Rapid Determination of Saponification Value and Polymer Content of Vegetable and Fish Oils by Terahertz Spectroscopy

Feng Ling Jiang¹, Ikuo Ikeda¹, Yuichi Ogawa² and Yasushi Endo^{3*}

¹ Graduate School of Agricultural Science, Tohoku University (Sendai 981-8555, Japan)

² Graduate School of Agriculture, Kyoto University (Kyoto 606-8502, Japan)

³ School of Bioscience and Biotechnology, Tokyo University of Technology (Tokyo 192-0982, Japan)

Abstract: A rapid method for determining the saponification value (SV) and polymer content of vegetable and fish oils using the terahertz (THz) spectroscopy was developed. When the THz absorption spectra for vegetable and fish oils were measured in the range of 20 to 400 cm⁻¹, two peaks were seen at 77 and 328 cm⁻¹. The level of absorbance at 77 cm⁻¹ correlated well with the SV. When the THz absorption spectra of thermally treated high-oleic safflower oils were measured, the absorbance increased with heating time. The polymer content in thermally treated oil correlated with the absorbance at 77 cm⁻¹. These results demonstrate that the THz spectrometry is a suitable non-destructive technique for the rapid determination of the SV and polymer content of vegetable and fish oils.

Key words: vegetable oil, fish oil, saponification value, polymer content, terahertz spectroscopy

1 Introduction

Determination of the saponification value (SV) of fats and oils is extremely important for estimating their degree of average molecular weights. SV determination is especially necessary for defining the quality of fish oils, because their fatty acid composition of fish oils varies with fishing season and area, and some fish oils may include not only triacylglycerol (TAG) but also wax ester or diacyl glyceryl ether (DAGE). Titration is generally used as the official method¹⁾ for determining the SV of fats and oils, but it is difficult to obtain reliable results because highly skilled analysts are required to perform the titration and the conventional method takes more than 1 h to complete the analysis of a sample. The SV of vegetable oil can be calculated from the fatty acid composition estimated by gas liquid chromatography (GLC). However, the GLC method is time consuming and requires methylation pretreatment.

As polymers are formed during deep-frying with vegetable oils, the level of deterioration of the oil can be estimated by determining its polymer content. High-performance liquid chromatography (HPLC) is often used to determine the polymer content in fats and oils²⁾, but it requires the use of organic solvents such as tetrahydrofuran.

Recently, near-infrared (NIR) spectroscopy has become an accepted method for the determination of the quality of fats and oils because it does not require toxic organic solvents and because it is a rapid technique. Some researchers have applied the NIR spectroscopy for determining the values of free fatty acids^{3, 4)} and peroxide values^{5, 6)} of edible fats and oils. The McGill University IR Group^{7, 8)} developed the NIR method for determining the SV and iodine value (IV) of vegetable oils. In a previous study, we applied the NIR spectrometry to fish oils in the range of 2000 to 12000 cm⁻¹ to determine their SV and IV⁹⁾.

Our group has developed the terahertz (THz) spectrometry that enables the monitoring of the region between 10 and 400 cm⁻¹, and that can be applied to food components such as sugars¹⁰⁾ and drugs¹¹⁻¹³⁾. In a previous study¹⁴⁾, we also applied the THz spectroscopy to fatty acids and their analogues, and we found that all fatty acids had the same absorption peaks but the carboxylic group or the ester bond affected the spectra.

In this paper, we report the application of the THz method for determining the SV and polymer content of vegetable and fish oils, and then we discuss the efficiency of THz method for analyzing fats and oils.

* Correspondence to: Yasushi Endo, School of Bioscience and Biotechnology, Tokyo University of Technology, Tokyo 192-0982, Japan

E-mail: endo@bs.teu.ac.jp

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2 Materials and Methods

2.1 Vegetable and fish oils

Refined coconut, corn, linseed, palm kernel, rapeseed, soybean and high-oleic safflower oils were provided by Nisshin OilliO Group, Ltd. (Tokyo, Japan). Refined rosy soldier, sardine, squid liver, and tuna oils were provided by Nippon Suisan Kaisha, Ltd. (Tokyo, Japan). Shark liver oil was provided by NOF Corp. (Tokyo, Japan). All vegetable and fish oils were of commercial grade.

2.2 Measurement of SV¹⁾

The titration method to determine SV in fish oils and the “Standard Methods for the Analysis of Fats, Oils and Related Materials” published by The Japan Oil Chemists’ Society (1996)¹⁾ were employed.

