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## Microwave-assisted extraction of phytochemicals from *Cannabis sativa* L. inflorescences with 2-methyloxolane

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### ABSTRACT

2-methyltetrahydrofuran, also known as 2-methyloxolane (2-MeOx), was investigated for the extraction of phytochemicals from *Cannabis sativa* L. inflorescences. Dynamic maceration with 2-MeOx yielded comparable CBD (75.45 mg CBD/g DM) to ethanol (77.71 mg) and hexane (75.09 mg). The use of water-saturated 2-MeOx (4.5% water) increased the CBD recovery to 81.30 mg/g DM and the polyphenols content. 2-MeOx was also a good solvent for the recovery of hemp terpenes (87906–120485 mg/kg extract). Microwave-assisted extraction improved the CBD yield and shortened the time (by 3–30 times). Optimised microwave conditions resulted in higher CBD yields (84.18–86.76 mg CBD/g DM) compared to conventional extraction. Microwave-assisted decarboxylation-extraction with 2-MeOx was less effective than ethanol for CBDA decarboxylation (about 3–10 times lower). Finally, a single-mode microwave reactor for flow extraction was tested, achieving 75.48 mg CBD/g DM with 2-MeOx (3.65% water) at 60 °C in 10 min. Further optimisation is required for continuous microwave extraction with 2-MeOx.

### 1. Introduction

*Cannabis sativa* L. (family Cannabaceae), is a widespread plant species cultivated for various industrial uses, particularly for its medicinal properties and inedible fibre content (Valizadehderakhshan et al., 2021). *Cannabis sativa* L. is usually classified according to its  $\Delta^9$ -tetrahydrocannabinol (THC) content, including drug type, with a THC >0.3%, and industrial hemp, with a THC <0.3% (Liu et al., 2022; Pojić et al., 2014). The global hemp market has grown steadily over the last 5–10 years, fuelled by the therapeutic applications of cannabidiol (CBD) and other minor cannabinoids (Mazzara et al., 2022). The most common therapeutic indications for this plant are chemotherapy-induced nausea and vomiting, spasticity and seizure in multiple sclerosis, neuropathic pain, glaucoma, and sleep disorders (Casiraghi et al., 2018).

Cannabinoids are the most important bioactive compounds and are synthesised from cannabigerolic acid (CBGA) in the glandular trichomes of the female inflorescences of hemp (Liu et al., 2022). In addition to the approximately 125 types of cannabinoids, hemp is

**Abbreviations:** 2-MeOx, 2-Methyloxolane; CBD, cannabidiol; CBGA, cannabigerolic acid; DM, dried matrix; EI, electron-impact; EtOH, ethanol; IS, internal standard solution; L/S ratio, liquid to solid ratio; MADE, microwave-assisted decarboxylation-extraction; MAE, microwave-assisted extraction; MW, microwaves; SPME/GC-MS, gas chromatography and mass spectrometry; THC,  $\Delta^9$ -tetrahydrocannabinol.

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a rich source of non-cannabinoid phytochemicals such as flavonoids, alkaloids, phenols, and terpenes (AL Ubeed et al., 2022). THC and CBD are mainly present in their corresponding acidic and less pharmacologically active forms (THCA and CBDA) (Nuapia et al., 2021). Before extraction, these compounds are usually decarboxylated into their neutral form by heat treatment at temperatures above 100 °C, but below 230 °C, to avoid the formation of smoke toxins (Binello et al., 2023).

Extraction is the main step in the recovery of bioactive compounds from hemp inflorescences. Ethanol (EtOH), methanol, and hexane are the commonly used solvents for the recovery of cannabinoids, with EtOH proving to be more effective than other organic solvents in several studies (AL Ubeed et al., 2022). EtOH is considered a green solvent and GRAS (generally recognised as safe). However, its recovery is more energy-intensive than that of hexane due to the high latent heat of vaporisation (874 kJ/kg compared to 334 kJ/kg for hexane). In addition, the extraction of cannabinoids is significantly reduced by increasing the water content in EtOH (Szalata et al., 2022). Since EtOH is completely miscible with water, extractive distillation is required to obtain it in anhydrous form, which increases process costs.

Hexane has a good affinity for cannabinoids and is immiscible with water, so it does not need to be dehydrated after distillation and is suitable for the extraction of raw materials with an even higher moisture content (Song et al., 2023). However, like methanol, hexane is highly toxic and both solvents are classified as class 2 solvents in the guidelines of the European Medicines Agency (EMA ICH Q3C).

2-Methyltetrahydrofuran, also known as 2-methyloxolane (2-MeOx), is a food-grade bio-based solvent derived from lignocellulosic biomass (e.g., corncobs and sugarcane bagasse). The lignocellulosic biomass is first treated with sulphuric acid to obtain pentose and hexose sugars. The sugar mixture then undergoes several acid-catalysed reactions to produce levulinic acid (C<sub>5</sub>H<sub>8</sub>O<sub>3</sub>) and furfural (C<sub>5</sub>H<sub>4</sub>O<sub>2</sub>) (Hayes et al., 2005). After hydrogenation and dehydration reactions, 2-MeOx is obtained, which is then separated by distillation (Rapinel et al., 2020). The cradle to gate life cycle analysis showed that the use of 2-MeOx in an industrial scenario leads to a 97% reduction in emissions compared to solvents produced by conventional chemical means (Slater et al., 2016). 2-MeOx has a safer toxicological profile than hexane and was added to the list of permitted solvents for food and feed production in Europe on January 2023 (Directive, 2009/32/EC) (EUR-Lex). Moreover, 2-MeOx offers significant advantages over EtOH, including a lower latent heat of vaporisation (364 kJ/kg compared to 874 kJ/kg) and only a partial miscibility with water. These properties substantially lower the costs associated with solvent distillation and dehydration.

There is evidence that 2-MeOx is one of the most promising solvents for replacing petrochemical solvents in the extraction of oil seeds (Rapinel et al., 2020). Among these, hemp seeds are increasingly attracting consumer attention as one of the most nutritionally complete food sources (Wang et al., 2018; Tang et al., 2006). A recent study proved for the first time that 2-MeOx is a valuable solvent for the extraction of hemp seed oil. 2-MeOx was able to quantitatively extract the oil (< 0.4% of residual oil content), which also exhibited high quality properties (Cravotto et al., 2024). In addition, this solvent has already been tested on a semi-industrial and industrial scale (Rapinel et al., 2020; Bartier et al., 2024).

Due to the chemical-physical properties of 2-MeOx, which lie between those of hexane and EtOH (miscibility with water, partition coefficient, dipole moment), 2-MeOx could be a good solvent for the extraction of cannabinoids. This study investigates how effective 2-MeOx is in the extraction of CBD, terpenes, and polyphenols from hemp inflorescences.

Various conventional methods are still used today, such as dynamic maceration and Soxhlet extraction, but the need for long extraction times, considerable amounts of solvents and often many extraction steps has favoured the development of alternative technologies (Bitwell et al., 2023). Microwave- and ultrasound-assisted extraction, supercritical CO<sub>2</sub>, deep eutectic solvents, pressurised fluid extraction and liquid butane have been used as alternatives to conventional methods for the extraction of cannabinoids (Liu et al., 2022; Qamar et al., 2021; Brighenti et al., 2017; Nahar et al., 2021). Recently, Lustenberger et al. reviewed the advances in CBD extraction and purification, highlighting the benefits and limitations of both traditional and innovative techniques (Lustenberger et al., 2022).

Microwave-assisted extraction (MAE) in particular offers a number of advantages: rapid heating, shorter process times, lower solvent consumption, higher extraction rates and higher yields (Gunjević et al., 2021). Indeed, microwaves (MW) can penetrate the plant matrix and generate heat inside the cell to facilitate its disruption and increasing the dissolution of the target compounds in the extraction solvent (Xie et al., 2014). Dielectric heating strongly favours the solvation of the plant tissue, thus improving the extraction kinetics (Binello et al., 2023). The yield and selectivity of cannabinoids recovered with MAE depend on the following parameters: type of solvent, irradiation time and power, temperature, liquid/solid ratio and surface contact area (Valizadehderakhshan et al., 2021; Sagili et al., 2023; Addo et al., 2022a; Drinić et al., 2020a). Radoiu et al. described an example of MAE on an industrial scale in a continuous-flow extractor with a production capacity of more than 200 kg/h biomass input, which can work with different solvents (e.g. EtOH, PAH, pentane, PEG400) (Radoiu et al., 2020). It has also been reported that pressurised MAE systems can be used for the simultaneous extraction and decarboxylation of cannabinoids (Lewis-Bakker et al., 2019). Only a few studies have investigated the use of MW in extraction with 2-MeOx. Positive results have been reported for the extraction of avocado oil and palm seeds oil (Chimsook, 2017; Ben-Youssef et al., 2017). In both cases, MAE improved the yield compared to Soxhlet extraction and maceration and significantly reduced the extraction time.

