Research Article

Supercritical CO_2 Extraction of Active Components of Fenugreek (Trigonella foenum-graecum L.) Seed

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ABSTRACT

Use of supercritical fluids for the separation of analytes from the matrix of many samples has been of interest to researchers and industries now a day. This is because of the fact that, use of reagents of this type avoids many of the problems such as high solvent cost, environmental pollution, spent solvent impurity in the extract, longer duration of extraction etc. lying with conventional methods of extraction for organic liquid extractants. In this context, supercritical CO_2 extraction (SC-CO_2) of fenugreek (Trigonella foenum-graecum L.) seed was carried out in a bench scale plant. To investigate the effects of pressure and temperature on the solubility of oil and oil yield, and to optimize the operating conditions, three isobaric (100, 200, and 300 bar) and three isothermal (40, 50 and 60°C) extraction conditions were selected. The optimum extraction condition was 40°C /300 bar, and 85 min extraction time, where 3.65% oil yield was achieved. Chemical composition of the oil was investigated using gas chromatography–mass spectrometry in electron impact mode. Thirty numbers of different compounds are detected out of which 15 were fatty acids (58.44%). The major compounds are oleic acid (32.74%), heneicosane (9.50%) and sotolon (8.52%) and also contain some amount of Eucalyptol (0.85%).

Keywords: Fenugreek seed, fatty acids, GC-MS, operating conditions, supercritical CO_2 extraction

Received 3 June 2015 Received in revised form 20 June 2015 Accepted 27 June 2015

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INTRODUCTION

Supercritical fluid extraction (SFE) is a rapid, simple and inexpensive analytical separation technique for separation of the analyte or analytes from a sample matrix without loss or degradation, impurity and wastes that have to be disposed off. This method may be used for performing analytical separations on complex environmental, pharmaceutical, food and petroleum samples, thus attract many researchers and industries to work on it. It is a process separating one component (the extractant) from another (the matrix) using supercritical fluids as the extracting solvent. Extraction is usually from a solid matrix, but it can also be from liquids. SFE can be used as a sample preparation step for analytical purposes, or on a larger scale to either strip unwanted material from a product (e.g. decaffeination) or collect a desired product (e.g. essential oils). Different supercritical fluids used as solvents are Carbon dioxide, Water, Methane, Ethane and Methanol. Carbon dioxide (CO_2) is the most used supercritical fluid, sometimes modified by co-solvents such as ethanol or methanol. Extraction conditions for supercritical CO_2 are above the critical temperature of 31°C and critical pressure of 74 bar. Due to its low critical temperature 31°C, carbon dioxide is known to be perfectly adapted in food, aromas, essential oils and nutraceutical industries. Unlike other processes, the extraction process leaves no solvent residue behind. Moreover the CO_2 is non-toxic, nonflammable, odorless, tasteless, inert, and inexpensive. The density of the supercritical CO_2 at around 200 bar pressure is close to that of hexane, and the solvation characteristics are also similar to hexane; thus, it acts as a non-polar solvent. The solvation characteristics of supercritical CO_2 can be modified by the addition of an entrainer, such as ethanol, however some entrainer remains as a solvent residue in the product, negating some of the advantages of the “residue-free” extraction [1].

A number of studies on the supercritical fluid extractions of active molecules from different naturally occurring species have been reported in the literature and some of them are summarized in the following (**Table 1**).

