Hemp Microgreen Mineral Content, Cannabinoids, Total Phenolics, and Antioxidants

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HEMP MICROGREEN MINERAL CONTENT, CANNABINOIDs, TOTAL PHENOLICS, AND ANTIOXIDANTS

A Thesis
Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Master of Science

in

The School of Plant, Environmental and Soil Sciences

by
Cheston Tyler Schayot
B.I.S, Louisiana State University 2017
December 2021
ACKNOWLEDGEMENTS

With much gratefulness, thank you to my committee members. To Dr. Ted Gauthier, who is always respectful and professional in communication and has taught me many practical things in the areas of chemistry and biology, and to Dr. John Beaulieu, who has provided substantial guidance and thoughtful input during my time as a graduate student.

Thank you to my immediate and extended family who have been continually supportive throughout my college and professional career. Thank you to my girlfriend, Marina, for your love, support, and understanding which has uplifted me during challenging times.

Thank you to my superiors, colleagues, and university affiliates who have guided and assisted me as both an undergraduate and graduate student. Specifically, Mr. Holltman Florez, Mr. Rene Montellano, Ms. Kaylee Deynzer, Ms. Sandeep Sran, Ms. Celine Richard, Mr. Taylor Bryant, Mr. Calvin Glaspie, Mr. Robert Mirabello, Dr. Kathryn Fontenot, Dr. Edward “Ed” Bush, Dr. Heather Kirk-Ballard, Dr. Michael Stout, Dr. Gerald Myers, and the late Dr. Marc Cohn.

A special thank you to my major professor Dr. David Picha who had faith that I was up to the challenge of graduate school. You have acted as a mentor and guide to me for many years, and the wisdom you have imparted will assist me in life decision for years to come.
TABLE OF CONTENTS

ACKNOWLEDGEMENTS .......................................................................................................................... ii

LIST OF TABLES ........................................................................................................................................ iv

LIST OF FIGURES ...................................................................................................................................... v

ABSTRACT .................................................................................................................................................. vii

CHAPTER 1. LITERATURE REVIEW ........................................................................................................... 1
  1.1. Introduction ........................................................................................................................................ 1
  1.2. Economy .......................................................................................................................................... 3
  1.3. Nutritional Quality and Bioactive Compounds ................................................................................. 5

CHAPTER 2. MINERALS ............................................................................................................................ 8
  2.1. Introduction ...................................................................................................................................... 8
  2.2. Materials and Methodology .............................................................................................................. 9
  2.3. Results and Discussion ...................................................................................................................... 11

CHAPTER 3. CANNABINOIDs .................................................................................................................... 33
  3.1. Introduction .................................................................................................................................... 33
  3.2. Materials and Methodology ............................................................................................................ 37
  3.3. Results and Discussion .................................................................................................................... 38

CHAPTER 4. TOTAL PHENOLICS AND ANTIOXIDANTS ........................................................................... 43
  4.1. Introduction .................................................................................................................................... 43
  4.2. Materials and Methodology ............................................................................................................ 46
  4.3. Results and Discussion .................................................................................................................... 48

CHAPTER 5. CONCLUSIONS ..................................................................................................................... 53

LITERATURE CITED ................................................................................................................................. 55

VITA ............................................................................................................................................................ 66
LIST OF TABLES

Table 2.1. Percent fresh weight (% FW) of individual macronutrients (N, P, K, Ca, Mg, S) in fiber type Cannabis sativa microgreens grown for 7 or 12 days with or without supplemental lighting (SL) and/or one-quarter strength Hoagland solution fertilizer (F) ........................................12

Table 2.2. Percent fresh weight (% FW) of individual micronutrients (Fe, Mn, Zn, B, Cu) in fiber type Cannabis sativa microgreens grown for 7 or 12 days with or without supplemental lighting (SL) and/or one-quarter strength Hoagland solution fertilizer (F) ........................................23

Table 2.3. Dietary amount of minerals in fiber type Cannabis sativa microgreens compared to the DRI ........................................................................................................................................32

Table 2.4. Mineral content in Cannabis sativa microgreens compared to reported values by Xiao et al. (2016) and Li et al. (2021) ........................................................................................................................................32

Table 3.1. Parts per million (ppm) of analyzed cannabinoids (CBGA, CBG, CBDA, CBD, THCA) in fiber type Cannabis sativa microgreens grown for 7 or 12 days with or without supplemental lighting (SL) and/or one-quarter strength Hoagland solution fertilizer (F) ...............39

Table 4.1. Total phenolic content (TPC) represented by mg GAE/g and radical scavenging 
potential (DPPH inhibition %) in fiber type Cannabis sativa microgreens grown for 7 or 12 
days with or without supplemental lighting (SL) and/or one-quarter strength Hoagland 
solution fertilizer (F) ...........................................................................................................49
LIST OF FIGURES

Figure 1. Illustration of dramatic erosion in wholesale hemp prices from May 2019 to May 2020. ................................................................................................................................................4

Figure 2. Percent fresh weight (% FW) of total macronutrients (N, P, K, Ca, Mg, S) in fiber type Cannabis sativa microgreens. ..........................................................................................................................12

Figure 3. Percent fresh weight (% FW) of nitrogen in fiber type Cannabis sativa microgreens. .................................................................13

Figure 4. Percent fresh weight (% FW) of potassium in fiber type Cannabis sativa microgreens ........................................................................................................................................15

Figure 5. Percent fresh weight (% FW) of phosphorus in fiber type Cannabis sativa microgreens .................................................................................................................................17

Figure 6. Percent fresh weight (% FW) of magnesium in fiber type Cannabis sativa microgreens .................................................................................................................................18

Figure 7. Percent fresh weight (% FW) of calcium in fiber type Cannabis sativa microgreens .................................................................20

Figure 8. Percent fresh weight (% FW) of sulfur in fiber type Cannabis sativa microgreens .................................................................21

Figure 9. Parts per million (ppm) of total micronutrients (Fe, Mn, Zn, B, Cu) in fiber type Cannabis sativa microgreens ..............................................................................................................24

Figure 10. Parts per million (ppm) of iron in fiber type Cannabis sativa microgreens ........................................................................25

Figure 11. Parts per million (ppm) of manganese in fiber type Cannabis sativa microgreens ..................................................................................26

Figure 12. Parts per million (ppm) of zinc in fiber type Cannabis sativa microgreens ........................................................................28

Figure 13. Parts per million (ppm) of boron in fiber type Cannabis sativa microgreens ........................................................................29

Figure 14. Parts per million (ppm) of copper in fiber type Cannabis sativa microgreens ........................................................................31

Figure 15. Cannabinoid biosynthesis pathway leading to two major cannabinoids, THC and CBD ................................................................................................................................................35

Figure 16. Percent dry weight (% DW) of analyzed cannabinoids (CBGA, CBG, CBDA, CBD, THCA) in fiber type Cannabis sativa microgreens ........................................................................40

Figure 17. Percent dry weight (% DW) of analyzed individual cannabinoids (CBDA & CBD, THCA, CBGA & CBG) in fiber type Cannabis sativa microgreens ................................................................41
Figure 18. Total phenolics represented by milligrams of gallic acid equivalent (mg GAE/g) in fiber type *Cannabis sativa* microgreens .................................................................49

Figure 19. DPPH inhibition (%) in fiber type *Cannabis sativa* microgreens ..........................51
ABSTRACT

Hemp (*Cannabis sativa*) L. microgreens were grown to 7 and 12 days with and without supplemental lighting and/or fertilizer. The principal macronutrient found in hemp microgreens was nitrogen (N), followed by potassium (K), phosphorus (P), magnesium (Mg), calcium (Ca), and sulfur (S). The principal micronutrient found in hemp microgreens was iron (Fe), followed by manganese (Mn), zinc (Zn), boron (B), and copper (Cu). Hemp microgreens were similar to other commonly grown microgreen species in minerals except for P and Mg, where they could potentially be an excellent source, and Zn, where they fell below previously reported amounts. The detectable amount of delta-9 tetrahydrocannabinol (THC) for all hemp microgreen samples was less than 0.03% dry weight. Supplemental lighting increased the levels of cannabinoids cannabigerol (CBG) and cannabidiol (CBD), as well as the acid form of cannabinoids cannabigerolic acid (CBGA), cannabidiolic acid (CBDA), and delta-9 tetrahydrocannabinolic acid (THCA). Levels of CBD, CBDA and THCA increased with time. In relation to the averages reported for other microgreens, hemp was found to provide slightly higher amounts of total phenolics and antioxidants.
CHAPTER 1. LITERATURE REVIEW

1.1. Introduction

Industrial hemp (*Cannabis sativa*) L. has been an increasingly popular plant for research activity in the United States since the allowance of hemp research pilot programs through the Agriculture Act of 2014, and subsequent federal legalization of hemp through the Agricultural Improvement Act of 2018. Hemp, as defined in the act, is any *Cannabis* plant or derivative with a 0.3% or less delta-9 tetrahydrocannabinol (THC) content on a dry-weight basis. The *Cannabis* plant has been used by civilizations since ancient times and is thought to originate from the Northern Tibetan plateau in North Central China (McPartland *et al*., 2019). Dating back 12,000 years ago, evidence of hulled and ground hempseed being used as pressed oil for food was discovered in Japan and China (Kobayashi *et al*., 2008). The *Cannabis* plant was further cultivated and grown in high density plantings for production of fibrous stalks which could be used in textiles dating back to 5,600 B.C. (Zhang and Gao, 1999) and medicinal/drug use as far back at 2,700 B.C. (Jiang *et al*., 2006; Russo *et al*. 2008).

The *Cannabaceae* family consists of only one genus, *Cannabis*. How many species exist in the *Cannabaceae* is a debated topic, with some researchers claiming that *Cannabis* contains multiple species of plants and others arguing that *Cannabis sativa* is a highly polymorphic, single species (Emboden, 1974; Hillig, 2004, 2005; Hillig and Mahlberg, 2004; Small 1975a,b; Small and Cronquist, 1976; Gilmore *et al*., 2003; Small, 2015). If *Cannabis* were to be divided, it would consist of three main species, namely *Cannabis sativa* characterized by a taller growth pattern and a high THC:cannabidiol (CBD) ratio, *Cannabis indica* characterized by a shorter, bushier growth pattern and a balanced THC:CBD or high CBD:THC ratio, and finally *Cannabis ruderalis* characterized by a small growth pattern with low amounts of THC and CBD and ability to
withstand rugged terrains such as poor soil conditions and extreme cold (Hillig, 2005; McPartland and Guy, 2004; Clarke and Merlin, 2013). The *Cannabis* plant is mostly a dioecious, although sometimes monoecious, hermaphroditic annual herb. The stem is straight and narrow and can have a hollow core if planted in high density. Leaves are alternate or opposite on the stem and compound palmate in structure. The flowering structure is a panicle and initiated by age or photoperiod (Turner, 1980). Flowering *Cannabis* plants have the potential to accumulate high amounts of unique cannabinoids, which are found in limited amounts in nature (Getzch, Pertwee, and Marzo, 2010). Various terpenes and terpenoids are also found in high amounts. In addition, cannabinoids and terpenes accumulate in glandular trichome structures of the plant.

