Cannabis Metabolomics: Comparison of Cannabis Products and Effect of Vaporization

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Abstract

Cannabis is widely consumed medically and recreationally due to the presence of cannabinoids, but the phytochemical complexity of different varieties and preparations is a major knowledge gap. This thesis investigated the phytochemicals present in thirteen different cannabis strains using untargeted and targeted phytochemical analysis to determine "strain" differences in cannabis tinctures and oils. In addition, the phytochemical differences between different oil products, namely oils extracted by ethanol and CO_2 supercritical fluid, were also determined to evaluate different processing methods. It was found that inter-strain variability was more significant than the preparation methods due to the strain-specific presence of major cannabinoids, specifically THCA and CBDA. Furthermore, a processing step like drying removed phytochemicals contributing to strain differences, most notably terpenes. The results suggested that consumers can expect different strains and products to have different chemical profiles, as CO_2 oils were found to be more chemically consistent across products than tinctures.

Cannabis can be consumed in many different ways, and one popular mode of delivery is vaporization. Vaporization extracts active principles of cannabis with heated gas and could lead to a different phytochemical profile compared to the original flower counterpart. Consequently, the product label based on the raw material may not be representative of what is phytochemically available during consumption. The results of this study showed a reduction in available chemicals after vaporizing flower and oils, and little new chemical formation through this process. Decarboxylated cannabinoids were the most significant contributors to differences between pre and post-vaporized samples, and different phytochemistry composition was observed after vaporization. The results also demonstrated that vaporization reduces inter-strain and inter-product

chemical diversity, but the content of the vapor can still be affected by the strain used. Furthermore, it showed that vaporization could extract phytochemicals differently from oils than flower material.

This thesis provides a new understanding of phytochemical differences, extraction and vaporization processes of cannabis, and provides novel insights into cannabis for producers and consumers. Understanding the differences in chemical content of different types of concentrates can better inform producers and consumers about the products they make, sell and use. In addition, this thesis supports the use of vaporization as a harm reduction method for the consumption of cannabis, and increases understanding of cannabis vaporization. The information from this thesis contributes novel insights into cannabis research and provides a foundation for further studies.

Acknowledgments

This thesis is an accumulation of knowledge and experiences I have gained during the last two years. I have so many people to thank for their time and expertise that has allowed me to become a better researcher and scientist.

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C	CO_2 Supercritical fluid extracted oil samples
CBC	Cannabichromene
CBD	Cannabidiol
CBDA	Cannabidiolic acid
CBE	Cannabielsoin
CBL	Cannabicyclol
CBN	Cannabinol
CBND	Cannabinodiol
CBG	Cannabigerol
CBGA	Cannabigerolic acid
CBT	Cannabitriol
E	Ethanol extracted oil samples
F	Flower material extracted with ethanol/ tincture samples
HPLC	High Performance Liquid Chromatography
HRMS	High Resolution Mass Spectrometry
MCT	Medium-chain triglyceride
OPLS-DA	Orthogonal Partial Least Square-Discriminant Analysis
PCA	Principal component analysis
SFE	Supercritical fluid extraction
THC	Δ9-tetrahydrocannabinol
TIC	Total Ion Current
Δ8-THC	Δ8-trans-tetrahydrocannabinol
THCA	Δ9-tetrahydrocannabinolic acid

Chapter 1: General introduction and literature review

1.1 Botany of cannabis

Cannabis sativa L. is a flowering plant in the family Cannabaceae, to which *Cannabis*, *Humulus* (commonly known as hops) and several genera formerly in the *Celtidaceae* are members (McPartland, 2018; Small & Cronquist, 1976). The taxonomy has been widely debated since some scientists consider the plant to be a polytypic genus with three subspecies, *sativa, indica* and *ruderalis*, and others consider these subspecies to be different species (Hillig, 2005; Schultes et al., 2012). This thesis follows the taxonomy of Small and Cronquist (1976) who recognized all taxa as belonging to the single *C. sativa* species. Phytochemically, *C. sativa* is recognized for its production of cannabinoids, of which Δ9-tetrahydrocannabinol (THC), the psychoactive component, is best known.

The species contains two chemically and morphologically distinct lineages: hemp and marijuana. Hemp is distinguished by long stalks and production of fibre, seed and oil (Hillig & Mahlberg, 2004). Hemp plants generally produce relatively low amounts of cannabinoids. Legally defined hemp in Canada is regulated to contain a maximum THC concentration of <0.3% weight/total weight (w/w%) for field-grown hemp (Government of Canada, 2019). Conversely, marijuana is noted for its psychoactive properties and high levels of THC. The material from the flowering part of the unfertilized female plant is commonly harvested for consumers for medicinal and recreational use because the phytocannabinoids are found in highest concentration in the inflorescences (Small, 2017). This is due to the presence of glandular trichomes, which are epithelial outgrowths comprised of cells that can store or excrete secondary metabolites (Huchelmann et al., 2017). They are of high interest as studies have suggested that

biosynthesis and storage of cannabinoids is concentrated in the glandular trichomes of cannabis (Rodziewicz et al., 2019).

The classification of *C. sativa* is confounded by many factors such as historical cultural factors, geographic dispersal and artificial selection by humans (Sawler et al., 2015). Both subspecies *sativa* and *indica* have been developed into marijuana and hemp while some taxonomists believe that *C. sativa* ssp. *ruderalis* represents feral populations of the species.

1.2 Traditional use and ethnobotany

Historically, all parts of the *C. sativa* plant were used, such as the leaves for fodder and mulch, stalks for fibre and tow, seeds for food and producing oil, and resin glands and flowers for traditional medicines and ceremonies (Clarke & Merlin, 2016). *C. sativa* is hypothesized to have originated from Central Asia or northern South Asia, and was first cultivated in China on a large scale for fibre and seed production (Clarke & Watson, 2007). The oldest record of *C. sativa* use is from ancient Chinese texts dated around 2800 BCE, where it is indicated that Emperor Shen Nung instructed the Han people to cultivate hemp for fibre (Clarke & Merlin, 2013). Evidence supports that cannabis was farmed throughout history as a major food crop, also providing textiles, rope, paper and oil as well as used extensively for medicinal, recreational and spiritual use (Hand et al., 2016; Small, 2015). Whereas the cultivation, sale and possession of marijuana type *C. sativa* has been illegal in most parts of the world for the past several decades, a global movement toward decriminalization or legalization for medicinal purposes and, to a lesser extent, non-medicinal purposes has emerged in recent years (Fischer et al., 2015). These legal and regulatory shifts follow both scientific and medical advances in cannabinoid research and changing sociocultural norms.

1.3 Phytochemistry and chemotypes

Published literature (ElSohly & Slade, 2005) indicates that cannabis contains over 500 known phytochemical compounds from a diverse range of biosynthetic classes including terpenes, hydrocarbons, steroids, and flavonoids, to name a few. However, the genus is noted for its high concentration of cannabinoids (Table 1-1): the chemicals mainly recognized for the medical and psychoactive effects of cannabis (Lynch et al., 2016; Pomahacova et al., 2009). THCA (Δ9-tetrahydrocannabinolic acid) and CBDA (cannabidiolic acid) and their pharmacologically active analogues Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD) are the most widely studied phytocannabinoids in the plant.

A chemotype classification of medical and recreational cannabis has been proposed based upon the major cannabinoids, THCA and CBDA, leading to three main chemotypes: high THCA:CBDA ratio (>>0.1) (chemotype I), intermediate ratio (0.5-2.0) (chemotype II), and low ratio (<<0.1) (chemotype III) (Small & Beckstead 1973). Additional chemotypes were also proposed where it was defined by CBGA: THCA ratio (<1) (chemotype IV) and total cannabinoid content <0.02% (chemotype V) (Fournier et al., 1987; Mandolino et al., 2003).

The three main chemotypes were further supported by genetic analyses that demonstrated that the chemotype was determined by the presence at the B locus of two co-dominant alleles (B_D and B_T), which are responsible for the CBDA and THCA occurrences in the plant (De Meijer et al., 2003). Specifically, chemotype I plants contained B_T/B_T alleles, chemotype II plants contained B_T/B_D alleles, chemotype III plants contained B_D/B_D alleles. Others such as the B_{T0}, B_{Dw}, B_{D01}, and B_{D02} alleles were suggested to be associated with production of other cannabinoids thus defining chemotype IV (Onofri et al., 2015). While only two genes, THCA synthase and CBDA synthase are positively correlated with THCA and CBDA contents in

cannabis, a study suggests that multiple THCA synthase and CBDA synthase sequences are scattered along multiple loci that can interact on the chromosome (Weiblen et al., 2015).

1.3.1 Cannabinoids

Phytocannabinoids are products naturally found in *C. sativa* that share a typical C_{21} terpenophenolic skeleton (ElSohly et al., 2017). In the plant, acid-forms of cannabinoids are typically naturally found, such as THCA, where decarboxylation of the 2-COOH by heat or light lead to the neutral cannabinoids, such as THC (Figure 1-1) (Dussy et al., 2005; Yamauchi et al., 1967). This thermal conversion is a process common to all acid-form cannabinoids.

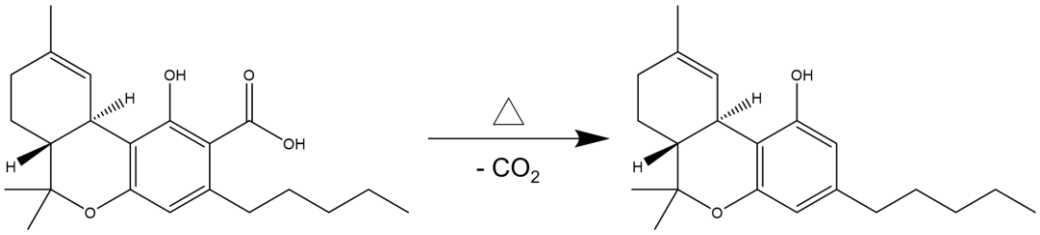


Figure 1-1: Chemical structures for the decarboxylation reaction of THCA

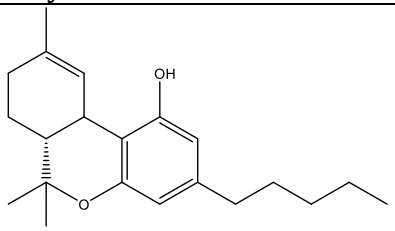
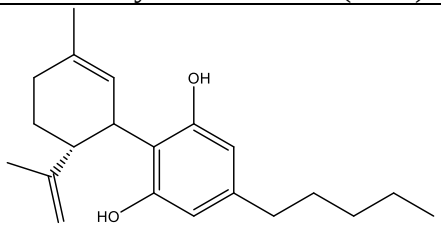
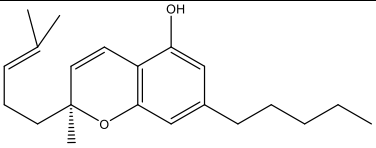
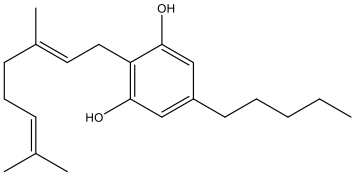
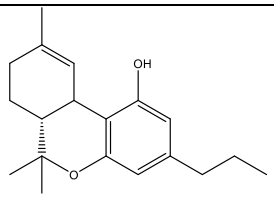
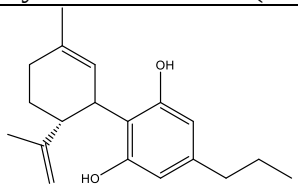
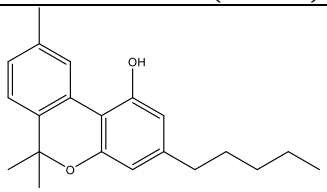
To date, 120 cannabinoids have been isolated, which has led to classification by 11 types: (-)-Δ9-trans-tetrahydrocannabinol (Δ9-THC), (-)-Δ8-trans-tetrahydrocannabinol (Δ8-THC), cannabigerol (CBG), cannabichromene (CBC), cannabidiol (CBD), cannabinodiol (CBND), cannabielsoin (CBE), cannabicyclol (CBL), cannabinol (CBN), cannabitriol (CBT), and miscellaneous (ElSohly et al., 2017).

THCA and CBDA are typically the most abundant cannabinoids found in cannabis, while the majority the other cannabinoids are not present in meaningful quantities in commercial

cannabis (Atakan, 2012). Still, other cannabinoids have shown interesting pharmacological properties (Table 1-1). THC is well known for its psychotropic effects and CBD has shown evidence for therapeutic activity without the psycho-euphoric properties (Groce, 2018). The psychoactive and therapeutic effects of cannabis has led to selective breeding for these compounds resulting in some cannabis strains with dried inflorescences with over 30% cannabinoid content (w/w %) (Swift et al., 2013).

The ecological functions of phytocannabinoids in nature are not well understood, but it has been proposed that the *C. sativa* plant produces them for biotic and abiotic defence, and attraction of seed dispersers (Lynch et al., 2016).

Table 1-1: Common cannabinoids and their pharmacological activity.Listed pharmacological activities have been reported in clinical studies, animal or *in vitro* systems (refer to Russo 2011).

Phytocannabinoid structure	Pharmacological activity
 Δ9-tetrahydrocannabinol (THC)	Analgesic Antioxidant Bronchodilatory Decrease symptoms associated with Alzheimer disease Benefit on duodenal ulcers Muscle relaxant Antipruritic, cholestatic jaundice
 Cannabidiol (CBD)	Antioxidant Anti-anxiety Anticonvulsant Cytotoxic versus breast cancer Effective versus MRSA Decreases sebum/ sebocytes Treatment of addiction
 Cannabichromene (CBC)	Anti-inflammatory/ analgesic Antifungal AEA uptake inhibitor Antidepressant in rodent model
 Cannabigerol (CBG)	TRPM8 antagonist prostate cancer GABA uptake inhibitor Anti-fungal Antidepressant rodent model Analgesic Effective versus MRSA
 Tetrahydrocannabivarin (THCV)	Anti-hyperalgesic Treatment of metabolic syndrome Anticonvulsant
 Cannabidivarin (CBDV)	Inhibits diacylglycerol lipase Anticonvulsant in hippocampus
 Cannabinol (CBN)	Sedative Effective versus MRSA TRPV2 agonist for burns Decrease keratinocytes in psoriasis Decrease breast cancer resistance protein

Studies evaluating biosynthesis of acid-cannabinoids have concluded that production of CBGA can lead to formation of THCA, CBDA, and CBCA via THCA synthase, CBDA synthase and CBCA synthase, respectively (Figure 1-2) (Russo, 2011; Taura et al., 1995; Thomas & ElSohly, 2015). Furthermore, through degradation and exposure to air, it was suggested that THC can oxidize to produce CBN (Mechoulam, 1970; Mechoulam & Gaoni, 1965). However, Citti et al. (2018) recommended that cannabinoid ratios between acid-form and neutral are better indicators of cannabis degradation.

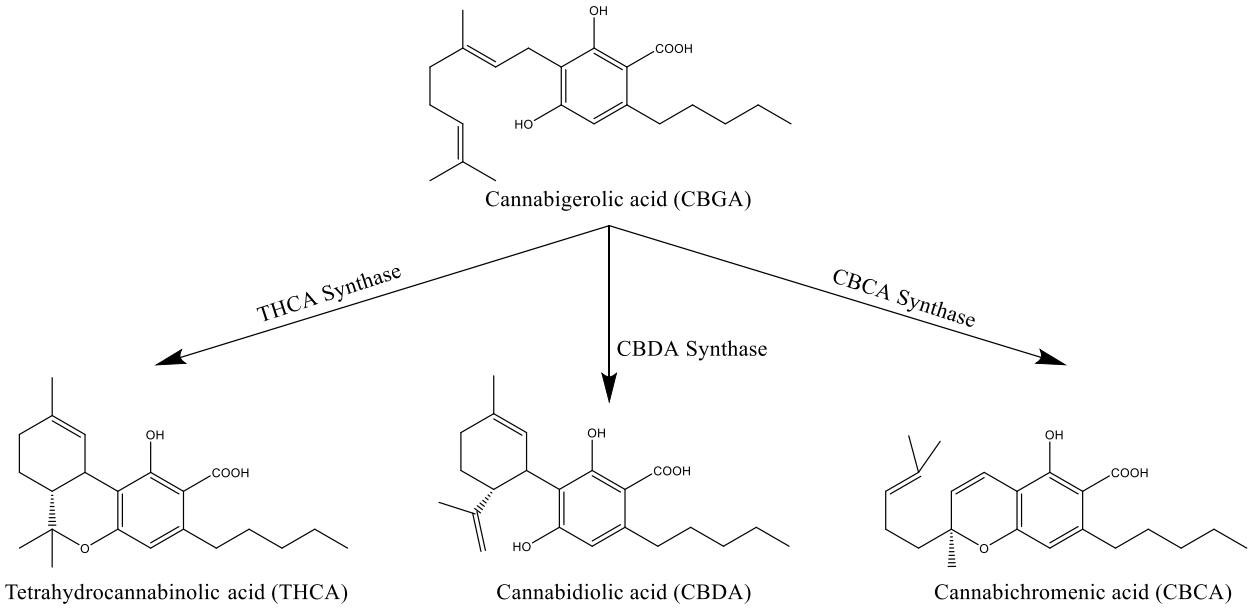


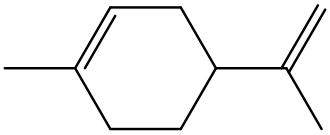
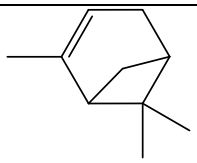
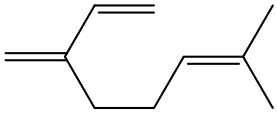
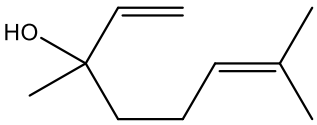
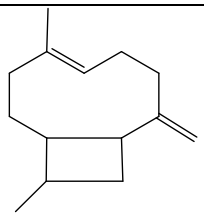
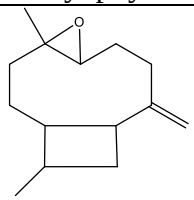
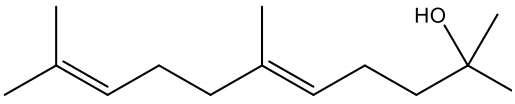
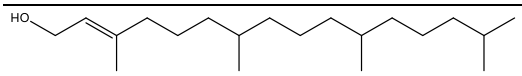
Figure 1-2: Biopathway of phytocannabinoids (adapted from Thomas & ElSohly 2015; Taura et al. 1995)

1.3.2 Terpenoids

Another class of compounds of high interest in cannabis is terpenes, where monoterpenes and sesquiterpenes are the most common classes (Clarke & Watson, 2007). Terpenes are small and aromatic volatile compounds found in many different plants derived biosythetically from mevalonate or the MEP pathway. Many of these compounds have shown promising therapeutic

properties such as anti-proliferative, anti-inflammatory or immune-stimulant effects (refer to review by ElSohly & Gul, 2015). A survey showed that the top factor for medical cannabis users for selecting their strain is by smell (Sexton et al., 2016), which is typically attributed to the terpenes present. Table 1-2 shows a list of common terpenes found in cannabis and their pharmacological properties. It was observed that plants of different chemotypes were differentiated by their terpene content where terpenes, such as β-eudesmol, γ-eudesmol, guaiol, α-bisabolene, α-bisabolol, or eucalyptol, were more pronounced in chemotype III plants (low THCA:CBDA), whereas γ-selinene, β-selinene, α-gurjunene, γ-elemene, selina-3,7(11)diene, and β-curcumene were characteristic of the chemotype I plants (high THCA:CBDA) (Aizpurua-Olaizola et al., 2016). Their correlation analysis showed that these terpenes were positively correlated with THCA and CBDA. This suggests that terpene profiles can vary between different strains of cannabis and, because consumers will select based on aroma, breeding has also selected for terpene content.

