Determination of *Cis*- and *Trans*-18:1 Fatty Acid Isomers in Hydrogenated Vegetable Oils by High-Resolution Carbon Nuclear Magnetic Resonance

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ABSTRACT: Carbon nuclear magnetic resonance (^{13}C NMR) methods for determining the composition of *cis*-/*trans*- and positional isomers in hydrogenated vegetable oils were developed to reduce analytical time. By selecting appropriate olefinic carbon peaks and by measuring individual peak areas subsequent to the identification of isomeric peaks on the NMR spectrum, compositional results of the isomers coincided well with those obtained by conventional gas chromatography (GC). Therefore, it is highly beneficial to choose the ^{13}C NMR method when analysis time is limited. Though the proposed ^{13}C NMR method is promising, further development is needed. For the time being, combination with the traditional GC method is still encouraged for precise compositional analysis of *cis*-/*trans*- and positional isomers.
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KEY WORDS: ^{13}C NMR spectroscopy, fatty acid isomers, hydrogenated vegetable oils.

In oleochemical industries, quality control for hydrogenated fats is usually carried out through measurement of iodine value and total *trans*-fatty acid content. Koseoglu and Lusas (1) reviewed recent advances in hydrogenation of canola oil. He compared the iodine values, total *trans*-fatty acid contents, and solid fat contents of several canola oils under various hydrogenation conditions. Unfortunately, not only isomerization of *cis*-fatty acids to *trans*-fatty acids but also side reactions, such as shifting of double bonds, occur during hydrogenation. These deleterious side reactions are affected by hydrogen gas pressure and reaction temperature in particular. Formation of conformational isomers and positional isomers often generates unacceptable odor and undesirable properties. Oxidative stability is often influenced by these isomers. Therefore, it is important to evaluate composition of isomers in addition to total *trans*-fatty acid content in the hydrogenated products. Several reports (2–4) pertain to the analysis of conformational and positional isomers by gas chromatography (GC). However, most GC methods need *cis* and *trans* isolation by silver nitrate-impregnated thin-layer chromatography (Ag^+-TLC) after preparation of isopropyl esters because peaks of *cis*- and *trans*-isomers otherwise overlap. Preparation of Ag^+-TLC plates is complicated, requiring impregnation with silver nitrate on silica gel in the dark.

Gunstone (5,6) demonstrated applications of carbon nuclear magnetic resonance (^{13}C NMR) for semiquantitative analysis of several hydrogenated fats and synthesized triglycerides that contained isomers. However, he neither examined the conditions of NMR in detail for this analytic method nor made comparisons with other workers.

The intent of our study is to develop a ^{13}C NMR method to a viable level to analyze *cis*-/*trans*- and positional isomer compositions.

EXPERIMENTAL PROCEDURES

Materials. Extra-pure reagent-grade triolein and trielaidin were purchased from Nacarai Tesque Inc. (Kyoto, Japan). Hydrogenated palm, soybean, and rapeseed oils and commercial vegetable oils for deep frying were products of Ajinomoto Co., Inc. (Tokyo, Japan).

Nine types of partially hydrogenated soybean oils were prepared in our laboratory as follows. Three of them were hydrogenated at 180°C and 1.0 kg/cm^2. Another three were hydrogenated at 200°C and 0.1 kg/cm^2. The remaining three were hydrogenated at 140°C and 3.0 kg/cm^2. Nickel catalyst (0.3%) was used for these hydrogenations.

NMR spectroscopy. NMR spectroscopy was performed on a Gemini 2000 at 300 MHz (Varian Instrument Co., Palo Alto, CA). Approximately 120 mg of sample was dissolved in 0.6 mL $CDCL_3$ that contained 0.03% trimethylsilane (Nacarai Tesque Inc.), and the resulting solution was placed in a 5-mm ϕ NMR tube. Iodine values were determined by ^{1}H NMR, which was developed in our laboratory (7). Analytical conditions were as follows: sample concentration, 20%; pulse angle, 7.5°; recovery delay, 2.5 s; number of scans, 32. Total *trans*-fatty acid contents were determined by ^{13}C NMR, which was also developed in our laboratory (8). Analytical conditions were as follows: sample concentration, 20%; pulse angle, 90°; recovery delay, 6.0 s; number of scans, 100.

