Sustainable Chemistry and Pharmacy 36 (2023) 101303

Contents lists available at ScienceDirect

Sustainable Chemistry and Pharmacy

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

A new prototype reactor for the fast microwave-assisted decarboxylation and extraction of cannabinoids in olive oil from *Cannabis* inflorescences

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ARTICLE INFO

Handling editor: Fabio Aricò

The authors dedicate this manuscript to the memory of the late Prof. Farid Chemat, who was a creative scientist, invaluable colleague and sincere friend.

Keywords:
Cannabis
Microwave reactor prototype
Phytocannabinoid decarboxylation
Microwave-assisted extraction
Olive oil

ABSTRACT

Several different *Cannabis*-based pharmaceutical preparations have been approved for medical treatments. Standardization to ensure specified phytocannabinoid contents and to maintain the integrity of the product is therefore required. Olive oil is clearly a safe and edible solvent suitable for the galenic preparation of ready-to-use *Cannabis* extracts. Pharmacists can use several non-standard procedures for this purpose, most of which involve the heat treatment of the plant matrix (i.e. the female inflorescences) for 2 h. In order to allow the "neutral" and more biologically active forms of the major phytocannabinoids to be extracted, prior thermal treatment of the plant matrix is desirable. This work investigates a new prototype microwave (MW) reactor (Ethos Lean) specifically designed for the decarboxylation of the acidic cannabinoids in *Cannabis* inflorescences and their subsequent extraction into oil. Both of the steps to produce the oily extract were carried out in the Ethos Lean's cavity with special accessories; a rotating drum for the decarboxylation process and a glass reactor with a stirrer for the extraction step. The variables considered in the process optimization were time and temperature, according to which the instrument automatically calibrated the power output. In collaboration with a hospital pharmacy, the efficiency of the device has been evaluated by comparing the results obtained with those of exhaustive decarboxylation in a conventional oven and ethanol extraction. A comparison was also made with conventional procedures in olive oil. Thanks to the rotating drum, which is sensitive to dielectric heating, the complete and homogeneous decarboxylation of phytocannabinoids was rapidly achieved (30 min, 120 °C), even with discrete quantities of inflorescences (150 g) and without releasing the characteristic intense odor into the laboratory. The exhaustiveness (100% yields of both CBD and THC) of MW-assisted extraction process in olive oil was achieved with 30 min irradiation at 90 °C. Furthermore, the possible decarboxylation occurring during MW-assisted extraction in oil and the effect of cooling time on the final yields have also been evaluated.

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https://doi.org/10.1016/j.scp.2023.101303
Received 14 August 2023; Received in revised form 23 September 2023; Accepted 7 October 2023
Available online 21 October 2023

1. Introduction

Cannabis sativa L. is a plant belonging to the genus *Cannabis*, a dicotyledonous angiosperm plant family of *Cannabaceae*. In recent years, there has been increasing interest in the potential therapeutic effects of this plant, particularly with regard to the treatment of neuropathic pain, chemotherapy-induced nausea and vomiting, multiple sclerosis (spasticity and seizures), glaucoma and sleep improvement (Casiraghi et al., 2018). This importance in the medical field is mainly attributed to the content of numerous secondary metabolites, including terpenes, phenolic compounds and especially phytocannabinoids. The latter are mostly present in the plant's trichomes, thin, hair-like outgrowths with a defensive function in female inflorescences (Appendino et al., 2011). It is worth noting that a so-called "entourage effect" in the raw extract has been described in the pool of other secondary metabolites (Anand et al., 2021), although the few existing preclinical and clinical studies show contradictory results and, to date, there is a lack of solid evidence to support the existence of such an effect (Christensen et al., 2023).

The main phytocannabinoids, Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD), are present in the plant in their corresponding acid and less pharmacologically active forms (THCA and CBDA, respectively), meaning that it is necessary to convert them into their decarboxylated and commonly named "neutral" structures before their extraction from the plant matrix. For this purpose, heat treatment capable of performing the decarboxylation step must be carried out at a temperature above 100 °C, but below 230 °C, to avoid the formation of smoke toxins (Veress et al., 1990; Nahar et al., 2021). The extraction of these types of compounds is performed via various conventional (e.g., ethanol maceration and Soxhlet extraction) and non-conventional methods, such as supercritical CO_2, pressurized fluid extraction (PFE), liquid butane, deep eutectic solvents, ultrasound and microwaves (MW) (Brighenti et al., 2017; Chang et al., 2017; Qamar et al., 2021; Valizadehderakhshan et al., 2021; Addo et al., 2022; Liu et al., 2022). In addition, an *in situ* decarboxylation pressurized liquid extraction system that achieves selective CBD extraction, while suppressing THC amount has been described (Nuapia et al., 2021).

The volumetric dielectric heating used in the microwave-assisted extraction (MAE) of plant material is one of the most efficient non-conventional energy sources used for the recovery of bioactive natural products (Chemat and Cravotto, 2013). The vibrational energy absorbed by the water, salts and other polar compounds in the matrix leads to an increase in intracellular pressure, which results in the rupture of cell walls and the release of metabolites of interest into the solvent. MAE enables the extraction of both essential oils and non-volatile compounds, as reported by Ferreira et al. (2020). Dielectric heating strongly promotes the solvation of plant tissues and extraction kinetics are consequently improved (Xie et al., 2014). The final extraction yield is essentially a function of the following parameters; matrix/solvent ratio, solvent choice, extraction time, irradiation power, temperature and contact surface area (Routray and Orsat, 2012; Addo et al., 2022). This technology has numerous advantages over conventional heating methods, particularly in terms of time savings and energy efficiency, as it can dramatically reduce the temperature gradient between the outside and the inside of the treated matrices (Valizadehderakhshan et al., 2021). Some studies have reported applications of MAE for *C. sativa*, particularly in obtaining CBD-enriched essential oils (Fiorini et al., 2020; Micalizzi et al., 2021; Mazzara et al., 2022). An example of continuous-flow MAE has been proposed as a means to facilitate the intensification of the extraction process of raw ground *Cannabis* biomass using solvents such as ethanol, isopropanol, pentane and PEG400 with the prospect of industrial-scale protocols (Radoiu et al., 2020). Moreover, MAE processes have been reported to result in the decarboxylation of cannabinoids (Lewis-Bakker et al., 2019), and the use of dielectric pre-heating of plant material prior to conventional extraction or distillation techniques has been demonstrated to have a favorable effect (Fiorini et al., 2019). We have recently reported an efficient pilot-scale MAE protocol for the sequential recovery of a terpene-rich hydrodistillate, an enriched polyphenol fraction from the undistilled water, and phytocannabinoids, with a high degree of decarboxylation (Gunjevic et al., 2021).