Herein, the efficient extraction of cannabinoids from hemp inflorescences using MAE as an innovative technique and 2-MeOx as green solvent is reported. In addition, the effects of solvent hydration, irradiation power and extraction time were investigated and optimised using response surface methodology. Preliminary results on MAE flow extraction in a new monomodal reactor are also reported.

## 2. Materials and methods

### 2.1. Solvents and standards

EtOH (ACS grade,  $\geq 99\%$ ), *n*-hexane (ACS grade,  $\geq 97\%$ ) and 2-methyloxolane (ACS grade,  $\geq 99\%$ ) used for cannabinoid extraction, acetonitrile (ACS grade,  $\geq 99\%$ ) used for HPLC analysis, and certified CBD standard in methanol (ACS grade,  $\geq 99\%$ ) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Milli-Q H<sub>2</sub>O was obtained in the laboratory using a Milli-Q Reference A + System (Merck Millipore, Darmstadt, DE, USA). Information on 2-MeOx quality were provided by the supplier: purity (GC, area%)  $\geq 99.0\%$ , density (d 20 °C/4 °C) 0.848–0.858 g/mL, free acid (as CH<sub>3</sub>COOH)  $\leq 0.002\%$ , peroxide (as H<sub>2</sub>O<sub>2</sub>)  $\leq 0.01\%$ , water content (Karl Fischer)  $\leq 0.1\%$ , identity (IR) passing the relevant test.

### 2.2. Plant material and sample preparation

*Cannabis sativa* L. inflorescences were kindly provided by the company Società Agricola F.lli Podimani Ss (Ragusa, Italy) with the certificate of analysis for THC content ( $< 0.3\%$ ). The inflorescences were decarboxylated in an oven at 130 °C for 30 min covered with aluminium foil. The raw material was then finely ground in a laboratory blender (Waring Commercial, CT, USA) and stored in a sealed glass container at room temperature.

### 2.3. Conductor-like screening model for real solvents (COSMO-RS)

The conductor-like screening model for real solvents (COSMO-RS) was used to predict the solubilities of main cannabinoids in EtOH, *n*-hexane, 2-MeOx and 2-MeOx 4.5% water. Parameters such as  $\sigma$ -surface area,  $\sigma$ -profile and  $\sigma$ -potential can be used to predict solvent compatibility for a given solute. Molecules structures and charge density surfaces are shown in Fig. S1. Calculations were performed using COSMOTermX software (18.0.2, COSMOlogic version GmbH & Co., Leverkusen, Germany). The standard quantum chemical method with polarised triple valence basis set (TZVP) was used, and the temperature chosen for solubility prediction was 25 °C (room temperature) and solvents boiling temperatures. The results were expressed as  $\log_{10}(x_{\text{solub}})$ , the logarithm of the molar fraction of the solute in the solvent. The closer the value of  $\log_{10}(x_{\text{solub}})$  is to zero, the greater the predicted solubility of the solute.

### 2.4. Conventional extraction

Hemp (1 g) was extracted in 50 mL of solvent (EtOH, *n*-hexane, 2-MeOx and 2-MeOx 4.5%) under reflux in a round bottom flask placed in an oil bath for 1 h under magnetic stirring. The oil bath was kept at 90 °C. The suspension was then cooled to room temperature, filtered through filter paper with a Buchner funnel and the solvent was removed under vacuum. A 10-min stream of nitrogen was used to remove residual solvent. The yield was calculated gravimetrically and expressed as g extract/100 g of dried matrix (DM). The extractions were carried out in triplicate and the results expressed as average  $\pm$  standard deviation (SD). The dry extracts were resuspended in absolute EtOH (10 mL), placed in an ultrasonic bath for 5 min, then centrifuged at 4200 rpm for 5 min and diluted with EtOH before HPLC analysis.

### 2.5. Microwave-assisted extraction (MAE)

The MW reactors used in this study are shown in Fig. S2. The MAE was performed in an ETHOS X (Milestone, Bergamo, Italy), a multimode MW reactor, with a maximum power of 1800 W (Fig. S2B). All extractions were performed in a 250 mL round-bottomed flask under magnetic stirring (100%) connected to a condenser, using a fixed liquid to solid (L/S) ratio of 50 mL/g as in conventional extraction. To investigate the influence of the key process parameters, 17 extracts were prepared under different extraction conditions (solvent water content, irradiation power and extraction time) according to the experimental design presented below. For each extraction, the sample was mixed with 2-MeOx at the desired water content (dry, 2.25% and 4.5% w/w water). The total extraction time was 2, 11 or 20 min and the MW power was set to 300, 650 or 1000 W. The extracts were filtered through filter paper with a Buchner funnel. The solvent was removed under vacuum and a 10-min nitrogen stream removed the residual solvent.

### 2.6. Single step microwave-assisted decarboxylation-extraction (MADE)

The raw inflorescences were freeze-dried and then cryomilled in a laboratory blender (Waring Commercial, CT, USA) to obtain a powder. The matrix (0.5 g) was weighed into MW vials (35 mL); the solvent (25 mL) and a magnetic stir bar were added, then the vial was capped. The microwave-assisted decarboxylation-extraction (MADE) was performed in a SynthWAVE reactor (Milestone, Bergamo, Italy), as shown in Fig. S2A. The vials were placed in a 1 L pressure-resistant PTFE cavity (up to 200 bars) equipped with a 5-position vials rack. This reactor can reach a high-power density (1.5 kW/L) with the possibility of external inert gas supply (N<sub>2</sub>). For each test, N<sub>2</sub> purging was performed three times to remove oxygen from the system. The reaction chamber was then pressurised with N<sub>2</sub> (20 bars) to prevent solvent evaporation. The samples were heated at 150 °C with a maximum irradiation power of 1500 W. The temperature was maintained for 20 or 60 min with magnetic stirring at 325 rpm. The suspension was filtered through filter paper and the solvent was removed under vacuum. Each extraction was performed in triplicate. An extract of the raw cryomilled inflorescences was prepared using EtOH as solvent at 40 °C for 1 h under conventional heating and magnetic stirring. The CBDA content in this EtOH extract was considered to be the maximum content in the inflorescence. The decarboxylation rate of CBDA by MADE was calculated using the ratio of the corresponding chromatograms areas divided by the mass of the extracted matrix, as shown in the equation below (Eq. (1)):

$$\text{CBDA conversion (\%)} = (R_{\text{max}} - R_x) \cdot 100 / R_{\text{max}} \quad \text{Eq. 1}$$

where  $R_{\max}$  is the ratio between the area of CBDA and the extracted matrix (g) with EtOH as solvent at 40 °C;  $R_x$  is the ratio between the area of CBDA and the extracted matrix (g) with different solvents using MADE.

### 2.7. MicroChem SAIREM reactor

The MicroChem reactor (SAIREM, Décines-Charpieu, France) was used for the flow extraction of hemp inflorescences. MicroChem is a single-mode MW reactor that can operate in both batch and flow mode (Fig. S2C). The output power is up to 200 W with a 1 W step and a power rise time of less than 1 ms. The reactor software detects the reflected power and the MW absorption in the reactor cavity can be adjusted via a screw near the coaxial MW cable connector. The temperature was measured directly in the reactor using an optical fibre. Extractions were carried out at ambient pressure at a temperature of 60 °C to avoid excessive evaporation of the solvent. All tests were performed under temperature control using 2-MeOx 3.65% water and oven-decarboxylated inflorescences. A condenser was installed above the irradiation chamber to recover solvent vapours. A Masterflex L/S Easy-Load II peristaltic pump, Model 77200–52 (Masterflex SE, Gelsenkirchen, Germany), was used to ensure a constant flow. Extraction was carried out at a flow rate of 100 mL/min to ensure homogeneous mixing of the biomass. The extraction time was calculated from the residence time of the mixture in the MW irradiated section, which corresponds to 40 mL. The total volume of the system was 80 mL, so that the mixture was irradiated for 1 min every 2 min. After 10 min of MW irradiation, the system was emptied, the extract filtered and distilled. Yield, cannabinoids and polyphenols were quantified.

### 2.8. Total phenolic content determination

Total phenolic content of the extracts was determined by the *Folin-Ciocalteu* method (Gunjević et al., 2021). 250 µL of the extract solution in EtOH was diluted accordingly and added to a test tube containing 4 mL of deionised water. Sodium carbonate solution (10%, w/v) and *Folin-Ciocalteu* reagent (diluted 1:1 with deionised water) were added successively. The resulting solution was mixed thoroughly and stored at room temperature in a dark place. After 25 min, the absorbance was measured at 725 nm using a Cary 60 UV–vis spectrophotometer (Agilent Technologies, USA). Gallic acid was used as a standard. The polyphenol content was expressed as mg of gallic acid equivalents on the dry matrix (mg GAE/g DM). All analyses were performed in triplicate and the results expressed as the average ± SD.

