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Extraction and characterization of industrially valuable oil from *Eruca sativa* (L.) Mill. through FT-IR and GC-MS analysis

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Abstract

Oil isolated from seeds of *Eruca sativa* (L.) Mill was investigated for various physiochemical properties and fatty acid composition. The seed oil was also characterized using FT-IR and GC-MS analysis. The oil content was estimated as 38%. The physiochemical data obtained in the study is as follows: refractive index (1.499), specific gravity (0.92), yellowish color, iodine value (106.20g/100g), saponification value (180.6mg $KOHg^{-1}$), acid value (0.86mg g^{-1}) and peroxide value (8.5 meq kg^{-1}). In fatty acid profile, six major fatty acids were identified. Unsaturated fatty acid i.e. erucic acid was found as predominant fatty acid in Eruca seed oil. This study revealed potential and suitability of *E. sativa* seed oil for various industrial applications including biodiesel production.

Keywords

Eruca sativa, Oil Content, Erucic Acid, FT-IR, GC-MS

1. Introduction

Oil seeds have gradually gained increasing importance because of their numerous applications. Oils extracted from seeds have been used since ancient times and have been exploited in many ways. Depending on the nature, viz. edible and non-edible type, oils produced by seeds are often used as raw materials in chemical and industrial applications, as well as in medicines, pharmaceutical industries, in paper industry, and biodiesel production etc. Since more than 95% of the biodiesel is obtained from edible oils it is believed that large-scale production of biodiesel may bring global imbalance to the food supply and demand in market [1]. Because of the high demand and economic importance of oil to the industry, attention has therefore been focused on some non-edible oilseed crops.

Eruca sativa (L.) Mill. (family Brassicaceae) is one of most important oilseed crop, well recognized in therapeutic applications since ancient times for its valuable properties as an astringent, aphrodisiac, diuretic, digestive, emollient, tonic, depurative, laxative, rubefacient and stimulant [2]. However, *Eruca* is an underutilized oilseed crop and its oil is non-edible in nature, but nowadays there is tremendous rise in its demand at various industries as a lubricant, illuminating agent, bio-fuel, for soap-making, in body massage and in paper industry [3, 4]. Its oil possess therapeutic properties in the treatment of indigestion, ulcers, bacterial and fungal infections, respiratory and urinary tract infection, scurvy, hair loss and hair lice treatment [5, 6, 7].

The industrial significance of oil is due to the presence of high content of erucic acid (EA) in *E. sativa.* This makes the oil more competitive with the oil of *Jatropha curcas* during biodiesel production. Erucic acid is a predominant fatty acid and there is an escalating global demand for the amide of erucic acid, namely erucamide, depending on its use in cosmetics, detergents, and polymer production. The seed can yield upto 25-35% oil and 37% of protein [8]. Its oil could possibly be comprised of some important fatty acids such as palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, eicosenoic acid and erucic acid as it belongs to rapeseed

group. Presence of all these fatty acids in Eruca oil could be valuable potential raw material in various industries. The objective of the present study is to evaluate the physio-chemical properties of the oil extracted from *E. sativa* seeds for the purpose of global industrial utility.

2. Materials and Methods

2.1. Collection and Preparation of Sample

The seeds of *E. sativa* were procured from Plant Breeding Department at SKN College of Agriculture (SKN Agricultural University), Jobner, Rajasthan, India. The seeds were cleaned, dried in shed for a day and were crushed in order to weaken or rupture the cell walls to release fat for extraction.

2.2. Determination of Moisture and Oil Content

Initially, 100 g crushed seeds were dried in an oven at 80°C for 6 h. After every 2 h, the sample was removed from the oven, allowed to cool for 30 min. in a dessicator and then re-weighed. The % moisture in the seeds was calculated according to the following formula given by Akpan et al. [9]:

$$\%\,\text{Moisture} = \frac{W1 - W2}{W2} \times 100$$

Where

W1 = Initial weight of the sample before drying; W2 = Weight of the sample after drying

Total oil content was estimated through SOCS PLUS apparatus (SCS6, PELICAN EQUIPMENTS) by using petroleum ether as a solvent and percentage of oil extracted was determined as given below [9]:

$$\%\,\text{Oil content} = \frac{Fw - Iw}{W} \times 100$$

Where

Fw = Final weight of the beaker; Iw = Initial weight of the beaker; W= Weight of the sample in g.

