Quantitative Determination of Castor Oil in Edible and Heat-Abused Oils by ^{13}C Nuclear Magnetic Resonance Spectroscopy

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Application of ^{13}C nuclear magnetic resonance (NMR) spectroscopy for detection of castor oil (CO) in various edible oils, such as coconut oil, palm oil, groundnut oil and mustard oil, is described. Characteristic signals observed at δ 132.4, δ 125.6, δ 71.3, δ 36.8 and δ 35.4 ppm, due to C10, C9, C12, C13 and C11 carbons of ricinoleic acid (RA) in CO, were selected for distinguishing it from edible oils. Quantitative ^{13}C NMR spectra of oils were recorded in $CDCl_3$ with a gated decoupling technique. The minimum detection limits for qualitative and quantitative analyses were 2.0 and 3.0%, respectively. The proposed method is simple, nondestructive and requires no sample pretreatment. Its application to heat-abused oils has also been demonstrated successfully without any of the interferences observed in most other methods.

KEY WORDS: Adulteration of edible oils, ^{13}C NMR spectroscopy, castor oil, gated decoupling technique, heat-abused oils, quantitative determination, ricinoleic acid.

Triglyceride oils and fats play an important role in the human diet. It is a usual practice to adulterate higher-priced oils with cheaper ones. Castor oil (*Ricinus cummunis* L., Euphorbiaceae) (CO), a nonedible oil, contains a major amount of 12-hydroxy 9-*Z*-octadecenoic acid or ricinoleic acid (RA) (80–90%), is cheaply priced and is used as an adulterant in edible oils (1,2). Therefore, detection and quantitative estimation of CO in edible oils is important in order to differentiate inadvertent contamination from deliberate adulteration, as well as for detecting substandard quality materials arising from poor preharvest technology, improper storage, handling and processing (3,4).

Methods based on physical, chemical, spectroscopic and chromatographic techniques have been reported in the literature for detection of CO in edible oils (2–10). However, physical, chemical (2) and spectroscopic (5) methods are nonspecific, empirical and insensitive. Column chromatographic methods are tedious and time-consuming (6). A thin-layer chromatographic method developed by Mani and Lakshminarayana (4), where the separation of RA or triricinolein is the basis to detect CO adulteration with a detection limit of 1.0%, has been incorporated in the Indian Standard specifications (7). This method is qualitative and not applicable to oxidized or heat-abused oils when peroxy and other oxidized fatty acids are present (8,9).

Taneja *et al.* (10) reported a high-performance liquid chromatographic method with a triglyceride column, where the triricinolein peak in CO and its absence in other oils is used to identify CO. However, this method is unsatisfactory for oils that contain free fatty acids (FFA) above 0.2% and for heat-abused oils, because FFA and degradation products coelute with the triricinolein of CO. Gas–liquid chromatography can be used successfully when the detection is based on the separation, identification and estimation of component fatty acids present in an adulterated oil (11). However, the method is specific, requires sample pretreatment and is tedious for heated oils (12).

We studied the possible application of ^{13}C nuclear magnetic resonance (NMR) spectroscopy for detection and determination of CO in various edible and heat-abused oils.

EXPERIMENTAL PROCEDURES

Reagents and samples. d-Chloroform (99.6 atom % D) was purchased from Aldrich Chemical Co. (Milwaukee, WI). Coconut oil (CCO), palm oil (PO), groundnut oil (GNO), mustard oil (MO) and CO of refined grade were purchased from local industries (around Hyderabad, India). Adulterated samples of GNO were obtained from a local supermarket.

Heated oil samples. A mixture of GNO with 5% CO (200 g) was heated in a 250-mL glass beaker under laboratory conditions in an air oven at 180 ± 2°C for 64 h. The surface area-to-volume ratio of the beaker was 1:7.25 (13). Deep-fried GNO after 4 h of frying (chillie bajjis), was collected directly from the frying pan of a roadside restaurant.

Apparatus and procedures. The gated decoupled ^{13}C NMR spectra were recorded on a JEOL JNM FX 90 Q Fourier-transform NMR spectrometer (JEOL Ltd., Tokyo, Japan) operating at 22.50 MHz with the following analytical parameters: spectral width, 5000 Hz (222.2 ppm); acquisition time, 0.81 s; pulse angle, 90°; pulse width, 28 μs; pulse delay time (PDT), 10 s; and number of scans accumulated, 500. The concentration of the oil solution in $CDCl_3$ was 1:3 (vol/vol), and the outer diameter of the NMR tube was 10 mm. Spin-lattice relaxation times for selected carbons of GNO and CO were determined by the inversion recovery method (14,15).

