Synthesis and Analytical Study of Fatty Acid Methyl Ester of *Cannabis sativa* (Hemp) Seed Oil in the Presence of Triglycerides

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Research Article

Abstract: Hemp seed oil contains linoleic acid (LA) and linolenic acid (LNA) as its major omega-6 and omega-3 polyunsaturated fatty acids (PUFA), respectively It is necessary to apply transesterification procedure to convert fatty acids into more volatile compounds such as fatty acid methyl ester (FAME). Fatty acid methyl ester is made virgin or used vegetable oils (both edible and nonedible) and animal fats. Fatty acid methyl ester operates in compression ignition engines like petro-disel. Fatty acid methyl ester can be blended in any ratio with petroleum disel fuels. It can be stored just like the petroleum disel fuel. Petrodisel can be replaced by biodiesel due to its superiority. It has various advantages. The seed of cannabis sativa (Hemp) contain 32.21 % oil. Methyl ester were prepared from seed oil containing a high free fatty acid content by using a two stage process. Sulphuric acid was used as catalyst in an esterification reaction which was then followed by a transesterification reaction using hydroxide as the catalyst. It was found that methanol and sulphuric acid were suitable to perform the transesterificatin reaction. Fatty acid methyl ester was characterized by UV, IR and LCMS.

Keywords: Cannabis sativa (Hemp) seed oil, Transesterification, Fatty acids, Instrumental Analytical techniques

Introduction

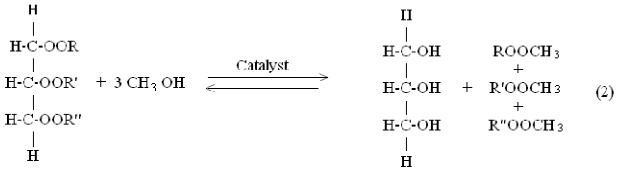
Approximately 44% of the weight of hempseed is edible oils, containing about 80% essential fatty acids (EFAs); e.g., linoleic acid, omega-6 (LA, 55%), alpha-linolenic acid, omega-3 (ALA, 22%), in addition to gamma-linolenic acid, omega-6 (GLA, 1–4%) and stearidonic acid, omega-3 (SDA, 0–2%). Proteins (including edest Approximately 44% of the weight of hempseed is edible oils, containing about 80% essential fatty acids (EFAs); e.g., linoleic acid, omega-6 (LA, 55%), alpha-linolenic acid, omega-3 (ALA, 22%), in addition to gamma-linolenic acid, omega-6 (GLA, 1–4%) and stearidonic acid, omega-3 (SDA, 0–2%). The 3:1 ratio of LA to LNA is alleged to be optimal for nutrition1-3. 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 4 . 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 5 .Reports of cannabinoid contamination have been focused primarily delta-9-tetrahydrocannabinol (THC) with THC levels in oil reported at up to 50 ppm5. 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 6.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* plant7 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 8.While many studies exist which base the nutritional value of hemp seed oil primarily on its fatty acid content. Fatty acids are seldom found as free molecules in nature but are almost often a part of a larger molecules called a triglyceride. Triglycerides consists of three membered carbon chain with fatty acid bonded to each of the three carbon atom in the glycerol backbone. The bond between

the fatty acid & the glycerol backbone is referred to as an ester linkage. Fatty acid methyl ester or ethyl ester obtained from vegetable acids or animal fats. Fatty acid methyl ester are conventionally produced by the transesterification of vegetable oils & fats using methanol in the presence of a suitable catalyst. High amount of free fatty acid (FFA) much higher than the maximum amount suitable to be used with basic homogenous catalysis, which woul otherwisw result in high amount of soap produced simultaneously with the transesterification reaction 10,12-16.To avoid this reaction alternative technologies should be employed 9,10,12,13,17 .. Essentially transesterification is the chemical reaction between triacyl glycerides & alcohol in the presence of a catalyst to produce monoesters. Both the straight & branced chain triacyl glyceride molecules are transformed. Transesterification consists of three consecutive reversible reactions. Conversion of triacylglycerides to diacyl glycerides then the conversion of diacyl glycerides to monoacyl glycerides. The monoacyl glycerides are finally converted into glycerol. Each step are molecule of ester & its reaction is reversible. For each molecule of triacylglyceride, three molecules of alcohol are required stoichiometrically. Transesterification, also called alcoholysis, is the displacement of alcohol from an ester by another alcohol in a process similar to hydrolysis, except than a alcohol is used instead of water. This process has been widely used to reduce the viscosity of triglycerides .

