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**Effect of heating on fatty acid composition of edible oils  
and fat**

MASTER'S THESIS

Prague 2019

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## **Declaration**

I, Filip Vojáček, hereby declare that I have done this thesis entitled “**Effect of heating on fatty acid composition of edible oils and fat**” independently, all texts in this thesis are original, and all the sources have been quoted and acknowledged by means of complete references and according to Citation rules of the FTA.

In Prague, 26th April 2019

.....

Filip Vojáček

## **Acknowledgements**

I would like to thank my supervisor, Ing. Klára Urbanová, Ph.D. for her support and help with laboratory work and the advice provided. She always found time for me whenever I needed some help with anything.

Besides my supervisor, I would like to also thanks to all from the laboratory colleagues for their advice whenever I needed and pleasant distraction during laboratory work.

Last but not least, I would like to really thank all of my classmates and of course, to my family. Their support during all of my study was crucial for me and without them, I would never have done it.

“Anything's possible if you've got enough nerve.” J.K. Rowling

## **Abstract**

Frying of food is one of the oldest methods of cooking. Oil is during the frying process subjected to aggressive conditions such as high temperature, and the presence of oxygen and water. These conditions cause thermal-oxidative degradation changes in the oil according to thermal-oxidative stability of the oil. These changes influence a fatty acid composition of the oil. In this study has been examined the influence of constant heating of oil on its fatty acid composition. Seven types of oil and one type of fat were heated up at 190 °C in a commercial fryer. The heating process steadily continued for 24 hours and after every 4th hour samples were collected. From the samples, the fatty acid methyl esters were prepared by transesterification according to the method of E.W. Hammon (2003). Was performed the GC-MS analysis in order to determine the fatty acid composition according to detected fatty acid methyl esters. There the relative representation of individual fatty acids was monitored. Was found significant decreasing in the representation of unsaturated fatty acids due to the decomposition of double bonds. This decomposition occurs significantly after the eighth hour of heating. The most influenced was the representation of linoleic acid and oleic acid. Their content in all tested samples was decreased after the heating period. The most significant increase occurred in stearic acid content. Was also found that during the heating process the oils and fat becoming be more saturated.

**Keywords:** GC-MS, transesterification, fatty acids, edible oil

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## List of the abbreviations used in the thesis

B.C.	Before Christ
CO	Copra oil
CPO	Crude palm oil
CULS	Czech University of Life Sciences Prague
EFA	Essential fatty acid
EPOA	European Palm Oil Alliance
ESI	Electrospray ionisation
FA	Fatty acid
FAME	Fatty acid methyl ester
FAO	Food and Agriculture Organization
FEDIOL	Federation for European Oil and Proteinmeal Industry
FFA	Free fatty acid
FID	Flame ionization detector
FTA	Faculty of tropical agriculture
FTIR	Fourier transform infrared spectroscopy
GC	Gas chromatograph
GC-MS	Gas chromatography-Mass spectrometry
HDL	High-Density Lipoprotein
HOSO	High-oleic sunflower oil
HPLC	High-performance liquid chromatography
HPSEC	High-performance size exclusion chromatography
IR	Infrared spectrometry
IUPAC	International Union of Pure and Applied Chemistry
KFC	Kentucky Fried Chicken
LCFA	Long-chain fatty acid
LDL	Low-Density Lipoprotein
LEAR oil	Low erucic acid rapeseed oil
MALDI	Matrix-assisted laser desorption/ionisation
MCFA	Medium-chain fatty acid
MS	Mass spectrometer
MUFA	Monounsaturated fatty acid
m/z	Mass-to-charge ratio

NCBI	National Center for Biotechnology Information
NEFA	Non-esterified fatty acids
NIR	Near-infrared spectroscopy
NIST	National Institute of Standards and Technology
NMR	Nuclear magnetic resonance
(n)h	n-hours heated sample
PKO	Palm kernel oil
PLOT	Porous Layer Open Tubular
PUFA	Polyunsaturated fatty acid
RBD	Refined, bleached and deodorised
SCFA	Short-chain fatty acid
SCOT	Support Coated Open Tubular
SFA	Saturated fatty acid
TAG	Triacylglycerol
TOF	Time-of-flight
UFA	Unsaturated fatty acid
USA	United States of America
U.S. HHS	U.S. Department of Health & Human Services
VCO	Virgin coconut oil
VLCFA	Very long-chain fatty acid
WCOT	Wall Coated Open Tubular
YMDB	Yeast Metabolome Database

## 1. Introduction

Edible fats and oils, humanity produces and consumes them for thousands of years, and the world production of them have been multiplied since the time before the Second World War (Belitz et al. 2009). They belong to a wide variety of lipids and may be of vegetable or animal origin. These substances are the primary source of energy, essential fatty acids, fat-soluble vitamins, cholesterol and phytosterol which are necessary for human life and good health. These types of lipids consist mainly of triacylglycerols which containing three fatty acids on their ester bonds (Rustan & Drevon 2005). The fatty acids composition in oils is essential for to ability to determine the effect of oil on human health (Petrović 2010). Oils and fats are used widely in gastronomy like frying oil. During frying, the food gets unique and delicious sensory characteristics (Sahin & Sumnu 2008). However, during the frying process, the frying oil is subjected to very aggressive conditions. These conditions can cause a thermal-oxidation of fatty acids (Zheljazkov et al. 2008). Fatty acids can lose some of their double bonds or part of their chain (Brühl 2014). These changes in fatty acids composition can be monitored by calculation of changing in their relative representation. For this calculation are extracted the fatty acid methyl esters from the oil. It can be achieved by acid- or base- catalysed transesterification. During this process, the fatty acids are removed from the triglycerides to form fatty acid methyl esters, glycerol and soap. After the transesterification process, Gas chromatography-Mass spectrometry (GC-MS) analysis is usually performed on extracted fatty acid methyl esters. The results of GC-MS analysis are compared with FAME standards for confirmation of correctness (Otera 1993; Schuchardt et al. 1998; Meher et al. 2006). Depending on the peak area, the relative content of each detected fatty acid in the oil is determined. Appropriate calculation methods or statistical analysis should analyse the result in order to observe the changing in fatty acid composition during the heating period (Moigradean et al. 2013).

## **2. Literature Review**

### **2.1. Lipids**

The term lipid comes from the Greek term lipos. This term was first time introduced in 1923 by a French pharmacologist, biochemist and bacteriologist M. Gabriel Bertrand and first appeared in the Journal of Biological Chemistry in 1926 in a paper by Warren M. Sperry (Belitz et al. 2009). Lipids are diverse natural substances of animal, plant and microbial origin (Metcalffe & Wang 1981; Grofová 2010). Lipids are formed of molecules of glycerol and fatty acids (FA).

According to a state of matter are generally called fats (solid) and oils (liquid) with several exceptions (e.g. coconut oil). According to physical properties, lipids are organic substances insoluble in water (Moigradean et al. 2013; Moulodi et al. 2015). Water insolubility caused the presence of large non-polar hydrocarbon structures in the molecules (Metcalffe & Wang 1981). Water insolubility is the analytical property used as the basis for their facile separation from proteins and carbohydrates which are usually hydrophilic and lipophilic (Moulodi et al. 2015). Lipids are soluble in non-polar organic solvents like are ether, chloroform, carbon tetrachloride and liquid hydrocarbons. (Moigradean et al. 2013). This definition applies only to simple lipids. The compound lipids may contain a polar component that gives them an amphiphilic character. It means that part of the molecule is hydrophobic and the second part is hydrophilic (Metcalffe & Wang 1981).

Lipids are macronutrients with the highest energy density. They contain the most of energy per gram unity (9 kcal/g or 37kJ/g) and are a source of essential FAs. They work as metabolic fuels. They are also necessary for the absorption of some fat-soluble vitamins (A, D, E, K). In organism serves mainly as a source and supply of energy. Some of the lipids are part of the cell membranes; some of them work as the organs cover or subcutaneous fat. Lipids are also protecting plant leaves from drying up. They are also a source of cholesterol or phytosterol. Lipids also serve as solvents for certain taste substances and numerous odour substances. Fats and oils influence the texture, taste and aroma of the dish. Overall, lipids are an important part of the nutrition of all organisms and an inherent pleasure for humans (Davídek et al. 1983; Moigradean et al. 2013). From a chemical point of view, lipids are esters of alcohol and higher (fatty)

carboxylic acids. They are various fats, oils, some vitamins, hormones, and components of biomembranes (Metcalf & Wang 1981).

### **2.1.1. Classification of lipids**

In general, lipids according to the state of matter and chemical composition are divided. In the first case, they are divided simply into fats and oils. In the second case, into homolipids, heterolipids and complex lipids. Furthermore, lipids into polar and neutral can be divided. Neutral lipids include glycerol esters (acylglycerols), sterols and their esters and free fatty acids (FFA). Polar lipids include phospholipids and other heterolipids. This partitioning system is mainly based on the behaviour of compounds during chromatographic separation (Belitz & Grosch 2009; Fahy et al. 2011).

#### **Classification by state of matter**

**Fats** contain predominantly saturated fatty acids; butter, lard, tallow.

**Oils** contain predominantly unsaturated fatty acids; rapeseed oil, sunflower oil, soybean oil, olive oil, sesame oil, and drying oils such as linseed oil and poppy seeds oil. However, there are exceptions which contain predominantly saturated fatty acids (e.g. coconut oil, palm oil). Among the oils are also ranked marine origin oils.

Overall, the term “fat” generally designates a solid at room temperature and “oil” a liquid. This division is inaccurate because some of the fats are neither solid nor liquid and some of the oils are solid at room temperature (e.g. coconut oil). However, this division is generally recognised (Gundstone 2002; Fahy et al. 2011).

#### **Classification by chemical composition**

**Homolipids** are esters of FAs and alcohols. The most common alcohol is glycerol. Other alcohols which are less common are glycerol ethers, higher aliphatic aldehyde hemiacetals, glycols, or higher monohydric aliphatic alcohols, aliphatic and alicyclic terpenoid compounds and various steroid compounds. Generally, homolipids can be classified according to consistency to waxes (hard, non-greasy), fats (plastic, mushy, greasy), oils (liquid). From a chemistry point of view, are divided according to the structure of bound alcohol to esters of monohydric alcohols (waxes), esters of glycerol (fats and oils), ethers of glycerol, esters of polyhydric alcohols (Velíšek & Cejpek 2006; Belitz et al. 2009).

**Heterolipids** are lipids containing fatty acids, alcohols and other components that give them a partially polar character. Heterolipids are divided most often into the following basic groups:

- Phospholipids – are similar in consistency to triacylglycerols (TAG). They contain glycerol backbone and fatty acids tail, but the third fatty acid is replaced by a phosphate group (hydrophilic head).
- Glycolipids – contain a sugar moiety in their molecule. They are lipids with a carbohydrate attached by a covalent bond.
- Sulfolipids – are lipids which contain bounded sulfuric acid.
- Lipoproteins – are lipid micelles and liposoluble compounds with proteins on the surface (Velíšek & Cejpek 2006; Belitz et al. 2009)

### **2.1.2. Edible fats and oils**

Vegetable oils and fats are a very important part of the human diet because they ensure nutritional function (Gharby et al. 2014). Adults consume approximately 85 g of fat daily (Rustan & Drevon 2005). They contribute to the energy supply, are essential sources of fatty acids (Gharby et al. 2014). Vegetable oils and fats consist mainly of TGAs (95 % – 99 %) which differ in their FA compositions to a certain extent (Dijkstra 2009; Gharby et al. 2014). They are also a source of cholesterol or phytosterol. Other constituents are the unsaponifiable fraction and some acyl lipids such as traces of FFAs, mono- and diacylglycerols. They also commonly contain fat-soluble vitamins (A, D, E, K), natural pigments and phospholipids (1 % – 5 %). FA composition of individual oil/fat may vary greatly according to variety, genetic aspects, breed, feed, fertilisers, environmental conditions and so on. Therefore, for comparison, average values use. The impact of individual fats and oils on human health should be assessed according to individual FAs because of their different influences on human health and risks of serious diseases (Gunstone 1996; Belitz et al. 2009; Gunstone 2011).

### **2.1.3. Vegetable oils**

Most of the edible oils are of vegetable origin. They are obtained by extraction of oilseeds (such as sunflower seeds, rapeseed seeds, soybeans etc.) or oleaginous fruits

like coconut or palm (Gharby et al. 2014). The world production of vegetable oils has multiplied since the time before the Second World War. There has been a significant rise in production since 1964 of soybean, palm and sunflower oils, as well as rapeseed oil (Belitz et al. 2009). Since the turn of the century, vegetable oils have gradually replaced animal oils as the primary source of food fat in the human diet. The exact composition of FA depends on genetic aspects, variety, and environmental conditions. Vegetable oils usually contain a higher amount of unsaturated fatty acids (UFA) than animal fats (Orsavova et al. 2015). The oils used in the experimental section are described below (coconut oil, palm oil, rapeseed oil, rice bran oil, soybean oil, sunflower oil and high-oleic sunflower oil).

**Coconut oil** is edible oil. This oil is obtained from the kernel of matured coconuts harvested from the coconut palm (*Cocos nucifera*) (Orsavova et al. 2015; Wallance 2018). Palm kernel and coconut oils are the two most commercially important oils in the lauric acid group. The characteristic of this group is a high content of SFAs (lauric acid, myristic acid) (Young 1983). In recent years, the popularity of this oil and products has grown considerably due to the perceived health effects of certain medium-chain fatty acids. Nowadays, coconut oil is consumed practically all over the world. The world's most significant are two types of this oil, copra oil (CO) and virgin coconut oil (VCO). Both of them have similar fatty acid profiles. However, the VCO contains higher amounts of some nutrients (e.g. vitamin E) and dietary bioactive compounds (e.g. polyphenols). These two types of oil differ in the extraction method. CO is produced by crushing dried coconut kernels to extract the oil. The extracted oil is then typically refined, bleached, and deodorised (RBD process). This oil is commonly used in shortening and for frying applications. VCO is processed by pressing shredded wet coconut kernel to squeeze out the oil and coconut milk, which form an emulsion that is then separated by various techniques. VCO is not refined and thus is not subjected to the high temperatures of FFA distillation and deodorisation, which can volatilize and otherwise destroy many heat-labile components. This fact is the reason why VCO has become increasingly popular in recent years. RBD-CO and VCO are classified as a source of saturated fat because they are contained approximately 92 % saturated FAs (Moigradean et al. 2013; Wallance 2018).



**Palm oil** is an oil extracted from the ripened mesocarp of the fruits of an oil palm tree (*Elaeis guineensis*). Leading producers of this oil are Indonesia, Malaysia, Thailand, Colombia, and Nigeria. The palm oil's economic attractiveness lies in the highest yield of oil per unit area of cultivated land. Every one hectare of oil palm plantation can produce up to 10 times more oil than other leading oilseed crops. From palm fruit, comes two distinct types of oils. Crude palm oil (CPO) produced from the mesocarp and palm kernel oil (PKO) produced from the inside kernel. The mesocarp of palm fruits contains about 56 – 70 % edible oil (Mba et al. 2015). CPO is used mainly for cooking and frying. Approximately 90 % of all palm oil is utilised in the food industry like edible oil, and the remaining 10 % is utilised in oleochemical manufacturing (Gourichon 2013; Mba et al. 2015). Palm oil has a unique FA and TAG profile with almost 50 – 50 composition of SFA and UFA (EPOA 2016). Therefore, it makes it naturally semi-solid at room temperature. It is rich in palmitic acid (44 %) and oleic acid (40 %). The other major FAs are linoleic acid (10 %) and stearic acid (5 %) (Che Man et al. 1999; Kritchevsky 2000). Palm oil is also a good source of vitamin E and is nature's richest source of  $\beta$ -carotene and lycopene (Cassiday 2017). These carotenoids and the vitamins are powerful natural antioxidants. They are the reason for good oxidative stability to palm oil during frying. Palm oil also contains partial acylglycerols which cause cloudiness when are contained in a concentration above 10 % and the temperature of oil drops under 20 °C. According to Choe & Min (2007), the range of TGAs in CPO is 94 – 98 % and range of FFAs is 2 – 5 %. Palm oil is the best oil among frying oils because of its unique FA composition, high smoking point (230 °C) and strong thermal-oxidation resistance (Mba et al. 2015).

**Rapeseed oil** comes from *Brassica napus*, also known as rape. It is a plant identified in 2000 B.C. and it is one of the oldest known sources of vegetable oils. It has grown annually in temperate climates such as Europe, Canada and China (Lin et al. 2013). Rapeseed can yield up to 45 % of rapeseed oil. Currently, rapeseed is considered to be the main source of both edible and technical vegetable oil as well as a protein-rich food and is also an excellent source of protein for the animal feed industry (Sakhno 2010). Fatty acid methyl esters of rapeseed are also commonly mixed with diesel fuel (PREOL 2018). Rapeseed oil is interesting for its richness in omega-3 fatty acids, and its low content in SFAs (6 %) in comparison to other edible oils and fats (FEDIOL 2011). It is high in MUFAs, respectively in oleic acid (56 %). In rapeseed oil is also the

relatively high content of PUFAs such as linoleic acid (26 %) and Linolenic acid (10 %) which negatively influences its thermal-oxidative stability (Gunstone 1996). But, it is a rich source of natural antioxidants, including tocopherols, polyphenols, and phytosterols which has a reverse effect. Rapeseed oil can contain up to 54 % of erucic acid. This acid is damaging to a cardiac muscle of animals. Therefore, were bred several cultivars low in erucic acid content, such as low erucic acid rapeseed oil (LEAR oil) or canola oil also known as rapeseed “00” oil. According to regulations is maximal content of erucic acid in this oil 2 % in the USA and 5 % in the EU (European Commission 1980; U.S. HHS 2018). The rapeseed oil is widely used for frying. Is characterised that does not transfer taste and has a high smoke point (204 °C – 230 °C). If it is not hot enough it quickly absorbs into the product (Maszewska et al. 2018).

**Rice bran oil** is obtained by extraction from the germ or inner husk of fresh rice bran of *Oryza sativa* or *Oryza glaberrima*. Widely consumed is rice bran oil (RBO) in the USA and Asia, mainly in Japan, Korea, and Thailand. Major producers of RBO are India, China and Myanmar (Gaopala Krishna 2013). RBO can be extracted by the process of solvent extraction by n-hexane, by using ohmic heating or can be extracted mechanically. Then it is usually refined by chemical refining or physical refining (Sharma et al. 2015). It is suitable for deep frying, roasting, and use in salad dressings. The rice bran contains 15 – 25 % oil depending on the cultivar, agricultural practices, and the extent of polishing (Gaopala Krishna 2013). Rice bran oil is commonly called healthy oil because of is believed that have the ability to reducing LDL cholesterol and withal increasing HDL cholesterol in the body (Oluremi et al. 2013). Unfortunately, there exist also several aspects which negatively affected the health of consumers of RBO. The one major problem concerned with RBO is the absence of  $\omega$ -3 FA and the presence of high excess of  $\omega$ -6 FA acids which could be detrimental to health in this amount, it can cause may increase breast cancer and prostate cancer. According to studies RBO also can cause some digestive tract problems and lowers the blood calcium in the body (Nayik et al. 2015). RBO has an almost similar FA composition as peanut oil. The FA composition of RBO is formed mainly by oleic acid 44 % and linoleic acid 30 %. There is also a relatively high content of palmitic acid (15 %)(Kangabam et al. 2014). RBO has a high level of unsaponifiable matter and gamma oryzanol content. Oryzanol enhances stability at a higher temperature. The low viscosity of RBO allows lower oil uptake by foodstuff during frying. RBO also has a high smoking point and

provides the required flavour of fried food. Therefore, it is a suitable oil for frying or cooking (Latha & Nasirullah 2014; Muhammad et al. 2015).

**Soybean oil** is extracted from soybeans which come from legume crop (*Glycine max*). It is food crop comes from Eastern Asia and probably has been discovered by humans thousands of years ago. Soybeans are oval shaped, and their sizes are variety dependent. The major importer and consumer of this oil are Japan. Primarily cultivated are soybeans for oil and protein production for human and livestock consumption. Soybean plants are important for soil fertility because they fix atmospheric nitrogen by symbiosis with microorganisms. Soybean is a dominant oilseed crop in the world due to its favourable agronomic characteristics, its valuable edible oil, and its high-quality protein (Islas-Rubio & Higuera-Ciapara 2002; Gunstone 2011; Kim et al. 2016). The content of protein in soybean is much greater than in other oilseeds, that is the main reason for the increasing popularity of this crop in the last years (Hammond et al. 2005). It is produced in the second largest amount after palm oil (Gunstone 2011). On the other hand, there is a relatively low oil content of the seeds, just about 20 % on a moisture-free basis. The oil content is influenced according to variety and growing conditions (Islas-Rubio & Higuera-Ciapara 2002). Most of the soybean oil is extracted by using solvent hexane or using mechanical extraction from the beans (Gunstone 2011). Often is used as an edible oil in cold cuisine. It is also usually hydrogenated for use as a margarine stock or frying oil (Islas-Rubio & Higuera-Ciapara 2002). The crude soybean oil consists typically of 96 % TAGs, 2 % phospholipids, 1.6 % unsaponifiable, 0.5 % FFAs and trace amounts of carotenoid pigments. This oil contains a high content of linoleic acid (44 % – 51 %) and a lower level of linolenic acid (7 %). These FAs are important for human dietary because they are essential FAs. Overall, it is rich in PUFA (52 – 58 %) (Gunstone 1996). The FA composition of soybean oil is relatively considerably influenced according to maturity and seed oil deposition. The high content of linoleic acid strongly negatively influences the thermal-oxidative stability of this oil because of double bonds (Hammond et al. 2005). In recent decades were made numerous attempts to manipulate the FA composition of soybean oil to improve its oxidative stability. Generally, hydrogenation and lipid modification through traditional plant breeding or genetic transformation are used (Gunstone 2011).

**Sunflower oil** comes from a plant (*Helianthus annuus*) originates from central and North America (FAO 2019). It is cultivated mainly in the area covered by Russia, Ukraine, Turkey and adjoining countries in Europe. It is also grown in Argentina (Gunstone 2011). It is one of the main crops used for edible oil production over the world because of its ability to grow in large semi-arid regions without irrigation (Ismail & Arafat 2014). Sunflower oil has a broad range of applications. It is used in the food industry, in biodiesel production or commercial products (Zheljazkov et al. 2008). The sunflower oil is beneficial for human consumption and production of biodiesel (Ismail & Arafat 2014). The FA composition significantly depends on climatic conditions of the field, on the temperature of seed growth, on the genetic profile of plant and other environmental conditions. For example, oleic acid values can range between 10 % – 50 %. Are produced several types of edible sunflower oil. Main of them are oils with a high content of linoleic acid (~69 %) or oleic acid (~82 %), and oils with medium content of oleic acid (Zheljazkov et al. 2008). In past years was developed sunflower oil high in stearic acid (~18 %) in reaction on the controversy of palm oil to create a healthier and more sustainable alternative to palm oil. The oil containing a high level of oleic acid is preferred in nutritional use whereas that having higher linoleic content is preferred by paint or fuel industry. This oil also has a high content of tocopherols which lead to increased oxidative stability (Ismail & Arafat 2014).

In order to improve the thermal-oxidative stability and positive effects on the health of sunflower oil, the **high-oleic sunflower oil** (HOSO) has been developed. The sunflower seeds have been modified in FA composition. This modification reduced less stable linoleic acid and increased oleic acid content. Therefore, this oil is suitable for frying (AŞkin et al. 2016). Recent has been developed HOSO that exceed 89 % oleic acid content. HOSO is naturally stable and therefore does not need to be hydrogenated. On the other hand, the nutrition value of HOSO is lower because of less content of  $\omega$ -6 FAs than common sunflower oil (Ismail & Arafat 2014).

#### **2.1.4. Animal fat**

Animal fats are rendered tissue fats. Generally, are divided into several groups according to their origin, type of processing and use (lard, edible tallow, oleo-stock,

caul fat, leaf fat, chicken fat, rendered pork fat, inedible tallow and greases, et cetera). The exact composition of FA depends on kind, breed, and feed of animal. The animal fats usually contain predominantly SFAs and very little of the PUFAs, such as linolenic acid and linoleic acid. Therefore, are in contrast with most of the vegetable oil usually solid at room temperature (Sharma et al. 2013). The animal fat should have better oxidative stability because of its saturation, but despite this fact, the animal fat is not always more stable than vegetable oil. It is because of the fact that vegetable oils often contain some natural antioxidants. Necessary material for animal fat production is raw animal tissue usually from domestic animals, such as hogs and cattle. Hog lard is fat from the kidneys and back. Hog lard as animal fat is interesting in the fact that contain fewer SFAs and cholesterol and more UFAs than an equal amount of butter. It is often combined with butter in pastries for its shortening properties. The hog lard has a relatively high smoke point so is commonly used for quick frying (Marcus 2013; Oroian & Petrescu-Mag 2017).

#### **2.1.5. Marine oil**

Primary sources of these oils are sea mammals, seals, whales and fish of the herring family. Marine oils typically contain highly UFAs with 4 – 6 alkyl groups. These oils are not utilised directly as edible oils. It is possible only after hydrogenation of double bonds and refining. Since their highly UFAs are susceptible to autoxidation. Interesting is the occurrence of about 1 % branched methylated FAs (e.g. 12-methyl- and 13 methyl-tetradecanoic acids, or 14 methyl-hexadecanoic acids) in marine oils (Belitz et al. 2009).

## **2.2. Fried foods and thermal stability of oils**

Fried food has become an integral part of human nutrition. It is one of the oldest methods of cooking. This method was probably first discovered by ancient Egyptians around the 6th century B.C. Also, the Romans used this method and called it “boiling in oil”. The frying is also one of the fastest methods of food cooking (Tabee 2008; Mba et al. 2015). Frying is in nowadays one of the most important processes in the food

industry because it has several advantages over other cooking methods (Gerdev 2006). Almost in every culture, we can find food which is prepared by the frying process. The main reason why this way of processing food has become so popular is that after this process the food gets unique and delicious sensory characteristics. It is because of the presence of fats or oils which are essential parts of the frying process and play important functional and sensory roles in food products. Fats and oils enrich the nutritional quality and provide the desired texture, specific mouthfeel, and satisfactory aroma (Sahin & Sumnu 2008). The hot frying fat/oil also penetrates into the fried food and replaces part of the water it contains. Absorbed fat cause creation of a crust and made the food considerably more palatable (Ghidurus et al. 2010). Fundamentally, the frying can also be described as a dehydration process at high temperature (Tabee 2008). The final flavour and consistency of fried food depend on the product that is being fried and on the oil/fat used as the frying medium. When is set optimal temperature and time of frying and the oil absorption is optimal the food get golden brown colour and crispness. Compared to other ways of cooking are fried foods cooked faster (Gerde 2006). Besides, can be foods prepared by deep frying. In deep frying, the food is completely immersed in the hot fat or oil heated to a relatively high temperature. The oil/fat acts as a medium of heat and mass transfer (Sahin & Sumnu 2008).

However, during the frying process, a lot of changes in nutrients composition occurs (Zheljazkov et al. 2008). Oil is subjected to aggressive conditions such as high temperature, and the presence of oxygen and water (Gerde 2006; Ghidurus et al. 2010). These conditions according to thermal-oxidative stability of the oil cause the oxidative-degradation changes of oil. It manifests in chemical and physical changes (Mba et al. 2015). As the food enters the hot oil, is oxygen introduced into the oil, which leads to oxidation. Oxidation products include hydroperoxides, aldehydes, ketones, acids, hydrocarbons and many polymeric compounds. As the food absorbs frying fat, the food lipids, as well as colour pigments, are solubilised and released into the frying fat (Lillard 1982). These degradation processes affect the texture, taste, and overall flavour perception of the food and the nutrient value of food. The unsaturated oils are much less stable during the heating process. It is because of the presence of double bonds in the FAs chain. These double bonds increase the breakdown susceptibility of oils. Under certain conditions oxidation or gurgling occurs. This reaction takes place on the double bonds of UFAs which are then spread out. Thermal-oxidation which occurs on the

double bonds of UFAs results in the formation of hydroperoxides. Oils and fats rich in polyunsaturated fatty acids (PUFA) have high chemical reactivity (Demirbas 2007). Therefore, the less stable oils are oils rich in PUFAs. According to Szterk et al. (2010), the presence of two double bonds in the structure of the FA may cause 10 – 40 times faster oxidation than the presence of one double bond. Animal fats which are usually rich in SFAs are much more stable. Cis- FAs are also thermodynamically less stable than the trans- FAs (Rustan & Drevon 2005). Therefore, it has been developed a hydrogenation process. During this process, are formed trans FAs isomers. The main disadvantage of trans FAs oils is that it has been associated with health problems such as the increased risk of coronary heart disease and increased cholesterol levels in human blood serum. The composition of FAs is changing according to a time of heating. These changes can change the nutrient value of fried food. Therefore, it is necessary to track these changes. Moisture is released from the frying food, which results in hydrolysis of TAGs to form FFAs, diglycerides, monoglycerides and glycerol. The volatile degradation products are released from the oil with the steam as smoke. The thermal-oxidation lead to a change in colour, sensory characteristics, loss of essential nutrients and micronutrients and sometimes to change in texture. Also, vitamin E is significantly losing along with the oxidation of UFAs during heating (Zheljazkov et al. 2008; Ghidurus et al. 2010). Heating of oil to high frying temperatures also leads to the formation of polymers as the reaction products condense. These changes in result lead to in deterioration of the oil (Lillard 1982).

