

ADDIS ABABA UNIVERSITY  
COLLEGE OF HEALTH SCIENCE  
SCHOOL OF MEDICINE  
DEPARTMENT OF BIOCHEMISTRY



**TITLE: ASSESSMENT AND EVALUATION OF FATTY ACID CONTENT  
AND TYPE IN VARIOUS TYPES OF IMPORTED OILS IN ETHIOPIA,  
USING GAS CHROMATOGRAPHY**

A thesis submitted to the school of graduate studies of Addis Ababa University in partial fulfillment of the requirements for the degree of Master of Science in Medical Biochemistry

**BY: ZEWDU GEDEFAW FENTAHUN**

February, 2019

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COLLEGE OF HEALTH SCIENCE  
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# Declaration Sheet

This is to certify that this master's thesis entitled: Assessment and Evaluation of Fatty acid Content and type in various types of imported oils in Ethiopia, using Gas Chromatography and Submitted to the department of Biochemistry for partial fulfillment of the requirements for the degree of Masters of science in Medical Biochemistry complies with the regulation of university and meet acceptance standards with respect to originality and quality.

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## **Abbreviations and acronyms**

<b>CVD:</b>	Cardio vascular disease
<b>CHD:</b>	Coronary heart disease
<b>DHA:</b>	Docosahexanoic acid
<b>EPA:</b>	Eicosanopentoic acid
<b>FAME:</b>	Fatty acid methyl ester
<b>FAO:</b>	Food and agricultural organization
<b>GC:</b>	Gas chromatography
<b>GC-MS:</b>	Gas chromatography coupled with mass spectroscopy
<b>HDL-c:</b>	High density lipoprotein cholesterol
<b>HPLC:</b>	High performance liquid chromatography
<b>ISO:</b>	International organization for standardization
<b>KOH:</b>	Potassium hydroxide
<b>LCPUFA:</b>	Long chain poly unsaturated fatty
<b>LDL-c:</b>	Low density lipoprotein cholesterol
<b>MUFA:</b>	Mono unsaturated fatty acid
<b>NF-<math>\kappa</math>B:</b>	Nuclear factor –kappa B
<b>NIST:</b>	National institute of standards and technology
<b>PUFA:</b>	Poly unsaturated fatty acid
<b>RT:</b>	Retention time
<b>TAG:</b>	Triacylglycerol
<b>TC:</b>	Total cholesterol
<b>TLR 4:</b>	Toll- like receptor 4
<b>USA:</b>	United States of America
<b>VLDL-c:</b>	Very low density lipoprotein cholesterol
<b>WHO:</b>	World health organization

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## **Definition of some terms**

Codex standard – collection of internationally recognized standards, codes of practice, guidelines, and other recommendations relating to foods, food production, and food safety.

P/S index- is the ratio of poly unsaturated fatty acids to saturated fatty acids. It indicates the relationship between saturated and polyunsaturated fatty acids content.

GC-MS - analytical technique that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample. GC can separate volatile and semi-volatile compounds with great resolution, but it cannot identify them. MS can provide detailed structural information on most compounds such that they can be exactly identified, but it cannot readily separate them.

Transesterification - the general term used to describe the important class of organic reactions where an ester is transformed into another through interchange of the alkoxy moiety.

## **Abstract**

Edible oil is a vital component of our diet. It is composed of different types of fatty acids. The type and proportion of fatty acid in oil determines the nutritional value, physical and chemical properties of edible oil and is also implicated in its quality and applicability for consumption.

The main objective of this study was to assess and evaluate the fatty acid content and their type in imported edible oils widely consumed in Ethiopia. Five edible oils were analyzed for their fatty acid composition by Gas chromatography coupled with mass spectrometric detector (GC-MS). Fatty acid profile was determined after fatty acids were transformed to their corresponding fatty acid methyl esters (FAMES). The result of this study showed that Chief palm oil, Hayat palm oil and Mona palm oil contain the highest percentage of total saturated fatty acids, 69.97%, 53.53%, and 50.11% respectively. Palmitic acid and stearic acid were the predominant saturated fatty acids in these oils. Among the evaluated oils, the highest content of unsaturated fatty acids were determined in sunflower oil (84.41%), and soyabean oil (79.55%) with the predominant presence of linoleic acid and oleic acid, compared to total unsaturated fatty acid in Mona palm oil (48.89%), Hayat palm oil (46.29%) and Chief palm oil (28.53%). The fatty acid composition of soyabean oil contains a healthy mixture or proportion of all the types of saturated and unsaturated fatty acids. In addition, it contains balanced proportion of omega-6 to omega-3 fatty acid and P/S index value greater than one. Therefore, soyabean oil presented the healthiest fatty acid profile followed by sunflower oil and Mona palm oil, while Chief palm oil presented the least healthy fatty acid profile.

Key words: edible oil, saturated fatty acid, monounsaturated fatty acid, poly unsaturated fatty acid, cardiovascular disease, Gas chromatography.

# 1. INTRODUCTION

## 1.1 Background

Lipids are a family of biochemical molecules that are stored in plants and animals. Lipids include fats, oils, cholesterol, waxes and others. Fats are lipids which are solid at room temperature, where as oils are liquid at room temperature. Fat and oil provide our bodies with energy. Although we can get energy from other nutrients in our diet, we need some fat and oil as they provide essential fatty acids that our bodies cannot make. Fats and oils are also carrier of the fat-soluble vitamins and involved in their absorption.

Vegetable oils are naturally occurring esters, mainly composed of triacylglycerols (TAG), which are about (95 - 98%) and variety of minor components (Aluyor *et al.*, 2009). Triglycerides are formed when three molecules or units of fatty acids are covalently attached to single glycerol back bone. Fatty acids constituting triglycerides are classified as saturated and unsaturated. Saturated fatty acids contain single bond between carbon atoms; whereas unsaturated fatty acids contain one or more double bond between carbon atoms. The latter is further classified as monounsaturated (MUFA) and poly unsaturated (PUFA)(Chowdhury *et al.*,2007). Among unsaturated fatty acids linolenic and linoleic acid are essential, which are not synthesized endogenously and must be supplied in the diet. Unsaturated fatty acids exist as cis or trans configuration in which the former naturally exists but the latter is formed as a result of partial hydrogenation during processing. Partially hydrogenated vegetable oils become solid at room temperature (Strayer *et al.*, 2006; Zambiasi *et al.*, 2007; Orsavova *et al.*, 2015)

The type and proportion of fatty acid in oil determines the nutritional value, physical and chemical properties of edible oil and is also implicated in its quality and applicability for consumption. Thus it is essential to know fatty acid content and type of oils to identify their properties and to determine the possible adulteration, as well as to know the stability (Zambizi *et al.*, 2007)

Evidences from studies revealed that characterizing the fatty acid composition in edible oils and setting recommended daily intake will reduce the risk factors of cardiovascular diseases (CVD). This is because fatty acid content of edible oil influences blood lipid profile of consumers. Edible oils rich in unsaturated fatty acids are recommended currently because they increase

concentration of high density lipoprotein cholesterol (HDL-c)- is the moving cholesterol in HDL particles and decrease low density lipoprotein cholesterol (LDL-c)- is a fat that circulates in the blood, moving cholesterol around the body to where it is needed for cell repair and depositing it inside of artery walls. Oils rich in saturated fatty acids are stable chemically but reported to increase the concentration of LDL-c and TC. High levels of HDL-c have preventive effect against the incidence of CHD through stimulating endothelial nitric oxide, preventing inflammation and promoting antiatherogenic and antioxidant effect (Ahn and Kim, 2016; Ali *et al.*, 2012). High level of LDL-c triggers the development of atherosclerosis through reducing bioavailability of nitric oxide, promoting endothelial dysfunction and enhancing the expression of adhesion molecules (Badimon and Vilahur, 2012) which can trigger cardiovascular complications (FERENCE *et al.*, 2017).

Even though, local production of edible oil in Ethiopia is rising it is insufficient compared to existing homeland consumers need associated with the increase in urbanization and modernization. The main produced oil is Niger seed oil. But, production hardly covers the demand, thus the country remains highly dependent on imports to supply its market (unpublished result). The fatty acid profile of edible oil is determined after sample derivatization to fatty acid methyl ester (which is more volatile than the original fatty acids) using gas chromatography.

## **1.2 Statement of the problem**

The consumption of edible oil in Ethiopia is rising exponentially according to the food and agricultural organization (FAO), and Ethiopia Food Balance Sheet (2007). The country mainly imports large amount of tropical oil from abroad that contains high level of fatty acids which are atherogenic and associated with the risk of CVD.

In Ethiopia: Ethiopian food, medicine and health control authority has not yet made any attempt to regulate the analytical content of fatty acids in edible imported oils. Consumption of imported vegetable oil is continuing without knowing its composition and suitability to frying at high temperatures. However now a days, many consumers started questioning and complaining the use of imported vegetable oils. This project was conceived to assess and evaluate the fatty acid profile of imported edible oils largely consumed in Ethiopia.

### **1.3 Significance of the study**

This study is focused on evaluation of fatty acid composition of imported oils which are highly consumed by peoples of Ethiopia, with low and medium income. It helps to identify the constituents of oils in order to reduce the risk of developing CVD. This study is also expected to give some insight in changes brought about in the serum lipid profile as a consequence of consumption of imported oils in the Ethiopian population. Furthermore, it creates public awareness about the potential health benefits and risks of specific types of fatty acids and may provide a good input for policy makers and researchers to protect the community from taking unacceptable oil items.

## **1.4 OBJECTIVE**

### **1.4.1 General objective**

The general objective of this study is to assess and evaluate the fatty acid content and type in various imported oils largely consumed in Ethiopia.

### **1.4.2 Specific objective**

- ✓ To characterize fatty acid profile of imported oils using GC.
- ✓ To determine relative concentration or percentage of fatty acids in imported oils.
- ✓ To determine total saturated and unsaturated fatty acids in edible imported oils.
- ✓ To categorize suitable varieties of imported oils rich in essential fatty acids.

## 2. LITERATURE REVIEW

### 2.1 Oil

Oils are composed of diverse group of molecules that are insoluble in water but, soluble in non polar solvents. They are liquid at room temperature and less dense than water. This time, oils are the principal dietary component. Soybean, canola, palm, cottonseed, olive, coconut, peanut, sunflower, corn, palm kernel, safflower and blended oil are mostly used for food preparation around the world (Strayer *et al.*, 2006).

