5-2020

A Comparison of Antioxidant Potential, Total Phenolic Content, and Cannabidiol (CBD) Content of Cannabis Infused Hemp, MCT, and Olive Oils

Alexa Gruschow
Montclair State University

Abstract

The *Cannabis* industry has seen immense growth in recent years and research on this plant and its constituents has been growing to keep up with industry demand. The majority of research has focused on commercial-scale products and industrial processing, but there is a lack of research on the smaller scale manufacturing side of the *Cannabis* industry that includes homemade *Cannabis* products. Popular *Cannabis* products are oil-based tinctures that are made by infusing *Cannabis* plant material in a heated source of edible oil. The types of oils used for this process vary, and there is not an established standardized oil type that has been shown to be the optimal choice for reaping the most benefits from *Cannabis* infusion. The goal of infusing *Cannabis* in oil is to extract the desirable potentially neurologically active cannabinoid plant molecules that also serve as antioxidants, specifically cannabidiol (CBD). To determine the effect of oil type on extraction ability of *Cannabis*, different oil types were used to infuse a high-CBD strain of *Cannabi*s and measure antioxidant potential, total phenolic content, and CBD content of the resulting oils. Hemp oil, MCT oil, and olive oil were used as infusion solvents for the ground decarboxylated *Cannabis* flowers. Consistency in the protocol was followed for the strain of *Cannabis*, decarboxylation process, grinding process, heated infusion process, and storage conditions. Additionally, control standards were established by implementing the heating process for the oils without *Cannabis* infusion. Antioxidant potential was assessed using Trolox Equivalent Antioxidant Capacity (TEAC) assay, and total phenolic content was assessed using Gallic Acid Equivalence (GAE) assay. CBD content of the CBD oils was assessed using high-performance liquid chromatography with ultraviolet detection (HPLC-UV). For antioxidant potential, hemp CBD oil had the greatest antioxidant potential, but the other CBD oils had a significant increase in antioxidant potential compared to their control oils whereas hemp CBD oil

did not. For total phenolic content, olive CBD oil had the highest total phenolic content. For CBD content, hemp CBD oil and olive CBD oil had the highest CBD content.

MONTCLAIR STATE UNIVERSITY

A Comparison of Antioxidant Potential, Total Phenolic Content, and Cannabidiol (CBD) Content of *Cannabis* Infused Hemp, MCT, and Olive Oils

by

Alexa Gruschow

A Master's Thesis Submitted to the Faculty of

Montclair State University

In Partial Fulfillment of the Requirements

For the Degree of

Master of Science

May 2020

College/School: College of Education and Human Services

Department: Nutrition and Food Studies

Thesis Committee:

[redacted]

Dr. Adrian Kerrihard

Thesis Sponsor

[redacted]

Dr. Douglas Murray

Committee Member

[redacted]

Dr. John Specchio

Committee Member

A COMPARISON OF ANTIOXIDANT POTENTIAL, TOTAL PHENOLIC CONTENT, AND CANNABIDIOL (CBD) CONTENT OF *CANNABIS* INFUSED HEMP, MCT, AND OLIVE OIILS

A THESIS

Submitted in partial fulfillment of the requirements

For the degree of Master of Science

by

ALEXA GRUSCHOW

Montclair State University

Montclair, NJ

2020

Acknowledgements

I would like to sincerely acknowledge the individuals who have assisted me in fulfilling my thesis credentials during my graduate career. First, I would like to thank my parents, Janet and Matt Gruschow, who have supported me throughout my graduate experience and have continually encouraged me to pursue my passion of food science.

It is my pleasure to acknowledge my thesis sponsor, Dr. Adrian Kerrihard, for his incredible assistance and dedicated time and effort throughout my thesis process. I gained an abundance of knowledge working with Dr. Kerrihard, and I give my upmost appreciation to his character as a professor. I would also like to acknowledge Dr. Douglas Murray and Dr. John Specchio for doing the honor of serving as my thesis committee. I appreciate the continuous support of Dr. Murray and Dr. Specchio and instilling their trust in me in the exploration of novel research within our department.

I would like to acknowledge the Department of Nutrition and Food Studies for the assistance and contributions to this research. My requests within the laboratory were always acknowledged and fulfilled with upmost respect.

Additionally, I would like to thank Rick Richmond of Blessed Land Farm for donating all *Cannabis* samples used in this study and for expressing support and enthusiasm and offering valuable input to this research.

Table of Contents

1. Introduction

1.1 *Cannabis* Background

The *Cannabis* plant is one of the oldest plants cultivated for human use, dating back to its use for fiber and rope in 12,000 BCE in central Asia. Additional uses of *Cannabis* were documented as treatments of human disease and herbal remedies beginning in 2700 BCE China (Friedman & Sirven, 2017). Over thousands of years, *Cannabis* has been continuously used as a medicinal plant, most commonly in the form of a tincture. Through centuries of breeding and selection, 700 varieties of *Cannabis* have emerged with differing compositions of hundreds of compounds, including cannabinoids and terpenes. There are 113 identified cannabinoids found in *Cannabis* plants with the two main cannabinoids being cannabidiol (CBD) and delta-9-tetrahydrocannabinol (THC) (Klumpers & Thacker, 2019). Cannabinoids are concentrated in resin glands in the tips of secreting hairs on *Cannabis* flowers, and these glands excrete a resin substance in the form of droplets. Cultivating this substance from *Cannabis* plants allows the pharmacologically active compounds of the plant to be collected for use (Zuardi, 2008). The extracted cannabinoid composition is modulated through the use of varying extractive conditions and sample pretreatment in hopes to optimize the desired cannabinoid profile (Fiorini et al., 2019).

The *Cannabis* industry has become a rapidly growing force within recent years because of the realization that the beneficial cannabinoids of *Cannabis* can be present without the psychoactive effect. The *Cannabis* industry has branched off into a subindustry of cannabidiol, or CBD, one of the main therapeutic components of *Cannabis*. There is a high desire to reap the benefits of CBD without being accompanied by the cognitive “high” that is experienced from naturally-occurring delta-9-tetrahydrocannabinol (THC) in the *Cannabis* plant. The study of

CBD and THC began in the 1940s, but is a recent, and growing, phenomenon to create consumption products containing CBD (Klumpers & Thacker, 2019) (Friedman & Sirven, 2017).

1.2 *Cannabis* Composition

The three distinct species of the *Cannabis* plant with varying concentrations of cannabinoids are known as *Cannabis sativa*, *Cannabis indica*, and *Cannibas ruderalis* (Fares, 2018). All classes of cannabinoids are derived from precursor cannabigerol (CBG) compounds. Cannabinoids originate in the *Cannabis* plant due to the condensation of olivetolic acid in the polyketide pathway and geranyl pyrophosphate in the methylerythritol pathway to form cannabigerolic acid (CBGA) (Elkins et al., 2019). CBD and THC are derived from CBGA through synthesization of CBGA into cannabidiolic acid (CBDA) and tetrahydrocannabinolic acid (THCA) and decarboxylation of CBDA and THCA into CBD and THC, respectively. *Cannabis* plants contain quantities of CBDA and THCA which determines the relative quantities of CBD and THC that will be present after decarboxylation (Citti et al., "Analysis of Cannabinoids…", 2018). All strains contain levels of CBDA and THCA, but the amount of each depends on the strain of the species. The vast amount of *Cannabis* strains have varying ratios of CBDA to THCA, and certain strains are targeted for use based on the cannabinoid content that is desired (Fares, 2018).

CBD and THC are fatty compounds with a slight difference in their respective chemical structures, providing differing psychotropic properties. The hydroxyl group in CBD characterizes CBD as non-psychotropic whereas the cyclic ring in THC characterizes THC as psychotropic. Other notable cannabinoids found in *Cannabis* are cannabichromene (CBC), cannabigerol (CBG), cannabinol (CBN), cannabielsoin (CBE), cannabicyclol (CBL), cannabivarin (CBV), cannabicitran (CBT), and tetrahydrocannabivarin (THCV) (Klumpers & Thacker, 2019).

In addition to cannabinoids, *Cannabis* contains compounds known as terpenes which are responsible for the smell and taste of the plant. The most commonly identified terpenes in *Cannabis* include α-pinene, myrcene, limonene, β-caryophyllene, and linalool. Terpenes are a major component of *Cannabis* resin and there are more than 100 different types in *Cannabis*. Each strain of *Cannabis* has a unique terpene type and composition, and terpene content plays a key role in differentiating the effects of various *Cannabis* strains. Other compounds include hydrocarbons, nitrogen-containing compounds, carbohydrates, flavonoids, fatty acids, non-cannabinoid phenols, alcohols, and esters (Klumpers & Thacker, 2019).

1.3 *Cannabis* Preparation and Processing

When *Cannabis* is grown for the purpose of extracting cannabinoids, it undergoes the processes of harvesting, drying, and decarboxylation. Once *Cannabis* is grown and harvested, the flowers are dried to start the decarboxylation process of converting THCA to THC and CBDA to CBD. The biosynthesis process of converting CBG to THCA and CBDA will also continue after harvest during the drying cycle. After drying, the flowers of the *Cannabis* plant are heated to further induce the process of decarboxylation. Exposure to heat causes cannabinoids to undergo decarboxylation in which they convert from an acidic to a neutral form and become active (Żuk-Gołaszewska & Golaszewska, 2018). The precursors, CBDA and THCA, are not capable of passing the blood-brain barrier and will remain inactive within the body, whereas CBD and THC are active within the body, hence the need for the decarboxylation step (Elkins et al., 2019). Research has shown that the two main bioactive substances of CBD and THC are found in low concentrations in fresh *Cannabis* flowers as compared to those that have been heated. The decarboxylation technique has been shown to increase the levels of these two cannabinoids within the flowers (Grijó et al., 2018). Once the flowers undergo decarboxylation, the

cannabinoids can be extracted from the resin glands. For the production of cannabinoids, female crops are preferred as they produce much higher quantities of cannabinoids as compared to their male plant counterpart. Additionally, the amount of CBD and THC in the flowers differs amongst the species and strains of *Cannabis*. For CBD oil extraction, strains with a high-CBD and low-THC content are used (Chandra et al., 2017).

1.4 Extraction Methods

There are several methods that can be used to extract cannabidiol (CBD) from the plant material including CO_2 extraction, alcohol extraction, and oil infusion. CO_2 extraction is the preferred method based on its efficiency to extract the highest content of CBD in large scale extractions and its safety in producing a pure CBD oil without toxins. CO_2 is identified as a supercritical solvent in the supercritical fluid extraction method of extracting CBD. In this process, pressurized warm CO_2 gas is pumped through a chamber that contains *Cannabis* and it allows cannabinoid compounds to dissolve in the gas. The material is mixed with CO_2 under extreme pressures for several hours. The CO_2 carries the *Cannabis* particles to a lower pressure chamber which causes the cannabinoids to precipitate out of the gas, forming an oil-like substance (Chandra et al., 2017). The oil is collected in a separate vessel without any additional solvents (Rovetto & Aieta, 2017). The concentrated extract of *Cannabis* consists of a sticky and viscous oil with a concentrated cannabinoid content (Romano & Hazekamp, 2013).

