

Structural Analysis of Triacylglycerols and Edible Oils by Near-Infrared Fourier Transform Raman Spectroscopy

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Fourier transform Raman spectroscopy was employed for structural analysis of triacylglycerols and edible oils. Raman spectra sensitively reflected structural changes in oils. Even slight structural fluctuation between triacylglycerols and free fatty acids led to obvious differences in Raman bands as shown by C–O–C stretching from 800 to 1000 cm^{-1} and the band at 1742 cm^{-1} . Structural difference in geometric isomers was easily distinguished as proved by C=C stretching at 1655 cm^{-1} (*cis*) shifting to 1668 cm^{-1} (*trans*) and by =C–H in-plane bending at 1266 cm^{-1} in *cis* disappearing in the *trans* isomer. Raman intensity at 1266, 1302, and 1655 cm^{-1} changed concomitantly with the change of double-bond content in oils. It showed that FT-Raman was capable of precisely reflecting the content of double bonds in oils. A linear correlation with high consistency between the Raman intensity ratio (ν_{1655}/ν_{1444}) and the iodine value was obtained for commercial oils. Based on the results, FT-Raman spectroscopy proved itself a simple and rapid technique for oil analysis since each measurement could be directly completed in 3 min without any sample modifications.

Index Heading: Triacylglycerols; Edible oils; Unsaturation; Raman spectroscopy.

INTRODUCTION

Edible oils play an important role in nutrition and food processing. They provide energy and essential fatty acids, increase the palatability of food, and react with other food constituents to enhance food quality. Since the properties and functionalities of edible oils show great variance from their original plant or animal sources, the development of a simple, fast, and direct technique to characterize their behaviors is of great importance. Usually, the quality evaluation and nutritional values of edible oils are determined by the type and the degree of unsaturation in fatty acids. The determination of the degree of unsaturation has been frequently performed with the standard iodine value (IV), which is relatively tedious and inaccurate because it is conducted according to classical titration methods.¹ More advanced techniques such as gas chromatography (GC),^{2–5} high-performance liquid chromatography (HPLC),⁶ and infrared spectroscopy (IR)⁷ have been tried in comparison with the traditional IV method and have also shown promising results in the quality analysis of oil and fat processing. While most of these advanced methods suffer from sample modifications and pollutant solvent usage, Raman spectroscopy offers nondestructive and direct measurement free from the unfriendly impact of reagents.

Dispersive Raman spectroscopy using visible wavelength excitation has been applied to the quantitative

analysis of oils.⁸ Although Raman spectroscopy exciting by visible wavelength is relatively inert to water interference and is simple for sample preparation, it suffers from fluorescence interference from the sample and the impurities present in it. Particularly, carotenes, coloring ingredients, and some nutrient supplements are usually added in oils and margarines that might enhance fluorescence interference during dispersive Raman measurements.⁹ The development of Fourier transform Raman spectroscopy (FT-Raman)¹⁰ by the use of Nd:YAG lasers (excited at 1064 nm), Michelson interferometers, and high-sensitivity GaAs detectors (liquid nitrogen cooled) has enlightened its application in oils. The advantages of FT-Raman spectroscopy in oil characterization include the following: (1) it is free from fluorescence interference, (2) it has less photodecomposition compared to classical dispersive Raman measurement, (3) it allows direct sample measurement with no sample destruction, and (4) there is no use of unfriendly reagents. Recently, numerous studies of FT-Raman in edible oils have been reported, including adulteration detection,¹¹ classification of oil and fat,¹² determination of unsaturation,^{9,13} and quantitative analysis.^{14,15} Most of these studies were directly correlating the Raman response to the traditional IV methods or to the GC and HPLC analysis upon estimating the degree of unsaturation in oils. As mentioned above, the traditional methods are relatively tedious and inaccurate since either the Wijs method or the Hanus method, the two most common procedures for measuring iodine value, must follow a series process of redox reaction and back-titration.¹ Thus, to further consolidate the Raman technique in lipid applications, we present here a more profound and systematic study on triacylglycerols (oil standards), whose investigations are still lacking. First of all, structural characterization of various triacylglycerols and their free fatty acids with different degrees of unsaturation was carried out by FT-Raman spectroscopy to reconfirm the consistency with previous studies. Secondly, the oil standards with different types (*cis/trans*) of unsaturation were characterized from their spectral difference. Thirdly, comparison of the Raman spectroscopic method with the conventional IV method for oil analysis was carried out to clarify the feasibility and reliability of FT-Raman spectroscopy in oil applications. Finally, the quality of various edible oils from a local supplier was evaluated by FT-Raman spectroscopy.