2.3. Characterization of *E. sativa* Seed Oil for Various Physiochemical Parameters

Different physiochemical properties of the oil viz. refractive index, specific gravity, color, iodine value, saponification value, acid value and peroxide value were determined by using the standard procedures of AOAC [10].

2.3.1. Acid Value

Diethyl ether (20 ml) and ethanol (20 ml) were thoroughly mixed with carefully weighed 2 g oil. Then the contents were titrated with 0.1N alcoholic KOH solution using phenolphthalein as an indicator. A blank titration was also conducted simultaneously. The acid value of oil can be explained as mg KOH required to neutralize free fatty acids present in 1 g of oil.

2.3.2. Iodine Value

Iodine value was estimated by treating the known amount of oil (2 g) with known volume of standard solution of iodine monochloride (ICl) (25 ml). Ten ml of potassium iodide (KI) was used to determine the unused ICl by the amount of liberated iodine in the reaction. Liberated iodine was titrated with 0.1N sodium thiosulphate ($Na_2S_2O_3$) solution using starch as an indicator. The reaction was carried out in dark conditions. A blank titration was also conducted side by side.

2.3.3. Refractive Index

Refractive index of Eruca oil was determined by Abbe's refractometer. Few drops of the sample were transferred on the prism of the refractometer. Instrument was adjusted to determine the refractive index accurately. Refractive index of oil increases with the increase in unsaturation and also the chain length of fatty acid [11].

2.3.4. Specific Gravity

To determine the specific gravity or density of the oil sample, density bottle was used. It was calculated through the formula:

$$\text{S. G.} = \frac{(Fw - Iw)}{(W2 - Iw)}$$

Where

Fw = final weight of the bottle after filling up with oil; Iw = initial weight of the bottle; W2 = weight of the bottle, substituted with water.

2.3.5. Saponification Value

Reflux condenser was used to determine the saponification value of the oil. 25 ml of 0.1N ethanolic KOH was mixed with 2 g oil sample. Titration was done by 0.5 N HCl and phenolphthalein was used as an indicator. A blank reaction was carried out at the same time.

2.3.6. Peroxide Value

To 1 g of the oil sample, 1 g of KI and 20 ml of solvent mixture (glacial acetic acid: chloroform, 2:1) were added and the mixture was boiled for one minute. The hot solution was poured into a flask containing 20 ml of 5% KI. A few drops of starch solution were added to the mixture and the latter was titrated with 0.025 N $Na_2S_2O_3$ and the peroxide value was determined as follows [12]:

$$\text{Peroxide value} = \frac{SN10^3}{W}$$

Where

S = ml of $Na_2S_2O_3$; N = normality of $Na_2S_2O_3$; W = weight of oil sample (g).

2.4. FT-IR Analysis

For characterization and identification purpose, samples were analyzed by infrared spectroscopy. The spectra were recorded over scanning range of 400 to 4000 cm^{-1}.

2.5. GC-MS Analysis

2.5.1. Preparation of Fatty Acid Methyl Esters (FAMEs)

1. The oil sample (0.15 - 0.17 g) was taken in a test tube and10 ml of n-heptane was added and then vortexed.
2. Thereafter 4 ml of 3.5% methanolic KOH was added and vortexed again for 2 min. This solution was put in a water bath maintained at 70ºC for 2 min.
3. Thereafter the solution was vortexed 5 more times and the upper layer is drawn out in to a beaker and is evaporated till dried. Then 0.5 ml of n-heptane was added to the residue and mixed well. This constituted the fatty acid methyl esters extract for GCMS analysis.

2.5.2. Fatty Acid Content (FAC) Profile

Relative concentration of fatty acid (FA) from oil samples was measured as their corresponding methyl esters. One µl of the extract prepared as above was injected in GC-MS instrument (SHIMADZU QP-2010) equipped with MS detector. The column (0.10-0.25 mm) temperature was initially maintained at 140°C for 5 min, gradually increased to 180°C at 6°C/min., maintained for 2 min at 180°C, then further gradually increased to 240°C at 4°C/min and finally maintained for 15 min at 240°C. The carrier gas was helium at a flow rate of 1.21 ml/min. The injector and detector temperature were maintained at 230 and at 280°C, respectively and split ratio was 10:0.

