

The Composition of Hemp Seed Oil and Its Potential as an Important Source of Nutrition

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ABSTRACT. The fatty acid and natural product content of hemp seed oil was analyzed by GC-MS and LC-MS. The presence of linoleic acid (LA) and α -linolenic acid (LNA) were confirmed in their previously reported ratio of 3:1 LA:LNA. The presence of β -caryophyllene (740 mg/L), myrcene (160 mg/L), β -sitosterol (100-148 g/L) and trace amounts of methyl salicylate was observed in the oil which had not been previously reported. Trace amounts of cannabidiol (CBD) were also detected. Bioassays were performed with the oil to determine its effectiveness as an antimicrobial agent. Some bioactivity was observed during the primary screening. *[Article copies available for a fee from The Haworth Document Delivery Service: 1-800-342-9678. E-mail address: <getinfo@haworthpressinc.com> Website: <<http://www.haworthpressinc.com>>]*

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KEYWORDS. Hemp (*Cannabis sativa* L.) seed oil, essential fatty acid, linoleic acid, α -linolenic acid, β -sitosterol, cannabinoids, functional food

ABBREVIATIONS. AA, arachidonic acid; CBD, cannabidiol; CBDA, cannabidiolic acid; DGLA, dihomogamma linoleic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; GLA, γ -linolenic acid; LA, linoleic acid; LNA, α -linolenic acid; THC, Δ^9 -tetrahydrocannabinol

INTRODUCTION

Hemp (*Cannabis sativa* L.) seed oil is valued primarily for its nutritional properties as well as for the health benefits associated with it. Although its fatty acid composition is most often noted, with oil content ranging from 25-35%, whole hemp seed is additionally comprised of approximately 20-25% protein, 20-30% carbohydrates, and 10-15% fiber, along with an array of trace minerals (Deferne and Pate, 1996). With a complete source of all essential amino and fatty acids, hemp seed oil is a complete nutritional source. In addition, constituents exist within the oil that have been shown to exhibit pharmacological activity (Deferne and Pate, 1996; Erasmus, 1999).

Hemp seed oil contains linoleic acid (LA) and α -linolenic acid (LNA) as its major omega-6 and omega-3 polyunsaturated fatty acids (PUFA), respectively. These fatty acids comprise the most desirable contents of the oil, especially due to the ratios in which they exist. The 3:1 ratio of LA to LNA is alleged to be optimal for nutrition (Deferne and Pate, 1996; Callaway, Tennila & Pate, 1996; Erasmus, 1999). The additional presence of gamma-linolenic acid (GLA) in hemp seed oil ultimately makes its nutritional value superior to most comparable seed oils. The myriad of benefits reported to be attributable to omega-3 PUFA include anticancer, anti-inflammatory, and anti-thrombotic properties. In addition, dietary omega-3 PUFA help to increase general metabolic rates and promote the burning of fat (Erasmus, 1999; Simopoulos, 1994).

Cannabidiol (CBD) has been found to be present in hemp seed oil as well. Although not explicitly produced within the seed, traces of cannabinoid contamination have been reported to result from the pressing of the oil (Grotenhermen et al. 1998). Reports of cannabinoid contamination have been focused primarily on delta-9-tetrahydrocan-

nabinol (THC) with THC levels in oil reported at up to 50 ppm (Grotenhermen, Karus & Lohmeyer, 1998). The production and storage of both CBD and THC occur in the glandular structures of the plant and the concentrations of CBD are typically much higher than THC in most fiber and oil varieties of hemp. Therefore, it can be assumed that the concentration of CBD as a contaminant in the oil would be greater than the concentration of THC which has been reported in the literature. The presence of CBD is significant because it has documented anticonvulsive, anti-epileptic, and antimicrobial properties (Karler and Turkanis, 1973; Ferenczy, Gracza & Jakobey, 1958). Although the levels of CBD within the oil are typically small, many health benefits may still be gained from its presence.

Although previously identified only in the essential oils of the *Cannabis* plant (Hendriks et al., 1978), terpenoid compounds have been identified as being present within the seed oil. Health benefits may be gained from their presence even at concentrations similar to that of CBD. As is the case with CBD, the presence of these terpenes is most likely the result of contamination from glandular hairs during oil processing. Nevertheless, the major terpenes identified have been cited as having anti-inflammatory, anti-allergenic, and cytoprotective pharmacological properties (Tambe et al., 1996).

While many studies exist which base the nutritional value of hemp seed oil primarily on its fatty acid content, there are other constituents which are contained within the oil that possess beneficial properties as well. Natural products such as β -sitosterol and methyl salicylate complement the nutritious value of hemp seed oil and increases its effectiveness as a functional food. Even though the existing data on hemp seed oil clearly demonstrates its nutritional value, these additional compounds do add a marketable value, and need to be examined further for additional beneficial qualities and characterizations.

