

Determination of degree of unsaturation in edible oils using coherent anti-Stokes Raman scattering spectroscopy

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We demonstrate the use of coherent anti-Stokes Raman scattering (CARS) spectroscopy for determination of degree of unsaturation in edible oils. To this end, we have measured CARS spectra of six kinds of commercial edible oils having different degree of unsaturation, and it was found that the spectral intensity ratio ($I_{\text{C-H}}/I_{\text{C-H}}$) of two specific bands in C-H stretch region depends on the degree of unsaturation of sample. Based on this relation, the mass unsaturation ($N_{\text{C-H}}/N_{\text{C-H}}$) of six oils was estimated through the results of CARS spectroscopy in five fatty acids (FA), which are major component of edible oils. In addition, we obtained the calibration curve for determining iodine value (IV), and the result of our work was well matched with referred IV. Copyright © 2014 John Wiley & Sons, Ltd.

Keywords: coherent anti-Stokes Raman scattering; edible oils; fatty acids; lipids; degree of unsaturation

Introduction

Edible oils have been playing an important role in food industry as a widespread ingredient in dish as well as a source of nutrition, so that the oils used for food are directly linked with public health. Essential fatty acid in oils is well known for their health benefit, while unwholesome oils with low quality may cause cardiovascular disease such as arteriosclerosis.^[1] With increasing interest in public health, therefore, food scientists have paid attention to analysis of oil quality and production of high quality oil.^[2–5] For this, some characteristics of oils are investigated such as constituents, composition, and degree of unsaturation.^[6,7] Especially, degree of unsaturation, which represents how much carbon double bond has within the fatty acid chain, has been studied as a main property of oil (fat). The amount of unsaturation in fats has been described as iodine value (IV) indicating the mass of iodine consumed by 100 g of chemical substance, and is usually determined by chemical methods such as gas chromatography and like Wijs iodine method.^[8,9] However, these chemical methods have some limitations: time-consuming, tedious, and occurring errors during chemical experiments,^[10] so that the development of powerful analytical tools has been motivated. Therefore, spectroscopic techniques including IR, Raman spectroscopy has emerged, replacing traditional method.

The Raman technique, based on the nature that the scattered inelastic light contains the chemical information of specimens, has been paid attention as a tool for non-destructive chemically-selective *in situ* without any treatment of sample. Yet the spontaneous Raman scattering has its own set of problems: weak signal and interruption of fluorescence signal. To overcome these limitations, many advanced Raman techniques have been introduced: FT-Raman, visible spectral range Raman, and so on. Thanks to those, analysis of oil by means of Raman spectroscopy has been vigorously conducted, and the studies showed that Raman spectroscopy can determine IV as well as discriminate the genuine oil.^[11–16] More recently combined spectroscopic

techniques with microscopy, microspectroscopy has attracted high attention for oil research, providing quantitative and qualitative information.^[17–20] These trends suggest that Coherent anti-Stokes Raman scattering (CARS) microspectroscopy provides much usefulness in analysis of oils (fat).

As an advanced Raman technique, CARS is a four-wave mixing process requiring two laser beams called 'pump' (ω_p) and 'Stokes' (ω_s). When differences between wavenumber of two beams accord with the resonant wavenumber of sample, a strong anti-Stokes process occurs at wavenumber $\omega_{as} = 2\omega_p - \omega_s$, which is CARS signal. Because the strong CARS signal is only generated in small focal volume, it provides sub-micron spatial resolution at video rate.^[21] Besides, the anti-Stokes signal is free from fluorescence of the specimen because of spectrally blue-shifted signal, resulting to enhanced signal to noise ratio. Also, CARS has high sensitivity to aliphatic C-H vibrational mode, which are abundant in oils (fat).^[21] Although CARS microspectroscopy in fat research has been actively studied with the aforementioned advantages, such studies are mainly focused on bio imaging.^[22–25] This is because the CARS signal intrinsically contains nonresonant signal that arises from electronic contributions to the third-order susceptibility. The electronic contribution is not related with chemical structure of samples so that nonresonant signal interrupts quantitative analysis.^[26]

However, the quantitative CARS can be conducted by depressing the nonresonant signal through experimental approach or data processing.^[27] Recently, Heinrich *et al.* conducted CARS imaging to identify saturated and unsaturated fatty acids in micro-emulsions and cells using wide-field CARS.^[28] They used a nanosecond Nd:YAG laser pulse and an optical parametric

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oscillator pulse as Stokes and pump beams, respectively, and showed that a quantitative distinction of saturated and unsaturated fatty acids could be demonstrated within biological samples by using the CARS microspectroscopy.

