Tekniikan kandidaatintyö

Extraction and Purification of Cannabidiol

Lappeenranta 2020

Heini Teräsvalli

Työn ohjaaja: Prof. Tuomo Sainio

Tiivistelmä

LAPPENRANNAN-LAHDEN TEKNILLINEN YLIOPISTO
LUT School of Engineering Science
Kemiantekniikka

Heini Teräsvalli

Extraction and Purification of Cannabidiol

Kandidaatintyö

2020

36 sivua, 16 kuvaa, 3 taulukkoa.

Työn ohjaaja: Prof. Tuomo Sainio

Hakusanat: extraction, separation, purification, cannabidiol, cannabis, supercritical carbon dioxide, solid-liquid extraction, CBD, THC, kannabidioli, kiinteä-nesteuutto, ylikriittinen hiilidioksidiuutto

Kannabidioli (CBD) on hamppukasvista saatava ei-päihdyttävä yhdiste, jolla on lääkinnällisiä ominaisuuksia. Tämän työn tarkoituksena oli tarkastella ja arvioida kannabidiolin erotukseen ja puhdistukseen kaupallisessa mittakaavassa sopivia menetelmiä tieteellisten kirjallisuuslähteiden ja muiden, julkisten verkkoaineistojen avulla.

Markkinat CBD:tä sisältäville tuotteille ovat viime vuosina kasvaneet etenkin Pohjois-Amerikassa ja Euroopassa. Markkinoiden kasvu on tuonut alalle paljon uusia yrityksiä. Myös jo olemassa olevat kannabisalan yritykset ovat laajentaneet tuotevalikoimaansa CBD-tuotteisiin. Tärkeimmiksi kilpailuvalteiksi ovat nousseet tuotteen laatu ja turvallisuus, joiden perusteella usein valitaan sopivimmat erotus- ja puhdistusmenetelmät yrityskohtaisesti.

Kannabidiolia voidaan erottaa kasvimassasta, sekä tuotetta puhdistaa erilaisilla uuttomenetelmillä. Kuluttajatuotteiden valmistuksessa suosituimmat menetelmät kannabidiolin

erottamiseksi kasvimassasta ovat ylikriittinen hiilidioksidiuutto ja kiinteä-nesteuutto. Lisäksi on tutkittu syväeutektisia liuottimia vaihtoehtona kannabinoidien erotukseen. Liukoisuusparametriteorian mukaan etanoli on tehokkain liuotin kannabidiolille.

Vertailukelpoisen tieteellisen tutkimuksen määrä eri erotus- ja puhdistusmenetelmien tehokkuudesta on tällä hetkellä pieni, mutta joitakin päätelmiä voitiin tehdä. Ylikriittinen hiilidioksidiuutto on perinteistä kiinteä-nesteuuttoa selvästi hitaampi menetelmä. Seosliuottimien käyttö parantaa saantoa ja laskee hiilidioksidin kulutusta ja sitä kautta erotuksen kustannuksia. Hiilidioksidia pidetään muita liuottimia turvallisempana etenkin sisäisesti nautittavien tuotteiden valmistukseen. Haittapuolena menetelmän käytössä kaupallisessa mittakaavassa on laitteistojen korkea hinta verrattuna kiinteä-nesteuuttoon. Kokonaiskustannuksiltaan kiinteä-nesteuutto etanolilla on ylikriittistä hiilidioksidiuuttoa edullisempi menetelmä. Kannabidiolia voidaan puhdistaa edelleen saostamalla vahat ja lipidit alhaisessa lämpötilassa, tislaamalla, kiteyttämällä tai kromatografisesti. Tislaamalla voidaan tehokkaasti poistaa liuotinjäämät ja muut helposti haihtuvat yhdisteet. Isolaattia voidaan valmistaa kiteytyksen lisäksi kromatografisesti, joista CPC-kromatografia on uudehko menetelmä, joka sopii hyvin kaupalliseen käyttöön.

Abstract

LAPPEENRANTA UNIVERSITY OF TECHNOLOGY
LUT School of Engineering Science
Chemical Engineering

Heini Teräsvalli

Extraction and Purification of Cannabidiol

Bachelor’s Thesis
2020

36 pages, 16 figures, 3 tables.

Examiner: Prof. Tuomo Sainio

Keywords: extraction, separation, purification, cannabidiol, cannabis, supercritical carbon dioxide, solid-liquid extraction, CBD, THC, kannabidioli, kiinteä-nesteuutto, ylikriittinen hiilidioksidiuutto

Cannabidiol (CBD) is a non-psychoactive, medicinal compound found in the cannabis plant. The aim of this thesis was to examine and evaluate methods used to extract and purify cannabidiol in a commercial scale based on scientific literature and other public online sources.

The market for products containing CBD have grown in the last few years especially in North America and Europe, and therefore many new companies have entered the business. Companies already in the cannabis industry have also began producing CBD products. The most important competitive factors are the quality and safety of the product, and each company chooses the most suitable extraction and purification methods according to their own criteria.

Different extraction methods can be used to extract cannabidiol from the plant matter and to purify the product. Supercritical carbon dioxide and solid-liquid extraction are the most popular methods to extract CBD from plant matter when producing consumer goods. Deep eutectic solvents have also been studied as an alternative. According to the solubility parameter theory, ethanol is the most effective solvent for cannabidiol.

The number of comparable scientific studies about the effectiveness of different extraction and purification methods is low at this time, but some conclusions could be made. Supercritical carbon dioxide extraction is a significantly slower method than a traditional solid-liquid extraction. The use of co-solvents improved yield and lower CO_2 consumption, thus lowering the costs of the extraction. Carbon dioxide is seen as a safer solvent than most for the production of internally consumed products. A drawback of supercritical CO_2 is the high cost of commercial-scale equipment compared to solid-liquid extraction. The total cost of solid-liquid

extraction with ethanol is lower than supercritical CO_2. Cannabidiol can be purified further by solidifying and removing waxes and lipids in a low temperature, in process called winterization. Distillation, crystallisation and chromatographic techniques can also be used. Solvent residue and other volatile compounds can be efficiently removed by distillation. Isolate can be produced by crystallisation as well as chromatographically. Centrifugal partition chromatography (CPC) is a novel method of purification, which is suitable for commercial use.

Symbols & Abbreviations

$[A]_{mob}$	concentration of A in the mobile phase
$[A]_{stat}$	concentration of A in the stationary phase
BP	boiling point
CBD	cannabidiol
CBDA	cannabidiolic acid
CBN	cannabinol
CO2	carbon dioxide
CPC	centrifugal partition chromatography
δ	solubility parameter
DES	deep eutectic solvent
DME	dimethyl ether
EtOH	ethanol
GRAS	generally regarded as safe
HPLC	high performance liquid chromatography
K_d	partition coefficient
LCO2	liquid carbon dioxide
M:AA	menthol:acetic acid mixture
MeOH	methanol
$scCO_2$	supercritical carbon dioxide
SCF	supercritical fluid
SPT	solubility parameter theory
TFE	tetrafluoroethane
THC	(Δ^9) tetrahydrocannabinol
THCA	tetrahydrocannabinolic acid
U.S.	the United States
USD	the United States dollar

1 Introduction

For the last several years there has been a growing interest in cannabis-based products around the world. Products containing cannabidiol (CBD) have gained popularity among consumers which has attracted many new companies in to the business [1]–[3]. This has led to improvements in the methods and technology of extracting CBD from plant matter as well as methods for purifying the extracted oil. Some new methods have also been researched for possible implementation to large scale commercial use.

The information surrounding the commercial production of CBD is scarce and scattered, and scientific reports is lacking in many areas. The novelty of the industry and the desire of companies to keep trade secrets has enabled the spread of anecdotal information. More high quality scientific research needs to be conducted at a larger scale in order for the industry to benefit.

The goal of this thesis is to present and assess different extraction and purification techniques most commonly used in commercial scale production of consumer products containing CBD from industrial hemp by using literature and online sources. Some new promising extraction and purification methods are also evaluated. The CBD industry, and regulations surrounding the market is explored and assessed to further evaluate what is expected of the methods in terms of quality and quantity of the product.

2 Hemp and key components

The cannabis plant or hemp (*Cannabis sativa* L.) is believed to have been originated from Central North-East Asia [4]. It has been cultivated for around 5000 years and it is one of the oldest known domesticated plants [4]. The plant is dioecious which means that the male and female are typically a separate plant [5]. Cannabis was used to produce a wide variety of goods such as cordage, cloth, lighting oil and medicine [6, p. 8]. Today hemp fibre can be used in paper manufacturing, textile, cosmetics, pharmaceutical and food industries, and even to produce biofuel [4], [5].

There are two main types of cannabis plants, industrial hemp and marijuana. The main difference between the two is the amount of Δ^9-tetrahydrocannabinol (THC) in the plant. Marijuana, drug-type cannabis, has a higher level of THC. For example, some popular strains sold in Colorado 2017 had THC-levels between 17-28 % [7]. Industrial hemp has been bred for high fibre content or seed production and needs to have a low THC-level to be cultivated legally in many countries [5]. For example in the United States hemp needs to have a THC content of less than 0.3 % of dry mass according to the Agriculture Improvement Act of 2018, also known as the Farm Bill of 2018 [8]. In the European Union the limit for THC is 0.2 % [3]. Figure 1 shows female plants of industrial hemp.

Figure 1 Female industrial hemp plants and flowers [9].

Almost 500 different compounds can be found in the plant, such as terpenes, amino acids, fatty acids, hydrocarbons, flavonoids and sugars [10]. Terpenes are compounds responsible of the rather distinct aroma of the plant [11] and many of them are highly volatile [12]. Cannabinoids are a class of chemical compounds that are only found in the cannabis plant and more than 70 have been identified [10]. The two most important ones for their potential in medical uses are THC and cannabidiol (CBD). These compounds are naturally found especially in the unpollinated flowers of the female plant [6] mostly in their acidic forms, tetrahydrocannabinolic acid (THCA) and cannabidiolic acid (CBDA). The structures of THC, THCA, CBD and CBDA are shown in Figure 2.

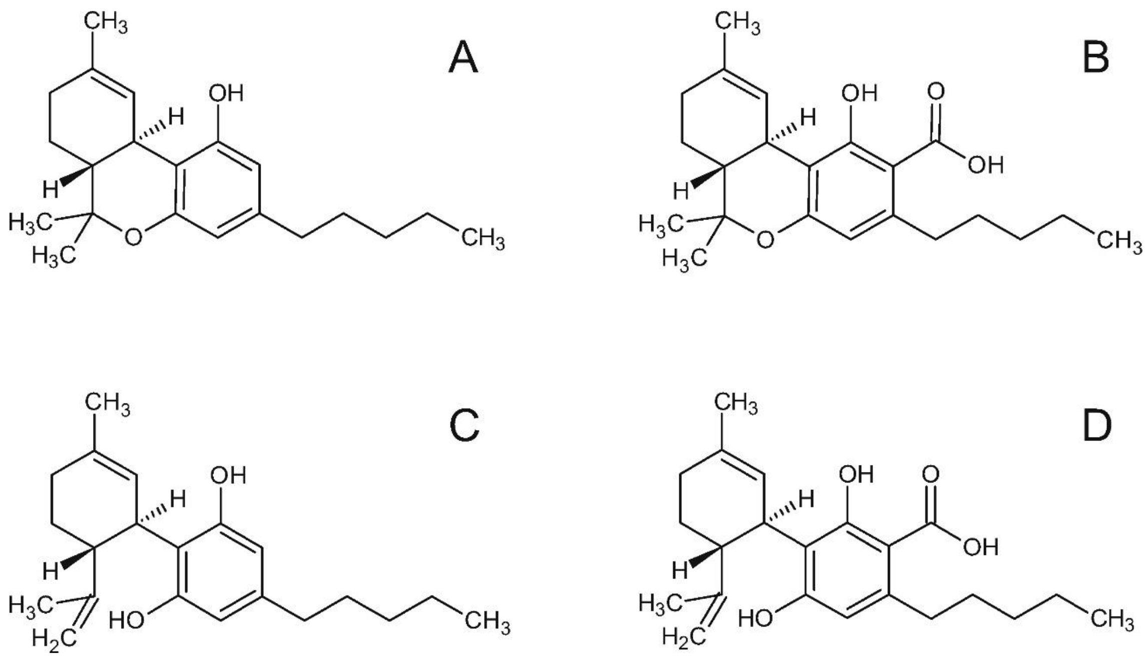

Figure 2 Structures of tetrahydrocannabinol (THC) (A), tetrahydrocannabinolic acid (THCA) (B), cannabidiol (CBD) (C) and cannabidiolic acids (CBDA) (D) [13].

The neutral and acidic forms of both THC and CBD differ only by the carboxyl group attached to the aromatic ring. CBD and CBDA have two hydroxyl groups, whereas THC and THCA have an ether group. When light, heat or alkaline conditions are introduced, the acidic compounds can convert to their neutral forms by spontaneous non-enzymatic decarboxylation, where a CO_2 molecule is released when the carboxyl group is removed [13], [14]. CBD has a molecular weight of 314.5 g/mol and quite a low melting point at 67 °C. THC on the other hand has the same molecular weight of 314.5 g/mol, but it is liquid at room temperature [15]. The two large alkyl groups make CBD non-polar and thus not soluble in water, but the two phenolic hydroxyl groups allow for the forming of hydrogen-bonds [5]. CBD has a boiling point of around 180 ℃ [16], sometimes said to be around 160-180 ℃, and THC 157 ℃ at atmospheric pressure [12].