The medical and therapeutic applications of *Cannabis* extracts require products whose main active ingredients (especially THC and CBD) are precisely dosed and whose extraction can be performed in highly efficient and reproducible processes. Since it has been estimated that up to 60% of the phytocannabinoids present in the female inflorescence may be lost during extraction (and possible subsequent purification step), the process applied to the plant matrix clearly plays a key role in the overall economy of the medical *Cannabis* market and thus requires the highest technological control, including that of the raw material, which must be certified and is often difficult to obtain (Rosenthal, 2014).

Currently, besides the commercially available cannabinoid-containing products approved for medical use, whether synthetic or derived from *Cannabis* extracts (e.g., Marinol, Sativex, Cesamet, Epidiolex) (Giacoppo et al., 2017; Badowski and Yanful 2018; Nielsen et al., 2018), there are also galenic preparations that pharmacists can prepare based on medical prescriptions. The latter case can involve several processes in which the dried female inflorescences are extracted in olive oil or ethanol (both pharmaceutical grade), with or without previously undergoing decarboxylation (Citti et al., 2016; Calvi et al., 2018). To date, however, there is still no standardized protocol for oily magistral preparations of *Cannabis* intended for medical use, with procedures being carried out essentially at home based on data published in the literature. Although the chemical structure of phytocannabinoids makes it possible to obtain their exhaustive extraction from dried inflorescences with ethanol (Giese et al., 2015), the use of vegetable oil (e.g., olive oil) as an extraction solvent may be considered a more environmentally friendly option. With regard to alternative protocols for obtaining *Cannabis*-based galenic products, preliminary results on very rapid MAE extraction in olive oil have recently been reported (De Vita et al., 2020).

In this piece of work, a new prototype of MW reactor (*Ethos Lean* by Milestone srl), specifically designed for the decarboxylation of *Cannabis* inflorescences and their extraction in olive oil (pharmaceutical grade), has been investigated, in collaboration with the hospital pharmacy at the San Luigi Gonzaga University Hospital Company (Orbassano, Turin, Italy). The first set of trials were carried out on industrial *Cannabis* (Seedlution Srl), which is easily available in the market, and was then followed by tests on medicinal *Cannabis* FM2 (Stabilimento Chimico Farmaceutico Militare, FI, Italy).

The aims of these new process and equipment for *Cannabis*-based preparations focus on time and energy savings, as well as the versatility of the application, which is principally due to the relatively large range of plant amounts that can be processed (5 g–150 g). The novel heating drum, where homogenous cannabinoid decarboxylation also occurs when large volumes of matrix are employed is worthy of note.

Our data have been compared, by applying previously optimized protocols, with those from matrix decarboxylation in a laboratory oven and exhaustive extraction in hot ethanol. Samples obtained from the conventional procedure were also mentioned in the discussion.

Process yields, in terms of the THCA, THC, CBDA and CBD content in the obtained samples, and the achievement of decarboxylation were verified using UPLC-MS/MS analysis.

The stability of the olive oil that was subjected to MAE with the decarboxylated inflorescences has been verified by ^{1}H NMR, and the shelf-life of the oily extract has been evaluated up to six months.

2. Materials and methods

2.1. Plant material

Two distinct types of dried inflorescences from hemp were employed for the phytocannabinoid extraction process: *C. sativa* for industrial use, variety Seedlution n.3 (8% CBD, 0.2% THC), produced by Seedlution Srl (Saluggia - VC, Italy); *C. sativa* for medical use FM2 (5–8% THC and 7.5–12% CBD), CINRO variety, produced by the Stabilimento Chimico Farmaceutico Militare in Florence (Italy) according to EU regulations, GMP certified and grown in GACP by cloning, in indoor greenhouses and with the germination method reported in the DM 9/11/15.

2.2. Reagents, solvents and instruments

HPLC-grade ethanol, HPLC-grade methanol, HPLC-grade acetonitrile, Trifluoroacetic acid ≥99.5% for HPLC, MilliQ Water, Acetone for analysis 99.5%, Ph. Eur. grade olive oil. Certified CBD standard in ethanol solution (50 mg/ml, 99.9%) (Lipomed AG Arlesheim Switzerland). Certified THC standard in ethanol solution (100 mg/ml, 99.7%) (Lipomed AG Arlesheim Switzerland). UPLC analysis using the Acquity TQD LC/MS/MS system (Waters Corporation, Milford, MA, USA). ^{1}H NMR analysis using a Jeol ECZ-R Spectrometer, 600 MHz (JEOL Ltd., Tokyo Japan).

2.3. Prototype of MW reactor (Ethos Lean)

The Ethos Lean (Milestone Srl, Sorisole, BG, Italy) is a professional MW oven equipped with a 1 kW magnetron and an infrared pyrometer for temperature measurement. Integrated software allows the setting and control of process parameters such as temperature, time and power. The software of the instrument enables automatic adjustment of the output to the selected temperature profile. An IR pyrometer continuously measures the temperature of the irradiated mixture. Ethos Lean does not require space under a fume hood as it is equipped with a built-in exhaust system. Both of the steps to produce the extract can be carried out in the furnace cavity using dedicated accessories; a rotary drum for the decarboxylation process and a glass reactor for the extraction process.

The rotating drum is made of a material that is sensitive to dielectric heating and can operate up to 120 °C. The *Cannabis* flowers come into contact with the internal stainless steel cylinder, which is easy to clean at the end of the decarboxylation process. As this procedure is usually an odor-intensive process for operators, a pump provides a continuous flow of air inside the drum to transport the volatile molecules to an aspiration system, such as a fume-extraction unit (used in this study) or an external trap system (not evaluated in this study). The drum can process up to 150 g of dry matrix per batch, and rotation around its axis ensures continuous mixing to maintain homogeneous conditions throughout the reactor.

After the decarboxylation step, the plant matrix can be transferred to the glass reactor together with the required volume of solvent. Ethos Lean is equipped with two types of glass reactor that are calibrated to work with different amounts of solvent, and thus matrix: a larger one for 25 mL to 1.5 L of solvent and a smaller one for 50 mL–250 mL of solvent. Finally, the reactor was equipped with a magnetic stirrer to ensure good mixing of the inflorescence suspension in oil.

