

1. Introduction

1.1. Lipid chemistry

Fats occur naturally in food and play a significant role in human nutrition. Fats are used to store energy in the body, insulate body tissues, cushion internal organs, and transport fat-soluble vitamins in the blood. Fats also play in an important role in food preparation: They enhance food flavor and food texture, make baked products tender, and conduct heat during cooking (Day, 2004).

Fats are the most prevalent class of compounds (in living systems) referred to as lipids. Lipids are found in all living molecules and play an essential role in the maintenance of life. Unlike carbohydrates and proteins, lipids are highly polymorphic and structurally difficult to define. As a result they are classified according to their major feature as water insoluble (or mostly insoluble) organic compounds found in biological systems. Lipids can be either amphipathic (having polar and non polar qualities) or hydrophobic (no polar) (Prosecus, 2006). Lipids are cellular compounds that are insoluble in water. Fats are soft, low-melting solids, with a density less than that of water. Fats and closely related oils are mixtures of compounds consisting of fatty acids combined with glycerol (commonly known as glycerin) via ester linkages. Fatty acids are long, straight chain carboxylic acids. A fat (or oil) is formed when three fatty acid molecules react with a glycerol molecule to yield a triglyceride (and three water molecules) (Figure1.) Fats in the body are transported and stored as triglycerides (Dupont, 2005)

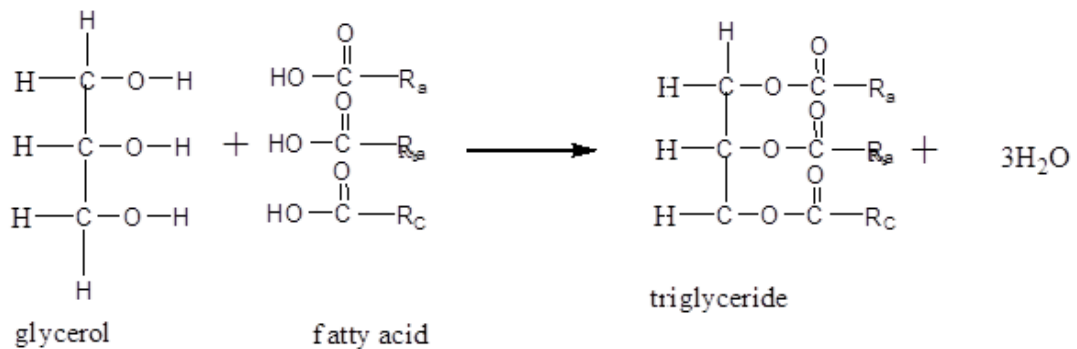


Figure 1: The formation of a triglyceride (Dupont, 2005).

This hydrolysis reaction produces glycerol and fatty acids, which are carboxylic acids derived from fats and oils. In the fatty acids, R_a , R_b , and R_c , represent groups of carbon and hydrogen atoms in which the carbon atoms are attached to each other in an unbranched chain.

The hydrolysis reaction is promoted by acids and by bases. When a strong base such as NaOH (lye) is used, the product contains salts of the fatty acids. These salts of fatty acids are the functional ingredient in soap. The ingredients lists of some soaps include sodium tallow ate, a generic name for the mixture of fatty acid salts obtained from tallow (animal fat), and sodium coco ate, obtained from coconut oil.

Lipids constitute a wide variety of naturally occurring substances in plant and animal tissues. They contain long-chain hydrocarbon groups in their molecules. Lipids can be classified based on their physical properties at room temperature; oils are liquids and fats are solids; the polarity-neutral lipids include fatty acids (FAs), glycerides and sterols, and polar lipids include glycerol phospholipids and glyceroglycolipids (glycolipids). The most common classification of lipids is based on their structure; simple or

complex. Simple lipids, composed of FAs and alcohol components, include acylglycerols, ether acylglycerols, sterols, and their esters that can be hydrolyzed to two different components; usually an alcohol and an acid. Complex lipids include Phospholipids are amphipathic molecules. The head of a lipid molecule is negatively charged phosphate group and the two tails are highly hydrophobic hydrocarbon chains. Phospholipid tails will congregate together to form a local hydrophobic environment. This leaves the charged phosphate groups facing out into the hydrophilic environment. Glycolipids, and sphingolipids, which yield three or more different compounds on hydrolysis. The fatty acids (FAs), i.e., carboxylic acids, are the main building blocks in lipid structures. Only a small portion of the total lipid fraction consists of free carboxylic acids, or free fatty acids (FFAs). Most of the carboxylic acids in the lipid fraction are found as esters of glycerol, i.e., as tri-acylglycerols (TAGs), commonly known as triglycerides (Figure 1) Triglycerides are esters of trihydroxy alcohols with three fatty acid molecules. They are the principal components of oils and fats. And the most common TAGs are those with long-chain carboxylic acids ($C_{14} - C_{22}$). Positions of carboxylic acids in TAGs are very important to their properties and utilization. The saturated forms of FAs are the most stable against oxidation and they also have high melting points in comparison with unsaturated FAs with the same number of carbon atoms. In plant TAGs, unsaturated fatty acids are predominantly located in the 2-positions. Monoacylglycerols (MAGs) have two FA chains at any FA at two positions (Lehner and Kuksis, 1996). However, the phosphoric acid residue of phosphoglycerides (PLs) and the first glycoside contain only one long-chain FA (saturated or unsaturated) at either the 1, or 2, or 3 positions. Similarly diacylglycerols (DAGs) have two FA chains at any of the two positions. However, the phosphoric acid residue of phosphor glycerides

(PLs) and the first glycoside residue of glycosylate glycerides (GLs) are always in position 3 (Dupont, 2005).

Triglyceride molecules contain mostly carbon and hydrogen atoms, with only six oxygen atoms per molecule. This means that fats and oils are highly reduced (that is, un-oxidized). They are, in this way, similar to the hydrocarbons in petroleum, and like petroleum they are good fuels. The main biological function of triglycerides is as a fuel. The normal human body stores sufficient energy in fat for several weeks' survival. This storage ability helps the organism deal with unpredictable variations in the food supply. Plants, too, store energy in fats and oils. Oils are particularly common in seeds, where the stored energy helps seedlings during germination, until they can exploit solar energy through photosynthesis (Mattson and Grundy, 1985).

Fatty acids contain an even number of carbon atoms, from 4 to 36, bonded in an unbranched chain. Most of the bonds between carbon atoms are single bonds. If all of these bonds are single bonds, the fatty acid is said to be saturated, because the number of atoms attached to each carbon atom is the maximum of four. If some of the bonds between carbon atoms are double bonds, then the fatty acid is unsaturated. When there is only one double bond, it is usually between the 9th and 10th carbon atom in the chain, where the carbon atom attached to the oxygen atoms is counted as the first carbon atom. If there is a second double bond, it usually occurs between the 12th and 13th carbon atoms, while a third is usually between the 15th and 16th (Senanayake, 2008).

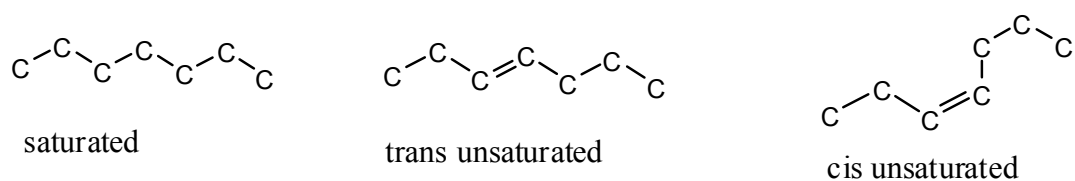


Figure 2: Isomerization of Fatty Acid (O'keefe, 2002).

Double-bond geometry is designated with the *cis-trans* or *E/Z* nomenclature systems, the *cis/trans* terms are used to describe the positions of atoms or groups connected to doubly bonded atoms. They can also be used to indicate relative positions in ring structures. Atoms/groups are *cis* or *trans* if they lie on same (*cis*) or opposite (*trans*) sides of a reference plane in the molecule. Some examples are shown in Figure 2. The prefixes *cis* and *trans* can be abbreviated as *c* and *t* in structural formulas (O'keefe, 2002).

Double bonds between carbon atoms in fatty acids can cause kinks in the chains of atoms. This is particularly true for *cis* double bonds. These kinks prevent the molecules from stacking together well. Because they do not fit together well, unsaturated fatty acids and triglycerides have lower melting points than saturated ones. Thus fats, which are solids, are usually more saturated than oils, which are liquids at room temperature.

Highly unsaturated oils undergo a chemical reaction in the presence of oxygen and light. In this reaction the unsaturated carbon atoms become saturated by reaction with oxygen. This reaction causes separate molecules of oil to become linked by oxygen atoms. Linking from molecule to molecule causes the oil to solidify. Oils that undergo this process are called drying oils and are used in oil paints. Oils that are rich in double bonds are most able to undergo this process. Among the most unsaturated of vegetable oils is linseed oil, the preferred medium for artists' oil paint.

Reactions of the fatty acyl moieties of lipids both at double bonds and at carboxyl groups are important both in biology and for industry. For

example, autoxidation can lead to spoilage of foods, and oxidation reactions can cause problems within living tissues. In contrast, a huge industry has developed that makes use of chemical reactions of fatty acyl residues of lipids to develop products main polar lipids. Other minor lipid constituents are for industry - from cosmetics detergents, etc (Frankel, 1984).

Waxes are esters formed by the combination of fatty acids with high molecular weight mono hydroxyl alcohols. Waxes minimize water loss on plants and act as waterproofing on animals. In most cases, waxes are hard, brittle substances with melting points. Liquid wax esters like those from jojoba seed and sperm whale oil are used in diverse commercial products as lubricants, cosmetics, solid wax coatings and biofuel additives. Similar wax esters can also be produced by microorganisms (Antonio *et al*; 2000; Wisniak, 1977)

1.1.1. Uses for vegetable oils

Vegetable oils are largely used in food materials; they are either utilized in cooking or frying. They are used for food products such as shortenings, salad and cooking oils, and margarines, large quantities serve feed and industrial needs (Bialy's, 2005). The latter applications include chemicals such as plasticizers, which add plasticity to plastics and other substances; stabilizers, which help other substances resist chemical change; emulsifiers, which enable the mixing of normally unmixable liquids; surfactants, which reduce the surface tension of liquids and are commonly used in detergents; and esters, nylons, and resins, which are basic ingredients in many industrial products. Besides detergents and plastics, products that contain chemicals derived from vegetable oils include lubricants, coatings, corrosion inhibitors, adhesives, cleaners, cosmetics, water repellants, and fuels (Hagemann, 1988).

Vegetable oils are too viscous and too reactive with atmospheric oxygen to establish significant markets for use in cosmetics, lubricants, and certain chemical additives. Fortunately, properties such as viscosity, pour point, freezing point, and reactivity can be decreased by chemically introducing branching groups or side chains on the straight-chained fatty acids. For example, derivatives of stearic acid, a byproduct of commercial dimer acid manufacture, can be used in many products textile lubricants, softeners, and antistatic agents; coupling agents; emulsifiers; greases; and synthetic lubricants for which the unmodified oil would be too reactive. Conversely, to make certain products, vegetable oils must be made more reactive. By changing domestic oil's physical properties, it can be made to resemble and replace imported Tung oil in coatings, resins, and plastics (Larsson, 1994).

1.1.1.1. Pharmaceutical and cosmetic preparations

The applications of lipids in the pharmaceutical field has been reported by (Antonio *et al*; 2000), different lipids used as excipients in cosmetics and medicines have been described. Many vegetable oils are used in this sense: almond oil, apricot oil, avocado oil, borage oil, coffee oil, safflower, sunflower oil, sesame oil ect for example sesame oil is used as solvent and skin and hair conditioner in cosmetics. From the animal source, fish oil and bird oil can be employed as excipients in cosmetic formulation. Fats and waxes may also be used for this purpose. A broad range of phospholipids are suitable for use in cosmetics, pharmaceuticals and diagnosis. These substances are used as vehicle for therapeutic substances, such as liposomes.

Lipids, as a function of their biological activity, as active substances for the elaboration of pharmaceuticals, cosmetics or nutritional supplements.

Carotenoids, retinoids, tocopherols are used for their antioxidant properties, that are important to health and diagnostic medicine (Ten-Wolde *et al*; 1997).

1.1.1.2. Bio Diesel

Vegetable oils have potential as reliable and renewable sources of fuel for compression ignition engines (diesel) a concept as old as the diesel engine itself. In fact, early engines were demonstrated running on peanut oil. Once cheap petroleum became readily available, the modern engine was designed to use petroleum fuel. Periodically, the alternative vegetable fuel concept has been reestablished, usually during petroleum shortages and as petroleum shortages and prices eased, interest in alternatives again waned. Consequently, scientists do not yet understand how best to change the chemical and physical properties of vegetable oils to allow their trouble-free use as a fuel source (Murray *et al*; 1982; Souza *et al*; 1991).

1-1-2.Oils and Nutrition

Lipids are the richest source of energy on a weight basis. They also play a significant role as barriers, such as for skin and in stabilizing biological membranes (Day, 2004). Appropriate intake of lipids is essential for health maintenance. However, a high consumption of fat, especially saturated FAs, may be connected with several chronic diseases, such as heart disease and obesity(Casimir and David, 2002), of the lipid components of a normal diet, the most important FAs are linoleic (n-6 PUFA) and *a*-linolenic (n-3 PUFA) acids, the two primary essential Fas (Fan and Chapkin, 1998; Jeffery *et al*; 1996). They play a role in stabilizing biological membranes by creating physical properties that are optimal for the transport of substances across the membrane and for the biochemical reactions occurring in the

membrane (McDonald and Eskin, 2006). Through metabolism, they are converted into a whole range of oxygenated compounds, which exert a range of profound physiological activities involving lowering plasma cholesterol, aggregating red blood cells, and smoothing muscle performance, all attributes that are required for good health. Since the human body is unable to synthesize them, they must be obtained from dietary sources (Kang *et al*; 1995; Fernandes and Venkatraman, 1993).

1.1.3. Vegetable oils

The principal constituents of vegetable oils are esters of glycerol and fatty acids along with partially glyceridic material such as lecithin and substances such as tocopherol. Their composition will vary according to the species and the use will depend especially upon the variety, type and proportion of fatty acids (Antonio *et al*; 2000). Vegetable oils are primarily extracted from the seeds of plants and processed in a number of ways to make it suitable for many different uses. Some vegetable oils are edible and some are not. Edible oils are plant based oils that are manufactured for human consumption. The term edible oils can also include oils derived from animal fat, but generally when one refers to edible oils, one usually means oils that are made by extracting the oil from certain plants (Casimir and David, 2002).

1.1.3.1. Processing of Fresh Oil

1.1.3.1.1. Agriculture

Oil is made by plants in a chemically complicated process in the farm. The agricultural steps are the determinant part, starting from seeds selection, soil preparation, plantation, irrigation up to harvesting and proper storage

avoiding heat and moisture to prevent pests and microbiological attacks (mainly aflatoxin).

1.1.3.1.2. Seed preparation

Cleaning of sand, plant residues, wood and other impurities. The oil seeds are carefully cleaned of these materials using magnets, screens and aspirator systems. The cleaned seeds are dried to remove moisture. Then the oil seeds are usually decorticated to remove the hull that surrounds the oilseed meat before being further processed (Lawrence and Johnson, 2002; Bialys', 2005).

1.1.3.1.3. Methods of oils extraction from oil seeds:

Crude vegetable oil and fats are obtained from oil bearing seeds and fruits by either of two methods: mechanical pressure or extraction by solvent. The effect of treatment by either method is to separate the oil more or less completely from the solid matter naturally associated with it. The residue latter is generally used for agricultural purposes either for stock feeding or fertilizer (Gunstone, 2000). The winning of the oil from the original materials is much more perfect by solvent extraction than by mechanical pressure though the difference varies greatly in different circumstances and with different materials, when mechanical pressures employed the residue contains approximately from 4 to 8% of oil, where it is only from 1 to 2% when the material is extracted by solvent. (Eljack, 1999) reported that the separation of oils and fats from various oil –bearing seeds , nuts and fruits constitutes a distinct and specialized branch of fat technology, there have been three extraction processes namely hydraulic pressing , continuous screw pressing and solvent extraction (Lawrence and Johnson, 2002).

1.1.3.1.4. Refining methods

Various processing steps impact the quality of freshly refined oil. Vegetable oils are refined principally by Physical refining method and Chemical refining method. Palm oil and coconut oil are refined by the physical citric refining method. The crude oil is bleached with acid-activated clay and acid at elevated temperatures under vacuum. The objective is to remove phosphorus (phospholipids), trace metals, oil decomposition products, and some of the color bodies from the crude oil. The volatile impurities in the bleached oil are then removed via steam distillation under very low absolute pressure and high temperature in a deodorizer (Bailey's, 2005).

Physical refining process is fairly simple and is environmentally friendly and more economical for palm oil, coconut oil, and palm kernel oil. The process may not remove the trace impurities if the bleaching step is not done properly (Bravo, 1995).

Seed oils are refined mostly by the chemical method where the crude oil is intimately mixed with a caustic (sodium hydroxide) solution under controlled conditions. The caustic primarily reacts with the free fatty acids to form soap (in this case, sodium soap of fatty acids). The soap is removed from the refined oil using a centrifuge. Aside from free fatty acids, some of the phosphor lipids trace metals, and some of the color bodies from the crude oil are also removed and they appear in the soap phase. The soap is processed further to regenerate fatty acids. The refined oil is water washed to reduce the soap and then bleached with acid-activated clay and citric acid at elevated temperatures under vacuum. The bleached oil is either deodorized to make liquid oil or is hydrogenated to make shortening and margarine and the formulated product is then deodorized (Lawrence and Johnson, 2002; Bailey's, 2005).

The schematic flow diagram for the chemical refining process is shown in Figure 3.

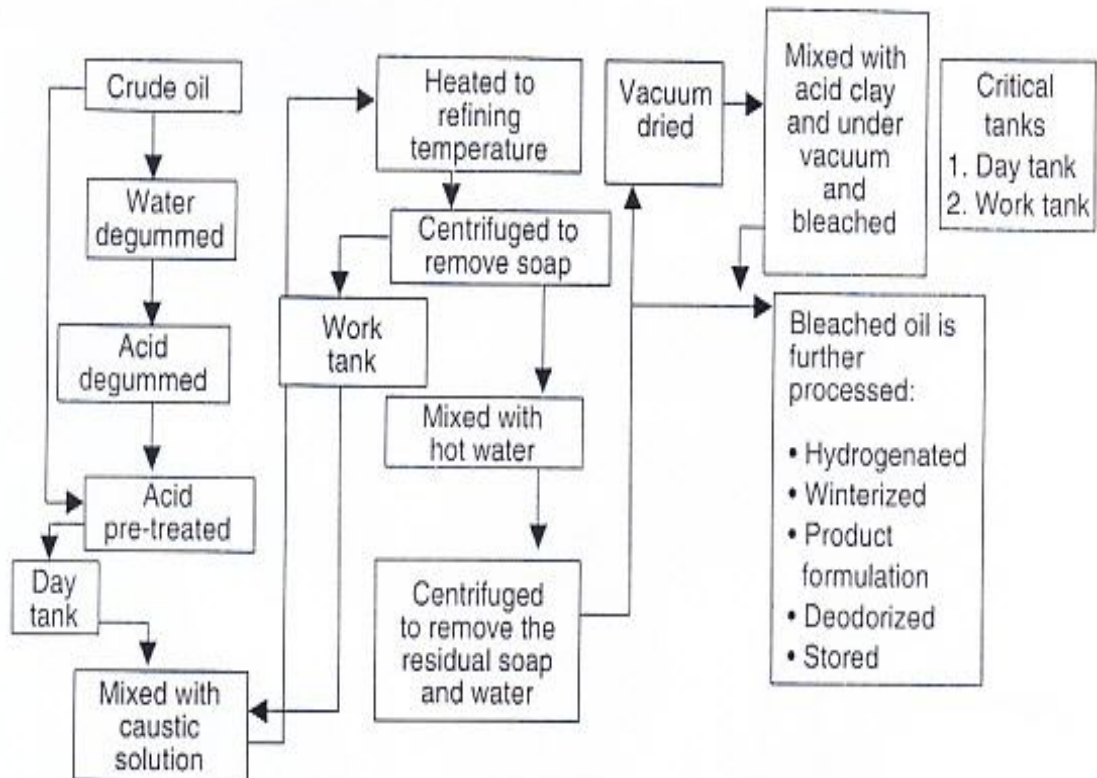


Figure 3: Chemical refining process (Bailey's, 2005).

1.1.3. 2. Vegetable oils (conventional and nonconventional)

Lipids in oilseeds are predominantly 90% TAG, or more of total lipids, and phospholipids being the main polar lipids. Other minor lipid constituents are FFA, sterols, hydrocarbons, etc. The FA composition“ of lipids is the main influence in terms of the “quality and the uses of the oil.

Among the common oilseeds, safflower, sunflower, and peanut oil contain high levels of unsaturated fatty acids. A high proportion of unsaturated FAs in edible oil, however, do decrease its storage stability and quality, due to oxidation reactions associated with the double bonds in unsaturated Fats.

1.1.3. 2.1. Conventional Groundnut oil

Groundnut is very important oil in Sudan beside cotton, sunflower and sesame seeds. Peanut cooking oil or groundnut oil is organic material oil extracted from peanuts which is scientifically named *Arachis hypogaea*. Pale yellow, golden in colour, this viscous oil has a nutty aroma and taste, however, is not overpowering. Peanut cooking oil has various uses right from cooking to being used as a source of fuel for diesel engine, as an ingredient in ear wax removing products, etc.

About 96% of Groundnut triglycerides are composed of 71% of oleic acid and 30% of linoleic acid. Moreover, it also contains some other fatty acids like lignoceric acid, palmitic acid, arachidonic acid, arachidic acid and behenic acid. Found 17 to 22% and 78 to 83 % saturated and unsaturated fatty acids respectively in Peanut oil. It is used in large quantities just as olive oil is used in the Mediterranean. Fatty acid composition of peanut oil as influenced by cultivation, maturity, storage, processing treatment and environmental condition.

Most of Sudanese people prefer the crude form, which were used in many forms like butter (dakoaa). Roasting in the shell, in confections, cooking oil and residue as animal feed (Balla, 2001). Groundnut oil contain up to 30 % linoleic acid, the essential fatty acid which plays a major part in human diet (Ihekoronye and Ngoddy, 1985). Groundnut seed are rich in oil 38- 50% which is used for cooking, salad, and manufacture of margarine soap and as lubricant. The high quality oil is used as well in pharmaceutical industry.

Peanut oil is often used in cooking, because it has a mild flavor and a relatively high smoke point. Due to its high monounsaturated content, it is considered more healthy than saturated oils, and is resistant to rancidity. There are several types of peanut oil including: aromatic roasted peanut oil, refined peanut oil, extra virgin or cold pressed peanut oil and peanut extract. In the United States, refined peanut oil is exempt from allergen labeling laws. Besides cooking benefit, peanut oil is quite healthy. It reduces the risks of heart diseases. Moreover, since peanut oil is high in saturated fats which when heated at normal temperatures does not turn to trans fatty acids, it is better off than other cooking oils.

1.1.3. 2.2. Conventional Sunflower Oil

Sunflowers are known worldwide for their beauty and vitality. A sunflower's large seeds are pressed and used to make sunflower oil. It is edible and economical and the uses for sunflower oil in many new products increase every day. Their flour sunflower oil has almost no flavour or odour it is a well known cooking oil for deep frying as it will not alter the flavour of the fried food. Sunflower oil only contains a small amount of saturated fats and contains high amounts of vitamin E and is therefore one of the best cooking oils for deep frying. Sunflower oil can also withstand higher cooking temperatures than other cooking oils.

Sunflower oil is characterized by its high concentration of linoleic acid (60-70%) followed by oleic acid (15-35%). Sunflower oil is next to safflower in having high levels of linoleic acid. The FA composition of safflower oil is similar to that of sunflower. The oil of commercial safflower cultivars contains 55-81% linoleic acid and 7-42% oleic acid as major FAs, followed by stearic (1-10%) and palmitic acid (2-10%) as minor FAs. In peanut oil, oleic and linoleic acids constitute 33-71% and 12- 46% of the total FAs, respectively. There are three types of sunflower oil available, namely Mid-Oleic, Linoleic and High Oleic sunflower oil. They all have different oleic levels, making the uses for sunflower oil vast (Tremolieres *et al*; 1982).

Sunflower oil provides more vitamin E than any other cooking oil and so also acts as a moisturizer by assisting the body retain water inside its cells. For this reason, sunflower oil is largely used in the manufacturing of cosmetic products. The benefits of sunflower oil with its large vitamin E content include the reduction of rheumatoid arthritis, asthma and colon cancer.

One of the benefits of sunflower oil is that it acts as an antioxidant. This means it neutralizes free radicals that cause cancer. Sunflower oil also contains high levels of essential fatty acids called polyunsaturated fats which provide energy and lower the risk of cardiovascular diseases. These essential fatty acids cannot produce in our bodies. Thus a major benefit of sunflower oil in our daily diet is that it reduces cholesterol and this decreases the risk of developing heart disease.

The uses for sunflower oil in beauty products is constantly increasing as sunflower oil is easily absorbed into the skin and provides deep nourishment and moisturizes the skin. Sunflower oil is also used in

aromatherapy practices and is a popular ingredient in lotions and creams. By applying sunflower oil to the face and necks severe acne can be reduced. Researchers have proven that sunflower oil provides a protective barrier and lowers infection when applied to the skin of premature babies whose skin is still much undeveloped (Max, 1977).

Other nutrients found in sunflower oil include, Palmitic acid, Stearic acid, Lecithin, Tocopherols, Carotenoids, Selenium, Proteins, Copper, Iron, Zinc, Calcium, Omega-6 fatty acids and folate or folic acid. Folic acid found in sunflower oil promotes the creation of new cells in the body (Sebedlo *et al*; 1988).

1.1. 3. 2. 3. Non-conventional (*Moringa oleifera*) oil

Moringa oleifera is considered one of the world trees most useful as almost every part of the *Moringa* tree can be used as food, or has some other beneficial properties. In the tropics it is used as forage in livestock, and *Moringa* in many countries, is used as micronutrient powder to treat indigenous diseases (Council, 2006).

In the Sudan, dry *Moringa oleifera* seeds are used as substitute of alum by rural woman to treat highly turbid Nile water (Jhan, 1986). A large number of reports on the nutritional qualities of *Moringa* now exist in both the scientific and the popular literature. It is commonly said that *Moringa* leaves contain more vitamin A than carrots, more calcium than milk, more iron than spinach, more vitamin C than orange, and more potassium than bananas, and that the protein quality of *Moringa* leaves rivals that of milk and eggs (Olson and Carlquist, 2001).

The seed oil contains all the fatty acids contained in olive oil, except linoleic and was used as its acceptable substitute (Morton, 1991). The seeds contain between 33 and 41% w/w of vegetable oil (Sengupta and Gupta,

1970). The oil is high in oleic acid (> 70%). Oils that are high in mono-unsaturated/oleic acid can be used as a healthier alternative to the more saturated and hydrogenated oils used in frying because of their stability. Consumption of saturated and partially hydrogenated fats and oils has been shown to increase the risk of coronary heart disease (Mattson and Grundy, 1985; Mensink and Katan, 1990).

Moringa oleifera is commercially known as “ben.oil” or “behen oil”, due to its content of behenic acid, possesses significant resistance to oxidative degradation (Lalas and Tsaknis, 2002). *Moringa* oil is good source of behenic acid (9%) in nature and is used as preservative in food industries; the oil is liquid at ambient temperature, translucent and pale yellow in colour. The oil does not turn rancid and also burns without smoke (Fuglie, 1999)

Figure 4 outlines important uses of various parts of the plant.

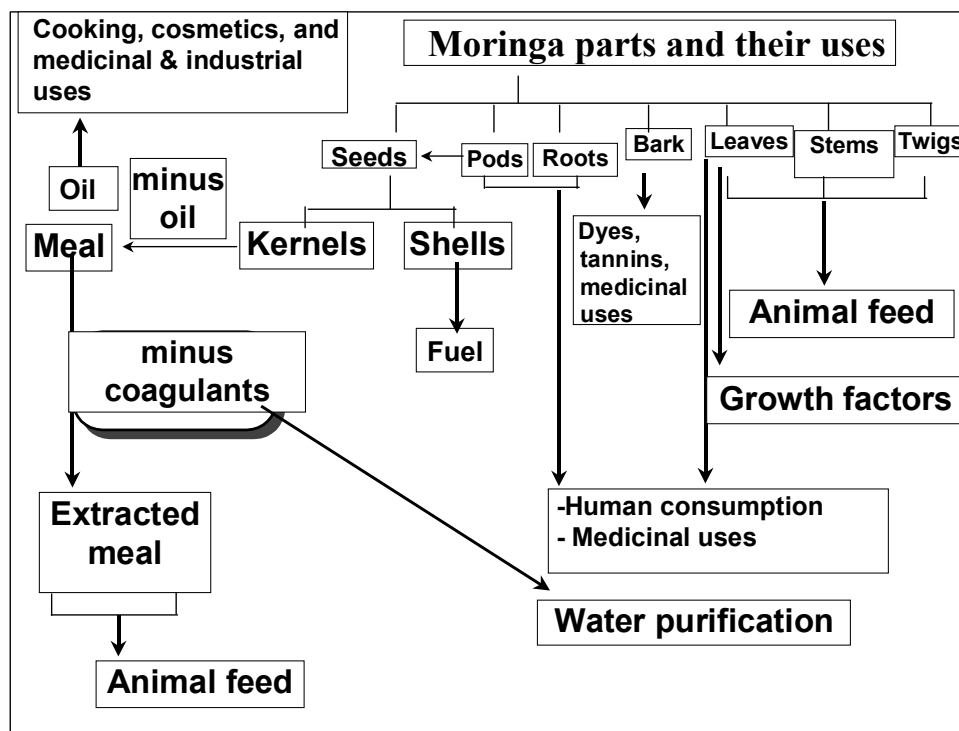


Figure 4: Uses of different parts of *Moringa oleifera* Source (Foidl et al; 2001).

The young leaves are edible and are commonly cooked and eaten like spinach or used to make soups and salads. They are an exceptionally good source of pro vitamin A, vitamins B, and C, minerals (in particular iron), and the sulphur -containing amino acids methionine and cystine. The composition of the amino- acids in the leaf protein is well balanced (Foidl et al; 2001).

The dry seeds can be ground to a powder and used for seasoning sauces. The roots from young plants can also be dried and ground for use as a hot seasoning base with a flavor similar to that of horseradish. This is why the *Moringa* tree has been given the name “Horseradish Tree”. A tasty hot sauce from the roots can also be prepared by cooking them in vinegar. The flowers can be eaten after being lightly blanched or raw as a tasty addition to salads. The resin from the trunk of the tree is also useful for thickening sauces.

The oil content of de-hulled seed (kernel) is approximately 42 %. The oil is brilliant yellow. It is used as a lubricant for fine machinery such as timepieces because it has little tendency to deteriorate and become rancid and sticky (Ramachandran *et al*; 1980). It is also useful as vegetable substances and is therefore valuable in the perfume industry for stabilizing scents. The free fatty acid content varies from 0.5 to 3 %.

1.1.4. Frying

In recent years, increasing attention has been paid to oils consumed by humans. The most popular vegetable oils contain large quantities of unsaturated fatty acids, most of all those that are necessary for a human consumption (Gromadzka and Wardenck, 2011).

It is well known that deep-fat frying is a prevalent and old food cooking method which can go back to 1600 BC. Although 180°C is usually recommended for frying foods, it is always higher than 180°C in the practical deep-fat frying (Firestone, 2004). Fast food processing, palatable taste of fried food and considerable economic benefit make the deep-fat frying become one of the most popular food cooking methods used. The fried food is endowed with attractive flavor, golden pellicle and crisp texture or mouth feel when it is fired under the appropriate conditions (Warner 2008; Qing Zhang *et al*; 2012).

Under the established conditions of fried material's natural properties corresponding sample handling, frying can involve all of the components to participate in a series of physical and chemical alterations. These changes not only include the decomposition reactions of the constituents such as the nutrients of raw material and triacylglycerols (TAGs) of frying oil, but also include the inter-actions among these constituents (Chu and Luo, 1994; Dobarganes *et al*; 2000). Moreover, deep-fat frying is a complicated physicochemical processes which simultaneously influenced by many factors such as the nature of fried material and frying oil, time, temperature, intermittent or continuous heating, fresh oil complement, fryer model and use of Filters (Kalogianni *et al*; 2010). Therefore, many products are formed due to these complex substrates and chemical conditions. Furthermore, frying with food and frying without food have a significant different chemical in reaction pathways (Qing Zhang *et al*; 2012).

Frying is one of the fastest, oldest and simplest methods of food cooking; it involves heating an edible oil or fat and using the hot oil to cook food. During deep – frying, oil is exposed to elevated temperatures in the presence of air and moisture. Under these conditions a number of chemical

reactions occur, including oxidation, hydrolysis and polymerization of unsaturated FA (Mariod *et al*; 2006; Choe and Min, 2007). Deep- fat frying enhances the sensory properties of food (color, texture and flavors); however, repeated use of frying oils produces undesirable constituents that may pose health hazards (Tyagi and Vasishtha, 1996).

Vegetable fats and oils are much used in deep frying operations. Frying temperature (130-190°C) affect the stability and, hence, quality of such oils particularly under repeated exposure to these conditions. The stability of oils is commonly affected by oxidation processes, which yields both primary and secondary oxidation compounds. Primary oxidation compounds, such as lipid hydroperoxides are formed in an initial stage of lipid oxidation. Later, lipid hydroperoxides are decomposed in secondary oxidation compounds such as aldehydes, ketones, acids and alcohols (Cameli Papuc *et al*; 2009). There is, thus, an increased risk of oxidative fat decomposition which is accelerated by thermal decomposition and pro-oxidants such as water, salt, bicarbonates, iron, copper and oxygen among others. The effect of frying on the physicochemical constants of fats and oils has been studied extensively. Unsaturated sites are the most susceptible sites for heat-induced and radical-based fat oxidation (Wagner and Gupta, 2005).

On the other hand, frying in vegetable oils is one of the simplest methods of turning raw food ingredients into palatable meals. Oils are refined prior to use to remove components that might adversely affect the quality of the oil. During this process, some unwanted side reactions may occur. Similarly, during frying, changes can occur in the frying oil, including oxidation, *cis* /*trans*-isomerization and cyclization. These have the potential to affect the taste and produce undesirable nutritional effects in consumers (Carmen, 2011).

During frying, a number of complex reactions take place in the oil, which depend not only on the biological composition of the raw material, but also on the choice of ingredients and processing conditions (frying method, heating time and temperature used during frying, type of fats and oils used etc.). Oxidation of food lipids is one of the main causes of deterioration of food quality in terms of sensory and nutritional values (Martins, 2010). It is well known that autoxidation is an important degradation reaction which is attributed to the rancidity of oil and fat.

It is as well the major reaction occurred during frying along with the increase of temperature. The mechanism of thermal oxidation is principally similar with the autoxidation mechanism, the difference only in the reaction speed (Houhoula *et al*; 2003). During the frying treatment, except the absorbed parts by both fried material and frying oil, these compounds volatilize out of the frying system due to the high-temperature and their volatility. On the other hand, the oxygen content decreases with the proceeding of high temperature treatment. In addition, when the state of oxygen-free occurs, many other thermal reactions would take place. However, the reaction speed of oxidation increases during the frying treatment under the condition of high temperature (Qing Zhang *et al*; 2012)

Heating in the presence of air causes partial conversion of fats and oils to volatile chain- scission products, non volatile oxidized derivatives, and dimeric, polymeric, or cyclic substances. There is some evidence that high oxidized and heated fats may have carcinogenic properties because of potentially toxic substances (Tyagi and Vasishtha, 1996; Warner, 1996). Therefore, the measurement of oxidative stability and oxidation products is essential to determine shelf life, acceptability, and nutritional quality of edible oils (Yildiz *et al*; 2001).

There are numerous studies that report changes in fats and oils after heating or frying procedures (Che Man and Jaswir, 2000; Gertz, 1996; Takeoka *et al*; 1997). Most of them conclude that such changes depend on the temperature, the heating cycles, the surface/ volume and food/oil ratios, the fatty acid and the antioxidant composition of the oils (Melton *et al*; 1994).

1.1.4.1. Physiochemical changes during heating and frying

Deep-fat frying is a process of cooking and drying in hot oil with simultaneous heat and mass transfer. As heat is transferred from the oil to the food, water is evaporated from the food and oil is absorbed by the food (Figure 5). Many factors affect heat and mass transfer, including thermal and physical properties of the food and oil, shape and size of the food, and oil temperature (Warner, 2002). In general, deep-fat frying increases foaming, color, viscosity, density, the amount of polymeric and polar compounds and the free fatty acid content of frying oils (Alireza Serjouie *et al*; 2010)

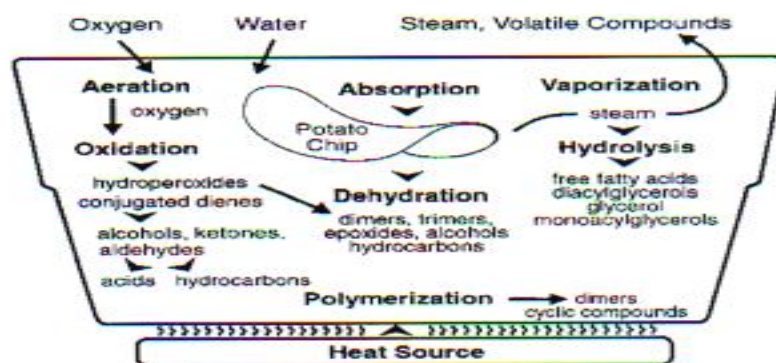


Figure 5: Physical and chemical reactions that occur during frying (Warner, 2002).

During deep frying, fats and oils are exposed to elevated temperatures and a variety of reactions take place. In the presence of oxygen, food moisture

and high temperature, the oil undergoes three main reactions: hydrolysis, oxidation and thermal alteration (Elham, 2008).

Frying oils not only transfer heat to cook foods but also help to produce distinctive fried-food flavor and, unfortunately, undesirable off-flavors if deteriorated oil is used. During deep-fat frying various deteriorative chemical processes (e.g., hydrolysis, oxidation, and polymerization) take place, and oils decompose to form volatile products and nonvolatile monomeric and polymeric compounds (Figure 5). With continued heating and frying, these compounds decompose further until breakdown products accumulate to levels that produce off-flavors and potentially toxic effects, rendering the oil unsuitable for frying. The amounts of these compounds that are formed and their chemical structures depend on many factors, including oil and food types, frying conditions, and oxygen availability (Warner, 2002; Velasco *et al*; 2008). In the presence of oxygen moisture, trace elements and free radicals physiochemical reaction such as thermoxidation, hydrolysis, polymerization, isomerization or cyclization take place at the high temperatures of the frying process, thus leading to the decomposition of frying oil and formation of monomeric, polymeric, primary and secondary oxidative compounds, thereby affecting the quality of oil and fried product (Gertz, 1996; Alireza Serjouie *et al*; 2010). also these processes may reduce the amount of antioxidants in the oil, decrease its stability and produce new products which are responsible for loss of the nutritional value and quality of the oil (odour, flavour, absorption, etc). The oxidized products of fatty acids give off-flavours and odors (hydrolytic rancidity) to the medium and fried foods . In addition, these chemical reactions hydrolysis, oxidation, and polymerization are interrelated producing a complex mixture of products (Warner, 2002). The individual

processes of hydrolysis, oxidation, and polymerization and their degradation products are described below.

1.1.4.1.1. Hydrolysis

Hydrolysis is one of the major reactions occurring during deep frying due to the presence of moisture introduced with the food and the relatively high temperatures used. Hydrolysis of ester bonds in the lipids results in the formation of free fatty acids (FFA), mono- and di-acylglycerols and glycerols (Perkins, 2006).

When food is fried in heated oil, the moisture forms steam, which evaporates with a bubbling action and gradually subsides as the foods are fried. Water, steam and oxygen initiate the chemical reactions in the frying oil and food. Water, a weak nucleophile, attacks the ester linkage of triacylglycerols and produces di- and monoacylglycerols, glycerol, and free fatty acids. Free fatty acids contents in frying oil increase with the number of frying (Chung *et al*; 2004; Warner, 2002) as shown in (Figure 6). Free fatty acid value is used to monitor the quality of frying oil. Thermal hydrolysis takes place mainly within the oil phase rather than water–oil interface. Hydrolysis is more preferable in oil with short and unsaturated fatty acids than oil with long and saturated fatty acids because short and unsaturated fatty acids are more soluble in water than long and saturated fatty acids. Water from foods is easily accessible to short-chain fats and oils for hydrolysis. Water hydrolyzes the oil faster than steam (Min and Choe, 2007), large contact between the oil and the aqueous phase of food increases hydrolysis of oil.

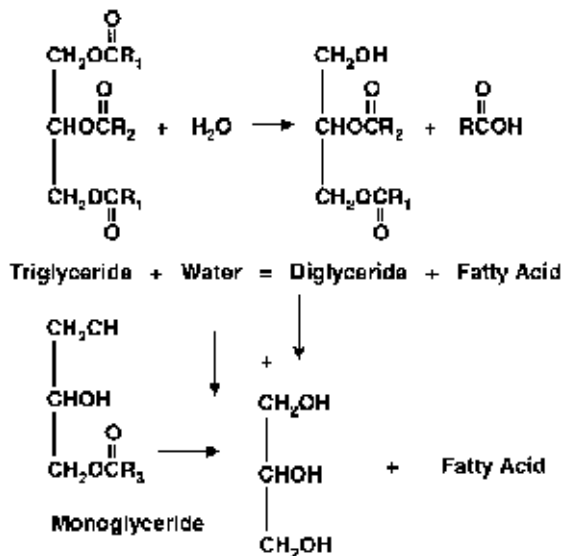


Figure 6: Hydrolysis reactions in frying oils (Warner, 2002).

Oil and fat is a mixture of TAGs which are composed of one glycerol and three groups of saturated or unsaturated fatty acids with different carbon numbers. Not only the natures of fatty acid, but also the various combination positions of fatty acids to glycerol molecule would impact the reaction activity of TAG. Therefore, the TAG degradation products mainly result from the breakages occurred in the carbon-carbon double bond ($\text{C}=\text{C}$) of aliphatic chains and ester bond. These compounds have a smaller molecular weight compared with that of the parent TAG and almost possess of volatility such as the decomposition compounds of lipid oxidation and TAG hydrolysis (Qing Zhang *et al*; 2012).

Free fatty acids and their oxidized compounds produce off-flavor and make the oil less acceptable for deep-fat frying. Di- and monoacylglycerols, glycerol, and free fatty acids accelerate the further hydrolysis reaction of oil. Glycerol evaporates at 150°C and the remaining glycerol in oil promotes the production of free fatty acids by hydrolysis (Naz *et al*; 2005; Stevenson *et al*; 1984) suggested that the maximum free fatty acid content for frying oil is 0.05% to 0.08%.

