Measurement and modeling of optical properties of heated adipose tissue in the terahertz range

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Abstract

Measurements and modeling of the optical properties of adipose tissue and its components in the terahertz range with a change in tissue temperature were carried out. It was shown that the optical density (OD) of adipose tissue samples decreases with increasing temperature, which can be mainly associated with dehydration of the sample. We can also expect some contribution to the decrease in the OD of suppression of THz wave scattering when matching the refractive indices of scatterers and their environment due to the intake of free fatty acids secreted by adipocytes due to thermally induced cell lipolysis. It is shown that in the experimental model, the difference between the THz absorption spectra of water and oil allows us to estimate the water content in adipose tissue. A comparison of the measurement results and molecular modeling in the terahertz region confirmed the hypothesis about the reasons for the change in the optical properties of heated adipose tissue.

Keyword list: terahertz spectroscopy, modeling, absorption spectra, adipose tissue, oleic acid, water, dehydration

1. INTRODUCTION

Terahertz (THz) spectroscopy allows one to determine the complex refractive index of the medium under study, which is important for creating a functional THz -tomography with high sensitivity to changes in the concentration of metabolites and accurate marking of the boundaries of the pathological lesions. Therefore, the development of spectroscopic methods for studying biological tissues in the THz -frequency range, providing detection and imaging of metabolic and pathological processes, has caused great interest in recent years, especially as an additional channel for obtaining information in multimodal systems in combination with optical methods.[1,2] The contrast between healthy and diseased tissue for THz wave probing is due to differences in water content[3-6] as well as in optical properties of adipose and muscle tissues and their structure.[7-10] Lipids weaken THz radiation less strongly than polar molecules. Absorption rate for all lipids increases with frequency and reaches a maximum for about 2 THz.[11-14] The difficulty of interpreting results of measurements and the transition from *in vitro* measurements to *in vivo* diagnostics is caused by uncontrolled environment, for example, diffusion into a sample of saline during tissue storage, changes in the level of hydration during the measurement, effects of scattering.[15]

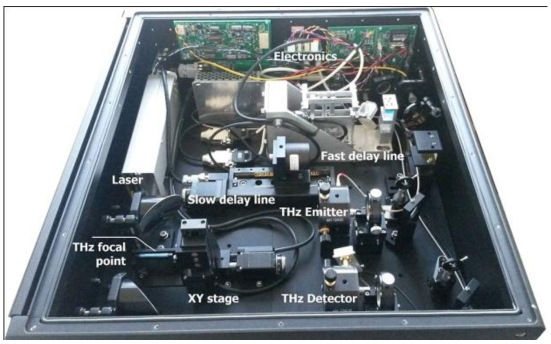
The study molecular mechanisms of biophysical processes is currently almost impossible without methods of computer modeling. The method of density functional theory (DFT) is well suited for theoretical estimation of absorption spectra of many biologically important substances.[16]

Terahertz Photonics, edited by Mona Jarrahi, Sascha Preu, Dmitry Turchinovich, Proc. of SPIE Vol. 11348, 1134817 · © 2020 SPIE · CCC code: 0277-786X/20/$21 · doi: 10.1117/12.2555656

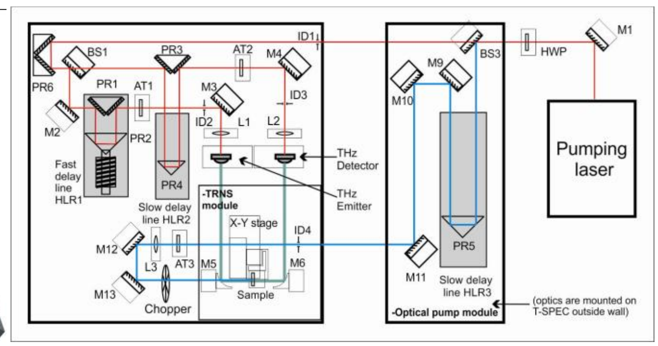
This study is aimed for creating a model of optical properties of adipose tissue and its components in the terahertz range taking into account measurements for different temperatures.

