Investigation of thermal degradation of vegetable oils by spectroscopic methods and its mathematical analysis

M. Tobolova, V. Kresalek, H. Vaskova and M. Buckova

***Abstract*—**In this paper, relatively new spectroscopic methods were used for investigation of edible oils and their thermal degradation. There were included the samples of refined rapeseed and sunflower oil and the same oils heated for specified time interval at the temperature 200 °C. The optical characteristics of oils have been investigated with Terahertz Time Domain Spectroscopy (THz-TDS). The refractive indices were calculated in the specified terahertz frequency range, processed and statistically evaluated. The Wilcoxon matched pairs test was carried out for comparison of unheated and heated oil samples. The results measured by THz-TDS were supplemented by Raman spectra analysis. Both methods seem to be capable analyzing tool for oil investigation.

***Keywords*—**heating the oils, Raman spectroscopy, thermal degradation, THz time domain spectroscopy, vegetable oil.

I. INTRODUCTION

LAST few decades years, a big boom came in the propagation of healthy eating habits and healthy food preparation, because series of common diseases come from the bad lifestyle, diet and stress. These “modern” problems form the basis of diseases of civilization that were not so common in previous generations. We could mention the heart diseases, obesity, diabetes mellitus, cancer illness and some chronic diseases. The greatest blame is often given to excessive consumption of fats and sugars that are usually not of the natural origin. These are often contained in calorie bombs that generally miss any really necessary components for our metabolism. On the other hand, we cannot forget the fats are also important because of some unique substances contained. Therefore, it depends both on the quantity and quality. Moreover, the oil is necessary component for the preparation of most meals whether it is cooking, baking, frying or flavoring. Especially frying is very popular but also the least healthy method. To a certain extent, we can manage the quality of the meal by using the appropriate oil, i. e. to distinguish the oils for cold and hot meals, and by following the recommended conditions for storage and also the principles of their preparation, especially thermal processing. It is known that some changes occur during heating the oil.

For hot dishes, a variety of oils may be used. Their physical and chemical properties influence the reactions that occur during frying, but there are other multiple factors playing the role; the way the food is prepared, applied temperature, length of heating, and type of food being heated [1]. At relatively high temperatures, the lipid oxidation occurs and produces compounds that affect the food flavor but also its quality. The oxidative changes are associated with a decrease in the unsaturation of the fat and with increases in the free fatty acid content, content of polar compounds and polymeric material, foaming, color, and viscosity.

Quality of fats can be evaluated by standard physical (molecular weight, smoke point, refractive index, chromatic parameter, viscosity, surface tension, dielectric constant) and chemical methods (iodine value, saponification value, free fatty acid content, peroxide value etc.). The degree of oxidation could be also tested by spectroscopic methods (infrared and nuclear magnetic resonance technique), and chromatography provides both qualitative and quantitative evaluation of oxidative changes. In order to prevent oxidation, the natural and synthetic antioxidants are added in fats and fat-containing food.

Generally, antioxidants increase the oxidative stability of the oil and the antioxidant activity can be also measured [2]. Commonly used methods are quite time-consuming and expensive. The aim of this study is to find out if the newest spectroscopic methods, terahertz time domain spectroscopy and Raman spectroscopy, are suitable alternative methods for investigation of the changes caused by thermal degradation during heating the oil. Some first experiments were made in [3] and [4].

THz-TDS is a new vibrational-rotational spectroscopic method enabling material characterization. It uses terahertz

This paper is supported by the European Regional Development Fund under the project CEBIA-Tech No. CZ.1.05/2.1.00/03.0089.

Marie Tobolova is with the Department of Electronics and Measurements, Tomas Bata University, Zlin, Czech Republic (corresponding author, phone: +420 576 035 228; e-mail: tobolova@fai.utb.cz). She is at the same time the Ph.D. student of the Department of Biomedical Engineering, Brno University of Technology, Brno, Czech Republic.

Vojtech Kresalek is with the Department of Electronics and Measurements, Tomas Bata University, Zlin, Czech Republic (e-mail: kresalek@fai.utb.cz).

Hana Vaskova is with the Department of Electronics and Measurements, Tomas Bata University, Zlin, Czech Republic (e-mail: vaskova@fai.utb.cz).