### **2.3. Fatty acids**

First FAs have been isolated and named by French chemist M. E. Chevreul at 1818. He called them “graisse acide” or “acide huileux” (Dijkstra 2009). FAs make up the main component of lipids and are the main compounds in edible oils (Moulodi et al. 2015; Grofová 2010). FAs are colourless liquids or solids (Velíšek 2014). In biochemistry, FAs are higher, branched monocarboxylic acids having at least two carbon atoms. They are the source, storage and transporter of energy for the organism. FAs represent 30 – 35 % of total energy intake in many industrial countries. The most important dietary sources of FAs are vegetable oils, meat and dairy products, grain, and fatty fish or fish oils (Rustan & Drevon 2005; IUPAC 2014). Consumption of FAs is

crucial for the correct development of the central nervous system (Grofová 2010). Nowadays we recognise more than 100 species of FAs in nature. Most of them with even number of carbons because their biosynthesis takes place by the addition of an acetate having two carbons. Most of the FAs are aliphatic monocarboxylic acids and have a linear and also a long unbranched chain. In food lipids, FAs are found mainly as esters in the form of TAGs (fats and oils) and glycerophospholipids (Velíšek & Cejpek 2006).

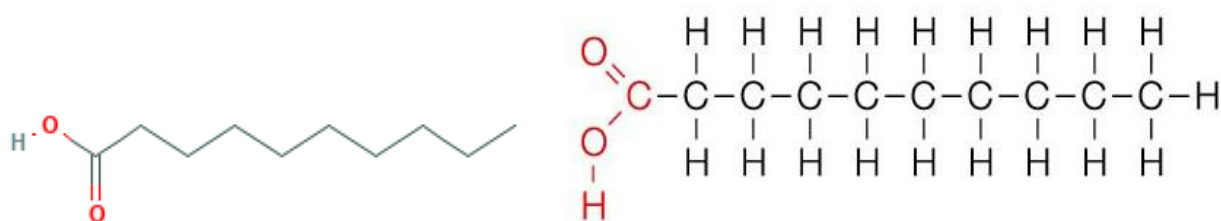
FAs are divided according to various criteria, like is chain length or saturation. According to saturation, we recognise saturated FAs (SFA), monounsaturated FAs (MUFA), polyunsaturated FAs (PUFA) and unsaturated FAs (UFA). According to chain length, we recognise short-chain FAs (SCFA) with the number of carbons <6, medium-chain FAs (MCFA) with 6 – 12 carbons, long-chain FAs (LCFA) with 14 – 20 carbons and very-long-chain FAs (VLCFA) with >20 carbon atoms. The most natural FAs have an unbranched chain with the number of carbon atoms from 4 to 28. Single bonds link some carbon atoms (-C-C-; SFA), and double bonds link others (-C=C-; UFA, MUFA, PUFA). Double bonds can under certain conditions react with hydrogen to form single bonds (Rustan & Drevon; Belitz et al. 2009; IUPAC 2014). The melting point of FAs depends on the number of carbon atoms in the chain. Together with increasing chain length, the melting point of FAs is increasing. For chains longer than 20 carbon atoms the melting point does not change significantly. The reverse effect has an increasing number of double bonds. Also, the solubility of FAs in water decreases with increasing chain length. Higher FAs are non-volatile, and lower FAs are volatile at atmospheric pressure. Lower FAs can be found in butter, and higher FAs with long hydrocarbon chain predominantly are found in waxes. With increasing chain-length, the boiling point is also increasing. Double bonds have very little influence on the boiling point. According to the configuration of double bonds, we can recognise cis- and trans- FAs (Žák 2011; Velíšek 2014).

### **2.3.1. Saturated fatty acids**

SFAs are saturated by hydrogen. Most of the SFAs have an even number of carbon atoms in the molecule. SFAs have an unbranched, linear chain (Belitz et al. 2009). The most common SFAs contain 12 – 22 carbon atoms. They are chemically



stable and only change when they are heated up. In nature, SFAs make up 10 – 40 % of the total fatty acids (Davídek et al. 1983; Velíšek & Hajšlová 2009). Saturated fat tends to solidify at room temperature. SFAs are usually of animal origin. Lard and butter are the most typical examples of saturated animal fats (Grofová 2010). However, there exist also several plant origin oils which contain mainly SFA it is, for example, coconut oil or palm oil. The most common SFA in vegetable fats and oils is palmitic acid (Rustan & Drevon 2005). Epidemiological and clinical studies found that the dietary fats containing high levels of SFAs (usually more than 15 % of total energy) induce an increase in plasma total- and low-density lipoprotein (LDL) - cholesterol concentrations in humans (Rioux et al. 2005). An example of one of SFAs is shown in Figure 1.



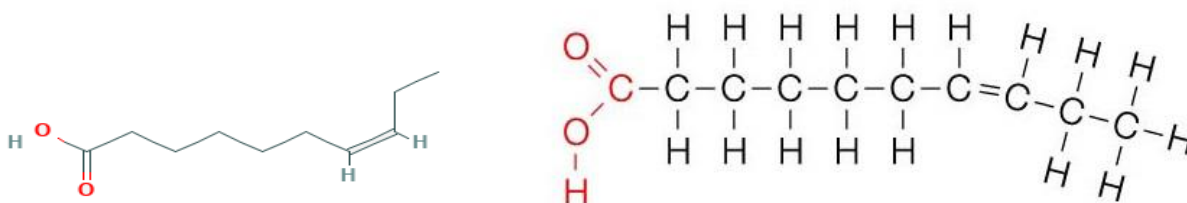
**Figure 1.** Structure of decanoic acid  
(Source: ChemSpider 2019)

### 2.3.2. Unsaturated fatty acids

UFAs have one or more double bonds in their hydrocarbon chain. They have a linear chain that contains 10 – 36 carbon atoms in a molecule. Most of them have a chain length of 16 – 22 carbon atoms. According to the number of double bonds are further divided into MUFAs and PUFAs. The presence of double bonds causes a restriction in the mobility of the acyl chain at that point. Oils which contain UFAs predominantly are liquid at room temperature, and they turn solid when they are chilled. UFAs are contained mainly in oils of plant origin and marine oils (Rustan & Drevon 2005; Belitz et al. 2009).

### 2.3.3. Monounsaturated fatty acids

MUFAs have just one double bond in the fatty acid chain. The remaining carbon atoms have a single bond. An example of one of MUFAS is shown in Figure 2. Most common sources of MUFAs are avocados, nuts and olive oil. MUFAs can help lower the LDL cholesterol level. It can lower the risk of stroke or heart disease risk (Gunstone 1996; Rustan & Drevon 2005).

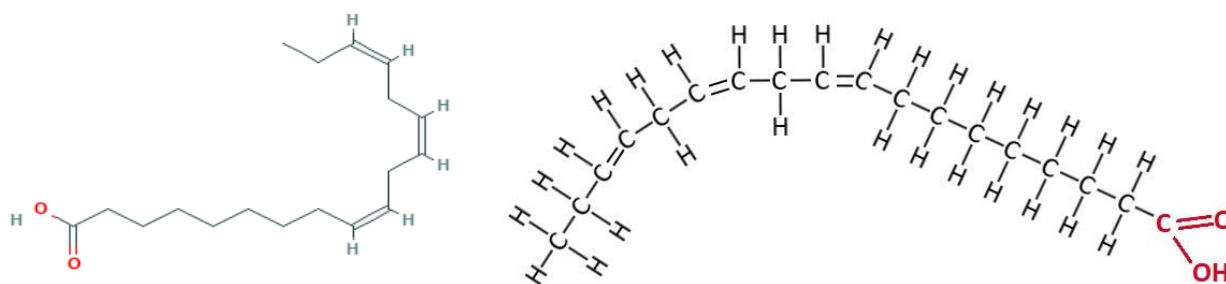


**Figure 2.** Structure of (7Z)-dec-7-enoic acid

(Source: PubChem Database 2019)

### 2.3.4. Polyunsaturated fatty acids

Chemically, PUFAs belong to the simple lipids. PUFAs have two and more double bonds in the fatty acid chain. The first double bond may be found between the third and the fourth or between sixth and seventh carbon atom from the  $\omega$  carbon. These FAs are called  $\omega$ -3 FAs (n-3 FA) in the first case, or  $\omega$ -3 FAs (n-6 FA) in the second case. These fatty acids are not convertible and have very different biochemical roles. Both of n-3 FAs and n-6 FAs are essential FAs (EFA). The predominant sources of n-3 fatty acids are vegetable oils and fish. The primary sources of n-6 fatty acids are vegetable oils (safflower oil, sunflower oil, and soybean oil). PUFAs are a necessary part of a diet to all higher organisms, including mammals and fish. They are produced by plants and phytoplankton (Rustan & Drevon 2005; Benatti et al. 2004; Gunstone 1996). An example of one of PUFAs is shown in Figure 3.



**Figure 3.** Structure of (9Z,12Z,15Z)-octadeca-9,12,15-trienoic acid ( $\alpha$ -linolenic acid)

(Source: PubChem Database 2019)

### 2.3.5. Free fatty acids

FFAs are FAs which are not bonded with glycerol. They are not esterified; therefore are also sometimes called as non-esterified fatty acids (NEFAs). They occur for example in plasma. The hydrolysis of oils and fats produces FFAs. The content of FFAs depends on moisture content, temperature or time. Level of FFAs can also be influenced by processing such as heating or frying (Mahesar et al. 2014).

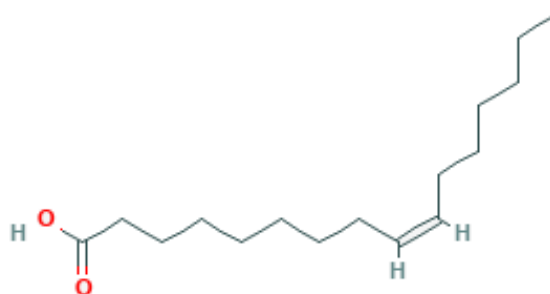
### 2.3.6. Essential fatty acids

Essential fatty acids (EFAs) are acids which the human body cannot synthesise them. Therefore, they must be consumed in the food diet. An omega-6 ( $\omega$ -6) linoleic acid and an omega-3 ( $\omega$ -3)  $\alpha$ -linolenic acid are the main EFAs. (Di Pasquale 2009).

### 2.3.7. Configuration of double bonds

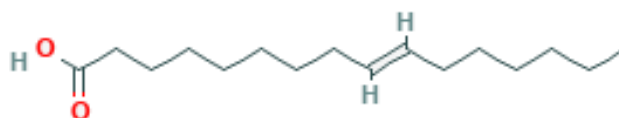
Each double bond may have two types of spatial arrangement. It may contain both hydrogens on the double bond on the same side (cis- bond) or different sides (trans- bond). This spatial arrangement results in a considerable change in the shape of the molecule. Trans-UFAs have a similar form like SFAs. They have a straight chain. Cis-UFAs have a bent chain. This fact has of great importance in enzyme reactions and in the formation of membranes where these acids are most widely used (Gunstone 1996; Benatti et al. 2004; Rustan & Drevon 2005; Belitz et al. 2009).

**Cis- configuration** – This is the most common structure. Two hydrogen atoms adjacent to the double bond stick out on the same side of the chain. It is shown in Figure 4. The more double bonds in cis- configuration make FA more curved and less flexible. FAs which contain this configuration are commonly called like “good fats”. The cis-FAs have lower melting points than the trans- FAs or the SFAs (Rustan & Drevon 2005). The number of bonds in cis- configuration also decreasing oxidative stability of oils/fats. The most of naturally occurring UFAs have this configuration of double bonds (Gunstone 1996; Benatti et al. 2004; Rustan & Drevon 2005).



**Figure 4.** cis- configuration ((9Z)-hexadec-9-enoic acid)  
(Source: PubChem Database 2019)

**Trans- configuration** – A trans- configured FAs have the adjacent two hydrogen atoms lie on opposite sides of the chain like is shown in Figure 5. These double bonds do not cause the chain to bend much. Therefore, trans- FAs have a similar shape to SFAs. Most FAs in the trans- configuration are the result of human processing. Trans isomers are made through the industrial chemical process of hydrogenation of unsaturated oils or in the gastrointestinal tract of ruminants. Hydrogenation solidifies liquid oils. This process increases the shelf life and the flavour stability of oil and the products which contain it. Fats which contain trans- bonds are called trans fats. According to research, trans fats drive up the LDL cholesterol which increases the risk of coronary artery heart disease and stroke. It is also often associated with a higher risk of developing type 2 diabetes. It is the main reason why these fats are called “bad fats” (Velíšek & Cejpek 2006; Belitz et al. 2009; Velíšek & Hajšlová 2009; Valenzuela et al. 2011; Orsavova et al. 2015).



**Figure 5.** trans- configuration (9E)-hexadec-9-enoic acid  
(Source: PubChem Database 2019)

### 2.3.8. Major fatty acids

The major fatty acids, according to Belitz et al. (2009), are described in the following lines. For better clarity, the common names of these FAs are used. In a table (Table 1) are shown their structures.

**Palmitic acid** (C16:0), discovered by E. Frémy in 1840, is the most common saturated fatty acid found in the human body (Wisniak 2013; Carta et al. 2017). Its chemical formula is C<sub>16</sub>H<sub>32</sub>O<sub>2</sub>. The carbon number of palmitic acid is 16 and has no double bonds. It is therefore ranked among LCFAs. It can be synthesised endogenously from other FAs, carbohydrates, and amino acids or can be provided in the diet. Palmitic acid is occurring in many natural oils and most commonly is produced from palm oil. This acid represents 20 – 30 % of FAs in membrane phospholipids, and adipose TAGs (Carta et al. 2015; Carta et al. 2017). Palmitic acid makes 44 % of total fats of palm oil, of 26 % cocoa butter, or 8 – 20 % of olive oil. A significant amount of this acid can also be found in meat and dairy products where makes 50 – 60 % of total fats. According to Innis (2016), palmitic acid is present also in breast milk where represent 20 – 30 % of total fats. In addition to the food industry, it is ordinarily used to produce soaps, cosmetics, and industrial mould release agents (YMDB 2017).

**Stearic acid** (C18:0) is saturated fatty acid with 18 atoms of carbon in the chain. Its IUPAC name is octadecanoic acid, and its chemical formula is C<sub>17</sub>H<sub>35</sub>CO<sub>2</sub>H. The esters of stearic acid and salts are called stearates. Stearic acid can be obtained from fats and oils by the saponification of the TAGs using hot water followed by distillation. Like all SFAs also this acid is contained mainly in animal fat. Notable exceptions are cocoa butter or shea butter where the content of stearic acid in the form of TAGs is 28 – 45 % (Belitz et al. 2009; Livesey 2014).







**Myristic acid** (C14:0) is one of the saturated fatty acids. This acid is abundantly represented in copra oil (15 – 23 %), palmist oil (15 – 17 %) or milk fat (7 – 12 %). There is a presumption that myristic acid to increase animal and human blood cholesterol concentrations more than other FAs (Rioux et al. 2005).

**Oleic acid** (C18:1) is an unsaturated fatty acid that is the most widely distributed and abundant FA in nature (NCBI 2019). It occurs naturally in animal and vegetable fats and oils. This FA contains 18 carbon atoms with one double bond in the chain. Therefore, oleic acid is in chemical terms classified as an LCFA and MUFA. Its chemical formula is  $C_{18}H_{34}O_2$ . Salts of oleic acid are called oleates. This acid is used commercially in the preparation of plates and lotions and as a pharmaceutical solvent (Campos et al. 2012; NCBI 2019).

**Linoleic acid** (C18:2) is doubly unsaturated fatty acid so is rank among PUFAs. It belongs to the Omega-6 fatty acids group. Its chemical formula is  $C_{18}H_{32}O_2$ . Linoleic acid occurs widely in plant glycosides. It is an essential FA in mammalian nutrition. It is even used in the biosynthesis of prostaglandins and cell membranes. In the human body, linoleic acid is used to make other types of  $\omega$ - 6 FAs. Foodstuff rich in linoleic acid is sunflower seeds, flax seeds, hemp seeds, poppy seeds, sesame seeds, soybean, corn or nuts (Whelan & Fritsche 2013; NCBI 2019; Jandacek 2017).

**Linolenic acid** (C18:3) is PUFA. This acid can be found in plants and involved in the formation of prostaglandins. It is an essential fatty acid belonging to the omega-3 FAs group. Linoleic acid is highly concentrated in certain plant oils. Has been reported that this acid inhibits the synthesis of prostaglandin resulting in reduced inflammation and prevention of certain chronic diseases. Its chemical formula is  $C_{18}H_{30}O_2$  (Kapoor & Huang 2007; Stark et al. 2008).

**Table 1.** Major fatty acids and their structures according to Belitz et al. (2009)

Abbreviated designation	Structure <sup>a</sup>	Common name	Proportion (%) <sup>b</sup>
14:0		Myristic acid	2
16:0		Palmitic acid	11
18:0		Stearic acid	4
18:1(9)		Oleic acid	34
18:2(9,12)		Linoleic acid	34
18:3(9, 12, 15)		Linolenic acid	5

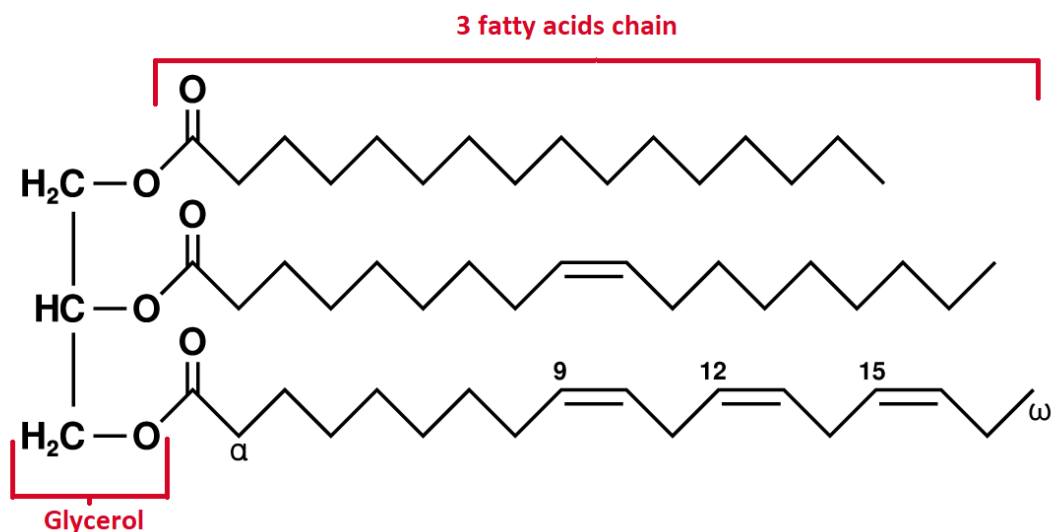
<sup>a</sup> Numbering of carbon atoms starts with carboxyl group-C as number 1.

<sup>b</sup> A percentage estimate based on world production of edible oils.

(Source: Belitz et al. 2009)

### 2.3.9. Triacylglycerols

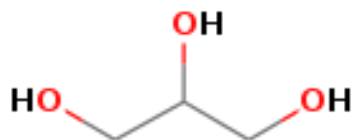
TAGs are esters of FAs with trihydric alcohol glycerol. In other words, TAGs consisting of one glycerol molecule bonded with three FA molecules as is shown in Figure 6. They are the main part of lipids. In TAGs, the hydroxyl groups of the glycerol join the carboxyl groups of the FAs to form ester bonds. The esters bonds are bonds between the molecules and are covalent. The three bonded FAs are a usually different type (Belitz et al. 2009; Velišek & Cejpek 2006). The charges are equally distributed around the molecule. Therefore, hydrogen bonds do not form with water molecules, and TAGs have hydrophobic character. In naturally occurring TAGs most of the FAs have 16, 18 or 20 carbon atoms in a chain. Most fats and oils contain a complex mixture of individual TAGs. Therefore, they have a wide range of melting point. Unusual is cocoa butter which is composed only by few types of TAGs, and most of them are composed of palmitic, oleic, and stearic acids (Lipp et al. 2001). TAGs are the main constituents of body fat in the human body, animal body, and vegetable fat. They also occur in the blood where they enable the bidirectional transference of adipose fat and blood glucose from the liver (Lampe et al. 1983; Alfred et al. 2015).



**Figure 6.** Structure of triacylglycerol  
(Source: Soult 2018)

### 2.3.10. Glycerol

Glycerol, or also glycerin is one of the most versatile chemical substances known to man. Swedish chemist Carl W Scheele identified glycerol in 1779 (Pagliaro & Rossi 2008). It is the second most important component of fats and oils. Glycerol is an essential organic compound because it forms an integral part of natural oils in the form of its esters. Glycerol contains three -OH hydroxyl groups (Figure 7) that can combine with up to three FAs to form monoglycerides, diglycerides, and TAGs. Glycerol has a melting point of 18.2 °C and a boiling point of 290 °C under normal atmospheric pressure, accompanied by decomposition. Natural glycerol is obtained hydrolytically from fats and oils during soap and FA manufacture and by transesterification (Pagliaro & Rossi 2008; Quispe et al. 2013).



**Figure 7.** Structure of glycerol  
(Source: PubChem Database 2019)



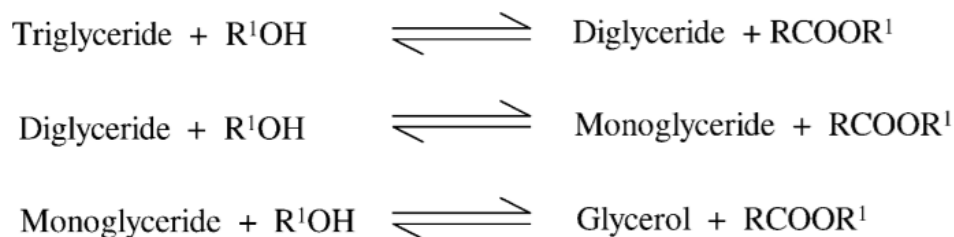
### **2.3.11. Determination of fatty acids**

Two methods are commonly used for the determination of FAs composition in a sample. It is infrared spectrometry (IR) and gas chromatography (GC). Method IR is easier but method GC is much more accurate (Moigradean et al. 2013). However, before the analysis of the composition of FAs they must be extracted from TGAs. FAs are easily extracted with nonpolar solvents from solutions or suspensions by lowering the pH to form the uncharged carboxyl group (Rustan & Drevon 2005).

## **2.4. Transesterification and GC-MS**

### **2.4.1. Transesterification**

It is an organic reaction and a method widely used to prepare FAMES from TAGs to enable their chromatographic analysis. This process is similar to hydrolysis, except that alcohol is used instead of water (Meher et al. 2006). If methanol is used in this process, it is called methanolysis. It is processed during which is an ester transformed into another through an interchange of alkoxy moiety. In the transesterification of vegetable oils, TAGs react with an alcohol in the presence of a strong acid or base. It produces a mixture of fatty acids alkyl esters and glycerol (Meher et al. 2006; Otera 1993; Schuchardt et al. 1998). The transesterification is an equilibrium reaction. The transformation occurs fundamentally by mixing the reactants. For acceleration, the transesterification process is usually catalyst used. In organic chemistry, it is typically used strong acid or base catalyst. Insufficient amount of catalyst can cause a formation of soap. The product of the transesterification is fatty acid alkyl esters and glycerol and the intermediates diglycerides and monoglycerides. This process is shown in Figure 8. The process of transesterification is affected by several aspects such as the presence of FFAs, moisture, catalyst type, catalyst concentration, the molar ratio of alcohol to oil, type of alcohol, reaction time, temperature, mixing intensity or using organic cosolvents. (Schuchardt et al. 1998; Hammond 2003; Wang et al. 2014; Meher et al. 2006; Prošková et al. 2009). According to Freedman et al. (1986) transesterification can happen at different temperatures, depending on the oil used.



**Figure 8.** The general equation for transesterification of triglycerides  
(Source: Meher et al. 2006)

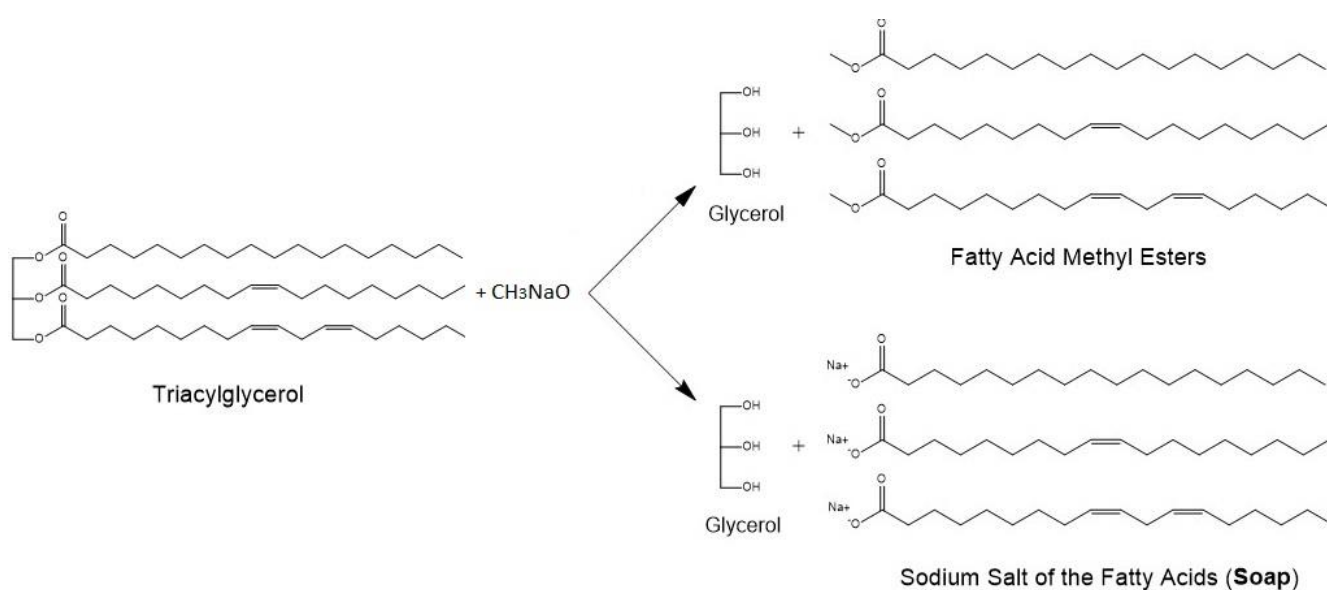
#### 2.4.2. Acid-catalysed transesterification

In this method, the acid catalyst is used. Acid-catalysed transesterification gives very high yields in alkyl esters. On the other hand, this reaction is significantly much slower than base-catalysed. It takes more than 3 hours for a complete conversion. There is also required high temperature (typically above 100 °C). The protonation of the carbonyl group of the ester leads to the carbonation, which after a nucleophilic attack of the alcohol produces a tetrahedral intermediate. The intermediate eliminates glycerol to form a new ester and to regenerate the catalyst. During acid-catalysed transesterification, the protonation of the carbonyl group of the ester leads to the carbonation. It produces a tetrahedral intermediate after a nucleophilic attack of the alcohol. The intermediate eliminates glycerol to form a new ester and to regenerate the catalyst. This type of transesterification is appropriate if the oil has a higher content of FFAs than 3 % and higher water content. (Meher et al. 2006; Bhatti et al. 2008; Ejikeme et al. 2010).

#### 2.4.3. Base-catalyzed transesterification

Base-catalyzed means that the alkali catalyst is used for transesterification. This technique is very rapid but produce FAMEs only from glycerol esters and not from any FFA components. As well is much less corrosive. The most used catalysts are sodium methoxide, sodium hydroxide, or potassium hydroxide (Metcalf 1981). Was observed that transesterification is faster when it is base-catalysed. In the first step of base-catalysed transesterification the attack of the alkoxide ion to the carbonyl carbon of the

TAG molecule. This causes formation of a tetrahedral intermediate. In the second step, the reaction of this intermediate with alcohol produces the alkoxide ion. In the last step, the rearrangement of the tetrahedral intermediate gives rise to an ester and a diglyceride. This method is appropriate for transesterification of fats and oils with low FFA content. The content of FFAs should be lower than 3 %. The higher FFA content of the oil causes the lower the conversion efficiency (Meher et al. 2006; Bhatti et al. 2008; Ejikeme et al. 2010). Base-catalysed transesterification by sodium methoxide ( $\text{CH}_3\text{NaO}$ ) is represented in Figure 9.



**Figure 9.** Triacylglycerol and products of base-catalysed transesterification by sodium methoxide  
(Source: Gebremariam & Marchetti 2017)

#### 2.4.4. Analytical method

Can be used several analytical methods for the identification of FAMES in oil. They are gas chromatography (GC), gas chromatography-mass spectrometry (GC-MS), high-performance liquid chromatography (HPLC), high-performance liquid chromatography-mass spectrometry (HPLC-MS), high-performance size exclusion chromatography (HPSEC), nuclear magnetic resonance (NMR), Raman spectroscopy, Fourier transform infrared spectroscopy (FTIR) or near-infrared spectroscopy (NIR). However, for the determination of individual profiles of FAs in oils and fats is the most widely used is the GC-MS technique. The second most widely used technique is the

GC–FID method. There are no significant differences between both methods. Overall, GC techniques are the most useful technique for identification of odd carbon number FAs (Wang et al. 2014; Petrović et al. 2010).