2. METHODS AND MATERIALS

THz spectral measurements were made using a real-time T-SPEC terahertz spectrometer (EKSPLA, Lithuania) working in the frequency range 0.25-1 THz with a software THz Spectrometer 2D (see Fig. 1). Calibration was performed first on air, then this signal was removed from the signal received at passing through 2 plates of fluoroplastic (refractive index is 1.41) of thickness 1 mm and surface area of 2×1.5 cm^2 each, served as a tissue sample holder. This signal was taken as the reference. Measurements were carried out in the transmission mode. The temperature was varied from 25°C to 70°C. A thermistor build in the spectrometer was used to heat samples. The thermistor was placed on the instrumental stage in contact with tissue sample holder.



(a)



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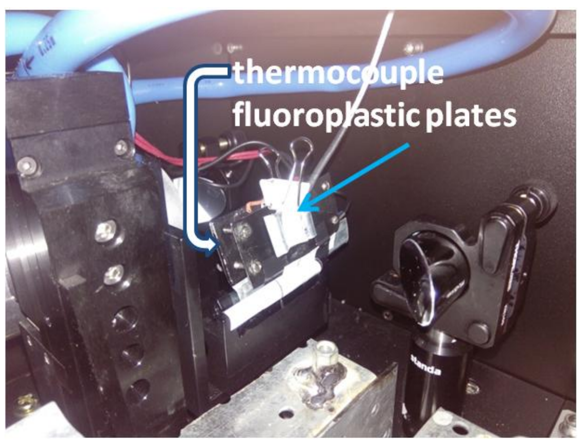


(c)

Figure 1. General view (a) and schematics (b) of T-SPEC terahertz real-time spectrometer (EKSPLA, Lithuania); sample holder with heating arrangement (c).

Abdominal porcine adipose tissue samples were used in the study. Totally ten samples were investigated. The thickness of the samples were about 1 mm, their area was amounted to 1 cm^2. After tissue freezing, sections were made using a microtome. To evaluate terahertz wave attenuation (absorption) coefficient, rather precise knowledge of sample thickness is needed. Therefore, thickness measurements were provided for each sample, which was placed between two glass slides, and measurements were performed at several points of the sample by a digital micrometer. The error of each measurement was approximately 10 μm. The obtained thicknesses were averaged.

To monitor changes of terahertz radiation transport in adipose tissue, the temperature was ranged from 25°C to 70°C with increment of 1°C. For heating of samples a thermistor was used, which heating ability was controlled by changing of applied voltage (Fig. 1c).

The transmittance spectra of porcine abdominal adipose tissue samples were obtained at various temperatures (25 - 70°C) and the temperature dependency of optical density (OD) at a frequency of 1 THz was evaluated.

In experiments on observing changes in the weight of porcine abdominal adipose tissue samples during heating, samples of 2×2 cm and a thickness of 1 mm were used. The error in the measurement of weight was 0.01 g. Time was measured using a stopwatch. This time corresponded to the time during which the sample lost its weight. The first measurement was the longest until the sample was completely warmed up, then changes occurred within 1 min. The measurements were completed when changes in the weight of the samples did not occur for several consecutive measurements after reaching a certain temperature.

Optical density (OD) of H_2O at different thickness and oleic acid (LLC and commercial firm "NIZHEGORODKHIMPRODUKT", Russia) was measured in THz range at room temperature.