Martina Buckova is with the Department of Food Technology, Faculty of Technology (buckova@ft.utb.cz).

region of the electromagnetic spectrum which lies between microwaves and infrared; frequency range 100 GHz – 10 THz in particular. When interacting with the sample, the vibrational modes of molecules are activated, the THz pulse is modulated and specific response of the sample can be detected. Two time signals are measured and compared – the reference, usually ambient air, and the sample. These signals are transformed into the frequency domain using Fourier transform and optical parameters describing investigated sample are calculated from amplitude and phase spectra. The method brings many advantages. Because of low energy of the radiation, it is non-invasive and non-destructive type of spectroscopy. The measurement is quite fast, usually has not any special requirements for sample preparation and does not need any addition of chemical substances. It has very good time resolution in order of picoseconds; therefore it could be used for studying the dynamics of systems. THz radiation is sensitive to water because of strong absorption therefore it is necessary to minimize the water content in measured sample. Especially dry solid materials, such as polymers and powders, or hydrophobic liquids such as oils are ideal for measurement [5].

As a second method for studying the thermal degradation, the Raman spectroscopy was used. It is a vibrational type of spectroscopy which provides specific chemical “fingerprint” of single chemical substance in the form of the Raman spectrum. It is based on the Raman scattering phenomenon, inelastic scattering resulting from photon–molecule interaction. Photons of inelastic scattering have slightly changed wavelengths that are characteristic for specific bonds in sample [3].

Both mentioned techniques bring advantages such as non-destructiveness, no special requirements for sample preparation, rapidity or contactless measurements. For the present, they are rather experimental methods that find many applications especially in chemistry, biomedicine, pharmacy, arts, materials science, forensic science and security.

II. MATERIALS AND METHODS

A. Instrumentation

For analyzing the thermal degradation of oils, the latest spectroscopic techniques were used: Raman spectroscopy and Terahertz time domain spectroscopy (THz-TDS).

THz-TDS system TPS Spectra 3000 by TeraView Company was used as an analyzing device in this experiment. TPS Spectra 3000 is the world's first commercial terahertz spectrometer capable of performing both transmission and attenuated total reflection measurements. It utilizes powerful, ultra-fast Ti-Sapphire laser source and semiconductor based detection system to supply full access to the 0.06 – 4 THz spectral region. Furthermore, this spectrometer does not require liquid helium cooling or any vacuum systems for its operation, enabling results to be obtained in a minute or less, all while operating under ambient temperature conditions [6]. It works on the traditional pump and probe principle and the delay between pump and probe pulse is realized mechanically by rotating mirrors.

InVia Basis Raman microscope by Renishaw was used for recording Raman spectra of investigated oils. Measurements were performed using 785 nm NIR diode laser with maximum output power 300 mW. A Leica DM 2500 confocal microscope with the resolution up to 2 μm was coupled to the Raman spectrometer. All measurements were collected at 5x magnification, with 20 seconds exposure time and 5 accumulations. Measurements from different spots of the same sample gave corresponding results. The samples were scanned in range 700 to 1800 cm^{-1} with 2 cm^{-1} spectral resolution as this area is the crucial for characteristics of oils.

B. Samples

Two types of edible oil were the objects of this study. There were included refined oils made from sunflower seeds and rapeseed. These oils are widespread in hot meals preparation. Three samples of each oil were prepared: one taken directly from the bottle marked with a suffix “_bottle” and two heat-treated. The heat-treated samples were prepared in laboratory conditions by heating the oil to the temperature 200 °C. When the temperature of oil reached required temperature, the sample marked with a suffix a “_0min” was taken. This temperature was maintained for 15 minutes and after this period the sample marked with a suffix “_15min” was taken. The sample was continually stirred. This procedure was repeated three times and finally nine samples of each oil were available.

C. Experimental Design

Samples of oils were prepared in a liquid form at room temperature for all measurements. For Raman spectra measurement, only a small drop of the oil was placed on the slide. When laser irradiates the sample, the vibrations of specific molecules occur. After Rayleigh scattering filtration, a diffraction grating disperses the light onto a detector. The resulting Raman spectrum gives the information about molecule bonding.

For THz-TDS measurement, a plastic cuvette was filled with the amount of 700 μl of just investigated oil. The average thickness of the cuvette was 5740 μm and the thickness of the oil layer inside was 1962.2 μm. The experimental adjustment had standard transmission geometry. THz pulse passes through the investigated sample (oil in cuvette) and the signal reflecting the interaction with sample is detected. THz-TDS relies on comparing this THz response with the same THz pulse transmitted only through the air (reference measurement). Measurement of each sample was repeated six times.

