RESEARCH PAPERS
FACULTY OF MATERIALS SCIENCE AND TECHNOLOGY IN TRNAVA
SLOVAK UNIVERSITY OF TECHNOLOGY IN BRATISLAVA

2017 Volume 25, Number 40

GAS CHROMATOGRAPHIC DETERMINATION OF FATTY ACIDS IN OILS WITH REGARD TO THE ASSESSMENT OF FIRE HAZARD

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Abstract

The aim of the paper was to study and research the application of processing gas chromatographic method for the rapid and accurate determination of the composition of different types of oils, such as substances with the possibility of an adverse event spontaneous combustion or self-heating. Tendency to spontaneous combustion is chemically characterized mainly by the amount of unsaturated fatty acids, which have one or more double bonds in their molecule. Vegetable oils essentially consist of the following fatty acids: palmitic, stearic, oleic, linoleic, and linoleic. For the needs of assessment, the fire hazard must be known, in which the double bond is present, as well as their number in a molecule. As an analytical method, GCMS was used for determination of oils content. Three types of oil were used - rapeseed, sunflower, and coconut oil. Owing to the occurrence of linoleic acid C18:2 (49.8 wt.%) and oleic acid C18:1 (43.3 wt.%) with double bonds, sunflower oil is the most prone to self-heating. The coconut and rapeseed oils contain double bond FAME in lesser amount, and their propensity to self-heating is relatively low.

Key words

Gas chromatography, oil, fatty acid, ester, oxidation, saturation, fire

INTRODUCTION

Fats or lipids consist of numerous chemical compounds, including monoglycerides, diglycerides, triglycerides, phosphatides, cerebrosides, sterols, terpenes, fatty alcohols, and fatty acids. Fatty acids constitute the main component of phospholipids, triglycerides, diglycerides, monoglycerides and sterol esters. Fatty acids consist of the elements, such as

carbon, hydrogen, and oxygen that are arranged as a linear carbon chain skeleton of variable length with a carboxyl group at one end (1).

The fire hazards associated with the use, storage, and shipment of combustible liquids are so familiar as to need no discussion, and the tremendous and varied consumption of such liquids carries the problem of protection against these hazards. Fatty oils are a quite common cause of fires caused by spontaneous combustion.

Typical composition of fatty acids, present as glycerides esters, consists of 6 – 7 wt.% of palmitic acid (C16:0), 3 – 6 wt.% of stearic acid (C18:0), 14 – 24 wt.% of oleic acid (C18:1), 14 – 19 wt. % of linoleic acid (C18:2) and 48 – 60 wt.% of linolenic acid (C18:3) (5), where the first number in each ratio corresponds to the length of the carbon chain and the second to the number of double bonds in the compound (5).

Fatty acids (FA) can be *saturated* (no double bond), *monounsaturated* (one double bond), or *polyunsaturated* (two or more double bonds), and are essential (1).

The saturated fatty acids begin with methanoic (formic) acid. Methanoic, ethanoic, and propanoic acids are uncommon in natural fats and are often omitted from definitions of lipids. However, they are found nonesterified in many food products. Omitting these fatty acids based on their water solubility would argue for also eliminating butyric acid, which would be difficult, given its importance in dairy fats. The simplest solution is to accept the very short chain carboxylic acids as fatty acids while acknowledging the rarity in natural fats of these water-soluble compounds (2). The unsaturated FA may contain one or more double or triple bonds.

The self-heating phenomenon of vegetable oils originates in the oxidation of the fatty acids present in them. Because of a high proportion of carbon-carbon double bonds they are prone to oxidation.

Because this oxidation reaction is exothermic, it generates heat directly in the material. In turn, the heat released may be sufficient to provide the activation energy necessary to bring the material or its substrate to its auto-ignition temperature. The auto-oxidation of vegetable oils is a process that can be described as occurring in three different steps: initiation, propagation, and termination.

The initiation step consists of the reaction of the fatty acid chain with a free radical (R•). The origin of the free radical is rarely explained in the literature; however, some authors have reported the natural presence of hydroperoxides (ROOH), which decompose to form free radicals. . The radical attacks a C–H bond, most likely one of the methyl group present between two double bonds (most common configuration). The resulting chain is stabilized in a resonance configuration (4).

