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Physicochemical Characterization of Cucumis sativus L. seed oil

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Abstract: ***Cucumis sativus L. seeds oil was extracted by an organic solvent and analyzed for its physicochemical properties i.e. acid value, iodine value, moisture content, density and refractive index. The acid value, moisture content and iodine value were 1.581 mg KOH/g oil, 0.108% and 125.26 gI_2/100 g respectively. The density and refractive index of the oil were found to be 0.8977 g/cm^3 and 1.4685 respectively. Gas chromatographic analysis of Abelmoschus esculentus seeds oil showed that unsaturated fatty acids such as linoleic acid (54.31 wt.%) and oleic acid (22.27 wt.%) account for more than 70% of total fatty acids. The prominent saturated fatty acid present is palmitic acid (19.43 wt.%).***
Key words: ***Cucumis sativus L., unsaturated acid, Musa balbisiana Colla, transesterification***

I. INTRODUCTION

Cucumis sativus L. (locally called 'Tioh') is an important vegetable and one of the most popular members of the Cucurbitaceae family. They are found to exhibit a wide spectrum of activity including antioxidant and amylolytic [1]. The crop is the fourth most important vegetable after tomato, cabbage and onion in Asia, the second most important vegetable crop after tomato in Western Europe [2]. It is an annual climber growing 2 m. Leaves are hairy and have 3–5 lobes, branched tendrils at leaf axes support climbing. Plants are usually monoecious (male and female flowers on separate plants), but varieties show a range of sexual systems. Female flowers are yellow with 5 petals, and develop into a cylindrical fruit, which may be as large as 60 centimeters (24 in) long and 10 centimeters (3.9 in) in diameter. In this paper, the physicochemical properties of Cucumis sativus L. seed oil is reported.

Fig.1. Cucumis sativus L. seed

II. EXPERIMENTAL SECTION

A. *Materials*

Cucumis sativus L. seeds were collected from Kamrup and Barpeta Districts of Assam, India during its availability of the season. The seeds were selected according to their condition where damaged seeds were discarded before seeds in good condition were cleaned, de-shelled and dried at high temperature of 100-105^{o}C for 35 min. Seeds were grounded using grinder prior to extraction.

Solvents and other chemicals used were of analytical grade, and they were procured from commercial sources and used as such without further treatment.

B. Instruments used

^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ at 300 and 75 MHz, respectively using Bruker Avance III 300 MHz/54 mm NMR spectrometer. FT-IR spectra were obtained on a Perkin Elmer RX I FT-IR spectrometer. The colour of the oil sample was determined by observation using several independent competent individuals. Oil colours were correlated using colour charts. Refractive index was determined by using the Abbe Refractometer (AW-24) at room temperature (28°C). The acid value was determined following established procedure of AOAC [3]. Iodine value was estimated by applying Wijs method [4, 5]. Moisture content was determined by oven drying a known quantity of the oil in the oven at 105°C for 24 hours after which the percentage moisture was calculated as follows:

$$\%Moisture = \frac{Initial\ weight\ of\ oil - Final\ weight\ of\ oil}{Initial\ weight\ of\ oil} \times 100$$

C. Oil Extraction

Extraction of oil was done by solvent extraction technique on the crushed kernel using petroleum ether as the solvent. Crushed kernel in petroleum ether (bp 40-60°C, 10 mL/g) was magnetically stirred at room temperature (28-29°C) for 3 h, solvent was removed at 45°C using a rotary vacuum evaporator to yield the crude oil. The process was repeated 2-3 times with the seed cake using fresh solvent each time in order to extract most of the oil. The oil was purified prior to transesterification done, by column chromatography over silica gel (60-120 mesh) using a mixture of petroleum ether and ethyl acetate (20:1) as the eluent.