Another method of CBD extraction involves the use of organic solvents such as methanol, ethanol, chloroform, butane, and hexane. In this method, a solvent is mixed with the *Cannabis* flowers to separate the cannabinoids from the plant material. Mixing the extraction solvent with the flowers will allow the solvent to dissolve cannabinoids from the plant. This mixture is strained to remove the plant solids and heated to evaporate the solvent, leaving behind

the plant extracts in an oil form. The solvent extraction method poses risks because many of the solvents are known to be of high toxicity to humans. This is dangerous if the solvent is not completely evaporated during the process and remains in the final oil product that is used for consumption. Additionally, solvent extraction causes valuable terpenes to be excluded from the final product (Křížek et al., 2018).

CBD extraction can also be accomplished by a relatively simple non-standardized procedure that involves infusing the *Cannabis* in an existing oil. This method does not require particular laboratory instruments or materials, and it has become a popular procedure that can be accomplished in an at-home kitchen (Romano & Hazekamp, 2013). A standard cooking oil such as olive oil can be used to extract cannabinoids from *Cannabis* flowers using heat. This method lacks a standard protocol, but it has been shown to extract a significant amount of CBD from dried decarboxylated *Cannabis*. A common method that has been adopted involves adding dried *Cannabis* flowers to olive oil and placing this solution in a heated water bath. The water bath serves as the decarboxylation step, and this is followed by a filtration step that separates the oil from the plant matter. Infusing the *Cannabis* in heated oil for a prolonged period of time allows the cannabinoids to dissolve into the oil base thus producing an olive oil that contains CBD (Deidda et al., 2019).

A CBD extraction procedure without a standardized protocol allows for variations among the decarboxylation and extraction methods including differences in equipment used, heating procedure used, and extraction oil used. These procedures tend to lack scientific data or research studies and are used by small-scale individual sellers. There is variance seen in suggestions to use an oven for the decarboxylation step or a water bath, both with varying temperature suggestions. Additionally, there is variance noted for the extraction method such as using a

double boiler method, placing the *Cannabis* in heated oil and water, or placing the *Cannabis* in heated oil. The oil used for extraction varies as well amongst internet suggestions including oils such as MCT, olive, sunflower, hemp seed, and avocado oil. It is also unknown which extraction oil may have the highest potential to extract the highest concentration of CBD from the *Cannabis*. Determining the best oil for extraction of the highest concentration of CBD is necessary in order to improve efficiency and avoid wasting plant product. More research is needed to find the ideal extraction oil for CBD.

1.5 Properties of Infusion Oils

In order to create a topical CBD oil or a consumable CBD tincture, edible oils are commonly infused with *Cannabis* extracts (Maida & Daeninck, 2016). Since CBD is nonpolar, the nonpolar property of oil provides a complimentary environment for CBD to leech into the oil from the plant material. Different oils are characterized by different profiles of saturated and unsaturated fatty acids, antioxidant potential, and phenolic content amongst other factors. Oils are composed of a fatty acid profile of saturated, polyunsaturated, monounsaturated, or a combination of these types of fatty acids (Aeschbach, et al., 1994). Differing oil types used in CBD extraction may result in CBD oils that vary in terms of antioxidant potential, phenolic content, and CBD content, and it is undetermined which oil type may result in the maximum amount of each of these components.

1.6 Identification of CBD

Cannabinoid content, specifically CBD, can be determined through the use of gas chromatography coupled with mass spectrometry. Headspace gas chromatography involves heating the *Cannabis* extraction in a gas chromatograph to a specific temperature in order to cause the volatile components in the sample to escape into the headspace above the sample.

Helium, hydrogen, and nitrogen gas are pumped through the headspace causing the *Cannabis* volatiles to move into the gas chromatograph fibers which separates the volatiles based on size and polarity. The separated volatiles are then passed through a mass spectrometry that can identify the components that make up the volatile chemistry of the sample. The mass spectrometer will give a full cannabinoid profile of the sample by identifying compounds such as CBD, THC, THCA, CBDA, CBGA, etc. Additionally, the mass spectrometer will identify terpenes that are present and their corresponding quantity in parts per million (ppm) (Lachenmeier et al., 2004).

Liquid chromatography is another method for the identification of the cannabinoid profile in a sample. In high-performance liquid chromatography (HPLC), the *Cannabis* oil is pumped at high pressure through a column with chromatographic packing material. The carrier gas, consisting of helium or nitrogen, is able to carry the sample through the chromatographic column while separating the sample compounds. The chromatographic column consists of a granular material of solid particles that interact with and absorb the sample components and cause a degree of separation. Compound bands will be displayed in the column absorbent material based on flow rates for the various components. A detector will identify the separation of compounds and the amount of the components that emerge from the column (Citti et al., "Pharmaceutical and Biomedical…", 2018). Ultraviolet detection is the most frequently used detection method for the analysis of cannabinoids in plant materials. This method involves the identification of the structural elements of cannabinoids by passing UV light through a sample and measuring the absorption of the different wavelengths that pass through the sample. The amount of light absorbed by the sample allows for the identification of the chemical markers that signify and quantify cannabinoid properties. When HPLC is coupled with UV detection it is

known as HPLC-UV. (Citti et al., “Pharmaceutical and Biomedical…”, 2018) (Brighenti et al., 2017).

1.7 Antioxidants and Phenolics

The cannabinoid CBD expresses antioxidant activity based on its chemical structure. Antioxidants are able to donate an electron to a free radical without damaging their own structure. CBD possesses a phenol group that can protect cells against oxidative stress. Cannabinoids are able to donate an electron to an unpaired electron in a free radical to prevent the radical from stealing an electron from cell DNA thus damaging the cell (Tura et al., 2019). CBD has been shown to exhibit pleiotropic activities including antioxidant and anti-inflammatory effects. A study by Tura et al. determined that CBD has the potential to neutralize free radicals, thus serving as an antioxidant (2019). Additionally, this study showed that CBD has a greater antioxidant potential than α-tocopherol (vitamin E), likely due to the presence of two hydroxyl groups in the CBD molecule. It was shown that CBD suppresses a known free radical called 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Tura et al., 2019). Research by Hampson et al. determined that CBD had similar antioxidant potential compared to the antioxidant butylated hydroxytoluene, and CBD had a greater antioxidant effect than other dietary antioxidants, α-tocopherol or ascorbate (1998).

CBD is classified as a phenol based on its aromatic ring molecular structure and bioactive plant phenols are classified as antioxidant sources. The antioxidant content of a *Cannabis* extraction can be determined based on its total phenolic content; total phenolic content of plants is an important parameter for their antioxidant properties (Sahin et al., 2012). The measurement of phenolic content can be used in conjunction with the measurement of antioxidant potential to

determine the antioxidant behavior of a substance based on the quantity of antioxidants present (Andre et al, 2016).

1.8 Endocannabinoid System

Cannabinoids are able to elicit responses in the human body due to the endocannabinoid system. CBD and THC have the most interaction with the endocannabinoid system compared to other cannabinoids and therefore research is centered around these two compounds (Fares, 2018). Cannabinoid receptors (CBR) are present on neurons and they are known as CB1R and CB2R. Endocannabinoids are naturally produced in the body and they have the ability to bind to and active CB1 and CB2 receptors. Endocannabinoids are a factor in neural development, inflammation, appetite and metabolism, immune function, pain, memory, psychiatric disease, reproduction, and many other physiologic and pathophysiologic processes (Zou & Kumar, 2018). The endocannabinoid system is a neuroregulatory system that modulates the release of excitatory and inhibitory neurotransmitters upon activation of cannabinoid receptors. Cannabinoids, specifically CBD and THC, have the ability to influence CB receptors and raise the synaptic levels of endocannabinoids. Assisting in the regulation of endocannabinoids increases the physiologic actions of the endocannabinoid system thus contributing to homeostasis. Endocannabinoids assist the endocannabinoid system in communicating with all other systems in the body and their regulation is crucial to this system. Cannabinoids can help to modulate the activity of the endocannabinoid system thus giving potential to offer therapeutic benefits for various ailments such as mental health disorders, neurological and movement disorders, pain, autoimmune diseases, spinal cord injury, cancer, cardiometabolic disease, stroke, and others (Corroon & Felice, 2019).

Cannabis research suggests that the benefits of *Cannabis* revolve around a concept known as the 'entourage effect'. The entourage effect proposes that a full spectrum extraction from *Cannabis* allows for more biological activity rather than a *Cannabis* isolate. A full spectrum extraction includes all cannabinoids and terpenes whereas a CBD isolate only includes cannabidiol. Research has supported that cannabinoids and terpenes may offer complimentary physiological activities that may improve the therapeutic index of *Cannabis* extracts (Russo, 2011). Full spectrum CBD oil is represented on commercial shelves as well as with homemade products.

1.9 Uses of CBD

CBD oil is used in an expansive variety of consumer products ranging from topical oils and balms to consumable oil tinctures and consumable food products that contain CBD oil. CBD products are used for therapeutic purposes and not psychoactive purposes, thus they are authorized to contain a maximum THC content of only 0.3%. CBD oil is used topically to reduce inflammation, pain, and muscle soreness whereas CBD oil that is ingested is intended to have disease-fighting actions (Freeman et al., 2019). CBD oil has entered the food system and is featured in consumer products such as chocolate, baked goods, coffee, cooking oils, and many others. Additionally, consumers are engaging in practices in which they extract their own CBD oil from home. More research is needed to verify the efficiency of at-home CBD extraction in terms of the most efficient method to extract the most CBD along with its antioxidant abilities.

1.10 CBD Rules and Regulations

The FDA established *Cannabis* regulations in the 2018 Farm Bill relating to the production and marketing of *Cannabis*. Any *Cannabis* product is subject to the same authorities and requirements as any other FDA-regulated products containing a substance. CBD products

cannot be sold as dietary supplements or added to food included in interstate commerce which would violate the Federal Food, Drug, and Cosmetic Act; the FDA does not harness any evidence that CBD is generally recognized as safe (GRAS) for its use in food or any food additive regulation that authorizes the use of CBD as a food ingredient. Additionally, the FDA has not approved the marketing of CBD products for therapeutic purposes (Commissioner, 2020). Despite these regulations by the FDA, CBD products are widely available in health food shops, dietary supplement stores, grocery stores, and on the internet via independent *Cannabis* companies. Similar to other herbal remedies and supplements, non-medicinal CBD products lack quality assurance in which they are not scheduled or regulated as medicines, oftentimes showing variance and inaccuracy in its declared contents. The amount of CBD in these products tends to be significantly lower than amounts recorded in clinical trials (Freeman et al., 2019). The FDA is continuously working to update and enforce *Cannabis* regulations and eliminate misconceptions within this industry.