MATERIALS AND METHODS

GC-MS Analysis of Hemp Oil Constituents

The analysis of the total fatty acid composition of hemp oil was performed using standard techniques and reagents. The hemp oil sam-

ples (40 μL) were saponified and methylated as described by Sasser, 1990. The samples were manually injected in the splitless mode into a gas chromatograph (model 5890, Hewlett-Packard)/mass spectrometer (model 5971, Hewlett-Packard) equipped with a 30-m \times 0.25 mm DB-5MS fused silica capillary column (J&W Scientific, Folsom CA). Chromatographic parameters were as follows: injection temperature at 280°C, initial oven temperature at 50°C for 5 min followed by a ramp at 5°C/min to 280°C. Fatty acid standards of palmitic, oleic, stearic, linolenic, linoleic and gamma-linolenic acids (Sigma, Saint Louis, MO) were processed and analyzed simultaneously for purposes of identification and quantification. Hemp oil samples were typically diluted in hexane 1:1 for natural products analysis. The concentrations of myrcene and β -caryophyllene within the oil were based on standards obtained from Sigma, St. Louis, MO. The trace amounts of methyl salicylate were identified by GC retention time and mass fragmentation pattern.

LC-MS Analysis of Hemp Oil Constituents

Unmethylated total fatty acids and free fatty acids were also analyzed using a Waters Integrity® LC-MS system consisting of a 616 pump, 717 plus autosampler, 996 photodiode array detector and a Thermabeam® EI-MS detector. The Thermabeam Mass Detector operates with standard electron impact ionization energy of 70 eV and operated in the scanning mode from 45 to 700 m/z producing library searchable spectra. Spectral data was managed by the Millennium v. 2.21 LC-MS software. A Waters semi-microbore Nova Pak C₈ column (2 mm \times 150 mm) was equilibrated with 0.5% acetic acid:acetonitrile (95: 5, v/v) with a flow of 0.25 mL/min. After injection, a gradient to a final solvent composition of 5:95, v/v, was established over 25 min. The solvent composition will then be returned to initial over 2 min and equilibrated for 15 min prior to subsequent injections. Mass fragmentation pattern searches using the Wiley® registry for mass spectral data, 6th edition were used for the identification of the fatty acid and chemical constituents of the oil in addition to the use of chemical standards. Analysis of natural products by LC-MS was performed with hemp oil diluted in isopropanol. Concentrations of β -sitosterol, α/γ -tocopherol, and CBD were then quantified on the basis of standards supplied by Sigma, St. Louis, MO.

Activity Bioassay

Assay screenings were performed using hemp seed oil diluted into several solvents. Sample solutions were prepared using 500 μL hemp oil diluted 1:5 into either 80% methanol or 100% methanol, or 1:1, 1:3, and 1:5 in hexane (Sigma, St. Louis). Complete solubility was achieved exclusively in hexane and emulsions were formed with other solvents. The oil sample emulsions of 80% and 100% methanol were vortexed for 10 seconds and then centrifuged at $10,000 \times g$ and 21°C for 5 minutes to separate into distinct layers. The supernatant layer was then tested for activity.

The oil/solvent solutions were evaluated for their ability to inhibit the growth of organisms representing the major pathogenic classes: *Aspergillus niger* (mycelium-forming fungi), *Escherichia coli* (Gram-negative), *Staphylococcus aureus* (Gram-positive), *Saccharomyces cerevisiae* (yeast, single cell fungi), and *Pseudomonas aeruginosa*. Bacterial (*E. coli* and *S. aureus*) cultures were cultivated and maintained on solid agar medium (LB Agar, Miller). Before performing each assay, bacteria was transferred into liquid medium and cultivated for 12 hours at 37°C on a shaker. Preliminary studies show that this cultivation results in cell density values of 10^5 - 10^6 , which is sufficient for antimicrobial activity evaluation. *S. cerevisiae* was cultivated and maintained on potato dextrose medium. Prior to testing, yeast cells were transferred into liquid medium and cultivated for 48 hours at 30°C on a shaker.

Sterile, plastic microplates containing 24 wells (4×6) were used for testing. Two milliliter of freshly sterilized LB agar medium (for antibacterial tests), or 2 mL of potato dextrose agar (for antifungal tests), were dispensed in each well of the 24-well sterile microplates under sterile conditions at a temperature of 40 - 50°C . Ten microliter aliquots of oil/solvent samples were injected into each well in triplicate, the fourth well being used as a control with 10 μL of solvent. This was repeated for each microorganism for a total of 25 (5×5) rows of testing. The plates were left open for a few minutes in the laminar flow hood, so that the solvent of the 10 μL sample partly diffused and partly evaporated from the surface, after which 30 μL of the previously prepared bacterial suspension, or fungal spore suspension was plated on the agar surface of each well. Plates were then closed, marked, and transferred in an incubator for 24 hours at 30°C . After incubation, the plates were examined for cells/spores growth inhibition zones.

A smaller variety of microorganisms were used in the secondary screening, as the primary screening eliminated possible activity against certain microbes. The oil/solvent solutions were re-tested for activity against *Staphylococcus aureus*, *Escherichia coli* and *Saccharomyces cerevisiae* using the same methods as described in the primary screening.

RESULTS AND DISCUSSION

Fatty Acids in Hemp Oil

The hemp seed oil used in this study was pressed from Canadian grown seed of the French variety Fedora-19 and was provided by CGP Canada, Ltd. The results of fatty acid analysis are shown in Table 1. These results further strengthen previous reports that the relative ratios and composition of hemp oil fatty acids are ideal for human nutrition.

Benefits of Essential Fatty Acids

While there are many sources for omega-3 PUFA in the diet, hemp seed oil is exceptionally rich in these compounds, which are usually present in the nutritionally optimal ratio of omega-6 to omega-3 PUFA (LA to LNA) of 3:1 (Erasmus, 1999). As shown in Table 1, LA concentrations ranged from 52-62% of total fatty acid composition while LNA concentrations ranged from 12-23%. The range of concentrations of fatty acids results from the natural variation of individual samples of the Fedora hemp oil being tested. Several factors, including processing and storage methods, as well as age of the samples being tested, could contribute to the variability of the fatty acid profile.