D. Transesterification of Seed Oil

Transesterification of the purified oil was carried out at room temperature with MeOH. The catalyst used for transesterification was prepared in the laboratory from the trunk of Musa balbisiana plant [6]. A mixture of oil, methanol (10mL/g of purified oil) and catalyst (20wt% of oil) was stirred magnetically in a round bottom flask at room temperature (30-32°C). Reaction was monitored by TLC. After completion of the reaction, the product mixture was partitioned between water and petroleum ether and the combined organic layers was washed with brine, dried over anhydrous Na_2SO_4 and the solvent removed under vacuum to yield the crude FAME
mixture. The product was purified by column chromatography over silica gel (60-120 mesh) using a mixture of petroleum ether and ethyl acetate (20:1) as the eluent. The purified product was further subjected to high vacuum to remove the last traces of solvents to yield pure FAME.

E. Analysis of FAME

The fatty acid methyl esters were identified using Perkin-Elmer Clarus 600 GC-MS analyzer. The column used was Elite 5 MS with dimension 30.0m x 250µm. The oven temperature was initially held at 140°C for 5 minutes, increased to 240°C at 4°C/min and finally held for 5 min at 240°C. The injector, transfer and source temperatures were 250°C, 200°C and 150°C respectively. Helium was used as the carrier gas. The mass spectrum was scanned from 20 to 400 Da. For identification of FAME library search was carried out using NIST, NBS and Wiley GC-MS library. Fatty acid profile of FAME from *Cucumis sativus* L. seed oil is reported in Table 2.

III. RESULTS AND DISCUSSION

The results of the physical characteristics of oil obtained from the seeds of *Cucumis sativus* L. are shown in Table1.
The oil content of the seed is found to be 29.21%.

Moisture content of Cucumis sativus L. seed oil is found to be very low (0.108). It indicates a long storage life for the seed oils. Besides, it also indicates that the oils are of good qualities and are not easily subjected to contamination. High moisture content in plant seed oils usually leads to increase in microbial load as well as lipid oxidation resulting in rancidity. A higher value of moisture play a retarding role on the transesterification of glycerides [7, 8, 9].

Table 1 : Some physicochemical properties of *Cucumis sativus* L. seed oil

S/N	Parameters	Observed values
1	Colour	Light green
2	Oil content (%)	29.21
3	Density (g/cm^3)	0.8977
4	Acid value (mg KOH/g)	1.581
5	Iodine value ($gI_2/100$ g)	125.26
6	Refractive index	1.4685
7	Moisture (%)	0.108

The acid value is the measure of quantity of fatty acids in the oil. A higher fatty acid value (1.581) was observed in Cucumis sativus L. oil. This reflects the high fatty acid content of the oil. This indicates that these oils are not favourable source for production of biodiesel as high free fatty acid (FFA) content causes formation of soap during transesterification reaction of glycerides with alcohol and makes it extremely difficult to separate the products [10, 11]. Iodine value measures the unsaturation of fats and oils. The iodine value of Cucumis sativus L. seed oil was found to be 125.26. This suggests that seed oil is highly unsaturated. The refractive index (1.4685) of the oil is in close range with the values obtained for some conventional oils such as palm kernel oil (1.449-1.451), Soya bean oil (1.466-1.470) etc [12, 13, 14]. Since the refractive index of the oil is greater than that of water (1.330) at room temperature, this property suggests the use of the oil in studies relating to optics. Fatty acid profile of the FAME from Cucumis sativus L. seed oil was determined by GC-MS analysis. The individual peaks of the gas chromatogram (Fig. 2) were analyzed and the fatty acids were identified using MS data base. Relative percentage of fatty acid esters were calculated from total ion chromatography by computerized integrator. The fatty acid composition of Cucumis sativus L. seed oil are presented in Table 2. The results show that Cucumis sativus L. seed oil consists mainly of linoleic (54.31 wt.%), oleic (22.27 wt.%) and palmitic (19.43 wt.%) acids. Unsaturated fatty acids such as linoleic and oleic acids account for more than 75% of total fatty acids. Saturated fatty acids on the other hand, account for approximately 22% of total fatty acids. Among them, palmitic acid appears to be the significant one while small amount of stearic acid (3.12 wt.%) is also detected.