Oils are mainly composed of triacylglycerols - a tri ester molecule which is composed of three units of fatty acids and one molecule of glycerol alcohol. The fatty acids composing this molecule may be identical (commonly called simple triglyceride) or different (commonly called complex triglyceride). In addition to TAG oils are also composed of minor components like mono and diacylglycerols, free fatty acids, phosphatides, sterols, tocopherols, carotenoids, and fat soluble vitamins (Strayer *et al.*, 2006; Johnson *et al.*, 2009).

In the 1960s, Ancel Keys revealed that high intake of lipid is a risk factor for the development of atherosclerosis and CVD. Based on this study many countries developed dietary guidelines that reduce fat consumption. In contrast to this conception, Puskas *et al.* (2004) reported that the type of fat may be more important than the quantity of fat in reducing CVD risk. Several published sources documented the negative effect of trans and saturated fatty acids available in oils in the risk of developing atherosclerosis and CVD. On the other hand, essential fatty acid in oils play significant role in reducing the risk of various diseases like CVD, cancer, osteoporosis, and diabetes. Therefore, proper dietary intake giving special attention to what type of fatty acid composes fats and oils could be therapeutic option in prevention and treatment of many diseases (Orsavova *et al.*, 2015).

### 2.2 Fatty acid

Fatty acids are carboxylic acids, in which 4 -36 carbon atoms are bonded to each other with one carboxylic functional group. Fatty acids are the main constituent of triglycerides obtained when triglyceride molecule is hydrolyzed. The type and content of the component fatty acids and their distribution through the glycerol molecule influence the properties of oils. Even though, small amounts of branched and cyclic fatty acids are found in oils, saturated and unsaturated fatty acids

with an even number of carbon atoms and single carboxyl group predominates in oil (Strayer *et al.*, 2006).

## **2.2.1 Classification of fatty acid**

According to the degree of saturation, fatty acids in oil are classified as saturated and unsaturated. In addition, fatty acids can be classified in terms of number of carbon atoms (as short chain, medium chain, long chain, and very long chain) and spatial arrangement of hydrogen atoms around the double bond (as cis and trans configuration)(Strayer *et al.*, 2006).

### ***2.2.1.1 Saturated fatty acid***

Saturated fatty acids are fatty acids with no carbon-carbon double bonds but contain carbon to carbon single bond-which makes them more stable chemically. Saturated fatty acids are dominantly found in solid fats and some oils (Johnson *et al.*, 2009). The common saturated fatty acids found in edible oils are listed in Table 1 below.

Large set of evidence is documented on the effect of high intake of saturated fatty acids on blood lipid profile. Saturated fatty acids present in vegetable oils, are associated to the incidence of heart disease by increasing TC and TAG plasmatic levels. previous studies revealed that high intake of saturated fatty acid heighten the level of LDL-c, which are very atherogenic, partly by reducing receptor mediated uptake (Mensink and Katan, 1992; Denke and Grundy, 1992). In addition, a recent study revealed that TC is elevated in coconut oil fed rats, which mainly contains saturated fatty acids (Elsayed *et al.*, 2015), and in line with this, a recent rat model study reported that serum LDL-c to HDL-c is higher in rats fed hydrogenated vegetable fat and butter than those fed margarine with low content of PUFA, high PUFA and soybean oil, which may be explained by high content of saturated fatty acid, as well as dietary cholesterol and trans fatty acid (Angelis-Pereira *et al.*, 2017). Another consistent study done by Mekonnen *et al.* (2018) revealed that the high content of saturated fatty acids in imported palm oil is responsible for the significant increase in TC, HDL-c, LDL-c and TAG in rats fed imported palm oil. Moreover, recently Hruby and Hu, (2016) reported that replacing 5% of energy from saturated fatty acids with equal amount of PUFA reduces risk of CVD by 25% and of MUFA reduces by 15%.

Saturated fatty acids have direct or indirect atherogenic effect. The indirect atherogenic role of saturated fatty acid is through elevating serum concentration of LDL-c (Hruby and Hu, 2016).

On the other hand, saturated fatty acid possesses direct atherogenic role through activating the inflammatory process. This is mediated by Toll-like receptor 4 (TLR-4)/ nuclear factor kappa B (NF- $\kappa$ B) pathway, in which saturated fatty acids activate TLR 4; this in turn activates NF- $\kappa$ B (Poledne, 2013). Moreover, saturated fatty acid consumption increases the risk of developing inflammation associated diseases like metabolic syndrome than MUFAs. Van Dijk *et al.* (2009) concluded that consumption of saturated fatty acid diet resulted in a proinflammatory “obesity-linked” gene expression profile, whereas, consumption of a MUFA diet caused a more antiinflammatory profile. Therefore, recommended daily intake of saturated fatty acids may alleviate or reduce its health effect.

Table 1: Common saturated fatty acids found in edible oils (source: Johnson *et al.*, 2009).

Carbon chain	Chemical name	Common name	Melting point ( $^{\circ}$ c)
C <sub>10:0</sub>	Decanoic acid	Capric acid	31.6
C <sub>12:0</sub>	Dodecanoic acid	Lauric acid	44.2
C <sub>14:0</sub>	Tetradecanoic acid	Myristic acid	54.4
C <sub>16:0</sub>	Hexadecanoic acid	Palmitic acid	62.9
C <sub>17:0</sub>	Heptadecanoic acid	Margaric acid	60.9
C <sub>18:0</sub>	Octadecanoic acid	Stearic acid	69.6
C <sub>20:0</sub>	Eicosanoic acid	Arachidic acid	75.4
C <sub>22:0</sub>	Docosanoic acid	Behenic acid	80

### ***2.2.1.2 Unsaturated fatty acid***

Unsaturated fatty acids are carboxylic acids containing at least one carbon– carbon double bond; monounsaturated fatty acids contain one double bond, whereas polyunsaturated fatty acids contain more than one double bond. The position of the double bond relative to the omega end determines whether a PUFA is an n-3 (omega 3) or an n-6 (omega 6) fatty acid. Unsaturated fatty acids are more reactive than saturated fatty acids and can be altered by hydrogenation and halogenation. This is accounted by the presence of one or more double bonds. Unsaturated fatty acids are dominantly found in vegetable oils (Laposata, 1995). Unsaturated fatty acids commonly found in edible oils are listed in Table 2 below.

Unsaturated fatty acid prevents from the risk of developing CVD. It augments the level of HDL-c but, reduce total cholesterol and LDL-c (which is commonly referred as bad cholesterol)(Lopes *et al.*, 2016). Earlier study indicates that humans taking PUFA rich diet show a significant decrease in LDL-c and TC compared to those taking palmitic acid and trans fatty acid (Pedersen *et al.*, 2005). Another study reported that, normal rats fed oils rich in PUFA show a significant increase in HDL-c and HDL-c to TC ratio than control group (Mohamed *et al.*, 2011). In line with this, rats fed locally manufactured highly unsaturated Niger seed oil show decrease in TC, LDL-c and TAG compared to those fed imported palm oil (Mekonnen *et al.*, 2018).

Another recent comparative study indicates rats treated with high cholesterol and 12 % olive oil diet experienced a decrease in the serum LDL-c, VLDL-c and TC levels compared to those fed with high cholesterol and sunflower oil diet, this is because MUFA diet had high absorption velocity in the acinar zone 1 of the liver compared to the PUFA diet (Duavy *et al.*, 2017). On the other hand, the reduction of serum TAG by consumption of higher MUFA diet compared to saturated fatty acids may be associated to the increased secretion of chylomicrons by oleic acid (Dinicolantonio and Keefe, 2018).

The consumption of diets enriched in unsaturated Fatty acid as diet therapy was highly pronounced in the last three decades against increased plasma TC and LDL-c levels, and hence substitution of saturated fatty acid with equivalent amount of unsaturated fatty acid could be used as nutritional alternative to prevent lipid disturbances and risk of developing CVD. A recent systematic review and meta analysis study by Hannon *et al.* (2017) revealed that replacing saturated fatty acid with unsaturated fatty acid in metabolically healthy adults with overweight and obesity was marginally effective in improving lipid profile, particularly significant reduction in serum total cholesterol. Another recent systematic review and regression analysis by Mensink (2016) revealed that replacement of saturated fatty acids with cis MUFA or PUFA (for each 1% dietary energy) resulted significant reduction in total cholesterol, LDL-c, TAG, and TC to HDL-c ratio. In addition, a recent review by Wang and Hu, (2017) reported that isocaloric substitution of saturated fatty acids by unsaturated fatty acids, especially PUFAs bring significant reduction in the incidence of CVD risk. According to Dinicolantonio and Keefe, (2018) consumption of MUFA rich meal than high saturated fatty acids result many health benefits like increased secretion of less atherogenic chylomicrons, faster clearance of postprandial lipids, and greater

reduction of fasting TAG level. Furthermore, substitution of saturated fatty acid rich diet with MUFA-rich diet can prevent and reduce the risk of developing inflammation related disease like metabolic syndrome. This is because the former induces expression of proinflammatory genes, where as the latter resulted more antiinflammatory gene expression profile (van Dijk *et al.*, 2009)

Table 2: Common unsaturated fatty acids found in oils (source: Johnson *et al.*, 2009).

Carbon chain	Chemical name	Common name	Melting point( <sup>0</sup> c)
C <sub>16:1</sub>	9-hexadecenoic acid	Palmitoleic acid	-
C <sub>18:1</sub>	9-octadecenoic acid	Oleic acid	16.3
C <sub>18:2</sub>	9,12-octadecadienoic acid	Linoleic acid	-6.5
C <sub>18:3</sub>	9,12,15-octadecatrienoic acid	Linolenic acid	-12.8

### 2.2.1.3 Essential fatty acid

Our body has the capability of synthesizing fatty acids from carbohydrate and other sources. But essential fatty acids are provided in the diet only, because human beings lack the enzyme ( $\Delta$ 12- and  $\Delta$ 15-desaturases) responsible for manufacturing these fatty acids. However, the enzyme is found in plants and marine algae, and hence availability of essential fatty acids for human being depends from these dietary intakes. Essential fatty acids are poly unsaturated fatty acid (PUFA), which includes linolenic (is also called as Omega-3) and linoleic (is also called as Omega-6)(Syadati *et al.*, 2013 ).

