solvent stream, also help to enhance fluidity of the extract through the equipment lines and collector vessels.

Two different types of experiments were carried out: with constant flow of ethanol and with ethanol supplied to the process in the form of pulses.

For the experiments with constant flow of ethanol, quantities of co-solvent increasing from 0 to 10% by mass were investigated for supercritical extraction from Sample B plant material. Extraction conditions of 34 MPa and 328 K were kept constant for all the experiments. Fig. 6 shows the obtained extraction curves with increasing co-solvent addition to the supercritical phase for cannabinoids extraction; accumulated mass from each separator (CS) as well as the total mass of cannabinoids extract are depicted for 0, 2.5, 5 and 10 wt% of ethanol. By comparing the collected mass for all the experiments, it becomes evident that most of the ethanol supplied as a co-solvent precipitates in CS2. It can be observed in Fig. 6(b) that the extraction curve corresponding to CS2 for the 2.5 wt% of ethanol case, does not reach a constant final value towards the end of the

extraction but a constant slight increment is perceived. The extraction experiment with 5 wt% ethanol addition shows in Fig. 6(c) that the final collected amount in CS2 is higher than the collected material from CS1, and also does not reach a constant final value, a steady increment is detected. This observation is even more evident for the case of 10 wt% of co-solvent in Fig. 6(d) with a notorious increment on the collected mass from CS2. In this case, after 40 min of extraction (equivalent to 8 kg of CO_2 consumed) and towards the end of the process the flow of ethanol was discontinued, and a decrease on the accumulated mass from CS2 was observed.

As a consequence of the presence of dissolved ethanol in the mass collected from CS2, the final total yield of the extraction cannot be calculated by the addition of the mass collected from the three separators; co-solvent interferes with the calculation giving erroneous values as can be seeing in Fig. 6.

In order to be able to compare the extraction results, only the weight of the extracted material from the first separator (CS1) was considered, where the presence of co-precipitated ethanol can be neglected. From Fig. 6(a), it can be observed that the plant material was almost exhausted after 50 min of extraction (i.e 10 kg of CO_2, and S/F = 20), no significant weight change was observed in the mass collected from CS1 after this time.
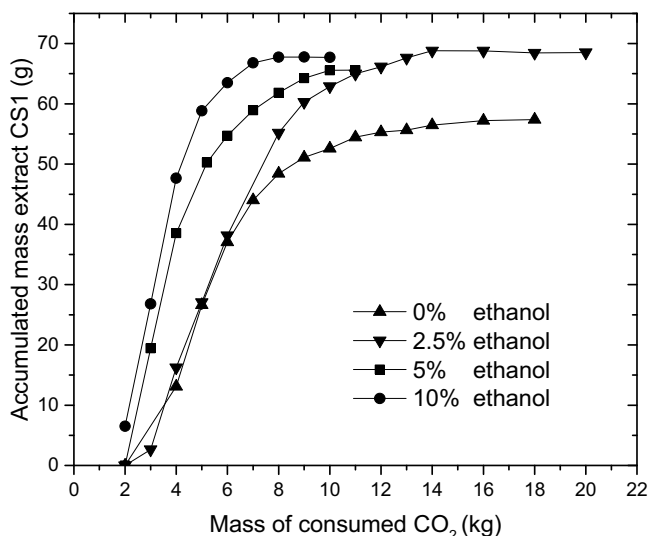
Fig. 7. Comparison of extracts collected from CS1 from sample B extractions, with constant flow of co-solvent at increasing concentrations (0–10 wt%).

The amount of collected material from CS1 from all the ethanol constant flow experiments was used to evaluate the extraction performance; such information is depicted in Fig. 7. The accumulated mass in CS1 for the three extractions with ethanol addition yields few grams more of extract, this fact plus the observation of a more fluid cannabinoids extract suggest that some small quantity of ethanol is solubilized within the mass of cannabinoids extract; however this is not consider as an important interference for the analysis. When comparing the obtained extraction curves results at the same extraction conditions with pure CO_2 and with increasing amount of ethanol as a co-solvent, no significant effect over the extraction rate (slope of Fig. 7) is observed for the experiment with 2.5 wt% of ethanol added as co-solvent; but, an improvement is observed when more than 5 wt% of ethanol at constant flow is used during the extractions. Results obtained with 5 and 10 wt% of ethanol concentration are almost coincident, thus, for a similar effect the lower amount of co-solvent is advised for the cannabinoids extraction. The use of ethanol as co-solvent improves the overall extraction rate for cannabinoids extracts.