### 2.9. Experimental design

The Box-Behnken design consisted of 17 experiments with three variables at three levels (−1, 0 and 1) and five replicates at the central point. The ranges of the variables, namely 2-MeOx water content (0–4.5% w/w of water), irradiation power (300–1000 W) and extraction time (2–20 min), were selected to assess the optimal extraction conditions. The water content range was chosen based on the solubility limit of water in 2-MeOx, with 4.5% w/w water (water-saturated) as the upper limit. The power range was selected considering the operational limits of the MW system. Both time and power ranges align with values reported in previous studies on MAE of hemp inflorescences (Addo et al., 2022b; Drinić et al., 2020b).

Statistical analysis was performed using Response Surface Methodology (RSM) and Design-Expert version 13 (Design-Ease, Inc., Minneapolis, MN, USA). The actual and coded values of the independent variables are shown in Table 1.

Three output variables (responses) were analysed: yield, CBD content, CBD selectivity. The model used in the RSM was the second-order polynomial equation shown below (Eq. (2)):

$$Y = \beta_0 + \sum_{i=1}^3 \beta_i X_i + \sum_{i=1}^3 \beta_{ii} X_i^2 + \sum_{i < j=1}^3 \beta_{ij} X_i X_j \quad \text{Eq. 2}$$

where  $Y$  is the estimated response and the expressions  $\beta_0$ ,  $\beta_i$ ,  $\beta_{ii}$  and  $\beta_{ij}$  are the equation constant ( $y$ -intercept) and the regression coefficients for linear, quadratic and interaction terms respectively. The results were statistically tested by analysis of variance (ANOVA). The adequacy of the models was evaluated by the coefficient of determination ( $R^2$ ), the coefficient of variance (CV) and the  $p$ -value for the model and lack-of-fit testing.

### 2.10. Cannabinoids analysis

Identification and quantification of CBD was performed using a HPLC binary pump 1525 linked to a 2998 photodiode array detector (PDA) and a 2707 automatic sampler (Waters Corp., Milford, CT, USA). A Kinetex C18 column (5.0 µm, 150 mm × 4.6 mm i.d.) with a guard column (0.5 µm depth filter × 0.1 mm) (Phenomenex, Torrance, CA, USA) was used, with 0.1% HCOOH in Milli-Q H<sub>2</sub>O (A) and 0.1% HCOOH in acetonitrile (B) as mobile phases. The gradient elution was modified as follows: 0–4 min 60% B, 4–16 min

**Table 1**  
Actual and coded values of independent variables.

Independent variables	Unit	Symbol	Levels		
			Low (−1)	Middle (0)	High (1)
Water	% w/w	A	0	2.25	4.5
Power	W	B	300	650	1000
Time	min	C	2	11	20

from 60% to 100% B, which was kept for 10 min. The equilibration time was 12 min. The flow rate was 0.8 mL/min. The sample injection volume was 20  $\mu$ L. The UV three-dimensional data were acquired in the range 200–450 nm, while the monitored wavelengths were 210 nm (for decarboxylated cannabinoids) and 220 nm (for cannabinoic acids) (Brighenti et al., 2017). The calibration curve was prepared with a standard solution of CBD in EtOH in the range of 6.25–200  $\mu$ g/mL. The calibration curve was linear in the concentration range analysed ( $R^2 > 0.999$ ).

### 2.11. Terpenes analysis

The terpenes were analysed by solid phase microextraction in combination with gas chromatography and mass spectrometry (SPME/GC-MS), according to the method recently published by Boffa et al. (2024). GC analyses were performed using an Agilent 6850 gas-chromatograph equipped with a split/splitless injector and an SPME injector liner (0.75 mm ID) coupled to an Agilent 5973 N Mass Selective Detector (MS). The installed capillary column was a Mega-5MS 5% Phenyl Methyl (length 30.0 m, ID 0.25 mm, film thickness: 0.25  $\mu$ m, MEGA S.r.l., Legnano, Italy). SPME was performed with a Supelco DVB/CAR/PDMS fibre with a length of 1 cm and a film thickness of 50/30  $\mu$ m (fused silica 24 Ga, gray). An internal standard solution (IS) was prepared by weighing 2-undecanol (approx. 10 mg) in a vial and then adding the sunflower oil used for the terpenes (approx. 5 g). The concentration of the final solution was 1.92 mg/g IS in sunflower oil. To ensure that the aromatic profile of the oil did not interfere with the IS and cannabis terpenes, a blank sample was analysed. Samples were prepared by adding 0.2 g of the IS solution to ~10/20 mg of extracts in a headspace vial. Analyses were performed in triplicate and the results expressed as the average  $\pm$  the SD. Samples were equilibrated for 10 min at 40  $^{\circ}$ C with magnetic stirring, and the SPME fibre was then exposed to the sample headspace for 40 min at 40  $^{\circ}$ C with magnetic stirring. Finally, the fibres were desorbed at the inlet of the GC oven at 270  $^{\circ}$ C for 5 min (sample injection). The analysis was performed with the following programmed elution temperature: 60  $^{\circ}$ C for 2 min, ramp from 5  $^{\circ}$ C/min to 275  $^{\circ}$ C, held for 5 min. Inlet: split mode with a split ratio of 5:1, temperature of 270  $^{\circ}$ C, carrier gas helium with a constant flow of 1.2 mL/min. Average velocity of 40 cm/s. Mass detector: temperature of 300  $^{\circ}$ C, MS Source 230  $^{\circ}$ C, MS Quad 150  $^{\circ}$ C. Detection mode: Scan. Resulting EM Voltage: 1612. Mass range: 50–500. Compounds were considered positively identified if the electron-impact (EI) mass spectra matched the Wiley7n and NIST11 libraries with a minimum quality of 90%. The identification of  $\alpha$ -pinene,  $\beta$ -pinene, limonene,  $\gamma$ -terpinene,  $\alpha$ -terpinolene, L-fenchone, linalool, D- $\alpha$ -fenchyl alcohol, L-borneol,  $\alpha$ -terpineol, *trans*- $\beta$ -caryophyllene,  $\alpha$ -humulene and  $\beta$ -eudesmol was based on standard retention times (Cannabis terpene mix A and B, Certified reference material from Sigma-Aldrich), while  $\beta$ -myrcene, terpinen-4-ol,  $\alpha$ -copaene,  $\alpha$ -farnesene,  $\alpha$ -bergamotene, *trans*- $\beta$ -farnesene, aromadendrene,  $\gamma$ -muurolene,  $\beta$ -selinene, valencene,  $\alpha$ -selinene,  $\beta$ -bisabolene, isodene,  $\beta$ -guaiane,  $\alpha$ -gurjunene,  $\alpha$ -bisabolene,  $\gamma$ -selinene, selina-3,7(11)diene, guaiol,  $\delta$ -selinene, and  $\gamma$ -eudesmol were identified by library comparison. Semi-quantitative analysis of terpenes in the analysed extracts was performed based on the amount of 2-undecanol (mg) used as IS using the following formula (Eq. (3)):

$$\mu\text{g FC} = \mu\text{g IS} \cdot \text{Area FC}/\text{Area IS} \quad \text{Eq. 3}$$

Quantitative results were expressed as mg/kg extract.

### 2.12. Statistical analysis

Prior to applying statistical tests, the normality of data distribution was assessed using the Shapiro-Wilk test.

All results not related to the experimental design were analysed by one-way ANOVA. Multiple comparison of means (where applicable) was carried out using Tukey's HSD test at 5% level of significance and results were presented as mean and standard deviation.

## 3. Results and discussion

### 3.1. COSMO-RS preliminary theoretical evaluation

COSMO-RS is a powerful tool for the molecular description and screening of solvents based on a quantum chemical approach. In this work, COSMO-RS was used to predict the solubility of the main cannabinoids of hemp inflorescences in four solvents: EtOH, *n*-hexane and 2-MeOx (dry and water saturated solvent). Cannabidiol and  $\Delta^9$ -tetrahydrocannabinol in both their neutral (CBD, THC) and acidic forms (CBDA, THCA) were selected for theoretical calculations.

The results are presented in Table 2, expressed as  $\log_{10}(x_{\text{solub}})$ , the logarithm of the mole fraction of the solute in the solvent. The solubility was predicted at two different temperatures: at room temperature (25  $^{\circ}$ C) and at the boiling temperature of the solvent. The latter is the temperature reached during reflux extraction.

**Table 2**

COSMO-RS predicted solubility at 25  $^{\circ}$ C and at solvents boiling point of major cannabinoids in EtOH, *n*-hexane, 2-MeOx and 2-MeOx 4.5% water.