1.1.4.1.2. Oxidation

Lipid oxidation is one of the important causes of food spoilage. Oxidation, which is accelerated at the high temperature used in deep frying, creates rancid flavors and reduces the organoleptic characteristics of fried food. Hydro peroxides are the major initial reaction products of lipid oxidation. However, they are not stable and decompose spontaneously to form other compounds such as aldehydes, ketones, alcohols, acids, hydrocarbons, *etc* (Nawar, 1996).

Edible fats containing unsaturated molecules are susceptible to attack by molecular oxygen. This process is referred to as lipid oxidation and can give rise to undesirable volatile flavor compounds, potentially toxic oxidation products and a general deterioration in the quality of the fat (Grootveld *et al*; 2001). Fat oxidation is influenced by a range of parameters, including light exposure, temperature, and presence of prooxidant metals, presence of antioxidant compounds, and the degree of unsaturation of the fat (Min and Boff, 2002).

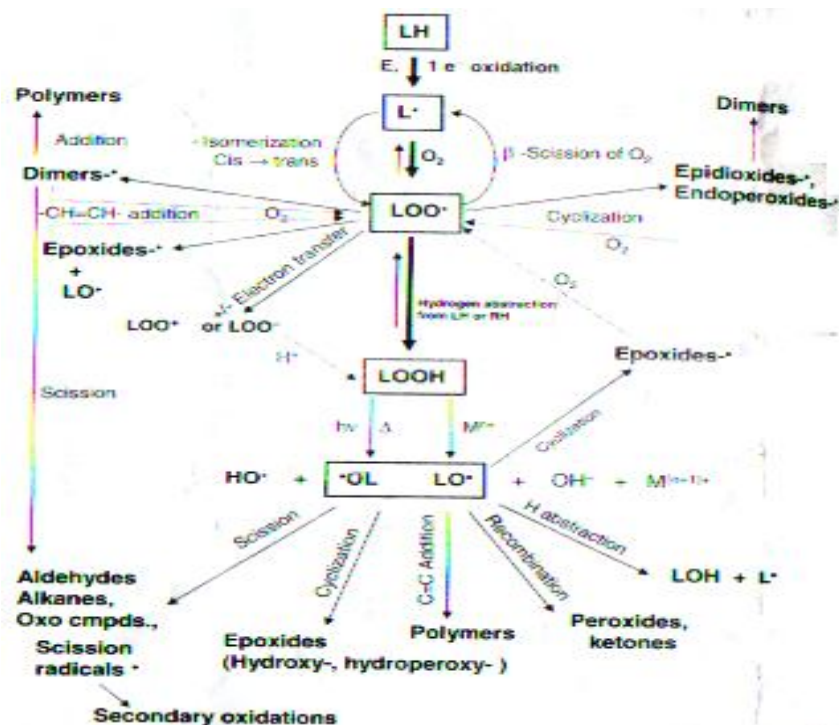


Figure 7: Integrated scheme for lipid oxidation (Bailey s, 2005).

On the other hand oxygen, which is present in fresh oil and is introduced into the frying oil at the oil surface and by addition of food, activates a series of reactions involving formation of free radicals, hydroperoxides, and conjugated dienoic acids. The chemical reactions that occur during the oxidation process contribute to the formation of both volatile and nonvolatile decomposition products figure 7. The volatile degradation products are usually saturated and monounsaturated hydroxyl, aldehydic, keto, and dicarboxylic acids; hydrocarbons; alcohols; aldehydes; ketones; and aromatic compounds are primarily responsible for undesirable oxidized (rancid) flavors (Warner, 2002; Parkin and Damodaran, 2003).

Vegetable oils undergo extensive oxidative deterioration during storage, marketing, or deep fat-frying. Oxidative stability of oils is the resistance to oxidation during processing and storage. Oxidative stability is an important indicator to determine oil quality and shelf life (Hamilton, 1994) because low-molecular weight off-flavor compounds are produced during oxidation. The off-flavor compounds make oil less acceptable or unacceptable to consumers or for industrial use as a food ingredient (Choe and Min 2009). Oxidation of oil also destroys essential fatty acids and produces toxic compounds and oxidized polymers. Oxidation of oil is very important in terms of palatability, nutritional quality, and toxicity of edible oils (Choe and Min, 2009).

The composition of oleic, linoleic and linolenic acids in oil has been an affect the oxidative stability. Sunflower oil has approximately 70% linoleic acid and is highly susceptible to lipid oxidation. Heating speeds up the oxidative reaction, which is a major concern for deep fat frying operations (Stephanie *et al*; 2007) reported that high oleic sunflower oil may decrease the risk of coronary heart disease by decreasing low density lipoprotein (LDL) cholesterol susceptibility to oxidation. Genetic modification of

sunflower oil, to decrease linoleic acid and increase oleic acid, could increase the oxidative stability during storage and deep fat-frying, as well as improve the health benefits. However, there is not much information on the oxidative stabilities of modified vegetable oils, especially sunflower oil, available. Therefore, the oxidative stabilities of the oils are influenced by the amount and type of metals, natural antioxidants, phospholipids, free fatty acids, mono- and di-glycerides, polymers and the number of double bonds in the oil (Martins, 2010).

Different chemical mechanisms are responsible for the oxidation of edible oils during process, storage, and cooking. Depending upon the types of oxygen; two types of oxygen can react with edible oils. (Figure 8 and 9) one is called atmospheric triplet oxygen, $^3\text{O}_2$, and the other is singlet oxygen, $^1\text{O}_2$ (Choe and Min, 2006; 2009). Triplet oxygen, $^3\text{O}_2$ reacts with lipid radicals and causes autoxidation, which is a free radical chain reaction. The non-radical electrophilic singlet oxygen does not require radicals to react with; it directly reacts with the double bonds of unsaturated fats and oils with high electron densities, which is called type photosensitized oxidation (Choe and Min, 2005; 2009).

Comparison of Singlet and Triplet oxygen (Min and Boff, 2002).

Factor	$^3\text{O}_2$	$^1\text{O}_2$
Energy level	0	22.4kcal/mol
Nature	Diradical	Highly electrophilic
Reaction	Radical compound	Electron-rich compounds

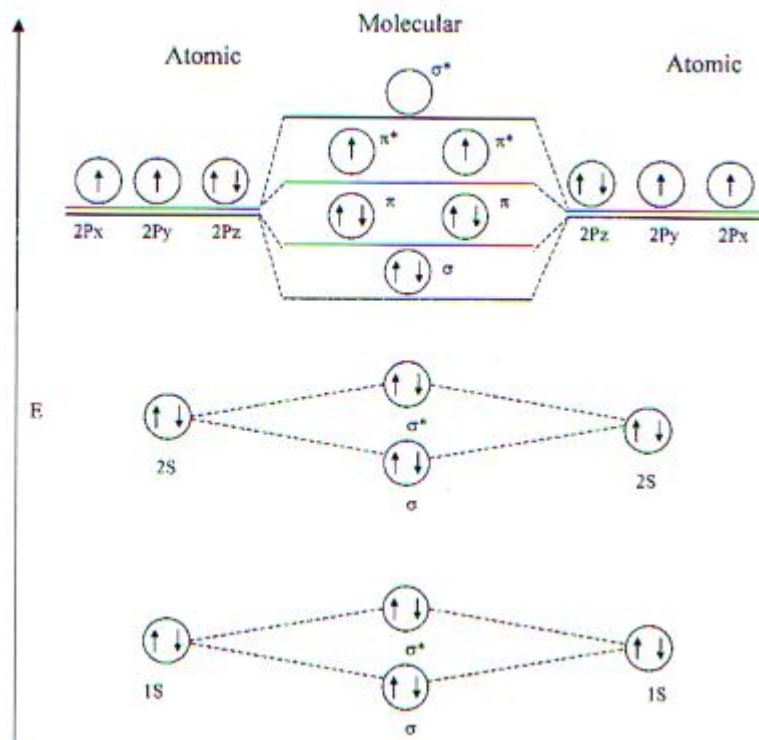


Figure 8: Molecular orbital of triplet oxygen (Min and Boff, 2002).

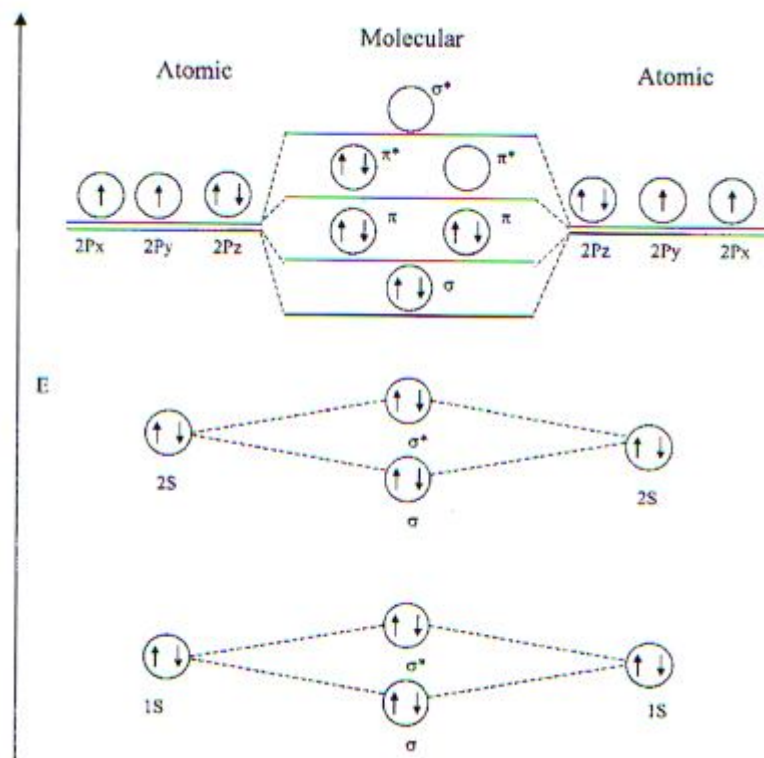


Figure 9: Molecular orbital of singlet oxygen (Min and Boff, 2002).

Fats and oils should be in radical forms to react with triplet oxygen in autoxidation. Lipids are normally in nonradical singlet state and heat, metals, or light accelerates their radical formation. Allylic hydrogen, especially hydrogen attached to the carbon between 2 double bonds, is easily removed due to low bond dissociation energy (Min and Boff, 2002; Choe and Min, 2005). The carbon and hydrogen dissociation energies are the lowest at the bis-allylic methylene position. Bis-allylic hydrogen at C11 of linoleic acid is removed at 75 to 80 kcal/mol. The energy required to remove allylic hydrogen in C8 or C14 of linoleic acid is 88 kcal/mol, and 101 kcal/mol is necessary to remove alkyl hydrogen from C17 or C18 shown in figure 10. (Min and Boff, 2002; Choe and Min, 2005). Upon formation of lipid radicals by hydrogen removal, the double bond adjacent to the carbon radical in linoleic and linolenic acids shifts to the more stable next carbon, resulting in conjugated diene structures. The shifted double bond mostly takes the more thermodynamically stable *trans* form.

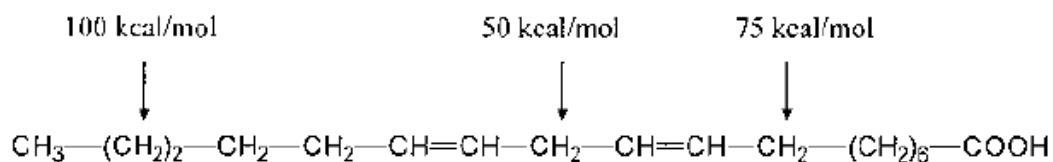


Figure 10: Energy required for hydrogen removal from linoleic acid (Min and Boff, 2002).

The lipid radical reacts with triplet oxygen very quickly at normal oxygen pressure (2 to 8×10^9 M/s) and forms lipid peroxy radical. The lipid peroxy radical abstracts hydrogen from other lipid molecules to form lipid hydroperoxide and another lipid radical. The radicals automatically catalyze the reaction and the autoxidation is called free radical chain reaction. When radicals react with each other, no radical species are produced to stop the reaction. Rate of oxidation is dependent on several factors, including temperature, presence of inhibitors or catalysts, and nature of the substrates.

Unsaturated fatty acids are more susceptible to oxidation than saturated fatty acids, a property that is primarily due to the lowered activation energy in the initiation of free radical formation for triplet oxygen autoxidation. Hydroperoxides formed by singlet oxygen oxidation are at positions that formerly contained double bonds. Singlet oxygen produced conjugated and nonconjugated hydroperoxides from linoleic and linolenic acids, but the triplet oxygen produced only conjugated hydroperoxides from linoleic and linolenic acids. The reaction rates of singlet oxygen and triplet oxygen with linoleic acid are $1.3 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ and $8.9 \times 10^1 \text{ M}^{-1} \text{ s}^{-1}$, respectively (Min and Boff, 2002).

1.1.4.1.3. Conjugated Dienes

Oxidation of polyunsaturated fatty acids is accompanied by an increase in the ultraviolet absorption of the product. Lipids containing methylene-interrupted dienes or polyenes show a shift in their double-bond position during oxidation due to isomerization and conjugate formation (Van den Berg *et al*, 1995). The resulting conjugated dienes exhibit an intense absorption at 234 nm; similarly conjugated trienes absorb at 268 nm.

Mossoba *et al*; 1991) have described an alternate spectroscopic method to Determine lipid oxidation of stored oils. In this assay, hydroperoxides of polyenoic fatty acids as well as hydroxy and carbonyl compounds derived from them are converted to more conjugated chromophores by two chemical reaction steps, namely reduction and then dehydration (Figure 11). These yield *conjugable oxidation products* (COPs), which are measured and expressed as COP values. Which results in the disappearance of the characteristic ultraviolet absorption of carbonyl compounds of oxidized polyenoic fatty acids (oxodienes). The decrease in the absorption at 275 nm is known as *oxodiene value*. The next step of the COP assay involves changes in the spectrum of the reduced compound to its dehydrated

counterpart which exhibits absorption maxima at 268 and 301 nm. The sum of these absorbance changes at 268 and 301 nm yields the COP value whereas their relative proportions define the COP ratio.

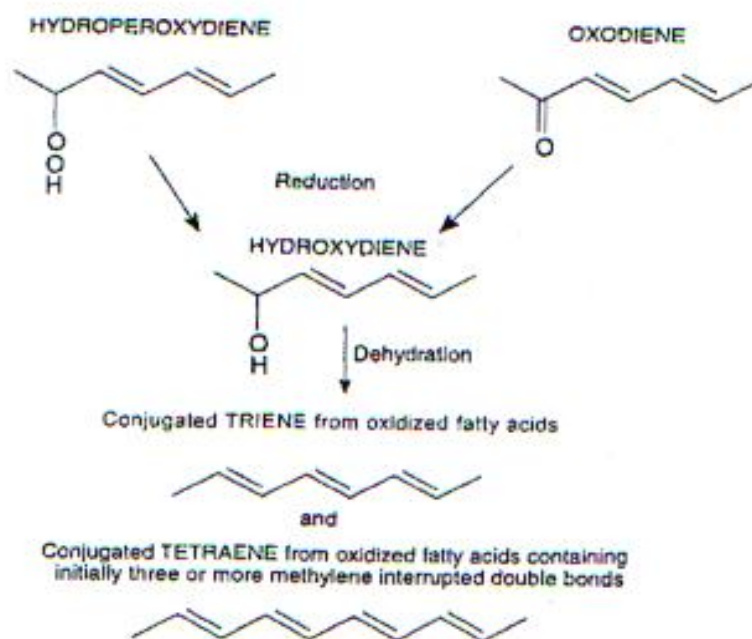


Figure 11: Chemical reaction steps in the assay of conjugable oxidation products (Shahidi and Wanasundara, 2002).

Edible oil undergoes autoxidation and photosensitized oxidation during processing and storage. The oxidation of edible oil produces off-flavor compounds and decreases oil quality. Free fatty acids, mono- and diacylglycerols, metals, chlorophylls, carotenoids, tocopherols, phospholipids, temperature, light, oxygen, oil processing methods, and fatty acid composition affect the oxidative stability of edible oil. To minimize the oxidation of edible oil during processing and storage, it is recommended to decrease temperature, exclude light and oxygen, and remove metals and oxidized compounds, and use appropriate concentrations of antioxidants such as tocopherols and phenolic compounds (Min and David, 2006).

1.1.4.1.4. Thermal oxidation

During frying, oils are degraded from thermal oxidation to form volatile and non-volatile decomposition products. The chemical changes in frying oil also result in changes in the quality of fried food (Pambou Tobi *et al*; 2010). The fatty acid composition of the frying oil is an important factor affecting fried food flavor and its stability; therefore, it should be low level of polyunsaturated fatty acid such as linoleic or linolenic acids and high level of oleic acid with moderate amounts of saturated fatty acid (Kiatsrichart *et al*; 2003; Mehta and Swinburn, 2001). As a result, the quality of frying oil is important because of absorbed oil of fried products during deep frying. Soybean oil has a good nutritional profile due to high level of unsaturated fatty acid but less oxidative stability. The chemistry of lipid oxidation at the high temperatures of food processes like baking and frying is highly complex since both oxidative and thermal reactions are involved simultaneously. As the temperature increases, the solubility of oxygen decreases drastically, although all the oxidation reactions are accelerated (Dobarganes *et al*; 1993). Figure 12 shows the well-known scheme of the oxidation process. It proceeds via a free radical mechanism of chain reactions, where RH represents here the triacylglycerol molecule undergoing oxidation in one of its unsaturated fatty acyl groups (Velasco *et al*; 2008).

In the high temperatures during the frying process, triacylglycerols undergo decomposition reactions such as isomerisation, cyclisation and polymerisation. Dimeric and polymeric glycerides acids also can be formed by the thermal and oxidative combination of free radicals (Dobarganes and Marquez-Ruiz, 2006) shown figure 12.

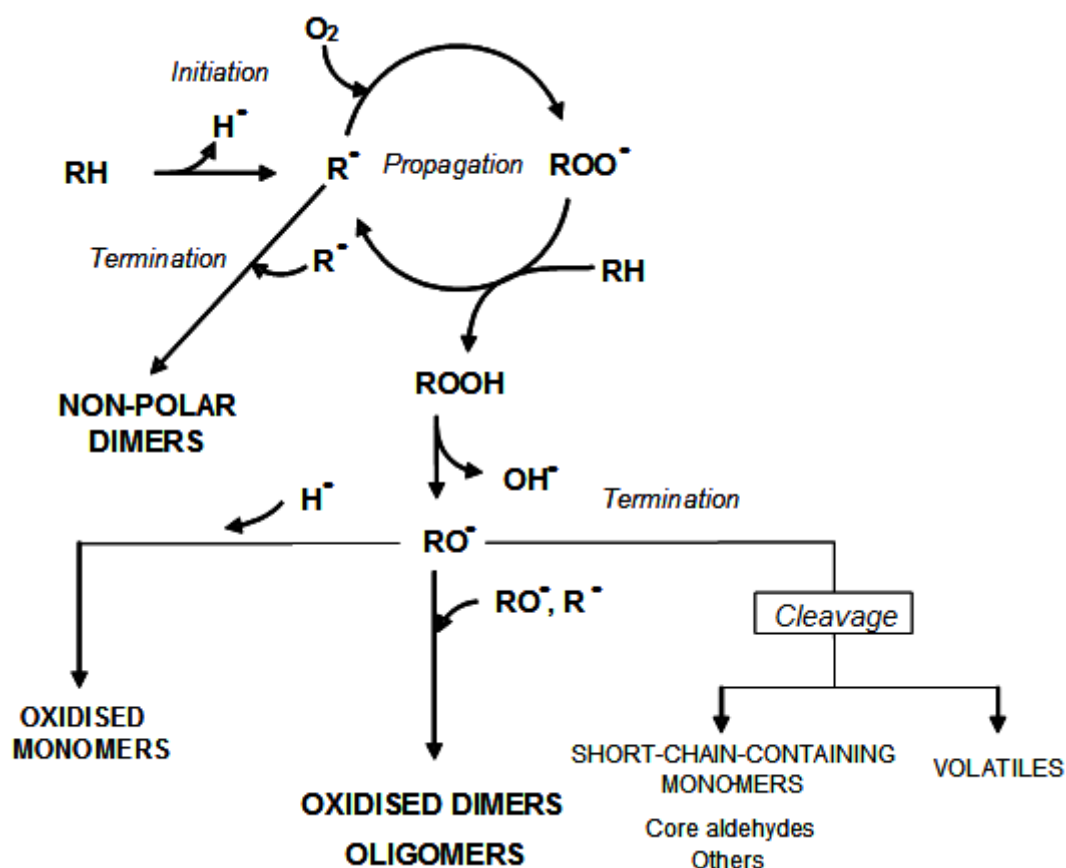


Figure 12: Simplified scheme of thermal oxidation (Min and Boff, 2002; Dobarganes and Marquez-Ruiz, 2006).

Heating of oil produces various chemical changes including oxidation. The chemical mechanism of thermal oxidation is basically the same as the autoxidation mechanism, but rate of thermal oxidation is faster than the autoxidation, and the unstable primary oxidation products, hydroperoxides, are decomposed rapidly into secondary oxidation products such as aldehydes and ketones (Choe and Min, 2009; Min and Choe 2007).

Decomposition of lipid hydroperoxides constitutes a very complicated process and produces a multitude of materials that may have biological effects and cause flavor deterioration in fat-containing foods (Frankel, 1984). Thermal oxidation of oil produces many volatiles and non-volatiles. Volatiles such as aldehydes, ketones, short-chain hydrocarbons, lactones,

alcohols, and esters are produced from decomposition of hydroperoxides by the same mechanisms as the autoxidation is shown in Figure 13. The most likely decomposition pathway of hydroperoxide is the cleavage between the oxygen and the oxygen of the R-O-O-H, i.e., R-O-O-H → R-O + O-H instead of R-O-O-H → R-O-O + H. (Min and Boff, 2002) reported that the activation energy of the cleavage of R-O-O-H was 44 kcal/mol, compared with the cleavage between the oxygen and hydrogen of R-O-O-H, which has high activation energy (90 kcal/mol). Therefore, the hydro-peroxide groups are cleaved by homolysis to yield an alkoxy and a hydroxy radical, as shown in (Figure 13).

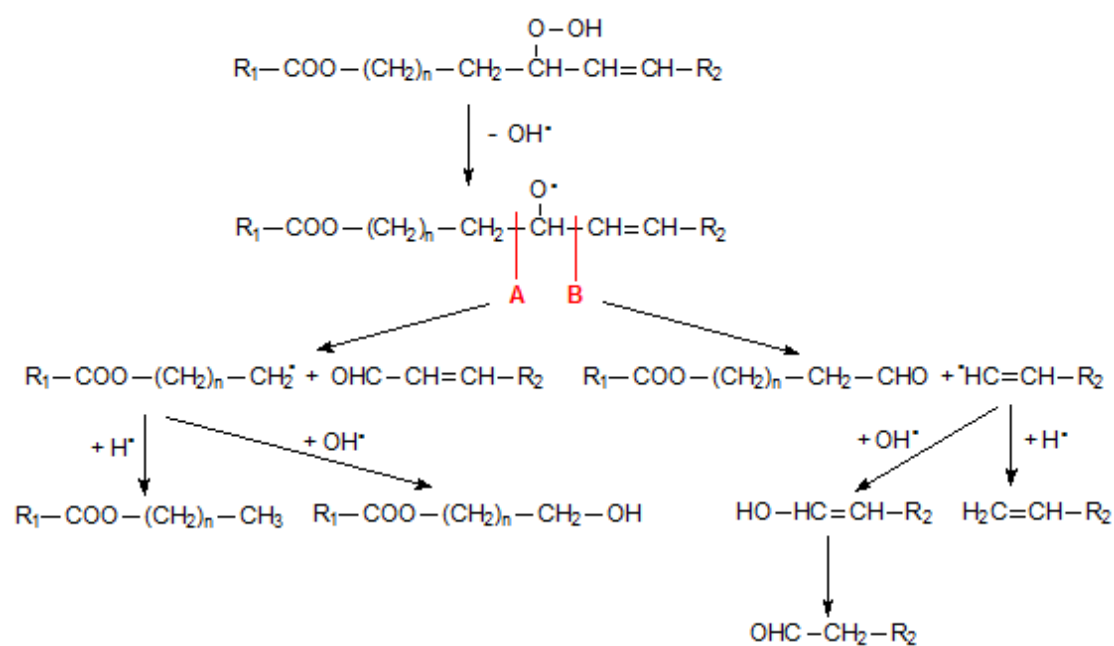


Figure 13: Decomposition of hydroperoxides to produce volatile and short – chain compounds (Min and Boff, 2002; Shahidi and Wanasundara, 2002)

The alkoxy radical formed from hydroperoxide is cleaved by the hemolytic β - scission of a carbon–carbon bond to produce oxo compounds and an alkyl or alkenyl radical. The hemolytic β - scission is an important free radical reaction that produces volatile compounds in edible oils during

oxidation (Dobarganes and Marquez-Ruiz,1996). The unsaturated alkoxy radical can be cleaved by β - scission in two mechanisms of cleavage (A and B of Figure 13). Scission of the carbon–carbon bond on the side of the oxygen-bearing carbon atom will result in formation of unsaturated oxo compounds and an alkyl radical, while scission of the carbon–carbon bond between the double bond and the carbon atom bearing the oxygen will produce a 1-olefin radical and an alkyl oxo compound (Min and Boff, 2002; Dobarganes, 2009).

1.1.4.1.5. Polymerization

There are two types of polymers formed in the fryer oil (Nawar, 1985).These include: Oxidative polymers and Thermal polymers. Oxidative polymers are formed in autoxidation when the free radicals terminate each other as under autoxidation. When a triacylglycerol molecule breaks down during autoxidation, the partial triacylglycerol molecules are not removed in the deodorization process and can react with each other, forming dimers, trimers, or polymers.

These oxidative polymers do not always impart an off flavor to the freshly fried food. However, an off flavor in the packaged product might be observed within a few days after production and may exhibit oxidized or rancid flavor in the product before expiration of the code date on the package. This is because of the following events that might occur; the oxidative polymers are strong free radicals and can decompose while the fried product is in storage. Some of oxidative polymer molecules may contain a higher amount of oxygen than the triacylglycerol molecule. When these oxidative polymers decompose, they produce free radicals and release some oxygen (Frankel, 1985). The free radicals and the released oxygen can continue the autoxidation process in the product during storage.

Polymerization of oil occurs under heat with or without the presence of oxygen. Heat can cleave the oil molecule or fatty acid. These cleaved compounds can then react with each other, forming large molecules. These polymers are referred to as thermal polymers. In the frying processes excessive fryer heat and excessive fryer down time can produce high levels of thermal polymers. Thermal polymers can be detected in the fresh product by expert panelists because they generally impart a bitter aftertaste to the fried food. Volatile compounds are extremely important to the flavor qualities of frying oil and fried foods, but volatile contents in total decomposition products of frying oil are present at the concentration of part per million levels (Nawar, 1985). The major decomposition Products of frying oil are nonvolatile polar compounds and triacylglycerol dimers and polymers. The amounts of cyclic compounds are relatively small compared to the nonvolatile polar compounds, dimers, and polymers (Frankel *et al*; 1984; Sanchez-Muniz *et al*; 1993; Takeoka *et al*; 1997; Dobarganes *et al*; 2000).

The most likely decomposition pathway of hydro peroxide is the cleavage. Many nonvolatile polar compounds and triacylglycerol dimers and polymers are produced in thermally oxidized oil by radical reactions. Dimerization and polymerization are major reactions in the thermal oxidation in oil. Dimers and polymers are large molecules with a molecular weight range of 692 to 1600 Daltons and formed by a combination of $-C-C-$, $-C-O-C-$, and $-C-O-O-C-$ bonds (Min and Choe, 2007).

Dimers or polymers are either acyclic or cyclic depending on the reaction process and kinds of fatty acids consisting of the oil (Takeoka *et al*, 1997; Tompkins and Perkins, 2000). Dimerization and polymerization in deep-fat frying are radical reactions. Allyl radicals are formed preferably at methylene carbons α to the double bonds. Dimers are formed from the

reactions of allyl radicals by C–C linkage. The formation of acyclic polymers from oleic acid during heating is shown in Figure 14. Triacylglycerols react with oxygen and produce alkyl hydroperoxide (ROOH) or dialkyl peroxides (ROOR). They are readily decomposed to alkoxy and peroxy radicals by RO-OH and ROO-R scission, respectively. Alkoxy radicals can abstract hydrogen from oil molecule to produce hydroxy compounds, or combine with other alkyl radicals to produce oxydimers. Peroxy radicals can combine with alkyl radicals and produce peroxy dimers (Figure 14). Formation of dimers and polymers depends on the oil type, frying temperature, and number of frying. The oil rich in linoleic acid is more easily polymerized during deep-fat frying than the oil rich in oleic acid (Tompkins and Perkins, 2000; Bastida and Sanchez-Muniz, 2001).

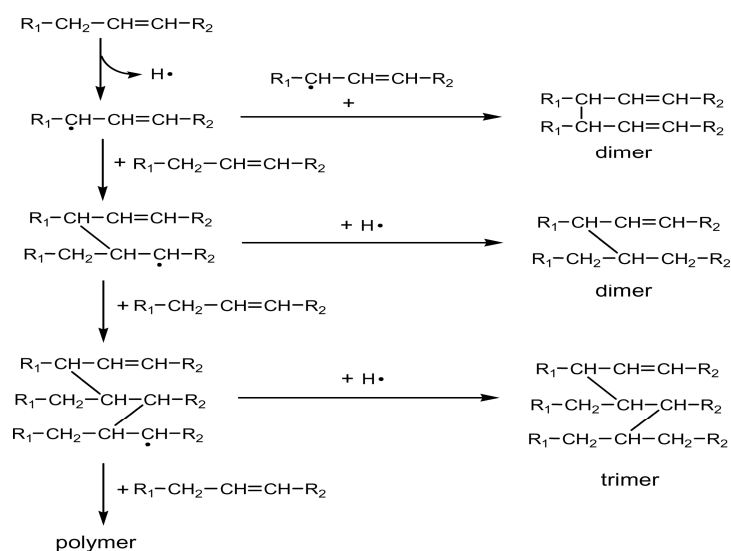


Figure 14: Acyclic polymer formation from oleic acid during deep-fat frying (Min and Choe, 2007).

Cyclic polymers are produced within or between triacylglycerols by-radical reactions and the Diels-Alder reaction (Figure 15). The formation of cyclic compounds in frying oil depends on the degree of unsaturation and the frying temperature. The formation of cyclic monomers and polymers

increased as the amount of linolenic acid increased (Tompkins and Perkins, 2000). The formation of cyclic monomers was negligible until the linolenic acid content exceeds about 20%. Cyclic compounds are not formed to a significant extent until the oil temperature reaches 200°C to 300°C. Soybean oil produced tricyclic dimers and bicyclic dimers of linoleate as well as cyclic monomers during deep-fat frying (Christopoulou and Perkins, 1989).

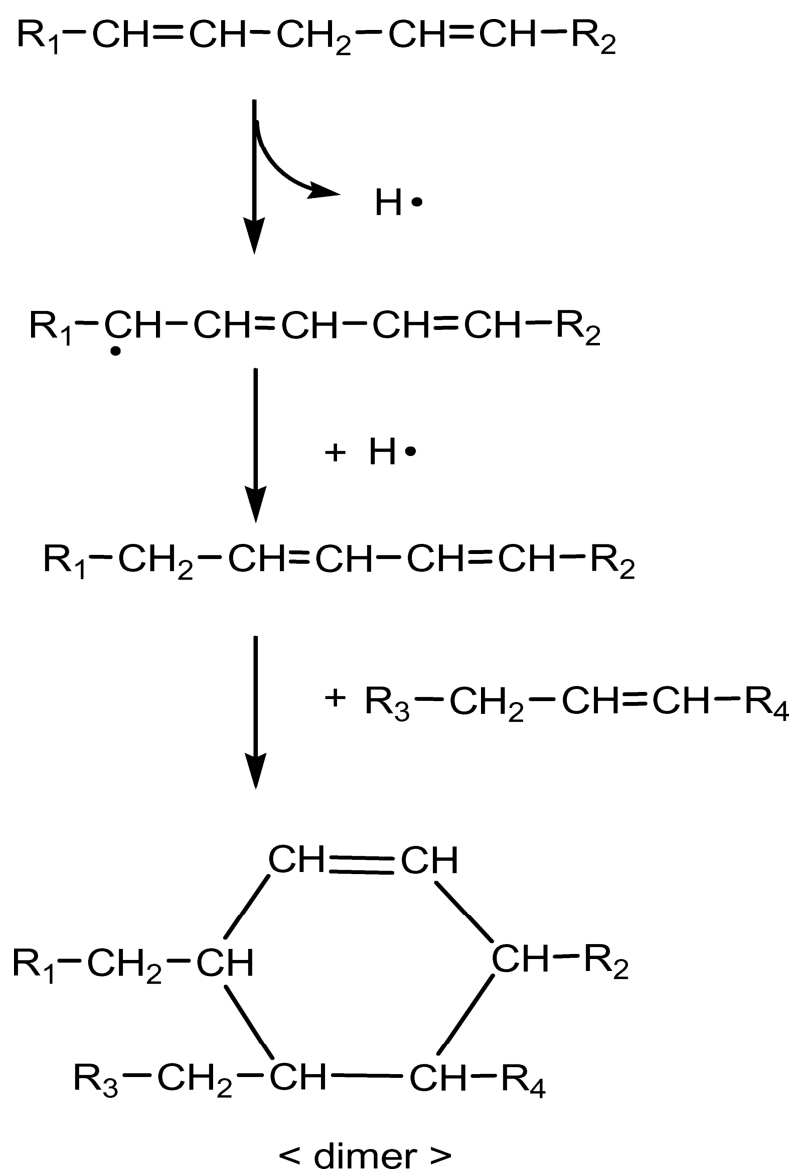


Figure 15: Cyclic compound formation from linoleic acid by Diels-Alder reaction during deep-fat frying (Min and Choe, 2007).

Polymers formed in deep-fat frying are rich in oxygen. (Yoon *et al*; 1988) reported that oxidized polymer compounds accelerated the oxidation of oil. Polymers accelerate further degradation of the oil, increase the oil viscosity (Tseng *et al*; 1996), reduce the heat transfer, produce foam during deep-fat frying, and develop undesirable color in the food. Polymers also cause the high oil absorption to foods.

Polymers are highly conjugated dienes and produce a brown, resin-like residue along the sides of the fryer, where the oil and metals come in contact with oxygen from the air. Resin-like residue is often produced when the oil does not release the moisture but keeps it trapped while also incorporating air (Lawson, 1995; Moreira *et al*; 1999).

Analysis of primary oxidation products, such as hydroperoxides, at any point in the frying process provides little information because their formation and decomposition fluctuate quickly and are not easily predicted (Choe and Min, 2009). During frying, oils with polyunsaturated fatty acids, such as linoleic acid, have a distinct induction period of hydroperoxides followed by a rapid increase in peroxide values, then a rapid destruction of peroxides. Measuring levels of polyunsaturated fatty acids, such as linoleic acid, can help determine extent of thermal oxidation (Warner, 2002) reported that oxidative degradation produced oxidized triglycerides containing hydroperoxide, epoxy, hydroxy, and keto groups and dimeric fatty acids or dimeric triglycerides. carbonyl groups, and -C-O-C- and -C-O-O-C- linkages (Dobarganes and Marquez-Ruiz, 2006).

1.1.4.1.6. Formation of cyclic fatty acids during Thermal oxidation

Cyclization can occur in both the fatty acyl chains in TAG and the decomposed fatty acids, as long as the occurrence of C- C. Then epoxy-TAGs, Cyclic fatty acid monomers (CFAM) ring with only carbon atom

also present in the deep-fat frying system. In spite of the low concentration of these cyclic monomers present in the frying products (Romero *et al*; 2000). The suspicious latent biological hazard to the health of consumer is a topic of worth exploring (Flickinger *et al*; 1997). Several vegetable oils have been used to investigate the influence thereof on the formation amount of CFAMs and the results indicated that frying oil with high oleic acid had well frying effect and lower CFAMs yield (Romero *et al*; 2003; Romero *et al*; 2006). It is well known that C- C is the essential for cyclization; however, the degree of cyclization, content and composition of the formed CFAMs during the deep-fat frying course could be varied according to the unsaturation degree, position and configuration of C- C in different unsaturated aliphatic chains of frying oil (Dobson *et al*; 1997; Dobson *et al*; 1996).

The mechanism proposed for the formation of cyclic fatty acids in heated oils involved a free radical reaction. However, if this were indeed to operate; it would probably produce more isomers than were in fact found. A recent publication by (Destailats and Angers, 2005) suggested an explanation that is more likely, i.e. thermally induced and prototropic migrations. As an illustration, the mechanisms for formation of two of the isomers from α -linolenate are shown in (Figure 16).

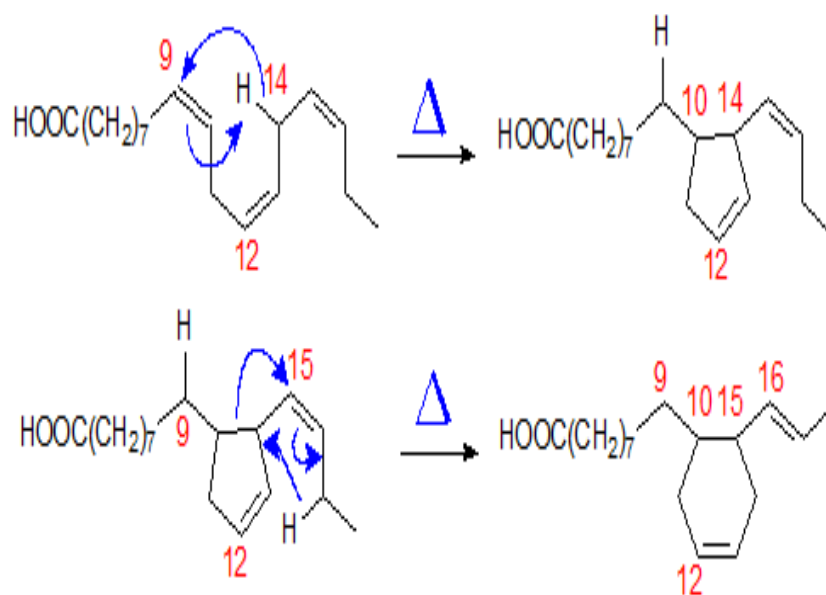


Figure 16: Proposed mechanism for the formation of cyclic fatty acids from α -linolenate (ed. William et al; 20011).

1.1.4.1.7. Tran's isomers

In fact, some of the aforementioned cyclic monomers referred to *cis* /*trans* isomerization belonged to the Trans isomers category. Except the trans cyclic compounds, there were several other kinds of *trans* isomers. It is well known that *trans* isomers of fatty acid have many adverse effects on human health such as coronary heart disease, sudden cardiac death and systemic inflammation (Kummerow, 2009). However, the source of *trans* isomers is very extensive in terms of both raw food materials and food products (Mozaffarian, 2006; Ledoux *et al*; 2007).

During the deep-fat frying, all the breakage, shift and formation of C - C involve the presence of *trans* configuration. Therefore, it is inevitable that the formation of *trans* fatty acid during vegetable oil heating or frying. Trans, trans-2,4-decadienal which related to the induction of low density lipoprotein oxidation was by-produced in fried potatoes (Boskou *et al*;

2006; Andrikopoulos *et al*; 2004). Fortunately, an ordinary frying process in suitable time using un-hydrogenated edible oils has little impact on intake of *trans* fatty acid from edible oils. With the increase of frying time, the amount of *trans* fatty acid increased but decreased when the frying system was added with butylated hydroxyanisole (BHA) or phenolic extracts of dry rosemary (Tsuzuki *et al*; 2010; Tsuzuki *et al*; 2008).

1.1.4.1.8. A free radical

Vegetable oils are largely used in food materials they are either utilized in cooking or frying. When used in frying they are subject to excessive heat. There is a chance for free radical production.

Free radicals can be defined as an atom or groups of atoms with unpaired electron. Such unpaired electrons make these species very unstable and therefore highly reactive with other molecules, due to the presence of pair their electrons and a more stable compound (Defeng and Cederbaum, 2003). There is a lot of controversy on their health effects.

Free radicals are now known to play an important role in many areas of biology and are therefore being actively investigated in connection with various human health problems. Free radicals are in general reactive species that can be of benefit to an organism, e.g.; the radicals produced during phagocytosis, as well as a liability, e.g.; in producing DNA damage, or lipid peroxidation. A hierarchy of free radical reaction exists, which can be exploited by organisms that generate these radicals during normal metabolic processes (Buettner, 1993).

Free radicals play important roles in living systems and have been implicated in the pathology of many human diseases including heart disease, ageing, and cancer, atherosclerosis and cellular damage associated

with ageing. Therefore, the consumption of non-oxidized foods and dietary antioxidants seems to play an important role in protecting against these degenerative events (Steven *et al*; 2003; Lisete Silva *et al*; 2010).

1.1.4.1.8.1. Sources of Free Radicals

Free radicals are formed whenever oil containing unsaturated fatty acids is heated in the presence of a metal initiator, (Nawar, 1985) such as iron, nickel, or copper. Free radicals are formed in the oil during frying. The metal initiator in the frying process can come from several sources such as the food being fried and the oil itself.

Trace metals are present in crude vegetable oils at parts per million (ppm) levels. Researchers have shown that soybean oil flavor can deteriorate from autoxidation, even at iron content as low as 0.3 ppm (List and Ericson, 1985) in the deodorized oil. Metal initiators initiate autoxidation in all vegetable oils and animal fats.

Trace metals in the crude oil are removed in the refining and, primarily, in the bleaching steps (Zschau, 2000; Basiron, 1996). Inadequate bleaching of the oil can leave trace metals at high concentration in the oil. This can promote autoxidation in the fryer. In addition, atmospheric bleaching, poor vacuum in the vacuum bleacher, high temperature in the bleacher, or poor vacuum in the deodorizer can produce free radicals in the fresh oil (Gupta, 1998). These free radicals can rapidly oxidize the oil in a fryer.

It is important to assess the oxidative degradation of fats and oils in the food industry, because free radical initiated oxidation is one of the main causes of rancidity. Free radicals are known to be responsible for the oxidation of food components, resulting in alterations of the major quality-control parameters, such as colour, flavour, aroma and nutritional value of

foodstuffs (Jose *et al*; 2002). Excessive free radical formation, contributing to the onset of certain pathologies, may demand a high dietary intake of fruits, which are rich in antioxidant vitamins and phenolics. That is a good reason to assess the amounts of these compounds in dietary oils and how different technological processes, such as frying, affect their availability.

