# Rapid Determination of Iodine Value by $^1$H Nuclear Magnetic Resonance Spectroscopy

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**ABSTRACT:** High-resolution $^1$H nuclear magnetic resonance ($^1$H NMR) has been found to be an effective tool for the direct, rapid, and automated determination of the iodine value (IV) of vegetable oils, including hydrogenated oils (IV = 45.9–140.2). The total time required to obtain the $^1$H NMR data is about 3 min per sample. The IV is calculated from the number of double-bonded protons and the average molecular weight derived directly from the spectrum. The average of olefinic protons and allylic plus divinyl protons area was used to calculate the absolute number of double-bonded protons. The $^1$H NMR results were compared with those obtained by the traditional Wijs–cyclohexane methods. The correlation coefficient between traditional IV and the novel $^1$H NMR method was $r^2 = 0.9994$ for the regression equation $Y = 0.9885X + 2.8084$, where $X$ was the result given by the traditional method. With the proposed regression equation, IV calculated by the $^1$H NMR method was within an error of ±1 unit of the result obtained by the traditional method. The proposed method is practically viable if one can afford to have the NMR system.
*JAOCS 75,* 15–19 (1998).

**KEY WORDS:** $^1$H NMR spectroscopy, hydrogenated oil, iodine value, vegetable oil.

Iodine value (IV), which shows the degree of unsaturation of oils, often has been used to predict the chemical and physical properties of fats and oils, such as oxidative stability and melting point. However, the traditional IV method is time-consuming. Recently, near-infrared reflectance (NIR) spectroscopy has been proposed as a useful tool to determine IV (1). Though this NIR method can give IV data within a couple of minutes, it cannot be applied to *trans* fatty acid-containing fats and oils. Therefore, it is impossible to measure the IV of fats and oils with hydrogenated fatty acids.

NMR has become one of the most promising methods to determine organic structures. There have been a few other reports that pertain to the analysis of oils by NMR (2–9). Brosio et al. (7) discovered that there is a correlation between the IV of corn oils and the proton T1 relaxation time. Also Matsui *et al.* (8) have shown that there is a linear relation between IV and the number of olefinic protons in oils determined by $^1$H NMR. IV was calculated indirectly through an external proton standard (dioxane), and as a result, erros of ±3 unit were unavoidable. The present study shows the quantitative condition of $^1$H NMR viable for vegetable oils, including hydrogenated products.

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## EXPERIMENTAL PROCEDURES

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

*Wijs–cyclohexane methods.* Traditional IV was measured according to AOCS Method Cd 1-25 with slight modification. Cyclohexane was used instead of carbon tetrachloride (10).

*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 $CDCL_3$, which contained 0.03% trimethylsilane (Nacalai Tesque Inc.), and the resulting solution was placed in a 5 mm ϕ NMR tube. The proton T1 relaxation time was acquired on the triolein by using the standard inversion recovery T1 pulse sequence provided in the Varian NMR software GRIDE.

## RESULTS AND DISCUSSION

*Assignment of proton signals.* The $^1$H NMR spectra of triolein and trilinolein are shown in Figure 1. The spectrum signals are divided into eight groups from A to H. These groups are assigned as follows (11): A, olefinic protons and methine proton in glyceryl group; B, the four methylene protons in the glyceryl group; C, the six α-methylene protons adjacent to carbonyl carbon; D, allyl methylene protons; E, the six β-methylene protons from carbonyl carbon; F, methylene protons on saturated carbon atoms; G, the nine terminal methyl protons [the different chemical shift (0.95 ppm) is observed for ω-3 fatty acid]; H, divinyl methylene protons.

