

Multivariate study of Raman spectral data of edible oils

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Abstract: - The study of structural differences and thermal degradation of edible oils during heating is presented in this paper. Five types of edible oil were used for the study: extra virgin olive oil, pomace olive oil, canola, sunflower and palm oil. The data for evaluation were acquired by Raman spectroscopy as this innovative method offers rapid, nondestructive, reagent free measurements, provides specific information on chemical composition and structure of material and enables detection of structural changes. For the processing of large data sets multivariate analytical method Cluster analysis and Principal component analysis are applied to highlight the patterns within the data. The results show good agreement with the known ratio of saturated and unsaturated fatty acids in the vegetable oils. The evaluation of thermal degradation process is also based on the major decomposition product of oxidized linoleate appearing in Raman spectra via band 1640 cm^{-1} . Thermal degradation was observed for all the studied oils. Mathematically processed data indicate the least effect of heating for olive oils, the greatest degradation and loss of unsaturation for sunflower oil.

Key-Words: - Thermal, Degradation, Stability, Edible, Oil, Raman Spectra, Multivariate, Principal Component Analysis, Cluster Analysis.

1 Introduction

The important role of edible oils in human diet is generally known. The edible oils are not only source of energy and source of taste and texture in food, but they are also very important for the intake of essential fatty acids and minor compounds content as are tocopherols, carotenoids, chlorophylls, phytosterols and others. Many people became to use predominantly vegetable oils in their diet trying to replace animal fat sources according to well-known health recommendations. They use vegetable oils for preparing many kinds of food in cold state but very often for many culinary purposes including high temperature application - as cooking, baking or frying.

Edible oils are commonly used for preparing many kinds of food and high temperatures are often applied. It is already known that extra virgin oils are recommended for preparing "cold" meals, because of their content of bioactive substances, minerals, vitamins, etc. Others, mainly refined, are suitable for frying or baking at higher temperature. However, even the edible oils that are relatively thermal resistant should be controlled because of formation of oxidation products. Therefore, the thermal stability of edible oils is a topic of research studies using various methods.

The conventional analytical methods for edible oils quality determination require pre-treatment of samples, reagents and solvents using as well as lab equipment and skills of the lab technicians. That is why new rapid and simple methods are still demanded. Raman spectroscopy seems to be very promising technique combining rapid and relatively simple approach with sensitivity for detection of changes in molecular structure. Moreover, Raman spectra can be obtained via transparent glass or plastic vials and this measurement can be done as on-line analysis and/or by portable Raman instruments. The increasing number of applications in recent years can be seen in the literature across number of scientific areas such as biochemistry, material science, medicine, also pharmaceutical, food technology or forensic and security purposes.

The objective of this study is to compare different types of edible oils and to explore their thermal stability using Raman spectroscopy. Methods that enable multivariate analysis such as Principal component analysis and Cluster analysis are used for data mining from the large spectral data sets. Chemometric methods combined with Raman spectroscopy seem to be very good tool for detection of authenticity of edible oils as mentioned elsewhere [1] and their characteristic behavior under thermal stress is completed in this study.

2 Motivation

For the process control of oils in terms of food technology, maintaining nutritional value and revealing significant structural changes leading to a reduction in quality of edible oils, it is desirable to look for innovative experimental methods offering especially rapid measurement independent of number of chemical reagents. Together with multivariate statistical methods that brings benefit of reduction the dimensionality of the problem, important similarities for further analysis can be observed.

The ability to obtain the required relevant information on edible oils and their thermal degradation is studied by a combination of Raman spectroscopic measurement with cluster analysis and principal component analysis.

3. Vegetable Oils

Vegetable oils consist of triglycerides in amount of 95 – 98 %. The rest is formed by minor compounds as carotenes, tocopherols, sterols, squalene and free acids [2, 3]. The triglycerides are composed of fatty acids with characteristic number of carbon atoms in chain and amount of saturated or unsaturated bonds that are responsible for behavior of edible oils. The main saturated fatty acids in vegetable oils are considered palmitic acid (C16:0) and stearic acid (C18:0). Oleic acid is the major monounsaturated fatty acid (C18:1). Linoleic acid (C18:2) and linolenic acid (C18:3) are the major polyunsaturated fatty acids. Each type of vegetable oil is characterized by its own specific fatty acids ratio content [3, 4].

