Quality Evaluation of Edible Oils by Proton Nuclear Magnetic Resonance Measurement

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1H-nuclear magnetic resonance (NMR) method using dioxane as the external proton standard was established to evaluate the quality of edible oils. The number of olefinic protons (OP) in oil was determined by the ratio of the integral value of olefinic protons (δ 5.33-5.47) versus that of methylene protons of dioxane (δ 3.4-3.5) using the solvent system of benzene-d6-30% MeOD (60°C). In this solvent system, good separation between the olefinic and methine protons from the triglyceride backbone was achieved. As a result, for various UV-irradiated oxidized rapeseed oils, the plots of the OP versus the iodine value and the plots of the number of divinylmethylene protons versus the 2-thiobarbituric acid value gave excellent linearity. In addition, the iodine values calculated by the proposed 1H-NMR method for five commercially available edible oils agreed with the experimental ones within ±3 iodine value error.

Keywords: 1H-NMR, external proton standard, edible oil

As is well-known, lipid oxidation in food during its processing and/or storage can bring about a marked deterioration in quality (rancidity), and the produced lipid hydroperoxides (HPO) during oxidation have been proved to have some harmful abilities to induce cell necrosis and degenerative arthritis (Kaneda, 1984). Therefore, various counterplans to retain the food quality against lipid oxidation, e.g., addition of radical or O2 quenchers such as α-tocopherol and butylated hydroxytoluene (BHT) (Karahadian & Lindsay, 1989), and purge the packaging with an inert gas (Benoualid, 1986) have been examined.

In order to check a quality of edible oil or lipids before use, a number of 2-thiobarbituric acid (TBA) methods have been proposed (Schmedes & Holmer, 1989; Pikul et al., 1989; Squires, 1990). However, the procedures are complicated and tedious, and skilled technicians are required for obtaining such data. To overcome these problems and correctly evaluate the oxidative degradation of lipids, a simple and alternative quantitative method would be advantageous.

Nuclear magnetic resonance (NMR) spectroscopy gives direct and useful information with respect to chemical composition and molecular dynamics. Thus, proton (1H) NMR measurement could serve to clarify the oil composition and estimate the quality change due to oxidative rancidity on the basis of induced proton signal changes of an unsaturated fatty acid residue. In this paper, we have tried to establish a simple quantitative method that is capable of checking the quality of edible oil using the 1H-NMR approach.

Materials and Methods

Materials Commercially available six edible oils (corn oil, rapeseed oil, soybean oil, cottonseed oil, sesame oil and olive oil) were purchased from Nacalai Tesque Inc., Ltd., Kyoto, Japan. These oils were all extra-pure reagent grade, but used without further purification. Fifty ml of rapeseed oil in a flask was UV-irradiated (300 W, λ = 254 nm) during bubbling using air at 25°C. Ten ml of oil was removed every 20 h (irradiation was done for 92.5 h) in order to prepare the oils with different degrees of oxidative rancidity. Samples of the irradiated rapeseed oils and other unirradiated edible oils were subject to the following Wijs-cyclohexane and TBA methods, and 1H-NMR measurement.

Wijs-cyclohexane method (iodine value) The iodine value (IV) of oil was determined by the modified Official Method Cd 1-25 of the American Oil Chemists’ Society using the solvent, cyclohexane, in place of carbon tetrachloride (Yukagaku, 1992). Samples of the oils (0.2 g in 10 ml of cyclohexane) were taken and mixed with 25 ml of Wijs solution for 60 min in the dark. After adding 20 ml of 10 wt% KI solution and 100 ml of water, the mixture was titrated with 0.1 M sodium thiosulfate to determine the IV.

