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Hemp (*Cannabis sativa* L.) Seed Oil: Analytical and Phytochemical Characterization of the Unsaponifiable Fraction

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ABSTRACT: Non-drug varieties of *Cannabis sativa* L., collectively namely as "hemp", have been an interesting source of food, fiber, and medicine for thousands of years. The ever-increasing demand for vegetables oils has made it essential to characterize additional vegetable oil through innovative uses of its components. The lipid profile showed that linoleic (55%), α-linolenic (16%), and oleic (11%) were the most abundant fatty acids. A yield (1.84−1.92%) of unsaponifiable matter was obtained, and the most interesting compounds were β-sitosterol (1905.00 ± 59.27 mg/kg of oil), campesterol (505.69 ± 32.04 mg/kg of oil), phytol (167.59 ± 1.81 mg/kg of oil), cycloartenol (90.55 ± 3.44 mg/kg of oil), and γ-tocopherol (73.38 ± 2.86 mg/100 g of oil). This study is an interesting contribution for *C. sativa* L. consideration as a source of bioactive compounds contributing to novel research applications for hemp seed oil in the pharmaceutical, cosmetic food, and other non-food industries.

KEYWORDS: *hemp, Cannabis sativa, vegetable oils, fatty acids, sterols, phytol, tocopherols*

INTRODUCTION

Cannabis is classified into the family Cannabaceae and comprises three main species: *Cannabis sativa*, *Cannabis indica*, and *Cannabis ruderalis*. *C. sativa* L., an annual herbaceous plant, is known by it long, thin flowers and spiky leaves. The plant is considered to be native of western and central Asia and has also been cultivated commercially in Europe and in parts of China, Japan, Canada, and the United States. Non-drug varieties of *C. sativa* L., collectively namely as "hemp", have been an important source of food, fiber, and medicine for thousands of years in the Old World.[1,2] Hempseed and all *Cannabis* varieties were eventually prohibited in the late 1930s in North America because of the presence of phytochemical drug δ-9-tetrahydrocannabinol (THC). Subsequently, the putative use and development of hempseed as a food or supplement for humans and domesticated animals were interrupted and have not been studied extensively. Nowadays, a low THC form is legal to grow (a 0.3% THC standard has been established by the European Union),[3] and the bast fiber from the stalk is used in the modern production of durable fabrics and specialty papers in some countries. Hemp is of economic and pharmaceutical importance throughout the world, with a global market for low THC valued at $100−2000 million annually.[4]

Hempseed has been documented as a folk source of food throughout recorded history, raw, cooked, or roasted, and hempseed oil (HSO) has been used as a food/medicine in China for at least 3000 years.[5,6] Hempseed has high levels of vitamins A, C, and E, minerals, and β-carotene.[7] It contains 20−25% protein, 20−30% carbohydrates, 25−35% oil, 10−15% insoluble fiber, and a rich set of minerals, particularly phosphorus, potassium, magnesium, sulfur, and calcium, along with modest amounts of iron and zinc, the latter of which is an important enzyme cofactor for human fatty acid metabolism.[8,9] HSO has a good taste and offers various advantages over other vegetables oils. It is considered to be perfectly balanced with regard to the ratio (3:1)[10] of two polyunsaturated fatty acids (PUFAs) essential for human nutrition, linoleic and linolenic acids. HSO, in addition to its nutritional value, has demonstrated positive health benefits, including lipid metabolism,[11,12] cardiovascular health,[13−15] immunomodulatory effects,[16] and dermatological diseases.[17]

The ever-increasing demand for vegetables oils, coupled with current awareness about the nutritional and functional roles of fats in human diets, has made it essential to characterize additional vegetable oil through innovative uses of its components and/or byproducts.[18] The beneficial effects of HSO are thought to be due to its balance between linoleic and linolenic acid content. Thus far, little effort has been focused to investigate the unsaponifiable fraction of HSO. The nonsaponifiable portion, about 1.5−2% of the oil, is an important source of interesting minor compounds.[19−22] As part of ongoing investigations on bioactive secondary plant metabolites in medicinal and food plants, our aim of the present study was to conduct a detailed analysis and phytochemical characterization to correlate with those of literature reports to accelerate efforts to establish a global database for this valuable oilseed crop.

MATERIALS AND METHODS

Material Samples, Reagents, and Standards. Samples of refined HSO were kindly provided by Botanica Nutrients (Seville, Spain) and guaranteed regarding botanical origin and quality grade. Samples were stored at 4 °C and protected from light prior to analysis. Standards of α-cholestanol, eicosanol, α-tocopherol, methyl heptadecanoate, and lauryl arachinodate (the purity of all standards were

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greater than 95%) and the rest of the reagents were provided by Sigma-Aldrich (St. Louis, MO).