Table 1-2: Common terpenes found in cannabis and their pharmacological activity. Listed pharmacological activities have been reported in humans, animal or *in vitro* systems (adapted from Russo 2011).

Terpenoid Structure	Pharmacological activity
 Limonene	Potent antidepressant/ immunostimulant via inhalation Anxiolytic Apoptosis of breast cancer cells Active against acne bacteria Dermatophytes Gastro-oesophageal reflux
 α-Pinene	Anti-inflammatory Bronchodilatory in humans Acetylcholinesterase inhibitor, aiding memory
 β-Myrcene	Block inflammation Analgesic, antagonized by naloxone Sedating, muscle relaxant, hypnotic Blocks hepatic carcinogenesis by aflatoxin
 Linalool	Anti-anxiety Sedative on inhalation in mice Local anesthetic Analgesic Anticonvulsant/ anti-glutamate
 β-Caryophyllene	Anti-inflammatory Gastric cytoprotective Anti-malarial Selective CB_2 agonist
 Caryophyllene Oxide	Decreases platelet aggregation Antifungal in onychomycosis Insecticidal/anti-feedant
 Nerolidol	Sedative Skin penetrant Potent antimalarial Anti-leishmanial activity
 Phytol	Breakdown product of chlorophyll Prevents Vitamin A tetratogenesis Increase GABA levels

1.4 Differences between varieties

As for all plants (and other organisms), both genetic and environmental factors affect the resulting phtyochemical phenotype. Cannabis has evolved under pressures of natural selection and was further selected by humans to provide fibre, seed or drug products (Clarke & Merlin, 2016). Through selective breeding for longer stronger fibre or bigger more oil-rich seeds (hemp), as well as more potent and aromatic inflorescences (marijuana), the genetic and chemical diversity of modern cannabis is immense.

Following pedigreed development of new varieties (as standard for other agricultural crops), there are now hundreds of registered hemp cultivars, 52 of which are approved for growing in Canada (Health Canada, 2019). For medicinal and recreational cannabis, varieties are named typically by their grower or breeder, and reflect properties of the plant such as tastes, colours and smells. It is also popularly thought that different varieties of cannabis can elicit different experiences (Sexton et al., 2016). Varieties of drug-type cannabis, commonly referred to as "strains", are not registered cultivars but largely the result of extensive breeding for specific pharmacological and somatosensory effects sought out by consumers. There are over 2900 different strains with the main parent categorization of sativa, indica and hybrids that have been described on Leafly, a website dedicated to rating and reviewing different strains of cannabis and cannabis dispensaries (Leafly, 2019). The product descriptions include flavors such as citrus or sweet, and effects such as relaxed or creative. Aside from relative cannabinoid concentration, these characteristics may be associated with presence or concentration of terpenoids. Previous research had compared 460 cannabis samples based on 44 major cannabinoids and terpenes, and differentiated between vernacularly labelled sativa and indica samples (Hazekamp et al., 2016). This analysis suggests that there exist different cannabis strains based on phytochemistry but it

does not fully describe the thousands of strains that currently exist. Many of the varieties publically listed may have different names but are the same, or a closely related, strain.

It was proposed by researchers that other cannabinoids and phytochemicals present in the plant can produce a synergistic benefit to the effect caused by THC (Russo, 2011; Russo & McPartland, 2001). Various cannabis terpenoids have been shown to display therapeutic benefit thus, in combination with cannabinoids, may produce a complementary effect. A study examining the anti-inflammatory effects of CBD suggested that the cannabis plant extract was more efficacious than pure CBD (Gallily et al., 2015).

1.5 Cannabis for medical use

There are many uses associated with cannabis including management of anxiety and stress-related symptoms (Crippa et al., 2009), stimulation of appetite (Mattes et al., 1994), pain relief (Karst et al., 2010), and promotion of sleep (Babson & Bonn-Miller, 2014). Many studies show these therapeutic effects are dose dependent and can show variable responses among users with researchers suggesting that further studies are required. Clinical evidence suggested that cannabinoids show therapeutic potential as analgesics in chronic neuropathic pain, appetite stimulants in debilitating diseases (cancer and AIDS), as well as in the treatment of multiple sclerosis (review by Hazekamp & Grotenhermen, 2010). These medicinal effects are largely mediated through interactions with the human endocannabinoid system. Endocannabinoids are substances that are produced by the body and bind to primary receptors CB1 and CB2 (Matsuda et al., 1990; Munro et al., 1993). Cannabinoids impact the immune system, where they suppress inflammatory and immune responses (Lu & MacKie, 2016). Some phytocannabinoids,

most notably THC, bind to the same receptors to activate the endocannabinoid system and produce therapeutic responses.

1.6 Legalized Cannabis in Canada

The Cannabis Act (Bill C-45) legalized access to recreational cannabis in Canada on October 17, 2018 (Canada Gazette, 2018). It permits selling of fresh and dried cannabis, cannabis plants and seeds, and cannabis oil by an authorized person with planned legislation to legalize cannabis edible products and concentrates the year following (Senate of Canada, 2018). Under this act, "cannabis" is defined as any part of a cannabis plant including the phytocannabinoids produced, any substance or mixture of substances that contains any part of the plant, and any substance that is identical to the phytocannabinoid produced by the plant. It excludes a non-viable seed of a cannabis plant, a mature stalk without any leaf, flower, seed or branch or a fibre derived from it, and the root of the plant. In terms of cannabinoid content, regulation requires quantification of only THC, THCA, CBD and CBDA.

1.6.1 Cannabis concentrates and oils

There are many cannabis concentrates for consumer use such as tinctures (alcohol-based extracts) and oils. These products can be mixed into food or orally ingested, and oils can also be vaporized. Cannabis concentrates like oils will be legalized in Canada near the end of 2019 (Government of Canada, 2019). Conversely, a cannabis concentrate mixed with a plant-based oil to a maximum of 3% THC, is currently legal and commercially available. Canadian licensed producers sell cannabis extracted resin mixed with a medium-chain triglyceride (MCT) oil or olive oil as cannabis oils (Aurora, 2019; HEXO, 2019; Tweed, 2019).

Cannabis concentrates are made by a variety of different methods. Two popular methods include ethanol extraction and supercritical fluid extraction (SFE) (Ramirez et al., 2018). Ethanol extraction is a commonly used technique to extract phytochemicals in plants (Altemimi et al., 2017), and is widely used by Canadian cannabis producers. According to Cannimed, their concentrate is produced by extracting dried cannabis flower using pharmaceutical grade ethanol and then removing the solvent by evaporation (Aurora, 2019). On the other hand, SFE is an extraction technique involving use of a supercritical fluid. A supercritical fluid is a substance at a temperature and pressure above its critical point which evokes unique chemical properties such as compressibility, homogeneity and a continuous change from gas-like to liquid-like properties (Williams et al., 2000). Typically, the diffusion coefficients in supercritical fluids are a magnitude higher than liquids resulting in faster transport in extraction. SFE using carbon dioxide is a popular commercial and industrial technique since CO_2 is nontoxic, non-flammable, and leaves no residues, making it attractive to the food industry (Small, 2017). Although CO_2 is non-polar, and popularly used to extract oily or lipophilic substances, it can be also used for extraction of many organic molecules including those with polar properties. Nevertheless, SFE using CO_2 can involve the use of a co-solvent to increase its affinity with polar solutes.

The preparation methods to produce different products may cause quantitative and qualitative phytochemical differences due to the different properties of the extraction solvent. It was previously observed that CO_2-SFE did not replicate flavor and fragrance of the original cannabis flower sample as the cannabinoid and terpene content was different (Sexton et al., 2018). Understanding the differences in chemical content of different types of concentrates, a knowledge

gap addressed in this thesis, can better inform producers and consumers about the products they make, sell and use.

1.7 Vaporization

There are many modes of delivery of cannabis including smoking, eating in food and vaporizing. According to a Health Canada survey conducted in 2017, the top two products used by cannabis users were dried flower, which is consumed by smoking and vaporizing, and edibles (Health Canada, 2017). When consuming cannabis flower or oils (with the exception of heat activated products), the mode of delivery must allow decarboxylation of the naturally found phytocannabinoids to form the neutral, more bioactive cannabinoids. It must be noted though, that the rate of conversion of THCA to THC through heat is variable and not 100% efficient. A study of smoking and combustion of cannabis found that up to 62% of the initial THC content may be delivered in the smoke under conditions common among cannabis users, although it was found in another study that only 30% THC is available (Dussy et al., 2005; Fehr & Kalant, 1972). A Canadian survey conducted in 2015 showed that vaporization was the most popular mode of delivery for medical cannabis followed by smoking (Shiplo et al., 2016). It was reported that vaporizing tasted better, had no smoke smell, and was more discreet. Vaporization is a method of ingestion that can extract active principles of cannabis with heated gas. This non-combustible mode of delivery can prevent some of the negative respiratory health effects associated with smoking as it reduces pyrrolic by-products, and serve as an effective harm reduction method for medical cannabis use (Abrams et al., 2007; Hazekamp et al., 2006). The Health Canada approved medical device, Volcano© vaporizer, is a commercial desktop product. A study comparing cannabis cigarette smoke and the Volcano vaporizer has concluded that the vapor consisted of

decreased level of degradation by-products (Pomahacova et al., 2009). In addition, since higher temperatures are associated with an increase in amount of desired cannabinoids, the vapor was able to provide double the quantity compared to cigarette smoke without compromising quality. Although common, this mode of consuming cannabis remains poorly studied and has high potential to show relatively different phytochemistry composition than the plant counterpart. This is another knowledge gap addressed in this thesis.

1.8 Metabolomics

Metabolites are defined to be the end products of cellular regulatory processes and regarded as the response of biological systems to genetic or environmental changes (Fiehn, 2002). The entire set of small molecules that are synthesized by the organism (e.g. cannabis), such as amino acids, carbohydrates, organic acids, nucleic acids and lipids are referred to as the metabolome. Metabolomics is the qualitative and quantitative study of all or a sample of the metabolites present (metabolome) in a biological sample under certain conditions and at a certain point in time. This experimental approach is advantageous as it can detect hundreds of compounds at a time and therefore provide a snapshot of a sample's chemical content. Comparative metabolomics usually refers to the untargeted profiling and comparison of metabolomes across variables such as species, time, and environmental/experimental conditions. Typical metabolomics workflows utilize High Resolution Mass Spectrometry (HRMS) or Nuclear Magnetic Resonance (NMR) to obtain data as these analytical techniques have high selectivity and sensitivity (Dunn & Ellis, 2005).

1.8.1 Targeted and untargeted approaches

Metabolomics strategies have been divided into two different approaches, targeted and untargeted. Targeted metabolomics refers to the measurement of specific group of chemically characterized and biochemically annotated metabolites typically using internal standards (Roberts et al., 2012). This approach combines understanding of metabolic enzymes, their kinetics and end-products, and their biochemical pathways. It often utilizes standards and/ or spectroscopic data libraries hand-in-hand with other technologies such as transcriptomics, genomics and proteomics. It aims to investigate hypothesis-driven research to quantify specific metabolites through optimizing extraction, separation and detection methods.

Untargeted metabolomics refers to generated data sets representing a broad chemical spectrum of a biological sample, which generates extensive amounts of raw data (Shulaev, 2006; Vinayavekhin et al., 2010). The coverage of the metabolome is vast but limited by the methodologies of sample preparation and specificity and sensitivity of the analytical technique used. Metabolomic approaches can be used to identify patterns or relationships from the datasets, metabolic fingerprinting, and novel target and pathway discovery. Untargeted studies are typically coupled with multivariate statistical approaches, to reduce the complexity of datasets, and subsequent putative identification of metabolites through spectral comparison with database entries.

Previous metabolomics studies of cannabis have compared the chemical composition of cannabis cultivars utilizing plant material. Hazekamp et al. (2016) performed a targeted metabolomics approach based on major cannabinoids and terpenes to demonstrate phytochemical diversity between cannabis samples while Choi et al. (2004) compared ^{1}H NMR metabolomic profiles of cannabis flowers and leaves.

1.9 Research rationale

Cannabis contains hundreds of phytochemicals with potential implications to human health. The extensive breeding of cannabis for specific pharmacological and somatosensory effects has led to production of different strains, and it is popularly thought that different strains of cannabis can elicit different experiences. However, strains are not registered cultivars and it is possible that the same or similar strains are being sold with different names. In addition, the alleged psychological effects are from personal testimonies and have little scientific backing. Producers report mainly cannabinoid and sometimes terpene content, but this information may not be reflective of the complex phytochemistry of cannabis. There are additional phytochemical compounds that are not evaluated, and it is possible that these compounds can synergistically contribute to a beneficial effect and/ or affect potency of cannabinoids.

In Canada, cannabis concentrates, topicals and edibles will be legalized for commercial sale in Canada on October 17, 2019; however, under current legislation, only flower and oils (as a mix of cannabis concentrate and MCT oil) are available. With different cannabis products available for consumer use such as flowers and oils, it is valuable to understand the differences between products and their preparation methods. Currently, flowers and oils are sold under the same strain name, although the methods to produce these cannabis products may cause quantitative and qualitative differences leading to different phytochemistry profiles. To fill some of the knowledge gaps, this research compared tinctures, ethanol extracted oils and CO_2-SFE oils using an untargeted and targeted phytochemical analysis. A better understanding of the chemical content in these concentrates can better support and inform consumers about different cannabis products as well as future developments in the cannabis industry.

A popular mode of delivery of medical cannabis in Canada is vaporization (Shiplo et al., 2016). Previous research suggested that it can act as an effective harm-reduction method due to the decrease in production of pyrollic by-products (Pomahacova et al., 2009); however, knowledge gaps still exist. As vaporization can cause chemical transformations that may lead to a different phytochemical profile than the pre-vaporized cannabis product, it is important to gain more information concerning the quantitative and qualitative phytochemical changes because the product label may not be representative of what is phytochemically available for consumption. Phytochemical changes due to vaporization using untargeted and targeted analysis of cannabis flowers, ethanol oils and CO_2 oils were therefore evaluated.

1.10 Objectives and hypotheses

This research project used untargeted metabolomics and targeted phytochemical analysis to explore quantitative and qualitative differences associated with different cannabis products, such as flower and oils, and the process of vaporization. The hypotheses and predictions of this research are as follows:

Hypothesis 1: Different strains will have different chemical profiles

Prediction: Multivariate metabolomics analysis and cannabinoids and terpene profiling will find significant differences between strains.

Hypothesis 2: Different extraction methods of the same raw material will yield phytochemical differences at the untargeted (metabolome) and targeted (cannabinoids and terpenes) levels.

Prediction 1: The drying process to produce oil will decrease total number of detectable phytochemicals due to the loss of volatiles such as terpenes.

Prediction 2: The different processes of producing oils such as ethanol extraction and CO_2 SFE will produce oils of significantly different phytochemical composition

Hypothesis 3: The process of vaporization will reduce total number of phytochemicals

Prediction: Untargeted metabolomics analysis will reveal qualitative reduction of detected variables in cannabis vapor compared to pre-vaporized samples.

Hypothesis 4: Vaporization will increase the relative concentration of decarboxylated cannabinoids.

Prediction: HPLC quantification will show lower concentration of acidic cannabinoids in cannabis vapor compared to pre-vaporized samples.

To test the hypotheses outlined above, this thesis is presented as two data chapters each with specific objectives. The first and second hypotheses were explored in Chapter 2 with the following specific objectives:

a) To evaluate 13 different cannabis strains as three different cannabis extracts (tincture, ethanol extracted oil, SFE using CO_2 oil) using an untargeted metabolomics approach and explore differences in qualitative phytochemical profiles

b) To quantify major cannabinoids, as they are the compounds of greatest interest to consumers and industry, to support and complement results from untargeted metabolomics data.
c) To quantify terpenoids and perform untargeted exploration to determine differentiation between strains and product types

The third and fourth hypotheses were explored in Chapter 3 with the following specific objectives:

a) To explore phytochemistry changes associated with vaporization of three different product types (flower, ethanol extracted oil, SFE using CO_2 oil) derived from 13 different cannabis strains using untargeted HRMS metabolomics
b) To quantify major cannabinoids to support and complement results from untargeted metabolomics data.
c) To quantify terpenoids and perform untargeted exploration to determine effect of vaporization

Chapter 2: Comparison between cannabis processing methods and impacts on phytochemical complexity of cannabis products

2.1 Introduction

Cannabis sativa L. is a flowering plant in the family Cannabaceae where the material from the flowering part of the unfertilized female plant is commonly harvested for medicinal and recreational use (Small, 2017). The phytochemicals mainly recognized for the medicinal and psychoactive effects of cannabis are the cannabinoids Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD), which, together with their acid forms THCA (Δ9-tetrahydrocannabinolic acid) and CBDA (cannabidiolic acid), are the most abundant, well-studied, and sought-after components in cannabis (ElSohly & Slade, 2005; Lynch et al., 2016). In addition to cannabinoids, cannabis contains hundreds of known chemical compounds from a diverse range of phytochemical classes including terpenes, hydrocarbons, steroids, and flavonoids.

In Canada, cannabis is a recently legalized substance where a variety of different licenced products are available for consumer selection and use. Licensed cannabis producers currently market products with brand or trade names with some producers also labelling the type of strain and main terpene content. Under the Cannabis Act (Government of Canada, 2018), the total THC and CBD content must be reported for all products. These products are primarily sold as flower/ bud or oils, among other formats. Two popular methods of cannabis oil production in the cannabis industry are ethanol extraction and supercritical fluid extraction (SFE) using CO_2 (Ramirez et al., 2018). Ethanol extraction uses pharmaceutical grade or food grade ethanol to extract phytochemicals from the flower material using a combination of sonicating and shaking (Citti et al., 2018; Romano & Hazekamp, 2013). The extract is further dried under vacuum to remove the solvent and produce oil. The SFE method uses solvents at a temperature and pressure

above its critical point which induces unique chemical properties such as compressibility, homogeneity and a continuous change from gas-like to liquid-like properties (Williams et al., 2000). Whereas SFE by CO_2 can yield solvent-free extracts, ethanol is often added during (co-solvent) or after (winterization) extraction to improve quality and efficiency (Ramire et al., 2018).

With extraction, the soluble components of the raw material are dissolved by the solvent (Pfennig et al., 2011). Therefore, the complexity of cannabis and the diversity of available phytochemicals with various polarities and other properties can result in selective extraction based on the solvent or method chosen for production. Ethanol is a polar organic solvent while CO_2 is a non-polar gas which can result in different selectivity of classes of molecules. In addition, the drying steps can result in changes to the extract's chemical composition by selectively removing or degrading certain classes of molecules (Shang et al., 2017). It was seen that drying fresh cannabis plant material reduced overall content of terpenes, where a greater loss of monoterpenes was observed relative to sesquiterpenes (Rossi & ElSohly, 1996).

In this chapter, the chemistry of different cannabis products was evaluated following different extraction protocols, which can yield quantitative and qualitative phytochemical differences. Specifically, 13 cannabis strains were processed as flower tincture, ethanol extracted oil and SFE CO_2 oil, representing different concentrates that can be used by consumers. An untargeted metabolomics along with targeted cannabinoid and terpene quantitative analysis were used to compare the phytochemistry of the three concentrates within and between the different cannabis strains. Currently, flowers and oils are sold under the same strain name, although the methods to produce these cannabis products may cause quantitative and qualitative differences leading to different phytochemical profiles. Changes in overall phytochemistry may potentially

change the bioactivity, therapeutic effects and/or consumer experience. Better understanding of the chemical content in these concentrates can support and inform consumers about different cannabis products as well as future developments in the cannabis industry.