During the propagation stage, the new radical reacts with oxygen to form a peroxyl radical (ROO•). In turn, this radical reacts either with another radical to form a nonradical species (a case of termination) or with a hydrogen atom from another fatty acid, thus forming a hydroperoxide (ROOH) and a new alkyl radical (R´•), as in the initiation stage. This last option occurs only rarely because its kinetics are several orders of magnitude lower than those of the reaction of oxygen (O_2) with an alkyl radical. Alternatively, a hydroperoxide can also lose a hydroxyl radical (•OH), thus leaving an oxy radical (RO•) (4).

In the termination step, two radicals react together to form a stable product. Because the resulting links take place between fatty acid chains, it is a cross-linking reaction. The bonds formed can be of either carbon-to-carbon (C–C), ether (C–O–C), or peroxide (C–O–O–C) types. Bonding can occur between two fatty acids from the same triglyceride, in which instance no polymerization of the oil takes place, or between fatty acids of different triglycerides, in which polymerization of the oil occurs. Because the auto-oxidation is initiated by the breakage of a C–H bond along the alkyl chain of fatty acids, the strength of that C–H bond significantly influences the occurrence and rate of the reaction. As a matter of fact, the strength of the C–H bond is, in and of itself, affected by the presence of double bonds on adjacent carbon atoms.

These double-bonded atoms weaken the C–H bonds of the adjacent carbon atoms, thus decreasing the bond dissociation energy necessary to break them (4).

The auto-oxidation of vegetable oils is highly dependent on the presence of double bonds. The easier the C–H bond breaks, the more likely the chain reaction is to take place. Thus, the more polyunsaturated fatty acids an oil contains, the greater its propensity to auto-oxidizing and thus, to self-heating (4).

One important parameter of different vegetable oils is the amount of unsaturation of the constituent fatty acids. This has been measured by the iodine value (IV), which is currently determined by the Wijs method. Although many methods have been developed, the Wijs method is the most widely used as a standard method. Major drawbacks of that method include the use of dangerous iodine trichloride (Wijs reagent) and the time-consuming procedures for reagent preparation and chemical analysis (3).

The most hazardous oils can self-heat when dispersed onto quite small amounts of material. The less reactive oils will present problems if dispersed on much large quantities of porous material, such as bales of wool. Under the right condition, self-heating of these dispersed oils will lead to the onset smouldering combustion.

Summary of the most common fatty acids is presented in Table 1. The table describes the number of carbons of the fatty acid and the structural formula, and shows the characterization of the binding saturation.

Table 1 List of fatty acids

Number of C	Fatty acids	Structural formula	Saturation
C_{12}	Lauric acid	$CH_3(CH_2)_{10}COOH$	saturated
C_{14}	Myristic acid	$CH_3(CH_2)_{12}COOH$	saturated
C_{16}	Palmitic acid	$CH_3(CH_2)_{14}COOH$	saturated
	Palmitoleic acid	$CH_3(CH_2)_5CH=CH(CH_2)_7COOH$	unsaturated: 1 *cis*-double bond (9)
C_{18}	Stearic acid	$CH_3(CH_2)_{16}COOH$	saturated
	Oleic acid	$CH_3(CH_2)_7CH=CH(CH_2)_7COOH$	unsaturated: 1 *cis*-double bond (9)
	Ricinoleic acid	$CH_3(CH_2)_5CH(OH)CH_2CH=CH(CH_2)_7COOH$	OH + 1 *cis*-double bond (9)
	Linoleic acid	$CH_3(CH_2)_4CH=CHCH_2CH=CH(CH_2)_7COOH$	unsaturated: 2 *cis*-double bonds (9,12)
	α-Linolenic acid	$CH_3CH_2CH=CHCH_2CH=CHCH_2CH=CH(CH_2)_7COOH$	unsaturated: 3 cis-double bonds (9, 12, 15)
C_{20}	Arachidonic acid	$CH_3(CH_2)_4(CH=CHCH_2)_4CH_2CH_2COOH$	unsaturated: 4 *cis*-double bonds (5, 8, 11, 14)

The main aim of the this paper was to qualitatively and quantitatively determine the methyl esters of fatty acids (FAME) by gas chromatography on a GC-MSD Agilent 5975 gas chromatograph with HP-5ms capillary column. Priority in identifying the represented fatty acid methyl esters was the prioritization of methyl esters containing multiple bonds.