Table 2 : Fatty acid profile of FAME from *Cucumis sativus* L. seed oil

Retention time (min)	FAME	Molecular ion peak (m/z)	wt.%
13.85	Methyl palmitate	270	19.43
17.56	Methyl linoleate	294	54.31
18.52	Methyl oleate	296	22.27
19.30	Methyl stearate	298	03.12

The mass spectra of methyl palmitate, methyl linoleate, methyl oleate and methyl stearate are shown in Figs 2.a to 2.d and their molecular ion peaks are observed at 270, 294, 296 and 298 respectively.

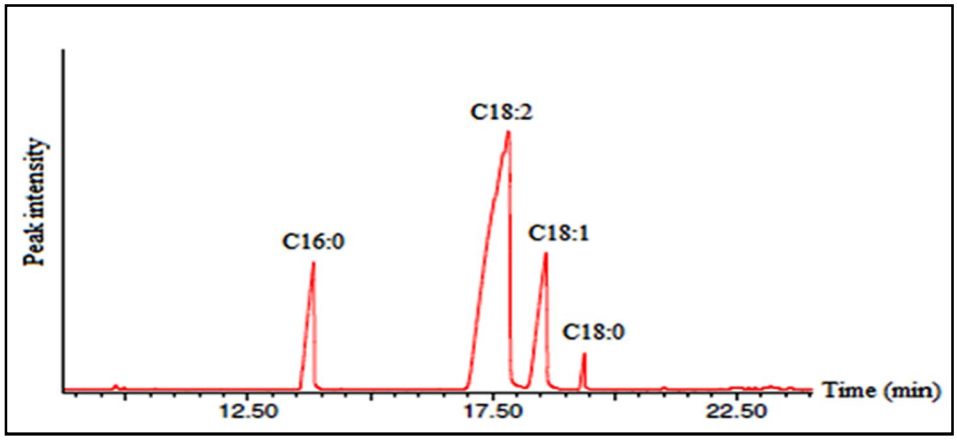

Fig. 2. Gas Chromatogram of FAME from Cucumis sativus L. seed oil

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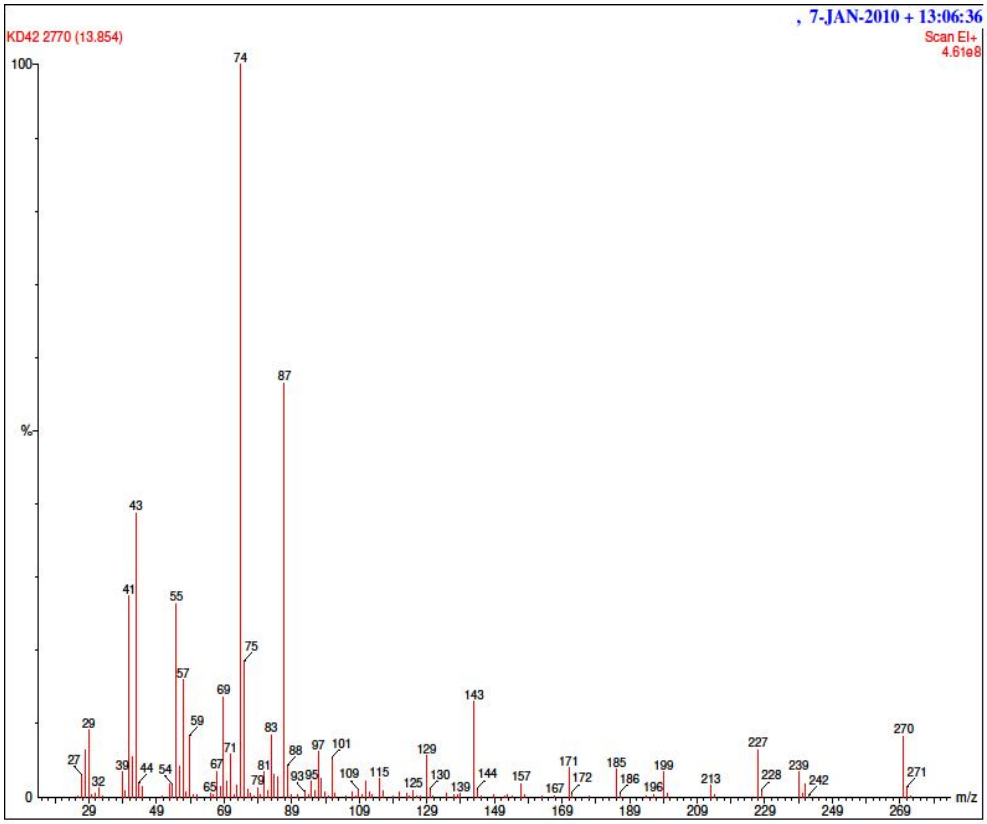