MATERIALS AND METHODS

Oil standards and free fatty acid standards with purity greater than 99%, including trioctadecanoin, *cis*- Δ^9 -trioctadecenoin, *trans*- Δ^9 -trioctadecenoin (trielaidin), and

Received 19 August 2002; accepted 13 December 2002.

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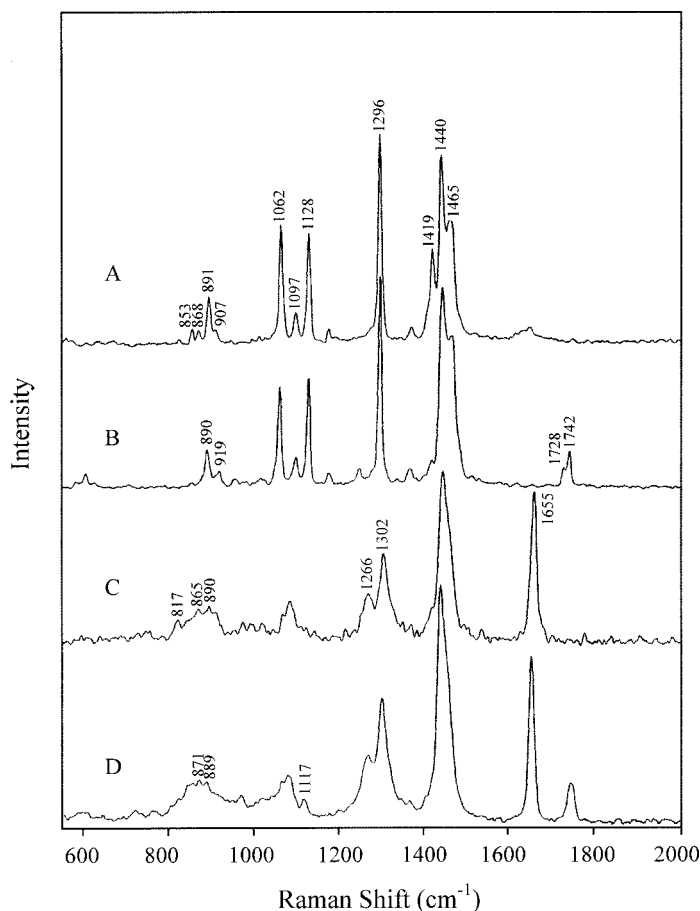


FIG. 1. FT-Raman spectra of triacylglycerols and free fatty acids: (A) hexadecanoic acid, (B) trihexadecanoin, (C) *cis*-9-hexadecenoic acid, and (D) *cis*- Δ^9 -trihexadecenoic acid.

cis,cis- Δ^9,Δ^{12} -trioctadecadienoin, *cis,cis,cis*- $\Delta^9,\Delta^{12},\Delta^{15}$ -trioctadecatrienoin, trihexadecanoin, *cis*- Δ^9 -trihexadecenoic acid, tripalmitelaidin, hexadecanoic acid, *cis*-9-hexadecenoic acid, all-*cis*-5,8,11,14,17-ethyl eicosapentaenoate, and all-*cis*-4,7,10,13,16,19-ethyl docosahexaenoate, were purchased from Sigma Co. (Nu-Chekprep, Elysian, MN). All reagents, CHCl_3 , Wijs solution (Merck KGaA, Darmstadt, Germany), $\text{K}_2\text{Cr}_2\text{O}_7$, HCl, $\text{Na}_2\text{S}_2\text{O}_3$ (Hayashi Pure Chem. Ind. Ltd., Osaka, Japan), KI, and starch (Wako Pure Chem. Ind. Ltd., Osaka, Japan), were analytical reagent grade. Edible oils including rice bran oil, soybean oil, sunflower oil, canola oil, sesame oil, tea seed oil (ku-char oil), blend oils, palm oil, chicken oil, and lard from different manufacturers were generously provided from a local supplier (Sei-I Oil company, Chiayi, Taiwan).

Fresh ku-char oil and soybean oil were also prepared directly from ku-char seeds and soybean in our laboratory. All sample oils were deposited in brown glass vials, sealed with a cap, and stored at -18°C pending further analysis.