Overview of the methods of determining the oil and the methyl esters of fatty acids by gas chromatography

Botinestean (2012) (6) analysed tomato seed oil by gas chromatography with mass detector (GCMS). For this analysis, a gas chromatograph HP with 6890 mass selective detector with a HP 5973 was used; helium was the carrier gas. As a column HP-5ms (30 m length, 0.25 mm ID, and film thickness 0.25 microns) was used. The column temperature was programmed from the initial temperature of 50 °C to 250 °C for 4°C/min. Injection volume was 2 μL. The basic principle of the preparation of FAMEs samples by (6) was the esterification of samples containing fatty acids with methanol. The resulting complex extraction solution was treated with 5 mL of methanol and BF3 solution. Then, the solution was heated for 2 minutes in a hot water bath. After addition of 5 mL of hexane, the solution was refluxed for another additional minute in a water bath. The solution was treated with 15 mL of saturated sodium chloride solution with vigorous stirring. The organic layer was separated and dried over anhydrous $CaCl_2$.

Naureen (2015) (7) analysed sunflower oil by GCMS with capillary column DB-5ms (30 m x 0.32 mm, 0.25 μm, and film thickness 0.25 microns). Transesterification was carried out using a 2000 mL round bottom flask equipped with a reflux condenser, magnetic stirrer, thermometer and sampling lead. 1L of vegetable raw oil was filtered and pre-heated to 120 °C to remove moisture. Transesterification of the sunflower oil is carried out in a ratio of 6:1 with methanol in the presence of sodium hydroxide as a catalyst.

In the case of fatty acid analysis in canola oil according to Aftaba (2014) (8), methyl esters of fatty acids (FAME) were prepared using standard method 2.301 (IUPAC, 1979). 100 mg of the sample was dissolved in 20 mL of methanol. To the sample was added 1 N KOH solution, which was refluxed for one hour. Then 10 mL of hexane was added to separate the organic layer. The final product was dissolved in chloroform for analysis by gas chromatography in conjunction with a mass detector.

Methyl esters of fatty acids are known for their activity and thermal lability. Incorrect injection conditions and incorrect liner selection result in non-linear responses. Late-elongated FAMEs exhibit a lower response than the nearly-elapsed FAME. This problem is overcome by the use of a glass wool liner, which greatly improves the uniformity of evaporation and reproducibility of the measurement. The liner surface is deactivated to increase its inertia.

Table 2 gives an overview of other methods of FAME determination by gas chromatography and the most widely used detection by mass spectrometry and flame ionization detection (FID) (9).

Table 2 Overview of methods of FAME determination by gas chromatography (GC)

Analyte	Derivatization	Column	Detector
Fatty acid (stearic, palmitic)	diazomethane	DB-ms 30 m x 0.25 mm x 0.15 μm	MS
$C_{16} – C_{18}$ fatty acids	BF_3/methanol	HP-5ms 30m x 0.25 mm x 0.25 μm	MS
Monocarboxylic and dicarboxylic acid	BF_3/methanol	HP-5 25m x 0.2 mm x 0.33 μm	MS
	BF_3/propan-1-ol	HP 30 m x 0.25 mm x 0.25 μm	FID, MS
$C_1 – C_{10}$ monocarboxylic acids	2,4´-dibromoacetofenon	DB-5 30m x 0.25 μm	FID