#### **2.4.5. Gas Chromatography-Mass Spectrometry**

Gas Chromatography (GC) is a physicochemical separation method. This method is generally used in analytical chemistry for separating and analysing of gases, liquids, and solids with a boiling point up to 400 °C (Poole, 2012). GC method is one of the most used chromatographic methods mainly for analysis of gases and solids. GC provides quantitative and qualitative analysis. The main advantage of GC is its speed of analysis. The analysis takes just several minutes and can be performed in a very small sample (Rozman, 2013). The method is based on dividing the analysed components between the two phases. The mobile phase and the stationary phase (Peroutková 2003).

**Gas Chromatograph** is a chemical analysis instrument. It was developed in the mid-1950s. A gas chromatograph utilises a flow-through narrow tube (column). Through column pass different chemical constituents of a sample. They pass in a gas stream at different rates depending on their chemical and physical properties and their interaction with a stationary phase. The main instruments of the gas chromatograph are carrier gas source, molecular sieves, a pressure regulatory system, an injector port, column, stationary phase, detector, thermostat, and data-processing unit. In the first phase of GC analysis is the sample in the form of a liquid or gas introduced via an injection port into the inlet (called injector). There are two major categories of injectors: vaporisation injector and on-column injector. Vaporisation injectors use rapid exposure of the sample to the high temperatures (200 – 300 °C). The sample is immediately vaporised and mixed with the flowing carrier gas. On-column injector omits vaporisation. The sample is not heated up and is deposit directly into the column (Cserhádi & Forgács 2003; Poole 2012).

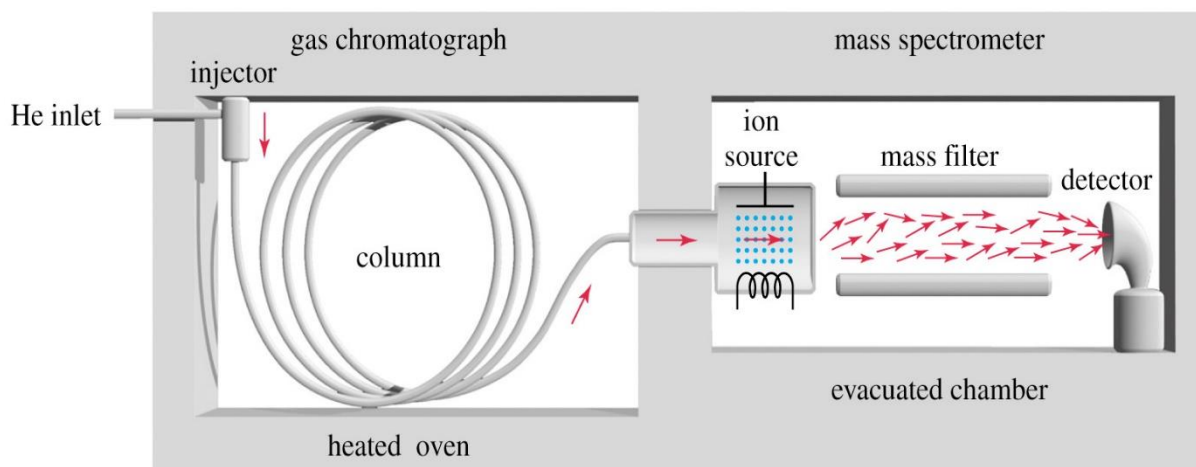
**Mobile phase** in gas chromatography is usually an inert gas called a carrier gas. The carrier gas acts as a background gas facilitating the detection. The most used gas is helium, nitrogen or argon. Nevertheless, in more than 90 % of the instruments is helium used. In some cases, it is necessary to add hydrogen and oxygen into the detector area, for instance when the flame ionisation detector is used. The carrier gas is transporting

the analyte through the column to the detector. The source of carrier gas are commonly pressure bottles. The carrier gas must be inert to the analyte and chromatographic environment. For cleaning of the gas are molecular sieves used. Sieves capture moisture and dirt in the carrier gas, removes traces of reactive oxygen that irreversibly damages the stationary phase in the column. The carrier gas flow is regulated by a regulation system. This system provides a steady or program-changing carrier gas flow. Different gases differ in their viscosity and price. Therefore, when selecting a suitable carrier gas, it is necessary to evaluate the importance and requirements for measuring accuracy and to choose the most efficient but also the most economical carrier gas (Stauffer et al. 2008; Stashenko & Martínéz 2014).

**Column and stationary phase** are the next most important parts of the GC. The stationary phase is on the inner side of the chromatography column. There, inside of a chromatographic column, the separation takes place. Two types of columns are commonly used. These are packed columns and capillary columns, also known as open tubular columns. The individual chromatography column differs both in the type of construction material and in their overall dimensions. Packed columns are steel or glass tubes of 1 – 5 m length and 2 – 8 mm in diameter. Packaged columns are densely packed with solid support, such as fluorocarbons, graphitised carbon black, diatomaceous earth, or glass beads. It is coated with a stationary phase, represented by a thin layer of high molecular weight polymer. Capillary columns are constructed of quartz glass and are covered with a polyimide layer. This layer gives the brittle column material flexibility and protects it from breaking. It also protects the column from high temperatures up to 350 °C. The internal diameters of the capillary are in the range of 0.10 mm – 0.53 mm and usually reach a length of 30 m but can be up to 100 m in length. There are three different types of capillary columns (WCOT, SCOT, PLOT). Wall Coated Open Tubular (WCOT) - the stationary phase is formed by a thin film on the inner wall of the capillary coated with the liquid stationary phase. To ensure sufficient contact between the mobile and stationary phases these columns must be very narrow. Support Coated Open Tubular (SCOT) - The inner wall of the capillary is lined by a thin layer of particulate support (e.g. diatomaceous earth). On to this support material, is then coated a thin layer of liquid stationary phase. It is an advantage of the SCOT columns as it provides them with the larger surface area and a thicker layer of stationary phase than WCOT columns. Porous Layer Open Tubular (PLOT) - There is a

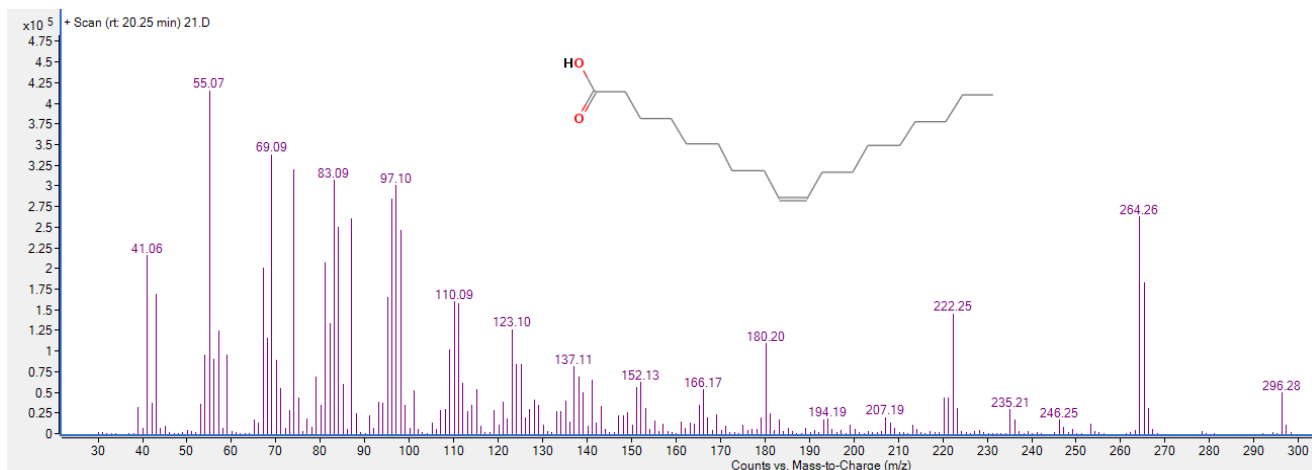
very thin layer of porous material (e.g. alumina) on the inside of the column. Capillary columns have higher resolutions, shorter analysis times, higher sensitivity and lower capacities compared to packed columns. Other types of chromatography column are a teflon column or older type copper column. The larger chromatography column diameter allows intake more amount sample. Conversely, a smaller column diameter limits capacity but increases efficiency. In general, it is not possible with certainty to determine if a capillary or packed column is better. During selecting process of a suitable column is need to take into account current needs, such as measurement requirements, the used sample and also, own experience. Therefore, it is necessary to have as much information as possible about the sample, such as its boiling point or structure before each analysis. The column is located in a chromatographic oven. The oven allows to heat the column on the required temperature. The temperature is precisely controlled electronically by a regulator (Stauffer et al. 2008; Matei et al. 2012; Rozman 2013, Sigma-Aldrich 2019).

**Mass spectrometry (MS)** is an analytical technique. It is a very accurate tool for identification of molecular structure. Main components of MS are inlet (GC), an ionisation source, mass analysers, mass-detector and data processing software. This technique ionised molecules. For ionisation are the most used matrix-assisted laser desorption/ionisation (MALDI) and electrospray ionisation (ESI) methods. After ionisation, the ionised molecules are transported to the mass-analyser (quadrupole, ion trap, orbitrap, time-of-flight (TOF)). There are sorted according to their mass-to-charge ratio ( $m/z$ ). (Pan et al. 2014; Vandel & Limbach 2017). Then are ions fragmented. These fragments are later further analysed in the second analyser. Ions leaving the last analyser are detected and measured. They are converted into signals according to their abundance. These electrical signals are processed by computer software. The result of this process is the mass spectrum. This spectrum is usually graphically illustrated like a bar graph. Bars demonstrated the individual ions with own ( $m/z$ ). The higher the bar, the higher the abundance of the ion. For illustration, a figure (Figure 11) shows the mass spectrum of methyl (9Z)-octadec-9-enoate. According to comparing of mass spectrum and retention time with a library of the national institute of standards and technology (NIST) can be molecule identified (Hoffman & Stroobant 2007; Stashenko & Martínéz 2014). Block diagram of a GC-MS with quadrupole mass analyser is shown in Figure 10.



**Figure 10.** Block diagram of a Gas chromatograph-Mass spectrometer with quadrupole mass analyser

(Source: Crasto 2014)



**Figure 11.** Mass spectrum of methyl (9Z)-octadec-9-enoate detected by GC-MS analysis

(Source: PubChem Database 2019)

### **3. Aims of the Thesis**

The diploma thesis was focused on the analysis of the change of fatty acids composition of edible oils and fat during heating. The main objective of the diploma thesis was to investigate the effect of long-term heating of oils and fat at a high temperature on the fatty acid composition. The secondary subject of interest of this research was to compare the thermal-oxidative stability of the sunflower oil against the high-oleic sunflower oil.

Seven different types of oils and one fat were heated up at 190 °C. The samples were taken, transesterified and analysed continuously every 4th hour of heating. The representation of individual fatty acids was monitored and compared within the same oil heated for different times. The recorded changes were compared with studies of a similar type.



## 4. Materials and methods

The experimental part was performed in the laboratory of the Czech University of Life Sciences Prague (CULS) of Faculty of Tropical Agriculture (FTA).

### Laboratory equipment

Fryer Tefal FF162131

Analytical balance Kern 572-30 accurate to 0.001g

GC/MSD System Agilent 7890B/5977A

Hamilton 500 µl Gastight syringe

Sterile silanized glass Pasteur pipettes

Laboratory vials (2 ml, 15 ml)

Other conventional chemical instruments (measuring cylinders, beakers, caps)

### Softwares

MassHunter Workstation Software; version: B.07.00

IBM SPSS Statistics; version: 25

Microsoft Office 365 ProPlus; version: 1902

### Chemicals

Toluene (C<sub>7</sub>H<sub>8</sub>) - Sigma-Aldrich

Sodium methoxide (CH<sub>3</sub>NaO) - Sigma-Aldrich

Acetic acid (CH<sub>3</sub>COOH) - Sigma-Aldrich

n-Hexane (C<sub>6</sub>H<sub>14</sub>) - Sigma-Aldrich

The standard mixture of fatty acids - FAME Mix 37 (Sigma-Aldrich, USA)

The composition of FAME Mix 37 is shown in the appendix (Appendix IV.)

#### 4.1. Material for analysis

For analysis, seven types of edible oils (coconut oil, palm oil, soybean oil, rapeseed oil, rice bran oil, sunflower oil, high-oleic sunflower oil) and one type of fat (hog lard) were used. These types of oils and the lard were chosen because they are the most conventional for cooking of food. All of the oils and the fat were bought in the local market in the Czech Republic. Detailed information about samples are shown in Table 2.

**Table 2.** Detailed information about analysed oils and fat

	High oleic sunflower oil	Rice bran oil	Coconut oil	Soybean oil	Palm oil	Rapeseed oil	Sunflower oil	Hog lard
<b>Raw material</b>	<i>Helianthus annuus</i>	<i>Oryza Sativa L.</i>	<i>Cocos nucifera L.</i>	<i>Glycine max L.</i>	<i>Elaeis guineensis</i>	<i>Brassica napus</i>	<i>Helianthus annuus</i>	<i>Sus scrofa domesticus</i>
<b>Fat content (per 100 ml)</b>	92 g	93 g	99.9 g	100 g	92 g	92 g	92 g	99 g
<b>of which:</b>								
- SUFAs	10 g	18.6g	79.7 g	17.3 g	32.3 g	6.7 g	11.3 g	31.2 g
- MUFAs	73 g	35 g	17.4 g	27 g	50.5 g	71 g	29.2 g	53.9 g
- PUFAs	9 g	39.4 g	2.8 g	55.8	9.2 g	14.3 g	51.5 g	13.9 g
<b>Brand</b>	Lukana	Basso	Franz Josef Kaiser	Emile Noël	Fabio produkt	Lukana	Clever	Brick
<b>Manufacturer</b>	Glencore Agriculture Czech s.r.o.	Basso Fedele e figli s.r.l.	Gaston s.r.o.	Huilerie Emile Noël	Fabio PRODUKT spol. s r.o.	Glencore Agriculture Czech s.r.o.	Glencore Agriculture Czech s.r.o.	Comperio s.r.o
<b>Country of origin</b>	Czech Republic	Thailand, China, India	Sri Lanka	France	Malaysia, Indonesia	Czech Republic	Czech Republic	Austria
<b>Others</b>	High heat resistance, Omega 9	Vitamin E	BIO	BIO	High heat resistance	---	---	---

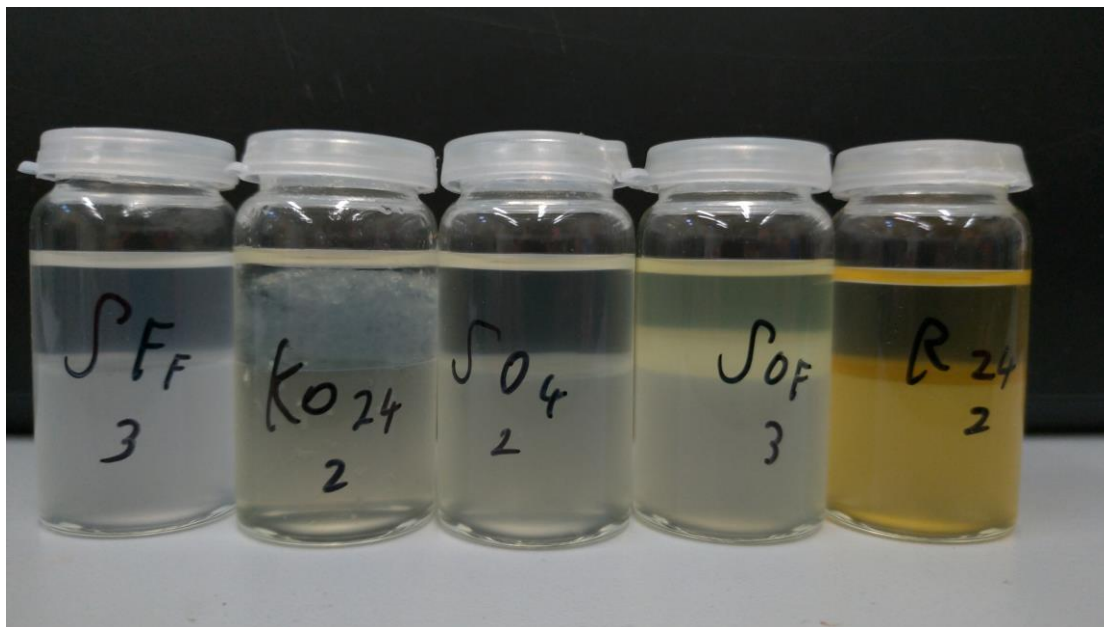
## **4.2. Heating of oils and fat**

From each testing oils and fat 3×1 ml of oil was collected for further analysis of a fresh sample. The transesterification process was performed immediately on these samples. One litre of the first of oil was poured into the kitchen fryer Tefal. The fryer was heated up on 190 °C and closed. This temperature was kept for all time of the heating process. 3×1 ml of heated oil was taken every 4th hour. As on fresh sample, the process of transesterification was immediately provided. The heating process was performed in three heating cycles of 8 hours. After each 8 h of heating, the oil was left at room temperature (25 °C) for the next day (16 h) and then reheated for next 8 h. The total heating time was 24 h. In total 168 samples were collected.

## **4.3. Transesterification**

FAMEs were extracted from all 168 of samples by the base-catalysed transesterification process according to Hammond (2003) method. This method is very rapid, but do not produce the FAMEs from FFAs. However, the amount of FFA is not significant in our tested oils and fat. Therefore, this method is appropriate. 100 mg of oil was weighted into the 15 ml laboratory vials by glass Pasteur pipette. The sample was diluted in 1 ml of toluene added by 500 µl Hamilton gastight syringe. Then, 4 ml of sodium methoxide was added by 5 ml laboratory measuring cylinder. The transesterification reaction started at this moment. The FAs are detached from triacylglycerols to form glycerin, soap, and FAMEs. The vial was capped, and the sample was gently shaken. The sample was allowed to rest for 15 minutes. Once the time was up the vial was opened, and 5 ml of 5 % acetic acid was added by 5 ml laboratory measuring cylinder to stop the transesterification reaction. In the next step, the sample was diluted in 5 ml of n-hexane. The sample was closed again by the cap and shook a lot. Over the next few minutes, glycerol began to settle. Three layers could be seen in the sample. The lower layer consists of glycerol, the middle formed by soap and mainly, the upper layer formed by dissolved FAMEs in n-hexane. The 500 µl of the upper layer was taken by the 500 µl gastight syringe and put to the 2 ml laboratory vial. The vial was closed by the cap and placed into the autosampler of GC for following GC-MS analysis. Every of vials were adequately marked according to the type of oil,

heating time, and order of collection. Examples of the transesterified samples are shown in Figure 12.



**Figure 12.** Base-transesterified oil samples

#### 4.4. GC-MS analysis

The subsequent GC-MS analysis was performed on an Agilent 7890B/5977A GC/MSD System (Agilent Technologies, USA) equipped with an HP-5 column (5 %-phenyl)-methylpolysiloxane with diameters 30 m length, 250  $\mu\text{m}$  internal diameter, 0.25  $\mu\text{m}$  film thickness. For the identifying of substances, Agilent Technologies 5977A mass spectrometer was used. Helium with a flow rate of 1 ml/min was used as the carrier gas. The optimised GC oven temperature program was 70  $^{\circ}\text{C}$  (2 min) to 280  $^{\circ}\text{C}$  at 10  $^{\circ}\text{C}/\text{min}$ , final temperature held for 10 min. The thermal program was set at 280  $^{\circ}\text{C}$ . The chromatography run was set to 33 minutes. The MSD transfer line temperature was maintained at 250  $^{\circ}\text{C}$  with the electron energy of 70 eV. Mass spectra were acquired in the mass range from  $m/z$  30 to 550, using a scan time of 1 s. Data were elaborated through MassHunter Workstation Software, Qualitative Analysis Version B.07.00, service pack 2, build 7.0.7024.29. This software generated integration peaks. Identification of FAME was based on comparing of mass spectra of detected substances

against mass spectra covered by the NIST/EPA/NIH library version 2.2. For accuracy, each detected fatty acid was compared with standard FAME Mix 37.

#### **4.5. Data analysis**

All data were sorted and saved in Microsoft Excel; version 1902 (build 11328.20158). Data were analysed by IBM SPSS Statistics; version: 25 and the Microsoft Excel. The average values of area peaks of each FA of each trinity of samples were calculated. According to of mean peaks area was calculated the percentage representation of each of FAMES in the sample. Then, according to the representation of FAMES in the fresh sample and the 24 hours heated sample was calculated the total change in the representation of each of FAMES. An overall change and relative change in composition were found. The progress of the change in the representation of each FAME was graphically represented. Also, the standard deviation, the relative standard deviation and Pearson correlation were calculated. All of these data are arranged in tables. The complete tables and charts are placed in the appendix of this thesis.

## 5. Results

The FAs composition in all of 168 oils and fat samples were determined after transesterification on the corresponding methyl esters (FAME) by GC-MS. All of the analysed FAs come from TGAs because the FFAs were not esterified by used base-catalysed transesterification method. Overall 27 different types of FAs were detected across all oils and fat. Rapeseed oil contained the most kinds of FAs (16). Coconut oil contained the least kinds of FAs (9). Palmitic acid, linoleic acid, oleic acid, stearic acid, arachidic acid were detected in all oils and the fat. In all tested oils and fat were detected SFAs, MUFAs and PUFAs. But, in the coconut oil, PUFAs were detected only in fresh, 4h, 8h, 12h, 16h and 20h samples. In 24h samples of coconut oil, no PUFAs were detected. It is because linoleic acid (C18:2) is the only PUFA representative in this oil and as a result of thermal-oxidation it changes the structure to oleic acid (C18:1). This process is presented in figure (Figure 14) where decreasing in the representation of methyl (9Z,12Z)-octadeca-9,12-dienoate (methyl linoleate) is obvious according to changes in the area of chromatographic peaks and slight increasing trend in the representation of methyl (9Z)-octadec-9-enoate (methyl oleate) until the 20th hour of heating. After 20th hour where methyl linoleate was no longer detected and also the representation of methyl oleate decreased. It is because of thermal-oxidation of oleic acid and further changes of its structure into the saturated FA stearic acid.

In all of the tested oils and fat, the content of FAs containing double bonds decreased. The most significant changes were obviously in the representation of methyl (9Z,12Z)-octadeca-9,12-dienoate. For example, in rice bran oils, this FAME content was decreased from 41.94 % (fresh) to 20.17 % (24h). Even, in the coconut oil, it was last detected in 20 hours heated (20h) samples. Then it was not detected. The opposite effect occurred at the methyl (9Z)-octadec-9-enoate (oleic acid) in some of the oils. This FAME has the same carbon number as the methyl (9Z,12Z)-octadeca-9,12-dienoate but contains just one double bond. In four of oils (rapeseed oil, rice bran oil, soybean oil, sunflower oil) was detected the increasing trend in the representation of this FAME. For example, in rice bran oil the content was increased by 15.54 % because of heating (from 35.93 % to 51.44 %). It is because of thermal-oxidation of double bonds of FAs. The reaction of oxygen with UFAs results in hydroperoxides, which immediately degrade in

further radical reactions at frying temperature. Overall, all tested oils and fat were more saturated after 24 hours of heating at 190 °C. The saturation all of the tested material is summarised in the table (Table 3).

The changes in the content of SFAs, MUFAs and PUFAs during frying in all tested oils and fat are shown in the Table 4. Figure (Figure 13) shows the total changes in the representation of PUFAs, MUFAs and SFAs. According to this figure (Figure 13.), it is obvious that decreasing the content of PUFAs directly influencing the content of MUFAs and SFAs. The same effect have changes in the representation of MUFAs on the representation of SFAs.

The significant correlation between FAs with the same numbers of carbons but with a different number of the double bond was detected. It is most apparent on the representation of FAs with carbon number 18. Correlations between all FAs in each of oils and fat are placed in the appendix of this thesis. The data with the representation of all detected FAs in all tested samples and their changes in representation during the heating period are shown in the tables (Tables 5, 6, 7, 8, 9, 10, 11, 12). The complete tables including graphs are placed in the appendix of this thesis. Total changes higher than 1 % are highlighted by green (positive) or red (negative) colour in the tables. For better clarity, the common names of FAs are used in the additional text.

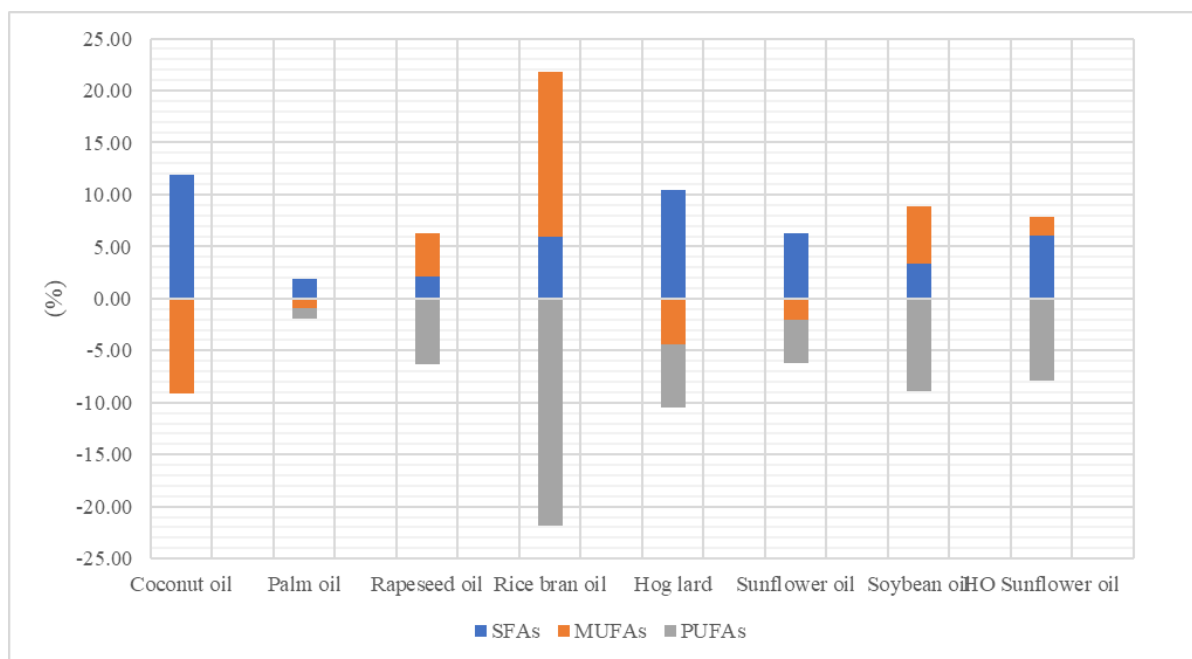
**Table 3.** Changes in SFAs, MUFAs and PUFAs in oils after 24 hours of heating at 190 °C in common fryer (%)

	SFAs	MUFAs	PUFAs
<b>Coconut oil</b>	11.96	-9.14	-
<b>Palm oil</b>	1.93	-0.93	-1.00
<b>Rapeseed oil</b>	2.07	4.27	-6.34
<b>Rice bran oil</b>	5.93	15.85	-21.79
<b>Hog lard</b>	10.48	-4.43	-6.06
<b>Sunflower oil</b>	6.23	-2.02	-4.21
<b>Soybean oil</b>	3.41	5.51	-8.92
<b>HO Sunflower oil</b>	6.02	1.82	-7.84

**Table 4.** Changes in the representation of SFAs, MUFAs and PUFAs during frying (%)

		<b>F</b>	<b>4h</b>	<b>8h</b>	<b>12h</b>	<b>16h</b>	<b>20h</b>	<b>20h</b>	<b>Total change</b>
<b>Coconut oil</b>	<b>SFAs</b>	79.79	80.46	79.81	82.00	87.66	89.81	91.75	<b>11.96</b>
	<b>MUFAs</b>	17.39	16.82	18.89	17.38	12.03	10.03	8.25	<b>-9.14</b>
	<b>PUFAs</b>	2.82	2.72	1.31	0.62	0.31	0.16	-nd-	-nd-
<b>Palm oil</b>	<b>SFAs</b>	35.13	35.91	35.08	36.35	35.99	37.17	37.06	<b>1.93</b>
	<b>MUFAs</b>	54.92	53.66	55.73	52.61	53.67	52.80	53.99	<b>-0.93</b>
	<b>PUFAs</b>	9.95	10.42	9.19	11.04	10.34	10.03	8.95	<b>-1.00</b>
<b>Rapeseed oil</b>	<b>SFAs</b>	7.26	8.87	6.72	11.91	9.43	10.76	9.33	<b>2.07</b>
	<b>MUFAs</b>	77.01	83.38	81.06	77.30	81.00	81.66	81.28	<b>4.27</b>
	<b>PUFAs</b>	15.73	7.76	12.23	10.79	9.58	7.58	9.39	<b>-6.34</b>
<b>Rice bran oil</b>	<b>SFAs</b>	20.12	20.40	19.62	20.84	23.99	25.04	26.05	<b>5.93</b>
	<b>MUFAs</b>	37.51	46.25	48.35	48.73	46.93	50.24	53.36	<b>15.85</b>
	<b>PUFAs</b>	42.38	33.35	32.03	30.43	29.08	24.72	20.59	<b>-21.79</b>
<b>Hog Lard</b>	<b>SFAs</b>	31.56	35.35	35.46	36.36	37.58	38.10	42.04	<b>10.48</b>
	<b>MUFAs</b>	54.41	52.80	53.98	53.34	52.27	52.57	49.98	<b>-4.43</b>
	<b>PUFAs</b>	14.03	11.85	10.56	10.30	10.15	9.33	7.97	<b>-6.06</b>
<b>Soybean oil</b>	<b>SFAs</b>	17.26	17.84	18.09	19.89	19.62	20.18	20.67	<b>3.41</b>
	<b>MUFAs</b>	26.96	26.22	28.61	25.60	27.98	28.70	32.47	<b>5.51</b>
	<b>PUFAs</b>	55.78	55.94	53.29	54.51	52.39	51.12	46.86	<b>-8.92</b>
<b>Sunflower oil</b>	<b>SFAs</b>	12.33	12.84	13.70	16.49	14.83	15.24	18.36	<b>6.02</b>
	<b>MUFAs</b>	31.74	32.24	31.77	29.34	33.36	36.18	33.56	<b>1.82</b>
	<b>PUFAs</b>	55.92	54.93	54.52	54.17	51.82	48.58	48.08	<b>-7.84</b>
<b>High-oleic sunflower oil (frying oil)</b>	<b>SFAs</b>	11.42	12.92	12.70	13.39	14.33	14.79	17.65	<b>6.23</b>
	<b>MUFAs</b>	79.15	79.10	80.09	80.38	78.71	79.82	77.14	<b>-2.02</b>
	<b>PUFAs</b>	9.42	7.98	7.04	6.23	6.97	5.38	5.21	<b>-4.21</b>