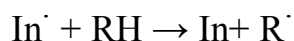
1.1.4.1.8.2. Free Radical Autoxidation

Autoxidation is an important degradation reaction which is attributed to the rancidity of oil and fat. Autoxidation depends on free-radical chain reactions, which involve the interaction of oxygen with free radicals generated at methylene groups adjacent to double bonds, especially at methylene groups between two double bonds (Damodaran, 2003). Autoxidation Access of atmospheric oxygen to unsaturated fatty acids or glycerides leads to deterioration through oxidation. Saturated and monounsaturated fatty chains oxidize very slowly and do not as a rule cause problems. Di -unsaturated chains, as in 18:2 n-6, oxidize more rapidly and polyunsaturated chains very rapidly (Shahidi and Wanasundara, 2002).

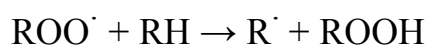
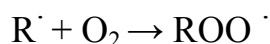
The reaction of oxygen with unsaturated lipids (RH) involves free radical initiation, propagation and termination processes (Qing Zhang *et al*; 2012). Initiation takes place by loss of a hydrogen radical in the presence of trace metals, light or heat. The resulting lipid free radicals (R[•]) react with oxygen to form peroxy radicals (ROO[•]). In this propagation process, ROO[•] react with more RH to form lipid hydroperoxides (ROOH), which are the fundamental primary products of autoxidation (Camelia *et al*; 2009). The primary products of lipid peroxidation are hydroperoxides (ROOH), which can dissociate into free radicals. Lipid hydroperoxides are stable at physiological temperatures, and a major role of transition metals is to catalyze their decomposition. Transition metal ions catalyze homolysis lipid

hydroperoxides that are cleaved to alkylperoxyl radicals (ROO \cdot) by metal ions in the oxidized state such as ferric ion, whereas reduced metal ions, such as ferrous ion, lead to alkoxy radicals (RO \cdot) (Gardner, 1989; Pratt and Porter, 2011). The free radicals produced in these processes are believed to stimulate the chain reaction of lipid peroxidation by abstracting further hydrogen from unoxidized lipids. If α -tocopherol is present in such conditions, it can trap these free radicals and terminate lipid peroxidation (Yamauchi, 2007) Lipid hydroperoxides can be dissociated into free radicals by heat during food processing (Gardner, 1989). α -Tocopherol can suppress further reactions of lipid hydroperoxides by donating a hydrogen atom to the free radicals. It has been reported that α -tocopherol can suppress the thermal decomposition of lipid hydroperoxides and inhibit the formation of volatile and nonvolatile decomposition products (Pratt and Porter 2011; Ned *et al*; 1995).

Initiation (homolytic covalent bonds cleavage)



Propagation (chain propagation)



Termination:

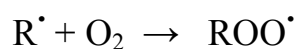


Figure 16: Chain sequence for free radical autoxidation (Shahidi and Wanasundara, 2002).

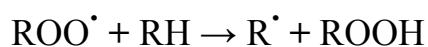
Propagation steps observed in autoxidation may be more complicated than the simple transfer and additions step shown in (figure 16). In general, propagation steps observed in free radical chain processes include radical coupling with oxygen, atom or group transfer, fragmentation, rearrangement, and cyclization (figure 17)

Molecular oxygen is a ground state triplet having two un-paired electrons; consequently, molecular oxygen is di-radical, and reaction with an organic free radical [figure 17 (a)] is essentially a radical- radical coupling process (Ned *et al*; 1995).

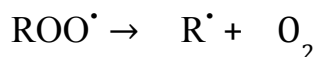
a) Radical coupling



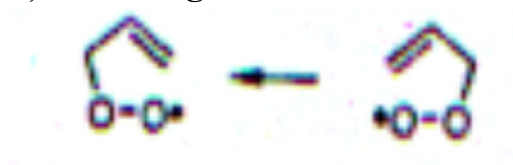
b) Atom transfer



c) Fragmentation



d) Rearrangement



e) Cyclization

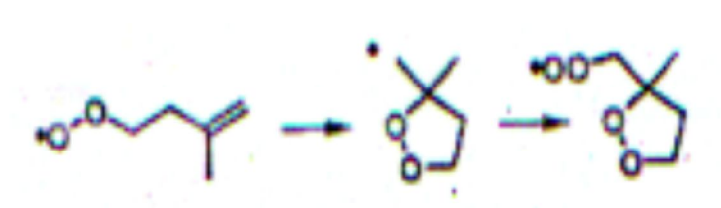


Figure 17: Propagation reactions in autoxidation (Ned *et al*; 1995)

1.1.4.1.9. Rancidity

Rancid oils are a major source of destructive free radicals in our diet. Exposure to air, heat, and light cause oils to oxidize, become rancid, and form free radicals. Saturated fats are not affected much by oxidation because they are very stable have a high degree of resistance to oxidation. Monounsaturated fats, since they have a pair of missing hydrogen atoms are somewhat vulnerable to oxidation. Polyunsaturated oils, which are missing several pairs of hydrogen atoms, are very unstable and highly reactive to oxidation (David, 1970).

Polyunsaturated oils are so vulnerable that even at room temperature and in subdued light oxidation occurs inside the bottle. All polyunsaturated vegetable oils sold at grocery stores have become rancid to some degree before you even bring them home. Because the oils have been highly refined and deodorized you can't smell or taste anything, but the free radicals are there, waiting to attack your body (Hamilton, 1994).

Fats undergo changes storage which result in the production of an unpleasant taste and odour, which is commonly referred to as rancidity. Rancidity is brought by action of air (oxidative rancidity) or by micro-organisms (ketonic rancidity). Oxidative rancidity is accelerated by exposure to heat and light, by moisture and by the presence of traces of certain metals (e.g. copper, nickel, iron). In general, the greater the degree of unsaturation (the higher the iodine value) the greater is the liability of the fat to oxidative rancidity. When the concentration of 'peroxides' reaches a certain level, complex chemical change occur and volatile products are formed which are mainly responsible for the rancid taste and odour (Bailey's, 2005).

1.1.5. Antioxidants

Edible oils naturally contain antioxidants such as α -tocopherols, tocotrienols, carotenoids, phenolic compounds, and sterols. Antioxidants are sometimes intentionally added to oil to improve oxidative stability. Antioxidants are compounds that extend the induction period of oxidation or slow down the oxidation rate. Antioxidants scavenge free radicals such as lipid alkyl radicals or lipid peroxy radicals, control transition metals, quench singlet oxygen, and inactivate sensitizers (Qing Zhang *et al*; 2012).

Antioxidants can donate hydrogen atoms to free radicals and convert them to more stable non-radical products (Dobarganes, 2009). The major hydrogen-donating antioxidants are monohydroxy or polyhydroxy phenolic compounds with various aromatic ring substitutions. Any compound whose reduction potential is lower than that of a free radical can donate hydrogen to that radical unless the reaction is kinetically unfavorable. Standard 1-electron reduction potentials of alkoxy, peroxy, and alkyl radicals of polyunsaturated fatty acids are 1600, 1000, and 600 mV, respectively (Min and david, 2006). The standard reduction potential of antioxidants is generally 500 mV or below. This clearly shows that antioxidants react with lipid peroxy radicals before the peroxy radicals react with other lipid molecules to produce another free radical. Any antioxidant radical produced from the reaction with lipid peroxy radical has lower energy than the lipid peroxy radical itself due to resonance structure.

Metal chelators such as phosphoric acid, citric acid, ascorbic acid, and EDTA (ethylenediaminetetraacetic acid) decrease oil oxidation in an indirect way. They can convert iron or copper ions into insoluble complexes or can sterically hinder the formation of the complexes between metals and lipid hydroperoxides.

Some antioxidants quench $^1\text{O}_2$ or excited sensitizers. $^1\text{O}_2$ is quenched physically and chemically. In physical quenching, $^1\text{O}_2$ is converted into $^3\text{O}_2$ by either energy transfer or charge transfer, and there is no oxidation of antioxidants. In chemical quenching, antioxidants react with $^1\text{O}_2$ and produce oxidized antioxidants (Min and Boff 2002).

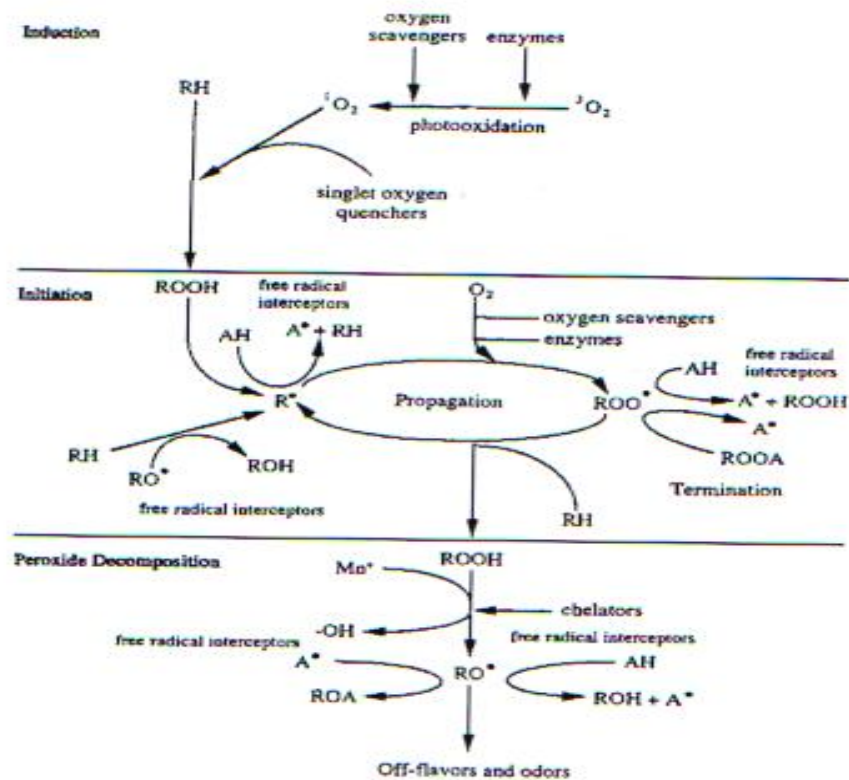


Figure 18: Overview of lipid oxidation and the interaction of antioxidants (Reische *et al*; 2002).

Singlet oxygen is a high energy molecule that is responsible for photooxidation of unsaturated fats and the subsequent generation of hydroperoxides. Singlet oxygen quenchers deplete singlet oxygen of its excess energy and dissipate the energy in the form of heat. Carotenoids, including β -carotene, lycopene, and lutein, are active singlet oxygen quenchers at low oxygen partial pressure. Figure 18 gives an overview of lipid oxidation and the interaction of antioxidants (Reische *et al*; 2002).

1.1.5.1. Prevention of Frying Oil Deterioration

The oxidation reaction can be inhibited by antioxidants that are naturally Present in the oils or added in order to increase the stability. Tocopherols and tocotrienols are natural antioxidants present in vegetable oils. Some Synthetic antioxidants are also available for increasing oil stability (Nawar, 1996). Antioxidant preparations typically contain mixtures of phenolic antioxidants, a synergist, and a solvent system. Some of the most important synthetic antioxidants are discussed in the following Structures are provided in Figure 19.

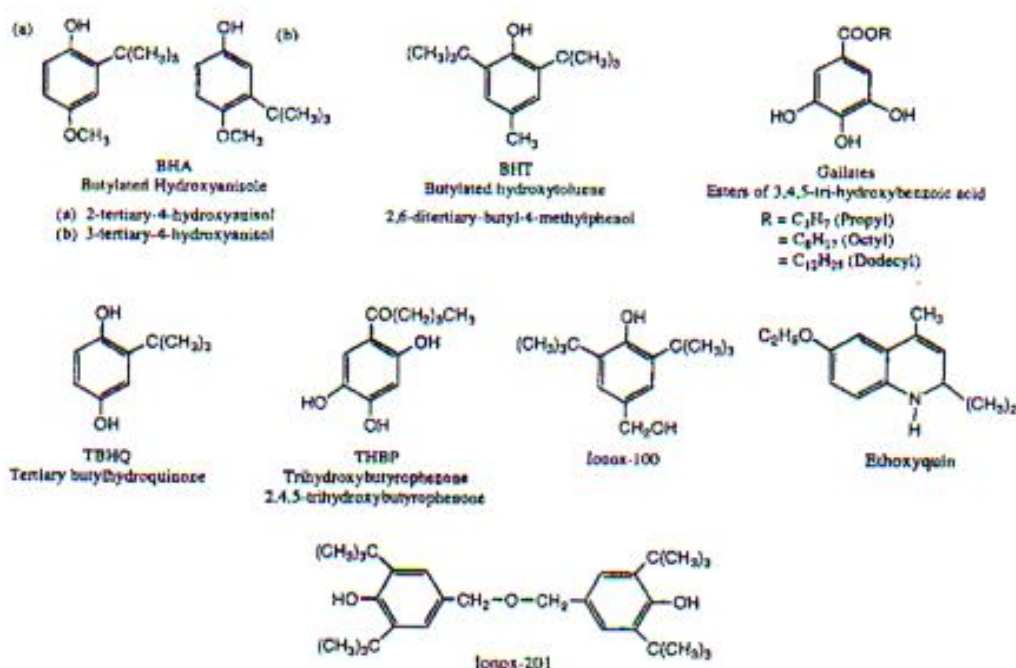


Figure 19: Structures of some synthetic antioxidants (Reische et al; 2002).

Antioxidants delay the oxidation reaction and inhibit the formation of free radicals by donating their phenolic hydrogen atoms to the free radicals. Investigations on the addition of tocopherols or tocotrienols to increase oil-stability at high temperature has shown that the antioxidant effect of these

compounds depends mainly on the fatty acid composition and the type and content of tocopherols in the oil (Wagner and Elmadfa, 2000; Wagner *et al*; 2001). The addition of tocopherols at high concentrations can act as a pro-oxidant and decrease the stability of fats or oils (Nogala-Kalucka *et al*; 2005).

Aim and objectives

It is important to assess the oxidative degradation of fats and oils in the food industry, because free radicals initiated oxidation is one of the main causes of rancidity. Free radicals are known to be responsible for the oxidation of food components, resulting in alterations of the major quality-control parameters, such as physiochemical properties, flavour, aroma and nutritional value of foodstuffs therefor the objectives of this study as following:

- 1) To detect and determine quantitative indicating products of free radical reactions of multiple frying using vegetable oils.
- 2) To Compare conventional (sunflower & groundnut) oils with non-conventional (*moringa oleifera*) oil, with regard to food-making.
- 3) To investigate the effects of free radical formation during multiple frying of vegetable oils and their physiochemical properties.

2. Materials and Methods

2.1. Materials

2.1.1. Chemicals

- . n-Hexane, LABA chemie PVT, LTD. Mumbai, India
- . Chloroform, 95.5%, LOBA chemie PVI, LTD. Mumbai, India
- . Petroleum ether 60-80°C, Avonchem, United Kingdom petroleum ether 60-80°C.
- . Methanol, 99.8%, Scharlau, made in the European Union - Spain.
- . Ethanol, 99%, Carl Roth + Co. KG, Germany.
- . Glacial acetic acid, Analar BDH Chemicals Ltd Blackpool, England.
- . Sulphuric acid, 98%, LR Company.
- . Potassium Hydroxide, 85%, Scharlau made in European Union-Spain.
- . Sodium Sulphate anhydrous, 95%, Metlap supplies Ltd, Hawarden Industrial park, Manon Lane, Clwyd, UK.
- . Sodium hydroxide, Scharlau made in European Union-Spain
- . Sodium thioSulphate anhydrous, 95%, Metlap supplies Ltd, Hawarden Industrial park, Manon Lane, Clwyd, UK.

2.1.2. Equipment

- **Fryer**

Electric fryer (Nikura corporation, Osaka, Japan)

- **Tintometer**

Tintometer type D, Made by: The Tintometer LTD, Salisbury England.

- **Refractometer**

Abbe 60 refractometer as double prism, Made in Japan

- **Viscometer**

Ostwald –U- tube viscometer (Made in France).

- **FTIR**

Fourier Transform Infrared Spectrophotometer (Model FTIR-8400S). Made in Japan (SHIMADZU).

- **Ultraviolet –visible spectrophotometry**

UV Spectrophotometer (Double Beam) Model UV-1800, (SHIMADZU).

- **GC**

Gas Chromatograph (Model GC- 2010), Made in (SHIMADZU).

- **Soxhlet extractor**

2.2. Methods

2.2.1. Sampling

Refined sunflower and ground-nut oils obtained from Arab-Sudanese Company for edible oils were sampled according to standard methods of sampling of liquids to give true representative samples.

2.2.2. Extraction of *moringa oleifera* seed oil

Crude vegetable oils and fat were obtained from oil bearing seeds and fruits, etc; by either method; solvent extraction and mechanical pressing.

2.2.2.1. Solvent extraction of *moringa oleifera* seed oil

Moringa oleifera seed oil was extracted from the seeds. The seeds were obtained from Seeds Center, Soba (Khartoum) with n-hexane, and petroleum ether (60-80°C), in Soxhlet apparatus for 6 h following the AOACS method. Solvent was removed by vacuum evaporation at 60° C for 1 h. The oil that stored at -4° C was used for frying.

2.2.2.2. Mechanical pressing of *moringa oleifera* seed oil

The extraction of the oil from *moringa oleifera* seeds was done in the manner described by (Balla, 2001) with minor modification. About 10 Kilogram seeds were weighed after removal of impurities, using mortar and pestle, the size of the seeds was reduced to increase the surface area for oil

extraction. The sample was transferred to cloth bag, and then the oil was extracted from seeds using hydraulic press in (Omdurman Industrial).

Adopting two frying techniques lead to increase the number of samples.

2.2.3. Frying Procedure

2.2.3.1. Frying for 6h

For each experiment 2 dm³ of oil was put into an electric fryer and heated to 175 ± 5 °C within 10 min .After each 1 h; 100g, 95g, 90g, 85g, 80g and 75g, respectively, of potatoes (5×5 mm in cross- section and 7 cm in length) were introduced into the hot oil and fried for 5 min .The oil was kept hot for 1 h before the next frying was carried out. Six batches of potatoes were fried within 6 h on one day for each oil. The total temperature load of the oils was 6 h. At the end each batch the oil was cooled, 100 cm³ was stored at - 4 °C until the day of analysis.

2.2.3.2. Frying for 36h

Frying was carried out following (Mariod *et al*; 2006) with some modifications. For each experiment 2 L of oil was put into an electric fryer and heated to 175 ± 5 °C within 10 min .After 1 hour, 100g of potatoes (4×4 mm in cross- section and 6 cm in length) were introduced into the hot oil and fried for 5 min .The oil was kept hot for 1 hour before the next frying was carried out. Each day for 6 h, six batches of potatoes were fried within 6 hours for each oil was used only for 6days, because the oil was no longer usable for deep-frying after that time .The total temperature load of the oils was 36 hours. At the end of each day the oil was cooled, 200 cm³ was stored at - 4° C until the day of analysis. The fryer was cleaned without removing any adhering gum and topped up with 200 cm³ of fresh oil ready for the next trial (Mariod *et al*; 2006).

2.2.4. Statistical analysis

Values represented are the means and standard deviations for three replicates. Statistical analysis was carried out by ANOVA using SPSS system. Significance was defined at $P < 0.05$, $P=0.000$ (high) used Lisd and Duncan's multiple range tests.

2.2.5. Physical Analysis of Oils

2.2.5.1. Viscosity (pa.s)

The viscosity is usually estimated by comparing the length of time it takes a given volume of oil (or melted fat) to flow through a tube of small bore, or through a small orifice, with the time it takes an identical volume of water.

The viscosity of the oil sample was detected using an Ostwald –U- tube viscometer. According to (Cocks and Van, 1966) the viscometer was suspended in a constant temperature bath ($32\pm 2^{\circ}\text{C}$) So that the capillary was vertical. The instrument was filled to the mark at the top of the lower reservoir with the oil by means of pipette inserted into the side arm, so that the tube above the mark was not wetted. the instrument was then left to stand for few minutes before reading in order to equilibrate the sample temperature with that of the instrument ($32\pm 2^{\circ}\text{C}$), By means of the pressure on the respective arm of the tube, the oil was moved into the other arm so that the meniscus was 1cm^3 above the mark at the top of the upper reservoir. The liquid was then allowed to flow freely through the tube and the time required for the meniscus to pass from the mark above the upper reservoir to that at the bottom of the upper reservoir to that at the bottom of the upper reservoir was recorded.

Calculation

$$\text{Viscosity of the oil} = \frac{T - T_o}{T_o}$$

Where

T: flow- time of the oil

To: flow-time of the distilled water.

2.2.5.2. Refractive index (RI)

The Refractive index of a substance is the ratio of the speed of light in a vacuum to the speed of light in the substance. For practical measurements the scales in the instrument indicate refractive indexes with respect to air rather than vacuum.

The refractive index (RI) was determined by Abbe 60 refractometer as described by the AOAC method (1990). A double prism was opened by means of screw head, few drops of oil were placed in prism. Then was closed firmly by tightening the screw head and the instrument was then left to stand for few minutes before reading in order to equilibrate the sample temperature with that of the instrument ($32 \pm 2^\circ\text{C}$). The prisms were cleaned between readings by wiping of the oil with soft cloth, then with petroleum ether and left to dry. The test was repeated three times.

2.2.5.3. Colour

This method determines the colour by comparison with glasses of known colour characteristics. The colour intensity of oils was recorded using a Lovibond Tintometer as units of (red, yellow and blue) in the manner described by (Balla, 2001). Samples of oils were filtered through filter paper immediately before testing. An appropriate 1 inches was filled with oil and placed in the tintometer in specific place. The instrument was

switched on and looked through the eyepiece. And then slides were adjusted until a colour match was obtained. The values obtained by matching were recorded as red and yellow.

2.2.6. Chemical Analysis of Oils

2.2.6.1. Free fatty acid

This method determines the free fatty acids existing in the sample. (m/m % expressed as Oleic, Palmitic or Lauric Acid).

Free fatty acid determined according to the AOAC method (2000; 1989). 7 g of cooled oil sample was weighed in a 250cm³ conical flask and 50 cm³ to 100 cm³ of freshly neutralized hot ethyl alcohol was added followed by about 1 cm³ of phenolphthalein indicator solution; the mixture was warmed for 5 minutes and was titrated while hot against standard alkali solution (Na OH), shaking vigorously during the titration. The weight of the oil was taken for the estimation and the strength of the alkali used for titration shall be such that the volume of alkali required for the titration must not exceed 10ml

$$\text{Free fatty acids oleic acid} = \frac{28.2 \times v \times N}{W}$$

Percent by weight

Calculation

Where:

V: Volume in ml of standard sodium hydroxide used

N: Normality of the sodium hydroxide solution

W: Weight in g of the sample

2.2.6.2. Peroxide value

Peroxide value (PV) is one of the methods for determination of hydroperoxides as the initial lipid oxidation products. The PV is expressed as mill equivalents oxygen per kg of fat/oil (Nawar, 1996).

The peroxide value (PV) of the oil sample was determined according to the AOAC method (2000) 5gm of the sample were weighed into a 250 cm³ stopper conical flask. 30 cm³ of acetic acid chloroform solvent mixtures were added and swirled to dissolve 0.5 cm³ saturated potassium iodide solution was added with a Mohr pipette, stood for 1 min in dark with occasional shaking, and then about 30 cm³ of water was added. Slowly the liberated iodine was titrated with 0.1N sodium thiosulphate solutions, with vigorous shaking until yellow colour was almost gone. 0.3ml starch solution as indicator was added and titration was continued with vigorous shaking to release all Iodine gas from CHCl₃ layer blue colour disappeared. If less 0.5 cm³ of 0.1 N Na₂S₂O₃ was used 0.01 N Na₂S₂O₃ was used to repeat the titration. Blank determination (must be less than 0.1 cm³ 10.1 N Na₂S₂O₃) was conducted AOCS method (1990).

Peroxide value expresses as mille equivalent of peroxide oxygen per kg sample (meq per Kg oil)

Calculation:

$$\text{peroxide} = \frac{\text{Titre} \times N \times 1000}{\text{Weight of the sample used}}$$

Where:

Titer = cm³ of sodium thiosulphate used (blank corrected)

N= Normality of sodium thiosulphate solution

2.2.7. Instrumental Techniques

2.2.7.1. Gas chromatography (GC)

Gas chromatographic methods have enabled used to determine the structure and amounts of the short-chain bound compounds in model systems of methyl oleate and linoleate, thermo-oxidized oils and used frying fats of different levels of degradation (Shahidi and Wanasundara 2002).

2.2.7.1.1. Determination of fatty acid composition

Total fatty acids were methylated according to (Abdulkarim *et al*, 2007). About 2cm³ of three oils were dissolved in 6cm³ of a solution of 2 N KOH in methanol and then 2 cm³ of n-hexane were added. The mixture was vigorously shaken with a vortex for 2 min, sodium sulfate was added and the mixture was shaken again. The sample (0.4μl) was injected 10 min later into a gas chromatograph (Model GC- 2010) equipped with a split-split less injector and a flame ionization detector. A DB-225, 30 x 0.25 mm ID and 0.15 μm column (J and W Scientific, Agilent) was used. The injector and detector temperatures were set at 250°C. The oven temperature was kept at 190°C for 1 min, then programmed from 190-210°C at 4°C min⁻¹, kept at 210°C for 5 min, then heated from 210-215°C at 3°C min⁻¹ and finally kept 18 min at the last temperature. Nitrogen was used as carrier gas at a flow rate of 1.0 mL min⁻¹. The

Peak identification was carried out by comparing the peak retention time with those of the standard mixture. An internal standard was used for the quantification of fatty acids. The GC response factor of each fatty acid was calculated by using the internal standard. The results were expressed as g fatty acid/100 g total fatty acids (%).

2.2.7.2. Fourier transform-infrared (FTIR) analysis

Vibrational spectroscopy, such as Fourier transform-infrared (FTIR) has been used to screen oils, providing data on fatty acids composition or free acidity in a few minutes (Alfred and Christy, 2006; Armenta and Guardia, 2007). Fourier transform infrared (FTIR) spectroscopy to assess the oxidative status or forecast the oxidative stability of oils (Shahidi and Wanasundara, 2002).

2.2.7.2.1. Sample preparation for FTIR analysis

5 μ of oil was placed between two plates of sodium chloride (salt). The plates were transparent to the infrared light. The drop formed a thin film between the plates, and then the plates were taken to FTIR instrument.

2.2.7.3. Ultraviolet –visible spectrophotometry

Spectrophotometric ultraviolet (UV) methods are among the oldest techniques for the analysis of oxidized lipids. Compounds showing an absorption maximum in the ultraviolet (UV) region usually contain one or several conjugated C=C, C=O, or C \equiv N double bonds. The position of the absorption maximum is shifted to higher wavelengths when the number of conjugated double bond increases (Farmer and Sutton, 2002).

2.2.7.3.1. Conjugated diene content determination

All frying oils were analyzed in duplicate for conjugated diene according to the modified version of the AOCS Official Method Ti 1a-64 (1980). In the modified AOCS method, a sample size of 10 μ was diluted to 5 cm³ and no additional dilutions were made. The conjugated diene content was determined at 233 nm, in (Duple Beam), using UV Spectrophotometer (Duple Beam) Model UV-1800 and was then converted to % conjugated dienoic acid.

3. Results:

Table (3.1) - (3. 14) show the changes in physiochemical properties, Gas chromatographic determination of Fatty acid composition (%) and FTIR spectra for sunflower, groundnut refined and *moringa oliefera* extracted vegetables oils respectively for six hours on one day and 36 hours on six days of frying oils at $175 \pm 5^\circ\text{C}$.

Table (3. 1)

Viscosity (m Pas) of different types of vegetable oil during frying at $175 \pm 5^\circ\text{C}$ for 6 h

Hours of frying	0	1	2	3	4	5	6
Sunflower oil	20.4±0.5	21.89±0.13	24.08±0.11	27.01±0.12	29.19±0.17	32.12±0.15	35.04±0.10
Groundnut oil	19.71±0.09	21.17±0.11	22.26±0.11	22.63±0.10	23.36±0.13	24.09±0.15	25.55±0.11
<i>Moringa Oleifera</i> seed oil	43.00	43.01±0.02	44.21±0.02	45.50±0.12	49.05±0.17	53.17±0.13	53.32±0.15

Mean values within each row followed by different letters (sunflower, groundnut and *moringa oliefera*) oils are significantly ($P < 0.05$) different.

Table(3.2)

Viscosity (s) of different types of vegetable oil during frying at $175 \pm 5^\circ\text{C}$ for 36h.

Hours of frying	0	1	2	3	4	5	6
Sunflower oil	20.40±0.15	25.23±0.03	31.19±0.04	36.01±0.12	41.45±0.15	47.31±0.13	53.46±0.20
Groundnut oil	19.71±0.03	23.63±0.07	28.55±0.13	32.49±0.03	36.01±0.04	41.54±0.05	45.55±10.12
<i>Moringa oliefera</i> oil	43.00±0.02	45.03±0.02	48.34±0.03	51.22±0.03	54.32±0.03	56.41±0.03	59.33±0.03

Mean values within each row followed by different oils (sunflower, groundnut and *moringa oliefera*) oils are significantly ($P < 0.05$) different (mean= 40.0086, standard deviation=1.2096E1).

Table (3. 3)

Refractive index (RI) of different types of vegetable oil during frying at $175 \pm 5^\circ\text{C}$ for 6h

Hours of frying	·	1	2	3	ε	5	٦
Sunflower oil	1.4650± 0.0002	1.4653± 0.0003	1.4656± 0.0001	1.4659± 0.0003	1.4662± 0.0003	1.4665± 0.0004	1.4670±0.0004
Groundnut oil	1.4625± 0.0002	1.4629± 0.0001	1.4633± 0.0002	1.46637± 0.0003	1.4641± 0.0001	1.4645± 0.0002	1.4649±0.0001
<i>Moringa oliefera</i> oil	1.4662± 0,0001	1.4663± 0.0002	1.4664± 0.0002	1.4665± 0.0001	1.4666± 0.0003	1.4667± 0.0004	1.4668±0.0003

Mean values within each row followed by different oils (sunflowe, groundnut and moringa oliefera) are significantly ($P < 0.05$) different. (mean= 1.4654, stander deviation=0.00138.

Table (3. 4)

Refractive index (RI) of different types of vegetable oil during frying at $175 \pm 5^\circ\text{C}$ for 36h.

Hours of frying	·	1	2	3	ε	5	٦
Sunflower oil	1.4650 ±0.000 2	1.4659 ±0.000 2	1.4664±0 .0001	1.4667±0 .0003	1.4673± 0.0002	1.4678± 0.0004	1.4682± 0.0005
Groundnut oil	1.4625 ± 0.0001	1.4635 ± 0.0002	1.4646±0 .0001	1.4654±0 .0003	1.4662±0 .0002	1.4667± 0.0005	1.4677± 0.0004
<i>Moringa oliefera</i> oil	1.4662 ± 0.0003	1.4664 ± 0.0002	1.4666±0 .0001	1.4668±0 .0003	1.467± 0.0003	1.4673± 0.0001	1.4675± 0.0003

Mean values within each row followed by different oils (sunflowe, groundnut and *moringa oliefera*) are significantly ($P < 0.05$) different (mean= 1.4664, stander deviation=0.00145.

Table(3. 5)

Color of different types of vegetable oil during frying at 175± 5°C for 6 h.

Hours of frying		0	1	2	3	4	5	6
Sunflower Oil	Yellow	3.0	3.2	4.5	5.3	6.4	7.3	8.1
	Red	0.4	0.5	0.6	0.7	0.9	1.2	1.6
Groundnut Oil	Yellow	1.5	1.6	1.6	2.1	3.7	4.6	5.3
	Red	0.3	0.3	0.3	0.4	0.7	0.9	1.2
<i>Moringa oleifera</i> seed Oil	Yellow	30.3	30.4	31.1	34.2	35.2	37.0	39.1
	Red	3.4	6.5	9.0	10.3	12.8	16.1	18.1

Table (3. 6)

Color of different types of vegetable oil during frying at 175± 5°C for 36h.

Hours of frying		0	6	12	18	24	30	36
Sunflower Oil	Yellow	3.1	3.3	7.1	15.0	23.0	31.0	34.0
	Red	0.4	1.7	2.3	5.7	7.23	8.14	9.62
Groundnut Oil	Yellow	15	3.5	5.7	8.3	10.5	11.4	12.7
	Red	0.3	0.5	2.03	2.92	3.45	5.36	6.77
<i>Moringa oleifera</i> seed oil	Yellow	30.0	35.2	39.6	43.1	46.3	47.6	51.4
	Red	3.4	10.0	13.0	16.5	20.0	22.0	26.0

Table (3. 7)

Free fatty acid (%) of different types of vegetable oil during frying at 175±5°C for 6h

Hours of frying	·	1	2	3	4	5	6
Sunflower oil	0.08± ·.15	0.42± ·.15	0.67±0.29	1.00±0.02	1.4±.01	1.70±0.03	2.01±0.02
Groundnut oil	0.04±0 0.03	0.23± 0.04	0.53±0.13	0.88±0.23	1.21±0.15	1.31±0.25	1.45±0.02
<i>Moringa oliefera</i> oil	.782± o.02	0.85± 0.03	0.94±0.02	1.03±0.04	1.04±0.07	1.25± 0.12	1.35±0.0.02

Mean values within each row followed by different oils (sunflower, groundnut and *moringa oliefera*) are significantly ($P < 0.05$) different. (mean= 0.9264, stander deviation=0.5166.

Table (3. 8)

Free fatty acid (%) of different types of vegetable oil during frying at 175±5°C for 36h

Hours of frying	·	٦	١٢	١٨	٢٤	٣٠	٣٦
Sunflower oil	0.08± ·.15	1.3± ·.25	2.45±0.02	3.74±0.03	4.89±0.03	5.75±0.25	6.89± 0.07
Groundnut oil	0.04± 0.03	1.12± 0.04	1.90±0.02	2.73±0.25	3.87±0.18	4.65±0.25	5.85± 0.04
<i>Moringa oliefera</i> oil	0.782± o.02	1.51± 0.02	2.01±0.03	2.02±0.03	2.05±0.03	2.21± 0.12	2.51± 0.13

Mean values within each row followed by different oils (sunflowe, groundnut and *moringa oliefera*) are significantly ($P < 0.05$) different. (mean= 2.7851, stander deviation=1.9104.

Table (3. 9)

Peroxide value (mg /Kg) of different types of vegetable oil during frying at 175± °C for 6h

Hours of frying	•	1	2	3	ε	5	ϕ
Sunflower oil	3.780± •.15	4.32± •.03	5.78±0.04	7.65±0. 12	8.98± 0.15	10.76± 0.13	12.75 ±0.20
Groundnut oil	2.39± 0.03	3.32± 0.07	10.43±0.13	4.34±0. 03	5.55± 0.04	6.45±0. 05	8.56± 10.12
<i>Moringa oliefera</i> oil	.996± o.02	1.21± 0.02	1.42±0.03	1.74±0. 03	1.94± 0.03	2.14± 0.03	2.39± 0.03

Mean values within each row followed by different oils (sunflowe, groundnut and *moringa oliefera*) are significantly ($P < 0.05$) different (mean= 2.7851, stander deviation=1.9104).

Table (3. 10)-

Peroxide value (mg/Kg) of different types of vegetable oil during frying at 175± 5 °C for 36h

Hours of frying	•	ϕ	ϑ	ϑ	ϑ	ϑ	ϑ
Sunflower oil	3.780± •.15	9.43± •.153	14.53±0.29	20.43±0.03	27.54±1.74	25.35 ±0.28	42.20± 1.82
Groundnut oil	2.39± 0.03	5.97± 0.04	10.43±0.13	16.31±0.23	22.35±0.18	19.32 ±0.25	34.11± 1.05
<i>Moringa oliefera</i> oil	.996± o.02	2.40± 0.02	4.23±0.03	5.89±0.04	6.94±0.07	8.79± 0.12	10.16± 0.13

Mean values within each row followed by different oils (sunflowe, groundnut and *moringa oliefera*) are significantly ($P < 0.05$) different.(mean= 13.977, stander deviation=1.1291E).

Table (3. 11)**Fatty acid composition (%) of different types of vegetable oil during frying at 175± 5°C for 6h**

Fatty acid composition	Frying time(h)	Sunflower oil	Groundnut oil	<i>Moringa oleifera</i> seed oil
Saturated fatty acids	0	13.239	6.734	0.459
	2	13.671	23.041	0.545
	4	14.545	23.999	4.095
	6	68.046	26.365	19.644
Monounsaturated fatty acids	0	10.095	89.292	98.212
	2	11.387	76.959	97.996
	4	14.996	75.669	95.625
	6	12.984	72.375	80.357
Polyunsaturated Fatty acids	0	76.951	3.974	1.330
	2	74.943	-	1.454
	4	70.459	0.332	0.2796
	6	18.972	1.260	-
Unsaturated fatty acids	0	87.046	93.266	99.542
	2	86.330	76.959	99.450
	4	85.455	76.001	95.342
	6	31.956	73.635	80.357

Table (3. 12)**Fatty acid composition (%) of different types of vegetable oil during frying at 175± 5°C for 6h**

Fatty acid composition	Frying time(h)	Sunflower oil	Groundnut oil	<i>Moringa oleifera</i> seed oil
Saturated fatty acids	0	13.239	6.734	0.459
	12	90.664	81.699	82.486
	24	91.409	81.792	87.911
	36	92.001	89.387	90.726
Monounsaturated fatty acids	0	10.095	89.292	98.212
	12	9.570	14.476	14.470
	24	8.660	14.595	11.169
	36	7.977	9.400	9.182
Polyunsaturated Fatty acids	0	76.951	3.974	1.330
	12	0.074	3.834	2.866
	24	0.126	3.723	0.885
	36	0.131	1.214	0.089
Unsaturated fatty acids	0	87.046	93.266	99.542
	12	10.544	18.310	17.336
	24	8.786	17.318	12.054
	36	8.108	10.614	9.261

Table (3. 1۳)

FTIR spectra [area (%)] of different types of vegetable oil during frying at 175± 5°C for 6h

Frying	Frying	Functional	Groups				3002.96 -		
Oils	Time(h)	1460.01	1650.95	1745.46	2845.45	2925.81	3006.82	3471.3	3583.49
Ground nut oil	0	5.32	-	9.12	11.03	29.41	-	-	-
	2	27.643	-	41.213	36.743	51.355	26.7	24.47	3.977
	4	34.64	8.608	54.514	45.845	69.99	40.765	22.107	4.581
	6	40.441	10.056	63.452	50.264	73.158	45.567	26.226	5.07
Sunflower oil	0	-	0.41	23.58	25.51	55.25	6.04	-	-
	2	28.164	9.631	45.666	35.821	44.605	30.126	30.903	4.671
	4	34.543	14.534	57.452	45.24	69.02	33.23	31.56	4.728
	6	57.636	16.317	90.045	77.636	133.263	55.806	34.193	4.991
<i>Moringa oleifera</i> oil	0	0.50	-	8.10	7.90	31.80	-	-	-
	2	3.90	-	13.0	16.20	43.0	-	-	-
	4	8.30	-	17.30	20.50	40.20	-	-	-
	6	20.0	2.47	44.39	43.28	79.80	16.44	1.26	-

Table (3. 14)**FTIR spectra [area (%)] of different types of vegetable oil during frying at 175± 5°C for 36h**

Frying Oils	Frying Time(h)	Functional Groups							
		1460.01	1650.95	1745.46	2845.45	2925.81	3002.96-3006.82	3471.3	3583.49
Groundnut oil	0	5.32	-	9.12	11.03	29.41	-	-	-
	12	37.382	8.90	63.189	55.157	96.818	40.435	22.107	3.48
	24	58.538	11.082	83.2.68	68.607	106.191	51.953	26.794	5.537
	36	72.651	18.585	107.154	94.968	157.749	63.98	29.457	-
Sunflower oil	0	-	0.41	23.58	25.51	55.25	6.04	-	-
	12	40.055	13.932	62.029	48.061	69.83	33.636	27.396	5.981
	24	85.68	17.532	115.298	95.105	128.0	76.819	31.951	-
	36	102.203	28.812	168.964	145.0	152.77	103.5	37.214	-
Moringa oleifera oil	0	0.50	-	8.10	7.90	31.80	-	-	-
	2	19.03	2.0	47.28	44.67	88.89	17.14	3.64	-
	4	28.64	6.82	67.07	60.88	113.51	23.76	6.33	-
	6	18.51	5.18	78.49	77.78	135.51	39.42	4.01	-

4. Discussion

4.1 Physical changes during frying oils

It is important to assess the oxidative degradation of fats and oils in the food industry, because free radicals initiated oxidation is one of the main causes of rancidity. Free radicals are known to be responsible for the oxidation of food components, resulting in alterations of the major quality-control parameters, such as colour, flavour, aroma and nutritional value of foodstuffs (Jose *et al*; 2002).

4.1.1 Changes in color and viscosity

Colour and viscosity are the most common physical parameters used to evaluate the extent of frying oil deterioration in commercial and household frying. Tables (3. 1), (3. 2), (3. 3), (3. 5) and (3. 6) show changes in color and viscosity for sunflower, groundnut refined oils and *moringa oleifera* seed oil respectively for (6h) on one day and (36h) on 6 days of frying. Color change in frying oils is a visual indication of the extent of oil deterioration caused by oxidation. Increase in the color intensity is due to accumulation of nonvolatile decomposition products such as oxidized triacylglycerols and free fatty acid FFA. All the oils darkened during the frying trial, and the rate of darkening is proportional to the frying time. In Tables 5 and 6 show a gradual increase from 1h to 6h for all frying oils. The initial rate of darkening was higher in *moringa oleifera* oil than refined sunflower and groundnut oils due to the presence of natural pigments of *moringa oleifera* oil. Table (3. 6) shows a rapid darkening after 12h and a steady increase in the rate of darkening throughout the rest of the frying trial. It was found that *moringa oleifera* seed oil showed the darkest color at the end of the frying trial followed by Sunflower and groundnut oils, for 36h.

Table (3. 1) and (3. 2) show that the viscosity of all the oils increased with increase of frying time due to the formation of high molecular weight polymers. The more viscous the frying oil, the higher the degree of deterioration. Free radicals formation of dimmers, trimers, epoxides, alcohols and hydrocarbons, during frying the oils all of them contribute to increases in viscosity.

4.1.2. Refractrometry

Table (3. 3) and (3. 4) show changes in refractive index(IR) for sunflower, groundnut oils and *moringa oleifera* seed oil respectively for 6h on one day and 36h on 6 days, of frying. In this work it was found that the refractive index increased showily for all the three oils from 0h to 6h and 36h as indicated by the detection of rancid odors. It was also claimed that the refractive index changes, in accordance with the three known stages of fat autoxidation. In the induction period when peroxide formation was low, the refractive index remained constant. During the secondary stage of relatively more peroxide formation, the refractive index increased sharply until the peroxide value reached a maximum. This increase in refractive index was attributed to conjugation, known to precede hydroperoxide formation. In the tertiary stage of peroxide decomposition, the refractive index continued to increase at a steady rate, the increase being less sharp than in the second stage. Polymerization of partially oxidized fats was thought to be responsible for this change in refractive index.

