Determination of Unsaturated Fatty Acid Composition by High-Resolution Nuclear Magnetic Resonance Spectroscopy

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ABSTRACT: High-resolution nuclear magnetic resonance (NMR) spectroscopy provides useful data for analyzing fatty acid compositions of edible vegetable oils. Quantitation of each fatty acid was carried out by evaluation of particular peaks. According to the ^{1}H NMR method, terminal methyl protons, divinyl protons, and allyl protons are useful to calculate linolenic acid, linoleic acid, and oleic acid, respectively. The ω-2 carbon, divinyl carbon, and allylic carbons were used for calculation of these acids by the ^{13}C NMR method. Compositional results obtained by NMR coincided well with those of the conventional gas chromatography (GC) method. Results from ^{13}C NMR were in better agreement with those from GC than were the results obtained by the ^{1}H NMR method.
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KEY WORDS: Fatty acid composition, ^{1}H NMR spectroscopy, ^{13}C NMR spectroscopy.

Fatty acid composition has commonly been determined by gas chromatography (GC). In most commonly used methods, lipids and/or oils must be converted into methyl esters before analysis by GC (1,2). Total time required to obtain the GC data is about 30 min.

Nuclear magnetic resonance (NMR) has become one of the most promising methods to determine organic structures. There have been several reports pertaining to the analysis of oils by NMR (3–10). Shoolery (3) showed a method for determining the percentages of the fatty acids with zero, one, two, or three double bonds in animal and vegetable oils by ^{13}C NMR (3). Olefinic carbon, ω-3 methylene carbon, allylic carbons, and β-methylene carbon from carbonyl carbon were used to calculate the $C_{18:3}$, $C_{18:2}$, $C_{18:1}$, and $C_{18:0}$ fatty acids, respectively. Sacchi *et al.* (4) presented the quantitation of n-3 polyunsaturated fatty acids in fish lipids by ^{1}H NMR. However, other fatty acids were not determined.

The intent of our study is selection of peaks for quantitative analysis of the unsaturated fatty acid composition by NMR.

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EXPERIMENTAL PROCEDURES

Materials. Extra pure, reagent-grade trilinolein was purchased from Nacalai Tesque Inc. (Kyoto, Japan). Palm, olive, rapeseed, soybean, safflower, and corn oils were products of Ajinomoto Co., Inc. (Tokyo, Japan). Other corn and safflower oils were obtained from a local market and used without further purification.

NMR spectroscopy. NMR spectroscopy was performed on a Gemini 2000 at 300 MHz (Varian Instrument Co., Inc., Palo Alto, CA). Approximately 120 mg of sample was dissolved in 0.6 mL of $CDCl_3$ that contained 0.03% trimethylsilane (Nacalai Tesque Inc.), and the resulting solution was placed in a 5-mm diameter NMR tube.

^{1}H NMR spectra were taken with 7.5-degree pulse angle, 2.5 s recovery delay, and 32 scan times. These conditions were developed for determining iodine value in our laboratory (11).

^{13}C NMR spectra were taken with complete proton decoupling. Measurement conditions were 90-degree pulse angle and 200 scan times. Recovery delay is discussed in the Results and Discussion section.

The proton T1 relaxation time was determined by using the standard inversion recovery T1 pulse sequence provided in the Varian NMR software GRIDE.

Capillary GC. Chromatograms were obtained with a GC-353 gas chromatograph (GL Sciences Inc., Tokyo, Japan), configured for capillary column operation with a split insert and flame-ionization detector. Ultra-high-purity helium was used as the carrier at a flow of 45.3 cm/s (6.0 mL/m). Samples (0.1 μL) were injected under the following conditions: column, BPX-70 (GL Sciences), 25 m × 0.53 mm i.d., 0.5-μm film thickness; injector temperature, 230°C; detector temperature, 230°C; column program, 85°C (2 min), 15°C/min to 160°C, 0.8°C/min to 170°C, 10°C/min to 230°C. Samples were methylated before injection to GC by Official Method Ce 2-66 of the American Oil Chemists' Society (12).

RESULTS AND DISCUSSION

Assignment of proton signals. The ^{1}H NMR spectrum of a rapeseed oil is shown in Figure 1. The spectrum signals are

annotated from a to h′. These groups are assigned as follows (4,9): a, olefinic protons and one methine proton in the glyceryl group; b, four methylene protons in the glyceryl group; c, divinyl methylene protons; d, six α-methylene protons adjacent to carbonyl carbon; e, allyl methylene protons; f, six β-methylene protons from carbonyl carbon; g, methylene protons on saturated carbon atoms; h, terminal methyl protons of saturated, monounsaturated, and n-6 polyunsaturated fatty acids; *h′*, terminal methyl protons of n-3 polyunsaturated fatty acids.