In this paper, we performed femtosecond CARS spectroscopy in six commercial oils and five FAs and showed that the IV can be obtained from the CARS intensity ratio ($I_{\text{C-H}}/I_{\text{C-H}}$) of specific two C-H stretch bands (=C-H , -C-H). An ultra-short CARS setup employing a femtosecond laser and a photonics crystal fiber was used to minimize the nonresonant signal.^[29,30] From the experiment, we observed the relation between the CARS intensity ratio and mass unsaturation of the oils and found the calibration curve for estimating IV of edible oils.

Materials and methods

Materials

A set of six commercial edible oils (sesame, rapeseed, perilla, olive, grape seed, and evening primrose) and five FAs (palmitic, stearic, oleic, linoleic, and linolenic acids), which are major constituents of edible oils, were used in this experiment. The edible oils were obtained from a local market, and five FAs were purchased from Sigma-Aldrich. Oleic (OA), linoleic (LA), and linolenic (LNA), which belong to unsaturated fatty acid group, and six oils are liquid state at room temperature (21°C), so that they did not need additional sample preparation procedures. Palmitic acid (PA) and stearic acid (SA), which belong to saturated fatty acid group, are solid states at room temperature, so they were melted on hot plate to achieve high CARS signal.

Coherent anti-Stokes Raman spectroscopy

Raman measurements were carried out using one kerr-lens mode-locked femtosecond laser with an output power of 450 mW, a repetition rate of 56 MHz, a pulse duration of 60 fs, and a center wavelength of 800 nm. The laser beam is divided into 3:1 to utilize as a pump and Stokes beam. The nonresonant signal in our setup was minimized using a chirped-photonics crystal fiber; the detailed experimental explanations are described in previous literatures.^[22,23] The data were collected at 4 cm^{-1} resolution and the acquisition time was 0.085 s.

Results and discussion

CARS spectroscopy in edible oils

In nature, the vegetable oils exist as triacylglycerol mixtures composed of a glycerol backbone and three FAs, and they can be characterized by the content and unique distribution of FAs. The six oils used for this experiment are composed of several FAs, and major FAs are PA, SA, OA, LA, and LNA; 16-carbons PA and 18-carbons SA are composed of carbon-carbon single bonds (C-C), and OA, LA, and LNA containing 18 carbons include one, two, and three double bonds (C=C), respectively. Because those FAs have a different degree of unsaturation, the unique distribution of FAs in some oil determines the total unsaturation of them. Therefore, the amount of unsaturation of oil can be used for characterizing oils, and this chemical property can be explored by CARS spectra.

Figure 1(a) shows the CARS spectra of six edible oils in C-H stretch region, and each spectrum was normalized at 2858 cm^{-1} . In repeated experiments, the normalized spectra of each sample

showed the same spectrum, and Fig. 1(a) shows one of them of each oil. Three main bands were observed near 2858, 2930, and 3031 cm^{-1} , which correspond to symmetric and asymmetric ν (C-H) vibration of the terminal chains of methylene (CH_2) groups of aliphatic molecules, and ν (=C-H) vibration of olefinic molecules (RCH=CHR), respectively. As shown in Fig. 1(a), the CARS spectra of oils represent the same kind of bands and spectral distinctions with regard to intensity ratio of those bands; this supports that edible oils are composed of the same molecular bonds with different concentration of those bonds. Many spectroscopic researches have demonstrated that the spectral intensity ratio at specific bands is dependent on the degree of unsaturation in specimen.^[19] Here, two spectral bands were used for this investigation; the bands near 2858 and 3031 cm^{-1} .