Fatty acid standards were procured from sigma (USA). The major peaks were found at RT 17.1, 21.54, 25.72, 30.01 min. Standard and sample peaks were identified with those of Wiley 8 and NIST 05 libraries. The area under the peak was used to calculate the percentage fatty acid content. All estimations were repeated three times. The percentage content of individual fatty acid is calculated from the ratio of the area under the corresponding peak to the sum of the areas under total peaks using the formula:

$$\text{Fatty acid content} = 100\frac{\text{Ax}}{\sum\text{A}}$$

Where

Ax = peak area for fatty acid x; ∑A = total of all the peak areas.

3. Results and Discussion

The percentage moisture content of *Eruca* seed oil was obtained as 5.23%, comparable with other reports [9]. Removal of moisture is quite essential for the extraction procedure of oil during handling of SOCS plus. On the other hand, the percentage of oil content in seeds was found to be 38%, which was higher than the oil content reported by Chakrabarti and Ahmad [8]. The oil of *E. sativa* appeared as yellowish liquid that has strong pungent smell. It was then subjected to estimation of physiochemical properties and fatty acid composition.

3.1. Physiochemical Analysis

The physiochemical properties of *E. sativa* oil were determined essentially to test the quality (Table 1). The low acid value of oil indicated that the triacylglycerols have not been hydrolyzed, which is an indication of good stability [13]. The oil shows a high iodine value due to its high content of unsaturated fatty acids. Therefore, the oil has attracted a wide interest in its potential utilization as a lubricant and in various industrial sectors. The refractive index value, 1.499 shows that the oil is thick. This value is also comparable to the refractive index of 1.484, obtained earlier by Mumtaz et al. [14]. The high saponification value of the oil indicates a high content of triacylglycerols demonstrating their potential to be used in the cosmetic and soap making industries.

Table 1. *Physiochemical properties of Eruca sativa seed oil*

Analytical parameter	Property / Values
Color	Yellow
Odour	Pungent
Moisture content	5.23%
Oil content	38%
Texture at 27º C	Liquid
Specific gravity	0.92±0.03
Refractive index	1.499
Iodine value	106.20 g/100g
Saponification value	180.6 mg KOHg-1
Acid value	0.86 mg g-1
Peroxide value	8.5 meq kg-1

3.2. FT-IR Analysis

FT-IR analysis was done for characterization and identification of various functional groups present in *Eruca* oil. The characteristic spectrum is shown in fig. 1, which shows the presence of important functional groups present in standard oil.

Table 2. *Major functional groups present in Eruca oil based on FT-IR analysis.*

Regions	Peak	Functional group
1	2921	CH2 asymmetry stretching
2	2852	CH2 symmetry stretching
3	1744	C=O stretching
4	1457	C=C stretching
5	1160	C-O stretching
6	721	CH2 bending

The five main regions are usually considered for the analysis of oil and fat samples, viz. 2805–3100 cm^{-1}, 1615–1770 cm^{-1}, 1420-1500 cm^{-1}, 1230-1345 cm^{-1}, and 850-1150 cm^{-1}. All the peaks obtained related to various stretching and bending vibrations (Table 2). The peak at 1744.04 cm^{-1} corresponds to the triglyceride ester groups. The peak at 1188-1200 cm^{-1} which is not present in oil, corresponds to O-CH_3 stretching, similarly Mumtaz et al. [14] also did not find this peak in *E. sativa* oil but it was detected in the biodiesel obtained from it. Results of our analysis are large in agreement to those reported by Mumtaz et al. [14].

3.3. GC-MS Analysis

GC-MS analysis (Fig. 2) was performed to identify the chemical ingredients in the sample and to evaluate the percentage content of individual fatty acid. In this study, FAMEs prepared from extracted oil were subjected to GC-MS apparatus along with the known standard solutions. The chromatogram showed several compounds at various retention times.

Determination of fatty acid content in *E. sativa* oil showed presence of six major fatty acids, palmitic acid (PA 16:0), stearic acid (SA 18:0), oleic acid (OA 18:1), linoleic acid (LA 18:2), linolenic acid (LN-A 18:3) and erucic acid (EA 22:1); the latter being the most abundant fatty acid (Table 3).