Fig. 2a. Mass spectrum of methyl palmitate

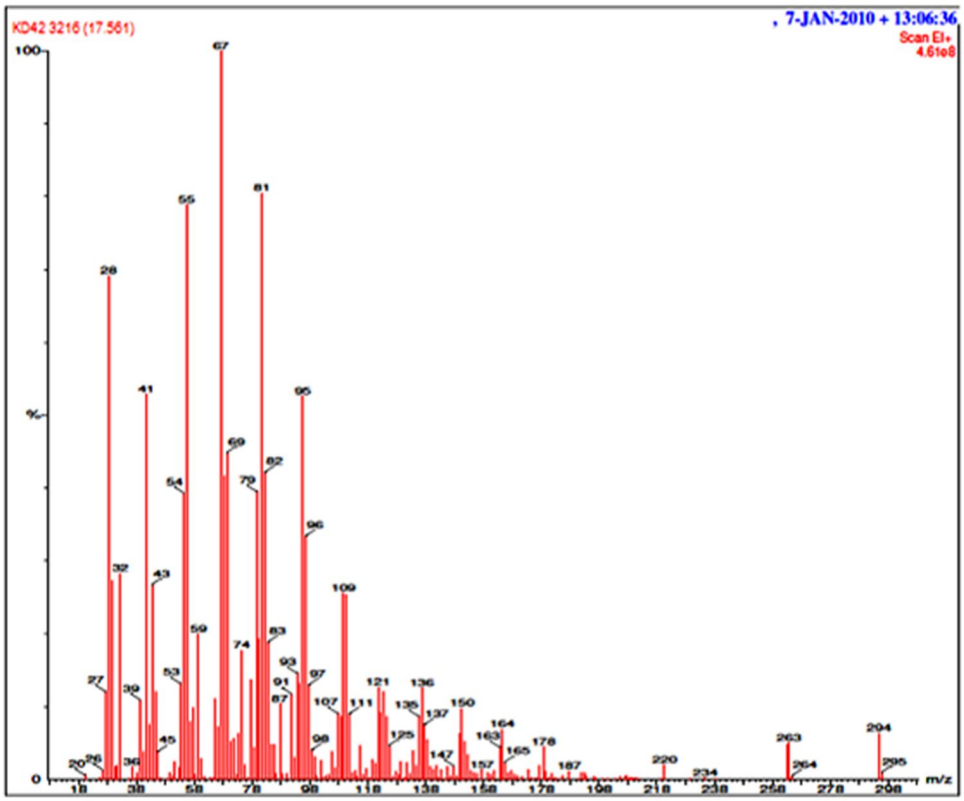

Fig. 2b. Mass spectrum of methyl linoleate

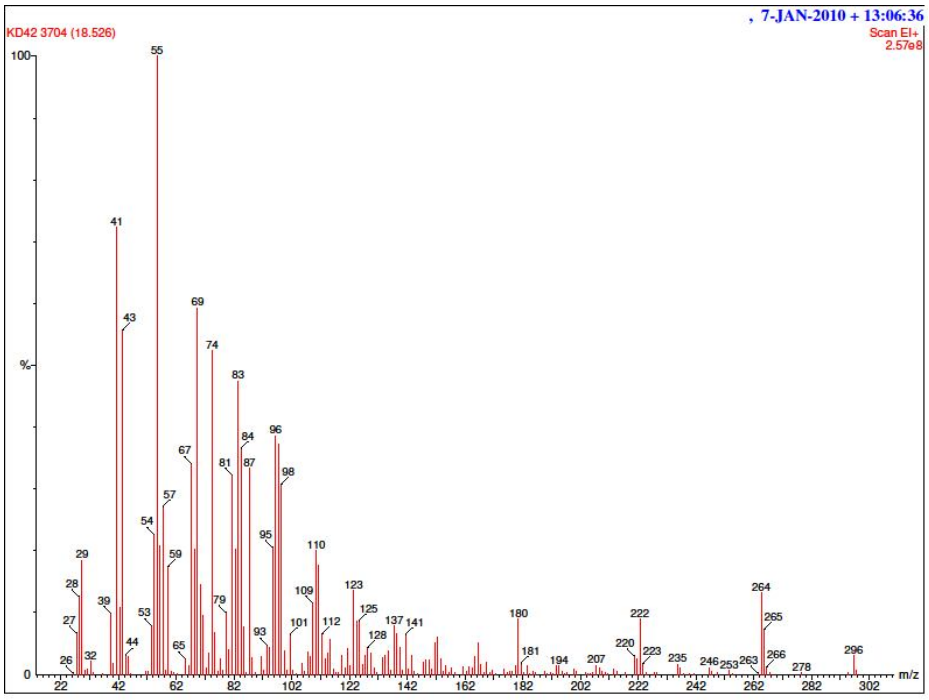

Fig. 2c. Mass spectrum of methyl oleate

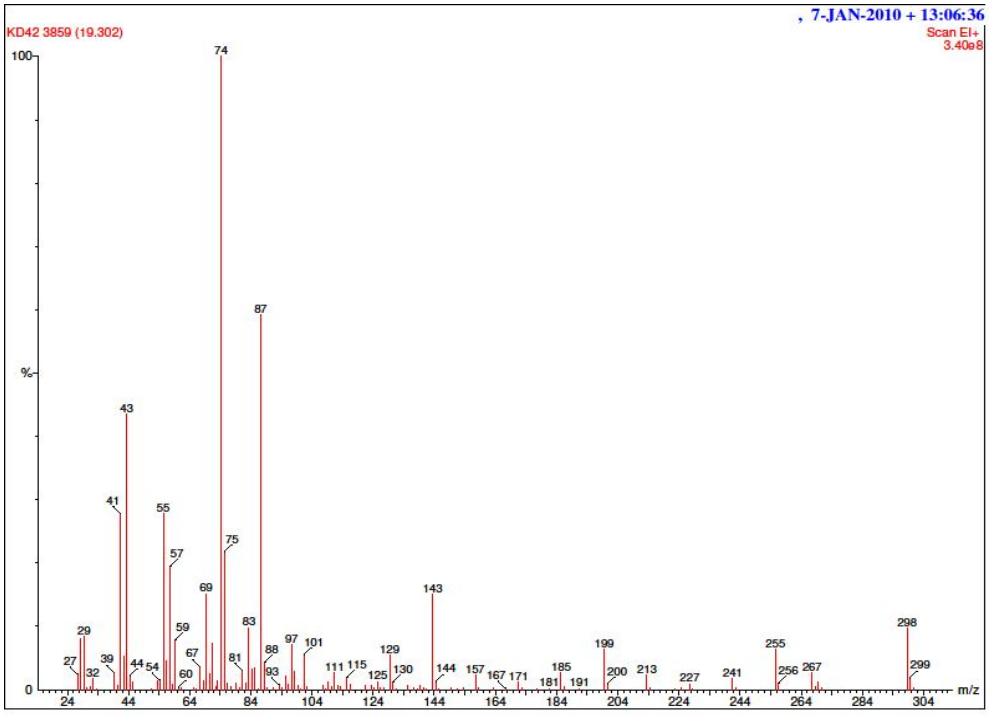

Fig. 2d. Mass spectrum of methyl stearate

The ^{1}H NMR spectrum of the FAME from Cucumis sativus L. oil is shown in Fig. 3. The multiplet at 5.25-5.30 ppm indicates the olefinic protons (-CH=CH-). A singlet signal at δ 3.61 ppm represents methoxy protons of the ester functionality of the FAME. The triplet at δ 2.28 ppm (t, ^{3}J=7.5 Hz) represents the α-methylene protons to ester (-CH_2-CO_2Me). The α-methylene protons to double bond (-CH_2-C=C-) is seen as a multiplet at δ 1.92-2.01 ppm. The β-methylene protons to ester (CH_2-C-CO_2Me) also appear as a multiplet at δ 1.51-1.59 ppm. The singlet signals at δ 1.27 and 1.23 ppm are due to the protons of backbone methylenes of the long fatty acid chain. The terminal methyl protons (C-CH_3) at δ 0.82-0.84 ppm appear as a multiplet.