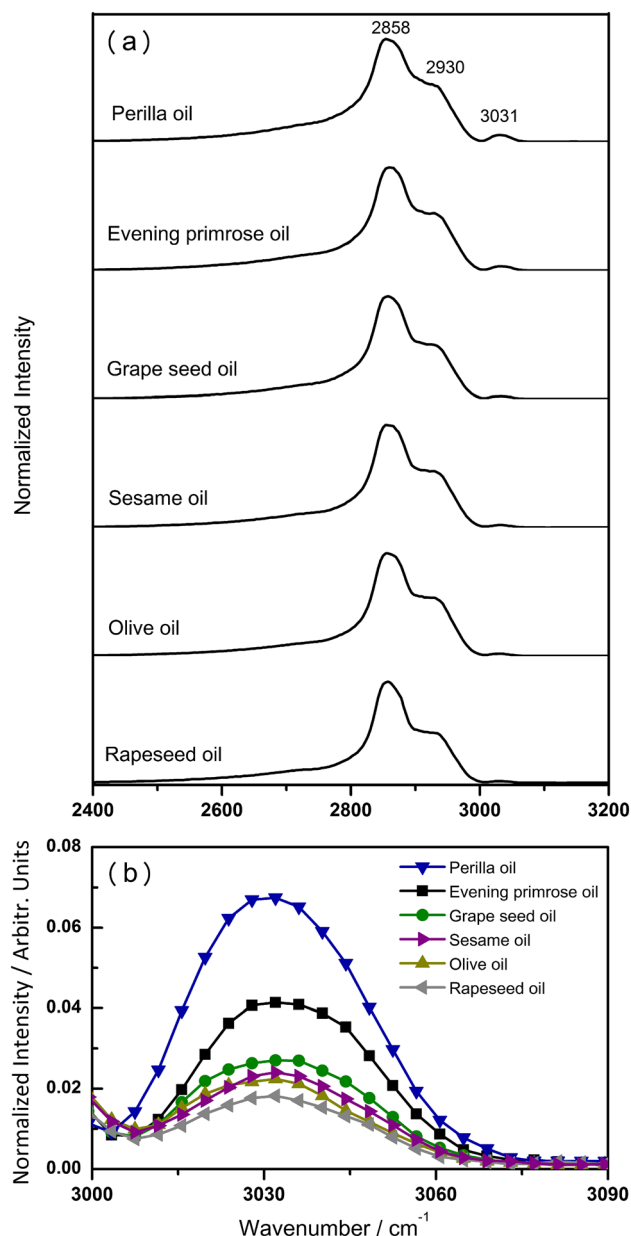


Figure 1. Normalized coherent anti-Stokes Raman scattering (CARS) spectra of six edible oils (a) in the region between 2400 and 3200 cm^{-1} . The spectra are separated in vertical direction for clarity. (b) The spectra are shown in the magnified region between 3000 and 3090 cm^{-1} for clear distinction of low CARS signal.

We also observed the change of the CARS signal at C=C vibration mode (1631 cm^{-1}) of the six oils. Intensity increased as the unsaturation degree increased. But there existed high nonresonant background signal, and the signal intensity was much weaker than C-H stretch mode. So we used C-H vibration region for the determination of the saturation degree.