Essential fatty acids play various biological roles. This includes, as structural component of the biomembranes (including cell membrane, endoplasmic reticulum, and mitochondrial membrane), source of energy, and precursor of eicosanoids (which are proinflammatory and anti-inflammatory). In addition, they exhibit wondered hypocholesterolemic effect, probably by blocking and reducing the activity of  $\beta$ -hydroxy-methylglutaryl coenzyme A reductase (Angelis-Pereira *et al.*, 2017). For instance, recent study reported that supplementation of omega-3 fatty acids to hypercholesterolemic rats result a significant decrease in TC and LDL-c, but increases HDL-c (Khan and Makki, 2017). Study by Mohamed *et al.* (2011) reported supplementation of 8 % flaxseed ( n-3, 51-55%) and evening primrose oil ( n-6, 65-80 %) to normal rats resulted

significant increase in HDL-c and reduction in LDL-c than control. Dietary PUFAs lower serum lipids by increasing both peroxisomal and mitochondrial  $\beta$ -oxidation of fatty acid in the liver.

The essential fatty acids, linolenic and linoleic are metabolized to long chain poly unsaturated fatty acids (LCPUFA) by the action of the enzyme delta-5 and delta-6 desaturase. Linolenic acid is converted to eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) whereas; linoleic acid is converted to arachidonic acid. EPA and DHA are precursor for eicosanoids which are anti-inflammatory but arachidonic acid is precursor for eicosanoids which are proinflammatory (Jump, 2002; Das, 2006). Linolenic and linoleic acids are catalyzed by the same enzyme (Regulska-Ilow et al., 2013) to their corresponding LCPUFA, which later result group of eicosanoids that have opposite effect (Gómez-Candela *et al.*, 2011).

The n-6/n-3 ratio is considered to be the key factor for balanced synthesis of eicosanoids and its nutritional importance. Adequate and balanced intake of omega- 6 and omega-3 fatty acids bring many health benefits. These include prevention and treatment of heart disease, diabetes, arthritis, inflammatory diseases, cancer, and improve or prevent some psychiatric and neurodegenerative diseases (Gómez-Candela *et al.*, 2011; Orsavova *et al.*, 2015). According to Simopoulos an ideal intake ratio of Omega-6 to Omega-3 fatty acids is the range of 5-10:1(Simpoulos, 2002)

### **2.3 Recommendation on daily fat consumption**

Several set of evidences link high consumption of fat with the risks of developing CVD. Different countries and health organizations set recommended daily intake of fatty acids to prevent this disease. According to the American heart association the daily energy obtained from fat consumption must be less than 30 % of total calories. The recommended dietary intake of fat; allowed by WHO and FAO are listed in Table 3. For an oil to be considered as healthy, it must contain balanced and recommended proportion of different types of fatty acids (Johnson et al., 2009).

Table 3: Recommended dietary allowance of fat in % from total calories (source: Johnson *et al.*, 2009; WHO, 2003)

Country/ organization	Total energy intake from fat	Total PUFA	Omega-6	Omega-3	Omega-6 /omega-3
WHO(2003)	15-30	6-10	5-8	1-2	5-10
FAO( 1994)	15-35	-	4-10	-	5-10

PUFA: poly unsaturated fatty acid

## 2.4 Types of imported oils mainly consumed in Ethiopia

### 2.4.1 Palm oil

Palm oil is a vegetable oil produced from fruit of tropical palm tree which is mainly cultivated in south East Asia particularly in Indonesia and Malaysia. In the unrefined form palm oil is reddish brown in color, and it has a semisolid consistency at ambient temperature. Malaysia and Indonesia produce large quantity of this oil for global export (Sambanthamurthi *et al.*, 2000). Ethiopia is one of the countries that imports huge amount of metric tons of palm oil from these countries per year. For instance in the year 2014 Ethiopia imported 340,000metric tons of palm oil.

Like other fats and oils, palm oil is mainly composed of triglycerides; however, phosphatides, sterols, pigments, tocopherols, tocotrienes, monoacyl and diacylglycerol and others are the minor constituents. According to published literatures, Palm oil consists of approximately equal amount of saturated and unsaturated fatty acids. Palmitic acid (44%) and stearic acid (5%) are the major saturated fatty acids, where as oleic and linoleic are the major unsaturated fatty acids (Sambanthamurthi *et al.*, 2000; Alias *et al.* 2002; Dauqan *et al.*, 2011)

Even though, large set of data was documented regarding the effect of palm oil consumption on serum lipid profile of consumers internationally. Yet, there is no study conducted on the effect of consumption of imported palm oil on serum lipid profile of Ethiopian consumers. But , rat model study done by Mekonnen *et al.* (2018) revealed that rats fed imported palm oil showed significant increase in total cholesterol, HDL-c, LDL-c and TAG compared with those fed

soyabean oil and Niger seed oil. Consumption of palm oil enriched diets resulted higher total cholesterol (Sambanthamurthi *et al.*, 2000). Another study revealed that oils rich in saturated fatty acid like palm oil cause an increase in TC and decrease in HDL-c level (Ibegbulem and Chikezie, 2012). In addition, similar study suggested that diet enriched with palm oil content (high saturated fatty acids) elevate serum LDL-c and TC compared to MUFA diet (Montoya *et al.*, 2002). Study conducted on Ivorian youth indicates the level of TAG and LDL-c increase in palm oil consumers than controls (Alexandre *et al.*, 2017). Furthermore, another study suggested that significant increase in the level of cholesterol, HDL-c and LDL-c was observed in palm oil fed rats (Karaji-Bani *et al.*, 2006).

However, contrary results are reported by recent studies. For instance, supplementation of palm oil doesn't affect serum lipid profile of rats (unpublished result). Another recent study conducted in Chinese population revealed that palm oil consumption has no effect in blood lipid profile of these populations (Sun *et al.*, 2017). The reported beneficial effect of palm oil consumption may be associated with hypothesis of sn- positioning. In palm oil, oleic acid is placed at sn-2 position and preferentially absorbed but, palmitic acid is placed at sn-1 and sn-3. The latter is partly excreted and hence its effect may be compromised (Tholstrup *et al.*, 2011).

#### **2.4.2 Soybean oil**

Soybean oil is one of the vegetable oil extracted from soybean mostly in USA, Brazil, and European Union and its consumption as edible oil is increased globally in the previous decade. Besides as oil source, soy bean is used as food for animal and human, and raw material in industrial food processing (Bormann *et al.*, 2008; Uhegbu *et al.*, 2013).

Like other oils, soybean oil is mainly composed of triglyceride and some minor components. It contains largely poly unsaturated fatty acid as its component. Even though a little bit difference is detected in the proportion of fatty acid in the oil produced from different countries. According to data from published literature, soy bean oil contains 15 % saturated fatty acid (out of it 70% is palmitic acid), 24 % monounsaturated and 61 % polyunsaturated fatty acids (53.2 % is linoleic acid and linolenic acid accounts 7.8 %) (Lecerf and Borgies, 2002; Uhegbu *et al.*, 2013).

Soybean oil has hypocholesterolemic effect since it decreases LDL-c but increase HDL-c. This is accounted by high ratio of PUFA to saturated fatty acids (Lecerf and Borgies 2002). Another

study revealed that supplementation of soybean oil to rats result significant decrease in LDL-c, TAG, TC, and significant increase in HDL-c compared to the control (Uhegbu *et al.*, 2013).

Consumption of soybean oil prevents from the risk of developing cardiovascular diseases. In addition, the essential fatty acids omega-6 and omega-3 in soy bean oil are metabolized to LCPUFA - precursors for synthesis of eicosanoids. Soybean oil contains balanced amounts of omega-6 and omega-3 fatty acids (Lecerf and Borgies, 2002; Uhegbu *et al.*, 2013), this is important to maintain the desired balance in different categories of eicosanods which have opposite function. Maintaining this balance also has potential to reduce the risk of atherosclerosis and heart attack (Gómez-Candela *et al.*, 2011). Soybean oil is composed of high amount of unsaturated fatty acids. However, it is highly vulnerable to oxidative deterioration and exhibit poor stability. Currently, unsaturated fatty acids are partially hydrogenated in soybean oil in order to increase its stability, which may produce trans fatty acids.

### **2.4.3 Sunflower oil**

Sunflower oil is widely consumed edible oil produced from sunflower seed. It is highly manufactured in Russia, Ukraine and Argentina. Sunflower oil is predominantly triglycerides. However, sterol, vitamin E (tocopherol), squalene and other components are found as minor constituent (Basak *et al.*, 2017; Saedi *et al.*, 2017). Sunflower oil is unsaturated oil in which the proportion of fatty acids varies in different families of the oil. For instance, mid oleic sunflower oil contains 43.1-71.8 % oleic acid followed by 18.7-45.3% linoleic acid, and small amounts of saturated fatty acids (Warner *et al.*, 2001).

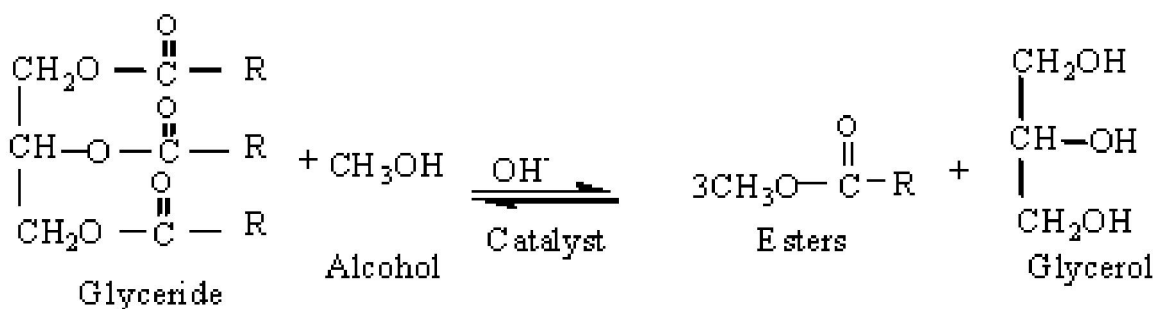
Sunflower oil contains mainly unsaturated fatty acids(dominantly oleic and linoleic acid), this makes it susceptible to oxidative deterioration which, is implicated in development of diseases like CVD and cancer. However, sunflower oil contains vitamin E (tocopherol) as minor component, which has antioxidant activity. It scavenges free radicals and reactive oxygen species that cause cellular damage and hence vitamin E in sunflower oil plays role in reducing the risk of developing atherosclerosis and CVD (Cuesta *et al.*, 2001; Kowalski, 2007).