RCOOR' + R"OH < > RCOOR" + R'OH (1)

General equation of transesterification

If methanol is used in the above reaction, it is termed methanolysis. The reaction of triglyceride with methanol is represented by the general equation:
transesterification, as shown in the equation below,



(2)

General equation for methanolysis of triglycerides where R1, R2, and R3 are long hydrocarbon chains, fatty acid chains.

Material and Method

Cannabis sativa (Hemp) seed oil, Methanol, NaOH, High purity water.

The instrument used were UV spectrophotometer with wavelength range 200-450 nm. IR spectra of FAME was taken in the range of 4000 cm-1 to 600 cm-1 on Perkin Elmer 221 IR Spectrophotometer using KBr pellet techniques & LCMS Chromatogram.

Experimental set up

The experimental set up as A 1000 ml three necked round bottom flask was used as a reactor. The flask was placed in heating mantle whose temperature about 55-60 0 C. two side necked was equipped with a condenser and other was used as a thermometer for temperature measurement.Stirrer place at centre for stirring the reaction mixture.

Pretreatment of oil

Cannabis sativa (Hemp) seed oil is first filtered to remove solid material then it is preheated at 1050C for 25 min to remove moisture (presence of moisture responsible for saponification in the reaction)9 .

Transesterification – Base catalysed reaction : Mixing of alcohol and Catalyst

Weight 6 kg of cannabis sativa (Hemp) seed oil and pour it into the reactor for preliminary heating to temperature of about 60-70°C.

In beaker dissolve 22.8 grams of NaOH (3.8grams per liter of oil, got by 3.5 grams stoichiometric equivalent and 0.3 grams for neutralizing FFA) in 1.2 L methanol (200 ml per liter of oil) add the NaOH slowly. This combined mixture makes sodium methoxide. Adding sodium methoxide in cannabis sativa seed oil Provide rigorous mixing with the use of a stirrer.The cloudy looking free fatty acids, called glycerine, will sink to the bottom and the methyl ester- a translucent liquid, will remain on top.When the separation appears not to be advancing any more, stop mixing. Let the mixture settle overnight. Meanwhile another batch can be started as the reactor is not being used. The liquid on top is fatty acid methyl ester, but before using it any remaining soaps or salts which have to be removed. The glycerin which has sunk to the bottom can be used in production of cosmetics

Rinsing Fatty Acid Methyl Ester

Fatty acid methyl ester was poured off into a separate clean container, where it was washed free of any remaining soaps and salts.Warm water was added to the methyl ester. It was stirred lightly and then allowed to settle. The warm water was heated in the main reactor itself. The water was drained out from the bottom. The process was repeated until the discarded rinse water reached pH level of 6-7 and no soap bubbles appeared in it. If the liquid remaining is cloudy, there is water being retained in the methyl ester and it will need to be reheated slowly to evaporate out the water. Any white substances forming at the bottom or any bubbles forming at the surface is a sign of soaps and should be removed or the liquid should be re-washed. The cleaned methyl ester is characterized by UV, IR and LCMS

Result and Discussion

Cannabis sativa (Hemp) seed oil shows density and viscosity 0.9021 gm/cc & 1.2362. Acid value is 0.38 mgKOH/gm. Iodine and saponification value 98g/100of oil, 168 mgKOH/g of oil respectively.Moisture content .001 % and ash content 0.04 wt%.A UV scan was performed at 207 nm wavelength . At 207 λmax absorbance was 2.836. IR spectra was done in the range of 4000 cm-1 to 600 cm-1 shows C-OH stretch at 1107.06, aromatic multiple bond at 1450.37, C=O steretch ketones at 1633.59, alkenes CHR=CH2 at 1643.24, R-COOH bonded on O-H stretch at 3251.76, primary amines free NH at 3475, and R-C=-N-OH at 3496.70 . LCMS is suitable analytical method for determining FAME of seed oil of *Cannabis sativa* (Hemp) shows 31.96 % at 0.525, 3.72% at 0.596, 34.30 % at 0.668, 3.38 % at 0.0798 , 6.48% at 0.97, 2.39 % at 1.06, 6.49 % at 2.46 , 0.94 % at 2.64.