2.2 Experimental methods

2.2.1 Plant materials

Thirteen different dried cannabis flower strains were received on May 9, 2018 from Hydropothecary (HEXO Corp), a Licensed Cannabis Producer located in Gatineau, Quebec. Two strains were noted as high CBD varieties (samples 2 and 10) while the rest were low CBD strains. In addition, two strains were provided as milled flower (samples 12 and 13) and the others as dried unprocessed inflorescence. Samples were stored according to security protocols approved by Health Canada.

2.2.2 Sample preparation

The samples were weighed and lyophilized overnight over 2 days until the mass of the samples did not change. The dried cannabis flowers were hand grinded using a herb mill (Storz & Bickel) until homogenous, while milled flowers were not grinded further. All samples were stored in the dark at room temperature in a desiccator until extraction.

Flower tincture

The tincture samples were prepared by an adapted method described by Mudge et al. (2017). Five grams of ground cannabis flower material were sonicated in 500 mL 99% ethanol for 10 minutes then the mixture was placed on shaker for 3 hours at 200 rpm. The

extracted plant material was removed by filtering through Whatman Grade A Filter Paper under vacuum. The extract was sampled and transferred through a 0.22 μm PTFE syringe filter into a HPLC vial and stored at -20°C until further analysis.

Ethanol oil extract

The remaining filtered 99% ethanol in the Flower tincture (described above) was removed under vacuum (rotary evaporator) at room temperature. The oil was transferred into a 50 mL falcon tube and further dried under vacuum at room temperature using a Centrivap Centrifugal Vaccum Concentrator (Labconco Centrivap, Model 7810014). The oil was reconstituted in 99% ethanol to a concentration of 1 mg/mL of total extract mass, filtered through a 0.22 μm PTFE syringe filter and stored at -20°C until further analysis.

CO_2 oil extract

The CO_2 extracted oil was prepared by the modified extraction procedure described by Rovetto and Aieta (2017). The lyophilized and hand ground cannabis flower was further ground into a fine powder using a Magic Bullet Blender®. Five grams of fine powder were extracted by CO_2 at 45°C, under 350 bar pressure, and 2.5 L/min flow then washed with 30 mL 99% ethanol. The ethanol was removed under vacuum at room temperature using a Centrivap. The oil was reconstituted in 99% ethanol to a concentration of 1 mg/mL and stored at -20°C until further analysis.

Sample C7 was prepared under a methodological error where not enough CO_2 was available during extraction. This extract was included for untargeted analyses and multivariate

analyses based on oil weight, but were excluded from quantitative comparisons calculated to dry plant weight due to extract recovery problems.

2.2.3 Cannabinoid quantification

1µL cannabis extract samples were analyzed using a high performance liquid chromatography system (Agilent 1100 HPLC) coupled with a photodiode array detector (G1315 series) and an autosampler (G1313 series). A Phenomenex Luna Omega Polar C18 column (100 × 2.1 mm; particle size 1.6 µm; pore size 100 Å) was used for separation. The mobile phase consisted of water (mobile phase A) and acetonitrile (mobile phase B). The gradient elution was the following: 0.0-1.0 min 75% B, 1.0-3.0 min 75-85% B, 3.0-4.0 85% B, 4.0-5.0 85-75%B, 5.0-10.0min 75%B followed by a 5-minute 75%B column rinse. The flow rate was set at 0.2 mL/min and the column temperature was maintained at 45°C. Calibration curves of THCA, THC, CBDA and CBD were prepared in 99% ethanol solution at concentrations of 250, 200, 150, 100, 50 and 25 µg/mL, and quantification was performed on chromatograms detected at 235 nm. Calibration curves were obtained each day with correlation coefficients greater than 0.99. The limit of detection (LOD) and the limit of quantification (LOQ) were evaluated as per ICH guideline Q2(R1) (International Conference on Harmonisation, 2005) based on standard deviation of the response and the slope. A low concentration cannabinoid mix was injected seven times to calculate the standard deviation (σ). The LOD was determined to be 3.3 σ/S where S is the slope of calibration curve, and LOQ was determined to be 10 σ/S.

In addition, total THC was defined as the sum of THC and 0.8772(THCA) since:

$$x\ \text{g THCA} \times \frac{\text{mol}}{358.478\ \text{g}}\text{THCA} \times \frac{1\ \text{mol THC}}{1\ \text{mol THCA}} \times \frac{314.469\ \text{g}}{\text{mol}}\ \text{THC} = x\ (0.8772)\text{g THC}$$

where complete conversion of THCA to THC was assumed. This was likewise when calculating total CBD.

2.2.4 Terpene quantification

Terpenes were quantified by an adapted method described by Supelco in their data packet. 2.5µL cannabis extract samples were injected on a gas chromatography instrument (Agilent 6890N Network GC) with an autosampler (7683 series) and dual injectors (7683B series) fitted with a flame ionization detector (FID) and SLB-5MS GC Column (30m x 0.25mm x 0.25µm) at a concentration of 1 mg/mL in 99% ethanol. The injector temperature was set to 230°C in splitless injection. The carrier gas was hydrogen set to a flow rate of 1.4 mL/min, and the oven was programmed for 60°C for 3.5 min, 3.5°C/min to 155°C, 30°C/min to 300°C with a post run at 340°C for 10 minutes. The needle was programmed to rinse before and after injection 3 times, at 1.5 uL volume in methanol.

Terpene standards (β-Pinene, 3-Carene, α-Terpinene, (R)-(+)-Limonene, γ-Terpinene, L-(-)-Fenchone, Fenchol, (1R) (+) Camphor, Isoborneol, Menthol, Citronellol, (+)-Pulegone, Geranyl acetate, (1S)-(+)-3-Carene, p-Cymene, Limonene, Terpinolene, Linalool, (1S)-(-)-Camphor, (+)-Borneol, (-)-α-Terpineol, Geraniol, α Cedrene, α Humulene, Nerolidol, (+)-Cedrol, (-)-α-Bisabolol, β-Caryophyllene, cis-Nerolidol, β-Eudesmol) were obtained from Sigma-Aldrich and calibration curves were prepared in 99% ethanol solution at concentrations of 250, 200, 150, 100 and 50 ng/mL. Calibration curves were obtained each day with correlation coefficients greater than 0.99. The data were processed on ChemStation using 0.25% error for peak detection. The LOD and the LOQ were evaluated as per ICH guideline Q2(R1) (International Conference on Harmonisation, 2005) based on standard deviation of the response and the slope. A low concentration cannabinoid mix was injected seven times to calculate the standard deviation (σ). The LOD was determined to be 3.3 σ /S where S is the slope of calibration curve, and LOQ was determined to be 10 σ/S.

2.2.5 Sample preparation for high resolution mass spectrometry

The flower tincture was prepared to 100 µg/mL in 99% ethanol by dry plant material weight while ethanol and CO_2 oils were prepared to 100 µg/mL by oil material weight. All samples were filtered through a 0.22 µm PTFE syringe filter then transferred into a HPLC vial and stored at -20°C until analysis.

2.2.6 High resolution mass spectrometry data acquisition

The samples were analyzed to yield technical replicates where they were injected as triplicates. In addition, it was analyzed along with cannabinoid standards (THC, THCA, CBD, CBDA, CBN, CBG, CBGA, CBC (Toronto Research Chemicals)). 5 µL aliquot of extracts were analyzed in random order using Thermo Scientific Dionex Ultimate 3000 UHPLC system coupled to a Thermo Scientific LTQ Orbitrap XL high resolution mass spectrometer. Chromatography was performed using a Phenomenex Kinetex C18 100Å column (2.1 × 50 mm, 1.7 µm) with a flow rate of 0.35 mL/min. The mobile phase consisted of water containing 0.1% formic acid (mobile phase A) and acetonitrile containing 0.1% formic acid (mobile phase B). The gradient started at 5% B and increased to 95% B over 4.5 min, held at 95% B for 3.5 min, returned to starting conditions over 0.5 min, and allowed to equilibrate for 6 min. The HRMS was operated in ESI^+ mode monitoring *m/z* 100-2000 using the following parameters: sheath gas (40), auxiliary gas (5), sweep gas (2), spray voltage (4.0 kV), capillary temperature (320˚C), capillary voltage (35 V), tube lens (100 V), maximum injection time (500 ms) and microscans (1). Ethanol blank samples were injected every 10 samples to evaluate sample carry over. In addition, reserpine was injected with every set run to evaluate mass accuracy and drift. The mass spectrometry data were exported as .raw files for analysis.

2.2.7 Untargeted Metabolomics Data Pre-Processing

Spectra were processed using MZmine 2.38 (Pluskal et al., 2010). Evaluation of the raw data files of the samples and 99% ethanol blank concluded an absolute chromatography and spectrometry noise level of 1E6 and 5E5. The *m/z* tolerance for all analysis done was set at 0.005 *m/z* or 10.0 ppm and the RT tolerance was set to 0.05 min. Raw data files were pre-aligned using a range of 5 horizontal scans, 1 max vertical alignment and minimum chromatogram height of 6E6. The mass detection using Exact mass Mass detector and the noise level was set at 5E5 to ensure all signals were detected, then the chromatogram builder was set with minimum height of 1E6. The chromatograms were then deconvoluted using the Local minimum search algorithm with an auto *m/z* center calculation. The parameters were set at 30% chromatographic threshold, 0.07 min minimum RT range, 9% minimum relative height and 5E5 minimum absolute height, 1 minimum ratio of peak top/edge, and peak duration range of 0.00-0.50 min. These values were determined by combing through all the collected spectra and ensuring that all relevant peaks were detected by the software. The data were de-isotoped by representing the isotope with the most intense peak with a maximum charge of 1. Peak alignment was performed using the RANSAC algorithm (Fischler & Bolles, 1981) with at least 25% of points required to consider the model valid, switching between polynomial and linear models. All spectra were gap-filled by applying a gap filling algorithm that filled detected variables that fell below the noise limit threshold during mass detection using their accurate retention time and *m/z* value. The duplicate peak filter method was performed using the new average filter mode. Variables corresponding to the cannabinoid standards were identified and labelled. This dataset was exported to csv file with peak area. A total of 979 features were detected and this data were further cleaned in later steps.

2.2.8 Untargeted Metabolomics Data Cleaning and Processing

The exported data matrix was further analyzed in R. The peak area average of the 99% Ethanol analytical blanks multiplied by 10 was subtracted from all samples to represent all discrete ions (RT; *m/z*) above a 10 signal-to-noise ratio from the noise. All negative values were substituted with zeros and all ions yielding no signal after this pre-processing step were removed. Normalization by total useful signal (MSTUS) and Pareto scaling was applied to the data, and a total of 816 ions were used for further analysis. Normalization, scaling and multivariate statistics were performed using the MUMA 1.4 R package (Gaude et al., 2013).

2.3 Results

2.3.1 Untargeted metabolomics: Variability between strains and products

The variability of strains within each product type was evaluated using untargeted metabolomics data. The HRMS analysis detected phytochemicals and their adducts, which represented unique discrete ions (RT; *m/z*). The Venn diagram (Figure 2-1) allowed for binary phytochemical comparisons by revealing presence and absence of ions in each product type where an ion was considered detected if it was present in at least two out of three replicates in at least one strain above 10 signal-to-noise (as described in experimental methods). 585, 463 and 626 discrete ions were detected in flower tinctures, ethanol oils and CO_2 oils, respectively. This suggested that CO_2 oils were the most phytochemically complex, followed by tincture and ethanol oil based on number of ions detected.

Overall, it was shown that the majority of discrete ions (RT; *m/z*) were detected in at least one sample of all three product types, where 421/ 816 ions were shared. These unique ions included all 10 cannabinoids (THC, THCA, CBD, CBDA, CBN, CBG, CBGA, CBC) that were evaluated.

This suggested that major cannabinoids were extracted by all methods and present in at least one of the tested strains. Still, differences in phytochemical profiles between the strains were observed. Strain-based differences were most pronounced in tinctures, where analyzing the number of unique ions shared across strains confirmed that different strains contained different phytochemical profiles since only 24% of ions were detected in >50% of strains and only 11% were detected in all tinctures. Strain differences were less pronounced in ethanol oil where 64% of ions were detected in >50% of strains and 45% were detected in all 13 oils. Despite their greater complexity, CO_2 oils showed the highest level of homogeneity as 77% of ions were detected in >50% of strains and 60% were detected in all 13 total ions across strains.

Comparing between tinctures and ethanol oil suggested that phytochemistry changes with drying, specifically rotary evaporation. The Venn analysis showed that removing solvent from the tincture to produce the oil decreased the detected number of discrete ions by 122/585 (21%), signifying that the drying process reduces the chemical complexity of ethanol extracted oil compared to tincture. The phytochemistry changes observed based on the number of discrete ions detected across 13 strains was determined to be statistically significant (Mann-Whitney-Wilcoxon test, p-value < 0.05). As mentioned earlier, strain differences were more pronounced in tinctures than oils which suggested that phytochemicals that contribute to strain variability were lost.

Ethanol oils and CO_2 oils were compared to evaluate differences related to oil production methods. Based on the number of discrete ions detected across 13 strains, it was determined that their differences were statistically significant (unpaired t-test, p-value < 0.05). Data demonstrated that CO_2 oils were the most chemically complex as there were 163 more unique ions detected than ethanol oils; however, there was less variability observed across strains in CO_2 oils where majority of ions were detected in all 13 strains.

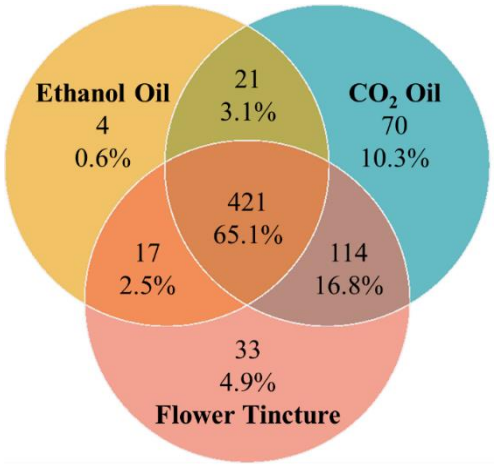


Figure 2-1: Venn Diagram of Flower tincture, Ethanol oil and CO_2 oils found in at least 1 sample out of 13 samples using unique discrete ions (total 585) detected in untargeted metabolomics data where it is considered present if present in at least two out of three technical replicates.

The untargeted metabolomics data were explored further by performing principal component analysis (PCA) using normalized and Pareto scaled data within each product type. PCA analysis of the 13 flower tinctures, ethanol oils and CO_2 oils were independently used to evaluate diversity within each product set as this analysis showed an unbiased distribution of the samples based on the detected features. In all three products, defined clustering of strains 2 and 10 from the other samples along PC1 was observed in the scores plot, where their corresponding loadings plots suggested that this clustering was due to CBDA (Figure 2-2). In addition, PCA of the flower tincture, ethanol oil and CO_2 oil samples was performed collectively and showed similar results with discrete grouping of samples 2 and 10 (Figure 2-3), suggesting that differences in cannabinoid content accounts for more of the observed chemical variability than product type or processing. Only PC1 and PC2 were presented as the point of separation as defined by the respective scree plot (Figure A-3a) determined PC1 described majority of the variance observed.

It should be noted that a second defined cluster of samples 9 and 11 along PC2 was observed among tinctures (Figure 2-2a) and, based on the loadings plot (Figure 2-2d), suggested related to THC and CBC; however, evaluating the raw chromatogram of F9 and F11 (Appendix A, Figure A-2) revealed that their largest peak, THCA, was larger by 25-fold but TIC was lower by 13-fold. In addition, Figure 2-3 showed F9 and F11 cluster with the oils along the PC2 suggesting that THC/THCA level in those samples was higher than in other tinctures. Because of this, it must be noted that separation along PC2 may be due to differences in phytochemical concentrations, most notably the cannabinoids, as sample F9 and F11 segregated with oil samples.

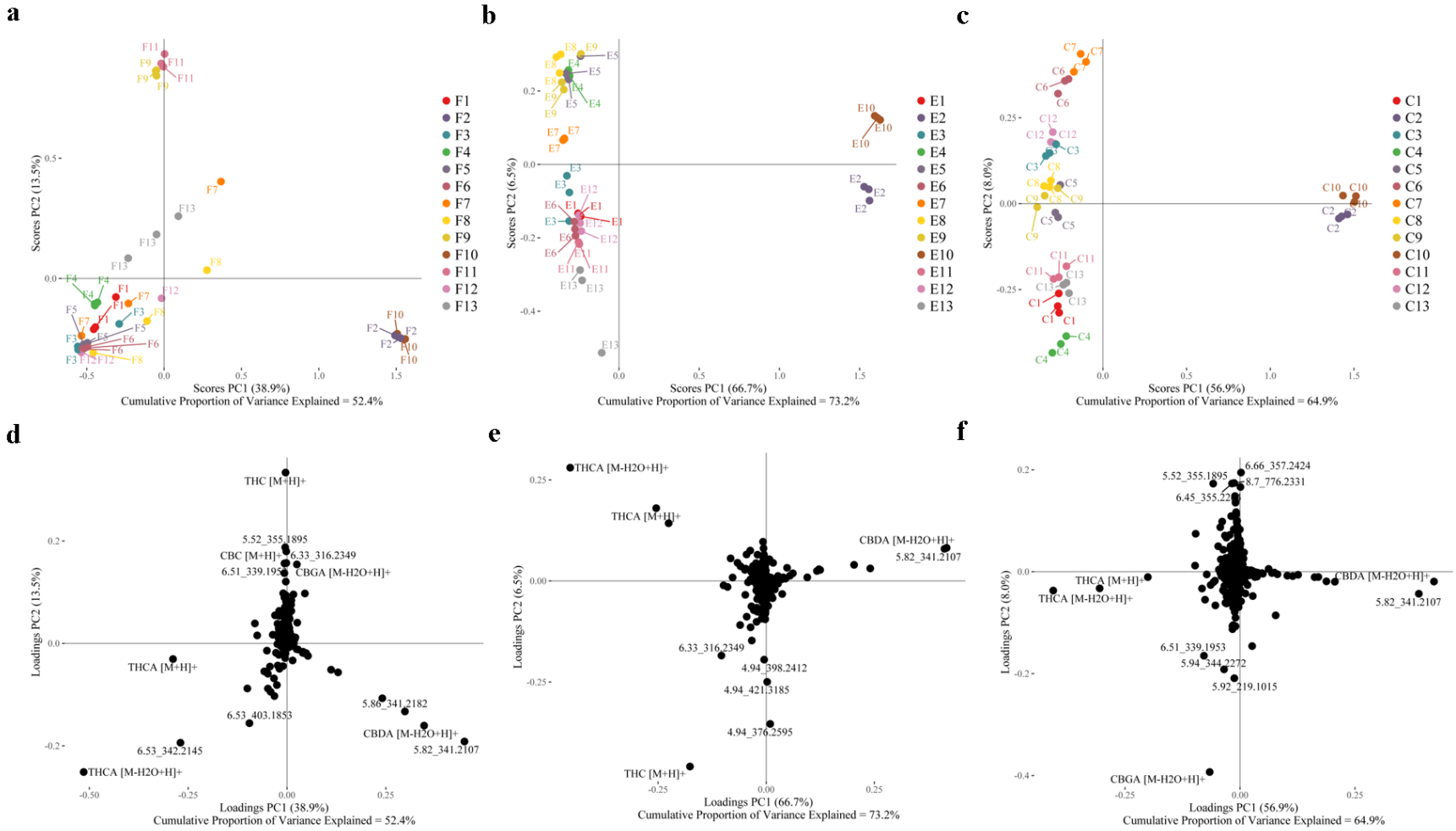


Figure 2-2: PCA of untargeted metabolomics data of flower tinctures, ethanol oils and CO_2 oils visualizing phytochemical diversity **(**a) PCA scores plot of flower tinctures where each dot represents a sample coloured based on strain type (**b** and **c** show the PCA scores plot of ethanol oil and CO_2 oil, respectively). (**d**) PCA loadings plot for the first two principal components of the flower tinctures (**e** and **f** show the PCA scores plot of Ethanol oil and CO_2 oil, respectively).