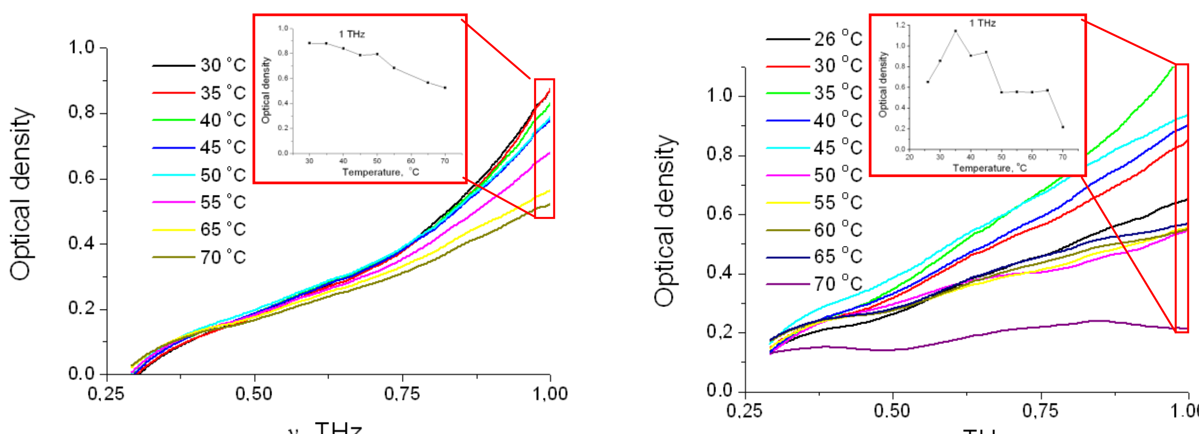
The structural models of five triglycerides of fatty acids (oleic, linoleic, palmitic, stearic, α-linolenic) are constructed using B3LYP/6-31G(d) method and the program from [17]. The vibrational wavenumbers and intensities in the THz spectra are calculated. The molecular model of porcine fat was constructed basing on five-component model of triglycerides of fatty acids. The THz spectra of porcine fat are simulated using the supermolecular approach. The content of these fatty acid triglycerides in the models is shown in Table 1. The halfwidth of all Lorentzian profiles was taken to be 10 cm^{-1}. For better agreement with experiment, the calculated vibrational wavenumbers were corrected using linear frequency scaling.[18]

Table 1.Melting temperatures of porcine fat (triglycerides) and free fatty acids (FFAs) and their concentrations in % by mass.[19]

Porcine fat (in solid state)	Melting temperature, °C	FFA, melting temperature, °C (concentration, % by mass)				
		Palmitic	*Stearic*	*Oleic*	*Linoleic*	*Linolenic*
Triglycerides	36-45	63 (27%)	70 (14%)	16 (45%)	-5 (5%)	-11 (5%)

3. EXPERIMENTAL RESULTS AND DISCUSSION

Typical attenuation spectra of abdominal adipose tissue samples for different temperatures are shown in Fig. 2.



(a) (b)

Figure 2. Two typical attenuation spectra of abdominal adipose tissue samples (a) and (b) at different temperatures.

Figures 3 shows the results on the kinetics of changes in the weight of the samples upon heating. Weight loss on heating adipose tissue samples was around 10%.

