J Am Oil Chem Soc (2011) 88:1675–1685
DOI 10.1007/s11746-011-1846-4

ORIGINAL ARTICLE

Estimation of Glycerides and Free Fatty Acid in Oils Extracted From Various Seeds from the Indian Region by NMR Spectroscopy

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Received: 23 June 2010 / Revised: 16 January 2011 / Accepted: 22 April 2011 / Published online: 8 May 2011

Abstract The present study is focused on the quantification of glycerides and free fatty acid in oils extracted from various seeds for biodiesel production from Indian territory. A new method based on ^{1}H- and ^{13}C-NMR spectroscopic techniques was developed in order to estimate triglycerides (TG), diglycerides, monoglycerides, free fatty acids (FFA) and various other components such as para-substituted phenols, silylated methyl esters, aromatic acids, naphthalenes, etc. Iodine values of the extracted oils were estimated using the developed ^{1}H-NMR spectroscopic method, which was correlated with the TG content present in the sample. Results by the NMR method were validated by the blend preparation, fatty acid composition determined by the GC and from the iodine value of the samples. The developed method is direct, rapid and no sample treatment is required. The results from these comprehensive studies indicated that NMR spectroscopic technique is useful for the quantification of extracted oils and can be used effectively for the development and monitoring of biodiesel production and determining the fuel quality.

Keywords Vegetable oil · Biodiesel · Glycerides · ^{1}H-NMR spectroscopy · ^{13}C-NMR spectroscopy

Introduction

Many vegetable oils have similar fuel properties to diesel fuel, except for their higher viscosity and lower oxidative stability [1]. Biodiesel production and the physico-chemical properties are directly linked to the characteristics of the vegetable oil from which it was produced. The use of domestic, renewable sources of energy reduces our dependence on imported oil, thereby improving our nation's energy security. The Indian biodiesel program is not only based on one kind of vegetable oil, because of the great social, economical and regional diversities of the country, many different sources of oils are under investigation. Due to the "food verses fuel" problem, the use of edible oil as a fuel source may not be a viable, long term solution. Non-edible oils such as Jatropha, Karanjia, as well as animal fat, tallow, etc., can replace vegetable oils as fuel sources. These non-edible oils usually contain glycerides (tri-, di- and mono-), free fatty acids (FFA), phospholipids, sterols, water, odorants and other impurities [2, 3]. The glycerides content and FFA content of these feed stocks is an important quality parameter. Several studies were carried out on the development and improvement of analytical methods for biodiesel production and determining the fuel quality. Analytical procedures reported in the literature include chromatographic methods [4–6] (GC, HPLC, GPC) and spectroscopic methods [7–12] (^{1}H, ^{13}C NMR and FTIR), which are extensively used for monitoring the production, synthesis and quality of biodiesel. High-resolution NMR (^{1}H, ^{13}C NMR) spectroscopy offers many advantages over existing analytical methods, because it allows the rapid, simultaneous, noninvasive and nondestructive study in the multi-component system of fat, oil and biodiesel. Previously, ^{1}H/^{13}C NMR were used to obtain the quantitative information about the determination of the unsaturated fatty acid composition [13], rapid determination of the iodine value [14, 15], solid fat content [16], FFAs [17], fatty acid composition of vegetable oils [18, 19], and vegetable seeds [20].

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The present studies are focused on the compositional analysis of oil extracts of different seeds in the context of biodiesel production and their development. Characterization and quantification of various types of oil extracted from different seeds namely; *Emblica officinalis* (Amla), *Carthamus tinctorius* (Kusum), *Guizotia abyssinica* (Niger), *Carum roxburghianum* (Ajmoda), *Azadirachta indica* (Neem), *Pongamia glabra* (Karanja), *Psoralea corylifolia* (Bawachi), *Tamarindus indicus* (Tamarind), *Semecarpus anacardium* (Bhilawa), *Terminalia belerica* (Baheda), *Terminalia chebula* (Hara), *Terminalia chebula* (Hara Kachariya), *Terminilia belerica* (Bahede Kachariya) were carried out using method based on high-resolution ^{1}H- and ^{13}C-NMR techniques. The following aspects were studied:

- Estimation of glycerides (TG, DG and MG)
- Estimation of free fatty acids
- Phenolic components
- Estimation of the iodine value

Experimental Section

Oil Extraction

Oils were extracted from the respective seeds by Soxhlet extraction using petroleum ether (bp 60–80 °C) at 80 °C for ~20 h [21]. The oil extracted seeds were again subjected to Soxhlet extraction under identical conditions for an extra 5 h in order to ensure complete oil extraction. The sample recovered after the extraction was monitored by Thin Layer Chromatography (TLC) using a mixed solvent system of petroleum ether (85 ml), diethyl ether (13.5 ml), glacial acetic acid (1.5 ml).

NMR Spectroscopic Studies

All the ^{1}H/^{13}C-NMR spectra were recorded on a Bruker ACP-300 MHz NMR spectrometer.

^{1}H-NMR Spectroscopy

The concentration of the sample used was 5–10% w/w in $CDCl_3$ for ^{1}H NMR containing tetramethylsilane (TMS) as an internal reference as per the following experimental conditions. Spectral width = 3,500 Hz (0.0–12.0 ppm), spectral size = 16 K, digital resolution = 0.305 ppm/point, 90° pulse = 18 μs, relaxation delay = 10 s and number of scans = 64.

^{13}C-NMR Spectroscopy

The quantitative ^{13}C-NMR spectra were obtained for a solution of approximately 30% w/w in $CDCl_3$. The quantitative ^{13}C-NMR spectra were obtained in the inverse gated mode using 0.1 M chromium acetylacetonate ($Cr(acac)_3$) as relaxation agent. Under these conditions, reproducible quantitative spectra were obtained. The spectral parameters were: spectral width = 15,000 Hz (0.0–200.0 ppm), spectral size = 16 K, digital resolution = 1.20 ppm/point, 90° pulse = 8.7 μs, relaxation delay = 5.0 s and number of scans = 10,000.