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GC. Chromatograms were obtained with a G-3000 gas chromatograph (GL Sciences, Inc., Tokyo, Japan), configured for capillary column operation with a split insert and flame-ionization detector. GC conditions were as follows: column, TC-70 (GL Sciences, Inc.), 120 × 0.25 mm i.d., 0.25-micron film; injector temperature, 250°C; detector temperature, 250°C; temperature program, from 170 (80 min hold) to 220°C at 5°C/min.

For total fatty acid composition analysis, chromatograms were obtained with a GC-353 gas chromatograph (GL Sciences Inc.). GC conditions were as follows; column, BPX-70 (GL Sciences, Inc.), 25 m × 0.53 mm i.d., 0.5-micron film; injector temperature, 230°C; detector temperature, 230°C; temperature program, from 85 (2 min hold) to 160°C at 15°C/min; to 170°C at 0.8°C/min; to 230°C at 10°C/min. Samples were methylated before injection into the GC by AOCS Method Ce 2-66 (9).

RESULTS AND DISCUSSION

Conditions for quantitative determination of cis- *and* trans-*isomers by NMR*. The quantitative effect of recovery delay beyond the T1 relaxation time of olefinic carbon or triolein and trielaidin was examined. As shown in Table 1, recovery delays longer than 6.0 s did not reduce errors substantially. The minimal number of scans that does not impair the signal/noise ratio under 6.0 s recovery delay and 20% sample concentration was examined to minimize the analytical time for NMR. Spectral width was reduced to 120–180 ppm with a digital filter device on the Gemini 2000. The increase in number of scans obviously increased the resolution of the ^{13}C NMR spectrum (Fig. 1). However, for the measurement for 10,000 scans, more than 12 h were required. Considering the time consumption, 1000 scan times seemed tolerable for avoiding large errors. Sample dilution did not improve the resolution of ^{13}C NMR, as is shown by comparing the spectra of Figures 1 and 2.

Olefinic carbon signal assignment. We compared the individual chemical shifts of olefinic carbon signals of $C_{18:1}$ isomers in the 10 kinds of hydrogenated fats of our materials and those of Gunstone (5) (Table 2). All our chemical shift data coincided well with those of Gunstone and gave only 0.03 ppm differences at most.

TABLE 1
Quantitative Effect of Recovery Delay on NMR Measurements[a]

Recovery delay (s)	Triolein	Trielaidin
Weight (gravimetry)	48.4	51.6
2	48.5 ± 1.4[b]	51.5 ± 1.4
4	48.2 ± 1.4[b]	51.8 ± 1.4
6	48.9 ± 0.4	51.1 ± 0.4
8	48.1 ± 0.3	51.9 ± 0.3
10	48.4 ± 0.5	51.6 ± 0.5
20	48.3 ± 0.4	51.7 ± 0.4
40	48.4 ± 1.0	51.6 ± 1.0

[a]Pulse angle 90°, sample concentration 20%, number of scans 96. NMR, nuclear magnetic resonance.
[b]Each *cis/trans* acid composition was compared after five runs.