1.11 Study Overview

Despite regulatory confusion within the industry, consumers are increasingly exploring the benefits and uses of CBD. Google searches in the United States that mentioned "CBD" or "cannabidiol" substantially increased from 2015-2019. Search volumes increased by over 100% in each year, and there were 6.4 million searches during April 2019 (Leas et al., 2019). Retail sales of CBD products in the United States reached $170 million in 2015 and $500 million in 2018 with a projected annual growth rate of 55% to reach over $1 billion in 2020 (Corroon & Phillips, 2018).

Outside of the commercial industry, there are small scale growers and product innovators that are using "at-home" methods to extract CBD from *Cannabis* plant material and

incorporating it into oil-based products for topical use or consumption. There are varying procedures being used to extract CBD using oil, and no standardized procedure has been created that proves highest efficiency (Romano & Hazekamp, 2013) (Deidda et al., 2019). There is a lack of research for non-commercialized/small-scale commercialized CBD products, and research is needed to assess the cannabinoid extraction potential of different oil bases in order to create a more standardized extraction method. The main objective of this research was to determine the effects of oil type on the antioxidant potential, total phenolic content, and cannabinoid content of *Cannabis* infused oils.

1.12 Limitations

One limitation in this study was that only one strain of *Cannabis* was used, and the findings will not be able to be generalized for all strains and *Cannabis* species. Another limitation was that one decarboxylation and extraction method was used, and the findings will not be able to be applied to other methods of decarboxylation and extraction. An additional limitation was that the antioxidant assessment methods used cannot identify which antioxidants are responsible for the antioxidant capacity and the phenolic content of the *Cannabis* sample. Another limitation was that only three oils were being assessed for the *Cannabis* extraction ability, and there are a larger variety of oils being used in the *Cannabis* industry that need to be assessed for extraction efficiency.

2. Manuscript I

Antioxidant Analysis and Total Phenolic Content of *Cannabis* Infused Oils

2.1 Abstract

Cannabinoids with antioxidant properties can be extracted from *Cannabis* by infusing decarboxylated *Cannabis* plant material in heated plant-based cooking oil. The antioxidant potential and total phenolics of the *Cannabis* infused oils can be measured in order to help determine the type of oil that is most efficient at extracting cannabinoids with antioxidant properties. Hemp oil, MCT oil, and olive oil were used as infusion solvents for a high-CBD low-THC strain of decarboxylated *Cannabis*. The resulting *Cannabis* oils, referred to as CBD oils, were assessed for antioxidant potential and total phenolic content using a Trolox Equivalent Antioxidant Capacity (TEAC) assay and Gallic Acid Equivalence (GAE) assay, respectively. Hemp CBD oil had the greatest antioxidant potential than the other CBD oils, and olive CBD oil had the highest total phenolic content than the other CBD oils. *Cannabis* infusion caused a significant increase in antioxidant potential and total phenolic content for all oils, except for the antioxidant potential of hemp oil.

2.2 Introduction

One of the most studied plants of interest, with continually growing research in recent years, has been the *Cannabis* plant. *Cannabis* is commonly cultivated for the extraction and use of its molecular constituents. The main constituents of focus are biologically active fatty acid compounds known as cannabinoids, including cannabidiol (CBD) (Klumpers & Thacker, 2019). Cannabinoids have been shown to possess beneficial characteristics such as antioxidant, anti-inflammatory, anti-fungal, and anti-bacterial abilities as well as therapeutic benefits for mental health disorders, neurological and movement disorders, pain, autoimmune diseases, spinal cord injury, cancer, cardiometabolic disease, stroke, and others (Andre, Hausman, & Guerriero, 2016) (Corroon & Felice, 2019).

Once heated and converted into their active form, known as decarboxylation, cannabinoids have the potential to neutralize free radicals, thus serving as antioxidants (Tura et al., 2019). Cannabinoids act as antioxidants because they are phenolic compounds. The antioxidant content of a *Cannabis* extraction can be determined based on its total phenolic content because total phenolic content of plants is an important parameter for their antioxidant properties (Sahin et al., 2012). The measurement of phenolic content can be used in conjunction with the measurement of antioxidant potential to determine the antioxidant behavior of a substance based on the quantity of antioxidants present (Andre, Hausman, & Guerriero, 2016).

The oil-based cannabinoids, specifically CBD, can be extracted from *Cannabis* flowers and used in products such as topical oils and consumable oil tinctures (Freeman et al., 2019). CBD extraction can be achieved through the use of high-tech methods such as supercritical CO_2 extraction and solvent-based extraction or a simpler method such as infusing *Cannabis* flowers in an edible plant-based oil (Romano & Hazekamp, 2013). CO_2 extraction has been shown to be

the most efficient method for extracting cannabinoids from *Cannabis* (Chandra et al., 2017), but this method is not available for all members of the *Cannabis* industry. The widely available method of infusing *Cannabis* in heated oil for a prolonged period of time has been shown to allow the cannabinoids to dissolve into the oil base (Deidda et al., 2019).

More research is needed to verify the efficiency of *Cannabis* extraction via oil infusion in terms of extracting the most cannabinoids along with its antioxidant abilities. Research is needed to compare the ability of different oils and different methodology to extract antioxidants, specifically cannabinoids, from *Cannabis*. This can be measured by evaluating antioxidant potential and total phenolic content of various plant-based oils that have been infused with *Cannabis*.

2.3 Materials and Methods

2.3.1 Plant Material and Oil Type

All *Cannabis* flowers used in this study were obtained from Blessed Land Farm. The species of plant is a *Cannabis sativa* dominant hybrid and the strain is Baox, recognized as a high-CBD low-THC strain. This farm is a registered grower in accordance with the Industrial Hemp Program beginning on the date of March 1, 2019. As part of registration, the farm is certified that the hemp seeds obtained for planting are a type and variety that do not exceed the maximum concentration of THC. Once received, the *Cannabis* flowers were vacuum sealed and stored at -81˚C until use. When needed, *Cannabis* packages were removed from the deep freezer and used immediately for oil infusion. The three infusion oils used in this study were 365 Everyday Value cold processed extra virgin olive oil, 365 Everyday Value MCT oil from fractionated expeller pressed virgin coconut oil, and Manitoba Harvest unrefined cold pressed hemp seed oil.

2.3.2 Cannabidiol (CBD) Oil Sample Preparation

Upon thawing from the freezer, *Cannabis* flowers were decarboxylated in an Isotemp oven set at 140˚C for 30 minutes, turning the buds over at 15 minutes. Decarboxylation methods were determined based on parameters for maximum CBD extraction as modeled in a previous study (Grijó, Osorio, & Cardozo-Filho, 2018). Stems were removed and the flowers were ground to a powder using a mortar and pestle. *Cannabis* powder was measured to 2.83g and placed in 94.6mL of designated oil. The oils containing *Cannabis* were heated in a glass beaker on a Corning hot plate set at 90˚C and agitated with a star bar at 200rpm for 3 hours. The oil was strained using Bolio organic hemp cloth #4 coffee filters to separate the oil from the *Cannabis* powder. The oil was centrifuged for 10 minutes at 1000rpm to separate the oil mixture and any remaining plant matter. The supernatant oil, referred to as CBD oil, was collected and stored in the deep freezer until further use. The extraction oils used were also heated and stored under the same parameters without *Cannabis* to be used as a control to compare to the *Cannabis* oil extracts. All samples were prepared in triplicate.

2.3.3 Trolox Equivalent Antioxidant Capacity (TEAC) Analysis

To analyze the antioxidant potential, a Trolox Equivalent Antioxidant Capacity (TEAC) analysis was performed on each sample. This method allowed for the measurement of antioxidant potential by evaluating the effectiveness of each CBD oil sample/control sample in slowing oxidative reactions compared to that of the measured effects of Trolox, a known powerful antioxidant. Therefore, the results of this assessment are described as a measure of "Trolox equivalency." The stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was used as a radical standard to assess the sample's capacity to neutralize the radical compared to a standard curve of Trolox.

An extraction and isolation protocol was used to isolate the antioxidants from the oil. Samples of *Cannabis* infused oil were removed from the deep freezer and thawed at room temperature. 0.6g of each oil was used and mixed with 4mL of acetone. Samples were vortexed for 5 minutes and placed in the centrifuge at 1,000g for 10 minutes. Once finished, the supernatants were removed and placed in different distilling spider flasks for evaporation. The rotary evaporator water tub was set to 40˚C and the spider flask rotated at 40rpm. The evaporation process began at approximately 307mBar for about 30 minutes, or until no more acetone was pulled off. The pressure was then decreased 20mBar every 5 minutes until reaching a final pressure of 100mBar. The system was held at 100mBar until no more acetone was being pulled off. The sample remaining in the spider flasks was weighed in milligrams and used for antioxidant analysis.

The DPPH solution (152.16075mM) was prepared by mixing 1.5mg of DPPH in 25mL of an 80/20 methanol/water solution and then sonicated for 4 minutes. The Trolox solution was prepared by creating 34mL of a 1:1 acetone/water solution and combining 16mL of this solution with 12mg of Trolox (3.0mM). A dilution series of Trolox solution was prepared by mixing the 3.0mM Trolox solution with the 1:1 acetone/water solution. A 0mM Trolox solution contained 0mL of 3.0mM Trolox solution and 5mL of 1:1 acetone/water solution. A 0.6mM Trolox solution contained 1mL of 3.0mM Trolox solution and 4mL of 1:1 acetone/water solution. A 1.2mM Trolox solution contained 2mL 3.0mM Trolox solution and 3mL of 1:1 acetone/water solution. A 1.8mM Trolox solution contained 3mL 3.0mM Trolox solution and 2mL of 1:1 acetone/water solution. A 2.4mM Trolox solution contained 4mL 3.0mM Trolox solution and 1mL of 1:1 acetone/water solution. A 3.0mM Trolox solution contained 5mL 3.0mM Trolox solution and 0mL of 1:1 acetone/water solution.

Samples were prepared for TEAC analysis in a microplate. A standard curve of Trolox for analysis was created by loading separate wells of the microplate with the following: 5µL 0mM Trolox solution with 295µL DPPH, 5µL 0.6mM Trolox solution with 295µL DPPH, 5µL 1.2mM Trolox solution with 295µL DPPH, 5µL 1.8mM Trolox solution with 295µL DPPH, 5µL 2.4mM Trolox solution with 295µL DPPH, and 5µL 3.0mM Trolox solution with 295µL DPPH. The isolated CBD oil samples were loaded into separate microplate wells according to the following: 5µL CBD olive oil with 295µL DPPH, 5µL CBD MCT oil with 295µL DPPH, and 5µL CBD hemp oil with 295µL DPPH. Each of the CBD oils were loaded into 3 separate wells in order to triplicate the data. The control oil samples followed the same procedure of loading 5µL of oil with 295µL of DPPH and done in triplicate. A VersaMax Microplate Reader with SoftMax Pro Software was used for microplate analysis. Absorbance was set to 517nm and the loss of absorbance was measured after 30 minutes of microplate incubation at 27˚C. The same procedure was repeated for control samples.