III. DATA ANALYSIS

A. *Refractive index value in THz*

Using TPS Spectra 3000, the refractive index of all oils were measured as optical parameters for oil characterization. The results needed other mathematical corrections because measured values were not the parameters of the oil itself but the cuvette included. All measured data were processed and statistically evaluated.

Because of the Fabry-Perot artifacts' influence, the frequency range of 0.5 – 2 THz was taken into account. Repeated measurements of each sample were averaged and are shown in the following figures. Fig. 1 shows the representative averaged signals of refractive index of fresh oils taken from bottles. At first sight, it seems the rapeseed oil has higher refractive index in the whole frequency range therefore the oils can be good distinguished.

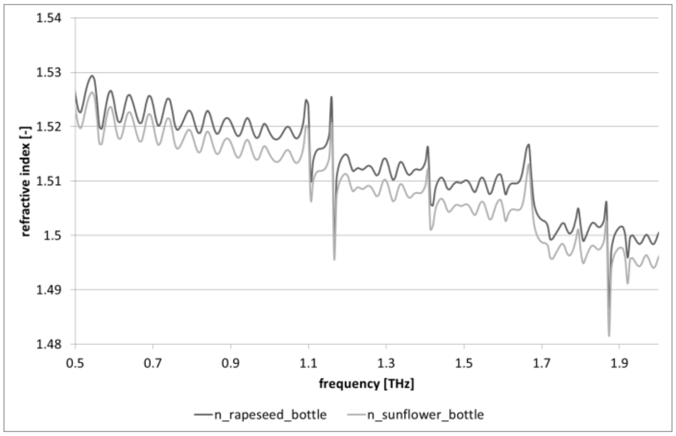

Fig. 1 Refractive indices of the fresh refined oils taken from the bottle in the frequency range of 0.5 – 2 THz

Fig. 2 and Fig. 3 show refractive indices of three samples of rapeseed and sunflower oil before and after heating. The refractive index changes with the frequency and has descending trend. It is obvious that the fresh rapeseed oil and oil heated for 15 minutes are significantly overlapped. For sunflower oil, this is not clear in the whole frequency range. Just heated oil in time 0 min. seems to be different from both fresh and heated forms.

For detailed analysis, there were chosen two frequencies (1 THz and 1.5 THz) for comparison the oil sample directly from bottle and its heated forms (just heated in time 0 min. and after 15 min. of heating). The descriptive statistics of the data is summarized in Table I for frequency 1 THz and Table II for frequency 1.5 THz. Box-Whisker plots show the refractive index distribution at chosen frequencies graphically; see Fig. 4 and Fig. 5.

The values were not normally distributed therefore the nonparametric statistics was used. For comparison of normal and heated forms of oils, the nonparametric paired design of the Wilcoxon test was used. The null hypothesis of no difference between the normal and heated form of each oil was tested at significance level 0.05. The results were summarized in Table III and Table IV for rapeseed oil, and Table V and Table VI for sunflower oil.

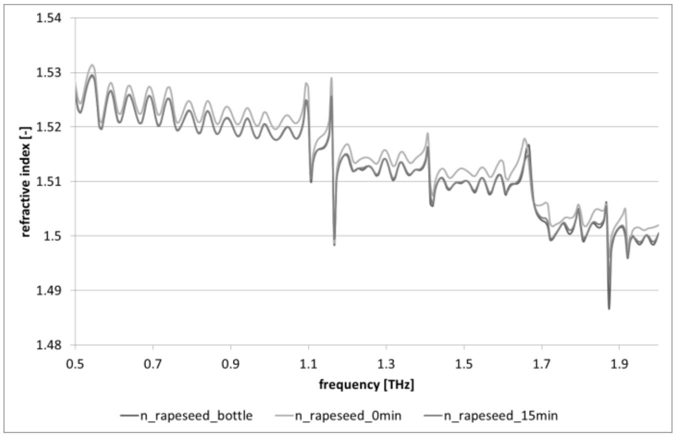

Fig. 2 Refractive indices of the fresh refined rapeseed oil (n_rapeseed_bottle) and its heated forms – just heated in time 0 minutes (n_rapeseed_0min) and after 15 minutes of heating (n_rapeseed_15min) in the frequency range of 0.5 – 2 THz