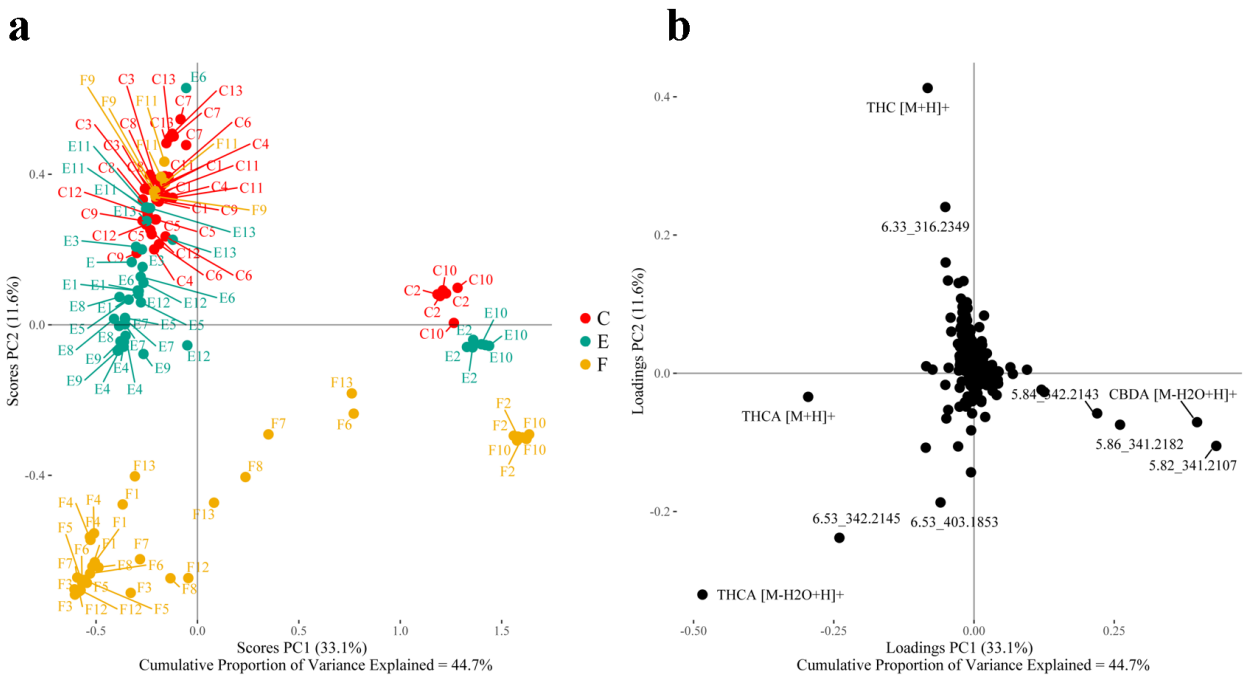


Figure 2-3: PCA of untargeted metabolomics data of all flower tinctures, ethanol oils and CO_2 oils samples visualizing phytochemical diversity.(**a**) PCA scores plot for the first two principal components (**b**) PCA loadings plot for the first two principal components

2.3.2 Targeted metabolomics: Cannabinoid and terpene variability between strains and products

Four major cannabinoids, CBDA, CBD, THCA and THC, were quantified as they were the main contributors of variance among samples. Although other cannabinoids were observed in the untargeted metabolomics data, these peaks were below the limit of quantification in the quantification method (Appendix B, Table B-1). Consequently, only cannabinoids >0.01% by plant weight were evaluated. The total THC and total CBD were evaluated for each strain in each product type to confirm that sample 2 and 10 were the only samples containing CBD(A), which contributed to their phytochemical differences from other samples (Figure 2-4). Overall, total cannabinoid concentration was similar between tinctures and ethanol oils, but lower in CO_2 oils. The concentration of THCA between tinctures and ethanol oils were found to be statistically

significant (paired t-test, p-value < 0.05), and between ethanol oils and CO_2 oils to be statistically significant as well (unpaired t-test, p-value < 0.05). Samples F9 and F11 contained CBD(A) levels above LOD but below 1% by dry weight plant material. The higher levels of CBD may have contributed to their clustering observed in Figure 2-2a.

The cannabinoid ratios of flower tinctures and ethanol oils were calculated to determine the changes associated with drying (Table 2-1). It was seen in general that the drying process decarboxylated the acid-form cannabinoids as an increase in CBD and THC ratios was observed with values 2.29 and 2.69, respectively, while a decrease in CBDA and THCA ratios were observed with 0.81 and 0.82, respectively. In addition, the ratio across the major cannabinoids of CO_2 oil to ethanol oil by flower weight showed a lower concentration, demonstrating that ethanol oil better extracted these cannabinoids than CO_2 oil (averages ranging from 0.61-0.82) (Table 2-2). CO_2 extraction, in contrast, yielded higher cannabinoid concentrations in oils but significantly lower total yield in oil (Tables 2-2 and Table 2-3). The cannabinoid ratio of CO_2 oil to ethanol oil by sample weight showed that CO_2 oils contained more cannabinoids than ethanol oils (averages ranging from 1.01-1.20 fold) which was indicative of an overall more cannabinoid rich or potent product.

Figure 2-4: Mean (SD) concentration of total THC and total CBD of 13 cannabis strains (**a**) Flower tincture (**b**) Ethanol oil and (**c**) CO_2 oil. THCA and CBDA concentrations were converted to THC and CBD equivalents using the described equation.

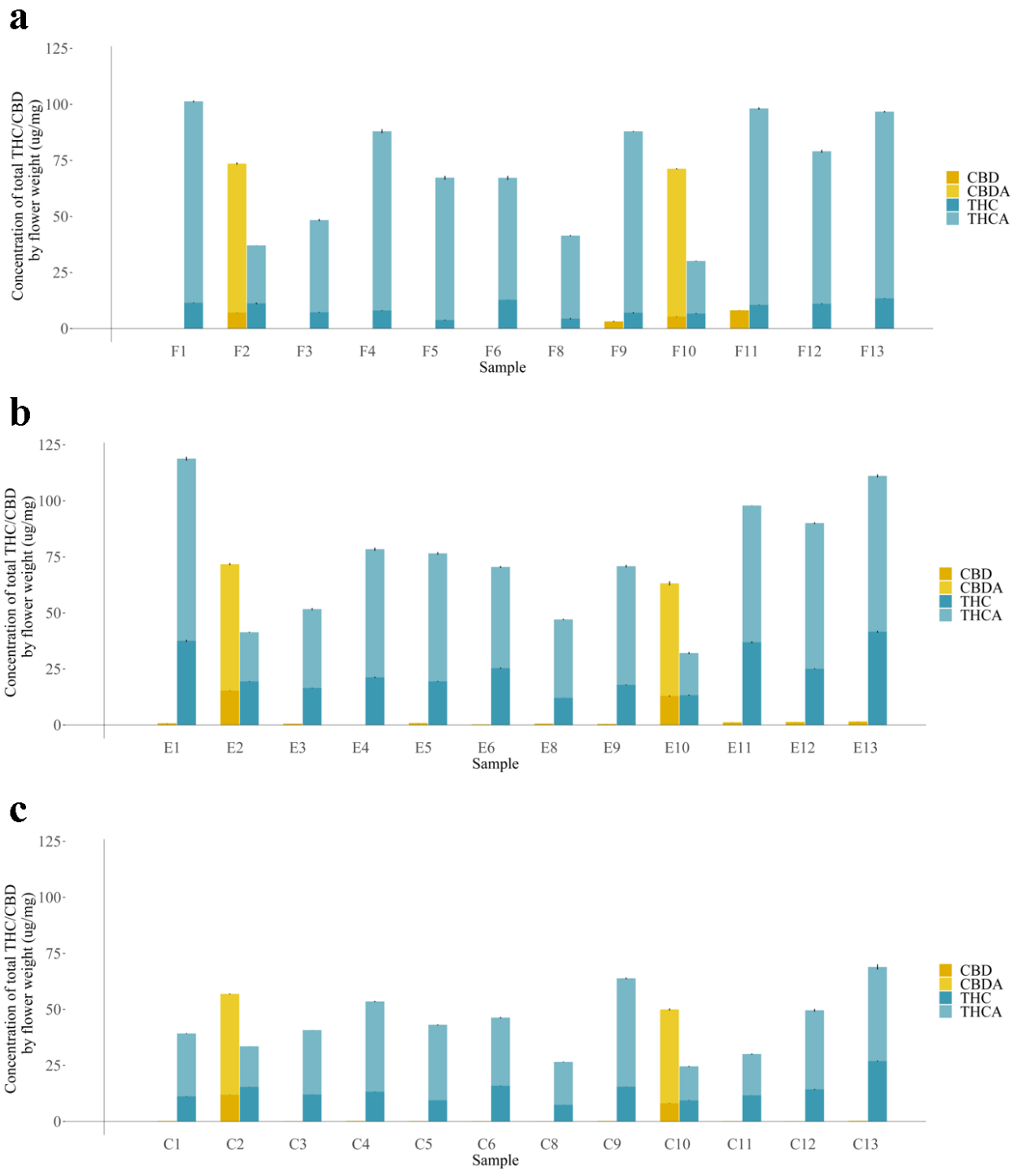


Table 2-1: Ratio of cannabinoid concentrations in ethanol oil compared to flower tincture. Calculated based on original flower weight to evaluate cannabinoids gained or lost in the drying process

Strain Number	CBDA	CBD	THCA	THC
1	-	-	0.90	3.27
2	0.85	2.16	0.85	1.73
3	-	-	0.85	1.73
4	-	-	0.85	2.28
5	-	-	0.72	2.64
6	-	-	0.90	5.13
8	-	-	0.83	1.98
9	-	-	0.65	2.54
10	0.76	2.43	0.80	2.00
11	-	-	0.70	3.51
12	-	-	0.96	2.27
13	-	-	0.83	3.09
Average	**0.81 ± 0.06**	**2.29 ± 0.19**	**0.82 ± 0.1**	**2.69 ± 0.92**

Table 2-2: Ratio of cannabinoid content in CO_2 oil compared to ethanol oil calculated by original flower weight and oil weight.

	By Flower Weight				By Oil Weight			
Strain Number	**CBDA**	**CBD**	**THCA**	**THC**	**CBDA**	**CBD**	**THCA**	**THC**
1	-	-	0.3	0.3	-	-	1.1	0.9
2	0.8	0.8	0.8	0.8	1.3	1.3	1.4	1.3
3	-	-	0.8	0.7	-	-	1.3	1.1
4	-	-	0.7	0.6	-	-	1.4	1.3
5	-	-	0.6	0.5	-	-	1.3	1.1
6	-	-	0.7	0.6	-	-	1.4	1.3
8	-	-	0.5	0.6	-	-	1.1	1.2
9	-	-	0.9	0.9	-	-	1.3	1.3
10	0.8	0.6	0.8	0.7	1.0	0.7	0.9	0.8
11	-	-	0.3	0.3	-	-	1.1	1.1
12	-	-	0.5	0.6	-	-	0.9	0.9
13	-	-	0.6	0.6	-	-	1.3	1.4
Average	**0.82 ± 0.03**	**0.70 ± 0.11**	**0.64 ± 0.19**	**0.61 ± 0.17**	**1.15 ± 0.27**	**1.01 ± 0.42**	**1.20 ± 0.19**	**1.14 ± 0.18**

* ND represents not detected

Table 2-3: Percent yield of cannabis oils produced by ethanol extraction and CO_2 supercritical extraction with standard deviation. The data were shown to be normally distributed by Shapiro-Wilk's test (p-value > 0.05) and the means were significantly different by unpaired t-test (p-value < 0.05).

Strain Number	Ethanol Percent Yield (%)	CO_2 Percent Yield (%)
1	29.0	9.3
2	25.3	15.1
3	16.6	10.7
4	25.4	12.6
5	24.5	10.9
6	21.3	10.4
8	14.8	7.7
9	23.2	15.9
10	22.5	19.7
11	26.3	7.5
12	24.0	14.7
13	27.1	12.5
Average	**23.7 ± 4.2%**	**11.4 ± 4.7%**

The phytochemical variance between samples based on terpene content was evaluated using PCA on terpene quantification data (Figure 2-5). Evaluating the scores plot (Figure 2-5a) suggested that more of the variance was attributed to differences across the 13 strains in tincture samples than the oil samples, and the loadings plot (Figure 2-5b) revealed that most terpenes had positive loadings along PC1. This suggested that terpenes were better conserved in tinctures than the oil products because more terpenes explained greater variance in tincture samples than oil samples. In addition, on average, the total monoterpene and sesquiterpene concentrations were higher in tincture samples than oil samples (Figure 2-6). Examining select monoterpenes and sesquiterpenes of tincture and ethanol oil revealed that drying under vacuum reduced certain

monoterpene concentration by over 80% and sesquiterpenes by 25-49% (Table 2-4). PCA also showed that CO_2 oil samples clustered into groups where a higher concentration of geraniol, terpinolene, L-(-)-fenchone and limonene were observed in select strains (Figure 2-5), but the same clustering of strains was not observed in ethanol oils or tinctures. This was confirmed when evaluating the terpene quantification results (data not shown).

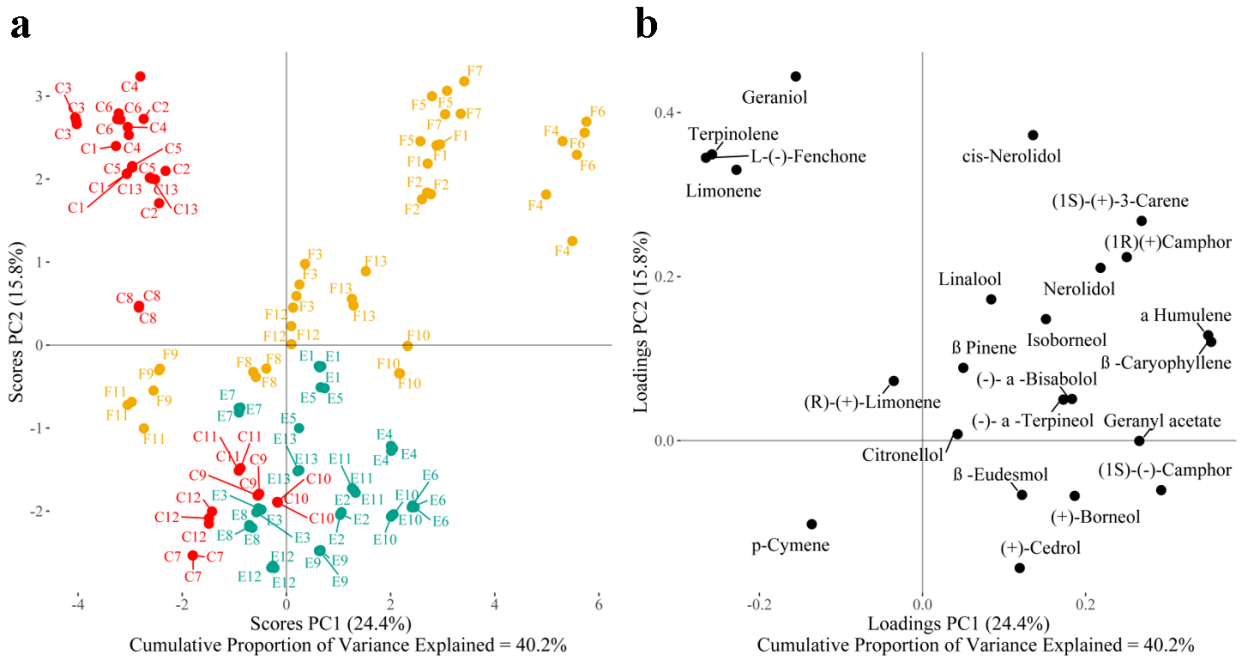


Figure 2-5: PCA on terpene quantification data of all 13 samples from flower tinctures, ethanol oils and CO_2 oils represented as F, E and C, respectively, visualizing terpene diversity between samples.(**a**) scores plot (**b**) loadings plot

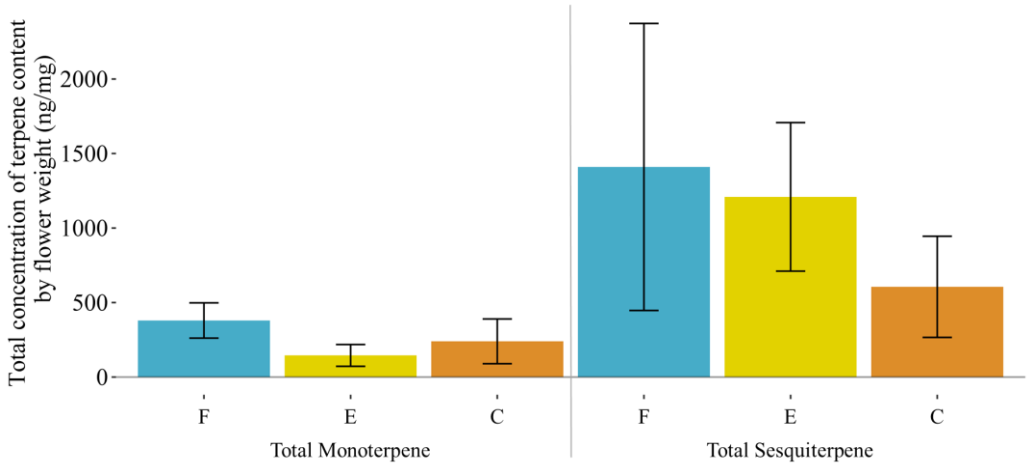


Figure 2-6: Mean (SD) of total monoterpene and total sesquiterpene in flower tincture, ethanol oil and CO_2 oil (n= 13).The data were shown to be not normally distributed by Shapiro-Wilk's test (p-value < 0.05) and Kruskal-Wallis test determined statistical different between the groups (p-value < 0.05). It was seen by Dunn test that the only statistically significance difference (p-value < 0.05) for total monoterpene concentration was between flower to ethanol oils, and no statistical significance was seen for total sesquiterpene concentration.

Table 2-4: Percentage of terpene concentration lost due to drying of select monoterpenes and sesquiterpenes. The data were shown to be not normally distributed by Shapiro-Wilk's test (p-value < 0.05) and Kruskal-Wallis test determined statistical different between the groups (p-value < 0.05). Dunn test determined statistical difference between the monoterpenes and sesquiterpenes (p-value < 0.05).