As a result of the change in dietary habits within the past century, the intake of *trans* fatty acids has increased dramatically. Studies have shown conclusively that *trans* fatty acids increase total cholesterol levels and diminish the levels of “good” high density lipoprotein (HDL). By supplementing the diet with high levels of unsaturated *cis* fatty acids, some of these negative effects can be reversed (Erasmus, 1999). With respect to modern diets, the amount of LA consumed compared to the amount of LNA consumed has increased exceptionally in the past 100-150 years (Simopoulos, 1994). This disparity has

TABLE 1. Hemp Seed Oil Macrocomposition

Components	Reported (Deferne and Pate, 1996; Callaway and Laakkonen, 1996)	Results
<i>Fatty Acids</i>	(% w/w)	(% w/w)
Linoleic Acid (18:2ω6)	50-70	52-62
α-Linolenic Acid (18:3ω3)	15-25	12-23
Oleic Acid (18:1ω9)	10-16	8-13
Palmitic Acid (16:0)	6-9	5-7
Stearic Acid (18:0)	2-3	1-2
γ-Linolenic Acid (18:3ω6)	1-6	3-4
Eicosanoic Acid (20:0)	0.79-0.81*	0.39-0.79
Eicosenoic Acid (20:1)	0.39-0.41*	0.51
Eicosadienoic Acid (20:2)	0.00-0.09*	0.00
<i>Natural Products</i>		
Cannabidiol	nr	10 mg/kg
Δ^9-tetrahydrocannabinol	50 mg/kg**	nd
Myrcene	nr	160 mg/L
β-Caryophyllene	nr	740 mg/L
β-Sitosterol	nr	100-148 g/L [‡]
α-Tocopherol	7-80 ppm ^{†,††}	tr
γ-Tocopherol	710-870 ppm ^{†,††}	468 mg/L
Methyl salicylate	nr	tr

* as reported for variety FIN-314

** as reported by Grotenherman et al. (1998)

† as reported by Blade (1997)

†† as reported by HYChem Corporation. Henry Yard, personal communication

‡ total sterol content measured as β -sitosterol

nr—not reported in cold pressed oil

nd—not detectable (lower limits of detection could not be determined without THC standard)

tr—trace amounts

disrupted the proper balance of dietary essential fatty acids that is considered nutritionally optimal. In addition to the lack of these essential fatty acids in the diet, factors such as stress and disease weaken the enzymatic activity that promotes the conversion of LA to GLA (Deferne & Pate, 1996). Therefore, a supplementation of LA can be helpful to alleviate this potential deficiency.

In an ideal diet, the daily consumption of fats should not exceed 15-20% of total caloric intake. Approximately one-third of these fats should be the essential fatty acids in their proper ratio. For a 2500 calorie/day diet, LA intake should be 9-18 grams/day, and LNA intake should be 6-7 g/day (Erasmus, 1999). This goal can easily be accomplished through the daily consumption of 3 to 5 tablespoons of hemp oil. Although these are the ideal amounts to maintain a healthy, bal-

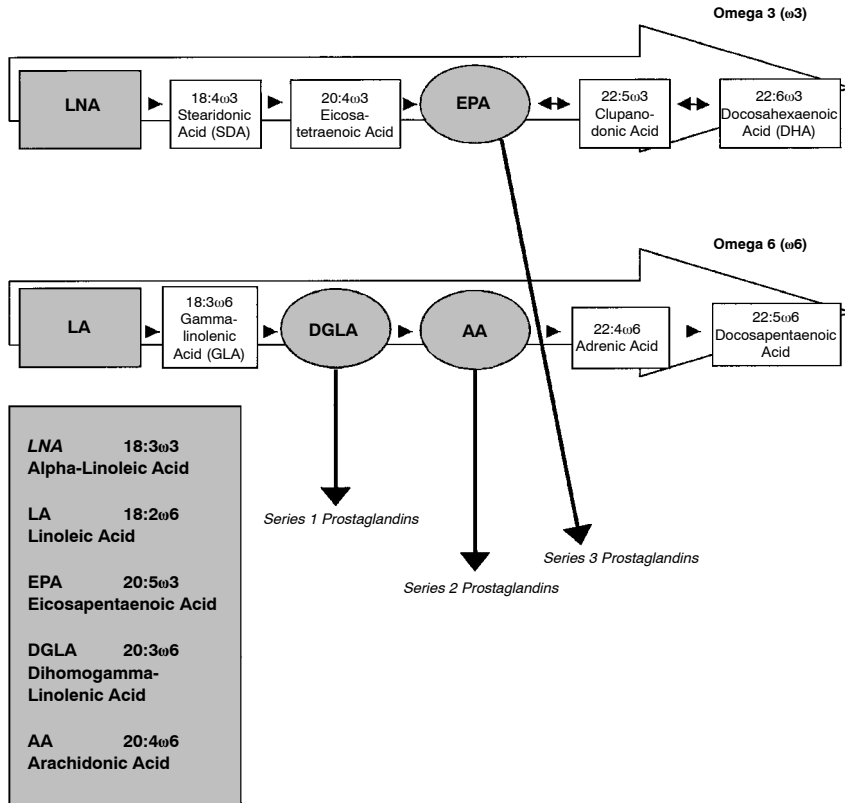
anced diet, certain stresses to the body warrant increased consumption of essential fatty acids, particularly the omega-3 PUFA such as LNA.