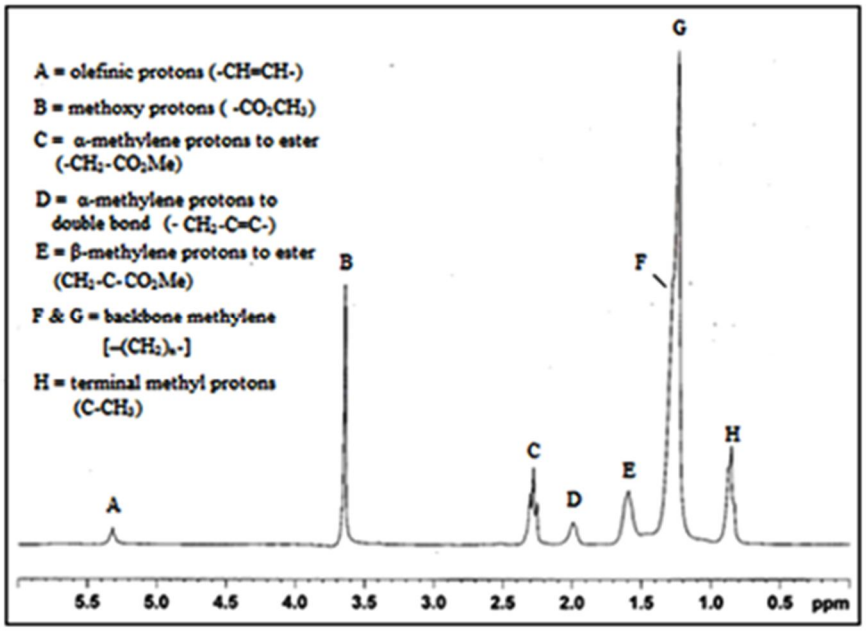

Fig. 3. ^{1}H NMR spectrum of FAME from Cucumis sativus L. seed oil

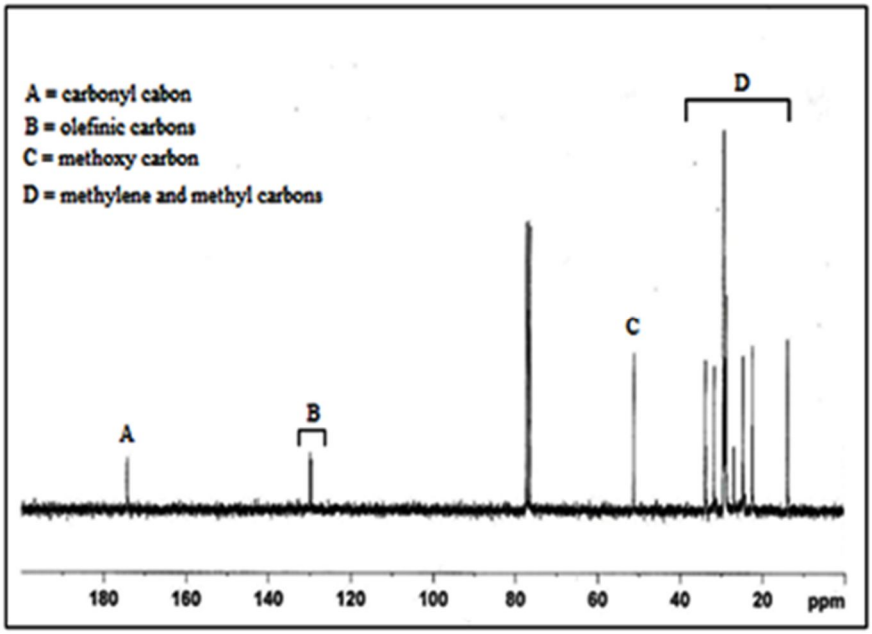

Fig. 4. ^{13}C NMR spectrum of FAME from Cucumis sativus L. seed oil

The ^{13}C NMR spectrum of FAME from Cucumis sativus L. seed oil is shown in Fig 4. The signal at δ 171.32 ppm indicates the carbonyl carbon of the ester molecules and the olefinic carbons appear at δ 129.61 and 129.85 ppm. The signal at δ 51.32 ppm in the ^{13}C NMR spectrum of FAME represents methoxy carbons of esters. The methylene and methyl carbons of fatty acid moiety appear in the range from δ 13.92 to 34.03 ppm. The IR spectrum of FAME from Cucumis sativus L. seed oil is shown in Fig 5. IR spectrum of the FAME shows a C=O stretching band of methyl esters at 1742 cm^{-1} and C-O stretching bands at 1112, 1171 and 1244 cm^{-1}. The weak signal at 1658 cm^{-1} may due to C=C stretching frequency. Strong and sharp