*Cannabis* has been an extensively used plant in the development of ancient and pre-modern civilization, being dispersed and cultivated throughout the old world for food, fiber, and medicinal purposes, and as a mind-altering drug. Only relatively recently has the plant been introduced into the New World. The first recorded shipments were through South America in the early 17th century and only as recently as the 1910’s was it introduced to the United States through the southwest region (Warf, 2014). *Cannabis*, regardless of the form, was quickly demonized and its production, possession, sale and consumption were soon outlawed. In 1937, Congress passed the Marihuana Tax Act, eliminating the economic viability of legally growing *Cannabis* and it was put under regulation and authority of the Drug Enforcement Agency (DEA). It was only during WWII that fibrous industrial hemp was allowed to be grown in the United States, in lieu of overseas imports of jute and sisal. Shortly after the war, hemp was again regulated by the DEA. In 1970, the Controlled Substances Act replaced the Marihuana Tax Act and listed *Cannabis*, regardless of the form, as a Schedule 1 drug along with heroine and LSD. As of 2021, *Cannabis* containing a THC content of greater than 0.3% is still considered to be a Schedule 1 controlled substance, whereas
federal legalization of *Cannabis* containing a THC content less than 0.3% on a dry-weight basis was put into law by the Agriculture Improvement Act of 2018.

1.2. Economy

Since ancient times it has been known that *Cannabis* is a versatile plant. In today’s economy, three main categories of products obtained from industrial hemp exist, which include hemp oil derived from the seed or flower, fiber from the stalk of the plant, or food from hemp seed (Johnson, 2018). Commercially available products either directly from or used in the manufacturing process include animal feed, animal bedding, insulation material, soap, cosmetic products, biofuels, paints, solvent paper, construction materials, automotive parts, packing materials, bioplastics, fabrics and clothing, rope, seed oil or flour for human consumption, and extracted CBD oil for medicinal/therapeutic uses. There are a number of additional products which are not listed here, and the potential for future uses of hemp in industrial/food products is unknown.

Of these products, CBD oil is expected to be the largest market of hemp derived products, projected to capture 34% of the market share by 2022 (Hemp Business Journal, 2018). With the complexities associated with forecasting in relatively new markets and uncertainties about future supply and demand mixed in with the dynamic legal environment surrounding these products, it is impossible to say what may or may not come from industrial hemp economically. Licensed hemp acreage saw massive increases in 2019 to a high of about 580,000 licensed acres of hemp, but by 2021, had dropped more than 80% to 108,000 licensed acres (Hemp Benchmarks, 2021). In line with licensed acreage, various CBD products dropped in value by approximately 80% (Figure 1). For example, price per kilogram of CBD isolate powder was $5000-$6000/kg in May 2019 and was $800-$1000/kg in May 2020 (Hemp Benchmarks, 2020).
The boom is over and farmers are now getting out of hemp growing altogether or are diversifying their growing to fill other market needs such as seed oil and fiber. Numerous breeding efforts by universities and private companies are now in development for hemp cultivars that are amenable to machine harvesting. Hemp, when planted at high density, will produce tall fibrous stalks with large apical buds. The bud is harvested for CBD extraction and the stalk is harvested for fibers. This seems to be the most economical and practical means of production for the United States, where farmers can use the same equipment to plant and harvest industrial hemp as they would to produce and harvest other major agronomic crops.
1.3. Nutritional Quality and Bioactive Components

Until now, little work has been conducted on the nutritional quality of hemp food products, particularly leafy hemp. It is becoming increasingly well known that pressed hempseed is a nutritious oil, having a high unsaturated fatty acid content and an ideal 3:1 ratio of omega-6 (linoleic) to omega-3 (alpha-linolenic) fatty acids (Callaway, 2004). Incorporating light to moderate amounts of unsaturated fatty acids into the diet is known to have numerous health benefits, including improving skin health, lowering blood pressure, promoting heart health, and reducing inflammation (Russo and Reggiani, 2013; Rodriguez and Pierce, 2010). Hemp seed oil also contains high amounts of antioxidants and bioactive molecules (Chen et al., 2012). Unlike the volatile CBD market, hempseed oil sales have steadily risen since 2017 (Food Additives and Nutricosmetics, 2020). Food products from hemp look to be a more predictable, increasingly popular market in the near future (Food Additives and Nutricosmetics, 2020).

An underserved potential market for hemp is its use as a microgreen food product. Microgreens are an increasingly popular produce item, with its main uses as a garnish, food additive, or freshly consumed item (Kyriacou et al., 2016; Pinto, Almeida, Aguiar, and Ferreira, 2015; Xiao, Lester, Luo, and Wang, 2012). Microgreens have been defined as “salad crop shoots harvested for consumption within 10-20 days of seedling emergence” (Lee et al., 2004). However, this definition is outdated as production of microgreens has expanded in recent years to include plants from many different crop groups and grown anywhere from 5-20 days (Xiao et al., 2016). Microgreens can encompass many colors, aromas, textures, and flavors which make them a unique food product.

Popular food items that have been on the market for many years are sprouts, such as alfalfa, brassicas, and mung bean. The main differences between sprouts and microgreens are plant components of harvest and growing environment. In general, sprouts are grown in a sprouting cell
or vessel covered to maintain a high relative humidity, watered several times a day, and grown for 5-14 days (Meyerowitz, 2010). Sprouts are harvested with the radicle in addition to the hypocotyl, cotyledons, and first true leaves, if they have emerged. Unlike sprouts, microgreens are grown in soil, hydroponically, or in a soilless substrate media and harvested at the substrate line without the roots. One area of research that has been studied in some detail is hemp sprouts - germinated seeds of hemp grown between three and five days. It is documented that sprouted seeds in general undergo phytochemical changes that may enhance their nutraceutical properties (Giorgetti et al., 2017). One scientific article analyzing hemp sprouts noted the development of novel prenylated flavonoids, cannaflavin A and cannaflavin B, as well as cannabinoids (terpene phenolic constituents) in hemp sprouts. The authors found high amounts of cannaflavin A and B present in sprouts (Werz et al., 2014), which had anti-inflammatory properties. In a second scientific article on bioactive and nutraceutical compounds of hemp sprouts, it was found that hemp sprouts were rich in phytochemical compounds, especially polyphenols (Frassinetti et al., 2018). In this article the author claims that hemp sprouts are a functional food, defined as “natural or processed foods that contains known or unknown biologically-active compounds; the foods, in defined, effective, and non-toxic amounts, provide a clinically proven and documented health benefit for the prevention, management, or treatment of chronic disease” (Martirosyan and Singh, 2015). In other words, hemp sprouts may contain bioactive compounds which aid in disease prevention and/or amelioration.

Microgreens are becoming increasingly known to contain high nutritional density and bioactive compound concentrations. In one of the earliest scientific papers focusing on the bioactive compounds found in microgreens, Xiao et al. (2012) documented that microgreen forms of red cabbage, cilantro, garnet amaranth, and green daikon radish had the highest gram per fresh
weight (g/FW) basis of carotenoids, ascorbic acid, phylloquinone, and tocopherols in comparison to mature vegetable counterparts. However, this is not always the case, as other researchers have shown that mature forms of vegetables can have higher nutritional and bioactive density than immature forms (Xiao et al., 2019; Klopsh et al., 2018; Niroula et al., 2018; Fuentes et al., 2019; Bulgari et al., 2019).

There are numerous studies highlighting the abundance of bioactive and nutraceutical compounds in microgreens. In 2013, Sun et al. documented a more diverse and expansive individual polyphenol profile in Brassica microgreens in comparison to mature plant counterparts. Subsequent studies found that carotenoids, ascorbic acid, phylloquinone, tocopherols, glucosinolates, and polyphenols were present in moderate to high amounts in Brassica microgreens (Xiao et al., 2019). In 2019 Kyriacou et al. reported high amounts of K and Mg in basil and Swiss chard. In addition, high levels of beta-carotene and polyphenols in green basil and coriander were reported, as well as ascorbic acid in purple basil microgreens. As an additive to bread, it was found that pea and lupin microgreens added significant amounts of flavonoids to the finished loaf (Klopsch et al., 2018). Microgreens are more commonly being recognized as functional foods which may provide health benefits beyond basic nutrition.

Microgreens are relatively easy to grow and have a quick turn-around in production, making them a produce item with good market potential for controlled environment growers. The popularity and consumption for microgreens is increasing and is expected to expand as a relatively new food product with many health benefits and unique sensory attributes (Charlebois, 2019; Riggio, 2019; Wood, 2019). The purpose of this research was to determine the mineral content, cannabinoids, total antioxidants, and total phenolic content in several cultivars of hemp microgreens.
CHAPTER 2. MINERALS

2.1. Introduction

An important aspect of well-being in life is proper nutrition. Humans require a certain amount of vitamins, minerals, protein, fat, and carbohydrates, which are acquired through food ingestion from the diet (Berdanier et al., 2013). The vast majority of minerals in the diet are obtained from eating plant-based and animal-based foods, or from drinking water (Higdon, 2001). The major elements of the human body are oxygen (O), hydrogen (H), carbon (C), nitrogen (N), calcium (Ca), phosphorus (P), potassium (K), sodium (Na), and magnesium (Mg). The minor or trace elements, are sulfur (S), iron (Fe), chlorine (Cl), cobalt (Co), copper (Cu), zinc (Zn), manganese (Mn), molybdenum (Mo), iodine (I), and selenium (Se) (Berdanier et al., 2013). Although many impoverished people worldwide suffer from malnutrition, excessive intake of any essential nutrient element can be just as dangerous. It is important to know the mineral content of food products in order to satisfy daily nutrient requirements (Welch et al., 1997).

Plants make up an important part of the diet and are the source of many vitamins and minerals (Higdon, 2001). The mineral content of a plant depends on multiple factors, including its genetics, environmental variations, soil characteristics, fertilizer use, plant stress, and maturity at harvest (Martinez-Ballesta et al., 2010). It has been shown that microgreens can be an excellent source of minerals (Kyriacou et al., 2019), as well as provide significantly higher amounts of minerals when compared to mature forms of the plant (Pinto et al., 2015). When environmental factors change, mineral content changes as well. Supplemental lighting can result in significant increases, decreases, or produce no effect on the mineral content of microgreens (Brazaityte et al., 2018, Kopsell and Sams, 2013). Fertilization of microgreens can also significantly affect their nutritional profiles (Petropoulos et al., 2001). It is reported that ground hempseed is an excellent source of...
dietary source of Cu, Mg, and Zn (Andrews et al., 2018), as well as P, K, Na, S, Ca, and Fe (Callaway, 2004), however information on the mineral content of hemp microgreens was not available.

The objective of this study was to quantify the mineral content of hemp microgreens and determine if maturity, supplemental lighting, and/or fertilizer has a significant effect on mineral content. The minerals analyzed were the principal plant macronutrients N, P, K, Ca, Mg, S, as well as the micronutrients Fe, Cu, Zn, Mn, and B.