2.4. Decarboxylation in the Milestone Ethos Lean prototype

A given amount of chopped vegetable matrix (Seedlution: 3 g; FM2: 5 g) was placed in the drum located within the cavity of the MW oven and then heated at 120 °C with rotation for 45 min. Electromagnetic radiation is absorbed by the outer surface of the rotating drum, causing it to heat up. The plant matrix is heated by conduction and convection from the inner hot wall of the rotating drum, as MW do not penetrate it. After cooling, the drum is removed from the oven cavity and the plant matrix is recovered and used for further transformations.

The procedure was repeated at least three times and the results are expressed as the mean value ± the 95% confidence interval (CI).

2.5. Decarboxylation in laboratory oven

A given amount of chopped plant matrix (Seedlution: 3 g; FM2: 5 g) was placed on aluminum foil, taking care to minimize the thickness of the powder, and then placed in an oven that was preheated to 120 °C. After 30 min, the matrix was collected and used for the next steps.

The procedure was repeated at least three times and the result expressed as the mean value ± the 95% CI.

2.6. Hot ethanol extraction

The crushed plant matrix was placed in a 100 ml flask and ethanol was added (12 ml ethanol/g matrix). The mixture was sonicated for 10 min (20 KHz, room temperature), and then heated to reflux for 2.5 h. After cooling, the mixture was filtered, the solid was washed with ethanol and the filtrate dried under vacuum to obtain a soft resinous extract.

The procedure was repeated at least three times and the result expressed as the mean value ± the 95% CI.

2.7. Conventional extraction in olive oil

1 g of dried inflorescence was placed in a 100 ml flask together with 10 ml of pharmaceutical grade olive oil. The mixture was heated in a silicone oil bath at 90 °C for 9–120 min with stirring. After cooling, the mixture was filtered under reduced pressure and the oil was recovered.

The procedure was repeated at least three times and the result expressed as the mean value ± the 95% CI.

2.8. Extraction in olive oil using the Milestone Ethos Lean prototype

A given amount of plant matrix (Seedlution: 3 g; FM2: 5 g) was placed in the smaller glass reactor (50–250 mL) together with pharmaceutical grade olive oil (matrix/solvent ratio 1:10) and heated under MW irradiation, with stirring, at 90 °C for the required time (3, 30, 60, 120 min). At the end of heating, an oil sample can be taken immediately, or can be allowed to cool for a certain time and then filtered under vacuum to recover the extract in oil.

The procedure was repeated at least three times and the result expressed as the mean value ± the 95% CI.

2.9. UPLC-MS/MS analytical method

Analyses were performed using a UPLC-MS/MS system equipped with a Waters BEH C8 (2.1 × 50, 1.7 μm) column. A linear gradient elution with water (solvent A) and acetonitrile (solvent B), both acidified with 0.1% trifluoroacetic acid, was performed at 40 °C. The gradient started from 50% solvent B, which was held for 0.44 min, then from 50% to 100% over 3.04 min, and then finally held for 1.73 min. The flow rate was set at 0.4 ml min^{-1} and the injection volume was 5 μL.The quantification of cannabinoids was performed in the APCI + ionization mode. CBD and THC were quantified in MRM mode following the transitions 315 -> 193 (quantification) and 315 -> 135 (qualification), using 28 eV as the collision energy and 30 V as the cone voltage for both transitions. CBDA and THCA were quantified in the MRM mode following the transitions 359 -> 341 (quantification, collision energy 18 eV, cone voltage 22 V) and 359 -> 219 (qualification, collision energy 25 eV, cone voltage 22 V).

The calibration curve of the UPLC-MS/MS method was obtained using standard solutions of CBD, THC, CBDA and THCA at 0.6, 1.2, 4.0, 6.0 and 40.0 ng/mL; the linear regression R2 was always greater than 0.98 and was obtained using Waters QuanLynx software. An LOD of 0.3 ng/mL and a LOQ of 0.6 ng/mL were determined for the standard solution.

Each sample was analyzed in triplicate and data are reported as mean value ± the 95% CI.

2.10. Extract in Ethos Lean stability control

FM2 extract stability was monitored by detecting THC and CBD concentration monthly for six months and no degradation was observed (UPLC-MS/MS and ^{1}H NMR).

3. Results and discussion

The optimization of a fast and efficient standardised process involving both the decarboxylation of the main phytocannabinoids (especially THCA and CBDA) and their extraction from the dried inflorescences is an important goal for *Cannabis*-based medicinal preparations. Our goal is to intensify the process and save energy to enable environmentally sound processes.

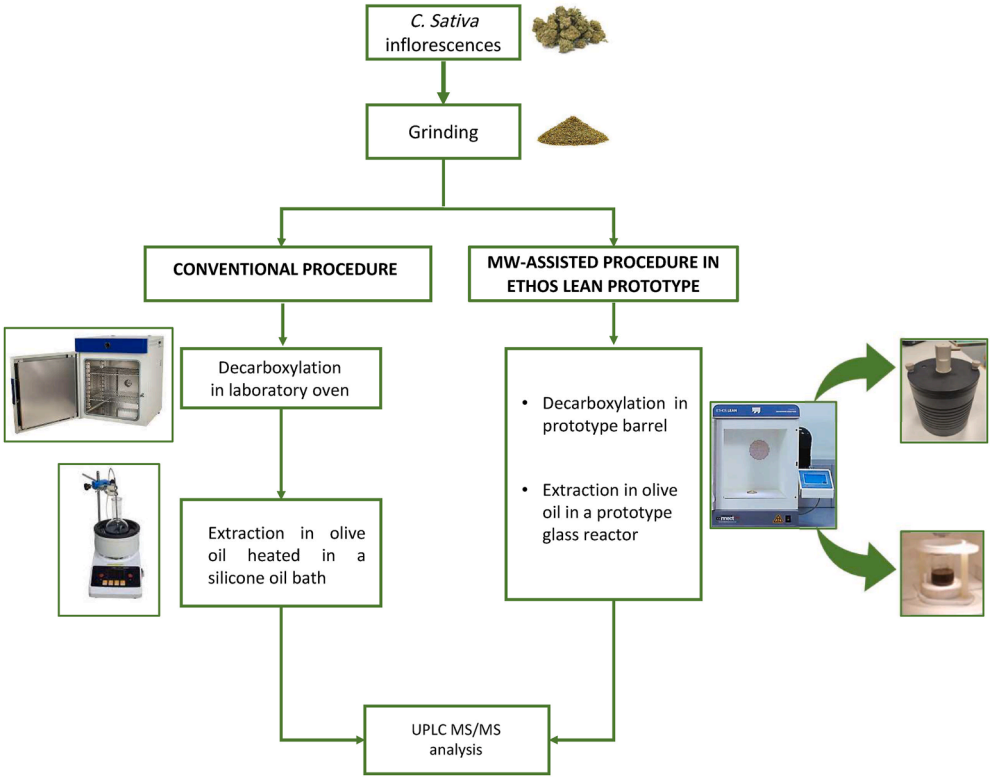
In order to extract cannabinoids from the plant matrix, it is necessary to use a solvent that is compatible with their lipophilic structure. Based on previous preliminary studies (unpublished data), we first developed a decarboxylation procedure in the laboratory (see paragraph 2.5.), an exhaustive extraction of the analytes of interest (THC and CBD) in hot ethanol under magnetic stirring (see paragraph 2.6.) and a qualitative/quantitative analytical method using UPLC-MS/MS (see paragraph 2.9.). The results obtained, in terms of dry weight of the plant matrix, served as a reference for the evaluation of the operating conditions of the Ethos Lean prototype and, consequently, of the efficiency of the decarboxylation and extraction of the inflorescences obtained with olive oil, which was used as a green solvent.