signals at 2842 and 2925 cm^{-1} indicate C-H stretching frequencies. The absorbance at 3461 cm^{-1} is due to the =C-H stretching frequency. The observation of an absorption peak at 720 cm^{-1} suggested the CH_2 rocking.

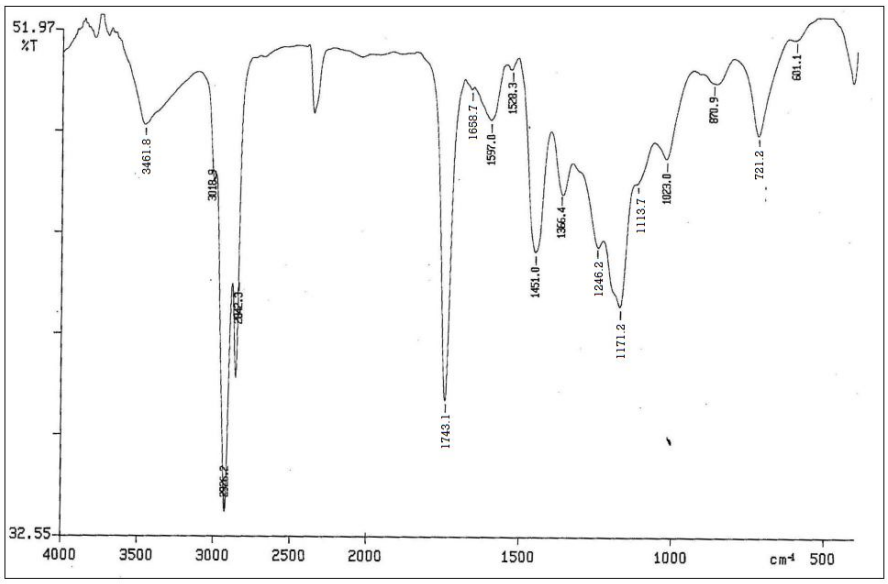

Fig 5. The IR spectrum of FAME from Cucumis sativus L. seed oil

IV. CONCLUSION

In this study, the physicochemical properties of Cucumis sativus L. seed oil was investigated. The study revealed that unsaturated fatty acids such as linoleic and oleic acids account for more than 75% of total fatty acids in Cucumis sativus L. seed oil. Linoleic acid is the major fatty acid found with 54.31 wt.% followed by oleic acid (22.27 wt.%) and palmitic acid (19.43 wt.%). The acid value, moisture content and iodine value were 1.581 mg KOH/g oil, 0.108% and 125.26 gI_2/100 g respectively. The density and refractive index of the oil were found to be 0.8977 g/cm^3 and 1.4685 respectively.