2.2. Materials and Methodology

Seeds of fiber type hemp cultivars Altair, Anka, and New West Genetics (NWG) 452 were grown in a polyacrylic bi-wall covered greenhouse at the LSU Plant Materials Center (30.36209, -91.17407) in 25.4 x 50.8 cm trays seeded at a 1.3 cm planting depth with a planting density of 1.3 seeds per cm². The seeds were sown in a peat moss:vermiculite media (1:1). Plants were grown with either reverse osmosis (RO) water (12-16µS/cm) or with RO water plus supplemental fertilizer added in the form of one-quarter strength Hoagland solution. The mineral concentration for standard Hoagland nutrient solution is (in ppm) N (210), K (235), Ca (200), P (31), S (64), Mg (48), B (0.5), Fe (5), Mn (0.5), Zn (0.05), Cu (0.02), and Mo (0.01). The irrigation (RO water) and fertigation (one-quarter strength Hoagland solution) treatments consisted of applying 650 mL to each tray once per day between 12:00-2:00 p.m. Plants were grown with either ambient lighting or ambient lighting plus supplemental lighting provided by 1000W high-pressure sodium lamps (P.L. Light Systems NXT2; Ontario, Canada). A common way to express light quantity is the daily light integral (DLI) reported in mol/m²/d or simply mol/d. A light sensor (Argus Titan Omni v4.0; Surrey, British Columbia) set to a 15 min recording window was used to measure photosynthetic photon flux density (PPFD) measured in µmol/m²/second. This was then converted to DLI, which
was determined to be 2.24 mol/d for 7-day old (7D) microgreens under ambient light, 3.66 mol/d for 12-day old (12D) microgreens grown under ambient light, 5.04 mol/d for 7D microgreens under supplemental light, and 7.82 mol/d for 12D microgreens under supplemental light. In general, seedlings are recommended to receive 6-10 moles of light/day (LED Tonic, 2021). Average outdoor luminous intensity was 59.2 W/m² for the first seven days of the experiment and 101.2 W/m² for twelve days following the start of the experiment.

The hemp microgreens were produced under four different growing conditions, ambient light with reverse osmosis water (AL), ambient light with fertigation (AL + F), supplemental lighting with reverse osmosis water (SL), and supplemental lighting with fertigation (SL + F). Planting was done on February 10, 2021 and harvest occurred at 7 days or 12 days post seeding.

Four replications (n=4) of each treatment were acquired by random sampling of two 10.1x10.1 cm sections from two separate trays. Each of the three cultivars were sampled separately, bringing the total amount of samples acquired for each treatment to n=12. Plants were harvested by cutting the microgreens immediately above the substrate line followed by placing into small Ziploc bags and transported on ice to J.C. Miller Hall on the LSU main campus (30.40825, -91.17648). Exactly 5.00 g of fresh plant tissue from each replication was dried in a forced-air drying oven (Model No. LO-136-E, Thermal Product Solutions, New Columbia, PA) set to 70 °C for 72 hr. Oven-dried plant tissue was then re-weighed and recorded for dry weight determination. The dried plant tissue was ground through a 20-mesh screen using a Wiley mill. Total nitrogen (N) was estimated based on the Dumas dry-combustion method (International Standards Organization [ISO] 16634-1, 2008). Dried plant material weighing 0.15 g was heated to 950 °C and analyzed using a LECO CHN 628 detector (LECO Corporation; St. Joseph, MI). All other minerals were analyzed using the method of Jones Jr. (2001). Briefly, 2.2 mL of deionized
(DI) water was added to 0.5 g of tissue, heated on a block at 125 °C followed by the addition of 5 mL concentrated nitric acid (HNO₃), and digested for 2.75 hr. Then, 3 mL of hydrogen peroxide (H₂O₂) was added to the digested tissue, cooled, and filled to a volume of 20 mL with DI water (0.5 g/20 mL). The digested tissue was analyzed using an Ametek Spectro Arcos Inductively Coupled Plasma-Optical Spectrometer (ICP-OES) (Berwyn, PA). Minerals analyzed were N, P, K, Ca, Mg, S, Zn, Cu, Fe, B, and Mn. The dry weight content of the plant material was converted back to its equivalent fresh weight for analysis and data reporting.

Data were analyzed with the statistical program SAS (version 9.4; SAS Institute, Cary, N.C.) Proc GLM with Tukey’s honest significant difference (HSD) test used for mean separation.

2.3. Results and Discussion

Differences in mineral content between cultivars were not significantly different, therefore the cultivar results were pooled together for simplicity of reporting. Total macronutrient content (N, P, K, Ca, Mg, S) was highest in 12D SL+F treated plants at 1.75% fresh weight (FW) (Table 2.1; Figure 2), however this was not statistically different than amounts found in 12 AL, 12D AL+F, 7D AL, and 7D AL+F. Supplemental light resulted in significantly lower amounts of macronutrients compared to the other treatments, especially in those without the use of supplemental fertilizer.

The principal macronutrient found in hemp tissue was N, followed by K, P, Mg, Ca, and S. Plant age and lighting had a significant effect on N content in hemp microgreens. Nitrogen significantly decreased with plant age as well as supplemental lighting, however supplemental fertilizer had no significant effect on N content (Table 2.1; Figure 3). The treatments resulting in the highest amount of N were 7D AL and 7D AL+F, which were significantly higher than the lowest amount of N found in 12D SL and 12 SL+F.
Table 2.1. Percent fresh weight (% FW) of individual macronutrients (N, P, K, Ca, Mg, S) in fiber type Cannabis sativa microgreens grown for 7 or 12 days with or without supplemental lighting (SL) and/or one-quarter strength Hoagland solution fertilizer (F).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N (% FW)</th>
<th>K (% FW)</th>
<th>P (% FW)</th>
<th>Mg (% FW)</th>
<th>Ca (% FW)</th>
<th>S (% FW)</th>
<th>Total (% FW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7D AL</td>
<td>0.94&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.26&lt;sup&gt;de&lt;/sup&gt;</td>
<td>0.26&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.15&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.02&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.06&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.70&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>7D AL+F</td>
<td>0.90&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.28&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.24&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.15&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.04&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.68&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>7D SL</td>
<td>0.78&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.23&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.20&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.15&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.04&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.05&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>1.46&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>7D SL+F</td>
<td>0.78&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.30&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>12D AL</td>
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<td>0.36&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.21&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.17&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.06&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>1.70&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
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<td>0.42&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.16&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.16&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.06&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.66&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>12D SL</td>
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<td>0.29&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.18&lt;sup&gt;g&lt;/sup&gt;</td>
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<td>0.10&lt;sup&gt;b&lt;/sup&gt;</td>
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</tr>
<tr>
<td>12D SL+F</td>
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<td>0.48&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.12&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>0.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.75&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Total macronutrient content is listed in the last column. Different letters suggest significant difference among means within a column indicated by the Tukey’s HSD test at p ≤ 0.05.

Figure 2. Percent fresh weight (% FW) of total macronutrients (N, P, K, Ca, Mg, S) in fiber type Cannabis sativa microgreens grown for 7 or 12 days with or without supplemental lighting (SL) and/or one-quarter strength Hoagland solution fertilizer (F). Different letters suggest significant difference among means indicated by the Tukey’s HSD test at p ≤ 0.05.
A measure of the crude protein content in a sample can be estimated by multiplying total N by a factor of 6.25 as outlined by the ISO protocol described in 16634 part 1 (International Standards Organization, 2016). This method has been used since the mid 1900’s, and is based on a quantification relating to the relative amount of N in amino acids at 16% (Jones, 1941). Hemp microgreens contain between 0.68-0.94% N, which equates to 4.25-5.88% crude protein. A serving of 100 g FW (roughly ½ cup) hemp microgreens would contain between 4.3-5.9 g protein.

Figure 3. Percent fresh weight (% FW) of nitrogen in fiber type Cannabis sativa microgreens. Top left shows nitrogen amount for plants grown for 7 or 12 days with or without supplemental lighting (SL) and with (Y) or without (N) one-quarter strength Hoagland solution fertilizer (F). Different letters suggest significant difference among means indicated by the Tukey’s HSD test at p ≤ 0.05. Other boxes show individual treatments with significance (p) value labeled in top right corner as compared with a Student’s t-test.
According to the Dietary Reference Intake (DRI) guide published by the Institute of Medicine the average man should consume 56 g protein/day and woman should consume 46 g protein/day (DRI, 2006). At a mean protein content of 5.0 g, a 100 g serving would provide 9.2% DRI protein for men and 11.2% DRI protein for women. At an average of 5% protein content, hemp microgreens cannot be considered high in protein, however the moderate amount is worth noting. In comparison to other leafy vegetables, this is a high amount. For example, mature spinach protein content is 2.9 g/100 g FW (USDA, 2018b). In a study done on the effects of fertilization on mineral content in microgreens, Li et al. (2021) found a N content of 0.20-0.37% N for various broccoli microgreens, which would equate to a crude protein content of 1.25-2.3 g/100 g FW. Hemp microgreens protein content was higher than the protein content reported for various leafy greens.

The mineral content of K, the second most abundant macronutrient, was significantly affected by fertilizer use, age, and lighting (Table 2.1; Figure 4). Fertilizer use and longer duration of growth both resulted in significantly higher amounts of K in plant tissue, with 12D SL+F being the treatment with the highest K amount. Supplemental lighting with fertilizer resulted in the highest increase in K, although supplemental lighting alone resulted in significantly lower amounts of K in hemp microgreens.

Hemp microgreens contain between 0.23-0.48% K. A serving of 100 g hemp microgreens would contain 230-480 mg K. According to the DRI the average man should consume 3,400 mg K/day and the average woman should consume 2,600 mg K/day (DRI, 2006). At a mean K content of 328 mg/100 g FW, this would provide 9.6% DRI K for men and 12.6% DRI K for women. Thus as with N, hemp microgreens can provide a moderate amount of K. In an extensive study of selected minerals in microgreens, Xiao et al. (2016) found a range of 176-387 mg/100 g FW K.
when comparing 30 different species of microgreens, with an average K content of 297 mg/100 g FW. The average hemp microgreen sample ranked slightly higher than the average amount found by Xiao et al. (2016).

Figure 4. Percent fresh weight (% FW) of potassium in fiber type Cannabis sativa microgreens. Top left shows potassium amount for plants grown for 7 or 12 days with or without supplemental lighting (SL) and with (Y) or without (N) one-quarter strength Hoagland solution fertilizer (F). Different letters suggest significant difference among means indicated by the Tukey’s HSD test at p ≤ 0.05. Other boxes show individual treatments with significance (p) value labeled in top right corner as compared with a Student’s t-test.

Hemp microgreens do show potential to be higher in K content when compared to other microgreens, as 12 AL+F and 12 SL+F plants produced 420-480 mg/100 g FW of tissue, leading
to the conclusion that under certain growing conditions, hemp microgreens can prove to be better sources of K than other microgreen species. A follow-up study comparing mineral content of hemp microgreens alongside other microgreens grown under similar conditions would need to be conducted, as other microgreen species may also contain more K under similar growing conditions.

Phosphorus is the third most abundant macronutrient found in hemp microgreens. Plant age, fertilizer use, and supplemental lighting all resulted in significant decreases in P amounts, with 12D SL+F having the lowest of any treatment (Table 2.1; Figure 5). The highest amount of P was found in microgreens grown under 7D AL treatment, which was significantly higher than all other treatments.