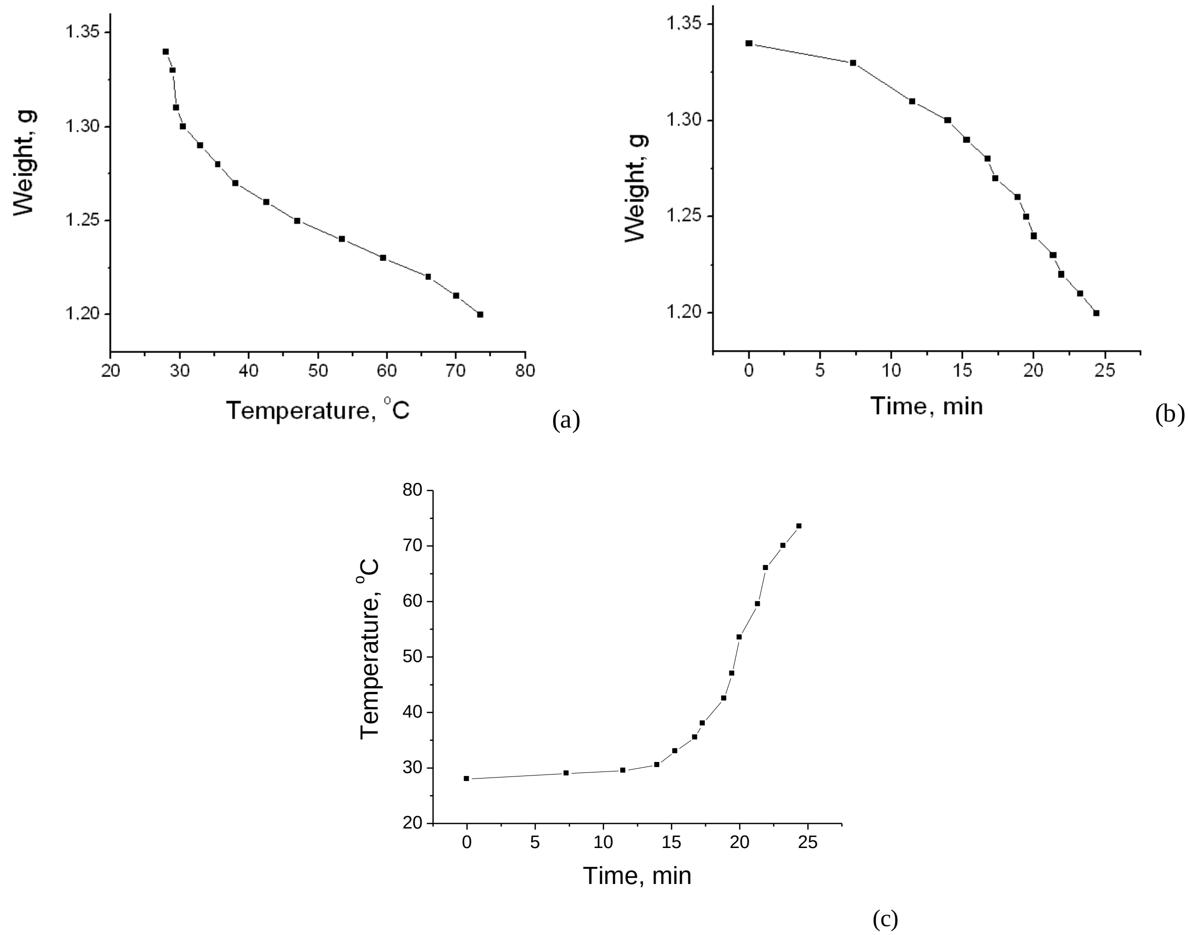


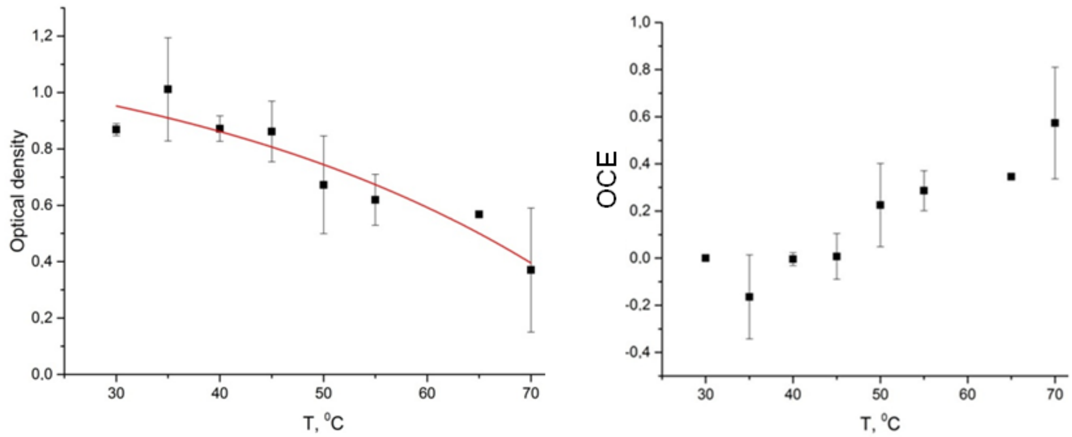
Figure 3. Temperature (a) and temporal (b) dependences of fat sample weight during heating from 25°C to 70°C. Temporal dependence of fat heating (c).