2.3.4 Total Phenolic Content (TPC) Analysis

To analyze the total phenolic content (TPC), a modified Gallic Acid Equivalence (GAE) Folin-Ciocalteu method (Szydlowskaczerniak et al., 2008) was performed on each sample. This method allowed for the measurement of total phenolic content by evaluating the quantity of phenols in each CBD oil sample/control sample compared to that of the measured content of gallic acid, a type of phenolic acid. Therefore, the results of this assessment are described as a measure of "gallic acid equivalency." The Folin-Ciocalteu reagent (FCR) and a sodium carbonate solution were used as reactive reagents to assess the sample's phenolic capacity compared to a standard curve of gallic acid.

An extraction and isolation protocol was used to isolate the phenolics from the oil. Samples of oils were weighed at 2.5g and extracted with methanol by mixing 2.5mL of methanol with the sample and extracting the methanolic portion after 2 minutes; this was performed three times. The methanolic extracts were left overnight and then 0.5mL of extract was transferred into 10mL calibration flasks.

0.25mL of Folin-Ciocalteu reagent was then added to the 0.5mL of sample extract and vortexed for 3 minutes. To the extract, 0.5mL of an 8% saturated sodium carbonate solution (2g sodium carbonate/25mL water) was added and the mixture was made up to the 2.5mL mark with water. The solutions were placed in the dark for 1 hour and then centrifuged at 1,000g for 5 minutes. The supernatant was used for TPC analysis.

The gallic acid solution was created by mixing 1mg of gallic acid with 10mL of water. 0.25mL of Folin-Ciocalteu reagent was added to the solution and vortexed for 3 minutes, and then 0.5mL of an 8% saturated sodium carbonate solution was added and mixed. A dilution series of gallic acid solution was prepared by mixing 5mL of the gallic acid solution with 5mL of water. 5mL was taken from this diluted gallic acid solution and mixed with 5mL of water. This dilution process was repeated to create a series of six gallic acid solutions in the concentration range 0.003-0.1mg/mL.

Samples were prepared for TPC analysis in a microplate. A standard curve of gallic acid for analysis was created by loading separate wells with 300µL of the gallic acid dilution series (0.003-0.1mg/mL) solutions. The supernatants of samples were loaded into separate microplate wells according to the following: 300µL CBD olive oil, 300µL CBD MCT oil, and 300µL CBD hemp oil. Each of the CBD oils were loaded into 3 separate wells in order to triplicate the data. The control oil samples followed the same procedure of loading 300µL of isolated oil into the

well and done in triplicate. A VersaMax Microplate Reader with SoftMax Pro Software was used for microplate analysis, and the absorbance at 765nm and 27˚C was measured. The same procedure was repeated for control samples.

2.3.5 Statistical Analysis

For each assay, Statistical Package for Social Sciences (SPSS) was used to perform one-way ANOVA with Tukey's Test to determine significant differences between samples. Additionally, a scatterplot was used to assess correlation between antioxidant potential and total phenolic content of the samples.

2.4 Results and Discussion

2.4.1 Antioxidant Potential Results

Trolox Equivalent Antioxidant Capacity (TEAC) results of all samples are shown in Figure I and Table I. Hemp CBD oil (M=1159.34) showed significantly greater antioxidant potential than olive CBD oil (M=1095.79) ($p<0.05$), which in turn showed significantly greater antioxidant potential than MCT CBD oil (M=966.75) ($p<0.001$). Hemp CBD oil was not significantly different from hemp control oil (M=1190.33) ($p=0.757$) since the hemp control oil had a high antioxidant potential on its own. MCT CBD oil showed significantly greater antioxidant potential than MCT control oil (M=283.81) ($p<0.001$), and olive CBD oil showed significantly greater antioxidant potential than olive control oil (M=910.43) ($p<0.001$). MCT oil showed the greatest increase in antioxidant potential from its control oil to its CBD oil, most likely due to the low antioxidant potential of its control oil. Hemp control oil showed significantly greater antioxidant potential than olive control oil ($p<0.001$) and MCT control oil ($p<0.001$), and olive control oil showed significantly greater antioxidant potential than MCT control oil ($p<0.001$). Oils that had the greater antioxidant potential in their starting control oil

also had the greater antioxidant potential after *Cannabis* infusion. CBD extraction via heated infusion oil significantly increased the antioxidant potential of olive oil and MCT oil, but not hemp oil. The data suggests that the antioxidants present in *Cannabis* are able to be absorbed by oil that is heated with decarboxylated *Cannabis*, thus increasing the preexisting antioxidant potential of the oil to a certain threshold depending on the oil type used.

2.4.2 Total Phenolic Content Results

Total phenolic content (TPC) results for all samples are shown in Figure II and Table II. Olive CBD oil (M=184.62) showed significantly higher total phenolic content than hemp CBD oil (M=121.37) ($p<0.001$), which in turn showed significantly higher total phenolic content than MCT CBD oil (M=34.06) ($p<0.001$). Hemp CBD oil showed significantly higher total phenolic content than hemp control oil (M=27.57) ($p<0.001$), MCT CBD oil showed significantly higher total phenolic content than MCT control oil (M=4.67) ($p<0.001$), and olive CBD oil showed significantly higher total phenolic content than olive control oil (M=110.68) ($p<0.001$). Hemp oil showed the largest increase in total phenolic content from its control oil to its CBD oil. Olive control oil showed significantly higher total phenolic content than hemp control oil ($p<0.001$) and MCT control oil ($p<0.001$), and hemp control oil showed significantly higher total phenolic content than MCT control oil ($p<0.005$). Oils that had the higher total phenolic content in their starting control oil also had the higher total phenolic content after *Cannabis* infusion. CBD extraction via heated infusion oil significantly increased the TPC of hemp oil, MCT oil, and olive oil. The data suggests that the phenolics present in *Cannabis* are able to be absorbed by oil that is heated with decarboxylated *Cannabis,* thus increasing the preexisting total phenolic content of the oil.

2.4.3 Total Phenolic Content vs Antioxidant Potential

TPC and TEAC values were plotted against one another, as seen in Figure III, to look for correlations between the total phenolic content and antioxidant potential of the oils. Olive CBD oil showed the highest total phenolic content values and showed antioxidant potential values between those of hemp CBD oil and MCT CBD oil. Hemp CBD oil showed the highest antioxidant potential values and showed total phenolic content values between those of olive CBD oil and MCT CBD oil. MCT CBD oil showed the lowest total phenolic content values and antioxidant potential values amongst the CBD oils. All CBD oils had significantly increased total phenolic content and antioxidant potential values from their respective control oils, except for the antioxidant potential values of hemp oil. It is possible that the antioxidant potential of the hemp CBD oil did not significantly increase because the hemp control oil already had a high antioxidant potential. This suggests that there may be a threshold to the antioxidant potential in an oil and adding an antioxidant source such as cannabinoids to an oil with high antioxidant potential may not cause a significant increase in antioxidant potential.

According to the correlation curve in Figure III, hemp control oil can be considered an outlier amongst the results. Hemp control oil had a lower total phenolic content than expected based on its high antioxidant potential. It was expected that a high antioxidant potential would correlate with a high total phenolic content, but this was not shown for the hemp control oil. The low total phenolic content of the hemp control oil was unexpected because hemp oil is known to have a high total phenolic content according to other studies (Yu, Zhou, & Parry, 2005) (Teh & Birch, 2013) even under heating conditions (Liang et al., 2018). Since different plant material oils requires different solvent type for maximum extraction of phenolic compounds (Venkatesan, Choi, & Kim, 2019) (Paradiso, et al., 2016), it is possible that the methanol solvent did not

provide optimal phenolic extraction of the hemp oil. Further research is needed to assess the use of various solvents for hemp oil phenolic extraction in order to determine if solvent type causes significant differences in total phenolic content results of hemp oil.

2.5 Conclusion

The results of the antioxidant potential and total phenolic content assays indicated that *Cannabis* infused in hemp oil had the greatest antioxidant potential than the other CBD oils, and *Cannabis* infused in olive oil had the highest total phenolic content than the other CBD oils. *Cannabis* infusion caused a significant increase in antioxidant potential and total phenolic content for all oils, except for the antioxidant potential of hemp oil. Further research to identify and compare the antioxidant profiles of the CBD oils to the control oils would allow for more insight as to which antioxidants in *Cannabis* are responsible for the increase in antioxidant potential and total phenolic content of the oils. Additionally, identifying the antioxidants may provide an explanation as to why the total phenolic content of hemp control oil is low compared to its high antioxidant potential.

2.6 Tables and Figures

Table I. Antioxidant Potential Results

Sample	TEAC (umol Trolox / 1L oil) +/- SD
Hemp CBD	1159.34**A** +/- 83.38
Hemp Control	1190.33**A** +/- 134.69
MCT CBD	966.75**B** +/- 79.44
MCT Control	283.83**C** +/- 97.81
Olive CBD	1095.79**D** +/- 78.78
Olive Control	910.43**B** +/- 106.43

Samples in rows without the same letter are significantly different ($p<0.05$). Significance determined with one-way ANOVA Tukey's Test with a consideration of oil type (n=3). Samples were assessed in triplicate and standard deviation (SD) is shown.