Hemp microgreens contain between 0.12-0.26% P. A serving of 100 g hemp microgreens would contain 120-260 mg P. According to the DRI the average adult should consume 700 mg P/day (DRI, 2006). At a mean P content of 193 mg/100 g FW, hemp microgreens can potentially provide a significant amount of the DRI of P at 27.6%. When comparing this to Xiao et al. (2016), of the 30 microgreen species analyzed, the range of P reported was between 52-86 mg/100 g FW with an average P content of 66 mg/100 g FW. Similarly Li et al. (2021) reported a P content of 45-106 mg/100 g FW for six microgreen species. The average hemp microgreen sample when comparing the mean P amount of all treatments is roughly three times the amount reported by Xiao et al. in 2016, and when comparing to the highest overall amounts in 7D AL and 7D AL+F, P content is roughly four times the amount. Phosphorus assimilation is common in new plant development (Flyman and Afolayan, 2008; Khader and Rama, 2003), and this would explain why P levels are higher in 7D vs. 12D treatments, however this does not explain the high levels in comparison to other microgreens. Foods similar in P content would be chickpeas or certain types of cooked beans (Dieticians of Canada, 2018).
Supplemental lighting and age resulted in significant increases in Mg levels (Table 2.1; Figure 6). The 12D SL+F and 12D SL treated plants had significantly higher amounts of Mg than all other treatments. When comparing all treatments, fertilizer use resulted in no significant differences in Mg concentration, except in 7D SL + F treated plants which had significantly higher amounts of Mg than any other 7D treatment. The 7D AL, 7D AL+F, and 7D SL plants had the lowest amount of Mg in hemp microgreens.
Figure 6. Percent fresh weight (% FW) of magnesium in fiber type Cannabis sativa microgreens. Top left shows magnesium amount for plants grown for 7 or 12 days with or without supplemental lighting (SL) and with (Y) or without (N) one-quarter strength Hoagland solution fertilizer (F). Different letters suggest significant difference among means indicated by the Tukey’s HSD test at \( p \leq 0.05 \). Other boxes show individual treatments with significance (p) value labeled in top right corner as compared with a Student’s t-test.

Hemp microgreens contain between 0.15-0.22% Mg. In a serving of 100 g, hemp microgreens would contain 150-220 mg Mg. The average man should consume 420 mg Mg/day and the average woman should consume 320 mg Mg/day (DRI, 2006). At a mean Mg content of 173 mg/100 g FW, hemp microgreens can potentially provide a substantial amount of the DRI of Mg for men at 41.2% and 54.1% for women. In Xiao et al. (2016), a range of 28-66 mg/100 g FW Mg was found when comparing 30 different species of microgreen, with an average Mg content of 44 mg/100 g FW. Similarly, Li et al. (2021) reported a Mg content of 29-60 mg/100 g FW for
six microgreen species. The mean Mg value for hemp microgreens is roughly four times the amount reported by Xiao et al. in 2016, and when comparing to the highest overall amounts in 12D SL and 12D SL+F, Mg content is roughly five times the reported amount. In other reports, Mg content in mature spinach and Swiss chard, both considered to be high sources of Mg, is 79-81 mg/100 g FW (USDA, 2018a). A follow-up study could be conducted comparing Mg content in hemp microgreens with other microgreens as well as mature spinach and Swiss chard.

Fertilizer use, plant age, and supplemental lighting all resulted in significant increases in Ca levels (Table 2.1; Figure 7). The 12D SL+F treated plants had the highest amounts of Ca. The 7D AL plants had significantly lower amounts of Ca than other treatments, whereas 12D SL+F treated plants had significantly higher amounts.

Hemp microgreens contain between 0.02-0.19% Ca. A serving of 100 g hemp microgreens would contain 20 mg-190 mg Ca. According to the DRI, an adult male should consume 1,000 mg Ca/day (DRI, 2006). At a mean Ca content of 81 mg/100 g FW, hemp microgreens can potentially provide a moderate amount of the DRI of Ca at 8.1%. In Xiao et al. (2016), a range of 39-98 mg/100 g FW Ca was found when comparing 30 different species of microgreens, with an average Ca content of 67 mg/100 g FW. Li et al. (2021) reported 28-135 mg/100g FW for six microgreen species. The Ca content is comparable to those reported by Xiao et al. in 2016 and Li et al. in 2021. However when comparing the highest amount found in 12D SL+F plants, a Ca content of 190 mg/100 g FW would nearly triple previously reported amounts. As previously mentioned, a follow-up study would need to be conducted in order to compare hemp microgreens with other species of microgreens grown under similar conditions in order to see if hemp microgreens do accumulate higher concentrations of Ca.
Figure 7. Percent fresh weight (% FW) of calcium in fiber type Cannabis sativa microgreens. Top left shows calcium amount for plants grown for 7 or 12 days with or without supplemental lighting (SL) and with (Y) or without (N) one-quarter strength Hoagland solution fertilizer (F). Different letters suggest significant difference among means indicated by the Tukey’s HSD test at $p \leq 0.05$. Other boxes show individual treatments with significance ($p$) value labeled in top right corner as compared with a Student’s t-test.

Sulfur was the plant macronutrient found in the lowest concentration in hemp microgreens. Supplemental lighting, plant age, and fertilizer resulted in significant differences in S levels (Table 2.1; Figure 8). Supplemental light without the use of fertilizer heavily reduced S levels in plant tissue, similar to the results for K. Fertilizer use resulted in either slightly higher or significantly higher amounts of S in all treatments. Hemp microgreen age resulted in significantly lower amount
of S. The highest amount of S was found in 7D AL+F treated plants, whereas the lowest S levels were found in 12SL and 7D SL plants.

Figure 8. Percent fresh weight (% FW) of sulfur in fiber type Cannabis sativa microgreens. Top left shows sulfur amount for plants grown for 7 or 12 days with or without supplemental lighting (SL) and with (Y) or without (N) one-quarter strength Hoagland solution fertilizer (F). Different letters suggest significant difference among means indicated by the Tukey’s HSD test at p ≤ 0.05. Other boxes show individual treatments with significance (p) value labeled in top right corner as compared with a Student’s t-test.

Hemp microgreens contain between 0.05-0.07% S. A serving of 100 g hemp microgreens would contain 50 mg-70 mg S. There is currently no DRI amount for sulfur, as S is readily obtained from the amino acids methionine and cysteine, whose amounts can vary depending on protein
source (Nimni, Han, and Cordoba, 2007). In one study by Li et al. (2021) it was found that the average S content in five microgreen species (radish, kale, broccoli, cabbage, and mustard) was 29-90 mg/100 g FW, suggesting that hemp microgreens have similar amounts of S when compared to certain other microgreens.

When compared to the amounts of macronutrients reported in other microgreens, hemp microgreens had substantially higher amounts of P and Mg (Xiao et al., 2016). In addition, N was found in higher amounts than previously reported amounts. Levels of K, Ca, and S were similar to other commonly grown microgreens and fell within the range of expected value.

Overall, there were many changes in macronutrient content based on microgreen plant age, fertilizer use, and supplemental lighting treatments. Plant age resulted in significantly lower amounts of N, P, and S with significantly higher amounts of K, Ca, and Mg. Fertilizer use resulted in significantly lower P amounts, had no significant effect on N and Mg content, and resulted in significant increases in K, Ca, and S. Supplemental lighting resulted in significantly lower amounts of N, P, and S, and significantly higher amounts of Mg and Ca, and K. These findings are supported by Samuoliene et al. (2019) who found that different forms of lighting can affect Mg and Ca levels in Brassicaceae microgreens. Li et al. (2021) found that fertilization can have significant impacts on overall N and P concentrations in various microgreens. Pinto et al. (2015) found that age can have a significant impact of overall and individual mineral content of plants within the same species.

The micronutrients analyzed included Fe, Zn, Mn, B, and Cu. Micronutrient content varied in amount depending on plant age, fertilizer, and supplemental lighting treatment. Overall, the highest concentration of micronutrients was found in 12D AL and 12 SL treated microgreens. The lowest amounts of micronutrients were found in 12D SL+F, 7D SL+F, and 7D SL treated plants.
The principal micronutrient found in hemp tissue was Fe, followed by Mn, Zn, B, and Cu.

Table 2.2. Percent fresh weight (% FW) of individual micronutrients (Fe, Mn, Zn, B, Cu) in fiber type *Cannabis sativa* microgreens grown for 7 or 12 days with or without supplemental lighting (SL) and/or one-quarter strength Hoagland solution fertilizer (F).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Iron (ppm)</th>
<th>Manganese (ppm)</th>
<th>Zinc (ppm)</th>
<th>Boron (ppm)</th>
<th>Copper (ppm)</th>
<th>Total Micronutrients (ppm)</th>
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<tr>
<td>7D AL</td>
<td>41.6&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>16.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.9&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>82.7&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>7D AL+F</td>
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<td>17.4&lt;sup&gt;b&lt;/sup&gt;</td>
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</tr>
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<td>2.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>68.7&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Different letters suggest significant difference among means within a column indicated by the Tukey’s HSD test at p ≤ 0.05.

Iron was the most abundant micronutrient found in hemp microgreen tissue. Plant age, supplemental lighting, and fertilizer all resulted in significant differences in Fe content (Table 2.2; Figure 10). Supplemental lighting resulted in major reductions in Fe levels, especially when combined with fertilizer use. Plant age had a slight but significant lowering effect on Fe content. The treatments with the highest amounts of Fe were 7D AL and 12D AL, whereas the lowest amounts of Fe were found in the 12D SL+F treatment.
Figure 9. Parts per million (ppm) of total micronutrients (Fe, Mn, Zn, B, Cu) in fiber type *Cannabis sativa* microgreens grown for 7 or 12 days with or without supplemental lighting (SL) and/or one-quarter strength Hoagland solution fertilizer (F). Different letters suggest significant difference among means indicated by the Tukey’s HSD test at $p \leq 0.05$.

Hemp microgreens contained between 19.5-43.7 ppm Fe. A serving of 100 g hemp microgreens would contain between 195-437 µg Fe. According to the DRI, the average adult male should consume 8.7 mg Fe/day and the average adult female should consume 14.8 mg Fe/day (DRI, 2006). At a mean Fe content of 348 µg/100 g FW, hemp microgreens can potentially provide a small amount of the DRI of Fe at 4% for men and 2.3% for women. Xiao *et al.* (2016) reported a range of 470-840 µg/100 g FW Fe in 30 different species of microgreens, with an average Fe content of 615 µg/100 g FW. Similarly Li *et al.* (2021) reported a range of 465-750 µg/100 g FW in six microgreen species. The average hemp microgreen Fe content is roughly half of that reported by Xiao *et al.* in 2016. When comparing hemp microgreens to leafy greens that are well known to provide high amounts of Fe, such as spinach at 2.7 mg Fe/100 g FW, hemp microgreens fall well below in nutritional value (USDA, 2018b).
Figure 10. Parts per million (ppm) of iron in fiber type Cannabis sativa microgreens. Top left shows iron amount for plants grown for 7 or 12 days with or without supplemental lighting (SL) and with (Y) or without (N) one-quarter strength Hoagland solution fertilizer (F). Different letters suggest significant difference among means indicated by the Tukey’s HSD test at $p \leq 0.05$. Other boxes show individual treatments with significance ($p$) value labeled in top right corner as compared with a Student’s t-test.