THC is the main psychoactive compound found in the plant and as it metabolises to 11-hydroxy-THC, it gives the user euphoric effects [17], but it is also used to relieve nausea during chemotherapy and to improve appetite of AIDS patients [5]. CBD on the other hand is non-psychoactive and has uses in treating a variety of central nervous system disorders, such as Alzheimer's disease [4], cancer and drug-resistant epilepsy [5]. CBD infused products have been marketed for treatment of other less severe ailments, such as insomnia, anxiety and different types of chronic pain [18].

3 CBD Market and Products

As interest in CBD has risen in the past few years, so has the number of new companies entering the business. The influx of new businesses has even been described as "a gold rush" by Bethany Gomez, a director of research from Brightfield Group, a market intelligence firm for the cannabis and CBD industry [19]. Brightfield has estimated in their report of the 2019 U.S. CBD market that the industry is worth over $4 billion which is a 562 % increase compared to 2018. The United States market could be worth $24.4 billion by 2025 [20] assuming the cannabis stock market recovers from the fall of 2019 that can be seen from the development of the North American Marijuana Index in Figure 14. The CBD market value in Europe was estimated to be worth $416 million (€373 million) in 2018 and to grow over 400 % by 2023 [21]. The CBD industry is discussed more in chapter 6.

The variety of products has broadened as the market size has grown, from full spectrum CBD oils to topical creams and infused food products, such as ice cream [19] and beauty products [22]. In 2017 most sales were online, but some products are already being sold in large, well-known retail chains, which promises growth for the business [20]. Some companies do not grow their own hemp, but instead buy CBD oils or isolates from other companies to be used in their own products [19]. The business models existing in the industry are discussed in more detail in chapter 6.1.

As mentioned above, the variety of CBD products is vast. The three forms are categorized by their cannabinoid profile as isolate, full spectrum and broad spectrum. Isolate is a crystallised form of CBD, and it is highly purified using for example, chromatographic methods such as centrifugal partition chromatography (CPC) which is introduced in chapter 5.2. As the name suggests, CBD isolate does not contain any other cannabinoids or terpenes. The product is tasteless and odourless [23]. For consumers that have concerns about the intoxication effect of THC or drug-testing, CBD isolate is recommended [24].

Full spectrum CBD extract contains other naturally occurring compounds in the plant, such as terpenes, other cannabinoids and sometimes a small amount of THC. The entourage effect of the terpenes and other cannabinoids may make the product more beneficial or produce the same effect with a lower dosage [23], [25]. Full spectrum CBD is usually the least expensive choice as it requires less processing. The products have a stronger flavour and smell compared to products made with isolate which results from the presence of terpenes. Broad spectrum CBD is equivalent to the full spectrum CBD, but all of the THC has been removed [23].

Most common ways of using CBD products are by ingestion, inhalation, mucosal delivery and topical application. CBD infused foods, capsules and oils are eaten, and CBD is absorbed through the digestive system. Inhaled products include dried cannabis flowers smoked in a joint or a pipe, and products designed for vaping. CBD can also be absorbed through the mucus membrane. The product, for example a tincture, is typically placed under the tongue for a short time and then swallowed. This method is considered faster than ingestion. Topically applied products, such as lotions and sprays, are applied directly to the skin of the area where the user experiences pain for example [26].

4 Methods for extracting cannabidiol from plant matter

Several different methods exist to extract CBD-rich oil from the plant. The seemingly most popular methods used include carbon dioxide (CO_2) extraction and other solvent-based extractions. For traditional solvent-based extraction, ethanol is the common choice as it is regarded as safe to use in both medical as well as edible products [10]. Regardless of the chosen method, the pre- and post-processing steps vary quite little.

Before the extraction, the plant matter is milled to reduce particle size. This allows for a more efficient and uniform extraction [10], [27]. After the milling process, the substance can be decarboxylated by introducing heat before the extraction. The material is then subjected to the chosen extraction process. The extracted crude oil is then mixed with a solvent, typically ethanol. The next step is called winterization or dewaxing, where the mixture is cooled down to -20 – -80 °C [10] and left to settle for 24-48 h to allow the components with a higher melting point, such as lipids and chlorophyll, to solidify. The cool mixture is then filtered or centrifuged to remove the waxes [28]. The solvent used in the winterization is removed with for example, a rotary evaporator, collected and recycled back to the process. If the product needs further purification, it can be distilled. To create isolates, the product can be purified chromatographically [10], [15], [17] or crystallised. Purification methods are discussed in more detail in chapter 5. Figure 3 shows the outline of the preliminary processing, extraction and winterization steps.

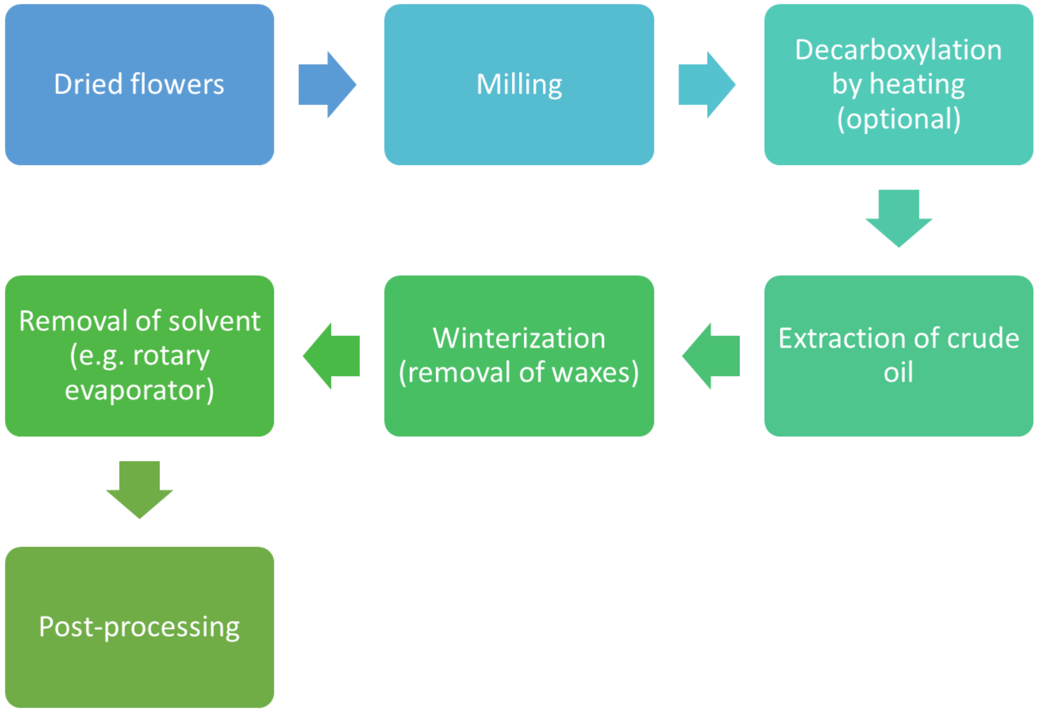

Figure 3 Simplified schematic of the preliminary, extraction and winterization steps commonly used to produce products containing cannabidiol.

Choosing the most suitable method for extracting CBD can be quite difficult as many variables need to be considered, such as solvent properties, temperature, pressure and time. Other factors to consider when choosing a solvent include environmental safety, toxicity and the cost from an economic standpoint. The complex nature of the plant's chemical makeup makes these decision increasingly hard; as mentioned before, there are many other often unwanted components present that tend to be extracted together with the target compound [29].

The solvent's capability to extract the desired compound or compounds as well as selectivity are crucial. As discussed before, hemp contains many different compounds, which differ in terms of solubility to different solvents. According to solubility parameter theory (SPT), a compound is optimally soluble and miscible with a solvent when their solubility parameters (δ) match or are close to each other. The further apart the solubility parameters are, the poorer the solubility. Molecular interactions between the solutes may enhance or inhibit the extraction of cannabis constituents, for which reason SPT only offers some guidance for choosing a solvent [17].

Solubility parameters are not always constant; for liquified fluid solvents, such as ethanol and liquid carbon dioxide (LCO_2), δ is dependent on temperature. For supercritical carbon dioxide ($scCO_2$), δ is a function of temperature and pressure. Figure 4 presents the δ-values of some cannabinoids, including CBD, THC and cannabinol (CBN), and some terpenes in the first column. The second column shows the δ-values of hydrocarbon fluids, namely propane, butane, tetrafluoroethane (TFE) and dimethyl ether (DME) at temperatures commonly used in extraction. The δ-values of ethanol (EtOH) and super- and subcritical CO_2 under varying conditions are shown in the third column [17].

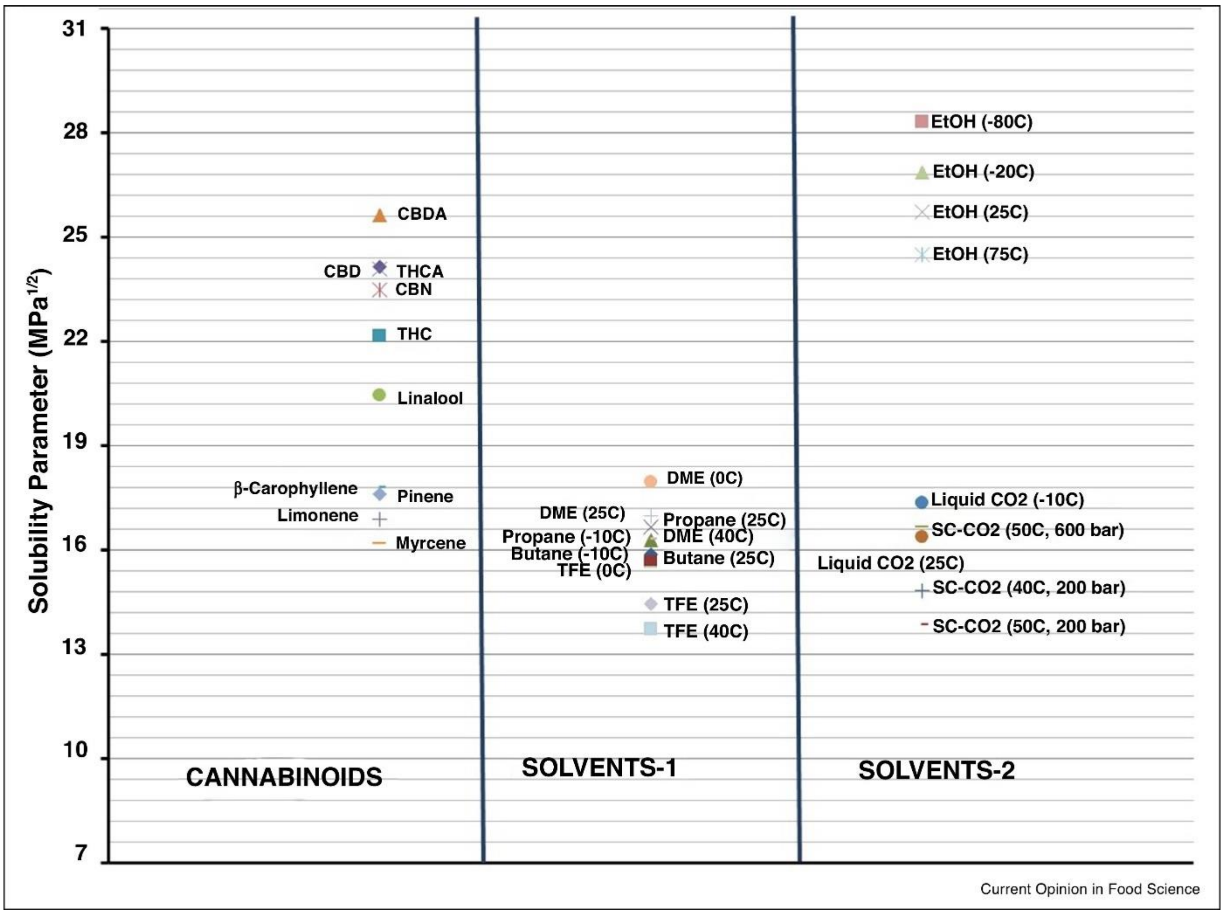

Figure 4 Solubility parameters of some cannabinoids, terpenes and solvents under varying extraction conditions [17].

The δ-values of main cannabinoids are 22-26 $MPa^{1/2}$, terpenes around 19 $MPa^{1/2}$. The δ-values of waxes are 16.0-16.6 $MPa^{1/2}$. According to SPT, ethanol is the most effective solvent for cannabinoids. Cold ethanol is a poor solvent for waxes which allows for less post-processing. Hydrocarbons like propane and butane can be used, but they are not as selective for cannabinoids as ethanol, and more terpenes and waxes are extracted. Considering the popularity of $scCO_2$ it is interesting to note that carbon dioxide is a poor solvent for cannabinoids. Extraction time and the number of solvent passes need to be increased in order to effectively extract CBD (or other cannabinoids) with $scCO_2$ [17]. Using ethanol as co-solvent together with $scCO_2$ has been reported to improve the solubility of cannabinoids [10], [17], this phenomenon is discussed in more detail in chapter 4.1.

The next chapters discuss two of the most common methods of extracting CBD from plant matter. Carbon dioxide extraction is discussed in chapter 4.1, and solid-liquid extraction options in chapter 4.2.