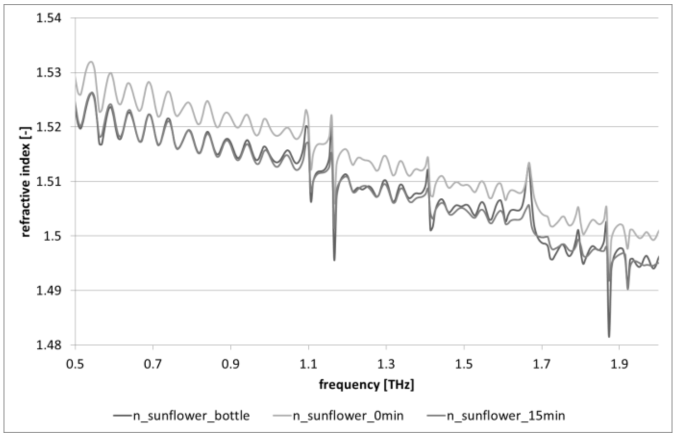

Fig. 3 Refractive indices of the fresh refined sunflower oil (n_sunflower_bottle) and its heated forms – just heated in time 0 minutes (n_sunflower_0min) and after 15 minutes of heating (n_sunflower_15min) in the frequency range of 0.5 – 2 THz

Table I Descriptive Statistics of refractive indices of oils at frequency 1 THz

	Mean	Median	Minimum	Maximum	Std.Dev.	Standard
rapeseed_bottle	1.513	1.506	1.505	1.526	0.009	0.003
rapeseed_0min	1.521	1.524	1.505	1.531	0.008	0.002
rapeseed_15min	1.519	1.521	1.499	1.531	0.010	0.003
sunflower_bottle	1.512	1.510	1.508	1.518	0.004	0.002
sunflower_0min	1.517	1.517	1.503	1.529	0.008	0.002
sunflower_15min	1.515	1.515	1.502	1.524	0.007	0.002

Table II Descriptive Statistics of refractive indices of oils at frequency 1.5 THz

	Mean	Median	Minimum	Maximum	Std.Dev.	Standard
rapeseed_bottle	1.503	1.496	1.496	1.518	0.010	0.003
rapeseed_0min	1.513	1.515	1.470	1.523	0.009	0.002
rapeseed_15min	1.511	1.513	1.489	1.524	0.010	0.003
sunflower_bottle	1.502	1.500	1.498	1.509	0.005	0.002
sunflower_0min	1.508	1.508	1.494	1.521	0.008	0.002
sunflower_15min	1.505	1.506	1.493	1.515	0.008	0.002

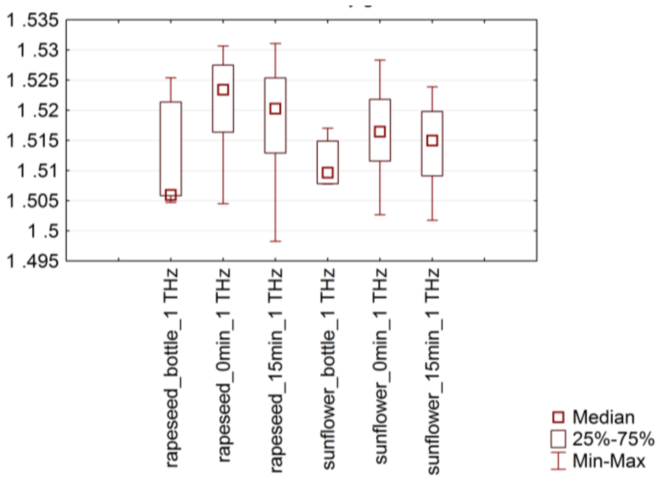

Fig. 4 Box-Whisker plot of refractive index distribution for oil samples at frequency 1 THz

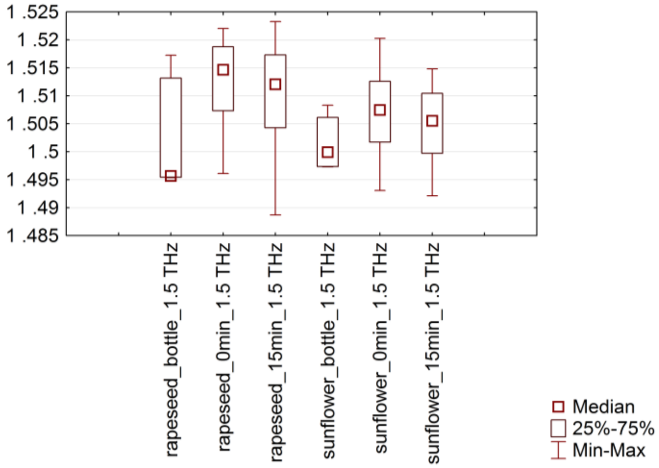

Fig. 5 Box-Whisker plot of refractive index distribution for oil samples at frequency 1.5 THz