Signal assignment of ^{13}C NMR spectra and calculation of mole percent of RA chains. Peak assignments of ^{13}C NMR spectra for individual fatty acids, their methyl and glyceryl esters have already been reported (16–28). Typical gated decoupled ^{13}C NMR spectra of GNO, a representative edible oil, and CO are shown in Figure 1. Chemical shift values and assignment of the peaks, as numbered in their respective spectra, based on the available literature for pure fatty acid esters, are given in Table 1. Figure 1 and Table 1 show that CO has five characteristic signals, when compared to GNO, at chemical shifts δ 132.4, δ 125.6, δ 71.3, δ 36.8 and δ 35.4 ppm, which are assigned to C10, C9, C12, C13 and C11 carbons of RA, respectively (28). CO and GNO possess almost identical chemical shifts that correspond to various carbons of identical molecular structures of the fatty acids present. Based on these data, the presence or absence of CO in edible oils can easily be estimated.

Quantitative determinations of percent RA in CO and edible oil–CO mixtures. For quantitative analyses, the ^{13}C NMR spectra were recorded by suppressing nuclear Overhauser enhancement by gated decoupling, in which the PDT is suggested to be at least five times the longest T_1, [where T_1 is the spin-lattice relaxation time of those carbons considered for calculation purposes (22,24,27)].

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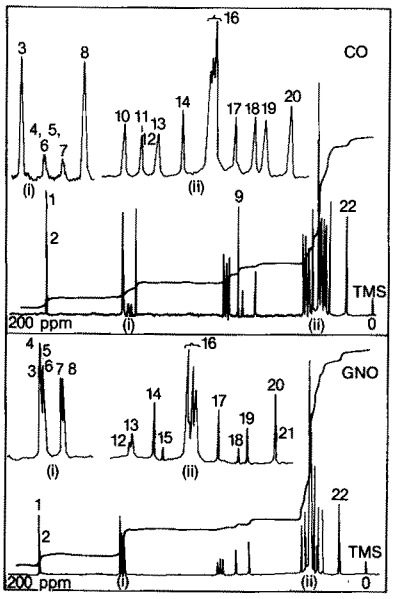

FIG. 1. Gated decoupled ^{13}C nuclear magnetic resonance spectra of groundnut oil (GNO) and castor oil (CO); (i) eight times X-axis expansion of unsaturated carbon region; (ii) eight times X-axis expansion of methylene carbon region. TMS, tetramethylsilane.

Table 2 gives the T_1 data for selected carbons of GNO and CO, which serve as a guide in setting the PDT for spectral accumulation. It is evident from Table 2 that the olefinic carbons of RA in CO have relatively shorter T_1 values than oleic and linoleic acids. It may be attributed to possible hydrogen bonding between RA chains of the same or different triricinolein molecules, which is responsible for decreasing the mobility of the carbon chain attached to unsaturated carbons of the RA chains (29).

The calculation of mole percent RA chains follows from the average integration of its five characteristic signals and of the peak at δ 24.8 ppm, which is assigned for the C3 carbon of all fatty acids in CO and edible oils:

$$\text{Total number of fatty acid chains (a)} = \frac{\alpha \text{ integration of the peak at } \delta \text{ 24.8 ppm}}{1} \quad [1]$$

$$\text{Number of RA chains (b)} = \frac{\alpha \text{ average integration of five characteristic peaks}}{(\delta\ 132.4 + \delta\ 125.6 + \delta\ 71.3 + \delta\ 36.8 + \delta\ 35.4)/5} \quad [2]$$

$$\text{Mole percent of RA chains} = (100\ \text{b})/\text{a} \quad [3]$$

At low concentrations of CO (below 6%) in edible oil mixtures, the step-height integrations of the RA peaks (peak areas) are too small to measure for a reliable integral value

TABLE 1

^{13}C Chemical Shifts (δ) and Their Assignments for Various Carbons of Groundnut (GNO) and Castor (CO) Oils[a]

Chemical shift region	Signal number in the respective spectra	GNO		CO	
		δ[b]	Assignment[c]	δ	Assignment[c]
Carbonyl carbons	1	172.85	[d]	173.00	[d]
	2	172.44	[e]	172.62	[e]
Unsaturated carbons	3	130.33	C13 L	132.36	C10 RA
	4	129.92	C10 O	130.03	C13 L
	5	129.81	C9 L	129.91	C9 L
	6	129.65	C9 O	129.86	C10 O
	7	128.13	C10 L	128.02	C9 O, C12 L
	8	127.97	C12 L	125.64	C9 RA
Carbon attached to hydroxyl group	9	—	—	71.33	C12 RA
Methylene chain carbons	10	—	—	36.84	C13 RA
	11	—	—	35.43	C11 RA
	12	34.13	C2 S, O	34.07	C2 S, O, RA
	13	34.02	C2 L	33.97	C2 L
	14	32.07	C16 S, O	31.91	C16 of all
	15	31.64	C16L	—	—
	16	29.08–29.20	[f]	29.60–29.00	[f]
	17	27.30	C8 O, L C11 O C14 L	27.36	C8 O, L C11 O C14 L
	18	25.73	C11 L	25.73	C11 L C14 RA
	19	24.97	C3 of all	24.86	C3 of all
	20	22.81	C17 S, O	22.64	C17 S, O, L, RA
	21	22.65	C17 L	—	—
Terminal methyl carbon	22	13.97	C18 of all	14.03	C18 of all

