chemeca CONFERENCE
30 September – 3 October 2018
Queenstown, New Zealand

***Paper no**. 133*
Chemeca 2018
30 September – 3 October 2018, Queenstown, New Zealand

Extraction and fractionation of cannabinoids from *Cannabis Sativa*

S. Tallon[1*] T.Moreno[1] F.Montanes[1] and M.Watson[2]

[1] *Callaghan Innovation, Integrated Bioactive Technologies, Industrial Research Limited, 69 Gracefield Road, 5040 Lower Hutt, New Zealand*

[2] *University of Canterbury, Department of Chemical and Process Engineering, Private Bag 4800, Christchurch 8140, New Zealand*

[*] *Corresponding author. Email: stephen.tallon@callaghaninnovation.govt.nz*

Abstract: There is a rapidly growing market for therapeutic and medicinal products containing cannabinoids from Cannabis, driven in part by a growing acceptance of their therapeutic benefits, and by global regulatory change that allows more widespread use of both recreational and prescribed products. There are a number of known and established methods for preparation and concentration of extracts containing cannabinoids and other natural compounds from the plant material; however, in many cases these are still poorly developed and understood from a large scale industrial manufacturing perspective. Product composition preferences and preferred formulation options are also still very much in development.

This paper gives a review of the current state of the art for extraction and fractionation of *Cannabis Sativa*. One of the emerging methods of choice is extraction using supercritical fluid CO_2 as it offers flexibility and selectivity in terms of the extract composition and is a low temperature solvent-free process that produces a high quality product. Here we present experimental data on the extraction of two New Zealand grown industrial hemp cultivars using supercritical CO_2 and subcritical (liquid) propane. The effect of decarboxylation of the plant material on the yields and extract composition is also presented.

Keywords: Cannabis, Cannabidiol, Hemp, Extraction, Supercritical CO_2, Propane.

1 Introduction

Cannabis is widely grown in New Zealand and globally to make legal as well as illicit products. Strains of cannabis that have less than 0.3% levels of the psychoactive cannabinoid THC are commonly referred to as hemp and are grown legally in New Zealand, under licence, for industrial fibre and edible seed products. Hemp seeds do not contain cannabinoids and the seed oil, made by cold pressing the seeds or solvent extraction, can be used as a culinary oil and the de-oiled seed meal has applications as a high protein flour. Hemp seed oil is currently manufactured and sold in New Zealand, and hemp seed meal products will soon be available for human consumption.

Products containing cannabinoids are a new growth area in New Zealand. Cannabinoids are known for the psychoactive high associated with recreational use of the plant, particularly strains high in THC content, but the various cannabinoids that are present in the plant also have a range of clinical and therapeutic applications. This includes prescription for pain relief, control of seizures or fits, and treatment of nausea following chemotherapy treatment [McPartland and Russo].

There are a wide range of identified cannabinoids in cannabis. The ones that are present at the highest concentration, and the most studied, include tetrahydrocannabinol (THC), cannabidiol (CBD), and to a lesser extent cannabigerol (CBG) [McPartland and Russo]. These compounds are present in the leaf and flower, and are in highest concentration in flowering parts of the plant. Total cannabinoid content can exceed 10% of the dry weight of flowering tops, and can vary in composition between high THC and low THC content.

Cannabinoids in their natural state exist in a carboxylated (acid) form. This form decomposes rapidly at high temperatures and cannabinoid products are often sold in de-carboxylated form. De-carboxylation can be carried out in a controlled way during industrial preparation of the product by holding the plant material, or extracts from the plant, at temperatures typically in the range 120 to 150C for up to an hour [Veress et al, Iffland et al].

Decarboxylation will occur at temperatures as low as 80-90C over much longer periods. Temperatures higher than 150 are generally avoided to minimise further decomposition of the cannabinoids. De-carboxylation will also occur during smoking or vaping of cannabinoid products.