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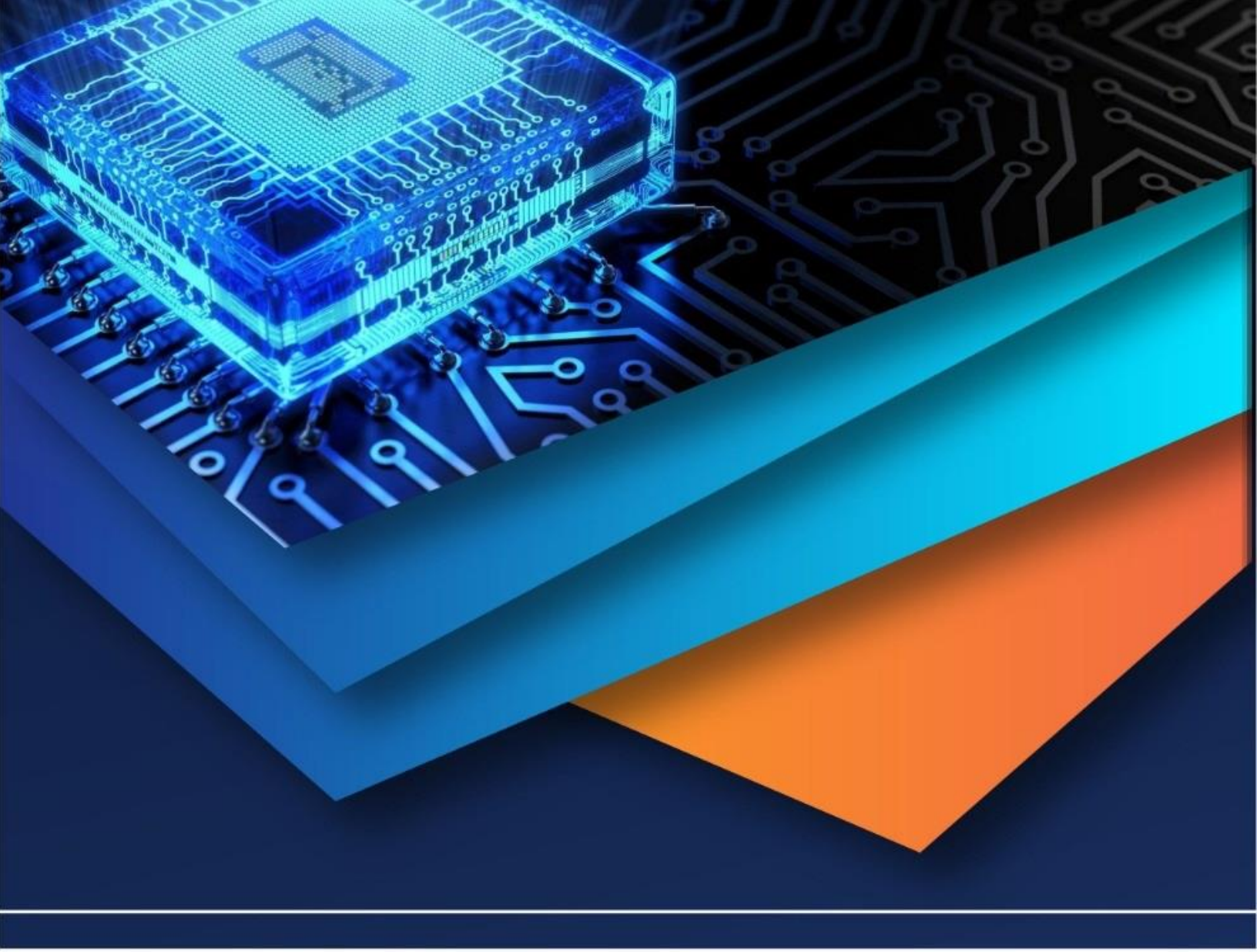

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