Table III Wilcoxon matched pairs test for rapeseed oil samples at frequency 1 THz (tests are significant at $p < 0.05$)

	T	Z	p-level
rapeseed_bottle & rapeseed_0min	1.000	2.981	0.003
rapeseed_bottle & rapeseed_15min	25.000	1.099	0.272

Table IV Wilcoxon matched pairs test for rapeseed oil samples at frequency 1.5 THz (tests are significant at $p < 0.05$)

	T	Z	p-level
rapeseed_bottle & rapeseed_0min	0.000	3.060	0.003
rapeseed_bottle & rapeseed_15min	24.000	1.177	0.240

Table V Wilcoxon matched pairs test for sunflower oil samples at frequency 1 THz (tests are significant at $p < 0.05$)

	T	Z	p-level
sunflower_bottle & sunflower_0min	14.000	1.962	0.050
sunflower_bottle & sunflower_15min	15.000	1.883	0.060

Table VI Wilcoxon matched pairs test for sunflower oil samples at frequency 1.5 THz (tests are significant at $p < 0.05$)

	T	Z	p-level
sunflower_bottle & sunflower_0min	14.000	1.962	0.050
sunflower_bottle & sunflower_15min	12.000	2.118	0.035

B. *Raman spectroscopy*

Using Raman spectrometer, the spectra of all samples were measured. The Raman spectra for rapeseed oil samples are shown in Fig. **6** and for sunflower in Fig. **7**.

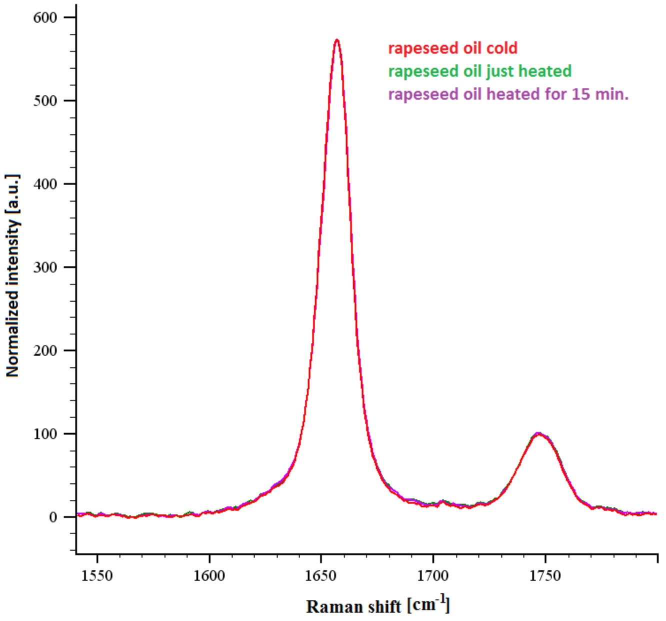

Fig. 6 Raman spectra of the refined rapeseed oil from bottle (rapeseed oil cold) and its heated forms – just heated in time 0 minutes and after 15 minutes of heating

Generally, the oils contain saturated and unsaturated fatty acids. Considering a variety of health benefits, the unsaturated fatty acids are known as “healthy fats” but they have lower stability and stronger predisposition to oxidation processes. For thermal degradation monitoring, the Raman band around 1640 cm-1 is crucial. This band is assigned to formation of trans, trans-2,4-decadienal, a major decomposition product of heated oxidized linoleate which corresponds to the C=C stretching vibration in conjugated system [7]. For better evaluation, there are shown details of the region of interest around the wavenumber 1640 cm-1 (see Fig. 8 and Fig. 9).