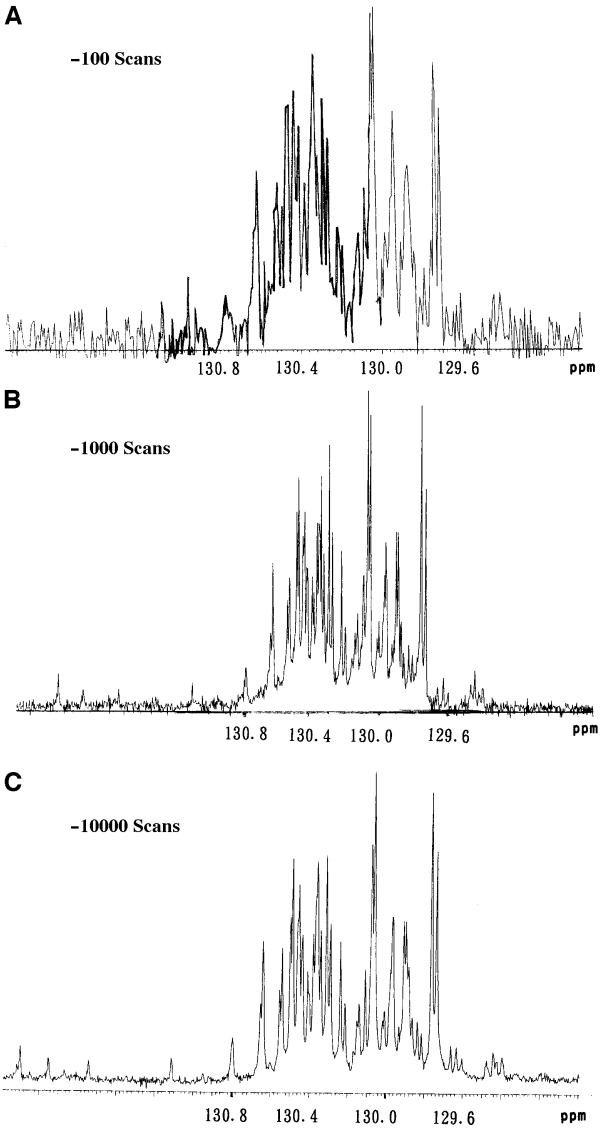

FIG. 1. Influence of number of scans on carbon nuclear magnetic resonance (^{13}C NMR) spectrum of partially hydrogenated soybean oil (iodine value = 66.2, sample concentration = 20%).

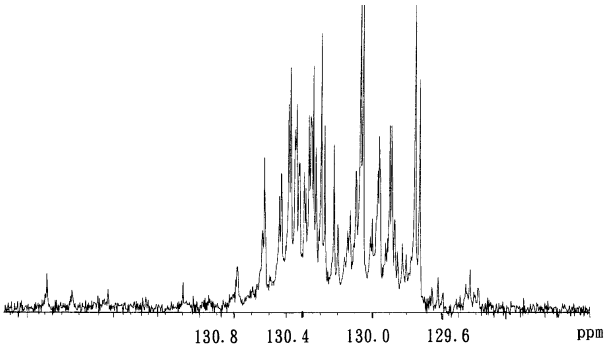

FIG. 2. Influence of sample concentration on ^{13}C NMR spectrum of partially hydrogenated soybean oil (same sample as Figure 1 was used except the sample was diluted to 5% from 20%). See Figure 1 for abbreviation.

TABLE 2
The Chemical Shifts for *cis*- and *trans*-Olefinic Carbon in $C_{18:1}$ Isomers

cis	Chemical shift (ppm)		*trans*	Chemical shift (ppm)	
Δ8	130.10	129.60	Δ8	130.76	129.80
Δ9	130.03	129.71	Δ9	130.60	130.07
Δ10	129.97	129.78	Δ10	130.50	130.19
Δ11	129.93	129.85	Δ11	130.42	130.31
Δ12			Δ12	130.39	130.33
Δ13	129.87	129.84	Δ13	130.37	

Comparison of compositional results of isomers between NMR and GC methods. The consistency of compositional isomeric results between NMR and GC were evaluated. Quantitative analysis of each isomer was carried out by dividing each peak area by that of total olefinic carbon in the NMR spectrum. Advantages of the NMR method were: *cis*- and *trans*-isomers could be obtained without any pretreatment, and positional isomers near the carbonyl group were resolved satisfactorily. But unfortunately, samples that contained *trans*-fatty acids below 3% were not measured satisfactorily because of the limitation in signal threshold.

It was necessary to select peaks to do the quantitative analysis by the NMR method because Δ9 and 12 of linoleic acid and Δ9 of linolenic acid overlapped seriously with *cis*-$C_{18:1}$ Δ10 or *trans*-$C_{18:1}$ Δ11 and 12 peaks. As shown in Figure 3, the GC method showed satisfactory resolution of *cis*-fatty acids that

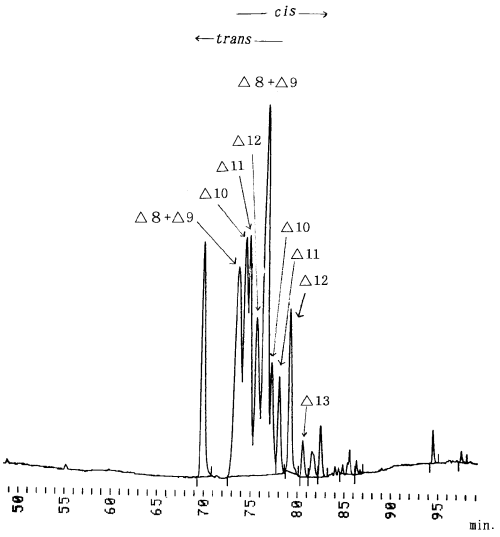

FIG. 3. Gas chromatogram of *cis*- and *trans*-$C_{18:1}$ methyl esters prepared from shortening.