4. 2. Chemical changes during frying oils

4. 2.1. Changes in free fatty acid content (FFA)

Free fatty acids (FFA) are a measure of the amount of fatty acids hydrolysed on the triacylglycerol backbone. They are used as a chemical marker for monitoring the quality of frying operations (Stier, 2001). This parameter is often used for assessment of the suitability of frying oils for

human consumption and a value of 2% is defined as the limit for oil rejection (Matthaus, 2006).

On the other hand, the amount of FFA in fats and oils can be used to indicate the extent of its deterioration due to hydrolysis of TAG and/or cleavage and oxidation of fatty acid bonds. Although the initial FFA values of the oils were different (0.08, 0.04 and 0.923% for refined sunflower, groundnut oils and *moringa oleifera* seed oil, respectively), at the end of the frying period for 6h on one day, the total changes in FFA values in final day of frying were found to be the lowest in *moringa oleifera* seed oil (1.406%), followed by refined groundnut oil (2.012%), and Sunflower oil (2.354%) as shown in Table (3. 7). Whereas, at the end of the frying period for 36h on six days, the total change in FFA values in the final day of frying were found to be the lowest in *moringa oleifera* seed oil (2.512%), followed by groundnut oil (4.345%), With the highest percentage found in Sunflower oil (5.954%) as shown in Table (3. 8), these free fatty acids contents in frying oil increased with the number of frying.

There are numerous studies that reported changes in fats and oils after refining techniques, (Yoon and Kim, 1994; Warner *et al*; 2003). Most of them conclude that changes in fats and oils after refining techniques could reduce color, unpleasant flavor, and content of free fatty acids in various edible oils. Unfortunately, some of the refining processes have been reported to decrease the contents of natural antioxidants such as tocopherols and oryzanols. Antioxidants are responsible for higher thermal oxidative stability of *moringa oleifera* seed oil, whereas, refined sunflower and groundnut oilshaving a lower antioxidant content, they will be more susceptible to thermal and oxidative degradation during deep-frying operations than *moringa oleifera* seed oil. The increase in free fatty acid values during frying could be mainly due to the hydrolysis of the oils,

although oxidative pathways can also result in the formation of free fatty acids. The initial FFA values of *moringa oleifera seed* oil was higher than other oils in this study but it is known to be one of the most resistant oil to oxidative rancidity among the vegetable oils, due to the natural antioxidants and that produce oils with an increased monounsaturated (oleic) acid content .

4. 2. 2. Change in Peroxide Value (PV)

The peroxide value was taken as a measure of primary oxidation compounds produced in the thermal oxidation of oils samples. The change in PV during frying for(6h) during, one day and(36h)during six days respectively are presented in Table (3. 9) and (3.10). The primary products of lipid oxidation are hydroperoxides which are generally referred to as peroxides. Therefore, it seems reasonable to determine the concentration of peroxides as a measure of the extent of oxidation. However, the peroxides are intermediate products in the formation of carbonyl and hydroxy compounds. One measure for the degree of autoxidation of oils is PV. However, in oils that contain polyunsaturated fatty acids (PUFA), the formation of peroxides takes place through the oxidation of free radicals obtained from abstraction of protons from methylene-interrupted fatty molecule. These peroxides decompose much faster by means of labile hydrogen, obtained from the active methylene group of another molecule, which causes free radical polymerization. (Bailey's, 2005) reported that the initial steps of lipid oxidation involve chain reactions of free radicals as important short-lived intermediates. Oxidation level of fats and oils can be measured directly by detecting the formation of radicals. Since, high temperature ($180\pm 5^{\circ}\text{C}$) was applied for the frying process; peroxides formed during oxidation might be decomposed to secondary oxidation products, as also reported in a previous study (Robards *et al*; 1988).

Lipid hydroperoxides may be partially polymerized during the reaction, thus leading to the formation of less reactive products. The PV represents the total hydroperoxide content and is one of the most common quality indicators of fats and oils during production and storage.

In general, the results indicated that the highest change in PV was observed in sample of sunflower oil, lowest high change of Groundnut oil, whereas the least change was shown by *moringa oleifera* oil Table (3. 10) this could be due to the presence of high oleic acid such as *moringa oleifera* seed oil At the end of last frying for (6h), the PV was found to be 12.75, 8.56 and 2.44 for refined Sunflower oil, groundnut oil and *moringa oleifera oil* respectively Table (3. 9). Whereas the PV for(36h), were found to be 42.20, 35.110 and 10.12 respectively for the same oils Table (3.10).These findings indicate that the frying time effects of degradation of frying oil is due to increases of peroxides.

The statistical analysis of the results of the physicochemical properties of the frying oils show that they were highly significantly ($P < 0.00$), among the PVs of all the samples of oils. The changes showed that there was an initial sharp increase in PV from (0h) to (36h) in all frying oils, the PV of frying medium *mringa oleifera oil* increased continuously from the first through 36h of frying Table (3. 10). Conversely, the rate became slower after (24h) of frying. The highest and least alterations in PV were shown by sunflower and groundnut oils, respectively Table (3.10). The results indicated that the PV of medium above decreased after fourth day of frying and then increased on the last day of frying. This observation could be explained by the fact that peroxides are unstable compounds and will break down to carbonyl and aldehyde compounds during deep-fat frying in presence of temperature, air and light. The higher temperature of deep- fat frying, the stronger the tendency for decomposition of peroxides.

4.3. Fourier transform-infrared (FTIR) analysis

Deep fried food is very popular nowadays and many health issues associated with the quality of the oil used for frying have been raised. Oxidation of the unsaturated fatty acids starts via a free-radical reaction, producing hydroperoxides (ROOH), which are the primary oxidation products. The hydroperoxides are very unstable and quickly decompose by C-C bond scission to form secondary oxidation products, such as aldehydes, alcohols or hydrocarbons (Tompkins and Perkins; 2000). Recent developments in Fourier transform infrared (FT-IR) spectroscopy instrumentation extend the application of this technique to the field of food research, facilitating particularly the studies on edible oils and fats.

FT-IR spectroscopy is of particular value in the recognition of unusual functional groups and in the study of fatty acids with *trans* double bonds. As the compounds formed during the oxidation of fats change, it is possible using FT-IR spectroscopy to follow the course of oxidation. In the oxidation experiments, in this work, sunflower oil, groundnut oil and *moringa oleifera* oil were heated for 6h and 36h at $175\pm 5^{\circ}\text{C}$ and the changes in the FT-IR spectra were observed. The appearance of bands at 3400 cm^{-1} and 3600 cm^{-1} indicated formation of hydroperoxides (OO-H stretching); the disappearance of the band at 3125 cm^{-1} indicated the replacement of the hydrogen on a double bond with some other radical, probably indicating polymerization; the appearance of additional bands at about 1748.25 cm^{-1} ester C = O stretching indicates the formation of aldehydes, ketones, or acids, This is in accordance with similar observations made by (Shahidi and Wanasundara, 2002). It is also interesting to follow the spectral changes in the CO region (1746 cm^{-1}). Here, the study shows a widening of the band for the heated samples. This observation is due to

production of saturated aldehyde functional groups or other secondary oxidation products that cause an absorbance at 1728 cm^{-1} , which overlaps with the stretching vibration at 1746 cm^{-1} of the ester carbonyl functional group of the triglycerides. The increase of the carbonyl compounds coincides with the decomposition of the hydroperoxides; through this mechanism two types of aldehydes (volatile aldehyde and esterified aldehydes) and an alkyl or alkenyl radical are produced. The appearance of bands at 3300 cm^{-1} - 3600 cm^{-1} and 1450 cm^{-1} indicated formation of alcohols and hydrocarbons respectively, due to further interaction of alkyl and alkenyl radicals with other low molecular weight radicals present in the oil (H^\cdot and OH^\cdot) contributes to their stabilization to form alcohols, and hydrocarbons. Changes in bands in the region 1050 to 900 cm^{-1} indicated *cis*, *trans* isomerization; the presence of *trans* isomers in samples oils might be due to formation of the free radical polymerization by decomposition of hydroperoxides (Tyagl and Vasishtha, 1996).

A band shift observed at 3009 cm^{-1} is assigned to the C–H stretching vibration of the *cis*-double bond, FT-IR spectra of various oil samples show that there exist notable differences in the band around 3006 cm^{-1} assigned to the C- H stretching vibration of the *cis*-double bond (CH). The oil composition affects the exact position of the band and yields shifts when the proportion of the fatty acid changes; the value of this frequency in non-oxidized oil samples varies significantly from 3009 to 3006 cm^{-1} ; sunflower oil and groundnut oil show a maximum absorbance at 3006 cm^{-1} comparing to *moringa oleifera* oil that has a maximum of absorbance at 3002 cm^{-1} - 3004 cm^{-1} . This is due to their composition, as vegetable oils (sunflower oil, groundnut oil) contain higher proportion of linolenic or linoleic acyl groups whereas *moringa oleifera* oil consists higher proportion of oleicacyl groups Table (3.11); this is in accordance with similar observations made by

(Vlachos *et al*; 2006). Furthermore, after heating, spectral changes appeared in the 3050–2800 cm^{-1} and 1745 cm^{-1} region, elevated temperatures and/or exposure to ultraviolet radiation, aid the oxidation process monitoring. The spectral region between 3050 and 2740 cm^{-1} undergoes several changes during the oxidation process. Above bands are observed in all oils when heated for 6h or 36h at $180 \pm 5^{\circ}\text{C}$. Olefin bonds in unsaturated fatty acids are oxidized with time or during high temperature processing resulting in the formation of hydroperoxides which leads to rancidity.

4.4. Changes in the ultraviolet spectrum

Changes in the ultraviolet spectrum have been used as a relative measure of oxidation. (Farmer and Sutton, 2002) indicated that the absorption increase due to the formation of conjugated dienes and trienes is proportional to the uptake of oxygen and formation of peroxides during the early stages of oxidation. Changes in the ultraviolet absorption at 233 and 269 nm are associated with the changes in the conjugated dienes and trienes that are produced due to the oxidation of polyunsaturated fatty acids. The resulting conjugated dienes exhibit an intense absorption at 233 nm; similarly the conjugated trienes absorb at 269 nm (Corpet, 2003). The changes in molar absorptivity (E) at 233 and 269nm in the conjugated dienes and trienes respectively during frying for sunflower, groundnut, and *moringa oleifera* oils are shown in appendixes (43- 63). The E at 233 - 269 nm for all the samples increased with frying time for 6h and 36h throughout the frying days (one and six). The levels of conjugated dienes throughout the frying period are lowest in *moringa oleifera* followed by groundnut, with highest levels found in sunflower. The levels of conjugated trienes are however lowest in *moringa oleifera* oil as compared to all the other oils. The low levels of both conjugated dienes and trienes in *moringa oleifera* oil are indications of good oxidative stability of the oil, and it is because of the

high percentage of monounsaturated/oleic acid it contains. The higher the Percentage of polyunsaturated acids in the oil, the higher the levels of conjugated dienes and trienes formed during frying. This was the reason why Sunflower oil, that contained high percentages of polyunsaturated acids (linoleic and linolenic), have accumulated more conjugated dienes and trienes. In contrast, *moringa oleifera* oil and Groundnut, that contain high percentages of saturated and monounsaturated acids, respectively, have lower levels of the conjugated dienes and trienes. The high percentage saturated fatty acid in *moringa oleifera* oil is, however, considered less desirable due to the increased risk of coronary heart disease it presents whereas the high monounsaturated acid in *moringa oleifera* oil is desirable because it is associated with decreased risk.

It was found that in all the samples, the levels of conjugated dienes are higher than trienes; this is indicated by the higher values at E at 233 nm. The rate of increment in the level of conjugated dienes is sharp during frying on day 1, 2, and 3, after which it decreased on day 4 and 5. Most of the dienes will be transformed into polymer compounds with increase in frying time.

4.5. Fatty acids composition

Table (11 and 12) shows the contents of saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids of the oils used for the frying experiments. The fatty acid composition was determined by gas - liquid chromatography of the methyl esters of fatty acids during deep-fat frying. Although the calculations were made by the integrator of the gas chromatograph on the basis of the fractional peak areas, there was an apparent increase in saturated fatty acid content and a decrease in polyenoic acid content as frying time increased (Tyagi and Vasishtha, 1996). The increase in saturated fatty acid content is very high for 36h than 6h and so a

decrease in polyenoic acid content as frying time increased. Thus, the ratio of polyunsaturated to saturated fatty acids (P/S) is considered to be a major factor affecting oil oxidation. The presence of a high content of polyunsaturated fatty acids increases the susceptibility of oil to oxidation (Quaglia and Bucarelli, 2001). Oil resistance to oxidation in the frying process depends mainly on the fatty acid composition and antioxidant content of the oil (Rossell, 2001; Sanches-Silva *et al*, 2003; Nogala-Kalucka *et al*; 2005; Przybylski and Eskin, 2006; Fatemi and Hammond, 1980).

Sunflower oil was very rich in PUFA (76.951%) by comparison groundnut oil (3.974%) and *moringa oleifera* oil (1.330%) show table (3. 11), mainly as linoleic acid and makes it particularly sensitive to oxidation. The most abundant PUFA, linoleic acid, was degraded when was heated for 36h Table (3. 12), during frying in the sunflower oil because of lipid oxidation. Thus, the deterioration of dienes in Sunflower oil was high because only these fatty acids could provide the active methylene groups or conjugated double bonds that could have a deteriorative effect on the fatty acid content. However MUFA content of the groundnut oil and *moringa oleifera* oil more stable toward oxidation reactions when they were heated for 6h.

Conclusion:

This study investigated the effects of free radical formation during multiple frying of vegetable oils and their physiochemical properties. It was found that frying temperature, numbers of frying, free fatty acids content, viscosity, color, refractive index and unsaturated fatty acids of oils decrease the oxidative stability and flavor quality of oils. The two refined oils; sunflower and groundnut generally exhibited the least chemical stability during the frying process and *moringa oleifera* oil the highest. Therefore the presence of polyunsaturated fatty acids in FA chain reduces evaporative loss but accelerates oxidative degradation. High oleic vegetable oils perform better than regular vegetable oils in terms of thermal and oxidative stability.

Oxidation level of fats and oils can be measured directly by detecting the formation of radicals. Alkyl and alkenyl radicals interact with other low molecular weight radicals (H^{\cdot} and OH^{\cdot}), both present in the oil, to form alcohols, and hydrocarbons which contributes to their stability. The detection of free-radical formation during multiple deep - fat frying could thus be confirmed by the detection and quantitative analysis of the indicating products of free - radical reactions, including aldehydes, ketones, acids, alcohols and hydrocarbons.

However, repeated use of frying oils produce undesirable constituents that may pose health hazards. The health hazards of multiple frying. Excessive free radical formation, contributes to the onset of certain diseases which may be protected by a high intake of food, which are rich in antioxidants vitamins and phenolics such as fruits and vegetables.

Recommendation:

Suggestions for Further work for free radical includes:

- may be applied for the study of free radicals produced by other oils.
- To try to detect the free radical itself instead of indicative products.
- Satisfy additives to counteract free radical produced during frying proses.

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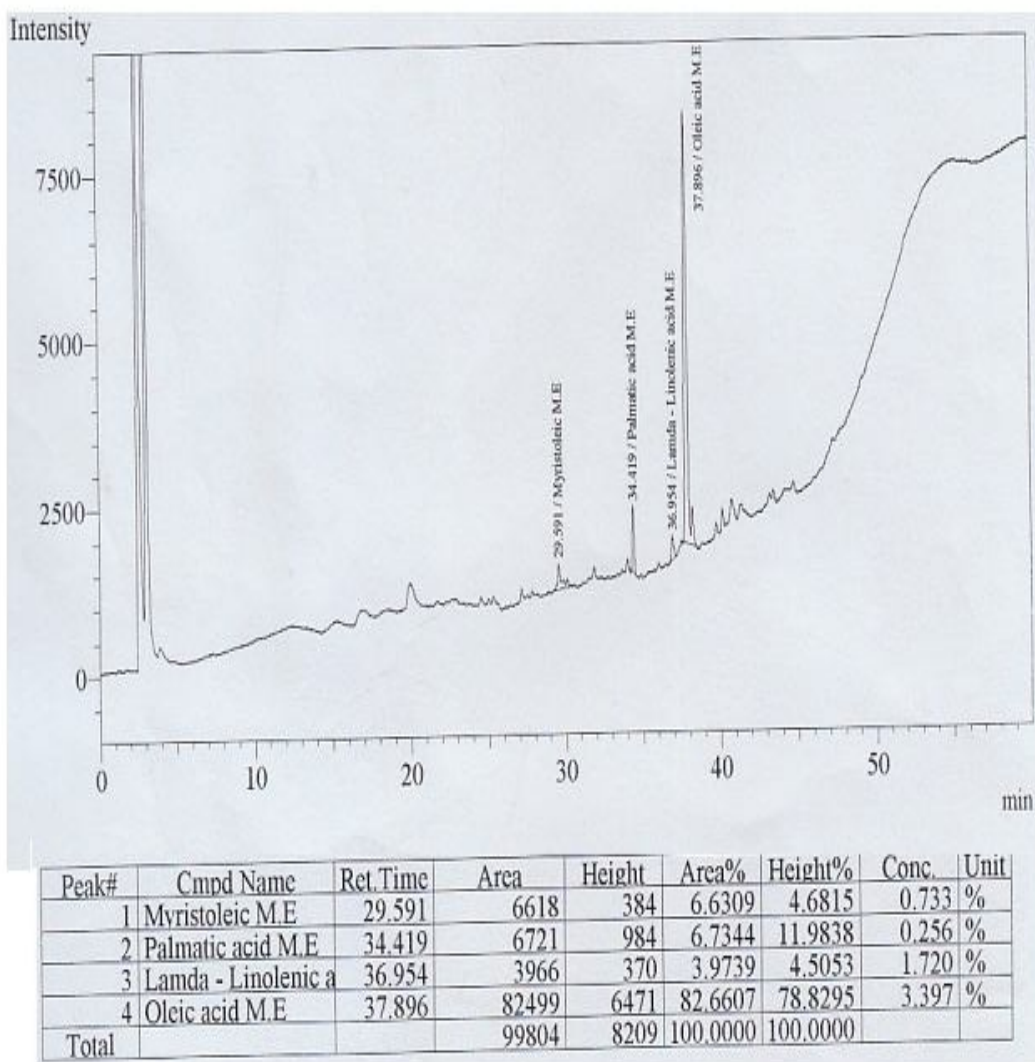
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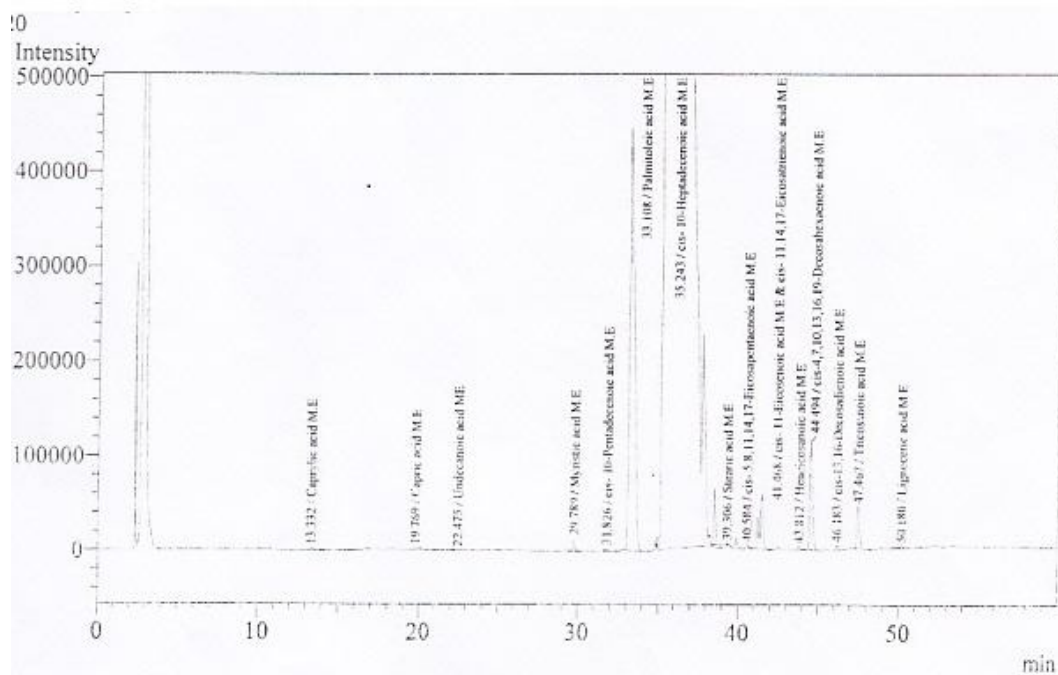
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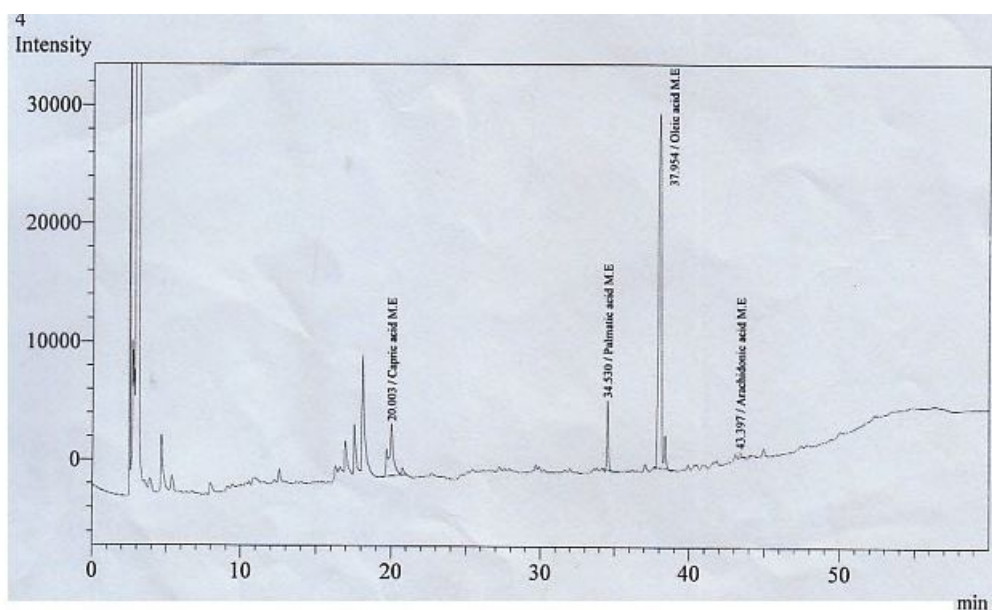


Appendix 1. Gas chromatographic determination of fatty acid composition of groundnut oil frying for (0h).



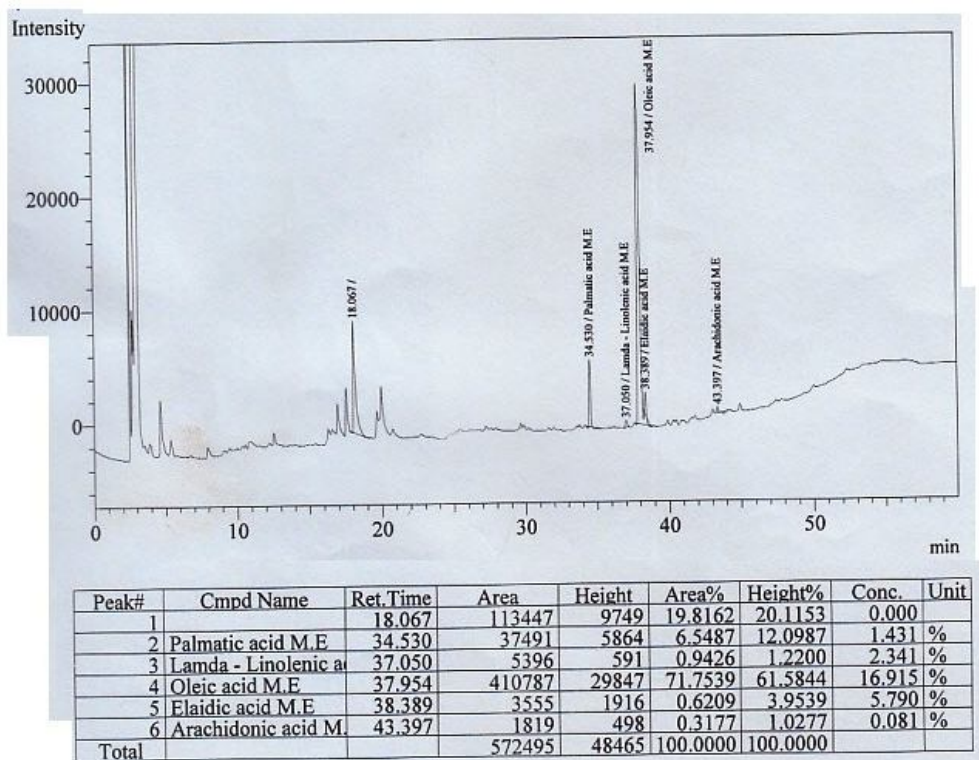
Peak#	Cmpd Name	Ret. Time	Area	Height	Area%	Height%	Conc.	Unit
1	Caprylic acid M.E.	13.332	28526	2327	0.0204	0.1051	1.954	%
2	Capric acid M.E.	19.769	61941	2316	0.0442	0.1046	3.399	%
3	Undecanoic acid M.E.	22.475	11160	669	0.0080	0.0302	0.625	%
4	Myristic acid M.E.	29.789	73011	13365	0.0521	0.6035	3.485	%
5	cis-10-Pentadecenoic acid M.E.	31.826	6828	2855	0.0049	0.1289	0.733	%
6	Palmitoleic acid M.E.	33.108	9242823	446570	6.5956	20.1639	868.037	%
7	cis-10-Heptadecenoic acid M.E.	35.243	127750643	1512459	91.1622	68.2920	2350.239	%
8	Stearic acid M.E.	39.306	18760	918	0.0134	0.0415	14.577	%
9	cis-5,8,11,14,17-Eicosapentenoic acid M.E.	40.584	48029	4500	0.0343	0.2032	3.665	%
10	cis-11-Eicosanoic acid M.E.	41.468	880942	58535	0.6286	2.6430	20.645	%
11	Heneicosanoic acid M.E.	43.812	43494	4251	0.0310	0.1920	16.060	%
12	cis-4,7,10,13,16,19-Heptacosanoic acid M.E.	44.494	1388410	113752	0.9908	5.1362	133.717	%
13	cis-13,16-Decosadioic acid M.E.	46.183	50155	5185	0.0358	0.2341	2.232	%
14	Tricosanoic acid M.E.	47.467	470240	44419	0.3356	2.0056	15.113	%
15	Lignoceric acid M.E.	50.180	60510	2573	0.0432	0.1162	73.443	%
Total			140135472	2214594	100.0000	100.0000		

Appendix 2. Gas chromatographic determination of fatty acid composition of groundnut oil frying for (2h).

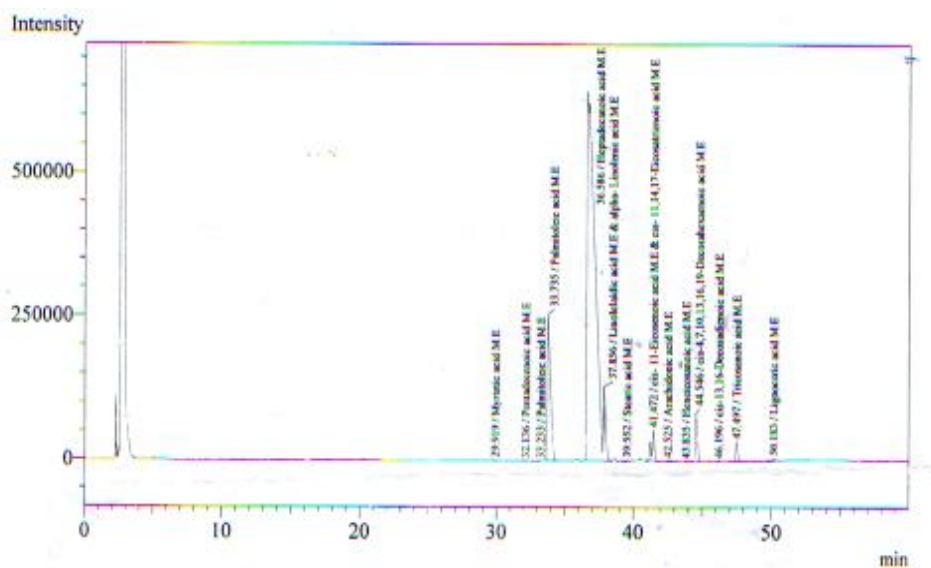


Peak#	Compd Name	Ret. Time	Area	Height	Area%	Height%	Conc.	Unit
1	Capric acid M.E	20.003	93920	4396	17.1521	10.8268	5.155	%
2	Palmitic acid M.E	34.530	37491	5864	6.8467	14.4407	1.431	%
3	Oleic acid M.E	37.954	414342	29847	75.6690	73.5058	17.061	%
4	Arachidonic acid M.	43.397	1819	498	0.3322	1.2267	0.081	%
Total			547572	40605	100.0000	100.0000		

Appendix 3. Gas chromatographic determination of fatty acid composition of groundnut oil frying for (4h).

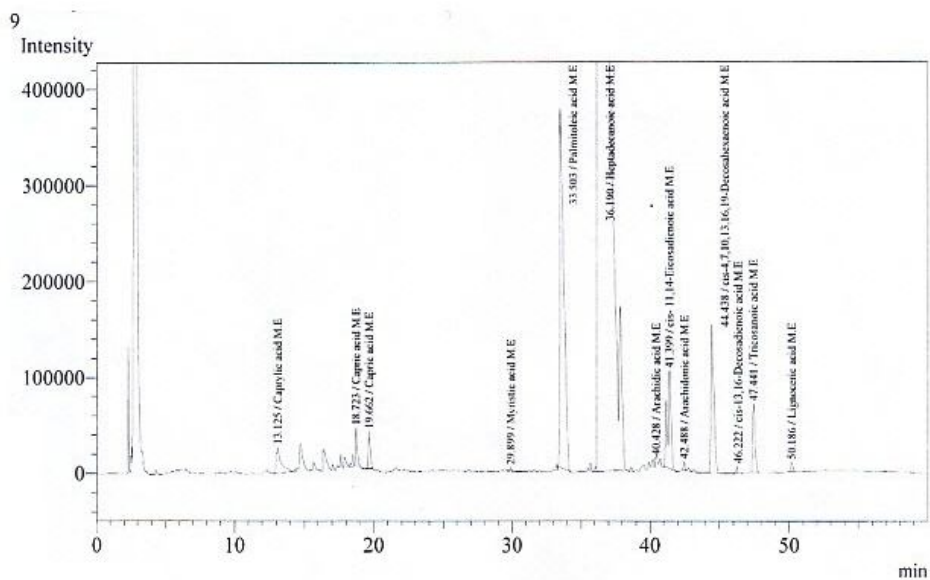


Appendix 4. Gas chromatographic determination of fatty acid composition of groundnut oil frying for (6h).



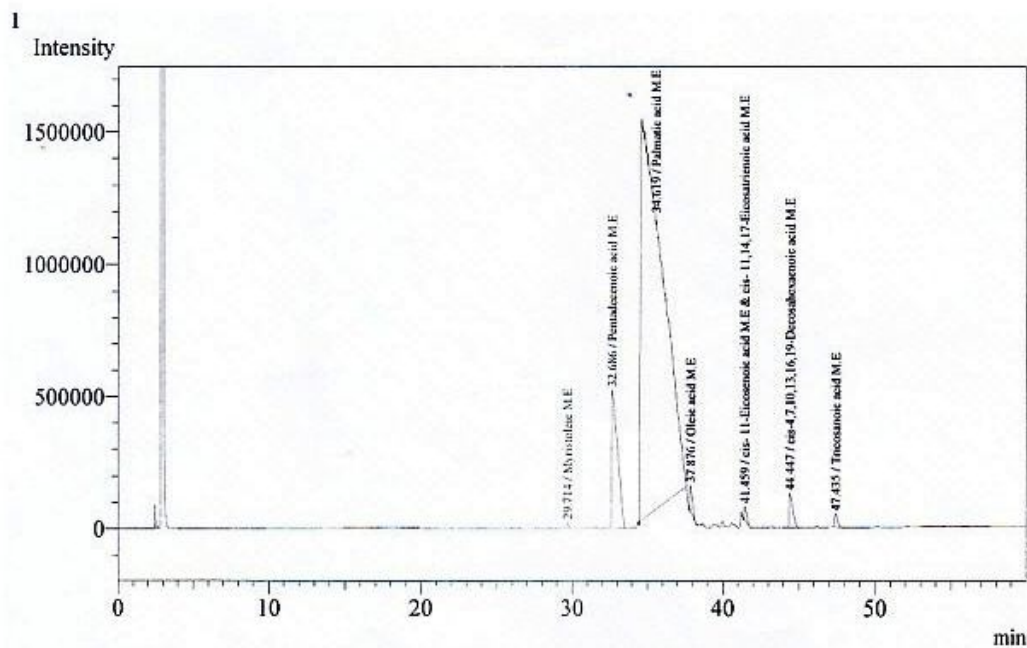
Peak#	Compd Name	Ret.Time	Area	Height	Area%	Height%	Conc.	Unit
1	Myristic acid M.E	29.919	10806	1749	0.0324	0.1505	0.516	%
2	Pentadecenoic acid M.E	32.136	327	626	0.0010	0.0539	0.014	%
3	Palmitoleic acid M.E	33.233	3550	396	0.0106	0.0341	0.333	%
4	Palmitoleic acid M.E	33.735	4075069	254707	12.2118	21.9209	382.709	%
5	Heptadecanoic acid	36.586	26831345	642196	80.4059	55.2694	1199.676	%
6	Linoleic acid M.E	37.856	323344	79022	0.9690	6.8009	6.284	%
7	Stearic acid M.E	39.552	29753	1812	0.0892	0.1559	23.120	%
8	cis-11-Eicosenoic acid	41.472	751603	54389	2.2523	4.6809	17.614	%
9	Arachidonic acid M.E	42.525	30559	2103	0.0916	0.1810	1.366	%
10	Heneicosanoic acid	43.835	6751	736	0.0202	0.0634	2.493	%
11	cis-4,7,10,13,16,19-Heptacosanoic acid	44.546	916038	83849	2.7451	7.2163	88.223	%
12	cis-13,16-Decosadienoic acid	46.196	9579	1286	0.0287	0.1107	0.426	%
13	Tricosanoic acid M.E	47.497	331379	34655	0.9930	2.9825	10.650	%
14	Lignoceric acid M.E	50.183	49784	4411	0.1492	0.3796	60.424	%
Total			33369887	1161937	100.0000	100.0000		

Appendix 5. Gas chromatographic determination of fatty acid composition of groundnut oil frying for (12h).



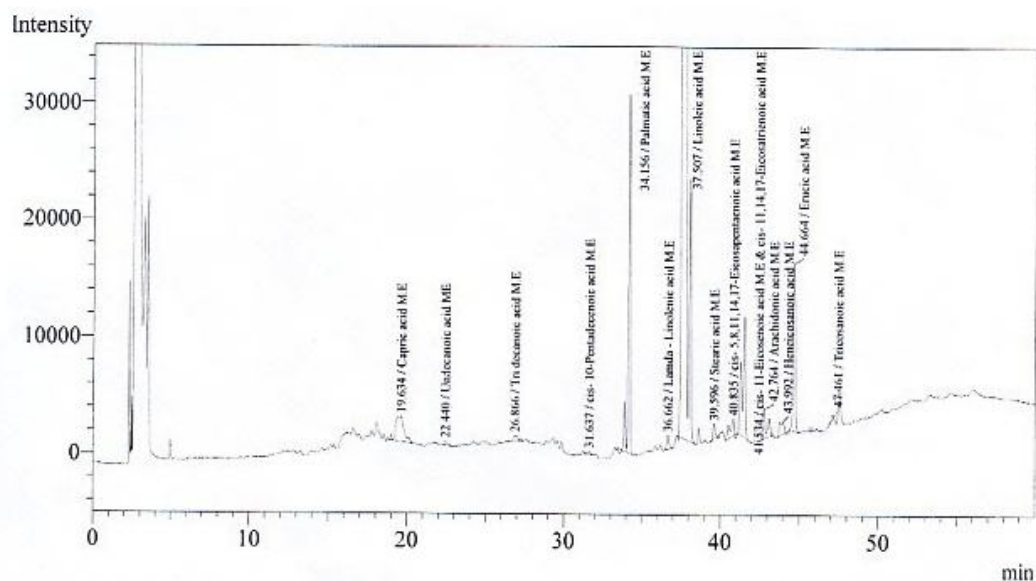
Peak#	Cmpd Name	Ret.Time	Area	Height	Area%	Height%	Conc.	Unit
1	Caprylic acid M.E	13.125	650514	27269	1.0034	1.5537	44.552	%
2	Capric acid M.E	18.723	466457	40009	0.7195	2.2796	25.600	%
3	Capric acid M.E	19.662	373061	33962	0.5754	1.9351	20.474	%
4	Myristic acid M.E	29.899	-2523	3538	-0.0039	0.2016	0.000	%
5	Palmitoleic acid M.E	33.503	7620405	374061	11.7537	21.3132	715.668	%
6	Heptadecanoic acid	36.190	50618174	924918	78.0733	52.6998	2263.227	%
7	Arachidic acid M.E	40.428	-70083	51	-0.1081	0.0029	0.000	%
8	cis- 11,14-Eicosadie	41.399	1842013	102233	2.8411	5.8250	180.788	%
9	Arachidonic acid M.	42.488	128909	9452	0.1988	0.5385	5.762	%
10	cis-4,7,10,13,16,19-	44.438	2256398	155150	3.4803	8.8401	217.312	%
11	cis-13,16-Decosadie	46.222	29695	3009	0.0458	0.1715	1.321	%
12	Tricosanoic acid M.	47.441	811420	71408	1.2515	4.0687	26.079	%
13	Lignoceric acid M.E	50.186	109717	10010	0.1692	0.5703	133.167	%
Total			64834157	1755070	100.0000	100.0000		

Appendix 6. Gas chromatographic determination of fatty acid composition of groundnut oil frying for (24h).



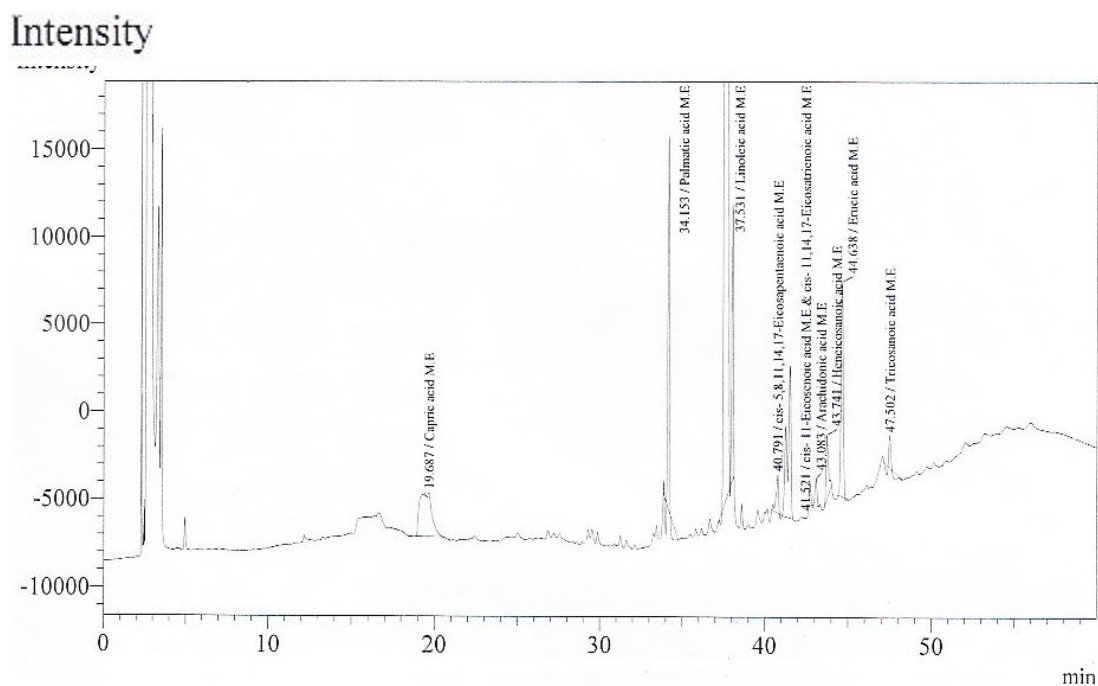
Peak#	Cmpd Name	Ret.Time	Area	Height	Area%	Height%	Conc.	Unit
1	Myristoleic M.E	29.714	147924	20157	0.0874	0.8439	16.373	%
2	Pentadecenoic acid M.E	32.686	14087374	522739	8.3243	21.8868	617.093	%
3	Palmitic acid M.E	34.619	150593803	1519796	88.9867	63.6332	5746.084	%
4	Oleic acid M.E	37.876	1078552	95236	0.6373	3.9875	44.411	%
5	cis-11-Eicosenoic acid M.E. & cis-11,14,17-Eicosatrienoic acid M.E	41.459	593741	51093	0.3508	2.1392	13.914	%
6	cis-4,7,10,13,16,19-Deocosanoic acid M.E	44.447	2053745	129068	1.2136	5.4040	197.795	%
7	Tricosanoic acid M.E	47.435	676721	50281	0.3999	2.1052	21.749	%
Total			169231860	2388370	100.0000	100.0000		

Appendix 7. Gas chromatographic determination of fatty acid composition of groundnut oil frying for (36h).