Cannabinoid	EtOH		<i>n</i> -Hexane		2-MeOx		2-MeOx 4.5% water	
	25 $^{\circ}$ C	BP	25 $^{\circ}$ C	BP	25 $^{\circ}$ C	BP	25 $^{\circ}$ C	BP
CBDA	-1.69	-0.94	-4.71	-2.88	-0.61	-0.30	-0.74	-0.41
CBD	-0.56	-0.37	-3.30	-1.98	0.00	0.00	0.00	0.00
THCA	-2.07	-1.22	-3.61	-2.18	-1.12	-0.58	-1.26	-0.73
THC	-1.98	-1.25	-2.23	-1.26	-0.48	-0.16	-0.76	-0.41

Results are expressed as: solubility index,  $\log_{10}(x_{\text{solub}})$ ; BP, boiling point: 80  $^{\circ}$ C (EtOH), 69  $^{\circ}$ C (*n*-hexane), 80  $^{\circ}$ C (2-MeOx), 71  $^{\circ}$ C (2-MeOx 4.5% water).



Values of  $\log_{10}(x_{\text{solub}})$  closer to zero correspond to a higher predicted solubility. The cannabinoids studied had a higher theoretical solubility in 2-MeOx, both dry and 4.5% water saturated. The temperature of 71 °C was chosen for 2-MeOx 4.5% water, because this is the boiling point of the minimum boiling azeotrope 2-MeOx/water. As shown in Table 2, the solubility of all tested cannabinoids increased with increasing temperature. This result is consistent with the experimental results, as presented below. Furthermore, a comparison of the different solvents showed that 2-MeOx (both dry and 4.5% water) is theoretically a better solvent for CBD, CBDA, THC and THCA than hexane and EtOH. In addition, 2-MeOx appears to be an ideal solvent for CBD, which has a  $\log_{10}(x_{\text{solub}})$  value of zero. These preliminary results are an important first achievement to determine the efficiency of 2-MeOx in the extraction of cannabinoids from hemp inflorescences.

### 3.2. Conventional extraction: solvent comparison

In this work, 2-MeOx was studied as an alternative green solvent for the extraction of hemp inflorescences. 2-MeOx has a slight miscibility with water, unlike hexane. This affects the entire industrial process, because after condensation and decantation (in the presence of a steam stripper), the mixture separates into an organic phase saturated with 4.5% water (at 55 °C). To obtain dry 2-MeOx, an additional distillation step is required, which increases the energy costs. To mitigate these additional costs, extraction using the water-saturated form of 2-MeOx (containing 4.5% water) was also explored.

In general, more polar solvents are preferable to non-polar solvents such as hexane, especially for the extraction of cannabinoid acids (Antunes et al., 2023). Conventional extraction of cannabinoids by dynamic maceration was more efficient with alcohols (MeOH and EtOH) than with less polar solvents such as acetone, MeOH/CHCl<sub>3</sub> 9:1 (v/v) and hexane (Brighenti et al., 2017). EtOH proved to be the most suitable solvent for cannabinoids solubilization, especially during hot maceration (Brighenti et al., 2017; Drinić et al., 2020a; Isidore et al., 2021). In addition, solvent selection and proper decarboxylation are crucial aspects to produce cannabis extracts with reproducible pharmacological activity (Moreno-Sanz et al., 2020).

In this study, dynamic maceration at room temperature resulted in lower CBD recovery than reflux extraction for both EtOH (73.25 vs. 77.71 mg CBD/g DM) and 2-MeOx (73.73 vs. 75.45 mg CBD/g DM). Therefore, dynamic maceration under reflux was chosen as conventional method.

2-MeOx afforded a CBD yield and selectivity comparable to that of anhydrous EtOH ( $p > 0.05$ ), as shown in Fig. 1. The extraction yield and CBD yield were higher with 2-MeOx 4.5% water (26.26 g extract/100 g DM; 81.30 mg CBD/g DM). Hexane resulted in a lower extraction yield (17.58 g extract/100 g DM) and CBD yield (75.09 mg CBD/g DM), which is consistent with the literature. However, the CBD selectivity with hexane was the highest among the studied solvents. This aligns with hexane's non-polar nature, which favours the extraction of non-polar compounds like cannabinoids while limiting the extraction of more polar compounds, such as polyphenols. The dried extracts obtained by conventional maceration under reflux are shown in Fig. S3.

Beyond extraction yields, energy efficiency and production costs are critical factors in determining the industrial competitiveness of various solvents. The price of 2-MeOx ranges from 7 to 9 €/kg, while food-grade hexane costs 0.8–1.0 €/kg (Rapinel et al., 2020), and EtOH 0.9–1.1 €/kg (Passos et al., 2014). However, economic simulations for industrial oil extraction show that 2-MeOx can compete with hexane in terms of cost under optimised recycling and process conditions (Rapinel et al., 2020). In fact, solvents are generally recycled with minimal losses in optimised systems, typically below 1 kg per ton of extracted biomass (Rapinel et al., 2020). Fur-

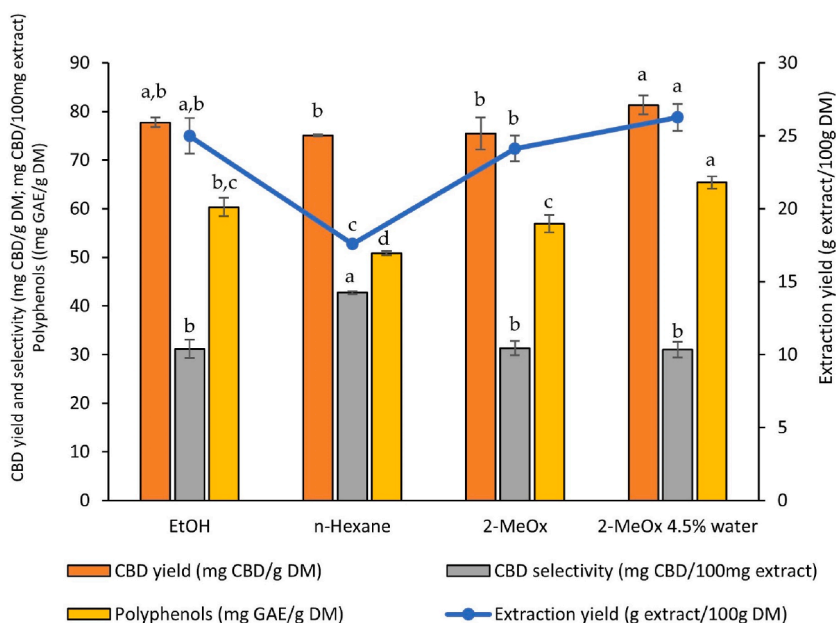


Fig. 1. Conventional extraction: yield, CBD yield and selectivity. CBD, cannabidiol; Means in the same row with different superscript letters are significantly different ( $p \leq 0.05$ ).

thermore, the main advantage of 2-MeOx over hexane is its safer toxicological profile and its bio-based origin (Chemat et al., 2022), which could lead consumers to accept a slightly higher price. EtOH-based extraction is generally more expensive than hexane, primarily due to higher energy demands for solvent distillation and the need for greater solvent volumes to achieve efficient extraction (Potrich et al., 2020). From an energy perspective, 2-MeOx offers significant benefits over EtOH, including a lower enthalpy of vaporisation and only partial miscibility with water, reducing costs associated with solvent distillation and dehydration (Bartier et al., 2024). For these reasons, CBD production with 2-MeOx is expected to be economically and energetically competitive compared to other solvents; however, further studies are still required.

Hemp is also an excellent source of valuable polyphenols (Brkljača et al., 2023). 2-MeOx 4.5% water resulted the most efficient solvent in the extraction of polyphenols (65.40 mg GAE/g DM), followed by EtOH (60.31), 2-MeOx (56.89) and finally hexane (50.82). Several studies have reported that 2-MeOx can extract a considerable amount of phenolic compounds from plants (black cumin seeds, basil seeds, olive pomace and soya flakes) in addition to the oil fraction (Bourgou et al., 2021; Claux et al., 2021; Cravotto et al., 2022). Furthermore, the extraction of these compounds is significantly improved by increasing the water content in the solvent.

Overall, dynamic maceration with 2-MeOx extracts a comparable amount of CBD as ethanol and hexane. In addition, the use of water-saturated 2-MeOx (4.5% water) increases the recovery of CBD and polyphenols.

Previous publications have identified about 120 terpenes in cannabis, namely 61 monoterpenes (C10 skeleton), 51 sesquiterpenes (C15 skeleton), 2 diterpenes (C20 skeleton), 2 triterpenes (C30 skeleton) and 4 miscellaneous compounds (Radwan et al., 2021). Terpenes are responsible for the characteristic aroma of the plant. Common terpenes found in cannabis inflorescence include limonene,  $\beta$ -myrcene,  $\alpha$ - and  $\beta$ -pinene,  $\alpha$ -humulene,  $\beta$ -caryophyllene, and the terpenoids linalool and  $\alpha$ -bisabolol (Giovannoni et al., 2023). The terpene profile of the dry extracts obtained by conventional extraction was analysed by SPME/GC-MS. Chromatographic profile is shown in Fig. S4 and the results are reported in Table 3.