$C_2 – C_{10}$ monocarboxylic acids	2,4´-dibromoacetofenon	DB-5MS, 30 m x 0.25 mmx 0.25 μm	MS
$C_5 – C_{32}$ monocarboxylic acids	BF_3/methanol	DB-5ms 60 m x 0.25 mm x 0.25 μm	MS
$C_7 – C_{28}$ monocarboxylic acids	BF_3/methanol	HP-5 30 m x 0.25 mm x 0.25 μm	MS
$C_{14} – C_{30}$ monocarboxylic acids	diazomethane	RTx-5ms 25 m x 0.25 mm x 0.25 μm	MS
$C_{26} – C_{28}$ monocarboxylic acids	BF_3/methanol	HP-ms 25 x 0.25 mm x 0.25 μm	MS
Dicarboxylic acid	BF_3/butan-1-ol	SPB 30 m x 0.32 mm x 1 μm	FID, MS
	BF_3/butan-1-ol	DB-5 30 m x 0.25 μm	MS
	diazomethane	CP Sil 8CB 30 m x 0.25 mm x 0.25 μm	MS
$C_5 – C_{10}$ dicarboxylic acid	BF_3/methanol	DB-5 60 m x 0.25 mm x 0.25 μm	MS
Acetic acid	-	HP-5 50 m x 0.2 mm x 0.5 μm	MS

MATERIALS AND METHODOLOGY

Instrumentation. Fatty acids were quantified by gas chromatography using the 7890 GC system (Agilent Technology, USA) and 5975C inert MSD with triple-Axis detector (Agilent Technology, USA). HP-5ms was used as column in GC/MS (5%-diphenyl, 95%-dimethylpolysiloxane, 30 m x 0.250 mm ID x 0.25 μm).

Methods. The temperature program was set up from 50 °C to 250 °C with 4 °C/min, both the injector and detector temperatures were 280 °C, and He was used as carrier gas. The injection volume was 2μL. Ionization energy EI of 70 eV was used for mass spectroscopy detector.

Samples. Three types of oil were used Vita - rapeseed oil (Brändle, Germany), Heliol - sunflower oil (Palma, Slovakia) and coconut oil (Health link, Czech Republic); all samples were purchased from a local store. For the determination by gas chromatography, the oil samples were prepared by esterification according to the equation:

$$oil + CH_3OH \xrightarrow{NaOH} methylester + C_3H_8O_3$$

Procedure of esterification: 50 mL of oil sample was heated to 55 °C, added was CH_3OH/NAOH, mixture was stirred for about 10 minutes. After reaction, solution was centrifuged to separate the layers. Sample of FAME was diluted with cyclohexene and prepared for GC analyses.

Calibration standards. A series of standard mixtures were prepared from AOCS Low Erucic Rapeseed Oil (Sigma-Aldrich) with analytical grade cyclohexane (Sigma-Aldrich) in concentration of solution from 1.5 – 10 mg/mL. For quantification of FAME in selected oil samples, quantification by external standard was made in duplicate. Since separation ability of

column HP-5ms, just six FAME calibration curves could be plotted. Individual calibration curves for selected FAME are in Table 3. Calibration curves were made as concentration dependence on the peak area of FAME. For the calculation of the peak area, the ChemStation Integrator was used. The amount of each FAME was calculated from the concerned calibration curve and mathematically recalculated to wt.%.

Table 3 Calibration curve and coefficient of determination of FAME

FAME	Saturation	Calibration range[mg/mL]	Calibration curve	Coefficient of determination R^2
Oleic acid methyl ester	C18:1	0.90 – 6.00	A = 2E+08c	0.9956
Methyl eicoseonate	C20:1	0.015 – 0.10		0.9834
Methyl linoleate	C18:2	0.18 – 1.20		0.9852
Methyl myristate	C14:0	0.015 – 0.10		0.9893
Methyl palmitate	C16:0	0.20 – 0.40		0.9843
Methyl stearate	C18:0	0.045 – 0.3	A = 3E+08c	0.9731

RESULTS AND DISCUSSION

Based on the comparison of the retention times measured by analysis of the analytical standard and the retention times measured at each sample of the analysed oil, it is possible to identify the fatty acids and verify the correctness of the measured results. In chromatogram of coconut oil, fatty acids not found in the calibration standard were identified by mass spectrometry detector. Table 4 represents percentage concentration of FAME in oils sample recalculated from calibration curve.