Many studies revealed that unsaturated fatty acids in sunflower oil increase HDL-c but, decrease LDL-c and total TAG. Recent study conducted on mice revealed supplementation of sunflower oil significantly increases HDL-c and decreases total triglyceride compared to control (Basak *et*

*al.*, 2017). Another study revealed that sunflower oil supplementation for patients with dyslipidemia, results significant decrease in TC, TAG, and increase HDL-c from the base line after six month (Saedi *et al.*, 2017). Like soybean oil, the hypocholesterolemic effect of sunflower oil is associated with the dominant essential fatty acids.

## 2.5 Transesterification of oils

Transesterification is the reaction of a triglyceride (fat/oil) with an alcohol to form esters and glycerol. It involves intermolecular interchange of the alkoxy moiety in methanol with alkoxy moiety in triglyceride (Schuchardt *et al.*, 1998). Even though, various methods of transesterification was reported in literature, (Christie, 1993) categorized into acid catalyzed, base catalyzed, and diazomethane catalyzed, which are commonly employed to prepare FAMES. Each of these methods have their own advantages and limitations. One step acid catalyzed transesterification widely uses sulfuric acid, boron trifluoride and hydrochloric acid with methanol. It esterifies free fatty acids but, it affects the compositions of fatty acids. Base catalyzed transesterification produces FAMES by utilizing potassium and sodium hydroxide with methanol. Even though, this method fails to derivatize free fatty acids, it is recommended if the oil is refined (since the refining process removes free fatty acids) and free fatty acid content is, 5 % mass fraction. This method is advantageous over acid catalyzed transesterification because, the reaction is rapid, mild and don't affect fatty acid composition (ISO, 2011; Wang *et al.*, 2015). However, (Wang *et al.*, 2015) revealed that two step transmethylation (sequential base – acid catalyzed transesterification) effectively improved the synthesis yield of FAMES in Silkworm pupae oil, conserved the agents and eliminated the usage of potential harmful reagents.



Scheme 1. General equation for a transesterification reaction of oil (source: Schuchardt *et al.*, 1998)

## **2.6 Determination of fatty acids**

Fatty acids in oils and fats are analyzed by the popular analytical chromatographic techniques: gas chromatography (GC), high performance liquid chromatography (HPLC) and gas chromatography coupled with mass spectroscopy (GC-MS). HPLC is appropriate for the analysis of short chain fatty acids and fatty acids with isomers (Sodamade *et al.*, 2013) whereas GC is used for the analysis of straight chain fatty acids with single carboxyl functional group. In all cases fatty acids are analyzed after they are transformed to FAME (which is more volatile than the former) by acid or base catalyzed transesterification method (Aluyor *et al.*, 2009).

## **3. MATERIALS AND METHODS**

### **3.1 Study design**

Laboratory based experimental study was conducted on palm oil, soybean oil and sunflower oil samples.

### **3.2 Study area**

The experiments were conducted in the laboratory of Ethiopian food, medicine and health control authority and in the laboratory of chemistry department (4 kilo campus) of Addis Ababa University

### **3.3 Chemicals and standards**

Dichloromethane (99.8%, sigma-Aldrich, USA) HPLC grade, anhydrous methanol (99.8%), anhydrous sodium sulfate (Fluka, Buchs, Switherland), and potassium hydroxide were used for analysis.

### **3.4 Sample collection**

Edible oil samples used for this study were three different brands of Palm oil (Hayat, Mona and Chief), Soya bean oil and sunflower oil as shown in Table 4. A total of five samples of edible vegetable oils imported from abroad were collected. Sample collection doesn't include locally produced edible oils because their share in the total edible oil consumption is very low compared to imported oils. According to report, for example in the year 2011, of the total domestic oil supply 65% is imported from abroad, mainly palm, soyabean and sunflower (Wijnands *et al.*, 2011). According to USDA Foreign agricultural service report (2016) 80 % of the total oil domestic supply is fulfilled from imported edible oil. Of this imported oil, more than 90 percent by volume was palm oil, most of which comes from Indonesia and Malaysia. So, imported edible oil is widely consumed by most of our population than locally produced oils.

Soya bean and sunflower (one L each) were purchased from Shoa super market found in Addis Ababa and three liter Hayat, Mona and Cheif Palm oil were purchased from co-operative shop

and these oil samples were packed in polyethylene bottle and stored in a Refrigerator. The five samples were methylated and analyzed separately.

Table 4: List of imported oil samples widely consumed in Ethiopia

Name of oil / brand name	Oil source	Country of origin
Mona	Palm	Indonesia
Hayat	Palm	Singapore
Chief	Palm	Malaysia
Sunflower oil	Sunflower	India
Soy bean oil	Soy bean	Egypt

### **3.5 Derivatization of fatty acids or preparation of fatty acid methyl esters (FAME)**

Fatty acid methyl esters were prepared by the transesterification method. Transesterification of oil samples were carried out in methanol using potassium hydroxide as a base and derivatization of fatty acid was performed according to the published procedure of international standardization for organizations (ISO 12966 /2011) with little modification.

Firstly, 1 g of market edible oils were weighed and transferred to round bottom flask. Each sample was warmed for 10 minutes at 50°C using water bath. Then 6.0 ml of 2% potassium hydroxide (KOH) methanolic base reagent (prepared by dissolving 2 g of KOH in 100 mL of methanol) was added to each sample and was placed in a heating bath and heated at 50°C for 60 min. The round bottom flasks were fitted with condensers and shaken continuously during heating process to facilitate the reaction. After heating was completed, the round bottom flasks were allowed to cool. Saturated sodium chloride solution (2 mL) was added to each mixture and transferred in to separatory funnel followed by adding 30 ml of hexane. The mixture was shaken vigorously and allowed to settle to form layers. The upper organic layer was separated from the aqueous layer by running out the bottom layer. The organic layer was dried over anhydrous sodium sulfate to remove trace amount of water and filtered. Each filtrate was concentrated on rotary evaporator leaving fatty acid methyl esters, and then weighed, labeled and stored in refrigerator until analyzed by GC-MS.

### **3.6 Analysis of fatty acid methyl esters**

FAMEs of each oil sample were injected to GC coupled with a mass spectrometer (Agilent technologies, 7890A GC-MS USA) and Agilent automatic detector. The chromatographic conditions were set with one  $\mu\text{L}$  of injection volume (splitless) and injector temperature was programmed at  $275^{\circ}\text{C}$  separation was achieved on HP-5MS 5% phenyl methyl silox capillary column (30 m x 250  $\mu\text{M}$ , film thickness 0.25 $\mu\text{m}$ ). An oven temperature condition was programmed as  $60^{\circ}\text{C}$  for initial and held for 2 min until temperature reached  $280^{\circ}\text{C}$ . The separation program was rate of  $10^{\circ}\text{C}/\text{min}$  until it reached  $200^{\circ}\text{C}$  and at rate of  $3^{\circ}\text{C}/\text{min}$  until it reached  $240^{\circ}\text{C}$  with zero hold time. Helium was used as carrier gas with a total flow rate of 52.665 mL/min and a pressure of 8 psi. Conditions of mass spectrometer were a source temperature of  $230^{\circ}\text{C}$ , scan range 40-650 m/z, and operated in positive electron impact mode with ionization energy of 70 electro volts. Chromatographic and mass spectral data were processed using the software (MS Chemstation: Agilent technologies, USA) and identification was achieved using chromatograph library.

## **4. RESULTS**

### **4.1 Identification of fatty acid profile of oil samples by GC-MS**

The fatty acid profile was identified by comparing the retention time (RT) and mass spectrometric fragmentation pattern corresponding to the various peaks in the sample total ion chromatogram with mass spectral database library called national institute of standards and technology (NIST). The GC-MS chromatogram of the fatty acid methyl esters of the evaluated oil samples are listed in Annex- I, from Fig 1-Fig 5 and mass spectrum of different fatty acid methyl esters are listed in Annex-II from Fig 1- Fig 6.

### **4.2 The content of saturated fatty acids in oil samples**

The type and quantity of saturated fatty acids found in the oil samples are summarized in Table 5. Four types of saturated fatty acids were found. Palmitic acid was the abundant saturated fatty acid in all oil samples which ranged from 7.62% to 63.85 %. The highest concentration was found in Chief palm oil and the lowest in sunflower oil. Except in sunflower oil, stearic acid was the second concentrated saturated fatty acid which ranged from 6.12 % in Chief palm oil to 7.52% in soyabean oil. Trace amount of myristic acid was detected in all palm oil samples, but not in Chief palm oil. In addition, o-veratramide, benzene methanol (benzyl alcohol) and 2-pentanamine were found in Chief and Hayat palm oil in trace amount respectively. The concentration of branched chain margaric acid found in sunflower oil was (6.72 %) but, trace amount of unusual 2-pentanamine and diphenyl ephrine were also found in sunflower oil. Yet, the toxicity of these un- important compounds were not reported.

Table 5: Saturated fatty acid composition of various types of imported oils (%)

Type of oil	Saturated fatty acids (%)				Predominant fatty acid
	Myristic acid (C <sub>14:0</sub> )	Palmitic acid (C <sub>16:0</sub> )	Margaric acid (C <sub>17:0</sub> )	Stearic acid (C <sub>18:0</sub> )	
Chief palm	-	63.85	-	6.12	Palmitic acid
Hayat palm	0.89	46.04	-	6.6	Palmitic acid
Mona palm	0.87	42.17	-	6.47	Palmitic acid
Sunflower	-	7.62	6.72	-	Palmitic acid
Soyabean	-	12.92	-	7.52	Palmitic acid

- denotes not detected

### 4.3 The content of unsaturated fatty acids in oil samples

The type and quantity of unsaturated fatty acids found in the oil samples are summarized in Table 6. Three types of unsaturated fatty acids of C<sub>18</sub> family were found. Oleic acid was found to be the only MUFA which ranged from 18.77% to 46.29%. The highest was found in Hayat palm oil and lowest in soyabean oil. Except, in Hayat and Chief palm oil, linoleic acid was the most concentrated poly unsaturated fatty acid in all oil samples, ranged from 9.73% to 57.86%. The highest was presented in sunflower oil, while the lowest in Mona palm oil. Linolenic acid, the other essential fatty acid, was only found in soya bean oil (5.5%).