Omega-3 PUFA have been reported to have an inhibitory effect on cancer and tumor growth. Increased consumption of omega-3 PUFA have not been shown to exhibit any negative side effects, but their beneficial qualities have been repeatedly confirmed. In addition to their anticancer properties, omega-3 PUFA have been shown to lower blood pressure and blood cholesterol levels, help normalize fat metabolism and decrease insulin dependence in diabetics, increase overall metabolic rate and membrane fluidity, and exhibit anti-inflammatory properties, specifically with regard to relieving arthritis (Erasmus, 1999). The benefits of omega-3 PUFA are not only present when taken in large quantities but the regular intake of recommended levels (2-2.5% of caloric intake/day) can be sufficient to provide many of its nutritional qualities.

The essential role of LA and LNA in the human diet is related to both the intermediary and end products that they become through several biochemical pathways. The fatty acid metabolism of LA and LNA is elucidated in Figure 1. LA is metabolized to GLA and subsequently arachidonic acid (AA). LNA is metabolized to both eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Simopoulos, 1994). EPA and AA are metabolized by the body into eicosanoids. These compounds ultimately become the prostaglandins which affect such varied functions as blood clotting, inflammation response, and immunoregulation (Erasmus, 1999).

During the synthesis of prostaglandins from AA and EPA, there is a biochemical competition within the cell membrane (Simopoulos, 1994). The AA has a tendency to move out of the cell membrane and form type 2 prostaglandins. EPA tends to promote the retention of AA within cell membranes, thereby preventing the formation of the unwanted type 2 prostaglandins (Erasmus, 1999). When the ratio of the initial starting compounds is shifted in favor of LA, however, it becomes more difficult for the products from LNA to sufficiently promote the retention of AA within the cell membrane. The resultant increase in type 2 prostaglandin production leads to increased platelet aggregation and inflammation (Erasmus, 1999). The benefits of having the proper ratios of fatty acids, with respect to the metabolized products of LA and LNA, are the production of the proper amounts of prostanoids and leukotrienes which have anti-thrombotic, anti-vasoconstrictive, and anti-inflammatory properties (Simopoulos, 1994).

FIGURE 1. Fatty Acid Metabolism (Adapted from Erasmus, 1999)



Natural Products in Hemp Oil

The results of the natural products analysis of the hemp oil are shown in Table 1. These results suggest that several natural products, such as cannabidiol, β -caryophyllene, myrcene, β -sitosterol, α/γ -tocopherol, and methyl salicylate may confer further health benefits to hemp oil in addition to fatty acids.

Cannabidiol

Pharmacological Properties of Cannabidiol. Cannabidiol (CBD) has been shown to possess several desirable pharmacological proper-

ties which are exhibited in absence of the psychoactive properties of THC (Karler & Turkanis, 1981), which are usually associated with the cannabinoids. Although the levels of CBD detected in the oil were low at 10 mg/kg, its presence could still provide some benefit. CBD has been reported to reduce tremors in dystonic movement disorders with minimal side effects (Consroe et al., 1986). Patients receiving doses of CBD ranging from 100-600 mg/day had tremor reductions of 20-50% (Consroe et al., 1986). The anticonvulsant and anti-epileptic activity of CBD has also been well documented (Karler et al., 1973; Karler & Turkanis, 1981). CBD has been found to be relatively selective with respect to the central nervous system (CNS), in contrast to THC (Karler & Turkanis, 1981). Its anticonvulsant activity is on the same order of magnitude of THC, but unlike THC, it lacks psychoactivity. CBD's added efficacy as an anti-epileptic, without the associated side effects of psychoactivity, give it great pharmacological potential.