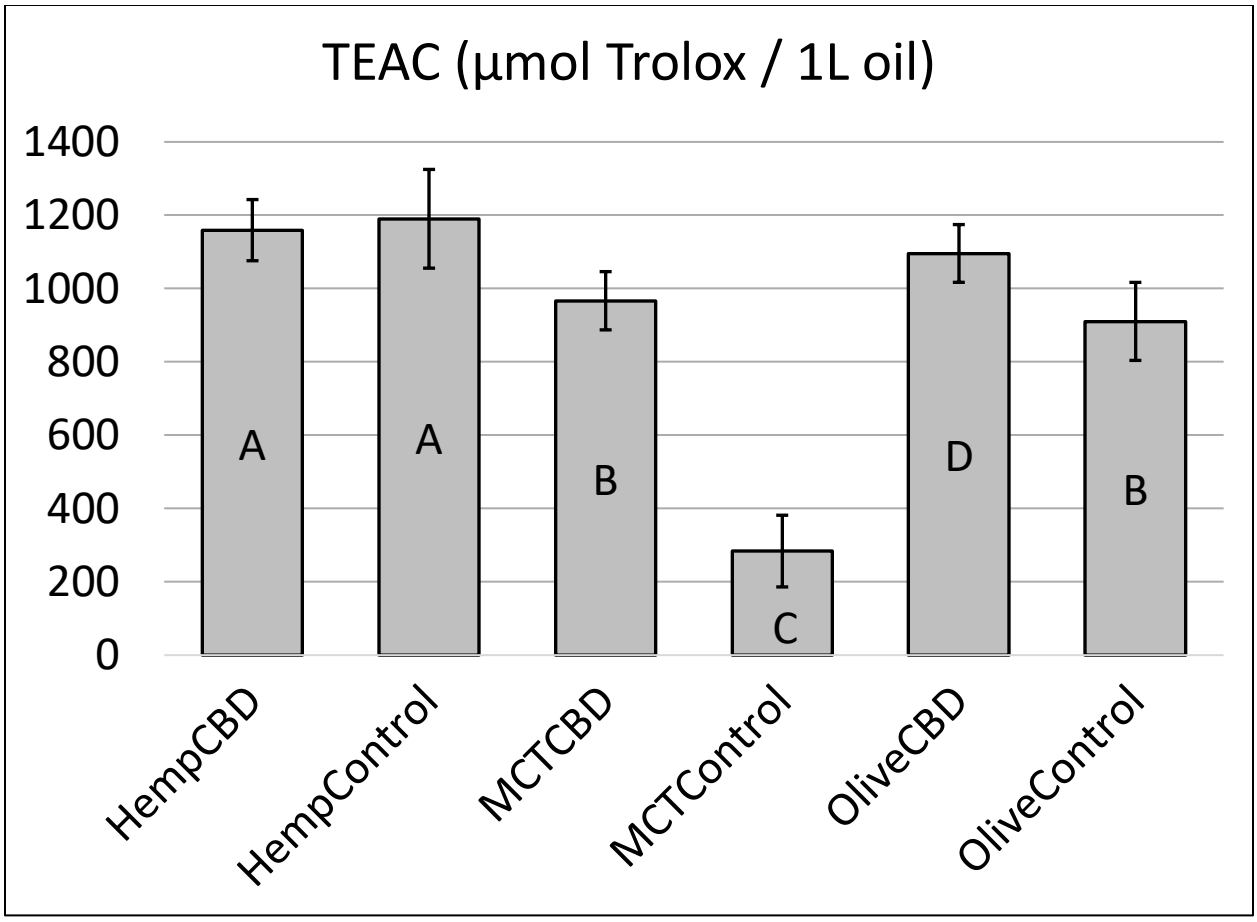

Figure I. Antioxidant Potential Results

Samples in columns without the same letter are significantly different ($p<0.05$). Significance determined with one-way ANOVA Tukey's Test with a consideration of oil type (n=3). Samples were assessed in triplicate and standard deviation (SD) is shown.

Table II. Total Phenolic Content Results

Sample	TPC (mg GAE / 1L oil) +/- SD
Hemp CBD	121.37**A** +/- 28.54
Hemp Control	27.57**B** +/- 3.21
MCT CBD	34.06**B** +/- 8.47
MCT Control	4.67**C** +/- 3.80
Olive CBD	184.62**D** +/- 29.19
Olive Control	110.68**A** +/- 16.83

Samples in rows without the same letter are significantly different ($p<0.05$). Significance determined with one-way ANOVA Tukey's Test with a consideration of oil type (n=3). Samples were assessed in triplicate and standard deviation (SD) is shown.

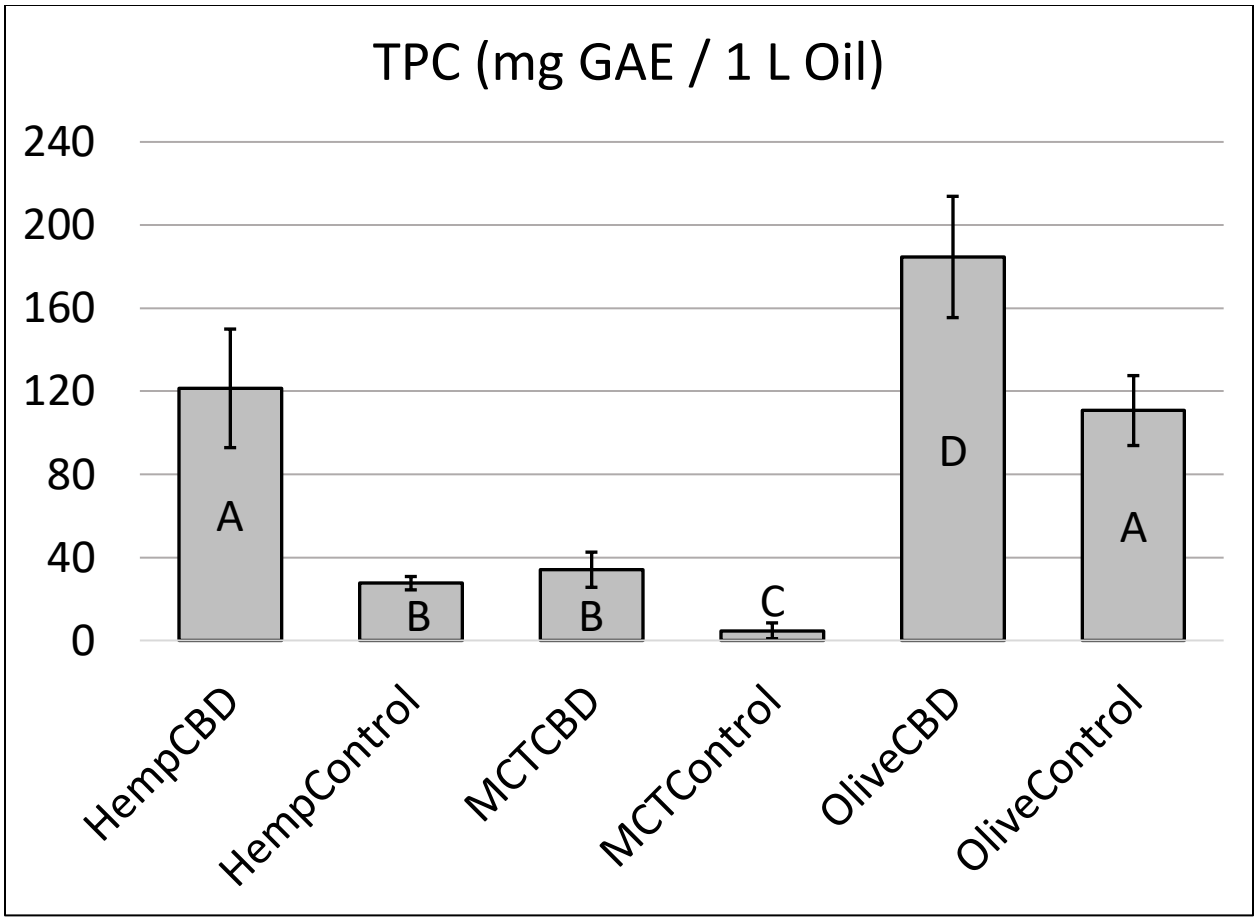

Figure II. Total Phenolic Content Results

Samples in columns without the same letter are significantly different ($p<0.05$). Significance determined with one-way ANOVA Tukey's Test with a consideration of oil type (n=3). Samples were assessed in triplicate and standard deviation (SD) is shown.

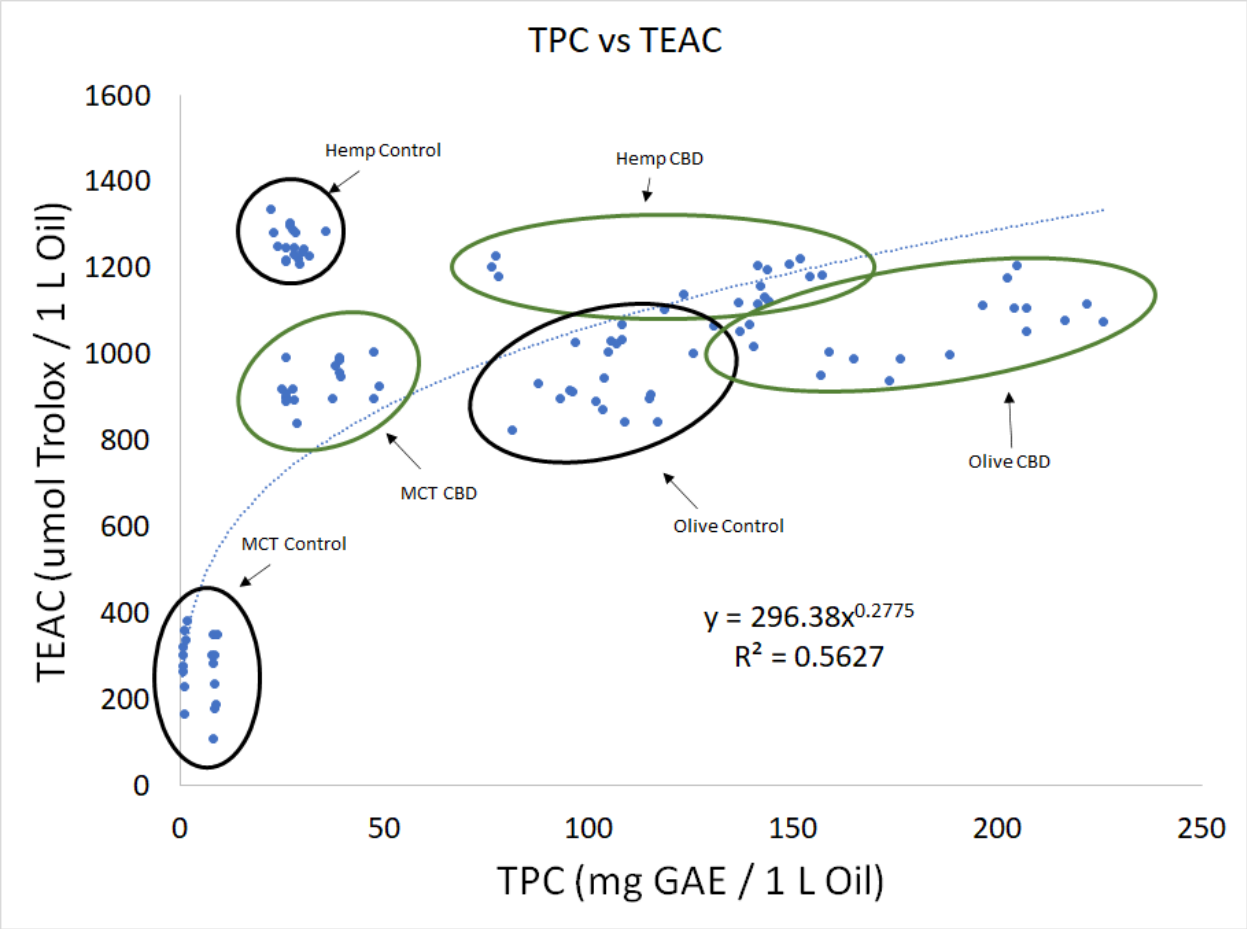

Figure III. Comparison of Total Phenolic Content (TPC) and Antioxidant Potential (TEAC)

A positive correlation curve is shown.