Table 4 Concentration of FAME in oils samples

	Concentration of FAME [wt.%]					
	Oleic acid methyl ester	**Methyl eicoseonate**	**Methyl linoleate**	**Methyl myristate**	**Methyl palmitate**	**Methyl stearate**
Rapeseed oil	68.50	0.08	15.79	-	5.14	1.26
Sunflower oil	43.3	-	49.86	-	1.05	1.06
Coconut oil	4.1	-	1.14	19.22	8.86	0.45

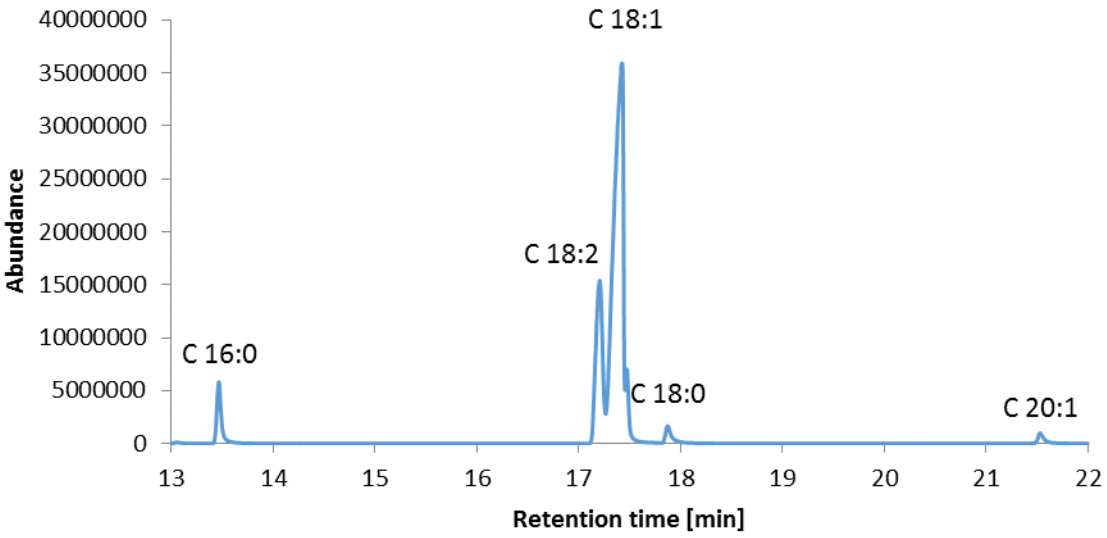

Fig. 1 *Chromatogram of rapeseed oil*

As can be seen from Figure 1, composition of rapeseed oil is best represented by oleic acid C18:1 (68.50 wt.%) and linoleic acid C18:2 (15.79 wt.%). Similar results are also presented in (10). In terms of susceptibility to self-ignition, linoleic acid is preferred, having two double bonds and oleic acid with one double bond. Other determinate FAME by this GCMS method are palmitic acid C16:0, stearic acid C18:0, and eicosenic acid C20:1. Their concentration is listed in Table 4.