In addition, the extraction of the decarboxylated matrix in olive oil was also carried out under conventional heating (oil bath) using time and temperature conditions comparable to those optimized using the Ethos Lean prototype. Due to the commercial availability of the plant matrix, preliminary tests were only conducted with a light Cannabis variety “Seedlution” (THC ≤0.2%), while medicinal Cannabis FM2 was only used to monitor the THCA decarboxylation process and evaluate theextraction efficiency of the MW-assisted optimized process.

A flowchart of the conventional and MW-assisted procedures is shown in Scheme 1.

3.1. Optimization of conventional decarboxylation process and phytocannabinoid extraction

According to Wang et al. (2015) decarboxylation should start at 90 °C, while the optimal temperature is 120 °C, although Casiraghi et al. (2018) considered 115 °C to be the most suitable temperature to achieve “neutral” cannabinoids in 40 min. Importantly, the heating time should be kept as short as possible, since overheating could lead to degradation processes that could alter the quality of the terpenes and cannabinoids contained in the phytocomplex and affect the “entourage effect”. Based on these assump-



Scheme 1. Conventional and MW-assisted procedures applied for decarboxylation and extraction in olive oil of *C. sativa* samples.

tions, an aliquot of finely chopped female inflorescences is introduced into a laboratory oven, which has been preheated to 120 °C. The vegetable matrix is then left for 15 and 30 min to determine the optimal thermal exposure period that can guarantee the complete decarboxylation of the main phytocannabinoids.

Tables 1 and 2 show process monitoring data for the Seedlution and FM2 samples, and the THCA and CBDA values detected in the unheated matrices. The percentage content of the different analytes was determined following the exhaustive extraction of the samples in ethanol (see paragraph 2.6.) and subsequent UPLC-MS/MS analysis.

In terms of the complete conversion of acidic phytocannabinoids to their respective "neutral" forms, the reported data show exhaustive treatment following heating at 120 °C for 30 min. Regarding the reaction kinetics, tests performed on FM2 displayed a faster decarboxylation rate for THCA than for CBDA, which is in agreement with a previous report by Moreno et al. (2020), performed under conventional conditions. It should be noted that, in this matrix, the obtained values of the two phytocannabinoids were lower than those commonly declared for FM2 (THC 5–8%, CBD 7.5–12%). This can be attributed to the fact that the vegetal matrix, from a 2016 harvest, was rather old and the long storage period therefore resulted in metabolite degradation processes, such as that of THC to cannabinol (Jaidee et al., 2022). As mentioned above, the content of the major phytocannabinoids in the FM2 and Seedlution ma-

Table 1
Percentage of CBD and CBDA in *C. sativa* Seedlution samples as a function of decarboxylation time in a laboratory oven at 120 °C.

Seedlution trials	Residence time (min) at 120 °C	CBD% ± CI[a]	CBDA% ± CI[a]
1	0	6.99 ± 0.61	4.49 ± 0.75
2	15	9.66 ± 0.10	3.87 ± 0.09
3	30	13.08 ± 0.11	<LOD

[a] CI calculated over 6 repetitions of the procedure.

Table 2
Percentage of CBD, CBDA, THC and THCA contained in FM2 samples subjected to decarboxylation time in laboratory oven.

FM2 trials	Residence time (min) at 120 °C	CBD% ± CI[a]	CBDA% ± CI[a]	THC% ± CI[a]	THCA% ± CI[a]
1	0	3.25 ± 0.27	1.27 ± 0.02	1.59 ± 0.04	1.44 ± 0.03
2	15	3.87 ± 0.18	0.42 ± 0.03	2.79 ± 0.06	<LOD
3	30	4.31 ± 0.34	<LOD	2.83 ± 0.07	<LOD

[a] CI calculated over 6 repetitions of the procedure.

trices under investigation, as such and after decarboxylation, was determined following an extraction in ethanol. The exhaustiveness of this procedure was verified by re-extracting the plant matrix residue under the same conditions and verifying the absence of cannabinoids in the new extract. For comparison, the Seedlution matrix was also subjected to a conventional olive-oil extraction protocol (90 °C, 2 h, matrix/solvent 1:10 w/w), giving a lower CBD yield (88.6%) than the ethanol procedure.

3.2. MW-assisted decarboxylation process

The Ethos Lean prototype decarboxylation method provided efficiency that is comparable to that of the laboratory oven when the procedure was carried out by heating the finely shredded Seedlution matrix inside the rotating drum for 45 min at a temperature of 120 °C. The drum was then left to cool for 5 min before sample collection. In this case, the entire process time was calculated starting from the cold instrument, while the 30 min indicated for decarboxylation in the laboratory oven were started once the matrix was placed into the oven after it had been heated to the desired temperature (oven pre-heating: about 20 min). It follows that the timescales of the two processes are comparable, with the particularity that the rotary barrel offers the possibility of subjecting larger quantities of matrix to efficient heat treatment than the laboratory oven, where it is necessary to arrange the inflorescences in a thin, uniform layer. Specifically, based on the calculated percentage of CBDA, decarboxylation reaches 14% in 15 min and 100% in half an hour in the hot (120 °C) laboratory oven while, using the Ethos Lean decarboxylation system, a 66% reduction of CBDA is observed in 15 min (calculated from reaching 120 °C) and, after 30 min of heating, decarboxylation resulted complete even with this system.