The FT-Raman measurement was accomplished by the Bruker instrument, RFS-100 FT-spectrophotometer (Bruker Optik GmbH, Lubeck, Germany). Continuous-wave near-infrared excitation at 1064 nm was provided by a diode laser pump (Nd:YAG laser, Coherent Lubeck GmbH, Lubeck, Germany), and the laser power at the sample position was about 100 mW. An aliquot (~ 0.5

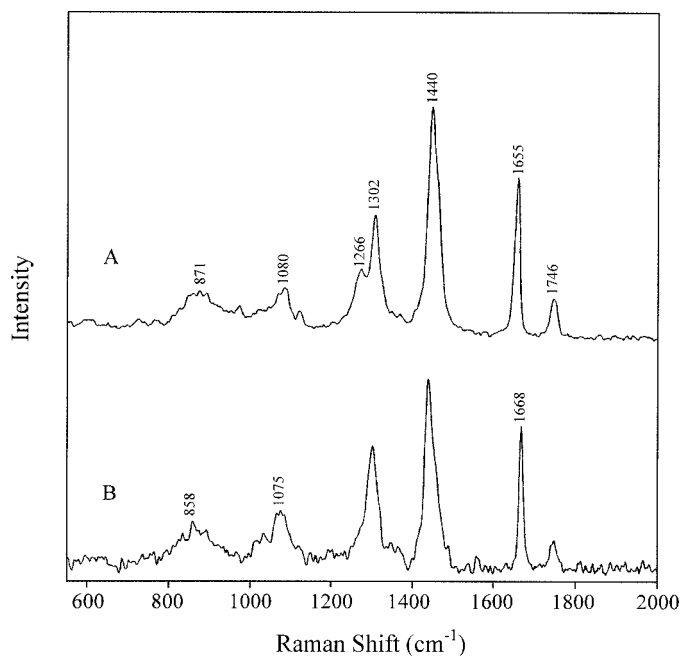


FIG. 2. FT-Raman spectra of oil standards with different types of unsaturation: (A) *cis*- Δ^9 -trioctadecenoin, and (B) *trans*- Δ^9 -trioctadecenoin.

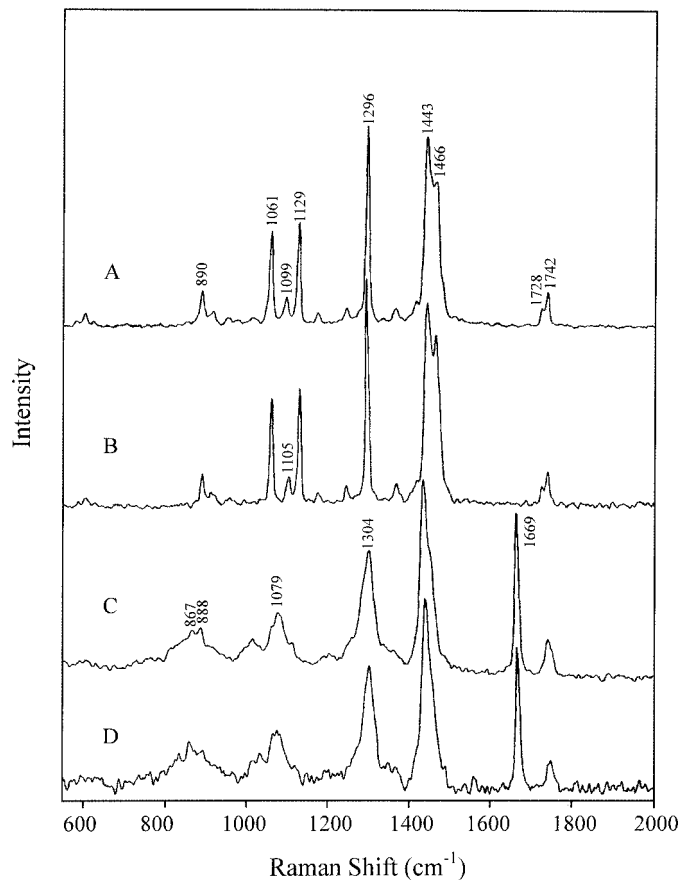


FIG. 3. FT-Raman spectra of oil standards with different numbers of carbon atoms: (A) trihexadecanoin, (B) trioctadecanoin, (C) tripalmitelaidin (C16-*trans*), and (D) trielaaidin (C18-*trans*).