Products containing only CBD (defined in New Zealand as CBD constituting >98% of total cannabinoids) can be imported and prescribed in New Zealand by registered practitioners and do not require Ministry of Health approval (https://www.health.govt.nz/our-work/regulation-health-and-disability-system/medicines-control/medicinal-cannabis/prescribing-cannabis-based-products). Other cannabinoid products may only be prescribed on a case by case basis if approved by the Ministry. One exception is the imported product Sativex (http://www.medsafe.govt.nz/profs/RIss/Sativex.asp) which contains CBD and THC in approximately 1:1 ratio and can be used without ministerial approval for treatment of Spasticity. Cannabinoids are classified as class B drugs in New Zealand and fall within the control of the Misuse of Drugs Act. Manufacture and export of cannabinoid products cannot currently be carried out in New Zealand.

Globally there are a variety of approaches for regulating cannabis use. Medicinal use is legal in an increasing number of countries, and personal use in many of these is either decriminalised or laws prohibiting its use are not enforced.

2 Extraction of cannabinoids

Extraction of a plant material is generally a balance between extraction of as much of the desired component as possible while minimising co-extraction of other compounds. Compounds in hemp that may extract along with the cannabinoids include terpenes, which are responsible for much of the aroma often associated with cannabis extracts, as well as a complex mix of other phenolics, waxes, chlorophylls, and other low polarity compounds. The degree of extraction of each of these depends on the solvent(s) and extraction conditions used. The solvent selected should also be non-toxic, and inexpensive. In practice, solvents that are practical to use include ethanol, carbon dioxide, light hydrocarbons (propane, butane). Hexane and other low polarity organic solvents like chloroform, acetone, and ethyl acetate will extract cannabinoids but are less desirable due to solvent toxicity concerns. Cannabinoids can also be extracted into other oils to make an infused oil product but these methods generally produce a product that contains only low concentrations of cannabinoids and a low yield of the cannabinoids present in the plant.

Medicinal use of cannabinoids typically requires preparation of a controlled and reproducible product with known composition. In practice this requires extraction and concentration of the cannabinoids and minimisation of other plant components. Increasing scale of production and pharmaceutical manufacturing regulations also create a need for dedicated industrial manufacturing facilities and good manufacturing practice to be employed.

There is a reasonable body of published work on analysis of cannabinoids, including methods for quantitative extraction of samples for analysis (see for example Citti et al), however there is limited published literature on industrial extraction of cannabinoids. Existing industrial knowledge is largely ad-hoc and small scale.

Eöry et al describes the effect of sieving milled plant material into different size fractions on extraction of THC using supercritical CO_2. Different size fractions were shown to contain different concentrations of extractable THC with the highest concentration in the 63 to 125 micron size range. Extraction was slower from larger particle size fractions, consistent with expectation.

Kitryte et al describe supercritical carbon dioxide, pressurized liquid, and enzyme-assisted extractions of cannabis plant material containing a mix of leaf, flower fragments and immature seed. CO_2 extraction conditions ranging from 35 to 70 °C and 100 to 500 bar were investigated. The highest total extraction yield of 8.3% was achieved at the highest pressure and temperature conditions tested, and using a solvent to feed ratio of approximately 50:1. CBD and (predominantly) CBDa constituted about 28% of the extract, and 93% of the available CBD and CBDa was extracted under these conditions. The concentration of CBD and CBDa was similar under other conditions however total yield was lower. Subsequent extraction was carried out by Kitryte et al using ethanol/water mixtures but was not targeted at extraction of cannabinoids.

The solubility of THC, and other cannabinoids, in supercritical CO_2 has been reported by Perrotin-Brunel et al [Perrotin-Brunel et al, 2010a, Perrotin-Brunel et al, 2010b]. Solubility was reported for temperatures ranging from 42 to 72 °C, and pressures from 113 to 251 bar. Molar solubilities of the order of 10^{-4} were reported, with solubilities for different cannabinoids generally increasing in the order THC, CBG, CBD, CBN.

Rovetto and Aeita describe extraction of four different high THC strains of *cannabis sativa* using supercritical CO_2. Conditions ranging from 170 to 340 bar at 55 °C were evaluated, and extraction using an ethanol co-solvent with CO_2 was also investigated. Total extract yields of up to 18% were reported, with higher yields at higher pressures. Extraction efficiency up to 92% of total THC was reported.

