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Cannabis extraction by supercritical CO_2: state of art and future perspectives

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ABSTRACT

The interest for *Cannabis sativa* extracts for medicinal purposes has been increased in the recent years. Scientific evidences are already available to support the therapeutic properties of the *Cannabis* constituents, including the treatment of multiple sclerosis, epilepsy, pain and several others. Among the *Cannabis* constituents, the cannabinoids named cannabidiol (CBD) and Δ^9-tetrahidrocanabidiol (THC) are highlighted as the most important. Supercritical fluid extraction (SFE) has been considered an important step to produce *Cannabis*-based medicines. Some patents describe the use of SFE for obtaining cannabinoids-rich extracts as part of the process to obtain purified cannabinoids. The literature also reports *Cannabis* extraction by SFE with results of oil composition, oxidative stability of the oil, antioxidant capacity, recovery of volatile compounds and determination of solubility parameters. However, considering the versatility of SFE process as well as the diversity of *Cannabis* species, more studies can still be carried out in order to optimize the specific process parameters to achieve the best global yield, target compound yield. This work aims to provide the current status of the use of SFE for *Cannabis*-based products and to discuss the future perspectives for its applications and industrial scaling.

INTRODUCTION

Although it has been known for thousand years, only for the last decades scientific advances has been achieved for *Cannabis sativa*. In the early 20th century, great companies such as Merck (Germany), Bristol-Meyers Squibb (USA) and Eli-Lilly (USA) produced *Cannabis* based medicine for a range of ailments. After several legal restrictions occurred in the USA, in 1941 *Cannabis* was officially removed from the American Pharmacopeia [1], which build a barrier to the scientific studies started with the discovery of THC structure identification in 1971 by Gaoni and Mechoulam [2]. However, in the early 1990's, the interest in studies about *Cannabis* was renewed, with the description and cloning of specific receptors for the cannabinoids in the nervous system and the subsequent isolation of anandamide, an endogenous cannabinoid [3] and numerous scientific studies about the *Cannabis* active compounds has bloomed [4, 5].

Cannabis sativa contains hundreds of chemical compounds produced by secondary metabolism including cannabinoids, terpenes and phenolic compounds, each one with potential interesting biological properties [6]. The term cannabinoid is traditionally used for the compounds with effect related to the cannabinoids receptors (CB1 and CB2). However, the cannabinoids can be further classified as: endocannabinoids to denominate non-vegetal substances of natural origin; phytocannabinoids to denominate the natural cannabinoids from vegetable matrices; and synthetic cannabinoids. Those cannabinoids include compounds that activate or inhibit cannabinoids receptors, reception and/or degradation inhibitors that increases endogenous cannabinoids levels, allosteric modulators which can fine regulates the receptors and standardized extracts containing phytocannabinoids [5].

The major phytocannabinoids, THC (Δ^9-tetrahidrocanabidiol) and CBD (cannabidiol) have been studied and tested. THC is already well-known for its psychotropic effects, however, daily intake therapeutic doses are possible at 30 mg THC, which may cause some adverse reaction but at the same level as other regular medicine such as benzodiazepine derivatives and other more serious reactions as the associated to abstinence crises with 5-6 times higher doses (150-180 mg THC/day). CBD is the most promising of the class of phytocannabinoids which are not psychotropic. It has been successfully tested and evidenced to exert multiple pharmacological effects via different mechanisms. Therapeutic effects of CBD includes treatment for psychosis, epilepsy, anxiety and insomnia, neuroprotection and neurodegenerative diseases, including Parkinson´s, Alzheimer´s and Huntington´s, cerebral and myocardial ischemia [6, 7].

Most of the research on cannabinoids therapeutic effects came up from the reports from *Cannabis* smokers to self-medicate for several aims, a method called reverse drug discovery. Initially, cannabinoids were synthetized, for example, Dronabinol – a THC synthetic version – which was tested and used treat appetite loss in AIDS patients and relieve of nausea associated to chemotherapy. Nowadays, based on evidences that the combination of different cannabinoids may present superior therapeutic effects the great interest came to be the phytocannabinoids with two basic approaches: (1) activate or suppress the primary target CB1 and CB2 or (2) turn phytocannabinoids into drugs [8]. The great challenge of *Cannabis*-based medicine use is the balance between its benefic therapeutic use and the adverse reaction, which has been observed by prolonged use at high doses [5].