Calculation method for ^1H NMR spectroscopy. The fatty acid composition can be calculated from the NMR spectrum. An outline of the calculation method is as follows:

$$\text{Area per proton } U = \frac{1}{3}\left[\frac{b}{4} + \frac{d}{6} + \frac{(h+h')}{9}\right] \quad [1]$$

The basis of the area per proton should not be influenced by the fatty acid composition. Because the signal of β-methylene protons from the carbonyl group is included with a small amount of water, the average signal of the other three peaks was used. The following three equations can then be derived:

$$\text{n-3 Polyunsaturated fatty acid } V \text{ (generally linolenic acid in vegetable oils) content } [\%] = \frac{h'/3}{U/3} \times 100 \quad (U = \text{area per proton}) \quad [2]$$

A different chemical shift was observed for the methyl resonance of n-3 polyunsaturated fatty acid (4).

$$\text{n-6 Polyunsaturated fatty acid } W \text{ (linoleic acid) content } [\%] = \frac{c/2 - 2h'/3}{U/3} \times 100 \quad [3]$$

This was calculated by use of the divinyl protons area.

$$\text{Monounsaturated fatty acid (oleic acid) content } [\%] = \frac{\frac{1}{2}(a - b/4)}{U/3} \times 100 - (2V + 3W) \text{ from olefinic protons} \quad [4]$$

and

$$\frac{e/4}{U/3} \times 100 - (V + W) \text{ from allylic protons} \quad [5]$$

As shown in Table 1, allylic protons gave better agreement with results from the GC method than values calculated from olefinic protons.

Determination of fatty acid composition. Consistency between the compositional results of unsaturated fatty acids between NMR and GC was evaluated (Table 2). Most NMR results showed good agreement with those obtained by the GC method. Unfortunately, with safflower oils, errors between the two methods were more than 5%, and low levels of linolenic acid were not detected.

Assignment of carbon signals. The ^{13}C NMR spectrum of the methyl and methylene regions of a rapeseed oil is shown in Figure 2. The spectrum signals are annotated from A to H. These groups are assigned as follows (3,10): A, three α-carbonyl methylene carbons; B, ω-3 carbons of saturated and monounsaturated fatty acids; B′, ω-3 carbons of n-6 polyunsaturated fatty acids; C, saturated methylene carbons; D, *cis* allylic carbons; E, divinyl methylene carbons; F, the three β-carbonyl methylene carbons; G, ω-2 carbons of saturated, monounsaturated, and n-6 polyunsaturated acids; G′, ω-2 carbons of n-3 polyunsaturated fatty acid; H, the three terminal methyl carbons.

Calculation method for ^{13}C NMR spectroscopy. Fatty acid compositions can also be calculated from the ^{13}C NMR spectrum. An outline of the calculation method is as follows:

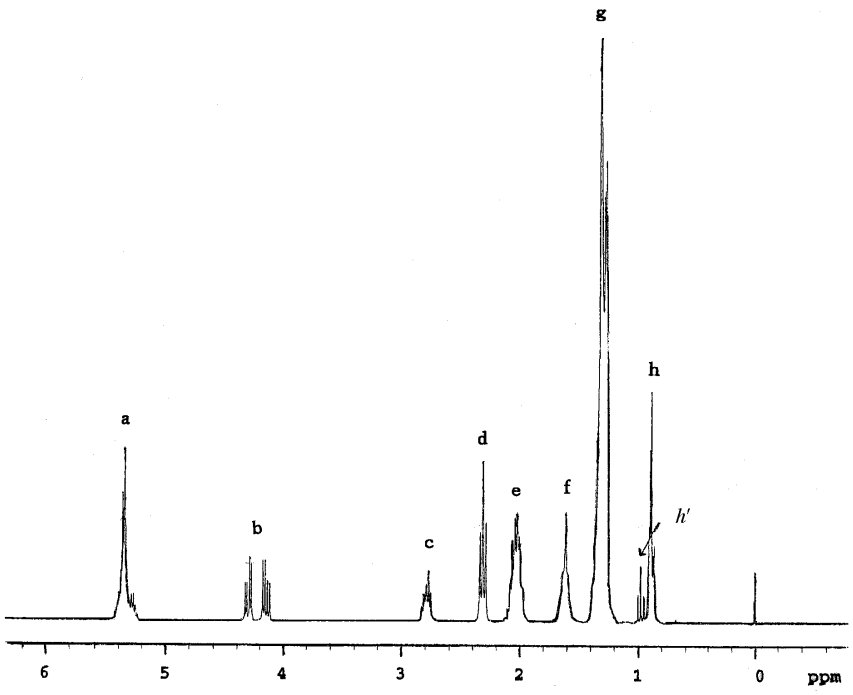

FIG. 1. ^1H Nuclear magnetic resonance spectrum of rapeseed oil.