All the ^{1}H- and ^{13}C-NMR spectra were integrated after base line correction and the mean of three integration values was taken for each calculation.

GC Analysis

A Perkin Elmer Clarus-500 gas chromatograph equipped with split/split less injector and flame ionization detector was used. Data were recorded and processed using Total Chrom workstation software. A packed stainless steel column of 10″ length and 10% coating of liquid stationary phase SP-2340 on support material Chromosorb-W mesh size 80–100 was used in the analysis using the following analytical conditions.

Injector and FID detector temperature: 300 °C, carrier gas: helium (UHP grade), sample volume: 0.2 μl, oven programming: 150 °C—6 °C—250 °C (30 min). The reference standards used were saturated and unsaturated methyl esters of C14:0 to C24:0 and C14:1 to C24:1 fatty acids.

The reagent and chemicals used were of high purity grade and were purchased from M/s Sigma-Aldrich.

Results and Discussion

The details of the oils extracted from various seeds are given in Table 1. The oil content of the respective seeds was calculated according to ISO 659:1998 (Revised 2009) test method. The total acid number (TAN) was estimated as per ASTM-D-664-09 (Table 1). Fatty acid composition as per ASTM-D-1983-90 (1995) by GC technique is given in Table 2. The GC data indicated that the oils 1 and 2 contain mainly C16:0 to C18:2 fatty acids, where as other oils 7, 9, 10, also contain C18:3, C20:0, C22:0 and higher fatty acids in small amounts.

^{1}H-NMR Spectral Features of Extracted Oils

The ^{1}H-NMR spectrum of a representative oil extracted from seeds as given in Fig. 1 shows all the characteristic signal of glycerides and fatty acids. The characteristics symmetrical multiplet at 4.10–4.40 ppm corresponds to the ester signal of terminal $–OCH_2–$ groups of the glyceride part of the fatty material. Thus, the integral intensities of

Table 1 Details of oils extracted from various seeds

Sample name	Seed	Botanical name	% Oil content	TAN mg KOH/g	Observation
Oil-1	Amla	*Emblica officinalis*	1.0	9.48	Dark green viscous liquid
Oil-2	Charota	*Cassia tora*	1.42	–	Dark brown liquid
Oil-3	Kusum	*Carthamus tinctorius*	30	9.75	Pale yellow viscous liquid
Oil-6	Ajmoda	*Carum roxburghianum*	1.08	35.3	Dark brown semisolid material
Oil-7	Neem	*Azadirachta indica*	33	9.93	Pale yellow viscous liquid
Oil-8	Unidentified	–	2.57	27.85	Brown viscous liquid
Oil-9	Karanja	*Pongamia glaibra*	34	3.96	Pale yellow viscous liquid
Oil-10	Bawachi	*Psoralea corylifolia*	5.0	6.73	Dark red viscous dye type material
Oil-11	Tamarind	*Tamarindus indicus*	0.87	–	Brown viscous liquid
Oil-13	Bhilawa	*Semecarpus anacardium*	19.0	24	Highly viscous black resin
Oil-14	Beheda	*Terminilia belerica*	15.6	11.02	Pale yellow viscous transparent liquid
Oil-15	Hara	*Terminalia chebula*	1.86	35.4	Brownish viscous liquid
Oil-16	Harra kachuriya	*Terminalia chebula*	0.33	–	Dark viscous product
Oil-18	Beheda kachuriya	*Terminilia berlerica*	0.71	–	Dark liquid
Oil-20	Nagarmotha	*Cyperus rotundus*	0.9	–	Dark viscous product

Table 2 Fatty acid analysis of oils extracted from various seeds as per ASTM-D-1983-90 (1995) by GC technique

Fatty acids	Oil-1	Oil-2	Oil-3	Oil-7	Oil-10	Oil-12	Oil-14	Oil-15
C16:0	32.2	6.5	22.0	15.8	21.3	19.4	19.97	20.6
C18:0	17.2	3.5	6.0	15.2	8.1	6.9	6.87	9.1
C18:1	29.4	17.4	19.0	50.9	18.6	24.5	42	34.9
C18:2	21.2	69.4	48.7	13.6	39.6	32	28.4	32.8
C18:3	–	1.3	1.5	1.2	11.1	1.9	1.5	0.7
C20:0	–	1.9	1.8	2.4	1.3	2.5	1.4	1.7
C22:0	–	–	1.0	–	–	13	–	–
Unidentified higher	–	–	–	0.9	–	–	–	–
Average alkyl chain length	17.4	17.9	17.6	17.8	17.6	18.3	17.7	17.6

the symmetrical multiplet ester signal at 4.10–4.40 ppm can be used to estimate the total triglyceride content in the extracted sample oil. The signal due to the third central ester group (–OCH–) is overlapped with the signals of unsaturated protons (–CH=CH–) at 5.00–5.40 ppm. In order to estimate the contribution of –OCH– group from the overlapped region, the spectrum between 5.0–5.4 ppm was expanded along with the integration. The unsaturated hydrogen content was estimated from the integral intensities of the region 5.00–5.40 ppm and total spectral region 0.5–9.0 ppm. The unsaturated hydrogen content is used to calculate the iodine value of the extracted oils [15]. Other signals are indicated at 2.28–2.38 ppm due to $–CH_2–$ attached to carbonyl group and a signal at 2.81–2.84 ppm due to $–CH_2–$ between the two unsaturated conjugated groups of fatty acids/esters having more than one double bond. The appearance of two resolved triplets at 0.95 and 0.85 ppm are due to the terminal methyl of polyunsaturated fatty acid (PUFA, $\geq$3 double bond) and all other fatty esters/acids, respectively (Fig. 1). Other signals in the sample are due to $–CH_2$ and $–CH_3$ groups of longer alkyl chain of fatty esters/acids. The highly enlarged spectrum in the region 6.80–8.20 ppm as shown in Fig. 1 indicates the presence of phenolic and naphthalene type aromatic compounds.