**Figure 13.** Graph of changes in SFAs, MUFAs and PUFAs in oils after 24 hours of heating at 190 °C in common fryer

**Table 5.** Changes in representation of fatty acids in coconut oil during frying at 190 °C in common fryer

		Coconut oil (%)								
Detected FAME	Common name of FA	Fresh	4h	8h	12h	16h	20h	24h	Total change	
Methyl octanoate	Caprylic acid	1.25	1.28	1.10	1.18	1.32	1.37	1.07	<b>-0.18</b>	
Methyl decanoate	Capric acid	1.65	1.62	1.03	0.82	1.67	1.82	1.29	<b>-0.35</b>	
Methyl dodecanoate	Lauric acid	23.61	25.79	19.76	18.02	24.10	25.24	24.27	<b>0.65</b>	
Methyl tetradecanoate	Myristic acid	20.21	20.06	20.43	18.84	22.78	23.38	24.43	<b>4.23</b>	
Methyl hexadecanoate	Palmitic acid	19.61	19.14	21.23	23.62	22.14	22.59	24.54	<b>4.93</b>	
Methyl (9Z)-octadec-9-enoate	Oleic acid	17.39	16.82	18.89	17.38	12.03	10.03	8.25	<b>-9.14</b>	
Methyl octadecanoate	Stearic acid	13.12	12.27	15.72	18.16	15.14	14.68	15.65	<b>2.53</b>	
Methyl icosanoate	Arachidic acid	0.35	0.30	0.53	1.36	0.50	0.73	0.50	<b>0.15</b>	
Methyl (9Z,12Z)-octadeca-9,12-dienoate	Linoleic acid	2.82	2.72	1.31	0.62	0.31	0.16	-nd-	---	

**Table 6.** Changes in representation of fatty acids in palm oil during frying at 190 °C in common fryer

<b>Palm oil (%)</b>									
Detected FAME	Common name of FA	Fresh	4h	8h	12h	16h	20h	24h	Total change
Methyl tetradecanoate	Myristic acid	0.35	0.59	0.51	0.53	0.47	0.54	0.44	<b>0.09</b>
Methyl (9Z)-hexadec-9-enoate	Palmitoleic acid	0.18	0.19	0.18	0.18	0.16	0.19	0.13	<b>-0.05</b>
Methyl hexadecanoate	Palmitic acid	27.84	28.85	27.62	29.10	28.20	29.61	28.85	<b>1.01</b>
Methyl heptadecanoate	Margaric acid	0.13	0.09	0.09	0.09	0.10	0.09	0.15	<b>0.02</b>
Methyl (9Z)-octadec-9-enoate	Oleic acid	54.37	53.04	55.00	52.06	53.17	52.21	53.40	<b>-0.97</b>
Methyl octadecanoate	Stearic acid	5.96	5.43	5.54	5.60	5.92	5.81	6.33	<b>0.37</b>
Methyl (11Z)-icos-11-enoate	Gondoic acid	0.36	0.44	0.54	0.36	0.34	0.40	0.46	<b>0.10</b>
Methyl octadecanoate	Arachidic acid	0.72	0.78	0.91	0.81	0.87	0.87	0.87	<b>0.15</b>
Methyl docosanoate	Behenic acid	0.13	0.17	0.41	0.21	0.43	0.24	0.43	<b>0.29</b>
Methyl (9Z,12Z)-octadeca-9,12-dienoate	Linoleic acid	9.95	10.42	9.19	11.04	10.34	10.03	8.95	<b>-1.00</b>

**Table 7.** Changes in representation of fatty acids in rapeseed oil during frying at 190 °C in common fryer

<b>Rapeseed oil (%)</b>									
Detected FAME	Common name of FA	Fresh	4h	8h	12h	16h	20h	24h	Total change
Methyl (9Z)-hexadec-9-enoate	Palmitoleic acid	0.34	0.20	0.16	0.25	0.18	0.21	0.16	<b>-0.18</b>
Methyl hexadecanoate	Palmitic acid	2.67	3.85	3.25	5.02	3.70	4.42	3.57	<b>0.90</b>
Methyl (10Z)-heptadec-10-enoate	---	0.05	0.08	0.07	0.10	0.09	0.10	0.08	<b>0.03</b>
Methyl heptadecanoate	Margaric acid	0.05	0.06	0.05	0.07	0.06	0.07	0.06	<b>0.01</b>
Methyl (9Z)-octadec-9-enoate	Oleic acid	73.30	79.59	78.38	72.01	76.51	76.71	76.92	<b>3.62</b>
Methyl octadecanoate	Stearic acid	2.36	2.63	2.40	3.45	2.77	3.08	2.98	<b>0.62</b>
Methyl (11Z)-icos-11-enoate	Gondoic acid	2.75	2.92	1.90	4.20	3.58	3.98	3.55	<b>0.80</b>
Methyl octadecanoate	Arachidic acid	1.11	1.36	0.03	2.17	1.87	2.13	1.72	<b>0.62</b>
Methyl heneicosanoate	Heneicosylic acid	0.05	0.02	0.09	-nd-	-nd-	-nd-	-nd-	---
Methyl (13Z)-docos-13-enoate	Erucic acid	0.33	0.35	0.32	0.43	0.35	0.40	0.33	0.00
Methyl docosanoate	Behenic acid	0.72	0.68	0.64	0.86	0.69	0.76	0.70	-0.02
Methyl tricosanoate	Tricosylic acid	0.02	-nd-	-nd-	-nd-	-nd-	-nd-	-nd-	---
Methyl (15Z)-tetracos-15-enoate	Nervonic acid	0.25	0.25	0.23	0.29	0.29	0.26	0.25	0.00
Methyl tetracosanoate	Lignoceric acid	0.29	0.28	0.25	0.34	0.33	0.30	0.30	0.01
Methyl (9Z,12Z)-octadeca-9,12-dienoate	Linoleic acid	15.18	7.25	11.74	10.38	9.21	7.26	9.10	<b>-6.08</b>
Methyl (9Z,12Z,15Z)-octadeca-9,12,15-trienoate	$\alpha$ -Linolenic acid	0.55	0.51	0.49	0.41	0.37	0.32	0.29	<b>-0.26</b>

**Table 8.** Changes in representation of fatty acids in rice bran oil during frying at 190 °C in common fryer

<b>Rice bran oil (%)</b>									
Detected FAME	Common name of FA	Fresh	4h	8h	12h	16h	20h	24h	Total change
Methyl tetradecanoate	Myristic acid	0.08	0.06	0.04	0.10	0.10	0.12	0.12	<b>0.04</b>
Methyl (9Z)-hexadec-9-enoate	Palmitoleic acid	0.19	0.22	0.13	0.19	0.21	0.22	0.21	<b>0.01</b>
Methyl hexadecanoate	Palmitic acid	14.14	13.80	11.91	13.82	16.59	17.18	17.64	<b>3.50</b>
Methyl (10Z)-heptadec-10-enoate	Margaroleic acid	0.02	0.05	0.04	0.03	0.02	0.02	0.02	<b>0.00</b>
Methyl heptadecanoate	Margaric acid	0.05	0.05	0.04	0.05	0.06	0.06	0.06	<b>0.01</b>
Methyl (9Z)-octadec-9-enoate	Oleic acid	35.93	44.58	46.97	47.01	45.49	48.47	51.44	<b>15.51</b>
Methyl octadecanoate	Stearic acid	3.03	3.14	3.10	3.32	3.76	3.80	4.07	<b>1.04</b>
Methyl (11Z)-icos-11-enoate	Gondoic acid	1.02	1.16	1.11	1.43	1.15	1.46	1.68	<b>0.66</b>
Methyl octadecanoate	Arachidic acid	1.53	1.77	1.67	1.91	2.05	2.31	2.42	<b>0.89</b>
Methyl (13Z)-docos-13-enoate	Erucic acid	0.35	0.24	0.11	0.07	0.07	0.06	0.02	<b>-0.33</b>
Methyl docosanoate	Behenic acid	0.52	0.62	1.15	0.65	0.57	0.65	0.68	<b>0.16</b>
Methyl tetracosanoate	Lignoceric acid	0.77	0.97	1.72	0.98	0.86	0.92	1.06	<b>0.29</b>
Methyl (9Z,12Z)-octadeca-9,12-dienoate	Linoleic acid	42.09	33.09	31.76	30.19	28.83	24.50	20.39	<b>-21.70</b>
Methyl (9Z,12Z,15Z)-octadeca-9,12,15-trienoate	$\alpha$ -Linolenic acid	0.28	0.26	0.27	0.25	0.25	0.23	0.20	<b>-0.09</b>

**Table 9.** Changes in representation of fatty acids in high-oleic sunflower oil during frying at 190 °C in common fryer (%)

<b>High-oleic sunflower oil (frying oil)</b>									
Detected FAME	Common name of FA	Fresh	4h	8h	12h	16h	20h	24h	Total change
Methyl (9Z)-hexadec-9-enoate	Palmitoleic acid	0.10	0.16	0.09	0.12	0.10	0.14	0.11	<b>0.01</b>
Methyl hexadecanoate	Palmitic acid	3.92	4.17	4.37	4.44	4.67	5.32	6.46	<b>2.55</b>
Methyl (10Z)-heptadec-10-enoate	Margaroleic acid	0.06	0.12	0.05	0.05	0.08	0.06	0.05	<b>-0.01</b>
Methyl heptadecanoate	Margaric acid	0.04	0.10	0.05	0.04	0.04	0.06	0.05	<b>0.01</b>
Methyl (9Z)-octadec-9-enoate	Oleic acid	78.39	77.22	79.18	78.87	77.41	77.79	75.94	<b>-2.45</b>
Methyl octadecanoate	Stearic acid	4.33	4.72	5.07	5.16	5.13	5.27	5.71	<b>1.37</b>
Methyl (11Z)-icos-11-enoate	Gondoic acid	0.60	1.60	0.77	1.34	1.11	1.83	1.04	<b>0.44</b>
Methyl octadecanoate	Arachidic acid	0.57	0.80	0.85	1.16	1.31	1.46	1.20	<b>0.63</b>
Methyl docosanoate	Behenic acid	1.94	2.53	1.78	1.95	2.39	2.01	3.03	<b>1.09</b>
Methyl tetracosanoate	Lignoceric acid	0.62	0.60	0.59	0.64	0.79	0.68	1.21	<b>0.59</b>
Methyl (9Z,12Z)-octadeca-9,12-dienoate	Linoleic acid	9.42	7.98	7.04	6.23	6.97	5.38	5.21	<b>-4.21</b>

**Table 10.** Changes in representation of fatty acids in hog lard during frying at 190 °C in common fryer

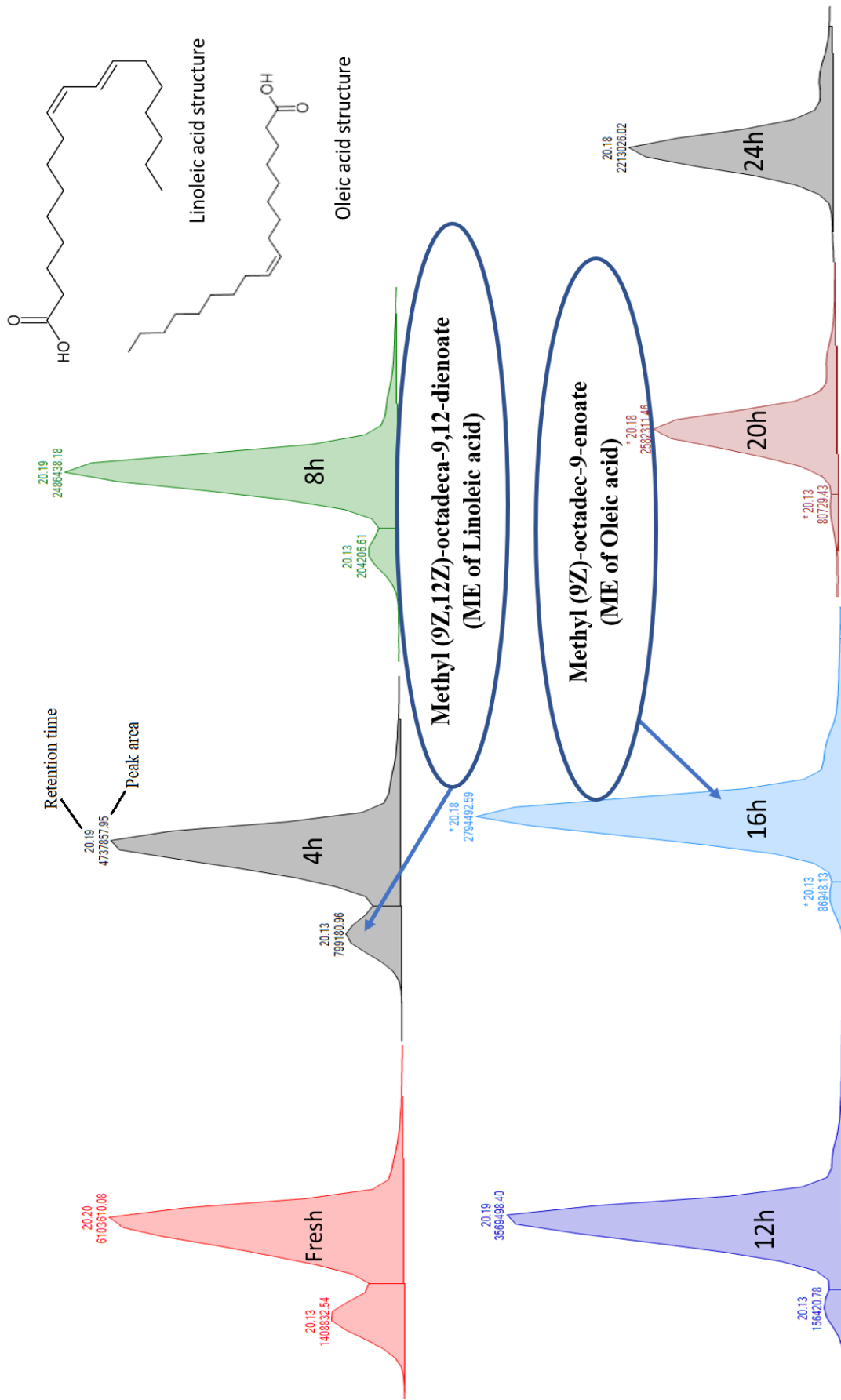
<b>Hog lard (%)</b>									
Detected FAME	Common name of FA	Fresh	4h	8h	12h	16h	20h	24h	Total change
Methyl tetradecanoate	Myristic acid	0.21	0.34	0.38	0.35	0.40	0.37	0.61	<b>0.40</b>
Methyl (9Z)-hexadec-9-enoate	Palmitoleic acid	1.64	1.36	1.38	1.33	1.30	1.25	1.07	<b>-0.57</b>
Methyl hexadecanoate	Palmitic acid	14.03	17.08	17.15	17.19	18.09	18.16	21.59	<b>7.56</b>
Methyl (10Z)-heptadec-10-enoate	Margaroleic acid	0.27	0.34	0.34	0.35	0.36	0.36	0.39	<b>0.12</b>
Methyl (9Z,12Z)-octadeca-9,12-dienoate	Linoleic acid	11.97	10.49	8.32	8.04	7.31	6.77	5.25	<b>-6.72</b>
Methyl (9Z)-octadec-9-enoate	Oleic acid	49.56	48.17	49.76	48.64	47.60	47.44	45.16	<b>-4.39</b>
Methyl octadecanoate	Stearic acid	16.89	17.45	17.41	18.24	18.29	18.77	18.99	<b>2.09</b>
Methyl (10Z)-nonadec-10-enoate	---	0.19	0.20	0.19	0.22	0.20	0.21	0.18	<b>-0.01</b>
Methyl nonadecanoate	---	0.08	0.05	0.05	0.06	0.06	0.05	0.04	<b>-0.03</b>
Methyl (5Z,8Z,11Z,14Z)-icosa-5,8,11,14-tetraenoate	Arachidonic	0.68	0.33	0.32	0.33	0.30	0.27	0.19	<b>-0.49</b>
Methyl (8Z,11Z,14Z)-icosa-8,11,14-trienoate	Dihomo-g - linolenic	0.15	0.32	0.50	0.78	1.21	1.24	1.48	<b>1.33</b>
Methyl (11Z,14Z)-icosa-11,14-dienoate	---	1.23	0.71	1.43	1.15	1.33	1.05	1.05	<b>-0.18</b>
Methyl (11Z)-icos-11-enoate	Gondoic acid	2.75	2.37	1.84	2.37	2.19	2.39	2.06	<b>-0.69</b>
Methyl octadecanoate	Arachidic acid	0.34	0.43	0.47	0.52	0.74	0.74	0.81	<b>0.47</b>

**Table 11.** Changes in representation of fatty acids in soybean oil during frying at 190 °C in common fryer

<b>Soybean oil (%)</b>									
Detected FAME	Common name of FA	Fresh	4h	8h	12h	16h	20h	24h	Total change
Methyl tetradecanoate	Myristic acid	0.03	0.25	0.01	0.02	0.03	0.02	0.04	<b>0.01</b>
Methyl (9Z)-hexadec-9-enoate	Palmitoleic acid	0.10	0.06	0.05	0.07	0.07	0.07	0.07	<b>-0.03</b>
Methyl hexadecanoate	Palmitic acid	8.13	7.53	8.25	9.79	9.28	9.78	9.69	<b>1.57</b>
Methyl (10Z)-heptadec-10-enoate	Margaroleic acid	0.09	0.09	0.09	0.09	0.10	0.12	0.09	<b>0.01</b>
Methyl heptadecanoate	Margaric acid	0.11	0.21	0.12	0.14	0.14	0.17	0.12	<b>0.01</b>
Methyl (9Z)-octadec-9-enoate	Oleic acid	26.28	25.52	27.59	24.66	26.38	27.52	31.80	<b>5.52</b>
Methyl octadecanoate	Stearic acid	7.23	7.91	7.77	8.07	8.42	8.36	8.49	<b>1.26</b>
Methyl (11Z)-icos-11-enoate	Gondoic acid	0.49	0.55	0.89	0.79	1.43	0.99	0.51	<b>0.01</b>
Methyl octadecanoate	Arachidic acid	0.72	0.83	0.83	0.88	0.85	0.99	1.07	<b>0.34</b>
Methyl henicanoate	---	0.11	0.07	0.16	0.06	0.07	0.05	0.08	<b>-0.03</b>
Methyl docosanoate	Behenic acid	0.71	0.79	0.71	0.70	0.84	0.81	1.18	<b>0.46</b>
Methyl tetracosanoate	Lignoceric acid	0.21	0.25	0.23	0.23	-nd-	-nd-	-nd-	---
Methyl (9Z,12Z)-octadeca-9,12-dienoate	Linoleic acid	55.78	55.94	53.29	54.51	52.39	51.12	46.86	<b>-8.92</b>

**Table 12.** Changes in representation of fatty acids in sunflower oil during frying at 190 °C in common fryer

<b>Sunflower oil (%)</b>									
Detected FAME	Common name of FA	Fresh	4h	8h	12h	16h	20h	24h	Total change
Methyl (9Z)-hexadec-9-enoate	Palmitoleic acid	0.07	0.08	0.08	0.10	0.10	0.10	0.11	<b>0.04</b>
Methyl hexadecanoate	Palmitic acid	4.57	5.17	5.46	5.55	6.00	5.87	7.31	<b>2.75</b>
Methyl (10Z)-heptadec-10-enoate	Margaroleic acid	0.04	0.04	0.04	0.05	0.05	0.05	0.06	<b>0.02</b>
Methyl heptadecanoate	Margaric acid	0.04	0.04	0.05	0.05	0.05	0.05	0.07	<b>0.02</b>
Methyl (9Z)-octadec-9-enoate	Oleic acid	31.17	31.74	31.01	28.38	32.06	34.98	31.96	<b>0.80</b>
Methyl octadecanoate	Stearic acid	4.89	5.00	5.24	7.66	5.50	5.78	7.19	<b>2.30</b>
Methyl (11Z)-icos-11-enoate	Gondoic acid	0.43	0.34	0.60	0.76	1.10	1.00	1.36	<b>0.93</b>
Methyl octadecanoate	Arachidic acid	0.49	0.52	0.65	0.74	0.90	0.86	1.09	<b>0.59</b>
Methyl docosanoate	Behenic acid	1.76	1.61	1.78	1.89	1.79	2.02	2.14	<b>0.38</b>
Methyl tricosanoate	Tricosylic acid	0.08	0.08	0.08	0.10	0.08	0.08	0.08	<b>0.00</b>
Methyl tetracosanoate	Lignoceric acid	0.54	0.46	0.51	0.53	0.57	0.63	0.55	<b>0.01</b>
Methyl (9Z,12Z)-octadeca-9,12-dienoate	Linoleic acid	55.92	54.93	54.52	54.17	51.82	48.58	48.08	<b>-7.84</b>



**Figure 14.** Chromatographic peaks of linoleic acidoleic acid. Illustrate changes in the representation of these FAs

**Table 13.** Pearson correlation between  $\alpha$  -linolenic acid, linoleic acid, oleic acid and stearic acid of rice bran oil

		$\alpha$ - Linolenic acid	Linoleic acid	Oleic acid	Stearic acid
$\alpha$ -Linolenic acid	Pearson Correlation	1	<b>.934**</b>	<b>-.819*</b>	<b>-.909**</b>
	Sig. (2-tailed)		0.002	0.024	0.005
Linoleic acid	Pearson Correlation	<b>.934**</b>	1	<b>-.949**</b>	<b>-.883**</b>
	Sig. (2-tailed)	0.002		0.001	0.008
Oleic acid	Pearson Correlation	<b>-.819*</b>	<b>-.949**</b>	1	0.697
	Sig. (2-tailed)	0.024	0.001		0.082
Stearic acid	Pearson Correlation	<b>-.909**</b>	<b>-.883**</b>	0.697	1
	Sig. (2-tailed)	0.005	0.008	0.082	

\*\*Correlation is significant at the 0.01 level (2-tailed).

\*Correlation is significant at the 0.05 level (2-tailed).

## 6. Discussion

In coconut oil, the absolute representation of MUFAs was decreased by about 9.14 %, but the representation of SFAs was increased almost by 12 % after 24 hours of heating. Oleic acid (-9.14 %), myristic acid (+4.23 %) and palmitic acid (+4.93 %) were the most influenced. Overall, the coconut oil after 24 hours of heating was more saturated by almost 12 % against to fresh sample and did not contain any detectable PUFAs. Koh & Long (2012) analysed the oxidative stability of coconut oil during deep-frying. They detected a little different composition of FAs in fresh coconut oil, but they used oil with a different origin (Indonesia) for analysis. They also detected changes in the representation of the same FAs as me but with lower intensity. However, they used a lower temperature (170 °C). After 24 hours of heating they also still detected 0.26 % of linoleic acid. I found last evidence of this FA in 20h samples. It is probably because of the different set of temperature and different origin of tested oil.

Composition of FAs in palm oil was not significantly affected in comparing with other oils. The content of UFAs fluctuated with a decreasing trend. Increasing in the representation of behenic acid was relatively the most significant. Content of other FAs fluctuated during the heating period. Overall, the content of SFAs was increased by 1.93 % after 24h of heating. The palm oil had the lowest increase of SFAs against to others tested oils and fat. Abiona et al. (2011) detected an increase in the representation of linoleic acid in palm oil (+0.71 %) heated at frying temperatures (160 – 180 °C) after 24 hours of heating. It is opposite effected than in my research where was detected a decrease in the content of this FA (-1.00 %). But, they used little bit lower temperature for their research, and also they measured the content only once after 24 hours. In my research, the content of this FA fluctuated with an increasing trend until 12 hours of heating. After that content of linoleic acid was started decreasing but still was higher than in the fresh sample. Only in 24h samples, the reduced content was detected. If we look at the development of the content of this FA and take into account the different temperatures, it is estimated that the content in 16 – 20h samples respond to 24h samples of Abiona et al. (2011). So, in result, this irregularity is attributed to the different temperature which was used. Sharoba & Ramadan (2012) detected a small number of trans- bonded FA C18:1 after 16 hours of heating at frying temperature. I did not detect any trans- bonded FA in my samples, but in their research, the peeled and



sliced raw potatoes also were in the oil fried. The compounds released from these potatoes probably caused the formation of trans- bonded FAs. Gharby et al. (2014) claim that their results indicated that palm oil has an excellent profile in terms of stability at high temperature. This statement I can confirm because the fatty acid composition in this oil was minimally affected in comparison with other tested oils and fat.

In rice bran oil the most affected was PUFAs which were decreased by 21.79 %. Is evident that most of PUFAs were changed the structure into MUFAs because these FAs increased overall content by 15.85 %. Specifically, the content of linoleic acid was decreased by 21.77 % against to increasing of oleic acid by 15.51 %. Also, the absolute content of SFAs was increased by 5.93 % after all heating period (from 20.12 % to 26.05 %). From SFAs the content of palmitic acid (+3.50 %) and stearic acid (+1.04 %) increased most. Latha and Nasirullah (2014) reported that heating did not cause any appreciable change in myristic acid and palmitic acids after 8 hours of heating at 180 °C. The statement about myristic acid agrees with my results, but the representation of palmitic acid was increased appreciable (+3.50 %). However, this FA strongly fluctuated in the first 12 hours of heating in almost all tested oil. Therefore according to Latha and Nasirullah (2014), which heated the oil only for 8 hours, it is not possible to claim that this FA is not significantly influenced by heating because the intensive changes start after this period. These changes are also strongly influenced by the content of palmitoleic acid which is during heating transformed to the palmitic acid due to saturation of double bonds. However, they did not detect any palmitoleic acid in their analysis but according to Akhter et al. (2015), Kang and Kim (2016), Dorni et al. (2017) as well according to my research, there is. Erucic acid which represents 0.35 % of the content in fresh samples was almost not detected in 24h samples (0.02 %). In rice bran oil the presence of a small amount of  $\alpha$ -linolenic acid (0.28 % in fresh samples) which was overall reduced to 0.20 % after 24 hours of heating was detected. This statement corresponds to the results of Latha and Nasirullah (2014).

The most of kinds of FAs were detected in the rapeseed oil (16). On the other hand, two of them (tricosylic acid and heneicosylic acid) were not detected all the time. Especially the tricosylic acid was detected only in fresh samples. Like in rice bran oil also in rapeseed oil the  $\alpha$ -linolenic acid in all samples was detected, and also it was

reduced after the heating period from 0.55 % to 0.29 % of the content. There a strong fluctuation in the content of linoleic acid with standard deviation  $\pm 2.8$  was detected. It is because of the low thermal stability of this fatty acid and contemporary presence of  $\alpha$  - linolenic acid. Representation of PUFAs was decreased from 15.73 % to 9.39 % (- 6.34 %). Compared to the content of MUFAs and SFAs it was increased (MUFAs +4.27 %; SFAs +2.07 %). These results correspond to the Gharby et al. (2014) which heated this oil for 30 hours at 180 °C. Aniołowska et al. (2016) also detected the presence of trans-FA C18:2, but they fried oil together with french fries, unlike my research. In the composition of other FAs was no significant difference against to my research.

In hog lard, 14 different FAs were detected. It is the only tested sample where FA with four double bonds (arachidonic) was detected. Also only in this fat eicosanoic acid with more than one double bond was detected. Overall, the composition of FAs in fresh sample correspond to Csap'ó and Salamon (2013). Interesting is mainly found increasing content of dihomo-g -linolenic during heating (+1.33 %). As in rapeseed oil, in hog lard was detected decreasing trend in the representation of linoleic acid (-6.72 %) and oleic acid (-4.39 %) and increasing trend in the representation of stearic acid (+2.09 %) and palmitic acid (+7.56 %). Representation of other FAs was not significantly affected by heating. The representation of MUFAs fluctuated during heating with a decreasing trend and representation of PUFAs was decreased from 14.03 % in fresh samples to 7.97 % in 24h samples. Overall, this fat was about 10.48 % more saturated after 24 hours of heating. Park and Kim (2016) 100 times fried chicken nuggets in lard (lard was heated up ten times per day for ten days). They detected very similar FAs composition of the used hog lard as me. They did not heat the lard constantly for ten days. They did not state the exact time of one cycle of heating but according to Ngadi and Adedeji (2010) is the optimal time for fried chicken nuggets about 16 minutes per cycle at 180 °C. Therefore we can estimate that Park and Kim (2016) overall heated this lard approximately 24 hours ( $\pm 2$ h) as well as me. They detected just a little bit lower content of MUFAs after heating period than me, but the disintegration of double bonds was affected by the presence of the chicken nuggets in their lard.