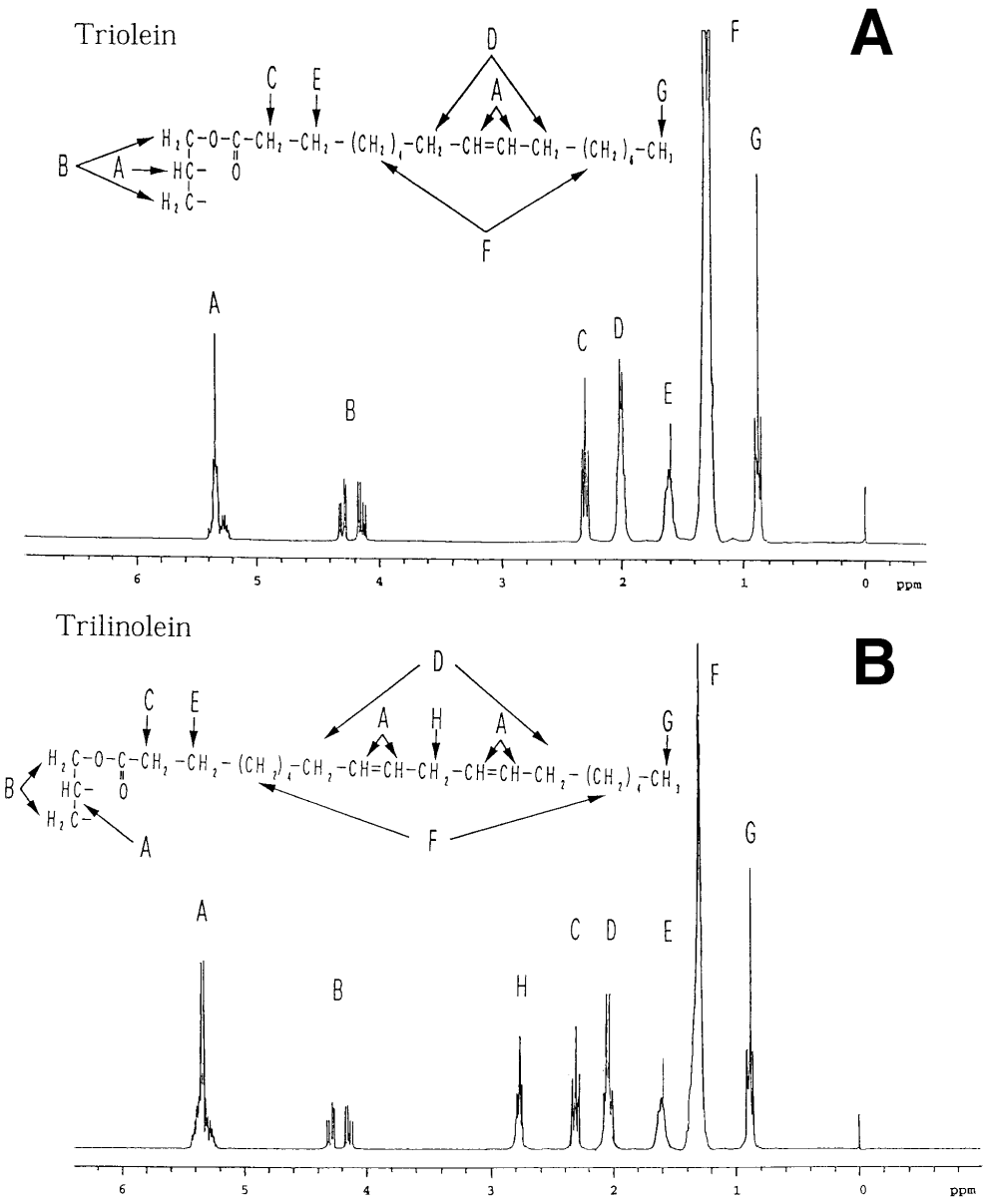


**FIG. 1.** $^1$H nuclear magnetic resonance (NMR) spectrum of triolein (A) and trilinolein (B).

*Conditions of NMR measurement.* Some experimental conditions that affect quantitative analysis on NMR were examined. Figure 2 shows the effect of sample concentration on constant pulse angle (90°) recovery delay (1.0 s), and number of scans (32 times). The relationship between sample concentration and the number of scans is one of the most important factors to obtain a quantitative result. Results show that a concentration of more than 5% (mg oil/mL $CDCL_3$) is required for quantitative determination (Fig. 2). Incidentally, methylene protons of the glyceryl group were used for the relative intensity because the glyceryl group's protons had a minimum T1 relaxation time compared to the other protons of oils. For example, the proton of the terminal $CH_3$ group can be derived as (the area of peak G/9)/(the area of peak B/4).

Proton T1 relaxation time of triolein was measured to get enough recovery delay for quantitative NMR analysis (Table 1). The longest T1 relaxation time was 2.236 s, which was for the terminal methyl protons. The quantitative effect of a recovery delay longer than the T1 relaxation time of the terminal methyl group, 2.236 s, was examined (Fig. 3). The result is shown by the relative intensity of the protons, which is calculated in the same manner with the relationship between sample concentration and the number of scans. Because a longer recovery delay had no effect on the relative intensity of protons, it was decided to select 2.5 s.