2.3 Measurement of polymer content²⁾

Thermally treated high-oleic safflower oils were dissolved in tetrahydrofuran (5 mg/mL) and a 25 μ L sample was injected into the HPLC. For HPLC analysis, two gel-filtration columns, Shodex GPC KF-8025 (300 mm $\times$ 8 mm i.d.) were used as columns. Polymers were eluted with tetrahydrofuran as a mobile phase at a flow rate of 1.0 mL/min at 40°C, and they were monitored with a refractive index detector.

2.4 Terahertz spectroscopy

A Fourier transform-infrared (FT-IR) spectrophotometer (JASCO Corp. Tokyo, Japan) was remodeled using a silicon broadband beam splitter and a deuterated L-alanine-doped triglycine sulfate (DLATGS) room-temperature detector, to obtain spectra in the range of 20 to 400 cm^{-1} . The light source was a high-pressure mercury lamp, which has a higher intensity than a ceramic lamp below 3 THz.

Vegetable and fish oils were dissolved in liquid paraffin at a concentration of 5% (w/w), and were placed in a polyethylene cell with a 1.0 mm path length and a volume of 1.0 mL. The polyethylene cell and liquid paraffin are well suited for THz spectrometry, as they have no notable absorption at 20 to 400 cm^{-1} . As a room-temperature reference, the THz absorption spectrum of the polyethylene cell was measured before the collection of the data for the fats and oils. The wavenumber resolution was 16 cm^{-1} and the

Fig. 1 The THz absorption spectra of vegetable oils.

accumulation was 128 times. The data represent the average of triplicate measurements.

3 Results and Discussion

3.1 Vegetable oil

Figure 1 shows the THz absorption spectra of coconut, corn, linseed, palm kernel, soybean and rapeseed oils as typical vegetable oils in the range of 20 to 400 cm^{-1} . All THz spectra were similar in spite of the different fatty acid compositions. Notable absorption peaks were found at 77 and 328 cm^{-1} , which may be due to the fatty acid, mainly the “-COO-”. These peaks were also observed for free fatty acid, but the absorption peak at 231 cm^{-1} detected in free fatty acids was not found in the spectra of the vegetable oils. Moreover, no peaks were found for aldehyde and ketone. The absorbance was seen to depend on the type of vegetable oils. Among six vegetable oils that were tested, coconut oil had the highest absorbance, followed by palm kernel oil, linseed oil, and soybean, corn, and rapeseed oils. **Table 1** shows the SV of vegetable oils, which were in the range of 182 to 252. The SV of coconut oil was 252 and the highest among 6 vegetable oils, whereas that of rapeseed oil was the lowest. These results suggest that the SV of vegetable oils could be related to the

Table 1 Saponification value of vegetable and fish oils.

Vegetable oil	Saponification value	Fish oil	Saponification value
Coconut	252	Tuna	191
Palm kernel	241	Sardine	183
Soybean	190	Squid liver	176
Linseed	189	Rosy soldier	113
Corn	189	Shark liver	20
Rapeseed	182		

absorbance in the THz region.

A correlation curve was made to demonstrate the relationship between the SV and the absorbance at 77 cm^{-1} . The value at 77 cm^{-1} was used as it could be derived from the “-COO-” in glyceride but not in methyl or ethyl esters, and the absorbance at 328 cm^{-1} could be markedly affected by the presence of moisture¹⁴). As shown in Fig. 2, a good linear relationship ($R=0.969$) was obtained between the SV and the absorbance.

3.2 Fish oil

Figure 3 shows the THz spectra of rosy soldier, sardine, shark liver, squid liver, and tuna oils as fish oils. All fish oils had the similar spectra in the range of 20 to 400 cm^{-1} , except for squid liver oil. They had notable absorption peaks at 77 and 328 cm^{-1} , and their spectral patterns were the same as those of the analyzed vegetable oils. These peaks may be also due to the “-COO-”. Squid liver oil had

Fig. 2 Plot of saponification value against THz absorbance at 77 cm^{-1} of vegetable oils.

Fig. 3 The THz absorption spectra of fish oils.

the highest absorption peak at 380 cm^{-1} . The intense red color of squid liver oil, due to its high vitamin A content might affect the THz absorption spectrum. The absorbance of fish oils also depended on the varieties. Squid liver oil had the highest absorbance among five fish oils that were tested, whereas rosy soldier oil had the lowest absorbance.

Table 1 shows the SV of the different fish oils. Fish oils cover a broad range SV(20-191). Among the five fish oils, the SV of sardine oil was the highest at 191, whereas rosy soldier oil had a lower SV(113). Rosy soldier oil had so much wax esters that the SV was lower than TAG-rich oil such as sardine or tuna oil. Shark liver oil had the lowest SV(20) among all fish and vegetable oils tested, as its main components are hydrocarbons. The effects of unsaponifiable components such as tocopherols and phytosterols on the THz absorbance of fats and oils were investigated. At concentrations below 1 wt%, they were seen to have no effect on the THz absorbance over the range of 20 to 400 cm^{-1} at the concentration below 1 wt % (data not shown).