**Table 3**  
Terpenes content (SPME/GC-MS analysis) of extracts obtained by conventional extraction.

Compound	EtOH		<i>n</i> -Hexane		2-MeOx		2-MeOx 4.5% water	
	Area %	mg/kg extract	Area %	mg/kg extract	Area %	mg/kg extract	Area %	mg/kg extract
$\alpha$ -pinene	NF	NF	1.2	1081	1.5	1354	0.7	842
$\beta$ -pinene	NF	NF	0.8	737	0.9	764	0.5	588
$\beta$ -myrcene	1.9	1180	14.2	12763	14.9	13065	8.1	9794
Limonene	2.3	1283	8.3	7409	6.5	5686	3.4	4104
L-fenchone	1.2	706	1.2	1025	0.5	407	NF	NF
Linalool	12.9	7692	11.9	10497	6.9	6108	3.7	4427
D- $\alpha$ -fenchyl alcohol	4.9	2866	3.3	2886	2.2	1927	1.1	1368
L-borneol	2.6	1582	1.8	1607	1.4	1260	0.8	958
Terpinen-4-ol	0.7	430	0.7	627	0.4	340	0.2	244
$\alpha$ -terpineol	7.2	4470	6.0	5245	4.4	3862	3.1	3683
$\alpha$ -copaene	NF	NF	0.3	235	0.3	259	0.2	266
$\alpha$ -farnesene	NF	NF	NF	NF	0.3	239	0.2	302
<i>trans</i> - $\beta$ -caryophyllene	28.0	18345	20.4	17734	27.4	24141	23.9	28865
$\alpha$ -bergamotene	2.5	1668	2.1	1787	2.7	2372	2.9	3478
<i>trans</i> - $\beta$ -farnesene	1.4	934	1.3	1094	1.8	1561	2.5	2985
$\alpha$ -humulene	7.8	5200	5.2	4547	8.9	7851	8.1	9780
Aromadendrene	0.9	587	0.8	684	NF	NF	0.9	1146
$\gamma$ -muurolene	1.0	795	0.8	701	1.0	881	1.1	1369
$\beta$ -selinene	1.4	973	1.1	950	1.3	1102	1.3	1622
Valencene	1.3	874	1.0	869	1.2	1083	1.5	1807
$\alpha$ -selinene	1.8	1246	1.4	1187	1.3	1170	1.6	1996
$\beta$ -bisabolene	4.8	3285	3.6	3076	4.3	3815	5.3	6413
Isoledene	1.4	964	NF	NF	NF	NF	NF	NF
$\beta$ -guaiene	NF	NF	1.0	876	NF	NF	2.2	2689
$\alpha$ -gurjunene	NF	NF	NF	NF	NF	NF	0.6	676
$\alpha$ -bisabolene	6.7	4840	5.3	4508	5.0	4363	11.3	13667
Selina-3,7(11)-diene	NF	NF	NF	NF	NF	NF	6.9	8111
Guaiol	2.1	1423	1.9	1634	1.8	1610	2.1	2477
$\delta$ -selinene	2.8	1872	2.4	2090	NF	NF	2.5	3017
$\gamma$ -eudesmol	NF	NF	NF	NF	1.6	1421	0.8	1024
$\beta$ -eudesmol	3.8	1170	2.8	2001	1.4	1266	2.3	2788
<i>Terpenes classes</i>								
Monoterpenes	4.2	2463	24.5	21990	23.8	20869	12.7	15327
Monoterpenoids	29.5	17745	24.8	21886	15.8	13903	8.9	10679
Sesquiterpenes	61.8	41582	46.5	40339	55.5	48837	73.1	88189
Sesquiterpenoids	6.0	2593	4.7	3635	4.9	4298	5.3	6290
Total		64384		87851		87906		120485

Values are expressed as relative percentage areas and mg/kg of extract. NF, not found.



*Trans*- $\beta$ -caryophyllene was the major compound in all extracts (20–28%), followed by  $\beta$ -myrcene, the main monoterpene in all extracts (8–15%) except the EtOH one (2%), linalool (3.7–13%),  $\alpha$ -bisabolene (5–11.3%),  $\alpha$ -humulene (5.2–8.9%), limonene (2.3–8.3%) and  $\alpha$ -terpineol (3.1–7.2%). These results are consistent with the literature (Isidore et al., 2021). All extracts showed a dominance of sesquiterpenes, that accounted for the 46.5–73.1% of the total terpenes. Pieracci et al. found that sesquiterpenes, both in hydrocarbon and oxygenated forms, represented the main compound class in 11 genotypes of *Cannabis sativa* L. (Pieracci et al., 2021).

$\beta$ -myrcene and limonene were the main monoterpenes detected, ranging from 1200 to 13000 mg/kg, while  $\alpha$ - and  $\beta$ -pinene were present in lower amounts (590–1350 mg/kg). As concerns monoterpenoids, linalool (4400–10500 mg/kg) and  $\alpha$ -terpineol (3700–5200 mg/kg) were the main compounds, together with L-fenchone, D- $\alpha$ -fenchyl alcohol and L-borneol in a lower quantity (1200–2900 mg/kg). The major sesquiterpenes were *trans*- $\beta$ -caryophyllene (18000–28000 mg/kg) and  $\alpha$ -humulene (4500–9800 mg/kg), which is consistent with Ascrizzi et al. (2020) and Menghini et al. (2021), as they are typical compounds of hemp cultivars. In addition,  $\alpha$ - and  $\beta$ -bisabolene were also present in high amounts (3000–4800 mg/kg), with the 2-MeOx 4.5% water extract showing the highest content (13667 and 6413 mg/kg, respectively). The amount of selinene-derived compounds ( $\alpha$ -selinene,  $\beta$ -selinene) was relatively high, ranging from 950 to 2000 mg/kg, while selina-3,7(11)diene was detected only in the 2-MeOx 4.5% water extract in a very high amount (8111 mg/kg). Oxygenated sesquiterpenes represented the minority class of terpenes in all extracts (4.9–6.0% of the total), with guaialol,  $\beta$ - and  $\gamma$ -eudesmol ranging from 1170 to 2800 mg/kg. These secondary metabolites are degradation products formed by the oxidation of the corresponding terpenes in air and are thought to be responsible for the antioxidant effect of many essential oils (Pieracci et al., 2021).

In general, the extract obtained with 2-MeOx 4.5% water showed a higher total terpenes content (120485 mg/kg), followed by the extracts in 2-MeOx and *n*-hexane (around 87906 and 87851 mg/kg, respectively). EtOH led to a lower recovery of terpenes (64384 mg/kg). In addition, the compound profile of the different extracts was different. *n*-Hexane and 2-MeOx afforded a higher recovery of monoterpenes in both absolute (21990 and 20869 mg/kg, respectively) and relative amounts (24.5 and 23.8%, respectively), mainly  $\beta$ -myrcene and limonene. Similarly, in an earlier study, the hexane extract contained a higher relative amount of monoterpenes than EtOH and the hexane-EtOH (7:3, v/v) extracts (Namdar et al., 2018). These differences could be due to the nature of the solvent and its different polarity. In addition, the evaporation process can considerably influence the recovery of monoterpenes by solvent extraction. Namdar et al. showed that drying methods using vacuum concentrators and rotary evaporators lead to the loss of significant amounts of monoterpenes (Namdar et al., 2018). In this study, the extracts were concentrated using a rotary evaporator and a gentle stream of nitrogen was used to remove residual solvent. EtOH has a higher enthalpy of vaporisation than hexane and 2-MeOx (874, 334 and 364 kJ/kg, respectively), so more energy is required for distillation (Rapinel et al., 2020; Abernathy et al., 2023). This aspect can have a negative effect on the recovery of more volatile terpenes. For sesquiterpenes, similar results were obtained with hexane, EtOH and 2-MeOx with a recovery of sesquiterpenes between 40339 and 48837 mg/kg extract. 2-MeOx 4.5% water yielded about twice the sesquiterpenes content compared to the other solvents tested. Sesquiterpenoids made up less than 6% of the total terpenes.

To summarise, preliminary studies on conventional extractions have shown that 2-MeOx is an effective solvent for the extraction of cannabinoids and polyphenols from hemp. 2-MeOx resulted in a similar or higher CBD yield than ethanol and hexane. It is also a very efficient solvent in its water-saturated form. These findings, coupled with the safer toxicological profile of 2-MeOx compared to hexane and its lower distillation costs relative to EtOH, position 2-MeOx as a promising solvent for the hemp inflorescence extraction industry. In addition, a higher recovery of terpenes can be achieved with both dry and water-saturated 2-MeOx.