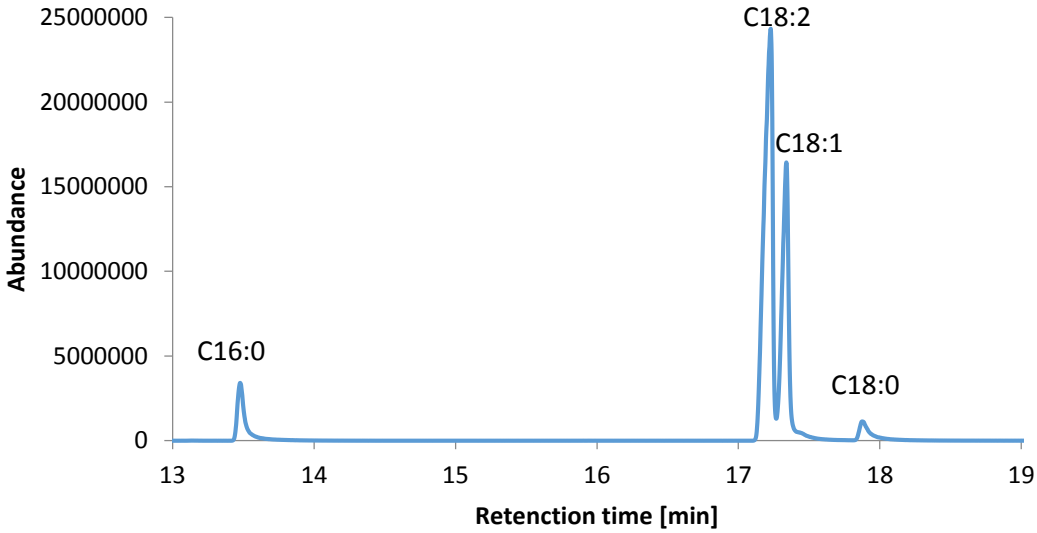

Fig. 2 *Chromatogram of sunflower oil*

The chromatogram of a sunflower Oil (Figure 2) shows the major presence of fatty acid methyl esters: linoleic acid C18:2 (49.8 wt.%) and oleic acid C18:1 (43.3wt.%). These two fatty acids contain multiple bonds and are the measure of the risk of self-ignition or self-heating of sunflower oil. In addition, it contains a smaller amount of palmitic acid C16:0 and stearic acid C18:0.

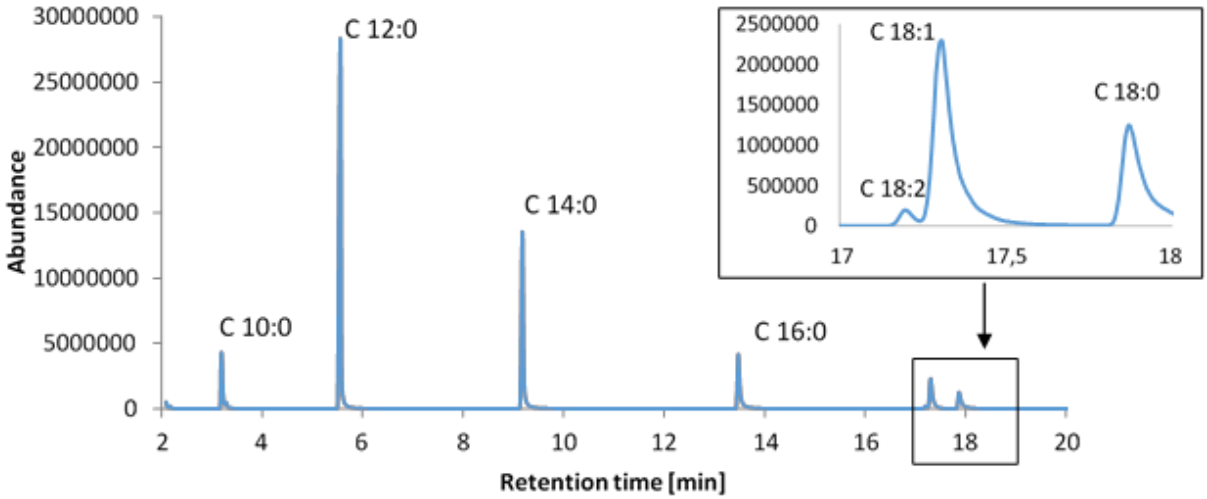

Fig. 3 *Chromatogram of coconut oil*

In the chromatogram of coconut oil (Figure 3), the major methyl esters of fatty acids are lauric acid C12:0 (saturated), myristic acid C14:0 (19.22 wt.%). In minor proportions, there are capric acid C10:0, palmitic acid C16:0 (8.86 wt.%), oleic acid C18:0 (4.1 wt.%), linoleic acid C18:2 (1.14 wt.%) , and stearic acid C18:0 (0.45 wt.%).

CONCLUSIONS

The purpose of the paper was to determine the methyl esters of fatty acids from rapeseed, sunflower and coconut oil samples, and to establish multiple linkages in fatty acid methyl ester chains as an indicator of the undesired self-heating and self-ignition event. The results of the chemical analysis show the presence of multiple fatty acid methyl esters of multiple bonds in all tested oils in different amounts.