TBA method (TBA value) The TBA value was determined as described by Squires et al. (1991). A weighed amount of oil (> 1 g) was dissolved in 0.5 ml of 0.05 wt% BHT (in methanol) and 5 ml of 5 wt% trichloroacetic acid, and heated to 80°C for 10 min. After cooling, 1 ml of 0.2 wt% TBA was added to 1 ml of the aqueous phase, followed by the heating at 95°C for 30 min. After cooling, the absorbance of the solution was measured at 532 nm. The TBA value (μg MDA/g oil) was calculated from the standard curve using the MDA standard (acid-hydrolyzate of malondialdehyde-dimethyl acetal): y = 0.006x1 - 1.28x10-5 (r = 0.9994), where y is the absorbance at 532 nm and x is the MDA content (μg MDA). All of the above reagents were analytical reagent grade from Nacalai Tesque Inc., Ltd., Kyoto, Japan and used without further purification.
Quality Evaluation of Oils by NMR Measurement

**1H-NMR measurement** Before preparing the deuterium-sample solution for NMR measurement, an appropriate choice of solvent for the oil and measuring conditions is needed to achieve complete separation of each proton signal. Our investigations with respect to solvent choice will be discussed later in detail. For preparing the sample solution, 0.5 ml of oil was dissolved in 1.0 ml of solvent, followed by the addition of 50.0 µl (±0.05 µl) of dioxane. Then, 0.7 ml of the mixture was placed in a 5-mm NMR sample tube. 1,4-Dioxane (Nacalai Tesque Inc., Ltd., Kyoto, Japan) was used as the proton standard (eight protons per mole) to determine the number of divinylmethylene and olefinic protons of oil from NMR data. 1H-NMR spectra were obtained using an A400 NMR instrument (400 MHz, JEOL Co. Ltd., Japan) with tetramethylsilane (TMS, 99.9%) from Nacalai Tesque Inc., Ltd., Kyoto, Japan as the internal standard. Accumulation was set at 32 scans without spinning and with 3.69 µs of pulse width (35'), and the pulsed delay was set at 10 s (resolution: 0.24 Hz). All of the deuterium solvents used in this study were purchased from E. Merck, Germany with the isotopic purity of 99.5 atom %D.

**Results**

**Optimization of NMR measurement** For direct monitoring of the quality of edible oils, we tried to establish a simple IV determination method by 1H-NMR. In this 1H-NMR measurement, successful determination of the number of olefinic protons may be essential, since IV is an index of the degree of unsaturation of oil and corresponds to the number of unsaturated bonds. Figure 1 shows the 1H-NMR spectra of rapeseed oil obtained using various deuterium solvents and operating temperatures. The solvent used was selected on the basis of the knowledge that a proton signal significantly shifts due to the degree of the solvent effect (dipole-dipole interaction between solvent and substrate) and temperature (Friebolin, 1991); CDCl₃ (25°C), CDCl₃-30% MeOD (25°C), pyridine-ᴅ₅ (25°C), benzene-ᴅ₆ (25°C), benzene-ᴅ₆-30% MeOD (25°C, 60°C). As shown in Fig. 1, CDCl₃ solvent systems unexpectedly gave little separation between olefinic protons and methine protons on the triglyceride backbone for rapeseed oil. Similar poor separations in CDCl₃ solvent systems were also observed for other edible oils used in this experiment (data not shown). For pyridine-ᴅ₅ (25°C), benzene-ᴅ₆ (25°C) and benzene-ᴅ₆-30% MeOD (25°C) solvent systems, adequate but not complete separation between both proton signals was observed. Only for the benzene-ᴅ₆-30% MeOD (60°C) solvent system, excellent separation was achieved due to the upfield shift of the methine protons (δ₆ 5.22-5.33) by the shielding of OH protons of the triglyceride backbone by methanol (olefinic protons; δ₆ 5.33-5.47).

**Estimation of IV by NMR measurement** The integral value obtained by 1H-NMR measurement is well known to be exactly proportional to the number of protons. Thus, we have attempted to establish the procedures to quantify the olefinic protons in edible oils using dioxane as an external proton standard (8 protons/mol). In this experiment, the number of olefinic protons in oil was determined by the ratio of the integral value of olefinic protons versus that of the methylene protons of dioxane (δ₆ 3.4-3.5) (Fig. 2). Namely, the number of olefinic protons (OP) could be accurately calculated by correcting the additional amount (50 µl/0.5 ml oil) of dioxane;

\[
\text{OP value (protons/ml oil)} = 8 \times \text{integral ratio} \times C \quad (1)
\]