Determination of the Fatty Acid Profile. *Sample Preparation.* The fatty acid profile was determinated by a gas chromatography procedure by methylation with cold methanolic solution of potassium hydroxide. In a 5 mL screw top test tube, 0.10 g of the HSO sample was weighed. A total of 3 mL of heptane and 500 μL of 2 N methanolic potassium hydroxide solution were added. The sample was shaken vigorously for 15 s, leaving it to stratify until the upper solution became clear. The upper layer containing the methyl esters was decanted.[23]

Chromatographic Conditions. The fatty acid methyl esters (FAMEs) were analyzed in a Hewlett-Packard 6890 gas chromatograph equipped with a flame ionization detector (FID), using a SP2380 silica capillary column (30 m × 0.32 mm internal diameter, 0.25 mm film). The initial column temperature was 165 °C, which was held for 10 min and programmed from 165 to 200 °C at 1.5 °C/min. The injector temperature was 210 °C, and the detector temperature was 250 °C. FAMEs were identified by retention time comparison to that of the corresponding standard peaks.

Unsaponifiable Extraction. The unsaponifiable matter of HSO was isolated following conventional procedures, and its components were analyzed following the International Union of Pure and Applied Chemistry (IUPAC) method.[24] A total of 5 g of HSO, 1 mL of internal standard (IS) solution (1 mg/mL α-colestanol and 0.5 mg/mL eicosanol in chroloform), and 50 mL of a 2 N potassium hydroxide solution in ethanol/water (80:20, v/v) were put into a 250 mL flask; saponification was carried out by boiling and stirring the sample for 1 h. After cooling, 100 mL of distilled water was added, and the sample was transferred to a separating funnel and extracted 3 times with 80 mL of ethyl ether. The ether extracts were pooled into a separating funnel (with 125 mL of distilled water) and washed with distilled water (125 mL each time), until the wash gave a neutral reaction. Then, the wash water was removed; the organic sample was dried with anhydrous sodium sulfate, filtered, and taken to dryness; and the residue was weighed.[25]

Separation of the Unsaponifiable Components. HSO unsaponifiable matter was by means of both flash and plate chromatography, providing fractions with superimposable profiles. Preparative thin-layer chromatography (TLC) was preferred over column chromatography because of its greater handiness and convenience of operation. The unsaponifiable fraction dissolved in chroloform was deposed on a 1 mm silica gel 60 F_{254} PLC plate, 20 × 20 (Merck, Darmstadt, Germany). The plate was placed into the developing chamber and eluted with hexane/diethyl ether (65:35, v/v). After development and solvent evaporation, the plate was developed with 2′,7′-dichlorofluorescein solution and important bands was evidenced.[25] The most important bands were scraped separately from the plate and extracted first with hot chloroform (10 mL) and then with ethyl ether (10 mL). The two solvent extracts were mixed and taken to dryness. The residue was finally dissolved and sylilated with 200 μL of a solution of pyridine−hexamethyldisilazane−trimethylchlorosilane to 15−30 mg of the insaponificables in glass-stoppered tubes.[20]

Chromatographic Conditions. Sterol and alcohol fractions were analyzed with a HP5890 Series II gas chromatograph (Boston, MA) equipped with a capillary column SPB5, 30 m length, 0.25 mm inner diameter, and 0.25 μm film thickness of poly(5% diphenyl/95% dimethyl siloxane) stationary phase. Carrier gas was hydrogen, with a head pressure of 110 kPa and 1:60 split ratio. The injector and detector temperature was 300 °C. The isotherm program to 255 °C was programmed to the sterol fraction for 30 min, and the oven temperature was programmed from 225 to 280 °C with a rate of 3 °C/min for the alcohol fraction for 50 min. The analysis was carried out following the amending Regulation (EC) 2568/91 on the characteristics of olive and the relevant method of analysis. The mass spectrometry (MS) analyses were performed using Kratos MS 80 mass spectrometer equipped with a NBSLIB2 data system. Scan time and mass range were 1 s and m/z 50−500, respectively. Fraction components were quantified by means of the ISs, and the total concentration in HSO was calculated as the sum of individual peak concentrations.

Determination of Hydrocarbon and Wax Contents. The wax esters, formed by the reaction of alcohols (aliphatic, triterpenic, methylsterols, and sterols) and free fatty acids, are present in seed and fruits. The usual procedure was followed by column chromatography. This method has been adopted by the European Union. A solution of lauryl arachinodate (0.1 mg/mL) and methyl heptadecanoate (0.1 mg/mL) in methanol was used as the IS. The wax esters eluted just before the triacylglycerols, thereby the addition of a colorant (Sudan I) with R_f similar to that of the triacylglycerols allows for visualization when the elution of wax esters ends. The fraction isolated contains also other minor components, such as the hydrocarbons. After isolation, this fraction was carried out by the gas chromatographic system (Agilent Technologies 6890N) equipped with an on-column injector and FID and using a short capillary-fused silica column (10 m) coated with 5% phenylmethylsilicone.[26]

Analysis of Tocopherols. The tocopherol content was analyzed by high-performance liquid chromatography (HPLC) in accordance with the IUPAC 2432 method.[27] A total of 1.5 g of HSO was dissolved in the solution (10 mL) of 0.5% isopropanol in *n*-hexane (mobile phase). The chromatographic separation was performed using a Perkin-Elmer liquid chromatograph equipped with an isocratic pump LC200 and an ultraviolet−visible (UV−vis) detector Lc295. A normal-phase column Lichrosphere Si60 (250 mm length, 4.6 mm inner diameter, and 5 μm particle size) was used with an injection volume of 20 μL and a flow rate of 1.0 mL/min. The absorbance was measured at 295 nm. The results were expressed as milligrams of tocopherol per 100 g of oil.