Lipids are important and irreplaceable part of the human diet as well as proteins and carbohydrates. The role of lipids in organism is essential. Hence the intake of vegetable oils is very important. Except saturated fatty acids they also contain a substantial proportion of monounsaturated and polyunsaturated fatty acids. The unsaturated fatty acids represent health benefit of edible oils in their consumption, however, also a source of instability. Vegetable oils undergo oxidative degradation that can be caused during storage, technological operation in food production or during meal preparing, what includes mainly frying and baking.

3.1 Thermal Degradation

Thermal stress speeds up oxidative processes concerning predominantly polyunsaturated fatty acids as linoleic and linolenic acids according to

high double bonds content. The major oxidation products are esters, alcohols, aldehydes, ketones, lactones and hydroxy compounds. Presence of these products strongly affects quality of edible oils (taste and aroma) and their nutritional value. Moreover, many of oxidation products are considered as potentially toxic in relatively low concentration [5]. In addition, frying of foods is one of sources of *trans* fatty acids (TFAs) and their isomers in frying oil. Regular intake of some of TFAs is considered as negative for human health because they can involve levels of lipoprotein fractions in human blood with tendency to increase risk of cardiovascular diseases [6, 7].

According to this health risk is necessary to detect formation of oxidative products during oil heating. Some of qualitative changes of vegetable oils can be reflected in Raman spectra.

4 Materials and Methods

Raman spectroscopy was used for the study of characteristics of oils and changes occurring during thermal degradation of oils. Obtained spectra were evaluated using multivariate analytical methods – Principal component analysis and Cluster analysis.

4.1. Materials

Five different types of edible oils, four refined and one extra-virgin were used for spectroscopic analysis: extra-virgin olive oil, olive pomace oil, canola, sunflower and palm. All of these oils were purchased from common markets.

At the beginning these oils were measured at lab temperature, later heated for up to 160 ± 5 °C and the temperature was maintained for 5 hours to monitor the thermal degradation process. The amount of 2 liters of each of oils was heated up. The first sample of 100 ml was taken right after reaching the temperature of 160 °C other samples after every 30 minutes in the same amount. Raman microscopy measurement followed.

4.2. Raman Spectroscopy

Raman spectroscopy provides very specific chemical “fingerprint” of every single chemical substance in the form of the Raman spectrum. In other words Raman spectroscopy reflects chemical composition and structure of materials what makes the method proper for material identification and study of structural changes. Moreover, considering the advantages of the method, Raman spectroscopy

becomes popular and valuable part of laboratories around the world in recent years.

The method is based on Raman scattering - an inelastic scattering resulting from an interaction of a photon and a molecule. Photons of inelastic scattering have slightly changed wavelengths (compared to incident laser photons), that are characteristic for specific bonds in surveyed material. Since most photons are on molecules scattered elastically (Rayleigh scattering without changing the wavelength), it is necessary to filter out of the spectrum of the strongly present wavelength of laser.

Raman spectroscopy brings many advantages as the method is relatively rapid, non-destructive, contactless, usable for measuring through transparent glass or polymeric covering layers or containers, applicable to all states of matter and different forms, without special requirements for sample preparation, usable as in situ analysis. The most often appearing drawback of the method is the fact that Raman scattering is a weak effect. Luminescence as much stronger quantum effect with bigger intensity can overlap Raman spectra and mask spectral information [8, 9].

4.2.1 Raman Instrumentation

InVia Basis Raman microscope from Renishaw was used for measurements. Raman instrument uses two lasers as light sources: argon ion laser with the excitation wavelength 514 nm and maximum output power of 20 mW and 785 nm NIR diode laser with maximum output power 300mW. Both were tested but more precise results were obtained using NIR laser.

A Leica DM 2500 confocal microscope with the resolution $2\mu\text{m}$ was coupled to the Raman spectrometer. All measurements were collected with 20 s exposure time and 5 accumulations. The samples were firstly scanned in range 100 to 3200 cm^{-1} with 1 cm^{-1} spectral resolution. After determining the principle peaks the spectral range was reduced approximately to the area 800 - 1800 cm^{-1} .