TABLE 1
Monounsaturated Fatty Acid Composition Determined by ^{1}H Nuclear Magnetic Resonance (NMR) from Olefinic Carbon, Allylic Carbons, and Gas Chromatography (GC)

	^{1}H NMR		
	I[a]	II[b]	GC
Palm	45.3	44.9	48.5
Olive	65.4	66.2	66.2
Olive/corn	55.7	55.0	56.5
Safflower (1)	41.8	43.7	45.2
Safflower (2)	11.3	15.0	13.2
Corn (1)	19.3	24.6	25.2
Corn (2)	24.6	27.7	27.9
RS (1)[a]	54.2	55.8	59.8
RS (2)	51.3	50.4	52.1
RS/SB[b]	44.1	46.2	45.5

[a]Calculated from divinyl carbon.
[b]Calculated from ω-3 carbons.
[c]RS, rapeseed.
[d]SB, soybean.

$$\text{Area per carbon } X = \frac{\frac{1}{2}(A+F)}{3} \quad [6]$$

Also, for the ^{13}C NMR method, the basis of the area per carbon should not be influenced by the fatty acid composition. However, the T1 relaxation time of 4.328 s was too long to allow use of the terminal methyl carbons as the basis of the area per carbon. The following equations can be derived:

$$\text{n-3 Polyunsaturated fatty acid (generally linolenic acid in vegetable oils) content } [\%] = \frac{G'}{X} \times 100 \quad [7]$$

TABLE 2
Unsaturated Fatty Acid Composition Determined by NMR and GC

	^{1}H NMR			^{13}C NMR			GC		
	Ln[a]	L[b]	O[c]	Ln[d]	L	O	Ln	L	O (%)
Palm	—	12.7	44.9	—	12.7	47.4	—	13.5	48.5
Olive (1)	—	13.6	66.2	—	13.6	67.2	—	15.0	66.2
Olive (2)	—	10.9	71.1	—	10.9	72.5	0.77	10.1	74.2
Olive/corn	—	27.8	55.0	—	26.5	54.9	1.33	27.8	56.5
Safflower (1)	—	42.5	43.7	—	43.7	47.3	0.39	44.9	45.2
Safflower (2)	—	15.4	70.3	—	14.5	80.5	0.33	15.4	75.6
Safflower (3)	—	43.0	43.1	—	43.5	48.5	0.31	46.2	43.8
Safflower (4)	—	70.6	15.0	—	74.6	13.9	0.36	75.6	13.2
Safflower (5)	—	70.7	14.3	—	77.5	12.9	0.38	77.5	12.5
Corn (1)	—	58.0	24.6	—	60.2	25.8	0.85	60.9	25.2
Corn (2)	—	54.3	27.7	—	58.0	29.1	1.33	56.6	27.9
Corn (3)	—	53.7	27.4	—	53.3	30.7	1.30	55.2	29.4
RS (1)	7.40	25.0	55.8	2.47	36.8	48.9	9.39	21.0	59.8
RS (2)	7.60	28.7	50.4	5.37	36.8	45.9	8.75	28.2	52.1
RS (3)	9.95	20.7	58.1	10.6	19.4	61.9	10.4	21.8	57.8
SB	6.94	49.9	24.0	6.71	52.7	22.1	7.36	53.7	22.8
RS/SB	8.78	31.9	46.2	2.08	40.6	50.1	9.21	32.2	45.5

[a]Ln, Linolenyl.
[b]L, Linoleyl.
[c]O, Oleyl.
[d]Linolenic acid composition was calculated from divinyl carbon. See Table 1 for other abbreviations.

TABLE 3
n-6 Polyunsaturated Fatty Acid Composition Determined by ^{13}C NMR from Divinyl Carbon, ω-3 Carbon, and by GC

	^{13}C NMR		
	I[a]	II[b]	GC
Palm	11.2	7.4	13.5
Olive	14.3	15.9	15.0
Olive and corn	26.5	24.6	27.8
Safflower (1)	43.7	40.5	44.9
Safflower (2)	74.6	69.4	75.6
Corn (1)	60.2	54.8	60.9
Corn (2)	58.0	54.2	56.6
Corn (3)	53.3	47.6	55.2

[a]Calculated from divinyl carbon.
[b]Calculated from ω-3 carbon. See Table 1 for abbreviations.

TABLE 4
Effect of Recovery Delay in Determining Linolenic Acid Composition by ^{13}C NMR

Recovery delay (s)	Linolenic acid composition[a] (%)
6	2.47
10	5.87
15	8.40
18	9.57
20	9.64

[a]Linolenic acid composition determined by GC was 9.39%. See Table 1 for abbreviations.