Statistical Analysis. All sample preparation and analyses of all of the classes of analytes (sterols, alcohols, hydrocarbons, waxes, fatty acids, phenols, and tocopherols) were performed in triplicate and were calculated with respect to respective ISs. Values are expressed as the mean, and standard error of the mean (SEM) is given. Each chromatogram is the most representative of three similar analyses.

RESULTS AND DISCUSSION

Industrial hemp is widely known to provide high-quality fibers and materials for textile, paper, or bio-building uses. Complementary products along with fibers should be taken into account in growing hemp because they might significantly enhance its competitiveness. The HSO and secondary metabolites, which can be found in the unsaponifiable fraction, are only some examples of potential complementary products that can be derived from industrial hemp.[28] The unsaponifiable fraction has received very little attention by researchers, despite the potential relevance that it may have in phytomedical technology, the discipline of studying the plant-derived pharmaceutical and nutraceutical compounds.[29]

In our analysis, the main fatty acids of HSO were linoleic acid (RT, 15.718), α- and γ-linolenic acids (RT, 18.223 and 18.323, respectively), and oleic acid (RT, 13.465), which together comprised 88% of the total fatty acids (Table 1). The fatty acid profile of HSO was similar to that reported in Canada[4,30] and for hemp cultivated in Europe with slightly higher linolenic acids.[31] The palmitic, stearic, and oleic acid contents of HSO were similar to those of evening primrose oil (EPO).[32] The PUFAs of the oil amounted to 76% of the total of fatty acids, while the monounsaturated and saturated fatty acids amounted to 12% each. The high amounts of α-linolenic acid (17%) may have favorable nutritional implications and beneficial physiological effects on the prevention of coronary heart disease and cancer.[33,34] The presence of γ-linolenic acid (3.4%) provides it with a high pharmaceutical value for degenerative chronic diseases.[35] The oil was characterized by a high polyunsaturated/saturated (P/S) ratio, which is regarded favorably for the

Table 1. Fatty Acid Profile (GC) in HSO

name	mean ± SEM (%)
palmitic acid (16:0)	5.62 ± 0.04
palmitoleic acid (16:1)	0.31 ± 0.02
estearic acid (18:0)	2.68 ± 0.12
oleic acid (18:1)	11.90 ± 0.35
linoleic acid (18:2, ω-6)	55.05 ± 0.47
eicosanoic acid (20:0)	2.50 ± 0.15
α-linolenic acid (18:3, ω-3)	16.70 ± 0.08
γ-linolenic acid (18:3, ω-6)	3.40 ± 0.12
eicosenoic acid (20:1)	1.44 ± 0.01
docosanoic acid (22:0)	0.40 ± 0.03
saturated fatty acids	11.20
monounsaturated fatty acids	13.34
polyunsaturated fatty acids	75.46
P/S ratio	6.7
ω-6/ω-3 ratio	3.5

Table 2. Amount of 4-Desmethylsterols (GC) in the Unsaponifiable Fraction from HSO

peak	retention time	name	mean ± SEM (mg/kg of oil)
1	7.824	colesterol	0.92 ± 0.03
2	8.362	α-colestanol (IS)	
3	9.298	24-methylene cholesterol	7.05 ± 0.14
4	10.310	campesterol	505.69 ± 32.04
5	10.811	estigmasterol	100.23 ± 7.47
6	11.499	clerosterol	58.39 ± 8.82
7	12.859	β-sitosterol	1905.07 ± 59.27
8	13.025	Δ_5-avenasterol	142.80 ± 7.68
9	13.384	Δ_5-24-estigmastadienol	31.97 ± 3.66
10	13.715	Δ_7-estigmasterol	21.74 ± 3.03
11	14.179	Δ_7-avenasterol	19.87 ± 2.18
total sterols			2793.73 ± 124.32

reduction of serum cholesterol and atherosclerosis and prevention of heart diseases.[36] Similarly, the ratio of ω-6/ω-3 fatty acids was 3.

On other hand, little effort has been expended to study other minor compounds present in HSO. The present work has isolated and characterized the different and interesting fractions from unsaponifiable HSO. A great amount of qualitative information was generated in every analysis. A yield (1.84−1.92%) of unsaponifiable matter was obtained.