4.1 Supercritical carbon dioxide extraction

This chapter discusses the use of supercritical carbon dioxide as a solvent for CBD extraction. The extraction process is explained as well as the use of a co-solvent. Different extraction conditions and regimes is explored. At the end of this chapter, some equipment currently available on the market are presented for comparison.

When matter reaches supercritical phase, it cannot be distinguished whether the matter is gas or liquid. Supercritical fluids, or SCFs, have a relatively high density, somewhere between a gas and a liquid, but have a low viscosity like gases. Typically, this state is reached at a high pressure, but the required temperature varies greatly between compounds. Carbon dioxide reaches its critical point at 7.37 MPa of pressure and at the temperature of 31 °C, and from this point forward it cannot be liquified regardless how much pressure is applied [30]. Subcritical conditions mean conditions under the critical point. Under ambient conditions CO_2 returns to gas which allows the final product to be solvent-free. The solvent strength and selectivity to some extent can be tuned by modifying temperature and pressure [10]. The supercritical state allows the solvent to act both as a gas and a liquid which has been proven a very useful property in various applications [30]. Typical uses for SCFs in general are the extraction of desired compounds, such as essential oils and other bioactive components, from vegetable matrices [10]. Some examples for supercritical CO_2 ($scCO_2$) uses are coffee and tea decaffeination, water-free dyeing of fabrics, extraction of natural flavours and perfumes as well as producing vegetable oils and rice with a long shelf-life [30].

Co-solvents can be used with $scCO_2$ to increase the solubility of the target compound by providing a specific chemical interaction with the desired solute. The co-solvent modifies the solvent properties of CO_2 allowing it to dissolve more polar compounds. Common co-solvent choices for CO_2 are low molecular weight alcohols such as methanol, ethanol and propanol, ketones (acetone, butanone), aldehydes (acetaldehyde), esters (ethyl acetate) [30], water and acids [10]. Many of the above-mentioned substances are toxic, irritating or flammable for which reason they are not used in any food-related applications but can be used to extract fragrances and flavours.

The phase conditions of the SCF mixture as well as the recovery and recycle possibilities of the co-solvent must be considered. Typically, the solvent mixture (CO_2 + co-solvent) is in a single phase, so pressure and temperature must be chosen accordingly. For example, alcohols are liquids at room temperature, but have a fairly low boiling point so they are easily removed and collected in a rotary evaporator after the winterization process, so using them as a co-solvent does not alter the process. As a downside, the use of a co-solvent might increase the solubility of unwanted compounds lowering the purity of the product [30], [10]. The removal of these unwanted compounds may increase the total processing time.

The extraction process (pre-processing and winterization included) of cannabinoids with $scCO_2$ follows the basic structure presented in Figure 3. After the pre-processing, the ground plant matter is placed in the extraction vessel (or vessels), and $scCO_2$ is pumped through the material extracting the cannabinoids and other compounds such as terpenes and waxes [17]. The extraction time for this method is long, starting from 2 hours at the low end [31] but is often 6 to 10 hours [32].

The solubility of CBD to $scCO_2$ changes with pressure and temperature. Figure 5 shows that solubility increases while pressure increases. With temperature the effect is not straightforward. At 61 ℃ the solubility is significantly lower that at 53 ℃. At an even lower temperature the solubility is also poorer that at a moderate temperature [15].

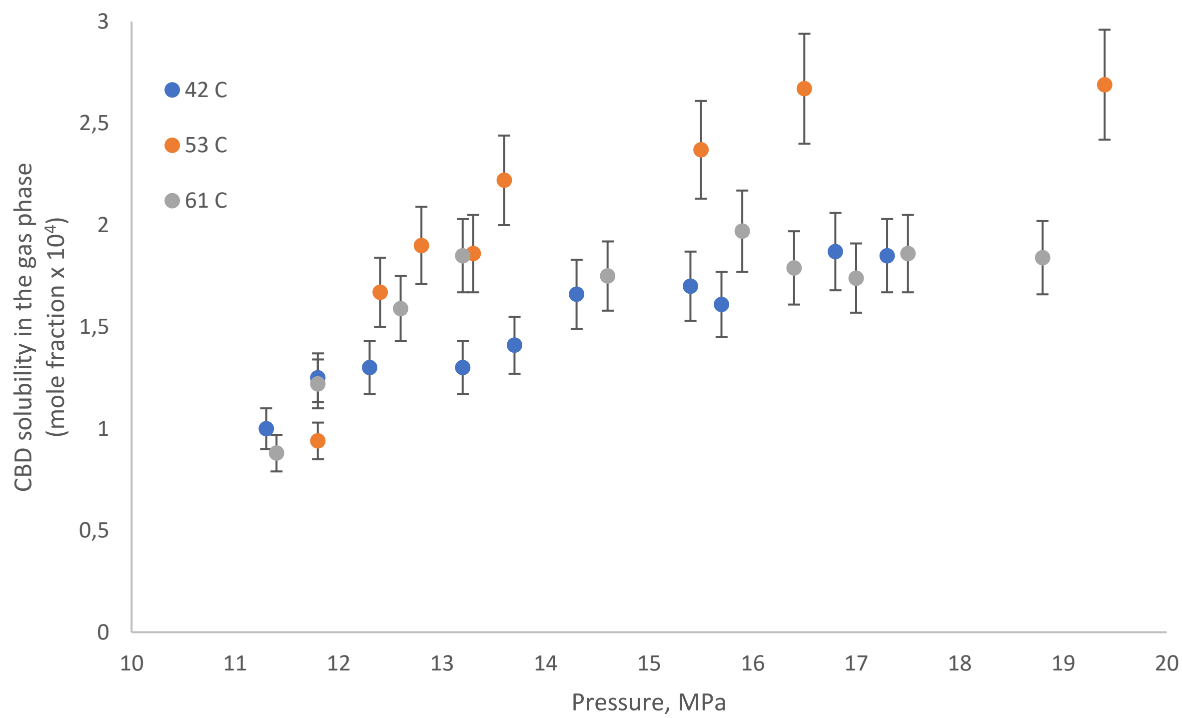

Figure 5 Molar solubility of CBD at different temperatures and pressures. Adapted from [15].

While there is a lack of quality scientific research on the effects of extraction parameters specifically in cannabis extraction, there are some general trends found through trial and error by some companies in the cannabis industry, some of which are supported by the limited research available (Figure 5). Most important parameters are temperature, pressure, flow rate and solvent-to-feed ratio, influencing yield, product quality, extraction time and overall cost efficiency [33].

Temperature can be adjusted throughout the process. The most important temperature is that in the extraction vessel. Increasing the extraction temperature decreases terpene content in the extract as terpenes are usually quite volatile. More waxes and resins as well as chlorophyll are extracted at a higher temperature, increasing the overall extract quantity. Decreasing the temperature reduces the amount of waxes extracted and increases the oil yield. Lower temperature is suggested to preserve volatile terpenes better [33].

Extraction pressure is also critically important. As with increase in temperature, increase in pressure also promotes wax and chlorophyll extraction. If the supply of CO_2 is not continuous, high pressures are difficult to maintain, leading to less predictable results [33]. More CBD can be extracted at a higher pressure as seen from Figure 5.

Flow rate of CO_2 has the most effect on yield and processing time. Low flow rate allows the solvent to be in contact with the material for longer. This increases yield, but extraction time is also increased. High flow rate can quicken the extraction process, but there are potential hazards. Dry ice can start to accumulate causing the lines to clog if no external heat is applied. At high pressures, maintain high flow rates can be challenging as the supply of CO_2 depletes over time [33].

Ethanol is a common co-solvent choice when extracting cannabinoids. Ethanol has been found to improve the extraction rate and lower the amount of CO_2 needed. If the co-solvent is administered in pulses instead of a constant stream, less CO_2 is required for efficient extraction. The amount of co-solvent needed is also decreased as well as the extraction time. The higher the concentration of ethanol is, the stronger are the perceived effects [10]. Solvent-to-feed ratio should be at a level where extraction is efficient in terms of quantity, thus minimising costs [33].

To summarize, extraction temperature and pressure should be moderate to promote CBD extraction and to lessen wax and chlorophyll extraction. Flow rate should be high enough to reduce extraction time without lowering the CBD yield. Ethanol as a co-solvent is recommended, supplied in pulses to minimize solvent consumption and to lower costs. The right feed-to-solvent ratio is the one most cost-efficient.

The equipment needed for scCO_2 extraction consists of a CO_2 reservoir, heat exchangers and a pump, the extraction vessel (or vessels), collection vessel (or vessels) and a number of valves and other instruments, such as pressure, flow-rate and temperature sensors. A separate reservoir and pump are needed for a co-solvent. The solvent flows through the plant material packed in the extraction vessel. The extract, i.e. crude oil, is collected, and CO_2 recycled back to the process [10], [29]. Figure 6 shows an example of a scCO_2 extraction equipment described above.

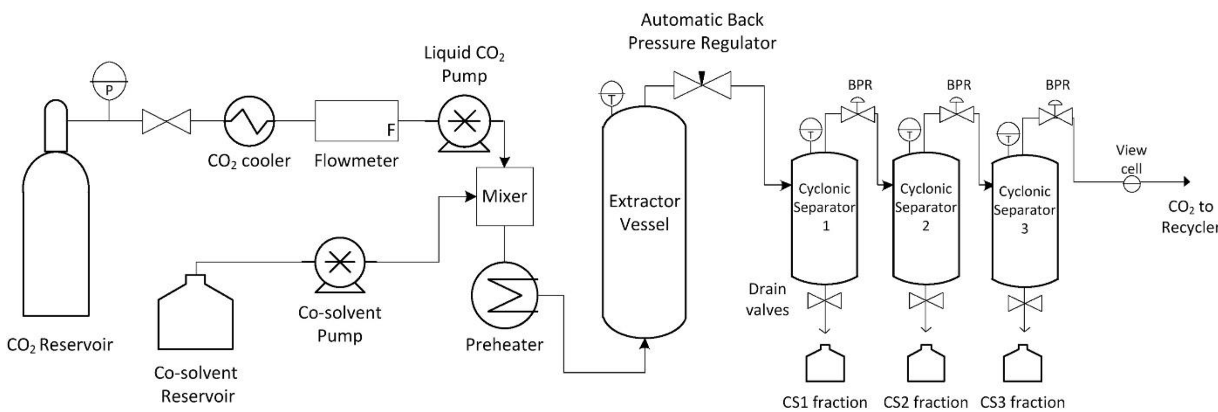

Figure 6 Schematic example of a supercritical carbon dioxide extraction equipment with possibility for co-solvent use [10].

One example of the equipment is presented in a patent [34]. The patent is currently active for example in the United States, Germany and Spain, but it has been filed in many other countries around the world with varying degrees of success. The apparatus is capable of extracting CBD as well as Δ^9-THC and Δ^8-THC [34]. The equipment is presented in Figure 7 and explained below in more detail.

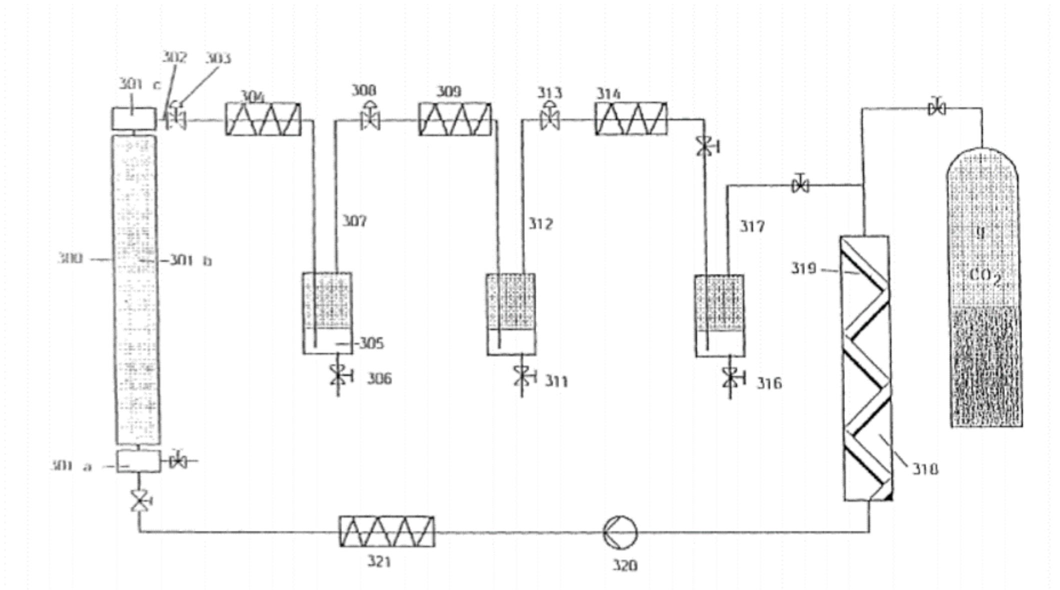

Figure 7 Schematics for equipment used to extract CBD and optionally Δ^9-THC and Δ^8-THC using super critical CO_2 as patented by Mueller. Extraction vessel is on the left, three collection vessels in the middle. On the right is a condenser and a CO_2 reservoir [34].

The extraction vessel consists of a bottom segment, a purification segment which is packed with silica gel, and a head segment. The extract then flows to the first separating vessel via a duct, a regulating valve and a heat exchanger to reduce pressure and temperature. The CBD is collected in this vessel. The extract continues through another set of a regulating valve and a heat exchanger to further reduce pressure and temperature. Δ^8-THC is collected in the second vessel. The third vessel and the components are identical to the first two, pressure and temperature again reduced. Δ^9-THC is collected in the third vessel. The remaining CO_2 is pumped to a liquefier equipped with a condenser coil. The liquid CO_2 is then pressurized with a pump and heated in a heat exchanger before it enters the extraction vessel again [34].