Extraction was also more rapid at higher pressure. Packed bed extractions were carried out and showed a constant extraction rate period in early stages of extraction from which an apparent solubility was calculated. Values ranging from 0.2% at 170 bar to 1.4% at 340 bar were reported, although the measured solubility values also varied substantially with plant variety.

Use of ethanol as a co-solvent gave higher total yields, mainly additional extraction of non-cannabinoid compounds, and more rapid extraction.

The use of ultrasound to assist solvent extraction is described by Agarwal et al. Extractions were carried out with different solvent ratios of ethanol in water, and for different time periods and ultrasound power levels. Only total phenolic and total flavonoid yields were reported. Ultrasound was demonstrated to give an improved yield, including qualitatively for major cannabinoids, under the conditions used.

There are a number of commercial suppliers of extraction equipment that are targeted towards extraction of cannabis, although many are still relatively small scale. These include ethanol extraction units sold under the trademarks COLDFINGER (Eden Labs, https://www.edenlabs.com/) and Ethos 4 (Capna Fabrication, http://capnafabrication.com/the-ethos-4). Cold ethanol extraction is often used to supress co-extraction of waxes and terpenes. The extraction unit is typically coupled with a vacuum evaporation step to remove ethanol.

Supercritical fluid CO_2 extraction is a well established industry, and established equipment manufacturers such as Natex (https://www.natex.at/), Supercritical Fluid Technologies (http://www.supercriticalfluids.com/), and Eden Labs (https://www.edenlabs.com/) market their equipment as suitable for extraction of cannabinoids from cannabis.

Concentration of cannabinoids, including separation of THC, can be achieved by a number of methods. These include crude separation methods such as winterisation to remove waxes and adsorption of minor compounds like chlorophylls, or the use of a selection of solvent systems to selectively separate terpenes before or after extraction of cannabinoids. More refined methods for concentrating cannabinoids include molecular distillation, chromatography, and selective adsorption on specialised resins (for example Molecularly Imprinted Polymers). Concentration methods are not discussed further here.

3 Extraction of Cannabinoids from NZ hemp varieties

We present here experimental data using CO_2 and propane for extraction of leaf and flower material from two hemp varieties that are currently grown in New Zealand. The varieties represented here are primarily grown for fibre and seed products and do not reflect newer varieties that are being established specifically for high concentrations of CBD. For commercial sensitivity reasons the varieties are referred to here only as Variety A and Variety B. The effects of decarboxylation are also explored.

3.1 Methods

Plant materials were received as air dried sample that were already separated into leaf and flower fractions. The flower buds were generally free of seed.

Supplied plant material was milled as received using a knife mill with a 2mm mesh screen. Milled material was then either extracted as-is or was first decarboxylated by placing the milled material in a convection oven at 150 C for 20 minutes. These conditions were sufficient to decarboxylate 60-80% of the THC and CBD acid forms (THCa and CBDa). Longer time or higher temperatures would have enabled higher conversion. A reduction in water content also occurred during decarboxylation.

The extractions described here were carried out using two different solvents: Supercritical CO_2 and subcritical propane. The method was similar for each solvent, but the scale of equipment varied. In each case, milled plant material was first loaded into a cylinder with porous sintered steel end plates, and the cylinder was then loaded into a pressure vessel. Packing density was approximately 230 kg/m^3.

Pressurised solvent was pumped through the vessel containing the plant material. After passing through the plant material, any extracted material dissolved in the solvent was recovered by depressurising and vapourising the solvent into a separator vessel in which dissolved solids precipitated and were recovered. In the case of CO_2

extraction the pressure was reduced in two stages and recovered from two separate separators. Results for the combined extracts are reported here.

After the separators the gas phase solvent was cooled to condense it and recirculated. Circulation of the solvent continued until no further extracted material was being observed in the separator.

Extraction using propane was carried out in a 2L extraction vessel at a pressure of 40 bar and 50 °C. Extractions using CO_2 were carried out at either 10L scale or 375mL scale, and results reported here were carried out at a pressure of 300 bar except where noted, and at a temperature of 50 °C.

Extract samples were analysed by HPLC-MS to determine the concentration of the main cannabinoids present, THC, CBD, CBG, CBN, and their acid forms. CBG, and in particular CBN, were only present at very low levels and are not discussed further here.