It is very interesting to note the symmetrical pattern of the glyceryl proton signal at 4.10–4.40 ppm indicating the presence of a triglyceride moiety. However, the unsymmetrical pattern indicates the presence of a diglyceride and monoglyceride along with the triglyceride as demonstrated by the standard spectra of mono-, di- and triglyceride shown in Fig. 2. Monoglyceride is also clearly distinguishable from other glycerides by the multiplets at 3.60–3.80 ppm (Fig. 2). The presence of multiplets at 3.60–3.80 ppm along with the unsymmetrical pattern at 4.10–4.40 ppm clearly reveals the presence of monoglyceride along with triglycerides in the oil-extracted samples (Fig. 3). Besides the spectral features corresponding to fats

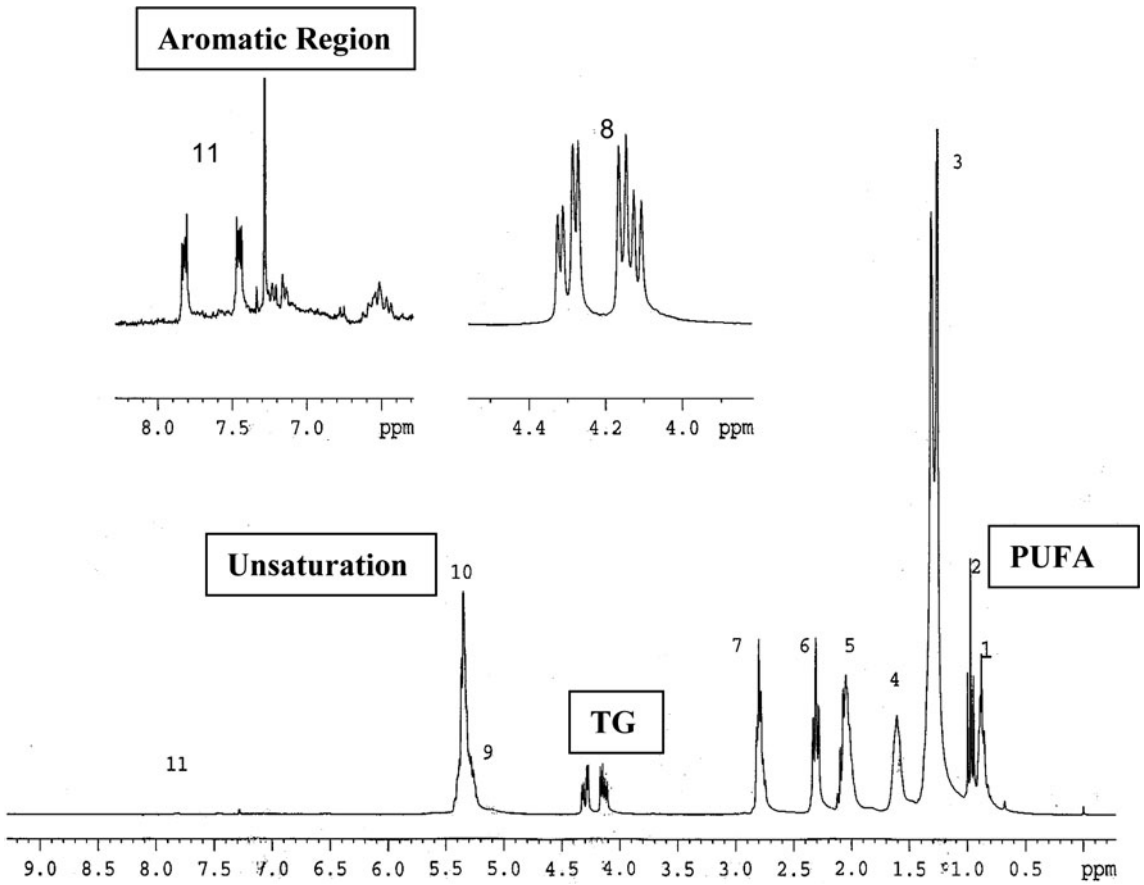

Fig. 1 ^{1}H-NMR spectrum of a representative oil extracted from seeds

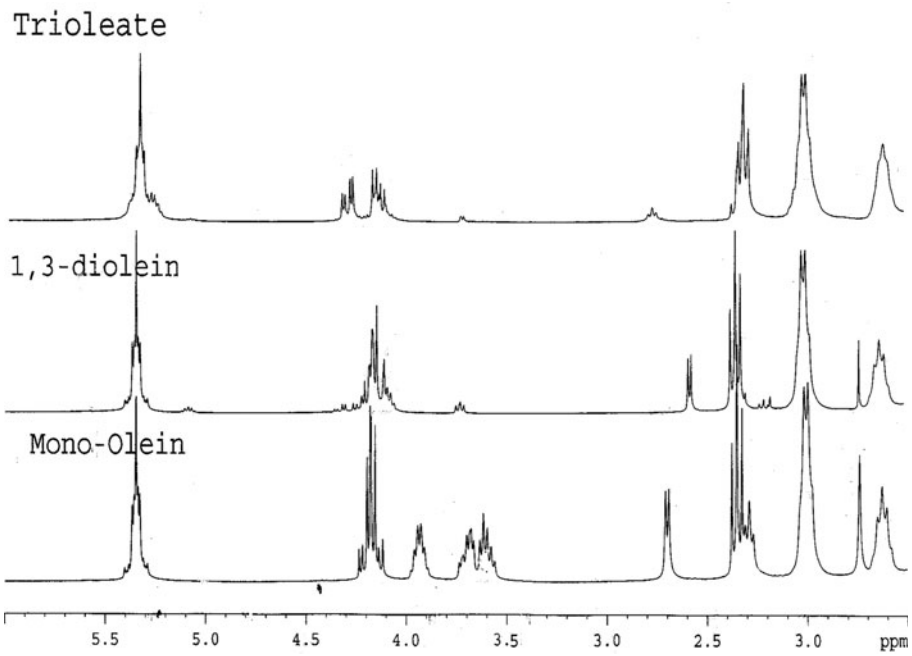

Fig. 2 ^{1}H-NMR spectra of glyceride moieties: mono-, di- and triglycerides

and fatty materials, it was found many resonances in aromatic region (6.8–8.8) ppm indicating the presence of substituted phenol type compound in the samples oil 10 and 16. The number of signals in region 4.6–6.8 ppm indicates that other than unsaturated fatty acid of glycerides, more complex nature of unsaturared compounds are