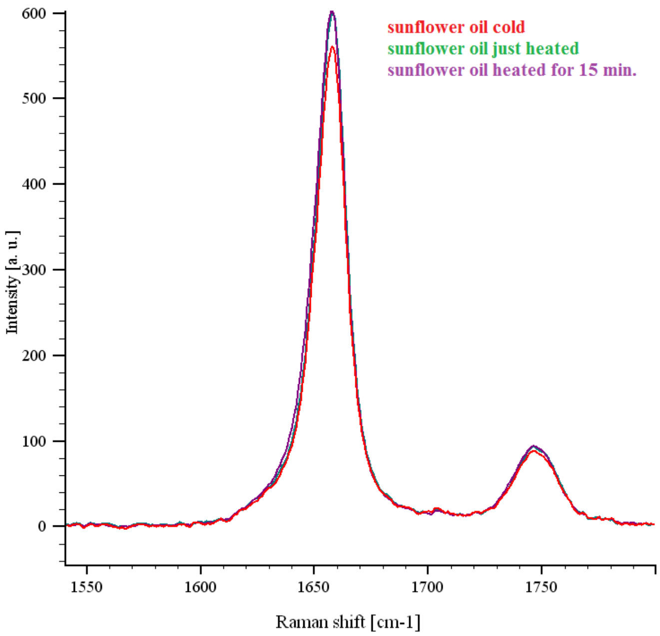

Fig. 7 Raman spectra of the refined sunflower oil from bottle (rapeseed oil cold) and its heated forms – just heated in time 0 minutes and after 15 minutes of heating

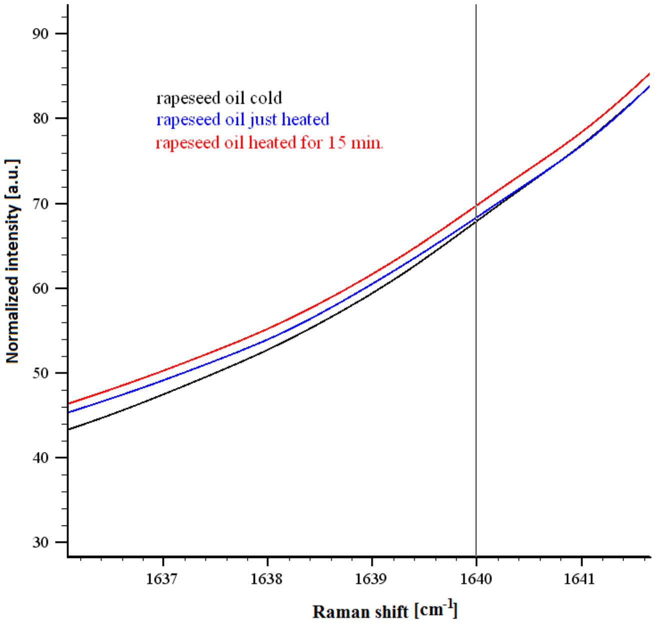

Fig. 8 Raman spectra of rapeseed oil in detail with the marked line at the wavenumber 1640 cm^{-1}

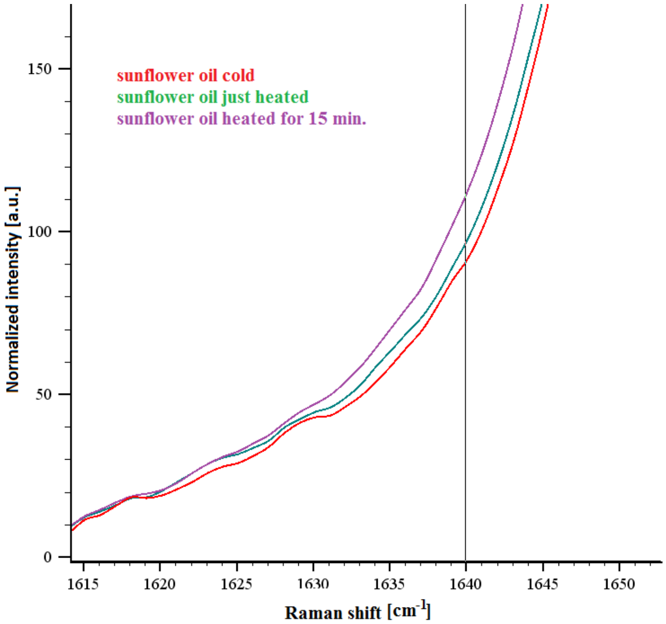

Fig. 9 Raman spectra of sunflower oil in detail with the marked line at the wavenumber 1640 cm^{-1}

IV. RESULTS

The aim of the whole experiment was to investigate if the thermal changes that occur during heating the oil are detectable by Raman and THz time domain spectroscopy. First, the averaged refractive indices of all measured unheated samples of the rapeseed and sunflower oil were plotted in specific frequency range to get the basic information about properties of investigated oils (see Fig. 1). As we can see, the shape of the curves of refractive index dependence at the frequency is practically identical for both oils in the whole frequency range. Around the frequencies 1.1 THz, 1.4 THz, 1.7 THz and 1.9 THz, we can recognize several peaks that can correspond to vibrations at specified frequency characteristic for some substances included in oil. Finding differences between the normal oils taken from the bottle and their heated forms is much more important.