In soybean oil, no interesting changes were detected. All changes were average against other oils. 13 FAMES here were detected. The representation of linoleic acid (-8.92 %) and oleic acid (+5.52 %) was the most affected. The content of both of these

FAs fluctuated during heating. It was also detected an increasing trend of stearic acid with final growth +1.26 %. The same effect reported Onal-Ulusoy (2005) which recorded decreasing content of linoleic acid and increasing content of oleic acid and stearic acid in soybean oil heated at frying temperatures. In research of Gerde et al. (2006) this trend even continued until the 9th day of heating of soybean oil. Gerde et al. (2006) also detected an increasing trend in the representation of palmitic acid such as was detected in this research (+1.57 %). I detected the presence of palmitoleic acid in all samples in a very small amount. Khan (2014), which investigated the effect of heating on soybean oil in 15 minutes intervals, detected this acid only in a fresh sample. After 15 minutes of heating in his research, it was no more detected. Also, the changes in the representation of other fatty acids were changed significantly faster in his research. However, he heated the oil to its boiling point, and boiling point of soybean oil is about 300 °C. It is about 58 % higher temperature than in my research. This fact shows that the higher temperature also causes the faster decomposition of double bonds in soybean oil. Hassanein et al. (2003) detected a much more rapid increase in the representation of palmitic acid (+0.8 % after 18 minutes), but he subjected oil to microwave heating. According to his results, it is evident that also the representation of other FAs is changed much more intensive when the oil is exposed to microwave heating. In result, heating of oil in fryer is much more thrifty to oil. These FAs constitute almost all changes in the representation. Therefore, the total decreasing in the representation of PUFAs (-8.92 %) is the same as decreasing in linoleic acid representation. The representation of MUFAs and SFAs was increased by 3.41 % and 5.51 %.

In sunflower oil (SO) and high-oleic sunflower oil (HOSO) were detected 12 and 11 types of FAs, respectively. As the manufacturer stated it, the HOSO contained a higher amount of oleic acid (78.39 %) than SO (31.17 %). In both of these oils, an increase in the content of palmitic acid about almost 3 % was detected. Also decreasing trend in the representation of linoleic acid in both of oils was detected. In sunflower oil, this decreasing effect was almost double higher. It is because of the content of linoleic acid in the fresh sample which was almost six times higher in SO (SO 55.92 %; HOSO 9.42 %). The similar effect has the representation of oleic acid. Because the content of this acid in HOSO was higher than the content of linoleic acid in the fresh sample during heating it was decomposed in a higher amount, and after 24 hours of heating it was overall decreased about -2.45 %. In SO the content of oleic acid (31.17 %) was

lower than the content of linoleic acid (55.92 %). Therefore, the content of oleic acid was increased by about 0.80 %, because of higher content and the fact that linoleic acid has lower thermal-oxidative stability than oleic acid. The representation of stearic acid had a growing trend in HOSO. In SO the representation fluctuated with a growing trend. Overall, the representation of stearic acid was increased by 1.37 % in HOSO and by 2.30 % in SO. The very similar results achieved Ali et al. (2013) under almost the same conditions (heating for 24h at 185°C ±5°C). The representation of other FAs was not significantly affected, and also there are no more interesting differences between these types of oils. The content of SFAs and MUFAs was almost equally affected in SO and HOSO. The only difference is in the content of PUFAs but only detected PUFA was above mentioned linoleic acid.

If the frying oil contains high amounts of PUFAs, the risk of cardiovascular and gastrointestinal diseases or cancer are that much higher (AŞKIN 2016). According to this statement and my results it is obvious that HOSO more appropriate for long term frying than SO. Because, the fresh sample of HOSO contained only 9.42 % PUFAs and the fresh sample of SO contained 55.92 % PUFAs. The overall final content of linoleic acid after 24 hours of heating is 48.08 % in SO and just 5.21 % in HOSO. In result, the HOSO is much more stable during the frying process which is evident by weaker changes in the representation of PUFAs.

## 7. Conclusions

According to results, it is evident that the best thermal-oxidative stability from all tested oils and fat has palm oil, followed by rapeseed oil. Therefore a mixture of this oil with sunflower oil or high-oleic sunflower oil is usually used for frying in fast food restaurants such as McDonalds or KFC. The least stable FAs composition has coconut oil and rice bran oil. These oils are therefore not suitable for long term frying. This research showed that the fatty acid composition of high-oleic sunflower oil has better thermal-oxidative stability than the fatty acid composition of sunflower oil. According to the discussion, it is also obvious that the changes in fatty acid composition differ significantly according to time of heating, set temperature and type of heating (microwave heating, fryer et cetera). Therefore, further researches in order to discover precisely the principles of decomposition of FAs during frying are needed. There it is needed also to inspect other substances which arise and disappear in oil during frying. The results show that in all tested oils and fat were the most affected the PUFAs and that oils become more saturated because of heating. Overall the composition of fatty acids is very variable, and a proportion of MUFAs usually fluctuated. According to results, it seems that the oils which contain fewer types of FAs are more stable than oils rich in differ FAs species. But there is needed to investigate a wider choice of oils in order to confirm this statement.

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# Appendices

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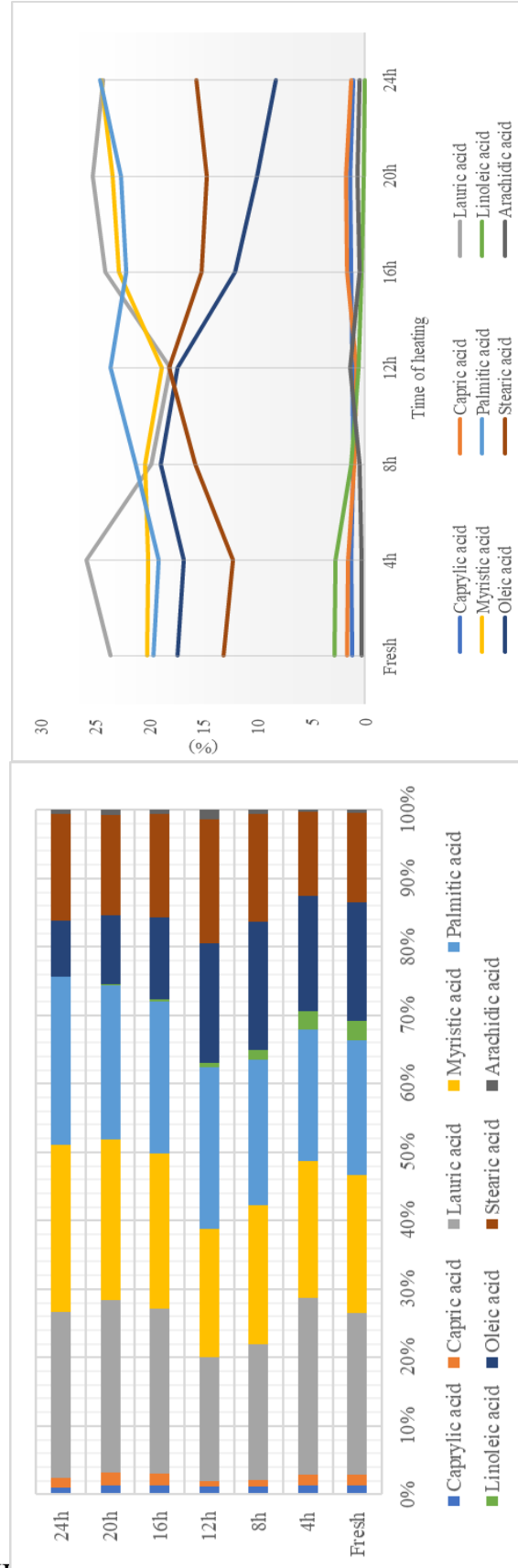
### Appendix IV.

<b>IVa.</b> Composition of standard FAME mix 37.....	XXI
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**Appendix I:** Tables of detected FAMES in oils and the fat during heating at 190 °C for 24 hours in common fryer including the graphs which illustrated changing in representation each of FA during the time; “-nd-“ not detected; “-” not enough data

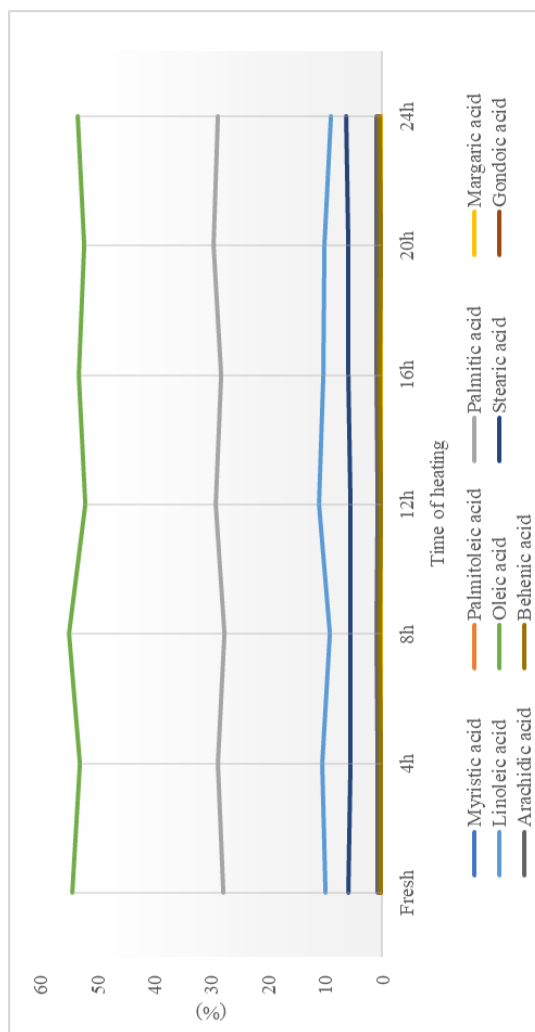
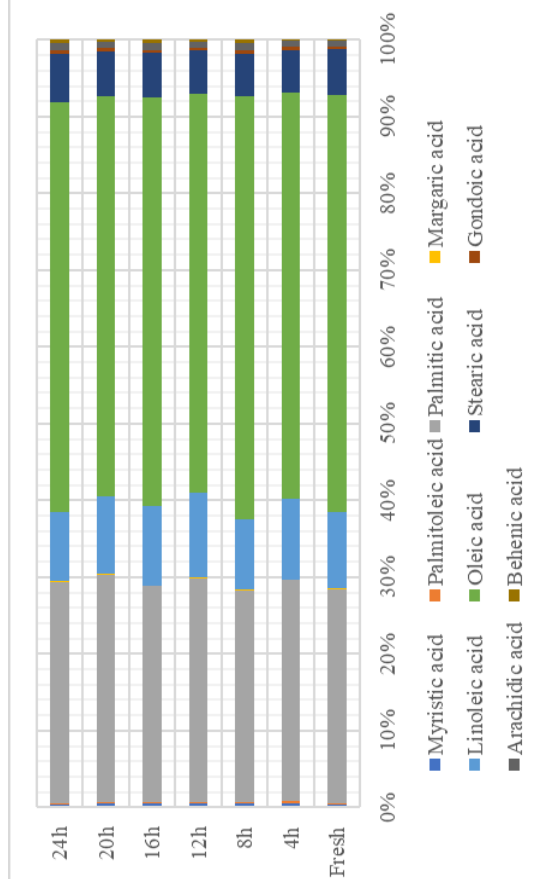
**Ia. Coconut oil**

Coconut oil															
MW	Detected FAME	Ab. Designation	Common name of FA	Average representation of FA over time ( Heating at 240 °C ) (%)								Change in representation (%)		σ	rel.σ (%)
				Fresh	4h	8h	12h	16h	20h	24h	Absolute (24h - F)	Relative			
158	Methyl octanoate	8:0	Caprylic acid	1.25	1.28	1.10	1.18	1.32	1.37	1.07	-0.18	-14.26	0.11	9.17	
186	Methyl decanoate	10:0	Capric acid	1.65	1.62	1.03	0.82	1.67	1.82	1.29	-0.35	-21.37	0.38	26.52	
214	Methyl dodecanoate	12:0	Lauric acid	23.61	25.79	19.76	18.02	24.10	25.24	24.27	0.65	2.77	2.95	12.83	
242	Methyl tetradecanoate	14:0	Myristic acid	20.21	20.06	20.43	18.84	22.78	23.38	24.43	4.23	20.91	2.07	9.64	
270	Methyl hexadecanoate	16:0	Palmitic acid	19.61	19.14	21.23	23.62	22.14	22.59	24.54	4.93	25.17	2.00	9.14	
294	Methyl (9Z,12Z)-octadeca-9,12-dienoate	18:2	Linoleic acid	2.82	2.72	1.31	0.62	0.31	0.16	-nd-	---	---	1.08	-nd-	
296	Methyl (9Z)-octadec-9-enoate	18:1	Oleic acid	17.39	16.82	18.89	17.38	12.03	10.03	8.25	-9.14	-52.57	4.21	29.24	
298	Methyl octadecanoate	18:0	Stearic acid	13.12	12.27	15.72	18.16	15.14	14.68	15.65	2.53	19.25	1.92	12.82	
326	Methyl icosanoate	20:0	Arachidic acid	0.35	0.30	0.53	1.36	0.50	0.73	0.50	0.15	43.50	0.36	58.91	
			SFAs	79.79	80.46	79.81	82.00	87.66	89.81	91.75	11.96	14.99	5.18	6.14	
			MUFAs	17.39	16.82	18.89	17.38	12.03	10.03	8.25	-9.14	-52.57	4.21	29.24	
			PUFAs	2.82	2.72	1.31	0.62	0.31	0.16	-nd-	---	---	1.08	-nd-	



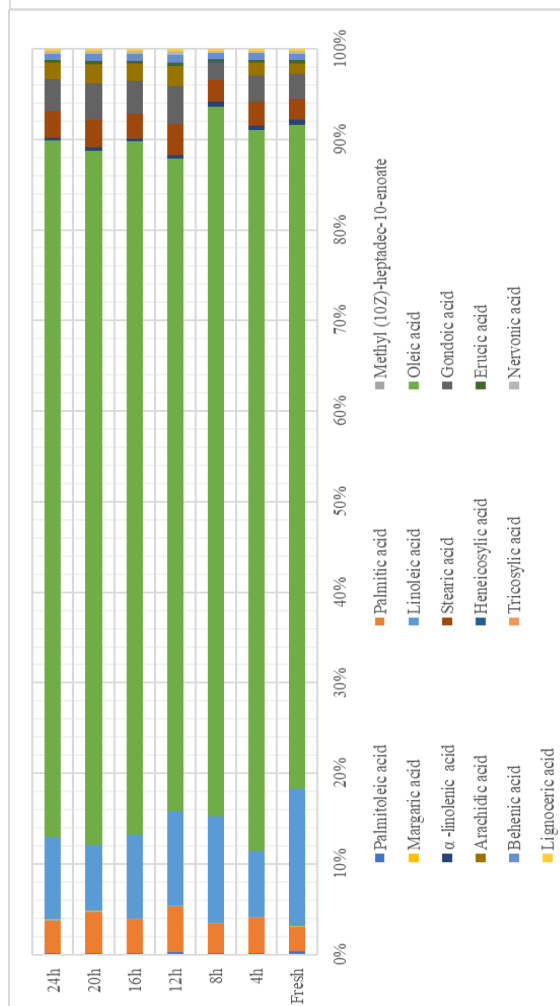
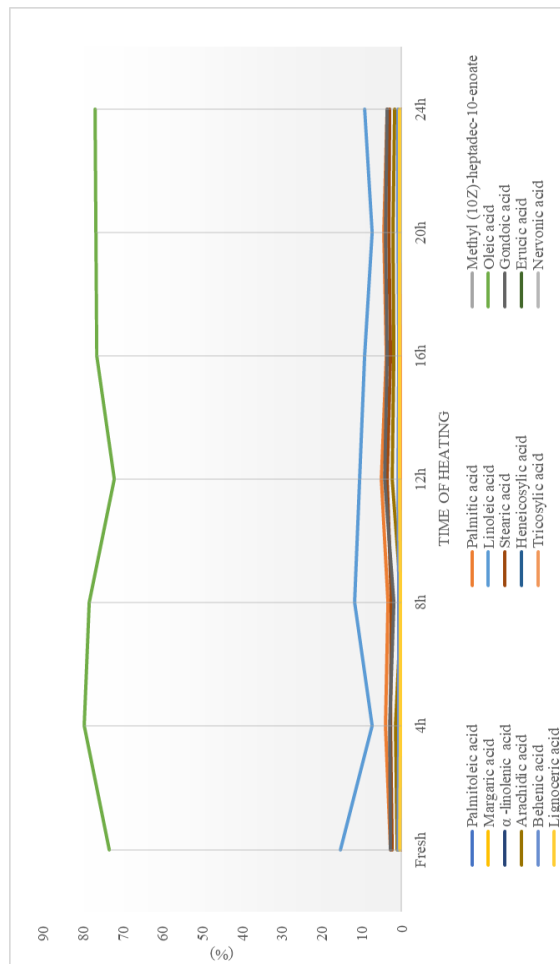
## Ib. Palm oil

Palm oil														
MW	Detected FAME	Ab. Designation	Common name of FA	Average representation of FA over time ( Heating at 240 °C ) (%)							Change in representation (%)		rel. $\sigma$ (%)	
				F	4h	8h	12h	16h	20h	24h	Absolute (24h - F)	Relative		
242	Methyl tetradecanoate	14:0	Myristic acid	0.35	0.59	0.51	0.53	0.47	0.54	0.44	0.09	24.41	0.083	16.85
268	Methyl (9Z)-hexadec-9-enoate	16:1	Palmitoleic acid	0.18	0.19	0.18	0.18	0.16	0.19	0.13	-0.05	-28.99	0.011	6.25
270	Methyl hexadecanoate	16:0	Palmitic acid	27.84	28.85	27.62	29.10	28.20	29.61	28.85	1.01	3.62	0.775	2.71
284	Methyl heptadecanoate	17:0	Margaric acid	0.13	0.09	0.09	0.09	0.10	0.09	0.15	0.02	17.49	0.016	14.80
294	Methyl (9Z,12Z)-octadeca-9,12-dienoate	18:2	Linoleic acid	9.95	10.42	9.19	11.04	10.34	10.03	8.95	-1.00	-10.06	0.613	6.13
296	Methyl (9Z)-octadec-9-enoate	18:1	Oleic acid	54.37	53.04	55.00	52.06	53.17	52.21	53.40	-0.97	-1.79	1.170	2.19
298	Methyl octadecanoate	18:0	Stearic acid	5.96	5.43	5.54	5.60	5.92	5.81	6.33	0.37	6.24	0.217	3.75
324	Methyl (11Z)-icos-11-enoate	20:1	Gondoic acid	0.36	0.44	0.54	0.36	0.34	0.40	0.46	0.10	27.08	0.074	17.94
326	Methyl octadecanoate	20:0	Arachidic acid	0.72	0.78	0.91	0.81	0.87	0.87	0.87	0.15	20.31	0.070	8.40
354	Methyl docosanoate	22:0	Behenic acid	0.13	0.17	0.41	0.21	0.43	0.24	0.43	0.29	221.73	0.126	43.43
			SFAs	35.13	35.91	35.08	36.35	35.99	37.17	37.06	1.93	5.49	0.83	2.31
			MUFAs	54.92	53.66	55.73	52.61	53.67	52.80	53.99	-0.93	-1.69	1.11	2.06
			PUFAs	9.95	10.42	9.19	11.04	10.34	10.03	8.95	-1.00	-10.06	0.72	7.24



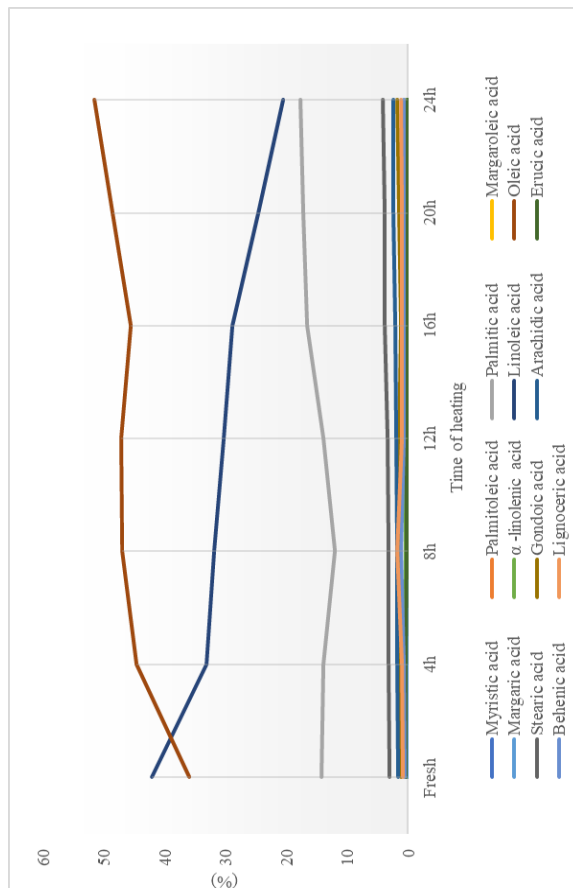
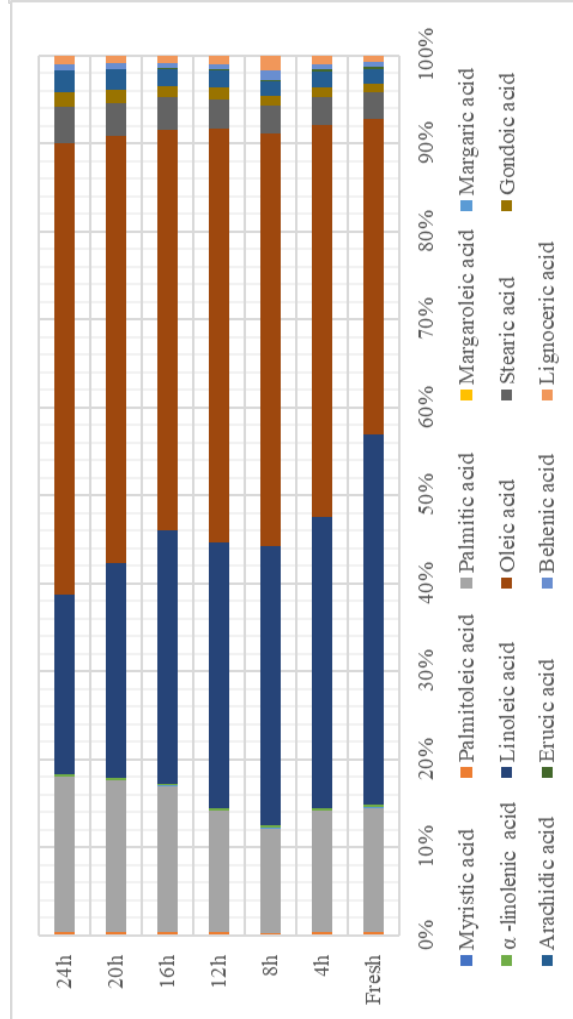
## Ic. Rapeseed oil

Rapeseed oil															
MW	Detected FAME	Ab. Designation	Common name of FA	Average representation of FA over time ( Heating at 240 °C ) (%)								Change in representation (%)	σ	rel. σ (%)	
				F	4h	8h	12h	16h	20h	24h	Absolute (24h - F)				Relative
268	Methyl (9Z)-hexadec-9-enoate	16:1	Palmitoleic acid	0.34	0.20	0.16	0.25	0.18	0.21	0.16	0.16	-0.18	-53.00	0.064	29.77
270	Methyl hexadecanoate	16:0	Palmitic acid	2.67	3.85	3.25	5.02	3.70	4.42	3.57	3.57	0.90	33.77	0.765	20.23
282	Methyl (10Z)-heptadec-10-enoate	17:1	---	0.05	0.08	0.07	0.10	0.09	0.10	0.08	0.08	0.03	49.29	0.018	21.72
284	Methyl heptadecanoate	17:0	Margaric acid	0.05	0.06	0.05	0.07	0.06	0.07	0.06	0.06	0.01	29.73	0.008	13.62
294	Methyl (9Z,12Z)-octadeca-9,12-dienoate	18:2	Linoleic acid	15.18	7.25	11.74	10.38	9.21	7.26	9.10	9.10	-6.08	-40.06	2.784	27.80
296	Methyl (9Z)-octadec-9-enoate	18:1	Oleic acid	73.30	79.59	78.38	72.01	76.51	76.71	76.92	76.92	3.62	4.94	2.680	3.52
292	Methyl (9Z,12Z,15Z)-octadeca-9,12,15-trienoate	18:3	α-linolenic acid	0.55	0.51	0.49	0.41	0.37	0.32	0.29	0.29	-0.26	-47.27	0.099	23.69
298	Methyl octadecanoate	18:0	Stearic acid	2.36	2.63	2.40	3.45	2.77	3.08	2.98	2.98	0.62	26.14	0.391	13.91
324	Methyl (11Z)-icos-11-enoate	20:1	Gondoic acid	2.75	2.92	1.90	4.20	3.58	3.98	3.55	3.55	0.80	29.05	0.797	24.38
326	Methyl octadecanoate	20:0	Arachidic acid	1.11	1.36	0.03	2.17	1.87	2.13	1.72	1.72	0.62	55.64	0.749	50.40
340	Methyl heneicosanoate	21:0	Heneicosylic acid	0.05	0.02	0.09	-nd-	-nd-	-nd-	-nd-	-nd-	---	---	-nd-	-nd-
352	Methyl (13Z)-docos-13-enoate	22:1	Erucic acid	0.33	0.35	0.32	0.43	0.35	0.40	0.33	0.33	0.00	0.39	0.041	11.49
354	Methyl docosanoate	22:0	Behenic acid	0.72	0.68	0.64	0.86	0.69	0.76	0.70	0.70	-0.02	-3.09	0.071	9.88
368	Methyl tricosanoate	23:0	Tricosylic acid	0.02	-nd-	-nd-	-nd-	-nd-	-nd-	-nd-	-nd-	---	---	-nd-	-nd-
380	Methyl (15Z)-tetracos-15-enoate	24:1	Nervonic acid	0.25	0.25	0.23	0.29	0.29	0.26	0.25	0.25	0.00	-1.40	0.022	8.67
382	Methyl tetracosanoate	24:0	Lignoceric acid	0.29	0.28	0.25	0.34	0.33	0.30	0.30	0.30	0.01	4.03	0.030	10.13
			<b>SFAs</b>	7.26	8.87	6.72	11.91	9.43	10.76	9.33	9.33	2.07	28.45	1.82	19.82
			<b>MUFAs</b>	77.01	83.38	81.06	77.30	81.00	81.66	81.28	81.28	4.27	5.54	2.35	2.92
			<b>PUFAs</b>	15.73	7.76	12.23	10.79	9.58	7.58	9.39	9.39	-6.34	-40.31	2.84	27.25



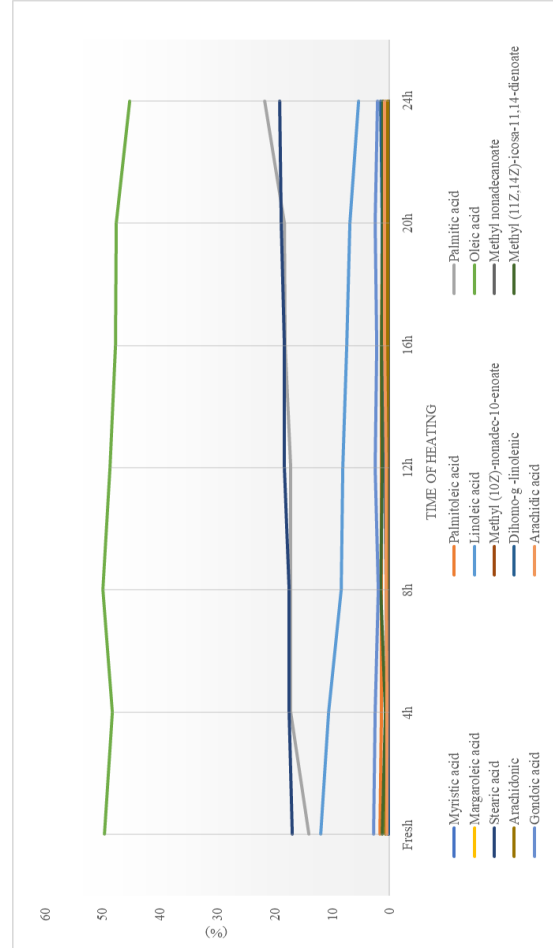
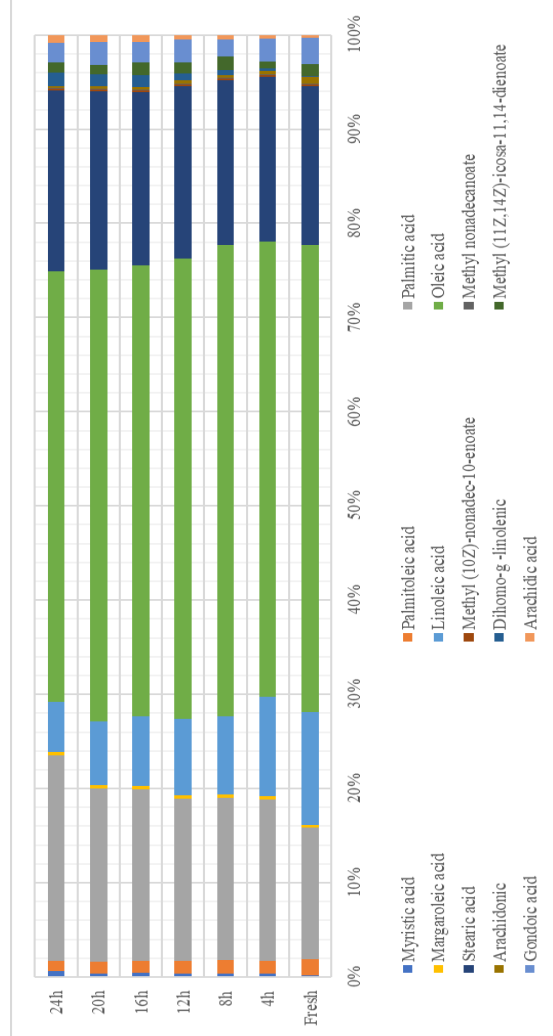
## Id. Rice bran oil