Many manufacturers offer $scCO_2$ equipment in varying sizes for different scales of operation. Some examples are compiled in Table I.

Table I Information of some supercritical carbon dioxide extraction systems available.

Product name	**The Force ®**	**The Bambino ®**	**E-180**	**Hi-FloTM FX2**
Manufacturer	Apeks Supercritical	Apeks Supercritical	extraktLAB	Eden Labs
Scale of production	Large scale commercial, industrial	Small scale commercial, R&D	Large scale commercial, industrial	Commercial
Price	Starting at $458 500	Starting at $89 500	Not disclosed	$175 000
Extraction vessel volume	80 L	5 L	4 x 20 L	20 L
Load capacity of dried plant matter per run	18 kg	1.4 kg	10-16 kg	4.5 kg
Max pressure (extraction vessel)	344 bar	137 bar	344 bar	344 bar
Temperature	Max 71 ℃	Max 71 ℃	25 – 100 ℃	-60 – 60 ℃
Flow rate of CO2	3.5-4.2 kg/min	0.4-0.8 kg/min	Not disclosed	2.2 L/min*
CO2 recovery	95 %	95 %	Not disclosed	Up to 95 %
Run time	Not disclosed	Not disclosed	Not disclosed	3-7 h
Other information	Fully automated, subcritical extraction possible	Fully automated, subcritical extraction possible	Subcritical extraction possible, automated process control	Extensive automation
Sources	[35], [36]	[37], [38]	[39]	[40], [41]

* Pressure not specified

As Table I shows, $scCO_2$ extraction equipment is quite expensive even at a small scale. This might deter some potential new companies that do not have access to adequate funding. The processing capacity, and thus yield, depends heavily on the density of the plant matter. Extraction conditions can be tuned at a large range based on the composition of the plant matter and the desired composition of the product. All the manufacturers mentioned above offer some form of training and support to customers. It should also be noted that some companies offer even larger extraction systems, as large as 3 x 2000 L (extraction vessel volume) [42].

4.2 Solid-liquid extraction

This chapter reviews traditional organic solvents and deep eutectic solvents in extraction of cannabidiol. Advantages and disadvantages are discussed. Select few commercial solid-liquid extraction equipment are presented in Table II for comparison.

Organic solvents, such as short-chain alcohols and hydrocarbons can be used to extract cannabinoids with high yields. These solvents are typically of low cost and require relatively simple equipment for the extraction, but many of these solvents pose risks to health, environment and safety [5], [15].

Hydrocarbons such as propane, butane and hexane are used in CBD extraction as well. The solubility parameter theory suggests that hydrocarbons are not very effective at extracting cannabinoids, as their δ-values are quite different (see Figure 4). An advantage with using hydrocarbons as solvent are their low boiling point (for example, butane -0.5 °C, propane -42.1 °C at 1 atm [43], [44]) which allows for an easy solvent recovery. Hydrocarbon extraction is quite heavily regulated, as these flammable compounds clearly pose safety concerns. Regulatory compliance adds to the overall cost of production [32], [45], [46].

As was discussed in chapter 4, ethanol is an effective solvent for CBD at a wide range of temperatures; from -80 °C to 75 °C (see Figure 4). For example, 70 % ethanol solution (30 °C, extraction time 40 min, 1:20 solid-liquid ratio) used to extract CBD from hemp leaves yields some 7 mg of CBD/g of hemp leaves powder [5]. With cold ethanol extraction, winterization process could be completely bypassed as the solubility of waxes and lipids in ethanol decreases in colder temperatures. After all, the winterization process is based on this phenomenon. As mentioned before, the δ-value of waxes is 16.0-16.6 $MPa^{1/2}$, which is significantly lower than that of cold ethanol, $\delta > 28$ $MPa^{1/2}$ [17] which means cold ethanol extract waxes and lipids poorly. Winterization is time-consuming process (24-48 h), so the production rate could be increased significantly. Higher ethanol concentration has been found to increase the yield of

CBD significantly. The hemp variety also has a major effect on the CBD concentration of the extract as CBD content varies between hemp strains [47].

Table II shows some solid-liquid extraction systems on the market. Ethanol is commonly used, most likely because ethanol is regarded as safe to use in medical and edible products by authorities such as the Food and Drug Administration (FDA) in the U.S.

Table II Technical information about solid-liquid extraction systems on the market.

Product name	**X10 MSE**	**ATLES**	**Ethanol Extraction Platform, High Performance**
Manufacturer	Precision Extraction Solutions	Capna Systems	Eden Labs
Scale of production	Small to medium commercial	Large commercial, industrial	Large commercial
Extraction vessel volume	24 L	200 L	379 L
Load capacity of dried plant matter per run	3.2-5.4 kg	40-180 kg (per hour)	29-36 kg
Solvent options	Ethanol, butane, propane	Ethanol	Ethanol
Temperature range	Not disclosed	-30 – -50 °C	down to -40 °C
Run time	55-85 min	Not disclosed	30-90 min
Solvent tank volume	50 L	Not disclosed	not included
Other information	-	Can be used to bypass winterization process	Ethanol requirement per run: 114-284 L. Can be used for warm or cold extraction
Sources	[48]	[49]	[50]

As Table II shows, solid-liquid extraction equipment is available for many scales of operation. Compared to $scCO_2$ equipment, larger amount of plant material can be processed faster (Table I).

In addition to traditional organic solvents, there has been interest in the use of deep eutectic solvents (DESs) to extract CBD and other cannabinoids. DESs are a mixture of two or three components, one which acts as a hydrogen bond acceptor and one acting as a hydrogen bond donor at room temperature [5]. DESs are made by mixing these two components together to form a eutectic mixture, meaning that the melting point of the mixture is lower than those of each component [51].

DESs have quite many favourable qualities compared to traditional organic solvents. For example, they are non-flammable, have negligible volatility and are therefore easy to store. As DESs are easy to prepare and handle, and no purification is needed, they are good candidates for large-scale use [51]. Many DESs are made from renewable and biodegradable compounds [13].

One study conducted by Křížek et al. compared several solvents, including methanol, ethanol, methanol:chloroform (9:1) with hydrophobic deep eutectic solvents. The cannabis plant material they used was from a marijuana seizure made by police, thus the THC content of the plant was high. There was no major difference between the solvent power (yield mg of substance per g of powdered plant), although a DES, menthol:acetic acid (9:1) mixture, performed slightly better as can be seen from Figure 8 [13].

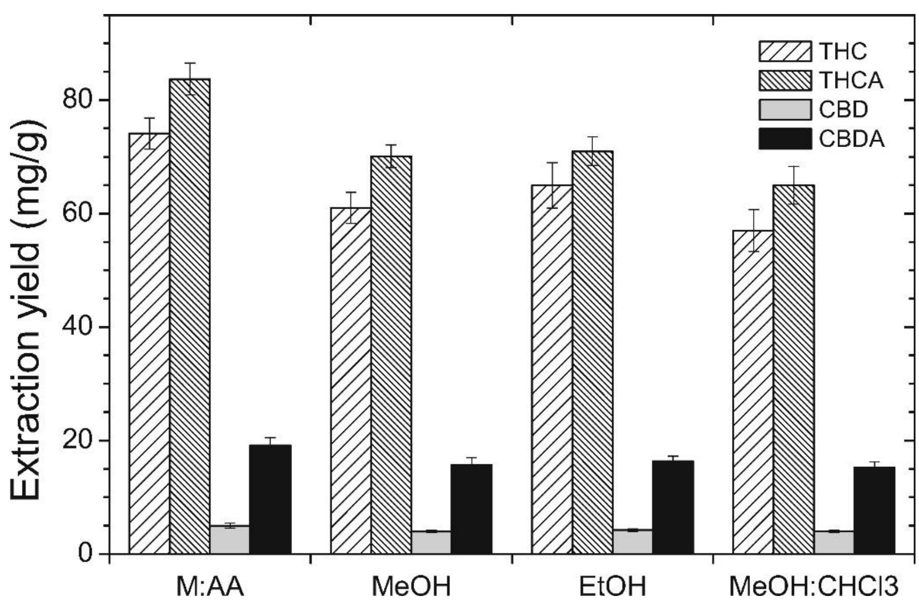

Figure 8 Comparison of cannabinoid yield using different solvents; menthol:acetic acid (1:1), methanol, ethanol, methanol:chloroform (9:1) [13].

Menthol:acetic acid mixture is also biodegradable and has “pharmaceutically acceptable toxicity” which could make it an acceptable alternative to other organic solvents for extracting cannabinoids [13]. DESs could be a viable option for cannabinoid extraction from hemp, but more research is needed.

5 Methods of purifying cannabidiol

After the initial extraction, the oil still contains many other components in addition to CBD. The objective of purification is to remove unwanted components, such as THC and remaining chlorophyll, present in the extract and to increase the CBD potency of the final product. Distillates can have up to around 90 % cannabinoid content and they can be used to produce for example edibles, topicals and products for vaping. Isolates can be produced with chromatographic techniques or by crystallisation. Figure 9 shows an example of some secondary processing steps that can be taken to produce certain products.

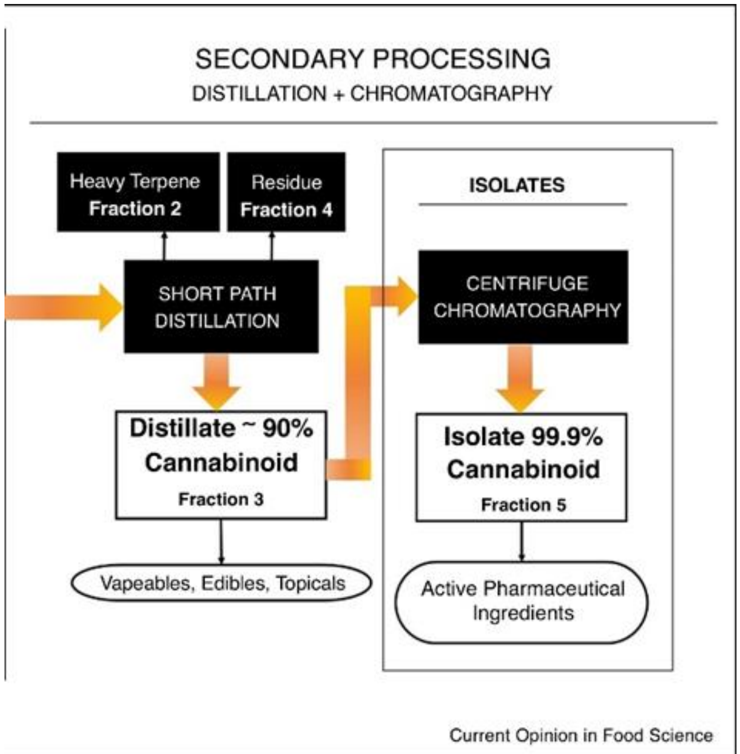

Figure 9 Secondary processing steps, distillation and centrifugal chromatography, used to produce certain popular product containing cannabinoids [17].

Crystallisation is used to produce CBD isolate by dissolving high purity oil (preferably at least 70 %) into a solvent. Pentane is commonly used for this purpose though other organic solvents, namely C4-8 alkanes or alkyl alcohols, can be used. The solvent is heated to 30 – 45 °C and the CBD oil is then added. After the oil has completely dissolved the mixture is cooled to -20 – -10 °C for at least 12 hours, to induce crystallisation. Once there are enough crystals, the crystals can be filtered and washed with cold solvent to wash away residue and to improve colour. The filtrate can be collected and reprocessed to improve yield [52], [53]. The collected CBD crystals can be further dried in e.g. a rotary evaporator, milled and dried again. With this method the composition of the isolate can be as high as 95 – 99.8 % CBD by weight of dry matter. THC content is 0 – 0.5 % when industrial hemp is used [52]. While time-consuming and arguably labour-intensive, crystallisation is a viable method to produce CBD isolate.

Chapter 5.1 discusses the commonly applied distillation methods and chapter 5.2 presents centrifugal partition chromatography for large scale CBD isolate production.

5.1 Distillation

Distillation is a process for separating a homogeneous liquid mixture into its components via the vapor phase. The mixture is heated to create two phases, gas and liquid. More volatile components transfer into the gas phase which can then be separated and condensed to collect the distillate. Essentially this means that the component with a lower boiling point (BP) evaporates first and is transferred into the gas phase [54, pp. 1–2]. With THC and CBD having BPs (160-180 ℃ and 157 ℃, respectively) very close to each other, complete removal of THC is difficult and inefficient [55].

As was shown in Figure 9, distillates can be used to produce many types of products, such as vapeables, topicals and edibles. Terpenes are frequently added back into the distillates as distillation may remove any variance between products made from different plant strains.

The three most common distillation methods used to create cannabis and hemp products are short-path distillation and wiped film distillation. Distillation is typically performed under reduced pressure as high temperatures may cause degradation of the cannabinoids [56]. In short-path distillation, the mixture is heated in a flask under vacuum. The vapor only travels a short distance before it is cooled in a condenser and collected. This method is quite effective and the equipment needed is very compact, so this method is commonly used by smaller scale operators [56], [57]. In wiped film distillation the initial mixture is fed into the machine from above, and wiper blades spread it across the surface of a heated tube [58]. Both wiped film and short-path distillation typically require a minimum of two passes to effectively purge unwanted compound from the product [59].