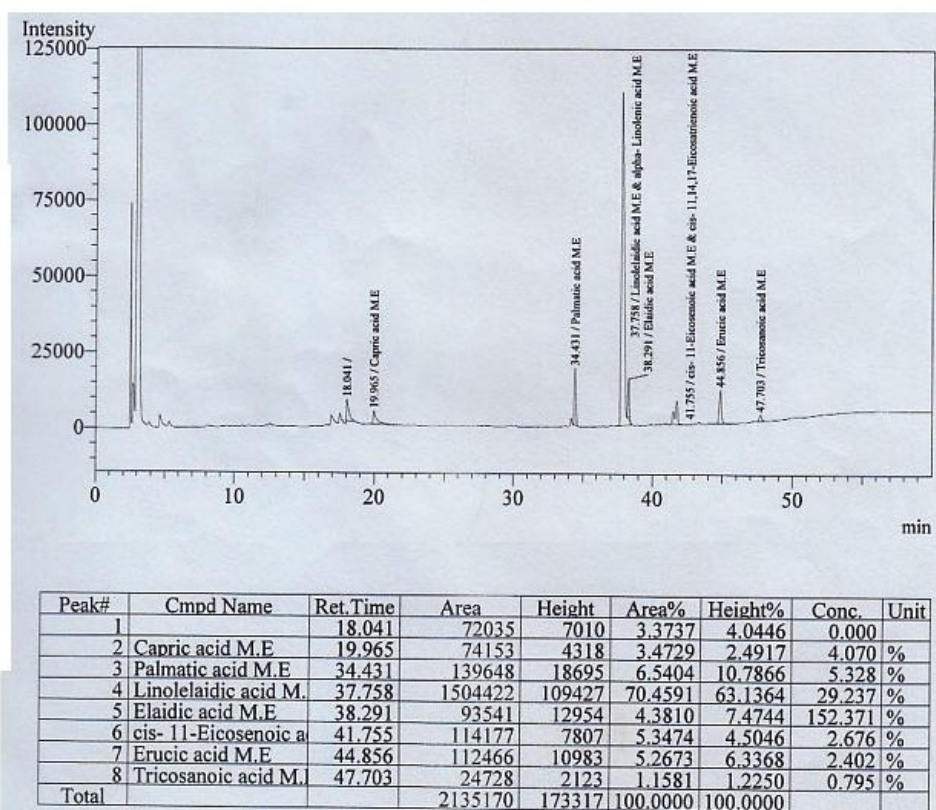
Peak#	Cmpd Name	Ret.Time	Area	Height	Area%	Height%	Conc.	Unit
1	Capric acid M.E	19.634	77370	2191	2.7157	1.0433	4.246	%
2	Undecanoic acid M.E	22.440	5536	393	0.1943	0.1870	0.310	%
3	Tri decanoic acid M.E	26.866	10256	527	0.3600	0.2511	0.451	%
4	cis- 10-Pentadecenoic acid M.E	31.637	-1223	233	-0.0429	0.1112	0.000	%
5	Palmitic acid M.E	34.156	254918	30649	8.9476	14.5976	9.727	%
6	Lamda - Linolenic acid M.E	36.662	12975	1231	0.4554	0.5864	5.628	%
7	Linoleic acid M.E	37.507	2115132	143742	74.2409	68.4616	1707.411	%
8	Stearic acid M.E	39.596	8843	1304	0.3104	0.6210	6.871	%
9	cis- 5,8,11,14,17-Eicosapentenoic acid M.E	40.835	23999	1473	0.8424	0.7014	1.831	%
10	cis- 11-Eicosenoic acid M.E	41.534	149483	10331	5.2468	4.9205	3.503	%
11	Arachidonic acid M.E	42.764	40244	2514	1.4126	1.1973	1.799	%
12	Heneicosanoic acid M.E	43.992	20267	1057	0.7114	0.5033	7.483	%
13	Erucic acid M.E	44.664	138123	14306	4.8481	6.8136	2.950	%
14	Tricosanoic acid M.E	47.461	-6911	10	-0.2426	0.0047	0.000	%
Total			2849012	209961	100.0000	100.0000		

Appendix 8. Gas chromatographic determination of fatty acid composition of sunflower oil frying for (0h).

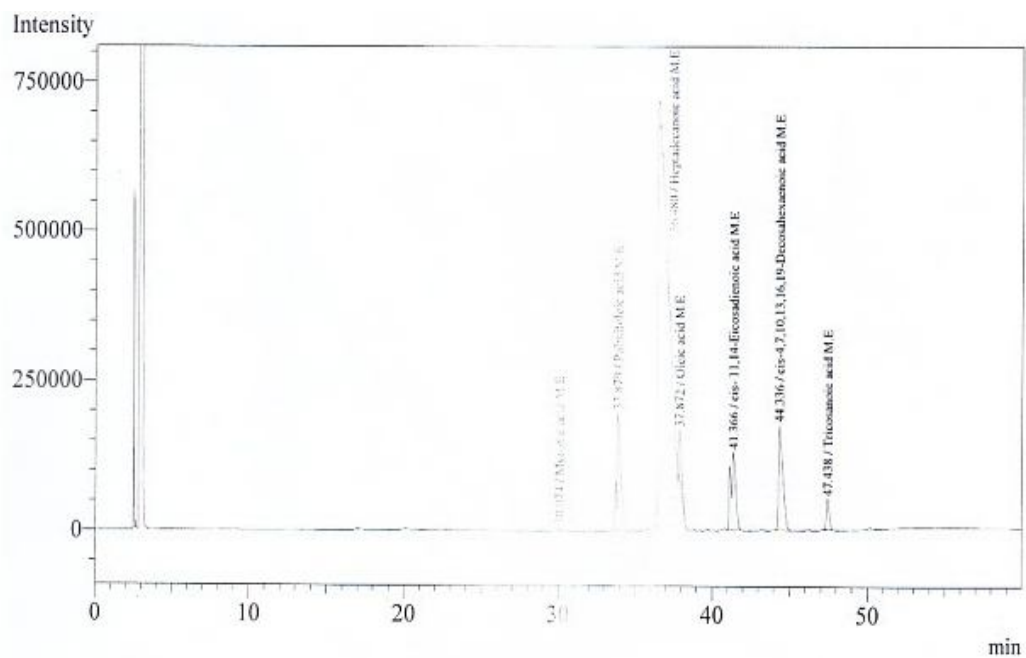


Peak#	Cmpd Name	Ret.Time	Area	Height	Area%	Height%	Conc.	Unit
1	Capric acid M.E	19.687	122987	2487	5.9709	1.4314	6.750	%
2	Palmitic acid M.E	34.153	116225	21248	5.6426	12.2297	4.435	%
3	Linoleic acid M.E	37.531	1494253	119667	72.5443	68.8751	1206.215	%
4	cis- 5,8,11,14,17-Eic	40.791	21119	2205	1.0253	1.2690	1.612	%
5	cis- 11-Eicosenoic a	41.521	131226	8728	6.3709	5.0235	3.075	%
6	Arachidonic acid M.	43.083	21269	1555	1.0326	0.8949	0.951	%
7	Heneicosanoic acid	43.741	36429	3694	1.7686	2.1260	13.451	%
8	Erucic acid M.E	44.638	112782	12278	5.4754	7.0668	2.409	%
9	Tricosanoic acid M.	47.502	3489	1883	0.1694	1.0836	0.112	%
Total			2059779	173745	100.0000	100.0000		

Appendix 9. Gas chromatographic determination of fatty acid composition of sunflower oil frying for (2h).

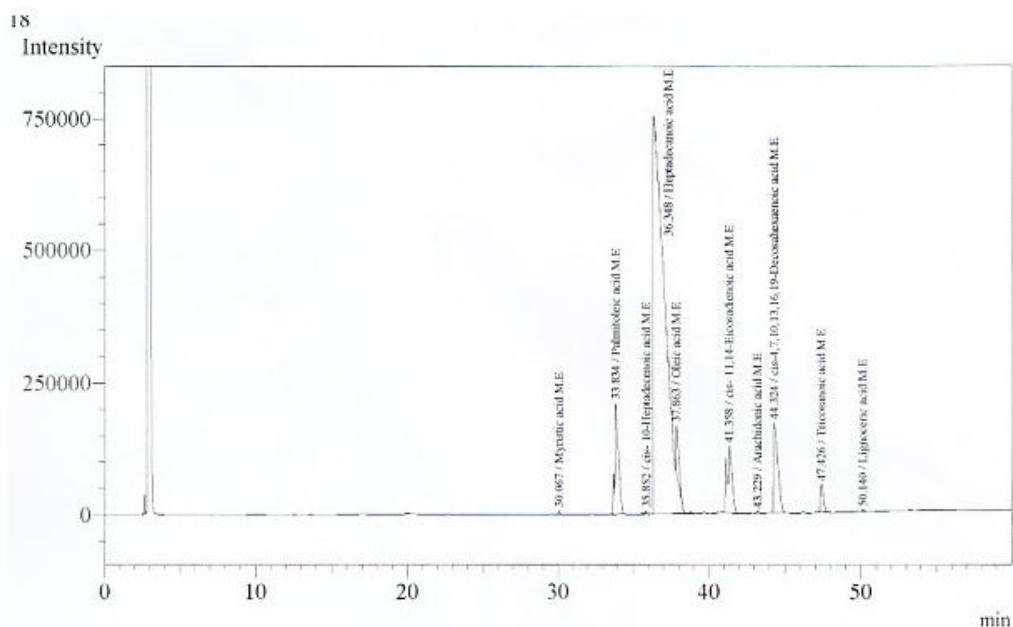


Appendix 10. Gas chromatographic determination of fatty acid composition of sunflower oil frying for (4h).



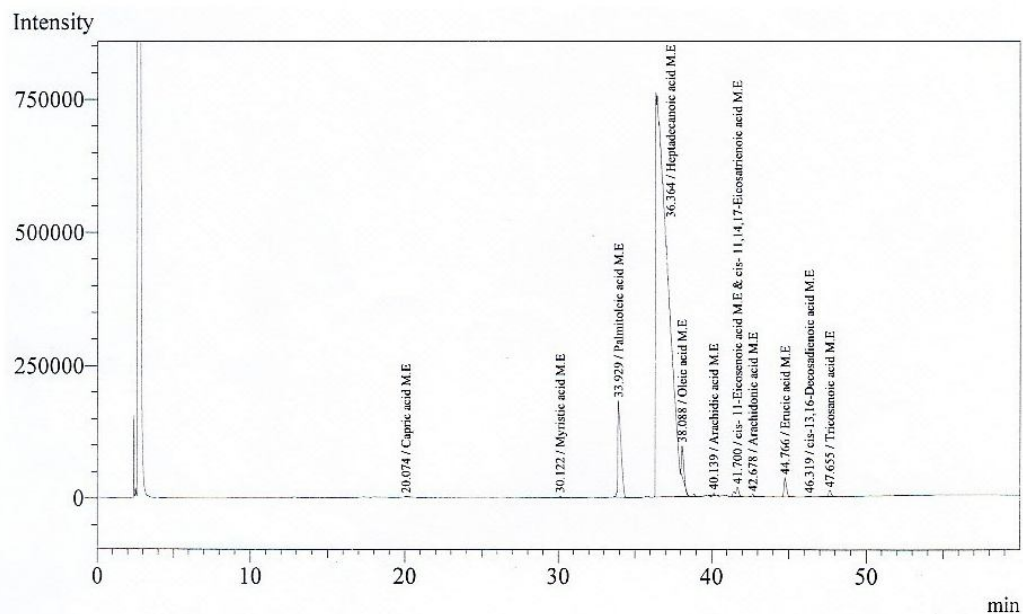
Peak#	Cmpd Name	Ret.Time	Area	Height	Area%	Height%	Conc.	Unit
1	Myristic acid M.E	30.074	40441	11228	0.4432	1.930	%	
2	Palmitoleic acid M.E	33.879	3447241	104665	14.9139	323.747	%	
3	Heptadecanoic acid	36.480	21726151	69650	51.6133	971.414	%	
4	Oleic acid M.E	37.872	829002	25170	5.8615	34.135	%	
5	cis-11,14-Eicosadie	41.366	3020725	91715	9.9188	296.475	%	
6	cis-4,7,10,13,16,19-	44.336	3227863	98004	13.3687	310.874	%	
7	Tricosanoic acid M.E	47.438	644476	19568	3.8807	20.713	%	
Total			32935899	100.0000	100.0000			

Appendix 11. Gas chromatographic determination of fatty acid composition of sunflower oil frying for (6h).



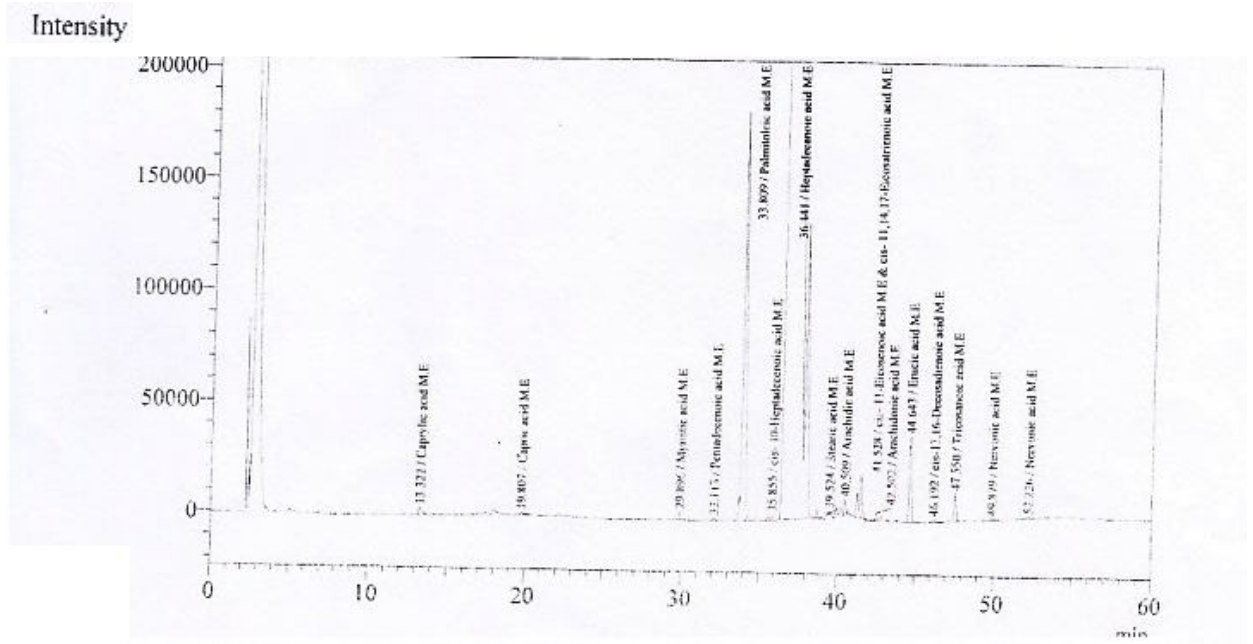
Peak#	Compd Name	Ret.Time	Area	Height	Area%	Height%	Conc.	Unit
1	Myristic acid M.E	30.067	42403	5836	0.0817	0.4108	2.024	%
2	Palmitoleic acid M.E	33.834	3695387	210237	7.1235	14.7998	347.051	%
3	cis- 10-Heptadeceno	35.852	54264	6064	0.1046	0.4269	5.246	%
4	Heptadecanoic acid	36.348	39896285	752253	76.9066	52.9557	1783.833	%
5	Oleic acid M.E	37.863	1277399	91876	2.4624	6.4677	52.598	%
6	cis- 11,14-Eicosadie	41.358	2958393	127220	5.7028	8.9558	290.357	%
7	Arachidonic acid M	43.229	18495	2472	0.0357	0.1741	0.827	%
8	cis-4,7,10,13,16,19-	44.324	3221680	169736	6.2103	11.9487	310.278	%
9	Tricosanoic acid M.	47.426	671074	51648	1.2936	3.6358	21.568	%
10	Lignoceric acid M.E	50.140	40918	3193	0.0789	0.2247	49.664	%
Total			51876298	1420535	100.0000	100.0000		

Appendix 12. Gas chromatographic determination of fatty acid composition of sunflower oil frying for (12h).



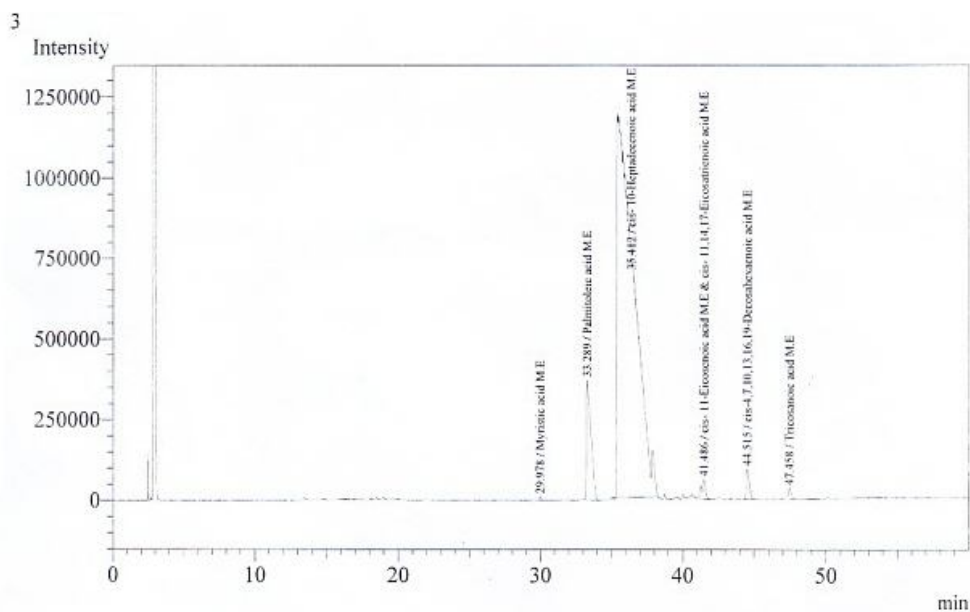
Peak#	Cmpd Name	Ret.Time	Area	Height	Area%	Height%	Conc.	Unit
1	Capric acid M.E	20.074	29129	1731	0.0644	0.1602	1.599	%
2	Myristic acid M.E	30.122	15693	3045	0.0347	0.2817	0.749	%
3	Palmitoleic acid M.E	33.929	2585876	181804	5.7135	16.8192	242.852	%
4	Heptadecanoic acid	36.364	41231990	759657	91.1021	70.2778	1843.554	%
5	Oleic acid M.E	38.088	493071	57258	1.0894	5.2971	20.303	%
6	Arachidic acid M.E	40.139	28617	5303	0.0632	0.4905	19.227	%
7	cis-11-Eicosenoic a	41.700	291091	18118	0.6432	1.6761	6.822	%
8	Arachidonic acid M.	42.678	41470	4502	0.0916	0.4165	1.854	%
9	Erucic acid M.E	44.766	394770	36491	0.8722	3.3758	8.431	%
10	cis-13,16-Decosadie	46.319	15480	1383	0.0342	0.1279	0.689	%
11	Tricosanoic acid M.	47.655	131928	11643	0.2915	1.0771	4.240	%
Total			45259115	1080935	100.0000	100.0000		

Appendix 13. Gas chromatographic determination of fatty acid composition of sunflower oil frying for (24h).



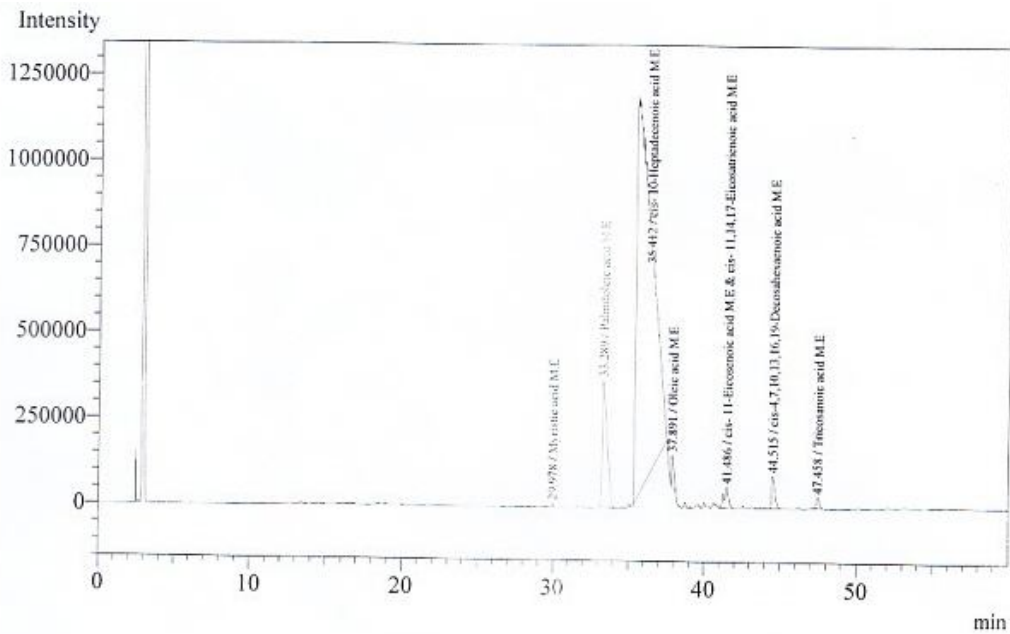
Peak#	Compd Name	Ret. Time	Area	Height	Area%	Height%	Conc	Unit
1	Caprylic acid M.E	13.322	36161	3130	0.0958	0.3067	2.477	%
2	Capric acid M.E	19.807	21093	1146	0.0559	0.1123	1.158	%
3	Myristic acid M.E	29.896	16540	3448	0.0438	0.3378	0.790	%
4	Pentadecenoic acid M.E	32.113	2429	982	0.0064	0.0963	0.106	%
5	Palmitoleic acid M.E	33.809	2377604	182800	6.3001	17.9115	223.292	%
6	cis- 10-Heptadecenoic acid M.E	35.855	31492	2834	0.0834	0.2777	3.044	%
7	Heptadecanoic acid M.E	36.441	34463596	743253	91.3208	72.8270	1540.928	%
8	Stearic acid M.E	39.524	-42695	1047	-0.1131	0.1026	0.000	%
9	Arachidic acid M.E	40.509	37135	5267	0.0984	0.5161	24.950	%
10	cis- 11-Eicosenoic acid M.E	41.528	253614	19583	0.6720	1.9189	5.943	%
11	Arachidonic acid M.E	42.502	41024	3309	0.1087	0.3242	1.834	%
12	Erucic acid M.E	44.643	345501	38453	0.9155	3.7678	7.379	%
13	cis-13,16-Decosadienoic acid M.E	46.192	8408	1325	0.0223	0.1298	0.374	%
14	Tricosanoic acid M.E	47.550	118617	12698	0.3143	1.2442	3.812	%
15	Nervonic acid M.E	49.879	16248	751	0.0431	0.0736	8.488	%
16	Nervonic acid M.E	52.226	12274	546	0.0325	0.0535	6.412	%
Total			37739041	1020572	100.0000	100.0000		

Appendix 14. Gas chromatographic determination of fatty acid composition of sunflower oil frying for (36h).



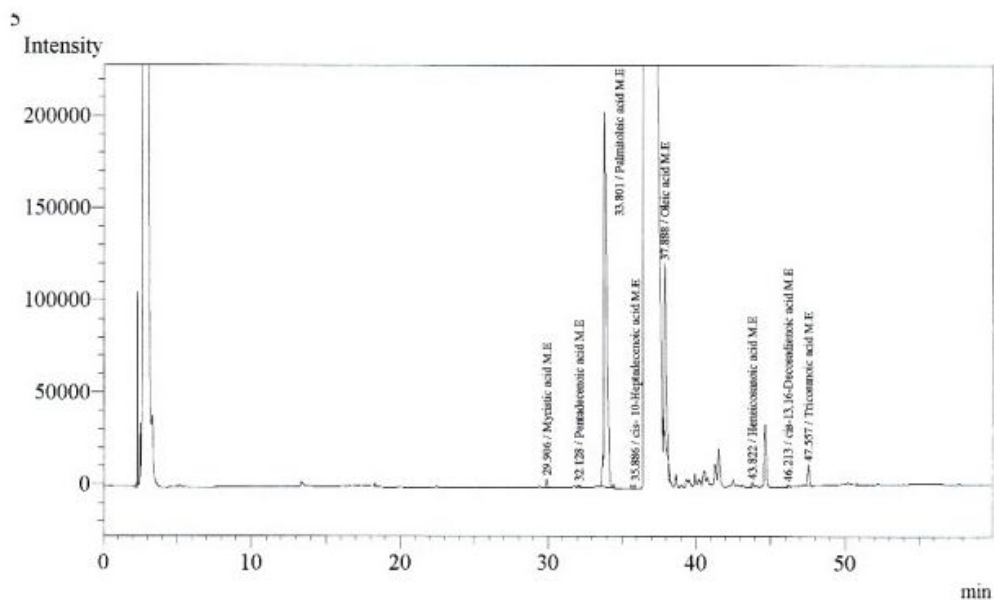
Peak#	Cmpd Name	Ret. Time	Area	Height	Area%	Height%	Conc.	Unit
1	Myristic acid M.E	29.978	75787	10864	0.0698	0.6182	3.618	%
2	Palmitoleic acid M.E	33.289	8022960	369812	7.3867	21.0429	753.474	%
3	cis- 10-Heptadeceno	35.412	97532415	1187826	89.7977	67.5891	9428.905	%
4	cis- 11-Eicosenoic a	41.486	1194658	60490	1.0999	3.4420	27.997	%
5	cis-4,7,10,13,16,19-	44.515	1352400	94578	1.2451	5.3816	130.249	%
6	Tricosanoic acid M.	47.458	435216	33851	0.4007	1.9262	13.988	%
Total			108613436	1757421	100.0000	100.0000		

Appendix 15. Gas chromatographic determination of fatty acid composition of *moringa oleifera* oil frying for (0h).



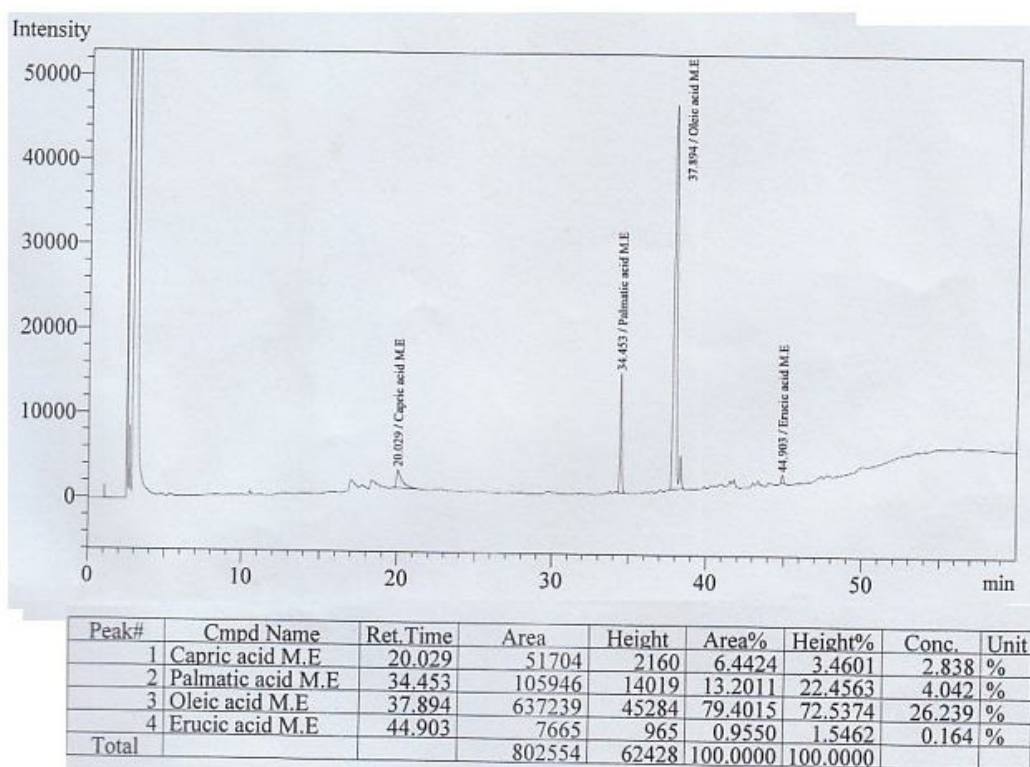
Peak#	Cmpd Name	Ret. Time	Area	Height	Area%	Height%	Conc.	Unit
1	Myristic acid M.E	29.978	75787	10754	0.0815	0.5908	3.618	%
2	Palmitoleic acid M.E	33.289	8022960	369112	8.6277	20.1099	753.474	%
3	cis- 10-Heptadeceno	35.412	80931373	1172353	87.0314	64.0229	7824.006	%
4	Oleic acid M.E	37.891	978613	92397	1.0524	5.0032	40.295	%
5	cis- 11-Eicosenoic a	41.486	1194658	60410	1.2847	3.2894	27.997	%
6	cis-4,7,10,13,16,19-	44.515	1352400	94578	1.4543	5.1430	130.249	%
7	Tricosanoic acid M.	47.458	435216	33651	0.4680	1.8408	13.988	%
Total			92991007	1838955	100.0000	100.0000		

Appendix 16. Gas chromatographic determination of fatty acid composition of *moringa oliefera* oil frying for (2h).

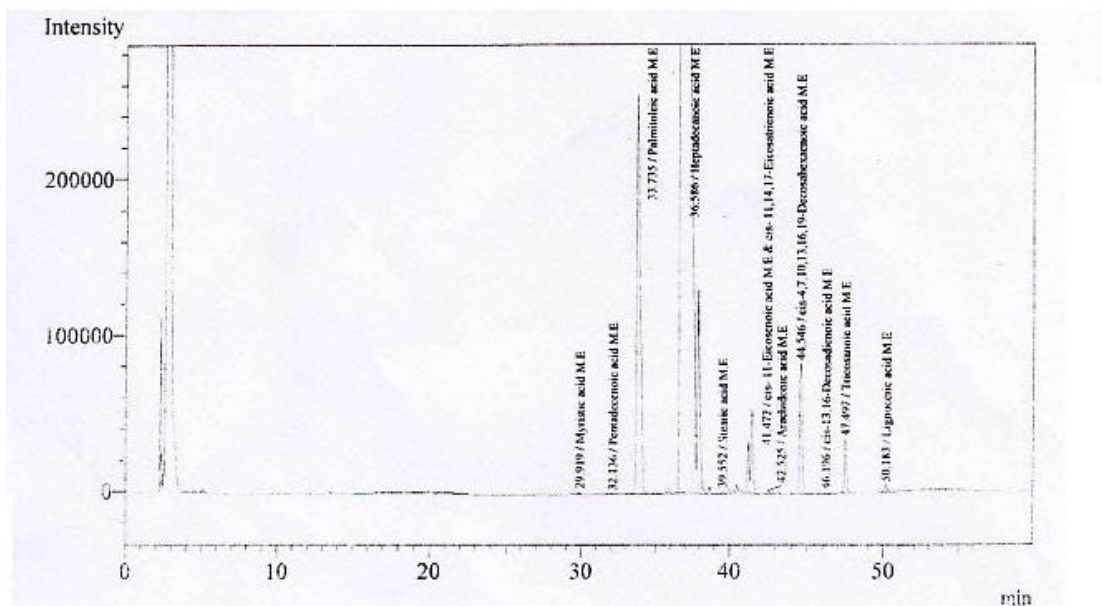


Peak#	Cmpd Name	Ret. Time	Area	Height	Area%	Height%	Conc.	Unit
1	Myristic acid M.E	29.906	27019	4610	0.7271	1.4508	1.290	%
2	Pentadecenoic acid M.E	32.128	3288	1118	0.0885	0.3519	0.144	%
3	Palmitoleic acid M.E	33.801	2783781	203078	74.9132	63.9156	261.438	%
4	cis- 10-Heptadecenoic acid M.E	35.886	25396	2477	0.6834	0.7797	2.455	%
5	Oleic acid M.E	37.888	740971	91650	19.9400	28.8454	30.510	%
6	Heneicosanoic acid M.E	43.822	26118	2253	0.7029	0.7090	9.644	%
7	cis-13,16-Decosadienoic acid M.E	46.213	10391	1197	0.2796	0.3767	0.462	%
8	Tricosanoic acid M.E	47.557	99046	11345	2.6654	3.5708	3.183	%
Total			3716010	317728	100.0000	100.0000		

Appendix 17. Gas chromatographic determination of fatty acid composition of *moringa oleifera* oil frying for (4h).

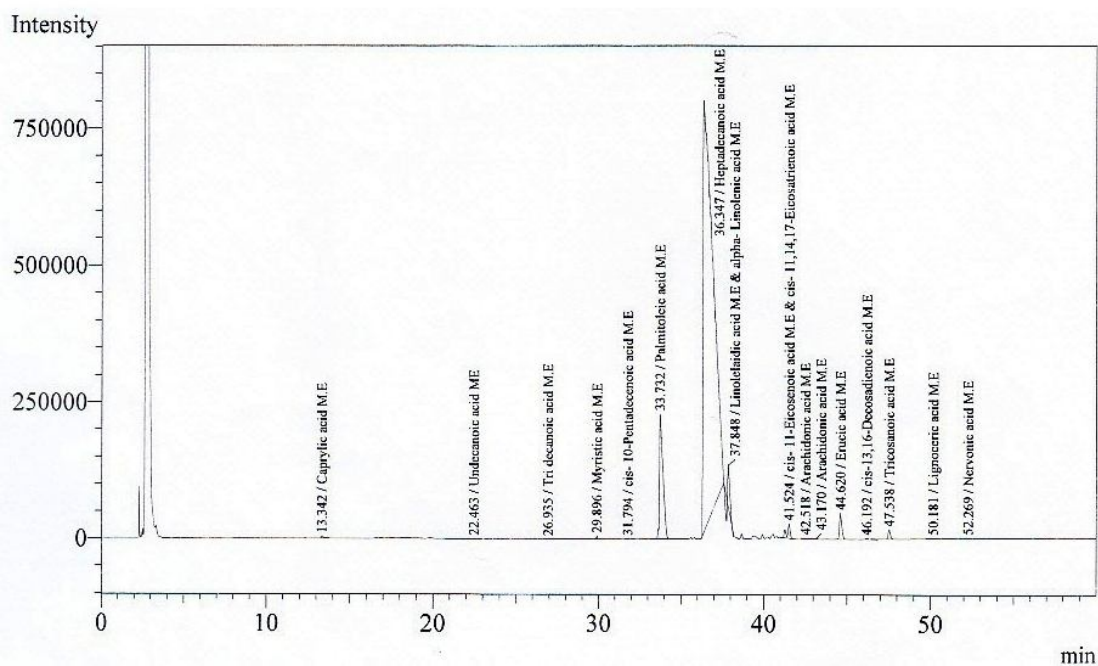


Appendix 18. Gas chromatographic determination of fatty acid composition of *moringa oliefera* oil frying for (6h).



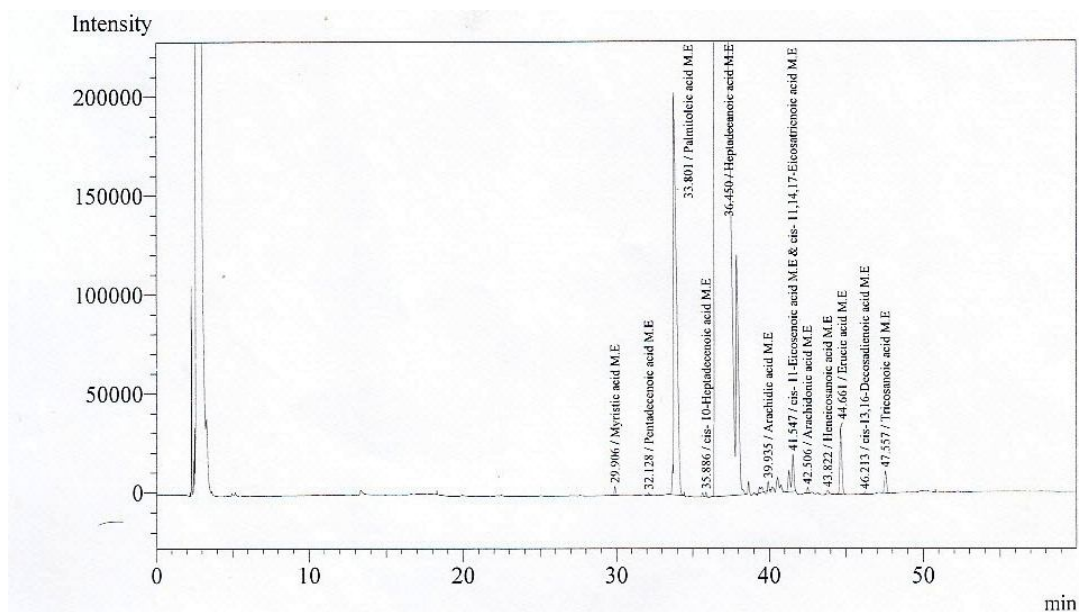
Peak#	Cmpd Name	Ret. Time	Area	Height	Area%	Height%	Conc.	Unit
1	Myristic acid M.E	29.919	10806	1749	0.0324	0.1617	0.516	%
2	Pentadecenoic acid M.E	32.136	327	626	0.0010	0.0579	0.014	%
3	Palmitoleic acid M.E	33.735	4075069	254707	12.2156	23.5451	382.709	%
4	Heptadecanoic acid M.E	36.586	27154689	642196	81.4000	59.3646	1214.134	%
5	Stearic acid M.E	39.552	29753	1812	0.0892	0.1675	23.120	%
6	cis-11-Eicosenoic acid M.E	41.472	751603	54389	2.2530	5.0277	17.614	%
7	Arachidonic acid M.E	42.525	30559	2103	0.0916	0.1944	1.366	%
8	cis-4,7,10,13,16,19-Decosahexenoic acid M.E	44.546	916038	83849	2.7460	7.7510	88.223	%
9	cis-13,16-Decosadienoic acid M.E	46.196	9579	1286	0.0287	0.1189	0.426	%
10	Tricosanoic acid M.E	47.497	331379	34655	0.9934	3.2035	10.650	%
11	Lignoceric acid M.E	50.183	49784	4411	0.1492	0.4077	60.424	%
Total			33359586	1081783	100.0000	100.0000		

Appendix 19. Gas chromatographic determination of fatty acid composition of *moringa oleifera* oil frying for (12h).



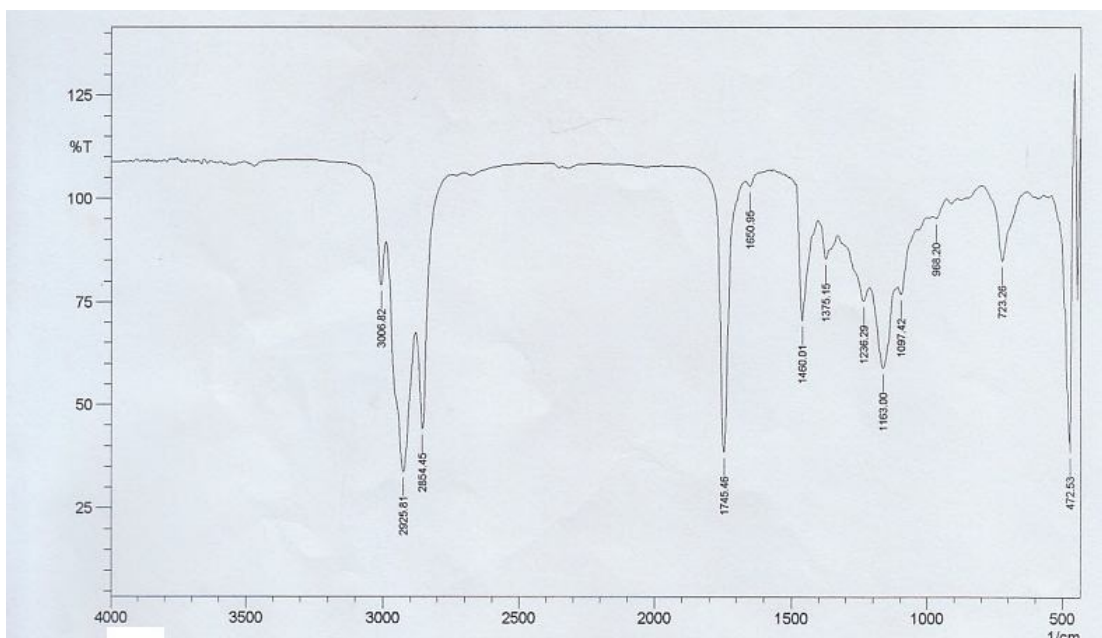
Peak#	Cmpd Name	Ret.Time	Area	Height	Area%	Height%	Conc.	Unit
1	Caprylic acid M.E	13.342	30665	2959	0.0834	0.2461	2.100	%
2	Undecanoic acid ME	22.463	13820	591	0.0376	0.0492	0.774	%
3	Tri decanoic acid M	26.935	4380	299	0.0119	0.0249	0.193	%
4	Myristic acid M.E	29.896	28403	4916	0.0773	0.4089	1.356	%
5	cis- 10-Pentadeceno	31.794	3619	438	0.0098	0.0364	0.388	%
6	Palmitoleic acid M.E	33.732	3332297	228928	9.0659	19.0404	312.952	%
7	Heptadecanoic acid	36.347	32057637	792482	87.2161	65.9125	1433.353	%
8	Linolelaidic acid M.	37.848	295392	72758	0.8036	6.0514	5.741	%
9	cis- 11-Eicosenoic a	41.524	314148	26874	0.8547	2.2352	7.362	%
10	Arachidonic acid M.	42.518	15221	2058	0.0414	0.1712	0.680	%
11	Arachidonic acid M.	43.170	6018	982	0.0164	0.0817	0.269	%
12	Erucic acid M.E	44.620	455305	48373	1.2387	4.0233	9.724	%
13	cis-13,16-Decosadie	46.192	10516	1617	0.0286	0.1345	0.468	%
14	Tricosanoic acid M.	47.538	161939	17350	0.4406	1.4430	5.205	%
15	Lignoceric acid M.E	50.181	13555	1067	0.0369	0.0887	16.452	%
16	Nervonic acid M.E	52.269	13626	632	0.0371	0.0526	7.118	%
Total			36756541	1202324	100.0000	100.0000		

Appendix 20. Gas chromatographic determination of fatty acid composition of *moringa oliefera* oil frying for (24h).