Beyond medicinal use of *Cannabis*-based products, there is a wild range of recreational purposes as well. Actually, with the legalization of *Cannabis* use in many countries in the world, great businesses have been developed nowadays. Recently, the second-biggest stock exchange in the world has given one *Cannabis* company the stamp of approval, bringing the industry of *Cannabis* out of the shadows and making it mainstream once for all [9].

Today, *Cannabis*-based products obtained by supercritical CO_2 have saturated almost every major market from consumables to pre-filled vaporizers to dabbing products and isolates. The world of *Cannabis* extraction has benefited greatly from the adoption of this technology, and no doubt, the future of CO_2 extraction technology will be positively impacted by its contributions to the *Cannabis* community. In this sense, this work aims to provide the current status of the use of SFE for *Cannabis*-based products and to discuss the future perspectives for its applications and industrial scaling.

Cannabis EXTRACTION BY SUPERCRITICAL CO_2

Phytocannabinoids are lipid-soluble chemicals present in the resin secreted from trichrome that are abundantly produced by female plants of the *Cannabis sativa* herb. All phytocannabinoids are uniquely found in *Cannabis*, with the total number of identified currently reported as over 100 [10]. From a pharmacochemical perspective, whilst THC and CBD have pentyl side chains, major homologues are Δ^9-tetrahydrocannabivarin (Δ^9-THCV) and cannabidivarin (CBDV) respectively, with propyl side-chains, derived from cannabigerovarin (CBGV). Despite only small differences in chemical structure, these compounds appear to exhibit markedly different pharmacological properties. Other phytocannabinoids, such as cannabinol (CBN), are considered to be oxidation products.

Considering the non-polar nature of the cannabinoids, extractions of such compounds from natural plants have been carried out by employing organic solvents. However, there is some safety concern about those techniques, especially when the product will be used for human consumption [11]. An alternative extraction method that have also been used for the *Cannabis* extraction is the supercritical CO_2 extraction (SFE-CO_2) [12], because its unique features, with special attention to its selectivity for non-polar compounds and as an environmentally friendly and sustainable processes [13].

SFE-CO_2 has been applied to *Cannabis* by several authors, who reported extraction from different parts of the plant, such as: seed [12, 14-18], leaves and buds [19] or flowers [20] (Table 1). As demonstrated in Table 1, other non-polar compounds beyond cannabinoids are also the focus of research, where results on oil composition [12, 16], oxidative stability of the oil [21], antioxidant activity [17] and volatile compounds recovery [20] has been reported. *Cannabis* seed oil is considered to be one of the best nutritional oil for health because it contains two polyunsaturated essential fatty acids from the "omega-6" family and α-linoleic from the "omega-3" family – which usually account for approximately 50-70% and 15-25%, respectively, of the total seed fatty acid content [14].

Table 1. Examples of conditions used for extraction of *Cannabis sativa L* by supercritical CO_2.

Material	Pressure (bar)	Temperature (°C)	CO_2 flow (kg/h)	Feed (g)	Bioactive Compound	Ref
Seed	300-400	40-80	10	300	fatty acids	[12]
Seed	300-400	40-60	1.94	100	chlorophyll, carotene, tocopherol	[16]
Seed	250-350	40-60	0.3	15	tocopherol	[15]
Seed	300	40	0.3	15	fatty acids	[14]
Flower	100-140	40	3	150	volatile	[20]
Flower	220	60	-	20	cannabinoids	[22]
Flower	230	40	6	45	cannabinoids	[23]
Leaves and buds	170-340	55	12	500	cannabinoids	[24]

Da Porto et al [12, 14, 21] have been developed interesting studies about SFE-CO_2 of *Cannabis* aiming to recover the oil from the seeds. These authors optimized process parameters temperature, pressure and solvent to feed ratio and obtained the highest oil yield of 22% corresponding to 72% recovery at 80 °C and 300 bar. This process condition also resulted the highest oxidation stability [12]. In another approach, response surface methodology (RSM) was

used to optimize temperature, pressure and particle diameter conditions regarding the oil extraction and stability. In this case, the highest oil yield (21.5%) was obtained when the SFE-CO_2 was carried out at 40 °C, 300 bar and 0.71 mm of particle size, while the maximum oil oxidation stability was obtained at 60 °C, 250 bar and 0.83 mm. [21].