Table 3. *Major fatty acids present in Eruca sativa seed oil*

Fatty acid	Content (%)
Palmitic acid	2.80
Stearic acid	30.8
Oleic acid	17.8
Linoleic acid	1.44
Linolenic acid	6.78
Erucic acid	47.0
∑ Saturated fatty acid	33.6
∑ Unsaturated fatty acid	73.0

The content of PA (2.80%) determined in the present study was lower than 10.2% reported by Chakrabarti and Ahmad [8], but same to the value (2.8%) obtained by Mumtaz et al. [14]. The amount of SA was found to be 30.8%, which was much higher than the values obtained by Sindhu and Kantharaj [15] and Chakrabarti and Ahmad [8] i.e. 0.93% and 1.60%, respectively. OA content obtained in our study was 17.8%, which was although lower but yet comparable to values of 19.88% and 22.8% reported by Sindhu and Kantharaj [15] and Chakrabarti and Ahmad [8], respectively. The content of LA (1.44 %) was also significantly lower than 10.3 % reported by Mumtaz et al. [14] and 9.23% by Sindhu and Kantharaj [15]. The content of LNA was 6.78% in our study, which was lower than the content (12.5%) obtained by Mumtaz et al. [14]. The EA content which is considered as the most important fatty acid was found 47.0% in our study, which was higher than the value reported by Chakrabarti and Ahmad [8], where as Mumtaz et al. [14] obtained a quite similar value of 47.7% in the seeds procured from Pakistan. Overall, *E. sativa* oil contains high percentage of unsaturated fatty acids which is about 73.02%.

4. Conclusion

Oils rich in erucic acid have desirable properties for a variety of applications because it confers several desirable superior characteristics, such as high lubricity, cold stability and fire resistance, on oils and derived compounds. The present study which has comprehensively analyzed various physiochemical characteristics of *Eruca* seed oil convincingly demonstrates the suitability of the oil towards many industrial applications including in biodiesel production. The introduction of high erucic acid rapeseed (HEAR) varieties in industries may lead to further expansion of the green market.