Figure 1(b) represents CARS spectra of six edible oils in magnified region between 3000 cm^{-1} and 3090 cm^{-1} . Because the spectra are normalized at 2858 cm^{-1} , spectral intensity in the figure indicates relative intensity to that of band at 2858 cm^{-1} . Thus, the intensity of band at 3031 cm^{-1} represents intensity ratio I_{3031}/I_{2858} ($I_{\text{C-H}}/I_{\text{C-H}}$). This value, $I_{\text{C-H}}/I_{\text{C-H}}$, can be used to the determination of the degree of unsaturation of edible oils with the following reasons. First, because the CARS intensity is square-dependent on density of corresponding molecular binding when the number density of sample is high, the intensity ratio of specific two bands also quadratically depends on density ratio of corresponding two molecular bindings. Consequently, the ratio I_{3031}/I_{2858} is associated with $N_{\text{C-H}}/N_{\text{C-H}}$ in oils where the notation N_k represents the number of k bonds per molecule. The details are given in chapter 3.3. Second, the ratio $N_{\text{C-H}}/N_{\text{C-H}}$ is related to mass unsaturation (degree of unsaturation). The value is numerically consistent with $N_{\text{C=C}}/N_{\text{CH}_2}$ because the number of C-H bonds in corresponding group is twice the number of those groups. Therefore, the ratio $N_{\text{C-H}}/N_{\text{C-H}}$ indicates the number of C=C bonds per methylene chain length, and it represents mass unsaturation.^[19] Therefore, we can speculate that the oils with strong intensity ratio $I_{\text{C-H}}/I_{\text{C-H}}$ is affected by high mass unsaturation (degree of unsaturation), and it shows the possibility to characterize them using those relations. The specific relation between two values, the CARS intensity and mass unsaturation, can be achieved by exploiting CARS spectroscopy in five FAs, which are major ingredients of organizing vegetable oils.

CARS spectroscopy in FAs

From CARS spectra of FAs, three major bands were observed as shown in Fig. 2(a). The bands located at 2874 cm^{-1} and 2930 cm^{-1} correspond to symmetric and asymmetric ν (C-H) vibration in methylene (CH_2) groups of aliphatic molecules, and the band at 3036 cm^{-1} exhibits the characteristic of the ν (=C-H) vibration; this band was observed in only unsaturated fatty acid group as shown in Fig. 2(b). The slightly shifted bands between the spectra of oils and FAs were caused by the structural difference between free FA and triacylglycerol. As shown in Fig. 2(a), the spectra of FAs show the high similarity with those of edible oils. In addition, the spectral change according to degree of unsaturation can be observed. For clear distinction about unsaturation indicated near 3030 cm^{-1} , the magnified spectra region between 3000 and 3090 cm^{-1} was shown in Fig. 2(b). The order of the intensity of band at 3036 cm^{-1} in FAs (LNA > LA > OA) is consistent with the order of $N_{\text{C=C}}$ as expected. With chemical information of FAs (listed in Table 1), more rigorous relation between CARS intensity ratio I_{3036}/I_{2874} ($I_{\text{C-H}}/I_{\text{C-H}}$) and $N_{\text{C-H}}/N_{\text{C-H}}$ can be obtained and is described in the next section.

Determination of degree of unsaturation

Figure 3(a) shows the dependence of CARS intensity ratio I_{3036}/I_{2874} ($I_{\text{C-H}}/I_{\text{C-H}}$) to $N_{\text{C-H}}/N_{\text{C-H}}$. The solid line in this figure indicates the fitting curve based on the CARS data of FAs; the function is given in the form