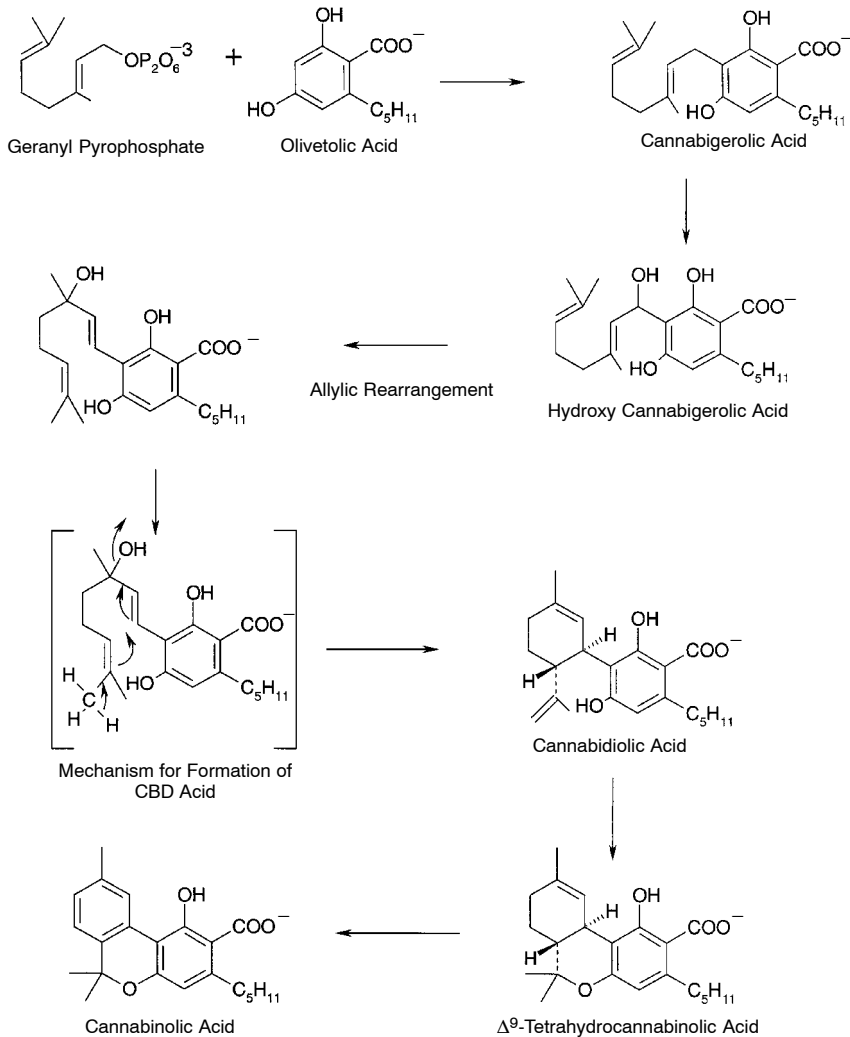
Analgesic and anti-inflammatory potential has been reported in animal studies with CBD as well (Formukong et al., 1988). CBD has been shown to inhibit both the induction of phenyl benzoquinone (PBQ) induced writhing and tetradecanoyl phorbol-acetate (TPA) induced erythema (Formukong et al., 1988). The mechanism by which CBD achieves its anti-inflammatory properties is possibly related to its effect on arachidonate metabolism (Formukong et al., 1988).

Antimicrobial activity has also been reported for CBD. Specifically, CBD has been shown to inhibit the growth in Gram-positive bacteria such as *Streptomyces griseus* and *Staphylococcus aureus* (Ferenczy et al. 1958). These organisms are particularly sensitive to extracts of *Cannabis* in slightly acidic culture medium even at dilutions as low as 5 ppm.

Biosynthesis. It is generally accepted that the biosynthetic pathway of the cannabinoids begins with the condensation of geranyl pyrophosphate with olivetolic acid (Clarke, 1981; Turner et al., 1980). As shown in Figure 2, the initial cannabinoid formed is cannabigerolic acid, which in turn is converted into cannabidiolic acid, tetrahydrocannabinolic acid, and ultimately cannabinolic acid. Several other cannabinoids are also formed in smaller quantities from side reactions. It has been reported that transient propyl and methyl forms of the cannabinoids exist as well as the predominant pentyl forms (Clarke, 1981).

The chemical structures of the cannabinoids in Figure 2 are depicted as their acid forms. These molecules do not possess any psy-

FIGURE 2. Cannabinoid Biosynthetic Pathway (Adapted from Clarke, 1981)



choactivity until they are decarboxylated. Decarboxylation occurs spontaneously or with the addition of heat (Clarke, 1981). The biosynthesis of the cannabinoids proceeds through the pathway with the molecules in their acid forms. It is the metabolism of these acid forms which will ultimately determine which cannabinoids will accumulate

(Clarke, 1981). The ratio of these specific cannabinoids is used to determine the gross chemotype of particular hemp plants. Evidence exists which shows the relation of chemotype to latitude of cultivation.

Through experimentation and observation, it has been determined that increasing ultraviolet (UV) radiation accelerates the “ripening” process of *Cannabis* (Turner et al., 1980). In tropical latitudes, *Cannabis* specimens tend to complete the ripening process with nearly complete conversion of CBD into THC. This is contrasted by *Cannabis* which is cultivated at more temperate latitudes where there is a higher proportion of CBD to THC within the plants. The increasing amounts of UV light at latitudes approaching the equator tends to accelerate production of THC from CBD, most likely due to an evolutionary advantage for THC accumulation as a protective agent of UV light (Pate, 1994).

The cultivation of modern day industrial hemp crops in more northern latitudes will show a gross chemotype of high CBD/low THC. With these “unripe” varieties, it will be possible to take advantage of the relatively high levels of CBD as compared to THC, and exploit the many benefits of CBD without risk of psychoactivity. The oil which was subjected to investigation here was Canadian grown. It has significant concentrations of CBD but no detectable THC. These results are consistent with the predicted cannabinoid content of northern-grown plants.

β-Sitosterol

Another component of hemp seed oil with several reported activities is β -sitosterol. Although studies have primarily demonstrated the efficacy of β -sitosterol in reducing hypercholesterolemia, additional antiviral, antifungal, and anti-inflammatory properties have been studied and observed (Malini & Vanithakumari, 1990).

Plant sterols have been known to affect plasma cholesterol levels by blocking cholesterol absorption through crystallization and coprecipitation (Mattson et al., 1982). Within the intestinal lumen, phytosterols reduce cholesterol solubility by excluding it from micelles, thereby preventing its absorption. In addition, competition exists between the sterols and cholesterol for uptake into the intestinal mucosa (Lees et al., 1977). A quantitative representation of this can be seen in human studies. Patients given 500 mg of cholesterol daily in their diets in addition to 1 g of β -sitosterol showed decreased cholesterol absorp-

tion. Mean reduction levels were 42%, demonstrating the efficacy of β -sitosterol even at low concentrations (Mattson et al., 1982). As shown in Table 1, sterol concentrations based on β -sitosterol were measured in sufficient quantities at 100-148 g/L. Although β -sitosterol was the predominant sterol, other minor sterols may have contributed to this measurement. At these levels, many of β -sitosterol's beneficial qualities will be obtainable.