*Calculation method.* The IV can be theoretically calculated by average molecular weight and absolute number of double bonds from the NMR spectrum. For an unknown sample, av-

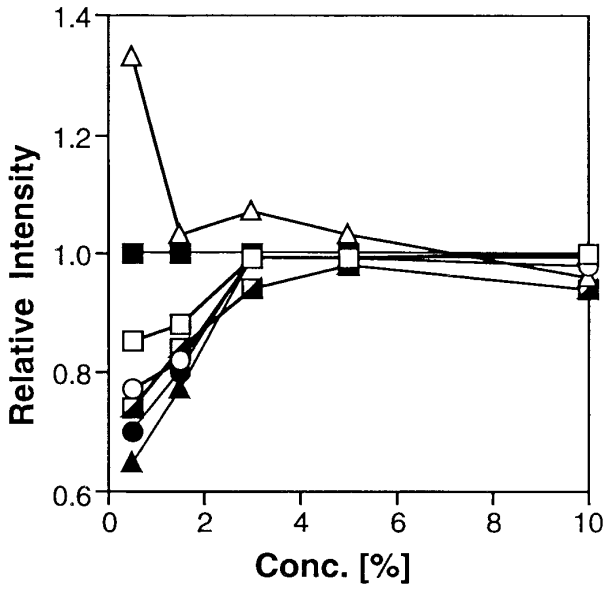


**FIG. 2.** Effect of sample concentration on the quantitativeness of the NMR spectrum. See Figure 1 for abbreviation. Relative intensity = (area of one peak)/(area of glyceryl group/4): □, olefin; ○, α-methylene; △, β-methylene; ◪, methyl; ■, glycerin; ●, allyl; ▲, methylene.

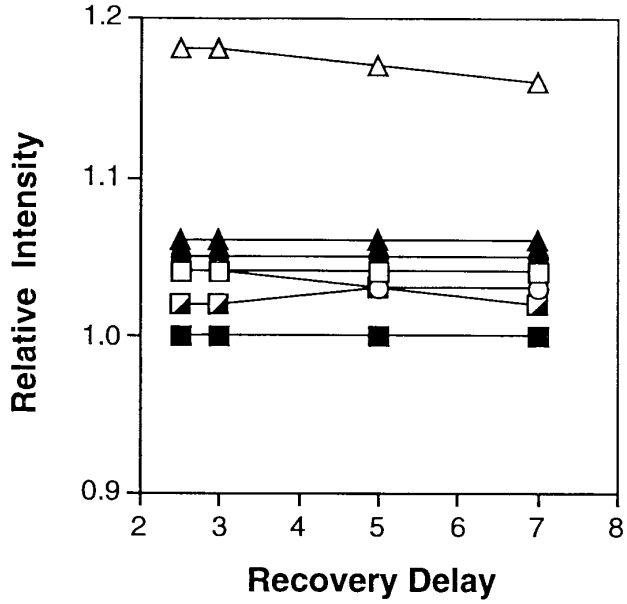


**FIG. 3.** Effect of recovery delay on the quantitativeness of the NMR spectrum. See Figure 1 for abbreviation. Relative intensity = (area of one peak)/(area of glyceryl group/4): □, olefin; ○, α-methylene; △, β-methylene; ◪, methyl; ■, glycerin; ●, allyl; ▲, methylene.

erage molecular weight must be determined prior to the NMR analysis to minimize error. In the following equations, the letters A–G represent the areas of the respective NMR signals (Equations 1–3):

$$\text{area per proton} = B/4 \quad [1]$$

$$\text{average mol. wt} = 15.034\ G/3/(1) + 14.026(C + D + E + F + H)/2/(1) + 173.100\ B/4/(1) + 26.016\ (A - B/4)/2/(1) \quad [2]$$

If oils include ω-3 fatty acids, their area of terminal methyl protons (0.95 ppm) is added to the area of G.

$$\text{iodine value} = 253.8\ (A - B/4)/2/100 \times \text{average ml. wt.}\ (253.8 = \text{mol. wt. of iodine}) \quad [3]$$

The standard deviation between the analytical and theoretical values was examined by 11 measurements of the standards, which were triolein and trilinolein (Table 2). The IV of 10 vegetable oils, obtained by $^1$H NMR and Wijs–cyclohexane methods, were compared (Table 3). Pulse angles (and

**TABLE 1**
**Proton T1 Relaxation Time of Triolein**

| Peak[a] | T1 (s) |
|---|---|
| A | 1.474 |
| B | 0.360 |
| C | 0.594 |
| D | 0.881 |
| E | 0.623 |
| F | 1.147 |
| G | 2.236 |

[a]Letters designate peaks in Figure. 1.