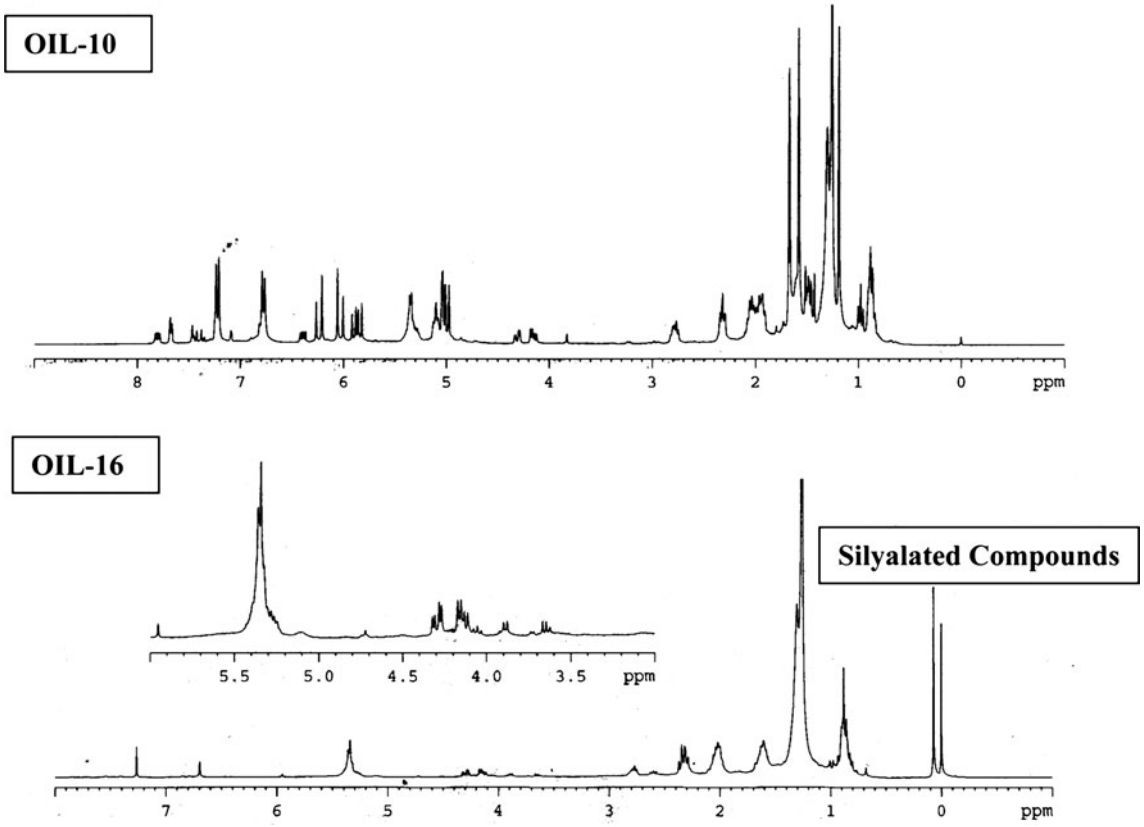

Fig. 3 ^{1}H NMR spectra of Oil-10 and Oil-16

present (Fig. 3). In samples oil 16, 17 and 20 presence of resonances at 0.07–0.09 ppm along with tetramethylsilane (TMS) resonance indicates the presence of silyalated compounds (Figs. 3, 6). These silylated compounds are part of extracted oils.

^{13}C-NMR Spectral Features of Extracted Oils

The ^{13}C-NMR technique is particularly useful in distinguishing between mono-, di- and tri-glycerides, a strong discriminating power to detect the specific components and free fatty acids. The ^{13}C-NMR spectrum of a representative oil extracted from seeds is shown in Fig. 4. The characteristic signal of each functional group like carbon–carbon double bonds, ester, acid and long alkyl chain were assigned. The C-1 and C-3 terminal carbons of the triglyceride moiety resonate at the same chemical shift, i.e. 61.99 ppm, whereas C-2 carbons resonate at 68.80 ppm (Fig. 5). In the 1,3 diglyceride moiety both C-1 and C-3 carbons resonate at the same chemical shift value, i.e. at 65.36 ppm and C-2 at 68.59 ppm, which are different from the chemical shift values obtained with the triglyceride moiety. The monoglyceride shows signals at 63.89, 65.37 and 70.48 ppm for C-1, C-2 and C-3 carbons, respectively (Fig. 5).