Table 1: Studies on the supercritical fluid extractions

Serial number	Raw materials	Extraction conditions	Major Component	Reference
1	Cynanchum paniculatum	CO_2+methanol, 150 bar, 55°C , 20 min static) + 90 min (dynamic)	Paeonol	Sun et al., 2008 [2]
2	Ramulus cinnamoni Cassia tora L. Seeds	CO_2, 230-410 bar, 40-50°C CO_2 + ethyl acetate (10%), 250 bar, 45°C	Volatile oil	Liang et al., 2008 [3]
3	Cardamom	CO_2, 300 bar, 35°C	Volatiles fatty acids, tocopherols	Hamdan et al., 2008 [4]
4	Rhodiola rosea roots	CO_2+water (10%),200 bar, 80°C, 3 h	Rosavin	IheozorjioforandDey,2009 [5]
5	Coriander (Coriandrum sativum L.)	CO_2, 80 bar, 35°C, 2h (dynamic)	Isocoumarins	Chen et al., 2009 [6]
6	Braccharis dracunculifolia	CO_2, 400 bar, 60°C, 20 min	Phenolics	Piantino et al., 2008 [7]
7	Vitex agnus castus	CO_2, 450 bar, 45°C, 4h (dynamic)	Diterpenes, triterpenes, casticin	Cossuta et al., 2008 [8]
8	Hyssop (Hyssopus officinalis L.)	CO_2, 90 bar, 40°C(dynamic)	Hyssop oil	Langa et al., 2009 [9]
9	Eugenia uniflora fruits	CO_2, 250 bar, 60°C, 120 min (dynamic)	Carotenoids	GenivalFilho etal., 2008 [10]
10	Garcinia mangostana	CO2 + ethanol (4%), 200 bar, 40°C	Xanthones	Zarena and UdayaSanka, 2009 [11]
11	Pinus sp.	CO_2 + ethanol (3%, v/v), 200 bar, 40°C	Flavonoids	Yesil-Celiktas etal., 2009 [12]
12	Hibiscus cannabinus	CO_2, 200 bar, 80°C, 150 min	Hibiscus oil	Chan and Ismail, 2009 [13]
13	Tomato	CO_2 40°C and 350 bar	Lycopene	Egydio et al., 2010 [14]
14	Eremanthuserythropappus	CO_2, 150 bar, 40°C (dynamic)	Bisabolol	de Souza et al. 2008 [15]
15	Vativeria zizanioides	CO_2 + ethanol (5%)200 bar, 40°C,5 h	Volatile oils	Talansier et al., 2008 [16]
16	Psidium guajava L.	CO_2+EtOH(10%), 30 MPa and 50°C	phenolic fraction	Castro-Vargas et al., 2010 [17]
17	Evodia rutaecarpa fruit	78 min, 62°C, 280 bar and co-solvent flow rate 0.4 ml/min	evodiamine rutaecarpine	Liu et al., 2010 [18]

The objective of present study includes the optimization of process conditions for the extraction of active components of fenugreek seed and characterization of the oil obtained at optimum condition for detail composition.

Fenugreek plant (Trigonella Foenum-Graecum), originating in the Mediterranean region and Asia, is one of the oldest herbs known. Its seeds were highly praised for their beneficial uses in ancient Egypt and India and later among the Greeks and Romans. As Fenugreek spread around the Mediterranean, ancient physicians learned that its seeds contained a great deal of mucilage and when mixed with water provided many health benefits. Fenugreek is the small stony seeds from the pod of a bean-like plant. The seeds are hard, yellowish brown and angular. Some are oblong, some rhombic, other virtually cubic, with a side of about 3mm (1/8"). A deep furrow all but splits them in two. The most common uses of Fenugreek today are culinary, such as providing a maple flavor for confectionaries, an ingredient of curry powders, and as an enhancement for meats, poultry and marinated vegetables. It is used as an appetizer, a tonic and an aphrodisiac, and it is included in many foods and beverages. Fenugreek has a long history of dubious indications, including fevers, colic, flatulence, dyspepsia, dysentery, cough, tuberculosis, edema, rickets, leg ulcers, gout, diabetes and baldness. There is little evidence to suggest the spice is toxic or that it has significant anticoagulant or hormonal effects [19].

MATERIAL AND METHODS

Dried Fenugreek seeds used in the experiment are procured from the grocery soap Burla, Odisha, India. The seeds are light tan, about 3-5 mm in size, very hard and irregularly shaped. They are pulverized to powered form using a household grinder and powered samples (**Figure 1**) are used as such in the experiment.

Figure 1: Fenugreek seed and powdered fenugreek sample

The extraction experiments were performed using a bench scale supercritical fluid extraction unit (**Figure 2**). The unit is a non-recirculating unit with a 3000 ml pressure vessel rated up to 300 bar pressure and 70˚C temperature operated with a flow rate of 20kg/hr of CO_2. Bone dry CO_2 with a purity of 99.8% was used as a supercritical fluid in the experiment.

Figure 2: Bench scale super critical fluid extractor

Next, one kilogram of the powdered sample was loaded into the extraction vessel. Before pressurization, the system was allowed to reach the preset operating temperature. In order to ensure a liquid feed to the diaphragm pump, CO_2 was fed from the cylinder into a chiller, where it was cooled to –5 °C. The chilled CO_2 was discharged from the pump into the bottom of the pressure vessel, and the pressure was adjusted to the desired operating pressure.