Table 6: Unsaturated fatty acid composition of various types of imported oils (%)

Unsaturated fatty acids (%)	Type of oils				
	Chief/palm	Hayat/palm	Mona/palm	Sunflower	Soyabean
Oleic acid (C <sub>18:1</sub> )	28.52	46.29	40.16	26.55	18.77
linoleic acid (C <sub>18:2</sub> )	-	-	9.73	57.86	55.28
Linolenic acid (C <sub>18:3</sub> )	-	-	-	-	5.50

#### 4.4 Poly unsaturated to saturated fatty acid and omega-6 to omega-3 ratio of oil samples

Poly unsaturated to saturated fatty acid ratio or P/S index of oil samples ranged from 0.49:1 to 4.04:1 as shown in Table 7 below. Sunflower oil presented the highest P/S index, followed by soyabean oil, where as the lowest was found in Mona palm oil. Omega-6 to omega-3 ratio was determined only for soyabean oil (10.05:1).

Table 7: The content of total saturated fatty acid, MUFA, PUFA (%), the values of P/S indexes and omega-6 to omega-3 ratio in various types of imported oils

Type of oil	Total saturated fatty acid (%)	MUFA (%)	PUFA (%)	Total unsaturated fatty acid %	P/S index	n-6/n-3 ratio
Chief/palm	69.97	28.53	-	28.53	Ud	Ud
Hayat/palm	53.53	46.29	-	46.9	Ud	Ud
Mona/palm	50.11	40.16	9.73	49.89	0.19:1	Ud
Sunflower	14.34	26.55	57.86	84.41	4.04:1	Ud
Soybean	20.44	18.77	60.78	79.55	2.97:1	10.05:1

MUFA- mono unsaturated fatty acid, PUFA- poly unsaturated fatty acid, P/S index- poly unsaturated to saturated fatty acid ratio, n-6/n-3 – omega 6 to omega 3 ratio, - denotes not detected, Ud- undetermined

## 5. DISCUSSION

This study was performed to evaluate the fatty acid composition of edible oils. The type and content of fatty acids ingested in the diet can have preventive or detrimental effect on our health. This is because dietary fatty acid composition influences serum and hepatic lipid profile, hemostatic function, oxidative stress, and inflammation, and hence knowing the content of fatty acids in our diet may be running one step in prevention and management of non communicable diseases (Thijssen and Mensink, 2005).

The results from this study showed that the distribution of fatty acids in the triglyceride molecule in the evaluated oils follow the general rule. The major fatty acids present in the vegetable oil or fat showed un branched and even number of carbon atoms, except, the presence of branched chain heptadecanoic acid in sunflower oil (Daniewski *et al.*, 2003).

The level of total saturated fatty acid ranged from 14.34% for sunflower oil to 69.97% for chief palm oil, with the predominant presence of palmitic acid and stearic acid. Except sunflower and soyabean oil- where total saturated fatty acid accounted below 1/4<sup>th</sup> of total fatty acid (saturated and unsaturated fatty acids), the other presented total saturated fatty acid content more than half of the total fatty acid content. Especially, total saturated fatty acid in Chief palm oil accounted more than 2/3 of the total fatty acid. The high saturated fatty acid content of Chief palm oil (69.97%) was in discordance with the reports for palm oil (Chowdhury *et al.*, 2007; Ajayi and Malachi, 2015) and also the level detected is higher than the appropriate range for total saturated fatty acid specified in the codex standard for named vegetable oils (codex standard, amended 2003, 2005). However, the total saturated fatty acid in Hayat palm oil (53.53%) and Mona palm oil (50.11 %) was close to the results reported in other researchers (Chowdhury *et al.*, 2007; Sengpracha *et al.*, 2012; Zambiazzi *et al.*, 2007). The highest saturated fatty acid content of palm oils make them semi solid at room temperature and suitable for deep frying process and applicable for margarine and bakery (Sadoudi and Ahmed, 2017).

Since 1960s, it has been believed that high consumption of fat increases the incidence of CVD. It is noteworthy that, not the quantity but the type of fatty acid matters, because different fatty acids have divergent effect on the risks of CVD. Consumption of high content of saturated fatty acids

in the diet is linked to the risk of developing CVD and metabolic syndrome (van Dijk *et al.*, 2009). In addition, study by Diniz *et al.* (2004) on rat model, revealed that saturated fatty acid rich-diet increases metabolic risk factors of CVD than PUFA rich-diet. However, systematic review and meta-analysis by Chowdhury *et al.* (2014) concluded that their findings do not support high consumption of total saturated fatty acids as a cardiovascular risk. Another meta-analysis of prospective epidemiologic study by Siri-Tarino *et al.* (2010) showed that there is no significant evidence for concluding that dietary saturated fat is associated with an increased risk of CHD or CVD. In contrary to these studies, various studies revealed that replacement of dietary saturated fatty acids with cis MUFA and PUFA reduce the incidence of CHD or CVD (Mensink, 2016; Hannon *et al.*, 2017; Wang and Hu, 2017; Dinicolantonio and Keefe. 2018; van Dijk *et al.*, 2009). These findings may indicate that isocaloric substitution of saturated fatty acid by unsaturated fatty acid is recommended as dietary guideline for prevention of CVD. In line with this, the Ethiopian ministry of health also recommends replacement of saturated fatty acid by unsaturated fatty acid (Minstry of Health, 2016).

In this study, Chief palm oil presented a conspicuously high palmitic acid concentration (63.85%), much higher than the reports for palm oil (Sengpracha *et al.*, 2012; Johnson *et al.*, 2009). Study conducted in Brazil on palm oil indicated that the palmitic acid content was 36.9 % (Bora *et al.*, 2003). The higher value of palmitic acid in Chief palm oil compared to previous studies may be explained by the refining process, genetic variability, and other environmental conditions (Sengpracha *et al.*, 2012). However, the level of palmitic acid in Hayat palm oil (46.04 %) and Mona palm oil (42.77%) was slightly similar to the report presented by Zambiasi *et al.* (2007). In addition, its level in sunflower (7.62%) and soyabean oil (12.92%) was very close to the result reported by Chowdhury *et al.* (2007). Although, palmitic acid has been found to compose 25-30 % of human adipose tissue fat (Kingsbury *et al.*, 1964), consumption of palmitic acid rich diet is negatively associated to coronary heart disease through increasing LDL-c and TC (WHO and FAO, 2003). In consistent to this, rat model study by Mekonnen *et al.* (2018) indicated that the high content of palmitic acid in imported palm oil is responsible for the significant increase in TC, LDL-c and TAG compared to the highly unsaturated soyabean and Niger seed oil. In addition, various studies indicated high content of palmitic acid in palm oil is responsible for the hyperlipidemic effects of palm oil consumption (Sambanthamurthi *et al.*, 2000; Montoya *et al.*, 2002; Karaji-Bani *et al.*, 2006; Alexandre *et al.*, 2017; Mekonnen *et al.*,

2018). On the other hand, study by Børsheim *et al.* (2006) concluded that consumption of high palmitic acid diet appears to lower the rate of oxygen consumption during and after exercise than high oleic acid diet.

The most intense saturated fatty acid following palmitic acid in all the oil samples, except sunflower oil was stearic acid. This is in line with the distribution of saturated fatty acid in nature (Gunstone *et al.*, 2007). Epidemiologic and clinical studies showed that consumption of stearic acid was found to be positively associated with reduction of CVD risk in comparison with other saturated fatty acids, through lowering LDL-c and TC to HDL-c ratio (Hunter *et al.*, 2010). In addition a meta-analysis of 60 controlled trials by Mensink *et al.* (2003) revealed that stearic acid consumption reduces TC to HDL-c ratio than palmitic acid. Furthermore, an isotope labeling study in humans by Emken (1994) revealed that the fraction of dietary stearic acid that oxidatively desaturates to oleic acid is 2.4 times higher than the fraction of palmitic acid analogously converted to palmitoleic acid. The above studies indicate that stearic acid is healthier than other saturated fatty acids. The highest stearic acid was found in soyabean oil, and the lowest in Chief palm oil. The level of stearic acid was slightly higher than the results presented by other studies (Chowdhury *et al.*, 2007; Zambiazzi *et al.*, 2007). On the other hand, stearic acid was not detected in sunflower oil in this study, but was reported in the results of other studies (Zambiazzi *et al.*, 2007; Chowdhury *et al.*, 2007)

The other saturated fatty acid that was present but in trace amount in Hayat and Chief palm oil was myristic acid. This is in line with the result reported by Sengpracha *et al.* (2012). The difference in fatty acid composition detected in the three brands of palm oil may be due to variation in species or strain, climate, degree of ripeness, harvesting, and chemical refining processes (Sengpracha, 2012). The presence of odd number fatty acids, branched margaric acid was determined only in sunflower oil (6.72%). However, the concentration of margaric acid was much higher than the result reported by Zambiazzi *et al.* (2007) -which was 0.04%. On the other hand, it was not detected on other findings (Kostik *et al.*, 2013; Dorni *et al.*, 2018).

As shown in Table 7 above, total unsaturated fatty acid in the evaluated oil varied from 28.53 % for Chief palm oil to 84.41% for sunflower oil. Study by Sadoudi and Ahmed, (2017) indicated that total unsaturated fatty acid in sunflower oil sold in Algeria was 88.886%. It seems that

sunflower oil is more unsaturated than soyabean oil in this study. Indeed, Kostik *et al.* (2013); Zambiasi *et al.* (2007) reported that sunflower oil contained more unsaturated fatty acids compared to soy bean oil; the values obtained were 86.5 % vs 91.2 % and 87.64 % respectively. The total unsaturated fatty acid in all samples of palm oil was below 50% - where the lowest was found in Chief palm oil (28.53%). These findings were in concordance with previous study (Dorni *et al.*, 2018)

The mono unsaturated fatty acid: oleic acid was the only concentrated MUFA found in all evaluated oil samples, as shown in Table 6. The concentration of oleic acid ranged from 18.77% to 46.29 %, the highest being observed in Hayat palm oil and the lowest (18.77%) in soyabean oil. The oleic acid content of Chief palm oil is below the range specified by the codex standard for oleic acid for palm oil (39.8-46%), but it is within the range for the others. It has been documented that consumption of MUFA reduces LDL-c and TC, while it increases HDL-c (Lopes *et al.*, 2016; Grundy, 1989). Oleic acid influences plasma level of hemostatic factors (Gebaue *et al.*, 2014), and is responsible for the hypotensive effect of olive oil (Terés *et al.*, 2008).