As it could be seen in Fig. 2, there is no clear boundary between normal rapeseed oil and its heated samples. While the just heated sample has slightly higher refractive index than unheated oil, the oil heated for 15 minutes is almost identical with the normal sample. Similarly, Fig. 3 compares the refractive indices of unheated and heated samples of sunflower oil. Also here, the difference of the just heated oil is obvious but we can see also some differences at specified frequencies between the unheated sample and sample heated for 15 minutes. Especially, the mentioned peaks are significantly flatter. It could seem that there are no visible changes of refractive index that would correspond to changes caused by heating the oil. But the question is, if the THz-TDS is really not capable to detect the oxidative changes in the oil or the time of heating the oil is so short that the changes are so weak. Using the statistical analysis of the measured data in combination with the results of Raman spectroscopy, we can try to answer this.

It is known that averaging can mask the real central value characterizing measured data because of outliers. Therefore, the detailed analysis was based on the original data from repeated measurements. For evaluation, two frequencies were chosen in measured THz range; 1 THz and 1.5 THz. It is obvious in Table I and Table II, that the mean value and median of all variables are not the same. Moreover based on the histograms and normal probability plots, the normal distribution of the data was not confirmed, therefore it is better to choose the median instead of the mean value for data representation. This is also shown in Box-Whisker plots (see Fig. 4 for 1 THz and Fig. 5 for 1.5 THz), where the non-normal distribution of the data is evident. Further, we can see the refractive index increase for both heated samples of rapeseed and sunflower oil; this is confirmed by concrete values in Table I and Table II.

The significance of mentioned differences at both frequencies was tested by nonparametric Wilcoxon matched pairs test. The results are shown in Table III and Table IV for rapeseed oil, and Table V and Table VI for sunflower oil. For rapeseed oil, the hypothesis of no difference between the normal sample of oil and sample heated for 15 minutes was accepted at significance level 0.05 at both frequencies, whereas this hypothesis was rejected in case of the normal sample and just heated sample. For sunflower oil, the situation is not the same. The Wilcoxon test confirmed a significant difference between unheated sunflower oil and both its heated samples at frequency 1.5 THz. At 1 THz, the unheated oil and sample heated for 15 minutes seem to be without any difference but this statement is quite strong because the p-value is very close to the significance level 0.05. Therefore we have to be careful in rejecting the hypothesis of no difference between these samples.

Fig. 6 and Fig. 7 show the Raman spectra of rapeseed oil and sunflower oil samples. The axis of frequency shift is zoomed in the region 1540 – 1800 cm^{-1}. We can see two dominant peaks: 1660 cm^{-1} is typical for C=C stretching mode of the cis unsaturated fatty acid and 1747 cm^{-1} for C=O stretching in an ester. We focused on band around 1640 cm^{-1} because at this wavenumber the changes caused by oxidization processes connected with heating the oil can be noticed as the first. Fig. 8 and Fig. 9 are zoomed in this area because the previous figures are difficult to read. For both oils, we can see an increasing Raman intensity at 1640 cm^{-1} dependent on time of heating. Intensities were acquired using normalization via 1747 cm^{-1} band and subtraction of the intensity at the ambient room temperature. The mentioned increase is more intensive for sunflower oil than rapeseed oil. There is also registered a general increase of intensity of the peaks at 1660 cm^{-1} and 1747 cm^{-1} for heated samples.

V. DISCUSSION

For this experiment, two most frequently used oils were chosen and purchased at the shop; refined sunflower oil, representing a high linoleic vegetable oil, and refined rapeseed oil as a high oleic vegetable oil. The sunflower oil contains up to 90% combination of oleic and linoleic acids (of which 60 – 75% of linoleic acid), and virtually no linolenic acid. Rapeseed oil typically contains 56% of oleic, 26% of linoleic, 10% of linolenic and traces of palmitic and stearic acids and less saturated acids than any other oil [2]. Obviously, there are considerable differences in the chemical composition but they have some common properties such as near smoke point (209 °C for sunflower, 218 °C for rapeseed) and iodine number (136 for sunflower, 120 for rapeseed) [8]. Further, the both oils contain antioxidants that can be natural or artificially added [9], [10]. Generally, the greater iodine number and lower smoke point as well as the lower content of antioxidants make the stability of the oil lower. Therefore, the rapeseed oil should have better stability and also better high temperature resistance. This hypothesis is also supported by the study [11].