$$y = a\sqrt{x} \quad (1)$$

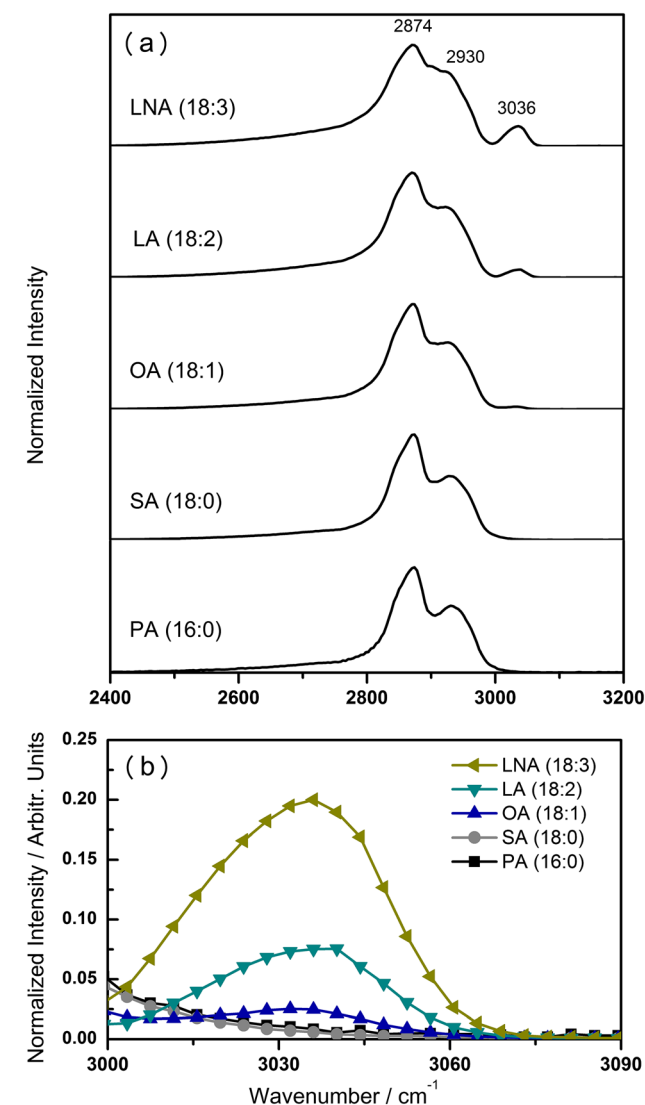


Figure 2. Normalized coherent anti-Stokes Raman scattering (CARS) spectra of five fatty acids (a) in the region between 2400 and 3200 cm^{-1} (b) in the magnified region between 3000 and 3090 cm^{-1} for clear distinction of low CARS signal.

Table 1. Parameters of fatty acids and experimental results

	$N_{\text{C-H}}$	$N_{\text{C-H}}$	$N_{\text{C-H}}/N_{\text{C-H}}$	$I_{\text{C-H}}/I_{\text{C-H}}$	IV
PA (16:0)	0	28	0.000	0.000	0
SA (18:0)	0	32	0.000	0.000	0
OA (18:1)	2	28	0.071	0.013	90
LA (18:2)	4	24	0.166	0.053	180
LNA (18:3)	6	20	0.300	0.150	273

The factors, $N_{\text{C-H}}$ and $N_{\text{C-H}}$, represent the number of =C-H bond and C-H bond of methylene (CH_2) group per molecule. $I_{\text{C-H}}/I_{\text{C-H}}$ indicates the CARS intensity ratio at specific two bands (=C-H, C-H), and iodine value is referred to Ham's work.^[9]

where x and y are $I_{\text{C-H}}/I_{\text{C-H}}$ and $N_{\text{C-H}}/N_{\text{C-H}}$, and it shows high correlation ($R^2 = 0.9932$) at $a = 0.75$. The fitting equation representing relation of mass unsaturation and CARS intensity ratio can be explained by CARS theory.