The ^{13}C-NMR spectral analysis provides a clear distinction between the signals of an acid and an ester moiety. The carbonyl signal of free fatty acids appears between 177.00–182.00 ppm, whereas ester signals in triglycerides resonate at 172.00–174.00 ppm. The polyunsaturated fatty ester (PUFE) shows a ^{13}C-chemical shift at 172.67 ppm in contrast to other fatty esters at 173.07 ppm. The specific signals at 142.73 and 143.72 ppm along with signals from 120.34 to 133.76 ppm in the aromatic region indicate the presence of hydroxyl fatty acids, such as 12-hydroxyoctadecanoic acid, 2-hydroxy-6-pentadecyl benzoic acid etc. The characteristic signals at 3.27 ppm (–OCH_2–), 6.62–7.20 ppm (aromatic-CH) in ^{1}H-NMR and 62.47 ppm (–OCH_2–), 135.71 ppm (–C–COOH), 161.86 ppm (–C–OH), 173.93 ppm (–COOH) in ^{13}C-NMR spectra (Fig. 6) clearly reveal the presence of aromatic hydroxyl acid. These two acids were found to be present in the sample oils 10, 13 and 16 (Table 3).

Quantitative Analysis

The quantitative analysis of various components present was carried out using integral intensities obtained under each signal from ^{1}H- and ^{13}C-NMR spectra.

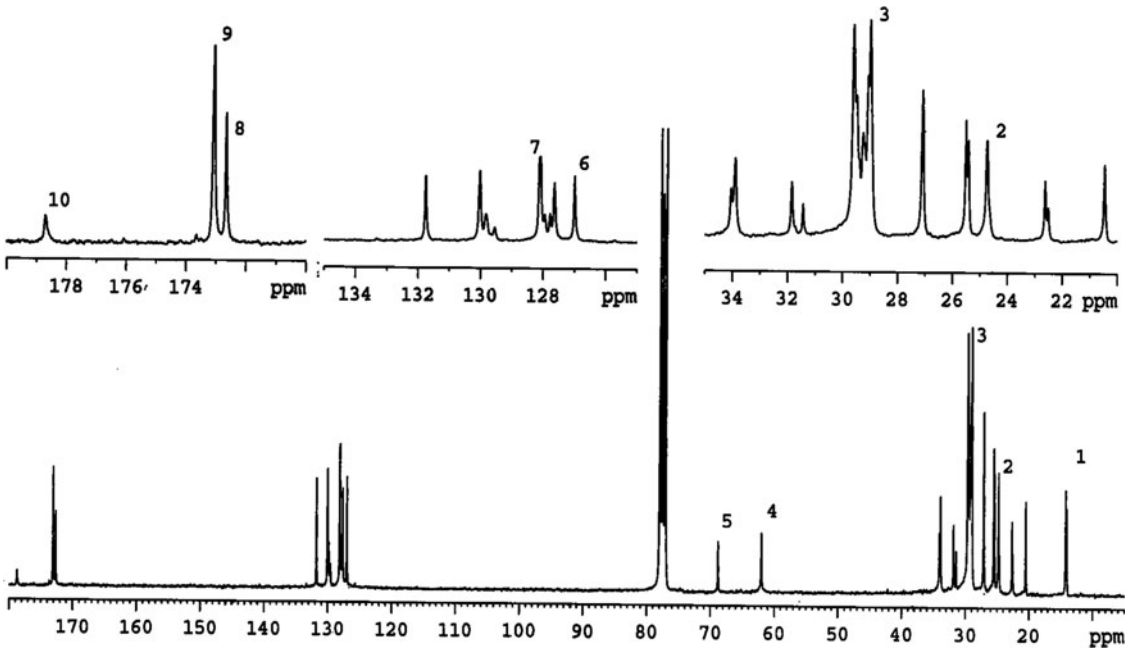

Fig. 4 ^{13}C-NMR spectrum of a representative oil extracted from seeds

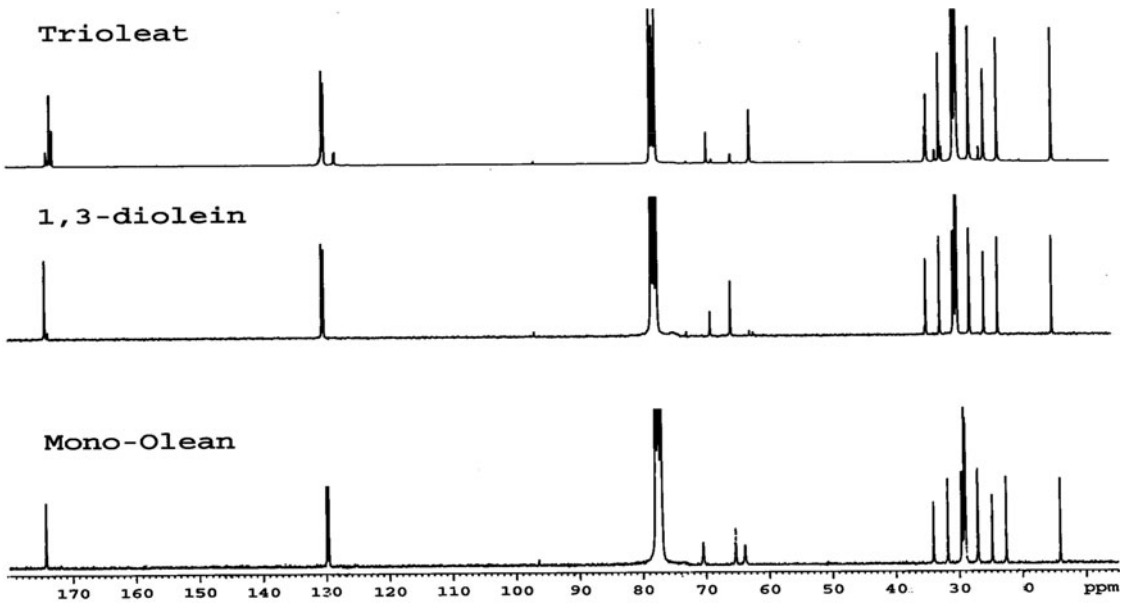

Fig. 5 ^{13}C-NMR spectrum of glyceride moieties: mono-, di- and triglycerides

Estimation of Triglycerides

The ^{1}H-NMR spectral region of 4.10–4.40 ppm (symmetrical pattern) corresponding to $-OCH_2$ protons is taken for the estimation of the triglyceride (TG) content in the oils extracted from seeds.