Analyzing the data measured by THz-TDS, sunflower and rapeseed oils have similar values of refractive index; 1.513 ± 0.009 for rapeseed and 1.512 ± 0.004 for sunflower oil at frequency 1 THz, and 1.503 ± 0.010 for rapeseed and 1.502 ± 0.005 for sunflower oil at 1.5 THz. According to results of Wilcoxon test, no fundamental change occurs during the 15 minutes of maintaining at the temperature 200 °C for rapeseed oil, unlike the sunflower oil. The difference of just heated samples from other samples at both oils remains unclear. It could be a subject of further studies but there is an idea that achieving the high temperature is connected with releasing vapours.

Generally based on the refractive index value, the rapeseed oil seems to be more stable than sunflower oil. The analysis of Raman spectra supports this statement. Using Raman spectroscopy, the spectral changes corresponding to C=C bond formation during oxidation and products of degradation are expected. The speed of oxidation products' formation is given by the content of antioxidants. It is known that the rapeseed contains natural antioxidants unlike the sunflower; therefore the antioxidants (especially vitamin E) are added to the sunflower oil synthetically. The content of these substances affects the Raman spectra. Just because of antioxidants, the increase of intensity at 1640 cm^{-1} in the Raman spectra is slow for rapeseed oil even after 15 minutes at the temperature 200 °C. Conversely, the intensity of the peak has clearly faster increase for sunflower oil. Based on the results of Raman spectroscopy and THz-TDS, we assume that the sunflower oil undergoes oxidation processes earlier than rapeseed oil.

VI. CONCLUSION

The experiment shows the capability of both methods (THz-TDS and Raman spectroscopy) to study the oils and their changes caused by high temperature. Based on the results, we present the possibility to use these spectroscopic methods as a relatively low-cost screening method for detecting the thermal changes and oxidation processes in oils.

REFERENCES

[1] Ch. Gertz, "Fundamentals of the frying process," *European Journal of* Lipid Science and Technology, vol. 116, April 2014, pp. 1 – 6.

[2] F. Shahidi and Y. Zhong, "Lipid oxidation: Measurement methods," in *Bailey's Industrial Oil and Fat Products*, 6th ed. vol. 6, F. Shahidi, Ed. New Jersey: John Wiley & Sons, Inc., 2005, pp. 357–385.

[3] H. Vaskova and M. Buckova, "Measuring and identification of oils," in *Proceedings of the 18th International Conference on Systems (part of CSCC'14)*, Santorini Island, 2014, pp. 211–215.

[4] M. Tobolova and V. Kresalek, "Investigation of thermal degradation of vegetable oils during frying using terahertz time-domain spectroscopy," in *Proceedings of the 18th International Conference on Systems (part of CSCC'14)*, Santorini Island, Greece, 2014, pp. 211–215.

[5] K.-E. Peiponen, J. A. Zeitler, and M. Kuwata-Gonokami, "Terahertz Spectroscopy and Imaging," Berlin: Springer, 2013. 641 pages.

[6] TPS Spectra 3000: User manual. TeraView, Ltd., Cambridge, United Kingdom, 2011.

[7] B. Muik, B. Lendl, A. Molina-Diaz, M. J. Ayora-Canada, "Direct monitoring of lipid oxidation in edible oils by Fourier transform Raman spectroscopy," *Chemistry and Physics of Lipids*, vol. 134, April 2005, pp. 173-182.

[8] M. Bockisch. "*Fats and Oils Handbook*," Hamburg: AOCS Press, 1998, pp. 90 – 120.

[9] M. Cvjetko. "Antioxidant properties of rapeseed," *Zbornik radova Tehnološkog fakulteta u Leskovcu*, vol. 19, September 2009, pp. 27 – 33.

[10] E. O. Aluyor and M. Ori-Jesu. "The use of antioxidants in vegetable oils – A review," *African Journal of Biotechnology*, vol. 7, issue 25, December 2008, pp. 4836 – 4842.

[11] Ch. Gertz, S. Klostermann, S. P. Kochhar. "Testing and comparing oxidative stability of vegetable oils and fats at frying temperature," *European Journal of Lipid Science and Technology*, vol. 102, issue 8 – 9, October 2000, pp. 543 – 551.