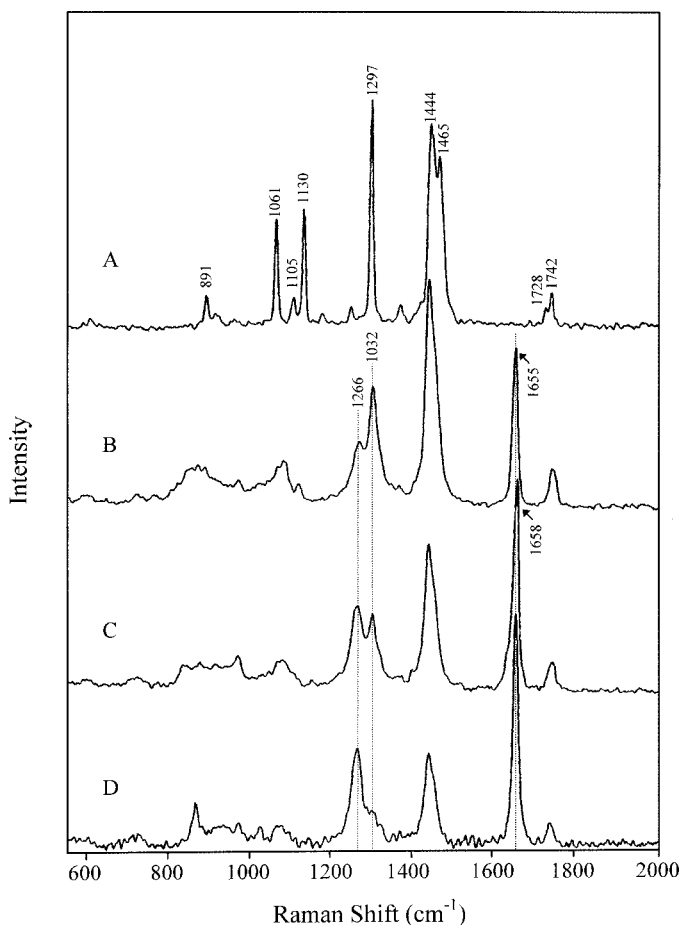


FIG. 4. FT-Raman spectra of oil standards with different degrees of unsaturation: (A) trioctadecanoin, (B) *cis*- Δ^9 -trioctadecenoin, (C) *cis,cis*- Δ^9,Δ^{12} -trioctadecadienoin, and (D) *cis,cis,cis*- $\Delta^9,\Delta^{12},\Delta^{15}$ -trioctadecatrienoin.

mL) of each sample was introduced into a capillary quartz tube, which was fixed by putting it onto a stainless steel holder, and the laser beam was focused on the sample. The scattered light from the sample was collected at 180° with an ellipsoidal mirror and was passed through the Michelson interferometer. A liquid nitrogen cooled GaAs detector was employed to provide superior signal-to-noise performance. Raman spectra were produced over the Raman shift $0\text{--}3500\text{ cm}^{-1}$. Typically, 50–100 interferograms were coadded at 4 cm^{-1} resolution with a sampling time of less than 3 min.

The calibration curve of the Raman intensity ratio (ν_{1655}/ν_{1444}) against the number of double bonds per each fatty acid was constructed from various oil standards with different double bonds. This curve might be used to estimate the total unsaturation of various edible oils. Each numerical calculation of the Raman intensity ratio was based on the average of triplicate measurements for each oil standard.

The determination of IV for various edible oils was analyzed in triplicate by the Wijs method.¹ Briefly, 0.15–0.30 g sample was dissolved in 10 mL CHCl_3 and 20 mL Wijs solution, then stored in a dark place for 2 h. After that, 10 mL of 15% KI was added into the solution, and the volume was adjusted to 100 mL with deionized, distilled water. The solution was then titrated with 0.1 N

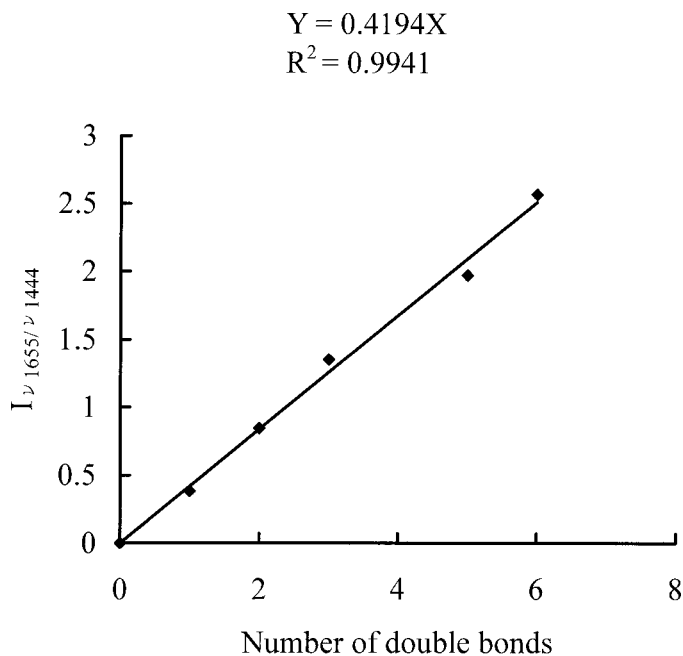


FIG. 5. Correlation of the Raman intensity ratio (ν_{1655}/ν_{1444}) to the number of double bonds for randomly selected oil standards and alkyl derivatives of fatty acids including trioctadecanoin, *cis*- Δ^9 -trioctadecenoin, *cis,cis*- Δ^9,Δ^{12} -trioctadecadienoin, *cis,cis,cis*- $\Delta^9,\Delta^{12},\Delta^{15}$ -trioctadecatrienoin, ethyl eicosapentaenoate, and ethyl decosahexaenoate.