Sterols were isolated from the HSO unsaponifiable matter and represented a 15.18% from this fraction. The identification of the different phytosterols has been made by gas chromatography (GC) and GC−MS comparing their relative retention time (RR_t) values and their MS fragments to literature values. Chromatographic analyses are shown in Figure 1 and Table 2. β-Sitosterol (1905.07 ± 59.27 mg/kg of oil) and campesterol (505.69 ± 32.04 mg/kg of oil) were predominant in the phytosterol fraction (2793.73 ± 124.32 mg/kg of oil). This proportion shows that EPO is twice as rich as olive oil in these natural products.[37] Plant sterols and stanols in fat matrices effectively lower low-density lipoprotein (LDL) cholesterol levels in hypercholesterolemic and diabetic patients and healthy human volunteers. Recent studies also show that dietary intake of plant sterols is related to a lower risk of myocardial infarction, lowering LDL cholesterol even when incorporated in non-fat matrices.[38] In addition, they may reduce biomarkers of oxidative stress and inflammation and modulate the atherosclerosis development.[20,39]

Another interesting fraction is the aliphatic alcohols.[40] Phytol (167.59 ± 1.81 mg/kg of oil) and geranylgeraniol (26.06 ± 0.08 mg/kg of oil) were predominant in the aliphatic alcohol fraction (226.94 ± 2.26 mg/kg of oil), and the abundant linear aliphatic alcohol was hexacosanol (16.17 ± 0.21 mg/kg of oil) (Figure 2 and Table 3). Chemical and nutritional studies have shown that mixtures of long-chain primary alcohols extracted from waxy materials from different sources, such as beeswax, rice bran, wheat germ, sugar cane, and grain sorghum, are able to exert several beneficial physiological effects, such as reducing platelet aggregation, endothelial damage, and cholesterol-lowering effects.[40,41] On the other hand, recent investigations

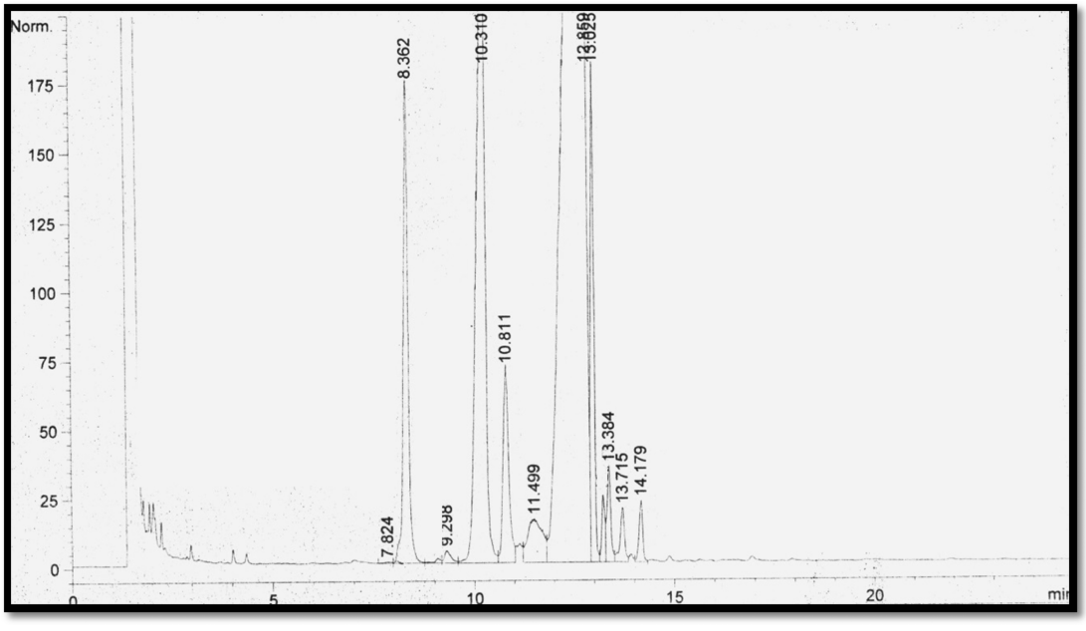

Figure 1. Gas chromatogram of the 4-desmethylsterol, erythrodiol, and uvaol fractions from unsaponifiable HSO.