3.2 Extraction results

Figure 1 shows the progress of the extraction with solvent throughput for extraction of type B milled leaf using CO_2 at 300 bar and 50 °C. Extraction of decarboxylated leaf begins to level off after a yield of approximately 15g/kg and use of a 70:1 CO_2 to feed mass ratio. The extraction curve for as-supplied leaf continues to increase above 40 g/kg yield however the majority of the additional extract is water from the leaf which continues to extract at a rate limited by the solubility of water in CO_2. Free water in the as-received extract was observed and was able to be decanted. The resulting yield, along with additional extract recovered from within the extraction plant after the run, was 3.9wt%. The decarboxylated leaf contained lower moisture content and no free water was observed in the extract. Final yield was 2.9wt%. Yields are reported here on a dry feed weight basis.

Figure 2 shows a comparison of extraction curves for extraction of flower heads using CO_2 and propane. Propane extraction occurs more rapidly than with CO_2, with the majority of the extraction occurring using less than 20:1 solvent to feed ratio. The propane extraction for type A flower shows a much higher yield than for type B, which is a reflection of the higher content of total extractable material in the type A flowers. CO_2 extraction was slower, with extract yield still increasing after use of 120:1 solvent to feed ratio. There is a lag in the early stages of the CO_2 extraction before extract is observed, which is due to the higher solvent residence time in the extraction plant used for CO_2 extraction compared to the propane extraction plant. The rate of extraction after this lag period however is a function of the solvent properties of CO_2.

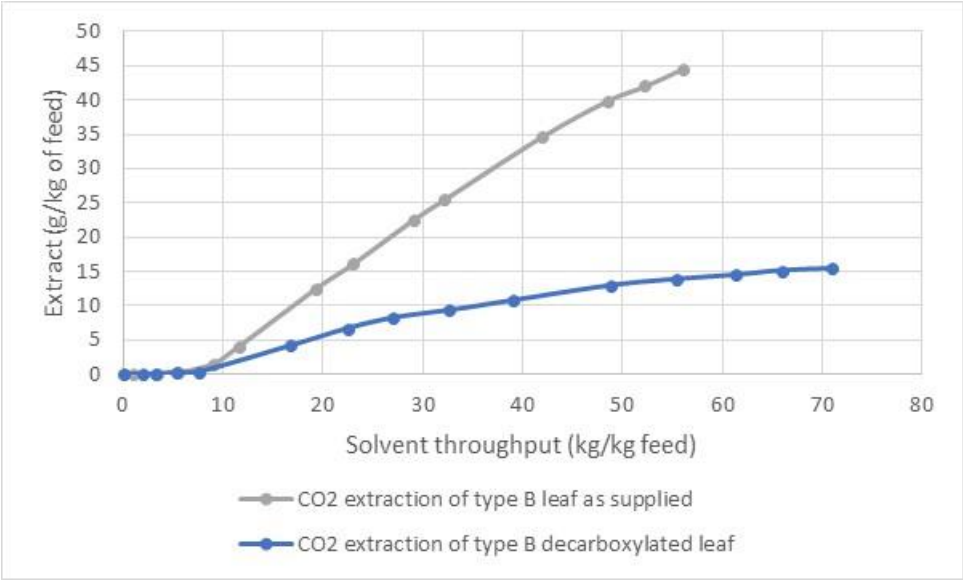

Figure 1: Extraction curve for CO_2 extraction of type B *cannabis sativa* leaf. Extract mass includes any co-extracted water.