Rice bran oil															
MW	Detected FAME	Ab. Designation	Common name of FA	Average representation of FA over time ( Heating at 240 °C ) (%)								Change in representation (%)		rel. $\sigma$ (%)	
				F	4h	8h	12h	16h	20h	24h	Absolute (24h - F)	Relative			
242	Methyl tetradecanoate	14:0	Myristic acid	0.08	0.06	0.04	0.10	0.10	0.12	0.12	0.12	0.04	48.33	0.030	33.62
268	Methyl (9Z)-hexadec-9-enoate	16:1	Palmitoleic acid	0.19	0.22	0.13	0.19	0.21	0.22	0.21	0.21	0.01	5.93	0.032	16.12
270	Methyl hexadecanoate	16:0	Palmitic acid	14.14	13.80	11.91	13.82	16.59	17.18	17.64	17.64	3.50	24.75	2.136	14.23
282	Methyl (10Z)-heptadec-10-enoate	17:1	Margaroleic acid	0.02	0.05	0.04	0.03	0.02	0.02	0.02	0.02	0.00	-15.60	0.012	39.85
284	Methyl heptadecanoate	17:0	Margaric acid	0.05	0.05	0.04	0.05	0.06	0.06	0.06	0.06	0.01	25.40	0.008	14.88
292	Methyl (9Z,12Z,15Z)-octadeca-9,12,15-trienoate	18:3	$\alpha$ -Linolenic acid	0.28	0.26	0.27	0.25	0.25	0.23	0.20	0.20	-0.09	-31.03	0.027	10.76
294	Methyl (9Z)-octadec-9-enoate	18:2	Linoleic acid	42.09	33.09	31.76	30.19	28.83	24.50	20.39	20.39	-21.70	-51.55	6.900	22.91
296	Methyl (9Z)-octadec-9-enoate	18:1	Oleic acid	35.93	44.58	46.97	47.01	45.49	48.47	51.44	51.44	15.51	43.18	4.843	10.60
298	Methyl octadecanoate	18:0	Stearic acid	3.03	3.14	3.10	3.32	3.76	3.80	4.07	4.07	1.04	34.35	0.411	11.89
324	Methyl (11Z)-icos-11-enoate	20:1	Gondoic acid	1.02	1.16	1.11	1.43	1.15	1.46	1.68	1.68	0.66	64.98	0.239	18.58
326	Methyl octadecanoate	20:0	Arachidic acid	1.53	1.77	1.67	1.91	2.05	2.31	2.42	2.42	0.89	57.90	0.329	16.86
352	Methyl (13Z)-docos-13-enoate	22:1	Eruic acid	0.35	0.24	0.11	0.07	0.07	0.06	0.02	0.02	-0.33	-93.42	0.119	90.74
354	Methyl docosanoate	22:0	Behenic acid	0.52	0.62	1.15	0.65	0.57	0.65	0.68	0.68	0.16	31.57	0.209	30.34
382	Methyl tetracosanoate	24:0	Lignoceric acid	0.77	0.97	1.72	0.98	0.86	0.92	1.06	1.06	0.29	37.96	0.314	30.12
			SFAs	20.12	20.40	19.62	20.84	23.99	25.04	26.05	26.05	5.93	29.50	2.65	11.88
			MUFAs	37.51	46.25	48.35	48.73	46.93	50.24	53.36	53.36	15.85	42.26	4.93	10.41
			PUFAs	42.38	33.35	32.03	30.43	29.08	24.72	20.59	20.59	-21.79	-51.42	6.89	22.70



## Ie. Hog lard

Hog Lard														
MW	Detected FAME	Ab. Designation	Common name of FA	Average representation of FA over time ( Heating at 240 °C ) (%)							Change in representation (%)		σ	rel. σ (%)
				F	4h	8h	12h	16h	20h	24h	Absolute (24h - F)	Relative		
242	Methyl tetradecanoate	14:0	Myristic acid	0.21	0.34	0.38	0.35	0.40	0.37	0.61	0.40	189.37	0.119	31.26
268	Methyl (9Z)-hexadec-9-enoate	16:1	Palmitoleic acid	1.64	1.36	1.38	1.33	1.30	1.25	1.07	-0.57	-34.65	0.170	12.79
270	Methyl hexadecanoate	16:0	Palmitic acid	14.03	17.08	17.15	17.19	18.09	18.16	21.59	7.56	53.87	2.231	12.66
282	Methyl (10Z)-heptadec-10-enoate	17:1	Margaroleic acid	0.27	0.34	0.34	0.35	0.36	0.36	0.39	0.12	42.96	0.037	10.75
294	Methyl (9Z,12Z)-octadeca-9,12-dienoate	18:2	Linoleic acid	11.97	10.49	8.32	8.04	7.31	6.77	5.25	-6.72	-56.12	2.271	27.34
297	Methyl (9Z)-octadec-9-enoate	18:1	Oleic acid	49.56	48.17	49.76	48.64	47.60	47.44	45.16	-4.39	-8.87	1.554	3.23
298	Methyl octadecanoate	18:0	Stearic acid	16.89	17.45	17.41	18.24	18.29	18.77	18.99	2.09	12.40	0.774	4.30
310	Methyl (10Z)-nonadec-10-enoate	19:1	---	0.19	0.20	0.19	0.22	0.20	0.21	0.18	-0.01	-4.86	0.013	6.77
312	Methyl nonadecanoate	19:0	---	0.08	0.05	0.05	0.06	0.06	0.05	0.04	-0.03	-44.54	0.013	22.92
318	Methyl (5Z,8Z,11Z,14Z)-icosa-5,8,11,14-tetraenoate	20:4	Arachidonic	0.68	0.33	0.32	0.33	0.30	0.27	0.19	-0.49	-71.78	0.155	45.05
320	Methyl (8Z,11Z,14Z)-icosa-8,11,14-trienoate	20:3	Dihomo-g-linolenic	0.15	0.32	0.50	0.78	1.21	1.24	1.48	1.33	863.01	0.511	62.99
322	Methyl (11Z,14Z)-icosa-11,14-dienoate	20:2	---	1.23	0.71	1.43	1.15	1.33	1.05	1.05	-0.18	-14.70	0.234	20.65
324	Methyl (11Z)-icos-11-enoate	20:1	Gondoic acid	2.75	2.37	1.84	2.37	2.19	2.39	2.06	-0.69	-25.01	0.288	12.64
326	Methyl octadecanoate	20:0	Arachidic acid	0.34	0.43	0.47	0.52	0.74	0.74	0.81	0.47	136.29	0.183	31.51
			SFAs	31.56	35.35	35.46	36.36	37.58	38.10	42.04	10.48	33.22	3.192	8.71
			MUFAs	54.41	52.80	53.98	53.34	52.27	52.57	49.98	-4.43	-8.14	1.45	2.74
			PUFAs	14.03	11.85	10.56	10.30	10.15	9.33	7.97	-6.06	-43.18	1.92	18.13



## If. High-oleic sunflower oil

Sunflower oil - High oleic (frying oil)														
MW	Detected FAME	Ab. Designation	Common name of FA	Average representation of FA over time (Heating at 240 °C) (%)						Change in representation (%)		σ	rel. σ (%)	
				F	4h	8h	12h	16h	20h	24h	Absolute (24h - F)			Relative
268	Methyl (9Z)-hexadec-9-enoate	16:1	Palmitoleic acid	0.10	0.16	0.09	0.12	0.10	0.14	0.11	0.00	2.23	0.025	21.21
270	Methyl hexadecanoate	16:0	Palmitic acid	3.92	4.17	4.37	4.44	4.67	5.32	6.46	2.55	64.99	0.868	18.21
282	Methyl (10Z)-heptadec-10-enoate	17:1	Margaroleic acid	0.06	0.12	0.05	0.05	0.08	0.06	0.05	-0.01	-10.58	0.026	37.00
284	Methyl heptadecanoate	17:0	Margaric acid	0.04	0.10	0.05	0.04	0.04	0.06	0.05	0.01	11.73	0.021	39.13
294	Methyl (9Z,12Z)-octadeca-9,12-dienoate	18:2	Linoleic acid	9.42	7.98	7.04	6.23	6.97	5.38	5.21	-4.21	-44.71	1.481	21.50
296	Methyl (9Z)-octadec-9-enoate	18:1	Oleic acid	78.39	77.22	79.18	78.87	77.41	77.79	75.94	-2.45	-3.13	1.106	1.42
298	Methyl octadecanoate	18:0	Stearic acid	4.33	4.72	5.07	5.16	5.13	5.27	5.71	1.37	31.67	0.434	8.59
324	Methyl (11Z)-icos-11-enoate	20:1	Gondoic acid	0.60	1.60	0.77	1.34	1.11	1.83	1.04	0.44	73.62	0.438	37.03
326	Methyl octadecanoate	20:0	Arachidic acid	0.57	0.80	0.85	1.16	1.31	1.46	1.20	0.63	109.43	0.317	30.17
354	Methyl docosanoate	22:0	Behenic acid	1.94	2.53	1.78	1.95	2.39	2.01	3.03	1.09	56.13	0.441	19.78
382	Methyl tetracosanoate	24:0	Lignoceric acid	0.62	0.60	0.59	0.64	0.79	0.68	1.21	0.59	95.85	0.221	30.28
	<b>SFAs</b>			11.42	12.92	12.70	13.39	14.33	14.79	17.65	6.23	54.52	1.99	14.36
	<b>MUFAs</b>			79.15	79.10	80.09	80.38	78.71	79.82	77.14	-2.02	-2.55	1.09	1.37
	<b>PUFAs</b>			9.42	7.98	7.04	6.23	6.97	5.38	5.21	-4.21	-44.71	1.48	21.50

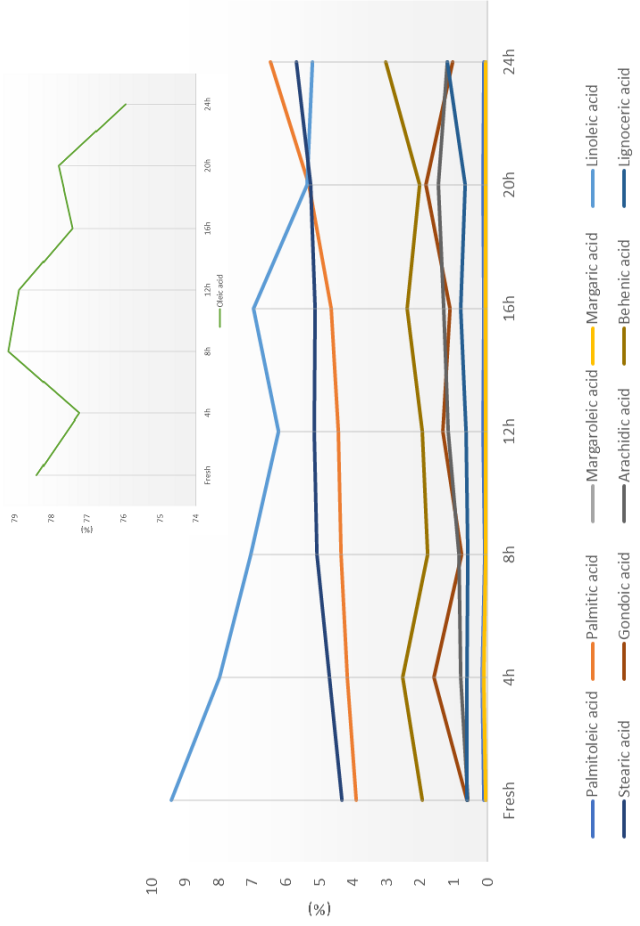
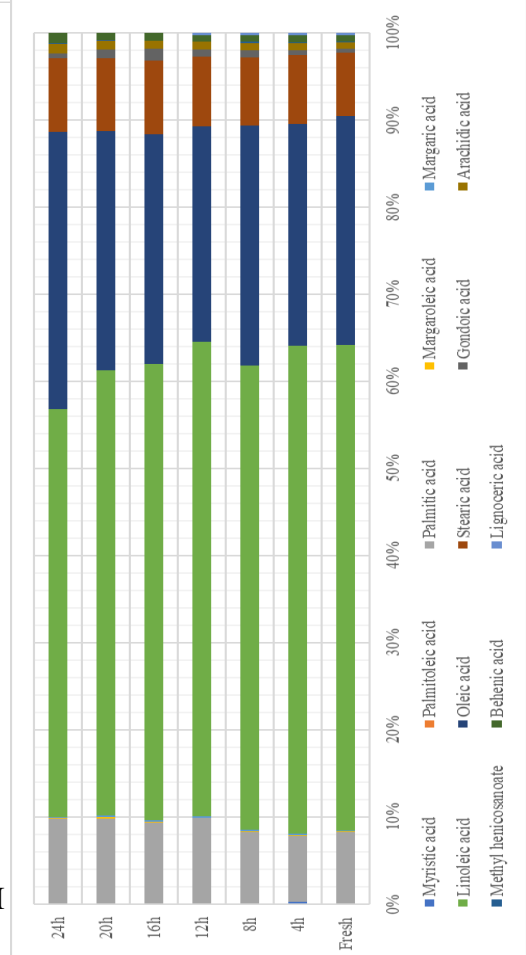
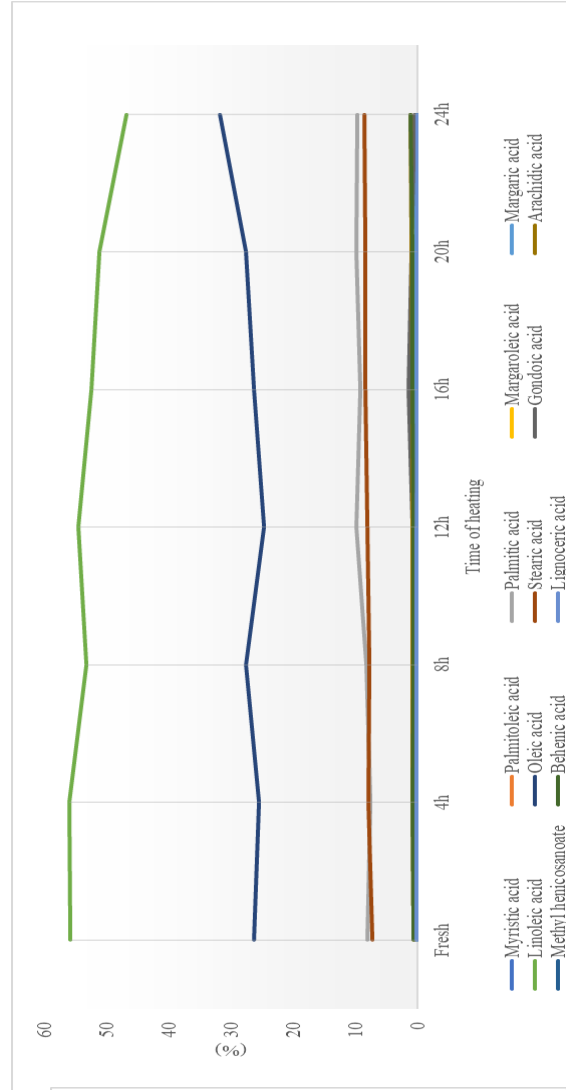


Fig. Soybean oil

Soybean oil														
MW	Detected FAME	Ab. Designation	Common name of FA	Average representation of FA over time ( Heating at 240 °C ) (%)							Change in representation (%)		σ	rel. σ (%)
				F	4h	8h	12h	16h	20h	24h	Absolute (24h - F)	Relative		
242	Methyl tetradecanoate	14:0	Myristic acid	0.03	0.25	0.01	0.02	0.03	0.02	0.04	0.01	45.55	0.086	148.57
268	Methyl (9Z)-hexadec-9-enoate	16:1	Palmitoleic acid	0.10	0.06	0.05	0.07	0.07	0.07	0.07	-0.03	-30.84	0.015	22.01
270	Methyl hexadecanoate	16:0	Palmitic acid	8.13	7.53	8.25	9.79	9.28	9.78	9.69	1.57	19.29	0.933	10.46
282	Methyl (10Z)-heptadec-10-enoate	17:1	Margaroleic acid	0.09	0.09	0.09	0.09	0.10	0.12	0.09	0.01	6.67	0.011	12.07
294	Methyl heptadecanoate	17:0	Margaric acid	0.11	0.21	0.12	0.14	0.14	0.17	0.12	0.01	7.93	0.035	24.14
294	Methyl (9Z,12Z)-octadeca-9,12-dienoate	18:2	Linoleic acid	55.78	55.94	53.29	54.51	52.39	51.12	46.86	-8.92	-15.99	3.167	5.99
296	Methyl (9Z)-octadec-9-enoate	18:1	Oleic acid	26.28	25.52	27.59	24.66	26.38	27.52	31.80	5.52	21.00	2.315	8.54
298	Methyl octadecanoate	18:0	Stearic acid	7.23	7.91	7.77	8.07	8.42	8.36	8.49	1.26	17.37	0.446	5.56
324	Methyl (11Z)-icos-11-enoate	20:1	Gondoic acid	0.49	0.55	0.89	0.79	1.43	0.99	0.51	0.01	2.73	0.338	41.87
326	Methyl octadecanoate	20:0	Arachidic acid	0.72	0.83	0.83	0.88	0.85	0.99	1.07	0.34	47.50	0.115	13.09
340	Methyl hencosanoate	21:0	---	0.11	0.07	0.16	0.06	0.07	0.05	0.08	-0.03	-25.94	0.038	43.44
354	Methyl docosanoate	22:0	Behenic acid	0.71	0.79	0.71	0.70	0.84	0.81	1.18	0.46	64.83	0.168	20.50
382	Methyl tetracosanoate	24:0	Lignoceric acid	0.21	0.25	0.23	0.23	-nd-	-nd-	-nd-	---	---	0.016	12.41
	SFAs			17.26	17.84	18.09	19.89	19.62	20.18	20.67	3.41	19.78	1.32	6.94
	MUFAs			26.96	26.22	28.61	25.60	27.98	28.70	32.47	5.51	20.43	2.27	8.07
	PUFAs			55.78	55.94	53.29	54.51	52.39	51.12	46.86	-8.92	-15.99	3.17	5.99

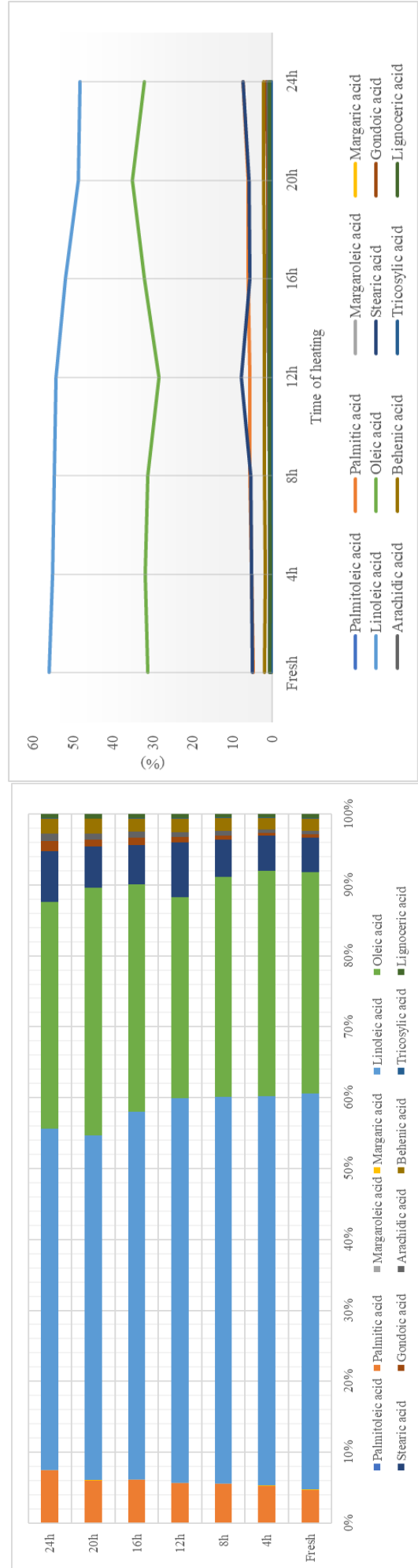




## Ih. Sunflower oil

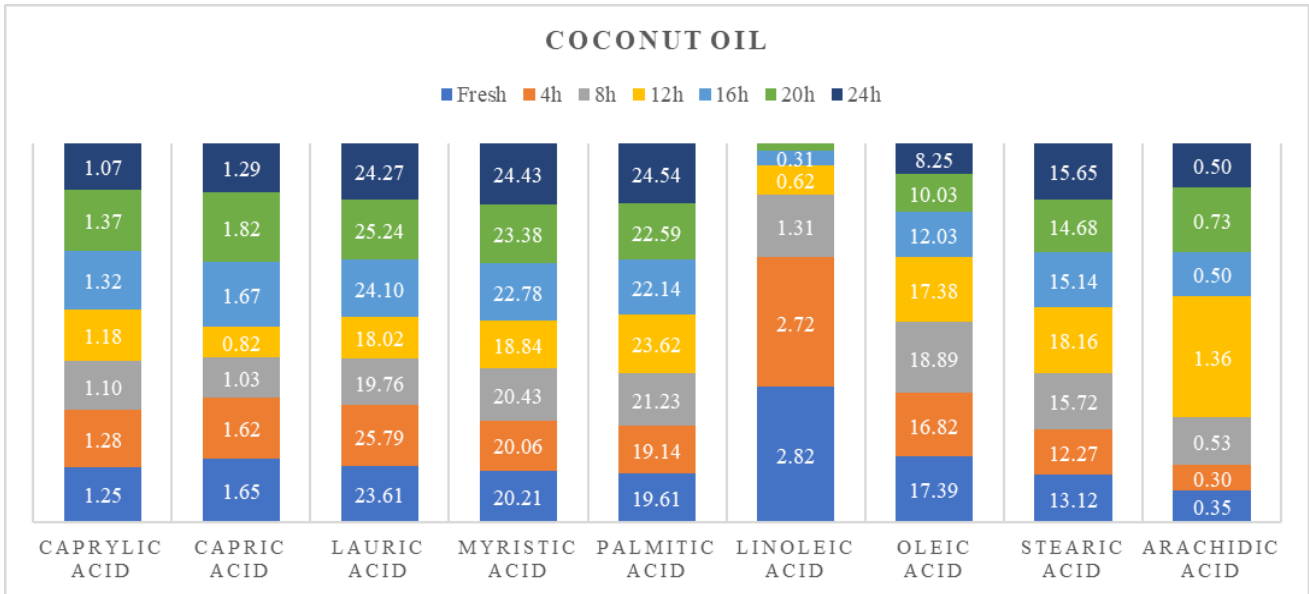
Sunflower oil															
MW	Detected FAME	Ab. Designation	Common name of FA	Average representation of FA over time ( Heating at 240 °C ) (%)								Change in representation (%)		σ	rel. σ (%)
				F	4h	8h	12h	16h	20h	24h	Absolute (24h - F)	Relative			
268	Methyl (9Z)-hexadec-9-enoate	16:1	Palmitoleic acid	0.07	0.08	0.08	0.10	0.10	0.10	0.10	0.11	0.04	68.15	0.015	16.18
270	Methyl hexadecanoate	16:0	Palmitic acid	4.57	5.17	5.46	5.55	6.00	5.87	5.87	7.31	2.75	60.17	0.851	14.93
282	Methyl (10Z)-heptadec-10-enoate	17:1	Margaroleic acid	0.04	0.04	0.04	0.05	0.05	0.05	0.05	0.06	0.02	51.91	0.008	16.66
284	Methyl heptadecanoate	17:0	Margaric acid	0.04	0.04	0.05	0.05	0.05	0.05	0.05	0.07	0.02	61.04	0.010	20.26
294	Methyl (9Z,12Z)-octadeca-9,12-dienoate	18:2	Linoleic acid	55.92	54.93	54.52	54.17	51.82	48.58	48.58	48.08	-7.84	-14.02	3.157	6.00
296	Methyl (9Z)-octadec-9-enoate	18:1	Oleic acid	31.17	31.74	31.01	28.38	32.06	34.98	31.96	31.96	0.80	2.55	1.944	6.15
298	Methyl octadecanoate	18:0	Stearic acid	4.89	5.00	5.24	7.66	5.50	5.78	7.19	7.19	2.30	47.03	1.096	18.58
324	Methyl (11Z)-icos-11-enoate	20:1	Gondoic acid	0.43	0.34	0.60	0.76	1.10	1.00	1.36	1.36	0.93	216.73	0.373	46.68
326	Methyl octadecanoate	20:0	Arachidic acid	0.49	0.52	0.65	0.74	0.90	0.86	1.09	1.09	0.59	120.47	0.216	28.82
354	Methyl docosanoate	22:0	Behenic acid	1.76	1.61	1.78	1.89	1.79	2.02	2.14	2.14	0.38	21.44	0.177	9.55
368	Methyl tricosanoate	23:0	Tricosylic acid	0.08	0.08	0.08	0.10	0.08	0.08	0.08	0.08	0.00	-1.71	0.008	9.48
382	Methyl tetracosanoate	24:0	Lignoceric acid	0.54	0.46	0.51	0.53	0.57	0.63	0.55	0.55	0.01	1.20	0.052	9.67
			SFAs	12.33	12.84	13.70	16.49	14.83	15.24	18.36	18.36	6.02	48.84	2.12	14.27
			MUFAs	31.74	32.24	31.77	29.34	33.36	36.18	33.56	33.56	1.82	5.72	2.10	6.45
			PUFAs	55.92	54.93	54.52	54.17	51.82	48.58	48.08	48.08	-7.84	-14.02	3.16	6.00

IX

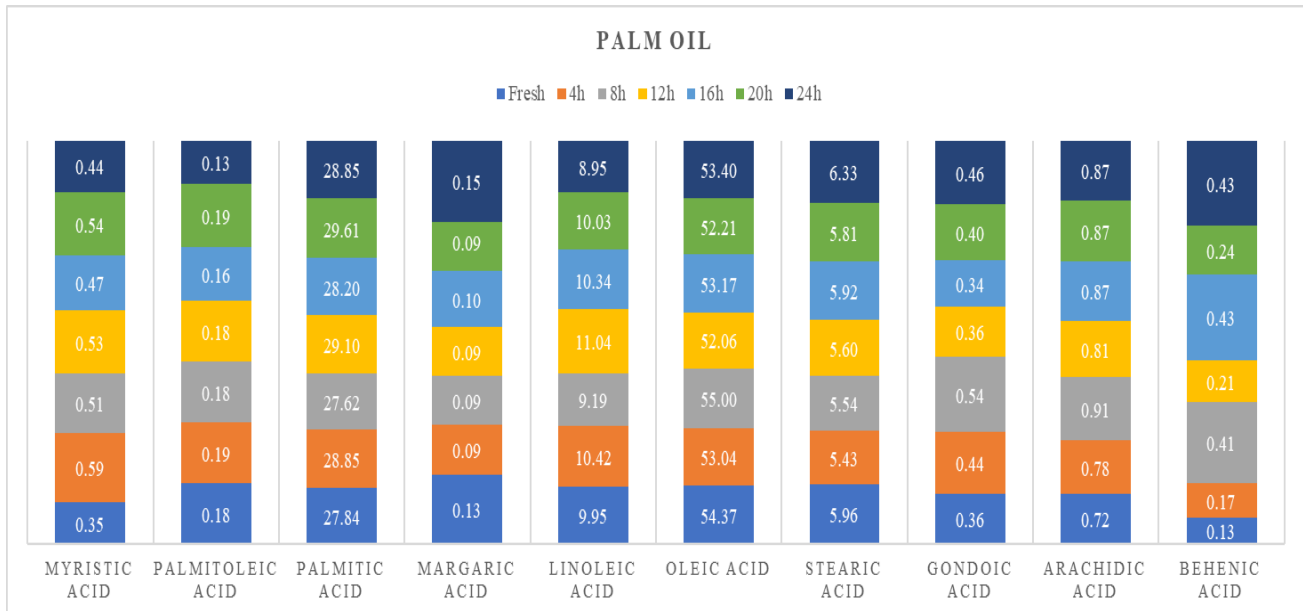


**Appendix II:** Graphically illustrate the changing in the relative representation of each FAs in all of tested oils and fat.