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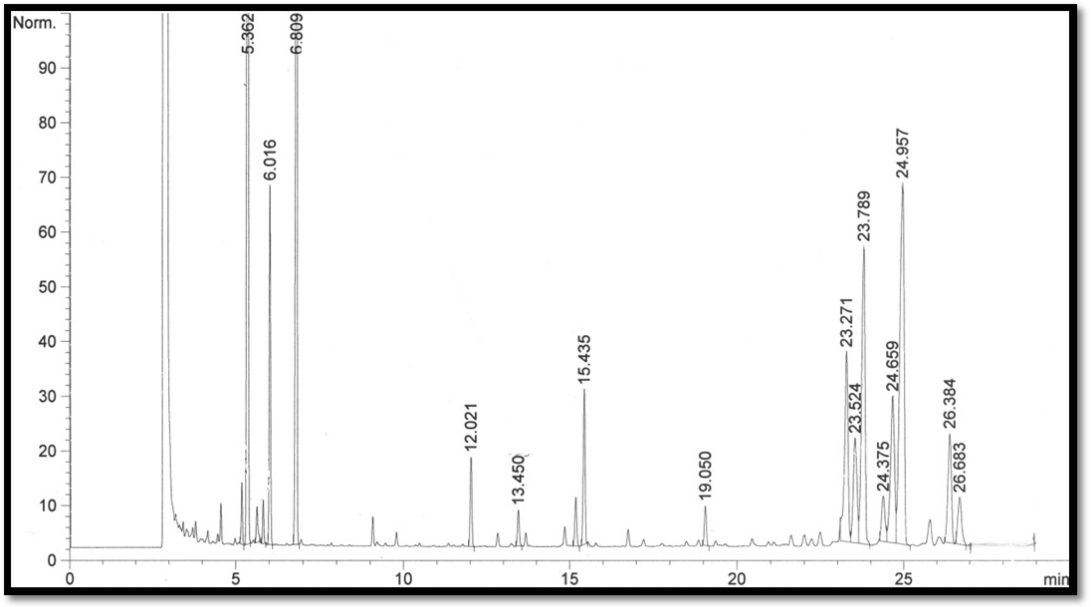

Figure 2. Gas chromatogram of linear aliphatic alcohol, triterpene alcohol, and 4-methylsterol fractions from unsaponifiable HSO.

Table 3. Amount of Linear Aliphatic Alcohols, Triterpene Alcohols, and 4-Methylsterols (GC) in the Unsaponifiable Fraction from HSO

peak	retention time	name	mean ± SEM (mg/kg of oil)
		Linear Aliphatic Alcohols	
1	5.362	phytol	167.59 ± 1.81
2	6.016	geranylgeraniol	26.06 ± 0.08
3	6.809	C_{20} (IS)	
4	12.021	C_{24}	8.78 ± 0.02
5	13.450	C_{25}	3.84 ± 0.06
6	15.435	C_{26}	16.17 ± 0.21
7	19.050	C_{28}	4.50 ± 0.08
total policosanols			226.94 ± 2.26
		Triterpene Alcohols	
8	23.271	dammaradienol	38.19 ± 1.07
9	23.524	taraxterol	22.18 ± 1.15
10	23.789	β-amirin	58.40 ± 2.03
11	24.375	butirospermol	8.97 ± 0.61
12	24.659	cycloeucalenol	34.03 ± 0.76
13	24.957	cycloartenol	90.55 ± 3.44
14	26.384	24-methylencycloartenol	21.75 ± 0.86
		4-Methylsterols	
15	26.683	citrostadienol	10.20 ± 0.42

Table 4. Amount of Squalene, Linear Hydrocarbons, and Waxes (GC) in the Unsaponifiable Fraction from HSO

name	mean ± SEM (mg/kg of oil)
squalene	80.52 ± 1.35
total linear hydrocarbons	451.77 ± 26.05
aliphatic waxes	0.98 ± 0.08
terpenic waxes	42.37 ± 0.61
total waxes	43.35 ± 0.69

performed by our group demonstrated that a similar linear alcohol fraction isolated from pomace olive oil (a byproduct of olive oil) has a protective effect on some mediators involved in the inflammatory damage development.[19] Phytol, a constituent of chlorophyll, is mainly found in human food, such as spinach, beans, raw vegetables, and asparagus, and has both anticancer and antioxidant actions.[42]

HSO did not prove be rich in squalene (Table 4), which had a presence of 80.52 mg/kg, if compared to other known plants rich in squalene (olive oil or *Amaranthus* oil).[43] The levels of saturated hydrocarbons were 451.77 ± 26.05 mg.

Tocopherols, another minor oil component, belong to a group of lipid-soluble compounds generally referred to as vitamin E, encompassing α-, β-, γ-, and δ-tocopherols. Because of their ability to scavenge free radicals involving a tocopherol−tocopheryl semiquinone redox system, tocopherols are the most important natural antioxidants. Moreover, these compounds were shown to exhibit various beneficial effects on degenerative diseases, such as atherosclerosis, cardiovascular disease, Alzheimer's disease, or certain types of cancer.[44] The major tocopherol in HSO was the γ isomer at 91 ± 1.4% of the total tocopherol (Table 5). Similar results of γ-tocopherol,

Table 5. Tocopherol Contents in the Unsaponifiable Fraction from HSO (mg/100 g) by HPLC

name	mean ± SEM (mg/100 g of oil)
α-tocopherol	3.22 ± 0.65
β-tocopherol	0.81 ± 0.16
γ-tocopherol	73.38 ± 2.86
δ-tocopherol	2.87 ± 0.83
total tocopherols	80.28 ± 4.50

accounting for 90% of the total, have been reported for *C. ruderalis* L.[45] Concentrations of tocopherol isomers from HSO were in the range reported for hemp cultivars grown in Canada.[4] α-Tocopherol is considered to be the predominant antioxidant in olive oil, sunflower oil, and EPO, while γ-tocopherol prevails in rapeseed oil.[46−48]

This study is an important contribution for *C. sativa* L. valorization as a source of bioactive compounds. Finally, the extraction of high-value bioactive compounds can be perfectly integrated with the large-scale applications of hemp, contribu-

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ting to research novel applications in the food, pharmaceutical, cosmetic, and other non-food industries.