After pressurizing the vessel, the static/dynamic valve was opened to a flow rate of approximately 20kg/hr. The extraction process was carried out by changing the pressure and temperature of the system in order to optimize the process condition for maximizing the oil yield. The CO_2 containing the extracted oil existed through the top of the vessel and passed through the static/dynamic valve. The unit is equipped with a restrictor valve, which is a valve that regulates the release flow rate of the CO_2. Due to the large decrease in pressure from inside the vessel to atmospheric pressure, the restrictor valve is heated to prevent the valve from freezing. Experiments were conducted in static/dynamic cycles, each interval being 10 minutes, for a total time of 90 minutes. The static interval allows the powered sample to soak so that the CO_2 can penetrate the matrix and extract the oil. During the dynamic interval, CO_2 carrying the oil flowed out of the unit and into a pre-weighed collection flask, where the CO_2 was vented to a fume hood.

A range of operating conditions was tested. Experimental temperatures included 40˚C, 50˚C and 60˚C, while operating pressures included 100, 200, and 300 Bar. The percent yield of fenugreek seed oil (% by weight) was determined gravimetrically, and was taken to be the mass of oil collected, divided by the mass of powdered sample loaded into the extraction vessel multiplied by 100.

The oil sample obtained at optimum condition was analyzed using GC/MS [20-25] which was carried out in Bureau Veritas Consumer Products Services India private limited laboratory, Chennai. The specification of GC/MS is summarized below.

Instrument: GC-MS-QP 2010 [SHIMADZU]
GC condition
Column Oven Temperature: 70°C, Injector Temperature: 200°C, Injection Mode: Split Split Ratio: 10, Flow Control Mode: Linear Velocity, Column Flow: 1.51ml/min,
Carrier Gas-Helium: 99.9995% purity
Column oven temperature program

Rate	Temperature (°C)	Hold Time (min)
-	70.0	2.0
10	300.0	7.0 (32 mins total)

Column: DB-5
Length: 30.0m
Diameter: 0.25mm
Film Thickness: 0.25µm

MS Condition

Ion Source Temperature: 200°C
Interface Temperature : 240°C
Start m/z : 40
End m/z : 1000

RESULTS AND DISCUSSION

Effect of operating conditions on oil yield

A number of experiments were carried out with different operating parameter combinations. In each experiment, a light yellow, transparent oil with the characteristic fenugreek scent was extracted. The resulting fenugreek cake varied in color ranging from a yellow-brown tone for operating conditions that removed only a small fraction of the oil to a brittle chalky white color for conditions that removed a large percentage of the oil content. A color gradient was observed in the packed bed, with the lighter colored fenugreek being at the bottom of the bed, and the more yellow tones at the top of the bed. This could be due to the fact that the equipment is an up flow unit and the fenugreeks at the bottom of the bed are more exposed to fresher CO_2 entering the vessel.

The experiment was carried out at different pressure and temperature conditions and it was found that the yield of fenugreek oil exhibited a strong dependence on temperature and pressure. (**Figure 3**) shows the yield of fenugreek oil verses temperature at constant pressures.

From the (**Figure 3**) it is clear that, the highest yield was obtained at temperature of 40°C and pressure 300 bar. This can be due to higher solubility of the components at those specific conditions. Lower yield of oil at higher temperature may be due to lower

solubility and/or degradation of the components. Cumulative yield of oil at the optimum condition is included in the (**Table 2**)**.** From the table it is clear that the oil yield was low at the beginning of the process which subsequently increased with increase in time of extraction. It may be due to soaking of the solvent in the dry powder.