Figure 10 illustrates the basic concept of wiped film distillation. The feed is fed into the evaporator and spread to the walls by the wiper blades. Highly volatile terpenes are vaporised early and can be condensed and collected separately. Cannabinoids are the next fraction to vaporise. They are condensed by the internal condenser and collected at the bottom. The heavier fraction, such as chlorophyll and remaining wax is collected from the outer surface of the evaporator. As the distillate is highly viscous, the added agitation by the wipers increase the mass transfer rate and thus quicken the overall distillation [55], [60].

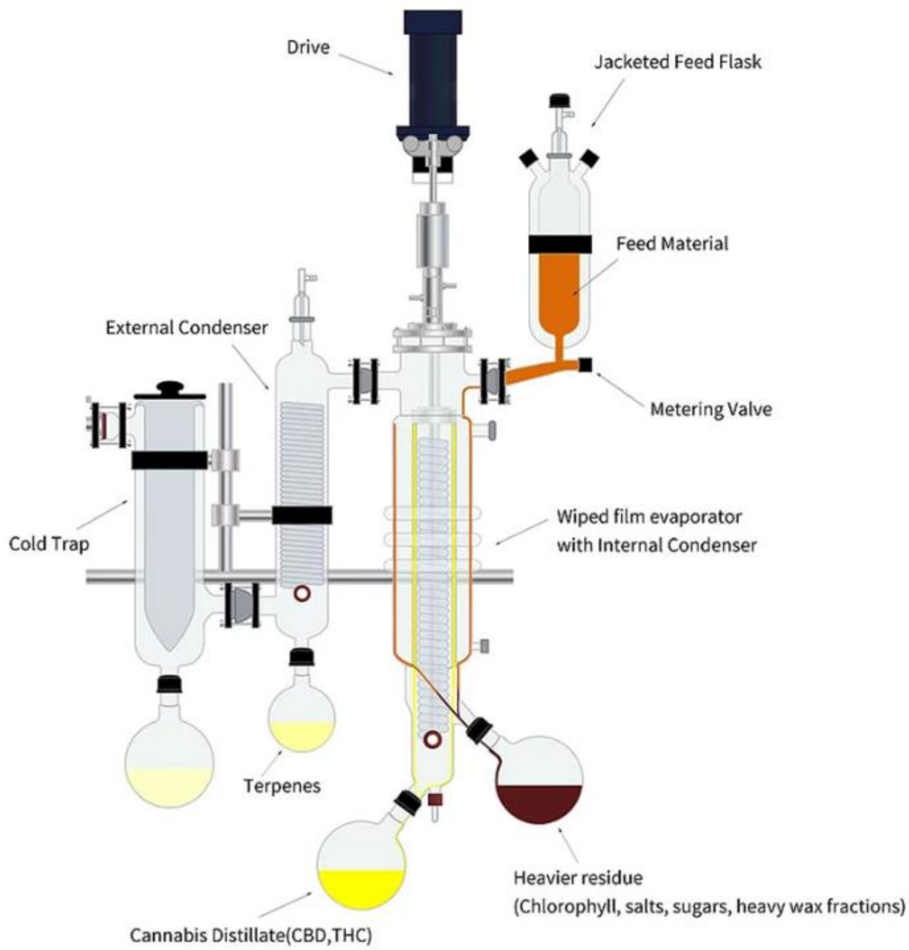

Figure 10 Basic concept of wiped film distillation [61].

Table III has some examples of distillation equipment available for comparison. There is no shortage of suitable equipment, these two examples were chosen as they were marketed specifically for the cannabis industry.

Table III Technical information about selected distillation equipment on the market.

Product name	clearSTILL	KD-30
Manufacturer	extraktLAB	Spectrum Technology
Distillation method	2 stage wiped film distillation	Short path distillation, 1-3 stages available
Processing capacity	4-6 L/h	15 L/h
Evaporator heating range	38 – 206 ℃	25 – 300 ℃
Evaporation area	0.18 m^2	0.30 m^2
Scale of production	Commercial	Medium to large scale industrial
Sources	[62]	[63]

Both of the distillation equipment presented in Table III are capable of processing large amounts of winterized oil. As distillation of CBD oil commonly requires at least two passes, both types of equipment are able to complete at least two passes in one cycle. This may shorten the overall processing time; systems only capable of a single pass should be cleaned before additional passes to avoid contamination.

5.2 Centrifugal Partition Chromatography

Centrifugal partition chromatography (CPC) is a counter-current liquid-liquid partitioning chromatography technique used for separation and purification purposes, where both the mobile and stationary phases are immiscible liquids [64]. The stationary phase is immobilised by centrifugal force and the mobile phase is pumped through at high flow rates. Components are partitioned between the stationary and mobile phases and are separated based on their partition coefficients K_d [65]. The coefficient can be calculated as shown in equation 1.

$$K_d = \frac{[A]_{stat}}{[A]_{mob}} \tag{1}$$

Where $[A]_{stat}$ concentration of A in the stationary phase

$[A]_{mob}$ concentration of A in the mobile phase.

The ideal value of K_d is 0.5-5. If the value is below 0.5, the analyte is retained in the mobile phase and no separation takes place, and if it is over 5, the analyte is retained in the stationary phase [66], [67].

The column consists of disks connected to a rotor in the centre which creates the centrifugal force. The disks have rotary seals at each end and over a thousand cells connected by a thin channel to allow the mobile phase to move through the cells. A valve is used to change the direction of the flow. This allows the system to work either in ascending or descending mode; in the descending mode, the stationary phase is the lighter liquid and the heavier liquid is the mobile phase. In the ascending mode, the lighter fluid is the mobile phase and the heavier is the stationary phase [64] [66]. The concept is illustrated in Figure 11.

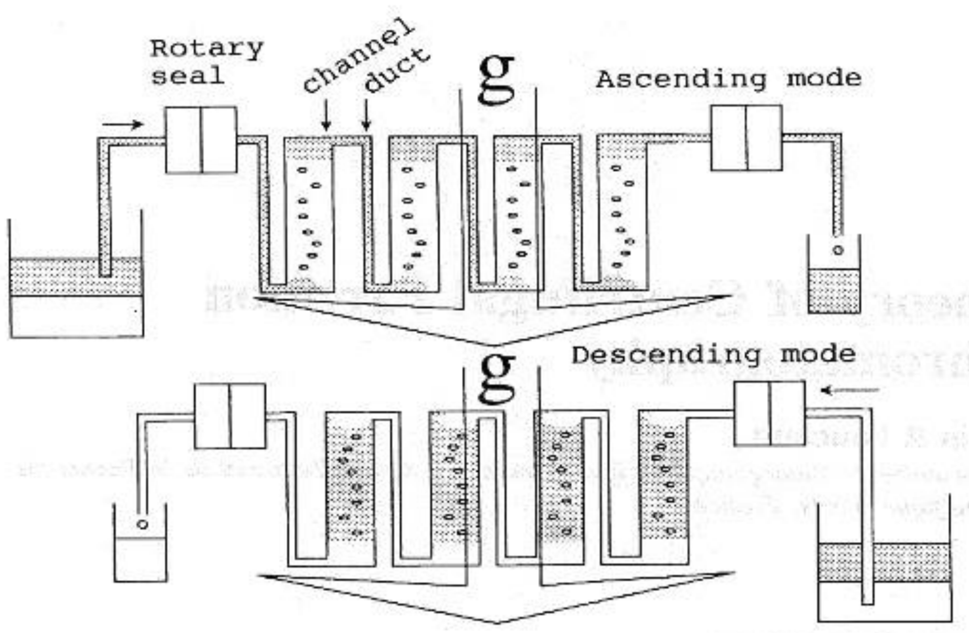

Figure 11 Concept of centrifugal partition chromatography. The mobile phase is pumped from the reservoir through the channels and cells and is collected. The droplets

represent the mobile phase. The arrow shows the direction of the centrifugal force [68].

Figure 12 and Figure 13 further illustrate how the disks are placed in the machine. Figure 13 shows how the cells are placed on the disks and packed on the central axis which is connected to the rotary seals.

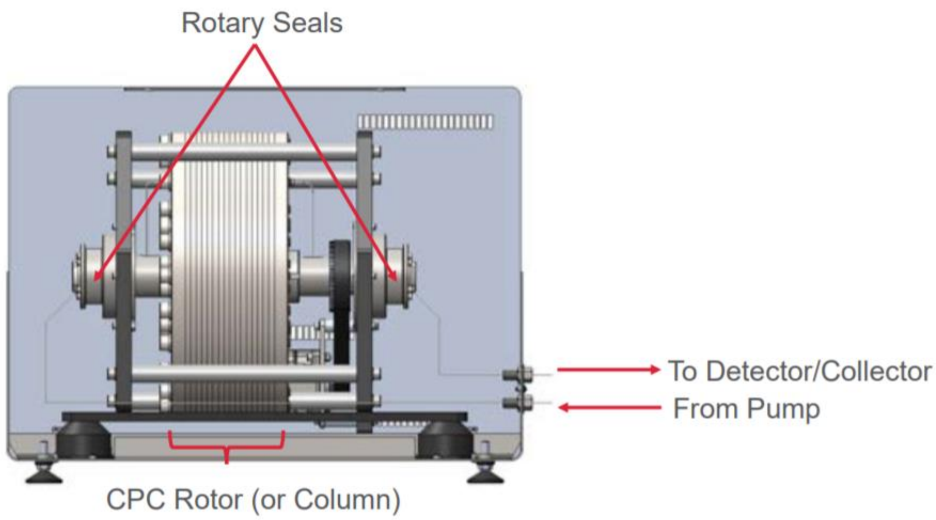

Figure 12 A basic diagram of a centrifugal partition chromatography system [69].

Figure 13 Centrifugal partition chromatography; the disks consist of cells connected via ducts. The disks are placed on a central axis, and together they form the CPC column (rotor) [69].

Many different liquids can be used to perform the purification. The possible toxicity of the liquids is considered, for example hexane, commonly used a solvent, is known to be a neurotoxin [70] whereas other options may be less dangerous if ingested. There is evidence that the purity of the product is very high, 70-99 %, after the removal of solvent, so the amount of residual solvent is low [67].

Some CPC systems can process up to 90 g of crude oil per hour and have a run-time of less than 20 min. One advantage of CPC when compared to High Performance Liquid Chromatography (HPLC) and Flash Chromatography, is the lack of needing to change the columns as they do not contain silica [71]. For example, a large industrial scale CPC system manufactured by RotaChrom Technologies could be potentially used to produce up to 8 kg of pure CBD per week [72] assuming enough crude extract is available.

As for disadvantages, CPC technology is still quite unknown among researchers. It is not an analytical technique so testing for product purity needs to be done with another method, such as analytical HPLC [73].

6 CBD Industry

The industry around CBD has been growing quickly as mentioned in chapter 3. The size of the market has been expected to soar in the near future to billions of dollars. With increasing competition and vague legislation, companies have discovered new approaches to market and sell their products, and to navigate the legislation. The CBD market is in many ways closely related to the marijuana market. Companies can focus on medical marijuana, recreational marijuana, CBD products or a combination of the previous. Branding is key to attract loyal customers in all business, and large companies with strong brands have dominated the cannabis market for the last several years. Different business models existing in the market are explained in chapter 6.1. Two CBD focused companies are presented in chapters 6.3 and 6.4 as small case studies. These companies were chosen because their brands are well recognised and there is a decent amount of information available about their production process and the company in general. Many companies offer a very limited amount of information, usually too general and unspecific. Many companies do not seem to disclose their extraction process or other source of CBD at all.

The United States and Canada have the largest cannabis markets. The North American Marijuana Index, started in January 2nd, 2015, follows the leading cannabis stocks in the U.S. and Canada. There are currently 47 companies included from various sectors, but all are strongly related to the cannabis or hemp industry. The index is equally balanced and rebalanced quarterly. Figure 14 shows the development of the index from January 2015 to March 2020 [74].

Figure 14 Development of the North American Marijuana Index from January 2015 to March 2020 [74].

The development of legalising medical or recreational marijuana can be clearly seen in Figure 14. Canada legalised recreational cannabis in October 2018 [75] causing a significant increase in value. The index capped at just over 350 points in late January 2018 but has since fallen to less than 100 points.

Few companies focused on CBD have listed, only a handful of the largest companies: CV Sciences, Cronos Group and Charlotte's Web Holdings Inc., to name the largest. The CBD market boom of late 2018 to early 2019 can be clearly observed from the stock price history from the last few years of the two first mentioned companies presented in Figure 15 and Figure 16. In late 2018 and early 2019 there was a major increase in CBD stock values, but the stock market bubble has since burst.