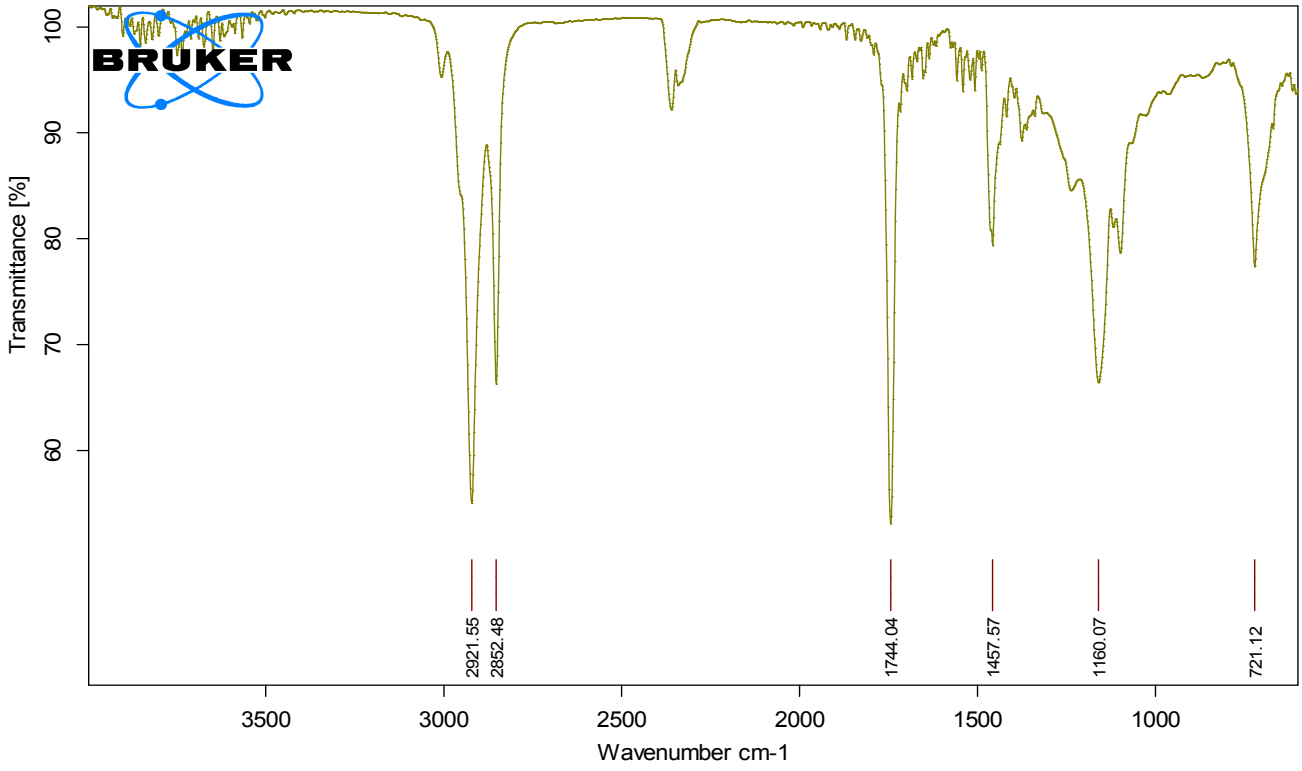

Fig. 1. *FT-IR spectrum of Eruca sativa seed oil*

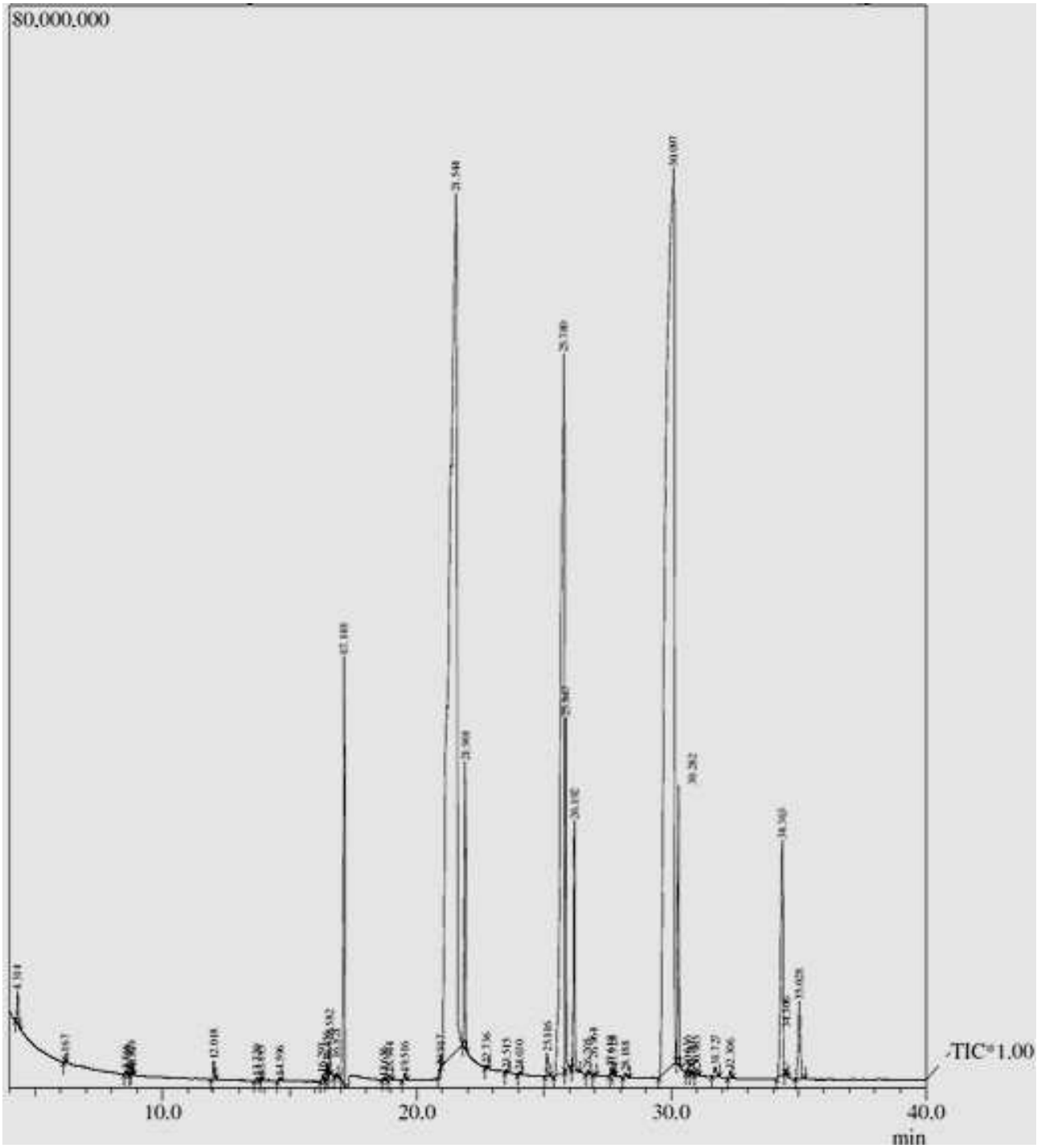

Fig. 2. *GC-MS profile of Eruca sativa seed oil*

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