[a]Signals for glycerol backbone carbons appeared at δ 68.9 ppm for α, α' carbons and δ 62.1 ppm for β-carbon.
[b]Chemical shifts are referred to as relative to internal tetramethylsilane (0.00 ppm).
[c]Abbreviations: S, saturates; O, oleate; L, linoleate; and RA, ricinoleate.
[d]Carbonyl carbon of all fatty acid chains attached to α, α' carbons of the glycerol backbone.
[e]Carbonyl carbon of all fatty acid chains to β-carbon of the glycerol backbone.
[f]Saturated carbons which are isolated from double-bond carbons, carbonyl carbons, hydroxy-attached carbon and terminal methyl carbon.

TABLE 2

Spin-Lattice Relaxation Time (T_1) in Seconds for Selected Carbons of GNO and CO[a]

Type of the carbon	T_1	
	GNO	CO
C9 O	1.40	1.60
C10 O	1.45	1.75
C9 L	1.80[b]	2.10[b]
C10 L	1.80[b]	2.10[b]
C12 L	2.90	3.10
C13 L	3.00	3.12
C9 RA[c]	—	0.80
C10 RA[c]	—	0.72
C12 RA[c]	—	1.03
C3 of all[c]	0.60	0.71
C11 L	2.30	2.50
C8, C11 O		
C8, C14 L, C8 RA[d]	1.60	1.50
C11 RA[c]	—	0.43
C13 RA[c]	—	0.56

[a]1:2 (vol/vol) solutions in $CDCl_3$ at 23°C. Abbreviations as in Table 1.
[b]Incompletely resolved pair.
[c]Analytical signals considered for quantitative estimation of CO.
[d]Peaks overlap.

when compared to the total fatty acid peak (δ 24.8 ppm). Hence, the corresponding digital peak intensities (peak heights) were used in the above calculations (22).

RESULTS AND DISCUSSION

To establish the detection limit and quantitative integrity of the estimation of CO in edible oils, several admixtures with CCO, PO, GNO and MO were prepared in different proportions, ranging from 1.0 to 20.0% on wt/wt basis. Table 3 gives the mole percent of RA chains obtained from the spectra, including that of pure CO. It is evident from the data that CO is detected quantitatively down to the level of 3.0%, whereas 2.0% is the minimum quantity that can be detected qualitatively in edible oils (Fig. 2).

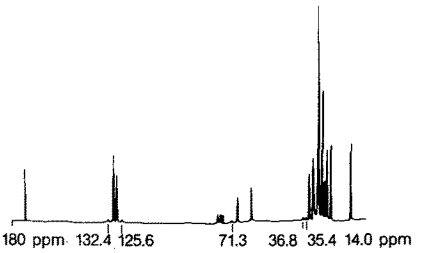

FIG. 2. Gated decoupled ^{13}C nuclear magnetic resonance spectrum for admixture of groundnut oil (98%) and castor oil (2%).

Furthermore, Figure 3 gives individual calibration graphs generated from the weight percent of CO added to various edible oils and the mole percent of RA chains calculated from ^{13}C NMR spectra. Figure 3 shows different slopes for calibration graphs of CCO-CO, PO-CO, GNO-CO and MO-CO mixtures. This is attributed to the marked differences in molecular weight of those edible oils, *i.e.*, CCO, 712; PO, 886; GNO, 903; MO, 1019; and CO, 965 (30).

To check the validity of the ^{13}C NMR spectroscopic method, a set of GNO samples (A-H), suspected as having been adulterated with CO, were analyzed. Turbidity tests showed that samples E, F and G gave a pearly-white precipitate, whereas the others showed only slight turbidity. Due to the various limitations of the turbidity test (3), the presence or absence of CO in samples A-D and H could not be unequivocally established.