β -Sitosterol seems to be particularly effective in cholesterol uptake inhibition, especially when delivered through dietary fats (Lees et al., 1977; Mattson et al., 1982). No appreciable decreases in efficacy were observed, even with long-term administration (Lees et al., 1977). In addition, lack of toxicity and little, or no side effects have been attributed to β -sitosterol, making it an attractive option for long-term cholesterol reducing therapy (Lees et al., 1977; Mattson et al., 1982).

Although not studied as extensively as its hypocholesterolemic properties, relevant antiviral and anti-inflammatory activities of β -sitosterol have been shown. Isolated ethanolic extracts of *Hedychium spicatum* containing β -sitosterol showed anti-inflammatory activity (Sharma et al., 1975). When purified, β -sitosterol fractions from *Artemisia annua* showed upwards of 80% virus inhibitory activity against tobacco mosaic virus (Abid Ali Khan et al., 1991).

Tocopherols

Antioxidant properties of tocopherols have been known and exploited for some time. Traditional supplementation of tocopherols has primarily focused on its α form. Many plants however, including hemp, tend to have significantly higher levels of γ -tocopherol. Although both exhibit antioxidant activity, their differing metabolic paths confer other specific activities to their respective isomeric forms.

α -Tocopherol is the primary (usually exclusive) tocopherol in formulated vitamin E supplements. It is preferentially secreted into plasma as opposed to γ -tocopherol which tends to be found in the intestine (Stone & Papas, 1997). It is α -tocopherol's concentration in the plasma that gives it properties other than that of an antioxidant. α -Tocopherol may induce increased membrane fluidity through intercalation between fatty acyl chains in the membrane bilayer (Berlin et al., 1992). Data suggests that there is a direct correlation between in-

creased fluidity and α -tocopherol content in the membrane (Berlin et al., 1992).

The biological activity of α -tocopherol tends to be significantly higher than γ -tocopherol as a result of its greater affinity to be secreted by the liver into very-low density lipoproteins (Stone & Papas, 1997). This increased bioactivity does not however make α -tocopherol a more effective antioxidant; γ -tocopherol inhibits phosphatidylcholine-hydroperoxide formation more effectively at low peroxy-nitrite concentrations than does α -tocopherol (Wolf, 1997). γ -Tocopherol has been shown to have significant antioxidant effects *in vitro* even at concentrations less than 50 ppm (Lampi, Hopia, & Piironen, 1997). In addition, γ -tocopherol is overall more effective in protecting against coronary heart disease, as compared to α -tocopherol supplementation (Wolf, 1997).

Perhaps the most interesting activity of γ -tocopherol which has not yet been widely studied, is its ability to act as an anticancer agent, specifically with respect to colon cancer. Because γ -tocopherol is secreted via the bile into the intestine and fecal material, it can inhibit lipid peroxidation and reduce the formation of mutagenic peroxidation products in the bowel (Stone & Papas, 1997). Ultimately, by being excreted into the colon, as opposed to being active in the plasma, γ -tocopherol is able to minimize DNA damage caused by reactive nitrogen oxide species (Stone & Papas, 1997).

Within hemp seed oil, γ -tocopherol is present in significantly higher quantities than α -tocopherol; the Fedora sample tested had 468 mg/L of γ -tocopherol with only trace amounts of α -tocopherol. They both however, play an important role as antioxidants in their respective physiological systems. The additional bioactive properties they possess add to their benefits as components of the seed oil.

Terpenes

The presence of several terpenes were confirmed in the seed oil, the most abundant of which were β -caryophyllene and myrcene which were found at 740 mg/L and 160 mg/L, respectively (Table 1). The terpene compounds, in general, are primarily found in the essential oil of *Cannabis* rather than in the seed oil (Hendriks et al., 1978) as a result of their production in the glandular structures on the aerial portions of the plant. These compounds are a component of the char-

acteristic aroma of *Cannabis* and may impart some of these properties to the seed oil. Additional benefits may be provided to the oil as well.

Some previously noted pharmacological properties of β -caryophyllene would include anti-inflammatory and cytoprotective activities which may too be active in the seed oil. In addition, it has been reported that myrcene exhibits antioxidant properties (Duke, 1999). The presence of β -caryophyllene and myrcene, even if only present as contamination components, add beneficial value to an already nutritionally important food product.

Methyl Salicylate (Oil of Wintergreen)

The medical benefits of plant salicylates have been enjoyed by people for centuries. Today aspirin or acetylsalicylic acid, a close relative of methyl salicylate, is one of the most widely used drugs in the world because of its antipyretic, anti-inflammatory and analgesic properties. Once injected, methyl salicylate can be hydrolyzed to salicylic acid, a common active ingredient of aspirin and most other salicylates. Thus, pharmacological effects of methyl salicylate are similar to those of aspirin. Also, millions of people regularly take low doses of salicylates (aspirin) to reduce the risk of heart attacks, strokes and cancer. Methyl salicylate deserves particular attention as a beneficial component of hemp oil, even if present in trace quantities.