Basing on the literature data for the absorption spectra of adipose tissue in the terahertz range of 0.25-1 THz there are no pronounced bands,[13,20,21] which is consistent with the results of this study. Figure 4a shows the temperature dependence of absorbance on frequency 1 THz. With increasing temperature, there is a decrease in the optical density, which can be associated with losing water as it was directly confirmed by data in Fig.3. Taking into account that fat tissue typically consists of approximately 67-93% of lipids, 2-3% of protein and 5-30% of water.[22] According to simple calculations, if absorption would decrease only due to tissue dehydration, then absorption would decrease of OD from approx. 1.0 to 0.75 is expected instead 0.4 that was obtained experimentally (see Fig.4a)). Some inclusion into OD decrease can be due to suppression of THz wave scattering at refractive index (RI) matching by FFAs released from adipocytes caused by thermally induced cell lipolysis (optical clearing effect).

The optical clearing efficiency (OCE) (see Fig.4b) was calculated as[18]

$$\Delta\alpha = (\alpha_0 - \alpha)/\alpha_0 = \mathrm{OCE},$$

where α_0 is the absorbance of the sample (at a frequency of 1 THz) for the initial temperature, and α is for current temperature.



(a) (b)

Figure 4. Temperature dependences of optical density of porcine adipose tissue samples at a frequency 1 THz (a) and the corresponding calculated relative change of absorbance Δα (averaged for six samples), i.e., optical clearing efficiency (OCE) (b).

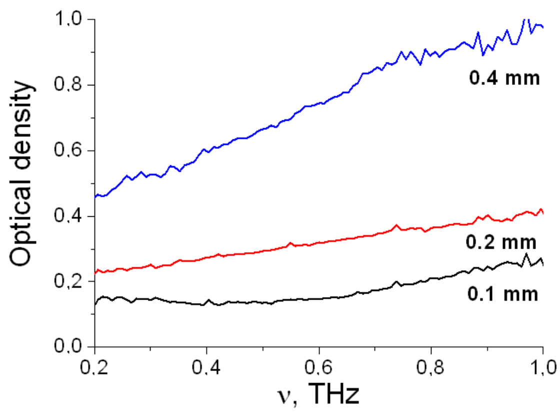


Figure 5. Optical density of liquid H_2O layer with different thicknesses in THz range. Temperature, 28°C.