Strain number	Linalool	Geraniol	α Humulene	β-Caryophyllene	Nerolidol
1	58%	47%	37%	37%	28%
2	89%	93%	60%	61%	28%
3	96%	95%	43%	35%	19%
4	91%	85%	49%	47%	26%
5	98%	59%	53%	44%	50%
6	79%	96%	42%	39%	96%
8	94%	94%	42%	28%	92%
9	94%	96%	-	-	-
10	76%	95%	65%	58%	48%
11	66%	71%	49%	-	-
12	78%	95%	50%	41%	89%
13	60%	48%	49%	44%	12%
Average	**82 ± 14%**	**81 ± 19%**	**49± 8%**	**43± 10%**	**25± 4%**

2.4 Discussion

Evaluating phytochemical differences between cannabis strains can provide both qualitative and quantitative insight on the degree of similarity and variability across strains. In addition, comparing cannabis products can determine phytochemicals recovered or lost during processing. This was accomplished by comparing untargeted and targeted metabolomics data among 13 different strains of cannabis between their corresponding flower tinctures and ethanol

oils to explore changes associated with drying, and between ethanol and CO_2 oils to explore differences related to methods of oil production.

2.4.1 Variability between strains within each product type

Currently, consumers can select from hundreds if not thousands of strains with allegedly different pharmacological and organoleptic properties (Leafly, 2019). These strains may be distinguished based on numerous factors such as parent lineage, appearance and geographical origin. Mudge et al. (2018) stated that the term "strain" was limited as it is difficult to define and can be labelled based on unique phytochemical profiles or different growers (among other factors), and questioned whether there are more appropriate definitions for this term. Cannabinoids are major constituents found in cannabis as they can be upwards of 30% by weight (Swift et al., 2013). Accordingly, cannabis strains have been classified by many researchers based on cannabinoid content, particularly the relative concentration of THC(A) and CBD(A) (Mudge et al., 2018). The presented data support these previous classifications as the main variance seen between strains was due to CBD(A) concentration, therefore, the two "classifications" observed in the 13 strains were high and low CBD(A) samples. Furthermore, samples clustered based on this classification rather than three clusters by product type, which suggested that cannabinoid profiling could be applied across products and the strain could be recognized despite the extraction methodology. The oils showed some separation from flower samples (with the exception of samples F9 and F11) based on THC content. This demonstrated that the process of making oils, whether through drying or SFE CO_2 extraction, decarboxylates cannabinoids to increase relative THC/THCA (and CBD/CBDA) content. Still, other phytochemicals, or non-cannabinoids, show some contribution to the variability of the samples, but not as notably as THCA and CBDA. Sequential PCs mainly

explained the variance across 13 strains in flower tinctures, and clustering of oils was observed (Appendix A, Figure A-4). This further suggested that variability in tinctures was better conserved than in oils.

Distinguishing cannabis strains based on cannabinoid content is limited as there exist significantly more "strains" than classifications. Some researchers have suggested that terpene profiling along with cannabinoid content is the best way to fingerprint and distinguish between different cannabis strains (Fischedick et al., 2010). While the number of tested strains was insufficient to detect clusters based on terpene content, PCA of terpene data across samples failed to reveal any consistent strain groupings between tinctures or either type of oils. In addition, our data demonstrated that not all terpenes were lost at the same rate, but they were volatile, which would complicate strain profiling. It may be premature to categorize distinct chemotypes at this point with a small sample of products, but the phytochemical differences observed here merits a larger study.

The data suggest that tinctures, or alcoholic extracts, would be best out of the extraction methods evaluated for the purposes of profiling and distinguishing cannabis stains since a higher degree of diversity was observed across 13 strains in untargeted and targeted data. In addition, intra-strain chemical diversity decreases with oil production, particularly SFE CO_2; this is counterintuitive given that the CO_2 oils were the most chemically complex cannabis products in the study. A study suggested that their CO_2-SFE cannabis extract contained cuticular waxes (Da Porto et al., 2014), thus the compounds uniquely detected in CO_2 oils may be lipophilic, primary metabolites and more conserved across strains. Some commercial operations use a winterization or dewaxing process (Raber et al. 2015) which is performed by chilling the vessel containing the plant material or re-dissolving the extract in cold ethanol to remove the fats and

waxes resulting in a more cannabinoid potent oil. Adding a winterization step in production of CO_2 oils may result in a qualitatively similar product to ethanol oils, but further studies are required to determine this.

2.4.2 Impacts of drying on phytochemistry

The process of producing oils typically involves a critical drying step where the solvent is removed from the cannabis product. A previous study determined that volatile compounds in cannabis were susceptible to loss during air drying where greater loss of monoterpenes were observed than sesquiterpenes (Rossi & ElSohly, 1996). This in accordance with the presented results, where overall terpene concentration decreased with more monoterpenes lost than sesquiterpenes. In addition, there were less overall compounds detected in ethanol oil samples than tincture samples. Variance between strain samples was also lost, which suggested that the phytochemicals contributing to strain differences were phytochemicals susceptible to loss with drying, and there were few signals observed in the ethanol oil that were not present in the tincture revealing that that the drying process resulted in very limited new chemistry. Furthermore, it was seen that the drying process decarboxylated the acid-cannabinoids; however, many companies heat-activate their plant material or oil prior to sale (Aurora, 2019) so this may not be a major concern to producers. Previous research on stability of acidic and neutral cannabinoids revealed that acidic species are more susceptible to degradation and, with proper storage conditions (-20ºC without light exposure), they determined THC concentration to be relatively stable (Lindholst, 2010).

2.4.3 Comparison of ethanol oils and CO_2 oils

Cannabis oils, concentrated products derived from cannabis flower, are popular among consumers, particularly medical users, as oil sales for medical purposes exceeded those for dried flowers in Canada in 2018 (Health Canada, 2019). Two popular methods of oil production include ethanol extraction and supercritical fluid extraction (SFE) (Ramirez et al., 2018).

In terms of extraction efficiency, ethanol extraction should be preferred as it yielded almost double the amount of oil by weight plant material, and more relative cannabinoids by flower weight. Similar to ethanol oils, the variability of the 13 samples in CO_2 oils was mainly attributed to cannabinoids instead of other phytochemicals; however, a larger number of phytochemicals were detected in CO_2 oil samples. This was hypothesized to be due to the presence and extraction of primary metabolites such as structural lipids rather than secondary metabolites, which are typically attributed to sample variance. Due to the lower polarity and other properties associated with supercritical fluid extraction using CO_2, it is likely CO_2-SFE extracted different compounds to ethanol. This was in accordance with Da Porto et al. (2014) who found cuticular waxes in their CO_2-SFE cannabis extract. In addition, since previous research observed that CO_2-SFE did not replicate flavor and fragrance of the original cannabis flower sample as cannabinoid and terpene content was different (Sexton et al., 2018), it was unsurprising that phytochemistry profiles differed between the extraction methods.

The data also suggested that CO_2 oil contained, on average, more total monoterpenes but less total sesquiterpenes than ethanol oils which could be attributed to a shorter drying process involved with producing CO_2 oils. It should also be noted that some industry practice involves additional solvents for SFE resulting in no drying step. It was suggested that terpenes should be maximally soluble at lower pressure (74 bar) in CO_2-SFE (Da Porto et al., 2014). As the CO_2 oil

extraction method included a single pressure and temperature (above the supercritical point for CO_2), it was not necessarily selective for these phytochemicals. Still, concentration of geraniol, terpinolene, L-(-)-fenchone and limonene was higher in CO_2 oil samples than ethanol oil samples which suggests that these compounds may be better extracted in the CO_2-SFE conditions.

2.5 Conclusions

This study was a novel approach to understand the phytochemical differences between "strains" and extraction methods. All thirteen strains of cannabis samples evaluated shared some phytochemicals (11%, 45% and 65% for tincture, ethanol oil and CO_2 oil, respectively), but phytochemical diversity was observed between different strains which further supports the need for additional research into the classification of "strains". Still, it demonstrated that an untargeted approach provided a finer lens than previous studies that only evaluated cannabinoids and terpenes for detecting differences and similarities. In addition, different cannabis products showed chemical differences within strains. The effects of drying on production of oils showed a reduction of phytochemicals but little new chemical changes. Furthermore, comparison between two different oil products demonstrated they yield quantitative and qualitative chemical differences. Based on observed sample variance, consumers can expect different strains and products will have different chemical profiles; however, consumers can expect CO_2 oils to be more chemically consistent across products than tinctures. Whether this variability will impact bioactivity and consumer experiences requires more study, but the multivariate analyses demonstrated that above all other variables, THC(A) and CBD(A) were the most influential factors. This supports the current focus on these cannabinoids by Health Canada and policy makers.

2.6 Acknowledgements

HRMS data collection was performed by Amanda Sproule from the Overy Lab. All 13 strains of CO_2 oils were extracted by Lyric Moses from the Harris Lab.

Chapter 3: Evaluation of phytochemistry changes associated with vaporization

3.1 Introduction

Cannabis flowers have long been used for recreational and medicinal purposes, eliciting a range of pharmacological and therapeutic responses that are mainly attributed to cannabinoids. In the plant, cannabinoids are synthesized as molecules containing carboxylic acid groups and heat is required for decarboxylation to their neutral forms, which yield the desired bioactivity (Dussy et al., 2005). For example, the acid-form cannabinoid Δ9-tetrahydrocannabinolic acid (THCA) produces the neutral cannabinoid Δ9-tetrahydrocannabinol (THC) with the addition of heat (Figure 3-1). This thermal conversion is a process common to all acid-form cannabinoids and essential to produce the typical psychoactive properties or medicinal benefits associated with cannabis.

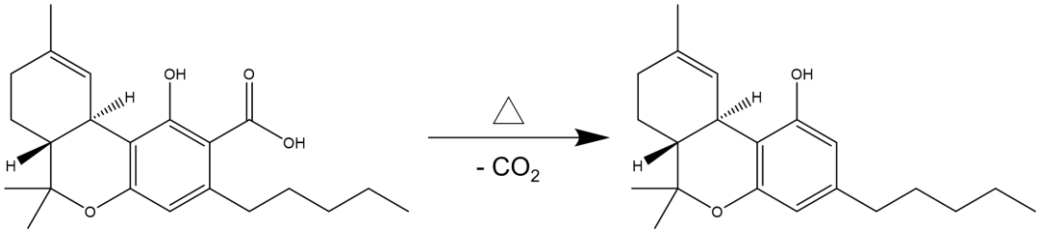


Figure 3-1: Chemical structures for the decarboxylation reaction of THCA

A Canadian survey conducted in 2015 reported that vaporization was the most popular mode of delivery for medical cannabis among patients, followed by smoking (Shiplo et al., 2016). It was reported that vaporizing tasted better, had less smoke smell, and was more discreet. Vaporization is a method of ingestion that extracts active principles of cannabis with heated gas and reduces pyrolytic by-products. Some research suggests this combustion-free mode of delivery can prevent some of the negative respiratory health effects associated with smoking as there were

no traces of ash or visible smoke, and suppression of benzene, toluene and naphthalene formation (Gieringer, 2001). In addition, an *in vitro* validation study revealed that electrical vaporizers showed almost complete decarboxylation of THCA leading to high recoveries of THC in the vapor without indications of combustion by-products (Lanz et al., 2016).

Vaporizers are considered a harm reduction alternative and promoted over smoking. The Volcano Medic vaporizer (Storz & Bickel) is a Health Canada approved medical device (Government of Canada, 2010), and used by many Canadians to consume medical cannabis. The Volcano utilises convection by passing heated air through the cannabis products to extract active and non-active compounds into vapor. A study comparing cannabis cigarette smoke and the Volcano vaporizer concluded that the vapor contained fewer and lower concentration of by-products (Pomahacova et al., 2009). In addition, higher temperatures were found to be associated with an increase in amount of desired cannabinoids released and were able to provide double the quantity of THC concentration by plant weight compared to cigarette smoke, without compromising quality. It must be noted, however, that the recovery of cannabinoids and the rate of conversion of THCA to THC through heat is variable and not 100% efficient. A study evaluating vaporizer devices demonstrated that the decarboxylation efficiencies of the Volcano Medic for THC and CBD were 97.3 and 94.6%, respectively, in the vapor with recovery of total THC and CBD relative to the pre-vaporized cannabis to be 58.4 and 51.4% (Lanz et al., 2016).

There is a lack of related research concerning this mode of consuming cannabis and there is a high potential that vapor can have a different chemical composition than what is found in the plant prior to vaporization. First, the process of vaporization can induce phytochemical transformations. This was already evident for cannabinoids where the acid-cannabinoids were found to be decarboxylated to form neutral-cannabinoids with heat; however, there may be other

heat-induced reactions that have yet to be documented. Second, vaporization can potentially selectively extract specific compounds since phytochemical extraction/vaporization is temperature dependent. Therefore, more information is needed concerning the quantitative and qualitative phytochemical changes associated with vaporization since the product label may not be representative of what is phytochemically available for consumption in vapor. Studies to date have looked at cannabinoids, specific smoke-related toxic compounds, or generalized by-products, but this research will evaluate the cannabinoids, terpenes and overall complexity of vapors: all of which impact consumer experience and, potentially, health and safety. In addition, previous studies on vaporization have focused on flower material, but this study was the first to evaluate chemistry of vapors in oils.

This chapter of the thesis investigated the phytochemical changes associated with vaporization using both untargeted and targeted metabolomic analytical approaches. In addition, the study evaluated differences in vaporization between three cannabis product types (flower, ethanol oil and CO_2 oil), which were previously described in Chapter 2.

3.2 Experimental methods

The description of the thirteen different strains of cannabis as well as the preparation of the three product types (pre-vapor samples) were described in Chapter 2. The flower tincture, ethanol oil and CO_2 oil previously described represent the pre-vapor samples of flower, ethanol oil and CO_2 oil, respectively. For each strain, approximately 5 g of dried inflorescence was hand ground using a herb mill (Storz & Bickel) until homogenous (particle diameter 2.00 mm) and stored in the dark at room temperature in a desiccator until vaporization trials.

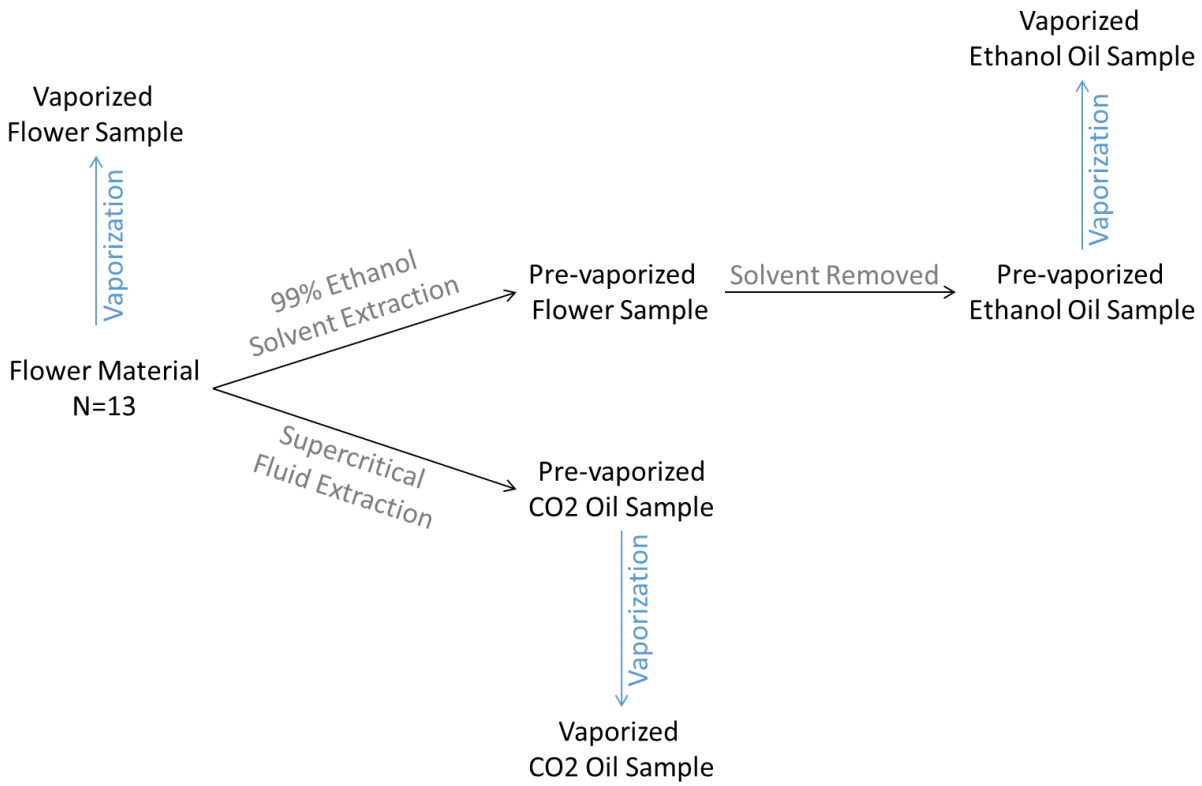


Figure 3-2: Methods visualization of preparation of non-vaporized and vaporized samples

3.2.1 Vaporization method

The samples were vaporized in triplicate using the Volcano© (Storz & Bickel). The system was heated to 210°C and the ground flower (250 mg) and oils (25 mg) were placed on the mesh screen and liquid pad, respectively, in the filling chamber. One balloon with mouthpiece (volume 6L determined by Pomahacova et al., 2009) was filled and the vapor was passed through a glass tube under vacuum leading to a porous glass frit immersed in 99% ethanol where the vapor condensed and solubilised into the solvent (Figure 3-3). The trap system was sonicated for 15 minutes and the solution was made up to 25 mL in volumetric flasks. In addition, four blanks were collected in 25 mL 99% ethanol from the vapor trap system: i) passing only air through the system without the vaporizer; ii) passing a bag filled with heated air from the vaporizer through

an empty filling chamber; iii) passing a bag filled with heated air from the vaporizer fitted with a screen in the filling chamber; and iv) passing a bag filled with heated air from the vaporizer fitted with a pad in the filling chamber. All extracts were stored at -20°C until analysis.

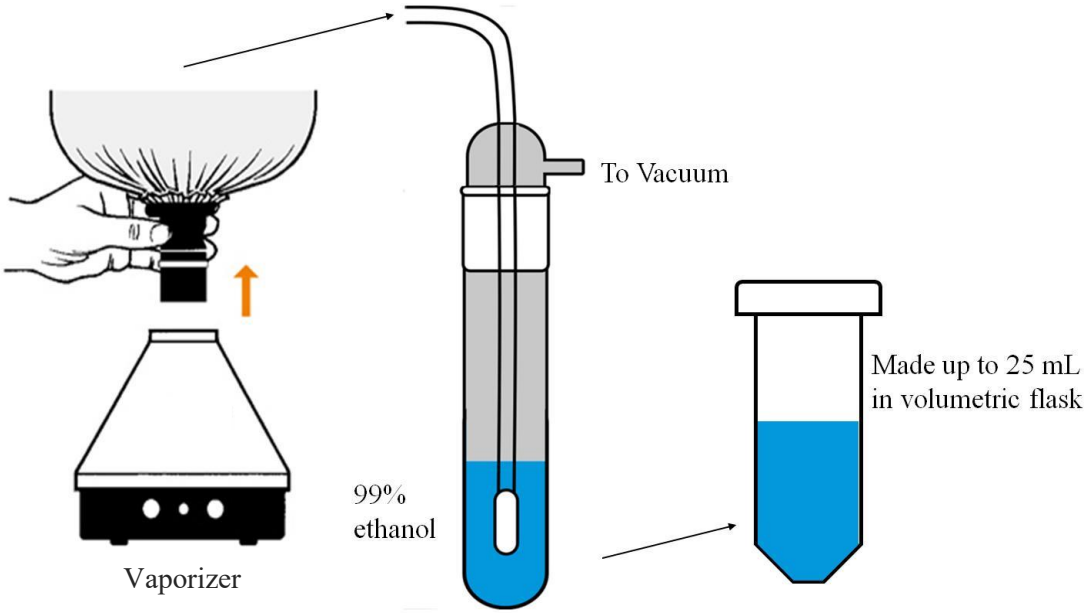


Figure 3-3: The system of capturing vapor collected by the Volcano balloon

The described vaporization and trap system parameters were optimized by vaporizing ground cannabis samples once (1 balloon), twice (2 balloons), and three times (3 balloons) and passing the collected vapor through single, double, and triple trap systems in series. Over 90% of total recovered THC was captured by passing the first balloon through the first trap system (Appendix C, Figure C-1).