The percentage fat content was estimated by Eq. 1

$$\% \text{ TG Content } = (I_{OS}/I_{OR}) \times 100 \quad (1)$$

where I_{OS} and I_{OR} are the percentage integral intensities in the region 4.10–4.40 ppm of the sample and the reference taken as C-18 triglyceride.

Estimation of I_{OR} and I_{OS}

It was observed that the average alkyl chain lengths '*R*' of the fatty acid part of the glycerides of all the samples under

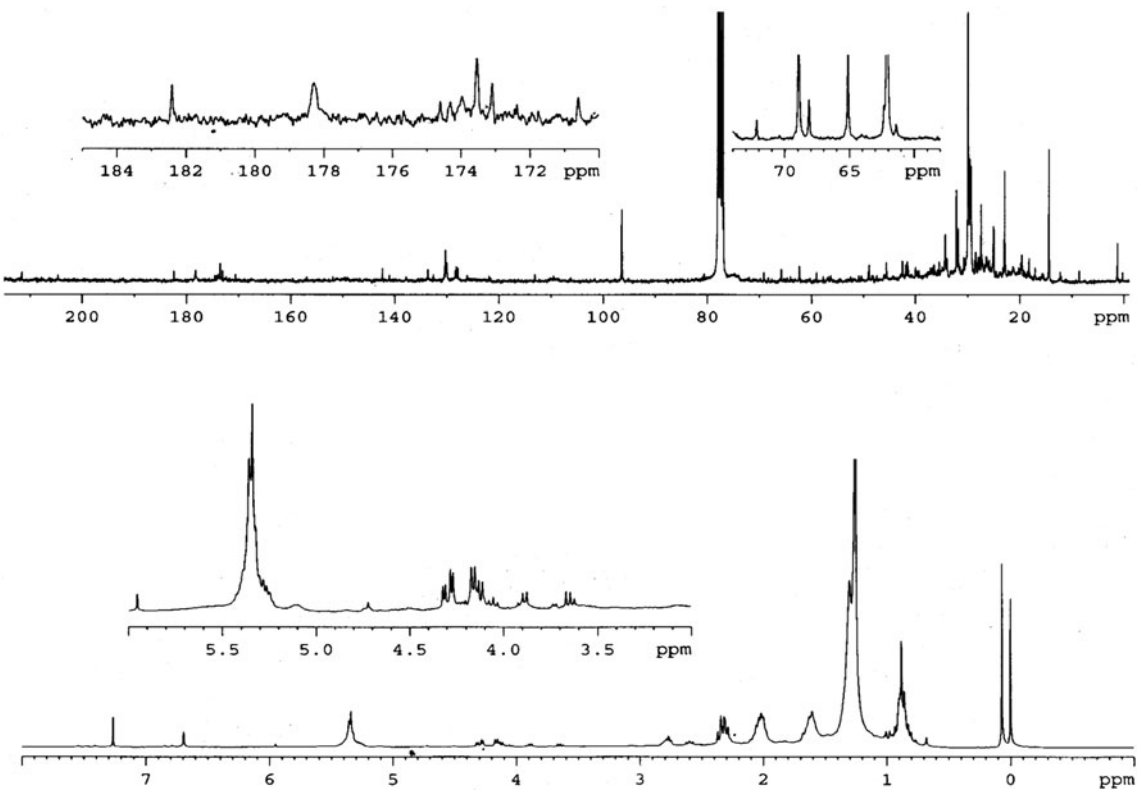

Fig. 6 ^{1}H- and ^{13}C-NMR spectra of Oil-20

Table 3 Quantitative analysis data from ^{1}H/^{13}C NMR spectra of oils extracted from various seeds

Sample name	% Wt.			
	TG	Free fatty acid	Other components	Iodine value
Oil-1	90.1	9.3 (PUFA)	0.6, Naphthalene aromatics	164.15
Oil-3	93.8	2.9	3.3, Naphthalen aromatics	140.44
Oil-6	nd	36.0	*p*-Substituted phenols, aromatic esters and others	–
Oil-7	88.0	8.0	4.0, Monoglycerides	55.60
Oil-9	93.2	1.7	6.8, Diglycerides, aromatic acids/esters	80.69
Oil-10	42.0	nd	58.0, *p*-Substituted phenols, aromatic hydroxy acid	47.3
Oil-11	83.6	10.0	6.4, Monoglycerides, naphthalene aromatics	99.81
Oil-13	21.5	7.0	71.5, Substituted catechol type and unsaturated hydroxy acid	96.64
Oil-14	93.5	2.0	4.5, Monoglycerides	82.26
Oil-15	94.3	5.7	nd	90.45
Oil-16	14.7	48.4	36.9, Substituted catechol types, silylated compound, unsaturated hydroxy acid, mono and diglycerides	74.69
Oil-18	41.4	36.2	22.4, Silylated compound, mono- and diglycerides	53.60
Oil-20	51.5	13.5	35.5, Silylated compound, mono- and diglycerides and other carbonyl type compounds	23.60

nd not detected

study and other commonly used oils for biodiesel purposes such as jatropha, rice bran, palm oil and coconut oil etc., are estimated to be between 17.0 and 18.5 from the ^{1}H-NMR spectral analysis. In order to make the method independent of the nature of the oil or fat, an average value of *R* as C-18 was taken for the estimation of I_{OR}. The fatty acid composition of various oils as determined by GC also estimates average values of '*R*' between 17.4 and 18.3. Thus, in order to make Eq. 1, universally applicable for all types of oils, the value of '*R*' taken as C-18 is considered quite

appropriate for the accurate estimation of the TG content. It is evident from the result of GC analysis (Table 2), that the C-18 component constitutes ~85–90% of the total fatty acid composition. Taking the average alkyl chain C-18 with one double bond as the reference for the triglyceride moiety, the total number of protons in the triglyceride reference appearing in the region 0.5–9.0 ppm are 104 and the protons of $-CH_2$ group of glyceryl moiety at 4.10–4.40 ppm is 4.

$$I_{OR} = (4/104) \times 100 = 3.85 \quad (2)$$

$$\%I_{OS} = (I_f / I_t) \times 100 \quad (3)$$

where I_f, I_t are integral values from the regions 4.10–4.40 and 9.0–0.5 ppm.