Although the increase in solvent power of the solvent phase by ethanol addition could enhance the co-extraction of other non-target plant material, the potential decrease in cannabinoids relative concentration can be neglected if a further purification of the cannabinoids is implemented.

In the search of finding a better extraction strategy with a minimum of ethanol used, the addition of co-solvent in the form of pulses is proposed. The same experimental procedure was carried out for samples B–D and designed to supply an equivalent mass of ethanol comparable with the experiments with ethanol 5 wt% constant flow. In the previous set of experiments with constant co-solvent flow, cannabinoids extraction from Sample B was considered almost complete after 60 min (12 kg of CO_2), therefore, 600 g of ethanol was also employed in this experiment. Such amount was divided in three pulses of 10 min each (10 wt% of ethanol in each pulse), and supplied in the following manner: first pulse, during pressurization of the extractor vessel before the extraction start, thus a 10 wt% of ethanol pulse was applied before time 0 (considered as the beginning of the extraction procedure). The second pulse of 10 wt% of ethanol was applied after 50 min of extraction, in other words, during the last 10 min of the first extraction hour the second pulse was applied. And following the same procedure, the third pulse could be applied after 110 min, i.e during the last 10 min of the following extraction hour. During all
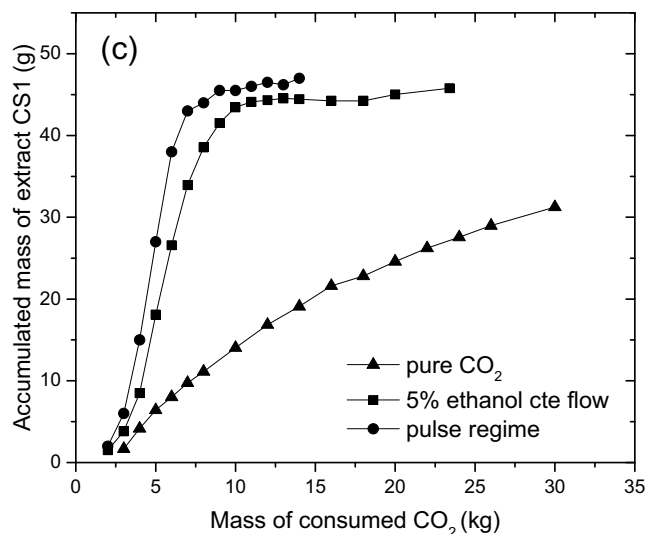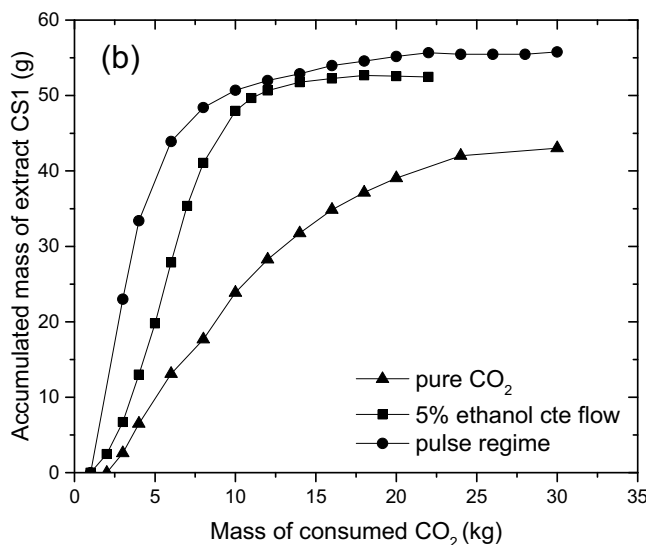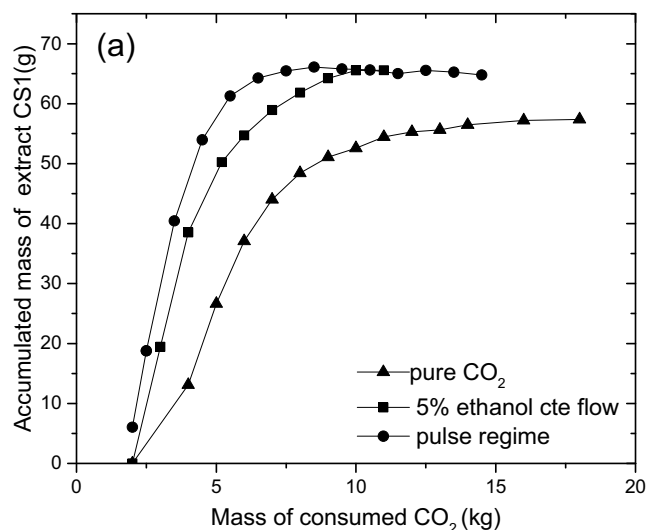