3.2.2 Sample preparation and data processing for untargeted metabolomics data

The pre-vapor samples were diluted to 100 µg/mL by sample weight in 99% ethanol while the vapor samples were diluted to 500 µg/mL by sample weight in 99% ethanol. All samples were

filtered through a 0.22 μm PTFE syringe filter then transferred into a HPLC vial and stored at -20°C until analysis. The parameters of the untargeted metabolomics data acquisition were described in Chapter 2. Pre-vapor samples were processed in triplicate to yield three replicates and vapor samples were processed in duplicate to yield six replicates. The untargeted metabolomics data were pre-processed as described in Chapter 2. In addition, to account for the potential metabolites associated with the vapor trap system, the 4 previously described vapor trap system blanks were accordingly removed from the aligned vapor sample data.

3.2.3 Cannabinoid and terpene quantification

All pre-vapor and vapor samples were made up to 1 mg/mL by sample weight in 99% ethanol. Cannabinoid concentration and terpene concentration were determined by the quantification methods described in Chapter 2.

3.3 Results

3.3.1 Untargeted metabolomics: Phytochemistry differences with vaporization

The phytochemical differences associated with vaporization in three product types (flower, ethanol oil and CO_2 oil) derived from thirteen different cannabis strains were evaluated using untargeted metabolomics data.

Overall, Venn diagrams of total discrete ions (RT; *m/z*) found in vapor for all products demonstrated that vaporization produced few new compounds, where 0.8%-8.5% new ions were detected in the vapor samples (Figure 3-4). These new ions were not found in all 13 strains, which indicated that they may be dependent on which strains were vaporized. In flower samples, approximately half of the phytochemicals were shared among pre-vapor and vapor samples with

51.9% total detected ions. In addition to the higher degree of shared ions among flower and flower vapor samples, flower vapor samples had the greatest chemical complexity and yielded the most new chemistry (Figure 3-4d). Conversely, in the ethanol and CO_2 oil samples, the majority of the ions were found in the pre-vapor samples and a lower number (36%) of discrete ions were shared among non-vaporized and vapor samples. This is further illustrated in Table 3-1, where 133 ions were detected in all 13 strains of vaporized flower samples while only 58 and 61 were detected in all 13 strains of vaporized ethanol oil and CO_2 oil, respectively. In terms of number of ions detected, vaporized ethanol oil and CO_2 oil showed similar results. Statistical tests (unpaired t-test or Mann-Whitney-Wilcoxon test) between the pre-vaped and vaporized samples for flower, ethanol oil and CO_2 oil determined significant differences when evaluating the number of total discrete ions detected.

Figure 3-4: Venn diagrams of detected discrete ions from untargeted metabolomics experiment. Each value represents an ion above 10 signal-to-noise that was detected in at least one of the thirteen samples where the ion was deemed present if detected in at least two out of three replicates in pre-vapor samples and present in at least four out of six replicates in vapor samples. (**a**) A Venn diagram comparing pre-vapor and vapor flower samples (**b** and **c** show the same comparison of ethanol oil and CO_2 oil, respectively) (**d**) A Venn diagram comparing the vapor samples of Flower, Ethanol oil and CO_2 oil.

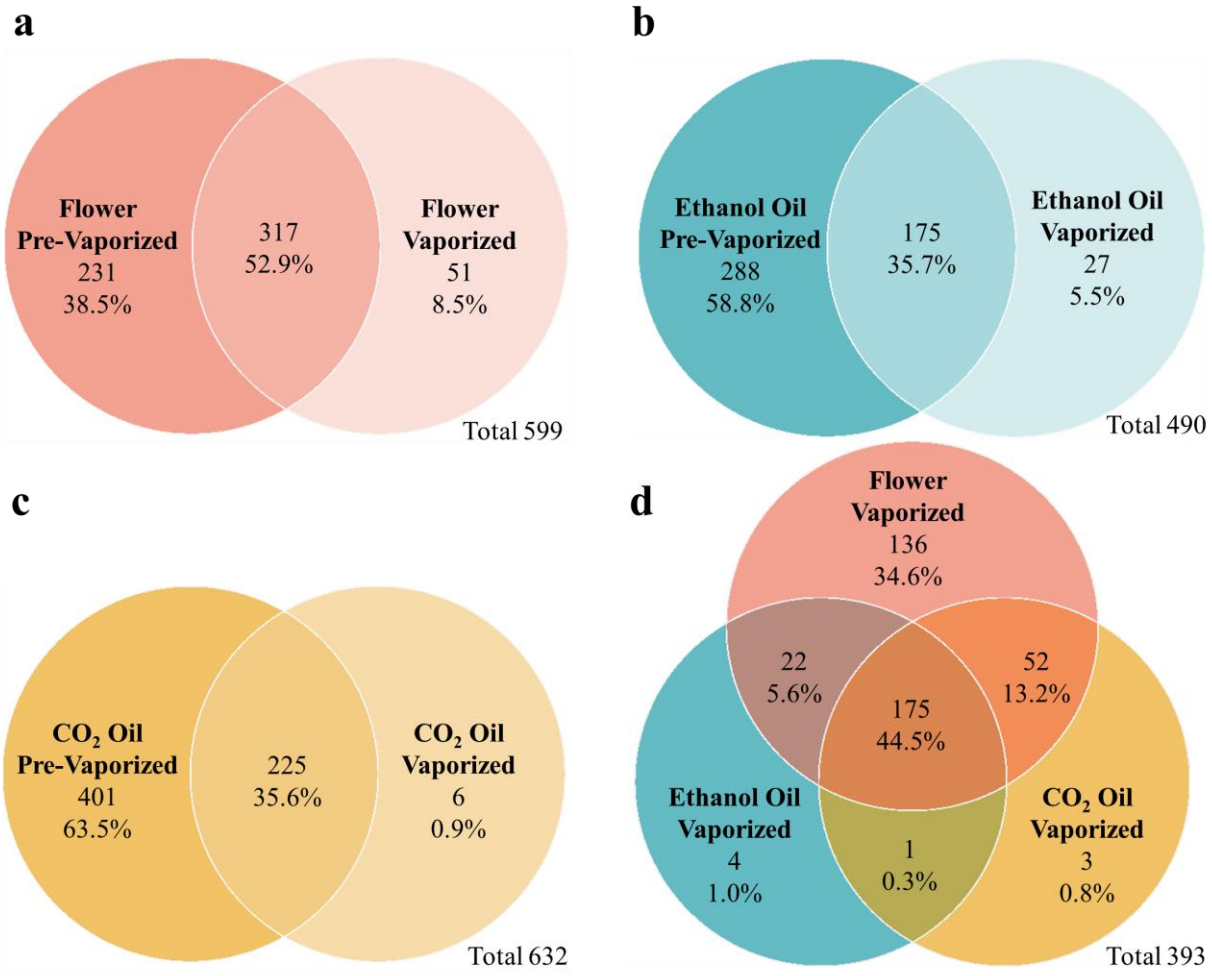
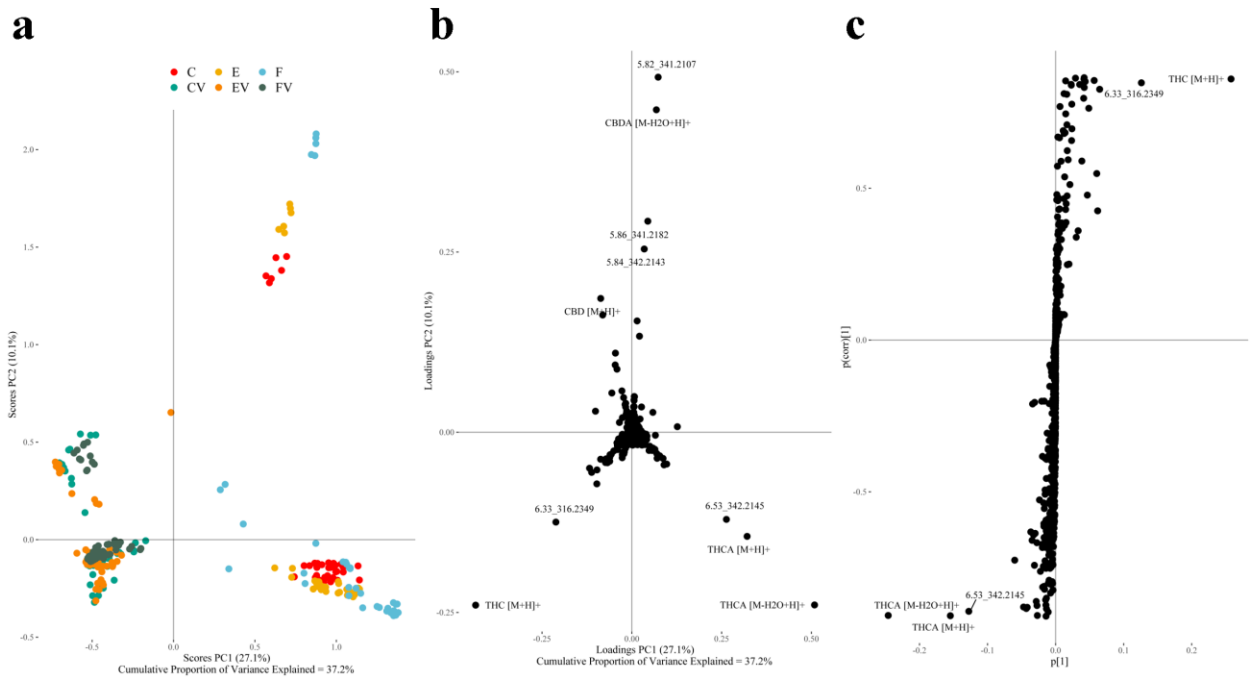


Table 3-1: A binary count of discrete ions shared among thirteen different strains within each vaporized sample type. The number of ions detected in at least one strain, in at least 7 out of 13 strains, and 13 out of 13 strains were reported. Percentages expressed relative to total detected per each product type.

	Flower Vapor	**Ethanol Oil Vapor**	**CO_2 Oil Vapor**
Total number of ions detected	393		
Ions found in at least 1 strain	368	202	231
Ions found in at least 7 strains	224 (58.2%)	106 (52.5%)	90 (39.0%)
Ions found in all strains	133 (34.5%)	58 (20.7%)	61 (26.4%)

Chapter 2 revealed that samples clustered based on THC(A) and CBD(A) concentration rather than by product type, so all samples were analyzed together. OPLS-DA, a supervised multivariate statistical method, was used to examine phytochemicals that showed differences between pre-vapor and vapor samples for flower, ethanol oil and CO_2 oil (Figure 3-5). The corresponding PCA showed that pre-vapor and vapor samples separated along PC1 with 27.1% variance explained and CBD(A) containing samples separated along PC2 with 10.1% variance explained. Only PC1 and PC2 were presented as the point of separation as defined by the respective scree plot (Figure A-3b) determined PC1 described majority of the variance observed. The PCA loadings plot and S-plot both suggested that the main variables discriminating between pre and post vaporized samples were THCA and THC, respectively, due to their high concentration levels. All significant ions above 10 signal-to-noise were reported even if not identified to chemical structure.

Figure 3-5: OPLS-DA analysis comparing variability between pre-vaporized and vaporized samples of untargeted metabolomics data of Flower (F: blue, FV: navy), Ethanol oil (E: yellow, EV: orange) and CO_2 oil (C: red, CV: turquoise) showing a (**a**) PCA scores plot (**b**) PCA loadings plot and (**c**) S-plot.



3.3.2 Targeted metabolomics: Cannabinoid and terpene concentration changes after vaporization

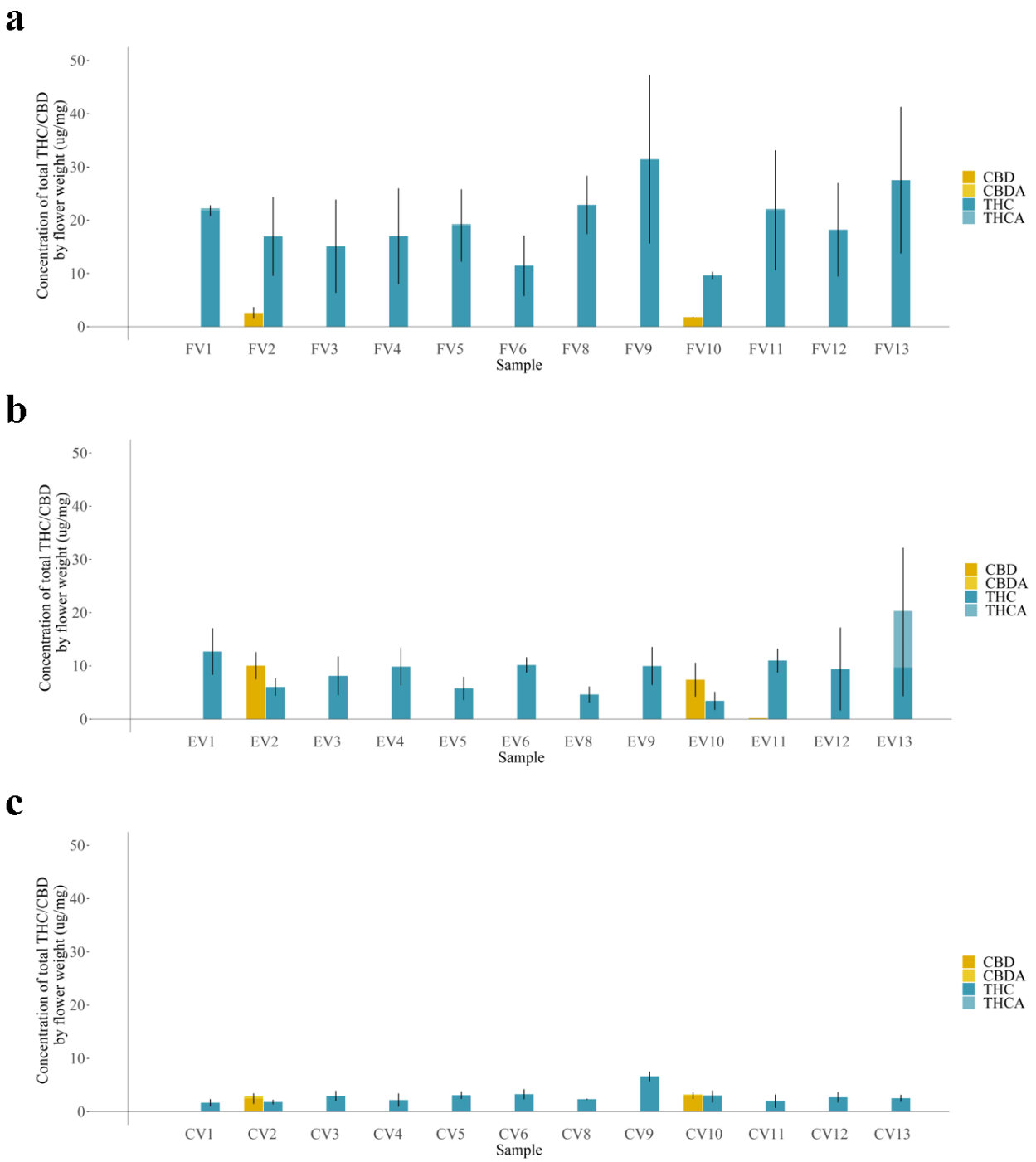
Four major cannabinoids, CBDA, CBD, THCA and THC, were quantified as they were the main contributors of variance among samples and the primary cannabinoids of interest to consumers, practitioners, and regulators. It was clear that the process of vaporization decarboxylated the phytocannabinoid acids since the THCA and CBDA content of pre-vaped samples was reduced to trace levels in vapor with corresponding increase in THC and CBD (Figure 3-6). In addition, the ratio of THCA/THC between pre-vaped and vaporized samples were found to be statistically significant (paired t-test, p-value < 0.05). The intra-sample variation (SD, error

bars in Figure 3-6) could be explained by the limits of vapor capture and temperature regulation by the cold-trap system and/or the density and surface area of the vaporized substance. Comparing relative concentration of THC quantified in vapor samples to total THC available in the pre-vapor samples revealed that at least 30% of available THC was activated and captured from flower samples while ethanol oil and CO_2 oil showed 12% and 8%, respectively (Table 3-2). Although quantitative, the results do not represent complete recovery as vaporization and collection of vapor were not exhaustive.

Table 3-2: Percentage of THC concentration by sample weight (µg/mg) captured in vapor following vaporization of flower, ethanol oil and CO_2 oil samples (n=3). The data were shown to be normally distributed by Shapiro-Wilk's test (p-value > 0.05) and one-way ANOVA determined statistical different between the groups (p-value < 0.05), and the difference between flower to both oils were significant (Tukey HSD, p-value < 0.05) while no statistical significance was determined between ethanol oil and CO_2 oil.

Strain Number	Flower	Ethanol Oil	CO_2 Oil
1	21%	11%	4%
2	46%	15%	5%
3	31%	16%	7%
4	19%	13%	4%
5	28%	8%	7%
6	17%	14%	7%
8	55%	10%	9%
9	36%	14%	10%
10	32%	11%	11%
11	22%	11%	6%
12	23%	10%	5%
13	28%	9%	4%
Average	**30 ± 11%**	**12 ± 2%**	**7 ± 2%**

Figure 3-6: Mean (SD) of total THC and total CBD content of 13 cannabis strains quantified based on µg cannabinoid by mg of original flower weight of (**a**) Vaporized flower tincture (**b**) Vaporized ethanol oil and (**c**) Vaporized CO_2 Oil.



The phytochemical variance between samples based on terpene content of pre-vapor and vapor samples were evaluated using PCA on terpene quantification data (Figure 3-7) to determine impact of product type relative to vaporization where vapor samples were projected onto the PCA of pre-vapor samples to compare to clustering observed in the initial PCA. The sample clustering and separation seen in pre-vapor samples (Figure 3-7a) was not observed in vapor samples (Figure 3-7b). Clusters previously observed such as CO_2 oil (samples 1, 2, 3, 4, 5, 6, 13) were weakened after vaporization.