3.3. Optimization of MW-assisted phytocannabinoid extraction in olive oil

In order to find an environmentally sustainable approach, olive oil was chosen as the extraction solvent to test the new Ethos Lean MW reactor. With good affinity for the target molecules under study, this non-polar solvent has the advantage of being biodegradable, produced from renewable sources and suitable for the safe and direct administration of the *Cannabis* extract to animals and humans.

MW-assisted oil extraction was mainly studied in terms of the extraction time and temperature. In addition, the scalability of the process was also evaluated.

Since most of the cannabinoids present in *Cannabis* inflorescences are located in the trichomes, i.e. thin hairy outgrowths that have a defensive function, it may be reasonable to assume that the simple washing of the vegetal matrix with an appropriate solvent can recover these metabolites, without requiring heat treatment. An evaluation of the extraction capacity of a non-conventional system, using a bio-sustainable solvent, must therefore start from the determination of the amount of cannabinoids that are recovered by simple washing/maceration. To this aim, the finely chopped inflorescences of Seedlution were soaked in olive oil at ambient temperature with two different contact times as references (3 and 30 min). As can be seen from Table 3, 3 min at room temperature led to the recovery of about 40% of the total CBD present in the matrix, with this value increasing to about 50% when the time was extended to 30 min. It is reasonable to assume that 3 min of treatment at room temperature does not involve an actual extraction process, but is limited to the removal of cannabinoids from the outer resinous corpuscles. The slightly increased yield achieved by extending the maceration time to 30 min is related to a better 'washing' of the plant surface, or partial extraction process.

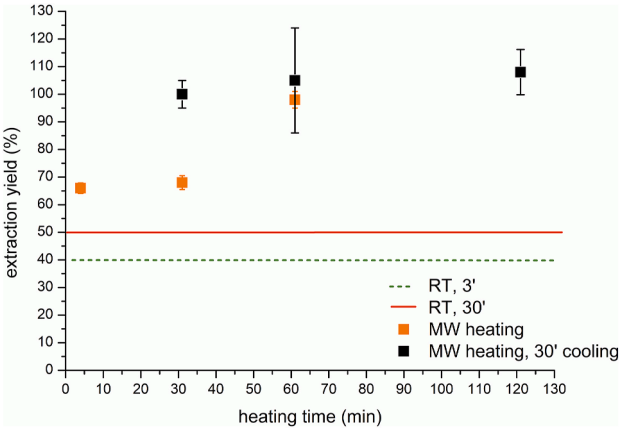
With this premise, and on the bases of literature data (Casiraghi et al., 2018; De Vita et al., 2020), a series of MAE experiments at 90 °C were then designed, with varying dwelling times (3, 30, 60 min) at this temperature under stirring to find the best operating conditions. Moreover, with the aim of reducing process times as much as possible, the time to reach the extraction temperature (ramp temperature) was set to 1 min.

An analysis of samples taken immediately after the end of the 4 min treatment (ramp 1 min, plus 3 min infusion) and after 31 min (ramp 1 min, plus 30 min infusion) shows that higher extraction yields were achieved here than under the cold procedure (Table 3). Graph 1 shows the evolution of the CBD extraction yield, which increases from 40% (3 min at room temperature) to about 67% after heating for 4 or 31 min. Hence, no significant differences are observed when extending the infusion time from 3 to 30 min. By cooling the extraction mixture for 30 min (always under stirring) after the previous heating treatment (31 min), it is possible to see an increase of the yields from 68% to 100%, thus demonstrating how the extraction process is still in progress at this stage. By prolonging the 90 °C heating process by up to 60 min, without taking into account the cooling time, the exhaustion of the hot procedure can also be achieved. It is therefore clear that, in order to reduce energy consumption, there is no point in extending the heating time beyond 30 min.

Table 3
Comparison of CBD-extraction yields after the MAE, conventional extraction and ambient temperature maceration of a previously decarboxylated Seedlution matrix. The % recovery of CBD was calculated on the basis of its concentration measured by exhaustive ethanol extraction.

Entry	Heating mode	Heating ramp (min) to 90 °C	Hold temperature (min)	Cooling time (min)	CBD yield (%) ± CI[a]
1	None (T amb)	–	3	–	40.2 ± 2.2
2	None (T amb)	–	30	–	49.8 ± 4.4
3	MW	1	3	0	66.3 ± 1.9
4	MW	1	30	0	67.7 ± 2.5
5	MW	1	30	30	100.0 ± 5.0
6	MW	1	60	30	105.0 ± 19.0
7	Conventional (oil bath)	6	3	0	51.8 ± 8.3
8	Conventional (oil bath)	6	3	30	75.3 ± 2.5
9	Conventional (oil bath)	6	30	30	85.8 ± 3.5

[a] The 95% CI was calculated over at least 3 repetitions.



Graph 1. Graphical representation of the CBD extraction yield on the previously decarboxylated Seedlution matrix under different experimental conditions. Green line: extraction at room temperature for 3 min; red line: extraction at room temperature for 30 min; orange squares: MW-assisted extraction at 90 °C (time includes 1 min to reach operating temperature) with sampling immediately after heating; black squares: MAE at 90 °C (time includes 1 min to reach operating temperature) with sampling after cooling for 30 min. The % recovery was calculated on the basis of its concentration measured by exhaustive ethanol extraction. (Error bars are calculated with a 95% CI over at least 3 repetitions).

The thermal stability of the analytes under investigation was demonstrated by further prolonging the heat treatment for up to 120 min (plus 30 min of cooling), and no change in concentrations was observed, also in terms of the ratio between the concentrations of acid and decarboxylated species when the crude vegetal matrix was considered. In fact, as can be seen in Table 4, the CBD amount in relation to the total CBD content (CBD + CBDA expressed as CBD %) remains more or less constant while the dielectric heating times vary. This result is in contrast with one report by De Vita et al. (De Vita et al., 2020), which showed that CBDA was converted into its decarboxylated form under MAE, and another by Fiorini et al. (2020), who assessed that the high MW energy penetration enhanced phytocannabinoid decarboxylation (1.1 W/g MW power values, ~115 min extraction times).

To assess the possible use of MW irradiation instead of conventional heating (conductive/convective), extractions were repeated by performing the procedure in an oil bath. In order to make the two procedures as similar as possible, the time required for the suspension (5 g of *Cannabis* in 50 ml of olive oil, placed in a flask immersed in a hot oil bath) to reach the operating temperature (90 °C) was measured first. Under these conditions, it takes 6 min. Obviously, MW irradiation is much more effective and is faster at raising the temperature to the operating values, taking only 1 min. Moreover, while this time can be considered constant in the extraction in the Ethos Lean, regardless of the volume of solvent used, and therefore of the matrix employed (n.b. matrix-solvent ratio 1:10), it certainly increases when conventional heating is utilized for higher matrix amounts.