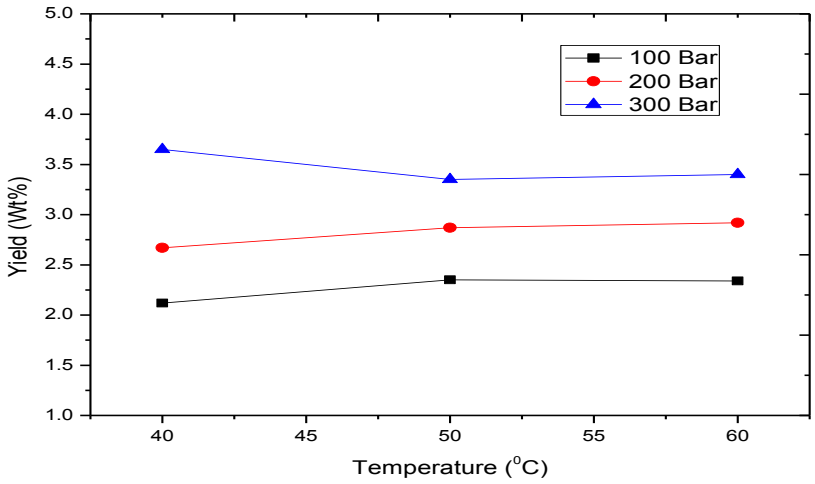

Figure 3: Effect of temperature and pressure on yield of oil

Table 2: Cumulative yield of oil (Temperature: 40˚C, Pressure: 300bar, Extraction time: 85 minutes Yield: 3.65%

Time	Temperature In ˚C	Pressure in bar Extractor	Separator	Pump	Yield%
5.15pm	40	300	40	310	0
5.40 pm	40	300	40	310	1.6
6.00 pm	40	300	40	310	2.9
6.20 pm	40	300	40	310	3.45
6.40 pm	40	300	40	310	3.65

Chemical identification of the fenugreek seed oil

The general properties of the oil are summarized in the (**Table 3**). The GC/MS of the fenugreek oil obtained at optimum temperature is taken to know the composition of the oil. (**Figure 4**) is the GC-MS plot of the oil and the components of the oil are identified by comparing the chromatogram of the oil with standard chromatographic data available in the NIST library and are listed in the (**Table 4**).

Table 3: General properties of the oil

Physical state	liquid oil
Colour	yellow – brown
Odour	strong maple like aroma
Density	0.976g/cm^3
Solubility	soluble in oil and insoluble in water

From the (**Table 4**), it is indicated that, the oil consists of 30 number of different compounds out of which 15 are fatty acids (58.44%). The major compound are oleic acid (32.74%), heneicosane (9.50%) and sotolon (8.52%) and also contains some amount of Eucalyptol (0.85%).Oleic acid is a fatty acid that occurs naturally in various animal and vegetable fats and oils. It is included in the normal human diet as a part of animal fats and vegetable oils.