Manganese is the second most abundant micronutrient found in hemp microgreen tissue. Age resulted in a significant increase in Mn content, whereas supplemental lighting or fertilizer use had no significant effect (Table 2.2; Figure 11).
Figure 11. Parts per million (ppm) of manganese in fiber type *Cannabis sativa* microgreens. Top left shows manganese amount for plants grown for 7 or 12 days with or without supplemental lighting (SL) and with (Y) or without (N) one-quarter strength Hoagland solution fertilizer (F). Different letters suggest significant difference among means indicated by the Tukey’s HSD test at $p \leq 0.05$. Other boxes show individual treatments with significance ($p$) value labeled in top right corner as compared with a Student’s t-test.

Hemp microgreens contained between 16.3-25.4 ppm Mn. A serving of 100 g hemp microgreens would contain 163-254 µg Mn. According to the DRI, the average adult male should consume 2.3 mg Mn/day and the average adult female should consume 1.8 mg Mn/day (DRI, 2006). At a mean Mn content of 202 µg in hemp microgreens would provide 8.8% of the daily amount for men and 11.2% of the daily amount for women (DRI, 2006). A range of 170-480 µg/100 g FW Mn was reported when comparing 30 different species of microgreens, with an average Mn content of 329 µg/100 g FW (Xiao *et al.*, 2016). Li *et al.* (2021) reported higher
average amounts at 223-921 µg/100 g FW. The mean Mn value from all hemp microgreen treatments is on the lower end, but still within the range reported by Xiao et al. in 2016, however outside of the range reported by Li et al. in 2021.

When comparing to the highest overall Mn amount in the 12D SL treated plants, 254 µg/100 g FW still falls below the average reported value. When comparing hemp microgreens to leafy greens that are well known to contain high Mn, such as spinach at 935 g Mn/100 g FW, hemp microgreens fell well below them in nutritional value (USDA, 2018b).

Plant age had no significant effect on Zn content, whereas fertilizer use resulted in significantly lower amounts of Zn (Table 2.2; Figure 12). Supplemental lighting in 7D treatments resulted in lower amounts of Zn, however this was not consistent in 12D treated plants. Fertilizer use had the largest overall effect on Zn content.

Hemp microgreens contain between 10.7-16.9 ppm Zn. A serving of 100 g hemp microgreens would contain 107-169 µg Zn. According to the DRI, the average adult male should consume 11 mg Zn/day and the average adult female should consume 8 mg Zn/day (DRI, 2006). At a mean Zn content of 136 µg/100 g FW, hemp microgreens would provide very little Zn, with a DRI amount of 1.2% for adult males and 1.7% for adult females per 100 g FW serving. In Xiao et al. (2016), a range of 220-510 µg/100 g FW Zn was reported when comparing 30 different species of microgreens, with an average Zn content of 350 µg/100 g FW. Li et al. in 2021 reported Zn at 331-536 µg/100 g FW in six microgreen species. The average hemp microgreen sample is lower than those amounts reported by Xiao et al. (2016) and Li et al. (2021). In general, fruits and vegetables do not have high levels of Zn, especially when compared to meats, nuts, and dairy products.
Figure 12. Parts per million (ppm) of zinc in fiber type *Cannabis sativa* microgreens. Top left shows zinc amount for plants grown for 7 or 12 days with or without supplemental lighting (SL) and with (Y) or without (N) one-quarter strength Hoagland solution fertilizer (F). Different letters suggest significant difference among means indicated by the Tukey’s HSD test at \( p \leq 0.05 \). Other boxes show individual treatments with significance (p) value labeled in top right corner as compared with a Student’s t-test.

Plant age and supplemental lighting resulted in significantly higher amounts of B in hemp microgreens (Table 2.2; Figure 13). In addition, fertilizer when combined with supplemental lighting resulted in higher amounts of B. The 12D SL+F treatment resulted in the highest amount of B whereas the 7D AL and 7D AL+F treatment resulted in the lowest amount.

Hemp microgreens contained between 4.6-15 ppm B. A serving of 100 g hemp microgreens would contain between 46-150 \( \mu \)g B, with a mean B value of 85 \( \mu \)g. Li *et al.* (2021) reported
amounts of B in plant tissue between 70-260 µg/100 g FW, slightly higher than amounts reported herein.

Copper was the micronutrient found in the lowest concentration in hemp microgreens. Supplemental fertilizer resulted in significantly lower amounts of Cu, especially in 12D treated microgreens or supplemental lighting treatments (Table 2.2; Figure 14). The 12 AL+F and 12
SL+F treated plants had the lowest amounts of Cu, whereas all other treatments were not significantly different from one another.

Hemp microgreens contain between 2.0-3.0 ppm Cu. A serving of 100 g hemp microgreens would contain 20-30 µg Cu. According to the DRI, the average person should consume 900 µg Cu/day (DRI, 2006). At a mean Cu content of 25 µg/100 g FW, hemp microgreens would provide minimal amounts of Cu, with a DRI amount of 2.8% at 100 g FW serving. In Xiao et al. (2016), a range of 4-13 µg/100 g FW Cu was reported when comparing 30 different species of microgreen, with an average Cu content of 7.4 µg/100 g FW. Similarly, Li et al. in 2021 reported a Cu content of 3-15 µg/100 g FW in six microgreen species. The average hemp microgreen sample when comparing the mean to all treatments is higher than those amounts reported by Xiao et al. in 2016 and Li et al. in 2021. In general, fruits and vegetables do not have high levels of copper. Grain and nut products would provide the vast majority of Cu in the average diet.

When compared to amounts reported in other microgreens, hemp microgreens had lower levels of total micronutrients (Xiao et al., 2016). The minerals Fe and Zn fell below the expected range, whereas Mn and B fell within the range previously reported. The only micronutrient in hemp microgreens found in higher amounts than previous reports was Cu.

The overall micronutrient content varied in hemp microgreens based on plant age, fertilizer use, and supplemental lighting treatments. Plant age resulted in decreased amounts of Fe, Mn, and Cu with increased amounts of B. Fertilizer use resulted in decreased amounts of Fe, Zn, and Cu and had no significant effect on Mn and B. Supplemental lighting resulted in decreased amounts of Fe, Zn, and Cu, with increased amounts of B in 7D treated plants. These findings are supported by Samuoliene et al. (2019) who found that different forms of lighting can affect Fe levels in Brassicaceae microgreens. Li et al. (2021) found that fertilization can have significant impacts on
overall Fe, Mn, Zn, B, and Cu concentrations in various microgreens. Pinto et al. (2015) found that age can have a significant impact of overall and individual mineral content of plants within the same species.

Figure 14. Parts per million (ppm) of copper in fiber type Cannabis sativa microgreens. Top left shows copper amount for plants grown for 7 or 12 days with or without supplemental lighting (SL) and with (Y) or without (N) one-quarter strength Hoagland solution fertilizer (F). Different letters suggest significant difference among means indicated by the Tukey’s HSD test at p ≤ 0.05. Other boxes show individual treatments with significance (p) value labeled in top right corner as compared with a Student’s t-test.
Table 2.3. Dietary amount of minerals in fiber type *Cannabis sativa* microgreens compared to the DRI.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Protein (g)</th>
<th>K (mg)</th>
<th>P (mg)</th>
<th>Mg (mg)</th>
<th>Ca (mg)</th>
<th>S (mg)</th>
<th>Fe (mg)</th>
<th>Mn (mg)</th>
<th>Zn (mg)</th>
<th>B (mg)</th>
<th>Cu (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DRI Men</td>
<td>56</td>
<td>3400</td>
<td>700</td>
<td>420</td>
<td>1000</td>
<td>N/A</td>
<td>8.7</td>
<td>2.3</td>
<td>11</td>
<td>N/A</td>
<td>0.90</td>
</tr>
<tr>
<td>DRI Women</td>
<td>46</td>
<td>2600</td>
<td>700</td>
<td>320</td>
<td>1000</td>
<td>N/A</td>
<td>14.8</td>
<td>1.8</td>
<td>8</td>
<td>N/A</td>
<td>0.90</td>
</tr>
<tr>
<td>Hemp Avg in 100 g FW</td>
<td>5</td>
<td>328</td>
<td>193</td>
<td>173</td>
<td>81</td>
<td>59</td>
<td>0.35</td>
<td>0.20</td>
<td>0.14</td>
<td>0.09</td>
<td>0.03</td>
</tr>
<tr>
<td>DRI Men in 100 g FW</td>
<td>8.9%</td>
<td>9.6%</td>
<td>27.6%</td>
<td>41.2%</td>
<td>8.1%</td>
<td>-</td>
<td>4%</td>
<td>8.8%</td>
<td>1.2%</td>
<td>-</td>
<td>2.8%</td>
</tr>
<tr>
<td>DRI Women in 100 g FW</td>
<td>10.9%</td>
<td>12.6%</td>
<td>27.6%</td>
<td>54.1%</td>
<td>8.1%</td>
<td>-</td>
<td>2.3%</td>
<td>11.2%</td>
<td>1.7%</td>
<td>-</td>
<td>2.8%</td>
</tr>
</tbody>
</table>

Table 2.4. Mineral content in *Cannabis sativa* microgreens compared to reported values by Xiao et al. (2016) and Li et al. (2021).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Protein (g)</th>
<th>K (mg)</th>
<th>P (mg)</th>
<th>Mg (mg)</th>
<th>Ca (mg)</th>
<th>S (mg)</th>
<th>Fe (mg)</th>
<th>Mn (mg)</th>
<th>Zn (mg)</th>
<th>B (mg)</th>
<th>Cu (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemp Avg in 100 g FW</td>
<td>5</td>
<td>328</td>
<td>193</td>
<td>173</td>
<td>81</td>
<td>59</td>
<td>0.35</td>
<td>0.20</td>
<td>0.14</td>
<td>0.09</td>
<td>0.03</td>
</tr>
<tr>
<td>Avg in Xiao et al. (2016)</td>
<td>-</td>
<td>176-387</td>
<td>52-86</td>
<td>28-66</td>
<td>39-98</td>
<td>-</td>
<td>0.47-0.84</td>
<td>0.17-0.48</td>
<td>0.22-0.51</td>
<td>-</td>
<td>0.004-0.013</td>
</tr>
<tr>
<td>Avg in Li et al. (2021)</td>
<td>1.25-2.3</td>
<td>65-253</td>
<td>45-106</td>
<td>28-60</td>
<td>28-135</td>
<td>29-90</td>
<td>0.47-0.75</td>
<td>0.22-0.92</td>
<td>0.33-0.53</td>
<td>0.07-0.26</td>
<td>0.003-0.015</td>
</tr>
</tbody>
</table>

Amounts in Li et al. (2021) were converted from dry weight to fresh weight.
CHAPTER 3. CANNABINOIDS

3.1. Introduction

Cannabinoids are a class of terpenophenolic compounds found in Cannabis sativa plants, concentrated in the glandular trichomes of flower tissue (Turner et al., 1978). What makes a compound a cannabinoid is that it binds to a cannabinoid receptor in the brain or body. Cannabinoids and endocannabinoids bind to A-G protein coupled receptors (GPCR) named cannabinoid receptor 1 (CB1) found mostly in the brain and cannabinoid receptor 2 (CB2) found mostly on white blood cells and in the tonsils and spleen (Matsuda et al., 1990; Munro et al., 1993; Shahbazi et al., 2020). The cannabinoids naturally produced in the body are anandamide and 2-arachidonoyl glycerol (2-AG) (Reggio, 2010; Devante, 1992; Stella, 1997). GPCRs are induced to conformationally change when bound to an activator, such as a cannabinoid, which causes activation of a G protein and initiates a specific cellular process (Latorraca et al., 2017; Weis and Kobilka, 2014). There are many cannabinoids (over 150 documented), each with a potentially unique relationship with CB1 and CB2 receptors including antagonistic or synergistic effects (Citti et al., 2019). In addition, other chemicals found in the Cannabis plant can alter the way cannabinoids interact with CB1 and CB2 receptors. For example, the terpenes β-myrcene and limonene have been found to alter the feeling one gets when exposed to THC (McPartland and Pruitt, 1999; McPartland and Russo, 2001, Russo, 2011; Russo, 2016).