$\text{Na}_2\text{S}_2\text{O}_3$, which was first standardized with solution containing 10 mL 10% KI, 5 mL HCl (35%), and 25 mL 0.1 N $\text{K}_2\text{Cr}_2\text{O}_7$. A correlative curve was then sketched between the Raman intensity ratio (ν_{1655}/ν_{1444}) and the IV value of various commercial oils to assure the consistency between the Raman spectroscopic method and the conventional IV method.

RESULTS AND DISCUSSION

The quality evaluation of fat or oil is usually carried out by determining its iodine value, which is a measure of degree of unsaturation, the number of carbon–carbon double bonds in relation to the amount of fat or oil. The Wijs method and the Hanus method have been the most common procedures to measure iodine value.¹ Briefly, a quantity of fat or oil is reacted with a measured amount of iodine. The excess amount of iodine left at the end of halogenation is then measured to calculate the amount of iodine consumed, which directly reflects the amount of unsaturation in oil and fat. Since both methods must follow a series of chemical reactions and back-titrations, they are relatively inaccurate and laborious. To develop a rapid analysis of fat and oil is of great importance in the food industry. Previous studies^{10–15} have successfully demonstrated that FT-Raman is an excellent alternative for the rapid analysis of oil and fat compared to the classical IV method. However, most previous Raman studies were basically correlating the Raman intensity of the alkyl derivative of triacylglycerol to IV. Whether the derivative processes will influence or disturb the quality of Raman analysis is not mentioned. To further confirm the potential of FT-Raman in the analysis of fat and oil, we made a more detailed study of free fatty acids, triacylglycerols (oil standards), and edible oil samples. How FT-

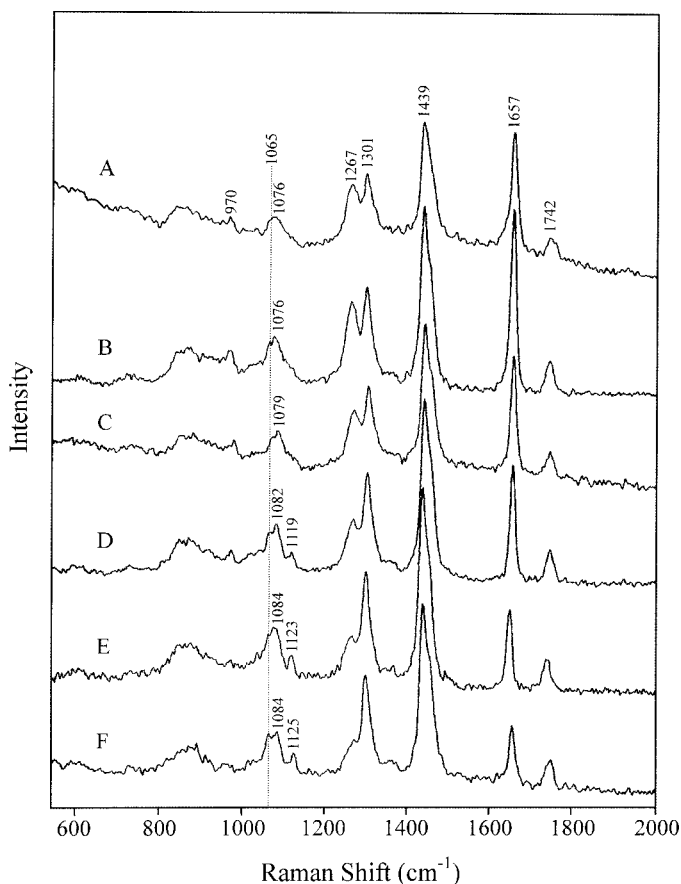


FIG. 6. FT-Raman spectra of some representative oils: (A) rice bran oil, (B) soybean oil, (C) sesame oil, (D) ku-char oil, (E) palm oil, and (F) lard.

Raman intensity responds accurately to the exact number of double bonds will be examined by measuring the high purity (greater than 99%) of free fatty acids and triacylglycerols with different contents of double bonds.