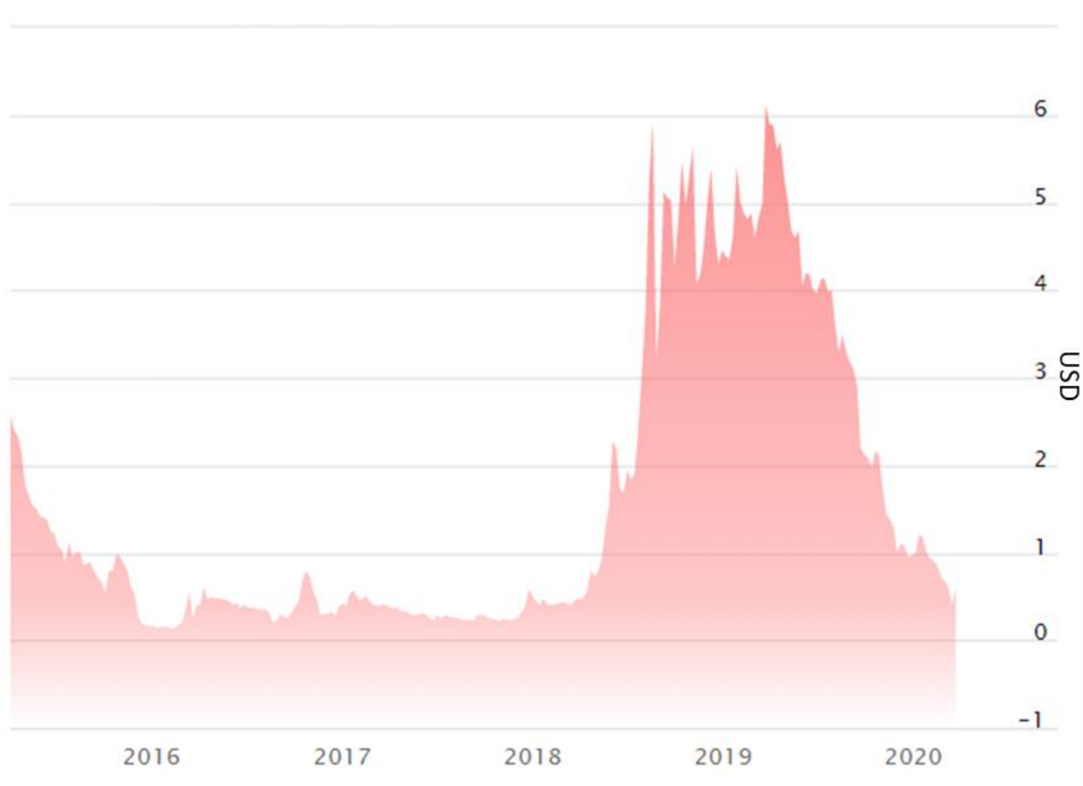

Figure 15 Stock price history of CV Sciences 6.4.2015 - 24.3.2020 in USD [76]

Figure 16 Stock price history of Cronos Group Inc. 2.3.2017 - 30.3.2020 in USD [77].

Both of stocks presented here have lost a significant portion of their value in a year while for example the S&P 500 index has grown. The S&P 500 measures the performance of 500 largest companies listed in the United States [78]. Possible reasons behind these developments in the stock market, as well as other challenges the industry has faced are discussed in chapter 6.2. The predictions made about the CBD market soaring in the early 2020s may have been overly optimistic. The industry is still new and there is room for further development in legislation and other industry standards.

6.1 Business Models

As in any industry, there are many types of companies in the CBD industry. Business models are developed and refined as competition increases with new companies entering the market. Possibly the most prominent ones are companies that sell exclusively CBD products. Many of these larger companies have been able to establish a good reputation among a diverse customer base [79]. Some companies grow their own hemp, extract the oil and refine it into the final product, while others buy the ready-made products in bulk and sell them under their own label. An example of CBD-only company is Florida based Green Roads which is presented as a short case study in chapter 6.3. Mile High Labs presented in chapter 6.4 is company that sells for example, CBD isolate in bulk to other companies to make their products. Mile High Labs also offers private labelling of their products [80].

Other often larger companies operating in the industry are cannabis companies that already have brand recognition, distribution networks, established supply chains and capital to expand to the CBD industry by creating their own product lines. These companies are often based in Canada or the United States [79]. For example, Curaleaf Inc. sells medical marijuana and CBD products in 17 states under several brand names. They grow their own cannabis plants and have 24 processing cites [81].

Some nutritional supplement brads have also launched their own CBD product lines. These companies are presented to have many advantages, such as existing connections to retail chains and a customer base that are suggested to be more prone to purchase CBD. These companies also have capital and experience in marketing similar products [79].

Multilevel marketing is a strategy some companies choose to utilise as it is seen to help reduce the stigma around cannabis-based products [79]. Multilevel marketing is a business model where the product is marketed and sold directly by individual people that buy the product from the company. These sellers are paid by what they earn in sales or by earning a commission on the wholesale purchases made by other sellers they have recruited under them. Kannaway LLC operating in North America as well as in Europe sells their products via direct sales (multilevel marketing [82]) as well as online [83].

Laboratories and processing equipment manufacturers specialised in cannabis and CBD are also an important part of the industry. Third party testing of the products is becoming more common as companies compete with quality [84], [85]. Equipment manufactures offer a wide range of capacities from very small operations to industrial size production. Many also offer extensive customer support together with on-site and online training on operating the equipment [35], [86].

6.2 Challenges in the industry

The CBD industry faces many challenges as it is quite new, and hemp still has a certain stigma around it; the CBD industry is closely tied with the rest of cannabis industry, namely recreational and medical cannabis, which both are polarising subjects. Lack of clear legislation around hemp-farming and production of hemp-derived products has been a major issue around the world, although new legislation has been passed recently.

The Agricultural Improvement Act, "the U.S. Farm Bill", was signed to a law December 20th, 2018. The Farm Bill made the production and sale of hemp and hemp-derived CBD products legal, and it had perhaps the biggest impact in the United States market [8]. Still the debate about the legal status of CBD products continued. The U.S. Food and Drug Administration (FDA) issued a statement for consumers on November 25th, 2019 that CBD can potentially cause harm and have side effects that might go unnoticed. As the FDA does not consider CBD a safe substance, CBD products cannot be marketed as a dietary supplement or be added to foods and beverages [87]. In January 13th, 2020 a bill was introduced to the House of Representatives, that would allow for this to change [88]. This has undoubtedly hindered the industry growth as consumers might be unsure about the legality of the products, as this question is raised on many frequently asked questions (FAQs) section of many CBD companies.

European countries have varying laws regarding CBD and cannabis in general. The European Union classified CBD and hemp flowers as novel foods in January 2020. Novel foods are foods that have not been consumed by people in the EU before May 15th, 1997 in significant quantities. The selling of novel foods requires a permit [3]. In Finland for example CBD is in a legal grey area, as The Finnish Medicines Agency (Fimea) considers CBD a prescription medicine and as Finnish Customs (Tulli) cooperates with Fimea, CBD products can be seized if it is imported without a prescription. The Finnish Food Safety Authority (Evira) considers CBD a novel food item according to the EU regulations. CBD can still be imported to Finland through loop-holes in the law [3] [89] as it is legal to sell cosmetics containing CBD. Some of these products are edible, but the consumer is not legally protected in cases of consuming these types of products as they are not meant to be consumed [3].

Mislabelling has also been a problem in the industry. [90], [91]. True CBD content may differ greatly from that advertised. Many products contain less CBD while others have more [92]. In autumn of 2018, the International Cannabis and Cannabinoid Institute (ICCI) based in Prague analysed 35 cannabidiol oils made by different companies. The results showed that some of the products contained THC over the legal limit of 0.3 % in Czech Republic (0.2 % is the general limit in the EU), and almost 90 % of the samples contained trace amounts THC, but this was not mentioned in the labels. The results also showed impurities in the oils, such as polyaromatic carbon compounds (PAH) which are known to cause cancer [3], [93], [94].

Contaminants found in CBD products include pesticides, heavy metals, moulds and bacteria. One major contaminant effecting CBD products specifically is residual solvents from the extraction process [28]. As companies compete with quality, third-party lab testing is advised. QR-codes in the packages increase transparency and thus consumer trust [85].

Funding, banking services and insurance are as well a major obstacle faced by many CBD companies. Lenders are hesitant to fund the companies because of vague legislation. Banking services may be unreliable, as some CBD companies have experienced suddenly closed accounts, and some credit card companies refusing to do business with them. Insurance may also be denied of companies in the hemp business [85]. These aspects might leave companies incapable to invest and expand their business.

6.3 Green Roads Inc.

Green Roads is a Florida based company founded in 2013, specialising in CBD products. It is one of the top companies currently in the business in the U.S. They sell a wide variety of ‘pharmacist-formulated’ products, ranging from topicals to edibles, in over 10 000 locations as well as online [95].

The hemp that they use to make their products is grown in America. The company does not extract their own CBD, instead they buy the extract from other facilities, and then formulate their own products [95]. The specific extraction method used is not explicitly stated, but they tell they are using CO_2 at a low temperature and under high pressure [96], suggesting they are using scCO_2 extraction even though exact conditions are not stated.

They rigorously test their raw material for contaminants such as heavy metals, pesticides, microbials and solvents. The products are tested in a third-party laboratory, and the results are made available for the consumers via a QR-code in the packaging. The company offers both broad and full spectrum products with varying amounts of CBD, prices ranging from just over $20 to well over $300. For example, their bestselling CBD oil is a 15 ml bottle containing 250 mg total of CBD which costs $42.95. serving size in 1 ml so about 17 mg of CBD per serving [95]. This product contains non-detectable amount of THC and no terpenes [97]. As distillation removes terpenes effectively, this product might have been made using a distillate, though this is not disclosed.

6.4 Mile High Labs International Inc.

Mile High Labs, founded in 2016, is a CBD manufacturing company based in Colorado. The company now has operations in Europe and New Zealand in addition to the United States. Unlike Green Roads for example, Mile High Labs produces CBD in bulk to be sold to other companies selling CBD consumer goods. They sell isolates and distillates, suitable for the manufacturing of many different types of products, for example water soluble CBD could be used in beverages and other infused food products. Mile High Labs also offers a possibility to create a private label line of CBD products directly with their products [80]. In 2020 the company will continue to expand despite the lay-offs of 20 people in January 2020, and is planning to release its own line of CBD products [98].

The company sources their hemp from across the U.S., but mainly from Oregon and Colorado. They use isopropyl alcohol (IPA) to extract CBD. Early 2019 a new modular, on-farm extraction site, “the Mile High Monster”, was introduced. The facility is located in eastern Colorado, and it increased the company’s production capacity by 500 %. The products are tested with a HPLC to verify the purity [80]. After the extraction with IPA, the alcohol is evaporated and collected. The crude then undergoes a number of other unspecified purification steps before it is distilled under vacuum, where the CBD is evaporated and collected, followed by crystallisation to produce isolate [99]. “The Monster” produces crude oil which is then shipped to another location for purification. Mile High Labs produces 25 % of isolate in the market. Another “Monster” is planned in southern Colorado [100].

7 Conclusion

The aim of this thesis was to present and assess different methods of extraction and purification when producing cannabidiol (CBD) from industrial hemp. Scientific literature and other online sources were used to evaluate these methods in terms of suitability for large scale production. Potential yield, quality of the product, time consumption and other factors were considered. The CBD industry and markets were studied to further determine what is desired of the extraction and purification methods, and what are some of the most commonly used methods in the industry today.

Supercritical carbon dioxide was found to be an effective extraction method, albeit extraction times are long compared to solid-liquid extraction. By tuning the extraction parameters, temperature, pressure and flow rate, the selectivity of the solvent can be highly modified, and the extraction of unwanted compounds decreased. The use of ethanol as a co-solvent has been found to increase yield and lower overall solvent consumption. Supercritical CO_2 extraction is a common choice for companies in the CBD industry as there is no solvent residue. The equipment is expensive, so production at a large scale requires substantial starting capital.

Many options are available for more traditional solid-liquid extraction. Butane, propane and ethanol are the most common choices as all of them generate high yields. Solid-liquid extraction is both effective and inexpensive, but many solvents pose environmental and health risks. Cold ethanol has the added benefit of extracting less waxes and chlorophyll which may speed up the overall processing as winterization step may be omitted. Compared to supercritical CO_2 extraction, solid-liquid extraction is faster.

Deep eutectic solvents (DESs) have been studied for cannabinoid extraction, but they are not yet used in commercial scale. DESs present less risks in terms of the environment and health compared to traditional solvents. They are also slightly more effective as solvents for cannabinoids. These features make DESs suitable for large scale production of CBD, but more comparative research to other extraction mediums should be conducted.

After the initial extraction of CBD from the plant matter and winterization, the oil is commonly purified by distillation, crystallisation or chromatographic techniques. Distillation is commonly performed as short path or wiped film distillation. Because the boiling points of CBD and Δ^9-tetrahydrocannabinol (THC), the main psychoactive compound in the plant, are very similar, the complete removal of THC by distillation is inefficient. To produce CBD isolate, chromatography or crystallisation is used. Crystallisation requires a great deal of time and labour but produces high quality isolate when successful. Centrifugal partition chromatography (CPC) is a promising novel method for producing isolate with high enough yields for commercial use. An advantage compared to traditional techniques is the lack of silica used which lowers the operational costs. To summarise, the best methods for extracting and purifying CBD often boils down to the company's marketing strategy. Supercritical CO_2 is considered safer than other solvents, but solid-liquid extraction with ethanol is cheaper. Crystallisation is also inexpensive, but chromatography as a high tech apparatus might make educated consumers trust the product more for example.

As the market for CBD grows and consumers become more educated, companies increasingly compete with quality. This may increase the demand for high quality equipment and research. Legislation is currently vague or restrictive on many aspects of the business. The leading problem concerning CBD products are strict regulation about the THC content. This presents opportunity for further research and development of effective extraction and purification methods.

The differences between hemp strains and the effects it has on the processing would be an interesting and important field of study. Extraction and purification techniques should be studied both individually and in combination to generate a good understanding about the true effects of all parameters have on the final product. Effectiveness of these methods in terms of yield is also limited. Currently a substantial amount of the information available is anecdotal and companies are forced to use trial and error. This together with lack of industry standards allows inconsistent products to enter the market and leaves consumers in a weak position.