Historically, the two most important cannabinoids found in Cannabis are THC and CBD. THC is important, as it is a cannabinoid that is a full agonist of CB1 receptors and can produce psychotropic effects, which have been described as hallucinogenic, psychedelic, psychotomimetic, illusinogenic, and psychodysleptic (Le Dain, 1972; Hua et al., 2016). Industrial hemp plants are regulated by the USDA under the Agricultural Improvement Act of 2018 and must fall below a...
threshold of 0.3% THC on a dry weight basis. Any samples found to contain higher than 0.3% THC are classified as marijuana and subject to destruction and loss of crop. Subsequently, only approved “hemp” seeds obtained from genotypes known to produce <0.3% THC on a dry-weight basis were evaluated in studies presented herein.

The non-psychoactive cannabinoid CBD is thought to have medicinal properties such as amelioration of pain, seizures, anxiety, and cognitive disorders (Shahbazi et al., 2020). Having a high affinity for agonistic binding of CB2 receptors, CBD is thought to be a negative allosteric modulator of THC and 2-AG agonism (Laprairie et al., 2015). In other words, CBD binds more strongly than THC to CB1 receptors throughout the body and is thought to negate the psychoactive effects of THC on CB2 receptors. To date, the U.S. Food and Drug Administration (FDA) has only approved one CBD derived medicine, Epidiolex, which is used for the treatment of seizures associated with Lennox-Gastaut syndrome, Dravet syndrome, or tubercular sclerosis in patients 1 year of age and older (FDA, 2021). In addition, the drugs Marinol and Syndros, whose active ingredient is synthetically derived THC, have been approved for the treatment of anorexia and weight loss in cancer patients.

In the plant, THC and CBD are mostly found in the forms of delta-9 tetrahydrocannabinolic acid (THCA) and cannabidiolic acid (CBDA), which are decarboxylated at high temperatures or age, resulting in THC and CBD, respectively (Turner et al., 1980). The molecular precursor to both THCA and CBDA is cannabigerolic acid (CBGA), whose decarboxylated form is cannabigerol (CBG) (Figure 15). Recent studies have shown that CBG has a lower affinity for CB1/CB2 receptor binding than THC or CBD, however may bind to other receptors in the body for which THC and CBD have no affinity (Pertwee, 2008; Cascio et al., 2010; Pollastro et al.,
Figure 15. Cannabinoid biosynthesis pathway leading to two major cannabinoids, THC and CBD (Grassi and McPartland, 2017).

The amount of cannabinoids present in the plant varies between biotypes in *Cannabis*. De Meijer (2014) describes three chemotypes: a marijuana “drug” type with a high THC:CBD ratio; a hemp type with moderate to high amounts of CBD and low THC; and a fiber type with little total cannabinoids at maturity. Chemotype II will be smaller and bushier than the other chemotypes, producing lots of flowers with numerous glandular trichomes which swell with CBD oil upon maturation. This type of seed is very expensive, with prices of hemp seeds with high CBD content...
at maturity at $328/lb. (Hemp Benchmarks, 2020). Fiber type hemp is becoming more readily available for purchase, with seed cost at $3.28/lb. (Hemp Benchmarks 2020). The most practical form of seed to use in a microgreen production system is chemotype III seeds, as they produce young vigorous plants that become readily established in high density plantings and are relatively inexpensive.

To date, the FDA only has three commercial hemp food products on the generally regarded as safe (GRAS) list which are hempseed oil, hulled hempseed, and hempseed protein powder. Per section 301(ll) of the Federal Food, Drug, and Cosmetics (FD&C) Act [21 U.S.C. § 331(ll)], “it is prohibited to introduce or deliver for introduction into interstate commerce any food (including any animal food or feed) to which has been added a substance which is an active ingredient in a drug product that has been approved under section 505 of the FD&C Act [21 U.S.C. § 355], or a drug for which substantial clinical investigations have been instituted and for which the existence of such investigations has been made public”. This includes THC and CBD, however, both of these compounds are found naturally in hemp microgreens. This poses a gray area, as hemp microgreens can be sold as a stand-alone food product in which THC and CBD occur naturally. Nevertheless, it has been noted that as of August 2021, upscale restaurants on the East Coast of the U.S. are paying up to $30/lb. for fresh hemp microgreens in order to be used as a garnish or salad mix (personal communication). Whether the FDA is taking action against companies selling hemp microgreens is unknown, however in the coming years CBD and THC food and beverage products may be made federally legal, which would allow for open competition of many new hemp products.

The objective of this study was to analyze different cultivars of fiber type Cannabis plants grown to a microgreen stage and quantify amounts of the two major cannabinoids and acid
precursors THCA, THC, CBDA, CBD, and the precursor CBGA, as well as the decarboxylated form CBG.

3.2. Materials and Methodology

Seeds of fiber type hemp New West Genetics (NWG) 2730 (Fort Collins, CO) were obtained in August, 2020 and grown in February 2021. The germination rate was determined to be 93.5% using the top of paper method derived from Rao et al. (2006). Eight replications of twenty-five seeds were placed in Petri dishes (Falcon 1007) lined with two sheets of Whatman no. 4 filter paper and moistened with 8 mL de-ionized (DI) water. Twenty-five seeds were placed on the moist paper for germination and a clear lid was placed on top of each Petri dish. Petri dishes were checked daily for germinated seeds and the filter paper was moistened with DI water as needed. Germination was determined by observing a visible radical or shoot. The number of germinated seed were recorded and discarded. Petri dishes were observed for 14 days. Mean time germination (MTG) was 1.97 days using the formula MTG = (∑ Ti Ni)/G where Ti is the day of germination, Ni is the number of seeds germinating on Ti, and G is the total number of germinated seeds (Hartmann et al., 1990).

The seeding and growing conditions for the hemp microgreens followed the same protocol as described in the Materials and Methods section of Chapter 2. In addition, the four growing conditions were similar to those described in Chapter 2 (AL, AL+F, SL, and SL+F).

Three replications (n=3) of each treatment were acquired by random sampling of one 10.1x10.1cm section from three separate trays. Plants were harvested by cutting the microgreens immediately above the substrate line and placing into small Ziploc bags which were kept on ice until return to J.C. Miller Hall on the LSU main campus (30.40825, -91.1764). The fresh plant tissue was then lyophilized at -65 °C for 72 hr. The freeze-dried plant tissue was ground with a mortar and pestle and extracted according to the Louisiana Department of Agriculture and Forestry
hemp extraction protocol. This consisted of adding 200 mg ± 0.5 mg of freeze-dried tissue to a 50 mL plastic centrifuge tube (Corning 430828) to which was added 25 mL of HPLC grade methanol. The mixture was vortexed for one minute (min) and sonicated for 15 min, stopping to vortex for one min per five min of sonication. The mixture was then centrifuged for 5 min at 1230 G. A portion of the supernatant was filtered into a 15 mL centrifuge tube (Corning 430790) using a 0.2 µm polyvinylidene difluoride (PVDF) syringe filter (Whatman 6873-2502). The filtered supernatant was then diluted at a ratio of 1:10 with 25% HPLC grade water/75% 0.1% formic acid and placed into autosampler vials.

Separation and quantification of the cannabinoids CBD, THC, CBG and the acid precursors CBDA, THCA, CBGA was done on a Thermo Fisher Q Exactive high performance liquid chromatography (HPLC) high resolution mass spectrometry (LC-HRMS) system. The column used was a 2.1x100 mm reverse-phase C-18 Accucore Vanquish, with a 1.5 µm particle size, (Thermo Scientific; Waltham, MA). Ten µl of filtrate was injected onto the column and the cannabinoids and acid precursors were eluted using a mobile phase consisting of 0.1% formic acid in water (A) and acetonitrile (B) with a gradient flow of 95% solvent A, 5% solvent B to 5% solvent A, 95% solvent B over a run time of 14.0 min at a 0.3 mL/min flow rate.

Data were analyzed with the statistical program SAS (version 9.4; SAS Institute, Cary, N.C.) Proc GLM with Tukey’s honest significant difference (HSD) test used for mean separation.

3.3. Results and Discussion

Percent dry weight (% DW) for the analyzed cannabinoids was highest in 12D hemp microgreens grown under supplemental lighting regardless of fertilizer use (Table 3.1; Figure 16). The highest amounts of cannabinoids were found in SL treated plants at 0.75% DW, however, this was not significantly different than 12D SL+F plants. Increasing plant age resulted in a significant
increase in total cannabinoids within all treatments. Amount of THC was below the machine detectable amount of 0.03%.

Table 3.1. Parts per million (ppm) of analyzed cannabinoids (CBGA, CBG, CBDA, CBD, THCA) in fiber type *Cannabis sativa* microgreens grown for 7 or 12 days with or without supplemental lighting (SL) and/or one-quarter strength Hoagland solution fertilizer (F).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CBDA (ppm)</th>
<th>CBD (ppm)</th>
<th>CBGA (ppm)</th>
<th>CBG (ppm)</th>
<th>THCA (ppm)</th>
<th>THC (ppm)</th>
<th>Total (ppm)</th>
<th>Total (% DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7D AL</td>
<td>532.5&lt;sup&gt;d&lt;/sup&gt;</td>
<td>10.5&lt;sup&gt;f&lt;/sup&gt;</td>
<td>53.3&lt;sup&gt;e&lt;/sup&gt;</td>
<td>10.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>81.1&lt;sup&gt;d&lt;/sup&gt;</td>
<td>n.d.</td>
<td>687.5&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.07</td>
</tr>
<tr>
<td>7D AL+F</td>
<td>1198.5&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>18.7&lt;sup&gt;f&lt;/sup&gt;</td>
<td>331.7&lt;sup&gt;e&lt;/sup&gt;</td>
<td>21.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>106.6&lt;sup&gt;d&lt;/sup&gt;</td>
<td>n.d.</td>
<td>1676.9&lt;sup&gt;de&lt;/sup&gt;</td>
<td>0.17</td>
</tr>
<tr>
<td>7D SL</td>
<td>1938.7&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>20.1&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>1353.2&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>69.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>218.7&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>n.d.</td>
<td>3600.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.36</td>
</tr>
<tr>
<td>7D SL+F</td>
<td>1209.9&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>31.2&lt;sup&gt;de&lt;/sup&gt;</td>
<td>937.5&lt;sup&gt;d&lt;/sup&gt;</td>
<td>59.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>143.7&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>n.d.</td>
<td>2382.1&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>0.24</td>
</tr>
<tr>
<td>12D AL</td>
<td>2237.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>49.4&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1061.5&lt;sup&gt;d&lt;/sup&gt;</td>
<td>67.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>258.6&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>n.d.</td>
<td>3674.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.37</td>
</tr>
<tr>
<td>12D AL+F</td>
<td>2524.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>40.5&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>249.7&lt;sup&gt;e&lt;/sup&gt;</td>
<td>26.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>161.9&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>n.d.</td>
<td>3002.2&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.30</td>
</tr>
<tr>
<td>12D SL</td>
<td>5509.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>63.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1502.9&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>71.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>329.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>n.d.</td>
<td>7476.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.75</td>
</tr>
<tr>
<td>12D SL+F</td>
<td>5139.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>87.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1733.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>67.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>324.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>n.d.</td>
<td>7352.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.74</td>
</tr>
</tbody>
</table>

Total percent dry weight (% DW) is listed in the last column. Different letters within each column denote a significant difference between means using Tukey’s HSD test at p ≤ 0.05. Not detectable amounts are indicated as n.d.