The poly unsaturated fatty acid, linoleic acid (omega -6) was the predominant essential fatty acid found in significant amount in sunflower oil (57.86%) and soyabean oil (55.28 %). The level of linoleic acid is in the range specified by the codex standard of linoleic acid for sunflower oil (48.3-74%) and soyabean oil (48-59%). A study conducted in Macedonia on sunflower oil indicated that the level of linoleic acid was  $59.5 \pm 7.5$  (Kostik *et al.*, 2013). On the other hand, a study conducted in Serbia on soyabean oil indicated that the level of linoleic acid was  $47.57 \pm 0.15$  (Ivanov *et al.*, 2010). From nutritional view point, the abundant presence of linoleic acid makes sunflower and soyabean oil the best salad oils. Linoleic acid (9.73%) was determined in Mona palm oil only among the evaluated palm oil samples. Consumption of PUFA plays vital role in the prevention of CVD (Dinicolantonio and Keefe, 2018; Lopes *et al.*, 2016 ). In addition, intake of PUFA is inversely associated with chronic kidney disease in patients with type 2 diabetes (Dos santos *et al.*, 2018). Furthermore, PUFA intake plays vital role in the prevention of atherosclerosis, coronary heart disease (CHD), cancer, type 2 diabetes, hypertension; further inflammatory, thrombotic and autoimmune disease (Ristić-Medić *et al.*, 2013).

The other poly unsaturated fatty acid, linolenic acid (omega -3) was found in soyabean oil only. The presence of linolenic acid (5.5%) and adequate and balanced amount of essential fatty acids make the fatty acid composition of soyabean oil unique than the evaluated oil samples. The level of linolenic acid is within the range specified by the codex standard for linolenic acid (4.5-11%) for soyabean oil. A study conducted in Serbia on soyabean oil indicated that the level of linolenic acid was  $12.11 \pm 0.17$  % (Ivanov *et al.*, 2010). The observed difference may be as a result of difference in soybean variety. Linolenic acid was not detected in sunflower oil in this study, which is consistent to the findings of Dorni *et al.* (2018). According to the findings of Chowdhury *et al.* (2007) and Zambiasi *et al.* (2007), it was detected below 0.5%. In addition, Linolenic acid (omega -3) was not found in the samples of palm oil. This is in accordance with the findings of Chowdhury *et al.* (2007). However, trace amount of linolenic acid was detected in palm oil localized in Thailand (Sengpracha *et al.*, 2012). Linolenic acid consumption may improve insulin sensitivity (Khan and Makki, 2017). In addition, linolenic acid intake has preventive role against the incidence of CVD (Pan *et al.*, 2012).

The omega-6/omega-3 ratio is one of the important parameter that determines the nutritional value of oil. Its value in soyabean oil (10.05:1) is within the ideal ratio range revealed by (WHO, 2003),( FAO,1994) and (Simpoulos, 2002). This makes soyabean oil nutritionally healthy than sunflower oil. This is because un balanced dietary intake of omega- 6 and omega-3 fatty acids promote the pathogenesis of heart disease, diabetes, arthritis, inflammatory diseases and cancer(Gómez-Candela *et al.*, 2011; Simopoulos, 2008). A study by Berger *et al.* (2017) conducted on young individuals with an ultra-high risk phenotype, revealed that high omega-6 to omega-3 ratio was causally associated to the increased incidence of mood disorder. In addition review by Simopoulos (2016) indicates elevated omega-6 to omega-3 ratio is associated to the increased risk of obesity. Thus, balanced dietary intake of omega-6 to omega-3 ratio is important for the prevention and management of non communicable diseases. .

The ratio of poly unsaturated fatty acids to saturated fatty acids (P/S ratio) is an index of dietary fatty acid composition. It indicates the relationship between saturated and polyunsaturated fatty acids content. P/S index is a vital parameter for determination of nutritional value of certain oils. Mainly, higher value of P/S index greater than one is an indicator for nutritionally healthy oil (Johnson *et al.*, 2009). Study by Kang *et al.* (2004) revealed that a diet with high P/S ratio lowers

the risk of developing CVD. In consistent to this, Guo *et al.* (2010) indicated that higher P/S ratio is very important to prevent coronary artery diseases. This is because high P/S ratio controls metabolic risk factors of CVD i.e. serum LDL-c, and TC. Furthermore, many studies revealed that higher P/S index is accompanied with lower deposition of lipids in the body (Lawton *et al.*, 2000). The value of P/S index is indicated in Table 7 above. Sunflower oil (4.03:1) followed by Soyabean oil (2.97:1) showed P/S index value greater than one, where as the lowest value was noticed in Mona palm oil (0.19:1). The P/S index value in sunflower and soyabean oil indicate the dominancy of unsaturated fatty acid over saturated fatty acids. The P/S index values were in line with data from literature (Johnson *et al.*, 2009).

## 6. CONCLUSION

Sunflower oil followed by soyabean oil presented the highest amount of unsaturated fatty acid (MUFA plus PUFA) than saturates, but Chief palm oil followed by Hayat palm oil and Mona palm oil presented low amount of unsaturates. Soybean oil is unique from others by containing adequate amount of linolenic acid (one of the essential fatty acid) and healthy proportion or mixture of saturated and unsaturated fatty acids. Even though, sunflower oil contains slightly higher level of total unsaturated fatty acids, soyabean oil appears superior because it contains balanced proportion of omega-6 to omega-3 fatty acid (10.05:1), very near to the range recommended by WHO (5-10: 1). P/S index- the important parameter that determine the healthiness of oil is greater than one for sunflower oil (4.03:1) and soyabean oil (2.97:1). The higher P/S index and total unsaturation of soyabean oil and sunflower oil make them favorable as salad oil and for mass consumption. Chief palm oil followed by Hayat palm oil and Mona palm oil showed the highest amount of total saturated fatty acids (> 50 %) than unsaturated fatty acids, which is mainly accounted by palmitic acid and stearic acid. The fatty acid composition of Mona palm oil is distinct than other brands of palm oil due to the presence of essential fatty acid linoleic acid. Among the evaluated palm oil brands, Mona palm oil contained the healthiest fatty acid profile followed by Hayat palm oil. Chief palm oil however, presented the least healthy fatty acid profile. .

## **7. RECOMMENDATION**

This study evaluated limited oil types commercialized on the market and a broader future study is recommended to include all available oil brands in the market. We would like to recommend Ethiopian food, medicine and health control authority to implement consolidated regulatory control to evaluate the quality of imported edible oil, in order to prevent consumption of unhealthy oils. In addition, further study is recommended to evaluate the trans fatty acid content of imported edible oils to identify possible adulteration and hydrogenation and to evaluate the effect of dietary intake of highly saturated palm oils. We also recommend consumers to incorporate alternative sources of essential fatty acids in their diet, if they consume highly saturated oils; they need to blend these oils with other highly unsaturated oils. Finally, it is suggested that unsaturated oils should be avoided by Ethiopian population for use in deep frying, but can be used as salad oil.

## **8. STRENGTH AND LIMITATIONS OF THE STUDY**

### **8.1 Strength of the study**

Assessment and evaluation of fatty acid composition of imported edible oil widely consumed in Ethiopia was attempted for the first time. Therefore, this study will provide baseline information for further studies to be conducted and to evaluate the fatty acid profile of indigenous edible oils and fats in Ethiopian settings.

### **8.2 Limitations of the study**

The main limitation of this study was financial shortage, which affected sample size and type of parameters being analyzed. In addition, the concentration of fatty acids were not reported as mean of data and the study didn't include locally manufactured edible oils and other important oils such as olive oil.

The sample size was small, due to the expensive cost of reagents and laboratory analysis of fatty acid profile by GC-MS. Moreover, trans fatty acid content and physiochemical parameters were not evaluated in this study.

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## 10. ANNEXES

### Annex I - GC-MS chromatograms of analyzed samples

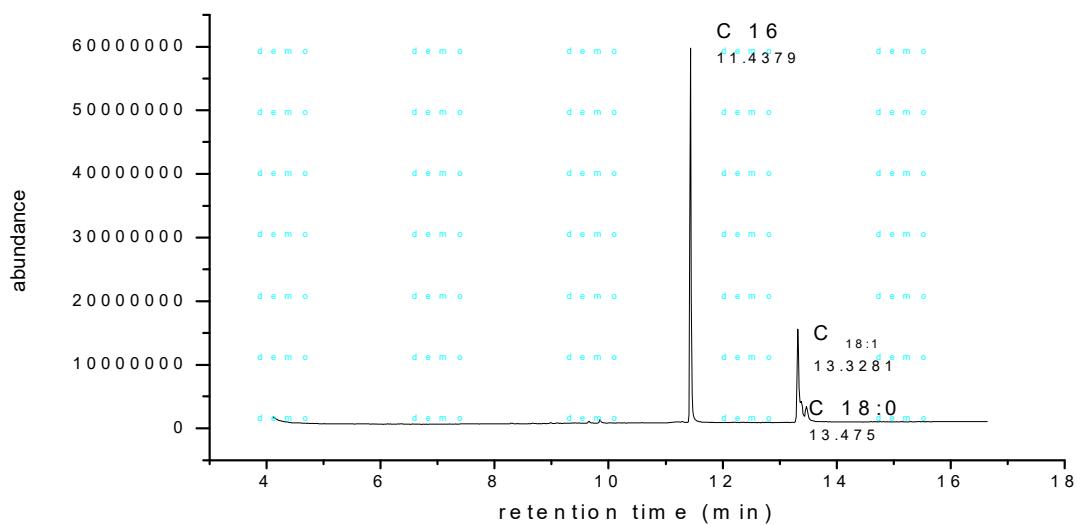


Fig 1: GC- MS Chromatogram of fatty acid methyl esters of Chief palm oil. The two prominent peaks correspond to palmitic acid and oleic acid.