Fig. 8. Effect of ethanol addition to supercritical CO_2 extraction from *Cannabis Sativa* L. samples B, C and D. Comparison between 5 wt% ethanol constant flow and pulse regime.

the extraction time the collected material in CS1 was weigh. The results are compared in Fig. 8 for samples B–D with the extraction curves obtained in experiments without co-solvent and when 5 wt% of ethanol was supplied at constant flow. Comparing the obtained data, the best extraction performance in terms of extraction rate was observed in all cases for the experiments carried out with ethanol supply in a pulse regime. Although results obtained showed no major difference on extraction rate between the 5 wt% constant flow and the pulse regime experiment, for all the samples the CS1 maximum yield was already achieved before the second pulse was applied. That means that only 33% of the projected ethanol consumption was required in the pulse regime experiments to achieve the same yield than those with constant 5 wt% ethanol flow. At the extraction point before the second pulse of ethanol i.e. after 50 min of extraction, only 200 g of ethanol were supply with the pulse regime, on the other hand with a 5 wt% constant flow, 500 g of ethanol were instead introduced into the system already. An operational improvement with the pulse regime was accomplished; a lower overall co-solvent consumption was required to achieve similar yield than with the constant co-solvent flow supply. In both cases and for the three samples analyzed, the addition of ethanol as co-solvent to the supercritical stream has a favorable effect over the extraction rate when compared with pure supercritical CO_2 extractions; the use of ethanol reduces the S/F ratio required to achieve same extraction yields. This effect has a predominant influence on extractions from plant material with lower cannabinoid concentration as can be observed in Fig. 8; the lower the plant material cannabinoids concentration, the highest the effect of ethanol addition as co-solvent for the supercritical extractions.

HPLC analysis of the plant material after extractions with ethanol as co-solvent gives an average of 1.03 ± 0.47 residual THC for all the experiments.

The use of ethanol as co-solvent improves the extraction process; in particular, the proposed mechanism of pulse regime to supply the co-solvent, allows achieving high yield in shorter time with the lowest co-solvent and solvent consumption. Of all the experiments carried out, the pulse of co-solvent shows the best extraction performance for this plant material.

4. Conclusions

Supercritical fluid extraction has been shown to be a viable technology for extraction of cannabinoids from drug-type *Cannabis Sativa* L., with high yield and efficiency. Different operation conditions and regimes had been evaluated in this study using a pilot plant scale set up. Extraction yield increased linearly with time at the initial stages of the extraction process, limited by the solubility of solute in the supercritical CO_2. The extraction rate increased with pressure, best results were observed at 34 MPa and 328 K if no co-solvent was used. At those operating conditions a more efficient extraction with higher yield and less solvent consumption was observed when compared with multistage pressure extraction. Concluding extraction results are highly dependent on the characteristics of the plant material used to perform the extractions. *Cannabis Sativa* L. strains with low concentration of cannabinoids showed lower extraction rate and extract apparent solubility for the samples analyzed in this study ($\sim$6–17% plant material *potency*). The three consecutive separators of the biobotanical extractor allowed the recovery of fractions with different composition. Cannabinoid concentration (THC and THCA) in the collection vessels and variations throughout the extraction process with supercritical CO_2 have been reported. In general, more than 60% of the total accumulated mass was recovered from the first separator. The obtained extracts presented similar cannabinoid profile distribution. The cannabinoids extract with the highest

THC potency was found in the first separator at 13 MPa and 328 K in all experiments analyzed, being at least 1.5 times higher in concentration than material from the second separator. Based on the cannabinoids profile distribution of the first two separators, THCA was preferentially precipitated in the first separator over the whole extraction procedure for the analyzed samples.

When ethanol was applied to modify solvent polarity and enhance the extraction process the extraction rate was improved and a lower solvent to feed ratio was required to achieve high yields. A new extraction strategy was proposed and investigated in this work: the supply of co-solvent in the form of pulses. This pulse regime showed a better performance than the traditional co-solvent at constant concentration in the solvent flow, with major impact on plant material with low cannabinoid concentration. The pulse regime reaches same extraction efficiency with lower solvent and much lower co-solvent consumption at a shorter extraction time. From the results obtained in this study, the pulse regime is the most recommendable operating procedure to obtain cannabinoids extracts with high yields in the shortest time.

The results presented in this work are the basis to develop more efficient extraction strategies and separation procedures to obtain extracts with high cannabinoids concentration. This work demonstrates the effectiveness of the supercritical extraction of cannabinoids with CO_2 while suggesting potential new strategies for co-solvent supply.

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