2.7 References

Andre, C.M., Hausman, J.F., & Guerriero, G. (2016). Cannabis sativa: the plant of the thousand and one molecules. *Frontiers in Plant Science, 7* (19), 1-17. doi: 10.3389/fpls.2016.00019

Chandra, S., Lata, H., Elsohly, M. A., Walker, L. A., & Potter, D. (2017). Cannabis cultivation: methodological issues for obtaining medical-grade product. *Epilepsy & Behavior, 70*, 302–312. doi: 10.1016/j.yebeh.2016.11.029

Corroon, J. & Felice, J. (2019). The endocannabinoid system and its modulation by cannabidiol (CBD). *Alternative Therapies in Health and Medicine, 25*(2), 6-14.

Deidda, R., Avohou, H. T., Baronti, R., Davolio, P. L., Pasquini, B., Bubba, M. D., Furlanetto, S. (2019). Analytical quality by design: Development and control strategy for a LC method to evaluate the cannabinoids content in cannabis olive oil extracts. *Journal of Pharmaceutical and Biomedical Analysis, 166*, 326–335. doi: 10.1016/j.jpba.2019.01.032

Freeman, T. P., Hindocha, C., Green, S. F., & Bloomfield, M. A. P. (2019). Medicinal use of cannabis based products and cannabinoids. *British Medical Journal, 365*(1141). doi: 10.1136/bmj.11141

Grijó, D. R., Osorio, I. A. V., & Cardozo-Filho, L. (2018). Supercritical extraction strategies using CO2 and ethanol to obtain cannabinoid compounds from Cannabis hybrid flowers. *Journal of CO2 Utilization, 28*, 174–180. doi: 10.1016/j.jcou.2018.09.022

Klumpers, L. E., & Thacker, D. L. (2019). A brief background on cannabis: from plant to medical indications. *Journal of AOAC International, 102*(2), 412–420. doi: 10.5740/jaoacint.18-0208

Liang, J., Aachary, A. A., Hydamaka, A., Eskin, N. A. M., Eck, P., & Thiyam-Holländer, U. (2018). Reduction of Chlorophyll in Cold-Pressed Hemp (Cannabis sativa) Seed Oil by Ultrasonic Bleaching and Enhancement of Oxidative Stability. *European Journal of Lipid Science and Technology, 120*(4), 1700349. doi: 10.1002/ejlt.201700349

Paradiso, V. M., Clemente, A., Summo, C., Pasqualone, A., & Caponio, F. (2016). Towards green analysis of virgin olive oil phenolic compounds: Extraction by a natural deep eutectic solvent and direct spectrophotometric detection. *Food Chemistry,212*, 43-47. doi:10.1016/j.foodchem.2016.05.082

Romano, L. L., & Hazekamp, A. (2013). Cannabis oil: chemical evaluation of an upcoming cannabis-based medicine. *Cannabinoids, 1*(1), 1–11. Retrieved from http://www.cannabismed.org/index.php?tpl=cannabinoids

Sahin, S., Isik, E., & Demir, C. (2012). Prediction of total phenolic content in extracts of prunella species from HPLC profiles by multivariate calibration. *ISRN Chromatography, 2012*, 1–7. doi: 10.5402/2012/120780

Szydlowskaczerniak, A., Dianoczki, C., Recseg, K., Karlovits, G., & Szlyk, E. (2008). Determination of antioxidant capacities of vegetable oils by ferric-ion spectrophotometric methods. *Talanta, 76*(4), 899–905. doi: 10.1016/j.talanta.2008.04.055

Teh, S.-S., & Birch, J. (2013). Physicochemical and quality characteristics of cold-pressed hemp, flax and canola seed oils. *Journal of Food Composition and Analysis, 30*(1), 26–31. doi: 10.1016/j.jfca.2013.01.004

Tura, M., Mandrioli, M., & Toschi, T. G. (2019). Preliminary study: comparison of antioxidant activity of cannabidiol (CBD) and α-tocopherol added to refined olive and sunflower oils. *Molecules, 24*(19), 1–15. doi: 10.3390/molecules24193485

Venkatesan, T., Choi, Y., & Kim, Y. (2019). Impact of Different Extraction Solvents on Phenolic Content and Antioxidant Potential of Pinus densiflora Bark Extract. *BioMed Research International,2019*, 1-14. doi:10.1155/2019/3520675

Yu, L. L., Zhou, K. K., & Parry, J. (2005). Antioxidant properties of cold-pressed black caraway, carrot, cranberry, and hemp seed oils. *Food Chemistry, 91*(4), 723–729. doi: 10.1016/j.foodchem.2004.06.044

3. Manuscript II

Cannabidiol (CBD) Content of *Cannabis* Infused Oils

3.1 Abstract

Cannabinoids, specifically cannabidiol (CBD), can be extracted from *Cannabis* by infusing decarboxylated *Cannabis* plant material in heated plant-based cooking oil. The CBD content of the *Cannabis* infused oils can be measured in order to help determine the type of oil that is most efficient at extracting physiologically beneficial CBD. Hemp oil, MCT oil, and olive oil were used as infusion solvents for a high-CBD low-THC strain of decarboxylated *Cannabis*. The resulting *Cannabis* oils, referred to as CBD oils, were assessed for CBD content via high-performance liquid chromatography with ultraviolet detection (HPLC-UV). Hemp CBD oil and olive CBD oil had higher CBD content compared to MCT CBD oil, thus hemp oil and olive oil had a greater ability at extracting CBD from *Cannabis* than MCT oil.

3.2 Introduction

One of the most studied plants of interest, with continually growing research in recent years, has been the *Cannabis* plant. *Cannabis* is commonly cultivated for the extraction and use of its molecular constituents. The main constituents of focus are biologically active fatty acid compounds known as cannabinoids, including cannabidiol (CBD) (Klumpers & Thacker, 2019). Cannabinoids have been shown to possess beneficial characteristics such as antioxidant, anti-inflammatory, anti-fungal, and anti-bacterial abilities as well as therapeutic benefits for mental health disorders, neurological and movement disorders, pain, autoimmune diseases, spinal cord injury, cancer, cardiometabolic disease, stroke, and others (Andre, Hausman, & Guerriero, 2016) (Corroon & Felice, 2019).

In recent years, the *Cannabis* industry has narrowed its focus on a particular cannabinoid known as cannabidiol or CBD. CBD is one of the most highly studied cannabinoids of *Cannabis* along with tetrahydrocannabinol or THC. The *Cannabis* industry has become a rapidly growing force because of the realization that the beneficial cannabinoids of *Cannabis* can be present without the psychoactive component of THC, which is illegal in most states. There is an abundant desire to reap the benefits of CBD without being accompanied by the cognitive "high" that is experienced from naturally-occurring THC in the *Cannabis* plant (Andre, Hausman, & Guerriero, 2016) (Klumpers & Thacker, 2019). There are certain strains of *Cannabis* that have a high CBD content and a very low THC content that are commonly used to make CBD products.

CBD can be extracted from *Cannabis* flowers and used in products such as topical oils and consumable oil tinctures (Freeman et al., 2019). CBD extraction can be achieved through the use of high-tech methods such as supercritical CO_2 extraction and solvent-based extraction or a simpler method such as infusing *Cannabis* flowers in an edible plant-based oil (Romano &

Hazekamp, 2013). CO_2 extraction has been shown to be the most efficient method for extracting CBD from *Cannabis* (Chandra et al., 2017), but this method is not available for all members of the *Cannabis* industry. The widely available method of infusing *Cannabis* in heated oil for a prolonged period of time has been shown to allow the CBD to dissolve into the oil base (Deidda et al., 2019).

More research is needed to verify the efficiency of CBD extraction via oil infusion in terms of extracting the most CBD content. Research is needed to compare the ability of different oils and different methodology to extract CBD from *Cannabis*. This can be measured by evaluating the CBD content that is present in various plant-based oils that have been infused with *Cannabis*.

3.3 Materials and Methods

3.3.1 Plant Material and Oil Type

All *Cannabis* flowers used in this study were obtained from Blessed Land Farm. The species of plant is a *Cannabis sativa* dominant hybrid and the strain is Baox, recognized as a high-CBD low-THC strain. This farm is a registered grower in accordance with the Industrial Hemp Program beginning on the date of March 1, 2019. As part of registration, the farm is certified that the hemp seeds obtained for planting are a type and variety that do not exceed the maximum concentration of THC. Once received, the *Cannabis* flowers were vacuum sealed and stored at -81˚C until use. When needed, *Cannabis* packages were removed from the deep freezer and used immediately for oil infusion. The three infusion oils used in this study were 365 Everyday Value cold processed extra virgin olive oil, 365 Everyday Value MCT oil from fractionated expeller pressed virgin coconut oil, and Manitoba Harvest unrefined cold pressed hemp seed oil.

3.3.2 Cannabidiol (CBD) Oil Sample Preparation

Upon thawing from the freezer, *Cannabis* flowers were decarboxylated in an Isotemp oven set at 140˚C for 30 minutes, turning the buds over at 15 minutes. Decarboxylation methods were determined based on parameters for maximum CBD extraction as modeled in a previous study (Grijó, Osorio, & Cardozo-Filho, 2018). Stems were removed and the flowers were ground to a powder using a mortar and pestle. *Cannabis* powder was measured to 2.83g and placed in 94.6mL of designated oil. The oils containing *Cannabis* were heated in a glass beaker on a Corning hot plate set at 90˚C and agitated with a star bar at 200rpm for 3 hours. The oil was strained using Bolio organic hemp cloth #4 coffee filters to separate the oil from the *Cannabis* powder. The oil was centrifuged for 10 minutes at 1000rpm to separate the oil mixture and any remaining plant matter. The supernatant oil, referred to as CBD oil, was collected and stored in the deep freezer until further use. The extraction oils used were also heated and stored under the same parameters without *Cannabis* to be used as a control to compare to the *Cannabis* oil extracts. All samples were prepared in triplicate.