Finally, extract stability is a crucial factor affecting product quality and shelf life. Several studies investigated the stability of cannabinoids in cannabis extracts (Kanabus et al., 2021). Cannabinoid stability is mainly influenced by light, temperature and oxygen availability (Lindholst, 2010). Moreover, the stability of acidic and neutral cannabinoids differs, with the acidic species being more susceptible to degradation. The choice of solvent is also crucial; for example, CBD is highly stable in methanolic and ethanolic extracts at low temperatures, but degrades rapidly in aqueous solutions (Fraguas-Sánchez et al., 2020). Based on previous findings, the stability of 2-MeOx extracts can be improved by promptly evaporating the solvent to eliminate any presence of water (water is distilled as 2-MeOx/water azeotrope, 10.6% water at 71 °C). Storing the dried extract in airtight, opaque containers at low temperatures (5 °C or below) will minimize exposure to light, oxygen, and heat. Additionally, the co-extraction of antioxidants using 2-MeOx may further enhance the extract's protection against oxidative degradation (Fraguas-Sánchez et al., 2020).

### 3.3. Microwave-assisted extraction (MAE): experimental design

Conventional extraction techniques are simple and can be carried out with affordable equipment, but have several disadvantages, especially long extraction times (AL Ubeed et al., 2022). Moreover, the treatment at high temperatures for a longer time during conventional extraction (e.g. Soxhlet) can accelerate the degradation of certain cannabinoids, such as THC, to cannabinol (CBN) (Wianowska et al., 2015). MAE has proven to be a viable alternative to conventional methods for cannabinoids extraction (AL Ubeed et al., 2022). The most important parameters in MAE are solvent polarity, extraction time, irradiation power, temperature, and contact surface area (Valizadehderakhshan et al., 2021). In this study, MAE was tested using 2-MeOx as solvent and optimised for the extraction of CBD. The influence of three key parameters (2-MeOx water content, irradiation power and extraction time) was analysed using a Box-Behnken experimental design, as reported in Table 4.

In this study, the CBD yield obtained with MAE was in the range of 79.46–88.18 mg CBD/g DM. The highest CBD yield was using 2-MeOx with 2.25% water, an irradiation power of 1000 W, and an extraction time of 20 min. The extraction selectivity, expressed as percentage of CBD in the extract, was between 29.04 and 40.43 mg CBD/100 mg extract. The highest extraction selectivity was

**Table 4**  
Box–Behnken experimental design and responses for extraction yield, CBD yield and selectivity.

Run	Water (%)	Power (W)	Time (min)	Yield (g extract/100 g DM)	CBD yield (mg CBD/g DM)	CBD selectivity (mg CBD/100 mg extract)
1	0	300	11	22.65	83.92	37.06
2	4.5	300	11	25.61	84.68	33.06
3	0	1000	11	20.79	84.05	40.43
4	4.5	1000	11	25.36	83.60	32.96
5	0	650	2	20.99	80.31	38.26
6	4.5	650	2	24.44	80.42	33.53
7	0	650	20	22.30	81.26	38.70
8	4.5	650	20	27.89	80.99	29.04
9	2.25	300	2	24.13	85.08	35.25
10	2.25	1000	2	24.04	79.46	32.58
11	2.25	300	20	25.37	83.28	32.83
12	2.25	1000	20	24.69	88.18	35.72
13	2.25	650	11	24.68	82.99	34.78
14	2.25	650	11	24.50	83.33	34.00
15	2.25	650	11	24.46	81.87	33.48
16	2.25	650	11	23.62	82.85	35.08
17	2.25	650	11	24.96	84.25	32.82

CBD, cannabidiol.

achieved with dry 2-MeOx at an irradiation power of 1000 W and an extraction time of 11 min. The results of the analysis of variance (ANOVA) of the experimental data and the test of statistical significance of the model terms are shown in Table S1.

All three models were significant ( $p \leq 0.05$ ). The  $p$ -values for lack of fit were not significant ( $p > 0.05$ ), indicating that the models were reliable and suitable for predicting the responses. The results of ANOVA showed that the linear and quadratic effects of 2-MeOx water content (A and  $A^2$ ) and the linear terms of extraction time (C) on extraction yield were significant ( $p \leq 0.05$ ). Based on the significance of the process parameters, the mathematical equation describing the extraction yield model is presented in Table 5.

The mathematical equation describing the extraction yield model resulted in an  $R^2 = 0.8815$ . The linear term of 2-MeOx water content (A) and time (C) show a positive influence on the extraction yield. This means that an increase in these parameters increases the yield, as shown in Fig. 2(A–C).

The linear term of the extraction time (C), the interaction of power and time (BC) and all quadratic terms ( $A^2$ ,  $B^2$ ,  $C^2$ ) have a significant influence ( $p \leq 0.05$ ) on the CBD yield. The interaction between power and time (BC) had the highest coefficient (2.63), showing that the combination of increased power and extended extraction times greatly enhances CBD extraction efficiency. The mathematical equation describing the CBD yield model resulted in an  $R^2 = 0.9033$ .

For the CBD selectivity, the linear and quadratic terms of 2-MeOx water content (A and  $A^2$ ), and the interaction of power and time (BC) have a significant influence ( $p \leq 0.05$ ). The mathematical equation describing the CBD selectivity model resulted in an  $R^2 = 0.8885$ . Factors with high coefficients indicate a higher level of influence on the variable (Soroush et al., 2021). According to Table 5, the 2-MeOx water content had the highest coefficient (−3.23) and consequently the higher negative effect on the response. This means that by increasing the solvent water content, the CBD selectivity was reduced (Fig. 2G–I). This is consistent with the higher extraction yields obtained by increasing the water content in the solvent, which is likely due to the extraction of other compounds besides cannabinoids, probably polyphenols and phospholipids (Esmailzadeh Kenari and Dehghan, 2020). Contour plots showing the combined effects of the studied parameters are shown in Fig. S5.

Overall, the solvent water content showed a complex impact, with higher water content increasing the overall extraction yield but reducing CBD selectivity, likely due to the co-extraction of non-cannabinoid compounds. MW power showed significant non-linear effects, with higher power levels improving CBD yield and selectivity but only when combined with optimised extraction times, as shown by the significant interaction between power and time. This highlights the importance of fine-tuning the extraction parameters to balance yield and selectivity for optimal cannabinoid extraction.

### 3.4. Microwave-assisted extraction (MAE): optimisation

The desirability function was used to optimise multiple responses, as it is one of the simplest and most popular approaches (Weremfo et al., 2023). The model was used to predict the optimal MAE conditions for maximum extraction yield, and maximum CBD

**Table 5**  
Designed equation models for the studied responses.

Responses	Equation	$R^2$	$R^2$ -adj	CV
Extraction Yield	$Y = 24.5 + 2.07A + 0.8315C - 0.7425A^2$	0.8815	0.8542	18.12
CBD yield	$Y = 83.05 + 0.0197A - 0.2077B + 1.05C + 2.63BC - 1.12A^2 + 2.13B^2 - 1.19C^2$	0.9033	0.8280	12.86
CBD selectivity	$Y = 34.06 - 3.23A + 0.4375B - 0.4176C + 1.39BC + 1.32A^2$	0.8885	0.8215	11.97

$R^2$ -adj,  $R^2$ -adjusted; CV, coefficient of variation.