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Pringle, H. Ice age community may be earliest known net hunters. *Science* **1997**, *277*, 1203−1204.

(2) Zias, J.; Stark, H.; Sellgman, J.; Levy, R.; Werker, E.; Breuer, A.; Mechoulam, R. Early medical use of cannabis. *Nature* **1993**, *363*, 215.

(3) Holler, J. M.; Bosy, T. Z.; Dunkley, C. S.; Levine, B.; Past, M. R.; Jacobs, A. Δ^9-tetrahydrocannabinol content of commercially available hemp products. *J. Anal. Toxicol.* **2008**, *32*, 428−432.

(4) Oomah, B. D.; Busson, M.; Godfrey, D. V.; Drover, J. C. G. Characteristics of hemp (*Cannabis sativa* L.) seed oil. *Food Chem.* **2002**, *76*, 33−43.

(5) Callaway, J. C. Hempseed as a nutritional resource: An overview. *Euphytica* **2004**, *140*, 65−72.

(6) de Padua, L. S.; Bunyaprafatsara, N.; Lemmens, R. H. M. J. *Plant Resources of South-East Asia: Medicinal and Poisonous Plants*; Backhuys Publishers: Leiden, Netherlands, 1999; Vol. *1*, No. 12, pp 167−175.

(7) Orhan, U. S.; kumenoglu, S.; Sener, B. GC−MS analysis of the seed oil of *Cannabis sativa* L. cultivated in Turkey. *J. Fac. Pharm. Gazi Univ.* **2000**, *17*, 79−81.

(8) Deferne, J. L.; Pate, D. W. Hemp seed oil: A source of valuable essential fatty acids. *J. Int. Hemp Assoc.* **1996**, *3*, 4−7.

(9) Yu, Y.; Cai, Z.; Zheng, J.; Chen, J.; Zhang, X.; Huang, X. F.; Li, D. Serum levels of polyunsaturated fatty acids are low in Chinese men with metabolic syndrome, whereas serum levels of saturated fatty acids, zinc, and magnesium are high. *Nutr. Res. (N. Y., NY, U. S.)* **2012**, *32*, 71−77.

(10) Scorletti, E.; Byrne, C. D. ω-3 fatty acids, hepatic lipid metabolism, and nonalcoholic fatty liver disease. *Annu. Rev. Nutr.* **2013**, *33*, 231−248.

(11) Prociuk, M. A.; Edel, A. L.; Richard, M. N.; Gavel, N. T.; Ander, B. P.; Dupasquier, C. M.; Pierce, G. N. Cholesterol-induced stimulation of platelet aggregation is prevented by a hempseed-enriched diet. *Can. J. Physiol. Pharmacol.* **2008**, *86*, 153−159.

(12) Schwab, U. S.; Callaway, J. C.; Erkkilä, A. T.; Gynther, J.; Uusitupa, M. I. J.; Järvinen, T. Effects of hempseed and flaxseed oil on the profile of serum lipids, serum total and lipoprotein lipid concentrations and haemostatic factors. *Eur. J. Nutr.* **2006**, *45*, 470−477.

(13) Kaul, N.; Kreml, R.; Austria, J. A.; Richard, M. N.; Edel, A. L.; Dibrov, E.; Hirono, S.; Zettler, M. E.; Pierce, G. N. A comparison of fish oil, flaxseed oil and hempseed oil supplementation on selected parameters of cardiovascular health in healthy volunteers. *J. Am. Coll. Nutr.* **2008**, *27*, 51−58.

(14) Al-Khalifa, A.; Maddaford, T. G.; Chahine, M. N.; Austria, J. A.; Edel, A. L.; Richard, M. N.; Ander, B. P.; Gavel, N.; Kopilas, M.; Ganguly, R.; Ganguly, P. K.; Pierce, G. N. Effect of dietary hempseed intake on cardiac ischemia-reperfusion injury. *Am. J. Physiol.: Regul., Integr. Comp. Physiol.* **2007**, *292*, R1198−R1203.

(15) Richard, M. N.; Ganguly, R.; Steigerwald, S. N.; Al-Khalifa, A.; Pierce, G. N. Dietary hempseed reduces platelet aggregation. *J. Thromb. Haemostasis* **2007**, *5*, 424−425.