Therefore, a correlation curve was made for fish oils between the SV and the absorbance at 77 cm^{-1} for the fish oils produced. As shown in Fig. 4, a good linear relationship ($R=0.962$) was also obtained for the fish oils.

These observations suggest that the absorbance at 77 cm^{-1} derived from the ester bond in vegetable and fish oils may follow the law of Lambert-Beer law.

3.3 Thermally treated oil

High-oleic safflower oil (800 g) was heated in a deep-frying pan at 180°C with spraying water for different durations up to 50 h, to prepare the thermally treated oil model. The high-oleic safflower oil was polymerized during heating, and the polymer content was found to be 15, 27, 36, 52, and 48% for 10, 20, 30, 40 and 50 h of heating time, respectively. Figure 5 shows the THz spectra of thermally

Fig. 4 Plot of saponification value against THz absorbance at 77 cm^{-1} of fish oils.

Fig. 5 The THz spectra of high-oleic safflower oils heated for 10, 20, 30, 40 and 50 h at 180°C.

treated oils, all showing similar patterns over the range of 20 to 400 cm^{-1} in spite of the different degrees of deterioration. All thermally treated oils and the fresh oils had notable absorption peaks at 77 cm^{-1} . However, the absorbance of thermally treated oils depended on the deterioration degree. The THz absorbance of high-oleic safflower oil increased with heating time.

A correlation curve showing the relationship between polymer content and absorbance at 77 cm^{-1} demonstrated an excellent linear relationship for the thermally treated oils (Fig. 6). The coefficient of determination was 0.990. This good correlation between THz absorbance and polymer content of thermally treated oil was also observed for corn oil (data not shown). It is known that the polymer-

Fig. 6 Plot of polymer content against the THz absorbance at 77 cm^{-1} of high-oleic safflower oils heated at 180°C.

ization of edible oils during deep-frying of edible oils may increase the SV²¹⁾, and in this work, the SV of thermally treated high-oleic safflower oil increased with heating time (data not shown). The polymerization and thermal oxidation may apparently enhance the THz absorbance of “-COO-”, although the presence of free fatty acids corresponding to acid value 3 formed by hydrolysis of TAGs did not affect the THz absorbance. The peak at 231 cm^{-1} due to free fatty acids was not found in the thermally treated oils. These results demonstrate that THz absorption spectrometry could be an effective technique for evaluating the quality of thermally treated oil.

It is well known that the NIR technique using the partial least squares (PLS) algorithm is effective for determining the chemical characteristics of edible oils⁷⁻⁹⁾. NIR analysis takes approximately 2 min per sample, making it much faster than the titration method. Moreover, the NIR technique does not require the use of harmful solvents.

THz spectroscopy is another non-destructive analytical method that needs no organic solvent. Thus, many researchers in the field of biophysics and biochemistry have adopted THz spectroscopy. There have been some reports concerning the THz absorption spectra of biomolecule and food component such as carbohydrates, amino acids, polypeptides, and vitamins¹⁵⁻²⁰⁾. However, there are few reports for fats and oils. Hu *et al.*²²⁾ observed that vegetable oils had broad peaks at 0.2 to 1.6 THz (7 to 53 cm^{-1}), although the THz region between 100 and 400 cm^{-1} was not measured for fats and oils. We observed THz absorption peaks for fats and oils at 77 and 328 cm^{-1} . Moreover, THz absorption spectra have not been discussed in relation to the chemical characteristics of fats and oils. In this paper, we demonstrated that the level of THz absorbance of edible oils at 77 cm^{-1} correlated with the SV. In previous work¹⁴⁾, we found that the THz spectral pattern of saturated fatty acids differed from those of unsaturated fatty acids, which suggested that the crystalline structure of fatty acids may affect the THz absorption spectra. In the future, we will investigate the relationship between the THz absorption spectrum and steric structure of fats and oils including TAGs.

4 Conclusions

A THz spectroscopy technique was applied for the determination of SV and polymer content of vegetable and fish oils. The THz absorption spectra were independent of the variety of vegetable and fish oils in the range of 20 to 400 cm^{-1} . However, the THz absorbance was well correlated with the SV of vegetable and fish oils. When THz spectrometry was applied to thermally treated oils, the THz absorbance was increased with polymer content in the oil. THz method is useful for SV and polymer content determination

in edible oil, as it does not need harmful organic solvent and the measurement time is only 2-3 min per sample.

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