8 References

[1] BDS Analytics and Arcview Market Research, 'U.S. CBD Market Anticipated to Reach $20 Billion in Sales by 2024', *bdsa.com*, May 09, 2019. [Online]. Available: https://bdsanalytics.com/u-s-cbd-market-anticipated-to-reach-20-billion-in-sales-by-2024/ (Accessed Feb. 27, 2020).

[2] J. Imrie, 'Warning for UK CBD industry as regulator sets approval deadline', *bdaily.co.uk*, Feb. 13, 2020. [Online]. Available: https://bdaily.co.uk/articles/2020/02/13/warning-for-uk-cbd-industry-as-regulator-sets-approval-deadline (Accessed Feb. 27, 2020).

[3] M. Leppänen, 'Ihmerohdoksi hehkutetun CBD-hamppuöljyn suosio kasvaa Euroopassa, mutta pullossa voi piillä terveysriski – Suomeen tuotteita tulee porsaanreiän kautta',

yle.fi, Jun. 09, 2019. [Online]. Available: https://yle.fi/uutiset/3-10769723 (Accessed Feb. 05, 2020).

[4] T. M. Attard, C. Bainier, M. Reinaud, A. Lanot, S. J. McQueen-Mason, and A. J. Hunt, ‘Utilisation of supercritical fluids for the effective extraction of waxes and Cannabidiol (CBD) from hemp wastes’, *Industrial Crops and Products*, vol. 112, pp. 38–46, Feb. 2018.

[5] C. Cai, W. Yu, C. Wang, L. Liu, F. Li, and Z. Tan, ‘Green extraction of cannabidiol from industrial hemp (Cannabis sativa L.) using deep eutectic solvents coupled with further enrichment and recovery by macroporous resin’, *Journal of Molecular Liquids*, vol. 287, Aug. 2019.

[6] R. C. Clarke, ‘Botany of Genus Cannabis’, in *Advances in Hemp Research*, CRC Press, 1999, p. 19.

[7] E. Stuyt, ‘The Problem with the Current High Potency THC Marijuana from the Perspective of an Addiction Psychiatrist’, *Mo Med*, vol. 115, no. 6, pp. 482–486, 2018.

[8] K. M. Conaway, *H.R.2 - Agriculture Improvement Act of 2018*. 2018.

[9] R. Boshart, ‘Iowa Senate OKs production of industrial hemp’, *thegazette.com*, Apr. 04, 2018. [Online]. Available: https://www.thegazette.com/subject/news/government/iowa-senate-oks-production-of-industrial-hemp-20180404 (Accessed Feb. 05, 2020).

[10] L. J. Rovetto and N. V. Aieta, ‘Supercritical carbon dioxide extraction of cannabinoids from Cannabis sativa L.’, *The Journal of Supercritical Fluids*, vol. 129, pp. 16–27, Nov. 2017.

[11] R. C. Clarke and D. P. Watson, *Marijuana and the cannabinoids. Chapter 1: Cannabis and Natural Cannabis Medicines*. Totowa, N.J: Humana Press, 2007.

[12] J. M. McPartland and E. B. Russo, ‘Cannabis and Cannabis Extracts’, *Journal of Cannabis Therapeutics*, vol. 1, no. 3–4, pp. 103–132, Jun. 2001.

[13] T. Křížek *et al.*, ‘Menthol-based hydrophobic deep eutectic solvents: Towards greener and efficient extraction of phytocannabinoids’, *Journal of Cleaner Production*, vol. 193, pp. 391–396, Aug. 2018.

[14] K. Leiman, L. Colomo, S. Armenta, M. de la Guardia, and F. A. Esteve-Turrillas, ‘Fast extraction of cannabinoids in marijuana samples by using hard-cap espresso machines’, *Talanta*, vol. 190, pp. 321–326, Dec. 2018.

[15] H. Perrotin-Brunel, M. C. Kroon, M. J. E. van Roosmalen, J. van Spronsen, C. J. Peters, and G.-J. Witkamp, ‘Solubility of non-psychoactive cannabinoids in supercritical carbon dioxide and comparison with psychoactive cannabinoids’, *The Journal of Supercritical Fluids*, vol. 55, no. 2, pp. 603–608, Dec. 2010.

[16] D. Fiorini, A. Molle, M. Nabissi, G. Santini, G. Benelli, and F. Maggi, ‘Valorizing industrial hemp (Cannabis sativa L.) by-products: Cannabidiol enrichment in the inflorescence essential oil optimizing sample pre-treatment prior to distillation’, *Industrial Crops and Products*, vol. 128, pp. 581–589, Feb. 2019.

[17] J. W. King, ‘The relationship between cannabis/hemp use in foods and processing methodology’, *Current Opinion in Food Science*, vol. 28, pp. 32–40, Aug. 2019.

[18] K. Askew, ‘CBD: Trending ingredient or ingredient trend?’, *foodnavigator.com*, Jan. 17, 2020. [Online]. Available: https://www.foodnavigator.com/Article/2020/01/17/CBD-Trending-ingredient-or-ingredient-trend (Accessed Feb. 05, 2020).

[19] K. Weisul, ‘The Next Gold Rush Is the $22 Billion CBD Business--and This Florida Company Is Ready to Win’, *inc.com*, Dec. 04, 2018. [Online]. Available:

https://www.inc.com/kimberly-weisul/best-industries-2019-green-roads.html (Accessed Feb. 05, 2020).

[20] Brightfield Group, ‘2019 US CBD Market’.

[21] Brightfield Group, ‘Europe’s CBD and Cannabis Market Shows Promise’, *brightfieldgroup.com*, Mar. 26, 2019. [Online]. Available: https://www.brightfieldgroup.com/press-releases/europe-cbd-market-through-2023 (Accessed Mar. 11, 2020).

[22] A. Bourque, ‘As Competition Increases, CBD Companies Need the Right Mentors To Survive’, *forbes.com*, Jun. 24, 2019. [Online]. Available: https://www.forbes.com/sites/andrebourque/2019/06/24/as-competition-increases-cbd-companies-need-the-right-mentors-to-survive/#58b12bb67b00 (Accessed Feb. 05, 2020).

[23] A. Jank, ‘Types of CBD’, *blog.brightfield.com*, Jan. 14, 2020. [Online]. Available: https://blog.brightfieldgroup.com/types-of-cbd (Accessed Feb. 05, 2020).

[24] K. Gresoski, ‘Types of CBD: Full Spectrum vs Broad Spectrum vs Isolate’, *hempsley.com*, Oct. 04, 2019. [Online]. Available: https://hempsley.com/learn/full-spectrum-vs-isolate (Accessed Feb. 05, 2020).

[25] E. B. Russo, ‘The Case for the Entourage Effect and Conventional Breeding of Clinical Cannabis: No “Strain,” No Gain’, *Front. Plant Sci.*, vol. 9, Jan. 2019, Accessed: Feb. 05, 2020. [Online]. Available: https://www.frontiersin.org/article/10.3389/fpls.2018.01969/full.

[26] K. Williams, ‘Ways to Use CBD’, *hempsley.com*, Aug. 22, 2017. [Online]. Available: https://hempsley.com/learn/methods (Accessed Feb. 05, 2020).

[27] WSPA 7News, *Inside Upstate CBD Oil Processing Facility ‘SC Botanicals’*. Dec. 8, 2018. [Video File]. Available:

https://www.youtube.com/watch?v=xGuauXXcMww&list=PLAA-DCrJjg7Y2_tmaTgLVVIaHyvQXhtMd&index=2&t=474s&has_verified=1 (Accessed Jan. 15, 2020).

[28] A. Hazekamp, ‘The Trouble with CBD Oil’, *MCA*, vol. 1, no. 1, pp. 65–72, 2018.

[29] J. Azmir *et al.*, ‘Techniques for extraction of bioactive compounds from plant materials: A review’, *Journal of Food Engineering*, vol. 117, no. 4, pp. 426–436, Aug. 2013.

[30] R. Smith Jr., H. Inomata, and C. Peters, *Introduction to Supercritical Fluids: A Spreadsheet-Based Approach*. Amsterdam: Elsevier, 2013.

[31] Precision Extraction Solutions, *Cannabis and Hemp Extraction Explained: Ethanol vs. Supercritical CO2 vs Hydrocarbon Extraction*. Jun. 3, 2019. [Video File]. Available: https://www.youtube.com/watch?v=pW66iiVNJtY&list=PLAA-DCrJjg7Y2_tmaTgLVVIaHyvQXhtMd&index=2. (Accessed Jan. 15, 2020).

[32] N. Shafer, ‘What is Hydrocarbon Extraction?’, *precisionextraction.com,* Aug. 01, 2019. [Online]. Available: https://precisionextraction.com/2019/08/what-is-hydrocarbon-extraction/ (Accessed Apr. 01, 2020).

[33] Supercritical Fluid Technologies, Inc., ‘Cannabis Extraction by Supercritical Carbon Dioxide – Effects of Extraction Parameters’. [Online]. Available: https://www.supercriticalfluids.com/wp-content/uploads/AP-01-Cannabis-Extraction-by-Supercritical-Carbon-Dioxide-Effects-of-Extraction-Parameters.pdf.

[34] A. Mueller, ‘Method for producing an extract from cannabis plant matter, containing a tetrahydrocannabinol and a cannabidiol and cannabis extracts’, U.S Patent 8895078B2, Nov. 25, 2014.

[35] Apeks Supercritical, ‘The Force’. [Online]. Available: https://www.apekssupercritical.com/extraction-systems/high-output-the-force/ (Accessed Mar. 11, 2020).

[36] Apeks Supercritical, ‘System specifications, The Force’. [Online]. Available: https://www.apekssupercritical.com/wp-content/uploads/2020/01/Force-Spec-Sheet-10.19.pdf. (Accessed Mar. 26, 2020).

[37] Apeks Supercritical, ‘The Bambino’. [Online]. Available: https://www.apekssupercritical.com/extraction-systems/introductory-the-bambino/ (Accessed Mar. 26, 2020).

[38] Apeks Supercritical, ‘System specifications, The Bambino’. [Online]. Available: https://www.apekssupercritical.com/wp-content/uploads/2020/01/Bambino-Spec-Sheet-10.19.pdf. (Accessed Feb. 26, 2020).

[39] extractLAB, ‘System specifications, E-180’. [Online]. Available: https://extraktlab.com/assets/images/supercritical-co2-extraction-180.pdf. (Accessed Mar. 26, 2020).

[40] Eden Labs, ‘System specifications, Hi-Flo FX2 High Performance Series’. [Online]. Available: https://www.edenlabs.com/wp-content/uploads/2019/09/EDL-Product_Flyer-Hi-FloFX2-071519.pdf. (Accessed Mar. 26, 2020).

[41] M. May, ‘A Guide to Cannabis Extraction Equipment and Machines’, *analyticalcannabis.com*, Mar. 21, 2019. [Online]. Available: https://www.analyticalcannabis.com/news/a-guide-to-cannabis-extraction-equipment-and-machines-311552 (Accessed Mar. 26, 2020).

[42] Eden Labs, ‘Industrial CO2 Extraction Platform’, *edenlabs.com*. [Online]. Available: https://www.edenlabs.com/co2-extraction/industrial-co2/ (Accessed Mar. 26, 2020).

[43] PubChem, ‘Butane’, Mar. 28, 2020. [Online]. Available: https://pubchem.ncbi.nlm.nih.gov/compound/7843 (Accessed Apr. 01, 2020).

[44] PubChem, ‘Propane’, Mar. 28, 2020. [Online]. Available: https://pubchem.ncbi.nlm.nih.gov/compound/6334 (Accessed Apr. 01, 2020).

[45] Apeks Supercritical, ‘How to Extract CBD Oil - The Extraction Process & How CBD Oil is Made’, *apekssupercritical.com*. [Online]. Available: https://www.apekssupercritical.com/cbd-extraction-process/ (Accessed Apr. 01, 2020).

[46] M. June-Wells and M. Lindback, ‘Your Guide To Hydrocarbon Extraction’, *cannabisbusinesstimes.com*, Nov. 01, 2019. [Online]. Available: https://www.cannabisbusinesstimes.com/article/your-guide-to-hydrocarbon-extraction/ (Accessed Apr. 01, 2020).

[47] M. Szalata and K. Wielgus, ‘Extraction of CBD and Δ9-THC from three varieties of fibre hemp by different concentrations of ethanol’, *New Biotechnology*, vol. 33, p. 162, Jul. 2016.

[48] N. Shafer, ‘X10 MSE Cannabis Extractor’, *precisionextraction.com*. [Online]. Available: https://precisionextraction.com/x10-mse-multi-solvent-extractor/ (Accessed Mar. 30, 2020).

[49] Capna Systems, ‘Ethanol Extraction with Atles’, *capnasystems.com*. [Online]. Available: https://capnasystems.com/equipment/atles (Accessed Mar. 30, 2020).

[50] Eden Labs, ‘System Specifications. ETHANOL EXTRACTION PLATFORM EXTRACTION, DISTILLATION & SOLVENT RECOVERY SYSTEMS’. Dec. 26, 2019, [Online]. Available: https://www.edenlabs.com/wp-content/uploads/2019/12/EDL-Product_Flyer-EthanolExt-122619.pdf. (Accessed Mar. 30, 2020).