Estimation of Free Fatty Acid

If free acid is present, the ^{1}H-NMR signals due to $-CH_2CO$ of acid part will merge in the $-CH_2CO$ of fat at 2.22–2.38 ppm as both have similar chemical shift values. The contribution of the acid part was calculated by utilizing the integral intensity of the ester signals ($-CH_2O-$) between 4.10–4.40 ppm. The contribution due to $-CH_2-CO$ of ester group was subtracted from the resonance at 2.28–2.38 ppm (I_{AS}) in order to get contribution of $-CH_2-CO$ of the acid moiety (I'_{AS}) as given below.

$$I'_{AS} = I_{AS} - (I_{OS} \times 6/4) \quad (4)$$

where I_{AS} and I_{OS} are the percentage integral intensities of signals at 2.28–2.38 and 4.10–4.40 ppm.

Hence the percentage of free fatty acids (%FFA) was estimated by the following equation:

$$\%\text{FFA} = \left(I'_{AS} / I_{AR}\right) \times 100 \quad (5)$$

Estimation of I_{AR}

In order to make the method independent of the nature of oil or fat, an average value of R as C-18 was taken for the estimation of I_{AR}, similar to that of I_{OR}. Taking the average alkyl chain C-18 with one double bond as the reference for free fatty acids (oleic acid), the total number of protons on an average in the free fatty acid will be 34. There are two protons of $-CH_2-CO$ group of acid moiety at 2.28–2.38 ppm, so

$$I_{AR} = (2/34) \times 100 = 5.88 \quad (6)$$

Estimation of the Iodine Value

The iodine value (I_V) of extracted oil was estimated using ^{1}H NMR from the developed methodology [15] as follows:

$$I_V = K \times U_H \quad (7)$$

where K is the proportionality constant and found to be 14.75. U_H is the unsaturation content of the sample given as:

$$U_H = (I_{OL} / I_T) \times 100$$

where I_{OL}, I_T are the integral intensities of olefinic and total spectral regions from 5.1 to 5.6 and 0.5 to 9.0 ppm, respectively.

The ^{13}C-NMR technique has a strong discriminating power to detect the specific components and is particularly useful in distinguishing between mono-, di- and triglycerides and free fatty acids. The mono-, di-, triglycerides and free fatty acid present in the sample were also estimated by ^{13}C-NMR spectra, from the respective integral intensity of the corresponding signal discussed above. The total quantitative analysis was estimated by the Eqs. 8, 9, 10 and 11.

$$\%\ \text{Tri-glycerides (TG) (w/w)} = A/G \times 100 \quad (8)$$

$$\%\ \text{FFAs (w/w)} = B/G \times 100 \quad (9)$$

$$\%\ \text{Mono-glycerides (MG) (w/w)} = C/G \times 100 \quad (10)$$

$$\%\ \text{Di-glycerides (DG) (w/w)} = D/G \times 100 \quad (11)$$

where A, B, C, D and G are the group molecular weights of triglycerides, FFA, mono-, diglyceride and total group molecular weight, respectively, taking the average alkyl chain length as C-18.

The total group molecular weight $(G) = A + B + C + D$

$$A = \text{Ia}/1 \times M_f, \quad B = \text{Ib}/3 \times M_a, \quad C = \text{Ic}/1 \times M_m, \quad D = \text{Id}/1 \times M_{fd}$$

where Ia, Ib, Ic and Id are integral intensities in the region 172–174 ppm, 177–182 ppm, 70.40–70.60 ppm and 68.40–68.60 ppm corresponding to triglycerides, FFA, mono- and diglyceride, respectively, which is divided by the number of carbons contributing to that signal to get M_f, M_a, M_m and M_d, that are average molecular weights of triglycerides, FFA, mono- and diglyceride, respectively, taking the average alkyl chain length as C-18.

The complete quantitative analysis data for all the seeds under study are tabulated in Table 3.

Validation of the NMR Data

The developed NMR methodology was validated by the two following methods:

Blends Analysis

Laboratory prepared blends using standard triglycerides, monoglycerides, oleic acid and dibutyl-*p*-cresol were analyzed by the developed NMR methodology and the results are given in Table 4.

Table 4 Validation of data (%TG by Blend vs. developed NMR method)

Blends	Tri-gly:mono-gly: FFAs:*p*-cresol (blends)	Triglyceride (developed method)
Bl-1	100.0:0:0:0.0:0.0	100.0
B-2	90.0:5.0:5.0:0.0	91.7
Bl-3	80.0:5.0:10.0:5.0	76.1
Bl-4	40.0:20.0:20.0:20.0	39.9
Bl-5	30.0:25.0:25.0:20.0	31.3
Bl-6	20.0:10.0:40.0:30.0	20.6