Hemp Oil Bioassay

Because of the relatively complex macrocomposition of hemp seed oil, numerous compounds within the oil have the potential to exhibit antibacterial and/or antimicrobial activity. Samples of the oil were diluted in various solvents and tested against several microorganisms, including bacteria and fungi.

A screening with an 80% methanol supernatant showed the ability of a component of the hemp oil to inhibit the growth of yeast in 2 of the 3 wells tested. It is unclear, however, if this activity is sufficient to characterize a constituent of the hemp oil as a significant inhibitor of yeast growth. The hemp oil dissolved 1:1, 1:3, and 1:5 in hexane without an initial extraction into other solvents also showed some bioactivity, significantly more so than the 80% methanol supernatant sample. There were clear zones of growth inhibition on the agar in the

three samples of hemp oil diluted with hexane, which would indicate a more significant inhibition of yeast growth.

Secondary screenings performed to support the results of the initial assays were inconclusive. The growth inhibition of yeast that was exhibited during the first screening did not yield the same results upon replication. Factors such as the concentration of antimicrobial compounds within the oil, or deterioration of the oil due to age could have played a role in the inability to replicate the initial results.

Even though it is unclear how significant the ability of hemp oil constituents to inhibit the growth of yeast is, some activity was detected which has never been previously reported in the literature. Therefore it is possible that the hemp seed oil may have an antimicrobial component, separate from CBD, which specifically prevents the growth of yeast, an activity which has not been previously demonstrated.

Although the screenings that were performed could not demonstrate antibacterial properties within the oil, reports of antibacterial properties of CBD have been documented in the scientific literature. In previous experiments, CBD was found to inhibit the growth of Gram positive bacteria (Ferenczy et al., 1958). Previous reports of antibacterial activity with respect to CBD have primarily focused on CBD concentrates taken from the resin of the plant. Because CBD is a contaminant in the seed oil as a result of oil processing techniques, and not actually produced within the seed according to the literature, the levels of CBD in the oil are most likely to be too low to exhibit antibacterial properties. It has been noted, however, that in previous screenings of CBD for antibacterial properties, there is a strong correlation between the plant's levels of cannabidiolic acid (CBDA) and antibacterial effectiveness (Radosevic, 1962). Plants which contained higher concentrations of CBDA displayed more pronounced antimicrobial activity. This also correlates with the observation that *Cannabis* plants from more northern latitudes have stronger antimicrobial activity than more tropical plants, most likely due to the specific cultivars' CBD/THC ratio.

SUMMARY

After detailed analysis of the macrocomposition of the hemp seed oil, several constituents which have not previously been reported within the oil have been detected, along with the major fatty acid components.

Hemp seed oil is comprised almost entirely of fatty acids, with an

essential fatty acid content of approximately 75%. As shown in Table 1, hemp oil is comprised primarily of LA and LNA in a 3:1 ratio. These results have been reported in the literature and were confirmed in this study. Other beneficial natural products such as β -sitosterol, which contributes hypocholesterolemic properties, and the tocopherols, which have both antioxidant and anticancer activities, are present in sufficient efficacious quantities. In addition, measurable amounts of terpenes, cannabinoids and phenolics were detected, including methyl salicylate which itself has many health benefits.

The reported health benefits of hemp seed oil, and especially the essential fatty acids, are well documented. When diets are supplemented with omega-6 and omega-3 PUFA in the proper 3:1 ratio, numerous benefits to health are achieved, including but not limited to greater resistance to cancer, inflammation, and blood clotting. A general increase in metabolism and lowering of overall blood cholesterol levels has also been observed.

In addition to all of these positive health benefits associated with the use of hemp oil, there seems to be a complete lack of negative effects from its consumption. To date, there has been no reported cases of toxicity from the ingestion of hemp seed oil. Toxicity has also not been observed with any of the other constituents that were found as contaminants, which are primarily the cannabinoids.

One reason for the lack of negative side effects from excessive ingestion of hemp oil is specifically related to the ratio of LA:LNA. Because most oils do not contain the optimum ratio of omega-6 and omega-3 PUFA, they tend to promote the accumulation of metabolic intermediates that in turn hinder fatty acid metabolism. The properly balanced hemp seed oil does not promote an over-accumulation of certain metabolic products and all of the fatty acid metabolic pathways have the necessary intermediates to work efficiently regardless of the quantities consumed.

The value of hemp seed oil is only beginning to be recognized in the marketplace. Its ideal fatty acid composition serves as only one of several potential beneficial qualities. A nutritionally complete food product that also exhibits several active pharmacological properties will undoubtedly have an appeal to a variety of potential markets and consumers. Although initially marketed to the natural foods consumer, the many benefits of hemp seed oil as an ideal food product and a nutritional supplement can be exploited providing interest to the mainstream consumer as well.