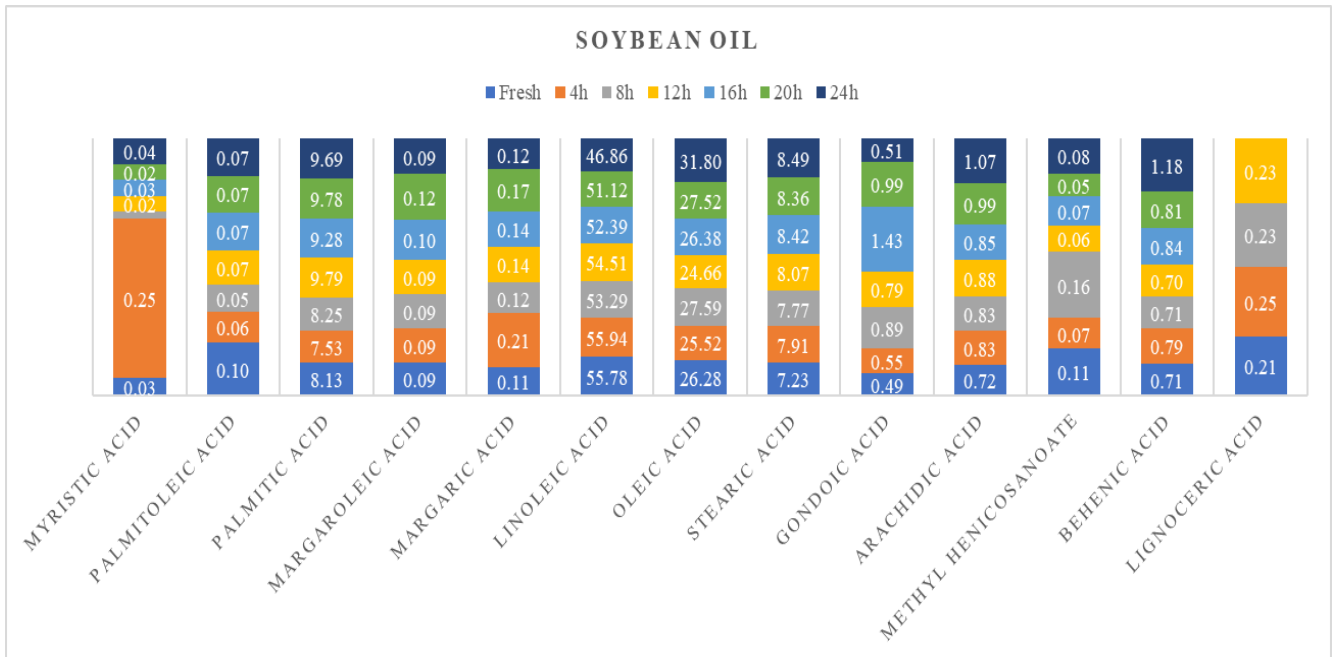
**IIa. Coconut oil**



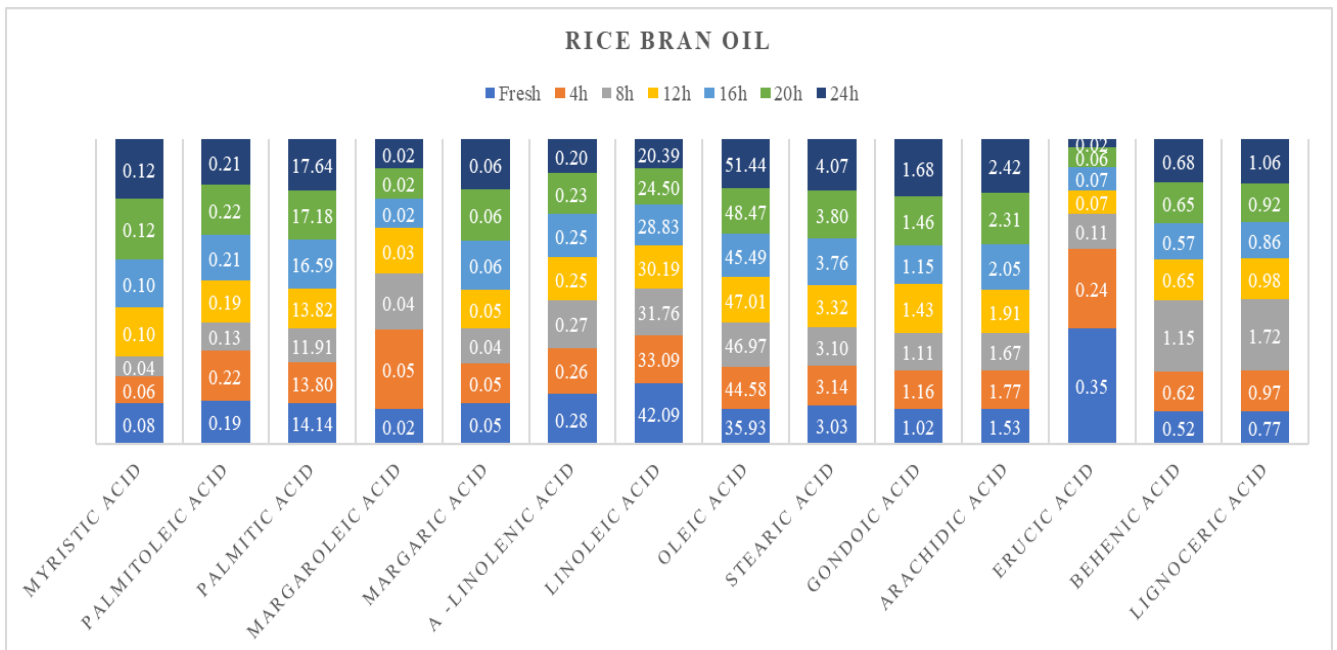
**IIb. Palm oil**



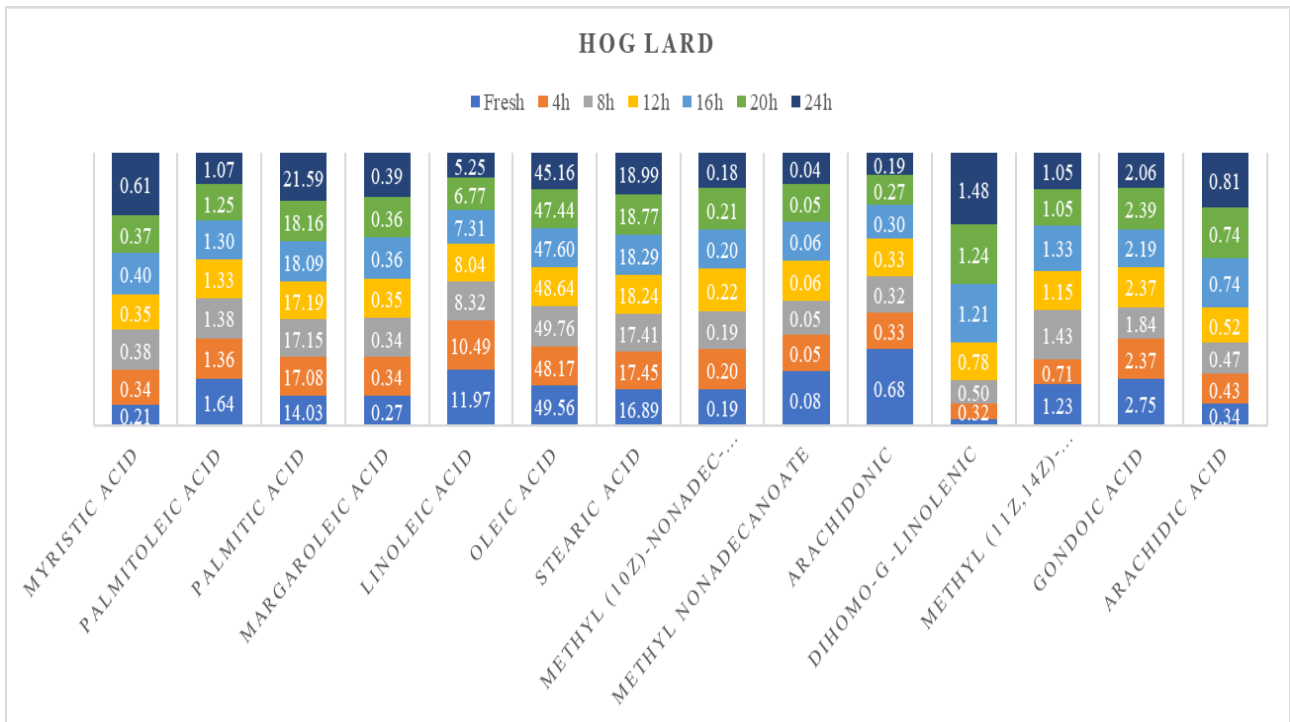
### IIIc. Soyabean oil



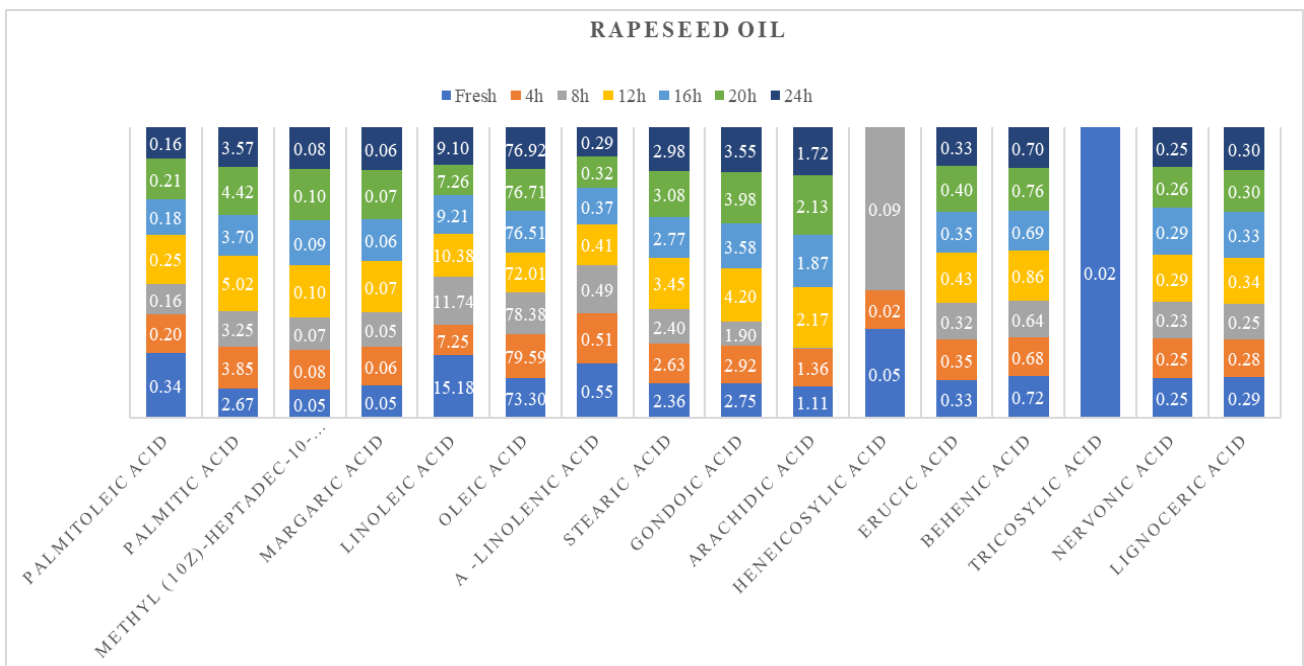
### III d. Rice bran oil



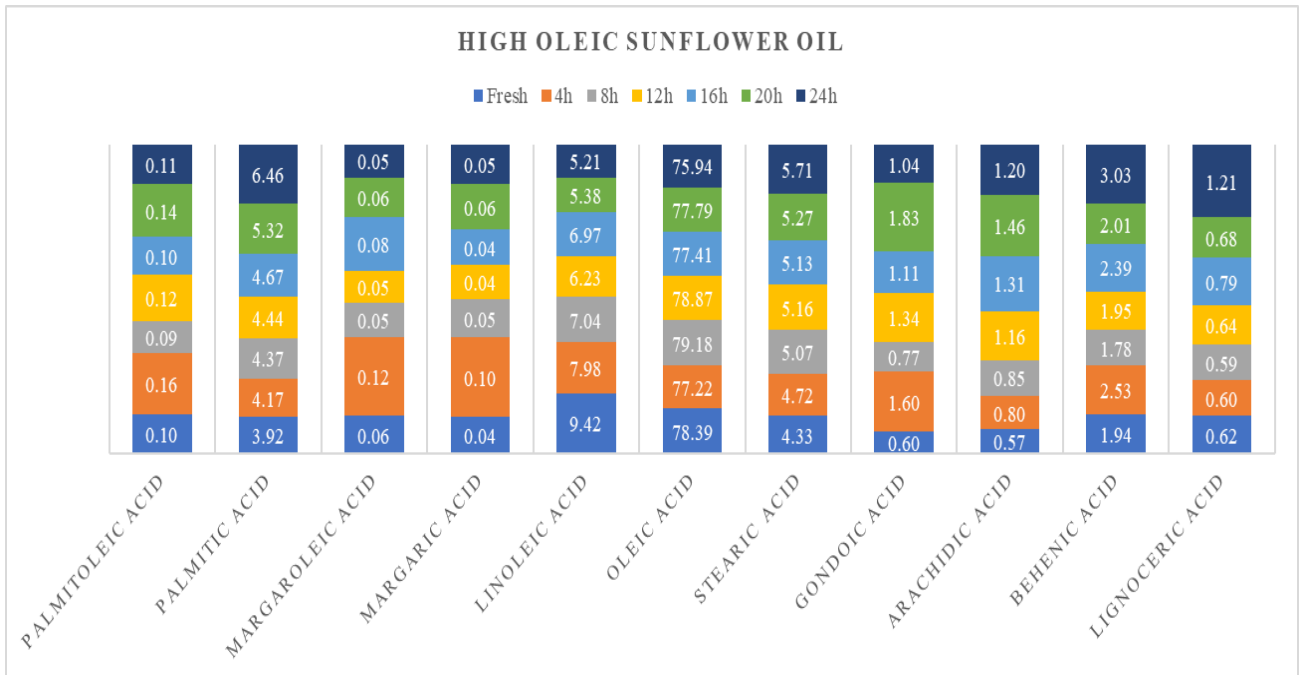
### IIe. Hog lard



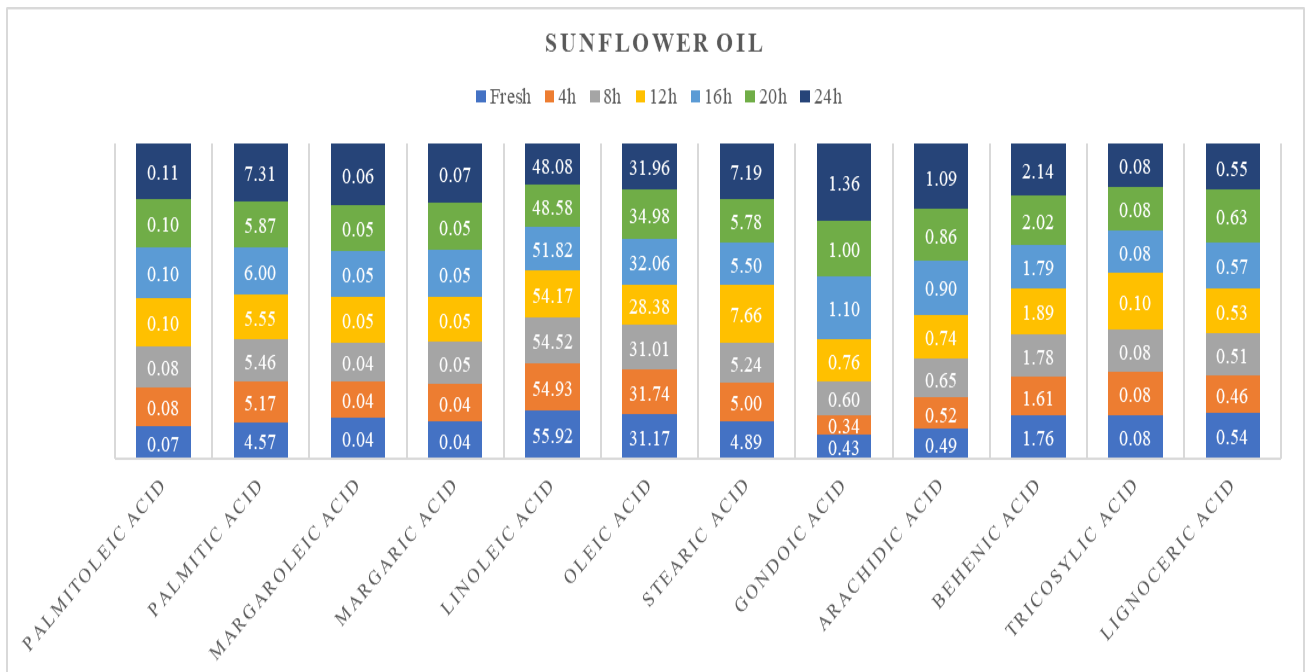
### IIIf. Rapeseed oil



**Iig.** High-oleic sunflower oil



**Iih.** Sunflower oil



### Appendix III: Correlation tables of fatty acids in all tested oils and the fat

#### IIIa. Coconut oil

Coconut oil correlation										
		Caprylic acid	Capric acid	Lauric acid	Myristic acid	Palmitic acid	Linoleic acid	Oleic acid	Stearic acid	Arachidic acid
Caprylic acid	Pearson Correlation	1	<b>.769*</b>	0.521	0.064	-0.361	-0.216	-0.142	-0.431	-0.093
	Sig. (2-tailed)		0.043	0.230	0.892	0.426	0.681	0.761	0.334	0.843
Capric acid	Pearson Correlation	<b>.769*</b>	1	<b>.905**</b>	0.461	-0.408	0.130	-0.420	<b>-.768*</b>	-0.622
	Sig. (2-tailed)	0.043		0.005	0.298	0.363	0.805	0.349	0.044	0.136
Lauric acid	Pearson Correlation	0.521	<b>.905**</b>	1	0.584	-0.298	0.250	-0.555	<b>-.791*</b>	-0.713
	Sig. (2-tailed)	0.230	0.005		0.169	0.517	0.633	0.196	0.034	0.072
Myristic acid	Pearson Correlation	0.064	0.461	0.584	1	0.502	-0.533	<b>-.944**</b>	-0.056	-0.304
	Sig. (2-tailed)	0.892	0.298	0.169		0.251	0.277	0.001	0.905	0.508
Palmitic acid	Pearson Correlation	-0.361	-0.408	-0.298	0.502	1	<b>-.919**</b>	-0.621	<b>.801*</b>	0.599
	Sig. (2-tailed)	0.426	0.363	0.517	0.251		0.010	0.137	0.031	0.155
Linoleic acid	Pearson Correlation	-0.216	0.130	0.250	-0.533	<b>-.919**</b>	1	0.628	-0.701	-0.598
	Sig. (2-tailed)	0.681	0.805	0.633	0.277	0.010		0.182	0.121	0.210
Oleic acid	Pearson Correlation	-0.142	-0.420	-0.555	<b>-.944**</b>	-0.621	0.628	1	-0.052	0.082
	Sig. (2-tailed)	0.761	0.349	0.196	0.001	0.137	0.182		0.912	0.861
Stearic acid	Pearson Correlation	-0.431	<b>-.768*</b>	<b>-.791*</b>	-0.056	<b>.801*</b>	-0.701	-0.052	1	<b>.855*</b>
	Sig. (2-tailed)	0.334	0.044	0.034	0.905	0.031	0.121	0.912		0.014
Arachidic acid	Pearson Correlation	-0.093	-0.622	-0.713	-0.304	0.599	-0.598	0.082	<b>.855*</b>	1
	Sig. (2-tailed)	0.843	0.136	0.072	0.508	0.155	0.210	0.861	0.014	

\*. Correlation is significant at the 0.05 level (2-tailed).

\*\*. Correlation is significant at the 0.01 level (2-tailed).

#### IIIb. Palm oil

Palm oil correlation											
		Myristic acid	Palmitoleic acid	Palmitic acid	Margaric acid	Linoleic acid	Oleic acid	Stearic acid	Gondoic acid	Arachidic acid	Behenic acid
Myristic acid	Pearson Correlation	1	0.389	0.521	<b>-.756*</b>	0.377	-0.494	-0.713	0.257	0.312	-0.063
	Sig. (2-tailed)		0.389	0.230	0.049	0.405	0.260	0.072	0.578	0.495	0.894
Palmitoleic acid	Pearson Correlation	0.389	1	0.053	<b>-.788*</b>	0.533	-0.096	<b>-.790*</b>	-0.102	-0.302	-0.664
	Sig. (2-tailed)	0.389		0.910	0.035	0.218	0.838	0.035	0.827	0.510	0.104
Palmitic acid	Pearson Correlation	0.521	0.053	1	-0.153	0.351	<b>-.910**</b>	0.027	-0.262	0.075	-0.241
	Sig. (2-tailed)	0.230	0.910		0.743	0.439	0.004	0.955	0.570	0.872	0.603
Margaric acid	Pearson Correlation	<b>-.756*</b>	<b>-.788*</b>	-0.153	1	-0.572	0.272	<b>.884**</b>	-0.017	-0.170	0.206
	Sig. (2-tailed)	0.049	0.035	0.743		0.180	0.555	0.008	0.971	0.716	0.657
Linoleic acid	Pearson Correlation	0.377	0.533	0.351	-0.572	1	-0.649	-0.510	-0.709	-0.452	-0.567
	Sig. (2-tailed)	0.405	0.218	0.439	0.180		0.115	0.242	0.075	0.309	0.184
Oleic acid	Pearson Correlation	-0.494	-0.096	<b>-.910**</b>	0.272	-0.649	1	0.049	0.553	0.016	0.249
	Sig. (2-tailed)	0.260	0.838	0.004	0.555	0.115		0.917	0.198	0.972	0.591
Stearic acid	Pearson Correlation	-0.713	<b>-.790*</b>	0.027	<b>.884**</b>	-0.510	0.049	1	-0.196	0.106	0.394
	Sig. (2-tailed)	0.072	0.035	0.955	0.008	0.242	0.917		0.674	0.821	0.382
Gondoic acid	Pearson Correlation	0.257	-0.102	-0.262	-0.017	-0.709	0.553	-0.196	1	0.483	0.378
	Sig. (2-tailed)	0.578	0.827	0.570	0.971	0.075	0.198	0.674		0.272	0.404
Arachidic acid	Pearson Correlation	0.312	-0.302	0.075	-0.170	-0.452	0.016	0.106	0.483	1	<b>.851*</b>
	Sig. (2-tailed)	0.495	0.510	0.872	0.716	0.309	0.972	0.821	0.272		0.015
Behenic acid	Pearson Correlation	-0.063	-0.664	-0.241	0.206	-0.567	0.249	0.394	0.378	<b>.851*</b>	1
	Sig. (2-tailed)	0.894	0.104	0.603	0.657	0.184	0.591	0.382	0.404	0.015	

\*. Correlation is significant at the 0.05 level (2-tailed).

\*\*. Correlation is significant at the 0.01 level (2-tailed).

### IIIc. Rapeseed oil

Rapeseed oil correlation																		
		Palmitoleic acid	Palmitic acid	(10Z)-heptadec-10-enoate	Margaric acid	Linoleic acid	Oleic acid	$\alpha$ -linolenic acid	Stearic acid	Gondoic acid	Arachidic acid	Heneicosylic acid	Erucic acid	Behenic acid	Tricosylic acid	Nervonic acid	Lignoceric acid	
Palmitoleic acid	Pearson Correlation	1	-0.139	-0.238	-0.091	0.626	-0.733	0.502	-0.072	0.103	0.140	-0.328	0.225	0.485	.-nd-	0.189	0.211	
	Sig. (2-tailed)		0.767	0.607	0.846	0.133	0.061	0.251	0.878	0.826	0.765	0.787	0.628	0.270	.-nd-	0.685	0.650	
Palmitic acid	Pearson Correlation	-0.139	1	<b>.949**</b>	<b>.947**</b>	-0.617	-0.222	-0.481	<b>.912**</b>	<b>.767*</b>	0.687	-0.412	<b>.923**</b>	0.743	.-nd-	0.588	0.548	
	Sig. (2-tailed)	0.767		0.001	0.001	0.140	0.632	0.275	0.004	0.044	0.088	0.730	0.003	0.056	.-nd-	0.165	0.203	
(10Z)-heptadec-10-enoate	Pearson Correlation	-0.238	<b>.949**</b>	1	<b>.973**</b>	-0.709	-0.158	-0.693	<b>.916**</b>	<b>.861*</b>	<b>.799*</b>	-0.452	<b>.859*</b>	0.658	.-nd-	0.633	0.641	
	Sig. (2-tailed)	0.607	0.001		0.000	0.074	0.735	0.084	0.004	0.013	0.031	0.702	0.013	0.108	.-nd-	0.127	0.121	
Margaric acid	Pearson Correlation	-0.091	<b>.947**</b>	<b>.973**</b>	1	-0.575	-0.329	-0.678	<b>.969**</b>	<b>.897**</b>	<b>.816*</b>	-0.585	<b>.909**</b>	<b>.787*</b>	.-nd-	0.617	0.669	
	Sig. (2-tailed)	0.846	0.001	0.000		0.176	0.471	0.094	0.000	0.006	0.025	0.602	0.005	0.036	.-nd-	0.140	0.100	
Linoleic acid	Pearson Correlation	0.626	-0.617	-0.709	-0.575	1	-0.546	0.586	-0.484	-0.467	-0.493	0.471	-0.343	-0.020	.-nd-	-0.143	-0.149	
	Sig. (2-tailed)	0.133	0.140	0.074	0.176		0.204	0.167	0.271	0.291	0.261	0.687	0.451	0.966	.-nd-	0.759	0.751	
Oleic acid	Pearson Correlation	-0.733	-0.222	-0.158	-0.329	-0.546	1	-0.024	-0.419	-0.460	-0.395	-0.072	-0.519	<b>-.795*</b>	.-nd-	-0.577	-0.638	
	Sig. (2-tailed)	0.061	0.632	0.735	0.471	0.204		0.960	0.350	0.299	0.380	0.954	0.233	0.033	.-nd-	0.175	0.123	
$\alpha$ -linolenic acid	Pearson Correlation	0.502	-0.481	-0.693	-0.678	0.586	-0.024	1	-0.673	-0.687	-0.636	-0.429	-0.328	-0.239	.-nd-	-0.312	-0.506	
	Sig. (2-tailed)	0.251	0.275	0.084	0.094	0.167	0.960		0.098	0.088	0.125	0.718	0.472	0.605	.-nd-	0.495	0.246	
Stearic acid	Pearson Correlation	-0.072	<b>.912**</b>	<b>.916**</b>	<b>.969**</b>	-0.484	-0.419	-0.673	1	<b>.899**</b>	<b>.811*</b>	-0.711	<b>.867*</b>	<b>.821*</b>	.-nd-	0.635	0.723	
	Sig. (2-tailed)	0.878	0.004	0.004	0.000	0.271	0.350	0.098		0.006	0.027	0.497	0.012	0.024	.-nd-	0.126	0.067	
Gondoic acid	Pearson Correlation	0.103	<b>.767*</b>	<b>.861*</b>	<b>.897**</b>	-0.467	-0.460	-0.687	<b>.899**</b>	1	<b>.981**</b>	-0.968	<b>.797*</b>	<b>.776*</b>	.-nd-	0.748	<b>.871*</b>	
	Sig. (2-tailed)	0.826	0.044	0.013	0.006	0.291	0.299	0.088	0.006		0.000	0.162	0.032	0.040	.-nd-	0.053	0.011	
Arachidic acid	Pearson Correlation	0.140	0.687	<b>.799*</b>	<b>.816*</b>	-0.493	-0.395	-0.636	<b>.811*</b>	<b>.981**</b>	1	-0.972	0.721	0.698	.-nd-	0.734	<b>.861*</b>	
	Sig. (2-tailed)	0.765	0.088	0.031	0.025	0.261	0.380	0.125	0.027	0.000		0.150	0.068	0.081	.-nd-	0.060	0.013	
Heneicosylic acid	Pearson Correlation	-0.328	-0.412	-0.452	-0.585	0.471	-0.072	-0.429	-0.711	-0.968	-0.972	1	-0.943	-0.543	.-nd-	-0.832	-0.688	
	Sig. (2-tailed)	0.787	0.730	0.702	0.602	0.687	0.954	0.718	0.497	0.162	0.150		0.216	0.635	.-nd-	0.375	0.517	
Erucic acid	Pearson Correlation	0.225	<b>.923**</b>	<b>.859*</b>	<b>.909**</b>	-0.343	-0.519	-0.328	<b>.867*</b>	<b>.797*</b>	0.721	-0.943	1	<b>.910**</b>	.-nd-	0.680	0.640	
	Sig. (2-tailed)	0.628	0.003	0.013	0.005	0.451	0.233	0.472	0.012	0.032	0.068	0.216		0.004	.-nd-	0.093	0.121	
Behenic acid	Pearson Correlation	0.485	0.743	0.658	<b>.787*</b>	-0.020	<b>-.795*</b>	-0.239	<b>.821*</b>	<b>.776*</b>	0.698	-0.543	<b>.910**</b>	1	.-nd-	0.661	0.705	
	Sig. (2-tailed)	0.270	0.056	0.108	0.036	0.966	0.033	0.605	0.024	0.040	0.081	0.635	0.004		.-nd-	0.106	0.077	
Tricosylic acid	Pearson Correlation	.-nd-	.-nd-	.-nd-	.-nd-	.-nd-	.-nd-	.-nd-	.-nd-	.-nd-	.-nd-	.-nd-	.-nd-	.-nd-	.-nd-	.-nd-	.-nd-	
	Sig. (2-tailed)			.-nd-	.-nd-	.-nd-	.-nd-	.-nd-	.-nd-	.-nd-	.-nd-	.-nd-	.-nd-	.-nd-		.-nd-	.-nd-	
Nervonic acid	Pearson Correlation	0.189	0.588	0.633	0.617	-0.143	-0.577	-0.312	0.635	0.748	0.734	-0.832	0.680	0.661	.-nd-	1	<b>.935**</b>	
	Sig. (2-tailed)	0.685	0.165	0.127	0.140	0.759	0.175	0.495	0.126	0.053	0.060	0.375	0.093	0.106	.-nd-		0.002	
Lignoceric acid	Pearson Correlation	0.211	0.548	0.641	0.669	-0.149	-0.638	-0.506	0.723	<b>.871*</b>	<b>.861*</b>	-0.688	0.640	0.705	.-nd-	<b>.935**</b>	1	
	Sig. (2-tailed)	0.650	0.203	0.121	0.100	0.751	0.123	0.246	0.067	0.011	0.013	0.517	0.121	0.077	.-nd-	0.002		

\*. Correlation is significant at the 0.05 level (2-tailed).  
 \*\*. Correlation is significant at the 0.01 level (2-tailed).