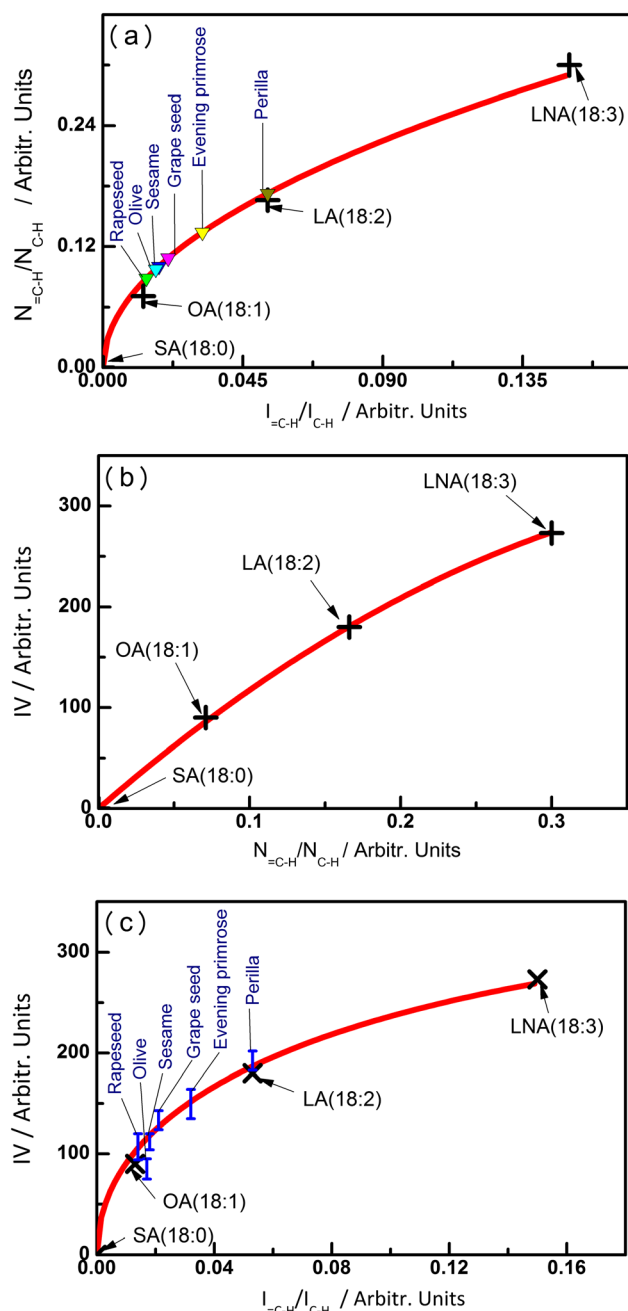


Figure 3. Relations to determine degree of unsaturation of oils from coherent anti-Stokes Raman scattering (CARS) data. (a) Dependence of the CARS intensity ratio (I_{C-H}/I_{C-H}) on the mass unsaturation (N_{C-H}/N_{C-H}) of fatty acids (FAs). The solid line indicates the fitting curve, which is $y = 0.75\sqrt{x}$ where x and y are I_{C-H}/I_{C-H} and N_{C-H}/N_{C-H} . (b) Dependence of IV on N_{C-H}/N_{C-H} in FAs. The solid line designates the parabola fitting curve, which is $z = -1304y(y-1)$ where the values y and z indicate N_{C-H}/N_{C-H} and IV. (c) Calibration curve estimating IV using CARS data and application of edible oils. The solid line designates the parabola fitting curve, which is $z = -733.5x + 978\sqrt{x}$ where the values x and z indicate I_{C-H}/I_{C-H} and IV.

In principle, CARS signal is proportional to square of the third-order susceptibility term, which is divided by the resonant and nonresonant term:

$$I_{CARS} \propto |\chi^{(3)}|^2 = |\chi_R + \chi_{NR}|^2 = |\chi_R|^2 + 2\text{Re}\{\chi_R\}\chi_{NR} + |\chi_{NR}|^2 \quad (2)$$

The nonresonant term (χ_{NR}) is assumed as the background contribution because it appears regardless of the wavenumber

combinations. On the other hand, the resonant term near j -th Raman mode ($(\omega_{pu} - \omega_s) \approx \Omega_j$) is given by

$$\chi_{Rj}^{(3)}(\omega_{as}) = \frac{A_j}{\Omega_j - (\omega_{pu} - \omega_s) - i\Gamma_j} = \frac{A_j}{\Omega_j - \delta - i\Gamma_j}, \quad (3)$$

where Ω_j , A_j , Γ_j are eigenfrequency, oscillator strength, and linewidth of j -th Raman mode, respectively. This relation shows that the signal experiences a resonant enhancement when the term Ω_j ($\omega_{pu} - \omega_s$) goes to 0, generating the strong CARS signal. By substituting χ_R to Eqn (3), the CARS intensity can rewrite as follows:

$$I_{CARSj} \propto \frac{A_j^2}{(\Omega_j - \delta)^2 - \Gamma_j^2} + 2\chi_{NR}^{(3)} \frac{A_j(\Omega_j - \delta)}{(\Omega_j - \delta)^2 - \Gamma_j^2} + (\chi_{NR}^{(3)})^2 \quad (4)$$

As shown in this equation, interpretation of CARS spectra is not easy because the total CARS signal is composed by several terms, which have different causes. However, when the resonant term is high enough compared with nonresonant term ($\chi_R \gg \chi_{NR}$), the first term is dominant and other terms can be neglected. Then the CARS intensity is square-dependent on oscillator strength. Therefore, the CARS intensity ratio of two spectral bands corresponds to the square of the relative concentration of those vibrational oscillators. Here, we regarded that the density of vibration oscillator at j -th mode affects the oscillator strength only at the mode.