4.3. Cluster Analysis

Raman spectral data are multivariate, since they reflect the composition of material and its structure. Raman peaks are assigned to the vibrations of chemical bonds in surveyed materials. For the evaluation it is advantageous to use multivariate

mathematics.

Cluster analysis is an explorative analysis offering patterns or structures recognition within the data. The method divides data into groups (clusters) in such a way, that objects in the same group are more similar to each other than to objects from other clusters.

4.4. Principal Component Analysis

Principal Component Analysis (PCA) is useful and powerful statistical method for data analysis. PCA finds application in various fields from chemistry via neuroscience to computer face recognition, image compression or social studies. Basically it finds and identifies patterns in datasets of high dimension and highlights hidden similarities and differences. It extracts relevant information from confusing data sets finding new variables for creation better imaginable mathematical space.

5 Results and Discussion

Samples of five cold edible oils and heated samples were measured on Raman microscope. Obtained Raman spectral data exhibit, due to their composition, similar distribution of characteristic peaks. However, each of oils has various ratios of saturated (SFA), monounsaturated (MUFA), polyunsaturated fatty acids (PUFA) and other components, what affects intensity and Raman shift of characteristic bands in spectra. Raman spectra of five oils are displayed in Fig. 1. The linear baseline correction and normalization according 1747 cm^{-1} band was applied. Raman band 1747 cm^{-1} (C=O ester carbonyl) was taken as an internal standard for normalization because it does not exhibit any changes in the structure. For the evaluation the essential molecular assignments for the measured spectral range are listed in Table 1 and are in accordance with literature [10, 11]. The attention was given firstly to cold oils, secondly to the heated samples.

The cluster analysis was performed for the Raman spectra of five cold oils, i.e. room temperature before heating. The hierarchical complete linkage based on correlation distance measure was used. There is the dendrogram in Fig. 2 of spectral area $(1200 - 1390)\text{ cm}^{-1}$, which refers to bands 1267 cm^{-1} and 1303 cm^{-1} . Intensities of these bands reflect the amounts of saturated and unsaturated fatty acids with straight aliphatic chains and predominantly with 16 or 18 number of carbon atoms in chains. Various types of oils differ in the