**TABLE 2**
**Iodine Value of Triolein (To) and Trilinolein (TLo)**

| | | To | TLo |
|---|---|---|---|
| Average molecular weight | | | |
| Theoretical value | | 884.45 | 879.39 |
| Analytical value ($n$ = 11) | max. | 900.6 | 891.5 |
| | min. | 873.1 | 869.0 |
| | av. | 884.0 | 878.0 |
| | S.D. | 4.3 | 6.2 |
| Iodine value | | | |
| Theoretical value | | 86.67 | 174.53 |
| Analytical value ($n$ = 11) | max. | 86.1 | 174.1 |
| | min. | 85.4 | 172.7 |
| | av. | 86.1 | 173.4 |
| | S.D. | 0.49 | 0.49 |

**TABLE 3**
**Iodine Value Compared for Wijs–Cyclohexane Methods and NMR Method for Two Pulse Angles and Sample Concentrations[a]**

| Oil Sample | Wijs | NMR (error) | |
|---|---|---|---|
| | | Pulse angle (degree) | |
| | | 15 | 7.5 |
| | | Sample conc. (% vol/vol) | |
| | | 5 | 20 |
| Palm | 64.6 | 62.4 (–2.4) | 64.8 (+0.2) |
| Olive | 85.6 | 83.2 (–2.4) | 86.3 (+0.7) |
| Olive/Corn | 100.4 | 99.5 (–0.9) | 99.4 (–1.0) |
| Rapeseed (1) | 115.7 | 116.5 (+0.8) | 116.3 (+0.6) |
| Safflower | 117.5 | 117.3 (–0.2) | 117.0 (–0.5) |
| Rapeseed (2) | 120.0 | 119.4 (–0.6) | 121.1 (+1.1) |
| Rapeseed/Soybean | 122.5 | 122.5 (+0.3) | 122.8 (+0.6) |
| Corn (1) | 125.3 | 123.5 (–1.8) | 125.1 (–0.2) |
| Corn (2) | 127.1 | 127.2 (+0.1) | 127.3 (+0.2) |
| Safflower | 140.8 | 141.6 (+0.8) | 140.2 (–0.6) |

[a]NMR, nuclear magnetic resonance.

**TABLE 4**
**Correlation Between Wijs and NMR methods[a]**

| Basis of proton area | Regression equation | Correlation coefficient |
|---|---|---|
| Olefinic $^1$H | $Y = 1.0034X + 0.8674$ | 0.9907 |
| Allylic $^1$H + Divinyl $^1$H | $Y = 0.9969X + 5.063$ | 0.9963 |
| Average[a] | $Y = 0.9914X + 2.432$ | 0.9986 |

[a]Average of olefinic $^1$H and allylic $^1$H. See Table 3 for abbreviation.

sample concentrations) were 15° (5%) and 7.5° (20%), respectively, for the $^1$H NMR method. The sample concentration was modulated to be able to analyze within 3 min. Each IV determined by $^1$H NMR is the result of triplicate runs. Errors between these two methods were from −2.4 to 0.8 and −1.0 to 1.1, respectively, and a 7.5° pulse angle was selected because the error of IV was smaller. When the errors between these two methods were more than ±1 unit, more examination was desirable to minimize errors.

To minimize errors, the basis of area per proton and the calculation method of the absolute number of double bonds were modified. The basis of area per proton should not be influenced by the fatty acid composition. Because the signal of β-methylene groups from the carbonyl group is included with a small amount of water, the average signal of the other three peaks was used. Allyl and divinyl methylene protons could be used to calculate the number of double bonds in place of the olefinic protons. Correlation between the Wijs–cyclohexane methods and the $^1$H NMR method was examined when the basis of protons was changed (Table 4). The combination of olefinic protons and allylic plus divinyl protons gave the best agreement with the Wijs–cyclohexane methods ($r^2$ = 0.9986). The calculation methods of area per proton and IV were revised as follows (the letters A–G represent the area of the respective NMR signals) (Equations 4,5):

$$\text{area per proton} = (B/4 + D/6 + G/9)/3 \quad [4]$$

$$IV = 253.8[(A - B/4)/2 + (D/4 + H/2)]/2/100 \times \text{average mol. wt. } (253.8 = \text{mol. wt. of iodine}) \quad [5]$$