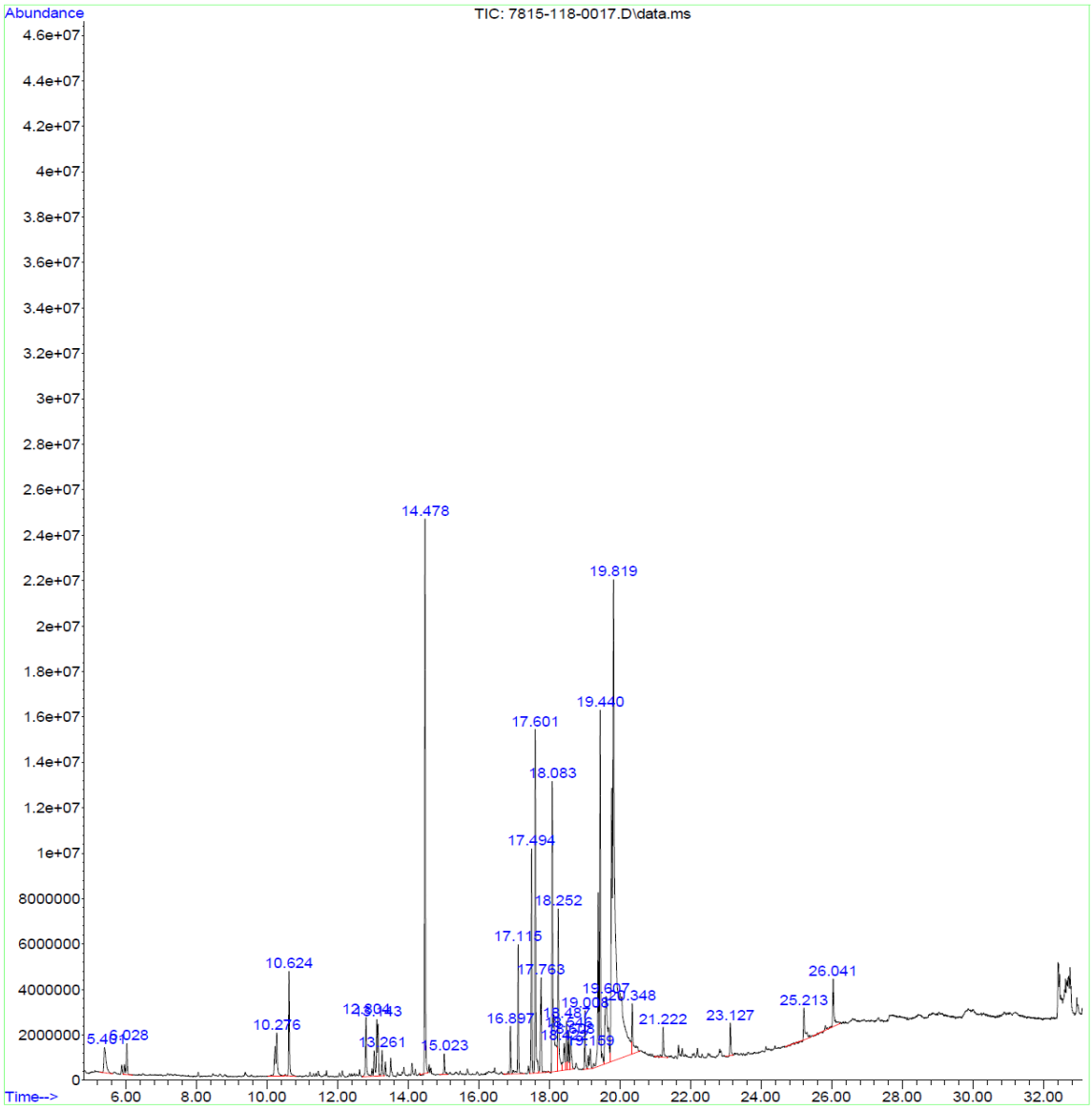

Figure 4: GC-MS of the fenugreek oil sample

Table 4: GC-MS composition of oil

Peak value	Retention time	Area percentage	Name of the compound	Molecular formula
1	5.404	1.16	2,4-Heptadienal, (E,E)-	$C_7H_{10}O$
			1,4-Hexadiene, 3-ethyl-	C_8H_{14}
2	6.028	0.85	Eucalyptol	$C_{10}H_{18}O$
3	10.277	1.78	2,4-Decadienal	$C_{10}H_{16}O$
4	10.626	1.91	2,4-Decadienal	$C_{10}H_{16}O$
			2,4-Nonadienal	$C_9H_{14}O$
5	12.803	1.29	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	$C_{15}H_{22}$
6	13.145	2.42	1,5-Cyclodecadiene, 1,5-dimethyl-8-(1-methylethenyl)-, [S-(Z,E)]-	$C_{15}H_{24}$
			8-Isopropenyl-1,5-dimethyl-cyclodeca-1,5-diene	$C_{15}H_{24}$
7	13.263	0.51	gamma.-Muurolene	$C_{15}H_{24}$
			Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-	$C_{15}H_{24}$