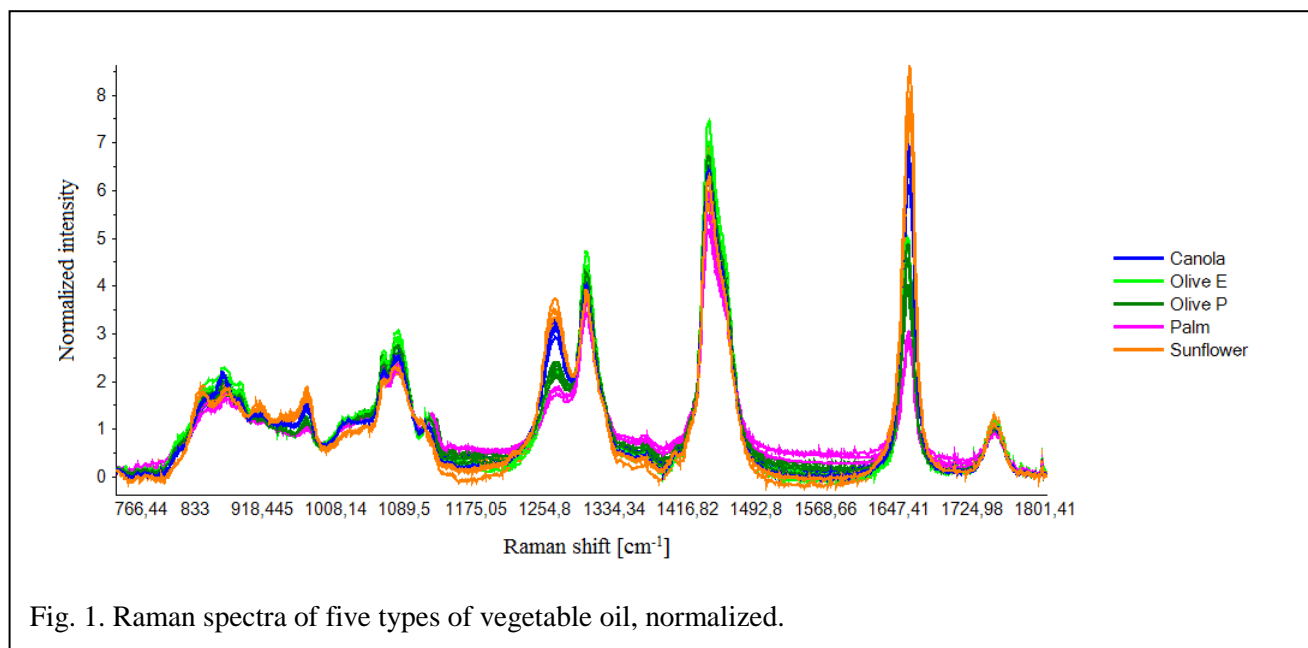


Fig. 1. Raman spectra of five types of vegetable oil, normalized.

proportion of these components, what can be used for their differentiation.

The relative intensity ratio of the bands at 1265 cm^{-1} and 1303 cm^{-1} is usually used to determine the degree of unsaturation in the oil [12]. The olive oils are the most similar. This group is enlarged by palm oil what is affected by the similar slight higher content of palmitic and stearic acids. Olive oil and palm oil contain higher share of saturated fatty acids [3, 5] compared to canola and sunflower oil. Canola and sunflower oil creates the second sub-cluster.

Table 1 Assignments for Raman bands.

Raman shift [cm ⁻¹]	Molecular vibration
1267	=C-H symmetric rocking
1303	CH ₂ in-plane twist
1442	CH ₂ scissoring
1526	C=C stretching of carotenoids (extra virgin oils)
1640	C=C <i>trans</i> , <i>trans</i> -2,4-decadienal
1658	C=C <i>cis</i> double bond stretching
1747	C=O ester-carbonyl stretching

In comparison to the first sub-cluster, sunflower and canola oils have rather higher amounts of polyunsaturated fatty acids mainly linoleic acid.

The context of saturated and unsaturated fatty acids in the composition of oils clearly illustrates Raman spectra in Fig. 1. at bands 1267 cm^{-1} and 1658 cm^{-1} (unsaturated) and 1303 cm^{-1} and 1442 cm^{-1} (saturated fatty acids).

Fig. 3 represents the dendrogram of spectral area ($1200 - 1800\text{ cm}^{-1}$). Here, the layout within clusters is alike, but the difference is in lower correlation, especially for the connection of palm oil to the olive oils. This area is more complex with the four major bands, but also less significant spectral characteristics, what affects the pattern creation.

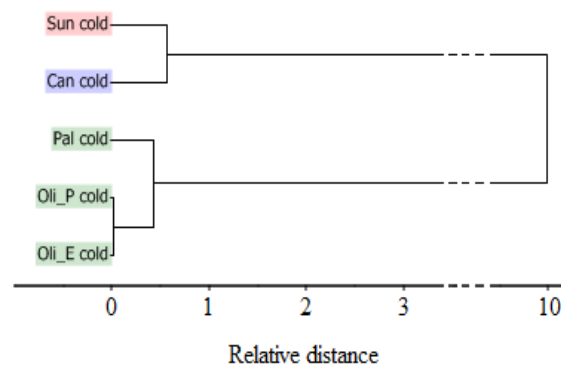


Fig. 2. Cluster analysis of cold oils in area ($1200 - 1390\text{ cm}^{-1}$).

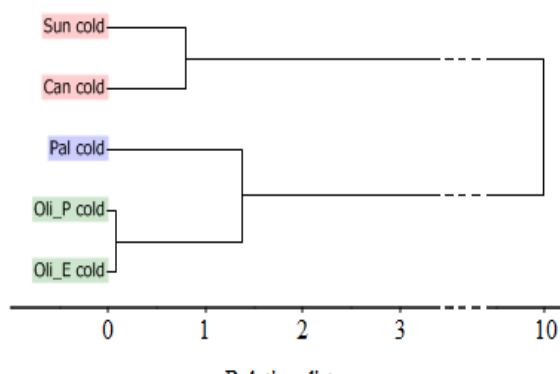


Fig. 3. Cluster analysis of cold oils in area 1200 - 1800 cm⁻¹.