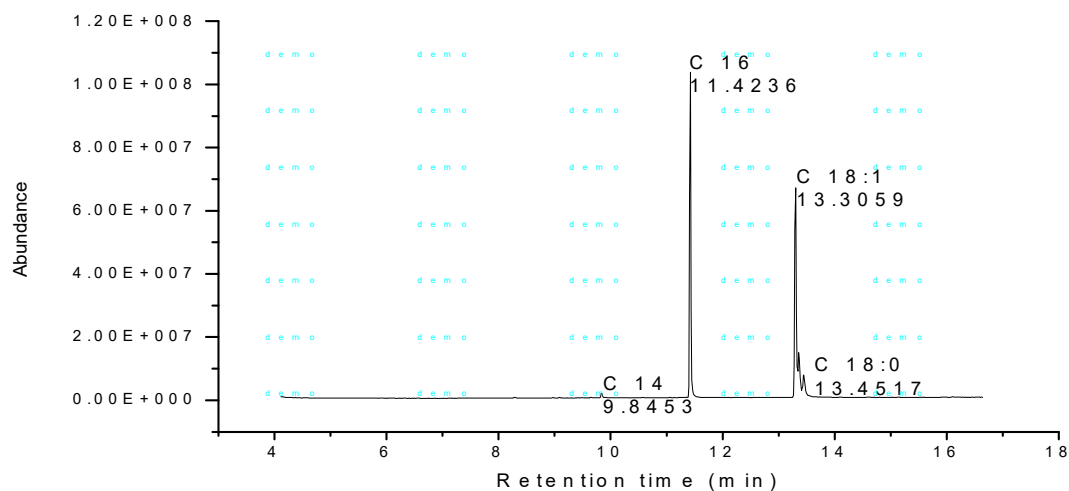


Fig 2: GC- MS Chromatogram of fatty acid methyl esters of Hayat palm oil. The two prominent peaks indicate the abundant presence of palmitic and oleic acid in Hayat palm oil.

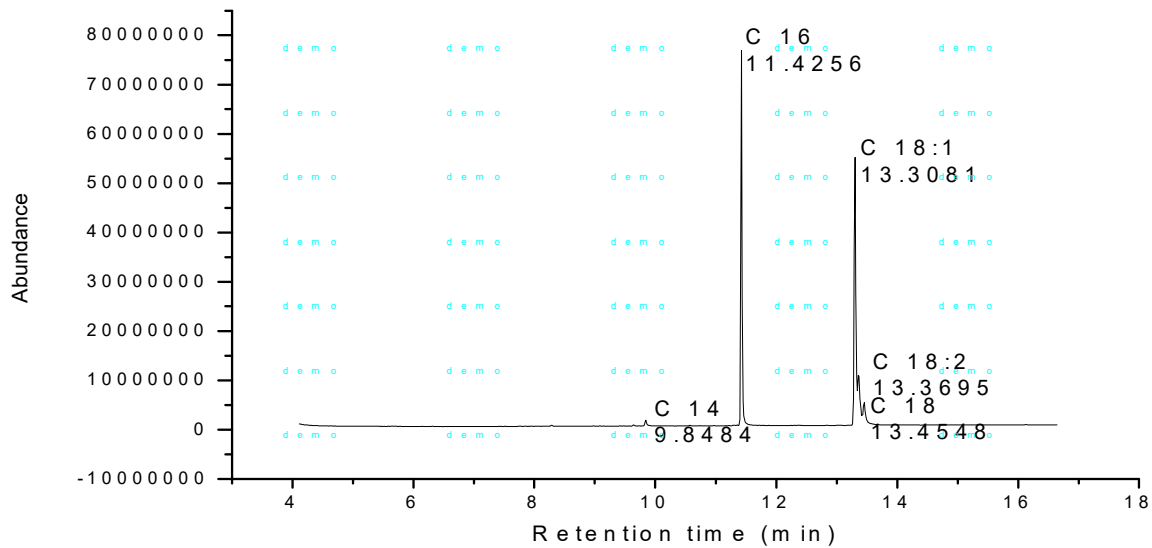


Fig 3: GC- MS Chromatogram of fatty acid methyl esters of Mona palm oil. The two prominent peaks indicate the abundant presence of palmitic and oleic acid in Mona palm oil.

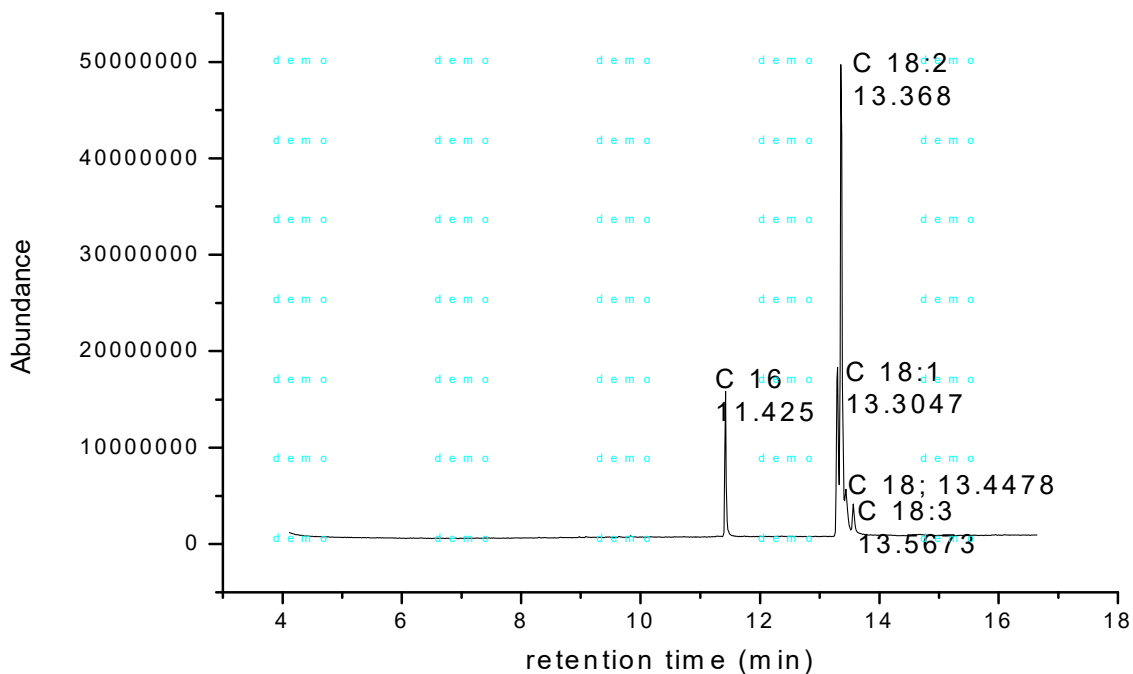


Fig 4: GC- MS Chromatogram of fatty acid methyl esters of soyabean oil. The two prominent peaks indicate the abundant presence of linoleic and oleic acid in soyabean oil.

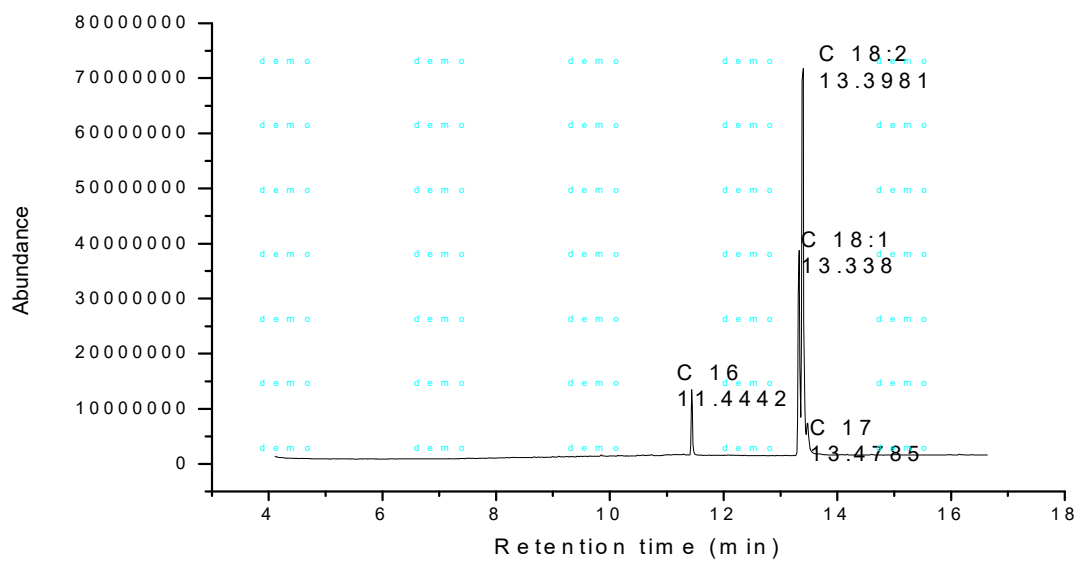


Fig 5: GC- MS Chromatogram of fatty acid methyl esters of sunflower oil. The two prominent peaks show the abundant presence of linoleic and oleic acid.