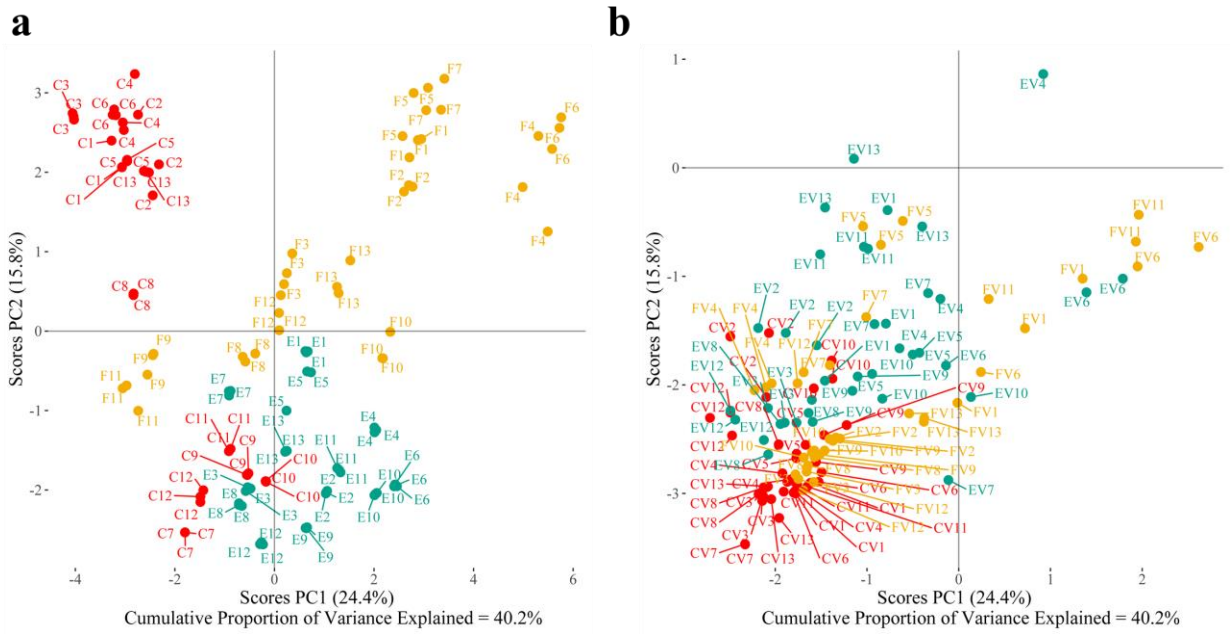


Figure 3-7: Principal component analysis (PCA) of terpene quantification data(**a**) PCA scores plot of pre-vapor samples (**b**) Projection of vapor samples onto PCA of pre-vapor samples

3.4 Discussion

3.4.1 Effect of vaporization

The phytochemical changes associated with vaporization were explored by evaluating untargeted and targeted metabolomics data. Vaporization produced a small number of new compounds and the number of detected discrete ions in vapor samples were diminished compared to pre-vapor samples, suggesting that all phytochemicals present in the flower or oil were not extracted through vaporization (at detectable levels). The results support Gieringer (2001) who proposed that vaporization was a promising alternative for smoke harm reduction; however, further research into identifying compounds uniquely found in vapor is needed to determine whether these compounds are harmful or of negligible risk to human health. In addition, vaporizing cannabis extracts like the oils may be preferred over ground flower material as it produced overall fewer new compounds and also released cannabinoids into vapor more slowly, but further study is required to determine if repeated vaporization of sequential balloons will result in greater cumulative recovery. It should be noted that the results are not reflective of cannabis oils currently legalized and available in Canada as they did not contain a plant-based oil or medium chain triglycerides. Moreover, medium chain triglycerides and other thinning agents or "carriers" (e.g. propylene glycol, vegetable glycerin, polyethylene glycol 400), which are common additives to vape pens and e-cigarettes, were found by Troutt & DiDontato (2017) to produce potentially dangerous carbonyl compounds such as acetylaldehyde and formaldehyde. In addition, these additives will become options for vape pens and concentrates with the coming amendment. Cannabis oils prepared with additives may produce a larger number of chemicals than the pure cannabis oil extracts evaluated here.

Supervised analysis of untargeted data showed that the cannabinoids were the major factor contributing to their phytochemical differences in all three products, which was previously seen in Chapter 2. The increased concentration of neutral cannabinoids such as THC through the decarboxylation of THCA was observed to drive the difference between pre and post-vaporized cannabis. This was confirmed based on cannabinoid quantitative data where vapor samples had a relatively larger concentration of decarboxylated cannabinoids than acid cannabinoids. Previous research evaluating cannabis vapor using the Volcano determined the total THC recovery relative to the pre-vaporized flower cannabis to be 58.4% (Lanz et al., 2016) but recovery in this experiment was seen to be 30 ± 11%. The discrepancy can be attributed to methodology where Lanz et al. vaporized 50 mg of cannabis ground in a mortar and aspirated the Volcano balloon through a SPE column (Polypropylene cartridges, Chromabond, 15 mL). A smaller amount of finer ground cannabis flower in the vaporizing chamber likely increased the surface area resulting in higher extraction efficiency. It was proposed by Pomahacova et al. (2009) that amount of the material would strongly influence extraction factors such as temperature distribution, contact surface and the kinetics of air that pass through the material. This effect may be observed here where a larger quantity of cannabis flower was vaporized, and a herb mill that is typically supplied along with the Volcano was used to grind the material. Since Pomahacova et al. also showed that THC levels in vaporized cannabis (between 50 mg to 500 mg) collected in one balloon were relatively constant, the THC recovery in cannabis ground by the herb mill (which is likely more used by consumers) may be more reflective of the results presented in this thesis.

3.4.2 Comparison of vaporization of different products

Evaluating strain differences and effect on diversity due to vaporization showed <35% of total chemistry would be consistent across the vapors of different strains. The results suggest that product choice will impact vapor chemistry considerably and consumers can expect different chemical content based on strain. However, quantification of terpenes, a class of compounds that have been attributed to influence strain variability (Fischedick et al. 2010), suggested that vapor samples showed more inter-strain and inter-product similarity than pre-vapor samples.

The majority of the detected ions in vaporized samples were found in flower vapor samples. This may suggest that phytochemicals that could be lost to drying, such as volatile terpenes, were lost in the processing of the oils, as observed in Chapter 2, leading to more volatile compounds appearing in flower vapor samples and less in oil vapor samples.

Comparing concentration of THC quantified in vaporized samples to total THC available in the pre-vaporized samples revealed that more THC became available when vaporizing flower than ethanol and CO_2 oils. The results may suggest that vaporization could extract phytochemicals differently from oils than from the ground flower material due to their matrix or consistency. Flower material was hand ground where trichomes were easily exposed to hot air stream which could circulate evenly through the lightly packed material. This was clearly observed where the vaporized flower material was evenly brown in colour compared to the original green-coloured material. Conversely, oils were denser, so air may only flow through and vaporize chemicals where the oil was “thinnest” resulting in more phytochemicals staying on the oil pad. Visually, after vaporization, the oils appeared to be absorbed by the pad where a much thinner oil layer was seen on the top of the pad. It is likely that more THC and CBD were left in the oil pad than the plant material. The low levels in oil vapor may reflect slower vaporization (since method was not

optimized to oil) where greater amounts would be recovered with subsequent balloons. An optimization experiment on vaporization and trap system parameters revealed that more phytochemicals (annotated ions) in flower material were detected with more balloons (Appendix C, Figure C-2), which could also be interpreted as a requirement for longer heating of cannabis oils to release compounds into vapor. Similarly, it may just take longer for phytochemicals in oils to be heated compared to flower material. Further studies need to be performed to conclude whether flower material is a more effective product for extraction of THC and CBD by vaporization than oils.

The similar number of compounds detected in the ethanol oil and CO_2 oil vapor samples suggested that the vapors were qualitatively chemically similar. Although the CO_2 pre-vapor samples were more complex to begin with, some of the compounds may not vaporize well making CO_2 oil vapor more similar to ethanol oil vapor. The phytochemicals detected in the CO_2 pre-vape samples may be waxy materials. The inclusion of these compounds in the oil does not appear to result in their appearance in the vapor but may have an effect on vaporization of cannabinoids as less THC was detected compared to ethanol oils. Many cannabis producers include a winterization step that removes wax esters, glycerides, unsaturated fatty acids (Ramirez et al. 2018; Bjorncrantz, 2015) therefore CO_2 oils processed with a winterization step may show similar results to ethanol oils. A study evaluating the factors that contribute to variability in nicotine delivery from e-cigarettes determined a positive correlation in nicotine concentration in aerosol with characteristics of the e-liquid such as base propylene glycol/ vegetable ratio and flavour (El-Hellani et al., 2018). This hints that even oils could vaporize differently based on components of the oil matrix, but further experiments are required to confirm this.

3.5 Conclusions

The results explored potential phytochemical changes associated with cannabis vaporization of three different cannabis products. It demonstrated that vaporization reduces inter-strain and inter-product chemical diversity, but the content of the vapor can still be largely affected by the strain used. In addition, vaporization could extract phytochemicals differently from oils than from the ground flower material due to their matrix or consistency resulting in different phytochemical profiles. Importantly, very few new compounds were produced via vaporization, supporting its use as a harm reduction method to consume cannabis. Supervised analysis revealed that decarboxylation of cannabinoids, mainly THCA, separated between pre and post-vaporized samples. The results also agreed with the proposition that recovery of cannabinoids through vaporization may be affected by the amount of material as well as the physicochemical properties, whether using flower or oil products. More research is required to determine whether and to what degree the observed variability in vapor chemical impacts bioactivity and consumer experience.

3.6 Acknowledgements

HRMS data collection was performed by Amanda Sproule from the Overy Lab. All 13 strains of CO_2 oils were extracted by Lyric Moses from the Harris Lab.

Chapter 4: General Discussion

4.1 Evaluation of hypotheses

The results obtained from these experiments validated the four hypotheses driving this research. The first hypothesis predicted that different strains will have different chemical profiles, so the phytochemistry profiles of thirteen cannabis strains were first evaluated. The untargeted metabolomics results presented in Chapter 2 demonstrated that all thirteen strains of cannabis samples evaluated shared some phytochemicals (11%, 45% and 65% for tincture, ethanol oil and CO_2 oil, respectively), with some observed variance within and between samples; however, the differences in phytochemistry content among product types (tincture, ethanol oil, CO_2 oil) did not strongly contribute to the overall variance between samples due to the high concentration of cannabinoids, namely THCA and CBDA. The overwhelming difference observed between the thirteen strains was determined by CBDA concentration, where strains would cluster based on low and high CBDA content. This was previously proposed where cannabis strains have been classified by based on cannabinoid content, particularly the relative concentration of THC(A) and CBD(A) (Mudge et al., 2018). Furthermore, some researchers have suggested that terpene profiling along with cannabinoid content is the best way to fingerprint and distinguish between different cannabis strains (Fischedick et al., 2010). Despite this, distinguishing cannabis strains based on cannabinoid content was limited as there were significantly more "strains" than classifications, and the results suggested that not all terpenes were lost at the same rate, but they were volatile, which would complicate strain profiling. In addition, the terpene data across samples failed to reveal any consistent strain groupings between tinctures or either type of oils. This suggests that further research is required to fully compare among different strain types as classification based on THC(A) and CBD(A) concentration and terpene profile is not complete. Unidentified unique

signals detected in the untargeted metabolomics data may also contribute to strain differences which may suggest that there exist other phytochemicals not yet examined for strain classification. It may be premature to categorize distinct chemotypes at this point with a small sample of products, but the phytochemical differences observed here merits a larger study.

The second hypothesis, that different extraction methods of the same material will yield phytochemical differences, was supported by results from Chapter 2. The two comparisons described in this chapter was the impact of drying (flower tincture vs. ethanol oil) as well as differences between oil extraction techniques (ethanol oil vs. CO_2 oil). First, the process of producing oils typically involves a critical drying step where the solvent is removed from the cannabis extract. The drying process to produce ethanol oil significantly decreased the total number of phytochemicals detected compared to flower tinctures due to the loss of volatiles such as terpenes. It was also seen that some strain variability was lost, suggesting that the phytochemicals contributing to strain differences were phytochemicals susceptible to loss with drying. Furthermore, there were few signals observed in the ethanol oil that were not present in the tincture revealing that that the drying process resulted in very limited new chemistry, but it was seen that the drying process partially decarboxylated the acid-cannabinoids. Second, phytochemical variance between oils produced by different extraction techniques was observed. The different properties of ethanol and CO_2 preferentially extracted different compounds where ethanol extraction yielded almost double the oil by weight cannabis material compared to SFE by CO_2, but a larger number of phytochemicals were detected in CO_2 oil samples. I hypothesize that this may be due to the presence and extraction of primary metabolites such structural lipids rather than secondary metabolites, which are typically attributed to sample variance, but further study is needed to confirm this. The data also demonstrated that CO_2 oil contained an average of more total

monoterpenes but less total sesquiterpenes than ethanol oils which could be attributed to a shorter drying process involved with producing CO_2 oils. Many cannabis producers include a winterization step where ethanol is added to the extract that removes wax esters, glycerides, unsaturated fatty acids (Ramirez et al. 2018; Bjorncrantz, 2015) so it is likely that winterization will remove some of the unique ions detected in CO_2 oil samples potentially leading to a product chemically similar to ethanol oils.

The third and fourth hypotheses, that the process of vaporization will reduce total number of phytochemicals and increase the relative concentration of decarboxylated cannabinoids was supported by results from Chapter 3. A significantly reduced number of compounds was detected in the vapor confirming the hypothesis, and few new compounds were produced. The results support Gieringer (2001) who proposed that vaporization can be utilized as a harm-reduction alternative for the uptake of cannabis. Future work should include identification of the chemicals that were newly detected after vaporization to determine whether they will negatively affect human health. As determined in Chapter 2, phytochemicals susceptible to loss by drying such as volatile terpenes were removed leading to more compounds being detected in vaporized flower samples and less in vaporized oil samples. The similar number of compounds detected in the ethanol oil and CO_2 oil vapor samples suggested that the vapors were qualitatively chemically similar. Although the CO_2 pre-vapor samples were more complex to begin with, some of the compounds may not vaporize well making CO_2 oil vapor more similar to ethanol oil vapor. In addition, the inclusion of these compounds in the oil may have an effect on vaporization of cannabinoids as a lower THC recovery was measured compared to ethanol oils. Further clean up steps such as winterization may lead to CO_2 oils vaporizing THC as efficiently as ethanol oils, but further experimentation is required to determine this. The quantitative data supported the fourth

hypothesis where the acid-cannabinoids were detected in trace concentrations. In addition, the process of vaporization changed terpene profiles where sample and product variance observed in pre-vapor samples was diminished after vaporization.

4.1 Original contributions

This was not only the first study to examine strain differences using untargeted metabolomics but the first to compare how these differences – together with targeted cannabinoid and terpene profiles – translate through processing into three different consumer product types. There are many cannabis products available for consumer use such as tinctures and oils. The preparation methods to produce them may cause quantitative and qualitative phytochemical differences (Sexton et al., 2018). Understanding the chemical content of these concentrates can better inform producers and consumers about their products. This research studied chemical changes associated with processing and product type across multiple different strains. A key finding was that CO_2 oils were the most chemically complex, but most consistent across strains while tinctures were the most chemically diverse. In addition, cannabinoids, namely THC(A) and CBD(A), were the phytocannabinoids present in highest abundance in all cannabis products. These compounds were of highest interest of consumers, producers and policy makers, and Health Canada requires all products to provide quantification of total THC and CBD content. This was supported by results from this study which determined the large influence on cannabinoid content on strain differences.

In addition, one popular mode of delivery of medical cannabis in Canada is vaporization (Shiplo et al., 2016) but there was a lack of research in this area. Vaporization can cause chemical transformations that may lead to a different phytochemical profile than the flower counterpart as

observed by the decarboxylation of acid-form cannabinoids to neutral-cannabinoids (Dussy et al., 2005). It is important to gain more information concerning the quantitative and qualitative phytochemical changes related to vaporization because the product label may not be representative of what is phytochemically available for consumption. Previous research has evaluated cannabinoids, specific smoke-related toxic compounds, or generalized by-products produced by vaporization (Gieringer, 2001; Lanz et al., 2016; Pomahacova et al., 2009), but this research was the first to profile this many terpenes in vapor, use untargeted metabolomics to assess thirteen different strains in vapor, and to study vaporization of cannabis oils. A novel finding was that relative chemical complexity and differences among samples diminish in vapor, which was observed in untargeted metabolomics data and terpene analysis. In addition, cannabinoids were recovered more efficiently from ground flower than oils.

4.2 Limitations

There are some possible limitations in this study. The samples presented in this study were not a complete picture of all cannabis products available to consumers, so the research should not be interpreted as such. First, all samples were obtained from the same growing facility so variability across sources was likely underestimated. In addition, only single batches of each cannabis sample was received resulting in one true biological replicate for each sample; however, at least 5 g of material was homogenized prior to sampling to ensure a representative sample. Second, only two extraction methods (ethanol extraction and CO_2 SFE) and one drying method for the production of oils were used. Although effort was made to mimic industry products, it is likely that the oil samples were not a true representative of what will be available on the market as results would likely vary based on different protocols and methods.

In terms of evaluating vaporization, the cold-trap system for collection of vapor metabolites was not a closed system and not a complete quantification of all phytochemicals. In addition, 99% ethanol may not capture all compounds found in the plant. An experiment comparing ethyl acetate, chloroform, hexanes and methanol as different trapping solvents was performed (Appendix D, Figure D-1). It was seen that majority of the discrete ions were extracted by all of the solvents tested, but 99% ethanol did not extract some compounds extracted by the other solvents. As well, some compounds can adsorb onto the balloon which could influence sample variance. Initial optimization experiments showed that as cannabinoids and easily vaporized compounds escape the plant material, other compounds may start to be released as observed in Appendix C, Figure C-1. Since a single balloon was used to collect vapor, the sample was not a complete representation of all chemicals that could be extracted by vaporization. In addition, a visible colour change was observed in the balloon over time, but experimental data were collected with a different balloon and mesh/ pad for each strain to mediate this effect and eliminate cross contamination. Lastly, it was previously seen that the amount of cannabis (and pre-treatment of plant material) can change the vaporization process (Lanz et al., 2016; Pomahacova et al., 2009). Although visually uniform, a hand mill may lead to different results depending on the plant material and the oil samples may vaporize differently depending on density or thickness of the oil on the pad. The results evaluated relative amounts and could not be used to determine complete collection of vapors, and as people can vaporize with different vaporizers at different temperatures, the findings may not be extended to all consumer cases.

Presence or absence of signals from replicates in the untargeted metabolomics data of the same sample may be related to concentrations near the LOD/LOQ, resulting in more measured variability than likely exists within the physical sample. This may also be due to potential

suppression of ionization in the LC/MS due to the presence of phospholipids and evaluation using a C18 column. It was observed in the raw data that some compounds remained on the LC column, especially between 7.0-10.0 minutes (Appendix A). In addition, the HRMS data were collected in only positive mode which is may not be a true reflection of all compounds present in the samples.

4.3 Implications and future research

This study was a novel approach to understand the extraction and vaporization process of cannabis. Observed chemical differences between strains, products and vapors may relate to bioactivity, therapeutic effects and/ or consumer experiences as different chemistry can predict different pharmacology. Future studies should investigate how these chemical differences can impact pharmacology. In addition, consumers can currently select different strains with the expectation that it may induce different experiences, but they should also expect that different products under a common name may elicit different effects. Industry should note that different extraction procedures will provide different chemistry profiles. There are trade-offs between extraction and drying practices that will impact the chemistry and pharmacology of final products. If they are mostly focused on extraction efficiency, ethanol oils may be preferred over CO_2 oils. More investigation into the winterization process on CO_2 oils is needed to determine whether CO_2 oils will result in a qualitatively similar product to ethanol oils.

Based on the results, regulators should continue to focus on THC and CBD; however, it should be understood that cannabis is chemically complex and other compounds could potentially affect health and safety. Also, since it was also seen that vaporization changes chemical profiles, future research should investigate other vaporizers for similar impacts on chemistry. The

information from this thesis can better inform policy and industry for future product development and effect of vaporization as well as provide a foundation for new questions and further studies.

