Journal of CO_2 Utilization 28 (2018) 174–180

Contents lists available at ScienceDirect

Journal of CO_2 Utilization

journal homepage: www.elsevier.com/locate/jcou

Supercritical extraction strategies using CO_2 and ethanol to obtain cannabinoid compounds from *Cannabis* hybrid flowers

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ARTICLE INFO

Keywords:
Cannabis
Cannabinoid
Supercritical extraction
Decarboxylation
Winterization

ABSTRACT

The genus *Cannabis* contains specific substances called cannabinoids that are found in high concentrations in the flowers of non-pollinated female plants. Cannabidiol (CBD) and Δ^9-tetrahydrocannabinol (Δ^9-THC) are the two main bioactive substances in this group with medicinal potential. However, these substances are found in low concentrations in fresh flowers. The decarboxylation technique can be applied to fresh flowers to promote an increase in the levels of these substances. Extracts with high levels of cannabinoids and without organic solvent residues have high medicinal and cosmetic potential. The purpose of this work was to present the results of cannabinoid extraction using pressurized fluids from two varieties of flowers of the genus *Cannabis*. The extractions were conducted using pure supercritical carbon dioxide ($scCO_2$) and with ethanol as a co-solvent, comparing the use of decarboxylation and winterization techniques. The chemical profiles of cannabinoids (CBD, Δ^9-THC and cannabinol (CBN)) and the essential oils in the extracts and in fresh flowers were analyzed using different chromatographic techniques. The decarboxylation technique employed maximized the levels of the cannabinoids of interest. The Sovová model used in the adjustment of the experimental kinetic extraction curves was adequate and satisfactory.

1. Introduction

Cannabidiol (CBD) and Δ^9-tetrahydrocannabinol (Δ^9-THC) are the main cannabinoids with medicinal potential present in plants of the genus *Cannabis* [1–3]. However, the highest concentrations of cannabinoids in fresh flowers are cannabidiolic acid (CBDA) and Δ^9-tetrahydrocannabinoic acid (Δ^9-THCA). The transformation of these cannabinoid acids into their respective neutral cannabinoids, CBD and Δ^9-THC, is possible by a decarboxylation reaction [4–6]. This reaction is favored by several factors such as storage time [7], heating [8] and the use of alkaline conditions [7]. Controlled heating is the simplest technique used to promote decarboxylation and prevent the degradation of desirable cannabinoids [9]. Upon degradation, CBD can be converted to Δ^9-THC [10] and/or cannabielsoin (CBE) [11] and Δ^9-THC is converted to cannabinol (CBN) and/or Δ^8-tetrahydrocannabinol (Δ^8-THC) [12].

The CBD and Δ^9-THC that form during decarboxylation are non-polar and soluble in supercritical carbon dioxide ($scCO_2$) [13]. However, the waxes present in the flowers are also extracted by $scCO_2$. The removal of these waxes through the "winterization" process can generate a desirable increase in the concentration of the cannabinoids in the extract. Syntactically, this process consists of suspending the extract in n-hexane and then decanting the waxes by severe cooling [14].

The purpose of this work is to present results of cannabinoid extraction using pressurized fluids from two varieties of flowers of the genus *Cannabis*. The extractions were conducted using pure $scCO_2$ and with ethanol as a co-solvent, comparing the use of the decarboxylation and winterization techniques. The chemical profile of cannabinoids (CBD, Δ^9-THC and CBN) and the essential oils in the extracts and in fresh flowers were analyzed using different chromatographic techniques. The Sovová model was used for the adjustment of the experimental kinetic curves of extraction. The decarboxylation technique was evaluated to maximize the pre-extraction contents of the cannabinoids of interest.

The extraction conditions used in this work on *Cannabis* flowers with pressurized carbon dioxide were defined from the solubility data of CBD and Δ^9-THC in $scCO_2$, as described in the literature [14–16].

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https://doi.org/10.1016/j.jcou.2018.09.022
Received 22 May 2018; Received in revised form 21 August 2018; Accepted 27 September 2018

2. Materials and methods

2.1. Chemicals

Table 1 presents some information on the chemicals used in this work. All chemicals were used without further treatment.

2.2. Plant material

The flowers used were of two hybrid varieties. The "GSC" variety called "Girl Scout Cookies" (The Cali Connection) consists of 60% *C. sativa* and 40% *C. indica* with an estimated chemical composition of approximately 25% Δ^9-THC. The "DMII" variety called "Durga Mata II CBD" (Paradise Seeds) consists of 10% *C. sativa* and 90% *C. indica* with a chemical composition estimated at 6.5% CBD and 7.5% Δ^9-THC.

2.2.1. Milling

The flower samples were milled using a knife mill (Solab, SL-30). Samples were classified using sieves (Bertel) of 10, 14, 20, 28, 35, 48, 65 and 100 mesh. The mean particle diameter of the samples was calculated using the Sauter equation (Eq. (1)) [17]:

$$D_{Sauter} = \frac{1}{\sum_{i=1}^{n}\left[\frac{\Delta \varnothing_n}{\bar{D}_n}\right]} \quad (1)$$

where $\Delta\varphi$ is the sample fraction retained in the sieve and $\bar{D}$ is the average of the sieve diameters.