### III.d. Rice bran oil

Rice bran oil correlation															
		Myristic acid	Palmitoleic acid	Palmitic acid	Margarolic acid	Margaric acid	$\alpha$ -linolenic acid	Linoleic acid	Oleic acid	Stearic acid	Gondoic acid	Arachidic acid	Erucic acid	Behenic acid	Lignoceric acid
Myristic acid	Pearson Correlation	1	0.605	<b>.889**</b>	<b>-.798*</b>	<b>.842*</b>	<b>-.763*</b>	-0.599	0.364	<b>.822*</b>	0.744	<b>.812*</b>	-0.490	-0.555	-0.584
	Sig. (2-tailed)		0.150	0.007	0.032	0.018	0.046	0.155	0.422	0.023	0.055	0.027	0.264	0.196	0.169
Palmitoleic acid	Pearson Correlation	0.605	1	0.691	-0.171	0.642	-0.387	-0.230	0.018	0.443	0.325	0.472	0.046	<b>-.865*</b>	<b>-.859*</b>
	Sig. (2-tailed)	0.150		0.086	0.714	0.120	0.392	0.620	0.969	0.320	0.477	0.285	0.922	0.012	0.013
Palmitic acid	Pearson Correlation	<b>.889**</b>	0.691	1	-0.704	<b>.990**</b>	<b>-.778*</b>	-0.670	0.404	<b>.921**</b>	0.640	<b>.870*</b>	-0.463	-0.533	-0.546
	Sig. (2-tailed)	0.007	0.086		0.078	0.000	0.039	0.099	0.368	0.003	0.122	0.011	0.296	0.218	0.205
Margarolic acid	Pearson Correlation	<b>-.798*</b>	-0.171	-0.704	1	-0.703	0.453	0.337	-0.099	-0.656	-0.389	-0.532	0.367	0.306	0.355
	Sig. (2-tailed)	0.032	0.714	0.078		0.078	0.307	0.461	0.833	0.109	0.389	0.219	0.418	0.504	0.434
Margaric acid	Pearson Correlation	<b>.842*</b>	0.642	<b>.990**</b>	-0.703	1	-0.743	-0.677	0.416	<b>.922**</b>	0.582	<b>.863*</b>	-0.484	-0.461	-0.480
	Sig. (2-tailed)	0.018	0.120	0.000	0.078		0.056	0.095	0.354	0.003	0.171	0.012	0.271	0.298	0.276
$\alpha$ -linolenic acid	Pearson Correlation	<b>-.763*</b>	-0.387	<b>-.778*</b>	0.453	-0.743	1	<b>.934**</b>	<b>-.819*</b>	<b>-.909**</b>	<b>-.958**</b>	<b>-.960**</b>	<b>.764*</b>	0.112	0.101
	Sig. (2-tailed)	0.046	0.392	0.039	0.307	0.056		0.002	0.024	0.005	0.001	0.001	0.046	0.812	0.830
Linoleic acid	Pearson Correlation	-0.599	-0.230	-0.670	0.337	-0.677	<b>.934**</b>	1	<b>-.949**</b>	<b>-.883**</b>	<b>-.863*</b>	<b>-.941**</b>	<b>.904**</b>	-0.126	-0.131
	Sig. (2-tailed)	0.155	0.620	0.099	0.461	0.095	0.002		0.001	0.008	0.012	0.002	0.005	0.787	0.780
Oleic acid	Pearson Correlation	0.364	0.018	0.404	-0.099	0.416	<b>-.819*</b>	<b>-.949**</b>	1	0.697	<b>.791*</b>	<b>.793*</b>	<b>-.920**</b>	0.357	0.370
	Sig. (2-tailed)	0.422	0.969	0.368	0.833	0.354	0.024	0.001		0.082	0.034	0.033	0.003	0.431	0.414
Stearic acid	Pearson Correlation	<b>.822*</b>	0.443	<b>.921**</b>	-0.656	<b>.922**</b>	<b>-.909**</b>	<b>-.883**</b>	0.697	1	<b>.778*</b>	<b>.966**</b>	-0.753	-0.222	-0.226
	Sig. (2-tailed)	0.023	0.320	0.003	0.109	0.003	0.005	0.008	0.082		0.040	0.000	0.051	0.632	0.626
Gondoic acid	Pearson Correlation	0.744	0.325	0.640	-0.389	0.582	<b>-.958**</b>	<b>-.863*</b>	<b>.791*</b>	<b>.778*</b>	1	<b>.878**</b>	-0.724	-0.080	-0.074
	Sig. (2-tailed)	0.055	0.477	0.122	0.389	0.171	0.001	0.012	0.034	0.040		0.009	0.066	0.865	0.874
Arachidic acid	Pearson Correlation	<b>.812*</b>	0.472	<b>.870*</b>	-0.532	<b>.863*</b>	<b>-.960**</b>	<b>-.941**</b>	<b>.793*</b>	<b>.966**</b>	<b>.878**</b>	1	<b>-.786*</b>	-0.170	-0.177
	Sig. (2-tailed)	0.027	0.285	0.011	0.219	0.012	0.001	0.002	0.033	0.000	0.009		0.036	0.716	0.704
Erucic acid	Pearson Correlation	-0.490	0.046	-0.463	0.367	-0.484	<b>.764*</b>	<b>.904**</b>	<b>-.920**</b>	-0.753	-0.724	<b>-.786*</b>	1	-0.295	-0.288
	Sig. (2-tailed)	0.264	0.922	0.296	0.418	0.271	0.046	0.005	0.003	0.051	0.066	0.036		0.521	0.531
Behenic acid	Pearson Correlation	-0.555	<b>-.865*</b>	-0.533	0.306	-0.461	0.112	-0.126	0.357	-0.222	-0.080	-0.170	-0.295	1	<b>.995**</b>
	Sig. (2-tailed)	0.196	0.012	0.218	0.504	0.298	0.812	0.787	0.431	0.632	0.865	0.716	0.521		0.000
Lignoceric acid	Pearson Correlation	-0.584	<b>-.859*</b>	-0.546	0.355	-0.480	0.101	-0.131	0.370	-0.226	-0.074	-0.177	-0.288	<b>.995**</b>	1
	Sig. (2-tailed)	0.169	0.013	0.205	0.434	0.276	0.830	0.780	0.414	0.626	0.874	0.704	0.531	0.000	

\*. Correlation is significant at the 0.05 level (2-tailed).

\*\* . Correlation is significant at the 0.01 level (2-tailed).



### IIIe. Hog lard

Hog Lard correlation															
		Myristic acid	Palmitoleic acid	Palmitic acid	Margaroleic acid	Linoleic acid	Oleic acid	Stearic acid	(10Z)-nonadec-10-enoate	nonadecanoate	Arachidonic	Dihomo-g-linolenic	(11Z,14Z)-icos-11,14-dienoate	Gondoic acid	Arachidic acid
Myristic acid	Pearson Correlation	1	<b>-.934**</b>	<b>.981**</b>	<b>.905**</b>	<b>-.867*</b>	<b>-.848*</b>	<b>.770*</b>	-0.395	<b>-.834*</b>	<b>-.832*</b>	<b>.789*</b>	-0.088	-0.688	<b>.782*</b>
	Sig. (2-tailed)		0.002	0.000	0.005	0.012	0.016	0.043	0.380	0.020	0.020	0.035	0.851	0.088	0.038
Palmitoleic acid	Pearson Correlation	<b>-.934**</b>	1	<b>-.983**</b>	<b>-.978**</b>	<b>-.929**</b>	<b>.862*</b>	<b>-.909**</b>	0.068	<b>.863*</b>	<b>.939**</b>	<b>-.874*</b>	0.223	0.600	<b>-.867*</b>
	Sig. (2-tailed)	0.002		0.000	0.000	0.002	0.013	0.005	0.885	0.012	0.002	0.010	0.631	0.155	0.011
Palmitic acid	Pearson Correlation	<b>.981**</b>	<b>-.983**</b>	1	<b>.951**</b>	<b>-.910**</b>	<b>-.892**</b>	<b>.862*</b>	-0.249	<b>-.846*</b>	<b>-.890**</b>	<b>.858*</b>	-0.176	-0.627	<b>.855*</b>
	Sig. (2-tailed)	0.000	0.000		0.001	0.004	0.007	0.013	0.591	0.016	0.007	0.014	0.705	0.131	0.014
Margaroleic acid	Pearson Correlation	<b>.905**</b>	<b>-.978**</b>	<b>.951**</b>	1	<b>-.933**</b>	<b>-.776*</b>	<b>.875**</b>	-0.020	<b>-.892**</b>	<b>-.972**</b>	<b>.863*</b>	-0.115	-0.691	<b>.859*</b>
	Sig. (2-tailed)	0.005	0.000	0.001		0.002	0.040	0.010	0.965	0.007	0.000	0.012	0.807	0.085	0.013
Linoleic acid	Pearson Correlation	<b>-.867*</b>	<b>-.929**</b>	<b>-.910**</b>	<b>-.933**</b>	1	0.749	<b>-.932**</b>	0.073	0.750	<b>.864*</b>	<b>-.946**</b>	-0.117	0.634	<b>-.923**</b>
	Sig. (2-tailed)	0.012	0.002	0.004	0.002		0.053	0.002	0.876	0.052	0.012	0.001	0.803	0.127	0.003
Oleic acid	Pearson Correlation	<b>-.848*</b>	<b>.862*</b>	<b>-.892**</b>	<b>-.776*</b>	0.749	1	<b>-.840*</b>	0.290	0.559	0.664	<b>-.836*</b>	0.372	0.233	<b>-.846*</b>
	Sig. (2-tailed)	0.016	0.013	0.007	0.040	0.053		0.018	0.529	0.192	0.104	0.019	0.411	0.615	0.017
Stearic acid	Pearson Correlation	<b>.770*</b>	<b>-.909**</b>	<b>.862*</b>	<b>.875**</b>	<b>-.932**</b>	<b>-.840*</b>	1	0.094	-0.609	<b>-.796*</b>	<b>.964**</b>	-0.126	-0.336	<b>.941**</b>
	Sig. (2-tailed)	0.043	0.005	0.013	0.010	0.002	0.018		0.841	0.147	0.032	0.000	0.788	0.462	0.002
(10Z)-nonadec-10-enoate	Pearson Correlation	-0.395	0.068	-0.249	-0.020	0.073	0.290	0.094	1	0.043	-0.110	-0.085	-0.268	0.267	-0.117
	Sig. (2-tailed)	0.380	0.885	0.591	0.965	0.876	0.529	0.841			0.927	0.814	0.857	0.562	0.803
nonadecanoate	Pearson Correlation	<b>-.834*</b>	<b>.863*</b>	<b>-.846*</b>	<b>-.892**</b>	0.750	0.559	-0.609	0.043	1	<b>.949**</b>	-0.569	0.190	<b>.842*</b>	-0.578
	Sig. (2-tailed)	0.020	0.012	0.016	0.007	0.052	0.192	0.147	0.927		0.001	0.182	0.683	0.018	0.174
Arachidonic	Pearson Correlation	<b>-.832*</b>	<b>.939**</b>	<b>-.890**</b>	<b>-.972**</b>	<b>.864*</b>	0.664	<b>-.796*</b>	-0.110	<b>.949**</b>	1	-0.751	0.192	0.729	-0.753
	Sig. (2-tailed)	0.020	0.002	0.007	0.000	0.012	0.104	0.032	0.814	0.001		0.052	0.679	0.063	0.051
Dihomo-g-linolenic	Pearson Correlation	<b>.789*</b>	<b>-.874*</b>	<b>.858*</b>	<b>.863*</b>	<b>-.946**</b>	<b>-.836*</b>	<b>.964**</b>	-0.085	-0.569	-0.751	1	0.051	-0.402	<b>.991**</b>
	Sig. (2-tailed)	0.035	0.010	0.014	0.012	0.001	0.019	0.000	0.857	0.182	0.052		0.914	0.371	0.000
(11Z,14Z)-icos-11,14-dienoate	Pearson Correlation	-0.088	0.223	-0.176	-0.115	-0.117	0.372	-0.126	-0.268	0.190	0.192	0.051	1	-0.320	0.017
	Sig. (2-tailed)	0.851	0.631	0.705	0.807	0.803	0.411	0.788	0.562	0.683	0.679	0.914		0.484	0.972
Gondoic acid	Pearson Correlation	-0.688	0.600	-0.627	-0.691	0.634	0.233	-0.336	0.267	<b>.842*</b>	0.729	-0.402	-0.320	1	-0.407
	Sig. (2-tailed)	0.088	0.155	0.131	0.085	0.127	0.615	0.462	0.563	0.018	0.063	0.371	0.484		0.364
Arachidic acid	Pearson Correlation	<b>.782*</b>	<b>-.867*</b>	<b>.855*</b>	<b>.859*</b>	<b>-.923**</b>	<b>-.846*</b>	<b>.941**</b>	-0.117	-0.578	-0.753	<b>.991**</b>	0.017	-0.407	1
	Sig. (2-tailed)	0.038	0.011	0.014	0.013	0.003	0.017	0.002	0.803	0.174	0.051	0.000	0.972	0.364	

\*. Correlation is significant at the 0.05 level (2-tailed).

\*\* . Correlation is significant at the 0.01 level (2-tailed).

### III. High-oleic sunflower oil

Sunflower oil - High oleic (frying oil) correlation												
		Palmitoleic acid	Palmitic acid	Margaroleic acid	Margaric acid	Linoleic acid	Oleic acid	Stearic acid	Gondoic acid	Arachidic acid	Behenic acid	Lignoceric acid
Palmitoleic acid	Pearson Correlation	1	-0.074	0.667	0.820	-0.035	-0.263	-0.135	0.831	0.101	0.207	-0.222
	Sig. (2-tailed)		0.875	0.102	0.024	0.941	0.568	0.773	0.020	0.830	0.656	0.633
Palmitic acid	Pearson Correlation	-0.074	1	-0.326	-0.170	-0.821	-0.722	<b>.883**</b>	0.212	0.633	0.677	<b>.897**</b>
	Sig. (2-tailed)	0.875		0.475	0.715	0.024	0.067	0.008	0.648	0.127	0.095	0.006
Margaroleic acid	Pearson Correlation	0.667	-0.326	1	<b>.879**</b>	0.352	-0.304	-0.351	0.410	-0.184	0.312	-0.246
	Sig. (2-tailed)	0.102	0.475		0.009	0.438	0.507	0.439	0.361	0.693	0.496	0.595
Margaric acid	Pearson Correlation	0.820	-0.170	<b>.879**</b>	1	0.206	-0.324	-0.246	0.519	-0.245	0.337	-0.195
	Sig. (2-tailed)	0.024	0.715	0.009		0.658	0.478	0.594	0.232	0.597	0.460	0.675
Linoleic acid	Pearson Correlation	-0.035	-0.821	0.352	0.206	1	0.354	<b>-.956**</b>	-0.497	-0.864	-0.331	-0.562
	Sig. (2-tailed)	0.941	0.024	0.438	0.658		0.436	0.001	0.257	0.012	0.469	0.189
Oleic acid	Pearson Correlation	-0.263	-0.722	-0.304	-0.324	0.354	1	-0.469	-0.241	-0.318	<b>-.962**</b>	-0.818
	Sig. (2-tailed)	0.568	0.067	0.507	0.478	0.436		0.289	0.603	0.487	0.001	0.024
Stearic acid	Pearson Correlation	-0.135	<b>.883**</b>	-0.351	-0.246	<b>-.956**</b>	-0.469	1	0.292	0.779	0.490	0.728
	Sig. (2-tailed)	0.773	0.008	0.439	0.594	0.001	0.289		0.524	0.039	0.265	0.064
Gondoic acid	Pearson Correlation	0.831	0.212	0.410	0.519	-0.497	-0.241	0.292	1	0.602	0.147	-0.087
	Sig. (2-tailed)	0.020	0.648	0.361	0.232	0.257	0.603	0.524		0.152	0.753	0.854
Arachidic acid	Pearson Correlation	0.101	0.633	-0.184	-0.245	-0.864	-0.318	0.779	0.602	1	0.229	0.400
	Sig. (2-tailed)	0.830	0.127	0.693	0.597	0.012	0.487	0.039	0.152		0.621	0.374
Behenic acid	Pearson Correlation	0.207	0.677	0.312	0.337	-0.331	<b>-.962**</b>	0.490	0.147	0.229	1	0.834
	Sig. (2-tailed)	0.656	0.095	0.496	0.460	0.469	0.001	0.265	0.753	0.621		0.020
Lignoceric acid	Pearson Correlation	-0.222	<b>.897**</b>	-0.246	-0.195	-0.562	-0.818	0.728	-0.087	0.400	0.834	1
	Sig. (2-tailed)	0.633	0.006	0.595	0.675	0.189	0.024	0.064	0.854	0.374	0.020	

\*. Correlation is significant at the 0.05 level (2-tailed).

\*\* . Correlation is significant at the 0.01 level (2-tailed).

### IIIg. Soybean oil

Soybean oil correlation														
		Myristic acid	Palmitoleic acid	Palmitic acid	Margaroleic acid	Margaric acid	Linoleic acid	Oleic acid	Stearic acid	Gondoic acid	Arachidic acid	Henicosan oate	Behenic acid	Lignoceric acid
Myristic acid	Pearson Correlation	1	-0.279	-0.622	-0.253	<b>.844*</b>	0.374	-0.245	-0.086	-0.367	-0.154	-0.232	0.001	0.753
	Sig. (2-tailed)		0.545	0.136	0.585	0.017	0.408	0.596	0.855	0.419	0.742	0.616	0.999	0.247
Palmitoleic acid	Pearson Correlation	-0.279	1	0.022	0.008	-0.421	0.124	-0.034	-0.414	-0.208	-0.296	-0.111	-0.015	-0.829
	Sig. (2-tailed)	0.545		0.962	0.987	0.347	0.791	0.942	0.355	0.655	0.519	0.812	0.975	0.171
Palmitic acid	Pearson Correlation	-0.622	0.022	1	0.552	-0.298	-0.663	0.319	0.715	0.362	0.719	-0.517	0.394	-0.251
	Sig. (2-tailed)	0.136	0.962		0.199	0.516	0.104	0.485	0.071	0.425	0.068	0.235	0.381	0.749
Margaroleic acid	Pearson Correlation	-0.253	0.008	0.552	1	0.211	-0.387	0.115	0.614	0.639	0.469	-0.535	0.131	0.759
	Sig. (2-tailed)	0.585	0.987	0.199		0.650	0.391	0.806	0.143	0.122	0.289	0.216	0.779	0.241
Margaric acid	Pearson Correlation	<b>.844*</b>	-0.421	-0.298	0.211	1	0.306	-0.369	0.193	-0.017	0.038	-0.503	-0.130	0.872
	Sig. (2-tailed)	0.017	0.347	0.516	0.650		0.505	0.416	0.678	0.972	0.936	0.250	0.782	0.128
Linoleic acid	Pearson Correlation	0.374	0.124	-0.663	-0.387	0.306	1	<b>-.902**</b>	-0.740	-0.078	<b>-.902**</b>	0.156	<b>-.878**</b>	-0.021
	Sig. (2-tailed)	0.408	0.791	0.104	0.391	0.505		0.005	0.057	0.867	0.005	0.739	0.009	0.979
Oleic acid	Pearson Correlation	-0.245	-0.034	0.319	0.115	-0.369	<b>-.902**</b>	1	0.427	-0.222	0.722	0.153	<b>.880**</b>	-0.187
	Sig. (2-tailed)	0.596	0.942	0.485	0.806	0.416	0.005		0.339	0.632	0.067	0.744	0.009	0.813
Stearic acid	Pearson Correlation	-0.086	-0.414	0.715	0.614	0.193	-0.740	0.427	1	0.469	<b>.843*</b>	-0.587	0.626	0.773
	Sig. (2-tailed)	0.855	0.355	0.071	0.143	0.678	0.057	0.339		0.289	0.017	0.166	0.133	0.227
Gondoic acid	Pearson Correlation	-0.367	-0.208	0.362	0.639	-0.017	-0.078	-0.222	0.469	1	0.022	-0.186	-0.195	0.194
	Sig. (2-tailed)	0.419	0.655	0.425	0.122	0.972	0.867	0.632	0.289		0.963	0.690	0.675	0.806
Arachidic acid	Pearson Correlation	-0.154	-0.296	0.719	0.469	0.038	<b>-.902**</b>	0.722	<b>.843*</b>	0.022	1	-0.429	<b>.790*</b>	0.676
	Sig. (2-tailed)	0.742	0.519	0.068	0.289	0.936	0.005	0.067	0.017	0.963		0.337	0.035	0.324
Henicosan oate	Pearson Correlation	-0.232	-0.111	-0.517	-0.535	-0.503	0.156	0.153	-0.587	-0.186	-0.429	1	-0.235	-0.304
	Sig. (2-tailed)	0.616	0.812	0.235	0.216	0.250	0.739	0.744	0.166	0.690	0.337		0.613	0.696
Behenic acid	Pearson Correlation	0.001	-0.015	0.394	0.131	-0.130	<b>-.878**</b>	<b>.880**</b>	0.626	-0.195	<b>.790*</b>	-0.235	1	0.725
	Sig. (2-tailed)	0.999	0.975	0.381	0.779	0.782	0.009	0.009	0.133	0.675	0.035	0.613		0.275
Lignoceric acid	Pearson Correlation	0.753	-0.829	-0.251	0.759	0.872	-0.021	-0.187	0.773	0.194	0.676	-0.304	0.725	1
	Sig. (2-tailed)	0.247	0.171	0.749	0.241	0.128	0.979	0.813	0.227	0.806	0.324	0.696	0.275	

\*. Correlation is significant at the 0.05 level (2-tailed).

\*\* . Correlation is significant at the 0.01 level (2-tailed).

### IIIh. Sunflower oil

Sunflower oil correlation													
		Palmitoleic acid	Palmitic acid	Margaroleic acid	Margaric acid	Linoleic acid	Oleic acid	Stearic acid	Gondoic acid	Arachidic acid	Behenic acid	Tricosylic acid	Lignoceric acid
Palmitoleic acid	Pearson Correlation	1	<b>.894**</b>	<b>.789*</b>	<b>.892**</b>	<b>-.809*</b>	0.134	0.730	<b>.916**</b>	<b>.948**</b>	0.728	0.189	0.458
	Sig. (2-tailed)		0.007	0.035	0.007	0.028	0.774	0.062	0.004	0.001	0.064	0.685	0.302
Palmitic acid	Pearson Correlation	<b>.894**</b>	1	<b>.883**</b>	<b>.985**</b>	<b>-.860*</b>	0.234	0.618	<b>.914**</b>	<b>.950**</b>	<b>.788*</b>	-0.099	0.324
	Sig. (2-tailed)	0.007		0.008	0.000	0.013	0.614	0.139	0.004	0.001	0.035	0.832	0.478
Margaroleic acid	Pearson Correlation	<b>.789*</b>	<b>.883**</b>	1	<b>.940**</b>	<b>-.759*</b>	0.056	0.745	<b>.870*</b>	<b>.873*</b>	<b>.864*</b>	0.111	0.404
	Sig. (2-tailed)	0.035	0.008		0.002	0.048	0.905	0.055	0.011	0.010	0.012	0.812	0.369
Margaric acid	Pearson Correlation	<b>.892**</b>	<b>.985**</b>	<b>.940**</b>	1	<b>-.857*</b>	0.180	0.689	<b>.939**</b>	<b>.961**</b>	<b>.862*</b>	-0.009	0.403
	Sig. (2-tailed)	0.007	0.000	0.002		0.014	0.699	0.087	0.002	0.001	0.013	0.986	0.369
Linoleic acid	Pearson Correlation	<b>-.809*</b>	<b>-.860*</b>	<b>-.759*</b>	<b>-.857*</b>	1	-0.622	-0.432	<b>-.897**</b>	<b>-.904**</b>	<b>-.853*</b>	0.223	-0.681
	Sig. (2-tailed)	0.028	0.013	0.048	0.014		0.136	0.333	0.006	0.005	0.015	0.631	0.092
Oleic acid	Pearson Correlation	0.134	0.234	0.056	0.180	-0.622	1	-0.398	0.317	0.291	0.273	-0.724	0.565
	Sig. (2-tailed)	0.774	0.614	0.905	0.699	0.136		0.377	0.489	0.526	0.554	0.066	0.186
Stearic acid	Pearson Correlation	0.730	0.618	0.745	0.689	-0.432	-0.398	1	0.583	0.624	0.691	0.708	0.210
	Sig. (2-tailed)	0.062	0.139	0.055	0.087	0.333	0.377		0.170	0.134	0.086	0.075	0.652
Gondoic acid	Pearson Correlation	<b>.916**</b>	<b>.914**</b>	<b>.870*</b>	<b>.939**</b>	<b>-.897**</b>	0.317	0.583	1	<b>.992**</b>	<b>.841*</b>	-0.049	0.635
	Sig. (2-tailed)	0.004	0.004	0.011	0.002	0.006	0.489	0.170		0.000	0.018	0.918	0.125
Arachidic acid	Pearson Correlation	<b>.948**</b>	<b>.950**</b>	<b>.873*</b>	<b>.961**</b>	<b>-.904**</b>	0.291	0.624	<b>.992**</b>	1	<b>.837*</b>	-0.024	0.567
	Sig. (2-tailed)	0.001	0.001	0.010	0.001	0.005	0.526	0.134	0.000		0.019	0.959	0.185
Behenic acid	Pearson Correlation	0.728	<b>.788*</b>	<b>.864*</b>	<b>.862*</b>	<b>-.853*</b>	0.273	0.691	<b>.841*</b>	<b>.837*</b>	1	0.126	0.676
	Sig. (2-tailed)	0.064	0.035	0.012	0.013	0.015	0.554	0.086	0.018	0.019		0.787	0.095
Tricosylic acid	Pearson Correlation	0.189	-0.099	0.111	-0.009	0.223	-0.724	0.708	-0.049	-0.024	0.126	1	-0.017
	Sig. (2-tailed)	0.685	0.832	0.812	0.986	0.631	0.066	0.075	0.918	0.959	0.787		0.972
Lignoceric acid	Pearson Correlation	0.458	0.324	0.404	0.403	-0.681	0.565	0.210	0.635	0.567	0.676	-0.017	1
	Sig. (2-tailed)	0.302	0.478	0.369	0.369	0.092	0.186	0.652	0.125	0.185	0.095	0.972	

\*. Correlation is significant at the 0.05 level (2-tailed).

\*\* Correlation is significant at the 0.01 level (2-tailed).

## Appendix IV.

### IVa. Composition of standard FAME mix 37

Methyl butyrate	400 µg/mL
Methyl hexanoate	400 µg/mL
Methyl octanoate	400 µg/mL
Methyl decanoate	400 µg/mL
Methyl undecanoate	200 µg/mL
Methyl laurate	400 µg/mL
Methyl tridecanoate	200 µg/mL
Methyl myristate	400 µg/mL
Methyl myristoleate	200 µg/mL
Methyl pentadecanoate	200 µg/mL
Methyl <i>cis</i> -10-pentadecenoate	200 µg/mL
Methyl palmitate	600 µg/mL
Methyl palmitoleate	200 µg/mL
Methyl heptadecanoate	200 µg/mL
<i>cis</i> -10-Heptadecanoic acid methyl ester	200 µg/mL
Methyl stearate	400 µg/mL
<i>trans</i> -9-Elaidic acid methyl ester	200 µg/mL
<i>cis</i> -9-Oleic acid methyl ester	400 µg/mL
Methyl linolelaidate	200 µg/mL
Methyl linoleate	200 µg/mL
Methyl arachidate	400 µg/mL
Methyl $\gamma$ -linolenate	200 µg/mL
Methyl <i>cis</i> -11-eicosenoate	≤ 200 µg/mL
Methyl linolenate	200 µg/mL
Methyl heneicosanoate	200 µg/mL
<i>cis</i> -11,14-Eicosadienoic acid methyl ester	200 µg/mL
Methyl behenate	400 µg/mL
<i>cis</i> -8,11,14-Eicosatrienoic acid methyl ester	200 µg/mL
Methyl erucate	200 µg/mL
<i>cis</i> -11,14,17-Eicosatrienoic acid methyl ester	200 µg/mL
<i>cis</i> -5,8,11,14-Eicosatetraenoic acid methyl ester	200 µg/mL
Methyl tricosanoate	200 µg/mL
<i>cis</i> -13,16-Docosadienoic acid methyl ester	200 µg/mL
Methyl lignocerate	400 µg/mL
<i>cis</i> -5,8,11,14,17-Eicosapentaenoic acid methyl ester	200 µg/mL
Methyl nervonate	200 µg/mL
<i>cis</i> -4,7,10,13,16,19-Docosahexaenoic acid methyl ester	200 µg/mL

(Source: Sigma-Aldrich 2019)