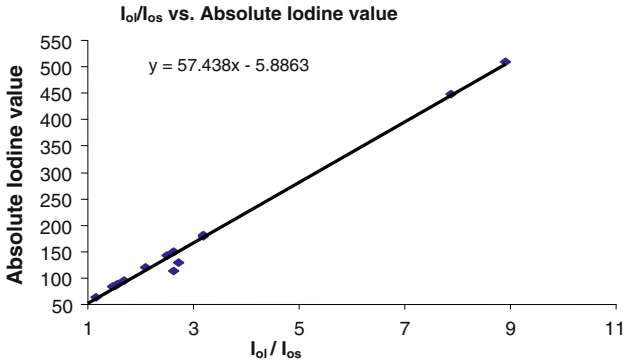

Fig. 7 Unsaturation H/Ester H versus Absolute Iodine Value of extracted oils

Iodine Value Estimation

The result of determination of glycerides content by the above-mentioned method was validated by the determination of the absolute iodine value (I_{AV}) of the sample. It was shown that the ratio of unsaturated proton signals at 5.2–5.4 ppm (I_{OL}) and the ester signals (–CH_2O–) at 4.10–4.40 ppm (I_{OS}) are directly proportion to their iodine value [15]. All samples under study were taken for the calibration curve in Fig. 7, showing a direct relationship between (I_{AV}) and the ratio of (I_{OL}/I_{OS}). The fat content was estimated by the following equation:

$$\% \text{ TG content} = I_V/I_{AV} \times 100 \quad (12)$$

The oil content of the sample was estimated as above, shows excellent correlation between both values in Table 5.

Repeatability of the Oil Extraction Procedure

In order to ensure that each extracted oil is a representative one and not an anomaly, the oil from the respective seeds were completely extracted using the above mentioned extraction procedure. Repeatability of oil content data from different seeds was also checked by experiments carried out using same method on identical test material. The statistical analysis was done on the basis of experimental results obtained. The calculated average yields of two seed samples were found to be 1.08 and 34.0%, respectively. The standard deviations calculated for different average values for the oil yields were found to be 0.11 and 1.0%.

Assessment/Repeatability of the NMR Method

Determination of TG, FFA by the NMR technique was subjected to ANOVA (Analysis of Variance) analysis, which is a statistical tool that separates and estimate possible causes of variation and hence systematic and random errors.

ANOVA analysis was carried out in order to check any variation in the NMR data separately due to (1) the operator, (2) the recording time during the day, (3) the instrumental operating parameters etc. The parameters taken into consideration, while recording 1H- and 13C-NMR spectra were:

1. Sample preparation/sample concentration.
2. Sensitivity/Resolution of spectrometer.

Table 5 Validation of data (%TG from absolute iodine value by ^{1}H NMR)

Sample	Unsat. H/ester H = I_{OL}/I_{OS}	Iodine value by NMR (Eq. 7)	%TG from NMR	Absolute Iodine Value I_{AV} (NMR)	% TG from iodine value
Oil-1	3.19	164.2	90.1	182.24	90.1
Oil-3	2.63	140.4	93.8	149.68	93.8
Oil-7	1.16	55.6	88	63.18	88
Oil-9	1.53	80.7	93.2	86.59	93.2
Oil-10	2.63	47.3	42	112.62	42
Oil-11	2.1	99.8	83.6	119.38	83.6
Oil-13	7.88	96.6	21.5	449.30	21.5
Oil-14	1.55	82.3	93.5	88.02	93.5
Oil-15	1.69	90.5	94.3	95.97	94.3
Oil-16	8.9	74.7	14.7	508.16	14.7
Oil-17	2.72	53.6	41.4	129.47	41.4
Oil-18	0.85	23.6	51.5	45.83	51.5

Table 6 ANOVA analysis of NMR data

Day	Sample oil-9	% TG by NMR		
		Morning	Noon	Evening
1.	A	93.2	90.2	93.0
2.	A	90.4	91.0	92.5
3.	A	91.7	93.5	91.8
4.	A	92.8	92.0	94.0
5.	A	93.0	92.4	90.6
1.	B	91.2	92.8	94.1
2.	B	93.5	90.7	92.7
3.	B	94.1	93.0	90.8
4.	B	90.6	93.6	93.2
5.	B	92.0	91.5	90.6

A, *B* Different operators

3. Shimming of instrument for homogeneity of field.
4. Base line correction.

The following criteria were used for judging the acceptability of results (95% probability). The repeatability of the NMR method was estimated on two numbers of samples having different average values of the total FFA, and TG content. A single operator recorded each of the samples at least three times on four different days of a week under similar experimental conditions on the same NMR equipment. Each time, a fresh sample solution was prepared in $CDCl_3$ in a new NMR tube as per standard protocol. The total FFA and TG contents were estimated from the recorded NMR spectra by using the developed equations. The value of the standard deviation (at the 95% confidence level) and repeatability by the NMR method for higher values of TG ($\sim$51.0–99.0) was found to be 1.3 and 4.7, respectively, and that for lower side (say $\sim$20.0–50.0) is 1.2 and 4.4%. Similarly, the value of the standard deviation (at 95% confidence level) and repeatability for higher values of FFA ($\sim$51.0–90.0) was found to be 1.3 and 4.8, respectively, and that for lower side (say $\sim$1.0.0–50.0) is 0.5 and 1.7%.

The data set shown in Table 6 indicated that there was no significant variation in the NMR data with respect to the operator and the time of analysis. Day-to-day variations, as well as variations due to operators, which effect the precision of the method were also found to be insignificant [F-calculated = 0.811, F-critical = 0.403, p ($T \leq t$) two-tail = 0.725, t critical two-tail = 2.145].

Conclusion

This paper presents NMR spectroscopic based measurements for rapid identification and quantitative determination of the organic components (glycerides, free fatty acids, phenolic components and other chemical constituents). NMR data were used to estimate iodine value of the extracted oils. The data presented are for a variety of twenty different seeds belonging to the Indian territory are very useful for selection and screening of oil extract from various seeds. The present methodology gives simultaneous, noninvasive and nondestructive analysis of a plethora of components present in extracted oils and multicomponent fatty material. The proposed ^{1}H-NMR based method for estimation of triglycerides and free fatty acids is less time consuming compared to the alternative analytical methods.

Acknowledgments The authors wish to acknowledge the Management of IOC, R&D Faridabad for allowing them to publish the present work.

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