			dimethyl-1-(1-methylethyl)-	
8	14.474	8.52	Sotolon	$C_6H_8O_3$
9	15.024	0.42	Ar-tumerone	$C_{15}H_{20}O$
			trans-Cinnamyl tiglate	$C_{14}H_{16}O_2$
10	16.896	0.78	2-Pentadecanone, 6,10,14-trimethyl	$C_{18}H_{36}O$
			2-Undecanone, 6,10-dimethyl-	$C_{13}H_{26}O$
11	17.119	2.17	1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester	$C_{16}H_{22}O_4$
			Phthalic acid, hept-4-yl isobutyl ester	$C_{28}H_{46}O_4$
12	17.490	3.39	Nonadecane	$C_{19}H_{40}$
			Tetradecane	$C_{14}H_{30}$
13	17.602	5.87	1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester	$C_{16}H_{22}O_4$
			Dibutyl phthalate	$C_{16}H_{22}O_4$
14	17.765	2.15	Phthalic acid, isobutyl nonyl ester	$C_{21}H_{32}O_4$
			Dibutyl phthalate	$C_{16}H_{22}O_4$
15	18.085	6.96	1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester	$C_{16}H_{22}O_4$
16	18.255	3.40	1,2-Benzenedicarboxylic acid, butyl cyclohexyl ester	$C_{18}H_{24}O_4$
			1,2-Benzenedicarboxylic acid, butyl octyl ester	$C_{20}H_{30}O_4$
			Dibutyl phthalate	$C_{16}H_{22}O_4$
17	18.419	0.91	2-(Propoxycarbonyl)benzoic acid	$C_{18}H_{18}O_4$
			Phthalic acid, 2-cyclohexylethyl cyclohexylmethyl ester	$C_{17}H_{22}O_4$
18	18.486	0.89	Eicosane	$C_{20}H_{42}$
			Hexadecane	$C_{16}H_{34}$
19	18.545	0.71	Diamyl phthalate	$C_{18}H_{26}O_4$
			1,2-Benzenedicarboxylic acid, diheptyl ester	$C_{22}H_{34}O_4$
20	18.605	0.81	1,2-Benzenedicarboxylic acid, butyl octyl ester	$C_{20}H_{30}O_4$
21	19.006	1.00	1,2-Benzenedicarboxylic acid, butyl cyclohexyl ester	$C_{18}H_{24}O_4$
			Dibutyl phthalate	$C_{16}H_{22}O_4$
22	19.162	0.67	Diamyl phthalate	$C_{18}H_{26}O_4$
23	19.437	9.50	Heneicosane	$C_{21}H_{44}$
			Pentadecane	$C_{15}H_{32}$
			Nonadecane	$C_{19}H_{40}$
24	19.607	4.11	Phytol	$C_{20}H_{40}O$
25	19.815	32.74	cis-13-Octadecenoic acid	$C_{19}H_{36}O_2$
			6-Octadecenoic acid, (Z)-	$C_{18}H_{34}O_2$
			cis-Vaccenic acid	$C_{18}H_{34}O_2$
26	20.350	1.53	Heptadecane	$C_{17}H_{36}$
			Nonadecane, 9-methyl-	$C_{20}H_{42}$
27	21.219	0.71	Tricosane	$C_{23}H_{48}$
28	23.129	0.63	Diisooctyl phthalate	$C_{24}H_{38}O_4$
29	25.216	0.86	Squalene	$C_{30}H_{50}$
30	26.041	1.37	2,2-Dimethyl-3-(3,7,16,20-tetramethyl-heneicosa-3,7,11,15,19-pentaenyl)-oxirane	$C_{29}H_{48}O$

Oleic acid as its sodium salt is a major component of soap as an emulsifying agent. It is also used as an emollient. Small amounts of oleic acid are used as an excipient in pharmaceuticals, and it is used as an emulsifying or solubilizing agent in aerosol products. Foods prepared with oleic acid will remain safe to eat for longer periods, even without refrigeration. Such foods include bakery goods such as breads, cakes and pies. It is also used as a softening agent, in creams, lotions, lipsticks and skin products [26].

Eucalyptol is used in flavorings, fragrances, and cosmetics. Cineole-based eucalyptus oil is used as a flavoring agent at low levels (0.002%) in various products, including baked goods, confectionery, meat products and beverages. It is also an ingredient in many brands of mouthwash and cough suppressant, as well as an inactive ingredient in body powder. It is also used as an insecticide and insect repellent [27].

Sotolon (also known as sotolone) is a lactone and an extremely powerful aroma compound, with the typical smell of fenugreek or curry at high concentrations and maple syrup, caramel, or burnt sugar at lower concentrations. Sotolon is the major aroma and flavor component of fenugreek seed [28].

The extracted oil does not contain the some important alkaloids and amino acids (4-hydroxyisoleucine) which can be explained by the fact that such compounds being polar are not soluble in super critical CO_2 liquid. Such compounds may be extracted by using co-solvents like ethanol along with super critical CO_2 during extraction.

CONCLUSION

The optimum conditions for SC-CO_2 to extract fenugreek seed oil were 40ºC temperature, 300 bar pressure, and 85 min extraction time, and the fenugreek seed oil contained approximately 59% of fatty acids with oleic acid, heneicosane, eucalyptol and sotolon as major constituents which have many greater applications. This method could successfully extract the different active component of the raw material. Therefore, SC-CO_2 represents a valuable alternative to the traditional extraction methods for the efficient extraction of fenugreek seed oil.

ACKNOWLEDGEMENT

The authors are thankful to Mr. Sasikant Tiwari, Managing Director, Gram Tarang Foods operating under Centurion University of Technology and Management, Parlakhemundi, Odisha for providing necessary support for carrying out the experiments.

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