The effect of ultrasound pre-treatment of *Cannabis* seeds on the SFE-CO_2 was also studied by Da Porto, Natolino and Decorti [14]. They observed that the maximum oil yield (24%) was obtained after 10 min of ultrasonic pre-treatment, however, the fatty acid composition of the oil was not significantly affected by the pre-treatment. *Cannabis* inflorescences were extracted by SFE-CO_2 at 40 °C and 100 bar followed by fractionation of the extract using two separators operating at Sep 1: 25 °C and 70 bar; Sep 2: 50 bar and 15 °C. Under these operating conditions, cuticular waxes were collected in the first separator and 100% of volatile compounds in the second.

Although cannabinoids are still the most valuable targets, at least considering therapeutic properties which might enable its production over several countries around the world, SFE-CO_2 applied to drug-type *Cannabis* is still scarce in the scientific literature.

Perrotin-Brunel et al [23, 25-27] performed a great study about the sustainable production of cannabinoids with SFE-CO_2. Firstly, the authors determined the solubility of THC [26], CBN [27] CBD and CBG (cannabigerol) [25] in supercritical CO_2 and observed that the solubility of the different cannabinoids in supercritical CO_2 increases at 53 °C in the following order: THC < CBG < CBD < CBN. Based on the solubility parameters, the authors explored the cannabinoids extraction and isolation by SFE-CO_2 and concluded that this process is superior to the conventional techniques (extraction with organic solvents) regarding the both ecological and economic point of view [23]. The THC yield (98%) was obtained at 40 °C and 230 bar using a CO_2 flow rate of 100 g/min and solvent to feed ratio of 400:1 [23]. The authors also suggested a two-steps process to selectively extract minor cannabinoids (CBN, CBD and CBG) in a first step at low pressure (~150 bar), and THC in a second step at higher pressure (~200 bar).

A most recent study conducted by Rovetto and Aieta [24] reported the evaluation of different *Cannabis* strains (with different cannabinoids content) exploring the effects of pressure, extraction time and ethanol as co-solvent over the THC concentration of the extracts and overall process yield. The best extraction conditions selected by the authors were 55 °C and 340 bar. They also observed that by applying pulses of 5% of co-solvent (ethanol) it was possible to reduce the solvent to feed ratio from 40 to 12. However, the use of ethanol enables the co-extraction of other compounds, such as chlorophyll.

Regardless of the rising popularity and usage of SFE-CO_2, the scientific literature about the SFE-CO_2 of *Cannabis* is relatively recent and there is very limited reported information about the efficiency of the SFE-CO_2 for *Cannabis*. However, Figure 1 present the number of publications found for the search using the keywords “cannabis” and “extraction” along the last 30 years, where it is evident the increasing interest in the subject, which means that more achievements related to the process design and optimization may are coming soon. It is still necessary to evaluate the parameters in order to determine the scaling up process as well as to evaluate the economic and environmental impacts of the process.

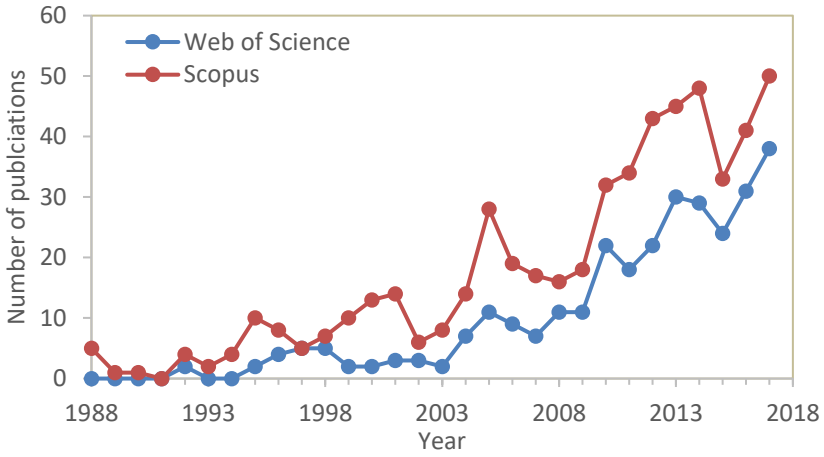

Figure 1: Number of publications resulted from the search using the keywords "cannabis" and "extraction" for the period 1988-2017. Bases consulted: Web of Science and Scopus.