(16) Li, Y.; Yang, R.; Hu, X.; Long, Z.; et al. Initial study of Hemp seeds protein on antifatigue and the immunomodulation effects in mice. *Wei Sheng Yan Jiu* **2008**, *37*, 175−178 (in Chinese).

(17) Callaway, J.; Schwab, U.; Harvima, I.; Halonen, P.; Mykkänen, O.; Hyvönen, P.; Järvinen, T. Efficacy of dietary hempseed oil in patients with atopic dermatitis. *J. Dermatol. Treat.* **2005**, *16*, 87−94.

(18) Anwar, F.; Latif, S.; Ashraf, M. Analytical characterization of Hemp (*Cannabis sativa*) seed oil from different agro-eocological zones of Pakistan. *J. Am. Oil Chem. Soc.* **2006**, *83*, 323−329.

(19) Fernandez-Arche, A.; Marquez-Martin, A.; De la Puerta-Vazquez, R.; Perona, J. S.; Terencio, C.; Perez-Camino, C.; Ruiz-Gutierrez, V. Long-chain fatty alcohols from pomace olive oil modulate the release of proinflammatory mediators. *J. Nutr. Biochem.* **2009**, *20*, 155−162.

(20) Montserrat-de la Paz, S.; Fernandez-Arche, M. A.; Angel-Martin, M.; Garcia-Gimenez, M. D. The sterols isolated from evening primrose oil modulate the release of proinflammatory mediators. *Phytomedicine* **2012**, *19*, 1072−1076.

(21) Ramos, P. A. B.; Guerra, A. R.; Guerreiro, O.; Freire, C. S. R.; Silva, A. M. S.; Duarte, M. F.; Silvestre, A. J. D. Lipophilic extracts of *Cynara cardunculus* L. var. *altilis* (DC): A source of valuable bioactive terpenic compounds. *J. Agric. Food Chem.* **2013**, *61*, 8420−8429.

(22) Montserrat-de la Paz, S.; Garcia-Gimenez, M. D.; Angel-Martin, M.; Pérez-Camino, M. C.; Fernandez-Arche, M. A. Long-chain fatty alcohols from evening primrose oil inhibit the inflammatory response in murine peritoneal macrophages. *J. Ethnopharm.* **2014**, *151*, 131−136.

(23) Cert, A.; Moreda, W.; Perez-Camino, M. C. Methods of preparation of fatty acid methyl esters (FAME). Statistical assessment of the precision characteristics from a collaborative trials. *Grasas Aceites (Sevilla, Spain)* **2000**, *6*, 447−456.

(24) International Union of Pure and Applied Chemistry (IUPAC). Determination of the unsaponifiable matter. Method 2401. In *Standard Methods for the Analysis of Oils, Fats and Derivatives*, 7th ed.; Paquot, C., Hautfenne, A., Eds.; Blackwell: Oxford, U.K., 1992.

(25) Caligiani, A.; Bonzanini, F.; Palla, G.; Cirlini, M.; Bruni, R. Characterization of a potential nutraceutical ingredient: Pomegranate (*Punica granatum* L.) seed oil unsaponifiable fraction. *Plant Foods Hum. Nutr.* **2010**, *65*, 277−283.

(26) Cert, A.; Moreda, W.; Perez-Camino, M. C. Chromatographic analysis of minor constituents in vegetable oils. *J. Chromatogr., A* **2000**, *881*, 131−148.

(27) International Union of Pure and Applied Chemistry (IUPAC). Determination of tocopherols and tocotrienols in vegetable oils and fats by HPLC. Method 2432. In *Standard Methods for the Analysis of Oils, Fats and Derivatives*, 7th ed.; Paquot, C., Hautfenne, A., Eds.; Blackwell: Oxford, U.K., 1992.

(28) Ranalli, P.; Venturi, G. Hemp as a raw material for industrial applications. *Euphytica* **2004**, *140*, 1−6.

(29) Nissen, L.; Zatta, A.; Stefanini, I.; Grandi, S.; Sgorbati, B.; Biavati, B.; Monti, A. Characterization and antimicrobial activity of essential oils of industrial hemp varieties (*Cannabis sativa* L.). *Fitoterapia* **2010**, *81*, 413−419.

(30) Blade, S. F. Industria hemp in Alberta. In *Alberta Hemp Symposia Proceedings*; Blade, S., Ed.; Alberta Agriculture, Food and Rural Development: Red Deer, Alberta, Canada, 1998; pp 2−11.

(31) Theimer, R. R.; Molleken, H. Analysis of the oils from different hemp (*Cannabis sativa* L.) cultivars—Perspective for economic utilization. In *Bioresource Hemp*, 2nd ed.; Nova Institute: Cologne, Germany, 1995; pp 536−545.

(32) Montserrat-de la Paz, S.; Fernández-Arche, M. A.; Ángel-Martín, M.; García-Giménez, M. D. Phytochemical characterization of potential nutraceutical ingredients from evening primrose oil (*Oenothera biennis* L.). *Phytochem. Lett.* **2014**, DOI: 10.1016/j.phytol.2013.08.008.