3.3.3 High-Performance Liquid Chromatography with Ultraviolet Detection (HPLC-UV)

To analyze CBD content of the *Cannabis* infused oil samples, high-performance liquid chromatography with ultraviolet detection (HPLC-UV) was used. HPLC-UV involves separation technology (HPLC) and detection technology (UV). Ultraviolet detection is the most frequently used detection method for the analysis of cannabinoids in plant materials (Crowley, 2020) (Citti et al., “Pharmaceutical and Biomedical…”, 2018) (Brighenti et al., 2017). This method allowed for the identification and quantification of CBD present in the oil samples. High pressure was used to move the CBD oil sample through a chromatographic column in order to separate the cannabinoids within the sample. Each cannabinoid was then exposed to UV light to detect

differences in molecular structure through varying absorption of UV wavelengths, thus allowing CBD to be detected and quantified.

3.3.4 Statistical Analysis

Statistical Package for Social Sciences (SPSS) was used to perform one-way ANOVA with Tukey's Test to determine significant differences between samples.

3.4 Results and Discussion

3.4.1 Cannabidiol (CBD) Content

CBD content results for all samples are shown in Figure I and Table I. MCT CBD oil showed significantly lower CBD content (M=4.23) than olive CBD oil (M=4.50) ($p<0.005$) and hemp CBD oil (M=4.45) ($p<0.01$), and the CBD content of olive CBD oil and hemp CBD oil were not significantly different ($p=0.553$). It is possible that the difference in CBD content extraction was due to the differing fatty acid profiles of the oils. Hemp oil and olive oil mainly consist of long-chain unsaturated fatty acids whereas MCT oil consists of medium-chain saturated fatty acids. The long length of fatty acids of hemp and olive oil have longer nonpolar hydrocarbon chains than the shorter nonpolar hydrocarbon chains of MCT oil; these chains also contain a polar end. A longer fatty acid chain has a higher proportion of nonpolar chain compared to polar end than a shorter fatty acid chain with a shorter nonpolar chain. Therefore, hemp and olive oil have a slightly lower polarity than MCT oil, making them a more complimentary solvent for nonpolar CBD (Borges, et al., 2013) (Cai, et al., 2019). The lower polarity of hemp and olive oil may allow a greater proportion of nonpolar CBD molecules to infuse into the oil based on the greater similarity in polarity compared to the more polar MCT oil (Aeschbach, et al., 1994). Differing polarities between MCT oil and CBD may inhibit CBD extraction success.

3.5 Conclusion

CBD content results showed that hemp oil and olive oil had significantly greater CBD extraction ability than MCT oil. Further research should be implemented to compare CBD extraction ability of hemp and olive oil to various other plant-based oils to determine an optimal oil for CBD extraction via infusion. Additionally, testing CBD extraction ability of oils of varying fatty acid profiles and chemical composition may provide insight into the most favorable oil composition for successful maximum CBD extraction. Various CBD oil preparation protocols, such as decarboxylation, infusion oil quantities, and heating temperature, should also be studied in order to determine a thorough protocol that results in optimal CBD content when infusing *Cannabis* in oil. This research is intended to promote a need for structured protocol for small-scale commercial/non-commercial CBD oil, which tends to be formulated by mixing *Cannabis* with heated oil. The homemade CBD oil that is sold on a smaller commercial scale is a contributing factor to the CBD industry, and more research is needed in this area.

3.6 Tables and Figures

Table I. Cannabidiol (CBD) Content Results

Sample	CBD (mg/g) +/- SD
Hemp CBD	4.45**A** +/- 0.10
MCT CBD	4.23**B** +/- 0.01
Olive CBD	4.50**A** +/- 0

Samples in rows without the same letter are significantly different ($p<0.05$). Significance determined with one-way ANOVA Tukey's Test with a consideration of oil type (n=3). Samples were assessed in triplicate and standard deviation (SD) is shown.

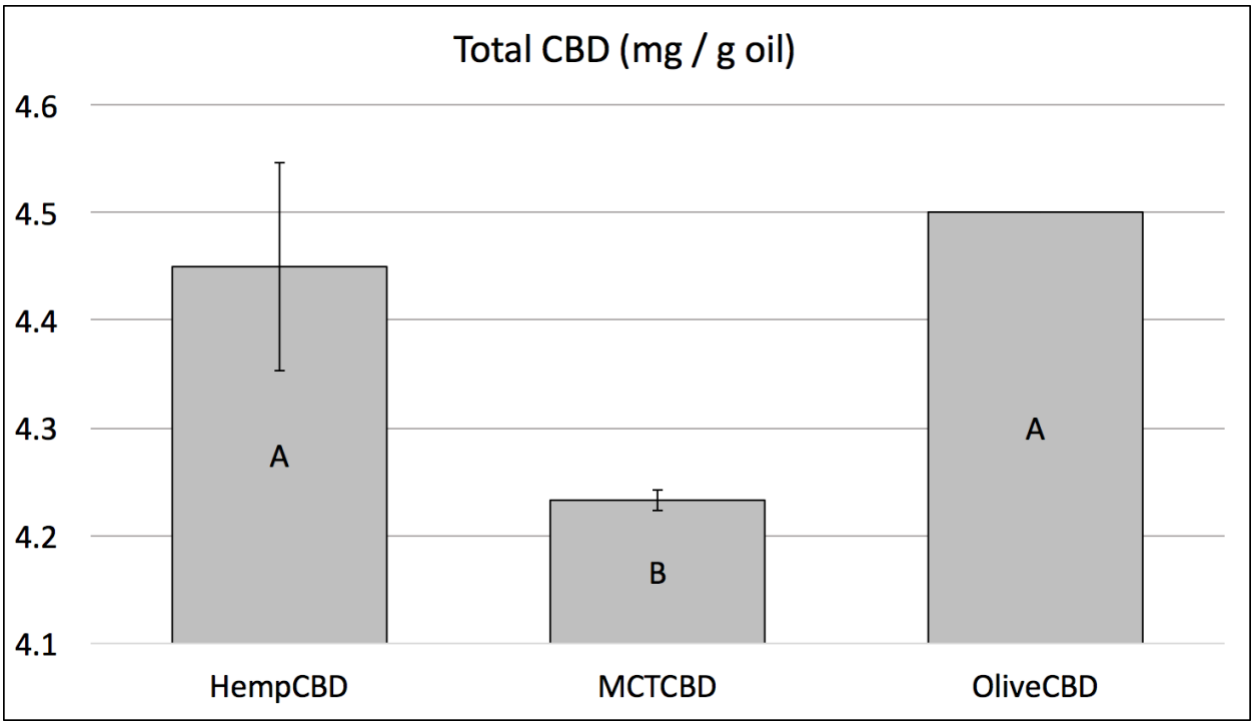

Figure I. Cannabidiol (CBD) Content Results

Samples in columns without the same letter are significantly different ($p<0.05$). Significance determined with one-way ANOVA Tukey's Test with a consideration of oil type (n=3). Samples were assessed in triplicate and standard deviation is shown.

3.7 References

Aeschbach, R., Büchler, R., Rossi, P., Sandoz, L., & Wille, H. J. (1994). Mechanical Extraction of Plant Antioxidants by Means of Oils. *Fett Wissenschaft Technologie/Fat Science Technology,96*(11), 441-443. doi:10.1002/lipi.19940961111

Andre, C.M., Hausman, J.F., & Guerriero, G. (2016). Cannabis sativa: the plant of the thousand and one molecules. *Frontiers in Plant Science, 7* (19), 1-17. doi: 10.3389/fpls.2016.00019

Borges, R., Batista, J., Viana, R., Baetas, A., Orestes, E., Andrade, M., Honório, K., Silva, A. D. (2013). Understanding the Molecular Aspects of Tetrahydrocannabinol and Cannabidiol as Antioxidants. *Molecules,18*(10), 12663-12674. doi:10.3390/molecules181012663

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

Cai, C., Yu, W., Wang, C., Liu, L., Li, F., & Tan, Z. (2019). Green extraction of cannabidiol from industrial hemp (Cannabis sativa L.) using deep eutectic solvents coupled with further enrichment and recovery by macroporous resin. *Journal of Molecular Liquids,287*, 1-9. doi:10.1016/j.molliq.2019.110957

Chandra, S., Lata, H., Elsohly, M. A., Walker, L. A., & Potter, D. (2017). Cannabis cultivation: methodological issues for obtaining medical-grade product. *Epilepsy & Behavior, 70*, 302–312. doi: 10.1016/j.yebeh.2016.11.029

Citti, C., Braghiroli, D., Vandelli, M. A., & Cannazza, G. (2018). Pharmaceutical and biomedical analysis of cannabinoids: A critical review. *Journal of Pharmaceutical and Biomedical Analysis, 147*, 565–579. doi: 10.1016/j.jpba.2017.06.003

Corroon, J. & Felice, J. (2019). The endocannabinoid system and its modulation by cannabidiol (CBD). *Alternative Therapies in Health and Medicine, 25*(2), 6-14.

Crowley, T. E. (2020). *Purification and characterization of secondary metabolites: a laboratory manual for analytical and structural biochemistry*. Academic Press.

Deidda, R., Avohou, H. T., Baronti, R., Davolio, P. L., Pasquini, B., Bubba, M. D., Furlanetto, S. (2019). Analytical quality by design: Development and control strategy for a LC method to evaluate the cannabinoids content in cannabis olive oil extracts. *Journal of Pharmaceutical and Biomedical Analysis, 166*, 326–335. doi: 10.1016/j.jpba.2019.01.032

Freeman, T. P., Hindocha, C., Green, S. F., & Bloomfield, M. A. P. (2019). Medicinal use of cannabis based products and cannabinoids. *British Medical Journal, 365*(1141). doi: 10.1136/bmj.11141

Grijó, D. R., Osorio, I. A. V., & Cardozo-Filho, L. (2018). Supercritical extraction strategies using CO2 and ethanol to obtain cannabinoid compounds from Cannabis hybrid flowers. *Journal of CO2 Utilization, 28,* 174–180. doi: 10.1016/j.jcou.2018.09.022

Klumpers, L. E., & Thacker, D. L. (2019). A brief background on cannabis: from plant to medical indications. *Journal of AOAC International, 102*(2), 412–420. doi: 10.5740/jaoacint.18-0208

Romano, L. L., & Hazekamp, A. (2013). Cannabis oil: chemical evaluation of an upcoming cannabis-based medicine. *Cannabinoids, 1*(1), 1–11. Retrieved from http://www.cannabismed.org/index.php?tpl=cannabinoids

4. Conclusion

This data showed that *Cannabis* infused in oil had greater magnitudes of antioxidant potential for hemp oil, higher magnitudes of total phenolic content for olive oil, and higher magnitudes of CBD content for hemp and olive oil. *Cannabis* infusion caused a significant increase in antioxidant potential, total phenolic content, and CBD content for all oils, except for the antioxidant potential of hemp oil. Further research into the identification of antioxidants in hemp oil would help to establish why hemp oil expressed the highest antioxidant potential but a low phenolic content. Additionally, research in this field should be continued to establish a standardized protocol for *Cannabis* infused oils that takes into consideration antioxidant potential, total phenolic content, and CBD content. This research is intended to provide insight on *Cannabis* infused oils and promote a need for structured protocol for CBD oil produced via this method.