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Appendices

Appendix A : Untargeted metabolomics data by HRMS

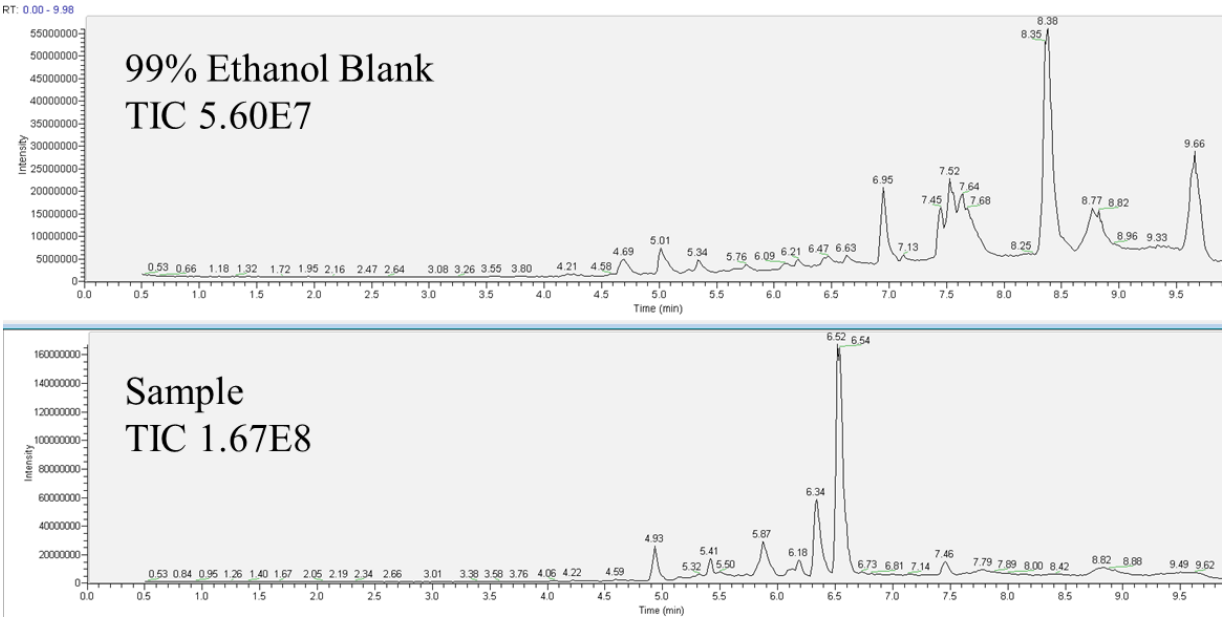

Figure A-1: Raw chromatogram from HRMS showing chromatographic differences in blank samples and cannabis samples.

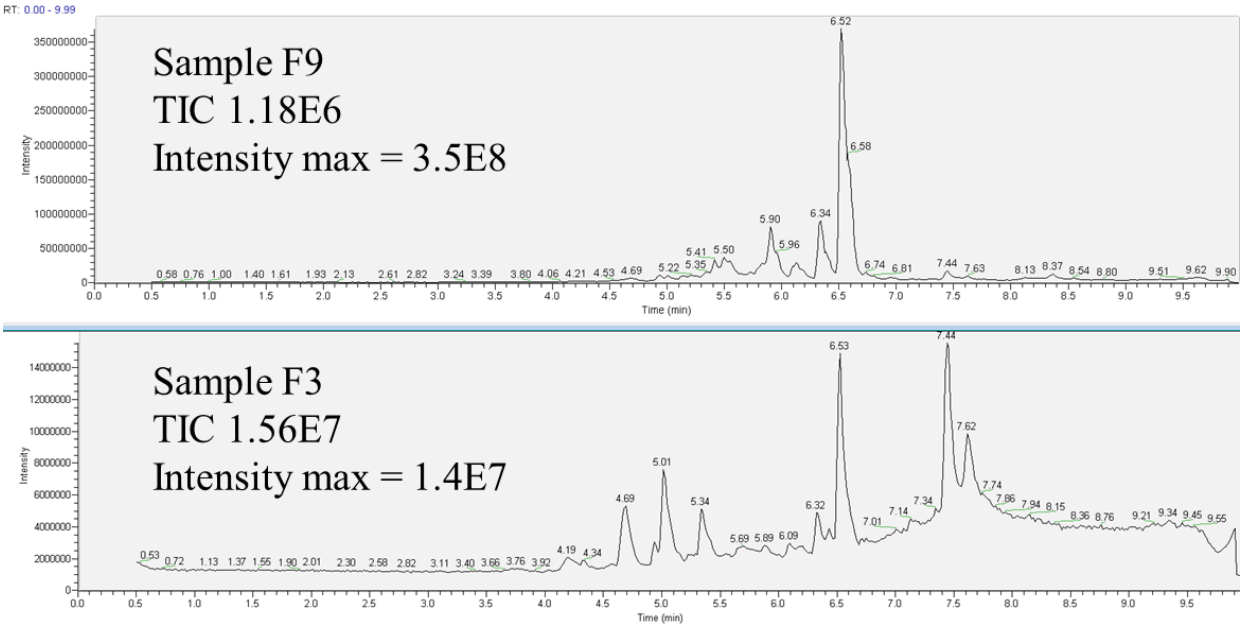

Figure A-2: Raw chromatogram from HRMS evaluating chromatographic differences between sample F9 and F3. This difference was also exhibited in sample F11.

Table A-1: Cannabinoid standards detected in metabolomics data matched with RT_*m/z*

Standard Name	Monoisotopic Mass	Unique Identifier	Adducts also detected
CBDV	286.1933	5.61_287.2002	
THCV	286.1933	5.96_287.2003	
CBN	310.1932	6.19_311.2004	293.19 $(M\text{-}H_2O+H)^+$
THC	314.2245	6.33_315.2318	
CBC	314.2245	6.48_315.2319	
CBD	314.2245	5.95_315.2319	
CBG	316.2402	5.92_317.2458	
CBDA	358.2144	5.87_359.2211	341.2107 $(M\text{-}H_2O+H)^+$
THCA	358.2144	6.53_359.2213	341.211 $(M\text{-}H_2O+H)^+$
CBGA	360.2301	5.91_343.2263	

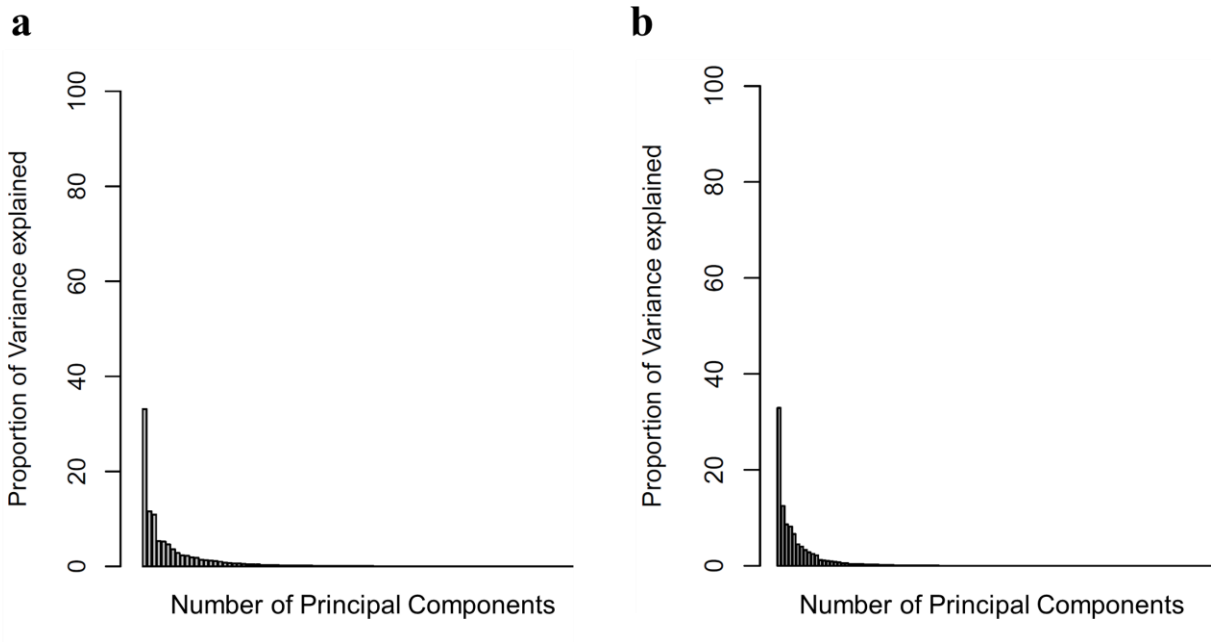

Figure A-3: PCA scree plot for (a) the comparison between three products types seen in Figure 2-3 and (b) the comparison of pre-vapor and vaporized samples in Figure 3-5

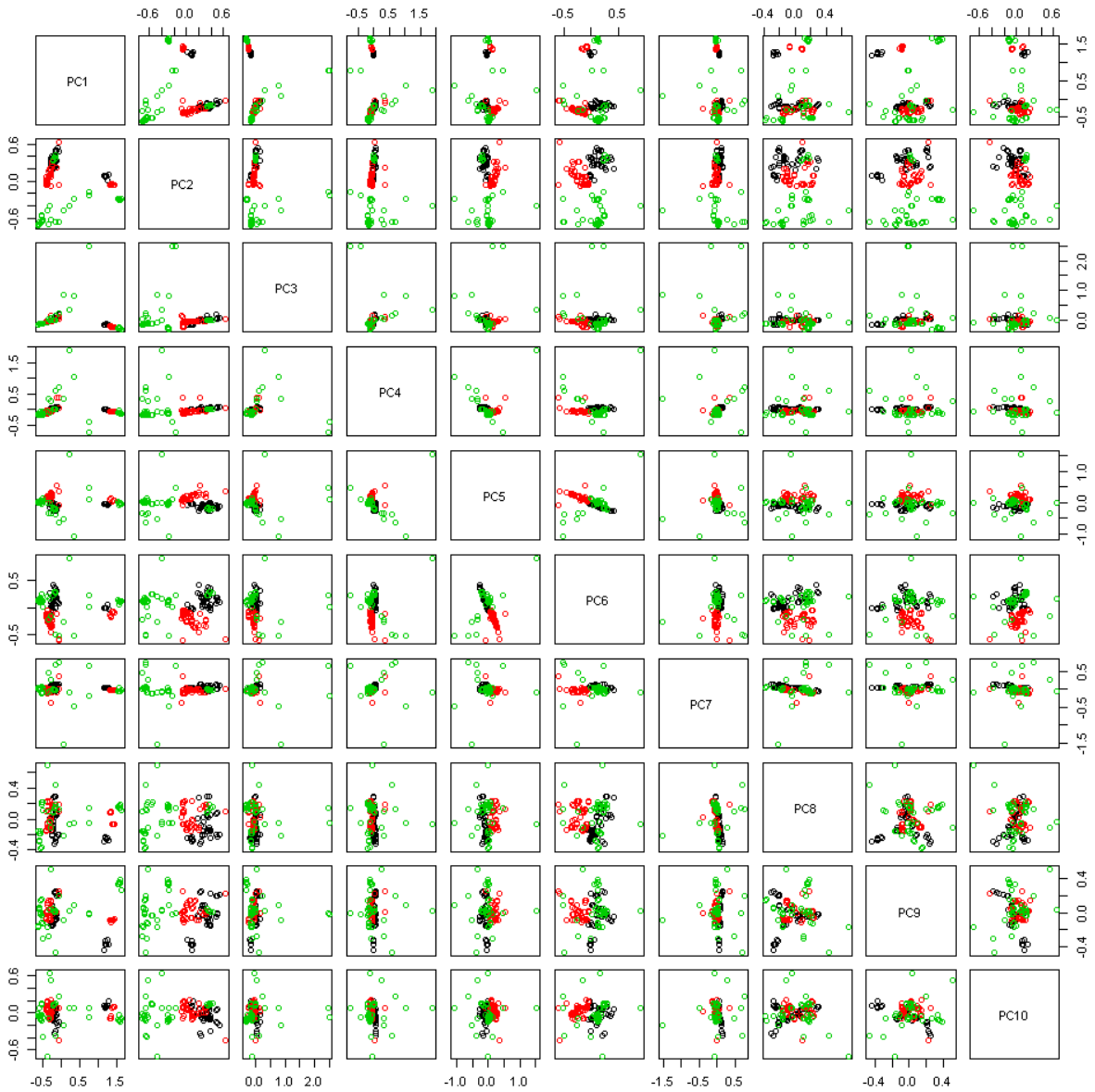

Figure A-4: First 10 principal components of PCA of flower tinctures, ethanol oils and CO_2 oils represented by colours green, red, and black, respectively.

Appendix B : Quantification LOD and LOQ

Table B-1: LOD and LOQ for cannabinoid quantification on HPLC

	CBDA (µg/mL)	**CBD (µg/mL)**	**THCA (µg/mL)**	**THC (µg/mL)**
LOD	0.15	0.38	1.13	0.37
LOQ	0.46	1.15	3.42	1.11

Table B-2: LOD and LOQ (ng/mL) for terpene quantification on GC-FID

	β-Pinene	**3-Carene**	**α-Terpinene**	**(R)-(+)-Limonene**	**γ-Terpinene**	**L-(-)-Fenchone**
LOD	17.04	16.14	11.56	5.45	4.08	1.96
LOQ	51.65	48.90	35.03	16.51	12.36	5.93

	Fenchol	**(1R) (+) Camphor**	**Isoborneol**	**Menthol**	**Citronellol**	**(+)-Pulegone**
LOD	1.33	3.27	1.89	2.66	4.71	5.34
LOQ	4.02	9.91	5.72	8.07	14.28	16.18

	Geranyl acetate	**(1S)-(+)-3-Carene**	**p-Cymene**	**Limonene**	**Terpinolene**	**Linalool**
LOD	2.17	11.78	3.31	4.10	2.79	1.16
LOQ	6.57	35.69	10.04	12.43	8.45	3.51

	(1S)-(-)-Camphor	**(+)-Borneol**	**(-)-α-Terpineol**	**Geraniol**	**α Cedrene**	**α Humulene**
LOD	2.38	1.16	1.78	7.44	2.06	2.87
LOQ	7.20	3.52	5.40	22.55	6.25	8.71

	Nerolidol	**(+)-Cedrol**	**(-)-α-Bisabolol**	**β-Caryophyllene**	**cis-Nerolidol**	**β-Eudesmol**
LOD	4.48	0.92	29.97	1.29	2.65	0.50
LOQ	13.58	2.79	90.81	3.92	8.04	1.52

3-Carene , α-terpinene and γ- terpinene were evaluated but not detected or below LOQ in all samples.

Appendix C : Vaporization trapping system optimization

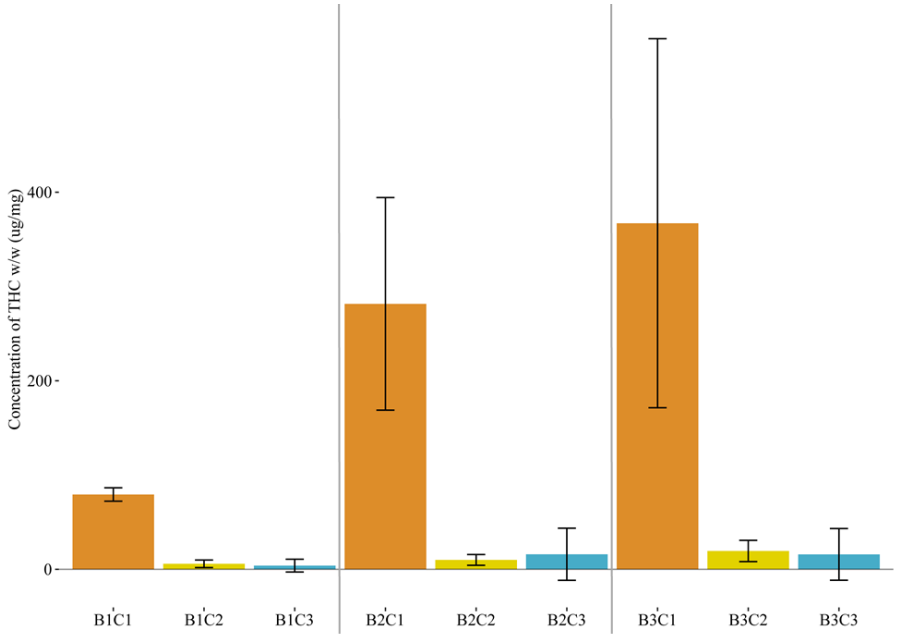

Figure C-1: Quantification of THC concentration cannabis vapor collected by Volcano balloon (B) and series of cold trap columns (C) with n=3 and error bars representing standard deviation.

	C1	C2	C3
B1	210	235	246
B2	288	122	176
B3	377	207	132

Figure C-2: Number of discrete ions of flower material detected by untargeted metabolomics binary data where at least 2 out of 3 replicates were detected in HRMS. The numbers represent the independent number of independent ions detected per balloon and column. B represents the sequential Volcano balloons used to collect vapor and C represents the series of cold trap columns to collect chemicals.

Appendix D : Comparison between extraction solvents

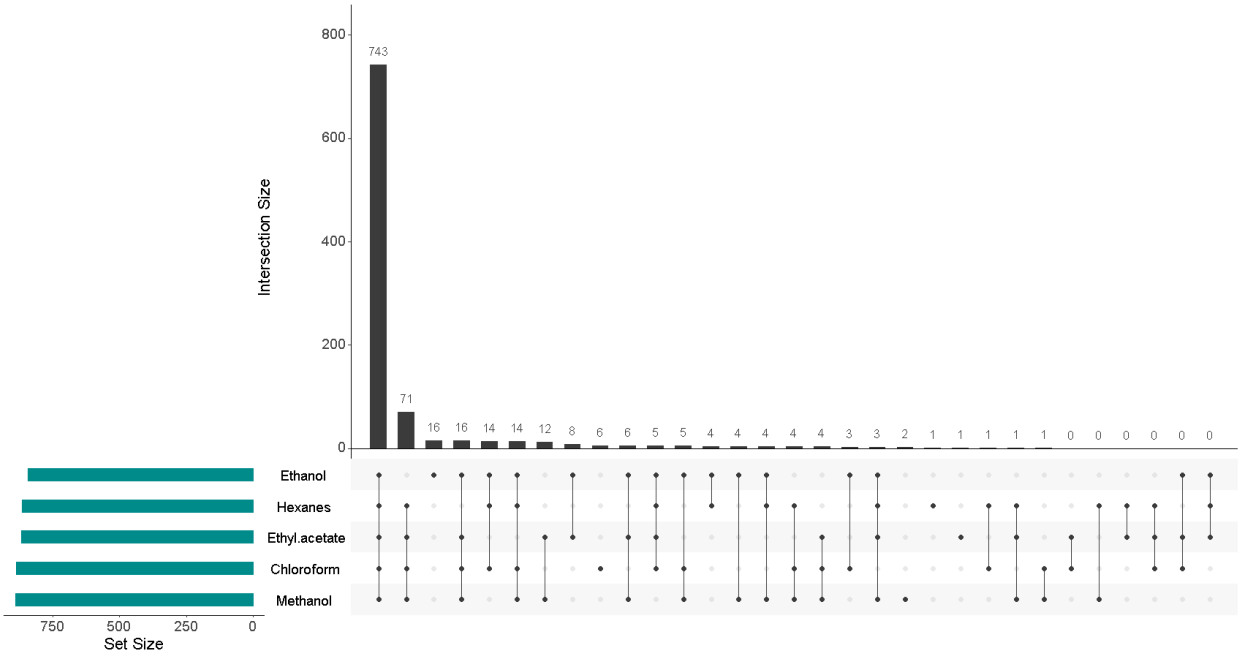

Figure D-1: Binary comparison of untargeted metabolomics data between 99% ethanol, hexanes, ethyl acetate, chloroform and methanol