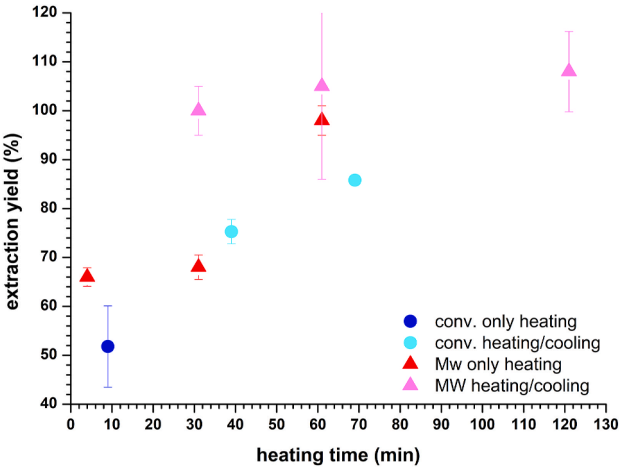
Three different tests were therefore carried out, each taking into account 6 min of heating ramp: in the first test, the oil was sampled after 3 min at 90 °C; in the second test, the oil was sampled after similar heat treatment followed by 30 min of cooling; in the third test, the oil was sampled after 30 min at 90 °C, followed by a further 30 min of cooling (Table 3, Graph 2).

A comparison of the CBD extraction yield obtained after 3 min of heating using the Ethos Lean prototype and conventional heating shows that the MW-assisted method has higher efficiency, as a yield of 66.3% ± 1.9 is obtained when the mixture is dielectrically heated for a total of 4 min (entry 3, Table 3), whereas 51.8% ± 8.3 was achieved in conventional mode after 9 min overall (entry 7, Table 3). The difference in extractive yield in the two cases is not very large, but is significant (Mann-Whitney *U* test, P = 0.95) and can probably be attributed to the intracellular thermal effect exerted by MW irradiation. Electromagnetic radiation acts directly on the water molecules contained in the plant cells, locally increasing their temperature and thus favouring the lysis of intracellular compartments and the rupture of cell walls. This is a phenomenon that does not occur, or occurs for other reasons and to a much lesser extent, when the mixture is heated conventionally, as reported by Fiorini et al. (2019), who observed a higher increase in CBD yield in *C. sativa* essential oil when a sample underwent MW-assisted pre-treatment of 1 min at 900 W than when heat treatment was performed in an unventilated oven at 120 °C for the same time. Moreover, the same authors (Fiorini et al., 2020) observed how MAE can be considered more efficient as a time- and cost-saving procedure than conventional techniques, such as hydro distillation, while test-

Table 4
Proportion of the decarboxylated form of CBD in relation to the total phytocannabinoid content in the Seedlution matrix as a function of irradiation time under MW. The operating temperature was set at 90 °C.

Exposure to MW irradiation (min)	% CBD in non-decarboxylated Seedlution matrix
3	19.72 ± 0.52
10	20.02 ± 0.63
20	18.44 ± 0.61
30	17.98 ± 0.55
60	20.06 ± 0.59
120	20.11 ± 0.67

The 95% CI was calculated over at least 3 repetitions.



Graph 2. Overall graphical representation of the evolution of the extraction yield as a function of time, with and without a 30-min cooling period. Circles: conventional heating (blue without cooling, light blue with cooling); triangles: MW heating (red without cooling, pink with cooling).

ing the Ethos X device's ability to produce CBD-enriched *Cannabis* essential oil. Further evidence of this phenomenon can be found by comparing MAE for 30 min plus 30 min cooling (entry 5, Table 3) with the similar test performed in conventional mode (entry 9, Table 3), as there is an increase in CBD extraction of about 15% when using the Ethos Lean prototype. This comparison makes it even clearer how effective MWs are in promoting the release of metabolites into the extractive solvent. On the other hand, maintaining the same solvent matrix ratio (1:10 w/w) without considering the effect of sample cooling time, Casiraghi et al. (2018), has been reported to give satisfactory extraction yields and process reproducibility when extracting medical *Cannabis* in oil under heating in an oil bath with magnetic stirring for 40 min at 100 °C. Moreover, it was indicated that the further prolongation of heating had no influence. It is worth noting that, thanks to the versatility of the extractor volume available, tests carried out on 150 g of matrix confirmed the prototype's efficiency in obtaining effective and homogeneous extracts.

Under the optimized conditions (30 min at 90 °C), the prototype was used for the extraction of FM2 medical *Cannabis*, meaning that the efficiency of the instrument has also been evaluated with regards to THC. The obtained data, which revealed effectiveness and reproducibility in both CBD and THC yields (97.3 ± 1.5% and 98.2 ± 0.9%, respectively), demonstrate the efficiency of the Ethos Lean system in the preparation of oily *Cannabis* extracts for medical use. Furthermore, tests performed on the stability of the obtained FM2 extract (stored up to 6 months both at room temperature and at 4 °C, shielded from the light) revealed no variation in phytocannabinoid profile.

In addition, the safety of using olive oil was demonstrated in a ^{1}H NMR analysis of this green solvent, before and after the extraction procedure with the prototype, as no degradation signal was detected following irradiation with MW in the spectrum obtained (Dais and Hatzakis 2013; Consonni and Cagliani 2019).

4. Concluding remarks

The efficient extraction and quantification of phytocannabinoids for medicinal use is essential to achieving required quality standards and to exploiting the so-called entourage effects of the phytocomplex. Herein, we have investigated the performance of a new MW reactor that has been developed to improve the galenic preparation of ready-to-use *Cannabis* extracts. The remarkable effect of dielectric heating on the decarboxylation of acidic cannabinoid derivatives and in promoting their extraction has been reported. Importantly, the tested device ensures the uniformity of treatment even with a plant matrix of up to 150 g. MAE was carried out using olive oil as the solvent. Its properties as a solvent from a renewable source, with low environmental impact, that is compatible with the direct administration of the extract to animals and humans make it particularly attractive for this application. The results obtained show that the process is more efficient than that carried out under conventional conditions, as it requires only 30 min of energy input, followed by another 30 min of maceration without irradiation. Under these conditions, extraction is considered exhaustive. Compared to conventional heating, MW irradiation enables time savings even more in bigger scale. In terms of energy consumption, the decarboxylation and subsequent extraction (both of which can be scaled to 150 g matrix at a matrix-solvent ratio of 1:10 w/w) also appear to have advantages over the conventional approach. The reproducibility of MAE and conventional methods is comparable, and MW irradiation does not degrade the olive oil under the conditions used. The prototype studied thus has advantages in terms of ease of operation, extraction yield (slightly higher than under conventional conditions) and the time required to obtain the final product. These features and in particular the standardisation of thermal decarboxylation and the subsequent MW-assisted extraction process in oil are highly appreciated by pharmacists, especially in hospital pharmacies. In terms of solvent stability and reproducibility of results, it does not differ from the conventional method.