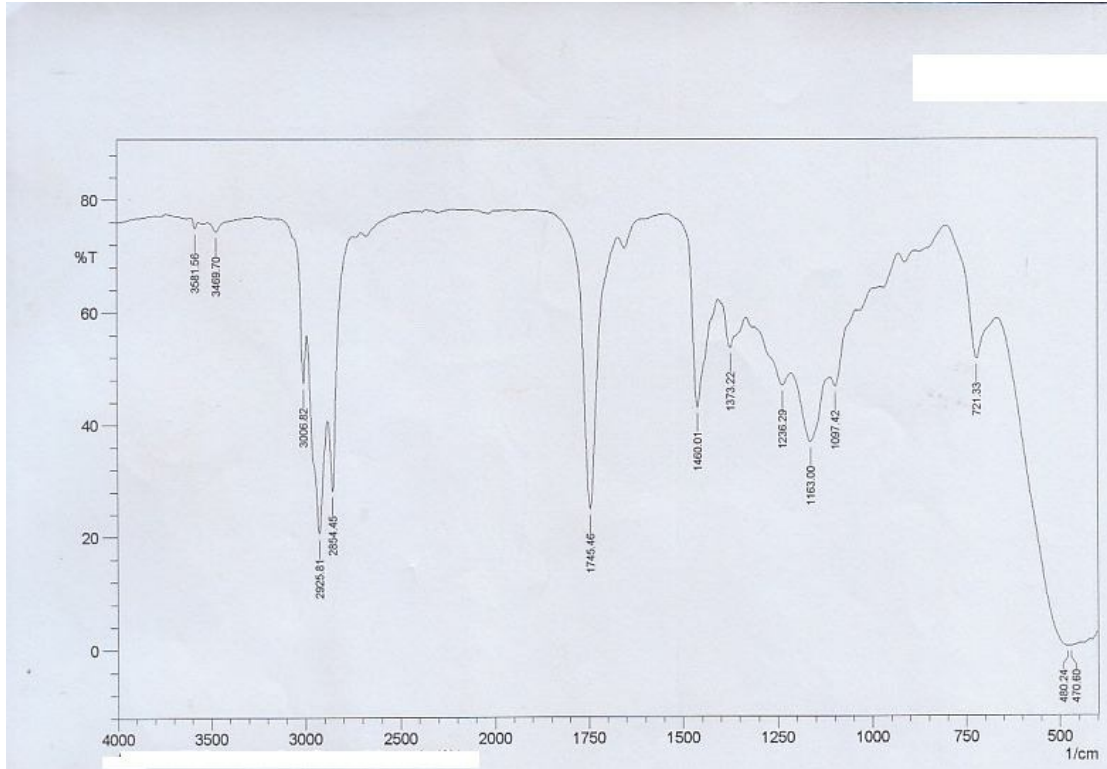
Peak#	Cmpd Name	Ret.Time	Area	Height	Area%	Height%	Conc.	Unit
1	Myristic acid M.E	29.906	27019	4610	0.0738	0.4513	1.290	%
2	Pentadecenoic acid M	32.128	3288	1118	0.0090	0.1095	0.144	%
3	Palmitoleic acid M.E	33.801	2783781	203078	7.6013	19.8812	261.438	%
4	cis- 10-Heptadeceno	35.886	25396	2477	0.0693	0.2425	2.455	%
5	Heptadecanoic acid	36.450	33029434	735026	90.1893	71.9584	1476.804	%
6	Arachidic acid M.E	39.935	45451	4580	0.1241	0.4483	30.537	%
7	cis- 11-Eicosenoic a	41.547	247229	19004	0.6751	1.8604	5.794	%
8	Arachidonic acid M.	42.506	22162	3008	0.0605	0.2945	0.991	%
9	Heneicosanoic acid	43.822	26118	2253	0.0713	0.2205	9.644	%
10	Erucic acid M.E	44.661	303042	33763	0.8275	3.3053	6.472	%
11	cis-13,16-Decosadie	46.213	10391	1197	0.0284	0.1172	0.462	%
12	Tricosanoic acid M.l	47.557	99046	11345	0.2705	1.1107	3.183	%
Total			36622357	1021459	100.0000	100.0000		

Appendix 21. Gas chromatographic determination of fatty acid composition of *moringa oliefera* oil frying for (36h).



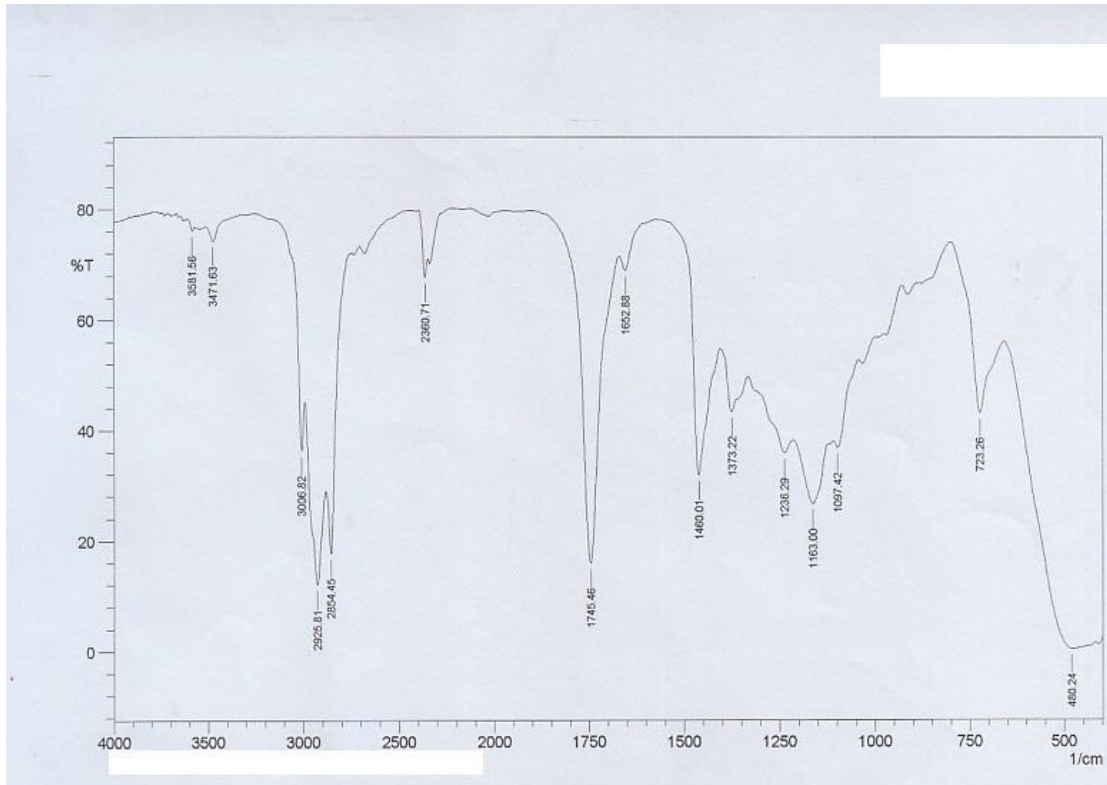
No.	name:ciprofloxac	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	472.53	38.7	13.05	474.46	455.17	1.28	-1.15
2	723.26	84.85	17.64	798.47	630.68	2.64	4.37
3	968.2	95.07	1.69	981.7	929.63	0.69	0.17
4	1097.42	76.82	4.45	1110.92	1041.49	5	0.18
5	1163	58.93	19.61	1213.14	1110.92	16.29	5.57
6	1236.29	75.23	5.75	1330.79	1213.14	9.56	0.99
7	1375.15	85.42	7.87	1404.08	1330.79	3.56	1.21
8	1460.01	70.45	30.41	1485.09	1404.08	5.32	4.99
9	1650.95	102.89	1.86	1664.45	1620.09	-0.84	0.13
10	1745.46	38.55	67	1853.46	1668.31	9.12	13.66
11	2854.45	44.13	31.06	2881.45	2746.44	11.03	1.15
12	2925.81	33.72	42.87	2989.46	2881.45	29.41	17.62
13	3006.82	79	12.25	3182.33	2989.46	-2.31	-3.44

Appendix 22. FTIR Spectrum of groundnut oil frying for (0h).

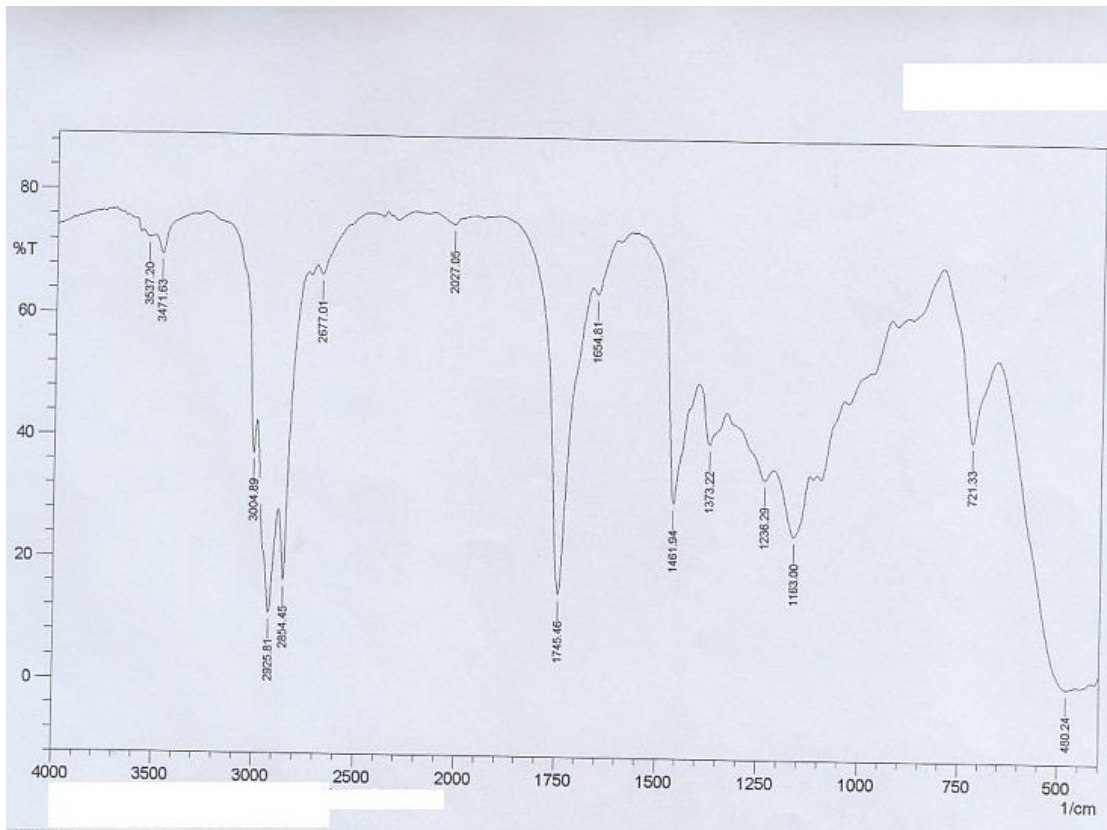


No.	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	470.6	0.464	0.04	472.53	439.74	70.853	0.401
2	480.24	0.423	1.823	665.4	474.46	168.692	1.542
3	721.33	51.495	13.78	802.33	667.32	27.957	3.96
4	1097.42	46.637	3.86	1110.92	1039.56	19.392	0.236
5	1163	36.716	11.857	1215.07	1110.92	38.208	5.545
6	1236.29	46.792	3.947	1311.5	1215.07	27.812	1.136
7	1373.22	53.526	7.127	1406.01	1332.72	17.69	1.677
8	1460.01	42.928	25.332	1539.09	1406.01	27.043	5.83
9	1745.46	24.879	50.015	1866.97	1672.17	41.213	17.377
10	2854.45	28.114	18.974	2881.45	2744.52	36.743	0.768
11	2925.81	20.537	26.438	2987.53	2881.45	51.355	17.038
12	3006.82	47.344	10.698	3157.25	2989.46	26.714	-4.173
13	3469.7	74.317	1.783	3514.06	3309.62	24.47	0.498
14	3581.56	74.869	1.5	3598.92	3566.14	3.977	0.142

Appendix 23. FTIR Spectrum of groundnut oil frying for (2h).

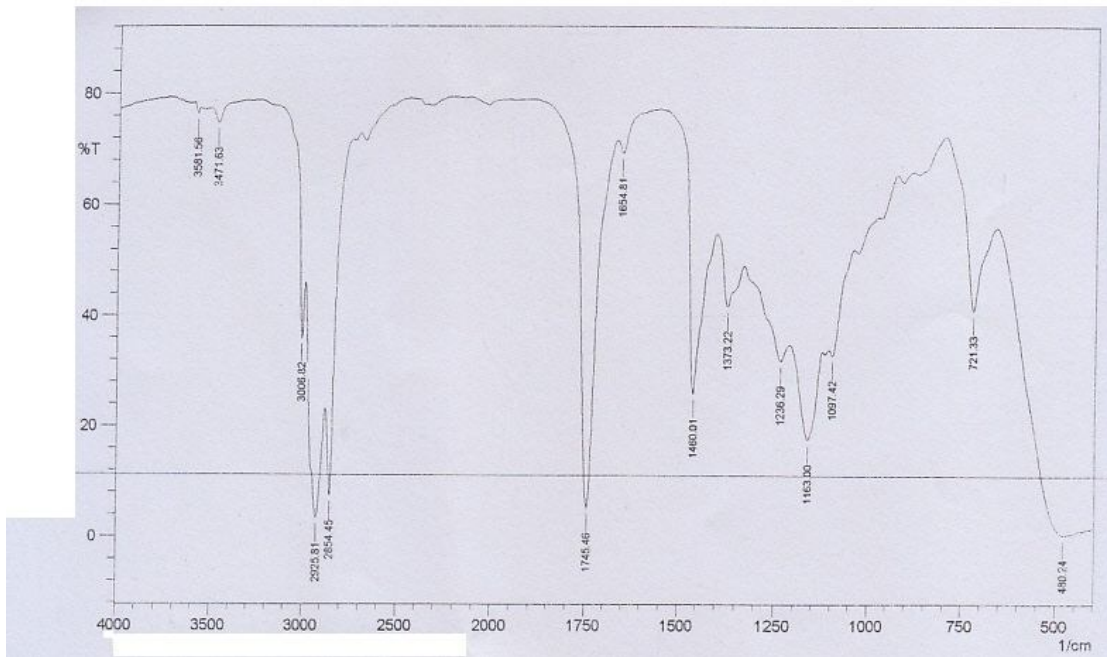


Appendix 24. FTIR Spectrum of groundnut oil frying for (4h).



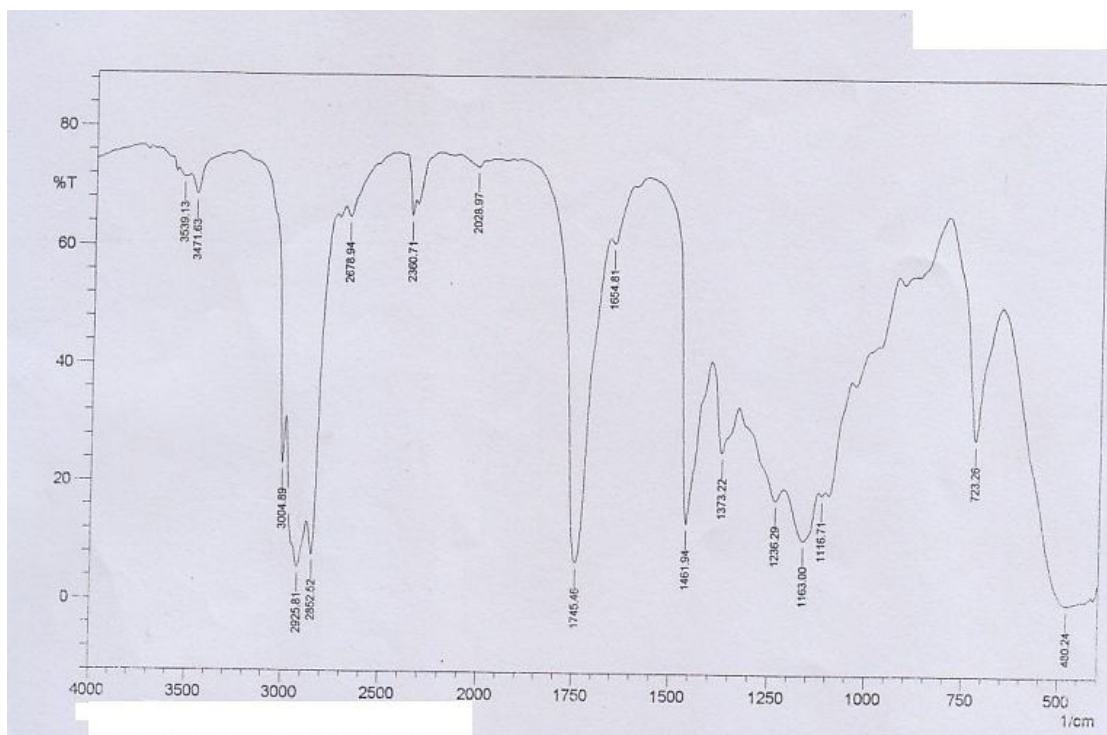
No.	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	480.24	0.279	5.963	659.61	459.03	209.689	12.299
2	721.33	40.182	20.268	798.47	659.61	38.38	8.394
3	1163	24.569	10.356	1215.07	1126.35	47.074	6.621
4	1236.29	33.712	3.428	1330.79	1215.07	47.7	1.412
5	1373.22	39.449	4.271	1400.22	1361.65	13.963	0.653
6	1461.94	29.73	30.27	1541.02	1400.22	40.441	9.508
7	1654.81	63.808	2.671	1666.38	1606.59	10.056	0.324
8	1745.46	14.619	54.047	1903.61	1668.31	63.452	27.857
9	2027.05	74.936	1.614	2129.27	1961.47	20.092	0.678
10	2677.01	66.532	2.292	2702.09	2432.07	37.305	-0.899
11	2854.45	16.353	18.822	2879.52	2746.44	50.264	6.707
12	2925.81	10.911	22.981	2989.46	2881.45	73.158	22.968
13	3004.89	37.113	7.408	3253.69	2989.46	45.567	-18.911
14	3471.63	69.566	3.708	3512.13	3313.48	26.226	0.609
15	3537.2	72.281	0.126	3569.99	3533.35	5.075	0.033

Appendix 25. FTIR Spectrum of groundnut oil frying for (6h).



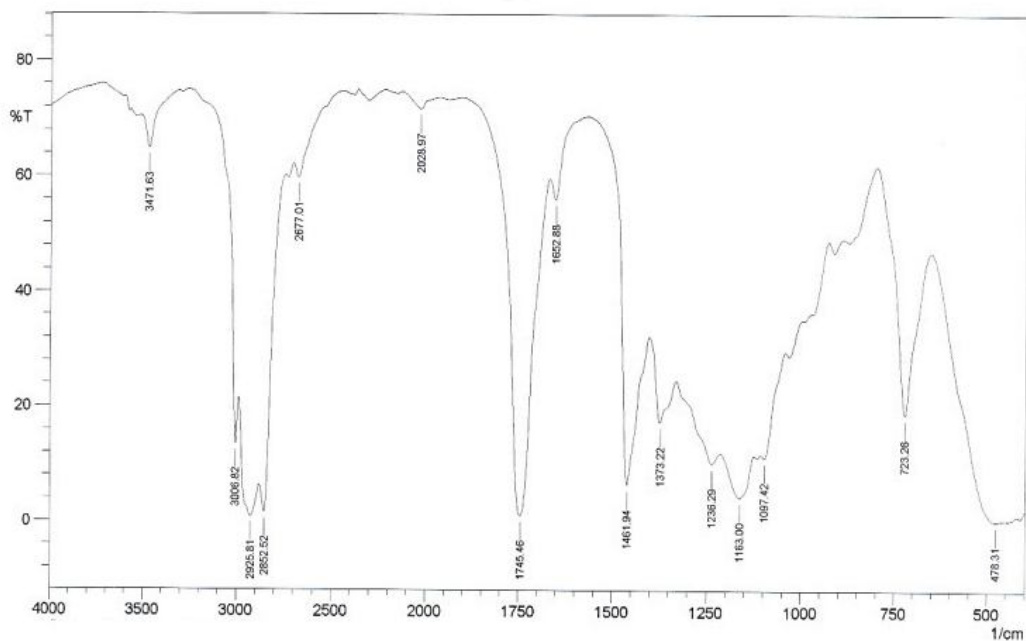
No.	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	480.24	0.342	4.016	659.61	466.74	189.464	5.167
2	721.33	40.695	22.102	800.4	661.54	35.4	7.925
3	1097.42	32.57	4.19	1108.99	1041.49	25.605	-0.002
4	1163	17.478	16.35	1213.14	1126.35	52.888	12.125
5	1236.29	31.652	5.572	1330.79	1215.07	46.799	2.164
6	1373.22	41.488	10.846	1402.15	1330.79	23.636	3.193
7	1460.01	25.714	38.275	1541.02	1402.15	37.382	11.306
8	1654.81	69.292	3.491	1670.24	1604.66	8.9	0.339
9	1745.46	5.119	68.946	1897.82	1670.24	63.189	35.047
10	2854.45	7.296	24.72	2879.52	2744.52	55.157	11.553
11	2925.81	3.234	29.277	2987.53	2881.45	96.818	44.669
12	3006.82	35.81	12.317	3251.76	2989.46	40.435	1.883
13	3471.63	74.685	2.974	3512.13	3311.55	22.178	0.606
14	3581.56	76.386	1.418	3598.92	3568.06	3.48	0.126

Appendix 26. FTIR Spectrum of groundnut oil frying for (12h).



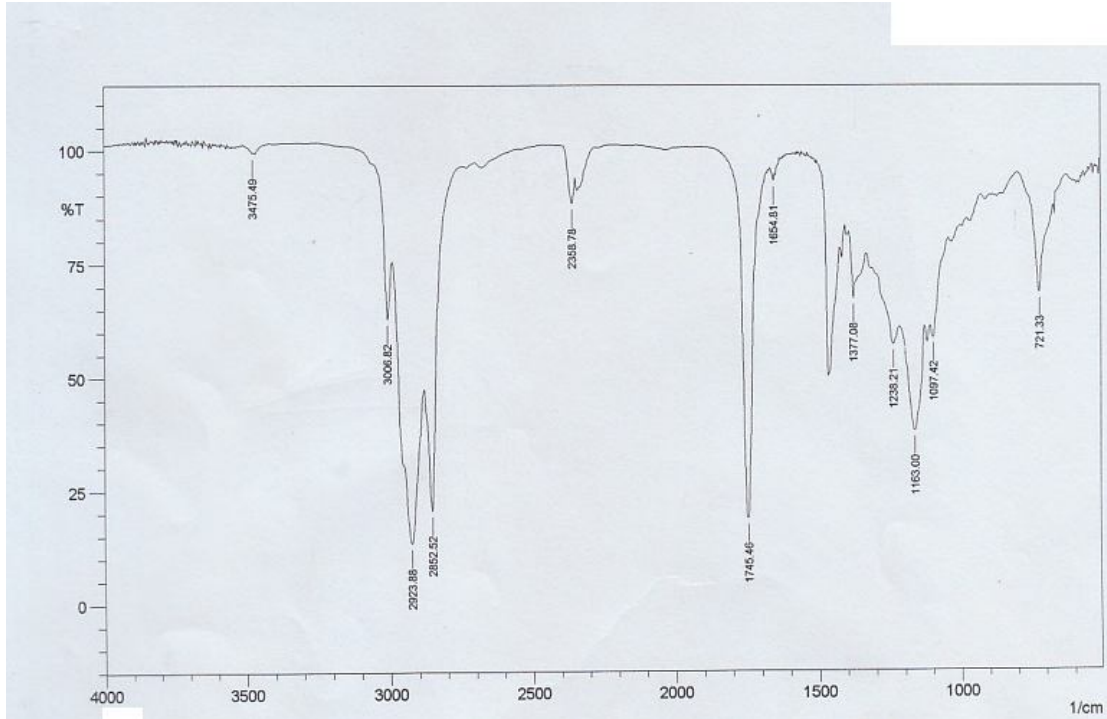
No.	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	480.24	0.239	6.854	655.75	445.53	241.451	26.409
2	723.26	28.033	29.827	798.47	655.75	47.769	13.748
3	1116.71	18.531	0.581	1126.35	1107.06	13.982	0.121
4	1163	10.793	8.506	1213.14	1126.35	73.77	11.826
5	1236.29	17.571	4.564	1330.79	1215.07	72.013	3.377
6	1373.22	25.596	12.367	1402.15	1330.79	36.245	5.453
7	1461.94	13.477	38.815	1568.02	1402.15	58.538	18.254
3	1654.81	60.868	2.593	1666.38	1606.59	11.082	0.304
9	1745.46	6.719	59.45	1901.68	1666.38	83.268	44.149
10	2028.97	73.573	1.652	2129.27	1969.19	20.383	0.683
11	2360.71	65.233	5.179	2393.5	2345.28	7.557	0.587
12	2678.94	64.988	2.528	2702.09	2437.86	38.104	-0.975
13	2852.52	7.627	16.162	2879.52	2746.44	68.607	9.209
14	2925.81	5.46	15.089	2989.46	2881.45	106.191	30.934
15	3004.89	23.055	10.133	3255.62	2991.39	51.953	2.06
16	3471.63	68.511	3.981	3512.13	3315.41	26.794	0.59
17	3539.13	71.379	0.354	3569.99	3531.42	5.537	0.069

Appendix 27. FTIR Spectrum of groundnut oil frying for (24h).



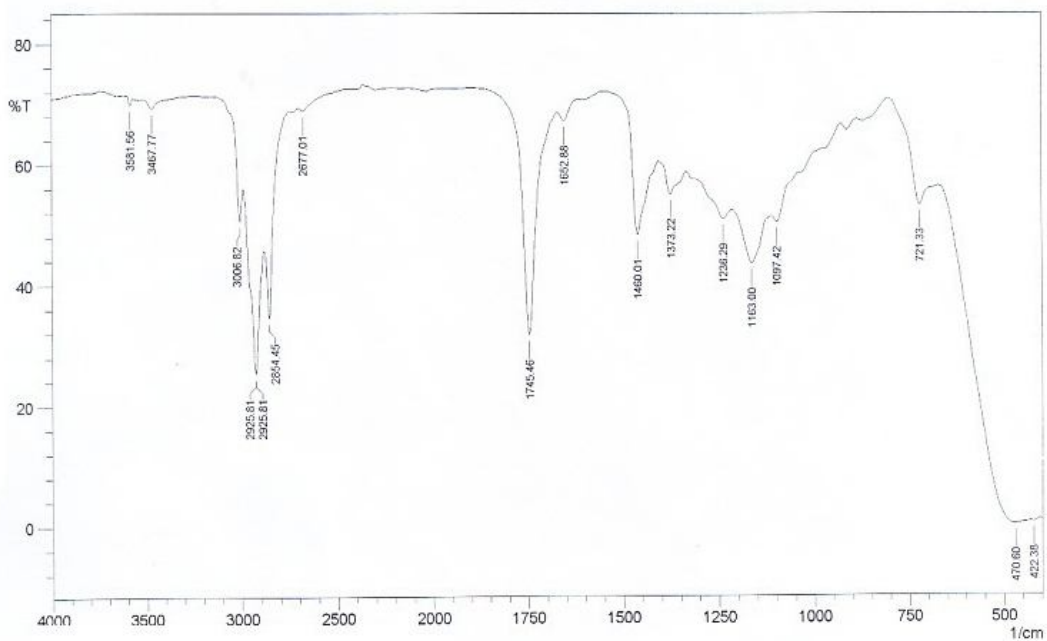
No.	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	478.31	0.266	5.308	651.89	457.1	219.754	11.916
2	723.26	18.661	35.308	798.47	653.82	60.771	21.655
3	1097.42	11.193	3.302	1107.06	1043.42	46.141	1.392
4	1163	4.314	7.504	1213.14	1126.35	99.85	19.437
5	1236.29	10.223	4.128	1330.79	1215.07	93.218	4.656
6	1373.22	17.369	11.662	1402.15	1332.72	45.358	7.003
7	1461.94	6.443	39.223	1568.02	1404.08	72.651	24.663
8	1652.88	55.954	5.552	1670.24	1569.95	18.585	-0.302
9	1745.46	1.061	63.121	1901.68	1670.24	107.154	65.972
10	2028.97	71.635	2.417	2131.19	1965.33	22.547	1.032
11	2677.01	59.74	3.694	2702.09	2443.64	41.901	0.901
12	2852.52	1.585	15.694	2879.52	2746.44	94.968	16.863
13	2925.81	0.853	11.74	2987.53	2881.45	157.749	58.043
14	3006.82	13.547	11.452	3257.55	2989.46	63.98	3.427
15	3471.63	64.799	6.534	3512.13	3313.48	29.457	1.793

Appendix 28. FTIR Spectrum of groundnut oil frying for (36h).



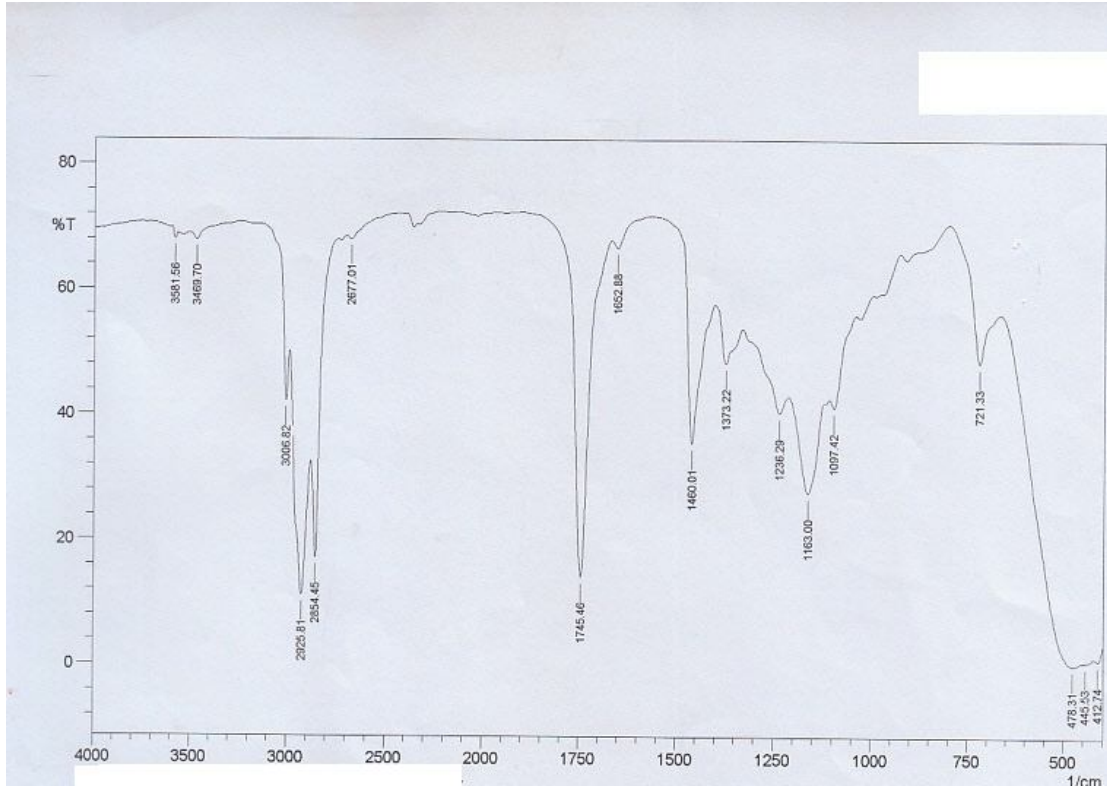
No.	name:ciprofloxac	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	721.33	68.14	22.22	796.55	671.18	9.67	4.55
2	1097.42	58.06	6.14	1108.99	1043.42	10.54	0.31
3	1163	37.48	23.12	1215.07	1128.28	27.92	9.03
4	1238.21	56.61	7.06	1311.5	1215.07	18.32	1.41
5	1377.08	66.93	13.65	1392.51	1330.79	8.4	2.2
6	1654.81	93.15	1.83	1668.31	1650.95	0.41	0.05
7	1745.46	18.43	77.05	1828.39	1687.6	23.58	21.06
8	2358.78	88.13	6.8	2407	2349.14	1.09	0.4
9	2852.52	20.39	36.49	2879.52	2742.59	25.51	2.08
10	2923.88	13.13	45.48	2989.46	2879.52	55.25	30.61
11	3006.82	62.77	15.55	3147.61	2989.46	6.04	-3.29
12	3475.49	99.44	0.28	3508.27	3471.63	-0.06	0.01

Appendix 29. FTIR Spectrum of sunflower oil frying for (0h).



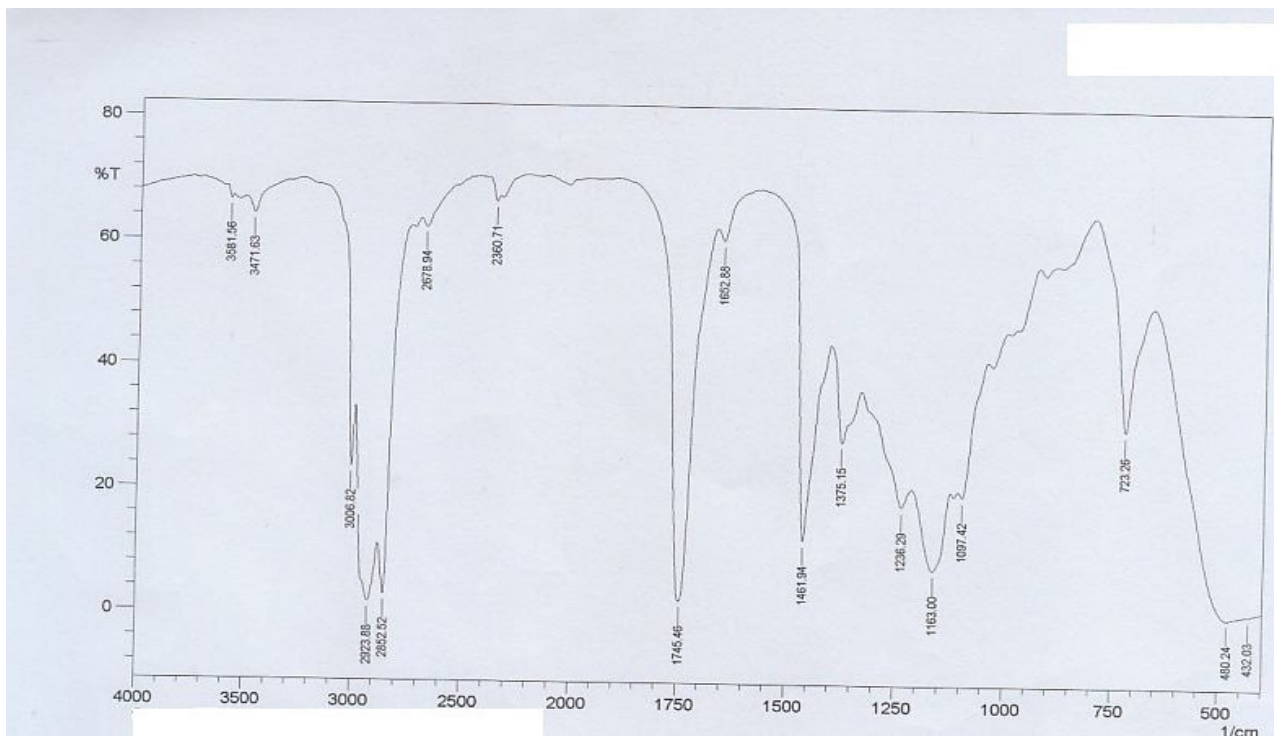
No.	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	422.38	0.481	0.241	430.1	406.95	50.521	1.798
2	470.6	0.066	9.505	671.18	432.03	286.342	36.903
3	721.33	53.164	8.706	802.33	671.18	28.472	2.301
4	1097.42	50.238	2.365	1110.92	1039.56	18.873	0.194
5	1163	43.428	8.306	1215.07	1110.92	33.181	3.368
6	1236.29	50.81	2.598	1309.58	1215.07	25.439	0.741
7	1373.22	54.92	4.675	1406.01	1332.72	17.619	1.092
8	1460.01	48.221	16.844	1541.02	1406.01	28.164	3.781
9	1652.88	67.147	2.041	1670.24	1610.45	9.631	0.243
10	1745.46	31.643	38.279	1892.04	1672.17	45.466	12.287
11	2677.01	68.926	0.702	2700.16	2437.86	38.828	0.151
12	2854.45	34.509	15.641	2881.45	2742.59	35.821	0.894
13	2925.81	25.363	24.573	2987.53	2881.45	44.203	12.659
14	2925.81	25.363	24.512	2989.46	2881.45	44.605	12.589
15	3006.82	50.557	6.973	3157.25	2989.46	30.126	-3.347
16	3467.77	69.325	1.607	3512.13	3307.69	30.903	0.583
17	3581.56	69.838	1.438	3598.92	3568.06	4.671	0.14

Appendix 30. FTIR Spectrum of sunflower oil frying for (2h).



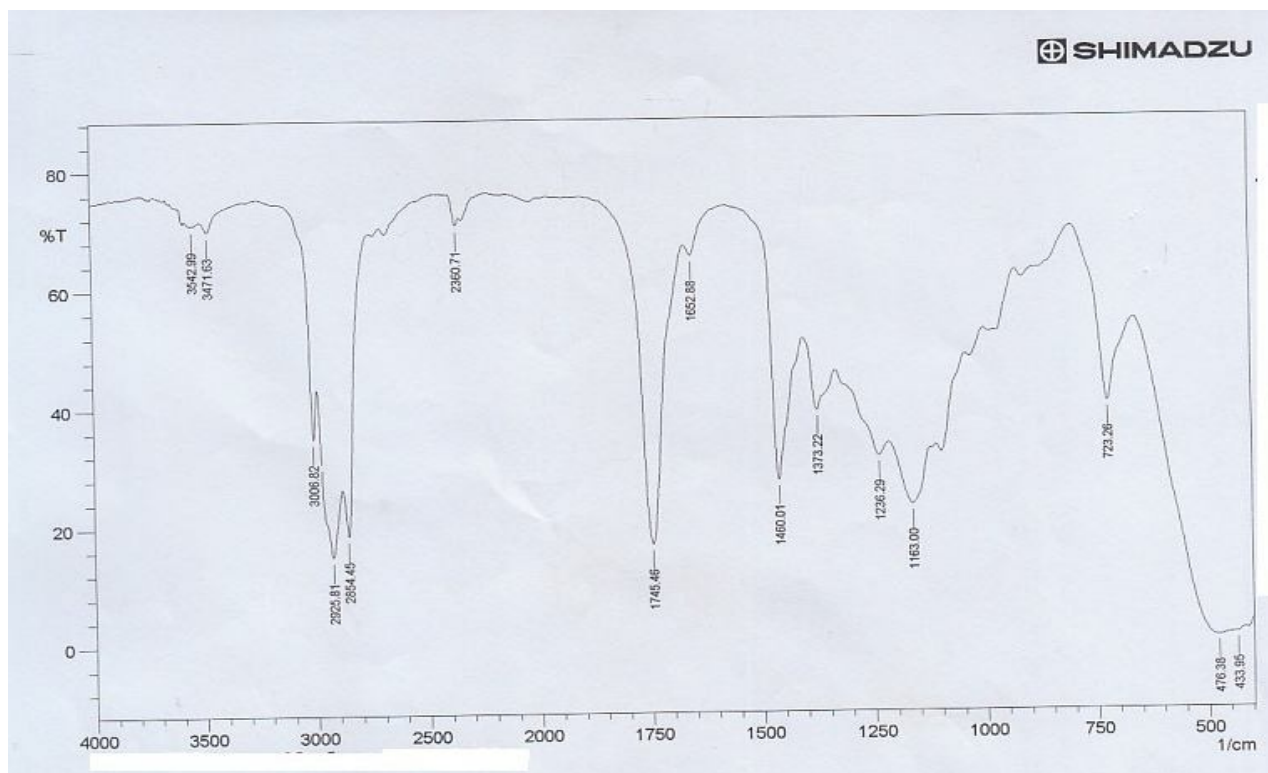
No.	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	412.74	0.905	1.394	422.38	399.24	42.193	4.242
2	445.53	0.661	0.129	449.38	424.31	51.65	1.381
3	478.31	0.162	7.449	667.32	451.31	232.931	22.248
4	721.33	48.25	13.444	802.33	669.25	30.616	3.774
5	1097.42	41.111	3.68	1108.99	1041.49	21.205	0.13
6	1163	27.584	14.737	1213.14	1124.42	41.155	8.072
7	1236.29	40.292	4.614	1330.79	1215.07	38.24	1.375
8	1373.22	48.147	7.859	1402.15	1330.79	20.259	2.072
9	1460.01	35.453	27.796	1544.88	1402.15	34.543	7.067
10	1652.88	66.541	1.914	1668.31	1575.73	14.534	0.033
11	1745.46	14.01	55.278	1899.75	1670.24	57.952	22.526
12	2677.01	67.903	0.89	2700.16	2430.14	40.882	0.185
13	2854.45	17	21.959	2879.52	2744.52	45.24	7.037
14	2925.81	10.964	28.813	2987.53	2881.45	69.025	26.94
15	3006.82	42.095	10.093	3157.25	2989.46	33.23	-4.764
16	3469.7	67.75	1.491	3510.2	3311.55	31.56	0.364
17	3581.56	67.877	1.375	3598.92	3569.99	4.728	0.13

Appendix 31. FTIR Spectrum of sunflower oil frying for (4h).



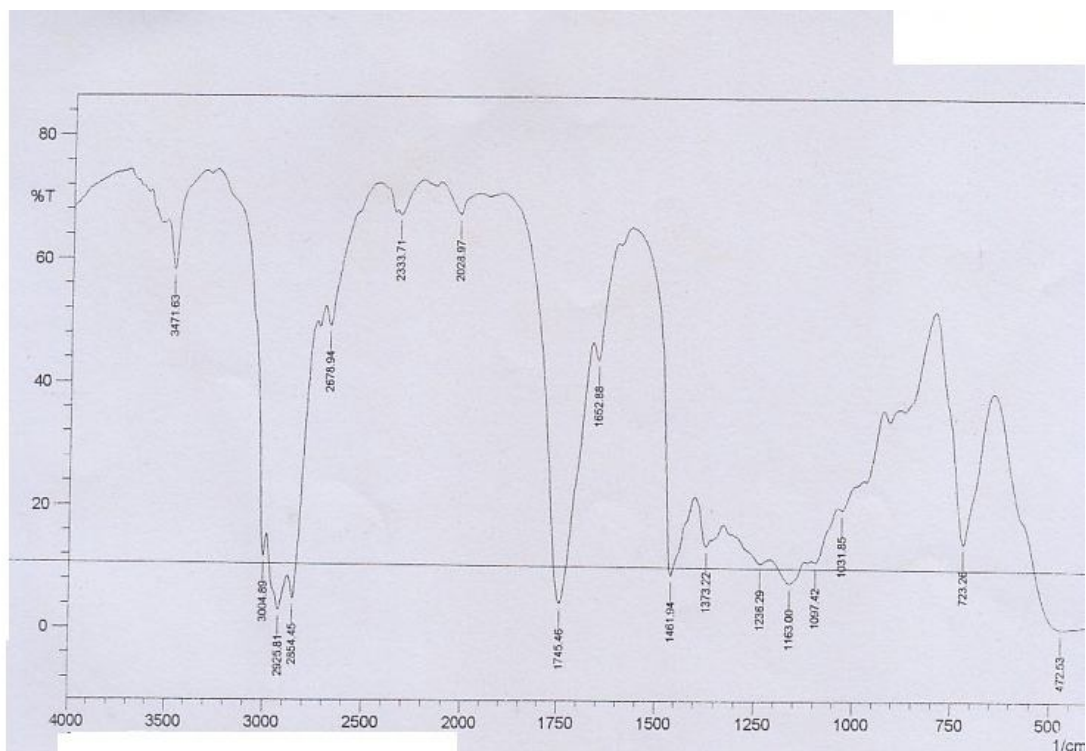
No.	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	432.03	0.758	0.134	435.88	399.24	73.06	1.409
2	480.24	0.183	10.207	657.68	437.81	263.634	33.105
3	723.26	30.382	26.652	798.47	659.61	44.956	11.151
4	1097.42	19.559	3.961	1107.06	1041.49	34.671	-0.501
5	1163	7.588	12.912	1213.14	1126.35	78.739	19.062
6	1236.29	17.884	5.79	1330.79	1215.07	68.312	3.552
7	1375.15	28.19	12.922	1402.15	1330.79	33.478	5.109
8	1461.94	12.197	40.521	1568.02	1404.08	57.663	18.05
9	1652.88	60.597	3.048	1668.31	1575.73	16.817	-0.075
10	1745.46	2.115	63.05	1899.75	1670.24	90.048	49.4
11	2360.71	66.531	1.866	2395.42	2345.28	8.301	0.208
12	2678.94	62.314	1.981	2702.09	2437.86	45.136	0.48
13	2852.52	2.768	18.619	2879.52	2746.44	77.636	14.874
14	2923.88	1.589	18.105	2989.46	2881.45	133.263	55.04
15	3006.82	23.546	11.75	3242.12	2991.39	55.806	2.371
16	3471.63	64.216	3.183	3512.13	3311.55	34.193	0.854
17	3581.56	66.486	1.203	3598.92	3569.99	4.991	0.115

Appendix 32. FTIR Spectrum of sunflower oil frying for (6h).