The developed ^{13}C NMR methodology has been adopted for confirming the presence of CO in the above-mentioned GNO samples. ^{13}C NMR spectra of GNO samples E, F and G have indicated the presence of CO, whereas it is absent in the spectra of other samples. The minimum detection limit and quantitative results for the

TABLE 3

Mole % of Ricinoleic Acid (RA) Chains Obtained by ^{13}C Nuclear Magnetic Resonance Spectroscopy for Various Edible Oil/CO Admixtures

Trainee mixtures of edible oil/CO (w/w)[a]		Mole % of RA chains	Trainee mixtures of edible oil/CO (w/w)[a]		Mole % of RA chains
Edible (%)	CO (%)		Edible (%)	CO (%)	
	100	85.60 ± 0.40	GNO		
CCO			98.00	2.00	DQ[b]
98.80	1.20	n.d.[c]	96.80	3.20	2.95 ± 0.18
96.00	4.00	3.00 ± 0.15	94.63	5.37	4.60 ± 0.12
93.80	6.20	5.10 ± 0.10	93.05	6.95	5.68 ± 0.10
89.07	10.93	8.63 ± 0.12	90.40	9.60	7.60 ± 0.10
86.06	13.94	11.00 ± 0.10	83.00	17.00	14.00 ± 0.08
81.96	18.09	13.90 ± 0.10	MO		
PO			97.00	3.00	2.40 ± 0.10
96.10	3.90	3.10 ± 0.16	95.40	4.60	4.40 ± 0.10
94.80	5.20	4.20 ± 0.10	92.30	7.70	6.60 ± 0.10
88.73	11.27	10.15 ± 0.10	90.00	10.00	8.71 ± 0.10
86.15	13.85	11.40 ± 0.15	84.80	15.20	12.70 ± 0.10
81.17	18.83	15.05 ± 0.10	81.00	19.00	17.40 ± 0.16

[a]CCO, coconut oil; PO, palm oil. GNO, groundnut oil; MO, mustard oil; CO, castor oil.
[b]DQ, detected qualitatively (see Fig. 2).
[c]n.d., Not detected.

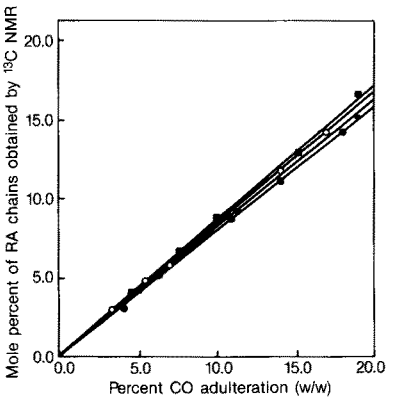

FIG. 3. Calibration graphs for castor oil (CO) adulteration in various edible oils. ●—●, Coconut oil; ▼—▼, palm oil; ○—○, groundnut oil; and ■—■, mustard oil. RA, ricinoleic acid; NMR, nuclear magnetic resonance.

TABLE 4

Results of Turbidity Test and Castor Oil (CO) Content in Adulterated and Heated Groundnut Oil (GNO)

Sample code	Turbidity test	Peroxide value	% RA by ^{13}C NMR[a]	% CO[b]
A	Positive	23.9	nil	nil
B	Positive	17.6	nil	nil
C	Positive	29.1	nil	nil
D	Positive	18.0	nil	nil
E	Positive	15.0	2.6	3.1
F	Positive	11.1	8.7	10.2
G	Positive	28.0	1.8	2.1
H	Positive	16.0	nil	nil
Heated GNO	Positive	12.5	4.1	4.8
Deep-fried oil	Positive	74.0	nil	nil

[a]RA, ricinoleic acid; NMR, nuclear magnetic resonance.
[b]Obtained from Figure 3.

presence of CO in samples E, F and G, obtained from the calibration graph, are presented in Figure 3 and Table 4, respectively. The positive response of the turbidity test for samples A–D and H is attributed to the presence of peroxy compounds, as indicated by high peroxide values (Table 4). Further, a synthetic sample of heated GNO with 5% CO and a sample collected from a frying pan of a roadside restaurant have been analyzed, and the results are recorded in Table 4. The ^{13}C NMR data (Table 4) show that CO is absent in the deep-fried oil, although the conventional turbidity test showed a positive response due to oxygenated compounds (3,8–10).

Though the minimum detection limit (2%) of CO in edible oils by the proposed ^{13}C NMR method is relatively low when compared to the existing methods, it does not rely on preliminary physical or chemical treatment of the sample. Further, FFA and oxygenated compounds in crude and in heat-abused oils do not interfere with CO because they are characterized by their own chemical environments, *i.e.*, C=O of FFA at ≈ δ 180.5 ppm and C-O-O- of peroxides at ≈ δ 90.5 ppm, respectively (20,31).

ACKNOWLEDGMENT

This paper is IICT Communication No. 3218.

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[Received May 13, 1993; accepted September 7, 1993]