A different chemical shift was observed for the ω-2 methylene carbon of n-3 polyunsaturated fatty acid.

$$\text{n-6 Polyunsaturated fatty acid (linoleic acid) content } [\%] = \frac{E-2G'}{X} \times 100 \text{ from divinyl carbons} \quad [8]$$

and

$$\frac{B'}{X} \times 100 \text{ from ω-3 carbons} \quad [9]$$

Divinyl carbons gave better agreement with the GC method than did the ω-3 carbon (Table 3).

$$\text{Monounsaturated fatty acid (oleic acid) content } [\%] = \frac{\frac{1}{2}D - G' - (E-2G')}{X} \times 100 = \frac{\frac{1}{2}D - E + G'}{X} \times 100 \quad [10]$$

TABLE 5
Unsaturated Fatty Acid Composition of Oil, Including Linolenic Acid, Determined by ^{13}C NMR and GC[a]

	^{13}C NMR			GC		
	Ln	L	O (%)	Ln	L	O (%)
RS (1)	8.89	20.0	60.9	9.39	21.0	59.8
RS (2)	7.29	28.0	51.0	8.75	28.2	52.1
RS (3)	9.39	21.4	60.9	10.4	21.8	57.8
SB	6.74	53.6	22.0	7.36	53.7	22.8
RS/SB	10.3	31.0	46.8	9.21	32.2	45.5

[a]See Tables 1 and 2 for abbreviations.

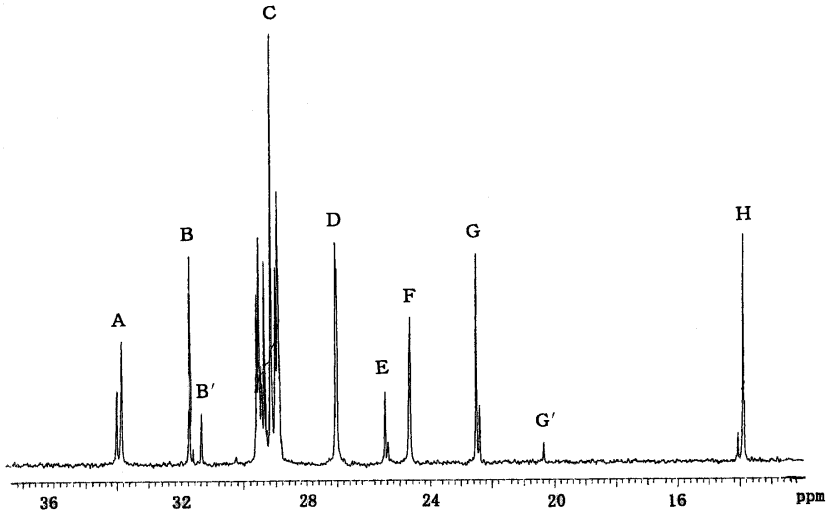

FIG. 2. ^{13}C Nuclear magnetic resonance spectrum of rapeseed oil.

Total monounsaturated fatty acid composition is easier to calculate from allylic carbons than from olefinic carbons, because stereo- and positional isomers in hydrogenated oils have their own olefinic signals.

Conditions for quantitative determination by ^{13}C NMR. Table 2 shows the results of the unsaturated fatty acid composition of 17 edible vegetable oils as measured by ^{13}C NMR and the traditional GC method. Most of the results were within an error of ±2%. Samples that contained more than 5% linolenic acid gave lower results from the ^{13}C NMR method than those from the GC method. We measured ^{13}C NMR at 6.0 s recovery delay, even though T1 relaxation time of ω-2 carbons was 3.572 s. This was not enough recovery delay for quantitative ^{13}C NMR analysis.

The effect of recovery delay in determining linolenic acid composition quantitatively was examined with a rapeseed oil (Table 4). The results show that 18 s are necessary to determine linolenic acid by ^{13}C NMR. The unsaturated fatty acid composition of vegetable oils with more than 5% linolenic acid was measured again and compared with results obtained by GC (Table 5). Though total time required to obtain the data is about 30 min per sample, the fatty acid composition determined by this ^{13}C NMR method coincided well with that obtained by the GC method. However, samples in which one of the unsaturated fatty acids was less than 5% were not satisfactory, because of the limitation in signal threshold.

In conclusion, the unsaturated fatty acid composition can be determined directly from allylic and divinyl carbons in ^{13}C NMR spectra. The discrepancy between the ^{13}C NMR results and GC results was at most ±5%. The ^{13}C NMR method developed in this study is therefore valid.

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