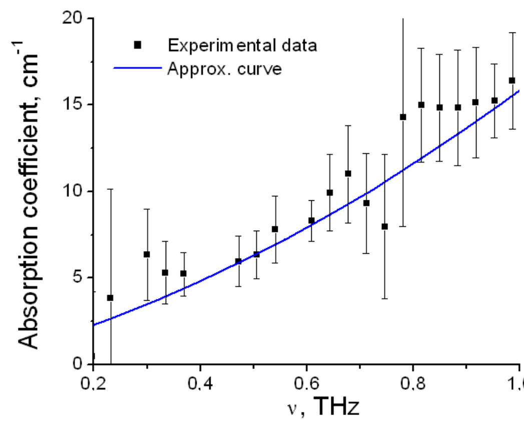
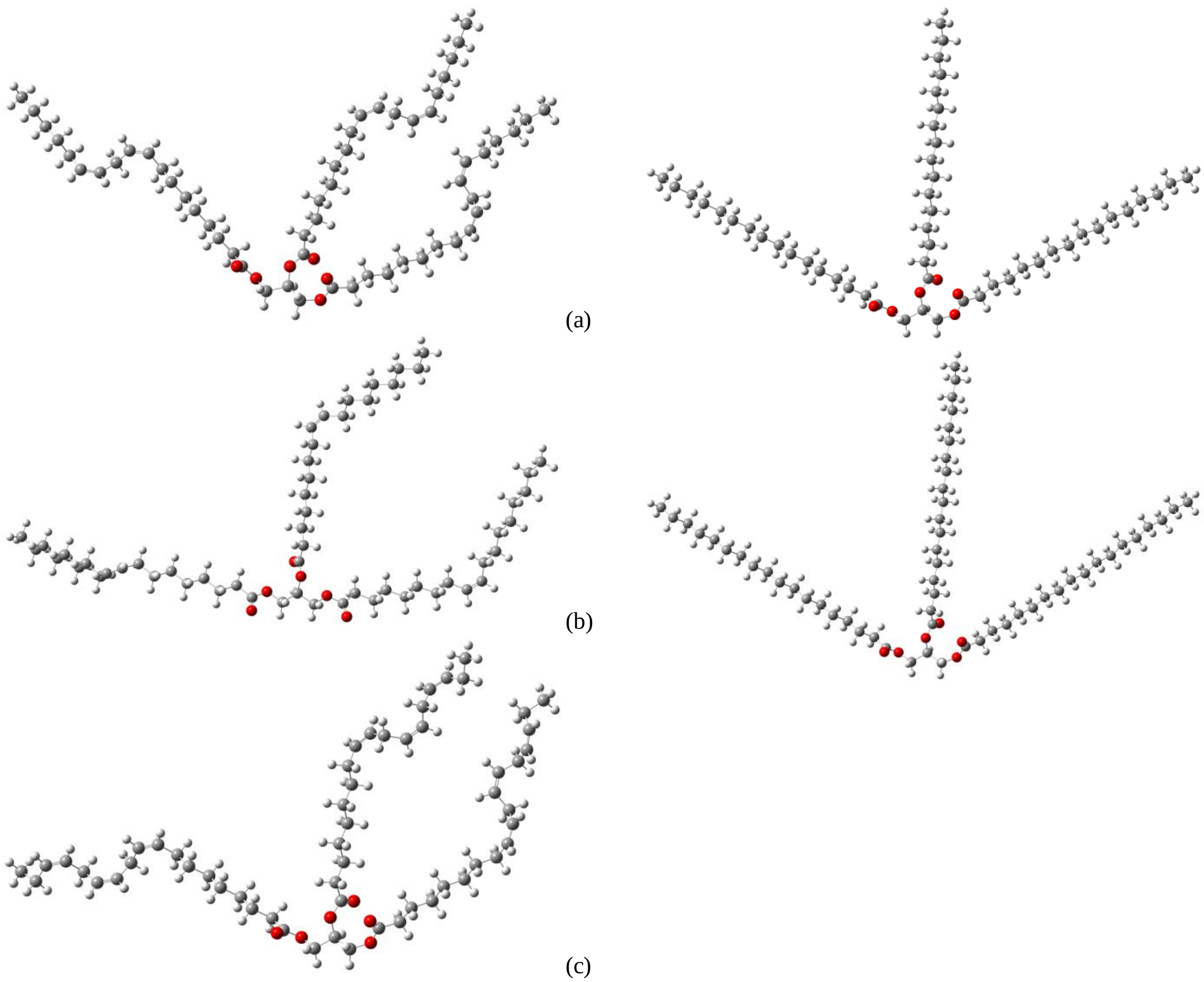


Figure 6. Absorption spectra of oleic acid at temperature of 28°C. Experimental data – dots, approximation curve – line.

To proof the concept that at heating fat tissue samples their terahertz spectra are changed due to water evaporation, absorption spectra of H_2O layers with different thicknesses in terms of OD (Fig. 5) and oleic acid as a major fat tissue component (Table 1) in terms of absorption coefficient in cm^{-1} (Fig. 6) were measured. The spectra obtained are in agreement with the results presented by other authors.[13,13,23] The absorption spectra of fat tissue and oleic acid presented in Fig. 2 and 6 are similar in this spectral range. This is due to the fact that the oleic acid predominates in porcine adipose tissue.

The fat cell size is in the range of 15–250 μm. The majority of the adipose tissue lipids are triglycerides. The size of its molecule, containing polyunsaturated fatty acids, is 1.5 nm. Triglyceride molecules can form various polymorphic forms. The most common forms are termed α, β' and β in order of increasing melting point, packing density and stability. The α form is the least stable and easily transforms to either the β' form or the β form.[24,25] Adipose tissue can be represented as a quasi-ordered structure due to crystal triglycerides. Since quasi-ordered media have scattering properties from both random and ordered structures, it is important to account even small local order of particles for estimating the scattering properties. For the quasi-ordered structures more comprehensive approaches such as generalized Mie solution or T-matrix formalism should be applied.[26] It was shown that sizes of triglyceride crystals are in the range from1-4 μm to larger than 100 μm.[24,25,27] As these structures are comparable with terahertz wavelengths, they are effective scatterers.[26,28]

Spatial configurations of the lowest energy conformers of five triglycerides of fatty acids (oleic, linoleic, palmitic, stearic, α-linolenic) are shown in Fig.7. Theoretical THz spectra of five triglycerides of fatty acids, taking into account their concentrations in porcine fat, are shown in Fig. 8.