The results agree with Danzinger (2021) who reported that lighting effects can significantly alter the cannabinoid contents of CBDA+CBD, THCA+THC, and CBGA in *Cannabis* plants, however this was analyzed on fully mature *Cannabis* plants. Oier *et al.* (2016) showed that overall amounts of CBDA were higher in 12D fiber type *Cannabis* plants, but CBGA and THCA remained stable. Saloner and Bernstein (2021) found that supplemental nitrogen resulted in an overall decrease in total cannabinoid content, however this was observed at high N fertilizer
concentrations (>150 ppm N) whereas other cannabinoids had mixed results at higher or lower concentrations of N under 150 ppm. The one-quarter strength Hoagland solution used for fertilization in this experiment had 52.5 ppm N. Phosphorus fertilizer was shown to cause a reduction in cannabinoids in leafy material of flowering Cannabis plants (Bernstein et al., 2019).

Figure 16. Percent dry weight (% DW) of analyzed cannabinoids (CBGA, CBG, CBDA, CBD, THCA) in fiber type Cannabis sativa microgreens grown for 7 or 12 days with or without supplemental lighting (SL) and/or one-quarter strength Hoagland solution fertilizer (F). Different letters suggest significant difference among means indicated by the Tukey’s HSD test at p ≤ 0.05.

Supplemental lighting resulted in significantly higher amounts of individual cannabinoids in all treatments (Figure 17). The highest % DW of cannabinoids CBDA+CBD, THCA, and CBGA+CBG were all found in 12D SL treated plants, however supplemental fertilizer did not result in significantly lower amounts of cannabinoids. Future research of interest would be to
Figure 17. Percent dry weight (% DW) of analyzed individual cannabinoids (CBDA & CBD, THCA, CBGA & CBG) in fiber type *Cannabis sativa* microgreens grown for 7 or 12 days with or without supplemental lighting (SL) and/or one-quarter strength Hoagland solution fertilizer (F). Different letters suggest significant difference among means indicated by the Tukey’s HSD test at \( p \leq 0.05 \).
investigate effects of higher concentrations of fertilizer (perhaps one-half strength and full strength Hoagland solution) on hemp microgreens to determine how this affects the cannabinoid content. Based on these results and findings from others, it would be expected that overall cannabinoid content would decrease with increasing fertilizer solution.
CHAPTER 4. PHENOLICS AND ANTIOXIDANTS

4.1. Introduction

The *Cannabis sativa* plant is a well-known source of numerous bioactive compounds with potential medicinal and therapeutic benefit (Elsohly and Slade, 2005). One class of chemicals known as phenolics is comprised of compounds having an aromatic ring with one or more hydroxyl groups and functional derivatives (Shahidi and Naczk, 2003). Phenolic compounds are produced via the phenylpropanoid pathway or from the aromatic amino acids phenylalanine and tyrosine. The functional role of phenolics and phenolic acid derivatives in plants is for defense against pathogen attack and to maintain cell wall integrity (Faulds and Williamson, 1999). Cannabinoids, a class of terpenophenolics, are perhaps the most unique and actively researched chemical class found in the *Cannabis* plant. Other types of phenolics such as spiro-indans, dihydrostilbenes, dihydrophenanthrenes, simple phenols, and various flavonoids are also found to naturally occur in the *Cannabis* plant (Radwan et al., 2021). These compounds were identified and/or quantified in various parts of the plant and at different stages of maturity, however not in the microgreen stage.

Consumption of phenolic compounds may safeguard the body from certain chronic diseases (Ames, 1983). Phenolic compounds act as antioxidants, scavenging free radicals and reactive oxygen species. Proper functioning of molecular DNA, lipids, and protein are all lessened by free radicals, which can result in long-term damage to the body in the form of cancer, neurodegenerative disorders, atherosclerosis, and diabetes (Ames, 1983; Aruoma, 1998). The body has natural defense mechanisms against free radicals, but overproduction in the body or external exposure to x-rays, ozone, industrial chemicals, and air pollutants can raise levels of free radicals to unmanageable levels (Bagchi and Puri, 1998). Under these circumstances, free radicals damage the body, unless sufficient amounts of free radical scavenging molecules are present.
Phenolics occur naturally in various foods and their benefit to human health is becoming more apparent. Total phenolic determination is generally done using a spectrophotometric method with several types of reagents being accepted as reliable indicators of phenolic content in food material. The Folin-type assays (Folin-Denis and Folin-Ciocalteu reagents) are most commonly used when quantifying total phenolics in fruits and vegetables. The reagents measure the ability of the mixture or extract to reduce the compounds phosphomolybdic acid and phosphotungstic acid resulting in a blue complex (Swain and Hillis, 1959). Although widely accepted as an accurate measure of total phenolics in a sample, these methods have their limitations. Estimation of total phenolics can be overestimated if the sample material is high in ascorbic acid, as this non-phenolic compound reacts with the Folin-Ciocalteu reagent (Shahidi and Naczk, 2003; Singleton et al., 1999). In addition, high amounts of sugar, especially fructose, can result in an overestimation of total phenolics (Appel et al., 2001). Nevertheless, Folin type assays continue to be used for estimation of total phenolics in fruits and vegetables. The phenolic content of hemp microgreens has not been previously reported.

Antioxidants are compounds that delay the oxidation of proteins, carbohydrates, lipids, and DNA (Sindhi et al., 2013). Many compounds fall into the category of antioxidants including some vitamins, minerals, enzymes, carotenoids, phenolics, terpenes, and others (Sindhi et al., 2013; Irshad and Chaudhuri, 2002). It is becoming increasingly known that some plant materials are good sources of antioxidants within a range of tissue types, including fruits, leaves, shoots, stems, and roots (Saritha et al., 2010; Kamat et al., 2000; Jiratnan et al., 2004; Shyamala and Jumana, 2010; Vijikumar et al., 2011; Rhattacharya et al., 2010; Ruberto et al., 2000). Manufacturers of health foods prefer to choose all natural sources of antioxidants, as synthesized compounds tend to be unstable at higher temperatures, have high variability between food samples in manufactured
products, contain potentially carcinogenic properties, and be unfavorable in consumer preference (Papas, 1999). Thus, discovering more natural plant sources high in antioxidants is an ongoing research initiative.

Microgreens may possess unique nutritional qualities that are only in the beginning stages of being discovered. The first major research focusing on the bioactive compounds in microgreens was published in 2012, where Xiao et al. showed that various microgreens including red cabbage, radish, amaranth, and cilantro microgreens contained significantly higher concentrations of certain vitamins, carotenoids, and phenolic compounds than mature plants. However, this is not the case with all microgreens, as it has been shown that kale, pea, and lupin microgreens possess lower levels of carotenoids than the mature plants (Xiao et al., 2019, Klopsh et al., 2018). The researchers did find that broccoli and cauliflower microgreens had higher levels of carotenoids than their mature plant counterparts (Xiao et al., 2019). Additionally, kale and mustard microgreens were shown to have lower amounts of ascorbic acid than mature kale and mustard plants (de la Fuente et al., 2019).

Even with mixed results in the published literature, it is known that microgreens can possess a unique array of antioxidants. Microgreens of the Brassicaceae family have been more thoroughly studied, and research has indicated they have moderate to excellent levels of ascorbic acid, phylloquinone, carotenoids, tocopherols, glucosinolates, and polyphenols, with one study finding 165 phenolic compounds present in Brassicaceae microgreens (Sun et al., 2013; Xiao et al., 2019). It should be noted that rapid degradation of bioactive compounds can occur (Polash et al., 2018), so consumption of fresh microgreens soon after harvest is the most desirable form of preparation.
The objective of this study was to determine the total phenolic concentration (TPC) and antioxidant amounts in hemp microgreens.

4.2. Materials and Methodology

Seeds of fiber type hemp cultivars Altair, Anka, and New West Genetics (NWG) 452 were obtained in August, 2020 and grown in May 2021. Seeds were grown in a polyacrylic bi-wall covered greenhouse at the LSU Plant Materials Center (30.36209, -91.17407) in 25.4 x 50.8 cm trays seeded at a 1.3 cm planting depth with a planting density of 1.3 seeds per cm². The seeds were sown in a peat moss:vermiculite media (1:1). Plants were grown with either reverse osmosis (RO) water (12-16µS/cm) or with RO water plus supplemental fertilizer in the form of one-quarter strength Hoagland solution added. The irrigation (RO water) and fertigation (one-quarter strength Hoagland solution) treatments consisted of applying 650 mL to each tray once per day between 12:00-2:00 p.m. Plants were grown with either ambient lighting or ambient lighting plus supplemental lighting provided by a JCBritw (Shenzhen, China) 60W LED grow light positioned 60 cm above an arrangement of six trays seeded with hemp microgreens.

Four growing condition treatments were established, including ambient light with RO water (AL), ambient light with one-quarter strength Hoagland solution (AL + F), supplemental lighting with RO water (SL), and supplemental lighting with one-quarter strength Hoagland solution(SL + F). Seeding was done on May 15, 2021 and harvest occurred at 7 days or 12 days post seeding.

Four replications (n=4) from each growing treatment were acquired by random sampling of two 10.1x10.1 cm sections from two separate trays. Each cultivar was sampled separately, bringing the total amount of samples acquired for each growing treatment to n=12. Plants were harvested by cutting the microgreens immediately above the substrate line followed by placing
into small Ziploc bags and transported on ice to J.C. Miller Hall on the LSU main campus (30.40825, -91.17648). Fresh plant tissue was lyophilized at -65 °C for 72 hr. The freeze-dried plant tissue was then ground with a mortar and pestle and 100 mg (± 0.5 mg) was placed in a 15 mL centrifuge tube and extracted in 10 mL of 80% methanol. The mixture was vortexed for one min and sonicated for 15 min, stopping to vortex for one min per five min of sonication. The mixture was then centrifuged for 5 min at 1230 G. The supernatant was used for total phenolics and total antioxidant determination.

A modified Folin-Ciocalteu (FC) method (Swain and Hillis, 1959) was used to determine TPC. Exactly 0.5 mL of supernatant was placed in a 25 mL test tube and mixed with 8 mL of megapure water (Barnstead MP-12A, Haverhill, MA) followed by the addition of 0.5 mL of Folin-Ciocalteu reagent. After 3 min, 1 mL of 1 N sodium bicarbonate (Na₂CO₃) was added and the solution was allowed to stand for 2 hr. at ambient temperature (~22 °C). Absorbance of the resulting blue complex was measured at 750 nm using a Lambda 35 UV/Vis spectrophotometer (Perkin Elmer Instruments, Norwalk, CT). Gallic acid was used as the standard, as it is a commonly used phenolic acid standard in many scientific articles analyzing TPC in leafy vegetables and microgreens. A standard curve of gallic acid (50-300 µg/mL concentration) was plotted. The total phenolic content was expressed as mg gallic acid equivalent/g dry weight (mg GAE/g).