Triacylglycerols, esters of glycerol with three fatty acid molecules, are the major components of fats in plant or animal cells. It is of great interest to understand the structural alteration during esterification. Figure 1 shows the FT-Raman spectra of fatty acid standards and triacylglycerol standards. The high similarity in the Raman spectra between saturated fatty acid (hexadecanoic acid, Fig. 1A) and saturated triacylglycerol (trihexadecanoin, Fig. 1B), or between unsaturated fatty acid (*cis*-9-hexadecenoic acid, Fig. 1C) and unsaturated triacylglycerol (*cis*- Δ^9 -hexadecenoin, Fig. 1D), was not a surprise due to their structural similarity. However, there remained significant marks revealing individual structural specialty, such as the Raman stretching of $\nu_{C=O}$ (1742 cm^{-1}) in $-\text{CH}_2-\text{COOR}$ clearly indicating the structural difference between triacylglycerol and free fatty acid, comparing Fig. 1A to Fig. 1B or Fig. 1C to Fig. 1D. Saturated and unsaturated structures of fatty acid and triacylglycerol were easily distinguished according to the characteristic Raman bands. The vibrational bands at 1742, 1655, 1444, 1302, 1266, and 1076 cm^{-1} were assigned to the C=O stretching, C=C stretching (*cis* form), CH_2 scissoring, CH_2 twisting, =C-H in-plane bending, and C-C stretching modes, respectively.¹² Both fatty acid and triacylgly-

TABLE I. Raman intensity of ν_{1655}/ν_{1444} and the estimated double bonds of various oils.^a

Type of oil	$I_{\nu_{1655}/\nu_{1444}}$	NDB ^b
Rice bran oil	0.681 ± 0.017	1.625 ± 0.041
Soybean oil (I)	0.580 ± 0.010	1.384 ± 0.023
Soybean oil (II)	0.566 ± 0.007	1.349 ± 0.016
Sunflower oil	0.516 ± 0.009	1.229 ± 0.022
Canola oil	0.496 ± 0.006	1.183 ± 0.015
Sesame oil	0.457 ± 0.011	1.090 ± 0.027
Blend oil ^c (I)	0.417 ± 0.015	0.994 ± 0.035
Blend oil ^c (II)	0.374 ± 0.012	0.891 ± 0.028
Tea seed oil (I)	0.361 ± 0.009	0.860 ± 0.020
Tea seed oil (II)	0.354 ± 0.005	0.845 ± 0.013
Blend oil ^c (III)	0.328 ± 0.000	0.782 ± 0.000
Blend oil ^c (IV)	0.304 ± 0.012	0.725 ± 0.029
Chicken oil	0.295 ± 0.004	0.703 ± 0.010
Palm oil (I)	0.261 ± 0.015	0.623 ± 0.036
Palm oil (II)	0.247 ± 0.013	0.588 ± 0.032
Palm oil (III)	0.240 ± 0.015	0.572 ± 0.036
Palm oil (IV)	0.236 ± 0.018	0.563 ± 0.043
Palm oil (V)	0.215 ± 0.009	0.512 ± 0.021
Lard	0.206 ± 0.018	0.492 ± 0.043

^a Each data is mean $\pm$ S.D. ($n = 3$).

^b NDB: the average number of double bonds per each fatty acid of edible oil. The value was calculated by linear regression based on the plot in Fig. 5.

^c Blend oils with different percentages of palm oil were provided from different manufacturers.

cerol displayed conspicuous differences between saturated and unsaturated structures as illustrated by the Raman bands at 1655, 1302, and 1266 cm^{-1} , which was consistent with previous studies.^{10–15} Besides, Raman fingerprints in the range 800 to 1150 cm^{-1} due to the vibrational stretching of C–O–C¹⁶ exhibited apparent variation between triacylglycerol and its free fatty acid that might provide valuable information for oil and fat analysis.

Fourier transform Raman spectra of *cis*- Δ^9 -trioctadecenoin and *trans*- Δ^9 -trioctadecenoin (Fig. 2) showed the property of *cis/trans* isomer in oil. Feature Raman bands showed significant difference in the *cis/trans* isomers such as C=C stretching at 1655 cm^{-1} for the *cis* and 1668 cm^{-1} for the *trans* isomer; the =C–H *cis* deformation at 1266 cm^{-1} disappeared in the *trans* isomer, while CH_2 twisting (1302 cm^{-1}) was greatly enhanced in the *trans* isomer. This would provide an effective method to characterize edible oil in food. Raman spectroscopic comparison of triacylglycerols with different fatty acid is interesting, but lacks study. It is of great importance to understand the property of different long-chain alkyl groups in various oil standards. Both saturated and unsaturated triacylglycerols with different fatty acid are examined in Fig. 3. The Raman spectrum of trihexadecanoin (Fig. 3A) was almost identical to that of trioctadecanoin (Fig. 3B), except that the vibrational stretching of 1099 cm^{-1} in trihexadecanoin was shifted to 1105 cm^{-1} in trioctadecanoin. Also, the unsaturated tripalmitelaidin and trielaidin showed great resemblance in the spectrum beyond the range of 1150 cm^{-1} . However, Raman fingerprints from 800 to 1150 cm^{-1} exhibited significant differences in the unsaturated triacylglycerol. The FT-Raman spectra of oil standards with different degrees of unsaturation (Fig. 4) indicated that saturated oil (trioctadecanoin) and unsaturated oils behaved very differently in their vibrational bands. However, unsaturated oils including *cis*- Δ^9 -trioctadecenoin, *cis,cis*- Δ^9 - Δ^{12} -trioctadecadienoin, and *cis,cis,cis*-