The rapeseed oil contains unsaturated oleic acid with one double bond in an amount of 68.50 wt.%, linoleic acid with two double bonds in an amount of 15.79 wt.%, and a trace amount of 0.08 wt.% eicosenoic acid with one double bond. The propensity to self-inflammation is relatively small.

Sunflower oil contained linoleic acid in an amount of 49.86 wt.% and oleic acid in the amount of 43.30 wt.%. Majoritarian representation of these two acids (93.16% by weight) with multiple bonds tends to self-ignite and the chemical tendency to self-ignition is more pronounced than that of rapeseed oil.

The coconut oil contained oleic acid in an amount of 4.15 wt.% and linoleic acid in an amount of 1.14 wt.% which means that the propensity to spontaneously ignite coconut oil is very small.

Analytical method of gas chromatography mass spectrometry detection appears to be appropriate for simple and rapid detection of multiple bond in fatty acid methyl ester chains in oils.

Acknowledgement

This contribution was written with the support of the Research and Development Operational Programme within the project: *Hybrid power source for technical and consulting laboratory*

use and promotion of renewable energy sources (ITMS 26220220056) financed from the resources of the European Regional Development Fund.

References:

1. COW, CH. K. 2007. *Fatty Acids in Foods and their Health Implications*. CRC Press ISBN: 9780849372612
2. AKOH, C., MIN, D.B. 2002. *Food Lipids Chemistry, Nutrition, and Biotechnology*. Marcel Dekker, Inc., ISBN: 0824707494
3. KYRIAKIDIS, N., KATSILOULIS, T. 2000. Calculation of Iodine Value from Measurements of Fatty Acid Methyl Esters of Some Oils: Comparison with the Relevant American Oil Chemists Society Method. *Journal of the American Oil Chemists' Society*, **77**(12), pp. 1235–1238.
4. BAYLON, A., STAUFFER, É., DELÉMONT, O. 2008. Evaluation of the Self-Heating Tendency of Vegetable Oils by Differential Scanning Calorimetry. *Journal of Forensic science*, **53**(6), pp. 1334–1343.
5. JUITA, DLUGOGORSKI, B.Z., KENNEDY, E.M., MACKIE, J.C. 2013. Roles of peroxides and unsaturation in spontaneous heating of linseed oil. *Fire Safety Journal*, Volume **61**, pp. 108 – 115.
6. BOTINESTEAN, C. et al. 2012. Fatty Acids Composition by Gas Chromatography – Mass Spectrometry (GC-MS) and most important physical- chemicals parameters of Tomato Seed Oil. *Journal of Agroalimentary Processes and Technologies*, **18**(1), pp. 89 – 94.
7. NAUREEN, R. et al. 2015. Synthesis, spectroscopic and chromatographic studies of sunflower oil biodiesel using optimized base catalyzed methanolysis. *Saudi Journal of Biological Sciences*, **22**(3), pp. 332 – 339.
8. AFTAB, A. et al. 2014. Gas chromatographic coupled mass spectroscopic study of fatty acids composition of Nigella sativa L. (KALONJI) oil commercially available in Pakistan. *International Food Research Journal*, 21, pp. 1533-1537.
9. KŘŮMAL, K., MIKUŠKA, P., VEČEŘA, Z. 2009. Referát. Zdroje, výskyt a analýza karboxylových kyselin v ovzduší. (Paper. Sources, occurrence and analysis of carboxylic acids in the air.) Brno: VUT. *Chemické listy (Chemical letters)*, 103, 287 - 283, pp. 280.
10. WROTIAK, M., REKAS, A. 2016. Nutritional value of cold-pressed rapeseed oil during long term storage as influenced by the type of packaging material, exposure to light & oxygen and storage temperature. *Journal of Food Science and Technology*, pp. 1338 – 1347.
11. JAŠO, I. 2016. Stanovenie vyšších mastných kyselín metódou plynovej chromatografie s hmotnostným detektorom. Diplomová práca. (Determination of the higher fatty acids by gas chromatography with mass detector. Diploma thesis. Supervisor: Alica Bartošová, PhD). Trnava: MTF STU, 85 p.

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