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Table 1: Physico-chemical properties of FAME of *Cannabis sativa* (Hemp) seed oil

Sr.No.	Properties	Unit	FAME
1)	Density	gm/cc	0.9021
2)	Viscosity at 400C	Cst	1.2362
3)	Acid value	mgKOH/gm	0.38
4)	Iodine value	g/100g of oil	98
5)	Saponification value	mgKOH/g of oil	168
6)	Moisture	%	0.001
7)	Ash content	Wt %	0.04

Table 2: IR of FAME of *Cannabis sativa* (Hemp) seed oil

Sr. No.	Frequency Wavenumber	Expected Elements
1	1016.42	Phosphrous compounds , P-O stretch
2	1107.06	Secondary alcohols C-OH stretch
3	1450.37	Aromatic multiple bond C=C
4	1633.59	C=O stretch ketones
5	1643.24	Alkenes (CHR=CH2) C=C stretch
6	2111.91	Alkynes
7	2842.88	Alkanes –CH2 -
8	2952.81	Alkanes –CH3
9	3251.76	R-COOH bonded OH
10	3286.48	O-H stretch
11	3361.00	N-H stretch
12	3475.02	Primary amines free NH secondary amines
13	3496.70	Oximes O-H stretch

Table 3: LCMS of FAME of *Cannabis sativa* (Hemp) seed oil

Sr.No.	Ret.Time	Area %
1	0.525	31.96
2	0.596	3.72
3	0.668	34.30
4	0.798	3.37
5	0.967	6.48
6	1.063	2.39
7	2.464	6.49
8	2.648	0.93

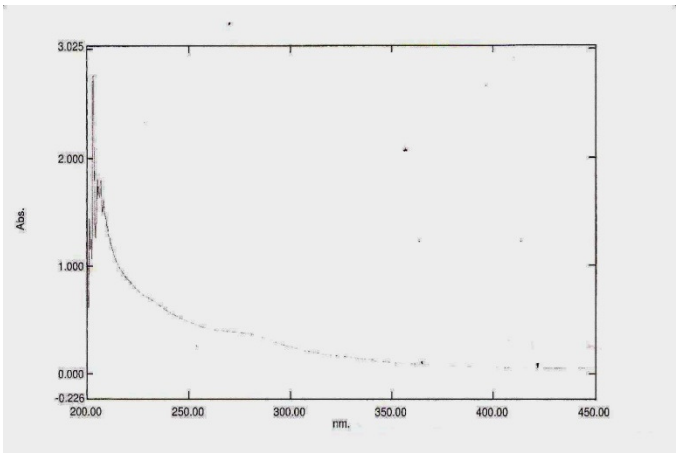

Figure 1: UV spectra of FAME of *Cannabis sativa* (Hemp) seed oil

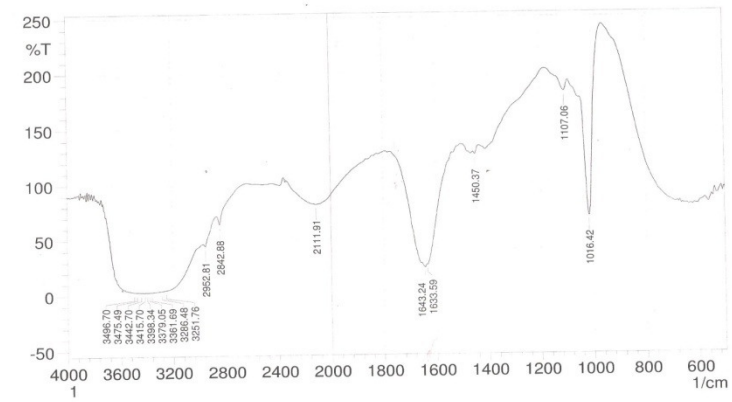

Figure 2: IR spectra of FAME of *Cannabis sativa* (Hemp) seed oil

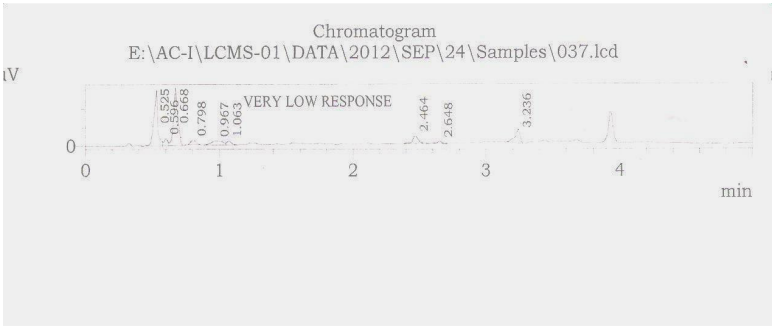

Figure 3: HPLC spectra of FAME of *Cannabis sativa* (Hemp) seed oil