The antioxidant activity was measured according to the method developed by Brand-Williams et al. (1995), with slight modifications. DPPH (1, 1-diphenyl-2-picrylhydrazyl) was used as the source of free radicals (Yu et al., 2003). The absorbance of free radicals at 517 nm disappears upon their reduction by an antioxidant. In this study, Trolox (6-hydroxy-2, 5, 7, 8-tetramethyl-chroman-2-carboxylic acid) was used as the standard antioxidant compound. Exactly 0.1 ml of hemp microgreen extract was diluted with an additional 0.4 mL of 80% methanol and added to a
1.5 mL amber colored centrifuge tube containing 0.5 mL of freshly prepared 80% methanol solution of DPPH (0.01577 g/100 mL). The resultant mixture was shaken in the dark for 2 min and then incubated for 30 min at room temperature in darkness. The percent inhibition of DPPH was calculated from the decrease in absorbance using the equation below (Zhao et al., 2008).

\[
I\% = \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \times 100
\]

Whereas \( A_{\text{blank}} \) = absorbance of control; \( A_{\text{sample}} \) = absorbance of samples.

The decrease in DPPH absorbance was measured at 517 nm using a Lambda 35 UV/Vis spectrophotometer (Perkin Elmer Instruments, Norwalk, CT). The hemp extract was replaced with 80% methanol in the control sample. A solution of 80% methanol without DPPH was used as the blank. The antioxidant activity was calculated from a standard curve made with known concentrations of Trolox.

Data were analyzed with the statistical program SAS (version 9.4; SAS Institute, Cary, N.C.) Proc GLM with Tukey’s honest significant difference (HSD) test used for mean separation.

4.3. Results and Discussion

Differences in TPC and percent DPPH inhibition between cultivars were not significantly different, therefore the results were pooled together for simplicity in reporting. There were no significant differences in total phenolic content in hemp microgreens from the four different growing treatments. (Table 4.1, Figure 18). The highest TPC was found in 12D SL treated plants at 134.0 mg GAE/g which was insignificantly higher than all other treatments. The lowest TPC was found in 7D AL treated plants at 115.5 mg GAE/g.
Table 4.1. Total phenolic content (TPC) represented by mg GAE/g and radical scavenging potential (DPPH inhibition %) in fiber type Cannabis sativa microgreens grown for 7 or 12 days with or without supplemental lighting (SL) and/or one-quarter strength Hoagland solution fertilizer (F).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total Phenolic Content (mg GAE/g)</th>
<th>DPPH Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7D AL</td>
<td>115.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>67.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>7D AL+F</td>
<td>121.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>56.1&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>7D SL</td>
<td>118.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>67.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>7D SL+F</td>
<td>130.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>52.8&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>12D AL</td>
<td>130.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>53.8&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>12D AL+F</td>
<td>125.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>49.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>12D SL</td>
<td>134.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>53.6&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>12D SL+F</td>
<td>119.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>52.3&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Different letters suggest significant difference among means indicated by the Tukey’s HSD test at p ≤ 0.05.

Figure 18. Total phenolic content (TPC) represented by milligrams of gallic acid equivalent (mg GAE/g) in fiber type Cannabis sativa microgreens. Top left shows amount of GAE for plants grown for 7 or 12 days with or without supplemental lighting (SL) and with (Y) or without (N) one-quarter strength Hoagland solution fertilizer (F). Different letters suggest significant difference among means indicated by the Tukey’s HSD test at p ≤ 0.05. Other boxes show individual treatments with significance (p) value labeled in top right corner as compared with a Student’s t-test.
The amounts of total phenolics present in hemp microgreens are comparable to other reports of the TPC in inflorescence hemp extracts using the FC method (Palmieri et al., 2020). The authors reported amounts of 110.3 mg GAE/g in hemp leaves compared to a mean TPC of 125.6 mg GAE/g in hemp microgreens. However, reported amounts of TPC in hemp was highly variable, with amounts of TPC ranging from 0.9-312 mg GAE/g in different studies depending on part of the plant analyzed (Teh and Birch, 2014; Moccia et al., 2020; Liang et al., 2018; Chen et al., 2012, Agrawal and Hoffman, 2018; Palmieri et al., 2020). When compared to similar studies done on microgreens, TPC of hemp microgreens at 125.6 mg GAE/g is markedly higher than those reported by Ghoora et al., (2020) who found a TPC of 21.4-73.6 mg GAE/g for onion, mustard, carrot, fennel, sunflower, roselle, and French basil microgreens. Kowitcharoen et al. (2021) noted highly variable TPC in fourteen different microgreens, ranging from 9.2-269.0 mg GAE/g. The mean for these samples was 98.7 mg GAE/g. Subsequently, hemp microgreens could potentially be a better source of total phenolics when compared to other microgreen species. Mature vegetable crops are also highly variable in TPC, ranging from substantially higher amounts than those found in hemp microgreens (Ismail et al., Marjan, and Foong, 2004) to substantially lower amounts (Aryal et al., 2019).

It would be appropriate to conduct a follow up study comparing hemp microgreens grown along with other microgreen species under similar condition in order to compare TPC.

Plant age and supplemental fertilizer resulted in significantly lower amounts of antioxidant potential of hemp microgreens, whereas supplemental lighting had no significant effect (Table 4.1; Figure 19). Antioxidant potential was highest in 7D microgreens grown under either lighting condition, as long as fertilizer was not added to the watering solution.
Figure 19. DPPH inhibition (%) in fiber type *Cannabis sativa* microgreens. Top left shows inhibition of free radical amount for plants grown for 7 or 12 days with or without supplemental lighting (SL) and with (Y) or without (N) one-quarter strength Hoagland solution fertilizer (F). Different letters suggest significant difference among means indicated by the Tukey’s HSD test at $p \leq 0.05$. Other boxes show individual treatments with significance ($p$) value labeled in top right corner as compared with a Student’s t-test.

Antioxidant potential, represented by percent DPPH inhibition, ranged from 49.4-67.5% in hemp microgreens. This was similar to reports of percent DPPH inhibition for hempseed and seed flour, reported at 46.8-74.0%, and higher than amounts reported for seed oil at 8.2-22.0% (Sianno *et al.*, 2019; Moccia *et al.*, 2020).

Reporting of DPPH inhibition is variable, as authors use multiple ways to express DPPH equivalent, such as Trolox equivalent on a mole basis (for example, mmol/100 g), Trolox
equivalent per gram or milligram of tissue (TE/g or TE/mg), and half maximum inhibitory concentration (IC50) of free radicals. When using the mean value of the hemp microgreen samples (22 mmol/100 g TE), hemp ranks fairly high in antioxidant levels compared to an extensive database consisting of more than 3,000 food products (Carlsen et al., 2010). When compared to all plant-based foods, hemp microgreens rank between the 75-90th percentile, which has a range of 4.1-24.3 mmol/100 g TE. In comparison to vegetables and vegetable products, hemp microgreens rank above the 90th percentile of 1.5 mmol/100 g TE, but below the maximum amount reported of 48.07 mmol/100 g TE. When compared to 1) spices and herbs, and 2) herbal and traditional plant medicines, hemp microgreens fell well below the mean reported values of 29.0 and 91.7 mmol/100 g TE, respectively.

As with the analysis of total phenolic compounds, it would be appropriate to conduct a follow up study comparing hemp microgreens grown along with other microgreen species under similar conditions in order to compare total antioxidant concentrations.
CHAPTER 5. CONCLUSIONS

Hemp has the potential to be a healthy and nutritious product choice in the expanding microgreens market. It was found that hemp microgreens contain higher than average amounts of overall minerals when compared to reported amounts in commonly grown microgreens, such as broccoli, radish, kale, and arugula (Xiao et al., 2016; Li et al., 2021). Specifically, N, Mg, P, and Cu were all found to be higher in hemp microgreens, whereas Zn and Fe fell below previously reported values. In addition, amounts of K, Ca, S, Mg, Mn, and B fell within the range when compared to other commonly grown microgreens. Plant age resulted in significantly lower amounts of N, P, and S with significantly higher amounts of K, Ca, and Mg. Fertilizer use resulted in significantly lower P amounts, had no significant effect on N and Mg content, and resulted in significant increases in K, Ca, and S. Supplemental lighting resulted in significantly lower amounts of N, P, and S, and significantly higher amounts of Mg and Ca, and K.

This research provides a good base of information regarding the complex interactions between plant age, fertilizer use, and supplemental lighting on the mineral content of hemp microgreens. Further studies should be conducted comparing how these treatments affect other plant species, and if the results found herein agree with those findings. In addition, a comparative study between hemp microgreens and other commonly grown microgreen species grown under the same conditions should be conducted in order to determine which microgreens are the most nutrient dense.

Total cannabinoid content for CBD, CBDA, THC, THCA, CBG, and CBGA in hemp microgreens was found to be < 0.8% DW for all treatments. The amount of THC found in all samples was less than the machine detectable amount of 0.03%. Amounts of cannabinoids CBDA+CBD, THCA, and CBG+CBGA significantly increased with age and supplemental lighting. Fertilizer application had no significant impact on both overall and individual
cannabinoid content. Future studies should be conducted on how supplemental lighting affects overall cannabinoid content throughout maturity of hemp plants, as well as the effect of stronger fertilizer solutions on cannabinoid content in hemp microgreens.

The amounts of total antioxidants and phenolics were found to be higher than the average amounts previously reported in other microgreens (Ismail et al., 2004) as well as other plant-based foods (Carlsen et al., 2010). The different growing condition treatments had no significant effect on TPC, however fertilizer had a significant reducing effect on microgreen antioxidant content. Future studies should be done in order to see what effect stronger fertilizer solutions have on overall antioxidant and TPC of hemp microgreens. In addition, a comparative study between hemp microgreens and other plant species grown under the same environmental conditions should be done in order to determine how hemp microgreen antioxidant and phenolic content ranks in comparison to other microgreens.

Although hemp microgreens are a minor commodity in relation to the wide array of Cannabis products available, in addition to the variety of soon-to-be discovered uses, the research and discoveries included in this thesis provide valuable information on the mineral, cannabinoid, phenolic, and antioxidant contents.
LITERATURE CITED


Smith, R., Cahn, M, and Hartz, T.K. (2016). Evaluation of N uptake and water use of leafy greens grown in high-density 80-inch bed plantings and demonstration of best management


VITA

Cheston Tyler Schayot, born in Metairie, Louisiana, worked for several years performing tree care and landscaping services throughout Southeast Louisiana preceding and during his time earning a bachelor’s degree in interdisciplinary studies from Louisiana State University (LSU) in 2017. Upon completing his undergraduate studies, he continued operating the business until beginning a master’s degree program at LSU researching hemp as a microgreen and its nutritional properties. He has worked closely with different departments at the university and acquired a knowledge base that will set him up for successful future academic endeavors. He will receive his master’s degree in December, 2021 and plans to continue operating the tree care and landscape business before starting a doctorate degree in the future.