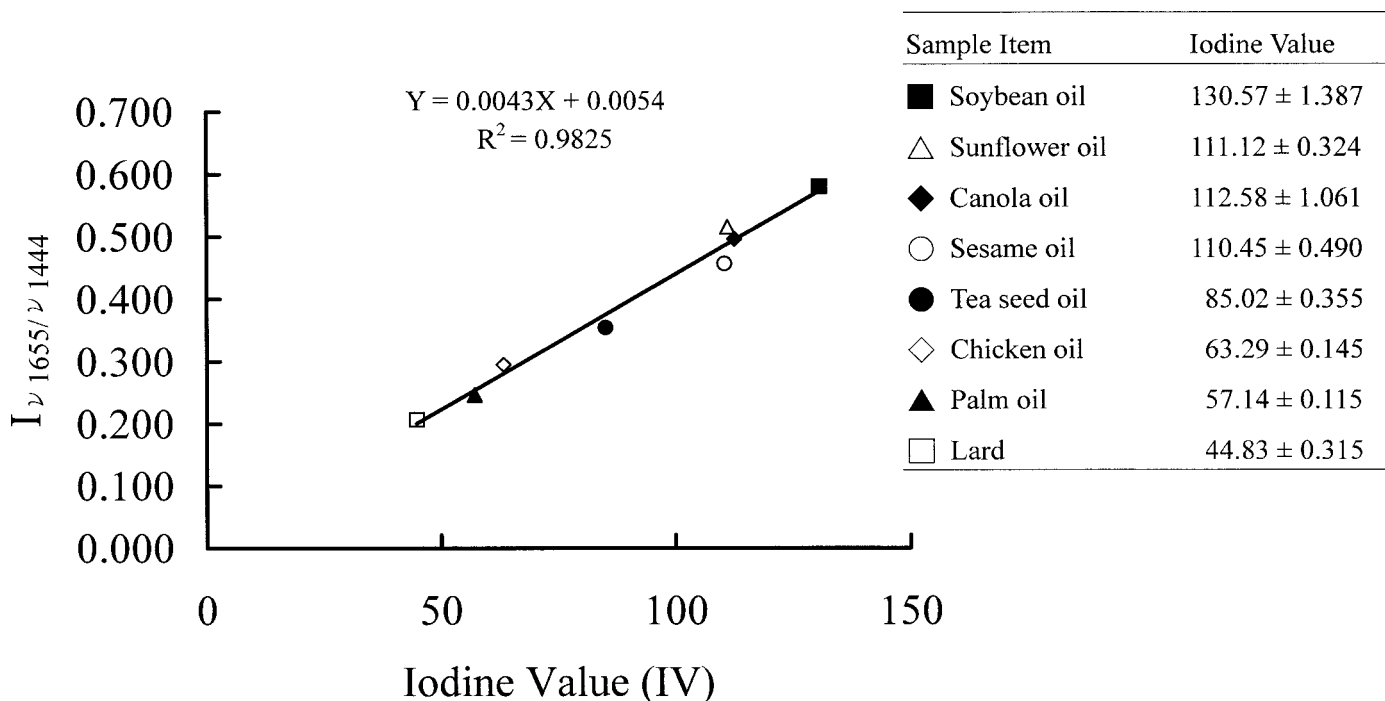


FIG. 7. Correlation of the Raman intensity ratio (ν_{1655}/ν_{1444}) to the iodine value of some commercial oils.

$\Delta^9\text{-}\Delta^{12}\text{-}\Delta^{15}$ -trioctadecatrienoin exhibited great similarity between their spectral patterns, except for the intensity of double bonds. The similarity in the spectral patterns is due to the similar major composition of unsaturated fatty acids. Carefully examining the Raman intensity at 1266, 1302, and 1655 cm^{-1} , it was obvious that the degree of unsaturation of oil standards reflected concomitantly the intensity change of these bands. The intensity of C=C (1655 cm^{-1}) and =C-H bending (1266 cm^{-1}) were exactly responding to the increase in unsaturation of oil standards. Contrarily, the intensity of CH_2 scissoring (1440 cm^{-1}) and CH_2 twisting (1302 cm^{-1}) decreased as oil became more unsaturated. This is consistent with previous studies;¹² therefore, we can easily detect the degree of unsaturation by determining the Raman intensity ratio ν_{1655}/ν_{1444} .