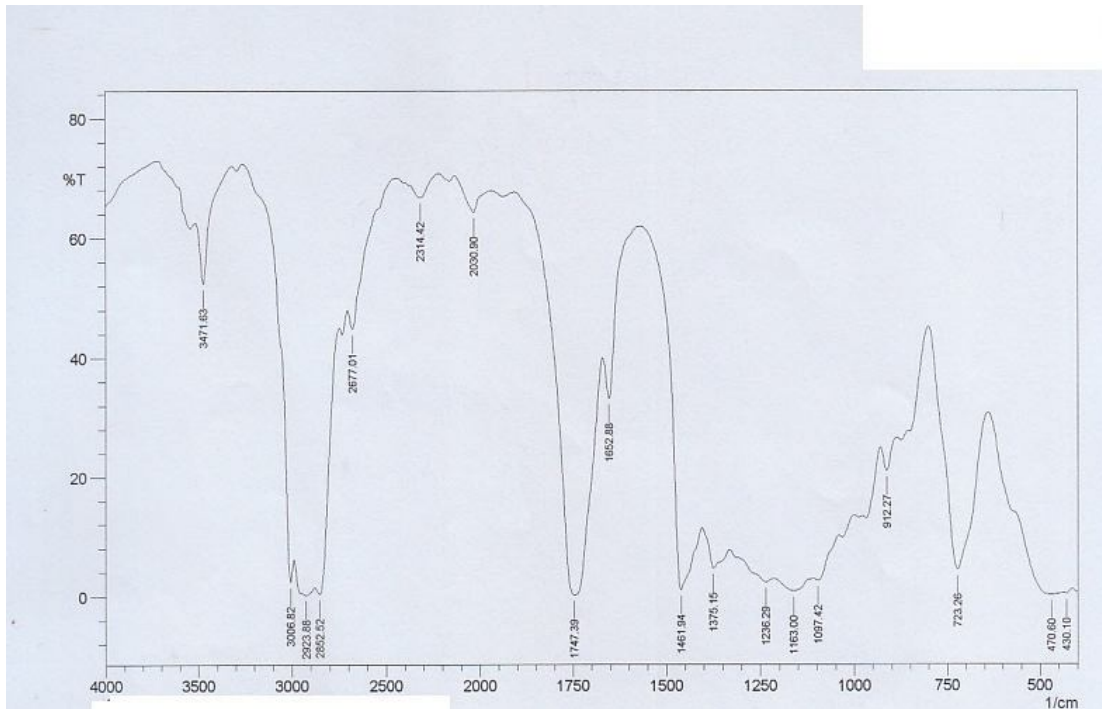
No.	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	433.95	0.875	0.187	439.74	416.6	44.474	0.484
2	476.38	0.379	8.961	659.61	441.67	244.549	24.976
3	723.26	40.013	21.029	800.4	659.61	37.712	7.782
4	1163	23.028	9.789	1213.14	1122.49	51.074	7.253
5	1236.29	31.19	4.426	1330.79	1215.07	49.391	2.045
6	1373.22	38.97	9.914	1402.15	1330.79	25.696	3.134
7	1460.01	27.257	32.55	1544.88	1404.08	40.055	11.053
8	1652.88	65.169	2.853	1668.31	1577.66	13.932	-0.035
9	1745.46	16.65	53.034	1901.68	1668.31	62.029	27.46
10	2360.71	70.587	2.729	2393.5	2343.35	6.905	0.316
11	2854.45	18.478	15.949	2879.52	2746.44	48.061	5.785
12	2925.81	14.947	18.348	2987.53	2881.45	69.83	19.439
13	3006.82	34.929	11.447	3159.18	2999.46	33.636	-8.172
14	3471.63	69.947	2.233	3508.27	3307.69	27.376	0.224
15	3542.99	70.887	0.389	3571.92	3531.42	5.981	0.062

Appendix 33. FTIR Spectrum of sunflower oil frying for (12h).



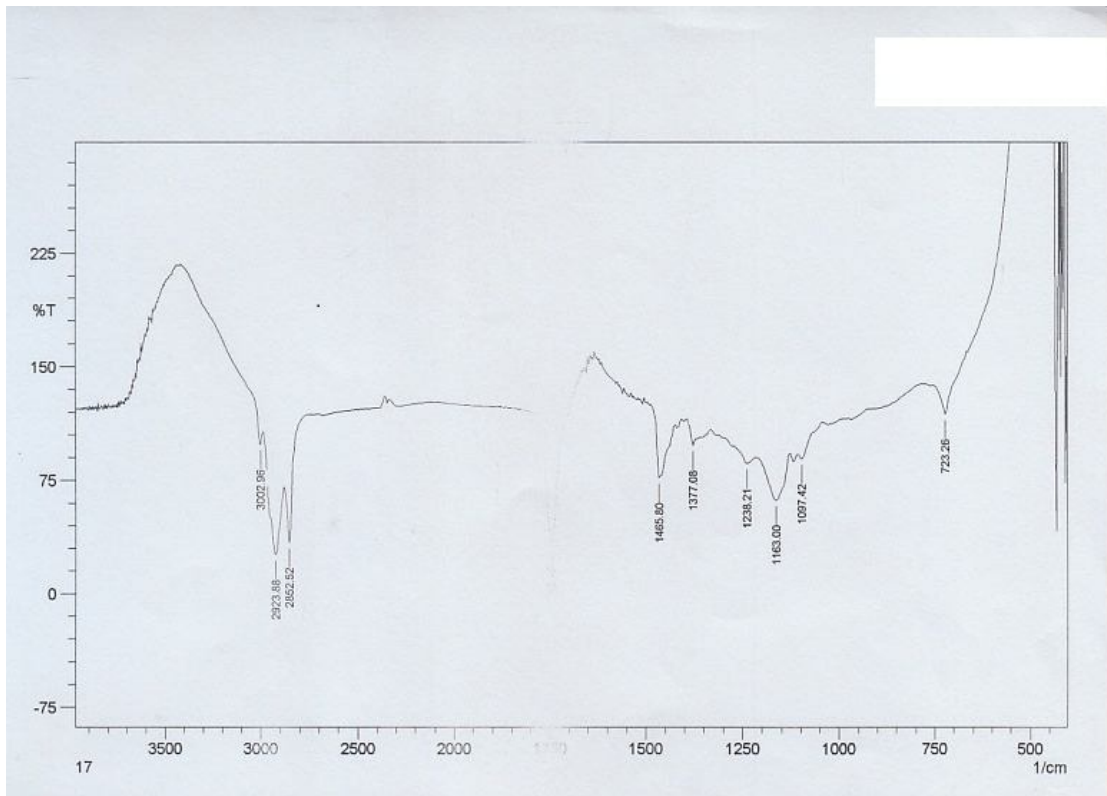
No.	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	472.53	0.164	3.688	644.18	455.17	233.618	9.475
2	723.26	14.078	31.098	798.47	646.11	83.283	29.727
3	1031.85	19.515	1.069	1041.49	977.84	41.928	0.404
4	1097.42	11.03	1.575	1107.06	1043.42	53.103	1.056
5	1163	7.601	3.751	1213.14	1126.35	89.712	7.77
6	1236.29	10.776	1.781	1330.79	1215.07	101.637	2.81
7	1373.22	13.576	6.15	1402.15	1332.72	55.106	5.259
8	1461.94	8.74	27.912	1571.88	1404.08	85.068	20.361
9	1652.88	43.673	6.958	1668.31	1806.59	17.532	1.047
10	1745.46	4.17	50.379	1903.61	1668.31	115.298	59.19
11	2028.97	67.341	4.108	2131.19	1963.4	26.155	1.856
12	2333.71	67.292	1.274	2349.14	2219.91	19.952	0.252
13	2678.94	49.138	5.082	2702.09	2443.64	52.496	1.484
14	2854.45	4.773	11.531	2879.52	2746.44	95.105	10.905
15	2925.81	2.933	8.416	2987.53	2881.45	128.154	27.906
16	3004.89	11.742	7.003	3259.47	2989.46	76.819	2.488
17	3471.63	58.153	9.701	3512.13	3317.34	31.951	1.928

Appendix 34. FTIR Spectrum of sunflower oil frying for (24h).



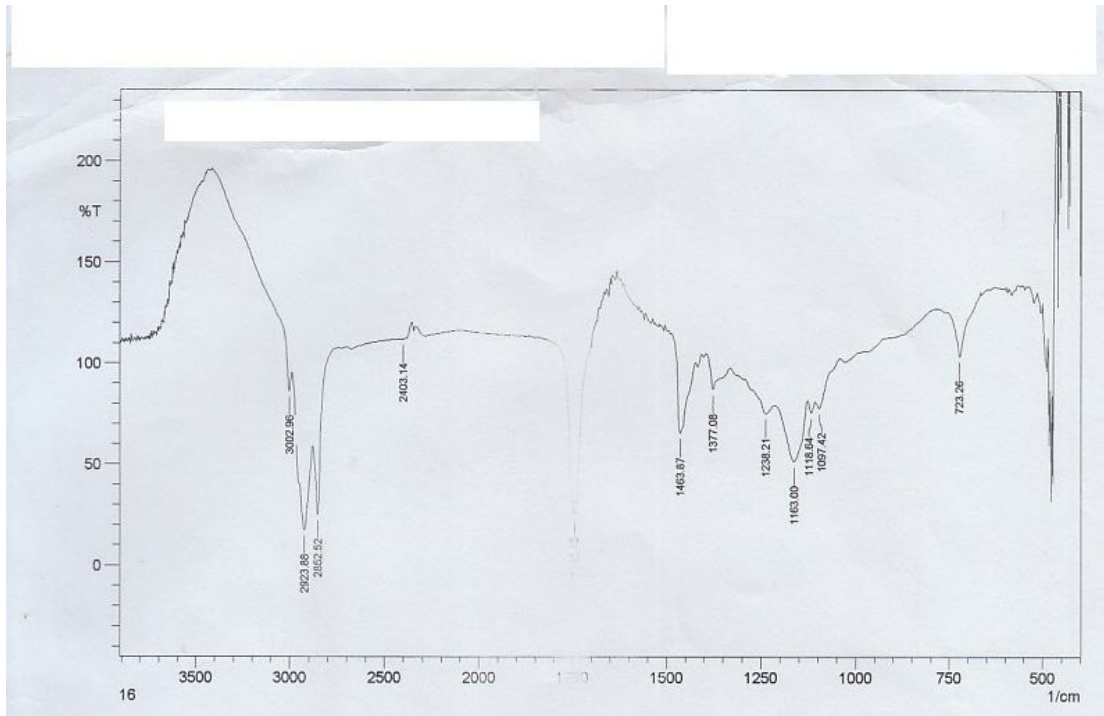
No.	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	430.1	0.431	0.365	435.88	416.6	41.623	2.141
2	470.6	0.261	5.271	638.39	437.81	280.611	27.45
3	723.26	4.499	33.691	798.47	640.32	123.836	55.677
4	912.27	21.026	4.524	929.63	885.27	27.707	1.545
5	1097.42	2.618	1.637	1108.99	1043.42	83.964	3.002
6	1163	0.953	2.037	1213.14	1110.92	179.576	23.763
7	1236.29	2.404	1.459	1330.79	1215.07	159.5	6.835
8	1375.15	4.722	5.272	1404.08	1332.72	83.484	10.214
9	1461.94	1.076	27.81	1568.02	1406.01	120.203	37.629
10	1652.88	33.104	10.879	1670.24	1575.73	28.812	0.13
11	1747.39	0.262	48.784	1905.54	1670.24	168.964	102.898
12	2030.9	64.282	4.607	2131.19	1967.26	28.449	2.196
13	2314.42	66.736	2.808	2368.42	2217.99	24.734	1.259
14	2677.01	44.875	5.009	2700.16	2441.71	58.196	1.454
15	2852.52	0.412	9.908	2879.52	2746.44	145.964	20.902
16	2923.88	0.28	0.639	2948.96	2881.45	152.77	16.097
17	3006.82	2.425	7.989	3257.55	2989.46	103.311	7.182
18	3471.63	52.397	12.097	3512.13	3317.34	37.214	3.476

Appendix 35. FTIR Spectrum of sunflower oil frying for (36h).



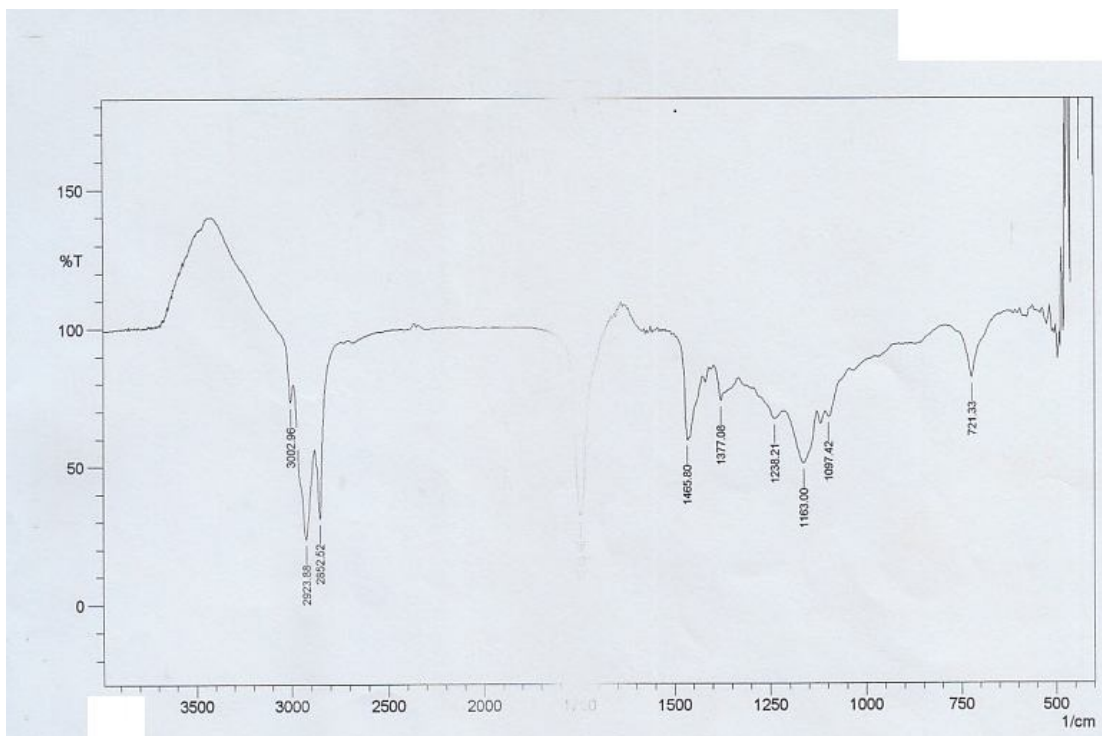
No.	Peak	Intensity	Corr. Intensity	Base (L)	Area	Corr. Area
1	723.26	117.9	27.4	667.32	-11.9	3.2
2	1097.42	88.6	6.5	1041.49	-0.3	0.3
3	1163	61.7	29.9	1128.28	10.8	7.4
4	1238.21	85.9	7	1215.07	2.5	0.9
5	1377.08	97.7	9.6	1365.51	-0.6	0.4
6	1465.8	76.3	43.5	1423.37	0.5	5.2
7	1745.46	33.8	84.3	1703.03	8.1	14.5
8	2852.52	33.6	46.5	2744.52	7.9	2.6
9	2923.88	25.6	59.7	2879.52	31.8	25.1
10	3002.96	98.2	12.4	2989.46	-92	-13.4

Appendix 36. FTIR Spectrum of *moringa oliefera* oil frying for (0h).



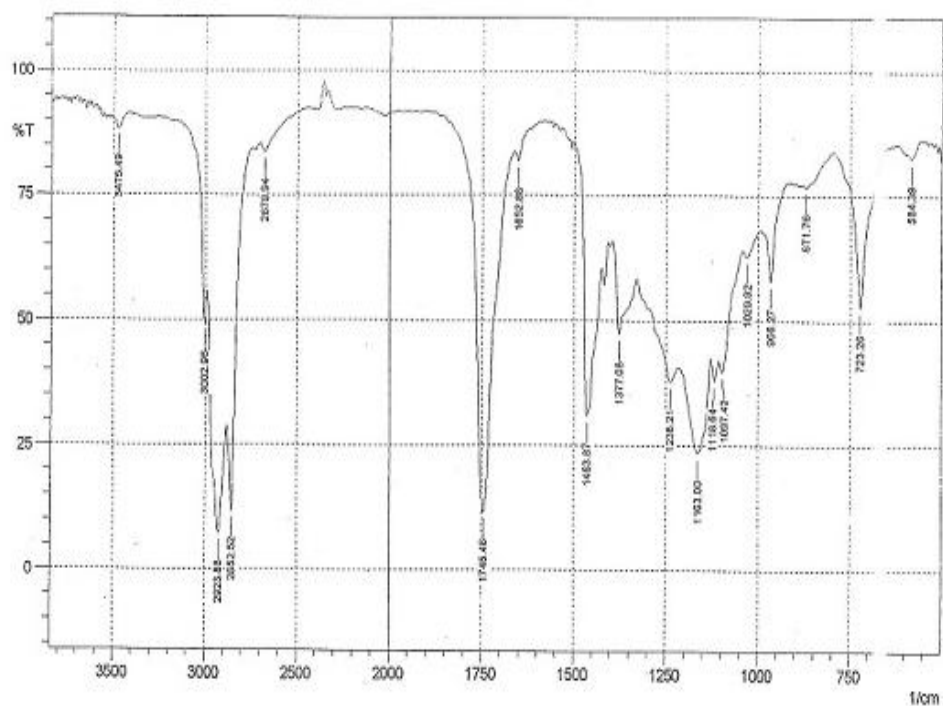
No.	Peak	Intensity	Corr. Intensity	Height (H)	Base (L)	Area	Corr. Area
1	723.26	103.3	27.9	10.75	667.32	-9.7	3.8
2	1097.42	77.8	6.5	10.06	1043.42	2.9	0.3
3	1118.64	75.6	5.7	10.28	1107.06	2.2	0.3
4	1163	51.6	29.1	10.07	1128.28	16.5	8.3
5	1238.21	75.2	7	10.86	1215.07	5.8	1
6	1377.08	86.9	15.8	10.44	1330.79	1.7	2
7	1463.87	65.6	43.6	10.94	1423.37	3.9	5.9
8	1745.46	25.7	87.5	10.75	1693.38	13	18.4
9	2403.14	111.8	1.3	10.85	2358.78	-2.9	0.4
10	2852.52	25	43.5	10.52	2742.59	16.2	2.6
11	2923.88	17.4	56.1	10.46	2879.52	43	29.2
12	3002.96	86	12.3	10.53	2989.46	-73.1	-15.1

Appendix 37. FTIR Spectrum of *moringa oliefera* oil frying for (2h).



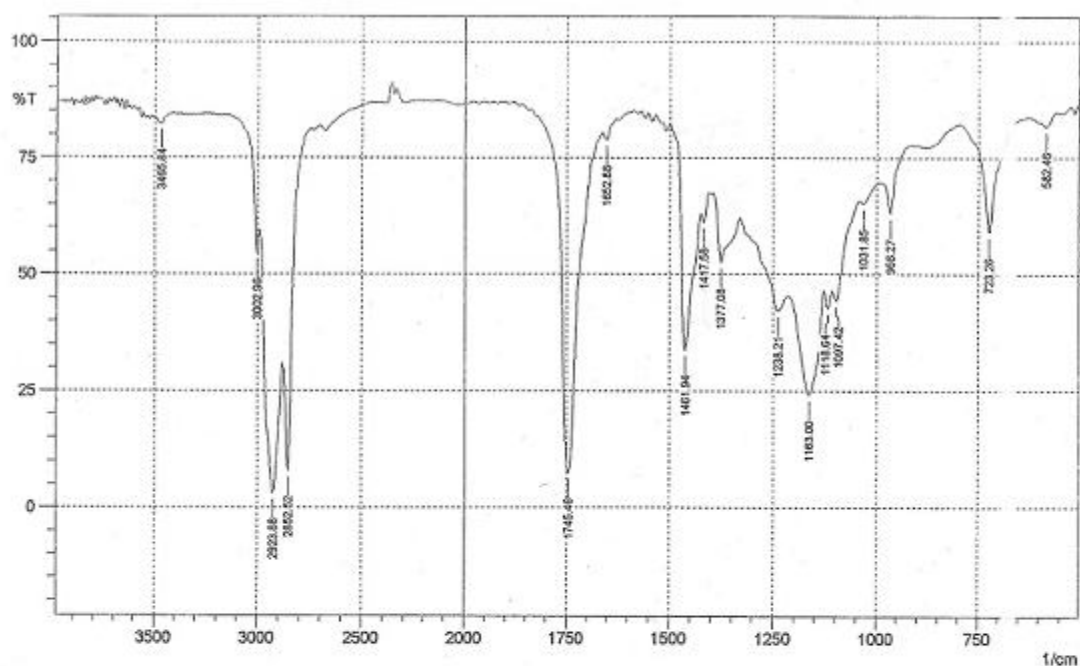
No.	Peak	Intensity	Corr. Intensity	Area (H)	Base (L)	Area	Corr. Area
1	721.33	81.5	19.8	7.1	669.25	2.9	3.5
2	1097.42	67.4	4.3	1.06	1043.42	7.5	0.2
3	1163	50.6	18.7	1.07	1128.28	19.7	5.9
4	1238.21	66.7	5.1	1.07	1215.07	12	1
5	1377.08	73.2	11.5	1.14	1330.79	6.7	1.8
6	1465.8	59	31.8	1.1	1425.3	8.3	4.9
7	1745.46	31.8	69	1.02	1670.24	17.3	17.8
8	2852.52	31	32.7	2.02	2740.66	20.5	1.5
9	2923.88	23.5	42.1	2.046	2879.52	40.2	21
10	3002.96	73.1	8.6	2.1	2989.46	-21.5	-11.3

Appendix 38. FTIR Spectrum of *moringa oliefera* oil frying for (4h).



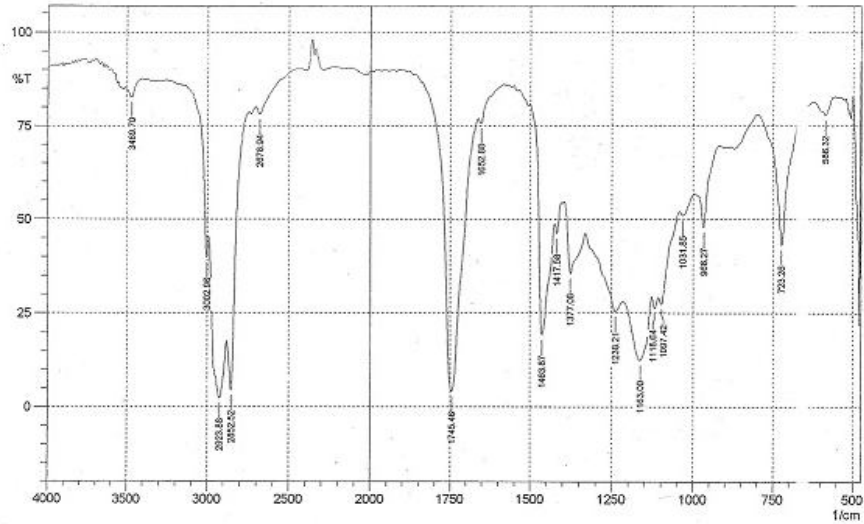
No.	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	594.39	82.58	3.46	624.89	557.39	5.01	0.57
2	723.26	52.82	30.63	798.47	665.4	18.03	7.6
3	871.76	76.82	1.16	891.41	798.47	8.1	0.28
4	966.27	57.94	13.76	993.27	919.98	11.67	1.57
5	1029.92	62.91	2.29	1043.42	993.27	9.29	0.28
6	1097.42	39.8	5.77	1107.06	1043.42	18.51	0.47
7	1118.64	38.04	4.38	1128.28	1107.06	8.4	0.5
8	1163	23.5	18.36	1215.07	1128.28	44.24	11.25
9	1238.21	37.84	6.49	1330.79	1215.07	38.3	2.26
10	1377.08	47.24	16.4	1396.37	1330.79	17.34	3.72
11	1463.87	30.94	42.33	1502.44	1425.3	20.89	8.98
12	1652.88	81.96	3.38	1664.45	1629.74	2.47	0.2
13	1745.46	11.53	75.06	1839.96	1664.45	44.39	33.57
14	2678.94	83.58	2.5	2704.01	2547.79	9.4	0.61
15	2652.52	11.72	28.04	2879.52	2742.59	43.28	1.21
16	2923.88	7.11	32.34	2991.39	2879.52	79.8	35.34
17	3002.96	49.04	9.06	3147.61	2991.39	16.44	7.19
18	3475.49	88.3	0.31	3485.7	3471.63	1.26	0.02

Appendix 39. FTIR Spectrum of *moringa oliefera* oil frying for (6h).



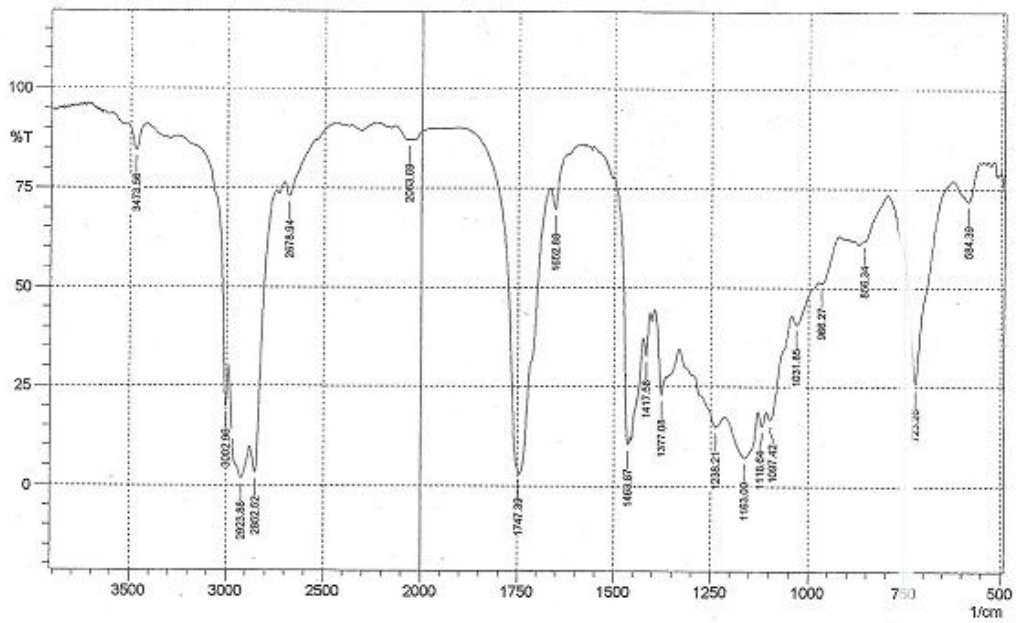
No.	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	582.46	81.69	2.75	626.82	559.32	5.47	0.45
2	723.26	59.06	23.82	796.55	626.82	19.75	6.01
3	966.27	63.06	9.51	995.2	914.2	11.75	0.96
4	1031.85	65.17	1.58	1043.42	995.2	8.35	0.2
5	1097.42	44.39	5.07	1107.06	1043.42	16.54	0.19
6	1118.64	42.8	3.79	1128.28	1107.06	7.42	0.38
7	1163	24.1	22.05	1215.07	1128.28	41.23	11.98
8	1238.21	42.18	6.59	1330.79	1215.07	33.65	1.87
9	1377.08	52.62	13.23	1396.37	1330.79	15.36	2.96
10	1417.58	61.13	3.67	1425.3	1406.01	3.8	0.22
11	1461.94	33.87	38.52	1500.52	1425.3	19.03	8.3
12	1652.88	78.84	2.46	1662.52	1629.74	3	0.16
13	1745.46	7.47	75.32	1838.04	1662.52	47.28	32.98
14	2852.52	7.84	33.1	2879.52	2742.59	44.67	3.69
15	2923.88	3.08	39.28	2991.39	2879.52	88.89	47.9
16	3002.95	54.52	7	3136.04	2991.39	17.14	-4.57
17	3455.84	82.37	0.41	3473.56	3427.27	3.64	0

Appendix40. FTIR Spectrum of *moringa oliefera* oil frying for (12h).



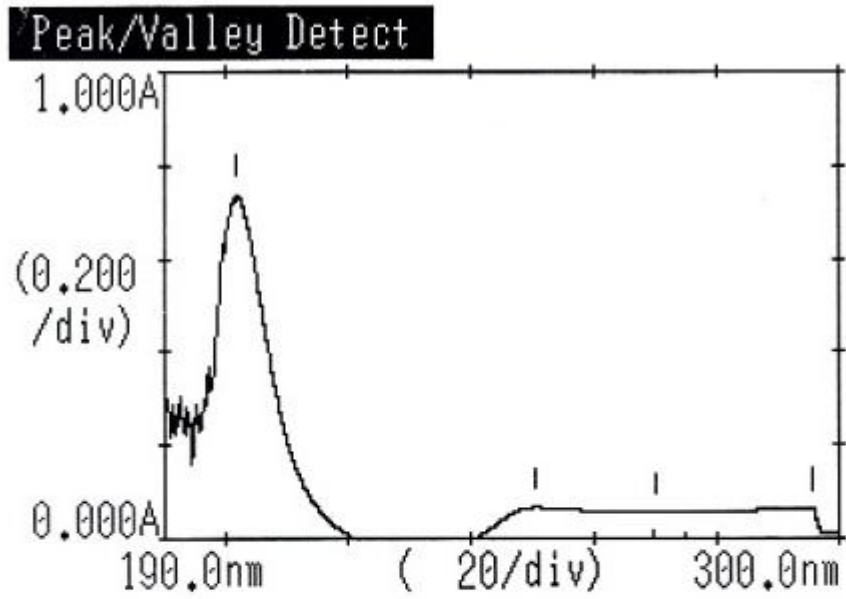
No.	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	566.32	77.74	2.28	595.96	561.25	3.42	0.21
2	723.26	43.17	36.15	800.4	646.11	26.33	10.8
3	968.27	47.8	13.91	995.2	918.05	17.52	2.03
4	1031.85	51.05	2.03	1041.49	995.2	12.59	0.37
5	1097.42	27.32	5.26	1107.06	1041.49	26.98	0.17
6	1118.64	26.11	3.17	1128.28	1107.06	11.82	0.5
7	1163	12.45	16.34	1215.07	1128.28	63.13	16
8	1239.21	25.45	6.19	1330.79	1215.07	54.23	2.88
9	1377.08	35.63	16.62	1396.37	1330.79	24.6	5.05
10	1417.58	46.12	4.78	1425.3	1404.08	6.45	0.35
11	1463.97	19.16	46.11	1500.52	1425.3	28.64	13.47
12	1662.88	75.61	2.49	1662.52	1583.45	6.82	-0.26
13	1745.46	4.12	77.78	1861.18	1662.52	67.02	50.99
14	2878.94	78.04	3	2704.01	2437.86	18.99	-0.4
15	2852.52	4.43	25.27	2879.52	2742.59	60.88	2.21
16	2923.88	2.37	26.3	2991.39	2879.52	113.51	52.23
17	3002.96	39.8	8.17	3172.68	2991.39	23.76	-13.27
18	3469.7	82.61	2.88	3496.7	3406.05	6.33	0.43

Appendix41. FTIR Spectrum of *moringa oliefera* oil frying for (24h).



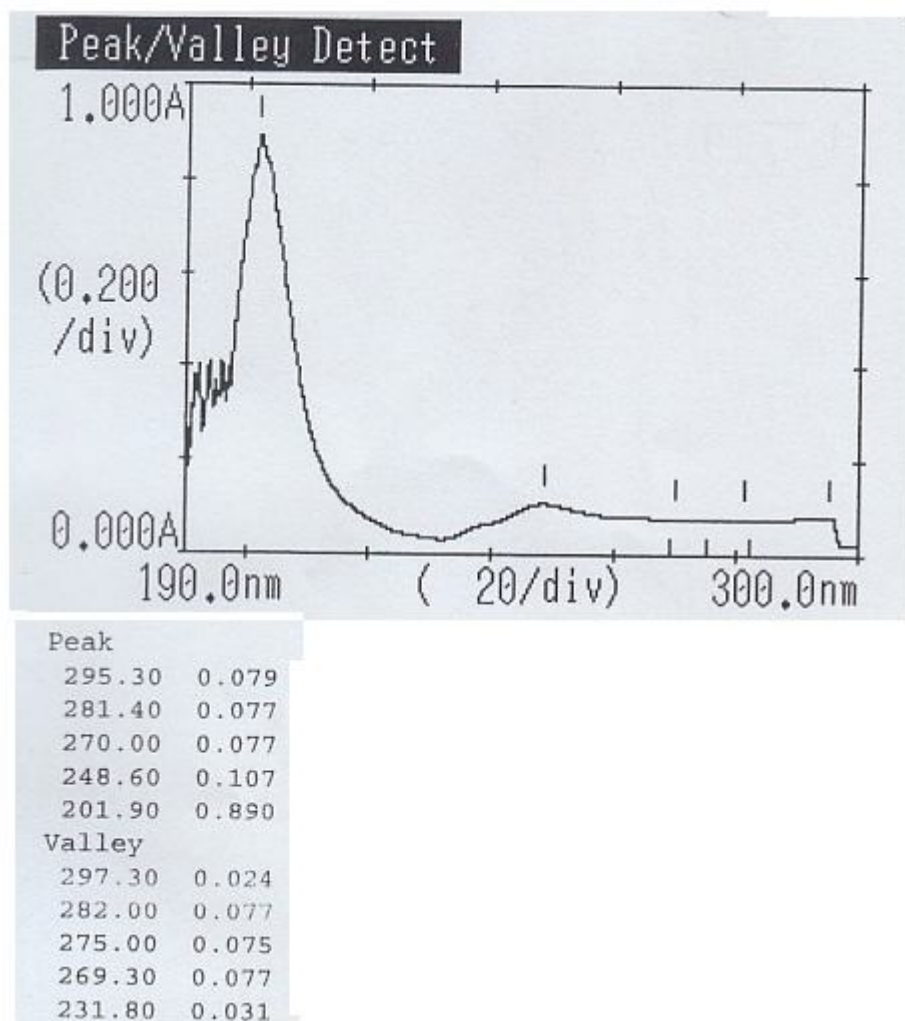
No.	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	584.39	71.85	0.76	566.32	555.46	3.54	-0.01
2	723.26	25.59	49.35	798.47	624.89	43.19	21.67
3	856.34	61.83	0.4	858.26	798.47	10.11	-0.13
4	966.27	51.18	2.16	973.99	923.84	12.67	0.45
5	1031.85	40.74	3.75	1043.42	973.99	23.56	0.87
6	1097.42	16.61	5.81	1107.06	1043.42	36.05	1.26
7	1118.64	15.03	3.75	1128.28	1107.06	16.44	1.06
8	1163	7.15	11.1	1215.07	1128.28	63.38	18.93
9	1238.21	14.82	4.63	1274.96	1215.07	44.86	2.91
10	1377.08	22.83	11.79	1396.37	1363.58	16.83	1.52
11	1417.58	32.68	7.48	1425.3	1407.94	7.52	0.66
12	1463.87	10.38	10.31	1504.37	1458.08	18.51	-4.83
13	1662.88	69.62	8.02	1666.38	1620.09	5.18	0.53
14	1747.39	2.93	77.77	1865.04	1666.38	78.49	81.04
15	2063.69	87.06	0.79	2135.05	2046.33	4.77	0.24
16	2678.94	72.88	4.94	2704.01	2439.78	20.53	-0.18
17	2852.52	3.05	19.34	2879.52	2742.59	77.78	-0.96
18	2923.88	1.81	16.11	2989.46	2879.52	135.51	51.08
19	3002.96	19.94	13.2	3249.83	2989.46	39.42	-35.83
20	3473.56	84.49	6.05	3506.35	3436.91	4.01	1.01

Appendix42. FTIR Spectrum of moringa oliefera oil frying for (36h).

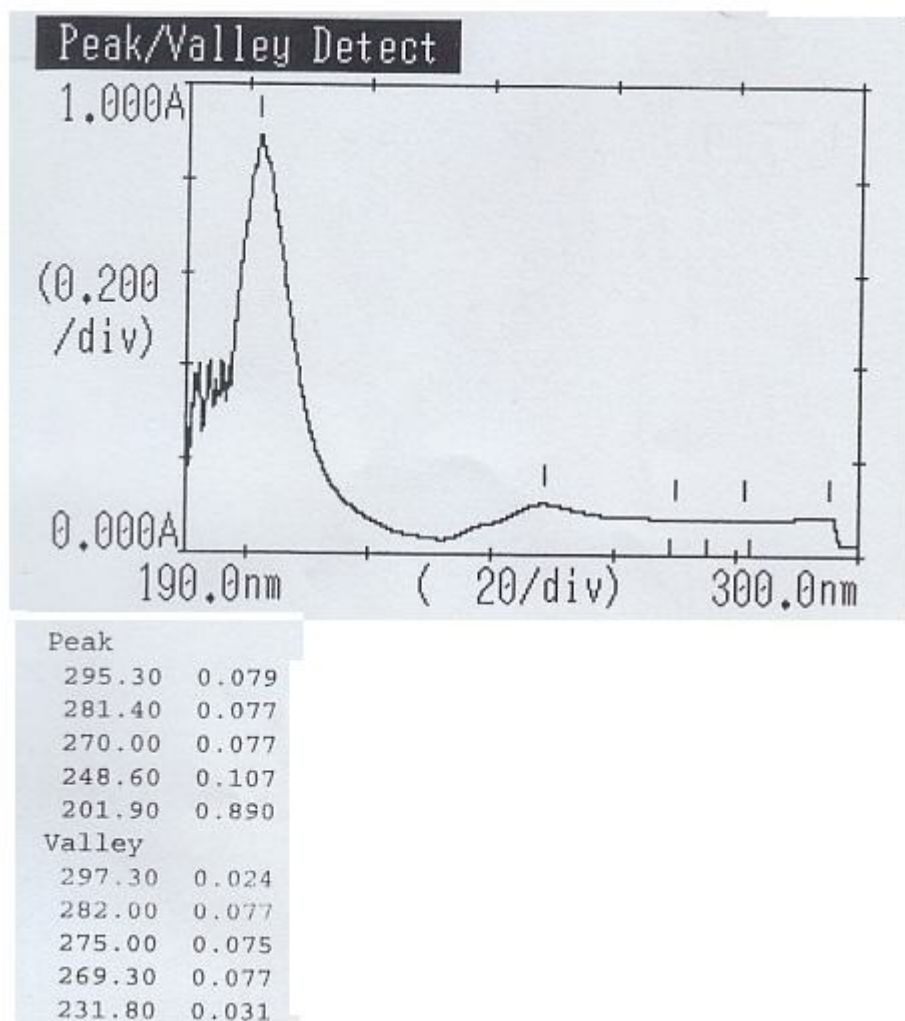


Peak	
295.70	0.064
270.00	0.057
250.50	0.066
201.90	0.738
Valley	
297.30	0.009
274.80	0.056
269.40	0.057
232.10	-0.045

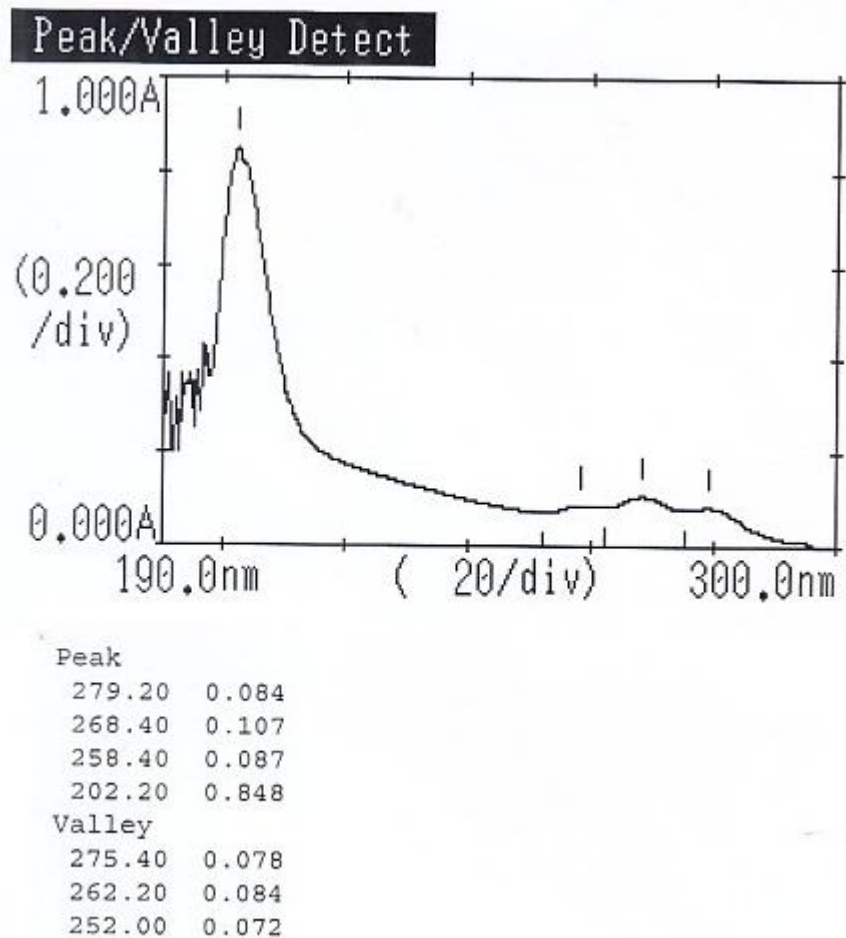
Appendix 43. UV Spectrophotometric determination of conjugating dienes and triene of Groundnut Oil Frying for (0h).