Thermal degradation was studied on all heated samples. Formation of the degradation product at 1640 cm⁻¹ assigned to *trans, trans*-2,4-decadienal is evident in Raman spectra of all heated oils. It is one of the oxidative products of heated oxidized linoleate which corresponds to the C=C stretching vibration in conjugated system [13]. This Raman

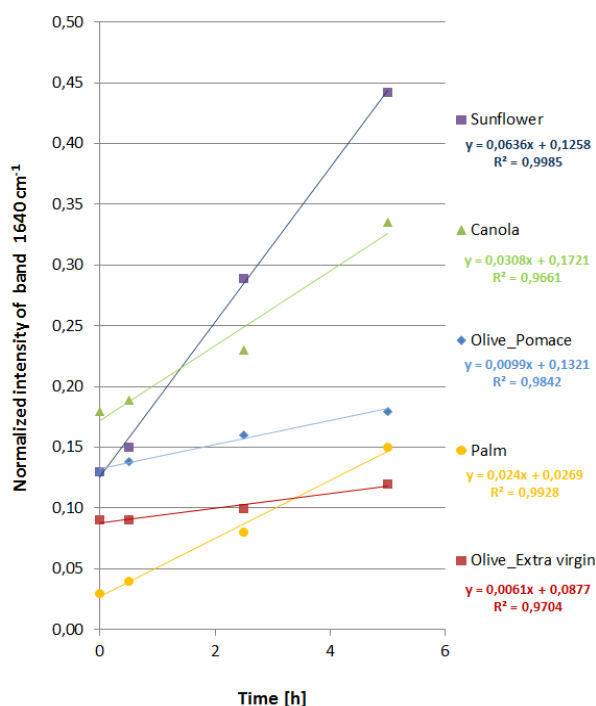


Fig. 4. Thermal degradation of edible oils according to normalized intensities of Raman band at 1640 cm⁻¹.

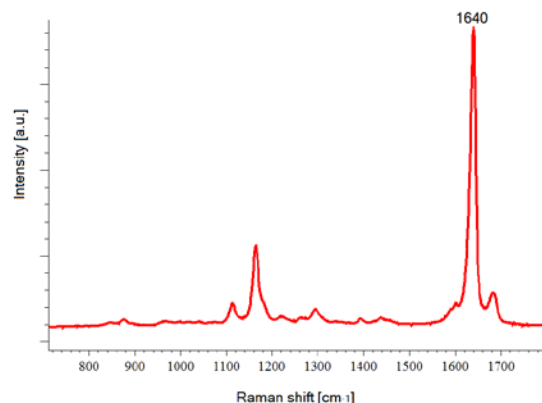


Fig. 5. Raman spectrum of the standard of *trans, trans*-2,4-decadienal.

band is confirmed by the Raman spectrum of standard of *trans, trans*-2,4-decadienal. The formation of degradation product in oils correspond to the most intensive band in *trans, trans*-2,4-decadienal standard, as is shown in Fig. 5.

The dependence of normalized intensities of band 1640 cm⁻¹ on heating time is shown in Fig. 4.

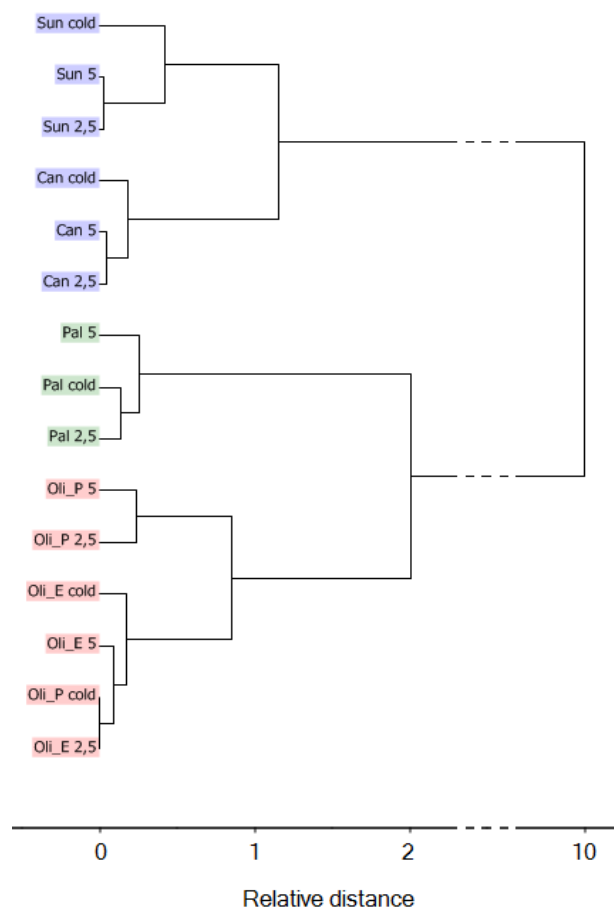


Fig. 6. Cluster analysis of cold and heated oils.