(a) (b) (c) (d) (e)

Figure 7. Spatial configurations of different triglycerides of fatty acids: linolic (a), oleic (b), α-linolenic (c), stearic (d) and palmitic (e) acids.

The THz spectrum of porcine fat model constructed using the supermolecular approach is shown in Fig. 9, and its interpretation is shown in Table 2. When compiling the interpretation, only those vibrations that make the main contribution to the formation of vibrational bands were considered.

Table 2. Interpretation of the theoretical THz spectra of porcine fat in the frequency range from 0 to 2.5 THz.

Frequency (THz)	Interpretation
0.72	Deformation (bending) oscillation of the left chain of palmitic acid triglyceride
1.20	Deformation (torsion) oscillation of the left chain of oleic acid triglyceride
1.41	Deformation (torsion) oscillation of the central chain of palmitic acid triglyceride and mixed deformation (torsion) oscillation of the side chains of oleic acid triglyceride
1.92	Deformation (bending) oscillation of the right chain of palmitic acid triglyceride and a similar oscillation of the central chain

Figure 9 also shows experimental THz spectra of one of the samples of porcine fat at different temperatures. It can be seen that with increasing temperature, the frequency of deformation vibrations of the chains of triglycerides of fatty acids increases. The explanation for this process may be as follows. Molecules of triglycerides of fatty acids are able to hold a certain number of water molecules on their surface using hydrogen bonds. These bonds are formed with oxygen atoms that are part of carbonyl groups and glycerol crosslinking of fatty acid triglycerides. When fatty acids are heated, the probability of breaking these hydrogen bonds increases. This leads to the fact that the weight of the chains decreases and their mobility increases. This, in turn, leads to an increase in the frequency of deformation vibrations of the chains of triglycerides of fatty acids.

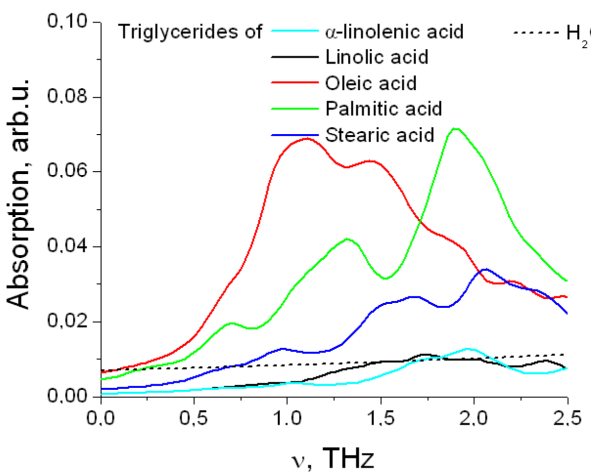


Figure 8. Absorption spectra of different triglycerides of fatty acids and free H_2O.

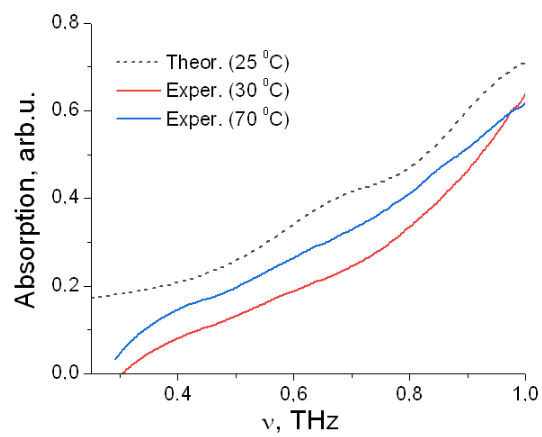


Figure 9. Theoretical and experimental THz spectra of porcine fat for different temperatures (all curves are normalized to value 1 on the ordinate axis).