where \(C\) (m) is the concentration of dioxane in oil. Figure 3 shows the relationship between the IV by Wijs-cyclohexane method and OP by the NMR method for various UV-irradiated oxidized rapeseed oils. Apparently, the plots of OP vs. IV gave a linear relationship with a correlation coefficient of 0.9998, and the obtained regression equation allows us to estimate IV of edible oil by 1H-NMR measurement without any complicated procedure: \(y = 1.684 \times 10^4 x - 14.473\) where \(x\) is the OP and \(y\) is the IV. In this experiment, the reproducibility of the 1H-NMR and IV measurements was high with a coefficient of variation of 0.64% (\(n\) = 10) and 0.39% (\(n\) = 6), respectively.

**Estimation of the oxidative rancidity of edible oils by NMR measurement** With attention to the protons in...
unsaturated fatty acid residues to which HPO is attached, we have attempted to estimate the oxidative rancidity of edible oil using the proposed \(^1\)H-NMR method. As shown in Fig. 2, the divinylmethylene proton was observed at \(\delta_1\) 2.68–2.84 in benzene-\(d_6\)-30% MeOD (60\(^\circ\)C), whose \(^1\)H-NMR intensity might decrease when HPO is attached or oil is oxidized. The benzene-\(d_6\)-30% MeOD (60\(^\circ\)C) solvent system was used in order to simultaneously evaluate the IV and oxidative rancidity in one measurement, although the divinylmethylene proton was completely separated in CDCl\(_3\). Figure 4 shows the relationship between the number of divinylmethylene protons (MP) calculated by Eq. (1) and the TBA value as an index of oxidative degradation. As a result, adequate linearity

![Dioxane](image)

**Fig. 2.** Typical \(^1\)H-NMR spectrum with dioxane as the external proton standard for unirradiated rapeseed oil. Solvent, benzene-\(d_6\)-30% MeOD. Operating temperature, 60\(^\circ\)C.

\[
y = 1.684 \times 10^4 x - 14.473 \\
(r = 0.9998)
\]

**Fig. 3.** Relationship between the number of olefinic protons obtained by the \(^1\)H-NMR method and the IV (C) for various UV-irradiated rapeseed oils. UV-irradiation time, 0; 47; 75; 92.5 h. Each datum was the mean of 6 replicates.

\[
y = -2.768 \times 10^4 x + 82.354 \\
(r = 0.9737)
\]

**Fig. 4.** Relationship between the number of divinylmethylene protons obtained by the \(^1\)H-NMR method and the TBA value (C) for various UV-irradiated rapeseed oils. UV-irradiation times are the same as in Fig. 3. Each datum was the mean of 6 replicates.
Table 1. Comparison of IV of commercially available edible oils by the proposed IH-NMR method with that of the conventional one.

| Oil          | IH-NMR method | Conventional method |
|--------------|---------------|---------------------|
| Corn oil     | 136.12±0.50   | 134.12±0.37         |
| Soybean oil  | 143.44±0.26   | 144.29±0.38         |
| Cottonseed oil| 121.56±0.88   | 124.72±0.81         |
| Sesame oil   | 117.58±0.51   | 120.17±0.43         |
| Olive oil    | 82.06±0.72    | 89.41±0.37          |

*Calculated by the obtained regression equation from the plots of OP (number of olefinic protons) vs. IV.

This IH-NMR method was also applied for evaluating the oxidative rancidity of edible oil. In this study, the oxidation of rapeseed oil by UV-irradiation may not reflect the practical hydroperoxidation process; during the first stage of autoxidation, the number of olefinic protons must not change, but our data were significantly affected. This may be caused by direct attachment of oxygen to the double bond, not to the vinyl methylene radical. However, adequate correlation between the number of divinylmethylene protons and the TBA value indicates that the proposed IH-NMR method could estimate the HPO oxidation from the decrease in divinylmethylene protons.

Consequently, the IH-NMR method with dioxane as the external proton standard has some advantages for the evaluation of the quality of food oils in terms of its high accuracy, reliability and rapidity without any complicated procedure.

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