5. References

Aeschbach, R., Büchler, R., Rossi, P., Sandoz, L., & Wille, H. J. (1994). Mechanical Extraction of Plant Antioxidants by Means of Oils. *Fett Wissenschaft Technologie/Fat Science Technology,96*(11), 441-443. doi:10.1002/lipi.19940961111

Andre, C.M., Hausman, J.F., & Guerriero, G. (2016). Cannabis sativa: the plant of the thousand and one molecules. *Frontiers in Plant Science, 7* (19), 1-17. doi: 10.3389/fpls.2016.00019

Borges, R., Batista, J., Viana, R., Baetas, A., Orestes, E., Andrade, M., Honório, K., Silva, A. D. (2013). Understanding the Molecular Aspects of Tetrahydrocannabinol and Cannabidiol as Antioxidants. *Molecules,18*(10), 12663-12674. doi:10.3390/molecules181012663

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

Cai, C., Yu, W., Wang, C., Liu, L., Li, F., & Tan, Z. (2019). Green extraction of cannabidiol from industrial hemp (Cannabis sativa L.) using deep eutectic solvents coupled with further enrichment and recovery by macroporous resin. *Journal of Molecular Liquids,287*, 1-9. doi:10.1016/j.molliq.2019.110957

Chandra, S., Lata, H., Elsohly, M. A., Walker, L. A., & Potter, D. (2017). Cannabis cultivation: methodological issues for obtaining medical-grade product. *Epilepsy & Behavior*, *70*, 302–312. doi: 10.1016/j.yebeh.2016.11.029

Citti, C., Braghiroli, D., Vandelli, M. A., & Cannazza, G. (2018). Pharmaceutical and biomedical analysis of cannabinoids: A critical review. *Journal of Pharmaceutical and Biomedical Analysis, 147*, 565–579. doi: 10.1016/j.jpba.2017.06.003

Citti, C., Pacchetti, B., Vandelli, M. A., Forni, F., & Cannazza, G. (2018). Analysis of cannabinoids in commercial hemp seed oil and decarboxylation kinetics studies of cannabidiolic acid (CBDA). *Journal of Pharmaceutical and Biomedical Analysis, 149*, 532–540. doi: 10.1016/j.jpba.2017.11.044

Commissioner, O. of the. (2020). FDA Regulation of Cannabis and Cannabis-Derived Products: Q&A. Retrieved from https://www.fda.gov/news-events/public-health-focus/fda-regulation-cannabis-and-cannabis-derived-products-including-cannabidiol-cbd

Corroon, J. & Felice, J. (2019). The endocannabinoid system and its modulation by cannabidiol (CBD). *Alternative Therapies in Health and Medicine, 25*(2), 6-14.

Corroon, J., & Phillips, J. A. (2018). A Cross-Sectional Study of Cannabidiol Users. *Cannabis and Cannabinoid Research,3*(1), 152-161. doi:10.1089/can.2018.0006

Crowley, T. E. (2020). *Purification and characterization of secondary metabolites: a laboratory manual for analytical and structural biochemistry*. Academic Press.

Deidda, R., Avohou, H. T., Baronti, R., Davolio, P. L., Pasquini, B., Bubba, M. D., Furlanetto, S. (2019). Analytical quality by design: Development and control strategy for a LC method to evaluate the cannabinoids content in cannabis olive oil extracts. *Journal of Pharmaceutical and Biomedical Analysis, 166*, 326–335. doi: 10.1016/j.jpba.2019.01.032

Elkins, A. C., Deseo, M. A., Rochfort, S., Ezernieks, V., & Spangenberg, G. (2019). Development of a validated method for the qualitative and quantitative analysis of cannabinoids in plant biomass and medicinal cannabis resin extracts obtained by super-critical fluid extraction. *Journal of Chromatography B, 1109*, 76–83. doi: 10.1016/j.jchromb.2019.01.027

Fares, M. (2018). Medical cannabis: where do we stand today? *American Medical Writers Association Journal, 33*(2), 63–67. Retrieved from https://www.amwa.org/

Fiorini, D., Molle, A., Nabissi, M., Santini, G., Benelli, G., & Maggi, F. (2019). Valorizing industrial hemp (Cannabis sativa L.) by-products: Cannabidiol enrichment in the inflorescence essential oil optimizing sample pre-treatment prior to distillation. *Industrial Crops and Products, 128*, 581–589. doi: 10.1016/j.indcrop.2018.10.045

Freeman, T. P., Hindocha, C., Green, S. F., & Bloomfield, M. A. P. (2019). Medicinal use of cannabis based products and cannabinoids. *British Medical Journal, 365*(1141). doi: 10.1136/bmj.11141

Friedman, D., & Sirven, J. I. (2017). Historical perspective on the medical use of cannabis for epilepsy: ancient times to the 1980s. *Epilepsy & Behavior, 70*, 298–301. doi: 10.1016/j.yebeh.2016.11.033

Grijó, D. R., Osorio, I. A. V., & Cardozo-Filho, L. (2018). Supercritical extraction strategies using CO2 and ethanol to obtain cannabinoid compounds from Cannabis hybrid flowers. *Journal of CO2 Utilization, 28*, 174–180. doi: 10.1016/j.jcou.2018.09.022

Hampson, A. J., Grimaldi, M., Axelrod, J., & Wink, D. (1998). Cannabidiol and (Δ) 9-tetrahydrocannabinol are neuroprotective antioxidants. *Proceedings of the National Academy of Sciences, 95*(14), 8268–8273. doi: 10.1073/pnas.95.14.8268

Klumpers, L. E., & Thacker, D. L. (2019). A brief background on cannabis: from plant to medical indications. *Journal of AOAC International, 102*(2), 412–420. doi: 10.5740/jaoacint.18-0208

Křížek, T., Bursová, M., Horsley, R., Kuchař, M., Tůma, P., Čabala, R., & Hložek, T. (2018). Menthol-based hydrophobic deep eutectic solvents: towards greener and efficient extraction of phytocannabinoids. *Journal of Cleaner Production, 193*, 391–396. doi: 10.1016/j.jclepro.2018.05.080

Lachenmeier, D. W., Kroener, L., Musshoff, F., & Madea, B. (2004). Determination of cannabinoids in hemp food products by use of headspace solid-phase microextraction and gas chromatography-mass spectrometry. *Analytical and Bioanalytical Chemistry, 378*(1), 183–189. doi: 10.1007/s00216-003-2268-4

Leas, E. C., Nobles, A. L., Caputi, T. L., Dredze, M., Smith, D. M., & Ayers, J. W. (2019). Trends in Internet Searches for Cannabidiol (CBD) in the United States. *JAMA Network Open,2*(10). doi:10.1001/jamanetworkopen.2019.13853

Liang, J., Aachary, A. A., Hydamaka, A., Eskin, N. A. M., Eck, P., & Thiyam-Holländer, U. (2018). Reduction of Chlorophyll in Cold-Pressed Hemp (Cannabis sativa) Seed Oil by Ultrasonic Bleaching and Enhancement of Oxidative Stability. *European Journal of Lipid Science and Technology, 120*(4), 1700349. doi: 10.1002/ejlt.201700349

Maida, V., & Daeninck, P. (2016). A user's guide to cannabinoid therapies in oncology. *Current Oncology, 23*(6), 398–406. doi: 10.3747/co.23.3487

Paradiso, V. M., Clemente, A., Summo, C., Pasqualone, A., & Caponio, F. (2016). Towards green analysis of virgin olive oil phenolic compounds: Extraction by a natural deep eutectic solvent and direct spectrophotometric detection. *Food Chemistry,212*, 43-47. doi:10.1016/j.foodchem.2016.05.082

Romano, L. L., & Hazekamp, A. (2013). Cannabis oil: chemical evaluation of an upcoming cannabis-based medicine. *Cannabinoids, 1*(1), 1–11. Retrieved from http://www.cannabismed.org/index.php?tpl=cannabinoids

Rovetto, L. J., & Aieta, N. V. (2017). Supercritical carbon dioxide extraction of cannabinoids from Cannabis sativa L. *The Journal of Supercritical Fluids, 129*, 16–27. doi: 10.1016/j.supflu.2017.03.014

Russo, E. B. (2011). Taming THC: potential cannabis synergy and phytocannabinoid-terpenoid entourage effects. *British Journal of Pharmacology, 163*(7), 1344–1364. doi: 10.1111/j.1476-5381.2011.01238.x

Sahin, S., Isik, E., & Demir, C. (2012). Prediction of total phenolic content in extracts of prunella species from HPLC profiles by multivariate calibration. *ISRN Chromatography, 2012*, 1–7. doi: 10.5402/2012/120780

Szydlowskaczerniak, A., Dianoczki, C., Recseg, K., Karlovits, G., & Szlyk, E. (2008). Determination of antioxidant capacities of vegetable oils by ferric-ion spectrophotometric methods. *Talanta, 76*(4), 899–905. doi: 10.1016/j.talanta.2008.04.055

Teh, S.-S., & Birch, J. (2013). Physicochemical and quality characteristics of cold-pressed hemp, flax and canola seed oils. *Journal of Food Composition and Analysis, 30*(1), 26–31. doi: 10.1016/j.jfca.2013.01.004

Tura, M., Mandrioli, M., & Toschi, T. G. (2019). Preliminary study: comparison of antioxidant activity of cannabidiol (CBD) and α-tocopherol added to refined olive and sunflower oils. *Molecules, 24*(19), 1–15. doi: 10.3390/molecules24193485

Venkatesan, T., Choi, Y., & Kim, Y. (2019). Impact of Different Extraction Solvents on Phenolic Content and Antioxidant Potential of Pinus densiflora Bark Extract. *BioMed Research International,2019*, 1-14. doi:10.1155/2019/3520675

Yu, L. L., Zhou, K. K., & Parry, J. (2005). Antioxidant properties of cold-pressed black caraway, carrot, cranberry, and hemp seed oils. *Food Chemistry, 91*(4), 723–729. doi: 10.1016/j.foodchem.2004.06.044

Zou, S. & Kumar, U. (2018). Cannabinoid receptors and the endocannabinoid system: signaling and function in the central nervous system. *International Journal of Molecular Sciences, 19* (833), 1-23. doi: 10.3390/ijms19030833

Zuardi, A. W. (2008). Cannabidiol: from an inactive cannabinoid to a drug with wide spectrum of action. *Revista Brasileira De Psiquiatria, 30*(3), 271–280. doi: 10.1590/s1516-44462008000300015

Żuk-Gołaszewska, K. & Gołaszewski, J. (2018). Cannabis sativa L. – cultivation and quality of raw material. *Journal of Elementology, 23*(3), 971–984. doi: 10.5601/jelem.2017.22.3.1500