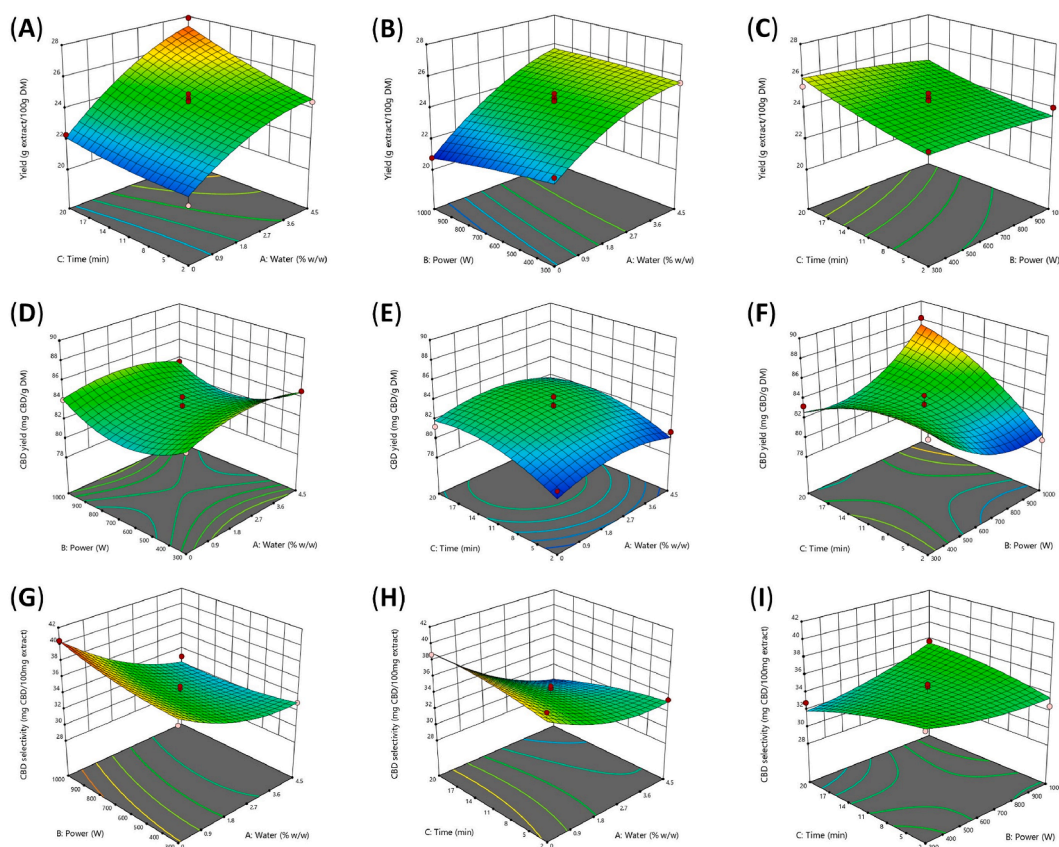


Fig. 2. 3D response surface plots showing the combined effects of 2-MeOx water content (% w/w), MW power (W) and extraction time (min) for MAE on extraction yield (A–C), and CBD yield (D–F) and selectivity (G–I).

yield and selectivity. The estimated optimal conditions and the predicted values of the studied responses are shown in Table 6. Finally, three experiments were performed using the optimal extraction conditions to validate the model.

The optimal MAE conditions with the highest desirability (option 1) were the use of 2-MeOx with 1.72% water, a power of 1000 W and an extraction time of 20 min. The desirability value was 0.699. The RSEs for the optimal conditions were less than 5% and the actual values were in good agreement with the predicted ones. However, these conditions required the highest MW power and extraction time. For this reason, a second, less energy-demanding option with lower desirability (0.568) was also studied, to reduce the MW power and extraction time. This alternative involved the use of 2-MeOx with a higher water content (3.65% water), a power of 300 W and an extraction time of 2 min. Although the yields were slightly lower compared to the first option, these conditions can lead to significant energy savings, while maintaining a high CBD yield and selectivity. As can be seen, a small increase in the water content (from 1.72 to 3.65%) leads to a significant reduction in extraction time and irradiation power, which is probably due to a higher MW absorption.

An overview of the results obtained with conventional extraction and optimised MAEs is presented in Table S2. For both optimised MAEs, the results for CBD yield were significantly higher compared to conventional extraction with EtOH, hexane and 2-MeOx ( $p \leq 0.05$ ), with the exception for 2-MeOx 4.5% water ( $p > 0.05$ ) for the second option. The CBD selectivity in the extracts was also

Table 6  
Predicted and actual response values for the optimal extraction conditions.

Option	Values	Desirability	Water (%)	Power (W)	Time (min)	Yield (g extract/100 g DM)	CBD yield (mg CBD/g DM)	CBD selectivity (mg CBD/100 mg extract)	Polyphenols content (mg GAE/g DM)
1	Predicted	0.699	1.72	1000	20	24.79	87.41	36.60	Not predicted
	Experimental		1.72	1000	20	23.97 ± 0.71	86.76 ± 1.82	36.20 ± 0.31	60.81 ± 1.38
	RSE (%)					3.42	0.75	1.10	
2	Predicted	0.568	3.65	300	2	24.67	85.36	34.70	Not predicted
	Experimental		3.65	300	2	23.72 ± 0.89	84.18 ± 1.37	35.51 ± 0.76	60.50 ± 0.14
	RSE (%)					3.99	1.40	2.29	

RSE, relative standard error.

significantly increased ( $p \leq 0.05$ ) compared to conventional extraction with EtOH, 2-MeOx and 2-MeOx 4.5% water. In addition, extraction times were reduced by up to 30 times with MAE.

The optimised MAE yielded a similar polyphenol content (60.81 mg GAE/g DM) as the conventional EtOH extraction (60.31 mg GAE/g DM), with an intermediate value between that obtained with 2-MeOx (56.89 mg GAE/g DM) and 2-MeOx 4.5% water (65.40 mg GAE/g DM).

In conclusion, the use of a MW reactor significantly improved CBD extraction yield (86.8–84.2 mg CBD/g DM) and selectivity (36.2–35.5 mg CBD/100 mg extract) compared to conventional extraction, and shortened extraction times.

Finally, the theoretical energy consumption for MAE optimised conditions was evaluated. Calculations were based on the extraction of 1 kg of matrix using 50 L of solvent, with results expressed in kWh/kg CBD (see Table 7). Option 1, which uses a higher power and longer extraction time, achieves the highest CBD yield, but at a significantly higher energy consumption (3.84 kWh/kg CBD). In contrast, option 2 required less power and a shorter extraction time, which drastically reduced energy consumption to 0.12 kWh/kg CBD. Overall, the MW energy requirement for option 2 was around 32 times lower than for option 1, while the CBD yield was comparable.

Additionally, the energy requirements for solvent distillation were calculated to estimate the kWh demand for distilling 50 L of 2-MeOx (both 1.72% and 3.65% water). For comparison, the energy demand for distilling an equivalent quantity of EtOH and hexane are also provided (Fig. 3). The calculations were carried out using the latent heat of vaporisation of solvents. The initial distillation temperatures were set as follows: 70 °C for 2-MeOx, 65 °C for hexane, and 75 °C for EtOH. The results indicate that distilling 50 L of 2-MeOx 1.72% water requires 4.9 kWh, slightly lower than that required for 2-MeOx 3.6% water (5.3 kWh), due to its lower water content.

However, a simulation of a continuous distillation process, including steam stripping and solvent regeneration, suitable for industrial production, showed a lower energy consumption for 2-MeOx 3.65% water. This was mainly due to the lower energy needs for solvent dehydration (see Supporting Information, Fig. S6). On the other hand, if further purification steps are required, steam stripping distillation could be avoided.

Among tested solvents, hexane showed the lowest energy demand, requiring only 3.2 kWh. In contrast, EtOH (96% and anhydrous) exhibited significantly higher distillation costs, reaching up to 10.1 kWh, due to its higher latent heat of vaporisation. This substantial difference highlights the energy-intensive nature of EtOH distillation compared to hexane and 2-MeOx. In conclusion, extraction with 2-MeOx is expected to significantly lower process costs for solvent recovery compared to EtOH protocol.

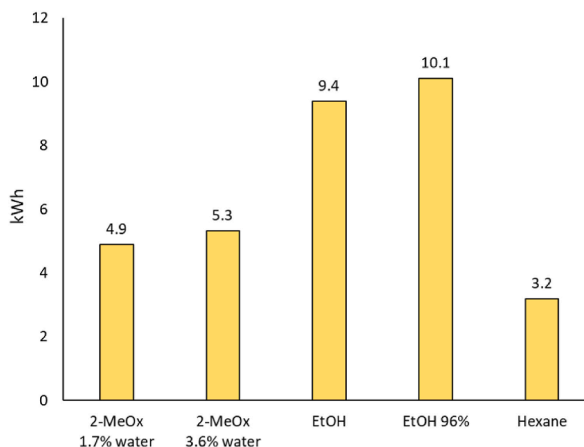
### 3.5. Single step microwave-assisted decarboxylation-extraction (MADE)

Cannabinoids are biosynthesised in the plant in acidic forms, the so-called acidic cannabinoids. These compounds must be converted into their neutral forms by a decarboxylation reaction to be therapeutically effective (Addo et al., 2021). During conventional decarboxylation, the hemp is subjected to a heating process before extraction (> 130 °C) (Binello et al., 2023). Decarboxylation in an oven at a certain temperature and for a certain time is relatively simple on a laboratory scale but becomes increasingly difficult with several tonnes of biomass (Radoiu et al., 2020). In addition, uncontrolled heating can cause combustion, affect terpenes profile, or

**Table 7**  
MW energy consumption of optimised conditions.

Option	Water (%)	Power (W)	Time (min)	MW energy (kWh)	CBD yield (g CBD/kg DM)	Energy demand (kWh/kg CBD)	Energy factor
1	1.72	1000	20	0.333	86.76	3.84	32.3
2	3.65	300	2	0.010	84.18	0.12	

Note: calculations were conducted assuming the extraction of 1 kg of matrix, with a L/S ratio of 50 L/kg.