REFERENCES

- Abid Ali Khan MM, Jain DC, Bhakuni RS, Zaim M, Thakur RS (1991). Occurrence of some antiviral sterols in *Artemisia annua*. *Plant Science* 75: 161-165.
- Berlin E, Bhatena S, Judd J, Nair P, Peters R, Bhagavan H, Ballard-Barbash R, Taylor P (1992). Effects of omega-3 fatty acid and vitamin E supplementation on erythrocyte membrane fluidity, tocopherols, insulin binding, and lipid composition in adult men. *Journal of Nutritional Biochemistry* 3: 392-400.
- Blade S (1997). How does hemp measure up? 1997 Low-THC Hemp Research Report. Alberta Agriculture, Food, and Rural Development. Project#'s 96CR03, 97NW13.
- Callaway JC, Tennila, Pate DW (1996). Occurrence of "omega-3" stearidonic acid (cis-6,9,12,15-octadecatetraenoic acid) in hemp (*Cannabis sativa* L.) seed. *Journal of the International Hemp Association* 3(2): 61-64.
- Calloway JC, Laakkonen TT (1996). Cultivation of *Cannabis* oil seed varieties in Finland. *Journal of the International Hemp Association* 3(1): 32-34.
- Clarke RC (1981). Marijuana Botany. An Advanced Study: The Propagation and Breeding of Distinctive *Cannabis*. Ronin Publishing, Berkeley, California.
- Consroe P, Sandyk R, Snider SR (1986). Open label evaluation of cannabidiol in dystonic movement disorders. *International Journal of Neuroscience* 30: 277-282.
- Deferne JL, Pate DW (1996). Hemp seed oil: A source of valuable essential fatty acids. *Journal of the International Hemp Association* 3(1): 1, 4-7.
- Duke J (1999). Phytochemical Database. Agricultural Research Service. www.ars-grin.gov/cgi-bin/duke/.
- Erasmus U (1999). Fats that Heal, Fats that Kill. Alive Books. Burnaby, British Columbia, Canada.
- Ferenczy L, Gracza L, Jakobey I (1958). An antibacterial prepartum from hemp (*Cannabis sativa* L.). *Naturwissenschaften* 45: 188.
- Formukong EA, Evans AT, Evans FJ (1988). Analgesic and anti-inflammatory activity of constituents of *Cannabis sativa* L. *Inflammation* 12(4): 361-371.
- Grotenhermen F, Karus M, Lohmeyer D (1998). THC limits for food: A scientific study. *Journal of the International Hemp Association* 5(2): 101-105.
- Grotenherman F, Karus M, Lohmeyer D (1998). Derivation of THC Limits for Food, Part II. nova-Institute. www.naihc.org/hemp_information/content/nova_report/part2.html.
- Hendriks H, Malingre TM, Batterman S, Bos R (1978). The essential oil of *Cannabis sativa* L. *Pharmaceutisch Weekblad*. 113: 413-424.
- Karler R, Cely W, Turkanis SA (1973). The anticonvulsant activity of cannabidiol and cannabinol. *Life Sciences* 13: 1527-1531.
- Karler R, Turkanis SA (1981). The cannabinoids as potential anti-epileptics. *Journal of Clinical Pharmacology* 21: 437S-448S.
- Lampi A, Hopia A, Piironen V (1997). Antioxidant activity of minor amounts of γ -tocopherol in natural triacylglycerols. *Journal of the American Oil Chemists Society* 74(5): 549-555.
- Lees A, Mok H, Lees R, McCluskey M, Grundy S (1977). Plant sterols as cholesterol-

- ol-lowering agents: clinical trials in patients with hypercholesterolemia and studies of sterol balance. *Atherosclerosis* 28: 325-338.
- Malini T, Vanithakumari G (1990). Rat toxicity studies with β -sitosterol. *Journal of Ethnopharmacology* 28: 221-234.
- Mattson F, Grundy S, Crouse J (1982). Optimizing the effect of plant sterols on cholesterol absorption in man. *American Journal of Clinical Nutrition* 35: 697-700.
- Molleken H, Theimer R (1997). Survey of minor fatty acids in *Cannabis sativa* L. Fruits of various origins. *Journal of the International Hemp Association* 4(1): 13-18.
- Pate D (1994). Chemical ecology of *Cannabis*. *Journal of the International Hemp Association* 1(2): 29, 32-37.
- Radosevic A (1962). Antibiotic activity of various types of *Cannabis* resin. *Nature* 195: 1007-1009.
- Sasser M (1990). MIDI: Identification of bacteria by gas chromatography of cellular fatty acids. Technical Note #101, Newark, DE, pp. 163-169.
- Sharma SC, Shukla YN, Tandon JS (1975). Alkaloids and terpenoids of *Ancistrocladus heyneanus*, *Sagittaria sagittifolia*, *Lyonia formosa*, and *Hedychium spicatum*. *Phytochemistry* 14: 578-579.
- Simopoulos AP (1994). Fatty acids. In I Goldberg, ed, *Functional Foods: Designer Foods, Pharmafoods, Nutraceuticals*. Chapman & Hall, New York, pp. 355-392.
- Stone W, Papas A (1997). Tocopherols and the etiology of colon cancer. *Journal of the National Cancer Institute* 89(14): 1006-1014.
- Tambe Y, Tsujiuchi H, Honda G, Ikeshiro Y, Tanaka S (1996). Gastric cytoprotection of the non-steroidal anti-inflammatory sesquiterpene, β -caryophyllene. *Planta Medica* 62(5): 469-470.
- Turner C, ElSohly M, Boeren E (1980). Constituents of *Cannabis sativa* L. XVII: A review of the natural constituents. *Journal of Natural Products* 43(2): 169-234.
- Weil A (1993). Therapeutic hemp oil. *Natural Health*. March/April, pp. 10-12.
- Wolf G (1997). γ -Tocopherol: an efficient protector of lipids against nitric oxide-initiated peroxidative damage. *Nutrition Reviews* 55(10): 376-378.
- Yard H (1999). HYChem Corporation, Wall, NJ, personal communication.