Industrial applications

Despite the scarce results reported in literature, SFE-CO_2 has been used to produce *Cannabis*–based products worldwide. Actually, according to specialized *Cannabis* market magazines, one of the big trends for the *Cannabis* industry is extracted products, which are used to provide alternatives to smoking or vaping cannabis flower, including edibles and concentrates. In some markets, the share of flower has declined to 50% with these derivatives making up the balance [28] . SFE-CO_2 has proved to be one of the most versatile and safest methods of producing *Cannabis* concentrates despite the lack of scientific information on optimized processes [29], which can be due to its unique features, such as:

- As far as non-polar solvents are concerned, CO_2 is among the safest. In fact, the FDA has labelled CO_2 safe for industrial extractions, making it a much less controversial solvent than petroleum based hydrocarbons such as butane or propane.
- The conditions that allow CO_2 to change from a fluid state to a supercritical state can occur without having to exceed temperatures above 31 °C, meaning there is less risk of compromising the natural volatile compounds found in *Cannabis.*
- The CO_2 solvent power is tunable with the operating conditions, allowing the fractionation of the many different types of biomolecules available in *Cannabis* strains, including the cannabinoids fractionation as well as terpenes and other compounds.

A quick look on the patents survey shows a quite higher number of results for *Cannabis* extraction by SFE-CO_2 compared to the scientific ones. This great industrial interest in *Cannabis*-based products can be a possible explanation for why *Cannabis* extraction processes optimization are not widely published in the open literature [30-37]. The patented processes stablish not only SFE-CO_2 as the main objective, but most of them report it as an important part of the whole process, which is the case of medicinal products that use purified compounds as active principle. Another aspect of the patented processes is that several of them perform the winterization step after the SFE-CO_2 to remove the waxes from the extract. Winterization is a really time consuming step that consists of mixing the extract with ethanol. This mixture is cooled and held for precipitating the waxes. Finally, the precipitated is removed from the extract by filtration.

An example is one of the process patented by GW Pharma, a big player in the *Cannabis* market. Its process describes the obtaining of crude extract by SFE-CO_2 at subcritical conditions (10 °C and 60

bar). The extraction process takes approximately 8 hours with a CO_2 flow rate of 1250 kg/h for a batch size of 60 kg. Subsequently, the winterization step is performed for removing the waxes from the extract. The winterization step takes approximately 48 hours. Finally, a semi-solid, viscous and brown extract containing 60% of cannabinoids. In this case, the inventors preferred to operate the extraction process at subcritical conditions to reduce the waxes content in the extract and, thus, improve the efficiency of the winterization step. However, as it can be observed, the whole process still very time consuming.

FUTURE PERSPECTIVES

Despite the advances in the *Cannabis*-based products field, some challenges need to be overcame to enable its full development, including not only aspects related to the SFE-CO_2 process itself, but to the raw materials too.

Although the regulatory issues still are a considerable barrier in many countries around the world, the discussion about the *Cannabis* regulation is hot and the tendency points at the relaxation to allow the medicinal use of *Cannabis* products. While some countries have allowed even the recreational use of *Cannabis* (as Uruguay, for example) several others keep strict laws, which may difficult the development of the *Cannabis* market.

Regarding the development of SFE-CO_2 process for *Cannabis* application, although it has already been commercially used, there are some concerns related to this industry that are similar to those faced by any other plant material. Among them, it is necessary to improve the knowledge on scaling up, economic and environmental assessment of the processes to effectively demonstrate the advantages of the compressed fluid technologies compared to the conventional [13]. In the current context, the warmed *Cannabis* market can accelerate the development of SFE-CO_2 for this application in principle, which can be further spread to other related plant materials. Considering the potential of *Cannabis* plant, the design of biorefinery platforms based on it could be a very promising perspective.

CONCLUSION

Cannabis-based products are already a case of success of SFE-CO_2 application, since it is a plant material full of non-polar compounds and SFE-CO_2 process is used with relatively good yields. The obtained products can be explored even for medicinal purpose or other commercial interest, always with a high added-value. The added-value of these products seems to be high enough to spare minimum details concerning improving the productivity or avoid losses even if the extraction is only a part of the whole production. Despite of this, there is still some work to do to improve process productivity, environmental and economics and, consequently to achieve the best goals.

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