The ability of fatty acids to retain water was not considered when constructing a model of porcine fat (see Figs. 8 and 9). When simulating various percentages of bound water, one to six water molecules were sequentially attached to the triglyceride model. A cluster of 27 water molecules was chosen as a model of free water. A shortened version of the saturated acid triglyceride $C_{15}H_{26}O_6$ was chosen as a fat model. The optimal geometry, wave numbers and intensities in the THz spectra of these models were calculated using the Gaussian program and the B3LYP / 6-311 + G (d, p) method. Based on the simulation results, it can be assumed that the release of free water from samples does not lead to structural changes in the terahertz spectra of fat. In the case of the release of bound water in the adipose tissue, structural changes take place and as a result, the spectrum changes in a more complex way. Note that the likely loss of water in a tissue sample at the beginning of heating (a decrease in the extinction coefficient due to a decrease in absorption) is due to free water first leaves the sample, and then bound water partly transfer to free water, which also leaves tissue. In this case, nonmonotonic behavior of the extinction coefficient of temperature is possible. Impact of THz waves scattering on tissue transmittance can be seen twice in the very beginning of heating when triglyceride crystals in cell lipid droplets are fused into liquid state (Table 1)and at higher temperatures when cell lipolysis with FFA release is important, which acts as immersion optical clearing with FFAs as OCAs.

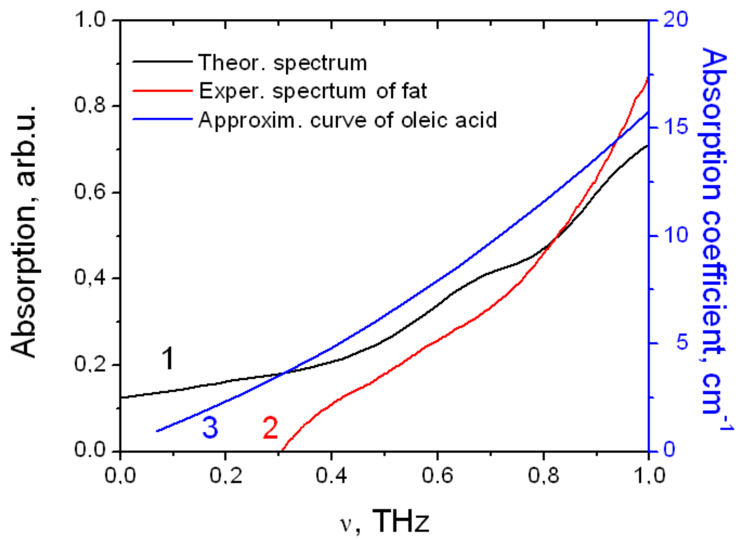


Figure 10. Theoretical and experimental THz spectra of porcine fat and approximation curve for oleic acid experimental data in one range.

It can be seen from Fig.10 that the shape of optical density curves of fat (theoretical and experimental data) and oil is similar, as evidenced by correlation analysis (Spearman's rank correlation coefficient between dependences of optical density for porcine abdominal fat (theoretical and experimental data) and oleic acid (approximation curve) is equal to 1).

4. CONCLUSION

Measurement and modeling of optical properties (OD) of adipose tissue and its components with temperature change in the terahertz range were done. The OD of adipose tissue samples was shown to decrease as temperature increased, which can be associated no more than 50 % with the dehydration of the sample. Some inclusion (50%) into OD decrease can be expected due to suppression of THz wave scattering at fusion of triglyceride crystals in cell lipid droplets are fused into liquid state and refractive index matching by free fatty acids released from adipocytes caused by thermally induced cell lipolysis. Using complex molecular simulation of the adipose tissue at temperature change using classical molecular dynamics and quantum chemistry, we found correlations between the results of measurements and modeling.

ACKNOWLEDGMENTS

The authors would like to acknowledge the support from RFBR grant No. 18-52-16025 (IYuYa, VVN, VIK and VVT), the National Research Tomsk State University Academic D.I. Mendeleev Fund Program (IYuYa, VVN, AVB, VIK, YuVK and VVT), Fundamental Research Program of the State Academies of Sciences for 2013-2020, the Government statement of work for ISPMS Project No. III.23.2.10 (VVN).

Authors are grateful to Dr. Kirill I. Zaytsev for discussion of results.

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