In our work, we reduced nonresonant signal using experimental methods.^[29,30] Also, the used samples have high concentration without solvent, so that the intensity ratio (I_{C-H}/I_{C-H}) theoretically depends quadratically on concentration of vibrational oscillators (N_{C-H}/N_{C-H}). In other words, mass unsaturation (N_{C-H}/N_{C-H}) is expressed by square root function of intensity ratio I_{C-H}/I_{C-H} , and it can well explain the fitting function given in Eqn (1). In addition, this relation shows that CARS intensity ratio can approximate mass unsaturation of edible oils, and triangles in Fig. 3(a) designate those of six oils.

Calibration curve for estimating IV

The experimentally attained mass unsaturation of oils can be converted to IV by adopting well-defined experimental IV of FAs.^[19,24] The obtained relation between IV and N_{C-H}/N_{C-H} is shown in Fig. 3(b), and the numerical relation of them was obtained using parabolic curve fitting method. The function was in the form

$$z = -by(y-1) \quad (5)$$

where the values y and z indicate N_{C-H}/N_{C-H} and IV. This fitting curve well matched ($R^2 = 0.9996$) to the data indicated in Table 1 where $0 \leq y \leq 0.3$ at $b = 1304$.

From the Eqns (1) and (5), the relation between I_{C-H}/I_{C-H} and IV is obtained as follows:

$$z = -a^2bx + ab\sqrt{x} \quad (6)$$

The values x and z are I_{C-H}/I_{C-H} and IV. The resultant calibration curve for evaluating the IV and the application of edible oils are represented in Fig. 3(c). This determined IV from this work was compared to the referred IV reported in previous literatures^[31–33] and websites (ec21). The referred IV values of the six oils which are listed in Table 2, are marked as blue vertical bars in Fig. 3(c). Here we used the x -values (I_{C-H}/I_{C-H}) of them in the figure with our experimental CARS data. As shown in Fig. 3(c), the estimated IV from calibration curve is well consistent with reference, while

Table 2. Referred^[31–33] and experimental iodine value of six edible oils

Edible oils	Referred IV	Our data
Perilla oil	180–205	187
Evening primrose oil	135–164	152
Grape seed oil	124–143	126
Sesame oil	104–120	118
Olive oil	75–95	115
Rapeseed oil	94–120	106

the referred IV of olive oil is deviated from our curve. It is not clear whether the result came from the intrinsic property of olive oil or was caused from the purity problem of the oil. We are planning to analyze that oil to obtain clear answer for result of olive oil. Although more studies should be carried out, the results show the potential of CARS spectroscopy in characterizing edible oils. In addition, the results suggest that this approach can be directly used to inspect the quality of oils by determining the degree of unsaturation.

Conclusions

We described utilization of CARS spectroscopy to determine the degree of unsaturation of edible oil. To estimate the degree of unsaturation of oils, we preformed CARS spectroscopy in six edible oils and FAs, and observed the relation between the CARS intensity ratio and mass unsaturation of sample. Additionally, calibration curve for estimating IV of edible oils was obtained, and the determined IV from our work was well matched with the referred IVs. Interestingly, only the IV of olive oil was deviated from the referred value, which requires the further investigation. And we plan to analyze the various kinds of olive oils and compare the IV between our approach and the other methods for more reliable characterization. Also, the analysis of raw material as well as refined oils will be studied with CARS imaging. We think this study shows the potential of CARS spectroscopy, which provides chemically-selective information of sample *in situ* at microscopic scale in three dimensions, as an analytical tool for oil analysis.

Acknowledgements

This work was supported by the National Research Foundation (NRF) of Korea funded by the Ministry of Science, ICT & Future Planning (2011–0015520, 2008–0062606) and by grants from the Bio Imaging Research Center, and the 'Top Brand project' research project through a grant provided by GIST.

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