**Fig. 3.** Energy requirement for solvents distillation: kWh for 50 L of solvent.

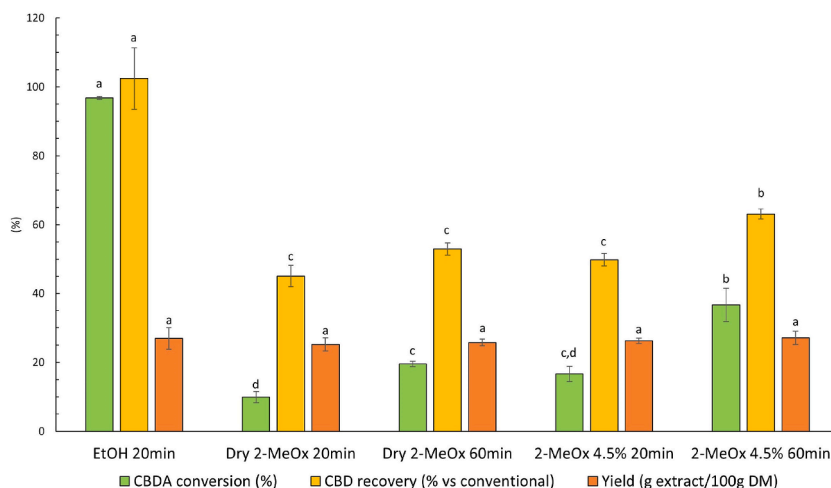


Fig. 4. Microwave-assisted decarboxylation-extraction (MADE) at 150 °C under pressure: CBDA conversion percentage, CBD recovery compared to conventional extraction, extraction yield.

have other undesirable effects that could reduce the quality or purity of the cannabis extract. For example, the decarboxylation process can lead to oxidative degradation or isomerisation of the cannabinoids (García-Valverde et al., 2022). A single decarboxylation and extraction step could reduce time and operational costs. Since the efficiency of MAE with 2-MeOx as solvent has been demonstrated, the possibility of simultaneously achieving decarboxylation and extraction directly in the MW reactor was investigated. MADE was performed in a pressurised MW reactor at a temperature of 150 °C for 20 min, as shown in Fig. 4. Lewis-Bakker et al. demonstrated that using EtOH under these conditions resulted in an extraction yield of 19.6–24.4% and a complete decarboxylation of the acidic cannabinoids (THCA and CBDA) into their respective neutral forms (THC and CBD) (Lewis-Bakker et al., 2019).

Accordingly, MADE with EtOH resulted in an extraction yield of  $26.90 \pm 3.14$  g extract/100 g DM and an almost complete decarboxylation of CBDA (~97%) to CBD. Chromatograms are shown in Fig. S7. The CBD yield was  $79.60 \pm 6.93$  mg CBD/g DM, which represents a quantitative recovery of CBD compared to conventional extraction with the same solvent. These results are consistent with previously published results (Lewis-Bakker et al., 2019). MADE with 2-MeOx (both dry and 4.5% water) resulted in a less efficient decarboxylation of CBDA than with EtOH. After 20 min of reaction time, the conversion of CBDA to CBD was only 10% with 2-MeOx and 16.7% with 2-MeOx 4.5% water. Consequently, the percentage recovery of CBD was less than 50% compared to conventional extractions. One possible explanation could be the slower decarboxylation kinetics in 2-MeOx. For this reason, MADE was carried out under the same conditions for 60 min. Extending the reaction time resulted in a significant increase in CBDA decarboxylation and CBD recovery with 2-MeOx 4.5% water, while this increase was not significant with the dry solvent. However, even with 2-MeOx 4.5% water, CBDA conversion (36.6%) and CBD recovery (63.1%) were lower.

In conclusion, the decarboxylation of CBDA was less efficient with 2-MeOx than with ethanol. Therefore, conventional decarboxylation before or after extraction with 2-MeOx is preferable. Further research is needed to investigate the chemical mechanisms behind the low decarboxylation rate with 2-MeOx, likely influenced by water contents, which can be partly due to the lower polarity of this solvent compared to EtOH, resulting in higher CO<sub>2</sub> solubility (Aigner et al., 2020).

### 3.6. Single-mode batch and continuous flow MAE

The MAE of hemp inflorescences was investigated using a single-mode MW reactor (MicroChem SAIREM, Décines-Charpieu, France) in batch and flow mode. Batch experiments were performed at 60 °C at two different times and L/S ratios. This temperature was chosen with a view to the future transposition into flow mode, to avoid solvent evaporation during the loop process.

2-MeOx with 3.65% of water was selected because it showed a high CBD yield at lower MW power and irradiation time, as above reported. Results are shown in Table 8.

Batch extractions yielded ~80 mg CBD/g DM for all tested conditions. These results account for approximately the 92% of CBD recovery compared to the optimised extraction conditions obtained for the MAE multimode system (86.78 mg CBD/g DM). Only slight increases in the CBD yield and selectivity were observed at 10 min compared to 5 min of extraction time. Moreover, there was not sig-

Table 8  
Single-mode MAE with 2-MeOx 3.65% water at 60 °C, batch and flow extraction.

Extraction setup	Time (min)	L/S ratio (mL/g)	CBD yield (mg CBD/g DM)	CBD selectivity (mg CBD/100 mg extract)
Batch	5	50	$79.83 \pm 0.37$	$32.80 \pm 0.15$
	10	50	$80.72 \pm 2.53$	$34.50 \pm 1.08$
	5	20	$79.93 \pm 2.48$	$32.75 \pm 1.02$
	10	20	$80.52 \pm 3.25$	$33.28 \pm 1.38$
Loop flow	10	50	$75.48 \pm 0.58$	$25.19 \pm 2.40$



nificant difference in the CBD recovery at the L/S ratio of 20 compared to 50. This is an interesting result, as when scaling up a process, reducing the use of solvents significantly reduces the energy required for distillation.

For this reason, a flow extraction with a L/S ratio of 20 at 60 °C was tested. The flow rate was kept constant using a peristaltic pump with adjustable speed. However, after a few min, the swelling of the biomass resulted in a less homogeneous flow, and partial accumulation of biomass at the pump inlet was observed. For this reason, L/S ratio was increased to 50. Under these conditions (loop flow), a recovery of approx. 75.5 mg CBD/g DM in 10 min at 60 °C was achieved (87–90% of CBD recovery under optimised batch MAE). Despite these results are promising, the difficult suspension of the matrix by peristaltic pumping was probably the main reason for this lower result compared to batch extraction. In fact, it was only possible to achieve homogeneity of the solid-solvent mixture at high flow rates. For this reason, it was not possible to test flow-through extraction, as the residence time in the reactor was too short at high pumping rates. In our opinion, future optimisation of the pump system and/or the addition of an in-flow agitation device may further improve the process.

#### 4. Conclusions

The extraction of phytochemicals from *Cannabis sativa* L. using green solvents and alternative technologies is a hot topic. In this context, the extraction efficiency of 2-MeOx for the recovery of CBD, terpenes, and polyphenols from hemp inflorescences was investigated. 2-MeOx extracted a similar amount of CBD (75.45 mg CBD/g DM) compared to EtOH (77.71 mg) and hexane (75.09 mg). In addition, the use of a water-saturated solvent (2-MeOx 4.5% water) resulted in the highest recovery of CBD (81.30 mg CBD/g DM), polyphenols and terpene compounds.

Furthermore, the use of a MW reactor significantly improved CBD extraction yield (86.8–84.2 mg CBD/g DM) and selectivity (36.2–35.5 mg CBD/100 mg extract) compared to dynamic maceration, and shortened extraction times (by 3–30 times).

Single step decarboxylation-extraction in a pressurised MW reactor was also investigated. However, the decarboxylation of CBDA was less efficient with 2-MeOx than with ethanol. Therefore, conventional decarboxylation before or after extraction with 2-MeOx is preferable.

Finally, a new single-mode MW reactor for flow extraction was tested. Approximately 75.48 mg CBD/g DM was extracted in flow-loop mode with 2-MeOx (3.65 % water) at 60 °C in 10 min, with a CBD recovery accounting for 87–90% of the amount obtained with the batch-optimised multimode MAE. Despite these results are promising, future optimisation of the pump system and/or the addition of an in-flow agitation device may further improve the process.

#### CRedit authorship contribution statement

**Christian Cravotto:** Writing – original draft, Validation, Data curation, Conceptualization. **Giorgio Grillo:** Writing – review & editing, Methodology, Investigation. **Luisa Boffa:** Writing – original draft, Validation, Methodology, Investigation. **Anne-Sylvie Fabiano-Tixier:** Writing – review & editing, Writing – original draft, Supervision. **Mickaël Bartier:** Writing – review & editing, Formal analysis. **Laurence Jacques:** Writing – review & editing, Conceptualization. **Silvia Tabasso:** Writing – review & editing, Supervision, Conceptualization.

#### Notes

The authors declare no competing financial interest.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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#### Data availability

Data will be made available on request.

#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scp.2024.101812>.



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