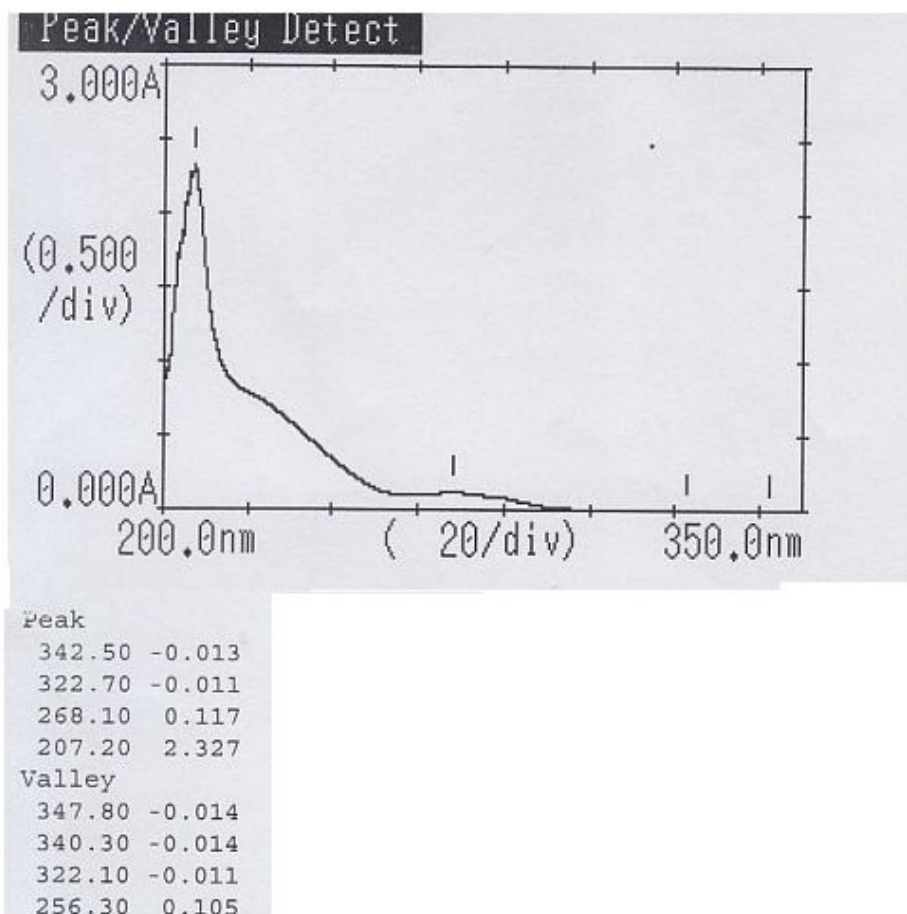
Appendix 44. UV Spectrophotometric determination of conjugating dienes and triene of Groundnut Oil Frying for (2h).



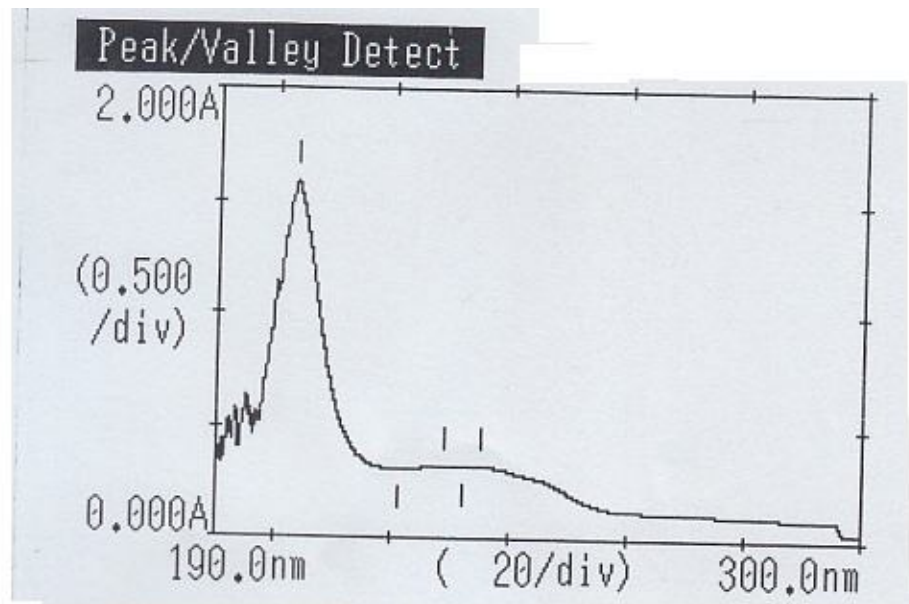
Appendix 45. UV Spectrophotometric determination of conjugating dienes and triene of Groundnut Oil Frying for (4h).



Appendix 46. UV Spectrophotometric determination of conjugating dienes and triene of Groundnut Oil Frying for (6h).

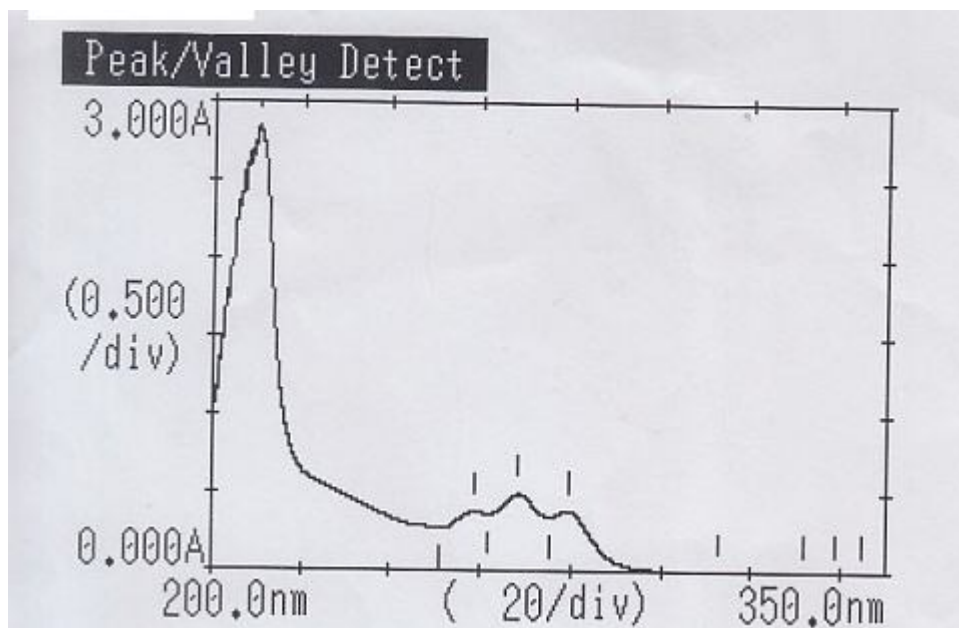


Appendix 47. UV Spectrophotometric determination of conjugating dienes and triene of Groundnut Oil Frying for (12h).



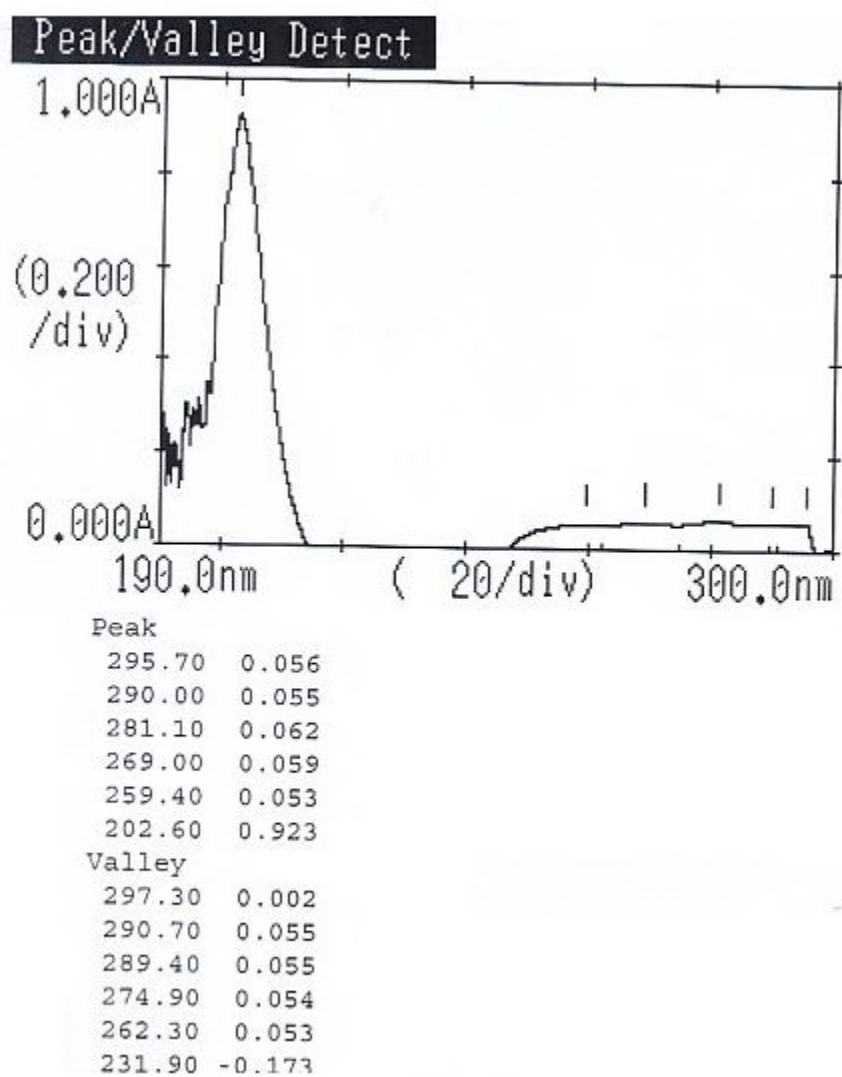
Peak	
235.20	0.322
229.30	0.322
203.40	1.588
Valley	
297.60	0.038
232.30	0.320
221.40	0.310

Appendix 48. UV Spectrophotometric determination of conjugating dienes and triene of Groundnut Oil Frying for (24h).

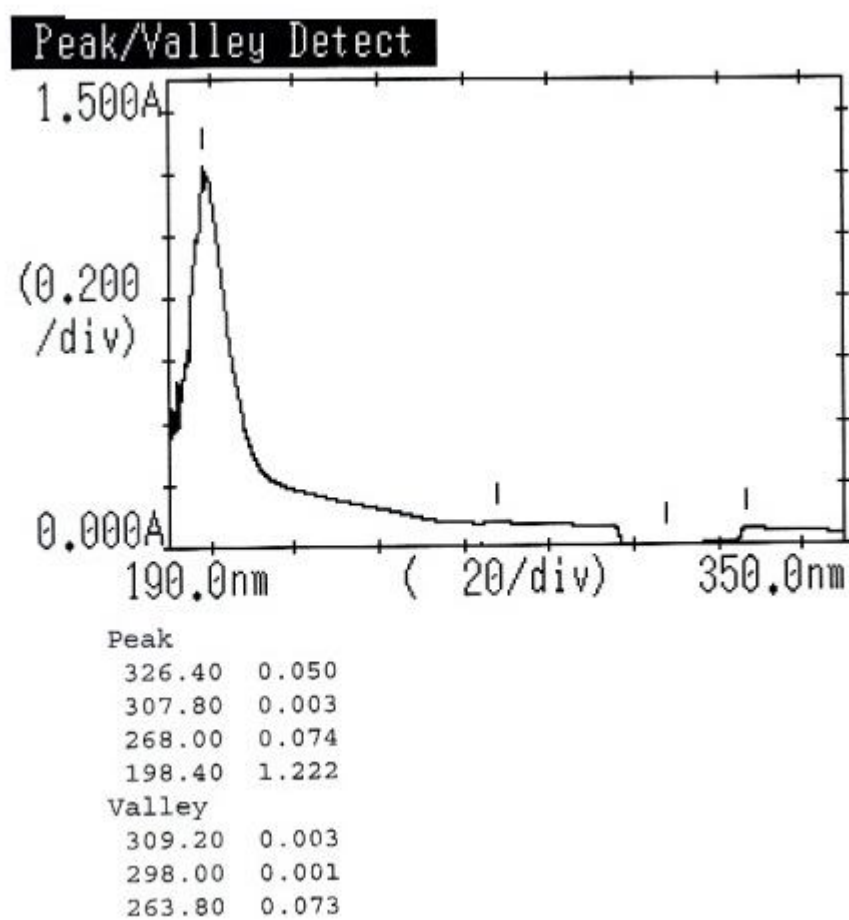


Peak	
344.80	-0.016
338.90	-0.016
331.40	-0.016
312.90	-0.012
279.20	0.381
268.30	0.492
258.80	0.379
210.00	2.840
Valley	
347.70	-0.016
340.30	-0.018
333.00	-0.016
329.90	-0.016
311.00	-0.012
275.20	0.344
261.80	0.366
251.20	0.272

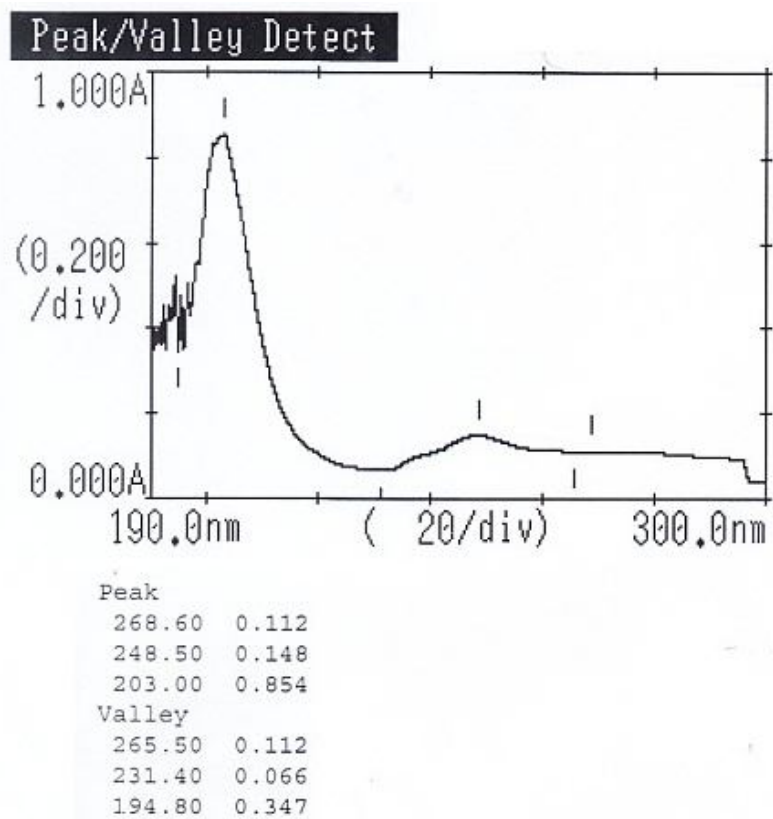
Appendix 49. UV Spectrophotometric determination of conjugating dienes and triene of Groundnut Oil Frying for (36h).



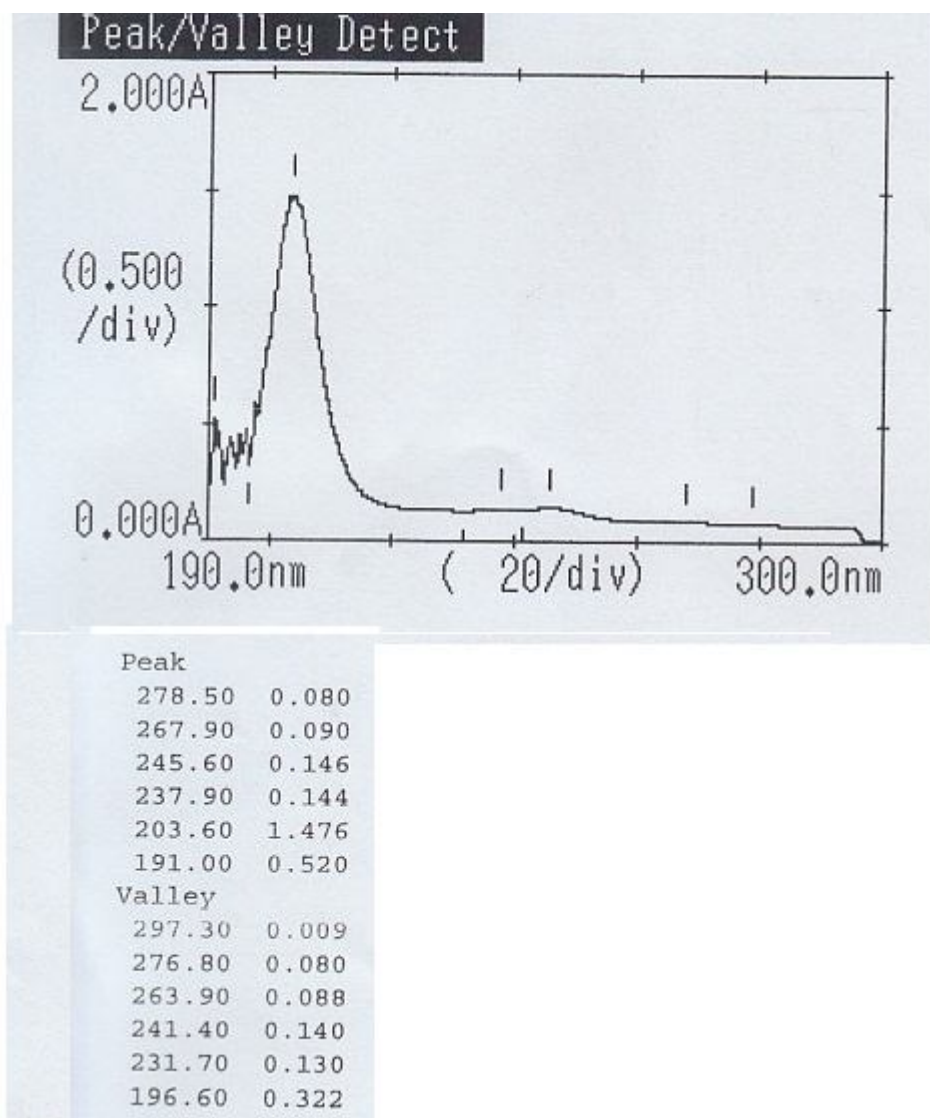
Appendix 50. UV Spectrophotometric determination of conjugating dienes and triene of sunflower Oil Frying for (0h).



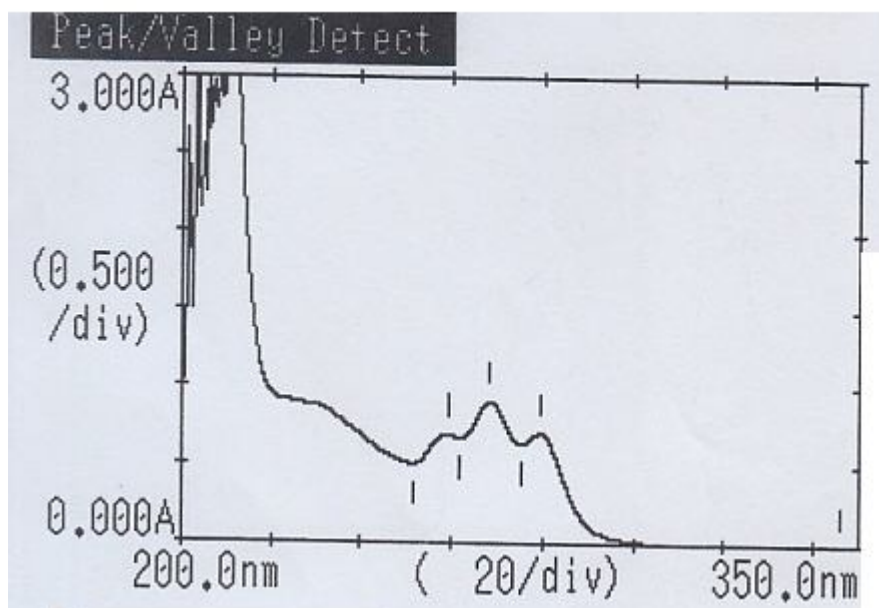
Appendix 51. UV Spectrophotometric determination of conjugating dienes and triene of sunflower Oil Frying for (2h).



Appendix 52. UV Spectrophotometric determination of conjugating dienes and triene of sunflower Oil Frying for (4h).

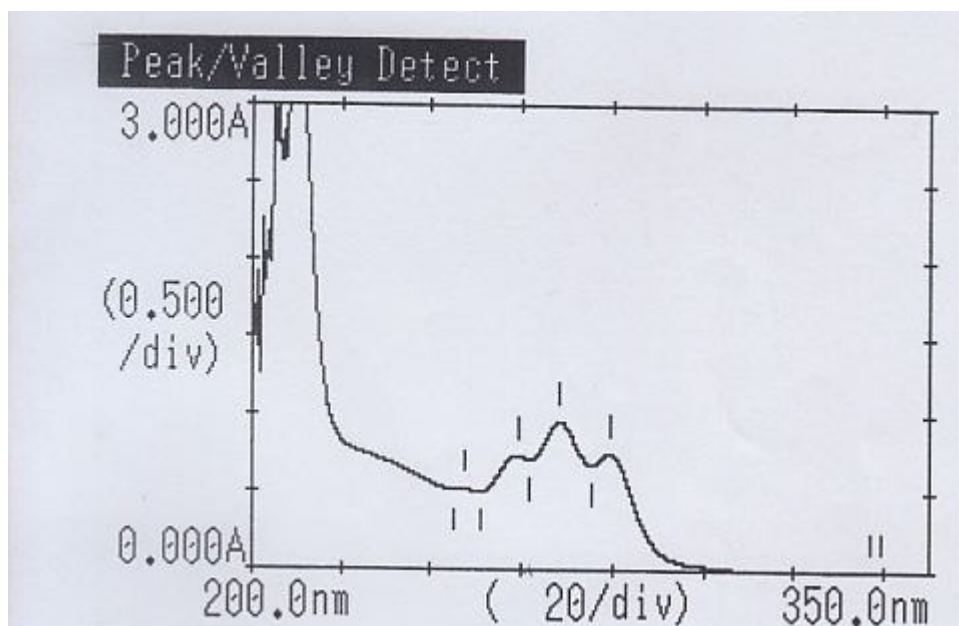


Appendix 53. UV Spectrophotometric determination of conjugating dienes and triene of sunflower Oil Frying for (6h).



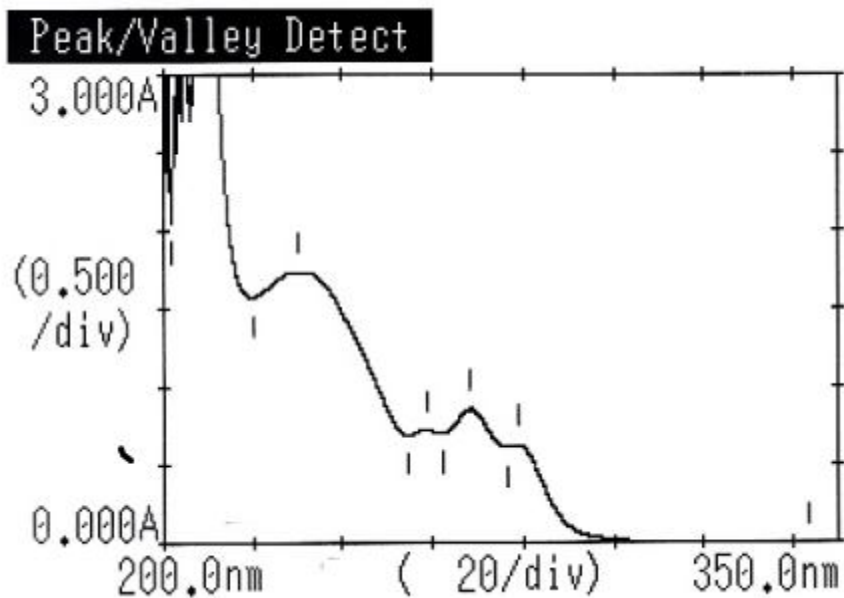
Peak	
345.60	-0.012
279.40	0.703
268.40	0.902
259.00	0.693
Valley	
340.20	-0.013
275.40	0.638
261.80	0.672
251.40	0.503

Appendix 54. UV Spectrophotometric determination of conjugating dienes and triene of sunflower Oil Frying for (12h).



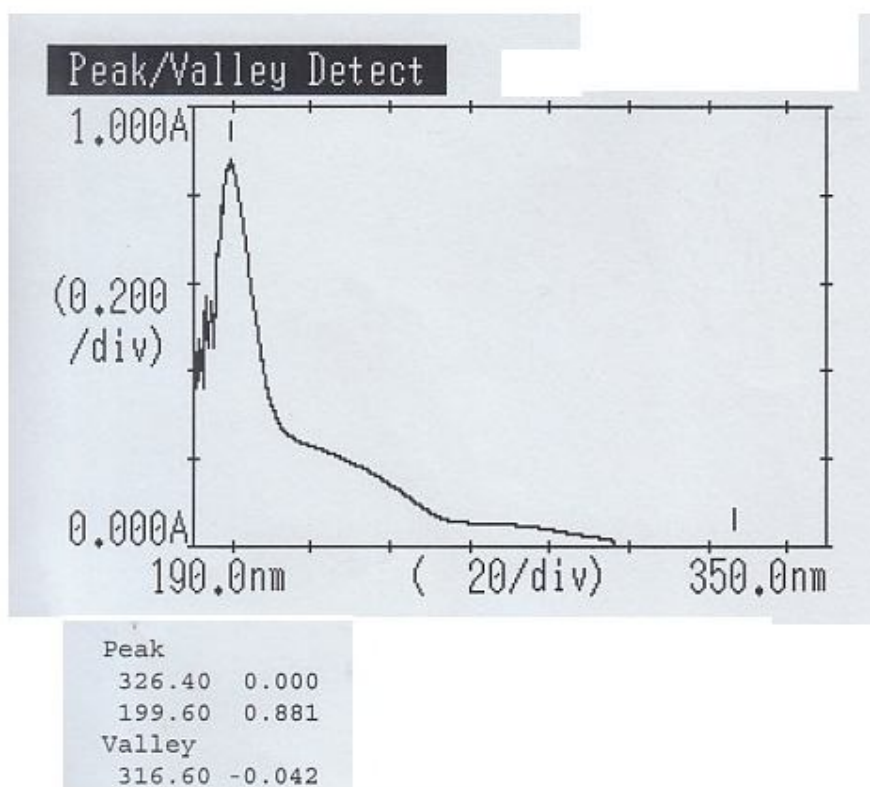
Peak	
339.20	-0.000
336.80	-0.001
279.20	0.745
268.40	0.952
259.00	0.725
247.40	0.513
Valley	
338.00	-0.001
333.40	-0.001
275.20	0.671
261.80	0.704
250.80	0.501
245.00	0.510

Appendix 55. UV Spectrophotometric determination of conjugating dienes and triene of sunflower Oil Frying for (24h).

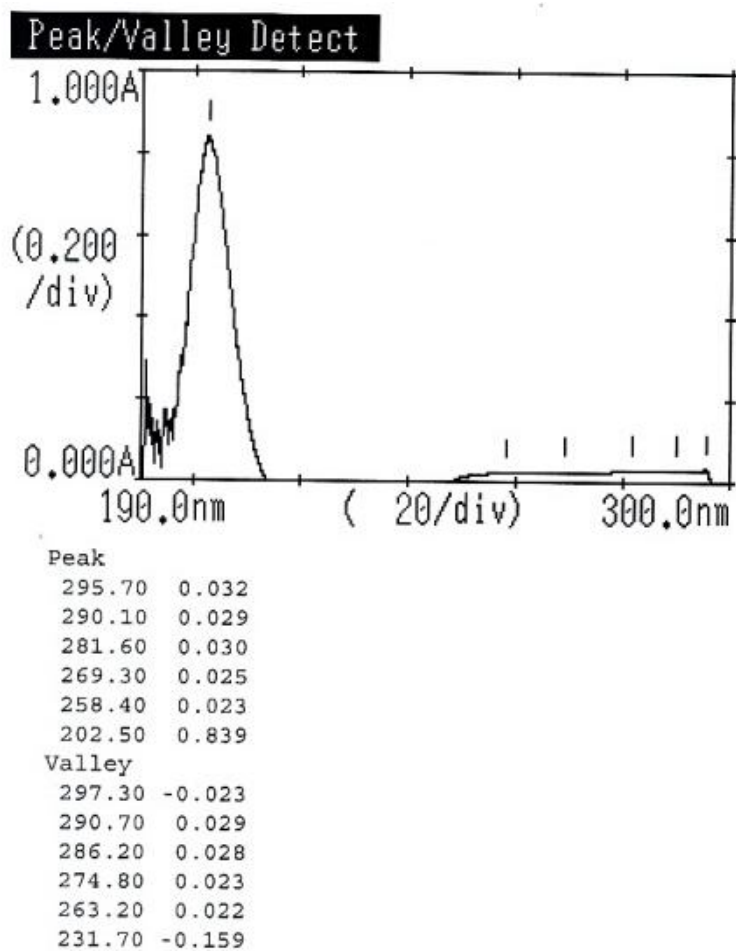


Peak	
343.30	-0.011
278.60	0.625
268.40	0.855
258.50	0.723
230.30	1.730
210.40	3.661
Valley	
340.30	-0.012
276.20	0.613
262.00	0.705
254.40	0.692
220.10	1.572
201.80	2.050

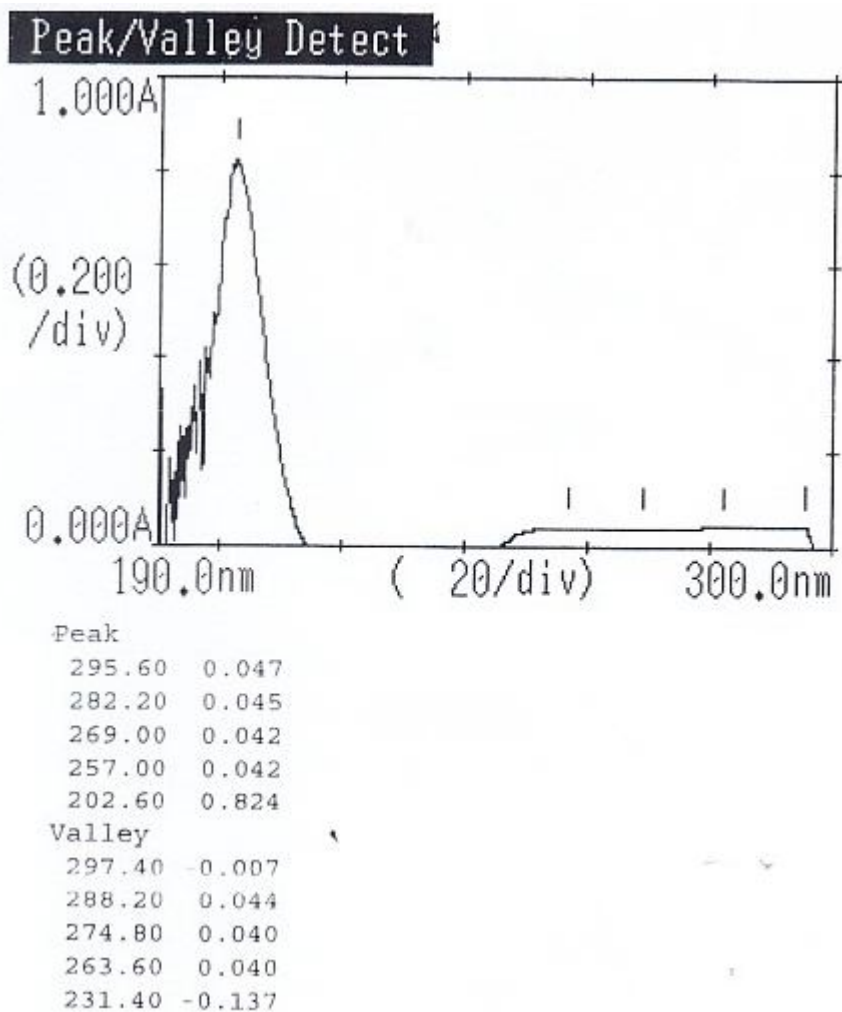
Appendix 56. UV Spectrophotometric determination of conjugating dienes and triene of sunflower Oil Frying for (36h).



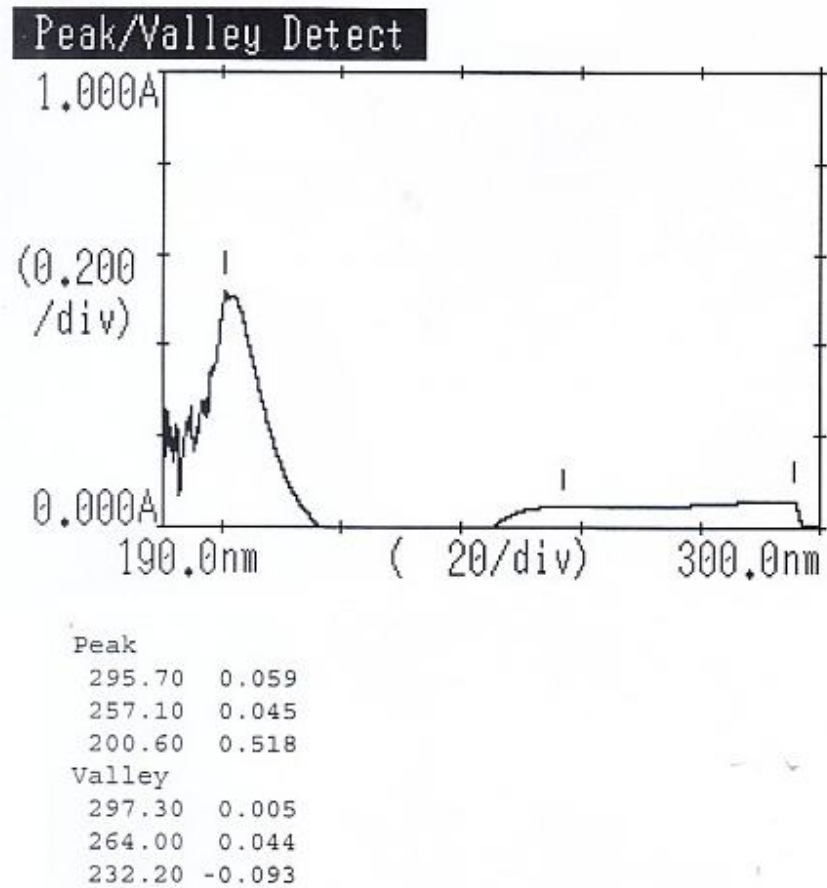
Appendix 57. UV Spectrophotometric determination of conjugating dienes and triene of moringa oliefera Oil Frying for (0h).



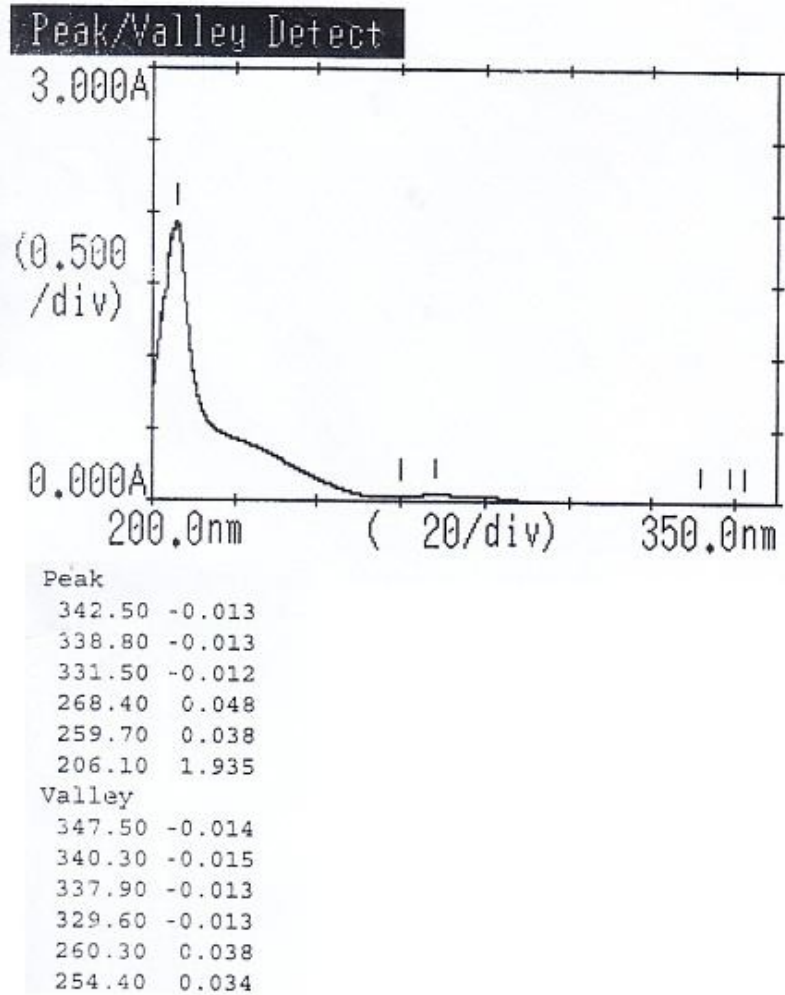
Appendix 58. UV Spectrophotometric determination of conjugating dienes and triene of moringa oliefera Oil Frying for (2h).



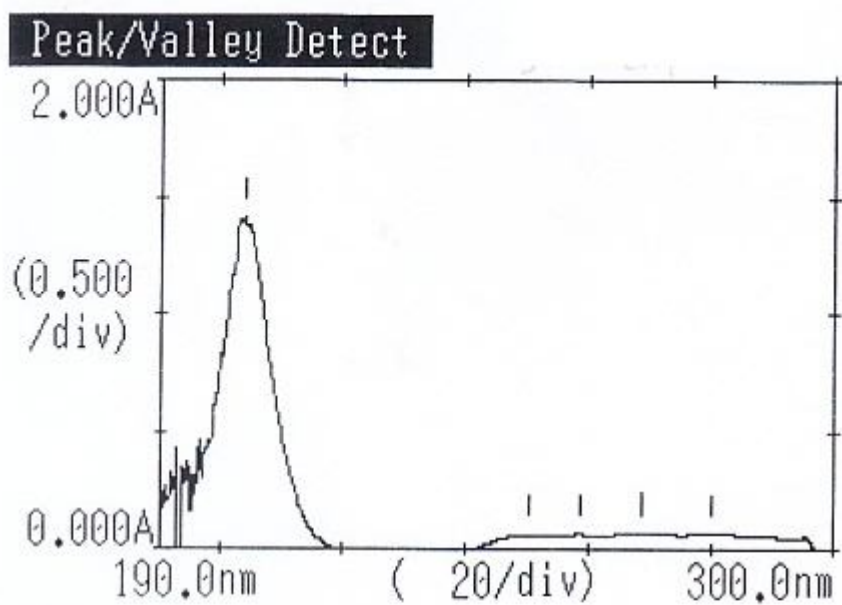
Appendix 59. UV Spectrophotometric determination of conjugating dienes and triene of moringa oliefera Oil Frying for (4h).



Appendix 60. UV Spectrophotometric determination of conjugating dienes and triene of moringa oliefera Oil Frying for (6h).

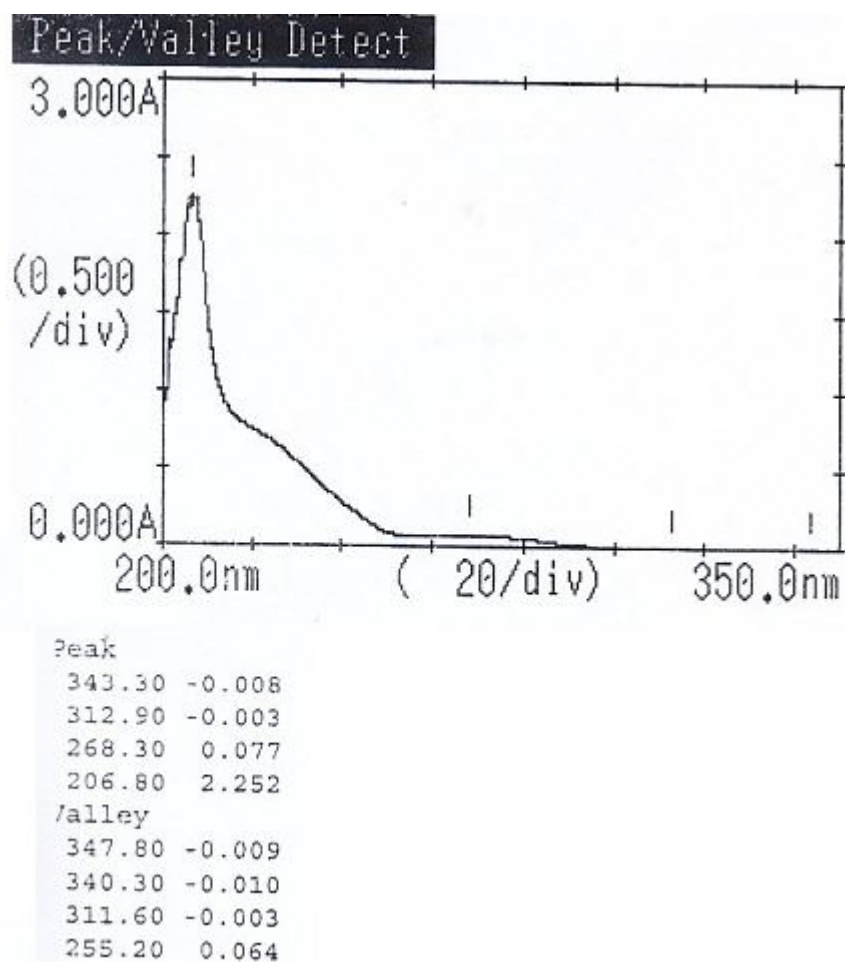


Appendix 61. UV Spectrophotometric determination of conjugating dienes and triene of moringa oliefera Oil Frying for (12h).



Peak	
280.20	0.069
268.80	0.071
258.60	0.063
250.40	0.062
203.80	1.409
Valley	
297.40	-0.003
275.00	0.063
262.00	0.062
253.60	0.061
231.40	-0.057

Appendix 62. UV Spectrophotometric determination of conjugating dienes and triene of moringa oliefera Oil Frying for (24h).



Appendix 63. UV Spectrophotometric determination of conjugating dienes and triene of moringa oliefera Oil Frying for (36h).