[51] Q. Zhang, K. D. O. Vigier, S. Royer, and F. Jérôme, 'Deep eutectic solvents: syntheses, properties and applications', *Chem. Soc. Rev.*, vol. 41, no. 21, pp. 7108–7146, Oct. 2012.

[52] C. M. Eiroa Martinez, D. Van Houten, M. V. Fernandez Cid, and G. F. Woerlee, 'Cannabidiol isolate from industrial-hemp and use thereof in pharmaceutical and/or cosmetic preparations', World Intellectual Property Organization Patent 2016153347A1, Sep. 29, 2016.

[53] B/R Instrument, 'How to Crystallize CBD - Make CBD Isolate', *brinstrument.com*, Mar. 13, 2019. [Online]. Available: https://brinstrument.com/blog/cannabis-distillation/how-to-crystallize-cbd-make-cbd-isolate/ (Accessed Mar. 28, 2020).

[54] J. G. Stichlmair and J. R. Fair, *Distillation: principles and practices*. New York: Wiley-VCH, 1998.

[55] Zhenhzhou Well-known Instrument and Equipment Co., Ltd., 'Cannabidiol Distillation: How Is CBD Oil Extracted From Hemp?', *labrotovap.com*, Nov. 09, 2019. [Online]. Available: https://www.labrotovap.com/cannabidiol-distillation-how-is-cbd-extracted-from-hemp/ (Accessed Apr. 03, 2020).

[56] K. Manning, 'Cannabis & CBD Vacuum Distillation', *provac.com*, Jul. 22, 2019. [Online]. Available: https://www.provac.com/blogs/news/cannabis-vacuum-distillation (Accessed Mar. 12, 2020).

[57] Capna Systems, 'Short-Path Distillation for Pure Cannabis Distillate', *capnasystems.com*, Oct. 22, 2019. [Online]. Available: https://capnasystems.com/blog/short-path-distillation/ (Accessed Apr. 03, 2020).

[58] Capna Systems, 'Falling Film Evaporators: The Ultimate Guide', *capnasystems.com*, Nov. 20, 2019. [Online]. Available: https://capnasystems.com/blog/falling-film-evaporators/ (Accessed Apr. 03, 2020).

[59] B/R Instrument, ‘Distillation Rates Comparison’, *brinstrument.com*, Nov. 24, 2018. [Online]. Available: https://brinstrument.com/blog/cannabis-distillation/distillation-rates-comparison/ (Accessed Apr. 03, 2020).

[60] T. McKenna, ‘Design model of a wiped film evaporator. Applications to the devolatilisation of polymer melts’, *Chemical Engineering Science*, vol. 50, no. 3, pp. 453–467, Aug. 1994.

[61] Zhengzhou Well-known Instrument and Equipment Co., Ltd., ‘Short Path Glass Wiped Film Molecular Distillation System For CBD Cannabis Hemp Oil’, *labrotovap.com*. [Online]. Available: https://www.labrotovap.com/portfolio-item/molecular-distillation-system-for-cbd-cannabis-hemp-oil/ (Accessed Apr. 03, 2020).

[62] extraktLAB, ‘clearSTILL Wiped Film Evaporator’, *extraktlab.com*. [Online]. Available: https://extraktlab.com/wiped-film-evaporator/ (Accessed Mar. 12, 2020).

[63] Spectrum Techology, ‘KD-30 Distillation’, *www.spectrumtechnology.co.uk*. [Online]. Available: https://www.spectrumtechnology.co.uk/distillation-systems/kd-30/ (Accessed Apr. 03, 2020).

[64] A. Berthod and D. W. Armstrong, ‘Centrifugal partition chromatography. I. General features’, *Journal of Liquid Chroatography*, vol. 11, no. 3, pp. 547–566, Mar. 1988.

[65] A. Hazekamp, R. Simons, A. Peltenburg-Looman, M. Sengers, R. van Zweden, and R. Verpoorte, ‘Preparative Isolation of Cannabinoids from Cannabis sativa by Centrifugal Partition Chromatography’, *Journal of Liquid Chromatography & Related Technologies*, vol. 27, no. 15, pp. 2421–2439, Sep. 2004.

[66] Gilson Purification Applications Laboratory, ‘Introduction To Centrifugal Partition Chromatography - TECHNICAL NOTE TN215’. [Online]. Available: https://www.aga-

analytical.com.pl/uploads/gilson/gilson_introduction_cpc-ccc-new-branding-tn215.pdf. (Accessed Jan. 20, 2020).

[67] A. Rutz, ‘CPC distribution chromatography of cannabinoids’, U.S. Patent 20180036278A1, Feb. 08, 2018.

[68] R. Sankar, ‘HPCPC [HIGH PERFORMANCE CENTRIFUGAL PARTITION CHROMATOGRAPHY], WHAT IS…’, Jun. 15, 2013, [Online]. Available: https://www.slideshare.net/banuman35/hpcpchigh-performance-centrifugal-partition-chromatography-what-is-hpcpc-classification-of-counter-current-chromatography-instrumentation. (Accessed Jan. 20, 2020).

[69] L. Pahnke, *Improving the Purification and Extraction of Natural Products with Centrifugal Partition Chromatography*. LCGC, 2019.

[70] PubChem, ‘Hexane’. U.S. National Library of Medicine, Feb. 29, 2020, [Online]. Available: https://pubchem.ncbi.nlm.nih.gov/compound/8058. (Accessed Mar. 10, 2020).

[71] Gilson Purification Applications Laboratory, ‘Cost-effective Approach to the Purification of Cannabinoids using CPC’. [Online]. Available: https://www.gilson.com/pub/static/frontend/Gilson/customtheme/en_US/images/docs/CPC_CostEffectiveApproachPurificationCannabinoids.pdf.

[72] RotaChrom Technologies, *RotaChrom - Industrial Scale Centrifugal Partition Chromatography*. Jul. 29, 2019. [Video File]. Available: https://www.youtube.com/watch?v=3yKiZU_VKfw. (Accessed Mar. 10, 2020).

[73] G. Audo, ‘Why Centrifugal Partition Chromatography is a Better Way to Extract Cannabidiol for Medical Research’, May 07, 2018.

[74] ManifestSeven, ‘North American Marijuana Index’, *The Marijuana Index For Publicly Traded Companies*. [Online]. Available: https://marijuanaindex.com/stock-quotes/north-american-marijuana-index-tracking-top-cannabis-stocks-in-north-america/ (Accessed Mar. 27, 2020).

[75] BBC News, ‘Canada legalises sale and use of cannabis’, *bbc.com*, Oct. 17, 2018. [Online]. Available: https://www.bbc.com/news/world-us-canada-45806255. (Accessed Mar. 27, 2020).

[76] Nasdaq, ‘CV Sciences Inc. (CVSI) Stock Quotes’, *nasdaq.com*. [Online]. Available: https://www.nasdaq.com/market-activity/stocks/cvsi (Accessed Apr. 01, 2020).

[77] Nasdaq, ‘Cronos Group Inc. Common Share (CRON) Stock Quotes’, *nasdaq.com*. [Online]. Available: https://www.nasdaq.com/market-activity/stocks/cron (Accessed Apr. 01, 2020).

[78] Kauppalehti, ‘INX indeksi’, *kauppalehti.fi*. [Online]. Available: https://www.kauppalehti.fi/porssi/indeksit/INX (Accessed Apr. 27, 2020).

[79] C. Skelly, ‘Top 5 CBD Companies’, *brightfieldgroup.com*, Jul. 15, 2019. [Online]. Available: https://blog.brightfieldgroup.com/top-5-cbd-companies (Accessed Feb. 16, 2020).

[80] Mile High Labs, ‘Mile High Labs Home Page’. [Online]. Available: https://milehighlabs.com/ (Accessed Feb. 18, 2020).

[81] Curaleaf, ‘About’, *curaleaf.com*. [Online]. Available: https://curaleaf.com/about-curaleaf/ (Accessed Mar. 11, 2020).

[82] Kannaway LLC, ‘Palkkiojärjestelmä’, *kannaway.com*. [Online]. Available: https://kannaway.com/pages/palkkiojaerjestelmae,254.html (Accessed Mar. 11, 2020).

[83] Kannaway LLC, 'Kannaway Home Page', *kannaway.com*. [Online]. Available: https://kannaway.com/index.html (Accessed Mar. 11, 2020).

[84] Best CBD Oils, '30 Best CBD Oil Companies Of 2019 for 2020', *www.bestcbdoils.org*. [Online]. Available: https://www.bestcbdoils.org/best-companies/ (Accessed Mar. 11, 2020).

[85] A. Uzialko, 'Starting a CBD Business: How to Enter the CBD Industry', *businessnewsdaily.com*, May 28, 2019. [Online]. Available: https://www.businessnewsdaily.com/15052-how-to-start-a-cbd-business.html (Accessed Mar. 11, 2020).

[86] Precision Extraction Solutions, 'Precision Extraction Home Page', *precisionextraction.com*. [Online]. Available: https://precisionextraction.com/ (Accessed Mar. 11, 2020).

[87] S. Williams, 'Congress and the FDA May Square Off Over CBD', *fool.com*, Jan. 18, 2020. [Online]. Available: https://www.fool.com/investing/2020/01/18/congress-and-the-fda-may-square-off-over-cbd.aspx (Accessed Feb. 05, 2020).

[88] M. Thompson, 'Bipartisan Bill for Federal Regulation of CBD in Foods and Dietary Supplements', *healthlawdiagnosis.com*, Jan. 28, 2020. [Online]. Available: https://www.healthlawdiagnosis.com/2020/01/bipartisan-bill-for-federal-regulation-of-cbd-in-foods-and-dietary-supplements/ (Accessed Feb. 05, 2020).

[89] Daily CBD, 'Guide to Buying CBD in Europe — Updated For 2020', *dailycbd.com*. [Online]. Available: https://dailycbd.com/en/europe/ (Accessed Feb. 05, 2020).

[90] J. Hasse, 'Goldilocks And The Three Bears: Report Shows CBD Products Often Have Too Much Or Too Little CBD', *Forbes*. [Online]. Available:

https://www.forbes.com/sites/javierhasse/2019/11/19/cbd-product-mislabeling/ (Accessed Mar. 28, 2020).

[91] CBS News, 'What's in your CBD? Buyer beware', *cbsnews.com*, Sep. 30, 2019. [Online]. Available: https://www.cbsnews.com/news/cbd-cbs-news-tests-cannabidiol-products-for-cbd-thc-and-impurities-dosages/ (Accessed Mar. 28, 2020).

[92] D. A. Freedman and A. D. Patel, 'Inadequate Regulation Contributes to Mislabeled Online Cannabidiol Products', *Pediatric Neurology Briefs*, vol. 32, p. 3, Jun. 2018.

[93] L. Holthaus, 'Legal Status Of CBD Around The World', *Plain Jane CBD*, Jan. 23, 2020. [Online]. Available: https://blog.tryplainjane.com/blog/legal-status-of-cbd-around-the-world/ (Accessed Mar. 28, 2020).

[94] CBD Oil Europe, 'Is CBD Legal in Czech Republic - updated August 2018', *cbdoileurope.com*, Aug. 24, 2017. [Online]. Available: https://cbdoileurope.com/cbd-legality/is-cbd-legal-in-czech-republic-updated-august-2018/ (Accessed Mar. 28, 2020).

[95] Green Roads, 'Green Roads Home Page', *greenroads.com*. [Online]. Available: https://greenroads.com/ (Accessed Feb. 16, 2020).

[96] Green Roads, 'Green Roads Wholesale FAQ'. [Online]. Available: https://wholesale.greenroadsworld.com/faq/ (Accessed Feb. 16, 2020).

[97] Evio Labs, 'Certificate of Analysis, Batch 406819.' Green Roads, May 13, 2019, [Online]. Available: https://greenroads-labsheets.s3.us-east-2.amazonaws.com/d7cd8yjcpblv37y73f03ik549i4s?response-content-disposition=inline%3B%20filename%3D%22250mg-CBD-Oil-Batch-406819.pdf%22%3B%20filename%2A%3DUTF-8%27%27250mg-CBD-Oil-Batch-406819.pdf&response-content-type=application%2Fpdf&X-Amz-Algorithm=AWS4-HMAC-SHA256&X-Amz-

Credential=AKIAQ26SGY37XOLWWFUC%2F20200328%2Fus-east-2%2Fs3%2Faws4_request&X-Amz-Date=20200328T101304Z&X-Amz-Expires=300&X-Amz-SignedHeaders=host&X-Amz-Signature=eda74ae63d09ae7b65f8cc18a59a964d272ee9e283c4660cf2ce42a171190566. (Accessed Mar. 28, 2020).

[98] T. Ricciardi, ‘Colorado CBD producer Mile High Labs lays off 20 employees just months after major expansion’, *denverpost.com*, Jan. 10, 2020. [Online]. Available: https://www.denverpost.com/2020/01/10/colorado-cbd-mile-high-labs-layoff/ (Accessed Feb. 18, 2020).

[99] Leafly, *How Hemp-CBD Products are Made - CBD Isolate*.Jul. 7, 2019. [Video File]. Available: https://www.youtube.com/watch?v=AVwtOOIgJMs&has_verified=1. (Accessed Feb. 18, 2020).

[100] Cannabis Tech, *A Tech Moment Episode 1 Mile High Labs*. Mar. 3, 2019. [Video File]. Available: https://www.youtube.com/watch?v=P2fLpP0jpyw&t=657s. (Accessed Feb. 18, 2020).