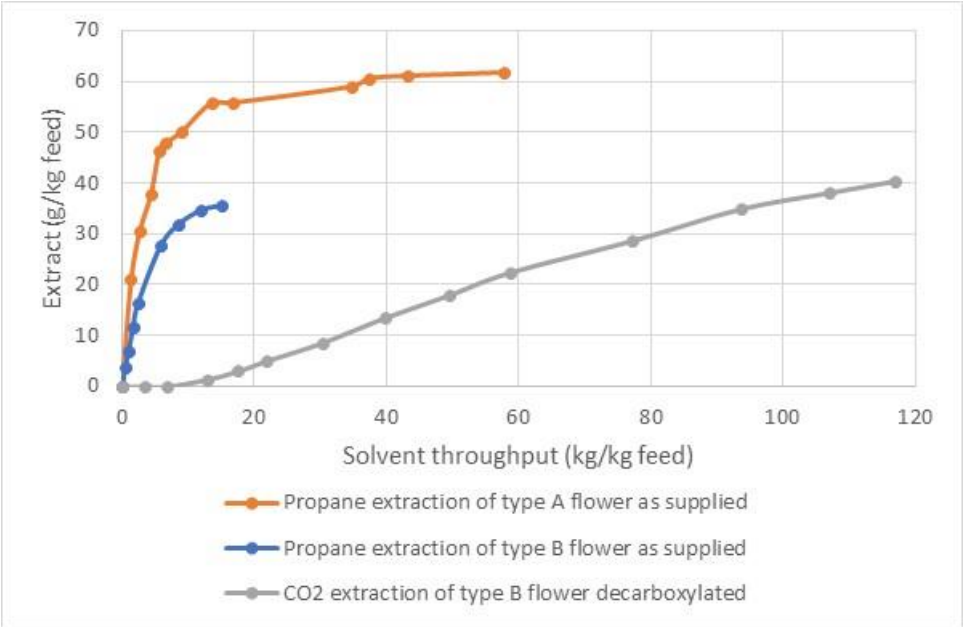

Figure 2: Extraction curve for solvent extraction of *cannabis sativa* flowers

3.3 Analysis results

Yield and analysis results are shown in Table 1. Total extract yields ranged from 2.7 to 3.9 wt% for leaf, through to 5.8 to 8.6 wt% for flower. Total extract yield and cannabinoid yields are higher from flower, due largely to the higher content present in the flowers compared to the leaf. Cannabinoid yields were higher for the type A variety, in both the leaf and flower.

Decarboxylation had a mixed effect on the total extracted yields observed here. The profile of cannabinoids in the extract however shifted towards the decarboxylated form, and the proportion of the total cannabinoids available in the feed material that was extracted increased. This is consistent with expectation as the decarboxylated form is more soluble in non-polar solvents than the acid form.

Extraction efficiency of up to 93% was observed for propane and 92% for CO_2.

Table 1: Total extract yield and yield of key cannabinoids

	Total extract	CBD	THC	CBDa	THCa	Total cannab.
	Wt% of dry feed weight					*Wt% of feed cannab.*
Type A leaf as received, Propane	2.7	0.22	0.02	0.16	0.00	83
Type A flower as received, Propane	8.2	0.31	0.05	3.43	0.13	93
Type A flower decarboxylated, Propane	8.2	2.59	0.15	0.71	0.01	80
Type A flower as received, CO_2	6.3	0.32	0.06	1.65	0.06	49
Type A flower decarboxylated, CO_2	8.6	3.17	0.18	0.60	0.01	92
Type B leaf as received, CO_2	3.9	0.03	0.01	0.27	0.01	62
Type B leaf decarboxylated, CO_2	2.9	0.17	0.02	0.21	0.01	75
Type B flower as received, CO_2*	7.6	0.37	0.06	0.59	0.02	58
Type B flower decarboxylated, CO_2*	5.8	1.15	0.10	0.20	0.00	88

* Extractions carried out at 500 bar.

4 Conclusions

Products containing cannabidiol and other cannabinoids from *Cannabis Sativa* are a growing global and New Zealand market opportunity, particularly for medicinal cannabis products. To meet this market new high yielding strains of cannabis are being developed along with improvement in scale and knowledge of industrial processing methods.

Results presented here demonstrate the ability of CO_2 and/or propane to produce a crude extract from milled plant material with a high overall recovery of available cannabinoids. Propane has a higher capacity to solubilise cannabinoids and extraction is more rapid than for CO_2. CO_2 however has the advantages of being non-flammable and provides some additional flexibility to adjust the composition of the extract.

Acknowledgement

The authors would like to acknowledge Hikurangi Enterprises for the preparation and supply of some of the raw materials used in this work.

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