(33) Arshad, A.; Al-Leswas, D.; Stephenson, J.; Metcalfe, M.; Dennison, A. Potential applications of fish oils rich in ω-3 fatty acids in the palliative treatment of advanced pancreatic cáncer. *Br. J. Nutr.* **2011**, *106*, 795−800.

(34) Fretts, A. M.; Mozaffarian, D.; Siscovick, D. S.; Heckbert, S. R.; McKnight, B.; King, I. B.; Rimm, E. B.; Psaty, B. M.; Sacks, F. M.; Song, X.; Spiegelman, D.; Lemaitre, R. N. Associations of plasma phospholipid and dietary α-linolenic acid with incident atrial fibrillation in older adults: The cardiovascular health study. *J. Am. Heart Assoc.* **2013**, *2*, No. e003814.
(35) Montserrat-de la Paz, S.; García-Giménez, M. D.; Ángel-Martín, M.; Marin-Aguilar, F.; Fernández-Arche, M. A. Dietary supplementation evening primrose oil improve symptoms of fybromyalgia syndrome. *J. Funct. Foods* **2013**, *5*, 1279−1287.
(36) Reena, M. B.; Lokesh, B. R. Hypolipidemic effect of oils with balanced amounts of fatty acids obtained by blending and interesterification of coconut oil with rice bran oil or sesame oil. *J. Agric. Food Chem.* **2007**, *55*, 10461−10469.
(37) Weihrauch, J. L.; Gardner, J. M. Sterol content of foods of plant origin. *J. Am. Diet. Assoc.* **1978**, *73*, 39−47.
(38) Klingberg, S.; Ellegård, L.; Johansson, I.; Jansson, J. H.; Hallmans, G.; Winkvist, A. Dietary intake of naturally occurring plant sterols is related to a lower risk of a first myocardial infarction in men but not in women in northern Sweden. *J. Nutr.* **2013**, *143*, 1630−1635.
(39) Lou-Bonafonte, J. M.; Arnal, C.; Navarro, M. A.; Osada, J. Efficacy of bioactive compounds from extra virgin olive oil to modulate atherosclerosis development. *Mol. Nutr. Food Res.* **2012**, *56*, 1043−1057.
(40) Seo, W. D.; Yuk, H. J.; Curtis-Long, M. J.; Jang, K. C.; Lee, J. H.; Han, S. I.; Kang, H. W.; Nam, M. H.; Lee, S. J.; Lee, J. H.; Park, K. H. Effect of the growth stage and cultivar on policosanol profiles of barley sprouts and their adenosine 5′-monophosphate-activated protein kinase activation. *J. Agric. Food Chem.* **2013**, *61*, 1117−23.
(41) Martino, F.; Puddu, P. E.; Pannarale, G.; Colantoni, C.; Martino, E.; Niglio, T.; Zanoni, C.; Barillà, F. Low dose chromium-polynicotinate or policosanol is effective in hypercholesterolemic children only in combination with glucomannan. *Atherosclerosis* **2013**, *228*, 198−202.
(42) Vetter, W.; Schröder, M.; Lehnert, K. Differentiation of refined and virgin edible oils by means of the *trans*- and *cis*-phytol isomer distribution. *J. Agric. Food Chem.* **2012**, *60*, 6103−6107.
(43) Ostlund, R. E. M.; Racette, S. B.; Stenson, W. F. Effect of trace components of dietary fat on cholesterol metabolism: Phytosterols, oxysterols and squalene. *Nutr. Rev.* **2002**, *60*, 349−359.
(44) Fromm, M.; Bayha, S.; Kammerer, D. R.; Carle, R. Identification and quantitation of carotenoids and tocopherols in seed oils recovered from different Rosaceae species. *J. Agric. Food Chem.* **2012**, *60*, 10733−10742.
(45) Ivanov, S. A.; Aitzetmuller, K. Tocopherol and tocotrienol composition of the seed lipids of a number of species representing the Bulgarian flora. *Fett/Lipid* **1998**, *100*, 348−352.
(46) Velasco, J.; Andersen, M. L.; Skibsted, L. H. Electron spin resonance spin trapping for analysis of lipid oxidation in oils: Inhibiting effect of the spin trap α-phenyl-*N*-*tert*-butylnitrone on lipid oxidation. *J. Agric. Food Chem.* **2005**, *53*, 1328−1336.
(47) Blekas, G.; Tsimidou, M.; Boskou, D. Contribution of atocopherol to olive oil stability. *Food Chem.* **1995**, *52*, 289−294.
(48) Koski, A.; Psomiadou, E.; Tsimidou, M.; Hopia, A.; Kefalas, P.; Wahala, K.; Heinonen, M. Oxidative stability and minor constituents of virgin olive oil and cold-pressed rapeseed oil. *Eur. Food Res. Technol.* **2002**, *214*, 294−298.