**TABLE 5**
**Iodine Values of Hydrogenated Oils**

| Sample | Wijs | NMR | |
|---|---|---|---|
| | | Raw data | After corr.[a] |
| Palm (1) | 48.0 | 46.5 | 48.5 |
| Palm (2) | 55.0 | 52.6 | 54.6 |
| Soybean (1) | 66.2 | 64.6 | 66.5 |
| Corn (1) | 71.0 | 68.6 | 70.4 |
| Corn (2) | 72.0 | 70.0 | 71.7 |
| Corn (3) | 72.7 | 70.5 | 72.3 |
| Rapeseed (1) | 84.6 | 82.3 | 84.0 |
| Rapeseed (2) | 87.7 | 85.1 | 86.8 |
| Rapeseed (3) | 100.5 | 98.1 | 99.6 |
| Soybean (2) | 120.0 | 118.9 | 120.3 |

[a]After adjustment with $Y = 0.9914X + 2.432$ ($X$ = raw data by $^1$H NMR spectrum). See Table 3 for abbreviation.

**TABLE 6**
**Iodine Values After Correction by Regression Equation**

| Sample | Wijs | NMR[a] | Sample | Wijs | NMR |
|---|---|---|---|---|---|
| Palm | 64.6 | 64.8 | Palm-H[b] (1) | 48.0 | 48.5 |
| Olive (1) | 85.6 | 85.6 | Palm-H (2) | 55.0 | 54.5 |
| Olive (2) | 85.9 | 85.4 | Palm-H (3) | 47.4 | 47.7 |
| Olive/Corn | 100.4 | 100.2 | Palm-H (4) | 45.9 | 45.7 |
| Corn (1) | 125.3 | 125.7 | Shortening (1) | 71.0 | 70.2 |
| Corn (2) | 127.1 | 126.3 | Shortening (2) | 72.0 | 71.6 |
| Corn (3) | 125.0 | 124.0 | Shortening (3) | 72.7 | 72.2 |
| Rapeseed (1) | 118.4 | 118.5 | Shortening (4) | 70.1 | 70.6 |
| Rapeseed (2) | 120.0 | 120.7 | Shortening (5) | 53.2 | 53.8 |
| Rapeseed (3) | 119.2 | 118.7 | Shortening (6) | 53.7 | 53.8 |
| Rapeseed (4) | 119.0 | 119.7 | Shortening (7) | 59.9 | 59.2 |
| Rapeseed (5) | 115.5 | 116.2 | Rapeseed-H (1) | 84.6 | 83.9 |
| Soybean | 132.9 | 132.9 | Rapeseed-H (2) | 87.7 | 86.7 |
| Safflower (1) | 117.5 | 117.5 | Rapeseed-H (3) | 100.5 | 99.6 |
| Safflower (2) | 92.9 | 93.2 | Rapeseed-H (4) | 68.7 | 69.5 |
| Safflower (3) | 118.6 | 118.4 | Rapeseed-H (5) | 70.4 | 70.5 |
| Safflower (4) | 140.8 | 140.9 | Rapeseed-H (6) | 69.5 | 69.8 |
| Safflower (5) | 144.0 | 143.2 | Soybean-H (1) | 66.2 | 66.4 |
| | | | Soybean-H (2) | 120.0 | 120.3 |

[a]After adjustment with $Y = 0.9885X + 2.8084$ ($X$ = raw data by $^1$H NMR spectrum.) See Table 3 for abbreviation.
[b]Hydrogenated oil.

*Linear regression equation.* Several vegetable oils were subjected to this novel $^1$H NMR method. The IV determined by this method coincided well with that obtained by the traditional Wijs–cyclohexane methods. We examined whether the IV of hydrogenated oils could be determined by the same NMR method (Table 5). Hydrogenated oils were included for regression analysis because their IV cannot be determined by NIR as general vegetable oils. The regression expression obtained from Table 6 was $Y = 0.9885X + 2.8084$ ($X$ = raw data from NMR method). The correlation coefficient was $r^2$ = 0.9994. The error between the traditional method and the proposed novel method was ±1 unit, when the range of IV was between 45.9 and 143.2.

In conclusion, IV was calculated directly from the absolute number of double-bonded protons and molecular weight from $^1$H NMR. Errors of IV between the $^1$H NMR and traditional methods were within ±1 by use of the proposed regression equation.

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[Received November 25, 1996; accepted August 30, 1997]