## Annex II- Mass spectrum of different fatty acid methyl esters

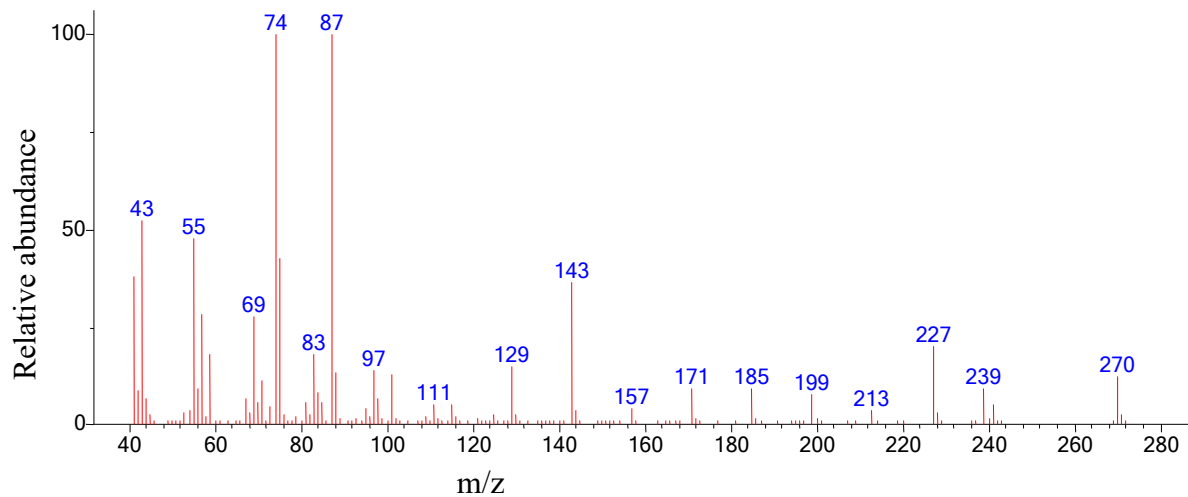


Fig 1: Mass spectrum of methyl palmitate

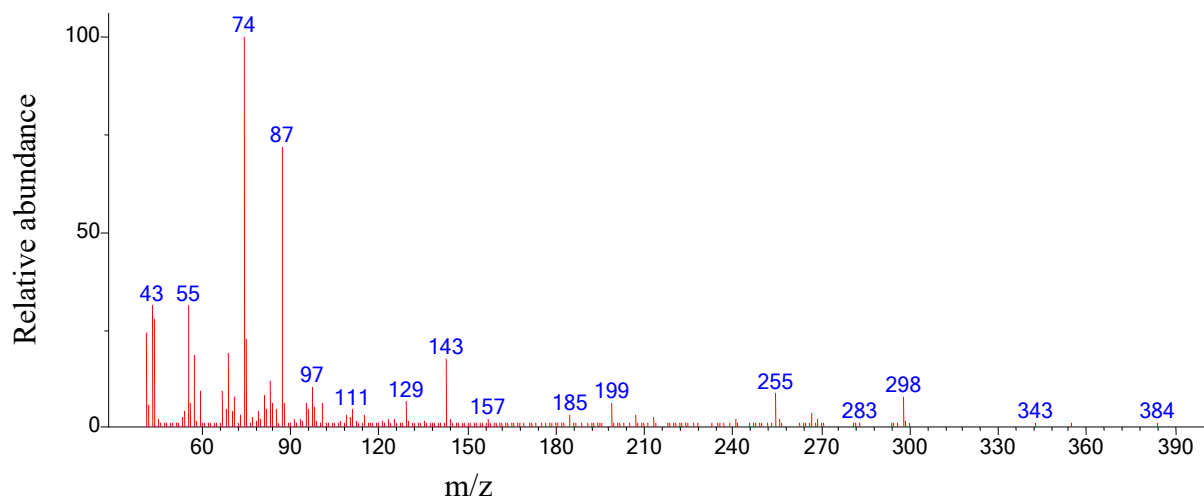


Fig 2: Mass spectrum of methyl stearate

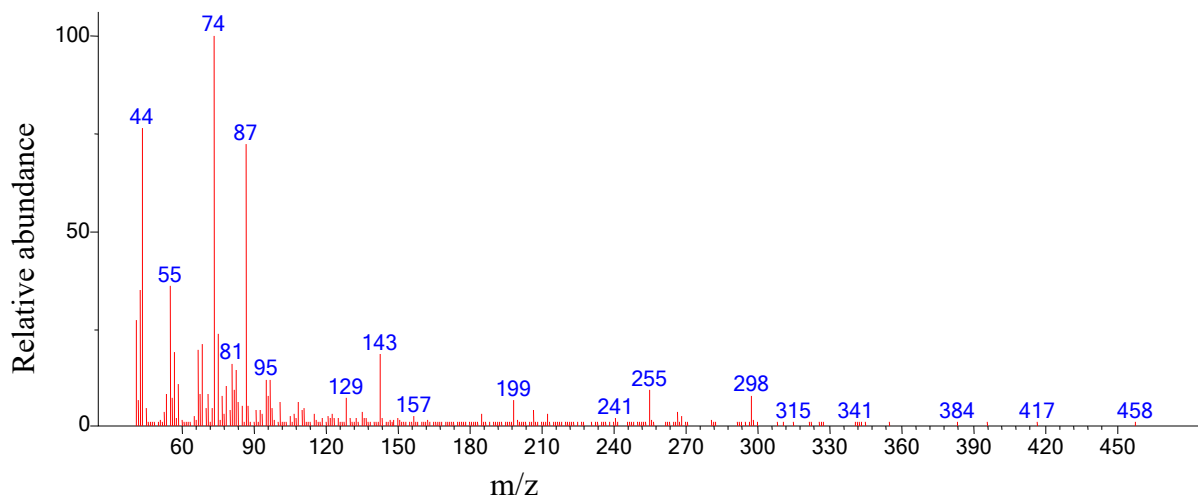


Fig 3: Mass spectrum of methyl margarate

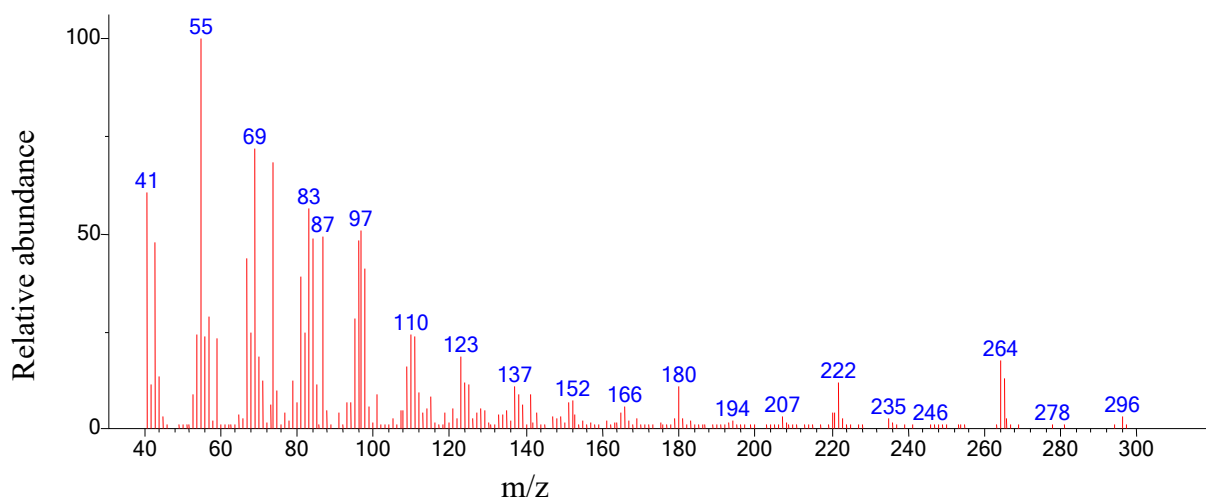


Fig 4: Mass spectrum of methyl oleate

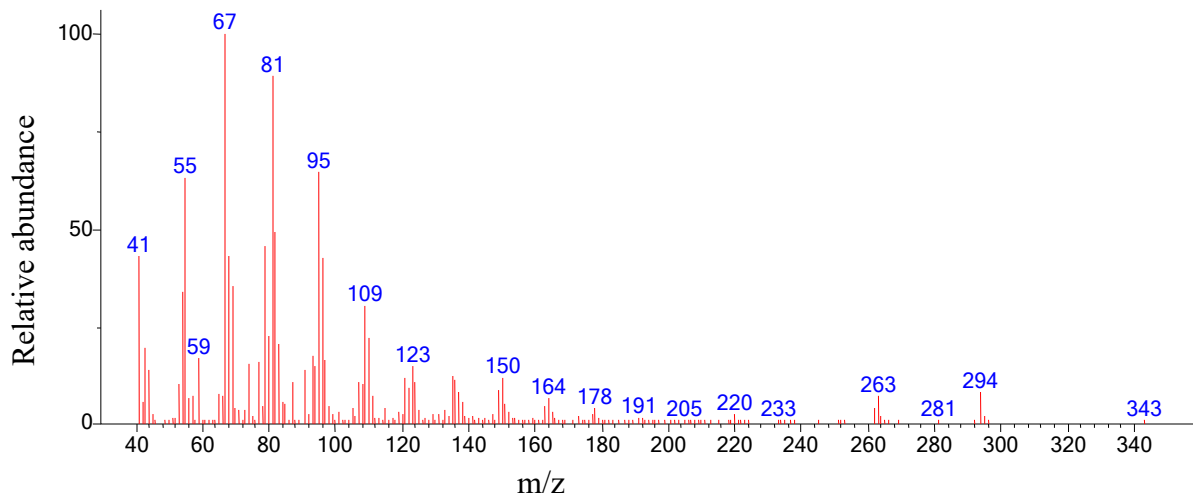


Fig 5: Mass spectrum of methyl linoleate

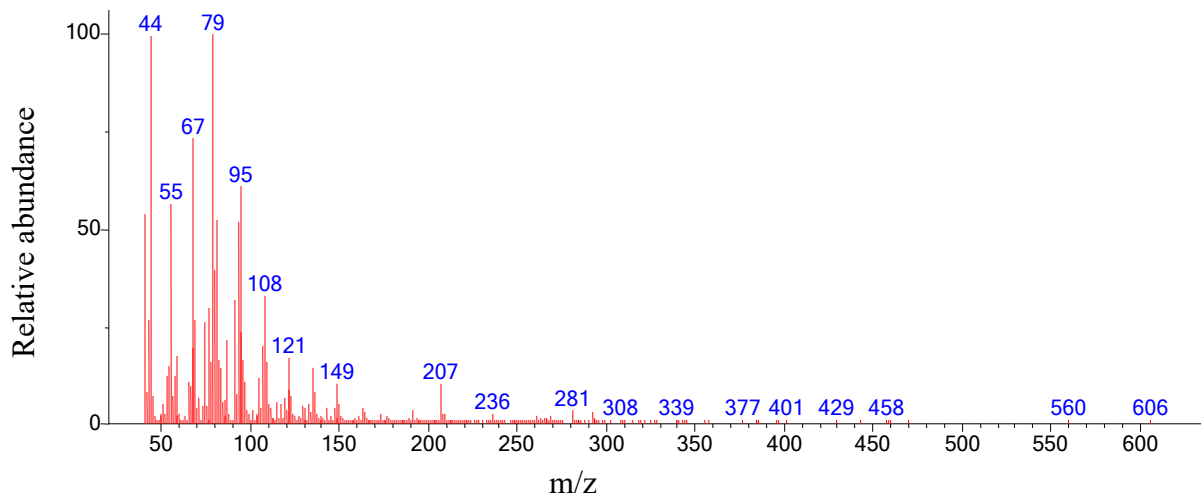


Fig 6: Mass spectrum of methyl linolenate