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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.

Data availability

No data was used for the research described in the article.

References

Addo, P.W., Sagili, S.U.K.R., Bilodeau, S.E., Gladu-Gallant, F.A., MacKenzie, D.A., Bates, J., McRae, G., MacPherson, S., Paris, M., Raghavan, V., Orsat, V., Lefsrud, M., 2022. Microwave- and ultrasound-assisted extraction of cannabinoids and terpenes from cannabis using response surface methodology. Molecules 27 (24), 8803. https://doi.org/10.3390/molecules27248803.

Anand, U., Pacchetti, B., Anand, P., Sodergren, M.H., 2021. Cannabis-based medicines and pain: a review of potential synergistic and entourage effects. Pain Manag. 11 (4), 395–403. https://doi.org/10.2217/pmt-2020-0110.

Appendino, G., Chianese, G., Taglialatela-Scafati, O., 2011. Cannabinoids: occurrence and medicinal chemistry. Curr. Med. Chem. 18 (7), 1085–1099. https://doi.org/10.2174/092986711794940888.

Badowski, M.E., Yanful, P.K., 2018. Dronabinol oral solution in the management of anorexia and weight loss in AIDS and cancer. Therapeut. Clin. Risk Manag. 14, 643–651. https://doi.org/10.2147/Tcrm.S126849.

Brighenti, V., Pellati, F., Steinbach, M., Maran, D., Benvenuti, S., 2017. Development of a new extraction technique and HPLC method for the analysis of non-psychoactive cannabinoids in fibre-type Cannabis sativa L. (hemp). J. Pharmaceut. Biomed. Anal. 143, 228–236. https://doi.org/10.1016/j.jpba.2017.05.049.

Calvi, L., Pentimalli, D., Panseri, S., Giupponi, L., Gelmini, F., Beretta, G., Vitali, D., Bruno, M., Zilio, E., Pavlovic, R., Giorgi, A., 2018. Comprehensive quality evaluation of medical *Cannabis sativa* L. inflorescence and macerated oils based on HS-SPME coupled to GC-MS and LC-HRMS (q-exactive orbitrap (R)) approach. J. Pharmaceut. Biomed. Anal. 150, 208–219. https://doi.org/10.1016/j.jpba.2017.11.073.

Casiraghi, A., Roda, G., Casagni, E., Cristina, C., Musazzi, U.M., Franzè, S., Rocco, P., Giuliani, C., Fico, G., Minghetti, P., Gambaro, V., 2018. Extraction method and analysis of cannabinoids in cannabis olive oil preparations. Planta Med. 84 (4), 242–249. https://doi.org/10.1055/s-0043-123074.

Chang, C.W., Yen, C.C., Wu, M.T., Hsu, M.C., Wu, Y.T., 2017. Microwave-assisted extraction of cannabinoids in hemp nut using response surface methodology: optimization and comparative study. Molecules 22 (11), 1894. https://doi.org/10.3390/molecules22111894.

Chemat, F., Cravotto, G., 2013. Microwave-assisted Extraction for Bioactive Compounds. Springer Science, Spring Street, New York, NY 10013, USA, p. 233.

Christensen, C., Rose, M., Cornett, C., Allesø, M., 2023. Decoding the postulated entourage effect of medicinal cannabis: what it is and what it isn't. Biomedicines 11, 2323. https://doi.org/10.3390/biomedicines11082323.

Citti, C., Ciccarella, G., Braghiroli, D., Parenti, C., Vandelli, M.A., Cannazza, G., 2016. Medicinal cannabis: principal cannabinoids concentration and their stability evaluated by a high performance liquid chromatography coupled to diode array and quadrupole time of flight mass spectrometry method. J. Pharmaceut. Biomed. Anal. 128, 201–209. https://doi.org/10.1016/j.jpba.2016.05.033.

Consonni, R., Cagliani, L.R., 2019. NMR studies on Italian PDO olive oils and their potential in olive-tree-derived products characterization. Eur. J. Lipid Sci. Technol. 121 (3), 1800174. https://doi.org/10.1002/ejlt.201800174.

Dais, P., Hatzakis, E., 2013. Quality assessment and authentication of virgin olive oil by NMR spectroscopy: a critical review. Anal. Chim. Acta 765, 1–27. https://doi.org/10.1016/j.aca.2012.12.003.

De Vita, D., Madia, V.N., Tudino, V., Saccoliti, F., De Leo, A., Messore, A., Roscilli, P., Botto, A., Pindinello, I., Santilli, G., Scipione, L., Costi, R., Di Santo, R., 2020. Comparison of different methods for the extraction of cannabinoids from cannabis. Nat. Prod. Res. 34 (20), 2952–2958. https://doi.org/10.1080/14786419.2019.1601194.

Ferreira, D.F., Lucas, B.N., Voss, M., Santos, D., Mello, P.A., Wagner, R., Cravotto, G., Barin, J.S., 2020. Solvent-free simultaneous extraction of volatile and non-volatile antioxidants from rosemary (Rosmarinus officinalis L.) by microwave hydrodiffusion and gravity. Ind. Crop. Prod. 145, 112094. https://doi.org/10.1016/j.indcrop.2020.112094.

Fiorini, D., Molle, A., Nabissi, M., Santini, G., Benelli, G., Maggi, F., 2019. Valorizing industrial hemp (Cannabis sativa L.) by-products: cannabidiol enrichment in the inflorescence essential oil optimizing sample pre-treatment prior to distillation. Ind. Crop. Prod. 128, 581–589. https://doi.org/10.1016/j.indcrop.2018.10.045.

Fiorini, D., Scortichini, S., Bonacucina, G., Greco, N.G., Mazzara, E., Petrelli, R., Torresi, J., Maggi, F., Cespi, M., 2020. Cannabidiol-enriched hemp essential oil obtained by an optimized microwave-assisted extraction using a central composite design. Ind. Crop. Prod. 154, 112688. https://doi.org/10.1016/j.indcrop.2020.112688.