TABLE 3
Isomeric $C_{18:1}$ Fatty Acid Composition Compared for GC Method with NMR Method

	S[b] (A)	SBH[c] (A)	S(B)	SBH (B)	SBH (C)	RSH[d] (A)	S (C)	S (D)
Total *trans*[a] (%)	3.0	10.2	11.2	19.9	26.2	34.6	43.3	43.8
NMR method								
$C_{18:1}$ *cis*[e] (%)	100.0	79.8	80.4	70.6	63.6	51.0	46.6	46.1
Δ8	—	—	—	—	4.5	4.1	5.4	2.2
Δ9	100.0	63.0	64.1	57.3	37.4	28.6	25.7	25.0
Δ10	—	—	6.2	4.3	1.7	3.3	4.5	5.9
Δ11 + Δ12	—	16.8	10.1	9.1	20.1	14.9	11.0	13.1
$C_{18:1}$ *trans*[f] (%)	—	19.3	19.6	29.4	36.4	49.0	53.4	53.9
Δ7	—	—	—	—	—	3.0	2.2	2.0
Δ8	—	2.5	3.5	5.9	2.3	5.6	9.3	5.9
Δ9	—	4.4	3.9	8.4	4.5	7.6	13.6	11.6
Δ10	—	6.5	5.5	9.0	4.5	13.9	14.4	14.6
Δ11	—	5.9	6.8	5.3	15.3	12.4	8.4	13.4
Δ12	—				7.6	3.9	3.5	4.2
Δ13	—	—	—	0.9	2.2	2.7	2.0	2.2
GC method[a]								
$C_{18:1}$ cis[a] (%)	93.5	79.0	75.1	65.7	65.9	54.5	45.6	46.6
Δ8 + Δ9	93.5	63.0	66.7	54.0	41.5	32.0	29.0	27.3
Δ10	—	1.4		4.7	0.9	5.2	6.2	5.6
Δ11	—	6.3	8.4[g]	5.4	11.2	4.9	4.7	4.2
Δ12	—	8.4		1.4	10.8	10.6	4.7	8.0
Δ13	—	—		0.2	1.5	1.8	1.0	1.5
$C_{18:1}$ *trans*[b] (%)	6.5	21.0	24.9	33.8	34.0	45.4	54.2	53.4
Δ7 + Δ8 + Δ9	6.5	5.4	10.4	13.9	8.7	13.8	25.2	19.6
Δ10	—	6.5		9.7	5.7	12.3	14.1	17.5
Δ11	—	5.9	14.6[h]	6.8	10.5	11.3	10.2	11.7
Δ12	—	3.2		3.4	9.1	8.0	4.7	4.6

[a]Total *trans* acid content/total fatty acid content.
[b]Shortening.
[c]Hydrogenated soybean oil.
[d]Hydrogenated rapeseed oil. GC, gas chromatography. See Table 1 for other abbreviation.
[e]Total $C_{18:1}$ *cis* acid content/$C_{18:1}$ fatty acid content.
[f]Total $C_{18:1}$ *trans* acid content/$C_{18:1}$ fatty acid content.
[g]Δ10 + Δ11 + Δ12 + Δ13.
[h]Δ10 + Δ11 + Δ12.

TABLE 4
Analysis of Various Hydrogenated[a] Soybean Oils

	1	2	3
Iodine value	112.3	93.2	74.6
Total *trans* content (%)	10.2	26.2	34.6
Fatty acid composition (%)			
$C_{16:0}$	10.5	10.6	10.5
$C_{18:0}$	5.65	8.72	12.4
$C_{18:1}$	37.1	48.8	61.7
$C_{18:2}$	43.5	30.7	15.0
$C_{18:3}$	3.30	0.88	0.38

[a]Hydrogenated at 180°C and 1.0 kg/cm^2 with 0.3% nickel catalyst.