Fatty acids in biological systems usually contain an even number of carbon atoms and the configuration of the double bonds in most unsaturated fatty acid is the *cis* form. To further confirm that Raman measurements were faithfully reflecting the degree of unsaturation in oils, we performed Raman measurements by choosing high purities of a certain type of oil standard (all in *cis* form) with different contents of double bonds. Then, we correlated the relationship between the Raman intensity ratios (ν_{1655}/ν_{1444}) and the number of double bonds. An extremely high correlative coefficient ($R^2 = 0.9995$) was obtained that further supported the capability of Raman application in the oil industry. Compared to previous studies,¹² in which methyl derivatives of fatty acids were used to evaluate Raman intensity vs. IV, we here show a more precise result without any interference from the process of sample derivatization. Since the oil standards chosen in this study were in *cis* form, the possibility of interference from the *trans* isomer was ruled out. Besides, the high resolution (4 cm^{-1}) of FT-Raman can definitely distin-

guish the C=C vibrational mode of the *cis* isomer (1655 cm^{-1}) from the *trans* isomer (1668 cm^{-1}). Generally, edible oils and fats usually contain mixtures of various fatty acids. To facilitate FT-Raman as a general tool for oil analysis, we purposely correlated the Raman intensity ratio (ν_{1655}/ν_{1444}) with the number of double bonds of various oil standards and with the methyl derivative of fatty acids, which was randomly selected. The application of FT-Raman in oil and fat analysis was quite reliable as evidenced by the high consistency ($R^2 = 0.9941$) in the correlation of the Raman intensity ratio to the number of double bonds (Fig. 5). The decrease of R^2 value, from 0.9995 to 0.9941, upon the test of various oil standards is understandable because of the sample variation in the latter.

Based on the fruitful results described above, characterization of various edible oils described in the Materials and Methods section was carried out by FT-Raman spectroscopy. The Raman spectra of some representative oils and fats including rice bran oil (Fig. 6A), soybean oil (Fig. 6B), sesame oil (Fig. 6C), ku-char oil (Fig. 6D), palm oil (Fig. 6E), and lard (Fig. 6F) were displayed. Apparently, the double bonds in most edible oils were in *cis* form, as evidenced by the Raman stretching of C=C at 1657 cm^{-1} . In addition to the different degrees of unsaturation, indicated by the change of Raman intensity at 1267, 1301, and 1657 cm^{-1} , there were obvious differences in the Raman fingerprints from 800 to 1200 cm^{-1} for various oils, which was mainly due to the vibrational stretching of C-O-C. The different behaviors in various oils might be closely related to their original sources. According to the plot of Fig. 5, the number of double bonds per each fatty acid was calculated from the equation $Y = 0.4194X$. The results obtained from linear regression are listed in Table I. It shows that the degree of unsaturation of most plant oils was higher than that of

chicken oil and lard, which were the two most popular animal fats in the local area. Among them, rice bran oil and soybean oil present higher degrees of unsaturation, while lard contained the least amount of unsaturated fatty acid. From the nutritional point of view, the intake of higher degrees of unsaturated oils instead of saturated fats can lower blood cholesterol levels and lessen the risk of atherosclerosis.¹⁷ Based on current Raman study, rice bran oil and soybean oil might provide better quality for consumer health. Both blend oils and palm oils showed some variation in the degree of unsaturation between different manufacturers. According to the product information provided by the local supplier, Raman detection of the unsaturation is able to correctly respond to the quality of these commercial oils. Figure 7 displays the relationship of the Raman intensity ratio to the conventional measurement of IV for some commercial oils. Again, a linear correlation with $R^2 = 0.9825$ was obtained. A little drop in the R^2 value in commercial oils compared to that of oil standards might result from the intrinsic variation among various oils or from the fluctuation of IV determination. However, the consistency of Raman measurement compared to conventional IV determination remains convincing and reinforces the application of Raman spectroscopy in the study of edible oils. Thus, FT-Raman spectroscopy is able to offer a quick probe of the quality evaluation in the oil industry. In summary, the high correlation coefficient of Raman intensity vs. the number of double bonds or the IV in this report proved that FT-Raman spectroscopy could accurately detect the degree of unsaturation in edible oils. Thus, the quality evaluation of edible oils is easily obtained by Raman measurement.

We demonstrated here that FT-Raman spectroscopy is qualified as a simple, easy, nondestructive, and rapid method in the characterization of oils and fats since each measurement could be completed directly in 3 min without any sample modifications.

ACKNOWLEDGMENTS

Financial support (NSC-90-2113-M-415-002) from the National Science Council, Taiwan, is much appreciated.

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