2.2.2. Moisture

The relative humidity of the samples (3 g) were determined in triplicate using a furnace (New Ethics, 400/4ND) with air circulation at 35 °C for approximately 20 h until the sample weight was constant.

2.2.3. Density

The density of the flower samples was determined in triplicate using a pycnometer (Quantachrome Instruments, 440-C Stainless Steel) of Helium gas at 20 psi.

2.2.4. Potential of cannabinoids

2.2.4.1. Calibration curves. The calibration curves for the concentrations of the cannabinoids CBD, Δ^9-THC and CBN were performed using six dilutions (0.001, 0.003, 0.005, 0.010, 0.050 and 0.100 mg mL^{-1}) in methanol using a Shimadzu HPLC 20 A with diode array detector at 220 nm and RP-8 column (SUPELCOSIL (TM): 250 x 4.5 mm, 5 µm) at 35 °C. The mobile phase used was a solution of acetonitrile and water (8:2 v/v) under isocratic conditions with a flow rate of 1 mL/min for 10 min [18]. The curves showed linearity (R^2) higher than 0.9999.

2.2.4.2. Decarboxylation. Samples of the flowers were heated at temperatures of 90, 110 and 140 °C for a period of three hours. The cannabinoid content of each sample was determined at the intervals of 30, 60, 120 and 180 min.

2.3. Organic solvent extraction

A 3 mL volume of a methanol: chloroform solution (9:1 v/v) was added to 150 mg of ground flower samples. The mixture was homogenized using an ultrasonic agitator (UltraCleaner, 1400 A) for 15 min at 37 Hz and 40 °C. Then, the mixture was centrifuged (CentriBio) for 10 min at 3000 rpm and the supernatant was collected. To determine the oil content in the sample without heating, the supernatant was dried in an oven (New Ethics, 400 /4ND) with air circulation at 35 °C for approximately 20 h until the sample weight was constant. For analysis of the cannabinoid composition, 200 µL of the supernatant was diluted in methanol to obtain a concentration of 1 mg of the solid sample used in 1 mL of solution [18].

2.4. Supercritical extraction

In order to potentiate the cannabinoids of interest (CBD and Δ^9-THC) two extraction strategies using supercritical carbon dioxide ($scCO_2$) pure and cosolvent were studied.

In the first technique, the samples were initially subjected to the decarboxylation process (heating) and in sequence subjected to the extraction process with $scCO_2$. In the second technique, the samples are extracted using $scCO_2$ and ethanol as a co-solvent.

Synthetically, the equipment used in the $scCO_2$ extractions consisted of a syringe pump (ISCO), a stainless steel extractor with a capacity of 58 mL (19.40 cm in height and 1.95 cm in diameter) and micrometric valves for depressurizing. The experimental extraction apparatus is described in several studies by our research group available in the literature [17]. The extraction conditions were defined from studies on the solubility of cannabinoids in $scCO_2$ [15,16].

2.4.1. With decarboxylation

The extraction experiments were conducted using 2 g of sample in each analysis at temperatures of 50, 60 and 70 °C and flow rate of 2.5 mL min^{-1}. The pressures used were 16.5, 20.7 and 24.9 MPa for the "GSC" variety and 12.8, 18.4 and 24.0 MPa for the "DMII" variety. The samples were submitted to the decarboxylation process at 140 °C for 30 min before extraction.

Table 1
Chemicals employed in this work.

Chemical	IUPAC Nomenclature	Molecular formula	Molar mass (g·mol^{-1})	Supplier (country)	Minimum purity[a] (%)
Carbon dioxide	Carbon dioxide	CO_2	44.01	Linde (Brazil)	99.99
Ethanol	Ethanol	C_2H_6O	46.07	PanReac (Brazil)	99.8
Acetonitrile	Acetonitrile	C_2H_6N	41.05	Sigma (Uruguay)	99.5
Water	Water	H_2O	18.02	Sartorius (Uruguay)	Ultra-pure
Helium	Helium	He	4.00	Linde (Brazil)	99.999
Chloroform	Chloroform	$CHCl_3$	119.37	Nuclear (Brazil)	99.8
Methanol	Methanol	CH_4O	32.04	PanReac (Brazil)	99.9
Hexane	Hexane	C_6H_{14}	86.18	PanReac (Brazil)	99.0
CBD	Cannabidiol	$C_{21}H_{30}O_2$	314.47	Grace Davison Discovery Science (United States)	99
Δ^9-THC	Δ^9-tetrahydrocannabinol	$C_{21}H_{30}O_2$	314.47		91
CBN	Cannabidiol	$C_{21}H_{26}O_2$	310.44		97.9

[a] Purities were provided by the manufacturers.