TABLE 5
Analysis of Soybean Oils Hydrogenated Under Various Conditions

	HP[a] 1	HT[b] 1	HP 2	HT 2	HP 3	HT 3
Iodine value	112.7	111.0	98.6	96.6	79.8	79.4
Total *trans* content (%)	8.4	16.4	25.3	17.1	23.9	34.5
Fatty acid composition (%)						
$C_{16:0}$	10.5	10.6	10.5	10.7	10.6	10.6
$C_{18:0}$	6.7	14.6	9.53	5.51	15.8	8.42
$C_{18:1}$	37.9	40.5	40.8	54.1	55.6	67.9
$C_{18:2}$	37.9	34.5	26.8	21.3	13.0	5.99
$C_{18:3}$	3.2	12.6	1.46	0.85	0.36	0.65

[a]Hydrogenated at 140°C and 3.0 kg/cm^2 with 0.3% nickel catalyst.
[b]Hydrogenated at 200°C and 0.1 kg/cm^2 with 0.3% nickel catalyst.

TABLE 6
Influence of the Degree of Hydrogenation[a] on Isomeric $C_{18:1}$ Fatty Acid Composition

Hydrogenation degree	1	2	3
cis (%)			
Δ8	—	—	4.41
Δ9	27.9	25.9	22.9
Δ10	—	1.11	3.57
Δ11 + Δ12	7.42	9.41	16.0
trans (%)			
Δ7	—	0.60	1.41
Δ8	1.97	1.37	2.76
Δ9	1.94	1.78	2.76
Δ10	2.87	6.99	3.29
Δ11 + Δ12	2.17	7.94	17.5
Δ13	—	2.89	5.80

[a]Hydrogenated at 180°C and 1.0 kg/cm^2 with 0.3% nickel catalyst.

have double bonds near the terminal methyl group. However, as previously mentioned, the GC method requires pretreatment of the samples. Because discrepancies between the NMR results and the GC results were ±5% at most (Table 3), the NMR method developed in this study can be considered valid.

The NMR method, with support of GC, provides promising quantitative data for $C_{18:1}$ fatty acid isomer analysis.

Analysis of the isomerization of $C_{18:1}$ fatty acids under different hydrogenation conditions. Three different partially hydrogenated soybean oils were prepared from the same original soybean oil by varying hydrogenation degree and/or hydrogen pressure and temperature. The iodine values, total *trans*-fatty acid contents, and fatty acid compositions are summarized in Tables 4 and 5. As shown in Tables 4 through 7, compositional changes in unsaturated fatty acid isomers observed were not small. Intact linoleic and linolenic acids can be identified from the Δ10, 12, and Δ10, 12, 13, 15 peaks of olefinic carbon, respectively, which exist at 127.0–128.4 ppm. Under low hydrogen gas pressure (0.1 kg/cm^2), *trans*-fatty acids as well as positional isomers were

TABLE 7
Influence of Hydrogenation Conditions on Isomeric $C_{18:1}$ Fatty Acid Composition

Conditions	HP[a]1	HT[b] 1	HP 2	HT 2	HP 3	HT 3
cis (%)						
Δ8	—	—	—	3.0	2.5	2.8
Δ9	23.1	19.9	17.6	19.9	16.5	20.3
Δ10	—	2.3	1.1	3.8	1.3	4.1
Δ11 + Δ12	6.5	7.9	5.2	8.1	8.7	9.3
trans (%)						
Δ7	—	0.9	0.6	1.5	1.8	2.2
Δ8	—	0.7	1.5	3.7	3.1	4.3
Δ9	2.9	2.3	3.9	3.4	5.0	4.1
Δ10	2.4	2.1	5.8	5.4	7.5	8.1
Δ11 + Δ12	3.0	4.7	4.1	7.2	9.2	10.3
Δ13	—	—	1.0	1.3	—	3.2

[a]Hydrogenated at 140°C and 3.0 kg/cm^2 with 0.3% nickel catalyst.
[b]Hydrogenated at 200°C and 0.1 kg/cm^2 with 0.3% nickel catalyst.

abundant, compared with the preparation at high hydrogen gas pressure.

The present results agree with the results of GC analysis, and therefore can be considered to be useful to analyze *cis-/trans-* as well as positional isomers.

A well-designed ^{13}C NMR method in combination with the GC method promises perfect analysis.

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