Intensities were acquired from fitting using normalization via 1747 cm^{-1} band and subtraction of the intensity at the room temperature. Results show the smallest structural changes for extra virgin olive oil followed by pomace olive oil. More distinct structural changes due to heating were recorded for palm oil, followed by canola oil. The most significant impact of oxidative change is observed for sunflower oil.

For the clarity of the outputs of multivariate analysis cold and heated oils for 2,5 hours and 5 hours were used. The dendrogram for named samples and the whole measured spectral area is displayed in Fig. 6. The first cluster consists of sunflower and canola oils which create sub-clusters with clear separation of cold and heated samples. The measures indicate more notable differences for the sunflower oil what is in accordance with the results shown in Fig. 4. Second cluster consists of two sub-clusters of palm oil samples and olive oils. Higher proximity exhibit cold and 2,5 hour heated palm oil in comparison to heated palm oil for 5 hour. Extra virgin and pomace olive oils are partly mixed. However, both, cold extra virgin olive oil and for 5 hours heated extra virgin olive oil are separated; heated pomace olive oil samples create another sub-cluster.

Spectral data of 5 cold and thermally degraded samples were analyzed also by the Principal component analysis. The result is shown in Fig. 7. The diversity is sharply distinguished from the other species of oils. The only one overlap appears for

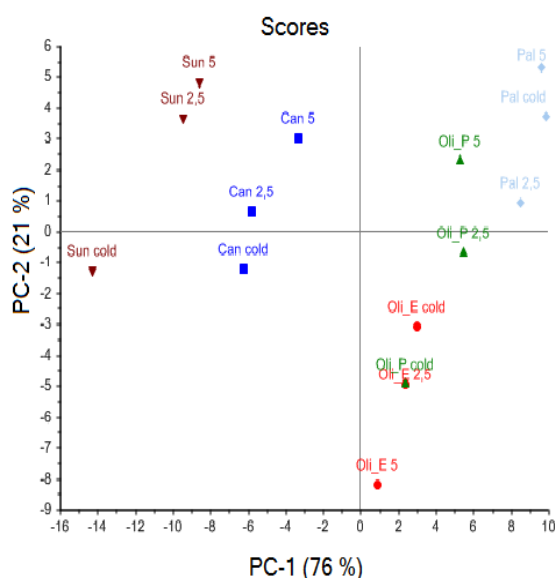


Fig. 7. PCA scores of cold and heated oils.

extra virgin and pomace olive oils, what supports the closeness to their composition over other oils. All four refined oils except of extra virgin olive oil show the same trends according to growing PC-1 and PC-2. This only one – extra virgin oil has the opposite trend. Another differentiation can be observed - the creation of two groups, the first containing sunflower and canola oils for negative PC-1 and the second one containing both types of olive oil and palm oil for positive PC-1, as well as for the results obtained by cluster analysis.

6 Conclusions

Data reflecting structural variations of 5 types of edible oils were acquired by modern spectroscopic method Raman spectroscopy. Multivariate statistical methods Cluster analysis and Principal component analysis were used for the evaluation of the datasets. The results show good agreement with the known ratio of saturated and unsaturated fatty acids in the oils. The assessment of thermal degradation can be done via amount of arising degradation products as *trans*, *trans*-2,4-decadienal. The most evident degradation was observed for sunflower oil, then for canola and palm oil. The best thermal stability show extra virgin olive oil followed by pomace olive oil. However, the thermal stress speeds up undesired decomposition of vegetable oils and promotes creation of unsafe and unhealthy oxidative products in all types of tested oils. This oxidative process is more intensive with the longer period of heating.

Acknowledgements

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