Giacoppo, S., Bramanti, P., Mazzon, E., 2017. Sativex in the management of multiple sclerosis-related spasticity: an overview of the last decade of clinical evaluation. Multiple Sclerosis Related Disorders 17, 22–31. https://doi.org/10.1016/j.msard.2017.06.015.

Giese, M.W., Lewis, M.A., Giese, L., Smith, K.M., 2015. Development and validation of a reliable and robust method for the analysis of cannabinoids and terpenes in cannabis. J. AOAC Int. 98 (6), 1503–1522. https://doi.org/10.5740/jaoacint.15-116.

Gunjevic, V., Grillo, G., Carnaroglio, D., Binello, A., Barge, A., Cravotto, G., 2021. Selective recovery of terpenes, polyphenols and cannabinoids from Cannabis sativa L. inflorescences under microwaves. Ind. Crop. Prod. 162, 113247. https://doi.org/10.1016/j.indcrop.2021.113247.

Jaidee, W., Siridechakorn, I., Nessopa, S., Wisuitiprot, V., Chaiwangrach, N., Ingkaninan, K., Waranuch, N., 2022. Kinetics of CBD, delta(9)-THC degradation and cannabinol formation in cannabis resin at various temperature and pH conditions. Cannabis Cannabinoid Res. 7 (4), 537–547. https://doi.org/10.1089/can.2021.0004.

Lewis-Bakker, M.M., Yang, Y., Vyawahare, R., Kotra, L.P., 2019. Extractions of medical cannabis cultivars and the role of decarboxylation in optimal receptor responses. Cannabis Cannabinoid Res. 4 (3), 183–194. https://doi.org/10.1089/can.2018.0067.

Liu, Y., Liu, H.Y., Li, S.H., Ma, W., Wu, D.T., Li, H.B., Xiao, A.P., Liu, L.L., Zhu, F., Gan, R.Y., 2022. Cannabis sativa bioactive compounds and their extraction, separation, purification, and identification technologies: an updated review. Trac. Trends Anal. Chem. 149, 116554. https://doi.org/10.1016/j.trac.2022.116554.

Mazzara, E., Carletti, R., Petrelli, R., Mustafa, A.M., Caprioli, G., Fiorini, D., Scortichini, S., Dall'Acqua, S., Sut, S., Nunez, S., Lopez, V., Zheljazkov, V.D., Bonacucina, G., Maggi, F., Cespi, M., 2022. Green extraction of hemp (*Cannabis sativa* L.) using microwave method for recovery of three valuable fractions (essential oil, phenolic compounds and cannabinoids): a central composite design optimization study. J. Sci. Food Agric. 102 (14), 6220–6235. https://doi.org/10.1002/jsfa.11971.

Micalizzi, G., Alibrando, F., Vento, F., Trovato, E., Zoccali, M., Guarnaccia, P., Dugo, P., Mondello, L., 2021. Development of a novel microwave distillation technique for the isolation of cannabis sativa L. Essential oil and gas chromatography analyses for the comprehensive characterization of terpenes and terpenoids, including their enantio-distribution. Molecules 26 (6), 1588. https://doi.org/10.3390/molecules26061588.

Moreno, T., Dyer, P., Tallon, S., 2020. Cannabinoid decarboxylation: a comparative kinetic Study.Ind. Eng. Chem. Res. 59 (46), 20307–20315. https://doi.org/10.1021/acs.iecr.0c03791.

Nahar, L., Uddin, S.J., Alam, M.A., Sarker, S.D., 2021. Extraction of naturally occurring cannabinoids: an update. Phytochem. Anal. 32 (3), 228–241. https://doi.org/10.1002/pca.2987.

Nielsen, S., Germanos, R., Weier, M., Pollard, J., Degenhardt, L., Hall, W., Buckley, N., Farrell, M., 2018. The use of cannabis and cannabinoids in treating symptoms of multiple sclerosis: a systematic review of reviews. Curr. Neurol. Neurosci. Rep. 18 (2), 8. https://doi.org/10.1007/s11910-018-0814-x.

Nuapia, Y., Maraba, K., Tutu, H., Chimuka, L., Cukrowska, E., 2021. In situ decarboxylation-pressurized hot water extraction for selective extraction of cannabinoids from cannabis sativa. Chemometric approach. Molecules 26, 3343. https://doi.org/10.3390/molecules26113343.

Qamar, S., Torres, Y.J.M., Parekh, H.S., Falconer, J.R., 2021. Extraction of medicinal cannabinoids through supercritical carbon dioxide technologies: a review. J. Chromatogr., B: Anal. Technol. Biomed. Life Sci. 1167, 122581. https://doi.org/10.1016/j.jchromb.2021.122581.

Radoiu, M., Kaur, H., Bakowska-Barczak, A., Splinter, S., 2020. Microwave-assisted industrial scale cannabis extraction. Technologies 8 (3), 45. https://doi.org/10.3390/technologies8030045.

Rosenthal, E., 2014. Beyond Buds: Marijuana Extracts-Hash, Vaping, Dabbing, Edibles & Medicines. Quick Trading Company, Oakland, CA, USA.

Routray, W., Orsat, V., 2012. Microwave-assisted extraction of flavonoids: a review. Food Bioprocess Technol. 5 (2), 409–424. https://doi.org/10.1007/s11947-011-0573-z.

Valizadehderakhshan, M., Shahbazi, A., Kazem-Rostami, M., Todd, M.S., Bhowmik, A., Wang, L.J., 2021. Extraction of cannabinoids from cannabis sativa L. (Hemp)-Review. Agriculture 11 (5), 384. https://doi.org/10.3390/agriculture11050384.

Veress, T., Szanto, J.I., Leisztner, L., 1990. Determination of cannabinoid acids by high-performance liquid-chromatography of their neutral derivatives formed by thermal decarboxylation .1. Study of the decarboxylation process in open reactors. J. Chromatogr. 520, 339–347. https://doi.org/10.1016/0021-9673(90)85118-F.

Wang, M., Wang, Y.H., Avula, B., van Antwerp, J., Parcher, J.F., ElSohly, M.A., Khan, I.A., 2015. Supercritical fluid chromatographic method for the analysis of cannabinoids in extracts of cannabis samples. Planta Med. 81 (11). https://doi.org/10.1055/s-0035-1556193. 878-878. .

Xie, D.T., Wang, Y.Q., Kang, Y., Hu, Q.F., Su, N.Y., Huang, J.M., Che, C.T., Guo, J.X., 2014. Microwave-assisted extraction of bioactive alkaloids from Stephania sinica. Separ. Purif. Technol. 130, 173–181. https://doi.org/10.1016/j.seppur.2014.04.026.